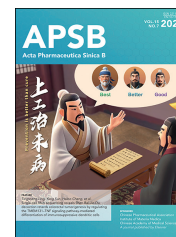




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ORIGINAL ARTICLE

Antisense oligonucleotides targeting IRF4 alleviate psoriasis



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Abstract Interferon regulatory factor 4 (IRF4) is a critical transcription factor that governs the differentiation of cluster of differentiation 4⁺ (CD4⁺) T cells. The pathogenesis and progression of psoriasis are primarily attributed to an immune imbalance stemming from the overproduction of interleukin-17A (IL-17A) by T lymphocytes. However, the role of IRF4 in psoriasis remains unexplored. In this study, we found that IRF4 activity is increased in the cutaneous lesions of patients with psoriasis in response to stimulation by IL-23A and IL-1 β . This IRF4 elevation heightens its binding to the E1A binding protein p300 (EP300) promoter, triggering the transcription of downstream retinoic acid receptor-related orphan receptor- γ t (ROR γ t) and increasing the secretion of IL-17A, thereby establishing the IL-1 β /IL-23A–IRF4–EP300–RORC–IL-17A inflammatory cascade in psoriasis. The alleviation of imiquimod

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(IMQ)-induced psoriatic-like symptoms was achieved through the creation of a *Irf4*^{-/-} gene deletion mouse model and pharmacological inhibition using antisense oligonucleotides targeted for *Irf4*. This amelioration was accompanied by a decreased number of IL-17A-producing CD4⁺ T cells in the skin. The findings of this study suggest that IRF4 plays a crucial role in the promotion of inflammation and exacerbation of IMQ-induced psoriasiform dermatitis. Consequently, IRF4 targeting could be a promising therapeutic strategy.

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1. Introduction

Psoriasis is a systemic autoinflammatory disorder that affects 2% of Europeans and North Americans and more than 6 million Chinese individuals. Its most prevalent phenotype is psoriasis vulgaris, which arises from nexuses of genetic predisposition (particularly that associated with human leukocyte antigen C*06:02 risk alleles) and environmental stimuli, including streptococcal infection, stress, tobacco use, obesity, and alcohol consumption¹. When genetically predisposed individuals are exposed to such environmental factors, dendritic cells (DCs) are activated and secrete cytokines such as interleukin-23 (IL-23) and tumor necrosis factor- α (TNF- α). These cytokines, in turn, activate and polarize helper T (Th) cells, driving their differentiation into Th17 and Th1 cells. The pro-inflammatory cytokines released by such cells act on keratinocytes and endothelial cells, triggering their activation, proliferation, and production of chemokines, adhesion molecules, and antimicrobial peptides (AMPs). These alterations lead to additional white blood cell recruitment and intensify the immune response in psoriasis²⁻⁵.

Several cytokine-targeting biologics, including brodalumab, secukinumab, and ustekinumab, have been developed in the past decade to treat psoriasis based on its underlying pathogenesis. Although significant advancements in the treatment of psoriasis have been made with the use of monoclonal antibodies, persisting challenges include the singular effects of these treatments, the high costs associated with them, and uncertainty regarding the long-term efficacy of single cytokine targeting. Debate is thus ongoing, and research is being conducted to further elucidate the dysfunction of T-lymphocyte subsets and the molecular mechanisms underlying psoriasis. Concurrently, researchers are searching for novel targets that can effectively exert multiple effects⁶⁻⁸.

Interferon regulatory factor 4 (IRF4) regulates multiple aspects of innate and adaptive immunity. It is synthesized exclusively by immunocytes, including lymphocytes, DCs, and macrophages, and promotes Th17 cell differentiation and IL-17 synthesis, which are key contributors to autoimmunity^{1,9-11}. It regulates various aspects of functional immune responses in an environment-dependent manner. Whereas the activity of other IRF family members is controlled by type-1 and -2 interferons, that of IRF4 is controlled by Toll-like receptors (TLRs) 4 and 9 and TNF receptors [*e.g.*, cluster of differentiation 40 (CD40)], which in turn are regulated by antigen receptor signaling and restricted categories^{12,13}. Abnormal IRF4 expression is linked to oncogenesis and autoimmunity¹⁴⁻²². T cells are activated rapidly upon T cell receptor (TCR) signaling, increasing the expression and activity of IRF4, which interacts with retinoic acid receptor-related orphan receptor- γ t (ROR γ t) to promote the differentiation of naïve CD4⁺ T cells into Th17 cells and

induce IL-17 synthesis²³⁻²⁷. IRF4 deficiency in CD4⁺ T cells eliminates anti-CD3 ϵ -induced IL-17A expression, indicating the necessity of IRF4 for TCR-mediated IL-17A expression by these cells^{2,28}. In systemic lupus erythematosus, IRF4 expression in T cells is upregulated and the ablation of T cell IRF4 synthesis ameliorates renal dysfunction by reducing Th17 cell differentiation^{13,29,30}. Similarly, IRF4 ablation has been found to reduce the *Rorc*-mediated synthesis of specific transcription factors in Th17 cells, decreasing the Th17 cell count^{9,31-33}. In addition, the inhibition of IRF4 regulates Th17 cell differentiation by reducing the DNA binding of basic leucine zipper ATF-like transcription factor (BATF), IRF4, and Jun B transcription complexes. Also, the inhibition of IRF4 inhibits CD4⁺ T cell proliferation, thereby downregulating *IL17* transcription to alleviate Sjogren's syndrome, autoimmune meningoencephalitis, and experimental autoimmune encephalomyelitis³⁴⁻³⁷.

IRF4 gene deletion or IRF4 antagonism may reduce Th17 cell-mediated autoimmunity³⁸. Our current understanding of this potential is based mainly on a study conducted by Ni et al.³⁹, which demonstrated that IRF4 expression in keratinocytes and inflammatory cells is significantly higher in psoriasis vulgaris lesion tissues than in normal control tissues. Nevertheless, the role of IRF4 overexpression, its regulatory mechanisms, and its physiological significance in psoriasis development *in vivo* remain poorly understood.

In the present study, IRF4 overexpression activated circulating and cutaneous Th1 and Th17 cells in patients with psoriasis and in a psoriasiform murine model by binding to its target promoter E1A binding protein p300 (EP300). This process stimulated *Rorc* transcription and increased IL-17A production, exacerbating psoriatic skin lesions. *Irf4* gene knockout and silencing through antisense oligonucleotides (ASOs) significantly reduced psoriasiform dermatitis in the murine model of imiquimod (IMQ)-induced psoriasis, indicating that IRF4 plays an essential role in the pathogenesis of this condition.

2. Materials and methods

2.1. Ethical considerations

The animal experiments performed in this study were approved by the Experimental Animal Ethics Committee of Anhui Medical University, Hefei, China (No. 2023327), and all procedures abided strictly by the Guide for the Care and Use of Laboratory Animals (8th ed., 2011), with every effort made to minimize animal suffering. The Biomedical Ethics Committee of Anhui Medical University approved all procedures performed with human

samples in this study (No. 20190195), which complied with the relevant provisions of the Declaration of Helsinki. All participants provided written informed consent.

2.2. Mice

Wild-type (WT; *Irf4*^{+/+}), *Irf4* systemic knockout (KO; *Irf4*^{-/-}), *Irf4*^{fllox/fllox} conditional KO (*Irf4*^{fl/fl}), and *CD4*-Cre C57BL/6J mice were purchased from Cyagen Biosciences Inc (Suzhou, China). The *Irf4*^{fl/fl} and *CD4*-Cre mice were hybridized to create *Irf4*^{fl/fl} *CD4*-Cre mice. All breeding was performed in the specific pathogen-free grade animal room of the Laboratory Animal Center of Anhui Medical University. Female mice were tested (Genotype identification) when they reached 8–10 weeks of age.

2.3. Human data sources

We analyzed data from a psoriasis patient database. Data were obtained from the Gene Expression Omnibus (GEO) repository (accession: GSE54456). Otherwise, blood and skin specimens from psoriasis patients were collected at the Department of Dermatology, the First Affiliated Hospital of Anhui Medical University. All patients met the following criteria: (1) diagnosis confirmed independently by 2 board-certified dermatologists; (2) no history of immunosuppressive drug use within the preceding 3 months. Healthy control blood samples were procured from the hospital's Physical Examination Center. Normal skin tissues were surgically obtained from osteoarthritis patients undergoing joint replacement in the Department of Orthopedics, with confirmed absence of immune-mediated diseases.

2.4. Psoriasis model establishment

We used an internationally recognized mouse model of IMQ-induced psoriasis and performed assessments using the Psoriasis Area and Severity Index (PASI). First, a 2.5 cm × 2.5 cm area on the back of each mouse (8–10 weeks of age) was scraped, and 62.5 mg 5% IMQ cream (Sichuan Mingxin Pharmaceutical, Chengdu, China) was applied to the dry area daily after PASI assessment. Vaseline (VAS) was applied to control mice. Vernier calipers were used to measure the thickness of back skin with lesions, and severity was determined based on this thickness according to the PASI standard. A dedicated laboratory technician scored the skin condition based on the presence of scales and erythema (0, none; 1, mild; 2, moderate; 3, severe; 4, very severe), using a typical mouse as a reference. The molding should be carried out for 4 consecutive days, and the follow-up treatment should be carried out. For *in vivo* ASO15 treatment, we injected ASO15 (50 mg/kg) diluted in 200 μ L 0.9% NaCl or 200 μ L 0.9% NaCl (control) subcutaneously into 5-week-old mice once a week for 4 weeks. IMQ molding occurred on the third to seventh day of the last week of injections.

2.5. *Irf4*^{fl/fl} *CD4*-Cre model establishment

To determine the intrinsic function of IRF4 in CD4⁺ T cells, we developed a conditional *Irf4* KO (*Irf4*^{fl/fl} *CD4*-Cre) murine model. We treated these mice and healthy controls with IMQ and evaluated dermatitis and gene expression.

2.6. Isolation of primary immune cells from mouse skin

To acquire primary immune cells, we first prepared Dulbecco's modified Eagle's medium containing 1 mg/mL collagenase IV (#C4-28-100 MG; Merck, Kenilworth, NJ, USA), 50 μ g/mL DNase I (#3750-OP; Merck), 10 mmol/L 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (#15630080; Gibco, Carlsbad, CA, USA), and 2% bovine serum albumin (#11020039; Gibco). Mouse skin samples were placed in Petri dishes and a small amount of the digestive fluid was added. The samples were then minced, transferred to 50-mL centrifuge tubes, and digested on an air bath shaker (Taicang Hualida Laboratory Equipment Co., Ltd., Suzhou, China) at 37 °C and 2.7 × *g* for 2 h. The tissue suspensions were then passed through 200-mesh filters into new centrifuge tubes, and phosphate-buffered saline (PBS) was added to 40 mL to terminate digestion. After centrifugation at 400 × *g* for 10 min, the cells were suspended with 3 mL 30% percoll (#17089101; GE Healthcare, Chicago, IL, USA) and added to 2 mL 70% percoll. The suspensions were centrifuged for 30 min at 22 °C and 400 × *g* with an acceleration setting of 6 and a deceleration setting of 2 [Acceleration level 6: Achieves maximum speed (400 × *g*) in approximately 60 s. Deceleration level 2: Returns to rest in approximately 30 s. Thermo Scientific Sorvall Legend X1R, Waltham, MA, USA]. Mouse skin immune cells, appearing as white membranous layers in the centers of the centrifuge tube contents, were then collected.

2.7. Flow cytometry sample preparation and analysis

Peripheral blood samples were collected from patients with psoriasis and healthy controls (Supporting Information Table S1) with the purple anticoagulant. They were diluted 1:1 with PBS (#70011044; Gibco) and transferred to new 15-mL centrifuge tubes, and ficoll was added at a volume ratio of 1:2. The samples were centrifuged at 400 × *g* for 30 min with an acceleration setting of 3 and deceleration setting of 0. The peripheral blood mononuclear cells (PBMCs; white membranous layers in the centers of the centrifugation tube contents) were then extracted and counted, and 1 × 10⁶ cells were resuspended in 1.5-mL Eppendorf tubes with an antibody mixture containing peridinin–chlorophyll protein complex (PerCP)-Cy5.5-CD45 (#40953, 2D1, 1:50 dilution; BD Biosciences, San Jose, CA, USA), fluorescein isothiocyanate (FITC)-CD3 (#349201, SK7, 1:50 dilution; BD Biosciences), and phycoerythrin (PE)-labeled mouse anti-human CD4 (#556616, M-T477, 1:50 dilution; BD Biosciences). The suspensions were mixed in a vortex, then incubated away from light at 4 °C for 30 min. They were then washed twice with PBS under centrifugation at 1856 × *g* and resuspended with 400 μ L Foxp3/transcription factor flow cytometry fixation and permeabilization breaking solution (#00-5523-00; eBioscience, San Diego, CA, USA). The membranes were broken for 1 h at 4 °C away from light. The cells were then resuspended with permeabilization buffer (#00-5523-00; eBioscience) diluted with double-distilled water under centrifugation twice at 1517 × *g*. Rabbit anti-human IRF4 (#48-9858-82, 3E4, 1:50 dilution; Thermo Fisher Scientific, Waltham, MA, USA) was then added, and the samples were incubated away from light for 1 h at 4 °C. Finally, the samples were centrifuged at 1517 × *g* and then examined using a CytoFLEX flow cytometer (Beckman Coulter, Brea, CA, USA) with CytExpert software (version 2.4; Beckman Coulter).

Immune cells were obtained from skin samples from patients with psoriasis and healthy controls (Supporting Information Table S2) using the same method, then incubated for 15 min at

4 °C with Human TruStain FcX Fc receptor blocking solution (#422302, 1:100 dilution; BioLegend, Beijing, China). They were then prepared for and subjected to flow cytometric analysis as described above.

For the examination of lymphocytes from mouse skin, spleen, and blood samples, isolated immune cells were first suspended in a Dulbecco's modified Eagle's medium and spread evenly in 12-well plates. Eppendorf tubes (1.5 mL) were prepared with a mixture of 100 ng/mL phorbol 12-myristate 13-acetate (#P8139; Merck), 50 ng/mL ionomycin (#I3909; Merck), and 5 µg/mL Brefeldin A (#B5936; Merck). The cells (1×10^6) were resuspended in the tubes with anti-CD16/32 and then an antibody mixture [PerCP-Cy5.5 rat anti-mouse CD45 (#550994, 30-F11, 1:50 dilution; BD Biosciences), EF506-CD3 (#69-0032-82, 17A2, 1:50 dilution; Invitrogen, Carlsbad, CA, USA), FITC-CD4 (#553046, RM4-5, 1:50 dilution; Invitrogen), allophycocyanin (APC)-labeled rat anti-mouse CD8b (#569870, YTS156.7.7.rMAb, 1/200 dilution; BD Biosciences), and PECy7-CD25 (#552880, PC61, 1:50 dilution; BD Biosciences)]. After incubation, the samples were prepared as described above, with incubation with an antibody mixture consisting of APC-interferon- γ (IFN- γ , #554413, XMG1.2, 1:50 dilution; BD Biosciences), PECy7-IL4 (#355008, G077F6, 1:50 dilution; BioLegend), and PE-IL17A (#39901, 1:50 dilution; Cell Signaling Technology, Boston, MA, USA). They were then examined by flow cytometry as described above.

Myeloid cells (1×10^6) from mouse skin, spleen, and blood samples were resuspended in 1.5-mL Eppendorf tubes with anti-CD16/32 and then incubated at 4 °C for 30 min with an antibody mixture [PerCP-Cy5.5-CD45 (#32125, 1:100 dilution; Cell Signaling Technology), PECy7-CD11b (#25-0112-82, M1-70, 1:50 dilution; Invitrogen), APC-Ly6C (#560595, AL-21, 1:50 dilution; BD Biosciences), PE-labeled hamster anti-mouse CD11c (#557401, HL3, 1:50 dilution; BD Biosciences), BV650 rat anti-mouse F4/80 (#743282, T45-2342, 1:50 dilution; BD Biosciences), PECy7 rat anti-mouse Ly-6G (#560601, 1A8, 1:50 dilution; BD Biosciences), major histocompatibility complex (MHC) class II rat monoclonal antibody (mAb, FITC conjugate; #42594S, M5/114.15.2, 1:50 dilution; Cell Signaling Technology). After incubation, the fragmented film buffers were centrifuged at $5157 \times g$, followed by flow cytometric analysis as described above. The gating strategies used are shown in Supporting Information Fig. S1.

2.8. Magnetic-activated cell sorting and cell treatment

Skin samples from seven to nine mice aged about 8 weeks and weighing about 18 g were pooled for CD4⁺, CD8⁺, CD11c⁺, CD19⁺, and F4/80⁺ cell sorting. CD4 nanobeads (#480070, 20 µL; BioLegend), a mouse CD8 T cell isolation kit (#480035; BioLegend), mouse CD19 nanobeads (#480002; BioLegend), and mouse CD11c nanobeads (#480078; BioLegend) were added to samples of 1×10^7 cells, followed by incubation at 4 °C for 30 min and adsorption using a magnetic-activated cell sorting (MACS) system (EasySep Direct, #19655; STEMCELL Technologies, Vancouver, Canada). A few cultured cells were incubated with beads distinct from the antibody clone numbers for the assessment of their purity by flow cytometry. Sorted cells with >90% purity was subjected to further experiments.

To further investigate IRF4-mediated IL-17A synthesis in CD4⁺ T cells, we explored the mechanism by which IRF4 affects the EP300-ROR γ t-IL-17A pathway. First, we assessed the inhibitory

efficiencies of ASO15 and SGC-CBP30, which are selective CREB-binding protein (CBP)/EP300 bromodomain antagonists that suppress Th17 responses. CD4⁺ T cells were treated with 25 µL anti-CD3/CD28 (#11363D; Thermo Fisher Scientific), 10 µmol/L ASO15, and 10 µmol/L of the EP300 inhibitor SGC-CBP30 (#HY-15826; Medchemexpress, Monmouth Junction, NJ, USA). The cells were collected after 12 h culture for the detection of mRNA expression levels and after 24 h culture for the assessment of protein expression and cytokine secretion in the supernatants.

After obtaining PBMCs from patients with psoriasis, CD4⁺ T cells in the samples were treated with MojoSort human CD4 nanobeads (#480070; BioLegend) and examined using the EasySep Magnet MACS system (#18000; STEMCELL Technologies). Flow cytometry was used to confirm that CD4⁺ T cell positivity rates exceeded 90%. The cells were then treated with ASO15 for 12 h for the assessment of mRNA expression and, after stimulation, for 24 h for the evaluation of protein expression and cytokine secretion in the supernatants.

2.9. Adoptive transfer

CD4⁺ T cells were isolated from spleens of *Irf4*^{+/+} (WT) or *Irf4*^{-/-} (KO) mice via magnetic-activated cell sorting (MACS, purity >95%). A total of 1×10^6 cells were resuspended in 200 µL sterile saline (0.9% NaCl) and adoptively transferred into *Rag2*^{-/-} mice (devoid of mature lymphocytes) via tail vein injection. As *Rag2*^{-/-} mice lack functional B and T cells, almost all observed immune responses were attributable to the adoptively transferred CD4⁺ T cells. 24 h post-transplantation, recipient mice were divided into 2 groups: *Rag2*^{-/-} + *Irf4*^{+/+} CD4⁺ T cells, *Rag2*^{-/-} + *Irf4*^{-/-} CD4⁺ T cells. Both groups received daily topical application of IMQ (62.5 mg/day) on shaved dorsal skin for 5 consecutive days to induce psoriatic inflammation. Skin pathology (thickness, erythema, scaling) was quantified daily using a modified Psoriasis Area Severity Index (PASI). On fifth day post-IMQ induction, mice were euthanized for collection of skin and lymphoid tissues.

2.10. RNA sequencing

LC Biotech Corporation (Hangzhou, China) performed the RNA sequencing (RNA-seq) and analysis of mouse dermal and epidermal samples. RNA was extracted from 1-cm² psoriatic lesion areas on *Irf4*^{+/+} and *Irf4*^{-/-} mice using Trizol (#15596018CN; Invitrogen). Its concentration and purity were assessed using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific). To ensure that RNA concentrations were ≥ 500 ng/µL, only samples with A260/A280 ratios of 1.8–2.0 were used. The RNA (1 µg) was used to construct a 300 ± 50 -bp cDNA library. The library was sequenced using a 2 $\times$ 150-bp paired-end protocol and an Illumina Novaseq 6000 device (Illumina, San Diego, CA, USA). Differential gene expression was analyzed using fold change thresholds of >2 and < 0.5. A Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis was performed for genes identified as differentially expressed.

2.11. RNA isolation and quantitative polymerase chain reaction

cDNA was synthesized from total RNA samples (<1000 ng) from *Irf4*^{+/+} and *Irf4*^{-/-} mouse lesions with sufficient concentrations and purity, as described above, using a reverse transcription kit (#AG11728; Accurate Biology, Changsha, China). It was diluted

10-fold and then subjected to a fluorescent quantitative polymerase chain reaction (qPCR; #AG11701; Accurate Biology). The $2^{-\Delta\Delta C_t}$ method was used to calculate the relative expression of the target and reference (*Gapdh*) genes. The primer and ASO sequences are listed in Supporting Information Tables S3–S5.

2.12. Chromatin immunoprecipitation sequencing and qPCR

Fifty psoriatic and untreated control mouse skin samples were pooled separately for CD4⁺ T cell sorting. Chromatin immunoprecipitation (ChIP)–qPCR was performed in our laboratory. Briefly, the cells were fixed in 4% formaldehyde for 10 min, and the chromatin was separated using a SimpleChIP kit (#9003; Cell Signaling Technology). After centrifugation, the supernatants were incubated with ChIP-grade anti-IRF4 antibody (#15106; Cell Signaling Technology) or homologous control at 4 °C for >12 h to obtain immunoprecipitation complexes. DNA fragments were obtained by washing, elution, and cross-linking, and the purified DNA was then subjected to qPCR using a SYBR green premix kit (#1420; Cell Signaling Technology). The $2^{-\Delta\Delta C_t}$ method was employed to calculate the relative expression of the target and reference (*Gapdh*) genes. The primer sequences are listed in Tables S4. As *Nos2*, *Igf1*, *Prkcb*, *Eloc*, *EP300*, and *Pgk2* were previously identified as differentially expressed genes, and as *EP300* regulates Th17 cell function and coactivates the transcription of multiple genes, we selected it as a target gene^{40,41}.

2.13. Luciferase assay

To validate the regulatory sequence essential for *Ep300* transcription, we established a reporter and gene construct driven by the 3-kb *Ep300* promoter. WT DNA fragments [3000–1 bp upstream of the EP300 transcription start site (TSS)] and fragments with WT binding sequences were subcloned into the pGL3-basic vector (Promega, Madison, WI, USA). Confluent HEK293T cells were then seeded into a 12-well plate (5×10^5 /well) and transfected at microscopically observed densities of 60%–70%. Four groups were transfected with 50 ng *Renilla* luciferase control reporter (pRL-TK) vector alone and pRL-TK vector with 1000 ng pGL3b luciferase vector, 1000 ng IRF4 overexpression plasmid, and a 3000-bp fragment of 1000 ng EP300, respectively. A 3000-bp fragment of 200 ng EP300 and 800 ng IRF4 overexpression plasmid were also used. The transfection solvent used was CTS Opti-MEM I medium (#A4124801; Gibco) and the transfection reagent used was LIP3000 (#L3000008; Invitrogen). The culture was terminated after 72 h. We prepared a 96-well blackboard, added 75 μ L suspended cells to each well, and then analyzed the samples using a dual-luciferase reporter assay system (#DD1205-01; Vazyme, Nanjing, China). The ratio of firefly to *Renilla* luciferase activity was calculated for each well.

2.14. Western blot

To explore whether proinflammatory cytokine and IRF4 expression is related in patients with psoriasis, we performed Western blot and immunohistochemical (see Section 2.16) analyses of skin tissue. For protein extraction, cell and tissue samples were lysed in radio-immunoprecipitation assay buffer (#P0013B; Beyotime, Shanghai, China) containing phenylmethylsulfonyl fluoride (#8553; Cell Signaling Technology), protease inhibitor cocktail (100c, #87786; Thermo Fisher Pierce), and Pierce protease and

phosphatase inhibitor mini tablets (#A32959; Thermo Fisher Pierce). After quantification, the protein was loaded onto 8%, 10%, and 12% sodium dodecyl sulfate polyacrylamide gel electrophoresis gels (25 μ g/lane; Tris-Gly, #P0452S, #P0456S, and #P0459S; Beyotime). The gels were then electrophoretically transferred onto nitrocellulose membranes, followed by incubation with the following antibodies: IRF4 (rabbit mAb #62834, 1:1000 dilution; Cell Signaling Technology), EP300 (#sc-48343, F-4, 1:2000 dilution; Santa Cruz Biotechnology, Dallas, TX, USA), anti-human/mouse IL-17A (#26163-1, 1:1000 dilution; Proteintech, Rosemont, IL, USA), β -actin (rabbit mAb, #81115-1, 1:10,000 dilution; Proteintech), anti-human/mouse GAPDH (#60004-1, 1:10,000 dilution; Proteintech), horseradish peroxidase (HRP)-conjugated Affinipure goat anti-mouse immunoglobulin G (IgG, #SA00001-1-A, 1:20,000 dilution; Proteintech), and HRP-conjugated Affinipure anti-rabbit IgG (#7074, 1:10,000 dilution; Cell Signaling Technology). They were detected using electrochemiluminescence (ECL) substrate (#32134; Thermo Fisher). After incubation with secondary antibodies, the target and internal reference bands were detected using an ECL Western blot substrate kit (#ab65623; Abcam, Cambridge, UK) and visualized with a FluorChem FC3 imaging system (Tanon, Minneapolis, MN, USA).

2.15. Enzyme-linked immunosorbent assays

IL-17 levels in mouse serum and splenic and human cell culture supernatants were measured using commercial enzyme-linked immunosorbent assay (ELISA) kits (#M1700 and #D1700; R&D Systems, Minneapolis, MN, USA).

2.16. Histological, immunohistochemical, and immunofluorescent assays

For hematoxylin and eosin (H&E) staining, mouse and human skin tissues were fixed with 4% paraformaldehyde (#P0099; Beyotime) for at least 1 day, paraffin embedded, and then cut into 4- μ m-thick sections. After dehydration, the nuclei and cytoplasm in the sections were stained with H&E (#C0105S; Beyotime). The slides were sealed and images were captured at 10 $\times$ magnification using an *ortho*-microscope (BX53; Olympus, Tokyo, Japan) and the cellSens software (version 1.5).

For immunohistochemical analysis, sections were obtained using the same method. To retrieve tissue antigens, they were boiled in sodium citrate buffer (0.01 mol/L, pH 6.0) with continuous heating for 1 min after boiling, cooled for 3 min, and heated again for 3 min until boiling and 1 min thereafter. The sections were then incubated with 3% hydrogen peroxide for 10 min and then blocked with goat serum at 37 °C for 30 min. They were incubated with the following primary antibodies: anti-mouse/human IRF4 (rabbit mAb #62834, 1/1000 dilution; Cell Signaling Technology), anti-human/mouse IL-17A (26163-1-AP, 1/1000 dilution; Proteintech), and anti-rabbit IgG (1:10,000 dilution). Immunostaining was performed with 3,3'-diaminobenzidine solution (#P0203; Beyotime). The slides were sealed and images were captured at 10 $\times$ and 20 $\times$ magnification using an *ortho*-microscope (BX53; Olympus) and the cellSens software (version 1.5).

For immunofluorescence analysis, non-coiled tissues were fixed for at least 1 day in 4% paraformaldehyde and then dehydrated in a 30% sucrose solution for 3 days until the tissues had

settled at the bottoms of the tubes. The tissues were embedded in optimal cutting temperature (OCT) compound, frozen, and cut into 5- μ m-thick slices. The slides were allowed to equilibrate at 20 °C for 15–20 min, and the remaining OCT compound was removed by washing three times with PBS. Then, the tissues were sealed with goat serum for 30 min and permeabilized with 0.02% Triton X-100 (#HFH10; Invitrogen) for 30 min at room temperature. After washing, the samples were incubated with anti-human/mouse IL-17A (#26163-1, 1:1000 dilution; Proteintech) and FITC anti-mouse CD4 (#100406, GK1.5, 1:50 dilution; BioLegend) at 4 °C away from light. The nuclei were stained with 4',6-diamidino-2-phenylindole (#4083S; Cell Signaling Technology). The slides were sealed and images were captured at 40 $\times$ using a confocal scanning microscope (TCS SP8; Leica, Hancock, MI, USA) and the LAS X software (version 3.5.1.18803).

2.17. ASO screening and ASO-mediated IRF4 inhibition

As the targeting of transcription factors with small molecules is problematic, we employed ASO-mediated inhibition to reduce the stability of *Irf4* transcripts. We developed an *in vitro* platform for selective IRF4 inhibition using primary mouse CD4⁺ T cells and screened 20 ASOs. To assess whether ASOs downregulate IRF4 expression, naïve murine CD4⁺ T cells were transfected with ASOs or a negative control antisense oligonucleotide (ASO-NC) and cultured under anti-CD3/CD28 stimulating conditions. We then assayed the mRNA expression of *Irf4*, *EP300*, and *Il17a* under ASO inhibition. To confirm the specificity of ASO15 inhibition, gene recovery experiments were conducted with IRF4-overexpressing CD4⁺ T cells. We used the *Irf4* inhibitor ASO15 to treat CD4⁺ T cells and constructed an IRF4 overexpression plasmid for complementation.

We also evaluated the inhibitory effect of ASO15 in CD4⁺ T cells of PBMCs collected from six patients with severe psoriasis. To reduce off-target effects, we selected another human and murine homologous ASO (ASO2) and repeated measurements of mRNA and protein expression and IL-17 secretion.

2.18. IRF4-targeted therapy

To investigate the efficacy of IRF4-targeted therapy for psoriasis, ASO15 or ASO-NC was injected subcutaneously into C57 mice at a dose of 50 mg/kg per week for 4 weeks. IMQ treatment was initiated on the third day of the fourth week and continued for 4 days. On the fifth day, we treated the mice and obtained samples for the experiments. Dermal single-cell suspensions were assayed to evaluate inflammatory cell infiltration in the ASO15 group and determine percentages of Th1 and Th17 cells.

2.19. Statistical analysis

The data are presented as mean $\pm$ standard deviation (SD) and were analyzed using GraphPad Prism 6. For the comparison of variables conforming to Gaussian distribution between groups, the unpaired Student's *t*-test was used; otherwise, the Mann–Whitney test was used. One-way analysis of variance was used for multi-group comparisons. Linear regression and correlation analyses were performed. *P* < 0.05 was considered to indicate significance.

3. Results

3.1. IRF4 is overexpressed in patients with psoriasis and mice with IMQ-induced psoriasiform dermatitis

The mRNA expression of *IRF4* was significantly elevated in lesional skin and blood samples from patients with psoriasis relative to controls (Fig. 1A and B). IRF4 expression in human psoriatic skin also exceeded that in non-lesional skin (Fig. 1C and D). Flow cytometric analysis revealed larger proportions of cutaneous and circulating IRF4⁺ immunocytes in patients with psoriasis than in healthy controls. The proportion of IRF4⁺CD4⁺ T cells in psoriatic lesion samples exceeded that in unaffected skin samples from patients with psoriasis, with no difference between unaffected patients and healthy control skin (Fig. 1E and F). Immunohistochemical analysis showed that IRF4 expression was also upregulated in IMQ-treated mouse skin relative to healthy mouse skin and was similar to that in the spleen (Fig. 1G, Supporting Information Fig. S2A). IRF4 expression was extremely low in naïve CD4⁺ T cells. As expected, IRF4 was overexpressed in psoriatic skin and CD4⁺ T cells from lesions of IMQ-treated mice (Fig. 1H and I). Cutaneous CD19⁺, CD8⁺, and CD11c⁺ cell populations were slightly increased in healthy skin from IMQ-treated mice compared with those in controls (Fig. S2B). Flow cytometry showed that IMQ treatment increased the proportions of cutaneous IRF4⁺ T cells and T cell subsets (CD4⁺ and $\gamma\delta$ T), but not those of CD8⁺ and Treg subsets, relative to the control (Fig. 1J, Fig. S2C). The proportions of circulating IRF4⁺ immunocytes, including T cells and T cell subsets (CD4⁺, CD8⁺, and Tregs), were also increased (Fig. 1L, Fig. S2D). Similar changes were observed among splenic T cells, except that the proportion of CD8⁺ cells was unchanged (Fig. 1K, Fig. S2D). These data suggest that IRF4 promotes the onset and progression of psoriasis and IMQ-induced lesions through the activity of CD4⁺ T cells.

3.2. IRF4 deficiency attenuates IMQ-induced psoriasiform dermatitis

Severity scores were lower and histopathological changes were lesser for *Irf4*^{−/−} mice than for WT mice on Days 3 and 4 of treatment (Fig. 2A and B, Supporting Information Fig. S3A). *Irf4*^{−/−} mice also exhibited less weight loss, associated with reduced inflammation (Fig. S3B). Histological analysis of IMQ-treated skin showed that acanthosis and dermal immunocyte infiltration were reduced in KO mice relative to WT mice (Fig. S3C and S3D).

RNA-seq analysis of skin samples from IMQ-treated *Irf4*^{+/+} and *Irf4*^{−/−} mice revealed the differential expression of genes associated with inflammation, chemokine signaling pathways, cytokine–cytokine receptor interactions, and Fc γ -mediated phagocytosis. qPCR revealed that the gene sets affected in psoriasis encode cytokines (*Il17a*, *Il17f*, *Il18*, *Il6*, and *Il17ra*), chemokines (*Ccl2*, *Ccl5*, *Ccl7*, *Ccl11*, and *Ccl17*), and AMPs (*S100a8* and *S100a9*). Chemokines and most cytokines were under-expressed in the *Irf4*^{−/−} group. CD4⁺ T cell-related IFN- γ and IL-17A expression were significantly lower in *Irf4*^{−/−} mice than in WT mice (Fig. 2C and D). Analysis of the specimens depicted in Fig. 1B revealed that the expression of IL-17A, but not that of IL-23 or IL-1 β , correlated positively with IRF4 expression (Fig. 2E, Fig. S3E). Furthermore, an ELISA revealed that IL-17 levels in the peripheral blood of IMQ-treated *Irf4*^{−/−} mice were markedly

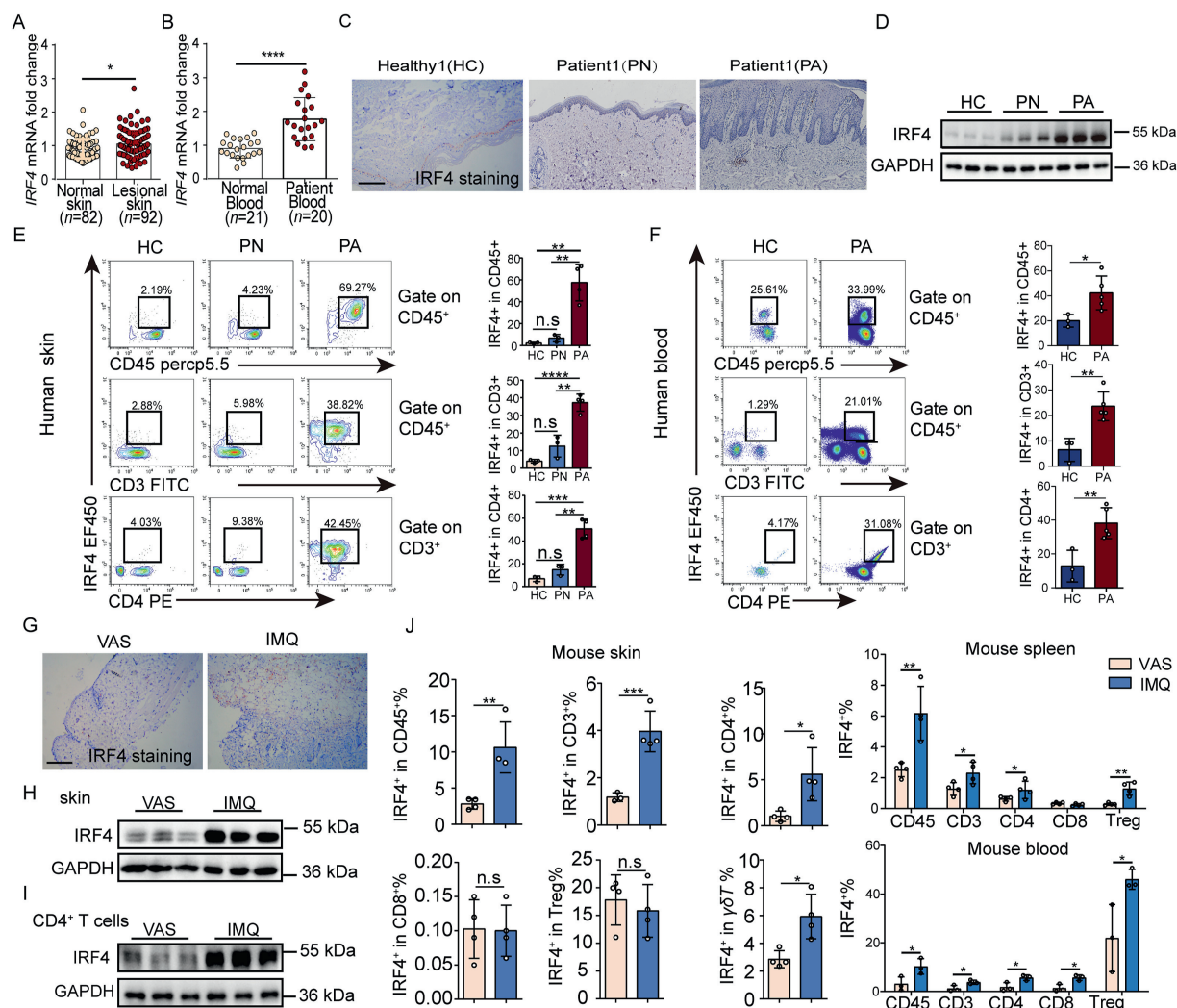


Figure 1 IRF4 expression is increased in lesional skin and blood from patients with psoriasis and mice with IMQ-induced psoriasis. (A) *IRF4* mRNA levels in normal skin ($n = 92$, GSE54456) and psoriatic skin ($n = 82$, GSE54456). (B) *IRF4* mRNA levels in peripheral blood cells from healthy controls ($n = 21$) and patients with psoriasis ($n = 20$); correlation between IL-17A and IRF4 expression in patients with psoriasis ($n = 3$). (C) Immunohistochemical staining for IRF4 in human psoriatic lesional skin ($n = 3$), non-lesional skin ($n = 3$), and healthy skin ($n = 3$). Scale bar = 100 μ m. (D) Western blots of IRF4 expression in human paired psoriatic lesional ($n = 3$) and non-lesional ($n = 3$) skin and healthy skin ($n = 3$). (E) Proportions of IRF4⁺ cells in T cell subsets from human paired psoriatic lesional ($n = 4$) and non-lesional ($n = 3$) skin and healthy skin ($n = 3$). (F) Proportions of IRF4⁺ cells in peripheral T cell subsets from healthy controls ($n = 3$) and patients with psoriasis ($n = 5$). (G) Immunohistochemical staining of mouse skin sections ($n = 3$) for IRF4. Vas, Vaseline. IMQ, imiquimod. Scale bar = 100 μ m. (H) Western blots of IRF4 expression in mouse skin sections ($n = 3$). (I) Western blot analysis of IRF4 expression in MACS-sorted CD4⁺ cells from mouse skin; >90% purity of sorted cells. (J) Proportions of IRF4⁺ cells in T cell subsets from mouse skin sections ($n = 4$). (K) Proportions of IRF4⁺ cells in T cell subsets from mouse spleen sections ($n = 4$). (L) Proportions of IRF4⁺ cells in T cell subsets from mouse blood samples ($n = 4$). Data are presented as mean $\pm$ SD. For (A, B, F, J), two-sided unpaired Student's *t*-test; for (E), one-way analysis of variance; for (K, L), two-sided Mann–Whitney test; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$; n.s., no significance.

decreased (Fig. 2F). The immunohistochemical analysis of skin specimens from IMQ-treated mice showed that the IL-17A⁺ cell population was much smaller in *Irif4*^{-/-} than in WT mice. Immunofluorescence revealed that CD4⁺ T cell levels of IL-17A were also reduced (Fig. 2G and H). The number of IL-17A⁺ $\gamma\delta$ TCR⁺ cells remained unchanged (Fig. S3F). These results suggest that *Irif4* KO does not affect the proportion of IL-17A⁺ $\gamma\delta$ T cells, but attenuates IMQ-induced dermatitis by decreasing the IL-17A⁺ CD4⁺ T cell population.

3.3. Cutaneous IL-17A⁺ CD4⁺ T cell populations are decreased in *Irif4*^{-/-} mice

The percentages of cutaneous F4/80⁺ CD11c⁺ cells and CD11b⁺ DCs in IMQ-treated mice were lower in the *Irif4*^{-/-} group than in the *Irif4*^{+/+} group. The percentages of MHC II⁺ macrophages were similar, whereas those of neutrophils differed significantly between groups (Fig. 2I, Supporting Information Fig. S4A and S4B). The percentage of cutaneous $\gamma\delta$ TCR⁺ cells in IMQ-

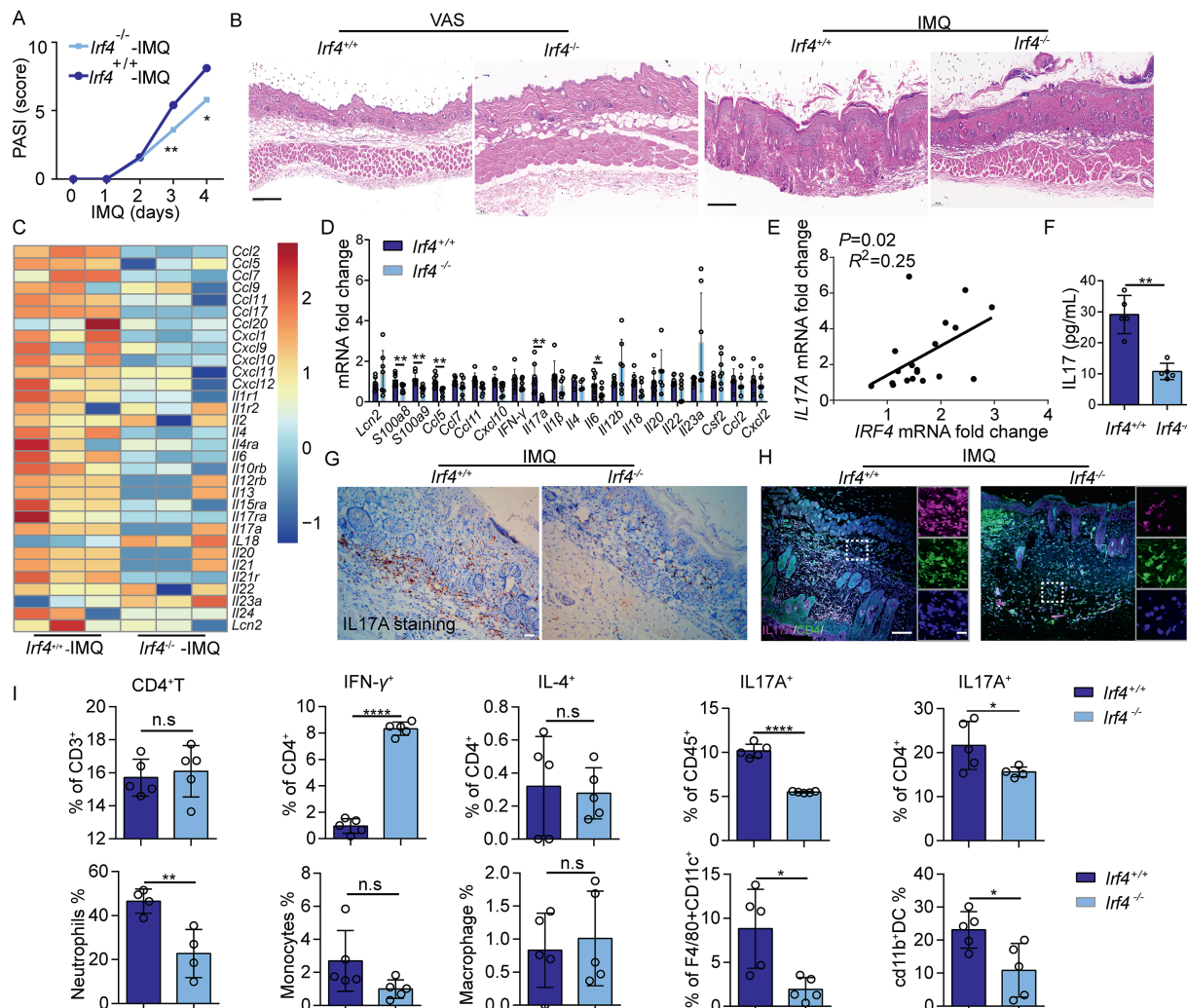


Figure 2 IRF4 deficiency alleviates the severity of IMQ-induced psoriasis. (A) Scoring curves for back skin thickness, scaling, and erythema. (B) H&E staining of skin sections. For (A, B), $n = 4-5$ /group, results from one independent experiment. (C) RNA-seq results for whole skin showing the differential expression of genes associated with cytokines, chemokines, AMPs, epidermis development, tissue repair, and phagocytosis between IMQ-treated *lrf4*^{+/+} and *lrf4*^{-/-} mice ($n = 3$ biologically independent samples). (D) qPCR results for pathogenic factors in skin from IMQ-treated mice ($n = 7$). (E) Correlation between *IL-17A* and *IRF4* mRNA expression in peripheral blood cells from patients with psoriasis ($n = 20$) and healthy controls ($n = 21$). (F) ELISA results for IL-17 in mouse serum ($n = 5$). (G) IL-17A immunostaining of mouse skin sections ($n = 3$). Scale bar = 200 μ m. (H) Immunofluorescent microscopy images of mouse skin sections stained with anti-CD4 (green), anti-IL-17A (pink), and DAPI (blue). The dashed rectangular box shows CD4⁺IL-17A⁺ cells in the dermis. Left: scale bar = 100 μ m; Right: scale bar = 20 μ m. (I) Flow data statistics for various types of cells. F and I, $n = 4-5$ /group, results from 2 independent experiments. Data are presented as mean $\pm$ SD. For (A), two-sided unpaired Student's *t*-test; for (D, F, I), two-sided Mann-Whitney test and two-sided unpaired Student's *t*-test; for (E), linear regression analysis. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$; n.s., no significance.

treated mice was lower in the *lrf4*^{-/-} group than in the *lrf4*^{+/+} group, and the imbalance of cutaneous CD4⁺ T cells seen in *lrf4*^{-/-} mice was not evident in the *lrf4*^{+/+} group (Fig. 2I, Fig. S4B). The proportions of IL-17A⁺CD45⁺ and IL-17A⁺CD4⁺ cells were smaller in the *lrf4*^{-/-} group, whereas those of IL-17A⁺ $\gamma\delta$ TCR⁺, IL-4⁺CD4⁺, and IFN- γ ⁺CD4⁺ cells did not differ between groups (Fig. 2I, Fig. S4B). The percentages of CD4⁺ T cells in IMQ-treated *lrf4*^{-/-} and WT mice were similar (Fig. 2I). However, compared with untreated controls, IRF4 was significantly overexpressed in Th17 cells but only slightly increased in Th1 cells, suggesting that it preferentially regulates Th17 and Th1 cell differentiation. As expected, clinical signs and histopathological findings of psoriasiform dermatitis were attenuated in *lrf4*^{-/-} mice compared with those in WT mice. The

KEGG enrichment analysis identified the IL-17A signaling pathway as the top-ranked inflammation-related pathway (Fig. S4C). The analysis of immune cell infiltration using RNA-seq data showed significant downward trends for mast cells and Tregs in *lrf4*^{-/-} mice compared with WT mice (Fig. S4D). These results suggest that IRF4 deficiency mitigates psoriasis by reducing CD4⁺ T cell-mediated IL-17A secretion.

3.4. *IL-1 β* and *IL-23A* stimulate *IRF4*'s initiation of the *Th17* pathway in psoriatic skin CD4⁺ T cells

The top 10 enrichment pathways for genes upregulated in murine cutaneous CD4⁺ T cells, identified by ChIP-seq and KEGG analyses, are shown in Supporting Information Fig. S5A. The

enrichment index for the hypoxia-inducible factor-1 pathway, involved in Th17 cell differentiation, ranked first, and immunoregulatory pathways were particularly relevant. *Ep300* had *Irf4* ChIP-seq peaks in the untreated and IMQ-treated groups, with about 37.5% at the annotated promoter TSS (Fig. S5B). Consistent with our expectation, samples from IMQ-treated mice displayed more *Irf4* ChIP-seq peaks than did those from the VAS group (Fig. 3A), as well as *Nos2*, *Igf1*, *Prkcb*, *Eloc*, and *Pgk2* peaks (Supporting Information Fig. S6A and S6B); these findings were confirmed by ChIP-qPCR (Fig. 3B). IRF4 was located in the proximal region of the *Ep300* promoter. The reporter construct demonstrated high luciferase activity in transfected HEK293T cells, indicating that the core promoter region was within 3000 bp upstream of the *Ep300* TSS (Fig. 3C). The transfected cells also

showed *EP300* mRNA and protein overexpression (Fig. 3D and E). The cutaneous expression of *EP300*, *ROR γ t*, and *IL-17A* in IMQ-treated and untreated groups was attenuated in *Irf4*^{-/-} mice compared with *Irf4*^{+/+} mice (Fig. 3F). Splenic CD4⁺ T cells from *Irf4*^{+/+} and *Irf4*^{-/-} mice exposed to the *EP300* inhibitor SGC-CBP30 showed almost no IRF4 expression and decreased *EP300* and *IL-17A* expression (Fig. S5C).

HOMER motif analysis revealed an enrichment of transcription factor detection peaks, with IRF motifs (GAAA, the IRF binding site), IFN-sensitive response elements (representing the tandem IRF motif occupied by two IRFs), and activating protein 1/IRF complex elements, usually occupied by IRF4 or IRF8 and BATF/JUN (Fig. 3G). Ets/IRF complex motifs were significantly enriched, suggesting that IRF4 and Ets family

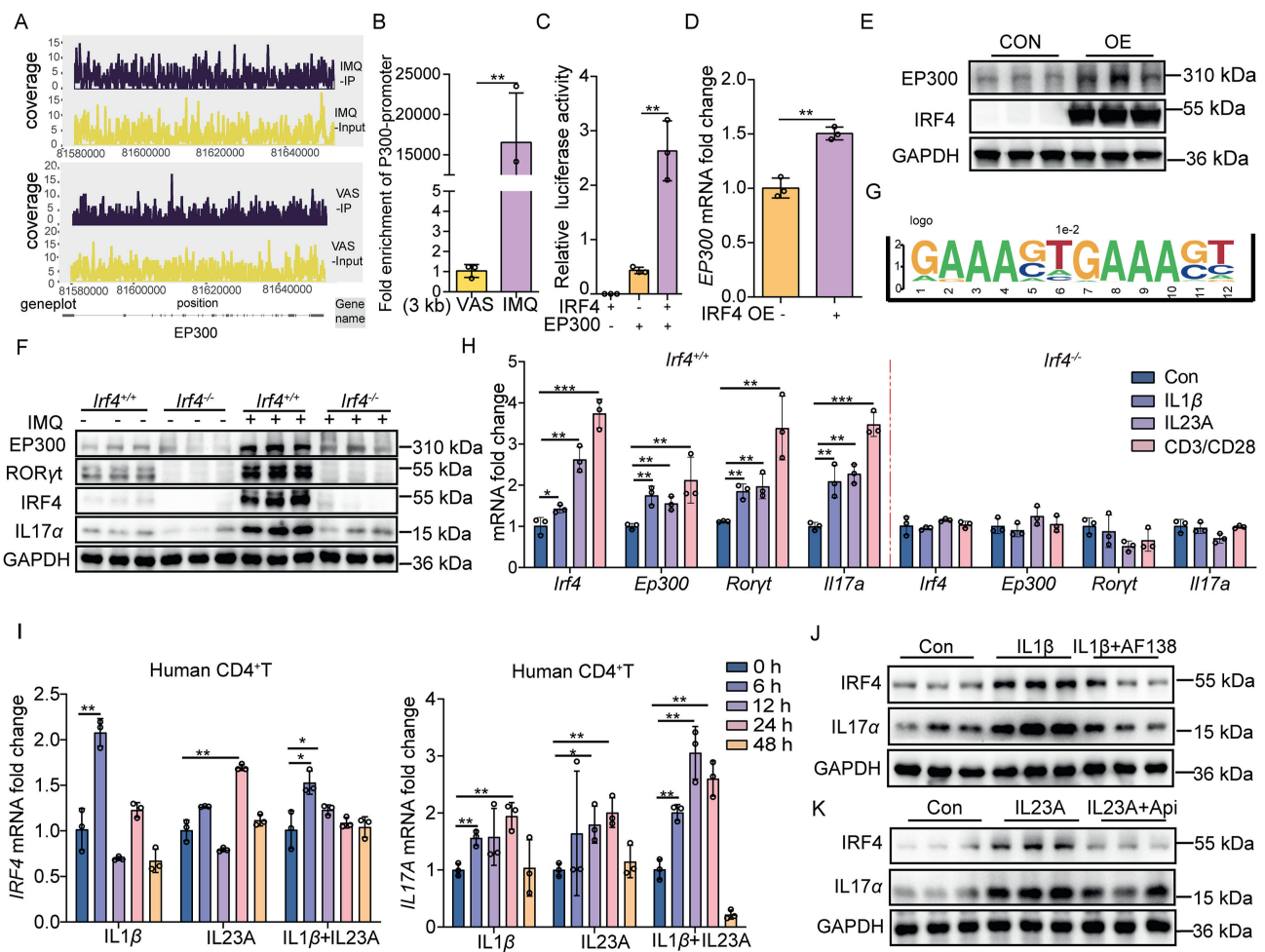


Figure 3 IL-1 β /IL-23A induces *Irf4* binding to the *Ep300* promoter to initiate Th17 pathway signaling in psoriatic skin CD4⁺ T cells. (A) ChIP-seq tracks for the indicated CD4⁺ T cell genes. (B) Fold change in *Ep300* mRNA from the sample shown in (A). (C) Luciferase activity in HEK293T cells transfected with luciferase reporter vectors and IRF4 overexpression plasmid. (D) qPCR results for CD4⁺ T cells treated with IRF4 overexpression plasmid. (E) Western blots of IRF4 and EP300 in CD4⁺ T cells treated with IRF4 overexpression plasmid. (F) Western blots of IRF4, EP300, ROR γ t, and IL-17A in mouse skin treated with IMQ. (G) The IRF4 binding motif. (H) qPCR results for *Irf4*, *Ep300*, *Rorc*, and *Il17a* in CD4⁺ T cells treated with IL-1 β , IL-23A, or anti-CD3/CD28 from the spleens of *Irf4*^{+/+} and *Irf4*^{-/-} mice. (I) qPCR results for *IRF4* and *IL-17A* mRNA in T cells treated with human IL-1 β or IL-23A for 0, 6, 12, 24, and 48 h. (J) Western blots of IRF4 and IL-17A in CD4⁺ T cells treated with mouse IL-23A and AF12198 (an IL-1R inhibitor). (K) Western blots of IRF4 and IL-17A in CD4⁺ T cells treated with mouse IL-23A and Apilimod (an IL-23 inhibitor). Data are presented as mean $\pm$ SD. For (B, D), two-sided unpaired Student's *t*-test; for (C, H, and I), one-way analysis of variance. **P* < 0.05, ***P* < 0.01, ****P* < 0.001.

members occupy these sites together⁴². Thus, the ChIP-seq, luciferase reporter assay, and Western blot results indicated that *EP300* is a direct target of IRF4 in CD4⁺ T cells from the murine psoriasis model.

Splenic CD4⁺ T cells activated with IL-1 β , IL-23A, and anti-CD3/CD28 showed slightly elevated *Irf4*, *Ep300*, *Rorc*, and *Il17a* mRNA expression in the *Irf4*^{-/-} group and normal expression in the *Irf4*^{+/+} group (Fig. 3H). Human peripheral-blood CD4⁺ T cells from healthy individuals stimulated with IL-1 β or IL-23A showed significantly increased IRF4 expression, which was more pronounced with IL-1 β stimulation. Changes in expression rates were time-dependent. During the 0–48 h period, the IL-1 β -induced expression of *IRF4* and *IL-17A* was greatest at 6 h, whereas IL-23A did not upregulate *IRF4* expression at the same time point. IL-23A-induced IRF4 expression increased primarily between 12 and 24 h. In addition, IL-1 β inhibition significantly reduced IRF4 expression. These data suggest that the upregulation of *IRF4* synthesis is increased by IL-1 β in the initial stage of psoriatic inflammation and sustained by IL-23A. These signals are significant for the *IRF4*-mediated early differentiation of CD4⁺ T cells. IRF4 expression decreased after 24–48 h (Fig. 3I), confirming that IRF4 expression requires continuous stimulation. Human peripheral blood-derived CD4⁺ T cells exposed to IL-1 β and IL-1R and their inhibitors showed increased IRF4 protein expression when stimulated and decreased expression when inhibited. IL-17 expression also followed this pattern (Fig. 3J), and IL-23A and IL-23 inhibitors yielded similar results (Fig. 3K). These results indicate that IL-1 β /IL-23A-induced IL-17A expression by CD4⁺ T cells in psoriasis requires the presence of IRF4.

3.5. *IRF4* deficiency in CD4⁺ T cells mitigates psoriasiform dermatitis

In the adoptive transfer experiment (Fig. 4A), the *Rag2*^{-/-} mice did not develop typical IMQ-induced lesions (Fig. 4B). IMQ-induced dermatitis and splenomegaly were significantly reduced in *Rag2*^{-/-} mice that received naïve CD4⁺ T cells lacking *Irf4* relative to those that received cells from WT mice (Fig. 4B–E, Supporting Information Fig. S7A and S7B). IL-17A retention was also decreased in mice that received *Irf4*-deleted CD4⁺ T cells (Fig. 4F, Fig. S7C). These results suggest that IRF4 overexpression dysregulates the CD4⁺ T cell activation and differentiation that drive the pathogenesis of psoriasis and that IRF4 deficiency in CD4⁺ T cells was the primary cause of attenuated psoriasiform dermatitis.

In the *Irf4*^{fl/fl}CD4-Cre mouse model, the loss of *Irf4* in CD4⁺ T cells attenuated IMQ-induced psoriasiform inflammation (Fig. 4G–J). Psoriasis-associated genes, including *S100a8*, *S100a9*, *Sprr2a*, *b*, *d*, *e*, *Ifng*, *Il17a*, *Il1b*, and *Il6*, were strongly downregulated (Fig. 4K). The cutaneous expression of IRF4, EP300, ROR γ t, and IL-17a was also decreased in IMQ-treated *Irf4*^{fl/fl}CD4-Cre mice. Dermal single-cell suspensions from *Irf4*^{fl/fl}CD4-Cre mice had significantly reduced percentages of IL-17A⁺ cells, whether from CD45⁺ or CD4⁺ T cells, relative to those from *Irf4*^{fl/fl} mice. We observed a smaller percentage of IFN- γ ⁺CD4⁺ T cells, no change in the percentage of IL-4⁺CD4⁺ T cells, and larger percentages of CD45⁺ and CD4⁺ T cells. Moreover, IMQ-induced psoriasiform dermatitis was attenuated in *Irf4*^{fl/fl}CD4-Cre mice compared with *Irf4*^{fl/fl} mice (Fig. 4M and L).

These findings suggest that the inhibition of IRF4 in CD4⁺ T cells represents an important therapeutic strategy.

3.6. *IRF4* binds to *EP300* promoters in CD4⁺ T cells, thereby upregulating *IL-17* synthesis and promoting the development of psoriasiform dermatitis

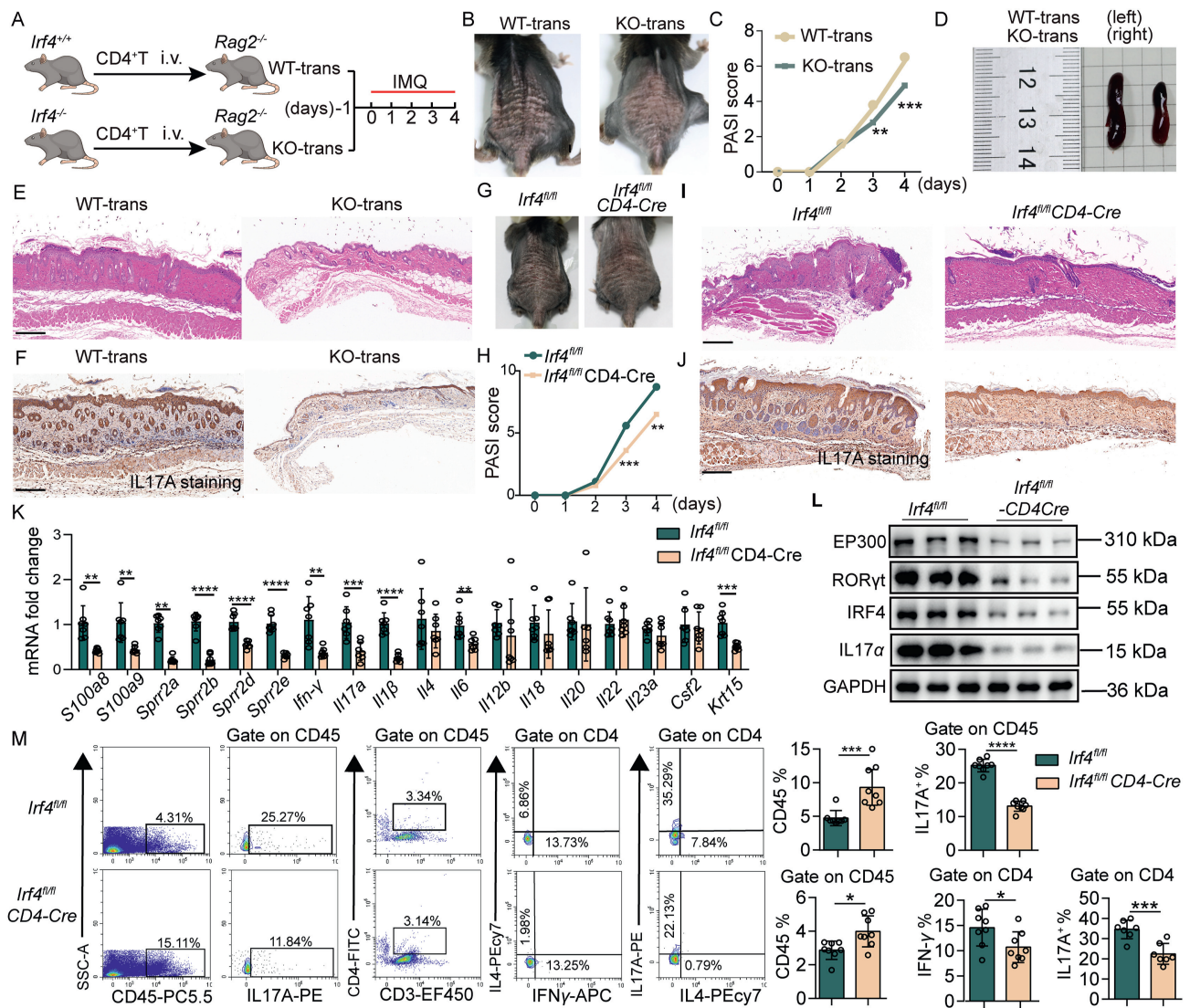
More than half of the ASO sequences screened significantly inhibited the mRNA expression of *Irf4*, *Ep300*, and *Il17a* (Fig. 5A). ELISA results for IL-17 secretion were similar for ASO1–ASO4, ASO11, and ASO15 (Supporting Information Fig. S8A). We chose to use ASO2 and ASO15, which are homologous to human and mouse sequences (BLAST data not shown; Fig. 5B, the sequence is shown in Table S5). *Irf4* levels decreased with increasing ASO15 concentrations (Fig. S8B). *Irf4* expression was upregulated in anti-CD3/CD28-treated CD4⁺ T cells relative to that in dimethyl sulfoxide-treated cells (Fig. 5C). These data demonstrated the preferential induction of IRF4 and showed that *Irf4* is inhibited indirectly by ASO15 in anti-CD3/CD28-treated CD4⁺ T cells.

EP300 protein levels in CD4⁺ T cells decreased with increasing SGC-CBP30 concentrations (Fig. S8B). The expression of IRF4, EP300, ROR γ t, and IL-17A was upregulated in anti-CD3/CD28-treated CD4⁺ T cells and was significantly reduced by ASO15 exposure (Fig. 5D). Exposure to SGC-CBP30 alone reduced the expression of EP300, ROR γ t, and IL-17A. IRF4 expression was also slightly reduced after SGC-CBP30 exposure, which may be related to the inhibitor's structure. Co-treatment with ASO15 and SGC-CBP30 reduced IL-17A expression. Congruently, supernatant levels of IL-17A were elevated following the stimulation of normal CD4⁺ T cells with anti-CD3/CD28, but were reduced in ASO15- or SGC-CBP30-transfected psoriatic CD4⁺ T cells (Fig. 5E). These findings revealed that IRF4 downregulates the EP300–ROR γ t–IL-17A pathway, thereby inhibiting IL-17A synthesis by CD4⁺ T cells. ELISAs of the corresponding cell culture supernatants showed that IL-17A secretion corresponded to the protein expression.

Results for ASO15- and anti-CD3/CD28-stimulated human CD4⁺ T cells were similar to those for the murine cells. Compared with untreated CD4⁺ T cells, ASO15-treated cells had reduced mRNA and protein levels of IRF4, EP300, ROR γ t, and IL-17A (Fig. 5F and G). IFN- γ , IL-4, IL-1, and IL-23A assays showed significant reductions of IFN- γ - and IL-1 β -encoding mRNA levels and almost no change in the IL-4 and IL-23A levels (Fig. S8C). ELISAs of the cell culture supernatants revealed that IL-17 secretion was significantly reduced (Fig. 5H). The results obtained with ASO2 were almost identical to those obtained with ASO15 (Fig. 5I–K). These results reflect the impact of IRF4 on CD4⁺ T cell differentiation.

3.7. Targeted *IRF4* inhibition attenuates psoriasiform dermatitis

On the fifth day of IMQ treatment in the assessment of IRF4-targeted therapy (Supporting Information Fig. S9A), cutaneous lesions and histopathological findings (*e.g.*, dermal inflammatory cell infiltration and acanthosis) were attenuated in the ASO15 group relative to the ASO–NC group (Fig. 6A–C, Fig. S9B and S9C). Immunohistochemical staining showed fewer IL-17A⁺ cell populations in the epidermis and dermis of lesional skin from



the ASO15 group than in that from the NC group (Fig. 6D, Fig. S9D).

The percentages of CD45⁺, CD3⁺, CD4⁺, and IL-17A⁺ cells, whether derived from CD45⁺, CD3⁺, or CD4⁺ cells, were smaller in dermal single-cell suspensions from the ASO15 group than in those from the ASO–NC group (Fig. 6E). In the EP300–ROR γ t–IL-17A pathway, the mRNA and protein expression levels of IRF4, EP300, and ROR γ t were decreased (Fig. 6F and H). An ELISA showed significantly decreased serum levels of IL-17 in ASO15-treated mice (Fig. 6G). These results

indicated that the downregulation of IRF4 alleviated IMQ-induced psoriasiform immunopathology and diminished disease severity.

4. Discussion

In this study, we elucidated a pathogenic mechanism of psoriasis involving IRF4. We showed that IRF4 expression is upregulated in psoriatic and psoriasiform lesions and circulating CD4⁺ T cells, and contributes to immunopathogenesis by binding to *Ep300* to

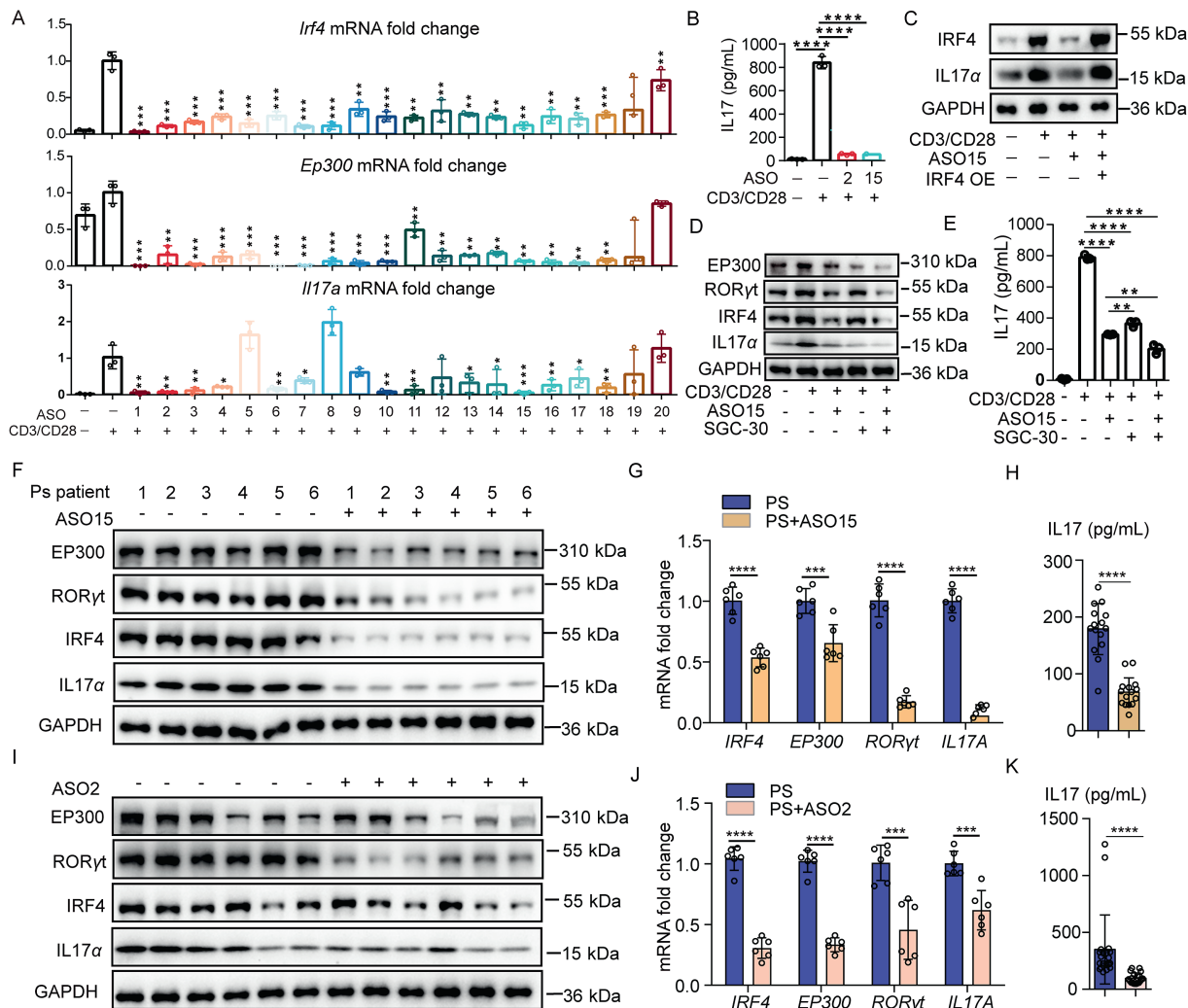


Figure 5 IRF4 inhibits IL-17A production in CD4⁺ T cells through the EP300, ROR γ t, and IL-17A pathways. (A) qPCR results for *IRF4*, *EP300*, and *IL-17A* mRNA expression as indicated ($n = 3$). (B) ELISA results for IL-17 in CD4⁺ T cell supernatants as indicated ($n = 3$). (C) Western blots of IRF4 and IL-17A in CD4⁺ T cells treated with CD3/CD28, ASO15, and an IRF4 overexpression plasmid. (D) Western blots of IRF4, EP300, ROR γ t, and IL-17A in MACS-sorted peripheral CD4⁺ T cells from patients with psoriasis. (E) ELISA results for IL-17 in CD4⁺ T cell supernatants as indicated in (D) ($n = 3$). (F) Western blots of IRF4, EP300, ROR γ t, and IL-17A in CD4⁺ T cells treated with CD3/CD28, ASO15, and SGC-CBP30. (G) qPCR results for *IRF4*, *EP300*, *RORC*, *IL-17A*, and inflammatory-related gene expression in MACS-sorted peripheral CD4⁺ T cells from patients with psoriasis ($n = 3$). (H) ELISA results for IL-17 in CD4⁺ T cell supernatants ($n = 3$). (I) Western blots of IRF4, EP300, ROR γ t, and IL-17A in CD4⁺ T cells treated with CD3/CD28, ASO2, and SGC-CBP30. (J) qPCR results for *IRF4*, *EP300*, *RORC*, *IL-17A*, and inflammatory-related gene expression in MACS-sorted peripheral CD4⁺ T cells from patients with psoriasis ($n = 3$). (K) ELISA results for IL-17 in CD4⁺ T cell supernatants ($n = 3$). Data are presented as mean $\pm$ SD. For (A, B, E), one-way analysis of variance; for (G, J), two-sided Mann–Whitney test; for (H, K), two-sided unpaired Student's *t*-test. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$.

upregulate IL-17A secretion *in vitro* and *in vivo*. We observed significantly larger numbers and greater activity of immune cells (including T cells, macrophages, and neutrophils) in psoriatic lesional skin compared with non-lesional areas, which likely induced IRF4 expression through various signaling pathways, including the IL-23/Th17 axis. The ablation and pharmacological inhibition of IRF4 mitigated dermatopathy in a psoriasis murine model.

IRF4 expression has been reported to be highly and specifically localized to immune cells and adipocytes⁴³. In psoriatic skin, it has been found to be increased in keratinocytes and immune cells; this study yielded similar findings, with a more pronounced increase in immune cells, likely due to differences in the clonal origins of the antibodies used³⁹.

The attenuation of dermatitis is related to the downregulation of pathogenic and inflammatory factors^{1,6}. Our KEGG analysis of differentially downregulated inflammatory factors in *Ir4* KO mice relative to WT mice revealed that these changes were concentrated in the TLR, C-type lectin receptor (CLR), chemokine, and nucleotide-binding leucine-rich repeat receptor signaling pathways and the Fc γ R-mediated phagocytosis pathway. The downregulation of CLRs can reduce TLR signaling pathway expression in keratinocytes and monocytes, thereby reducing DC maturation and activation and consequently diminishing Th17 cell function and differentiation^{44,45}. DCs from mice treated with an *Ir4*-silencing ASO upregulated the anti-inflammatory cytokine IL-10 and inhibited Th17 differentiation and infiltration and IL-17

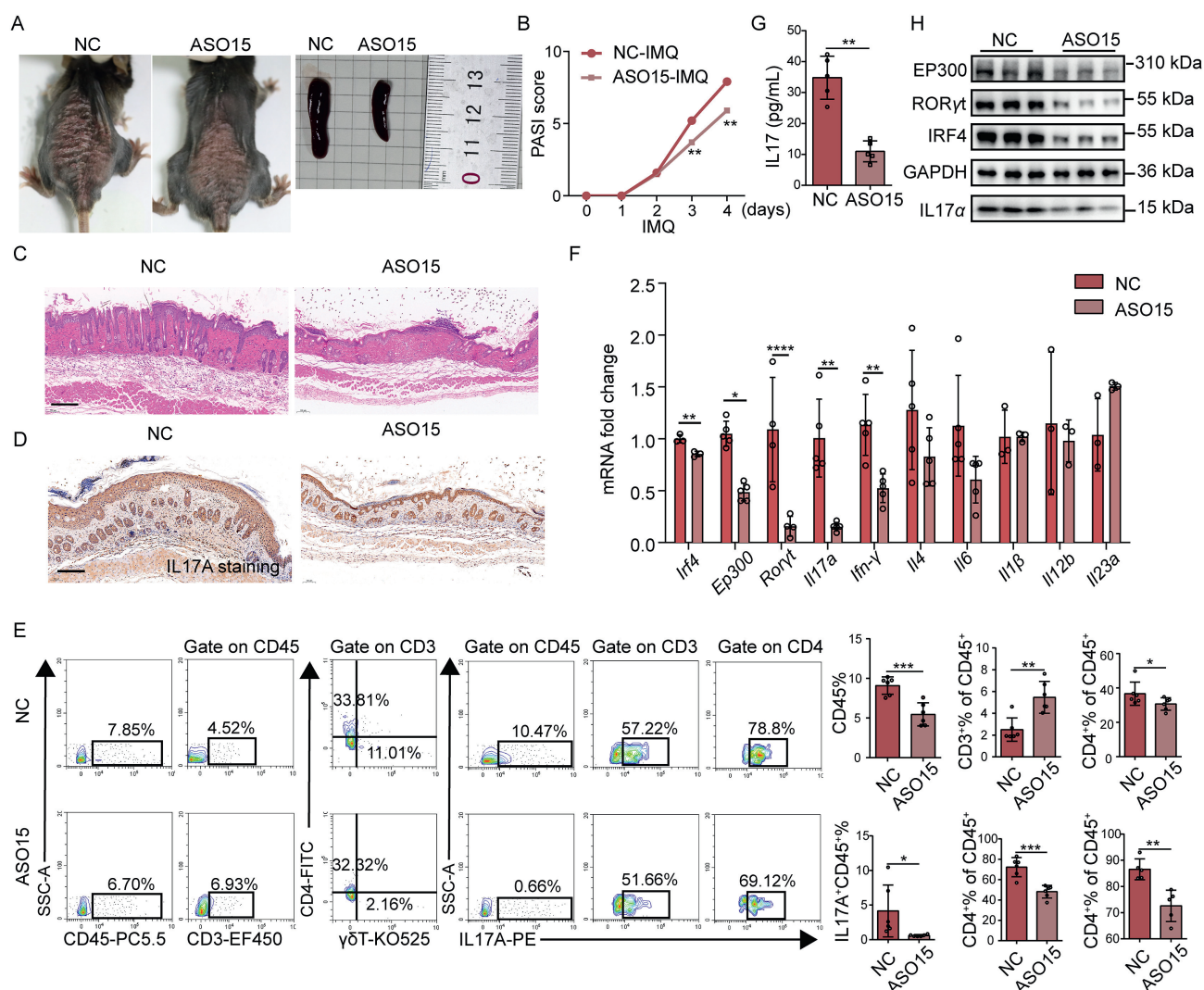


Figure 6 Targeted inhibition of IRF4 inhibits IMQ-induced psoriasis-like symptoms. (A) Representative images of mouse back skin and spleens. (B) Scoring curves for back skin thickness, scaling, and erythema. (C) H&E staining of skin sections. Scale bar = 200 μ m. (D) Representative images of IL-17A-stained mouse skin sections ($n = 3$). Scale bar = 200 μ m. (E) Representative flow cytometry plots and statistics for CD45⁺, CD3⁺, CD4⁺, $\gamma\delta$ T⁺, IL-17A⁺CD45⁺, IL-17A⁺CD3⁺, and IL-17A⁺CD4⁺. (F) qPCR results for *Irf4*, *Ep300*, *Rorc*, *Il17a*, and inflammatory gene expression in skin from the ASO-NC and ASO15 groups. (G) ELISA results for IL-17 levels in serum derived from the ASO-NC and ASO15 groups. (H) Western blots of IRF4, EP300, ROR γ t, and IL-17A in the skin from the ASO-NC and ASO15 groups. For (A–G), $n = 3$ –4/group from one independent experiment. Data are presented as mean $\pm$ SD. For (B), two-sided Mann–Whitney test; for (E, G), two-sided unpaired Student's *t*-test; for (F), two-sided Mann–Whitney test. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$; n.s., no significance.

release in an experimental autoimmune encephalomyelitis model^{5,46–49}. The reduction in IL-22 and IL-17 release by Th17 cells also decreases nuclear factor- κ B pathway activity, thereby reducing C–C motif chemokine ligand 20 secretion⁵⁰. Diminished Fc γ -R activation can weaken the phagocytic activity of macrophages and neutrophils, resulting in reduced TNF- α and IL-6 release^{51–53}. Notably, IL-17A expression, a crucial pathogenic factor that promotes the progression of psoriasis, is significantly reduced after IRF4 ablation¹. In psoriasis, IL-17A is secreted predominantly by CD4⁺ Th17 and $\gamma\delta$ T cells, with a significantly lesser contribution from natural killer cells^{54–57}. In this study, CD4⁺ T and IL-17A⁺ $\gamma\delta$ T cell counts in IMQ-induced lesions from *Irf4*^{−/−} and *Irf4*^{+/+} mice did not differ, but the number of Th17 cells was significantly reduced in the *Irf4*^{−/−}

group. IL-17A and IRF4 expression were correlated, corroborating previous findings regarding the effect of *Irf4* on *Il17a* transcription^{5,22,58}. Following adoptive CD4⁺ T cell transfer, *Rag2*^{−/−} mice did not exhibit characteristic IMQ-induced psoriasiform dermatitis and showed downregulated IRF4 expression in CD4⁺ T cells, especially through IL-17A hyposecretion. The reintroduction of *Rorc* and ROR α into IRF4-deficient CD4⁺ T cells incompletely restored defective IL-17 production. *Rorc* alone cannot initiate Th17 transcription; it must also antagonize related activation programs of other transcription factors, supporting the concept that IRF4 plays an additional and central role in Th17 differentiation. The expression level of IRF4 determines the differentiation and aggregation of Th17 cells, but is not critical for their survival. Thus, IRF4 does not affect the survival or number

of $CD4^+$ T cells, but is the most crucial element in the control of IL-17A secretion in psoriasisform dermatitis⁵⁹⁻⁶².

In this study, the DC population in the myeloid compartment was significantly reduced (Fig. 2I). IRF4 is essential for DC differentiation and plays a pivotal role in DCs' promotion of Th17 responses, as DCs secrete IL-23 to promote Th17 differentiation. The involvement of DCs in psoriasis is also well documented⁶³⁻⁶⁵. Our analysis of immune cell infiltration based on RNA-seq data provided further insights. As mast cells release IL-17 in psoriatic lesions, their reduced abundance likely contributes to the attenuation of the disease phenotype. In another study, Tregs lacking IRF4 predominantly maintained a naïve $CD62L^{hi}$ phenotype and exhibited decreased expression of effector and inhibitory molecules⁶⁶. Treg depletion or dysfunction is typically associated with autoimmunity and immunopathology, which may exacerbate psoriasis⁶⁷. Thus, this finding may explain why IRF4 depletion cannot eliminate psoriatic symptoms. Specifically, ASO, the IRF4 inhibitor employed in this study, targets IRF4 at the genetic level and acts non-specifically during the disease process. It reduces DC activation and Th17 differentiation in the early phase of psoriasis, disrupts the formation of the IL-23/IL-17/IL-36 immune feedback loop in the middle phase, and diminishes Treg generation, exacerbating the inflammatory response, in the late phase. The negative impact of IRF4 deficiency on Treg function has been overlooked in much research, including ours.

IRF4 regulates gene expression by binding to specific DNA-binding motifs and interacting with transcription factors⁶⁸. It directly binds and transactivates *Ii14* and *Ii21* promoters, and can bind directly to the *Ii17A* promoter and induce $CD4^+$ T cell expression of *Ii17a* and *Rorc*^{28,69}. The ChIP-seq and dual-luciferase assays performed in this study revealed that IRF4 binds directly to the *EP300* promoter to initiate its transcription in $CD4^+$ T cells from psoriatic skin. IRF4 and EP300 overexpression were directly related, and IRF4 reduction through *Irf4* ablation or pharmacological inhibition reduced the expression of EP300 and its downstream molecules *in vitro* and *in vivo*. These findings can be explained by the function of EP300 as a transcriptional coactivator, histone acetyltransferase, and histone acetylation reader. EP300 regulates gene expression, chromatin structure, transcription factor activity, and cellular processes, including differentiation, proliferation, and apoptosis. *Ep300* can bind directly to the *Irf4* promoter, upregulating the transcriptional activity of IRF4 and affecting the expression of IRF4-regulated downstream genes⁷⁰. This is the opposite of our findings that IRF4 binds to the *EP300* promoter and that EP300 inhibition slightly reduces IRF4 expression. Our findings are congruent with those of Abruzzese et al.⁶⁸, who showed that the binding of EP300 to *Irf4* affected *Irf4* expression. The interaction and functional regulation of IRF4 and EP300 may depend on the cell type, upstream and downstream events, and signaling pathways. EP300 can also regulate the differentiation and activity of Th cell subsets without interacting with IRF4, primarily because EP300 expression is usually increased during Th17 cell differentiation. EP300 complexes with *Rorc* and enhances *Rorc* transcription through deacetylation modification. EP300–*Rorc* interaction promotes *Rorc* binding to the enhancer regions of target genes and changes the chromatin structure, thereby upregulating transcription^{68,71}. This interaction can also upregulate the expression of Th17 cell-specific genes such as *Ii17a* and *Ii17f*^{72,73}. EP300 also complexes with other transcription and co-regulatory factors to jointly regulate Th17 cell differentiation and activity. For example, it interacts with signal

transducer and activator of transcription 3 to jointly promote Th17 cell differentiation and regulate the expression of related genes⁷⁴⁻⁷⁶.

Thus, the interactions between EP300 and IRF4 are complex, as they jointly participate in immune regulation, but their elucidation is important to fully understand the pathogenesis of diseases such as psoriasis. Further research is needed to elucidate autoinflammatory disease-specific and common pathways. In addition, the limitations of the IMQ-induced psoriasis model must be recognized. IMQ induction primarily recapitulates the acute inflammatory phase of psoriasis, failing to fully capture the chronic nature of the disease. Moreover, the resulting inflammation is confined to the local skin, mediated through TLR3/TLR7 activation, which does not fully replicate the systemic immune response observed in psoriasis⁷⁷. The differences between the human and murine immune systems further curtail the precision of therapeutic efficacy prediction. The findings of the present study were derived from the IMQ model. In future research, the combination of integration of multiple models is needed to better simulate human psoriasis, thereby supporting the development of more precise targeted therapies and, potentially, a cure for this condition.

5. Conclusions

Although mAbs targeting IL-23, IL-17, and IL-17RA can improve psoriasis, they cannot eliminate pathogenic T cells and thus allow recurrence due to keratinocyte repriming. As a downstream target of the proinflammatory cytokines IL-1 β and IL-23, IRF4 promotes Th17 function by targeting EP300 to upregulate IL-17A secretion, thereby dysregulating inflammatory responses in circulating immunocytes and psoriatic skin lesions. This study demonstrated that IRF4 is a key effector molecule in the immunopathology of psoriasis, and can serve as a therapeutic target.

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Author contributions

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Conflicts of interests

The authors declare no competing interests.

Appendix A. Supporting information

Supporting information to this article can be found online at <https://doi.org/10.1016/j.apsb.2025.05.004>.

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