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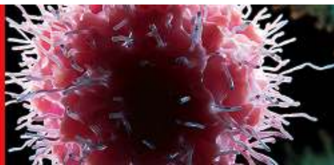

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MCPIP1/Regnase-1 Restricts IL-17A– and IL-17C–Dependent Skin Inflammation

Leticia Monin,* Johann E. Gudjonsson,† Erin E. Childs,* Nilesh Amatya,* Xianying Xing,† Akash H. Verma,* Bianca M. Coleman,* Abhishek V. Garg,* Meaghan Killeen,‡ Alicia Mathers,‡ Nicole L. Ward,§ and Sarah L. Gaffen*

The IL-17 family cytokines IL-17A and IL-17C drive the pathogenesis of psoriatic skin inflammation, and anti–IL-17A Abs were recently approved to treat human psoriasis. Little is known about mechanisms that restrain IL-17 cytokine-mediated signaling, particularly IL-17C. In this article, we show that the endoribonuclease MCP-1–induced protein 1 (MCPIP1; also known as regnase-1) is markedly upregulated in human psoriatic skin lesions. Similarly, MCPIP1 was overexpressed in the imiquimod (IMQ)-driven mouse model of cutaneous inflammation. Mice with an MCPIP1 deficiency (*Zc3h12a*$^{+/-}$) displayed no baseline skin inflammation, but they showed exacerbated pathology following IMQ treatment. Pathology in *Zc3h12a*$^{+/-}$ mice was associated with elevated expression of IL-17A– and IL-17C–dependent genes, as well as with increased accumulation of neutrophils in skin. However, IL-17A and IL-17C expression was unaltered, suggesting that the increased inflammation in *Zc3h12a*$^{+/-}$ mice was due to enhanced downstream IL-17R signaling. Radiation chimeras demonstrated that MCPIP1 in nonhematopoietic cells is responsible for controlling skin pathology. Moreover, *Zc3h12a*$^{+/-}$*Il17ra*$^{-/-}$ mice given IMQ showed almost no disease. To identify which IL-17RA ligand was essential, *Zc3h12a*$^{+/-}$*Il17a*$^{-/-}$ and *Zc3h12a*$^{+/-}$*Il17c*$^{-/-}$ mice were given IMQ; these mice had reduced but not fully abrogated pathology, indicating that MCPIP1 inhibits IL-17A and IL-17C signaling. Confirming this hypothesis, *Zc3h12a*$^{-/-}$ keratinocytes showed increased responsiveness to IL-17A and IL-17C stimulation. Thus, MCPIP1 is a potent negative regulator of psoriatic skin inflammation through IL-17A and IL-17C. Moreover, to our knowledge, MCPIP1 is the first described negative regulator of IL-17C signaling. *The Journal of Immunology*, **2017, 198: 767–775.**

In the past decade, IL-17 family members have emerged as drivers of inflammatory and autoimmune conditions, particularly psoriasis (1). Indeed, IL-17A–targeting Abs are approved for treatment of plaque psoriasis, psoriatic arthritis, and ankylosing spondylitis, underscoring the importance of IL-17A in these conditions (2, 3). IL-17A can be produced by a variety of immune cells, including Th17 cells, γδ T cells, invariant NKT cells, "natural" Th17 cells, and type 3 innate lymphoid cells (4–9). However, most studies indicate that IL-17 exerts its actions primarily on nonhematopoietic cells. IL-17A induces expression of a typical gene signature profile in target cells that is characterized by proinflammatory cytokines, chemokines, and antimicrobial peptides (1). Together, these factors provide important host defense against extracellular microbes but, when dysregulated, they can promote inflammation in autoimmunity.

In addition to IL-17A, the less-studied cytokine IL-17C is implicated in psoriasis. IL-17C is predominantly produced by epithelial cells and induces a similar set of downstream genes with inflammatory, antibacterial, and antiapoptotic functions. IL-17C appears to act in hematopoietic and nonhematopoietic cells, including intestinal epithelial cells, keratinocytes (KCs), and Th17 cells (10–13). IL-17C protein concentrations are ∼125-fold higher than IL-17A levels in psoriatic lesions, making it the most abundant IL-17 family member in human psoriasis (14). Additionally, KC-specific overexpression of IL-17C in mice causes a spontaneous psoriasis-like phenotype (14).

IL-17 family members signal through multimeric receptors composed of a common chain, IL-17RA, and a second chain that varies by ligand. IL-17A signals through IL-17RA and IL-17RC, and IL-17C was shown to act via an IL-17RA/IL-17RE receptor complex. Both cytokines use the adaptor and E3 ubiquitin ligase Act1 (also known as CIKS) to drive signaling, but little else is known about the positive activators of IL-17C downstream signaling (1, 15, 16).

Given the prominence of IL-17A in inflammation, numerous mechanisms have evolved to negatively regulate its signaling that are needed to limit collateral tissue damage during inflammatory processes. IL-17A is a key driver of psoriasis, but little is known about the factors that normally restrain IL-17A–dependent signal transduction in the skin. The endoribonuclease and deubiquitinase

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Abbreviations used in this article: BM, bone marrow; Ct, threshold cycle; EMEM, Eagle's MEM; GWAS, genome-wide association study; IMQ, imiquimod; KC, keratinocyte; MCPIP1, MCP-1–induced protein 1; qPCR, quantitative real-time PCR; WT, wild-type.

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MCP-1–induced protein 1 (MCPIP1; also known as regnase-1 and encoded by the *ZC3H12A* gene) is a vital regulator of inflammation. Its expression is induced by proinflammatory stimuli, including MCP-1, TLR ligands, IL-1β, and IL-17A (17–21). MCPIP1 regulates TLR signaling through cleavage of target gene mRNAs, including *Il6* (22–24), or deubiquitination of inflammatory mediators (25). In addition, MCPIP1 constitutively restricts TCR signaling, and MCPIP1 in T cells is inducibly degraded following T cell activation. MCPIP1 deficiency in $CD4^+$ cells was shown to enhance Th17 effector function (26, 27). We showed that MCPIP1 negatively regulates IL-17–dependent inflammation through the degradation of IL-17A–induced target gene transcripts and IL-17RA mRNA (18). In this study, we examined the impact of MCPIP1 on IL-17A and IL-17C signaling in a mouse model of acute psoriatic-like skin inflammation and in human psoriasis clinical samples. To our knowledge, our data identify MCPIP1 as the first recognized inhibitor of IL-17C signaling and establish this protein as a common regulator of IL-17 family members during skin inflammation.

Materials and Methods

Patients

Nine healthy nonpsoriatic controls and 10 patients with chronic plaque psoriasis were enrolled (psoriasis lesional plaque, psoriasis nonlesional skin, and healthy normal skin). Patients were off systemic treatment for ≥4 wk and off all topical treatments 2 wk prior to enrollment. Two 6-mm punch biopsies from uninvolved skin and two biopsies from lesional skin or two biopsies from normal skin were obtained under local anesthesia. One biopsy was fixed in 4% formaldehyde for immunohistochemistry, whereas the other was snap-frozen in liquid nitrogen and stored at −80°C until processing. Informed consent was obtained from all subjects under a protocol approved by the Institutional Review Board of the University of Michigan Medical School (HUM00087890). This study was conducted according to the Declaration of Helsinki Principles.

Mice

C57BL/6 and CD45.1 mice were from the Jackson Laboratory (Bar Harbor, ME). $Zc3h12a^{-/-}$ mice were provided by P. Kolattukudy (University of Central Florida) (28), $Il17ra^{-/-}$ mice were from Amgen (Seattle, WA), $Il17a^{-/-}$ mice were from Y. Iwakura (University of Tokyo) and $Il17c^{-/-}$ were from Genentech via the Mutant Mouse Regional Resource Center (Davis, CA). Experimental mice were female, matched for age, between 6 and 10 wk of age, and used in accordance with approved University of Pittsburgh Institutional Animal Care and Use Committee protocols and the National Institutes of Health's *Guide for the Care and Use of Laboratory Animals*.

Imiquimod-driven dermatitis

Dorsal skin was shaved with an electric razor and subsequently epilated with Nair cream to facilitate cutaneous absorption of treatment. Following a 48-h rest, 6.25 mg of 5% imiquimod (IMQ; 3M; Aldara) was applied daily to dorsal and ear skin. Contralateral ears or control groups were treated with a control cream (Vanicream; Pharmaceutical Specialties, Rochester, MN). Ear thickness was measured with a caliper (Mitutoyo). Gross pathology was recorded daily by investigators blinded to the experimental cohorts. Ten 4-mm punch biopsies from random sections of skin were taken after euthanasia; five were used for flow cytometry, and five were snap-frozen for RNA extraction.

Adoptive transfers

To generate bone marrow (BM) chimeras, mice were irradiated with two 500-rad doses delivered 4 h apart. A total of 1×10^7 BM mice cells was delivered i.v. 16 h postirradiation. Mice were given sulfamethoxazole (960 μg/ml) and trimethoprim (192 μg/ml) in drinking water for 2 wk to prevent infections. Chimeras were allowed to engraft for 45 d before experimentation.

RNA isolation and quantitative real-time PCR

Human biopsies were homogenized, and RNA was isolated with an RNeasy Plus Mini Kit (QIAGEN). For quantitative real-time PCR (qPCR), RNA was reverse transcribed using a High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems). qPCR was performed using a 7900HT Fast Real-Time PCR system with TaqMan primers (both from Applied Biosystems). Expression was normalized to *RPLP0*. Mouse skin was homogenized in QIAzol (QIAGEN), in gentleMACS M Tubes (Miltenyi Biotec), and total RNA was isolated using an RNeasy Lipid Mini Kit (QIAGEN). Cells were lysed in Buffer RLT, and RNA extraction was performed using an RNeasy Mini Kit (QIAGEN). cDNA was prepared using a Superscript III First-Strand Kit (Invitrogen). qPCR was performed with QuantiTect Primer Assays (QIAGEN) and SYBR Green FastMix, ROX (Quanta Biosciences, Gaithersburg, MD). Expression was normalized to *Gapdh* for mouse skin and *Rplp0* for KCs. Samples were analyzed on a 7300 Real-Time instrument (Applied Biosystems). Threshold cycle (Ct) values were obtained for target and housekeeping genes, and ΔCt values were calculated. Expression was normalized to *Gapdh* for mouse skin and *Rplp0* for KCs. Gene induction was transformed to a linear scale via calculation of $2^{-\Delta Ct}$ (relative expression).

Histology and immunohistochemistry

Immunohistochemistry was performed on 5-μm paraffin sections from human skin. Paraffin-embedded sections were heated at 60°C for 30 min, deparaffinized, and rehydrated. Slides were placed in Ag-retrieval buffer (pH 6) and heated at 95°C for 20 min. After cooling, slides were treated with 3% H_2O_2 (5 min) and blocked with 10% goat serum (30 min). Slides were incubated for 2 h at room temperature with MCPIP1 Abs (4.8 μg/ml, 1:100 dilution, catalog number 25009-1-AP; Protein Tech Group). Slides were washed and treated with 2˚C Ab and peroxidase (30 min) and diaminobenzidine substrate. Mouse skin samples were fixed in 10% formalin and embedded in paraffin, and H&E staining was performed at the University of Buffalo Histology Core (Buffalo, NY).

Flow cytometry

Skin biopsies were collected in media + GolgiStop. Samples were lysed in gentleMACS C Tubes (Miltenyi Biotec) with 1.5 ml of digestion mix (Liberase TM, DNase I, and GolgiStop) and incubated at 37°C for 80 min. Homogenates were filtered through 70-μm strainers. Single-cell suspensions were blocked with Fc and surface stained with Abs against CD45 (clone 30-F11), Ly6G (1A8), CD11b (M1/70), CD3 (145-2C11), and γδ TCR (GL3). Cells were permeabilized with Cytofix/Cytoperm (BD) and stained with anti–IL-17A Abs (TC11-18H10) or isotype control. Cells were acquired with a BD Fortessa and analyzed by FlowJo software (TreeStar).

Primary mouse KCs

KCs were prepared as described (29, 30). Briefly, skin was excised from postnatal day 2 skin, stretched in a 6-cm culture dish, and incubated for 2 h at 4°C. Skins were floated on 0.25% trypsin for 16 h at 4°C. Dermis was separated from epidermis and discarded, and undifferentiated cells were retrieved by vortexing in Eagle's MEM (EMEM) with 8% FBS + 1.4 mM Ca^{2+}. A total of 4×10^5 cells per well was plated in 12-well dishes in EMEM with 8% FBS + 0.3 mM Ca^{2+}. After 16 h, media were replaced by EMEM with 8% FBS and 0.05 mM Ca^{2+} to promote differentiation. KCs were rested for 3 d and stimulated for 16 h with mouse IL-17A (200 ng/ml), IL-17C (200 ng/ml), or TNF-α (2 ng/ml). Supernatants were collected for ELISA, and cells were scraped, pelleted, and snap-frozen for RNA analysis.

Statistics

Data are presented as mean ± SEM. Statistical analysis was performed using ANOVA, followed by the Bonferroni post hoc test or Mann–Whitney *U* test, with GraphPad Prism (La Jolla, CA). All experiments were performed a minimum of twice. For qPCR data, statistical analyses were run on ΔCt values. The p values < 0.05 were considered significant.

Results

MCPIP1 is expressed in psoriasis and limits disease severity in a mouse psoriasis model

To ascertain whether MCPIP1 is linked with psoriasis, we assessed *ZC3H12A* expression in lesional and nonlesional skin from psoriasis patients or healthy controls (healthy normal skin). *ZC3H12A* mRNA expression was elevated ∼10-fold in psoriasis lesions compared with nonlesional skin from the same patient or with skin from healthy controls (Fig. 1A). Immunohistochemical staining of skin samples indicated that MCPIP1 was expressed primarily in the epidermis, but it was also detectable in the inflammatory infiltrate (Fig. 1B), consistent with the known expression pattern of this protein (23).

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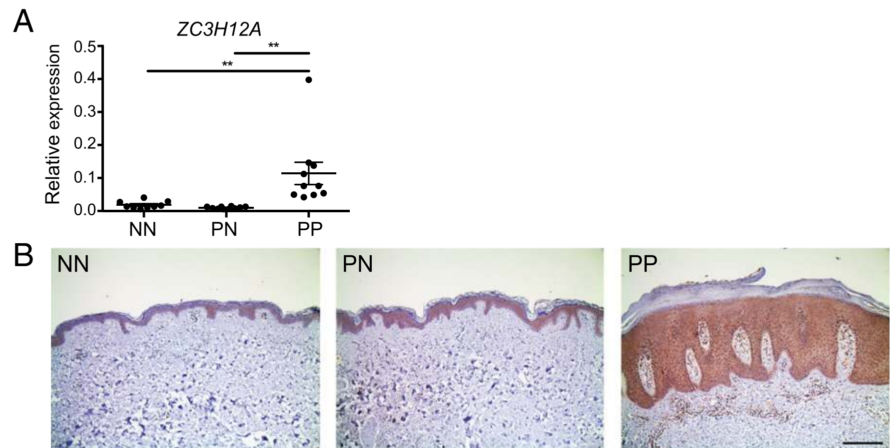

FIGURE 1. MCPIP1 expression is elevated in human psoriasis. (**A**) *ZC3H12A* expression in normal healthy skin (NN) or nonlesional (PN) and paired lesional (PP) skin samples from psoriasis patients (n = 9–10) was determined by qPCR. Expression was normalized to *RPLP0*. (**B**) MCPIP1 expression was detected by immunohistochemistry in formalin-fixed, paraffin-embedded sections from normal healthy skin (NN) or uninvolved (PN) and involved (PP) psoriasis skin. $^{**}p < 0.01$, Mann–Whitney U test. Scale bar, 100 μm.

In mice, topical application of IMQ leads to IL-17A– and IL-23–dependent development of skin lesions with the hallmarks of human psoriasis (31, 32). This system is considered a good model of the early events in psoriatic plaque formation (33). Consistent with the clinical samples, *Zc3h12a* mRNA expression was induced throughout the course of IMQ-driven psoriasiform dermatitis (Fig. 2A). Expression was detectable as early as 1 d following IMQ treatment. As expected, *Il17a* and *Il17c* were also upregulated during this time frame (Fig. 2B, 2C). *Il17c* expression increased rapidly, peaking by day 2 and remaining elevated. *Il17a* mRNA was induced somewhat later, with progressively increased levels seen over 5 d.

MCPIP1 negatively regulates several inflammatory stimuli, but its role in psoriasis is poorly defined (18, 34). *Zc3h12a*$^{-/-}$ mice cannot be used for experimentation because they exhibit severely shortened lifespans as a consequence of unrestricted TLR signaling and widespread inflammation (23, 28). To circumvent these confounding issues, we assessed disease in haploinsufficient mice (*Zc3h12a*$^{+/-}$) (18). Importantly, *Zc3h12a*$^{+/-}$ mice, unlike *Zc3h12a*$^{-/-}$ mice, did not present with exacerbated baseline inflammatory levels in skin, as determined by expression of proinflammatory cytokines, chemokines, and antimicrobial proteins (*Il17a*, *Ifng*, *Csf2*, *Lcn2*, *Cxcl1*, *Cxcl5*) (Fig. 2D). There also was no difference in cutaneous neutrophil infiltration in *Zc3h12a*$^{+/-}$ mice versus *Zc3h12a*$^{+/+}$ littermates (hereafter termed wild-type [WT]) (Fig. 2E). However, after 3 d of IMQ treatment, *Zc3h12a*$^{+/-}$ mice showed increased disease severity compared with WT, with enhanced erythema, epidermal thickening, skin scaling, and ear swelling (Fig. 2F, 2G). H&E staining of dorsal skin from IMQ-treated *Zc3h12a*$^{+/-}$ mice revealed increased neutrophil microclusters and parakeratosis compared with WT (Fig. 2H). Therefore, MCPIP1 restricts inflammation in IMQ-driven dermatitis.

MCPIP1 limits IL-17A– and IL-17C–mediated skin inflammation

In T cells, MCPIP1 deficiency is associated with increased TCR signaling and amplified Th17 differentiation (27). Thus, an explanation for the increased skin inflammation in *Zc3h12a*$^{+/-}$ mice could be increased IL-17A production in lymphocytes following IMQ treatment. V$\gamma 4^+$ $\gamma\delta$ T cells are a major source of IL-17A in IMQ-induced inflammation (35, 36). However, the levels of $\gamma\delta$ T cell–produced IL-17A did not differ between IMQ-treated *Zc3h12a*$^{+/-}$ mice and WT (Fig. 3A). Similarly, *Il17a*, *Il17f*, and *Il17c* mRNA levels were not statistically different in IMQ-treated *Zc3h12a*$^{+/-}$ mice compared with controls and neither was *Il23* or its receptor, *Il23r* (Fig. 3B).

Because the increased IMQ-induced skin inflammation in *Zc3h12a*$^{+/-}$ mice did not appear to be due to increased IL-17A or IL-17C expression, we hypothesized that it might instead be through increased IL-17R signaling. A hallmark of psoriasis is IL-17A–dependent neutrophil infiltration (31). Commensurate with their increased inflammation, *Zc3h12a*$^{+/-}$ mice showed increased neutrophil frequency, accompanied by increased expression of the neutrophil-attractive chemokine *Cxcl5* and IL-17A–associated genes *Lcn2* and *Il6* (Fig. 3C, 3D). To directly test the hypothesis that the enhanced susceptibility to IMQ-induced dermatitis in *Zc3h12a*$^{+/-}$ mice was due to enhanced downstream IL-17 signaling, *Zc3h12a*$^{+/-}$ mice were crossed to *Il17ra*$^{-/-}$ mice and treated with IMQ. *Zc3h12a*$^{+/-}$*Il17ra*$^{-/-}$ mice exhibited markedly reduced inflammation compared with *Zc3h12a*$^{+/-}$ mice, with milder skin pathology, reduced neutrophil infiltration (Fig. 4A, data not shown), and abrogated expression of *Il17a*, *Il17c*, and IL-17–dependent genes associated with psoriasis, such as *Il6* and *Lcn2* (Fig. 4B–E). In line with previous reports, *Il17ra*$^{-/-}$ mice also showed elevated IL-17A and IL-17C expression at baseline (Fig. 4B) (37, 38). These data verify that the MCPIP1-dependent phenotype is due to IL-17 signaling and not other cytokines associated with psoriasis.

IL-17RA is a shared subunit used by several IL-17 family cytokines, so the enhanced inflammation that we observed could be due to more than one ligand (11, 39). In particular, IL-17C is reported to signal through IL-17RA paired with IL-17RE, inducing a characteristic subset of inflammatory genes and AMP expression in KCs. To determine which ligands were affected by MCPIP1, *Zc3h12a*$^{+/-}$ mice were crossed to *Il17a*$^{-/-}$ and *Il17c*$^{-/-}$ mice. Following IMQ treatment, *Il17a* and *Il17c* expression levels did not differ among WT, *Zc3h12a*$^{+/-}$, *Zc3h12a*$^{+/-}$*Il17a*$^{-/-}$, and *Zc3h12a*$^{+/-}$*Il17c*$^{-/-}$ mice (Fig. 4B, 4C). Notably, *Zc3h12a*$^{+/-}$*Il17a*$^{-/-}$ mice and *Zc3h12a*$^{+/-}$*Il17c*$^{-/-}$ mice exhibited less skin inflammation than did *Zc3h12a*$^{+/-}$ mice, with decreased neutrophil accumulation and reduced *Lcn2*, *Il6*, and *Defb4* expression (Fig. 4). In addition, *Il17a*$^{-/-}$, *Zc3h12a*$^{+/-}$*Il17a*$^{-/-}$, *Il17c*$^{-/,-}$ and *Zc3h12a*$^{+/-}$*Il17c*$^{-/-}$ mice presented comparable levels of inflammation. These results demonstrate that the increased dermal inflammation seen with MCPIP1 deficiency can be partially rescued through elimination of IL-17A or IL-17C signaling and more strongly reversed in the absence of the shared subunit IL-17RA.

MCPIP1-driven regulation of IL-17 signaling is restricted to the stromal compartment

MCPIP1 regulates the effector mechanisms of IL-17A and its production by Th17 cells. To determine the relative contribution of MCPIP1 to the regulation of the immune and stromal compartments, we generated BM chimeric mice with MCPIP1 haploinsufficiency in hematopoietic or radioresistant cells and subjected them to IMQ dermatitis. Mice receiving *Zc3h12a*$^{+/-}$ BM developed gross skin inflammation that was indistinguishable from that of mice receiving

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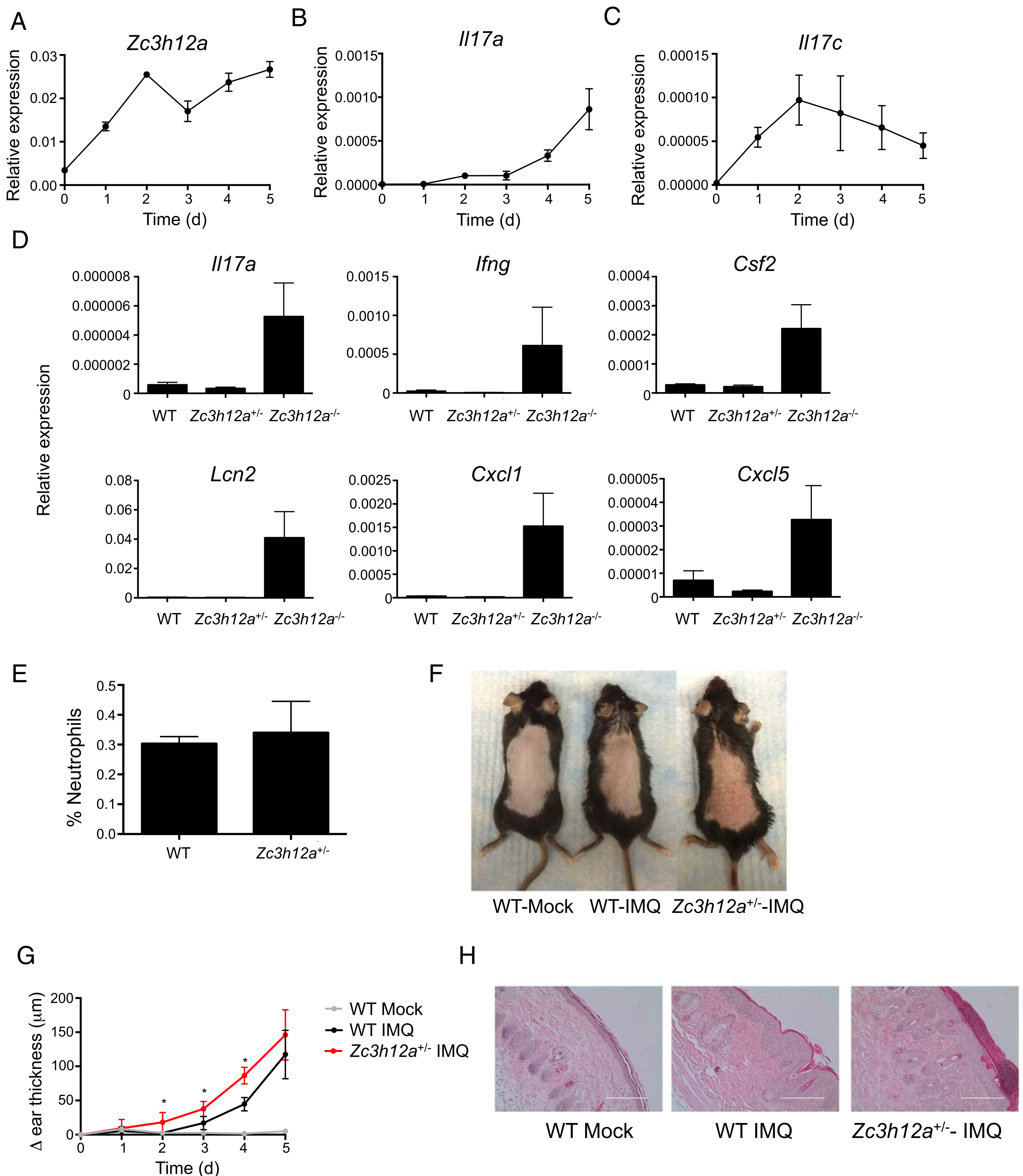

FIGURE 2. MCPIP1 expression is elevated in IMQ-driven dermatitis and limits disease severity. C57BL/6 (WT) mice (n = 3 per time point) were treated topically on dorsal skin with IMQ on days 0–4. Expression of *Zc3h12a* (**A**), *Il17a* (**B**), and *Il17c* (**C**) in skin was determined by qPCR. (**D**) Expression of the indicated genes was determined by qPCR in untreated *Zc3h12a*$^{+/+}$ (WT), *Zc3h12a*$^{+/-}$, and *Zc3h12a*$^{-/-}$ skin. (**E**) Cutaneous neutrophil infiltration was analyzed in skin by flow cytometry; percentage of neutrophils in the CD45^{+} gate is shown. (**F**) WT or *Zc3h12a*$^{+/-}$ mice (n = 3–5 per day) were treated topically with mock cream or IMQ on days 0–4. Gross skin pathology for a representative animal at day 3 is shown. (**G**) Change in ear thickness in treated and mock-treated ears was assessed daily in the indicated mice. (**H**) Representative images of H&E-stained formalin-fixed, paraffin-embedded mouse skin sections on day 3. Scale bars, 200 μm. *$p < 0.05$, Mann–Whitney U test.

WT BM (data not shown). Consistently, they showed comparable levels of skin neutrophil accumulation (Fig. 5A). In contrast, *Zc3h12a*$^{+/-}$ mice given WT BM developed exacerbated skin pathology with increased neutrophilia, comparable to those receiving WT BM (Fig. 5A). These data indicate that MCPIP1 regulates the skin phenotype specifically in stromal and radioresistant skin cells.

KCs are the most abundant cell type in the epidermis and serve a key role in the initiation and perpetuation of immune responses.

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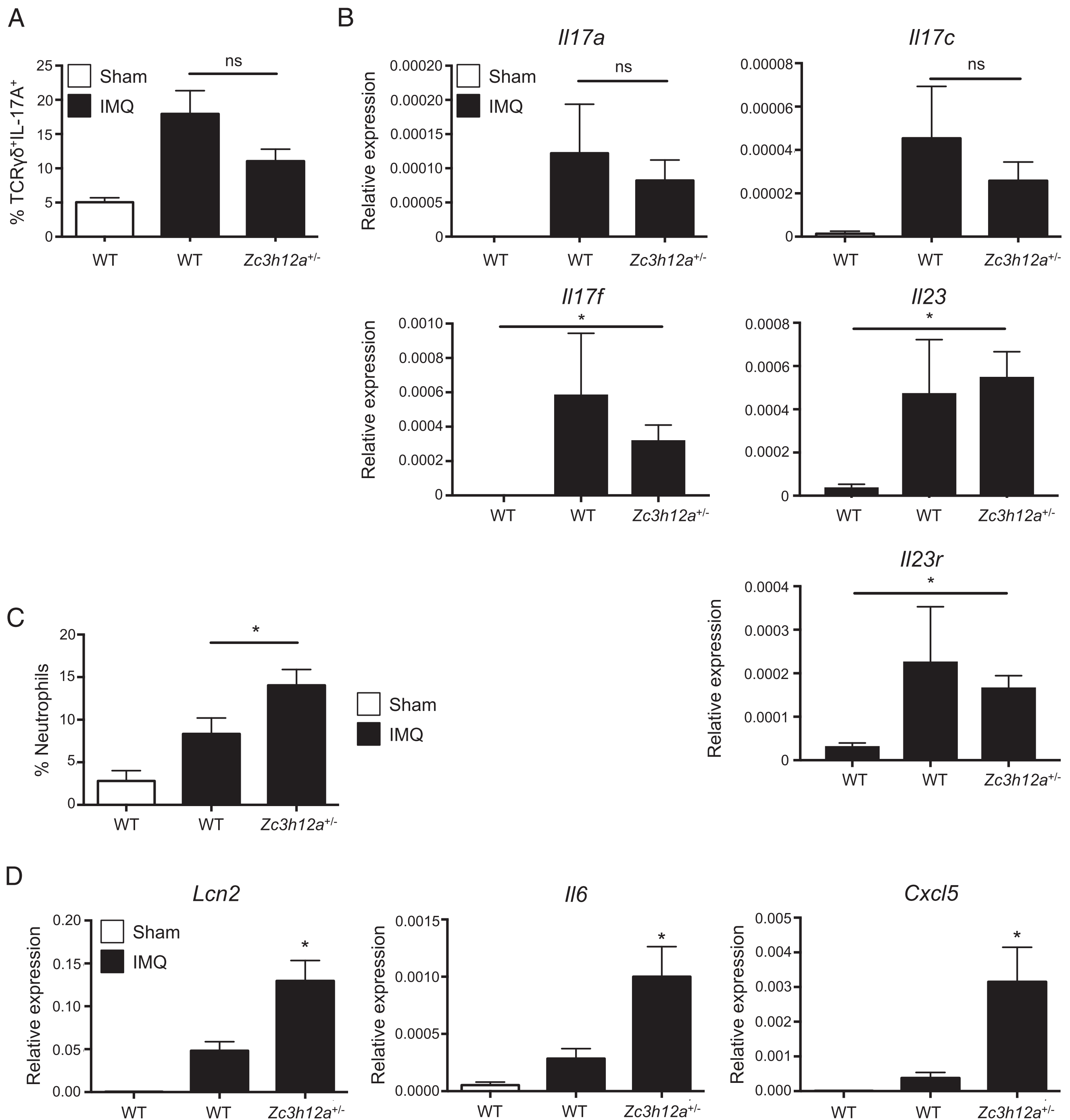

FIGURE 3. MCPIP1-deficient mice exhibit increased neutrophil infiltration and expression of IL-17 gene targets upon IMQ treatment. Mice (n = 5–8) were treated topically with IMQ daily. (**A**) IL-17 production in dermal γδ T cells was determined by flow cytometry. The percentage of IL-17A$^+$ cells within the CD45$^+$γδ-TCR^{int} lymphocyte gate is shown. (**B**) Expression of the indicated genes in dorsal skin was determined by qPCR. (**C**) Neutrophil infiltration in skin was evaluated by flow cytometry; the percentage of neutrophils in the CD45$^+$ gate is shown. (**D**) Expression of the indicated genes was determined by qPCR. *$p < 0.05$, Mann–Whitney U test. ns, not significant.

KCs are highly responsive to IL-17A and IL-17C (14, 40, 41). To determine whether MCPIP1 restricts IL-17R signaling in KCs, primary KC cultures from postnatal day 2 WT and *Zc3h12a*$^{-/-}$ mice were assessed for expression of various genes in response to IL-17A and IL-17C after 16 h of cytokine treatment. Consistent with a role for MCPIP1 as a negative regulator of signaling, *Zc3h12a*$^{-/-}$ KCs showed enhanced expression of IL-17A and IL-17C target genes, including *Cxcl1*, *Defb3*, and *S100a7* (Fig. 5B–D). MCPIP1 was shown to induce degradation of mRNA transcripts encoding IL-17RA and IL-17RC (18). Levels of *Il17re* mRNA were slightly, but not significantly, reduced in *Zc3h12a*$^{-/-}$ KCs (Fig. 5E). However, *Il17rc* and *Il17ra* mRNA levels were modestly, but consistently, elevated in *Zc3h12a*$^{-/-}$ KCs compared with WT (Fig. 5F, 5G), perhaps contributing to increased cytokine signaling.

Discussion

IL-17A and IL-17C play important roles in controlling infections, but they drive pathology in inflammatory conditions, especially psoriasis. Elevated levels of IL-17A and IL-17C occur in lesional human psoriasis skin samples (14, 39, 42), and human KCs

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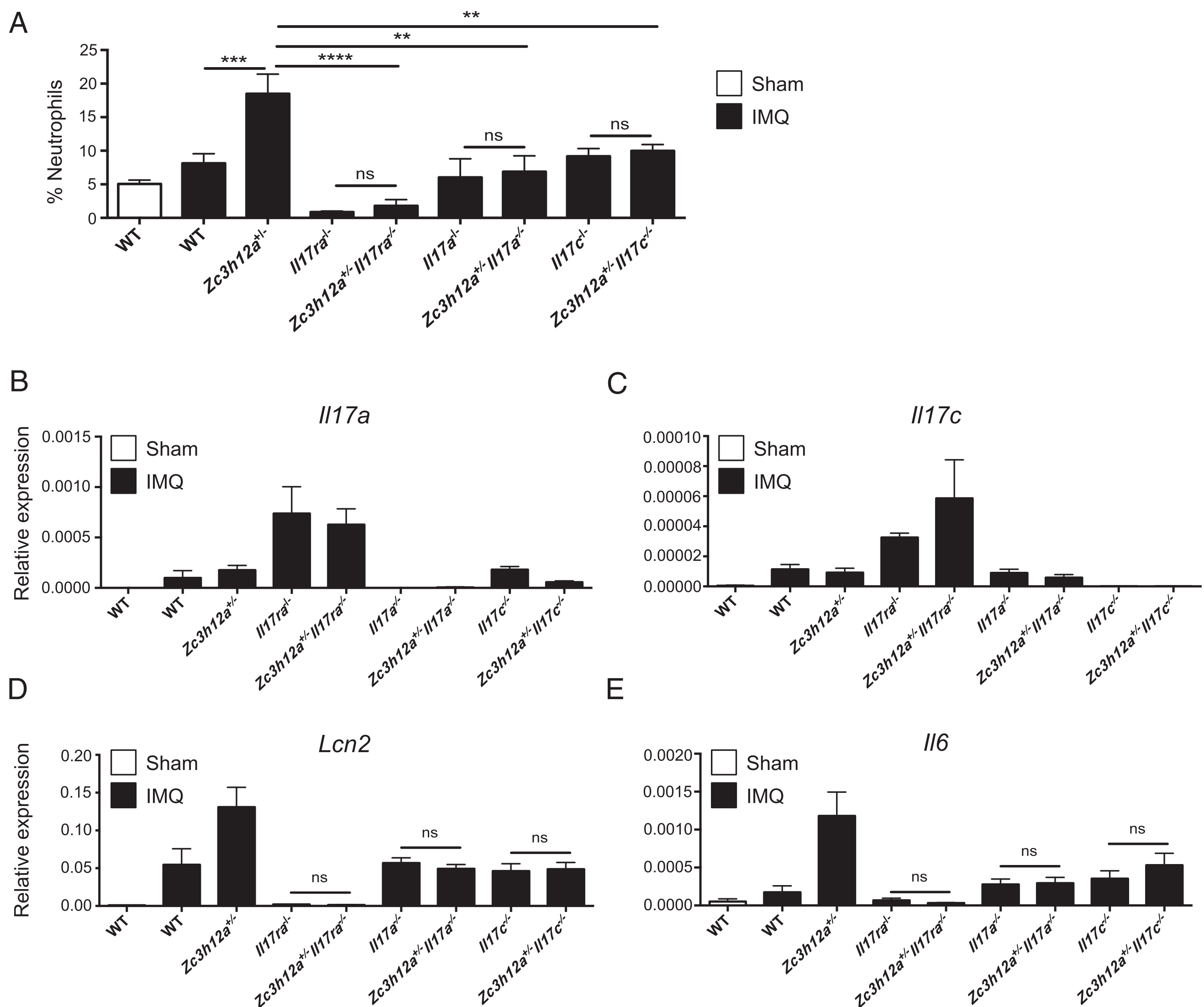

FIGURE 4. Increased IMQ-induced inflammation in MCPIP1-deficient mice is due to IL-17A and IL-17C signaling. Mice were treated topically with control cream or IMQ on days 0, 1, and 2. (**A**) Skin neutrophil infiltration was determined by flow cytometry; the percentage of neutrophils in the $CD45^+$ gate is shown. $n = 3–18$. Data are pooled from four independent experiments. (**B**–**E**) Expression of the indicated genes was determined by qPCR. $n = 3–10$. Data are pooled from two independent experiments. $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$, Mann–Whitney U test. ns, not significant.

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stimulated with IL-17A upregulate antimicrobial peptides and neutrophil-attracting chemokines (43, 44). Consistently, genome-wide association studies (GWASs) identified psoriasis-associated polymorphisms in genes critical for Th17 differentiation and IL-17A signaling, such as *IL23R* and *TRAF3IP2* (encoding the essential IL-17–signaling intermediate Act1) (45–49). Similarly, mouse preclinical models of psoriasis revealed a role for IL-17 family cytokines in mediating disease. In the IMQ dermatitis model, IL-17RA–deficient mice show dramatically reduced disease development (31). IL-17C intradermal application of mice led to enhanced leukocyte recruitment in skin, and IL-17C–deficient mice develop milder skin inflammation upon IMQ treatment (11). Additionally, a transgenic mouse overexpressing IL-17C in KCs develops spontaneous skin lesions with many features of human psoriasis (14). The importance of IL-17A–mediated inflammation in psoriasis was highlighted more recently by the clinical success of biologic drugs, including the IL-17A–blocking Abs secukinumab and ixekizumab and the IL-17RA–targeting Ab brodalumab (2, 50–53).

Given its prominent role in inflammation, it is not surprising that the IL-17A signaling pathway is tightly controlled. Indeed, recent studies elucidated several mechanisms by which IL-17A signaling is negatively regulated. For example, TRAF3 and TRAF4 interfere with receptor-proximal events by competing with Act1 or TRAF6 for IL-17RA occupancy (54, 55). The deubiquitinase A20 (*TNFAIP3*) is induced by IL-17A and mediates the removal of K63-linked ubiquitin chains on TRAF6, tempering activation of NF-κB and MAPK pathways (56). Similarly, USP25-mediated deubiquitination of TRAF5 and TRAF6 dampens IL-17A signaling (14). GSK-3β–mediated phosphorylation of the transcription factor C/EBPβ inhibits IL-17–dependent gene expression (57). Finally, MCPIP1 is a feedback inhibitor that degrades IL-17–induced target genes, including *Il6*, *Nfkbiz*, and transcripts encoding IL-17R subunits (18, 21, 58). A new report indicates that ABIN-1 (*Tnip1*) controls IL-17–induced pathology in IMQ-induced dermatitis (59). The concept that multiple inhibitors are needed to adequately control inflammatory cytokines was elegantly reviewed by Carpenter et al. (60). Despite its emerging role in inflammatory diseases (14), IL-17C signaling mechanisms are largely undefined, and no study has focused on negative regulation of IL-17C–dependent signal transduction.

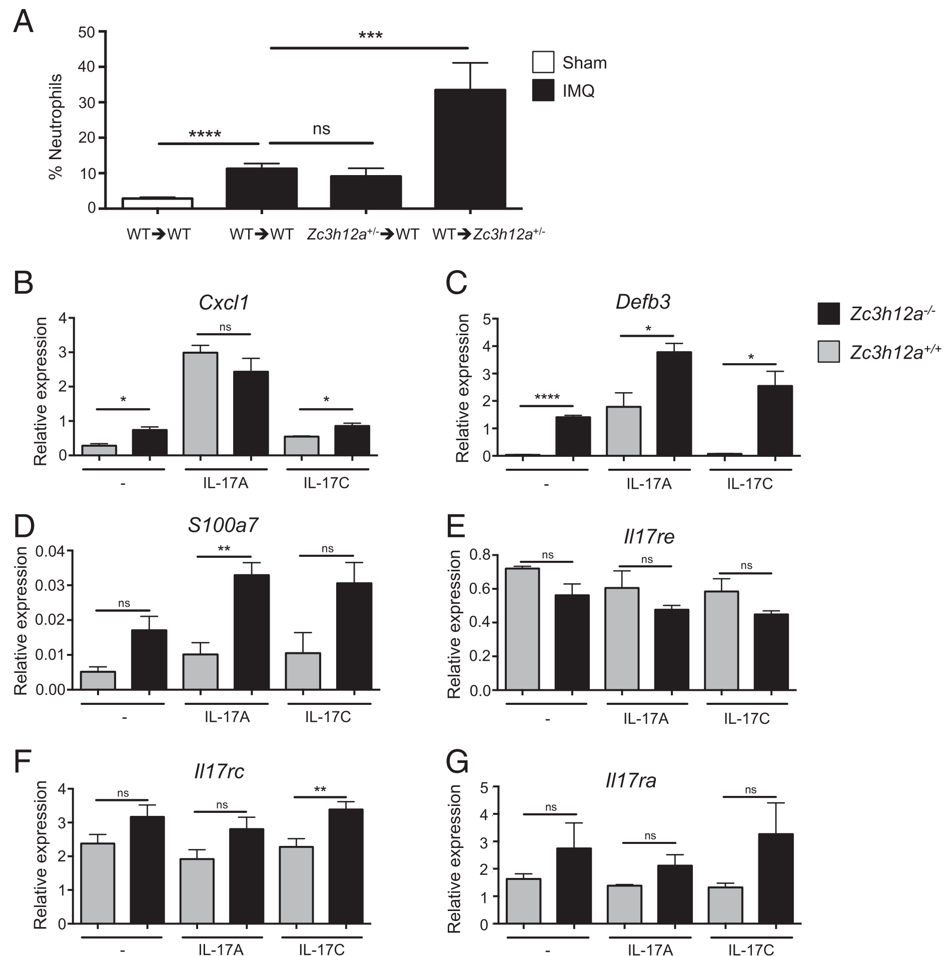

FIGURE 5. MCPIP1-dependent exacerbation of IMQ-driven dermatitis occurs through resident skin cells and is associated with elevated IL-17A and IL-17C signaling in KCs. WT or *Zc3h12a*$^{+/-}$ mice (n = 4–9) were lethally irradiated and reconstituted with WT or *Zc3h12a*$^{+/-}$ BM. After 6 wk to allow immune reconstitution, mice were treated topically with control cream or IMQ on days 0, 1, or 2. (**A**) Skin neutrophil infiltration was determined by flow cytometry; the percentage of neutrophils in the CD45^{+} gate is shown. (**B**–**G**) Primary mouse WT (gray bars) or *Zc3h12a*$^{-/-}$ (black bars) neonatal KCs were treated for 16 h with IL-17A or IL-17C, and expression of the indicated genes was assessed by qPCR (n = 3–5). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$, Mann–Whitney *U* test. ns, not significant.

In this article we show that MCPIP1 restricts IL-17A– and IL-17C–driven skin inflammation. Deficiency of one copy of the *Zc3h12a* gene was sufficient to render mice hypersusceptible to IMQ-driven psoriasis. We reported previously that the strong inflammatory phenotype in *Zc3h12a*$^{+/-}$ heterozygous mice is not due to impaired baseline levels of MCPIP1 in peripheral organs, such as kidney and lung; rather, haploinsufficient cells failed to show ligand-inducible upregulation of MCPIP1 (18). We verify in this study that *Zc3h12a*$^{+/-}$ mice also do not show exacerbated baseline inflammation in skin (Fig. 2). Notably, *Zc3h12a*$^{+/-}$ mice on an *Il17a*-deficient or *Il17c*-deficient background exhibited milder dermatitis than did *Zc3h12a*$^{+/-}$ mice (Fig. 4). However, the absence of *Il17ra* was required to render *Zc3h12a*$^{+/-}$ mice fully resistant to psoriasis, indicating a contribution of both IL-17 family members to skin pathology in this model system.

GWAS analysis of human psoriasis revealed associations with known regulators of immune signaling, including *TNFAIP3* (A20), *TNIP1* (ABIN-1, NAF1), and *NFKBIA* (IκBα) (61). Eleven nonsynonymous single-nucleotide polymorphisms were reported for the *ZC3H12A* gene, but none are associated with human disease (62). MCPIP1 belongs to a four-member family of CCCH-type zinc finger proteins. It is intriguing that polymorphisms in *ZC3H12C* (encoding MCP-1–induced protein 3) were identified in a GWAS study (48, 63). Similar to MCPIP1, MCP-1–induced protein 3 has endoribonuclease activity and curbs inflammatory responses in endothelial cells by suppressing NF-κB signaling (64). In addition, *ZC3H12C* and *ZC3H12A* are induced in LPS-treated macrophages (65), although it is not known whether *ZC3H12C* is an IL-17 target gene. Future studies are needed to establish how different MCP-1–induced protein family members regulate inflammation.

We found that *ZC3H12A* expression was markedly elevated in human psoriatic lesions compared with normal or uninvolved psoriatic skin (Fig. 1), consistent with published data (20, 66) and our data in mice (Fig. 2). Although this may seem counterintuitive given the role of MCPIP1 in restricting inflammation, it is common for feedback inhibitors like *TNFAIP3* and *TNIP1* to be associated with inflammation (67). It is likely that the increased *ZC3H12A* mRNA levels reflect the ongoing inflammatory milieu and particularly the high IL-17A levels found in diseased skin (18, 20, 22, 28).

BM chimeras demonstrated that MCPIP1 haploinsufficiency specifically in resident skin cells, and not in radiation-sensitive hematopoietic cells, is sufficient to exacerbate inflammation in the IMQ model (Fig. 5). This agrees with the restricted pattern of tissue expression of the IL-17RC and IL-17RE subunits, whereas IL-17RA is more broadly expressed. Prior studies in a model of autoimmune arthritis similarly demonstrated a role for IL-17RA only in the nonhematopoietic compartment (68). These results also agree with data showing that Act1 drives the development of dermal and epidermal pathology dominantly in cells of stromal origin (i.e., KCs, endothelial cells, and skin fibroblasts) (32). Previously, MCPIP1 was shown to negatively regulate TCR signaling and IL-17A production by Th17 cells (27). However, we saw that *Zc3h12a* haploinsufficiency did not cause increased IL-17A production at the mRNA or protein levels during IMQ inflammation (Fig. 2). Of note, γδ T cells are the main IL-17A–producing population in the skin in this model, whereas the

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frequency of IL-17$^+$ CD4$^+$ T cells was very low in *Zc3h12a*$^{+/-}$ mice. Accordingly, because this is an acute disease model in which IL-17 is made predominantly by γδ T cells, a role for MCPIP1 in conventional CD4$^+$ T cells may not be apparent.

In summary, we identified a novel role for the endoribonuclease and deubiquitinase MCPIP1 in restricting IL-17A– and IL-17C–mediated skin inflammation. To our knowledge this is the first report of a negative-regulatory element in the IL-17C signaling pathway, although it is not likely to be the last (60). Future studies will lend insight into the degree of conservation of MCPIP1-driven mechanisms of IL-17 cytokine family regulation.

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Disclosures

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References

1. Gaffen, S. L., R. Jain, A. V. Garg, and D. J. Cua. 2014. The IL-23-IL-17 immune axis: from mechanisms to therapeutic testing. *Nat. Rev. Immunol.* 14: 585–600.
2. Langley, R. G., B. E. Elewski, M. Lebwohl, K. Reich, C. E. Griffiths, K. Papp, L. Puig, H. Nakagawa, L. Spelman, B. Sigurgeirsson, et al; ERASURE Study Group; FIXTURE Study Group. 2014. Secukinumab in plaque psoriasis–results of two phase 3 trials. *N. Engl. J. Med.* 371: 326–338.
3. Hueber, W., D. D. Patel, T. Dryja, A. M. Wright, I. Koroleva, G. Bruin, C. Antoni, Z. Draelos, M. H. Gold, P. Durez, et al; Psoriasis Study Group; Rheumatoid Arthritis Study Group; Uveitis Study Group. 2010. Effects of AIN457, a fully human antibody to interleukin-17A, on psoriasis, rheumatoid arthritis, and uveitis. *Sci. Transl. Med.* 2: 52ra72.
4. Sonnenberg, G. F., and D. Artis. 2015. Innate lymphoid cells in the initiation, regulation and resolution of inflammation. *Nat. Med.* 21: 698–708.
5. Cua, D. J., and C. M. Tato. 2010. Innate IL-17–producing cells: the sentinels of the immune system. *Nat. Rev. Immunol.* 10: 479–489.
6. Ivanov, I. I., B. S. McKenzie, L. Zhou, C. E. Tadokoro, A. Lepelley, J. J. Lafaille, D. J. Cua, and D. R. Littman. 2006. The orphan nuclear receptor RORgammat directs the differentiation program of proinflammatory IL-17+ T helper cells. *Cell* 126: 1121–1133.
7. Kronenberg, M. 2005. Toward an understanding of NKT cell biology: progress and paradoxes. *Annu. Rev. Immunol.* 23: 877–900.
8. Marks, B. R., H. N. Nowyhed, J. Y. Choi, A. C. Poholek, J. M. Odegard, R. A. Flavell, and J. Craft. 2009. Thymic self-reactivity selects natural interleukin 17-producing T cells that can regulate peripheral inflammation. *Nat. Immunol.* 10: 1125–1132.
9. Villanova, F., B. Flutter, I. Tosi, K. Grys, H. Sreeneebus, G. K. Perera, A. Chapman, C. H. Smith, P. Di Meglio, and F. O. Nestle. 2014. Characterization of innate lymphoid cells in human skin and blood demonstrates increase of NKp44+ ILC3 in psoriasis. *J. Invest. Dermatol.* 134: 984–991.
10. Chang, S. H., J. M. Reynolds, B. P. Pappu, G. Chen, G. J. Martinez, and C. Dong. 2011. Interleukin-17C promotes Th17 cell responses and autoimmune disease via interleukin-17 receptor E. *Immunity* 35: 611–621.
11. Ramirez-Carrozzi, V., A. Sambandam, E. Luis, Z. Lin, S. Jeet, J. Lesch, J. Hackney, J. Kim, M. Zhou, J. Lai, et al. 2011. IL-17C regulates the innate immune function of epithelial cells in an autocrine manner. *Nat. Immunol.* 12: 1159–1166.
12. Song, X., H. Gao, Y. Lin, Y. Yao, S. Zhu, J. Wang, Y. Liu, X. Yao, G. Meng, N. Shen, et al. 2014. Alterations in the microbiota drive interleukin-17C production from intestinal epithelial cells to promote tumorigenesis. *Immunity* 40: 140–152.
13. Song, X., S. Zhu, P. Shi, Y. Liu, Y. Shi, S. D. Levin, and Y. Qian. 2011. IL-17RE is the functional receptor for IL-17C and mediates mucosal immunity to infection with intestinal pathogens. *Nat. Immunol.* 12: 1151–1158.
14. Johnston, A., Y. Fritz, S. M. Dawes, D. Diaconu, P. M. Al-Attar, A. M. Guzman, C. S. Chen, W. Fu, J. E. Gudjonsson, T. S. McCormick, and N. L. Ward. 2013. Keratinocyte overexpression of IL-17C promotes psoriasiform skin inflammation. *J. Immunol.* 190: 2252–2262.
15. Li, X. 2008. Act1 modulates autoimmunity through its dual functions in CD40L/BAFF and IL-17 signaling. *Cytokine* 41: 105–113.
16. Song, X., and Y. Qian. 2013. The activation and regulation of IL-17 receptor mediated signaling. *Cytokine* 62: 175–182.
17. Dhamija, S., R. Winzen, A. Doerrie, G. Behrens, N. Kuehne, C. Schauerte, E. Neumann, O. Dittrich-Breiholz, M. Kracht, and H. Holtmann. 2013. Interleukin-17 (IL-17) and IL-1 activate translation of overlapping sets of mRNAs, including that of the negative regulator of inflammation, MCPIP1. *J. Biol. Chem.* 288: 19250–19259.
18. Garg, A. V., N. Amatya, K. Chen, J. A. Cruz, P. Grover, N. Whibley, H. R. Conti, G. Hernandez Mir, T. Sirakova, E. C. Childs, et al. 2015. MCPIP1 endoribonuclease activity negatively regulates interleukin-17-mediated signaling and inflammation. *Immunity* 43: 475–487.
19. Jura, J., L. Skalniak, and A. Koj. 2012. Monocyte chemotactic protein-1-induced protein-1 (MCPIP1) is a novel multifunctional modulator of inflammatory reactions. *Biochim. Biophys. Acta* 1823: 1905–1913.
20. Ruiz-Romeu, E., M. Ferran, A. Giménez-Arnau, B. Bugara, B. Lipert, J. Jura, E. F. Florencia, E. P. Prens, A. Celada, R. M. Pujol, and L. F. Santamaria-Babí. 2016. MCPIP1 RNase is aberrantly distributed in psoriatic epidermis and rapidly induced by IL-17A. *J. Invest. Dermatol.* 136: 1599–1607.
21. Sønder, S. U., S. Saret, W. Tang, D. E. Sturdevant, S. F. Porcella, and U. Siebenlist. 2011. IL-17–induced NF-kappaB activation via CIKS/Act1: physiologic significance and signaling mechanisms. *J. Biol. Chem.* 286: 12881–12890.
22. Matsushita, K., O. Takeuchi, D. M. Standley, Y. Kumagai, T. Kawagoe, T. Miyake, T. Satoh, H. Kato, T. Tsujimura, H. Nakamura, and S. Akira. 2009. Zc3h12a is an RNase essential for controlling immune responses by regulating mRNA decay. *Nature* 458: 1185–1190.
23. Mino, T., Y. Murakawa, A. Fukao, A. Vandenbon, H. H. Wessels, D. Ori, T. Uehata, S. Tartey, S. Akira, Y. Suzuki, et al. 2015. Regnase-1 and roquin regulate a common element in inflammatory mRNAs by spatiotemporally distinct mechanisms. *Cell* 161: 1058–1073.
24. Liang, J., J. Wang, Y. Saad, L. Warble, E. Becerra, and P. E. Kolattukudy. 2011. Participation of MCP-induced protein 1 in lipopolysaccharide preconditioning-induced ischemic stroke tolerance by regulating the expression of proinflammatory cytokines. *J. Neuroinflammation* 8: 182.
25. Niu, J., Y. Shi, J. Xue, R. Miao, S. Huang, T. Wang, J. Wu, M. Fu, and Z. H. Wu. 2013. USP10 inhibits genotoxic NF-κB activation by MCPIP1-facilitated deubiquitination of NEMO. *EMBO J.* 32: 3206–3219.
26. Uehata, T., H. Iwasaki, A. Vandenbon, K. Matsushita, E. Hernandez-Cuellar, K. Kuniyoshi, T. Satoh, T. Mino, Y. Suzuki, D. M. Standley, et al. 2013. Malt1-induced cleavage of regnase-1 in CD4(+) helper T cells regulates immune activation. *Cell* 153: 1036–1049.
27. Jeltsch, K. M., D. Hu, S. Brenner, J. Zöller, G. A. Heinz, D. Nagel, K. U. Vogel, N. Rehage, S. C. Warth, S. L. Edelmann, et al. 2014. Cleavage of roquin and regnase-1 by the paracaspase MALT1 releases their cooperatively repressed targets to promote T(H)17 differentiation. *Nat. Immunol.* 15: 1079–1089.
28. Liang, J., Y. Saad, T. Lei, J. Wang, D. Qi, Q. Yang, P. E. Kolattukudy, and M. Fu. 2010. MCP-induced protein 1 deubiquitinates TRAF proteins and negatively regulates JNK and NF-kappaB signaling. *J. Exp. Med.* 207: 2959–2973.
29. Dlugosz, A. A., A. B. Glick, T. Tennenbaum, W. C. Weinberg, and S. H. Yuspa. 1995. Isolation and utilization of epidermal keratinocytes for oncogene research. *Methods Enzymol.* 254: 3–20.
30. Lichti, U., J. Anders, and S. H. Yuspa. 2008. Isolation and short-term culture of primary keratinocytes, hair follicle populations and dermal cells from newborn mice and keratinocytes from adult mice for in vitro analysis and for grafting to immunodeficient mice. *Nat. Protoc.* 3: 799–810.
31. van der Fits, L., S. Mourits, J. S. Voerman, M. Kant, L. Boon, J. D. Laman, F. Cornelissen, A. M. Mus, E. Florencia, E. P. Prens, and E. Lubberts. 2009. Imiquimod-induced psoriasis-like skin inflammation in mice is mediated via the IL-23/IL-17 axis. *J. Immunol.* 182: 5836–5845.
32. Ha, H. L., H. Wang, P. Pisitkun, J. C. Kim, I. Tassi, W. Tang, M. I. Morasso, M. C. Udey, and U. Siebenlist. 2014. IL-17 drives psoriatic inflammation via distinct, target cell-specific mechanisms. *Proc. Natl. Acad. Sci. USA* 111: E3422–E3431.
33. Wohn, C. T., S. Pantelyushin, J. L. Ober-Blöbaum, and B. E. Clausen. 2014. Aldara-induced psoriasis-like skin inflammation: isolation and characterization of cutaneous dendritic cells and innate lymphocytes. *Methods Mol. Biol.* 1193: 171–185.
34. Yang, C., S. Huang, X. Wang, and Y. Gu. 2015. Emerging roles of CCCH-type zinc finger proteins in destabilizing mRNA encoding inflammatory factors and regulating immune responses. *Crit. Rev. Eukaryot. Gene Expr.* 25: 77–89.
35. Pantelyushin, S., S. Haak, B. Ingold, P. Kulig, F. L. Heppner, A. A. Navarini, and B. Becher. 2012. Rorγt+ innate lymphocytes and γδ T cells initiate psoriasiform plaque formation in mice. *J. Clin. Invest.* 122: 2252–2256.
36. Cai, Y., X. Shen, C. Ding, C. Qi, K. Li, X. Li, V. R. Jala, H. G. Zhang, T. Wang, J. Zheng, and J. Yan. 2011. Pivotal role of dermal IL-17–producing γδ T cells in skin inflammation. *Immunity* 35: 596–610.
37. Nagata, T., L. McKinley, J. J. Peschon, J. F. Alcorn, S. J. Aujla, and J. K. Kolls. 2008. Requirement of IL-17RA in Con A induced hepatitis and negative regulation of IL-17 production in mouse T cells. *J. Immunol.* 181: 7473–7479.
38. Kumar, P., L. Monin, P. Castillo, W. Elsegeiny, W. Horne, T. Eddens, A. Vikram, M. Good, A. A. Schoenborn, K. Bibby, et al. 2016. Intestinal interleukin-17 receptor signaling mediates reciprocal control of the gut microbiota and autoimmune inflammation. *Immunity* 44: 659–671.
39. Johansen, C., P. A. Usher, R. B. Kjellerup, D. Lundsgaard, L. Iversen, and K. Kragballe. 2009. Characterization of the interleukin-17 isoforms and receptors in lesional psoriatic skin. *Br. J. Dermatol.* 160: 319–324.
40. Teunissen, M. B., C. W. Koomen, R. de Waal Malefyt, E. A. Wierenga, and J. D. Bos. 1998. Interleukin-17 and interferon-gamma synergize in the enhancement of proinflammatory cytokine production by human keratinocytes. *J. Invest. Dermatol.* 111: 645–649.
41. Chiricozzi, A., K. E. Nograles, L. M. Johnson-Huang, J. Fuentes-Duculan, I. Cardinale, K. M. Bonifacio, N. Gulati, H. Mitsui, E. Guttman-Yassky, M. Suárez-Fariñas, and J. G. Krueger. 2014. IL-17 induces an expanded range of downstream genes in reconstituted human epidermis model. *PLoS One* 9: e90284.
42. Wilson, N. J., K. Boniface, J. R. Chan, B. S. McKenzie, W. M. Blumenschein, J. D. Mattson, B. Basham, K. Smith, T. Chen, F. Morel, et al. 2007. Development,

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cytokine profile and function of human interleukin 17-producing helper T cells. *Nat. Immunol.* 8: 950–957.
43. Liang, S. C., X. Y. Tan, D. P. Luxenberg, R. Karim, K. Dunussi-Joannopoulos, M. Collins, and L. A. Fouser. 2006. Interleukin (IL)-22 and IL-17 are coexpressed by Th17 cells and cooperatively enhance expression of antimicrobial peptides. *J. Exp. Med.* 203: 2271–2279.
44. Nograles, K. E., L. C. Zaba, E. Guttman-Yassky, J. Fuentes-Duculan, M. Suárez-Fariñas, I. Cardinale, A. Khatcherian, J. Gonzalez, K. C. Pierson, T. R. White, et al. 2008. Th17 cytokines interleukin (IL)-17 and IL-22 modulate distinct inflammatory and keratinocyte-response pathways. *Br. J. Dermatol.* 159: 1092–1102.
45. Hüffmeier, U., S. Uebe, A. B. Ekici, J. Bowes, E. Giardina, E. Korendowych, K. Juneblad, M. Apel, R. McManus, P. Ho, et al. 2010. Common variants at TRAF3IP2 are associated with susceptibility to psoriatic arthritis and psoriasis. *Nat. Genet.* 42: 996–999.
46. Ellinghaus, E., D. Ellinghaus, P. E. Stuart, R. P. Nair, S. Debrus, J. V. Raelson, M. Belouchi, H. Fournier, C. Reinhard, J. Ding, et al. 2010. Genome-wide association study identifies a psoriasis susceptibility locus at TRAF3IP2. *Nat. Genet.* 42: 991–995.
47. Cargill, M., S. J. Schrodi, M. Chang, V. E. Garcia, R. Brandon, K. P. Callis, N. Matsunami, K. G. Ardlie, D. Civello, J. J. Catanese, et al. 2007. A large-scale genetic association study confirms IL12B and leads to the identification of IL23R as psoriasis-risk genes. *Am. J. Hum. Genet.* 80: 273–290.
48. Tsoi, L. C., S. L. Spain, J. Knight, E. Ellinghaus, P. E. Stuart, F. Capon, J. Ding, Y. Li, T. Tejasvi, J. E. Gudjonsson, et al; Collaborative Association Study of Psoriasis (CASP); Genetic Analysis of Psoriasis Consortium; Psoriasis Association Genetics Extension; Wellcome Trust Case Control Consortium 2. 2012. Identification of 15 new psoriasis susceptibility loci highlights the role of innate immunity. *Nat. Genet.* 44: 1341–1348.
49. Sønder, S. U., A. Paun, H. L. Ha, P. F. Johnson, and U. Siebenlist. 2012. CIKS/Act1-mediated signaling by IL-17 cytokines in context: implications for how a CIKS gene variant may predispose to psoriasis. *J. Immunol.* 188: 5906–5914.
50. Durham, L. E., B. W. Kirkham, and L. S. Taams. 2015. Contribution of the IL-17 pathway to psoriasis and psoriatic arthritis. *Curr. Rheumatol. Rep.* 17: 55.
51. Sanford, M., and K. McKeage. 2015. Secukinumab: first global approval. *Drugs* 75: 329–338.
52. Leonardi, C., R. Matheson, C. Zachariae, G. Cameron, L. Li, E. Edson-Heredia, D. Braun, and S. Banerjee. 2012. Anti-interleukin-17 monoclonal antibody ixekizumab in chronic plaque psoriasis. *N. Engl. J. Med.* 366: 1190–1199.
53. Baeten, D., J. Sieper, J. Braun, X. Baraliakos, M. Dougados, P. Emery, A. Deodhar, B. Porter, R. Martin, M. Andersson, et al; MEASURE 1 Study Group; MEASURE 2 Study Group. 2015. Secukinumab, an interleukin-17A inhibitor, in ankylosing spondylitis. *N. Engl. J. Med.* 373: 2534–2548.
54. Zhu, S., W. Pan, P. Shi, H. Gao, F. Zhao, X. Song, Y. Liu, L. Zhao, X. Li, Y. Shi, and Y. Qian. 2010. Modulation of experimental autoimmune encephalomyelitis through TRAF3-mediated suppression of interleukin 17 receptor signaling. *J. Exp. Med.* 207: 2647–2662.
55. Zepp, J. A., C. Liu, W. Qian, L. Wu, M. F. Gulen, Z. Kang, and X. Li. 2012. Cutting edge: TNF receptor-associated factor 4 restricts IL-17-mediated pathology and signaling processes. *J. Immunol.* 189: 33–37.
56. Garg, A. V., M. Ahmed, A. N. Vallejo, A. Ma, and S. L. Gaffen. 2013. The deubiquitinase A20 mediates feedback inhibition of interleukin-17 receptor signaling. *Sci. Signal.* 6: ra44.
57. Shen, F., N. Li, P. Gade, D. V. Kalvakolanu, T. Weibley, B. Doble, J. R. Woodgett, T. D. Wood, and S. L. Gaffen. 2009. IL-17 receptor signaling inhibits C/EBPbeta by sequential phosphorylation of the regulatory 2 domain. *Sci. Signal.* 2: ra8.
58. Somma, D., P. Mastrovito, M. Grieco, A. Lavorgna, A. Pignalosa, L. Formisano, A. M. Salzano, A. Scaloni, F. Pacifico, U. Siebenlist, and A. Leonardi. 2015. CIKS/DDX3X interaction controls the stability of the Zc3h12a mRNA induced by IL-17. *J. Immunol.* 194: 3286–3294.
59. Ippagunta, S. K., R. Gangwar, D. Finkelstein, P. Vogel, S. Pelletier, S. Gingras, V. Redecke, and H. Häcker. 2016. Keratinocytes contribute intrinsically to psoriasis upon loss of Tnip1 function. *Proc. Natl. Acad. Sci. USA* 113: E6162–E6171.
60. Carpenter, S., E. P. Ricci, B. C. Mercier, M. J. Moore, and K. A. Fitzgerald. 2014. Post-transcriptional regulation of gene expression in innate immunity. *Nat. Rev. Immunol.* 14: 361–376.
61. Harden, J. L., J. G. Krueger, and A. M. Bowcock. 2015. The immunogenetics of psoriasis: a comprehensive review. *J. Autoimmun.* 64: 66–73.
62. Cifuentes, R. A., P. Cruz-Tapias, A. Rojas-Villarraga, and J. M. Anaya. 2010. ZC3H12A (MCPIP1): molecular characteristics and clinical implications. *Clin. Chim. Acta* 411: 1862–1868.
63. Munir, S., S. ber Rahman, S. Rehman, N. Saba, W. Ahmad, S. Nilsson, K. Mazhar, and Å. T. Naluai. 2015. Association analysis of GWAS and candidate gene loci in a Pakistani population with psoriasis. *Mol. Immunol.* 64: 190–194.
64. Liu, L., Z. Zhou, S. Huang, Y. Guo, Y. Fan, J. Zhang, J. Zhang, M. Fu, and Y. E. Chen. 2013. Zc3h12c inhibits vascular inflammation by repressing NF-κB activation and pro-inflammatory gene expression in endothelial cells. *Biochem. J.* 451: 55–60.
65. Liang, J., J. Wang, A. Azfer, W. Song, G. Tromp, P. E. Kolattukudy, and M. Fu. 2008. A novel CCCH-zinc finger protein family regulates proinflammatory activation of macrophages. *J. Biol. Chem.* 283: 6337–6346.
66. Tian, S., J. G. Krueger, K. Li, A. Jabbari, C. Brodmerkel, M. A. Lowes, and M. Suárez-Fariñas. 2012. Meta-analysis derived (MAD) transcriptome of psoriasis defines the "core" pathogenesis of disease. *PLoS One* 7: e44274.
67. Ma, A., and B. A. Malynn. 2012. A20: linking a complex regulator of ubiquitylation to immunity and human disease. *Nat. Rev. Immunol.* 12: 774–785.
68. Lubberts, E., P. Schwarzenberger, W. Huang, J. R. Schurr, J. J. Peschon, W. B. van den Berg, and J. K. Kolls. 2005. Requirement of IL-17 receptor signaling in radiation-resistant cells in the joint for full progression of destructive synovitis. *J. Immunol.* 175: 3360–3368.

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