IL-20-Receptor Signaling Delimits IL-17 Production in Psoriatic Inflammation

Hye-Lin Ha¹, Hongshan Wang¹, Estefania Claudio¹, Wanhu Tang¹ and Ulrich Siebenlist¹

IL-17 cytokines, in particular IL-17A, are critical effectors in psoriasis. Antibodies that block IL-17A are highly efficacious in treating psoriasis. Likewise, disruption of IL-17 cytokines signaling, such as via the loss of the adaptor CIKS/Act1, ameliorates inflammation in mouse models of psoriasis. IL-17A promotes a cascade of effects, including the robust production of IL-19 in both humans and mice. IL-19, along with IL-20 and IL-24, signal via IL-20 receptors and comprise a subgroup within the IL-10 cytokine family. The role of these three cytokines in psoriasis is unresolved. They have been linked to inflammatory processes, including psoriatic pathology, but these cytokines have also been reported to suppress inflammation in other contexts. In this study, we demonstrate that signaling via IL-20 receptors, including in response to IL-19, delimited aspects of imiquimod-induced psoriatic inflammation. IL-20 receptor signaling suppressed the dermal production of the CCL2 chemokine and thereby reduced CCL-2-driven infiltration of inflammatory cells into the dermis, including IL-17A-producing γδT cells. This constitutes a negative feedback, since IL-17A strongly induces IL-19 in keratinocytes. The effects of IL-17 cytokines in this inflammatory setting are dynamic; they are central to the development of both dermal and epidermal hallmarks of psoriasis but also initiate a path to mitigate inflammatory damage.

INTRODUCTION

Psoriasis is a common chronic inflammatory skin disease characterized by thickening of the epidermis and scaling caused by the abnormal proliferation and differentiation of keratinocytes, as well as by microvascular changes and infiltration of leukocytes into the epidermis and especially the dermis. Although underlying mechanisms are still incompletely understood, the IL-23/IL-17A pathway plays a key role in pathogenesis (Boehncke and Schon, 2015). Importantly, monoclonal antibodies targeting IL-17A or its receptor are highly efficacious in treating psoriasis (Patel and Kuchroo, 2015). IL-17A (also known as IL-17) is the signature cytokine of the IL-17 family (IL-17A–F). The increased expression of IL-17A has been linked not only to psoriasis but also other inflammatory diseases (Miossec and Kolls, 2012). Dermal γδT cells are the primary producers of IL-17A in several acute mouse models of psoriasis (Cai et al., 2011; Ha et al., 2014), while dermal tissue-resident memory CD4⁺ T cells are the primary source of IL-17A in the chronic disease in humans (Matos et al., 2017). However, numbers of dermal γδT cells are also increased in human psoriatic lesions and may be critical during the initiation of the disease (Cai et al., 2011; Papotto et al., 2018). Regardless of the cellular source, IL-17A is the critical cytokine driving psoriatic inflammation in humans and in the IMQ mouse model. In addition to IL-17A, IL-17C and IL-17E may also contribute to human psoriasis and mouse models. They function downstream of IL-17A and are produced by and act on keratinocytes, with IL-17C, in particular, targeting genes largely overlapping with those of IL-17A (Ramirez-Carrozzi et al., 2011; Xu et al., 2018).

All members of the IL-17 family signal via heteromeric receptors composed of members of the IL-17 receptor family (RA–RE) (Monin and Gaffen, 2018). Upon ligand engagement, the adaptor CIKS (also known as Act1, Traf3ip2) is recruited for signal transmission; consequently, CIKS is critical for IL-17 cytokines-induced pathology in mouse models psoriasis, collagen-induced arthritis, lupus, and asthma (Claudio et al., 2009; Ha et al., 2014; Pisitkun et al., 2010; Pisitkun et al., 2012).

IL-19, IL-20, and IL-24 comprise a subgroup of cytokines within the IL-10 superfamily (Ouyang et al., 2011). All three cytokines signal via a receptor composed of IL-20RB and IL-20RA (IL-20R type1), while IL-20 and IL-24 can additionally signal via IL-20R type2 (IL-20RB and IL-22RA1). IL-19, IL-20, and IL-24 are produced primarily by epithelial, but also other cell populations, while their receptors are found primarily, but not exclusively, on non-hematopoietic cells. All three cytokines have been linked to psoriasis. Expression of IL-19 is highly up-regulated in human psoriatic skin lesions and has been associated with pathology in this context. This concept is largely based on in vitro studies in which IL-19, having negligible effects on its own, enhanced some effects of IL-17 on keratinocyte cultures but did not alter the proliferation, differentiation, or migration of these cells (Bissonnette et al., 2017; Witte et al., 2014). It remains to be established whether the IL-19/IL-20 receptor signaling is part of the pathogenic
IL-17 induces IL-19 in keratinocytes. (a) Relative mRNA expression of IL-20 family cytokines in the epidermis of IMQ- or control-treated ears from WT and CIKS ΔKC [K5-crtcTol313p2−/−] mice. (b) Relative mRNA expression of IL-19 in primary keratinocytes from WT and CIKS KO mice, HaCaT cells and WT ear explants stimulated with IL-17A for 6 hours (left, middle and right panels, respectively). *P < 0.05; mean ± SEM; n = 5-8, except n = 3 for primary CIKS KO keratinocytes. IMQ, imiquimod; WT, wild-type.

RESULTS

Loss of IL-19 does not ameliorate IMQ-induced psoriatic inflammation

We confirmed that IL-19 was highly induced in IMQ-induced psoriatic inflammation and that its expression was largely dependent on IL-17 cytokines signaling in keratinocytes. Strongly increased levels of IL-19 were noted in the epidermal ear layer (Figure 1a; left panel) of wild-type (WT), but not mutant mice specifically lacking CIKS in keratinocytes (CIKS ΔKC) (Ha et al., 2014). IL-24 was less prominently induced than IL-19 and trended slightly lower in the CIKS ΔKC mutant mice, while the expression of IL-20 was barely detectable (Figure 1a; middle, right panels). Similar results were obtained with dorsal whole skin samples (Supplementary Figure S1a). Prior data in human patients suggested keratinocytes to be the primary source of IL-19 (Steiniche et al., 2003). We found that IL-17A directly induced IL-19, as seen with primary keratinocyte cultures, the human keratinocyte cell line, HaCaT, and with mouse ear skin tissue explants (Figure 1b; left, middle and right panels, respectively).

To investigate the role of IL-19 in the IMQ model, we utilized IL-19-deficient (KO) mice. Following IMQ treatment, both WT and IL-19 KO mice developed psoriasis-like pathology, including epidermal thickening and infiltration of the immune cells into the skin; KO phenotypes tended to be slightly worse (Figure 2a). We noted a mild, albeit not significant, increase in the numbers of dermal γδT and monocytic cells in the skin of IMQ-treated IL-19 KO relative to WT mice (Figure 2b). IL-19 was therefore not required to execute pathologic effects of IL-17 in this model and may have had a mildly protective effect instead. IL-24 expression trended higher in IL-19 KO vs WT mice, which may have had a compensatory effect (Supplementary Figure S1b); this prompted us to investigate the combined action of these cytokines.

Exacerbated IMQ-induced psoriatic inflammation in IL-20 receptors-deficient mice

To delineate the combined contributions of IL-19, IL-20, and IL-24, we utilized IL-20RB-deficient mice (IL-20RB KO), which lack IL-20 receptors. Visual inspection of IMQ-treated WT and IL-20RB KO mice did not show obvious gross differences in psoriasis-like dorsal skin pathologies, but analysis of the sections revealed that the IL-20RB KO mice exhibited a notably increased thickening of the epidermis (with acanthosis and hyperkeratosis) (Figure 3a; consistent with increased proliferation in basal keratinocytes [Supplementary...
IL-17A, the IMQ-induced expression of IL-17A in WT mice (Supplementary Figure S2d and e). We also increased in the sDLNs of IL-20RB KO compared with WT mice (Figure 3b). Similarly, we observed more neutrophils and c). IL-12b mRNAs and IL-17A accumulation of dermal IL-17A in WT-19 KO mice (Supplementary Figure S2a). IL-20RB receptor signaling thus partially ameliorated the epidermal psoriatic phenotype.

To elucidate the mechanisms by which IL-19/IL-20RB limits the accumulation of IL-17A+ γδT cells in mice deficient in CCR2, we confirmed that CCR2 was highly expressed on dermal γδT cells and that IMQ failed to induce the accumulation of IL-17A+ γδT cells in mice deficient in CCR2 (Supplementary Figure S3a and b).

IL-19 limits the recruitment of IL-17A-producing dermal γδT cells
To assess whether IL-19/IL-20RB signaling could cap the accumulation of dermal IL-17A+ γδT cells, we injected IL-19 intradermally into the IMQ-treated ears of CIKS ΔKC mice. As shown, the IMQ treatment of CIKS ΔKC mice largely failed to generate IL-17, as IL-17 signaling into keratinocytes was blocked; furthermore, these mutant mice exhibited increased cellular infiltration, of IL-17A+ γδT cells, in particular (Ha et al., 2014). The injection of CIKS ΔKC mice with IL-19 significantly reduced the total numbers of IL-17A+ γδT cells in skin, along with other immune cell infiltrates (Figure 4a). Remarkably, IL-19 injection of the mutants led to an increase of IL-17A+ γδT cells in ear sDLNs in particular (Figure 4b).

This suggested that IL-19 curtailed the recruitment of IL-17A+ γδT cells from DLNs into skin following the IMQ treatments. To confirm, we administered FTY720, which blocks egress from the lymph nodes. In agreement with a prior report (Ramirez-Valle et al., 2015), FTY720 interfered with the IMQ-induced rise in IL-17A+ γδT cells in dorsal skin (Figure 4c), while at the same time retaining and increasing the numbers of these cells in sDLNs, along with other cells (Figure 4d). Ramirez-valle et al (2015) additionally posited that the IMQ-induced migration of IL-17A+ γδT cells from sDLNs to the skin was dependent on the chemokine receptor CCR2. We confirmed that CCR2 was highly expressed on dermal γδT cells and that IMQ failed to induce the accumulation of IL-17A+ γδT cells in mice deficient in CCR2 (Supplementary Figure S3a and b).

IL-19/IL-20RB limits the accumulation of IL-17A+ γδT cells via reduction of CCL2
To elucidate the mechanisms by which IL-19/IL-20RB signaling caps the accumulation of IL-17A+ γδT cells, we focused on CCL2, the primary CCR2 ligand. CCL2 mRNA expression was significantly higher in the dorsal skin of IMQ-treated CIKS ΔKC and IL-20RB KO compared with WT mice and trended higher in IL-19 KO mice (Figure 5a). Nearly all CCL2 expression in IL-20RB KO mice occurred in the dermal layer of ear skin (Figure 5b).

To confirm that CCL2 was essential for the increased accumulation of dermal IL-17A+ γδT cells in IMQ-treated CIKS ΔKC mice, we administered neutralizing antibodies to CCL2. Intradermal α-CCL2 injections in the ears of CIKS ΔKC mice led to significantly lower cellular infiltration into skin, including IL-17A+ γδT cells (Figure 5c). Simultaneously, α-CCL2 injections increased the numbers of IL-17A+ γδT cells in ear sDLNs, similar to IL-19 injections (Figure 5d). Thus, IL-19/IL-20RB dampened the IMQ-induced influx of IL-17A+ γδT from sDLNs into skin, at least in part by reducing CCL2 expression.

We investigated CCL2 and IL-19 expression during the course of the IMQ treatments of WT mice. CCL2 mRNA was
strongly induced in dorsal skin by 24 hours after the first IMQ application, but expression began to return to the baseline with subsequent applications. In contrast, IL-19 mRNA induction was delayed, peaking at day 3 of treatment; it gradually fell thereafter, remaining above pretreatment levels at the end of IMQ treatments. These results are consistent with the concept that IL-17-induced IL-19 dampened the induction of CCL2 after its rapid initial rise (Figure 6a).

**Figure 3. Exacerbated psoriatic inflammation in IL-20RB KO mice, including a rise in IL-17A^+γδT cells.**

(a) Representative hematoxylin and eosin-stained dorsal sections of IMQ- or control-treated WT and IL-20RB KO mice. Epidermal thickening was quantitated via measurement of the epidermal area from sections. (b, c) Representative flow cytometric analyses of IMQ- or control-treated dorsal skin cells from WT and IL-20RB KO mice analyzed for expression of markers as shown within the CD45^+ gate. (b) Numbers and percentages of the IL-17A^+γδT cells and (c) neutrophils generated from flow cytometric analyses. *P < 0.05; mean ± SEM; n = 8-12 mice per group. IMQ, imiquimod; WT, wild-type. Scale bar = 100 μm.
Measurements for the CCL2 protein in dorsal skin confirmed the early rise and subsequent decline (Figure 6b). CCL2 was primarily produced in the dermis, measured at peak times (Figure 6c). As noted above, the expression of CCL2 persisted in IL-20RB KO mice, and originated primarily in the dermis, assessed at the end of treatments.

Based on these findings, we investigated the effects of IL-19 and IL-24 on the expression of CCL2 in primary dermal fibroblasts, the primary cells in the dermis. CCL2 was induced upon stimulation with IFNγ and tumor necrosis factor-α; inflammatory cytokines also present in psoriatic inflammation, and the addition of IL-19 or IL-24 significantly reduced this induction, dependent on the presence of IL-20RB (Figure 6d). This suggests one mechanism by which IL-19 and IL-24 may impair CCL2 expression.

**DISCUSSION**

This study revealed that IMQ-induced psoriatic inflammation was not ameliorated in IL-19-deficient mice and instead, slightly worsened it. This finding calls into question the oft-surmised role of IL-19 as a critical downstream executioner of IL-17A-induced psoriatic pathologies. The most direct evidence for such a role stemmed from in vitro stimulations of keratinocyte cultures and explants, in which IL-19, though largely unable to elicit responses by itself, augmented some effects of IL-17A (Witte et al., 2014). However, whether the augmentation of IL-17A-mediated induction of, for example, anti-microbial proteins by IL-19 noted in the in vitro cultures translates to a pathologic role in psoriatic inflammation in vivo was not addressed. Our data indicate that IL-19 is likely to have a much more nuanced function in psoriatic inflammation, as we demonstrated an anti-inflammatory function. Injection of this cytokine dampened the excessive infiltration of leukocytes, including IL-17A+ γδT cells, into the skin seen in CIKS ΔKC mice. IL-17 cytokines cannot signal into keratinocytes in these mutant mice, and consequently, IMQ-induced IL-19 production is largely blunted. As shown previously, epidermal pathology was notably ameliorated in these mutants but not so in infiltration of leukocytes into the dermis, which instead was exacerbated compared with the WT mice (Ha et al., 2014). As documented here, intradermal injection of these CIKS ΔKC mice with IL-19 largely reversed excessive cellular infiltration, thereby delimiting the production of IL-17A by infiltrating dermal γδT cells. Since IL-19 is produced by keratinocytes in response to IL-17A, IL-19 functioned as a negative feedback regulator of IL-17A. Furthermore, the excessive leukocyte infiltration and IL-17A production observed in IMQ-treated CIKS ΔKC mice was thus owing, at least in part, to the severe drop in IL-19 in these mutant mice.

Based on these findings, it is not apparent why IL-19 deficient mice did not exhibit exacerbated dermal
infiltration in the IMQ model, including increased IL-17A production. This contrasts with CIKS Δ KC mice, in which the loss of IL-17-induced IL-19 exacerbated and intradermal injection of IL-19 reversed these pathologic phenotypes. Possibly, mice lacking IL-19 may have partially compensated the loss via IL-24 (IL-20 was barely detectable). This concept is supported by the exacerbated psoriatic pathology observed in mice deficient in IL-20 receptors (IL-20RB KO), the receptors required for signaling by all three cytokines. In these mutants, IMQ treatments resulted not only in increased leukocyte infiltration, including IL-17A-producing γδT cells and neutrophils, but also increased epidermal thickening. Furthermore, IL-24 was induced and trended higher in IMQ-treated IL-19 KO compared with the WT mice; it also suppressed CCL2 expression, similar to IL-19 (see further discussion below). Therefore, the combined action of these IL-20RB ligands exerted an overall anti-inflammatory effect, ameliorating several pathologic phenotypes associated with psoriatic inflammation, in distinction with the view that these cytokines help drive inflammation. It is important to note that signaling via IL-20 receptors, including in response to the highly induced IL-19, did not prevent IMQ-induced pathology but instead delimited the extent of inflammation. Future research will need to address which receptors and cell types mediate the effects of the IL-20RB ligands and whether they cross-regulate each other. 

Our findings demonstrate that IL-19/IL20RB signaling restrained cellular infiltration, at least in part by suppressing CCL2 in the skin. CCL2 is the primary ligand for CCR2. It attracts monocytes and neutrophils and is critical for dermal γδT cell recruitment into the skin from sDLNs under inflammatory conditions. This stands in contrast to homeostatic conditions, where the CCL20/CCR6 pathway is primarily responsible for recruitment (McKenzie et al., 2017; Ramírez-Valle et al., 2015). (CCL20 expression was not altered in IMQ-treated CIKS Δ KC vs WT [data not shown]). Importantly, CCL2 expression was notably elevated in CIKS Δ KC and IL-20RB KO mice and trended higher in IL-19 KO mice. Intradermal administration of IL-19 or CCL2-neutralizing antibodies reduced overall leukocyte infiltration in CIKS Δ KC mice, including IL-17A+ γδT cells. FTY720 more specifically prevented the infiltration of the latter cells in WT mice; they are stored in lymph nodes, unable to egress upon FTY treatment, while monocytes and neutrophils can readily enter from the circulation. Additional lines of investigation support the view that IL-19/IL20RB can limit the production of CCL2. CCL2 was rapidly induced in WT mice upon initial exposure to IMQ, but expression waned with subsequent exposures, coincident with the delayed rise in IL-19; expression of IL-19 fell only modestly thereafter and remained above the baseline. In contrast, CCL2 expression was sustained in IL-20RB KO mice at the end of the IMQ treatments, consistent with the loss of IL-19 signaling. We also noted that early IMQ-induced CCL2 production in WT mice was particularly evident in the dermis, as was sustained expression in IL-20RB KO mice.
are the most abundant CD45- cell type in the dermis, and we estimated the total dermal cells (data not shown). Fibroblasts also contributed, although they constitute only a small fraction of the total dermal cells. CCL2 induced by tumor necrosis factor-α was also present in psoriatic inflammation, and this may be one mechanism by which IL-19 (and IL-24) signaling via IL20-RB restricts the expression of CCL2, although additional mechanisms may exist.

Our findings show that IL-19/IL-20RB signaling does not function as a mere mediator of pathologic consequences to IL-17A in skin but has more nuanced roles, including specific anti-inflammatory effects, thus imposing a cap on IL-17A production in the IMQ model. The role of IL-20 receptors-signaling in skin is thus more in line with constraining, rather than eliminating psoriatic inflammation, and helping to promote barrier defenses. Whether such a role could also have long-term detrimental consequences in chronic psoriasis remains an open question. IL-20 receptors-mediated signals are integral to the communication between epidermal, stromal and immune cells in skin.

**MATERIALS & METHODS**

**Mice**

Mice strains used: [Il20rb-/-] (Zheng et al., 2008), [Il19-/-] (gifts from Genentech, S. San Francisco, CA), [Traf3ip2-/-] and [Traf3ip2-/-] (Pisitkun et al., 2012), [K5-cre; Traf3ip2-/-] (CIKS ΔKC) (Ha et al., 2014), and [Ccr2-/-] (Jackson Laboratories, Bar Harbor, ME) (all C57BL/6). Eight- to ten-week-old mice with littermate controls were used. All the mice were bred and housed in a NIAID facility, and all the experiments were performed with the approval of the NIAID Animal Care and Use Committee and in accordance with all relevant institutional guidelines.

**Experimentally induced psoriatic inflammation**

Aldara cream containing 5% imiquimod was applied to shaved dorsal skin or to ears for five consecutive days as described (Ha et al., 2014; van der Fits et al., 2009). Mice were harvested on day 6. In some experiments, mice were intradermally injected with IL-19 (1 μg/ml or phosphate-buffered saline) or with α-CCL2 (10 μg/ml or control IgG) on days 3–5 just prior to IMQ applications (reagents from R&D Systems, Minneapolis, MN; FTY720 (Cayman Chemical, Ann Arbor, MI; 1 mg/kg) was injected intraperitoneally every other day.

**Cellular analysis**

Lymph node cells were mechanically dissociated to obtain single-cell suspensions (Pisitkun et al., 2010) for the separation of the dermal and epidermal layers, the ears were split into dorsal and ventral halves; cartilage and fat was removed, and the halves were floated dermal side down in a 0.5 M ammonium thiocyanate solution (Sigma, St. Louis, MO), incubated at 37 °C for 20 minutes, washed in phosphate-buffered saline, and then the layers were separated with forceps. Single-cell suspensions from the dorsal skin were prepared as described (Ha et al., 2014) and stained with Aqua (Invitrogen, Carlsbad, CA) and antibodies against one or more of the following proteins: IL-17A, Ly6C(AL 21; BD Biosciences, San Jose, CA); TCRyβ(UCH-7-13D5), IL-17A(eBio1B78), and IL-17F(eBio18F10); eBioscience, San Diego, CA); Ly6G (I8D), CD45.2(104), TCRyβ(GL3), CD11b(M1/70), TCRvγ4(UC3-10A6), MHCIIM5/114.15.2) and CD64(X54-5/7.1; Biolegend, San Diego, CA); CCR2

![Figure 6. IL-19 downregulates the expression of CCL2 in dermal fibroblasts.](image-url)
Histology, tissue analysis

Mouse dorsal skin tissues were fixed in 4% formaldehyde, stained with hematoxylin and eosin and visualized with an Olympus BX50. Epidermal areas were quantified on hematoxylin and eosin-stained slides from multiple mice with ImageJ software (NIH, Bethesda, MD). Sections were prepared for immunofluorescence as described (Ha et al., 2014) and stained with primary antibodies against K5 (1:100; Lifespan Biosciences, Seattle, WA) and Ki67 (1:100; BD Pharmingen, San Diego, CA). Secondary antibodies were labeled with guinea pig IgG (Alexa Fluor 546) and mouse IgG (Alexa Fluor 488:1:1000; Molecular Probes, Eugene OR). Slides were mounted with Vectashield without DAPI (Vector Labs, Burlingame, CA) and visualized with a Leica AF6000LX fluorescence microscope (Leica, Wetzlar, Germany). To prepare protein extracts from the skin, frozen tissue sections were homogenized with a protease inhibitor cocktail (Roche, Basel, Switzerland) in phosphate-buffered saline; extracts were analyzed for CCL2 with an ELISA assay kit (R&D Systems).

In vitro cultures

Keratinocytes and fibroblasts were isolated from neonatal mice and their culture conditions as described (Ha et al., 2014). HaCaT cells were obtained from Dr. Maria Morasso (NIH). For ear explants, the ears were washed with betadine solution, split to remove the cartilage and fat, and then the tissue was placed dermal side down in complete culture media in the presence or absence of cytokine overnight. Cultures were stimulated as indicated with one or more of the following cytokines: tumor necrosis factor-α (20 ng/ml), IFNγ (10 ng/ml; PeproTech, Rocky Hill, NJ); IL-17A (100 ng/ml), IL-19 (100 ng/ml; R&D Systems).

Quantitative real-time PCR

RNA was purified using TRIzol (Invitrogen) and an RNeasy kit (Qiagen, Valencia, CA); cDNA was generated with a cDNA synthesis kit (Qiagen), and quantitative real-time PCR was performed (Taqman protocol). The mouse primers for Gapdh, Il19, Il20, Il24, Ccl2, Il1a, Il1b, Il17a, Il17f, Il22, and Cxcl1 were obtained from Applied Biosystems (Foster City, CA). All the values were normalized to Gapdh.

Statistical analyses

All data are presented as the mean ± SEM. Student’s t test (two-tailed) was used to evaluate significance; P values < 0.05 were considered to be statistically significant and P values < 0.01 highly significant.

Data availability statement

No datasets were generated or analyzed during the current study.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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AUTHOR CONTRIBUTIONS

Conceptualization: HH, US; Investigation: HH, HW, EC, WT; Supervision: US; Writing - Original Draft Preparation: HH, US; Writing - Review and Editing: HH, EC, and US.

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(R&D Systems). For intracellular staining, cells were treated with cell stimulation cocktail (plus protein transport inhibitors; eBioscience) for 4 hours. Data were collected with FACSCanto and FACSCelesta (BD Biosciences) and analyzed using FlowJo (Tree Star, Ashland, OR).

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SUPPLEMENTARY MATERIALS

Supplementary material is linked to the online version of the paper at www.jidonline.org, and at https://doi.org/10.1016/j.jid.2019.06.127.


Ramirez-Valle F, Gray EE, Cyster JG. Inflammation induces dermal Vγ4+γδT17 memory-like cells that travel to distant skin and accelerate secondary IL-17-driven responses. Proc Natl Acad Sci U S A 2015;112:8046–51.


Supplementary Figure S1. mRNA levels of IL-20 and IL-1 family members. (a, b) Relative mRNA expression for the indicated genes in the dorsal skin of (a) WT, CIKS ΔKC and (b) IL-19 KO mice after IMQ or control treatments as indicated. *P < 0.05; mean ± SEM; n = 6 mice per group. IMQ, imiquimod; n.s, not significant; WT, wild-type.
Supplementary Figure S2. IMQ-induced epidermal proliferation, and levels of inflammatory gene mRNAs and inflammatory cells in wild-type and IL-20RB KO mice. (a) Immunofluorescence images of sections from IMQ- or control-treated dorsal skin from WT and IL-20RB KO mice stained with DAPI (blue), anti-K5 (red) and anti-Ki67 (green). Epidermal Ki67^+ cells were quantitated by counting fluorescent dots in 200×200 μm^2 areas from different sections per mouse (n=6-10 mice per group; Scale bar = 100 μm). (b, c) Relative mRNA expression for the indicated genes in the (b) dorsal skin and (c) skin-draining lymph nodes of IMQ- or control-treated WT and IL-20RB KO mice (n=8-12 mice per group). (d, e) Representative flow cytometric analyses of IMQ- or control-treated skin-draining lymph nodes cells from WT and IL-20RB KO mice analyzed for the expression of the markers as shown. Numbers and percentages of (d) IL-17A^+ γδ T cells and (e) neutrophils generated from flow cytometric analyses. *P < 0.05, **P < 0.01; mean ± SEM; n= 6-12 mice per group. IMQ, imiquimod; n.s, not significant; WT, wild-type.
Supplementary Figure S3. CCR2 expression and IMQ-induced levels of IL-17^+ TCRγδ T cells in wild-type and CCR2 KO mice. (a) CCR2 expression on DETC (TCRγδ^hi), dermal γδT cells (CD45^+, TCRγδ_intermediate, TCRγδ^4^+) from IMQ-treated WT and CCR2 KO mice. (b) Representative flow cytometric analyses of IMQ- or control-treated dorsal skin cells from WT and CCR2 KO mice analyzed for expression of markers as shown. Numbers and percentages of IL-17A^+ γδT cells generated from flow cytometric analyses. **P < 0.01; mean ± SEM; n = 3-6 mice per group. DETC, dendritic epidermal T cells; IMQ, imiquimod; WT, wild-type.