Ras homolog gene family H (RhoH) deficiency induces psoriasis-like chronic dermatitis by promoting T_H17 cell polarization

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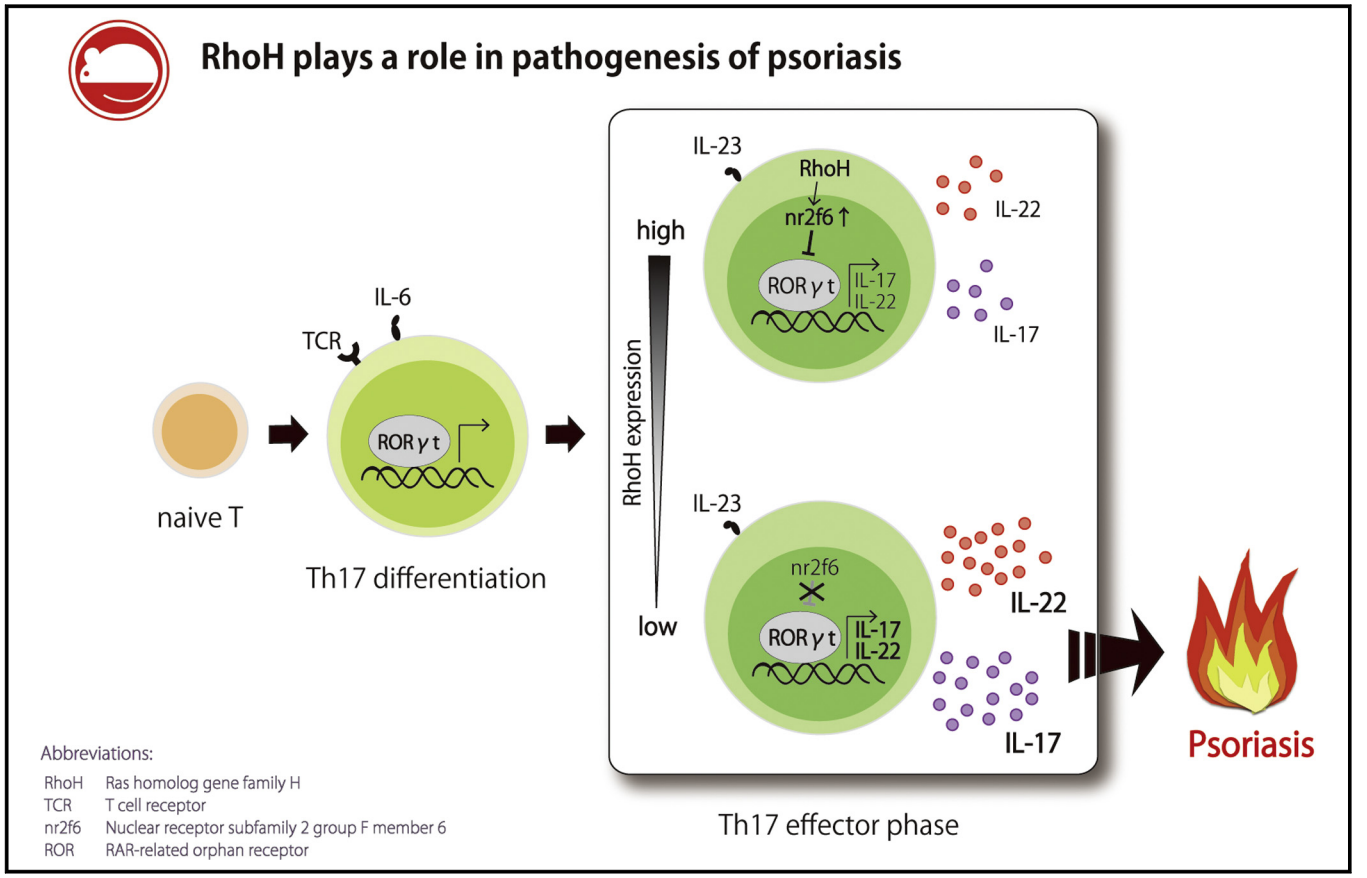
GRAPHICAL ABSTRACT



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Background: Ras homolog gene family H (RhoH) is a membrane-bound adaptor protein involved in proximal T-cell receptor signaling. Therefore RhoH plays critical roles in the differentiation of T cells; however, the function of RhoH in the effecter phase of the T-cell response has not been fully characterized.
Objective: We sought to explore the role of RhoH in inflammatory immune responses and investigated the involvement of RhoH in the pathogenesis of psoriasis.
Methods: We analyzed effector T-cell and systemic inflammation in wild-type and RhoH-null mice. RhoH expression in T cells in human PBMCs was quantified by using RT-PCR.
Results: RhoH deficiency in mice induced T_H17 polarization during effector T-cell differentiation, thereby inducing psoriasis-like chronic dermatitis. Ubiquitin protein ligase E3 component N-recognin 5 (*Ubr5*) and nuclear receptor subfamily 2 group F member 6 (*Nr2f6*) expression levels decreased in RhoH-deficient T cells, resulting in increased protein levels and DNA binding activity of retinoic acid–related orphan receptor γt. The consequential increase in IL-17 and IL-22 production induced T cells to differentiate into T_H17 cells. Furthermore, IL-22 binding protein/Fc chimeric protein reduced psoriatic inflammation in RhoH-deficient mice. Expression of RhoH in T cells was lower in patients with psoriasis with very severe symptoms.
Conclusion: Our results indicate that RhoH inhibits T_H17 differentiation and thereby plays a role in the pathogenesis of psoriasis. Additionally, IL-22 binding protein has therapeutic potential for the treatment of psoriasis. (J Allergy Clin Immunol 2019;143:1878-91.)

Key words: *Psoriasis, T cell, T_H17, IL-22 binding protein, retinoic acid–related orphan receptor γt, Nr2f6, T-cell receptor signaling*

Abbreviations used
cLN: Cervical lymph node
DC: Dendritic cells
EV: Epidermodysplasia verruciformis
Foxp3: Forkhead box protein P3
GFP: Green fluorescent protein
IL-22BP: IL-22 binding protein
LFA-1: Lymphocyte function-associated antigen 1
Nr2f6: Nuclear receptor subfamily 2 group F member 6
PASI: Psoriasis Area and Severity Index
Rag: Recombination-activating gene
RhoH: Ras homolog gene family H
ROR: Retinoic acid–related orphan receptor
STAT: Signal transducer and activator of transcription
γδT17: IL-17−producing γδT
T_C1: IFN-γ–producing $CD8^+$ T
TCR: T-cell receptor
Ubr5: Ubiquitin protein ligase E3 component N-recognin 5
ZAP: Zeta chain–associated protein

Ras homolog gene family H (RhoH) is an atypical small GTPase with no GTPase activity. Our group and others have reported that RhoH plays a critical role in immune cell function by acting as a membrane-bound adapter for various proximal protein kinases, including zeta chain–associated protein (ZAP) 70, spleen tyrosine kinase, lymphocyte-specific protein kinase, and C-terminal Src kinase, thereby modulating signal transduction pathways.[1-6] T-cell development and activation depend on RhoH. Because T-cell receptor (TCR) signaling is strongly inhibited by RhoH deficiency, human RhoH deficiency increases susceptibility to epidermodysplasia verruciformis (EV), a rare inherited skin disease.[7]

Psoriasis is a chronic inflammatory skin disorder characterized by formation of hard scaly plaques[8,9] and hyperproliferation of keratinocytes in the epidermis. Multiple factors contribute to the pathogenesis of psoriasis, including genetic, environmental, and immunologic triggers.[10] T cells are also recognized as a critical mediator of the pathogenesis of psoriasis because effective treatment of psoriasis has been reported uwith cyclosporine.[11,12] The current accepted model of the cause of psoriasis primarily involves the IL-23/IL-22 axis.[13] T_H17 cells activated by IL-23 produce IL-17 and IL-22, which in turn induce keratinocyte proliferation.

Although T cells seem to be important for the progression of psoriasis, the precise role of T cells in the pathogenesis of psoriasis is still unclear, partly because of the lack of a psoriasis mouse model with T-cell defects. The RhoH-deficient mice we generated represent a unique mouse model of psoriasis induced by the loss of a component of the TCR signal transduction pathway. In the current study we demonstrated that an increase in numbers of T_H17 cells led to chronic skin inflammation and clinical symptoms reminiscent of those observed in human psoriasis. We also found that IL-22 binding protein (IL-22BP) improved the psoriasiform dermatitis phenotype in $RhoH^{-/-}$ mice. Our findings offer novel insight into the pathogenesis of psoriasis and highlight the role of IL-22 as a promising target of therapeutic intervention.

METHODS

Mice

BALB/c mice were purchased from SLC Japan (Shizuoka, Japan). BALB/c recombination-activating gene 2 $(Rag2)^{-/-}$ mice (Model 601) were purchased from Taconic Farm (Germantown, NY). $RhoH^{-/-}$ and RhoH transgenic mice were backcrossed onto the BALB/c background strain for more than 10 generations. Conditional knockout mice lacking RhoH were generated by the targeting vector containing locus of X-over P1 (*LoxP*) sites flanking exons 2 and 3 of the *RhoH* locus and flippase flanked a premature stop codon and a neomycin cassette. Embryonic stem cell clones were expanded and injected into primary blastocysts. Generated mice were crossed to the germline flippase deleter to excise the flippase recognition target–flanked cassette. *Cd4-cre* transgenic and ER^{T2-}*cre* transgenic mice were gifts from Dr T. Ikawa (Riken, Wako, Japan). ER^{T2-}*cre* mice were treated daily for 3 days by means of intraperitoneal injection of 4 mg of tamoxifen dissolved in olive oil and allowed to rest for 1 day. $IL\text{-}22^{-/-}$ mice were generated by using CRISPR/Cas9 genome editing systems.

Single-guide RNA was designed for the target sequence (5′-CACAGATGTCCGGCTCATCG-3′) and prepared, as described previously.[14] Briefly, hCas9 mRNAs (100 ng/μL) and single-guide RNAs (50 ng/μL) were coinjected into the cytoplasm of pronuclear stage eggs obtained by means of *in vitro* fertilization from superovulated BALB/c female and male mice. Eggs were transferred into the oviducts of pseudopregnant female mice. The same phenotype of two *Il22* knockout mouse lines, which were identified either 157 or 53 bp deletion upstream from the Protospacer adjacent motif (PAM) sequence, were established from 2 independent embryonic stem cell lines. Mice were treated with intravenous injection of either vehicle (Solutol HS 15/ethanol/H_2O, 15/10/75) or 0.1 mg of lenalidomide.

All mice were bred and maintained under specific pathogen-free conditions in our animal facility. All animal experiments were approved by the Animal Care and Use Committee of the National Center for Global Health Medicine Research Institute (no. 23-Tg-31) and conducted in accordance with institutional procedures.

Flow cytometry and cell sorting

Lymphoid tissues were crushed and passed through a 42-μm-pore nylon mesh 3 times to obtain single-cell suspensions. Skin-infiltrating cells were prepared by digesting skin fragments with collagenase D (Roche, Mannheim, Germany) and DNase I (Roche). Skin lymphoid cells were corrected through a 40% to 70% Percoll step gradient. Cells (1×10^6) were stained with saturating concentrations of indicated antibodies for 30 minutes at 4°C and washed in 200 μL of staining medium (HBSS containing 0.1% BSA and 0.1% sodium azide). If needed, cells were then stained with saturating concentrations of appropriate fluorochrome-conjugated streptavidin or goat F(ab′)2 anti-rabbit IgG for 10 minutes at 4°C. For detection of intracellular cytokines, cells were incubated approximately 5 hours after initiation of phorbol 12-myristate 13-acetate plus ionomycin stimulation under brefeldin A treatment. Then cells were fixed with 2% paraformaldehyde for 30 minutes, permeabilized with 0.1% saponin, and stained with antibodies. Forkhead box protein P3 (Foxp3) staining was performed with the Foxp3 staining kit (eBioscience, San Diego, Calif), according to the manufacturer's protocol. Cells were analyzed on FACSCanto II (Becton Dickinson, San Jose, Calif). For cell sorting of naive, green fluorescent protein $(GFP)^+$, and $CCR6^+$ CD4 T cells, isolated cells were stained and sorted with the FACSAria III (Becton Dickinson).

Antibodies

Monoclonal antibodies specific for B220 (RA3-6B2), CD4 (GK1.5), CD8α (53-6.7), CD25 (PC61.5), CD62L (MEL-14), NK1.1 (PK136), TCRβ (H57-597), Foxp3 (FJK-16s), and retinoic acid–related orphan receptor (ROR) γt (B2D) were purchased from eBioscience. Monoclonal antibodies against CD3ε (2C11), CD4 (OKT4), CD11a (H155-78), CD44 (IM7), integrin β7 (FIB504), CCR6 (29-2L17, G034E3), TCRαβ (IP26), TCRγδ (GL3), IFN-γ (XMG1.2), IL-2 (JES6-5H4), IL-17A (TC11-18H10.1), IL-17F (9D3.1C8), and IL-22 (1H8PWSR) were purchased from BioLegend (San Diego, Calif). Monoclonal antibodies targeting CD3ε (2C11), CD28 (37.51), IL-4 (554435), IFN-γ (XMG1.2), and Ly-6G (1A8) were purchased from BD Biosciences. Polyclonal and monoclonal antibodies for Loricrin (Covance, Princeton, NJ), Ki67 (Abcam, Cambridge, United Kingdom), and keratin 14 (Covance) were used for immunohistochemistry.

Histologic analysis

Tissues were fixed overnight in 4% paraformaldehyde, dehydrated, paraffin embedded, sectioned (6 μm), and stained with hematoxylin and eosin. Frozen tissues embedded in OCT compound (Sakura Finetek, Torrance, Calif) were sliced into 5-μm-thick sections with a cryostat (Leica, Wetzlar, Germany), air-dried, fixed with acetone, and stained with hematoxylin and eosin or specific antibodies, as described previously. Multicolor images were obtained with a BZ-9000 fluorescence microscope (Keyence, Osaka, Japan).

Quantitative mRNA analysis

Total cellular RNA was reverse transcribed with Superscript III first-strand synthesis supermix using quantitative RT–PCR (Life Technologies, Grand Island, NY). Real-time PCR was performed with the SYBR Premix ExTaq (TaKaRa, Kusatsu, Japan) and StepOne Real-Time PCR System (Life Technologies).

Cytokine analysis

Cytokine measurement was performed with mouse plasma incubated with the Bio-Plex Pro Mouse Cytokine Group III 8-plex panel plate and processed according to the manufacturer's instructions (Bio-Rad Laboratories, Hercules, Calif). Data were collected and analyzed on a Bio-Plex 3D Suspension Array System (Bio-Rad).

In vitro cell differentiation

Sorted naive $CD4^+$ T cells were activated with antibodies against CD3 and CD28 (2 μg · mL^{-1}) in the presence of recombinant human TGF-β1 (0.5 ng · mL^{-1}; PeproTech, Rocky Hill, NJ), IL-1β (20 ng · mL^{-1}; 21-8012-U010; Tonbo Biosciences, San Diego, Calif), and IL-23 (20 ng · mL^{-1}; 14-8213-63; eBioscience) and recombinant murine IL-6 (40 ng · mL^{-1}; 406-ML; R&D Systems, Minneapolis, Minn) plus anti–IFN-γ (20 μg · mL^{-1}) and anti–IL-4 (20 μg · mL^{-1}) antibodies.

Chromatin immunoprecipitation assay

Chromatin immunoprecipitation assays were performed with the chromatin IP Assay kit (Millipore, Temecula, Calif). Briefly, cells were fixed in a formaldehyde solution and then resuspended in SDS lysis buffer. Lysates were sonicated with an E220 focused-ultrasonicator (Covaris, Woburn, Mass) to a desired fragment size distribution of 100 to 200 bp. Immunoprecipitation reactions were performed with anti-RORγt (eBioscience) or anti–nuclear receptor subfamily 2 group F member 6 (Nr2f6; GeneTex, Irvine, Calif) antibodies. Chromatin immunoprecipitates were quantified by using the real-time PCR system.

Human blood samples

Blood samples were collected from control subjects or patients with psoriasis before receiving any medical treatment. Informed consent was obtained from each participant. Sixteen patients with psoriasis between 23 and 84 years of age (average, 61.4 years; female, 37.5%) were included in this study. Eleven healthy volunteers who were 34 to 73 years of age (average, 54.0; female, 36.36%) and taking no medication served as control subjects. PBMCs were isolated from blood samples with a lymphocyte separating medium. Total RNA was extracted with RNeasy. These studies were approved by the Nagoya City University Institutional Review Board.

Purification of IL-22BP–Fc chimera

293T cells were transfected with the construct encoding the fusion protein IL-22BP (full-length)/Fc portion of human IgG and cultures in GIT medium. IL-22BP/Fc chimera protein were purified with protein A Sepharose beads.

Statistical analysis

Statistical analyses were performed with GraphPad Prism statistical analysis software (GraphPad Software, La Jolla, Calif). Group differences were analyzed by using the unpaired Student *t* test or 2-way ANOVA with multiple comparisons, followed by Tukey posttest comparisons, for 3 or more groups. *P* values of .05 or less were considered significant.

RESULTS

RhoH$^{-/-}$ BALB/c mice had a chronic skin disorder

The SKG mutation is a naturally occurring loss-of-function mutation in *ZAP70* that disrupts thymic T-cell selection and triggers autoimmune arthritis in BALB/c mice.[15] Although RhoH functions as an adaptor for ZAP-70, *RhoH*$^{-/-}$ mice never had arthritis or any signs of autoimmune symptoms, such as autoantibody production or intestinal degeneration, even after backcrossing mice to a BALB/c background for more than 10 generations (see Fig E1 in this article's Online Repository at

www.jacionline.org). Unexpectedly, however, after 10 weeks of age, *RhoH*$^{-/-}$ mice spontaneously had inflammation in the skin of the eyelid, ear, and tail (Fig 1, *A*) and exhibited mild weight loss (see Fig E2 in this article's Online Repository at www.jacionline.org). Immunohistochemistry staining for loricrin and keratin revealed signs of epidermal hyperplasia (acanthosis) and parakeratosis in the inflamed tissue (Fig 1, *C* and *E*). We also observed elongated capillaries with Ki67^{+} proliferating keratinocytes in the basal layer of the epidermis (Fig 1, *D*). *RhoH*$^{-/-}$ mice also exhibited intraepidermal microabscesses containing Ly-6G^{+} cells, indicating that neutrophils had been recruited to the inflamed lesions (Fig 1, *E*). We also found that CD11c^{+} dendritic cells (DCs) and CD4^{+} T cells had infiltrated the dermis of *RhoH*$^{-/-}$ mice (Fig 1, *E* and *F*). Furthermore, physiologic stimuli (tape-stripping) applied to the dorsal skin induced epidermal thickening reminiscent of what was observed in the eyelids of *RhoH*$^{-/-}$ mice (the Koebner phenomenon; see Fig E3 in this article's Online Repository at www.jacionline.org). We also found that repeated administration of zymosan, a molecule that promotes T_H17 cell differentiation,[16] accelerated and augmented the severity of dermatitis symptoms in *RhoH*$^{-/-}$ mice, even when the mice were young (see Fig E4 in this article's Online Repository at www.jacionline.org). Taken together, *RhoH*$^{-/-}$ mice exhibited multiple hallmarks of human psoriasis.

Loss of RhoH in T cells induced dermatitis

To investigate the role of lymphocytes in the pathogenesis of chronic dermatitis in *RhoH*$^{-/-}$ BALB/c mice, we outcrossed these mice to *Rag2*$^{-/-}$ mice. As shown in Fig 1, *G*, dermatitis did not develop in mice lacking lymphocytes, despite the presence of innate lymphoid cells, indicating that mature lymphocytes (T and/or B cells) are an absolute requirement for disease progression.

Next, we crossed *RhoH*$^{-/-}$ BALB/c mice to mice expressing RhoH-Tg under the control of the human CD2 promoter to restore T-cell development.[6] *RhoH*$^{-/-,\ RhoH\text{-}Tg}$ mice, which express RhoH exclusively in T cells, did not have dermatitis (Fig 1, *H*), indicating that RhoH deficiency in T cells was critical for the induction of dermatitis. In addition, total splenocytes transferred from BALB/c *RhoH*$^{-/-}$ mice into BALB/c *nu/nu* mice induced mild eyelid inflammation after 2 to 4 weeks (see Fig E5 in this article's Online Repository at www.jacionline.org). Although the Koebner phenomenon in *RhoH*$^{-/-}$ splenocytes transplanted into *nu/nu* mice was not tested, these results clearly demonstrated that the loss of RhoH in T cells induced dermatitis in BALB/c *RhoH*$^{-/-}$ mice.

Proportion of T_H17 cell and IFN-γ–producing CD8^{+} T cells increased in *RhoH*$^{-/-}$ mice

Because the significance of T_H17 cells in the pathogenesis of psoriasis is widely accepted,[17] we analyzed the T_H17 cell polarization in *RhoH*$^{-/-}$ mice. The T_H17-associated cytokines IL-17, IL-22, and IL-23 and the proinflammatory cytokine IL-6 were significantly upregulated in inflamed skin lesions and blood from *RhoH*$^{-/-}$ mice (Fig 2, *A* and *B*, and see Fig E6 in this article's Online Repository at www.jacionline.org). Plasma IL-21 and IL-2 levels were not changed in *RhoH*$^{-/-}$ mice. Accordingly, there was an increase in infiltration of IL-17A^{+}CD4^{+} T_H17 cells into dermal lesions in *RhoH*$^{-/-}$ mice (Fig 2, *C*). There was a general decrease in the number of peripheral mature T cells in *RhoH*$^{-/-}$ mice because of impaired T-cell development (see Fig E7, *A* and *B*, in this article's Online Repository at www.jacionline.org).[18,19] However, *RhoH*$^{-/-}$ mice with dermatitis symptoms exhibited an increase in the number of CD4^{+} and CD8^{+} T cells exclusively in the cervical lymph nodes (cLNs), which function as the draining lymph nodes of the eyelids (Fig 3, *A* and *B*). β2-(lymphocyte function-associated antigen 1 [LFA-1]) and β7-integrins were expressed at higher levels in T_H17 cells in cLNs of *RhoH*$^{-/-}$ mice (see Fig E8 in this article's Online Repository at www.jacionline.org). The proportion of T_H17 cells significantly increased in cLNs (Fig 3, *C* and *D*), inguinal LNs, mesenteric LNs, and spleens of *RhoH*$^{-/-}$ mice. T_H17 cells express the chemokine receptor CCR6 at the cell surface (see Fig E7, *C*).[20] The number of CD4^{+}CCR6^{+} cells increased in *RhoH*$^{-/-}$ lymph nodes and spleens (see Fig E7, *D*).

γδ T cells are an alternative source of IL-17, and IL-17–producing γδT (γδT17) cells are reported to be involved in patients with psoriasis-like dermatitis.[21] However, the number of γδT17 cells in the cLNs of *RhoH*$^{-/-}$ mice decreased compared with *RhoH*$^{+/+}$ mice (see Fig E7, *F*). Similar to T_H17-related cytokines, IFN-γ is considered a critical mediator of psoriasiform skin inflammation because IFN-γ–producing CD8^{+} T (T_C1) cells can initiate and orchestrate psoriasis-like skin inflammation.[22,23] Consistent with this finding, the number of T_C1 cells increased in the lymph nodes of *RhoH*$^{-/-}$ mice (Fig 3, *C* and *D*).

To test whether the increase in numbers of T_H17 and T_C1 cells was simply a consequence of chronic dermatitis, we analyzed young BALB/c *RhoH*$^{-/-}$ mice before development of dermatitis, as well as C57BL/6 *RhoH*$^{-/-}$ mice, which never have dermatitis. Even in the absence of inflammatory symptoms, the proportion of T_H17 cells increased in young BALB/c *RhoH*$^{-/-}$ mice and in C57BL/6 *RhoH*$^{-/-}$ mice (Fig 3, *G* and *H*). These findings demonstrated that the increase in numbers of T_H17 and T_C1 cells was not the result of inflammation but was a direct consequence of RhoH deficiency. Accordingly, the ectopic expression of RhoH exclusively in T cells in *RhoH*$^{-/-,\ RhoH\text{-}Tg}$ mice abolished the increase in numbers of T_H17 and T_C1 cells (Fig 3, *I* and *J*, see Fig E7).

To verify that the increase in T_H17 and T_C1 cells was due to T cell–intrinsic effects, we generated a conditional RhoH deletion in T cells by using mice harboring a Cre-recombinase transgene under the control of the CD4 promoter. Similar to global RhoH deficiency, the conditional deletion of RhoH in mature T cells also induced an increase in the number of T_H17 and T_C1 cells (see Fig E9, *A* and *B*, in this article's Online Repository at www.jacionline.org), indicating that this effect was a direct result of RhoH deficiency in T cells. Collectively, these results demonstrate that the absence of RhoH in T cells leads to an increase in the number of T_H17 and T_C1 cells *in vivo*.

T_H17 cells in *RhoH*$^{-/-}$ mice induced psoriasis-like dermatitis

T_H17 cells play a role in the pathogenesis of psoriasis progression. To investigate whether psoriasis-like dermatitis in *RhoH*$^{-/-}$ mice could be due to the observed increase in numbers of T_H17 cells, we transferred isolated CD4^{+}CCR6^{+} T_H17 cells from young BALB/c *RhoH*$^{-/-}$ mice into BALB/c *Rag*$^{-/-}$ mice. CD4^{+}CCR6^{+} cells from *RhoH*$^{-/-}$ mice promoted the

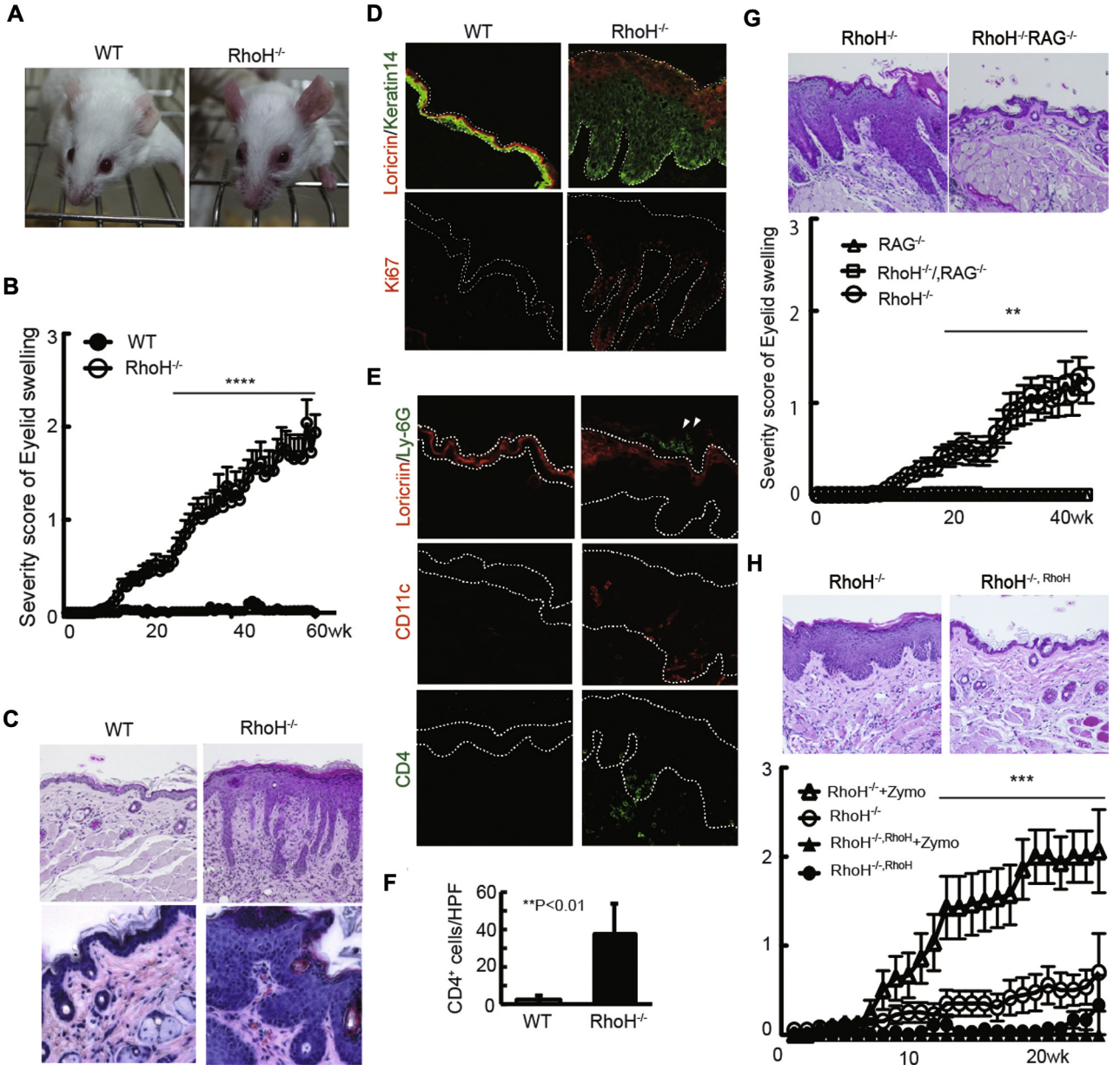

FIG 1. *RhoH*$^{-/-}$ mice have psoriatic lesions. **A** and **B,** Blepharophimosis in 1-year-old *RhoH*$^{-/-}$ mice (Fig 1, *A*) and dermatitis scores at the indicated periods of time (Fig 1, *B*). Disease severity was assessed once a week on a scale of 0 to 3: 0, no inflammation; 0.5, puffy eye; 1, eyelid swelling; and 1.5, eyelid erosion for each eye. Data shown are means from 51 mice (data points, >11). ****$P < .0001$, 2-way ANOVA and the Bonferroni test with multiple comparisons. **C,** Representative eyelid skin sections stained with hematoxylin and eosin. **D** and **E,** Immunohistochemical staining for loricrin-positive, keratin 14–positive, Li67^{+} keratinocytes; Ly6-G^{+} neutrophils; CD11c^{+} DCs; and CD4^{+} T cells. **F,** Migratory CD4^{+} T cells in mouse skin sections. **$P < .01$, unpaired *t* test. **G,** Eyelid sections (*top*) stained with hematoxylin and eosin and mean clinical scores (*bottom*) in *RhoH*$^{-/-}$, *Rag2*$^{-/-}$, and *RhoH*$^{-/-}$*Rag2*$^{-/-}$ mice with dermatitis. **H,** Hematoxylin and eosin-stained eyelid sections (*top*) and clinical scores in *RhoH*$^{-/-}$ and *RhoH*$^{-/-,\ RhoH\text{-}Tg}$ mice with dermatitis induced by 15 mg/kg zymosan (*bottom*). ***$P < .001$.

progression of skin inflammation in *Rag*$^{-/-}$ mice, whereas *RhoH*$^{+/+}$ CD4^{+}CCR6^{+} cells did not have any effect (Fig 4, *A* and *B*). IL-17 inhibition by mAbs targeting IL-17 (secukinumab and ixekizumab) and IL-17RA (brodalumab) have successfully shown significant improvement in patients with psoriasis. Administration of anti–IL-17A/IL-17RA antibodies ameliorated skin inflammation in *RhoH*$^{-/-}$ mice (see Fig E10 in this article's Online Repository at www.jacionline.org), indicating the importance of T_H17 cells in the pathogenesis of our dermatitis model. These results indicate that the loss of RhoH in T cells led to an increase in T_H17 cells *in vivo* and that the increase in T_H17 cells caused psoriasis-like chronic dermatitis.

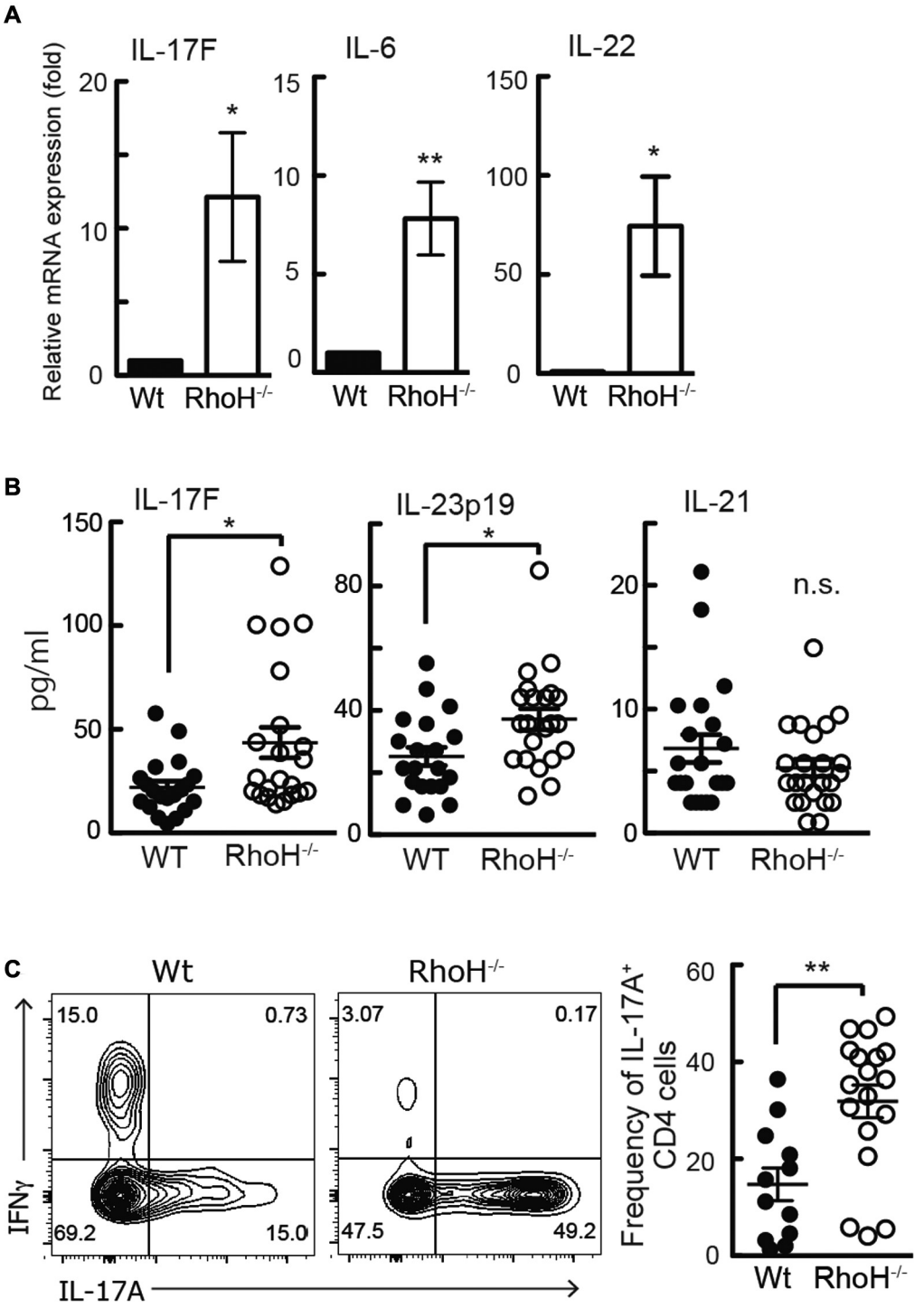

FIG 2. T cell–intrinsic immune responses in psoriatic inflammation in *RhoH*$^{-/-}$ mice. **A,** *Il17F*, *Il6*, and *Il22* mRNA levels in the eyelids of wild-type (*Wt*; n = 10) and *RhoH*$^{-/-}$ (n = 11) mice. **B,** Serum concentrations of IL-17 and IL-23p19 determined by using Bio-Plex. **C,** Representative fluorescence-activated cell sorting plots of intracellular IFN-γ versus IL-17A staining in CD4^{+} T cells that had infiltrated the eyelid (*left*). Numbers represent the proportion of stained cells in the total population of live CD4^{+} T cells. The scatter plot presents quantitative analysis of CD4^{+}IL-17A^{+} T_H17 cells with more than 12 mice per group. *$P < .05$ and **$P < .01$. *n.s.*, Not significant.

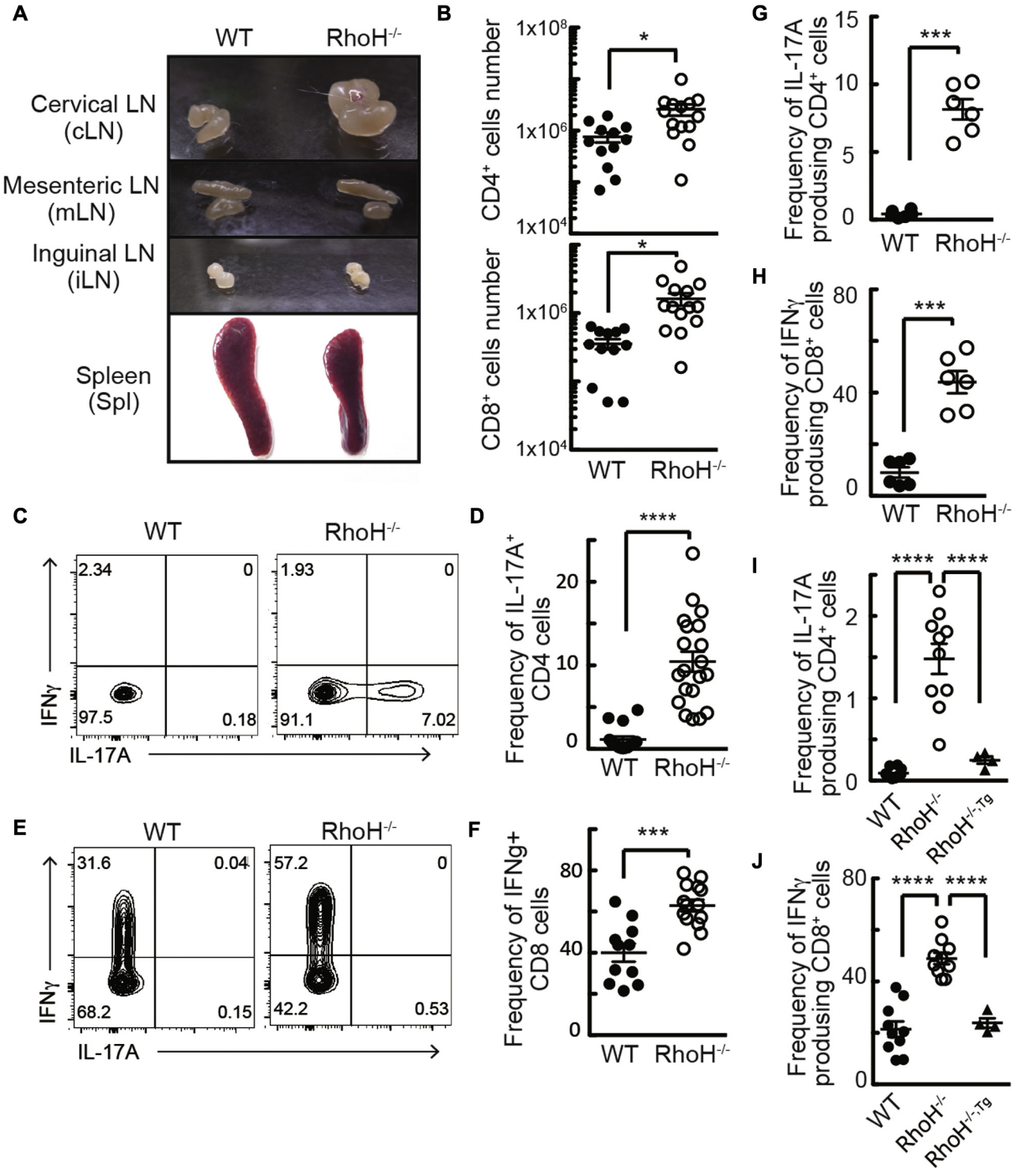

FIG 3. RhoH is essential for T_H17 cell development. **A,** Representative spleens and lymph nodes from *RhoH*$^{-/-}$ mice (*right*) and age-matched wild-type (*WT*) mice (*left*). **B,** CD4$^+$ T cells (*top*) and CD8$^+$ T cells (*bottom*) in cLNs from *RhoH*$^{-/-}$ and WT mice. **C** and **E,** Flow cytometric analysis of IFN-γ versus IL-17A production in CD4$^+$ (Fig 3, *C*) and CD8$^+$ (Fig 3, *E*) T cells from cLNs. **D** and **F,** Data represent means ± SEMs of IL-17$^+$CD4$^+$ cells (Fig 3, *D*) and IFN-γ$^+$CD8$^+$ T cells (Fig 3, *F*) from more than 11 mice per group. **G** and **H,** Proportion of IL-17A$^+$CD4$^+$ (Fig 3, *G*) and IFN-γ$^+$CD8$^+$ T cells (Fig 3, *H*) in wild-type and *RhoH*$^{-/-}$ C57BL/6 mice. **I** and **J,** Proportion of IL-17A$^+$CD4$^+$ (Fig 3, *I*) and IFN-γ$^+$CD8$^+$ (Fig 3, *J*) T cells in *RhoH*$^{-/-,\ RhoH\text{-}Tg}$ mice. **P* < .05, ****P* < .001, and *****P* < .0001.

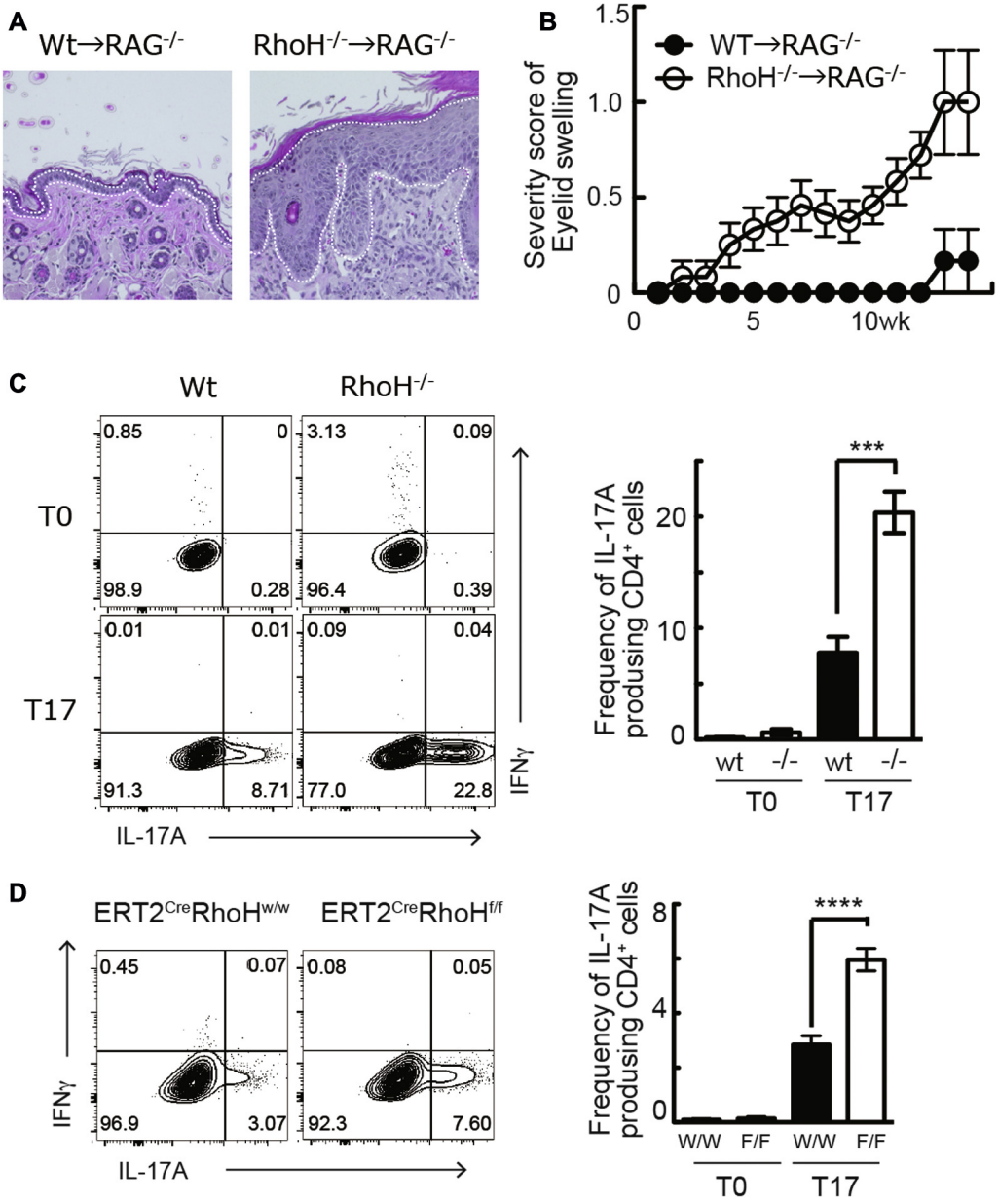

FIG 4. RhoH inhibits T_H17 cell differentiation. **A** and **B,** Histology and clinical scores of eyelid sections from mice with dermatitis induced by adoptive transfer of T_H17 cells. **C,** Proportion of IL-17A$^+$CD4$^+$ T cells in naive CD4 T cells grown under T_H17-polarizing conditions for 4 days. Cells stained for intracellular IL-17A and IFN-γ and were gated on live CD4$^+$ T cells. **D,** Proportion of IL-17$^+$ cells in naive CD4$^+$ T cells isolated from *RhoH$^{fl/fl}$ cre-ERT2$^+$* mice exposed to tamoxifen and grown under T_H17-polarizing conditions for 3 days. ***$P < .001$ and ****$P < .0001$. *WT,* Wild-type.

RhoH deficiency promotes T_H17 differentiation *in vitro*

Next, we sought to determine the mechanism underlying the increase in numbers of T_H17 cells in *RhoH*$^{-/-}$ mice. Because the increase in numbers of T_H17 cells appeared to be a T cell–intrinsic effect, we assessed the effect of RhoH deficiency on T_H17 differentiation *in vitro*. Although a subset of peripheral T cells in *RhoH*$^{-/-}$ mice had already skewed toward a T_H17 phenotype (Fig 3, *C*, *D*, *G*, and *I*), they exhibited a CD44^{hi}CD62L^{lo} activated phenotype, and the population of CD44^{lo}CD62L^{hi} naive T cells did not include IL-17–producing cells (see Fig E11 in this article's Online Repository at www.jacionline.org). Therefore we isolated naive CD62L^{hi}CD4$^+$ T cells from *RhoH*$^{-/-}$ mice and incubated them in T_H17 differentiation medium. RhoH is a critical component of the TCR signaling pathway, and therefore the response to TCR stimulation was strongly inhibited in *RhoH*$^{-/-}$ T cells.[6] As expected, T_H17 differentiation induced by stimulation with ordinary concentrations of anti-TCR (5–10 μg/mL monoclonal anti-CD3) was inhibited in *RhoH*$^{-/-}$ naive T cells (see Fig E12 in this article's Online Repository at www.jacionline.org). However, differentiation into T_H17 cells was enhanced in *RhoH*$^{-/-}$ T cells stimulated with higher concentrations of

anti-CD3 (100–500 μg/mL; Fig 4, C; see Fig E12). This enhancement was specific to T_H17 induction because differentiation into the T_H1, T_H2, and regulatory T lineages was comparable in cells treated with the stimulating antibody (see Fig E13 in this article's Online Repository at www.jacionline.org).

To exclude the possibility that the upregulation of T_H17 differentiation was due to changes in naive $CD4^+$ T cells resulting from impaired T-cell development, we analyzed mice in which RhoH was deleted after normal thymic differentiation by using a tamoxifen-inducible Cre-ERT2 system. The upregulation of T_H17 differentiation in *RhoH*$^{-/-}$ naive T cells was also observed in naive T cells isolated from mice with the conditional deletion of RhoH in mature T cells (Fig 4, *D*), indicating that this effect was not an indirect consequence of impaired T-cell development. In addition, lenalidomide-mediated inhibition of RhoH[24] also enhanced T_H17 cell differentiation in naive T cells (see Fig E14, *A* and *B*, in this article's Online Repository at www.jacionline.org).

Furthermore, we generated a conditional mutant in which RhoH was exclusively deleted in peripheral T cells by crossing mice harboring a *cd2-cre* transgene with *RhoH*$^{fl/fl}$ mice.[25] As shown in Fig E14, *A*, the loss of RhoH in peripheral T cells also promoted T_H17 differentiation (see Fig E14, *C*). Together, these results indicate that loss of RhoH in mature naive T cells promotes differentiation into T_H17 cells.

RhoH orchestrates RORγt-mediated transcription

To elucidate the role of RhoH in T_H17 differentiation, we evaluated T_H17-associated signal transduction. IL-6 receptor–mediated signaling is critical for the induction of RORγt, a master transcriptional regulator of T_H17 cell differentiation. Therefore we evaluated IL-6 receptor–mediated signal transducer and activator of transcription (STAT) 3 activation in *RhoH*$^{-/-}$ T cells. However, IL-6–induced STAT3 phosphorylation was comparable in *RhoH*$^{-/-}$ T cells and wild-type T cells (see Fig E15, *A*, in this article's Online Repository at www.jacionline.org).

Next, we evaluated expression levels of various T_H17-related genes. Consistent with upregulation of T_H17 differentiation, *Il17a/f* and *Il22* expression levels markedly increased in *RhoH*$^{-/-}$ T cells cultured in T_H17-polarizing conditions for 4 days (Fig 5, *A*). However, expression levels of IL-21, another pleiotropic cytokine produced by T_H17 cells, did not increase. Consistent with these findings, serum levels of IL-17 increased in *RhoH*$^{-/-}$ mice, whereas serum levels of IL-21 remained unchanged (Fig 2, *B*). Transcription of *Il17* and *Il22*, but not *Il21*, depends on RORγt. Therefore we predicted that *RORγt* expression levels could be increased in *RhoH*$^{-/-}$ T cells. However, levels of mRNA for RORγt and RORα decreased in *RhoH*$^{-/-}$ T cells in comparison with *RhoH*$^{+/+}$ T cells (Fig 5, *A*). In contrast, RORγt protein levels significantly increased in *RhoH*$^{-/-}$ T cells (Fig 5, *B*), suggesting that RhoH might be involved in the posttranscriptional regulation of RORγt.

Consistent with these results, TCR-induced expression of ubiquitin protein ligase E3 component N-recognin 5 (*Ubr5*), a gene encoding a ubiquitin E3 ligase for RORγt, decreased in *RhoH*$^{-/-}$ T cells (Fig 5, *A*). This finding indicated that the reduction in *Ubr5* expression enhanced RORγt stability, thereby increasing RORγt protein levels in *RhoH*$^{-/-}$ T cells.

In addition, TCR-induced expression of the gene encoding the nuclear orphan receptor Nr2f6 (also referred to as Ear2 or V-erbA-Related Protein2) was inhibited in *RhoH*$^{-/-}$ T cells (Fig 5, *A*). Nr2f6 inhibits RORγt binding to the IL-17 promoter, and therefore a reduction in *Nr2f6* expression levels could lead to an increase in RORγt binding to the IL-17 promoter region. Consistent with this hypothesis, chromatin immunoprecipitation experiments revealed that the recruitment of Nr2f6 to the IL-17 promoter was inhibited, and this effect was accompanied by a concomitant increase in RORγt binding to the IL-17 promoter (Fig 5, *C*). Furthermore, retrovirus-mediated overexpression of Nr2f6 in *RhoH*$^{-/-}$ T cells nearly abolished upregulation of T_H17 differentiation (Fig 5, *D*). Collectively, these results suggest that RhoH regulates the stability and DNA binding activity of RORγt during T_H17 cell differentiation, especially at the late effector phase.

RhoH expression in patients with psoriasis

Because RhoH deficiency in BALB/c mice induced psoriatic dermatitis by promoting the T_H17 lineage, we investigated the potential role of RhoH in the pathogenesis of human psoriasis. First, we analyzed *RhoH* mRNA expression in PBMCs from patients with psoriasis by using the quantitative PCR method (*RhoH* expression was divided based on T-cell lineage marker *CD3* expression to measure T-cell specific expression from PBMC samples). Although *RhoH* expression levels were similar in T cells from healthy donors and patients with psoriasis (data not shown), these differed between patients stratified by Psoriasis Area and Severity Index (PASI) scores. *RhoH* expression levels were significantly lower in patients with more severe disease (PASI > 20 [high PASI scores]) compared with those with less severe disease (PASI ≤ 20 [low PASI scores]; Fig 5, *E*). Therefore *RhoH* expression levels might be related to the severity of psoriatic symptoms.

We also found that *RhoH* expression was maintained at low levels in T_H17 cells isolated from RORγt-GFP knock-in transgenic mice. *RhoH* levels decreased in RORγt$^+$ $CD4^+$ T cells compared with GFP^-CD4^+ T cells (Fig 5, *F*), and similar results were observed in sorted human $CCR6^+CD4^+$ T cells (Fig 5, *G*).

IL-22BP suppresses progression of skin inflammation in *RhoH*$^{-/-}$ mice

Next, we evaluated the therapeutic potential of IL-22 inhibition in our RhoH knockout mouse model. IL-22 plays an important role in the pathogenesis of psoriasis,[26] and *Il22* expression levels strongly increased in the eyelid legion of *RhoH*$^{-/-}$ mice (Fig 2, *A*). An IL-22 deficiency generated by using the CRISPR/Cas9 system completely abolished the development of dermatitis in *RhoH*$^{-/-}$ mice (Fig 6, *A*), indicating that the development of dermatitis in this model was dependent on IL-22. A previous study demonstrated that blocking IL-22 activity by using an antibody against IL-22 was effective in psoriasis mouse model treatment.[27] Therefore we investigated the therapeutic effect of IL-22BP (IL-22RA), an endogenous soluble decoy receptor for IL-22. Repeated administration of a recombinant wild-type IL-22BP/Ig-Fc fusion protein partially inhibited dermatitis in RhoH$^{-/-}$ mice, whereas a mutant (A66/A118) version of IL-22BP, which does not bind to IL-22, did not have any effect (Fig 6, *B-D*). This finding suggests that neutralization of IL-22 by IL-22BP represents a potential therapeutic strategy for the treatment of psoriasis.

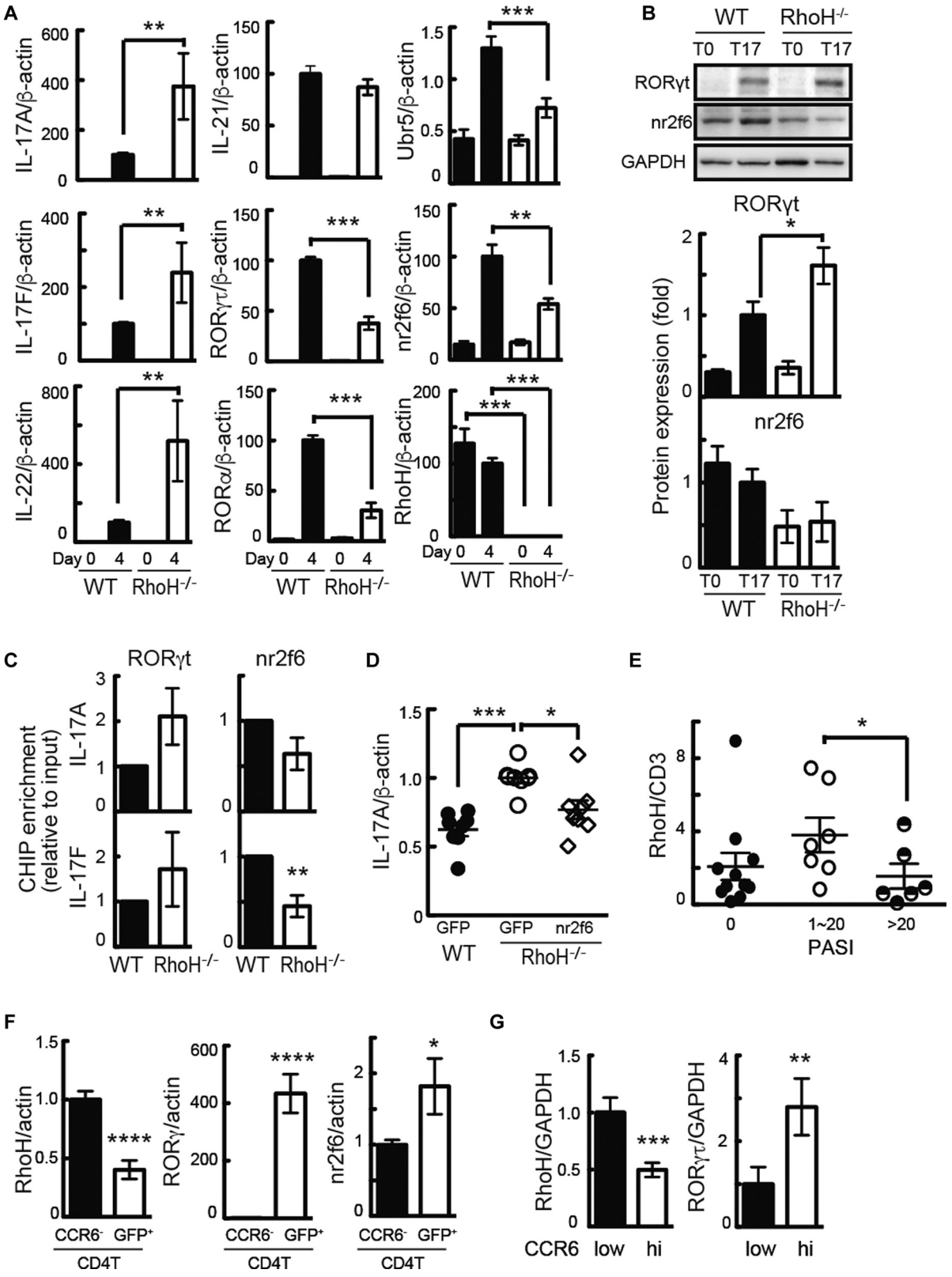

FIG 5. RhoH orchestrates T_H17 cell differentiation through Nr2f6 and Ubr5. **A,** Gene expression levels in naive T cells cultured for 4 days under T_H17-polarizing conditions were analyzed by using real-time PCR. **B** and **C,** Protein levels (Fig 5, *B*) and chromatin binding (Fig 5, *C*) in the IL-17 promoter region of RORγt and Nr2f6. **D,** Proportion of IL-17A$^+$CD4$^+$ T cells from *RhoH*$^{-/-}$ mice. **E,** Correlation between *RhoH* expression levels and PASI scores in patients with psoriasis. **F** and **G,** mRNA expression levels in T cells from GFP-RORγt mice (Fig 5, *F*) and in PBMCs from healthy human volunteers (Fig 5, *G*). Expression levels of *RhoH* were normalized to α-actin. **P* < .05, ***P* < .01, ****P* < .001, and *****P* < .0001.

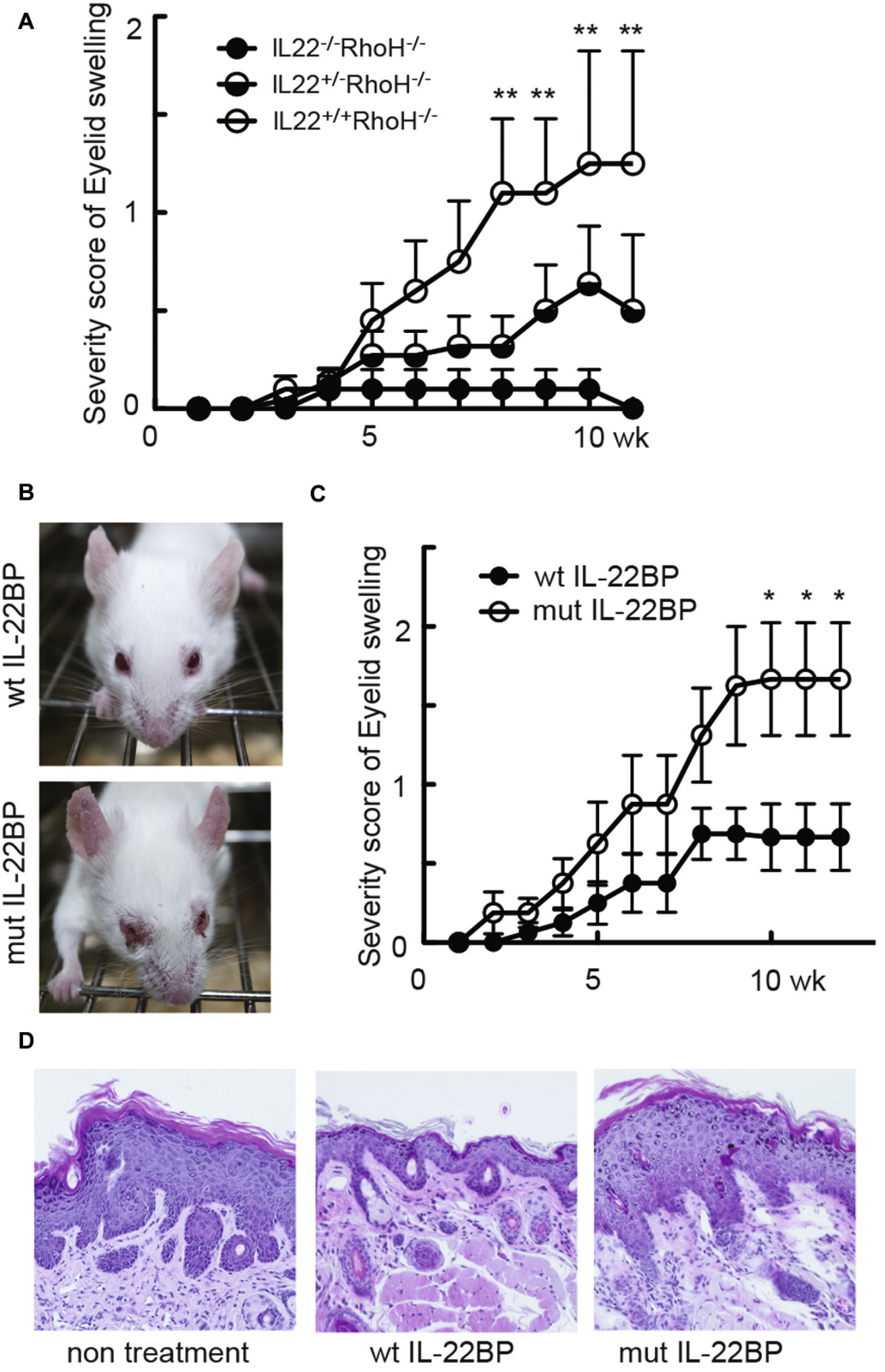

FIG 6. Inhibition of IL-22 attenuates psoriatic dermatitis in *RhoH*$^{-/-}$ mice. **A,** Clinical scores for eyelid swelling in *RhoH*$^{-/-}$, *RhoH*$^{-/-}$*IL-22*$^{+/-}$, and *RhoH*$^{-/-}$*IL-22*$^{-/-}$ mice. **B-D,** Representative images (Fig 6, *B*), clinical scores (Fig 6, *C*), and hematoxylin and eosin (H&E)–stained eyelid sections (Fig 6, *D*) from mice intravenously injected once a week with 25 μg of IL-22BP/Fc wild-type (*wt*) or mutant (*mut*) recombinant protein. *$P < .05$ and **$P < .01$.

DISCUSSION

Although multiple animal models of psoriasis have been reported,[28] few of them are associated with components of the TCR signal transduction pathway. Therefore it was unexpected that the loss of RhoH, an adaptor protein that functions in proximal TCR signaling, caused psoriatic dermatitis. However, RhoH can inhibit the transcriptional activity of RORγt by modulating RORγt stability and promoter-binding activity, thereby suppressing T_H17 cell polarization. For this reason, RhoH deficiency induces psoriasis-like dermatitis by upregulating T_H17 cell polarization. It is obvious that a simple increase in T_H17 cell numbers is not enough to induce psoriasis-like dermatitis because the symptom does not occur on the B6 background with the same increase in T_H17 cell numbers. Additionally, BALB/c-*RhoH*$^{-/-}$ mice did not have induced psoriatic arthritis or other systemic manifestations. Therefore multiple other factors are apparently important for the development of psoriasis.

Both IL-17 and IFN-γ play critical roles in the pathogenesis of psoriasis. IFN-γ expression is increased in psoriatic lesions compared with nonpsoriatic skin,[29] and it colocalizes with IL-17 expression in psoriatic lesions.[30] IFN-γ programs DCs to stimulate IL-17–producing T-cell expansion through IL-23 and IL-1. In patients with psoriasis, IL-17 and IFN-γ exhibit synergistic effects on the production of various proinflammatory cytokines, including IL-6 and TNF-α.[8] We found that the proportion of both IL-17–producing T_H17 cells and IFN-γ–producing T_C1 cells increased in *RhoH*$^{-/-}$ mice. β7-Integrin contributes tissue homing of T_H17 cells,[31] and RhoH inhibits expression of the β2-integrin LFA-1, which is also critical for entry into lymph nodes.[32] Given the increase in β7-integrin and LFA-1 expression in *RhoH*$^{-/-}$ T_H17 cells (see Fig E8), the increased integrin expression could partly account for the increase in numbers of T_H17 cells in lymph nodes of *RhoH*$^{-/-}$ mice.

Because IL-2 inhibits T_H17 cell differentiation,[33] reduced IL-2 production or reduced IL-2 response could enhance T_H17 cell differentiation. However, we observed comparable IL-2 production by *RhoH*$^{-/-}$ T cells in T_H17-inducing culture (see Fig E16 in this article's Online Repository at www.jacionline.org), as well as equivalent levels of IL-2 in sera of *RhoH*$^{-/-}$ mice (see Fig E6, *B*). Additionally, activation of STAT5 in T_H17-inducing culture was unaltered (see Fig E15, *B*), suggesting undisturbed IL-2 receptor–dependent signaling. Collectively, we concluded that increased T_H17 differentiation in *RhoH*$^{-/-}$ mice was independent of IL-2. On the contrary, *RhoH*$^{-/-}$ naive CD4$^+$ T cells produced more IL-2 in T0 conditions (see Fig E16, *A*). Because IL-2 enhances T_C1 cell differentiation,[34] increased regional IL-2 levels might contribute to the increase in numbers of T_C1 cells in *RhoH*$^{-/-}$ mice.

In addition to CD4$^+$ T_H17 cells, γδT17 cells, type 3 innate lymphoid cells, CD8$^+$ T_C17 cells, mast cells, and neutrophils are potential sources of IL-17.[35,36] The importance of γδ T17 cells in imiquimod-induced psoriatic dermatitis is well appreciated.[21] However, the proportion of γδT17 cells strongly decreased in the eyelids and cLNs of *RhoH*$^{-/-}$ mice (see Fig E7, *F*), indicating that dermatitis in *RhoH*$^{-/-}$ mice was independent of γδT17 cells. The SKG mutation in *Zap70* inhibits γδT17 cell development,[37] and therefore RhoH deficiency could have a similar effect on γδT17 cell development.[38] The results of our experiments using *Rag*$^{-/-}$, *RhoH*$^{-/-}$ double-knockout mice and *RhoH*$^{-/-,\ RhoH\text{-}Tg}$ mice indicated that RhoH depletion in αβ T cells induced dermatitis. Moreover, T_H17 differentiation was upregulated, even in mice in which RhoH was deleted after normal T-cell development (Fig 4, *D*, and see Fig E14). Therefore it can be inferred that upregulation of T_H17 cell differentiation was primarily a consequence of RhoH deficiency in T cells rather than due to indirect effects. Collectively, these findings indicate that non-αβ T cells are not likely to contribute to the pathogenesis of psoriasis-like dermatitis in *RhoH*$^{-/-}$ mice.

TCR-dependent signal transduction was strongly inhibited in *RhoH*$^{-/-}$ T cells. The strength of TCR signaling affects lineage commitment during effector T-cell differentiation. Because weak TCR signaling activity was reported to promote T_H17 differentiation,[39] this activity might account for the upregulation of T_H17 differentiation in *RhoH*$^{-/-}$ T cells. However, T_H17 differentiation was not strongly induced in *RhoH*$^{-/-}$ T cells exposed to lower concentrations of anti-CD3 (see Fig E12), indicating that weak TCR signaling activity cannot account for the tendency of T cells to differentiate into T_H17 cells in *RhoH*$^{-/-}$ mice.

RORγt plays central roles in the differentiation and function of T_H17 cells, and RORγt protein levels and DNA binding activity are regulated through multiple mechanisms. Recent studies have demonstrated that Ubr5 induces RORγt proteolysis and Nr2f6 competitively binds to RORγt response elements.[40,41] Therefore the balance among RORγt, Ubr5, and Nr2f6 is crucial for the dynamic regulation of T_H17 cell development. In our study expression levels of *Ubr5* and *Nr2f6* increased in response to TCR stimulation (Fig 5, *A*),[41] and this effect was inhibited in *RhoH*$^{-/-}$ T cells. Alterations in TCR signaling resulting from the absence of RhoH could account for insufficient levels of Nr2f6 and Ubr5 during T_H17 cell differentiation. Both proteins are induced at a relatively late phase during T_H17 differentiation, and they potentially mediate a negative feedback loop that inhibits excessive inflammatory responses. In contrast, STAT3 phosphorylation in the early stages of T_H17 cell differentiation and expression of various early responder genes, including *RORγt*, were not affected in *RhoH*$^{-/-}$ T cells, indicating that the initial stages of T_H17 cell differentiation were not affected by RhoH deficiency. Therefore RhoH might regulate T_H17 cell differentiation at a later phase, such as maturation of effector T_H17 cells.

Although there have been no reports of psoriasis-associated SNPs in the *RhoH* locus, a nonsense *RhoH* mutation (Y38X) identified in patients with persistent EV–human papillomavirus infection is associated with T-cell defects.[7] EV–human papillomavirus has been linked to psoriasis,[42,43] and it might play an important role in psoriasis progression. A recently identified psoriasis susceptibility gene, T-cell activation Rho GTPase-activating protein (*TAGAP*), has been shown to interact with RhoH and to inhibit its function.[44,45] This finding suggests that RhoH is potentially associated with the pathogenesis, progression, or both of psoriasis in human subjects. Consistent with this hypothesis, *RhoH* expression levels decreased in PBMCs from patients with high PASI scores compared with those with low PASI scores (Fig 5, *E*), indicating that *RhoH* expression levels are inversely correlated with the severity of psoriasis symptoms. A previous report demonstrated that the severity of psoriatic symptoms is directly correlated with the number of T_H17 cells.[27] We found that T_H17 cells expressed lower levels of *RhoH* than naive T cells (Fig 5, *F* and *G*), and therefore the decrease in *RhoH* expression in patients with high PASI scores could be due to an increase in the proportion of T_H17 cells. In contrast to a previous report,[27]

RORγt expression levels did not significantly differ between patients with high PASI and those with low PASI scores in the present study (see Fig E17 in this article's Online Repository at www.jacionline.org). However, despite the lack of a difference in the number of T_H17 cells, RhoH might affect psoriatic symptoms by modulating T_H17 cell function during the late phase of T_H17 cell differentiation.

Several cytokines produced by T_H1, T_H17, and T_H22 cells are implicated in the pathogenesis of psoriasis. In our *RhoH*$^{-/-}$ model *Il22* mRNA levels increased in inflamed lesions (Fig 6, *A*). We concluded that the increase in IL-22 in *RhoH*$^{-/-}$ mice was due to the increase in numbers of T_H17 cells. Involvement of T_H22, another IL-22 producer, seemed less significant because expression of *Ahr*, a master regulator for T_H22-differentiation,[46] was decreased in *RhoH*$^{-/-}$ T cells (see Fig E18 in this article's Online Repository at www.jacionline.org). IL-22 plays a critical role in the maintenance of epidermal homeostasis by promoting keratinocyte migration and antimicrobial peptide production, and therefore IL-22 upregulation can lead to epidermal hyperplasia and skin inflammation, 2 hallmarks of psoriasis.[47,48] A direct correlation between serum IL-22 levels and the severity of psoriatic symptoms was reported in clinical studies,[49] and genetic studies revealed that *Il22* polymorphisms are associated with susceptibly to psoriasis.[50,51] IL-22 has also been implicated in patients with several autoimmune diseases, including systemic lupus erythematosus and rheumatoid arthritis.[52,53] In our *RhoH*$^{-/-}$ model psoriasis-like dermatitis was strongly dependent on IL-22 (Fig 6, *A*), indicating that IL-22 plays an important role in the pathogenesis of psoriasis. Skin inflammation also depended on IL-17 in this model (see Fig E10); however, *Il17a/f*$^{-/-}$*RhoH*$^{-/-}$ triple-knockout mice had more severe dermatitis than *RhoH*$^{-/-}$ mice, with a tremendous increase in serum IL-22 levels (data not shown). Further extensive study is needed to characterize these surprising phenomena, but the double deficiency of IL-17A/F resulted in an unexpected increase in IL-22 levels.

Targeting cytokine crosstalk between T cells and keratinocytes is a promising approach for treating patients with psoriasis.[54] Several therapeutic trials evaluating treatments that block the IL-23/22/17 axis are currently being conducted. A clinical trial analyzing an antibody against IL-22 (ILV-094) was terminated in 2011.[55] Although inhibiting IL-22 with a neutralizing antibody did not exert substantial therapeutic effects in a large fraction of patients with psoriasis, a phase II trial of ILV-094 in patients with atopic dermatitis is still ongoing. We demonstrated that blocking IL-22 with chimeric recombinant wild-type IL-22BP/Fc partially attenuated eyelid swelling. IL-22BP is a soluble endogenous decoy receptor with a strong affinity (1 pmol/L) for IL-22, and therefore it is expected to exert a stronger inhibitory effect compared with an mAb against IL-22. A previous study demonstrated that retinoic acid, which is used as a therapeutic agent for psoriasis, strongly induced IL-22BP expression in DCs.[56] Furthermore, a recent study in mice demonstrated that IL-22 restored glycemic control in mice with diabetes.[57,58] Further studies should be conducted to investigate the role of IL-22 in metabolism and immunity. The therapeutic potential of upregulating IL-22BP by using a low-molecular-weight compound in patients with psoriasis and atopic dermatitis also merits investigation.

In the present study we demonstrated that RhoH suppresses psoriatic skin inflammation. Moreover, we substantiated that IL-22BP exerts therapeutic effects on the psoriasis mouse model, presumably by controlling keratinocyte proliferation. Therefore modulating IL-22 signaling might represent a potent therapeutic strategy for the treatment of chronic skin diseases.

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Key messages

- **RhoH deficiency induces T_H17 cell differentiation after psoriasis-like dermatitis in mice.**
- **In patients with severe psoriasis, RhoH mRNA expression in PBMCs is decreased in comparison with that in healthy subjects and patients with mild psoriasis.**
- **IL-22BP treatment to RhoH-deficient mice improves skin inflammation characterized by psoriasiform dermatitis.**

REFERENCES

1. Chae H-D, Siefring JE, Hildeman DA, Gu Y, Williams DA. RhoH regulates subcellular localization of ZAP-70 and Lck in T cell receptor signaling. PLoS One 2010;5:e13970.
2. Wang H, Zeng X, Fan Z, Lim B. RhoH modulates pre-TCR and TCR signalling by regulating LCK. Cell Signal 2011;23:249-58.
3. Oda H, Fujimoto M, Patrick MS, Chida D, Sato Y, Azuma Y, et al. RhoH plays critical roles in FcεRI-dependent signal transduction in mast cells. J Immunol 2009;182:957-62.
4. Oda H, Tamehiro N, Patrick MS, Hayakawa K, Suzuki H. Differential requirement for RhoH in development of TCR alpha beta CD8 alpha IELs and other types of T cells. Immunol Lett 2013;151:1-9.
5. Troeger A, Williams DA. Hematopoietic-specific Rho GTPases Rac2 and RhoH and human blood disorders. Exp Cell Res 2013;319:2375-83.
6. Tamehiro N, Oda H, Shirai M, Suzuki H. Overexpression of RhoH permits to bypass the Pre-TCR checkpoint. PLoS One 2015;10:e0131047.
7. Crequer A, Troeger A, Patin E, Ma CS, Picard C, Pedergnana V, et al. Human RHOH deficiency causes T cell defects and susceptibility to EV-HPV infections. J Clin Invest 2012;122:3239-47.
8. Nestle FO, Kaplan DH, Barker J. Mechanisms of disease: psoriasis. N Engl J Med 2009;361:496-509.
9. Griffiths CEM, Barker JNWN. Psoriasis 1—pathogenesis and clinical features of psoriasis. Lancet 2007;370:263-71.
10. Lowes MA, Suarez-Farinas M, Krueger JG. Immunology of psoriasis. Annu Rev Immunol 2014;32:227-55.
11. Ellis CN, Gorsulowsky DC, Hamilton TA, Billings JK, Brown MD, Headington JT, et al. Cyclosporine improves psoriasis in a double-blind-study. JAMA 1986; 256:3110-6.
12. Griffiths CEM, Powles AV, Baker BS, Fry L, Valdimarsson H. Topical cyclosporine and psoriasis. Lancet 1987;1:806.
13. Di Cesare A, Di Meglio P, Nestle FO. The IL-23/Th17 axis in the immunopathogenesis of psoriasis. J Invest Dermatol 2009;129:1339-50.
14. Nitta T, Muro R, Shimizu Y, Nitta S, Oda H, Ohte Y, et al. The thymic cortical epithelium determines the TCR repertoire of IL-17-producing gammadeltaT cells. EMBO Rep 2015;16:638-53.
15. Sakaguchi N, Takahashi T, Hata H, Nomura T, Tagami T, Yamazaki S, et al. Altered thymic T-cell selection due to a mutation of the ZAP-70 gene causes autoimmune arthritis in mice. Nature 2003;426:454-60.
16. Veldhoen M, Hocking RJ, Flavell RA, Stockinger B. Signals mediated by transforming growth factor-beta initiate autoimmune encephalomyelitis, but chronic inflammation is needed to sustain disease. Nat Immunol 2006;7:1151-6.
17. Nickoloff BJ. Cracking the cytokine code in psoriasis. Nat Med 2007;13:242-4.
18. Gu Y, Chae HD, Siefring JE, Jasti AC, Hildeman DA, Williams DA. RhoH GTPase recruits and activates Zap70 required for T cell receptor signaling and thymocyte development. Nat Immunol 2006;7:1182-90.
19. Dorn T, Kuhn U, Bungartz G, Stiller S, Bauer M, Ellwart J, et al. RhoH is important for positive thymocyte selection and T-cell receptor signaling. Blood 2007; 109:2346-55.

20. Annunziato F, Cosmi L, Santarlasci V, Maggi L, Liotta F, Mazzinghi B, et al. Phenotypic and functional features of human Th17 cells. J Exp Med 2007;204:1849-61.
21. Cai Y, Shen X, Ding C, Qi C, Li K, Li X, et al. Pivotal role of dermal IL-17-producing gamma delta T cells in skin inflammation. Immunity 2011;35:596-610.
22. Abdallah MA, Abdel-Hamid MF, Kotb AM, Mabrouk EA. Serum interferon-gamma is a psoriasis severity and prognostic marker. Cutis 2009;84:163-8.
23. Gunderson AJ, Mohammed J, Horvath FJ, Podolsky MA, Anderson CR, Glick AB. CD8(+) T cells mediate RAS-induced psoriasis-like skin inflammation through IFN-gamma. J Invest Dermatol 2013;133:955-63.
24. Troeger A, Johnson AJ, Wood J, Blum WG, Andritsos LA, Byrd JC, et al. RhoH is critical for cell-microenvironment interactions in chronic lymphocytic leukemia in mice and humans. Blood 2012;119:4708-18.
25. Zhu B, Symonds AL, Martin JE, Kioussis D, Wraith DC, Li S, et al. Early growth response gene 2 (Egr-2) controls the self-tolerance of T cells and prevents the development of lupuslike autoimmune disease. J Exp Med 2008;205:2295-307.
26. Kim JH, Hu Y, Yongqing T, Kim J, Hughes VA, Le Nours J, et al. CD1a on Langerhans cells controls inflammatory skin disease. Nat Immunol 2016;17:1159-66.
27. Ma HL, Liang S, Li J, Napierata L, Brown T, Benoit S, et al. IL-22 is required for Th17 cell-mediated pathology in a mouse model of psoriasis-like skin inflammation. J Clin Invest 2008;118:597-607.
28. Wagner EF, Schonthaler HB, Guinea-Viniegra J, Tschachler E. Psoriasis: what we have learned from mouse models. Nat Rev Rheumatol 2010;6:704-14.
29. Koga T, Duan H, Urabe K, Furue M. In situ localization of IFN-gamma-positive cells in psoriatic lesional epidermis. Eur J Dermatol 2002;12:20-3.
30. Kryczek I, Bruce AT, Gudjonsson JE, Johnston A, Aphale A, Vatan L, et al. Induction of IL-17(+) T cell trafficking and development by IFN-gamma: mechanism and pathological relevance in psoriasis. J Immunol 2008;181:4733-41.
31. Kleinschek MA, Boniface K, Sadekova S, Grein J, Murphy EE, Turner SP, et al. Circulating and gut-resident human Th17 cells express CD161 and promote intestinal inflammation. J Exp Med 2009;206:525-34.
32. Cherry LK, Li X, Schwab P, Lim B, Klickstein LB. RhoH is required to maintain the integrin LFA-1 in a nonadhesive state on lymphocytes. Nat Immunol 2004;5:961-7.
33. Laurence A, Tato CM, Davidson TS, Kanno Y, Chen Z, Yao Z, et al. Interleukin-2 signaling via STAT5 constrains T helper 17 cell generation. Immunity 2007;26:371-81.
34. Kalia V, Sarkar S, Subramaniam S, Haining WN, Smith KA, Ahmed R. Prolonged interleukin-2Ralpha expression on virus-specific CD8+ T cells favors terminal-effector differentiation in vivo. Immunity 2010;32:91-103.
35. Lin AM, Rubin CJ, Khandpur R, Wang JY, Riblett M, Yalavarthi S, et al. Mast cells and neutrophils release IL-17 through extracellular trap formation in psoriasis. J Immunol 2011;187:490-500.
36. Sutton CE, Mielke LA, Mills KHG. IL-17-producing gamma delta T cells and innate lymphoid cells. Eur J Immunol 2012;42:2221-31.
37. Wencker M, Turchinovich G, Di Marco Barros R, Deban L, Jandke A, Cope A, et al. Innate-like T cells straddle innate and adaptive immunity by altering antigen-receptor responsiveness. Nat Immunol 2014;15:80-7.
38. Muro R, Nitta T, Nakano K, Okamura T, Takayanagi H, Suzuki H. gamma delta TCR recruits the Syk-PI3K axis to drive proinflammatory differentiation program. J Clin Invest 2018;128:415-26.
39. Purvis HA, Stoop JN, Mann J, Woods S, Kozijn AE, Hambleton S, et al. Low-strength T-cell activation promotes Th17 responses. Blood 2010;116:4829-37.
40. Rutz S, Kayagaki N, Phung QT, Eidenschenk C, Noubade R, Wang X, et al. Deubiquitinase DUBA is a post-translational brake on interleukin-17 production in T cells. Nature 2015;518:417-21.
41. Hermann-Kleiter N, Klepsch V, Wallner S, Siegmund K, Klepsch S, Tuzlak S, et al. The nuclear orphan receptor NR2F6 is a central checkpoint for cancer immune surveillance. Cell Rep 2015;12:2072-85.
42. Favre M, Orth G, Majewski S, Baloul S, Pura A, Jablonska S. Psoriasis: a possible reservoir for human papillomavirus type 5, the virus associated with skin carcinomas of epidermodysplasia verruciformis. J Invest Dermatol 1998;110:311-7.
43. Ramoz N, Rueda LA, Bouadjar B, Favre M, Orth G. A susceptibility locus for epidermodysplasia verruciformis, an abnormal predisposition to infection with the oncogenic human papillomavirus type 5, maps to chromosome 17qter in a region containing a psoriasis locus. J Invest Dermatol 1999;112:259-63.
44. Tsoi LC, Spain SL, Knight J, Ellinghaus E, Stuart PE, Capon F, et al. Identification of 15 new psoriasis susceptibility loci highlights the role of innate immunity. Nat Genet 2012;44:1341-8.
45. Tamehiro N, Nishida K, Yanobu-Takanashi R, Goto M, Okamura T, Suzuki H. T-cell activation RhoGTPase-activating protein plays an important role in TH17-cell differentiation. Immunol Cell Biol 2017;95:729-35.
46. Basu R, O'Quinn DB, Silberger DJ, Schoeb TR, Fouser L, Ouyang W, et al. Th22 cells are an important source of IL-22 for host protection against enteropathogenic bacteria. Immunity 2012;37:1061-75.
47. Nograles KE, Zaba LC, Guttman-Yassky E, Fuentes-Duculan J, Suarez-Farinas M, Cardinale I, et al. Th17 cytokines interleukin (IL)-17 and IL-22 modulate distinct inflammatory and keratinocyte-response pathways. Br J Dermatol 2008;159:1092-102.
48. Moniaga CS, Egawa G, Miyachi Y, Kabashima K. Calcipotriol modulates IL-22 receptor expression and keratinocyte proliferation in IL-22-induced epidermal hyperplasia. J Dermatol Sci 2013;71:76-7.
49. Boniface K, Guignouard E, Pedretti N, Garcia M, Delwail A, Bernard FX, et al. A role for T cell-derived interleukin 22 in psoriatic skin inflammation. Clin Exp Immunol 2007;150:407-15.
50. Nikamo P, Cheuk S, Lysell J, Enerback C, Bergh K, Landen NX, et al. Genetic variants of the IL22 promoter associate to onset of psoriasis before puberty and increased IL-22 production in T cells. J Invest Dermatol 2014;134:1535-41.
51. Saeki H, Hirota T, Nakagawa H, Tsunemi Y, Kato T, Shibata S, et al. Genetic polymorphisms in the IL22 gene are associated with psoriasis vulgaris in a Japanese population. J Dermatol Sci 2013;71:148-50.
52. Pan H-F, Zhao X-F, Yuan H, Zhang W-H, Tang X-W, Li W-X, et al. Decreased serum IL-22 levels in patients with systemic lupus erythematosus. Clin Chim Acta 2009;401:179-80.
53. da Rocha LF Jr, Branco Pinto Duarte AL, Dantas AT, Mariz HA, Pitta IdR, Galdino SL, et al. Increased serum interleukin 22 in patients with rheumatoid arthritis and correlation with disease activity. J Rheumatol 2012;39:1320-5.
54. Nickoloff BJ, Xin H, Nestle FO, Qin JZ. The cytokine and chemokine network in psoriasis. Clin Dermatol 2007;25:568-73.
55. Pfizer. pipeline. 2011; https://clinicaltrials.gov/ct2/show/NCT00563524?term=nct00563524&rank=1.
56. Martin JCJ, Beriou G, Heslan M, Chauvin C, Utriainen L, Aumeunier A, et al. Interleukin-22 binding protein (IL-22BP) is constitutively expressed by a subset of conventional dendritic cells and is strongly induced by retinoic acid. Mucosal Immunol 2014;7:101-13.
57. Hasnain SZ, Borg DJ, Harcourt BE, Tong H, Sheng YH, Ng CP, et al. Glycemic control in diabetes is restored by therapeutic manipulation of cytokines that regulate beta cell stress. Nat Med 2014;20:1417-26.
58. Wang X, Ota N, Manzanillo P, Kates L, Zavala-Solorio J, Eidenschenk C, et al. Interleukin-22 alleviates metabolic disorders and restores mucosal immunity in diabetes. Nature 2014;514:237-41.