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Pharmacologic Inhibition of RORγt Regulates Th17 Signature Gene Expression and Suppresses Cutaneous Inflammation In Vivo

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IL-17–producing CD4$^+$Th17 cells, CD8$^+$Tc17 cells, and γδ T cells play critical roles in the pathogenesis of autoimmune psoriasis. RORγt is required for the differentiation of Th17 cells and expression of IL-17. In this article, we describe a novel, potent, and selective RORγt inverse agonist (TMP778), and its inactive diastereomer (TMP776). This chemistry, for the first time to our knowledge, provides a unique and powerful set of tools to probe RORγt-dependent functions. TMP778, but not TMP776, blocked human Th17 and Tc17 cell differentiation and also acutely modulated IL-17A production and inflammatory Th17-signature gene expression (*Il17a*, *Il17f*, *Il22*, *Il26*, *Ccr6*, and *Il23*) in mature human Th17 effector/memory T cells. In addition, TMP778, but not TMP776, inhibited IL-17A production in both human and mouse γδ T cells. IL-23–induced IL-17A production was also blocked by TMP778 treatment. In vivo targeting of RORγt in mice via TMP778 administration reduced imiquimod-induced psoriasis-like cutaneous inflammation. Further, TMP778 selectively regulated Th17-signature gene expression in mononuclear cells isolated from both the blood and affected skin of psoriasis patients. In summary, to our knowledge, we are the first to demonstrate that RORγt inverse agonists: 1) inhibit Tc17 cell differentiation, as well as IL-17 production by γδ T cells and CD8$^+$ Tc17 cells; 2) block imiquimod-induced cutaneous inflammation; 3) inhibit Th17 signature gene expression by cells isolated from psoriatic patient samples; and 4) block IL-23–induced IL-17A expression. Thus, RORγt is a tractable drug target for the treatment of cutaneous inflammatory disorders, which may afford additional therapeutic benefit over existing modalities that target only IL-17A. ***The Journal of Immunology*****, 2014, 192: 2564–2575.**

CD4$^+$ Th17 cells play a central role in the pathogenesis of many autoimmune diseases, including psoriasis, rheumatoid arthritis, inflammatory bowel disease, and multiple sclerosis (1–3). Th17 cells are characterized by their production of IL-17A (i.e., IL-17), IL-17F, and IL-22. In addition to promoting inflammatory tissue injury, these cytokines are important for host defense against mucosal bacteria and fungi (4). Fully human mAbs targeting IL-17 and the IL-17RA have shown clinical efficacy in psoriasis, rheumatoid arthritis, and uveitis (3, 5, 6). Like other effector T cell subsets, Th17 cells develop from naive precursors in an Ag- and cytokine-dependent manner. Th17 differentiation requires the retinoic acid receptor–related orphan nuclear receptor RORγt (7), which is induced in activated naive T cells upon stimulation with STAT3-activating cytokines, such as IL-6/IL-1β (8). In addition to Th17 cells, RORγt expression and IL-17A production have been described in CD8$^+$ Tc17 cells and subsets of γδ T cells characterized by IL-17 expression. Interestingly, each of these RORγt$^+$ IL-17$^+$ lymphocyte subsets has been shown to home to the skin and is implicated in the pathogenesis of psoriasis (9–11).

Another STAT3-activating cytokine, IL-23, regulates the pathogenic function of mature Th17 cells in in vivo models such as experimental autoimmune encephalomyelitis (EAE) (12), and also directly regulates cytokine production in IL-17$^+$ γδ T cell subsets (13, 14). IL-23–induced Th17 cells are pathogenic in adoptive transfer EAE studies in contrast with those Th17 cells induced by IL-6/TGF-β (15–17). Indeed, independent genome-wide association studies in autoimmune patient populations have found that *Il23r* polymorphisms are associated with several autoimmune diseases, including psoriasis (18–20). In addition, mAbs against IL-23p19 or IL-23/IL-12p40 have shown marked efficacy in clinical studies involving psoriasis patients (21).

The critical role of RORγt in the generation of IL-17–producing T cells led to the identification of small molecules targeting RORγt; these compounds not only show inhibitory activity against Th17 cell differentiation and IL-17 production, but have also demonstrated variable levels of efficacy in EAE studies (22–24). In this study, we describe a novel class of selective and potent RORγt inverse agonists, exemplified by a lead compound TMP778 and its structurally identical but functionally inactive diastereomer TMP776. The combined potency and inactivity of TMP778 and TMP776, respectively, provides a powerful tool for understanding

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Abbreviations used in this article: DBD, DNA binding domain; EAE, experimental autoimmune encephalomyelitis; FRET, fluorescence resonance energy transfer; GSK, GlaxoSmithKline; IMQ, imiquimod; LBD, ligand binding domain; MSD, Meso Scale Discovery; SRC1, steroid receptor coactivator 1.

the function of RORγt in settings of inflammation, which we apply in this article to define the cells and transcriptional circuits involved in cutaneous inflammatory disease.

Materials and Methods

Fluorescence resonance energy transfer assay

Biotinylated human RORγt protein was made at GlaxoSmithKline (GSK); biotinylated steroid receptor coactivator 1 (SRC1) was purchased from CPC Scientific; streptavidin-labeled allophycocyanin and europium were purchased from Perkin Elmer. Compounds were added into a fluorescence resonance energy transfer (FRET) mixture containing SRC1-europium and human RORγ-allophycocyanin for 1 h. The emissions at 516 and 665 nM were then read on a ViewLux in Lance mode for Europium/allophycocyanin. The percent of activation at each dose of compound was calculated and plotted using GraphPad Prism to determine the IC_{50}s.

IL-17F promoter assay

A Jurkat cell line stably expressing RORγt and an IL-17F promoter-luciferase reporter gene were generated. The promoter sequence for the reporter is the 1075 bp of 5′ untranslated region adjacent to the start codon of the IL-17F protein coding sequence. The Jurkat cells were cultured in RPMI 1640 media (Invitrogen) containing 10% FBS (Hyclone). The Jurkat cells were diluted at 5×10^5/ml and stimulated with anti-CD3 mAb (10 μg/ml; Biolegend) in the presence of compound for 20 h. The cultures were then mixed with Steadylite luciferase detection mix (Steadylite plus Reporter Gene Assay System, Perkin Elmer) for 30 min, after which luciferase activity was read on an EnVision Multilabel Plate Reader (PerkinElmer). The percent inhibition at each concentration of compound was calculated and plotted using GraphPad Prism to determine the IC_{50}s. We developed both IL-17A and IL-17F promoter cell lines but chose the IL-17F cell line because it gave us a better assay window for defining the structure–activity relationships of our compounds. In addition, the use of an IL-17F promoter for the first cell-based screening assay opened up the possibility of discovering RORγt inverse agonists that affect binding to both IL-17A and IL-17F promoters.

Nuclear receptor assays

The nuclear receptor ROR contains a ligand binding domain (LBD) and a DNA binding domain (DBD). Binding of an agonist or inverse agonist to the ROR-LBD induces a conformational change in the ROR-DBD that affects activity from the promoter. HEK293 cells were transiently transfected with Vector pBIND-GAL4-DBD/RORγt or RORβ or RORα-LBD and pG5 Luc, a reporter promoter containing 5 GAL4 binding sites upstream of a minimal TATA box with a luciferase reporter gene. Twenty-four hours posttransfection, compounds were added into the culture for an additional 18 h before luciferase activity was determined using an EnVision Multilabel Plate Reader (PerkinElmer). The relative light units at each concentration of compounds were plotted for IC_{50} determination.

T cell culture media

T cell culture medium was composed of IMDM, 2 mM L-glutamine, 0.1 mM NEAA, 1 mM Na pyruvate 1, 100 U/ml penicillin, 100 μg/ml streptomycin (all from Invitrogen) plus 10% FBS (Hyclone), and 0.05 mM 2-ME (Sigma). For naive T cell culture medium, we used X-VIVO 15 medium (LONZA) plus 2 mM L-glutamine, 1 mM Na pyruvate 1, 100 U/ml penicillin, 100 μg/ml streptomycin (all from Invitrogen). The ratio of anti-CD3/anti-CD28 Dynabead (Invitrogen) to cells was typically 1:1.

Blood, patient samples, and PBMCs

Peripheral blood buffy coats from healthy adult volunteer donors were commercially obtained from Research Blood Components. Matched peripheral blood and skin sections that underwent biopsy from patients with psoriasis undergoing surgery were commercially obtained from Tissue Solutions. Some psoriatic patient blood samples were also obtained from Bioreclamation. Human cord blood mononuclear cells were obtained as frozen stocks from All Cells. All patient samples were obtained in accordance with commercial vendor's approved institutional review board protocols. PBMCs were prepared from buffy coats by Ficoll-Paque density grade centrifugation (GE Amersham) and extensive washing with PBS.

Lentiviral transduction

Human RORγt lentiviral stocks were produced in HEK293T cells with 10 μg DNA transfected by calcium phosphate-mediated transfection following the manufacturer's protocol (Profection kit; Promega). Naive $CD4^+$ or $CD8^+$ T cells isolated from PBMCs using naive $CD4^+$ or $CD8^+$ T cell isolation kits (Miltenyi) were resuspended in naive T cell culture media (see the earlier *T cell culture media* section) at a concentration of 1×10^6 cells/ml. Anti-CD3/anti-CD28 Dynabead (Invitrogen) and RORγt-HSA or empty vector-HSA virus supernatant were added into the cells. RORγt inverse agonists were added at the time of transduction for some experiments. The culture plates were centrifuged for 30 min at 2000 rpm and then cultured under the indicated conditions for the specified number of days.

Intracellular staining

Cells used for intracellular cytokine staining were stimulated for 5 h at 37°C with PMA (10 nM) and ionomycin (1 μM) in the presence of brefeldin A (5 μg/ml) for the last 3 h (all from Sigma). Cells were fixed and permeabilized before staining for IL-17A and IL-17F. The data were acquired on a LSR FortessaII (BD Biosciences) and were analyzed using FlowJo software (Tree Star).

Naive T cell differentiation and treatment with RORγt inverse agonists

Naive $CD4^+$ T cells were isolated using a Naive CD4 T-cell enrichment kit (Miltenyi Biotec) following manufacturer's protocol. To study the effect of RORγt inverse agonists on Th17 differentiation, naive $CD4^+$ T cells (1×10^6 cells/ml) were stimulated with anti-CD3/anti-CD28 Dynabeads in naive T cell culture media under Th17 polarizing conditions (20 ng/ml IL-6, 3 ng/ml TGF-β, 20 ng/ml IL-23, 20 ng/ml IL-1β, 1 μg/ml anti–IL-4 mAb, 1 μg/ml anti–IFN-γ) in the presence of compound or DMSO control. After 6 d of culture, the supernatants were harvested and cytokine concentrations were determined using Meso Scale Discovery (MSD) assays. For Th1 or Th2 cell differentiation, cells were stimulated with anti-CD3/anti-CD28 Dynabeads and cultured with IL-12 (10 ng/ml)/anti–IL-4 mAb (2 μg/ml) or IL-4 (20 ng/ml)/anti–IL-12 mAb (2 μg/ml), respectively. After 1 wk of culture, the cells were restimulated with anti-CD3/anti-CD28 Dynabeads for 24 h. The supernatants were harvested and cytokine concentrations were measured by MSD assays.

PBMCs and memory $CD4^+$ T cells cytokine expression

PBMCs were prepared from buffy coat by Ficoll-Paque density grade centrifugation. Memory $CD4^+$ T cells were purified using a human Memory $CD4^+$ T cell Isolation Kit (Miltenyi Biotec). PBMCs, cord blood mononuclear cells, and memory $CD4^+$ T cells were cultured in T cell media plus the indicated concentration of compounds. PBMCs and cord blood mononuclear cells were stimulated with soluble anti-CD3 (1 μg/ml) and anti-CD28 (1 μg/ml) Abs with or without IL-23 (50 ng/ml) for 5 d, and the supernatants were then harvested to detect cytokine level by MSD. Memory $CD4^+$ T cells were stimulated with anti-CD3/anti-CD28 Dynabeads with or without IL-23 (50 ng/ml) for 2 d before the supernatants were harvested to detect cytokine level by MDS. The IC_{50} were calculated using XLfit software (IDBS) or GraphPad Prism.

RNA extraction, quantitative RT-PCR

Total RNA was extracted using RNeasy kits including the optional DNaseI digestion (Qiagen). cDNA synthesis and TaqMan Real Time PCR were performed as described previously (25–27). TaqMan quantitative PCR was performed on a 7900HT Real Time PCR System (Applied Biosystems). All TaqMan reagents were purchased from Applied Biosystems.

Microarray

After extraction of total RNA using an RNeasy mini kit, microarray assays were performed at the Boston University Microarray Resource Facility (Boston, MA). In brief, the RNA samples were amplified and labeled following Ambion WT Expression Kit Protocol (Life Technologies) and GeneChip Whole Transcript (WT) Sense Target Labeling Assay Manual (Affymetrix). The cRNA samples were then hybridized to Affymetrix human 1.0ST gene chips. Affymetrix data were extracted, normalized, and analyzed using GenePattern software (Broad Institute).

Imiquimod-induced cutaneous inflammation

Female BALB/c mice weighing 20 ± 2 g (10–12 wk) were purchased from Taconic Farms and housed in a pathogen-free barrier facility at Tempero Pharmaceuticals. All studies were conducted in accordance with the GSK Policy on the Care, Welfare and Treatment of Laboratory Animals and were reviewed the Institutional Animal Care and Use Committee either at GSK or by the ethical review process at the institution where the work was performed. TMP778 was dissolved in the following vehicle: 3%

dimethylacetamide, 10% Solutol, and 87% saline. Imiquimod (IMQ) was formulated at 50 mg/ml final concentration in ethanol/PBS/lactic acid (54:36:10%). Mice were anesthetized before applying IMQ solution on to the skin. TMP778 (20 mg/kg) or its vehicle was administered s.c. Treatment started at day 0 and continued twice a day for 10 d in the morning and afternoon with 8 h between the two doses. Ear thickness was measured daily using an engineer's caliper (Mitutoyo) before the application of IMQ.

Histology

Mouse ears were fixed in 10% formalin, sectioned, and stained with H&E.

γδ T cell isolation

Human γδ T cells were purified from PBMCs by negative selection using EasySep Human γ/Δ T Cell Isolation Kit (Stemcell Technologies) according to the manufacturer's protocol. The purified γδ T cells were stimulated with anti-CD3/anti-CD28 Dynabeads (1:2 cell-to-beads ratio) plus IL-1β (10 ng/ml), IL-6 (20 ng/ml), and IL-23 (50 ng/ml) for 2 wk to differentiate the cells to IL-17A–producing γδ T cells. Cells were then restimulated with anti-CD3/anti-CD28 beads plus IL-1β (10 ng/ml), IL-6 (20 ng/ml), and IL-23 (50 ng/ml) in the presence compounds for 5 d. The cytokine titers were then determined by MSD. Mouse γδ T cells were purified from draining lymph nodes and spleen of IMQ-treated mice using positive selection. Cells were stained with PE-labeled TCRγδ Ab and subsequently purified using an EasySep mouse PE selection kit (Stemcell Technologies). Cells were then treated with compound, IL-1β, and IL-23 for 20 h for gene expression analysis or 5 d for cytokine secretion by MSD.

Mononuclear cell isolation from skin sections of psoriatic patients

Five-millimeter punch biopsies from the skin of active psoriatic lesions were purchased from Tissue Solutions, U.K. The tissues were washed with buffer containing EDTA and digested with Liberase (Roche Diagnostics for 25 min at 37°C. The enzymatically digested tissues were then passed through a 70-μm filter (BD Falcon, San Jose, CA) to remove the debris. The filtered mononuclear cells were then washed twice and suspended in T cell medium. The cells were then stimulated with anti-CD3/anti-CD28 Dynabeads in a round-bottom plate and treated with compounds or DMSO as control. The supernatant was collected on day 5 to determine the IL-17A titers by MSD.

Statistics

For skin thickness, different groups were evaluated for statistical significance using the two-tailed unpaired Student *t* test.

Results

Identification of selective human RORγt inverse agonist TMP778 and its inactive diastereomer TMP776

To identify novel inhibitors of RORγt, we developed a FRET-based molecular screening assay using tagged recombinant ligand-binding domain of RORγt and a peptide corresponding to the endogenous RORγt cofactor SRC1 (28) capable of energy transfer upon interaction (Fig. 1A). Using this approach, we screened a diverse library containing ~2 million small molecules. Hits were identified and further optimized leading to the discovery of TMP536, a potent benzofuran derivative inverse agonist of RORγt with an IC_{50} of 0.023 μM (Fig. 1B). To assess the cellular potency of compounds, we established an RORγ*t*-dependent transcriptional promoter assay in Jurkat cells in which RORγt promotes luciferase expression through a native IL-17F promoter as described in *Materials and Methods*. TMP536 was active in this assay with an IC_{50} of 0.2 μM. Because TMP536 contains two chiral centers (Fig. 1B), we separated and tested its four diastereomers: TMP774, TMP776, TMP778, and TMP780 (Fig. 1B). Compared with TMP536, TMP778 demonstrated markedly improved potency in both the FRET and IL-17F promoter assays, whereas TMP776 displayed little to no inverse agonist activity (Fig. 1B–D). Collectively, this approach led to the discovery of >100 RORγt inverse agonists, all of which demonstrated IC_{50} potency between 0.01 and 0.04 μM in the FRET assay and 0.02 and 0.4 μM in the IL-17F promoter assay (manuscript in preparation).

To determine the specificity of these compounds within the ROR family of nuclear receptors, we established a series of ROR/Gal4 cell-based promoter assays in HEK293 cells in which transcriptional activity is driven specifically by RORα, RORβ, or RORγt. Whereas TMP778 potently inhibited RORγt promoter activity (Fig. 1E, 1F), it was inactive in the RORα and RORβ assays (Fig. 1F). In contrast, the diastereomer TMP776 was inactive in all three reporter assays (Fig. 1D–F). We next tested TMP778 against a panel of 22 distinct nuclear receptors to further determine its specificity; we found that TMP778 displayed no specific activity against any of the 22 nuclear receptors tested (i.e., $IC_{50} > 10$ μM; manuscript in preparation). In addition, we tested TMP778 against a panel of 45 kinases, GPCRs, transporters, and ion channels, and found >1000-fold selectivity against all of these targets (data not shown). Thus, TMP778 is a novel, potent, and highly selective inverse agonist of RORγt, whereas the structurally related diastereomer, TMP776, is inactive against RORγt. TMP778 and TMP776 thus provide elegant chemical probes useful for dissecting the specific functions of RORγt as a transcriptional regulator of inflammation.

IL-17– and IL-17–producing cells induced by ectopic RORγt expression in human T cells are inhibited by RORγt inverse agonists

RORγt is believed to be the master transcription factor for Th17 cell differentiation, and forced expression of RORγt in naive $CD4^+$ T cells induces the generation of IL-17A–producing cells (7). $CD8^+$ T cells can also be differentiated into IL-17A–producing Tc17 cells (29–31), and these cells play an important role in the pathogenesis of autoimmune diseases including psoriasis (9). To determine whether RORγt inverse agonist TMP778 could inhibit $CD4^+$Th17 cell differentiation and $CD8^+$ Tc17 cell differentiation, we compared the effect of TMP778 and its diastereomer TMP776 on RORγ*t*-induced Th17 and Tc17 cell differentiation. Naive $CD4^+$ T cells or $CD8^+$ T cells were transduced with RORγ*t*-expressing lentiviruses and expanded in the absence or presence of 0.1 μM compounds for 10 d before determining the frequency of $IL\text{-}17A^+$ cells by intracellular staining. TMP778, but not its diastereomer TMP776, impaired generation of IL-17A– and IL-17F–producing $CD4^+$Th17 cells (Fig. 2A) and $CD8^+$Tc17 cells (Fig. 2B). We also restimulated these cultures using anti-CD3/anti-CD28–coated beads for 48 h, and found that IL-17 secretion was reduced by TMP778, but not its diastereomer TMP776 (Supplemental Fig. 1).

T cells were transduced with RORγt lentivirus, differentiated into IL-17A–producing Th17 cells or Tc17 cells (as shown in DMSO controls in Fig. 2A, 2B, Supplemental Fig. 1A, 1B), and then restimulated with anti-CD3/anti-CD28 mAb beads in the presence of compounds or vehicle, to determine whether RORγt acutely regulates IL-17A production in already established Th17 cells. TMP778, but not TMP776, inhibited the acute IL-17A secretion by Th17 cells and Tc17 cells in a dose-dependent manner with IC_{50} values of 0.03 (Fig. 2C) and 0.005 μM (Fig. 2D), respectively. Thus, inhibition of RORγt function via TMP778, but not its inactive diastereomer TMP776, inhibits both Th17 and Tc17 differentiation, as well as acute IL-17A production by already established effector/memory Th17 and Tc17 cells.

Primary $CD4^+$ T cell Th17 cell differentiation and IL-17A production are blocked by RORγt inverse agonist

Our findings that TMP778, but not its diastereomer TMP776, regulates Th17 cytokine expression in cells forced to express RORγt using lentiviral transduction systems fails to account for the presence of Th17-inducing inflammatory cytokines, which may further regulate RORγt function in physiological settings of

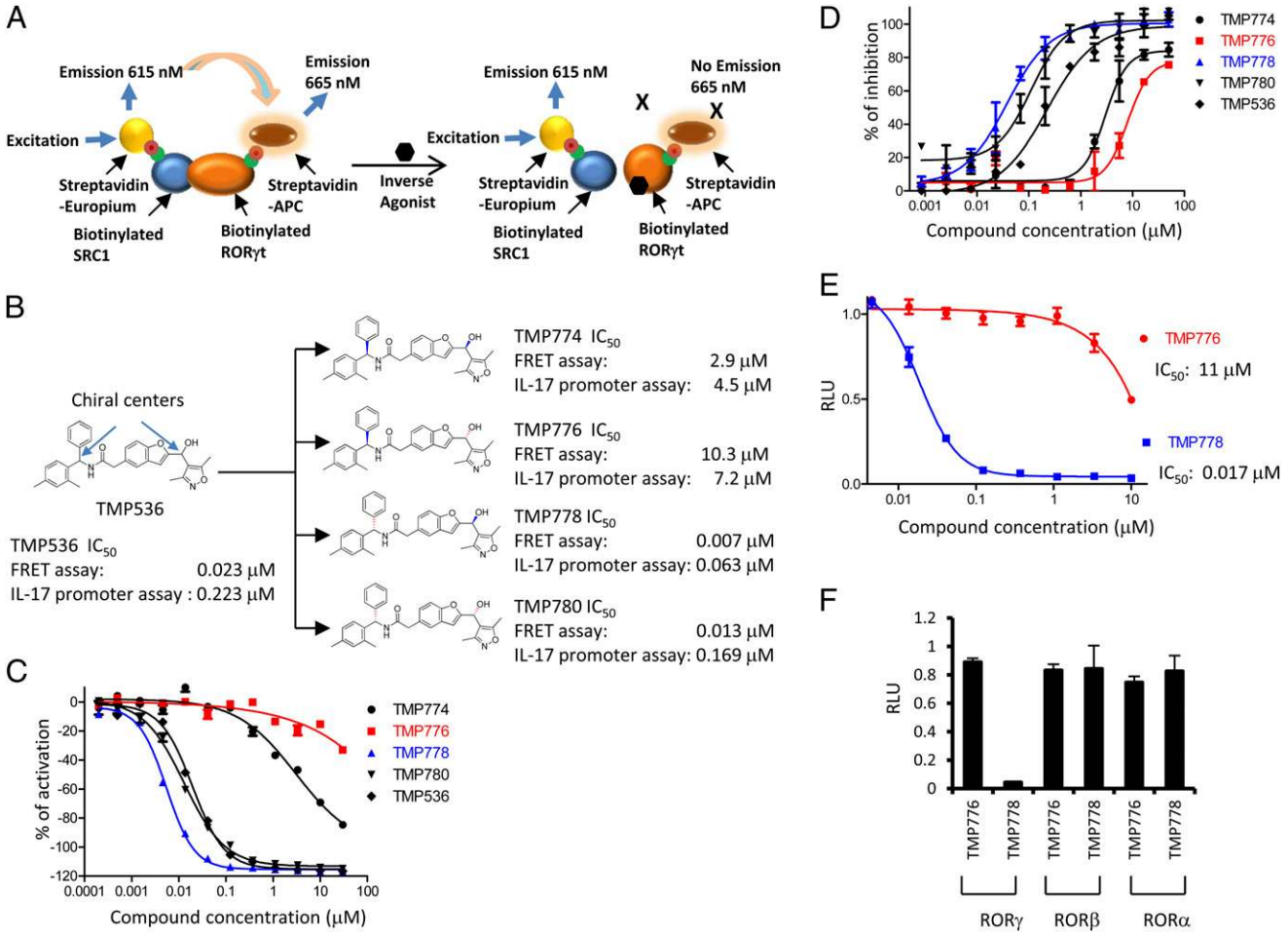

FIGURE 1. Identification of human RORγt inverse agonist TMP778 and its diastereomer TMP776. (**A**) FRET assay consisting of human RORγt ligand binding domain and cofactor peptide SRC1 was used to screen a proprietary small-molecule library to identify inverse agonists of RORγt. If a small molecule binds to RORγt, it could induce a conformational change in the RORγt protein, resulting in its inability to bind the cofactor SRC1. (**B**) Racemic TMP536 and its four diastereomers TMP774, TMP776, TMP778, and TMP780. Solid lines indicate the direction of the ring or hydroxyl is toward the reader, whereas red dashed lines indicate it is away from the reader. (**C**) FRET assay of TMP536 and its separated diastereomers TMP774, TMP776, TMP778, and TMP780; IC_{50}s are summarized in (B). (**D**) IL-17F promoter reporter assay in Jurkat cells. Jurkat cells were stably transduced with human RORγt and a human IL-17F promoter-luciferase reporter gene. The IC_{50}s, calculated as percent inhibition, are summarized in (B). (**E**) RORγ/Gal4 promoter activity in HEK293 cells. Cells were transiently transfected with Vector pBIND-GAL4-DBD/RORγ-LBD and pG5 Luc for 24 h. Compounds were then added into the culture for an additional 18 h before luciferase activity was determined. (**F**) TMP778 inhibits RORγ, but not RORβ or RORα activity. HEK293 cells were transiently transfected with Vector pBIND-GAL4-DBD/RORγt or RORβ or RORα-LBD and pG5 Luc for 24 h. Compounds were added into the culture at the final concentration of 1 μM for an additional 18 h before luciferase activity was determined. (B–D) Data are representative of 3–10 separate experiments. (E and F) Data are representative of two separate experiments.

inflammation. Therefore, we next asked whether TMP778 blocks Th17 cell differentiation and IL-17 production in human primary T cells cultured with Th17-inducing cytokines. We first tested the compounds in a classical Th17 skewing culture. TMP778 potently inhibited human IL-17 secretion with an IC_{50} of 0.005 μM (Supplemental Fig. 2A). In these cultures, TMP778 had no bearing on the expression of IFN-γ, IL-1β, IL-10, IL-12 p70, IL-13, IL-2, IL-4, IL-5, IL-8, and TNF-α in the Th17 culture (Supplemental Fig. 2B). We tested >10 additional RORγt inverse agonists, all of which demonstrated IC_{50} potency between 0.01 and 0.1 μM. Importantly, the diastereomer TMP776 had no effect on the differentiation of Th17 cells from naive $CD4^+$ T cells under classical Th17 skewing condition as demonstrated by IL-17A production (Fig. 3A). Although TMP778 potently inhibited Th17 cell differentiation, it did not affect the differentiation of Th1 and Th2 cells because there is no different effect observed on the production of IFN-γ, TNF-α, IL-2, IL-4, IL-5, IL-10, and IL-13 by cells treated with DMSO or TMP778 (Fig. 3B).

The role of RORγt in IL-17A expression by endogenous human memory T cells has not been widely studied. RORγt inverse agonists inhibited IL-17F promoter activity in Jurkat cells (Fig. 1D), indicating that RORγt can directly activate the IL17 promoter in addition to its role in Th17 cell differentiation. Indeed, RORγt inverse agonist TMP778 potently blocked IL-17A production by human primary memory $CD4^+$ T cells (Fig. 3C). The average IC_{50} from five different experiments was 0.1 μM. The inhibition was specific because TMP776 had no effect. In addition, TMP778 significantly inhibited IL-17A production, but not Th1/Th2 cytokine production, by human PBMCs (Fig. 3D) with IC_{50} of 0.04 μM (data not shown). Umbilical cord blood mononuclear cells were also used to test the effect of TMP778. Again, TMP778 significantly inhibited IL-17 production, but not Th1/Th2 cytokines, by umbilical cord blood mononuclear cells (Fig. 3E). In addition, we have discovered >25 different RORγt inverse agonists with IC_{50} potencies between 0.05 and 0.5 μM on IL-17A production by human PBMCs (manuscript in preparation). Thus,

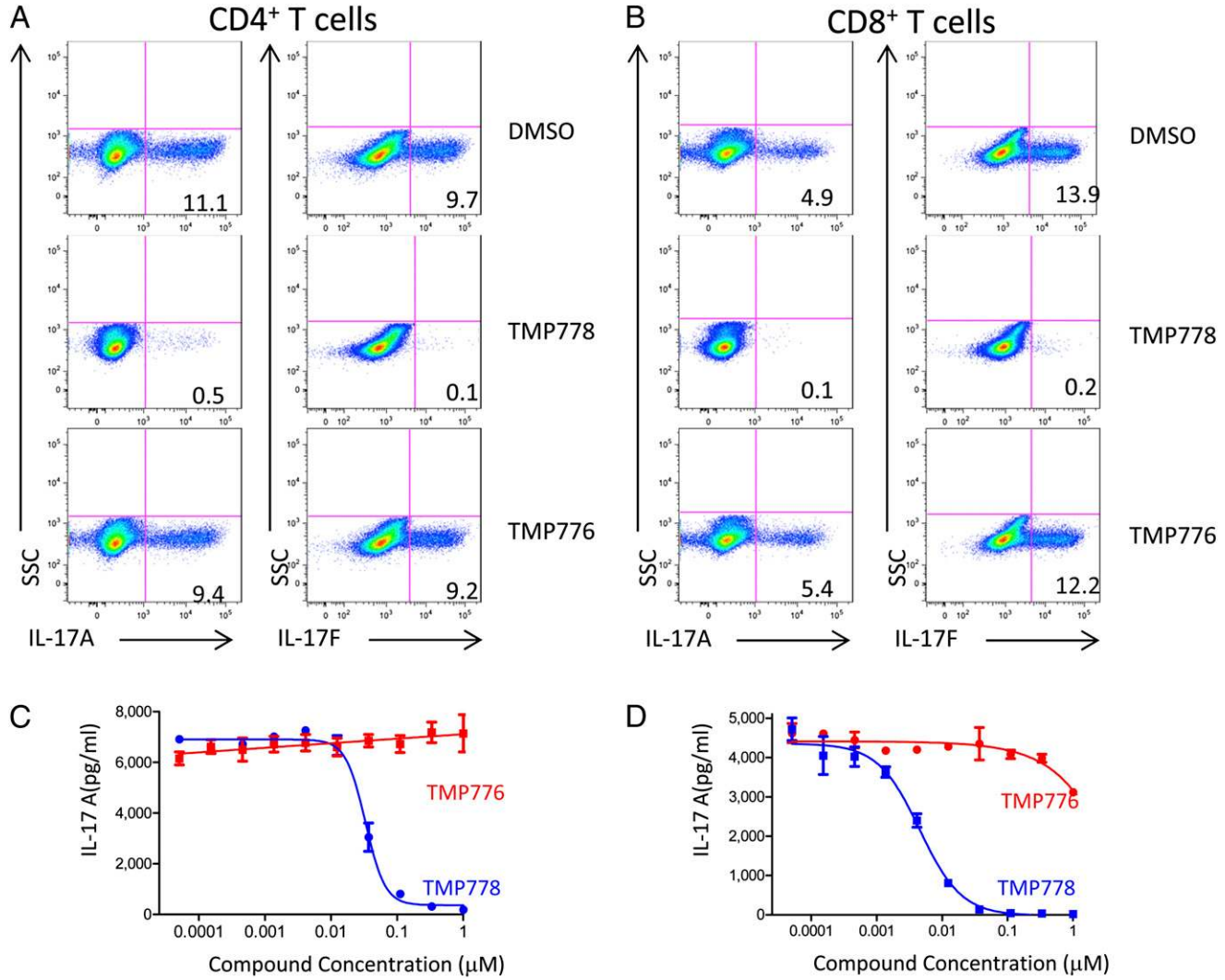

FIGURE 2. IL-17–producing cells and IL-17 expression induced by ectopic RORγt expression in human T cells are inhibited by an RORγt inverse agonist. (**A** and **B**) Naive CD4$^+$ T cells (A) or naive CD8$^+$ T cells (B) were transduced with RORγt lentivirus and then stimulated with anti-CD3/anti-CD28 mAb beads in the presence of 0.1 μM RORγt inverse agonist TMP778 or its diastereomer TMP776. Ten days after the infection, cells were restimulated with PMA/ionomycin in the presence of brefeldin A for 5 h before intracellular staining for IL-17. (**C** and **D**) Naive CD4$^+$ T cells (C) or naive CD8$^+$ T cells (D) were transduced with RORγt lentivirus and then stimulated with anti-CD3/anti-CD28 mAb beads without compounds for 10 d. Cells were then harvested and restimulated with anti-CD3/anti-CD28 beads in the presence of TMP778 or its diastereomerTMP776 at the indicated concentration for 48 h. IL-17 titers in the supernatants were determined by MSD. (A–D) Data are representative of two to three separate experiments.

RORγt inverse agonist TMP778, but not its diastereomer TMP776, inhibits acute IL-17A production by memory CD4$^+$ T cells and PBMCs.

RORγt inverse agonist TMP778 specifically blocks Th17-signature gene expression

Human memory CD45RO$^+$CCR6$^+$ CD4$^+$ T cells are enriched for Th17 cells compared with CD45RO$^+$CCR6$^-$CD4$^+$ T cells, which contain mostly IFN-γ–producing Th1 memory cells (31, 32). TMP778 significantly reduced the number of CCR6-expressing CD4$^+$ T cells in naive CD4$^+$ T cells transduced with RORγt (Supplemental Fig. 3). As expected from increased IL-17A protein levels, human memory CCR6$^+$ CD4$^+$ T cells also expressed high levels of many Th17-signature genes *Rorc (Rorγt)*, *Il17(Il17a)*, *Il17f*, *Il22*, *Il26*, *Ccl20*, *Ccr6*, and *Il23r* (Fig. 4A). Next, we examined the impact of TMP778 on the transcriptional signature of memory CCR6$^+$ CD4$^+$ T cells. We stimulated memory CD4$^+$ T cells with anti-CD3/anti-CD28 mAb beads in the presence of 1 μM TMP778 or 1 μM TMP776 for 20 h. Microarray analysis revealed that TMP778 inhibited the expression of *Il17a*, *Il22*, *Il26*, *Ccl20*, *Ccr6*, and *Il23r* mRNAs (Fig. 4B). In addition to these Th17 signature genes, TMP778 also affected the expressions of several other genes. TMP778 upregulated the gene expression of *Ifng*, *Il3*, *Cd27*, *Pdxdc2*, *Lgmn*, *Mx1*, *Lrrfip1*, *Rsad2*, *Herc5*, *Oasl*, *Cmpk2*, *Ifi44*, *Ifi44l*, *Ifit1*, and *Ifit3*, and downregulated the gene expression of *Snrpg*, *Ctsh*, *Adam12*, *Alox5ap*, *Ripk2*, *Ltb*, *Hsp90aa6p*, *Kif27*, *Cxcl9*, *B3galt2*, *Hrh4*, *Hnrnpa3*, and *Egln3* (Fig. 4B). In contrast, TMP776 had no effect on the expression of these genes (Fig. 4C). We confirmed the gene expression of *Il17a*, *Il22*, *Il26*, *Ccl20*, *Ccr6*, and *Il23r* using TaqMan real-time PCR (Fig. 4D). Neither TMP778 nor TMP776 treatment influenced the expression of *Rorc* itself. Furthermore, we found that TMP778 treatment also reduced Th17-associated gene expression in CCR6$^-$ memory CD4$^+$ T cells transduced with RORγt-expressing lentiviruses (Fig. 4E), whereas untransduced CCR6$^-$ memory CD4$^+$ T cells displayed low levels of Th17-associated mRNAs, and TMP778 had little to no effect on gene expression in these cells (data not shown). The expression of other possible RORγt-dependent genes including

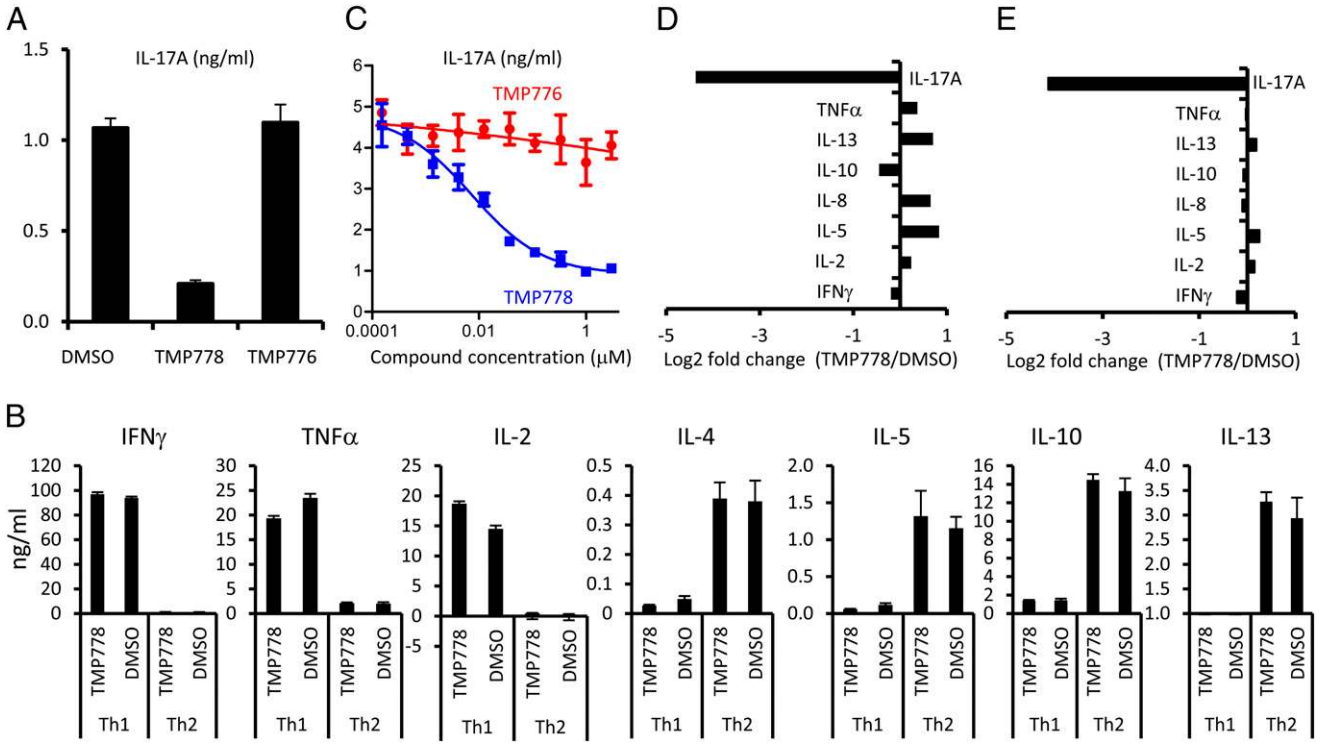

FIGURE 3. Th17 cell differentiation and IL-17 production by human primary CD4$^+$ T cells are blocked by RORγt inverse agonist. (**A**) Naive CD4$^+$ T cells were cultured under the Th17 skewing conditions as described in *Materials and Methods* in the presence of 0.1 μM compounds for 6 d before IL-17 titers in the supernatants were determined by MDS. (**B**) Naive CD4$^+$ T cells were cultured under Th1 or Th2 skewing conditions in the presence of 1 μM TMP778 for 6 d. Cells were then harvested, washed, and restimulated with anti-CD3/anti-CD28 mAb beads for 48 h. Cytokine titers in the supernatants were determined by MSD. (**C**) Memory CD4$^+$ T cells were stimulated with anti-CD3/anti-CD28 mAb beads in the presence of RORγt inverse agonist TMP778 or its diastereomerTMP776 for 48 h, after which IL-17 titers in the supernatants were determined by MSD. The average IC_{50} from five different experiments was 0.1 μM. (**D**) Human PBMCs were stimulated with soluble anti-CD3 and anti-CD28 mAbs in the presence of 1 μM TMP778 or DMSO control for 5 d. Cytokine titers in the supernatants were determined by MSD. Data are shown as the log2 fold change of TMP778/ DMSO. (**E**) Human cord blood mononuclear cells were stimulated with soluble anti-CD3 and anti-CD28 mAbs in the presence of 1 μM TMP778 or DMSO control for 5 d. Cytokine titers in the supernatant were determined by MSD. Data are shown as the log2 fold change of TMP778/DMSO. (A–E) Data are representative of at least three separate experiments.

Cd161, *Il4r*, *Il12rb2*, and *Cd28* was not affected by TMP778 treatment (Fig. 4E). The expression level of *Ii4i1*, however, was slightly reduced by TMP778 treatment (Fig. 4E). Cell-surface protein expression of CD161, IL-4R, and CD28 was not significantly affected by TMP778 treatment (Supplemental Fig. 3B). Using TaqMan real-time PCR, we confirmed that the gene expression of *Il12rb2* was not significantly affected, whereas expression of *Il4i1* was again slightly reduced by TMP778 treatment (Supplemental Fig. 3C).

We further asked whether TMP778 inhibits Th17 cell differentiation through its inhibition of Th17-signature genes expressed by naive CD4$^+$ T cells under Th17 skewing conditions. After naive CD4$^+$ T cells were stimulated under Th17 skewing conditions in the presence of 1 μM TMP778 or 1 μM TMP776 for 62 h, the expression of *Il-17a*, *Il-17f*, *Il-22*, *Il-26*, *Ccl20*, *Ccr6*, and *Il-23r* mRNAs were all significantly inhibited by TMP778, but not its diastereomer TMP776 (Fig. 4F, 4G).

The earlier whole-genome transcriptional profiling studies demonstrate that RORγt inverse agonist TMP778, but not its inactive diastereomer TMP776, inhibits Th17-signature gene expression in memory CD4$^+$ T cells and naive CD4$^+$ T cells under Th17 skewing conditions. Of equal importance, RORγt inverse agonists had little to no effect on gene expression not associated with the Th17 cell transcriptional signature (Fig. 4B–F). Thus, the highly selective effect of inhibiting RORγt on T cell gene expression suggests that TMP778 may display reduced toxicity in clinical applications vis-à-vis other less selective inhibitors.

TMP778 selectively regulates γδ T cell IL-17A production in addition to CD4$^+$ Th17 cells and CD8$^+$ Tc17 cells

IL-17A–producing γδ T cell subsets have been implicated in the pathogenesis of human psoriasis (33) and animal models of skin inflammation and psoriasis (34, 35). IL-17A–producing γδ T cells have been found in psoriatic regions of patient skin, but not in unaffected normal skin (10). To explore the role of RORγt in the regulation of inflammatory cytokine gene expression in γδT cells, we evaluated the impact of TMP778 treatment on γδ T cells. Human γδ T cells were purified from PBMCs and expanded in the presence of IL-1β, IL-6, and IL-23. The cells were then restimulated with anti-CD3/anti-CD28 mAb beads plus IL-1β, IL-6, and IL-23 in the presence or absence of compounds for 5 d, and IL-17A production was analyzed. TMP778, but not TMP776, significantly inhibited IL-17A production by human γδ T cells (Fig. 5A). In contrast, TMP778 had no effect on the production of IFN-γ and TNF-α by human γδ T cells (Fig. 5A). TMP778 also inhibited the expression of *Il-17a*, *IL-17f*, *Il-26*, *Il-23r*, *Ccr6*, and *Ccl20* by human γδ T cells (data not shown).

TMP778 attenuates IMQ-induced skin inflammation

Our studies demonstrate that RORγt inverse agonist TMP778 blocks Th17 and Tc17 cell differentiation, as well as the acute expression of IL-17A and Th17-signature genes by different subsets of T cells including CD4$^+$, CD8$^+$, and γδ T cells. Each of these cells is involved in the pathogenesis of autoimmune psoriasis.

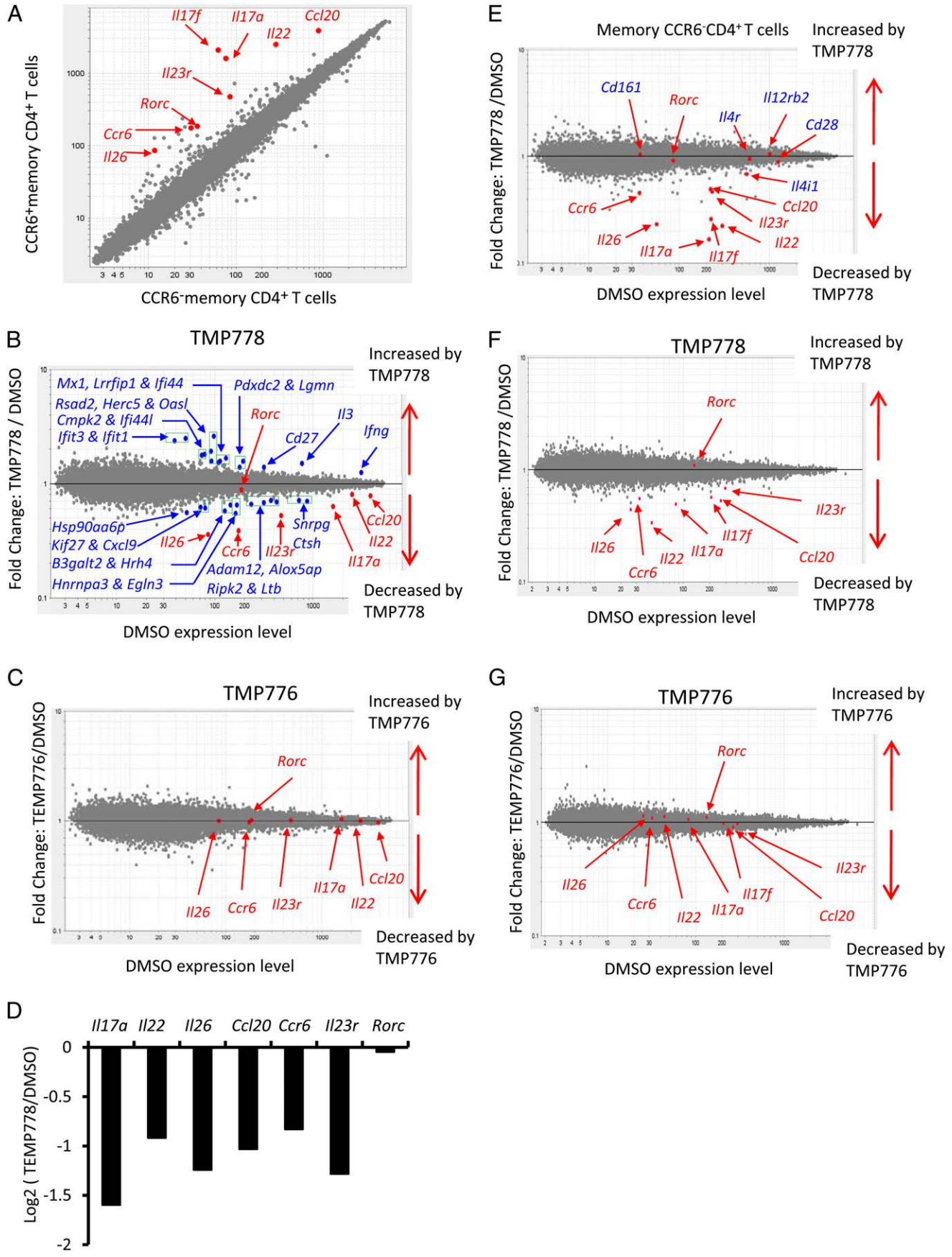

FIGURE 4. The effect of genome wide transcription by RORγt inverse agonists. (**A**) Human CCR6$^+$ and CCR6$^-$ memory CD4$^+$ T cells were stimulated with anti-CD3/anti-CD28 mAb beads for 20 h. Total RNA was extracted for microarray analysis using Affymetrix human Gene Arrays 1.0ST. The genes in the upper left were increased in CCR6$^+$ memory CD4$^+$ T cells, whereas the genes in the lower right were increased in CCR6$^-$ memory CD4$^+$ T cells. (**B** and **C**) Memory CD4$^+$ T cells were stimulated with anti-CD3/anti-CD28 mAb beads in the presence of 1 μM TMP778 (B) or 1 μM *(Figure legend continues)*

In humans, IMQ has been shown to induce psoriasis-like skin inflammation (36). IMQ-induced skin inflammation is mediated through IL-23 and IL-17A (37); it is also γδ T cell dependent (34). We purified γδ T cells from IMQ mice to >95% purity (Fig. 5B). The cells were then stimulated with different combinations of the cytokines IL-1β, IL-6, and IL-23 without TCR stimulation. γδ T cells produced high level of IL-17A after stimulation with IL-1β and IL-23 (Fig. 5C), which is consistent with previous studies (13, 14). IL-6 did not have a significant effect on the IL-17A production. We hypothesized that IL-17A production by IMQ-primed γδ T cells would be blocked by our RORγt inverse agonists. Indeed, mouse IL-17A production by γδ T cells from IMQ-treated mice was blocked by TMP778, but not its diastereomer TMP776 (Fig. 5D). Interestingly, TMP778 treatment also reduced TNF-α production ex vivo, whereas IFN-γ expression was slightly elevated (Fig. 5E). TMP778 treatment had no effect on the production of other Th1/Th2 cytokines (Fig. 5E).

Next, we asked whether RORγt inverse agonists could inhibit IMQ-induced skin inflammation in vivo. In this study, IMQ was applied on the skin once a day, whereas TMP778 was s.c. injected into mice twice a day for 10 d. The ear thickness of the TMP778-treated group was significantly reduced compared with the vehicle-treated group (Fig. 5F). Histological analysis showed that the TMP778-treated group displayed reduced epidermal hyperplasia and inflammatory cell influx (Fig. 5G). In addition, TMP778 significantly reduced IL-17A–producing γδ T cells in vivo in mice treated with IMQ (Supplemental Fig. 4A). We also isolated the skin infiltrate cells, stimulated the cells with PMA/ionomycin for 2 h, and performed RT-quantitative PCR. We found that the Th17 signature gene expression of *Ccl20*, *Il23r*, *Ccr6*, *Il17f*, *Il22*, *Il17a* were significantly inhibited by TMP778 treatment, whereas *Il2*, *Ifng*, *Il13*, and *Tgfb* gene expression were only slightly reduced. The level of *Il6*, *Tnfa*, and *Il10* mRNA seems lightly enhanced, whereas the level of *Stat3* and *Il1b* was not changed (Supplemental Fig. 4B).

Suppression of Th17 signature gene expression and IL-17A production in primary cells from psoriatic patients

Recent clinical trials have established that IL-17A is a critical factor in the pathogenesis of human psoriasis (3, 38, 39). Given our findings described earlier showing that inhibition of RORγt modulates cutaneous inflammation in mouse models, we next tested our RORγt inverse agonists in clinical samples obtained from psoriatic patients. We first examined gene expression in PBMCs from psoriatic patients after anti-CD3/anti-CD28 mAb stimulation. TMP778, but not its diastereomer TMP776, significantly inhibited the expression of *Il-17a*, *Il-17f*, *Il-22* and *Il-23r*, and *Ccl20* (Fig. 6A). Further, IL-23–enhanced expressions of Th17-associated mRNAs from psoriatic PBMCs were significantly inhibited by TMP778 (Fig. 6B). IL-17 protein production by PBMCs from psoriasis patients was also significantly reduced in TMP778- but not TMP776-treated cells (Fig. 6C); although in this setting, there was no significant inhibition of the production of IFN-γ, TNF-α, IL-2, IL-5, IL-8, IL-10, and IL-13 (Fig. 6D).

Because inflammatory T cell subsets present in autoimmune patients may be different in the blood and target tissue(s), we next purified mononuclear cells from affected skin of psoriasis patients and determined the influence of RORγt inhibition on these cells. We stimulated skin-derived mononuclear cells with anti-CD3/anti-CD28 mAb for 5 d in the presence or absence of compounds and determined Th17/Th1/Th2 cytokine protein levels. TMP778, but not TMP776, reduced production of IL-17A by psoriatic skin mononuclear cells (Fig. 6E), whereas no effects were observed on production of other non-Th17 cytokines, including IFN-γ, TNF-α, IL-2, IL-5, IL-8, IL-10, and IL-13 (Fig. 6E). These data demonstrate that our RORγt inverse agonists inhibit IL-17A production and Th17 signature gene expression in both PBMCs of psoriasis patients and mononuclear cells of psoriatic regions of skin.

Discussion

Several RORγt small-molecule inhibitors have been discovered and shown to block Th17 cell differentiation and IL-17A production, as well as demonstrate efficacy in EAE studies (22–24). In this article, we report the discovery of RORγt inverse agonist TMP778 and its functionally inactive diastereomer TMP776. This discovery has provided us with a unique and powerful set of tools to study RORγ*t*-dependent functions.

The discovery and characterization of TMP778 and its diastereomer TMP776 were first based on our unique FRET-based molecular assay and two cell line reporter assays (IL-17F promoter and RORγ-LBD/Gal4-DBD promoter assays). Use of pairs of diastereomeric RORγt inverse agonists that are functionally different can separate the direct effect of the compound on RORγt protein from nonspecific adverse compound effects on other proteins and cells. TMP778, but not TMP776, potently inhibited not only human $CD4^+$Th17 cell, but also human $CD8^+$Tc17 cell differentiation induced by RORγt ectopic expression. The acute IL-17A production by human $CD4^+$Th17 cells, $CD8^+$Tc17 cells, memory $CD4^+$ T cells, and PBMCs were potently blocked by TMP778, but not its diastereomer TMP776. Genome-wide transcription profiling studies demonstrated that TMP778, but not its diastereomer TMP776, blocks Th17 signature gene expression. In addition, TMP778 had very limited effects on the expression of other genes, indicating that the compound may have low off-target toxicity issues in clinical development. Indeed, TMP778 did not show detectable activity against broad panels of nuclear receptors, GPCRs, kinases, ion channels, transporters, Herg, Cyp panel, and genotoxicity.

Targeting RORγt could be beneficial for several autoimmune diseases such as rheumatoid arthritis, psoriasis, multiple sclerosis, and inflammatory bowel diseases. Treatments with mAbs against IL-12/IL-23p40, IL-17A, IL-17R, or IL-23p19 have resulted in clinical efficacy in psoriasis patients. Genome-wide association studies found that *Il23r* polymorphisms are associated with several autoimmune diseases, including psoriasis (18, 19). IL-23 has been shown to be critical for the differentiation of pathogenic Th17 cells (15–17). Our RORγt inverse agonist TMP778 blocks the differentiation of IL-17A–producing Th17 cells and Tc17 cells, as

TMP776 (C) for 20 h. Total RNA was extracted for microarray analysis. Data are shown as the fold change of TMP778/DMSO or TMP776/DMSO versus DMSO expression level, indicating genes increased or decreased by compound treatment. The gene symbols related with the dots in the green boxes were labeled in the order of highest fold change to lower fold change. (**D**) Log2 fold change of gene expression by TaqMan real-time PCR from samples in (B). (**E**) Memory $CCR6^-CD4^+$ T cells were transduced with RORγt lentivirus and then stimulated with anti-CD3/anti-CD28 mAb beads in the presence of 1 μM TMP778 or DMSO vehicle control for 62 h. Total RNA was extracted, and Affymetrix microarray studies were performed. Data are shown as the fold change of TMP778/DMSO versus DMSO expression level. (**F** and **G**) Naive $CD4^+$ T cells were cultured under Th17 skewing condition in the presence of 1 μM TMP778 (F) or 1 μM TMP776 (G) for 62 h before total RNA was extracted for microarray studies. Data shown are the fold-change decrease or increase by TMP778 or TMP776. (A–G) Data are representative of two separate experiments.

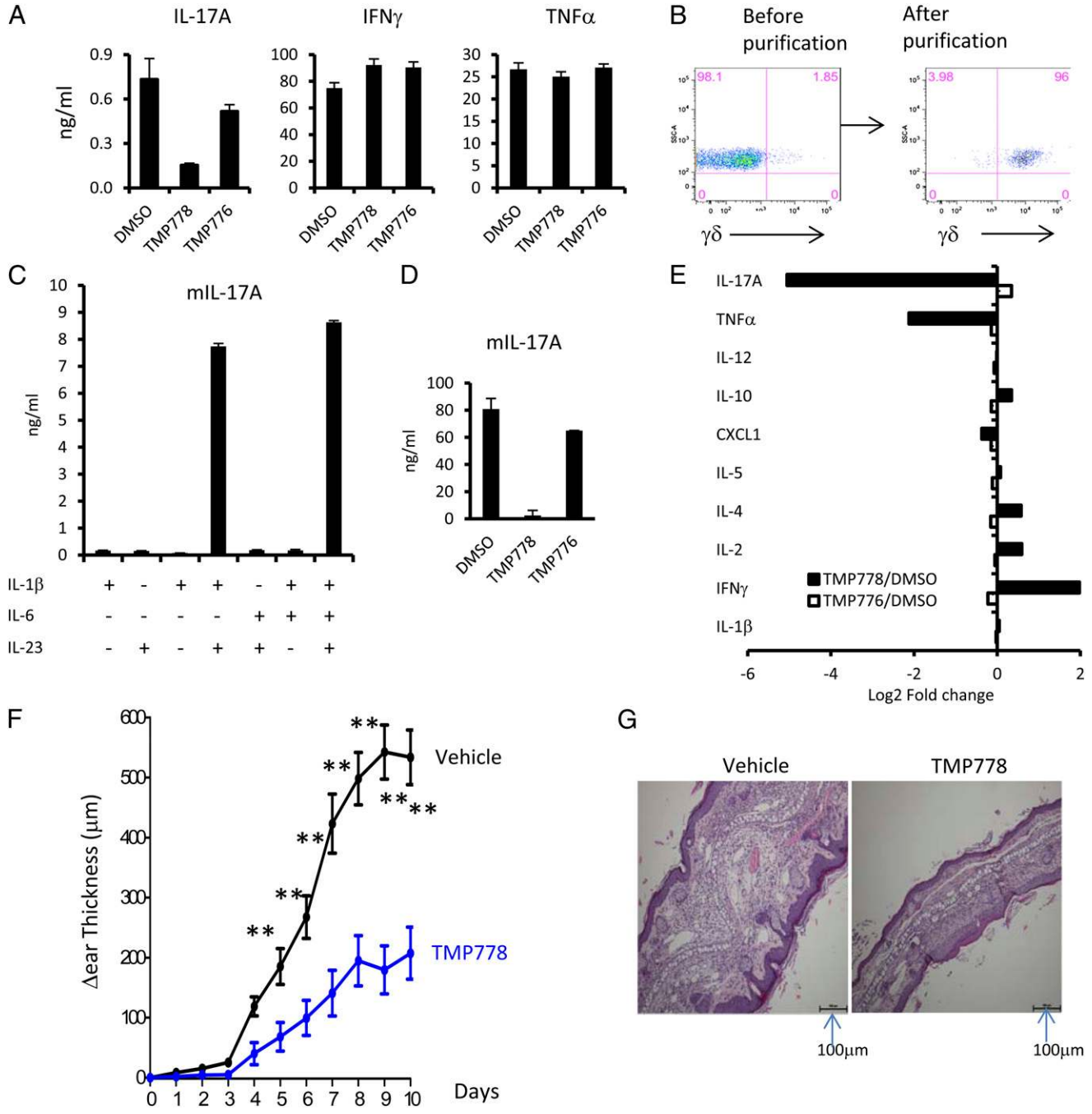

FIGURE 5. IL-17 production by γδ T cells and IMQ-induced skin inflammation are blocked by RORγt inverse agonist TMP778. (**A**) Human γδ T cells purified from PBMCs were first differentiated into IL-17–producing cells. Cells were then restimulated with anti-CD3/anti-CD28 mAb beads plus IL-1β, IL-6, and IL-23 in the presence of 1 μM compounds for 5 d. Cytokine titers in the supernatants were determined. (**B**) Mouse γδ T cells were purified from lymph node cells of mice treated topically on the skin with IMQ for 10 d. Purity of γδ T cells was consistently >95%. (**C**) Mouse γδ T cells were stimulated with different combination of cytokines for 5 d, and IL-17 titer in the supernatant was determined by MSD. (**D**) Mouse γδ T cells were cultured with IL-1β, IL-23, and TMP778 (1 μM) or its diastereomer TMP776 (1 μM) for 5 d. (**E**) Th1/Th2 cytokine titers in the supernatants of cell culture in (C) were determined by MSD. The comparison of TMP778/DMSO and TMP776/DMSO is illustrated as log2 fold changes. (**F**) IMQ was applied to the skin of BALB/c mice daily for 10 d, whereas TMP778 (20 mg/kg) or DMSO control was s.c. injected twice a day for the same 10 d. The change in ear thickness was determined as Δ value (Δ = current ear thickness − day 0 ear thickness). Ten to 15 mice were used in each group in each experiment. $^{**}p < 0.0001$. (**G**) After 10 d of treatment in (F), mouse ears were harvested from vehicle- and TMP778-treated groups, fixed in 10% formalin, and stained with H&E for histological examination. (A–G) Data are representative of at least three different experiments.

well as acute IL-17A production and acute Th17 signature gene expression. Furthermore, TMP778 inhibits IL-23–induced IL-17A production by PBMCs of psoriatic patients. In addition to αβ T cells, γδ T cells, especially IL-23–responsive IL-17A–producing dermal γδ T cells, also play an important role in the pathogenesis of psoriasis (34). TMP778 significantly blocks the IL-17A production of γδ T cells after stimulation with IL-1β and IL-23. These attributes of our RORγt inverse agonist TMP778 indicate its clinical potential for the treatment of psoriasis. One clinical form of psoriasis is triggered by TLR7 agonist IMQ (36). Application of IMQ on mouse skin induces psoriasis-like skin inflammation via the IL-23/IL-17A axis (37). In this study, the skin

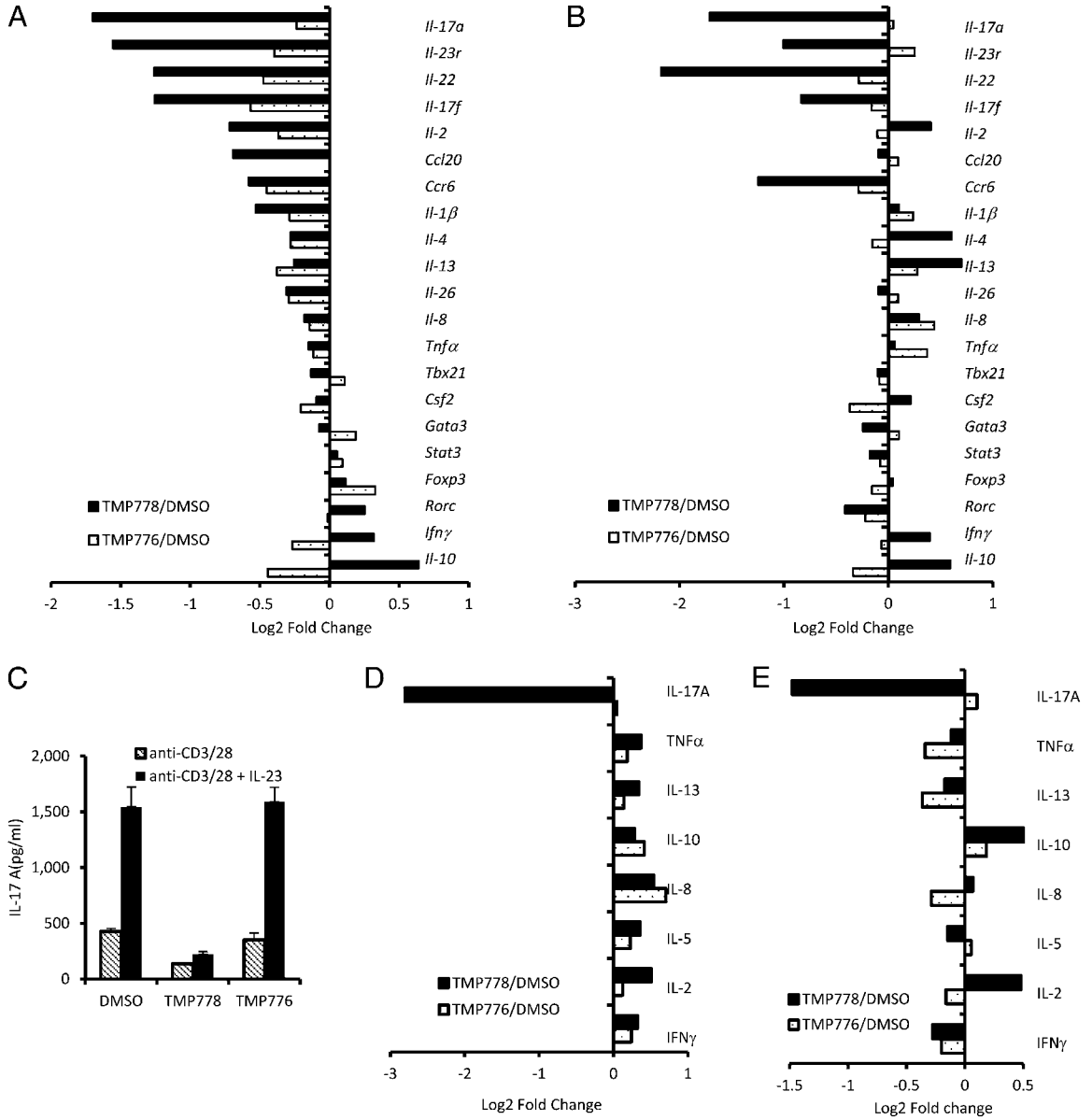

FIGURE 6. Th17 signature gene expression and IL-17 production by primary cells from psoriatic patients are suppressed by RORγt inverse agonist TMP778. (**A** and **B**) PBMCs from psoriatic patients were stimulated with soluble anti-CD3/anti-CD28 mAb (A) or soluble anti-CD3/anti-CD28 mAb plus IL-23 (B) in the presence of 1 μM compound or DMSO for 20 h. Cells were then harvested for RNA extraction and RT real-time PCR studies. The mRNA comparison of TMP778/DMSO and TMP776/DMSO are illustrated as log2 fold changes. (**C**) PBMCs from psoriatic patients were stimulated with anti-CD3/anti-CD28 mAb with or without IL-23 in the presence of 1 μM compound. IL-17 titers in the supernatants were determined by MSD after the cells were cultured for 5 d. (**D**) Supernatants from IL-23–treated cells in (C) were used to determine Th1/Th2 cytokine levels. The comparison of TMP788/DMSO and TMP776/DMSO are illustrated as a log2 fold change. (**E**) Mononuclear cells purified from psoriatic skin of four separate patients were stimulated with anti-CD3/anti-CD28 mAb for 5 d. IL-17 and Th1/Th2 cytokine titers in the supernatants were determined by MSD. The comparisons of TMP778/DMSO and TMP776/DMSO are illustrated as log2 fold changes. Data are representative of at least three different experiments.

inflammation induced by IMQ in mice was significantly reduced by TMP778 treatment. Of note, TMP778, but not TMP776, blocks IL-17A production by PBMCs and skin mononuclear cells of psoriasis patients, whereas Th1/Th2 cytokine production was not affected. The mRNA expression of Th17 signature gene *Il-17a*, *Il-17f*, *Il-23r*, *Il-22*, and *Ccr6* from psoriasis patients was significantly inhibited by TMP778, but not TMP776. Finally, and most importantly, TMP778 blocks Th17 signature gene expression by human whole blood of normal donors, as well as psoriasis patients.

The significant blockage of the acute gene expression of *Il-17a*, *Il-17f*, *Il-23r*, *Il-22*, and *Ccr6*, as well as the blockage of differentiation of IL-17A–producing cells by TMP778, indicate the potential for better efficacy in the clinic over treatment with mAbs against a single cytokine such as IL-17A or IL-23. For example, in addition to IL-17A and IL-23, IL-22 has been shown to be important in IL-23–induced skin inflammation (40) and IMQ-induced psoriasiform skin inflammation (41). It should be noted that blocking Th17 signature gene expression could have adverse effects such as increased risk for infectious diseases. For example, IL-22 and IL-17A are important for the host defense against mucosal microbiota, although they play a critical role in the pathogenesis of cutaneous inflammation. However, despite the fact that TMP778 significantly inhibits the expression of Th17 signature genes, it does not completely abolish their expression; therefore, TMP778 might have limited effect on host defense

because there should be still some level of Th17 signature gene expression. The transcriptional profiling studies further demonstrate that TMP778 does not broadly affect gene expression, reducing its safety risk in the clinic. Of course, it is still very important to monitor the adverse effects including infections such as *Mycobacterium tuberculosis* and *Candida albicans* in clinical studies. Thus, the combined significant, but not complete, inhibition of gene expression of *Il-17a*, *Il-17f*, *Il-23r*, *Il-22*, and *Ccr6* by TMP778 supports the concept that RORγt inverse agonist TMP778 should have better efficacy than targeting only one cytokine in the treatment of cutaneous inflammation such as psoriasis, whereas having a limited effect on host defense to microbiota.

Of note, use of psoriatic patient samples such as PBMCs and skin mononuclear cells in the evaluation of RORγt inverse agonist TMP778 has provided us preclinical proof of concept for the use of TMP778 in clinical cutaneous inflammation. Further, TMP778 inhibits the IL-17A production by colon tissues of Crohn's disease patients, indicating its role in other Th17-related autoimmune diseases.

Digoxin, SR2211, SR1001, and ursolic acid are a variety of small-molecule RORγt inhibitors that have been recently discovered (22–24, 42). These compounds inhibit Th17 cell differentiation in vitro and reduce severity of EAE, a mouse model of multiple sclerosis. In this study, we have shown that RORγt inverse agonist TMP778 inhibits Th17 cell differentiation both in vitro and in vivo (Figs. 2A, 3A, Supplemental Fig. 2A), and additionally reduces the severity of EAE (manuscript in preparation). We further describe that TMP778 blocks the differentiation of $CD8^{+}$Tc17 cells (Fig. 2B). Digoxin, SR1001, and ursolic acid suppress IL-17A production by memory $CD4^{+}$ T cells and are believed to be important for the maintenance of Th17 cells (22–24, 43). In this study, we demonstrate that RORγt inverse agonist TMP778 directly inhibits IL-17F promoter activity, acute IL-17A production, and Th17 signature gene expression, indicating that RORγt is not only important for the maintenance of Th17 cells, but also for the acute expression of Th17 signature genes. The use of an IL-17F promoter instead of IL-17A promoter for the first cell-based screening assay opened up the possibility of discovering RORγt inverse agonists, which affect binding to both the IL-17A and the IL-17F promoters. We further demonstrate that TMP778 blocks IL-17A production by human and mouse γδ T cells, as well as IL-23–enhanced IL-17A production by human PBMCs. Of note, we studied the role of TMP778 in human psoriasis PBMCs, blood, and skin lesion, and these studies indicated that targeting RORγt using RORγt inverse agonists such as TMP778 could be beneficial for the treatment of psoriasis patients. Because TMP778 targets the lineage of IL-17A–producing cells and acute expression of Th17 signature genes, it is predicted that TMP778 will have better efficacy than targeting a single Th17 cytokine or receptor. Genome-wide transcriptional studies and preclinical safety evaluation suggests that TMP778 could be a safe molecule in clinic. Thus, pharmacologic inhibition of RORγt can be beneficial for the psoriasis patients and TMP778 could be a candidate for the clinical treatment of psoriasis.

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Disclosures

All authors are present or former employees of Tempero Pharmaceuticals, Inc. or GSK, as indicated in the affiliations.

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