



A Critical Role of the IL-1 β –IL-1R Signaling Pathway in Skin Inflammation and Psoriasis Pathogenesis

Yihua Cai^{1,7}, Feng Xue^{2,7}, Chen Quan², Minye Qu¹, Na Liu², Yuan Zhang³, Chris Fleming¹, Xiaoling Hu¹, Huang-ge Zhang¹, Ralph Weichselbaum³, Yang-xin Fu⁴, David Tieri⁵, Eric C. Rouchka⁶, Jie Zheng² and Jun Yan¹

The IL-1 signaling pathway has been shown to play a critical role in the pathogenesis of chronic, auto-inflammatory skin diseases such as psoriasis. However, the exact cellular and molecular mechanisms have not been fully understood. Here, we show that IL-1 β is significantly elevated in psoriatic lesional skin and imiquimod-treated mouse skin. In addition, IL-1R signaling appears to correlate with psoriasis disease progression and treatment response. IL-1 signaling in both dermal $\gamma\delta$ T cells and other cells such as keratinocytes is essential to an IMQ-induced skin inflammation. IL-1 β induces dermal $\gamma\delta$ T cell proliferation and IL-17 production in mice. In addition, IL-1 β stimulates keratinocytes to secrete chemokines that preferentially chemo-attract peripheral CD27⁺CCR6⁺IL-17 capable of producing $\gamma\delta$ T cells ($\gamma\delta$ T17). Further studies showed that endogenous IL-1 β secretion is regulated by skin commensals to maintain dermal $\gamma\delta$ T17 homeostasis in mice. Mouse skin associated with *Corynebacterium* species, bacteria enriched in human psoriatic lesional skin, has increased IL-1 β and dermal $\gamma\delta$ T17 cell expansion. Thus, the IL-1 β –IL-1R signaling pathway may contribute to skin inflammation and psoriasis pathogenesis via the direct regulation of dermal IL-17–producing cells and stimulation of keratinocytes for amplifying inflammatory cascade.

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INTRODUCTION

Psoriasis is an autoinflammatory skin disease affecting 2%–3% of all individuals in the United States (Rachakonda et al., 2014) and is a serious global problem, with at least 100 million individuals affected worldwide. Recent studies have shown that cytokines such as TNF- α , IL-23, and IL-17 play critical roles in psoriasis pathogenesis (Lowes et al., 2014). Antibodies against TNF- α , IL-23, and IL-17 have been approved for the treatment of patients with moderate to severe plaque psoriasis. The cytokine IL-1 family has also been implicated to be a key player in psoriasis pathogenesis

(Renne et al., 2010). Previous studies have shown that IL-1 is a dominant cytokine in patients with generalized pustular psoriasis (Johnston et al., 2017). In addition, whole-exome single nucleotide polymorphism array identified the *IL1RL1* gene as one of the new susceptibility loci for psoriasis (Zuo et al., 2015). Further genetic studies showed that polymorphisms in the IL-1B gene can be used to differentiate patients with psoriasis of early and late onset (Hebert et al., 2014). The IL-1 cytokine family is a growing group of cytokines including IL-1 α , IL-1 β , and IL-1 receptor antagonist (Jensen, 2010). IL-1 α has been shown to be essential in the induction of Munro microabscess formation in imiquimod (IMQ)-induced murine psoriasis-like model (Uribe-Herranz et al., 2013). IL-1 α also stimulates human keratinocytes to induce potent proinflammatory responses (Yano et al., 2008). However, IL-1 α mRNA level is decreased in psoriatic skin (Cooper et al., 1990), and IL-1 α levels in plasma and skin show an inverse correlation with Psoriasis Area Severity Index score (Tamilselvi et al., 2013) before and after treatment with methotrexate, posing a question about the importance of IL-1 α in human psoriasis pathogenesis. IL-1 β has a well-documented role in the pathogenesis of psoriasis (Lowes et al., 2014). IL-1 β inhibitors have been tested in clinical trials for the treatment of psoriasis. Recently, there was a case report showing that a patient with severe pustular psoriasis treated with anti-IL-1 β canakinumab had complete skin clearance with no recurrent systemic manifestations (Skendros et al., 2017). IL-1 β is known to be critical in IL-17–producing T-cell differentiation and activation (Cai et al., 2014; Ghoreschi et al., 2010; Sutton et al., 2009). Despite these studies, how IL-1 β

¹Department of Medicine and Department of Microbiology and Immunology, James Graham Brown Cancer Center, University of Louisville, Louisville, Kentucky, USA; ²Department of Dermatology, Ruijin Hospital, School of Medicine, Shanghai Jiaotong University, Shanghai, China; ³Department of Radiation and Cellular Oncology, University of Chicago School of Medicine, Chicago, Illinois, USA; ⁴Department of Pathology, UT Southwestern Medical Center, Dallas, Texas, USA; ⁵Department of Anatomical Sciences and Neurobiology, University of Louisville, Louisville, Kentucky, USA; and ⁶Department of Computer Engineering and Computer Science, University of Louisville, Louisville, Kentucky, USA

⁷These authors contributed equally to this work.

Correspondence: Jun Yan, Tumor Immunobiology Program, James Graham Brown Cancer Center, University of Louisville School of Medicine, 529 South Jackson Street, Louisville, Kentucky 40202, USA. E-mail: jun.yan@louisville.edu or Jie Zheng, Shanghai Jiaotong University School of Medicine, 197 Ruijin Second Road, Luwan District, Shanghai 200020, China. E-mail: jie-zheng2001@126.com

Abbreviations: $\gamma\delta$ T17, IL-17–producing $\gamma\delta$ T17 cell; DC, dendritic cell; GF, germ-free; IL-1R, IL-1 receptor type 1; IMQ, imiquimod

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production is regulated in healthy skin and psoriatic lesional skin and the exact cellular and molecular mechanisms by which the IL-1 β –IL-1 receptor type 1 (IL-1R) signaling pathway regulates skin inflammation have not been fully understood.

In this study, we show that IL-1 β mRNA and protein expression levels are significantly elevated in psoriatic lesional skin and predominately secreted by macrophages, dendritic cells (DCs), and keratinocytes in human. Neonatal thymocytes/bone marrow chimeric mouse studies show that IL-1 signaling in $\gamma\delta$ T cells and other radio-resistant cells is required to induce IMQ-mediated skin inflammation. IL-1 β not only activates dermal $\gamma\delta$ T cells for IL-17 secretion but also stimulates keratinocytes to secrete chemokines, which chemo-attract IL-17 capable of producing T cells. Endogenous IL-1 β secretion in the skin is regulated by skin commensals in mice and is associated with dermal IL-17–producing $\gamma\delta$ T ($\gamma\delta$ T17) cell homeostatic expansion and activation. Altered skin microbiome dysbiosis in psoriasis patients may result in an exaggerated IL-1 β production, thus priming skin for inflammation. These findings identify pivotal roles of the IL-1 β –IL-1R signaling pathway in maintaining skin homeostasis in the steady state and in the induction of inflamed skin such as psoriasis.

RESULTS

IL-1 β is significantly elevated in human psoriatic skin and IMQ-treated mouse skin

It is debatable whether IL-1 β protein is produced in the skin (Mizutani et al., 1991). We first examined the mRNA level of IL-1 β in psoriatic lesional skin and skin tissues from healthy individuals. Consistent with previous studies (Dombrowski et al., 2011), IL-1 β mRNA level was significantly increased in psoriatic lesional skin versus normal skin (Figure 1a). To further determine IL-1 β protein expression in skin tissues, immunofluorescent staining was performed. IL-1 β protein expression was enhanced in psoriatic lesional skin, although healthy control skin did express a low level of IL-1 β (Figure 1b). IL-1 β protein was largely co-localized with keratinocytes in psoriatic lesional skin (Figure 1c). CD11c⁺ DCs and CD163⁺ macrophages also secreted IL-1 β (Figure 1c). We next used RNA-based next-generation sequencing to analyze lesional skin samples from patients initially effectively treated with glucocorticoid but in whom disease recurred after stopping treatment. Gene set enrichment analysis showed that IL-1R signaling pathway-related genes were down-regulated in patients effectively treated with glucocorticoid, whereas these genes were up-regulated when the disease recurred (Figure 1d). This was confirmed by real-time PCR analysis. These data suggest that the IL-1 β –IL-1R signaling pathway is associated with disease progression and treatment response.

We next examined IL-1 β expression levels in an IMQ-induced psoriasis-like mouse model. Similarly, the mRNA and protein expression level of IL-1 β was significantly increased in IMQ-treated skin (Figure 1e and f). However, IL-1 β mRNA was mainly expressed in DCs and macrophages. CD45-negative cells such as keratinocytes did not express appreciable level of IL-1 β mRNA (Figure 1e). This was confirmed by immunofluorescent staining showing that IL-1 β protein was co-localized with DCs and macrophages but not

with keratinocytes (Figure 1g), highlighting the differences between mouse and human skin.

IL-1R on $\gamma\delta$ T cells is essential to induce skin inflammation in mice

Cytokine IL-1 β exhibits its biological functions through IL-1R, and IL-1R is expressed on many types of cells in the skin. Our previous studies have shown that IMQ- or IL-23–induced skin inflammation is significantly decreased in IL-1R–knockout mice (Cai et al., 2014). In addition, dermal $\gamma\delta$ T cells play a critical role in these two models (Cai et al., 2011; Pantelyushin et al., 2012). We hypothesized that IL-1R expression on dermal $\gamma\delta$ T cells is necessary to mediate skin inflammation. We established neonatal thymocytes/bone marrow chimeric mice in which IL-1R was deficient only in $\gamma\delta$ T cells to test this hypothesis. As additional controls to verify the chimeric mice, chimeric mice from IL-1R–knockout mice (see Supplementary Figure S1a online) indeed showed a reduced erythema and scales (see Supplementary Figure S2a online). Histopathologically, these mice had decreased epidermal thickness and neutrophil infiltration compared with chimeric mice from wild-type mice (Figure 2a). We then used chimeric mice in which IL-1R is deficient in $\gamma\delta$ T cells (see Supplementary Figure S1b). Upon IMQ topical treatment, these mice showed a reduced erythema and scales (see Supplementary Figure S2b). In addition, epidermal thickness from $\gamma\delta$ T IL-1R–knockout chimeric mice was significantly decreased compared with that in IL-1R–intact mice (Figure 2b). The mRNA expression levels of IL-17, TNF- α , and IL-6 were also significantly decreased. These data suggest that IL-1R expression on $\gamma\delta$ T cells is essential in IMQ-induced skin inflammation.

IL-1R on other cells also contributes to IMQ-induced skin inflammation

We next examined whether IL-1R on other cells, particularly radio-resistant cells such as keratinocytes, is also necessary for IMQ-induced skin inflammation. We established chimeric mice in which all other cells do not express IL-1R except $\gamma\delta$ T cells (see Supplementary Figure S1c). Upon IMQ topical treatment, these mice showed a reduced erythema and scales (see Supplementary Figure S2c). Histopathologically, these mice also showed decreased epidermal thickness compared with IL-1R intact mice, although infiltrated neutrophils and the mRNA expression levels of IL-17, IL-22, TNF- α , and IL-6 were not significantly altered (Figure 3). These data suggest that IL-1R signaling on other cells, possibly keratinocytes, also contributes to IMQ-induced skin inflammation, particularly for epidermal hyperplasia.

IL-1 β stimulates dermal $\gamma\delta$ T-cell proliferation and IL-17 production and stimulates keratinocytes to secrete chemokines

IL-1 β is critical in IL-17–producing T-cell differentiation and activation. Consistent with previous studies (Cai et al., 2014), IL-1 β induced dermal $\gamma\delta$ T cell proliferation and synergized with IL-23 for IL-17 production (Figure 4a). In contrast, IL-23 induced minimal $\gamma\delta$ T-cell proliferation (Figure 4b). However, IL-23–induced dermal $\gamma\delta$ T-cell IL-17 production was abrogated in IL-1R–knockout mice (Figure 4a and b), suggesting that IL-1R signaling is required for IL-17 production in dermal $\gamma\delta$ T cells.

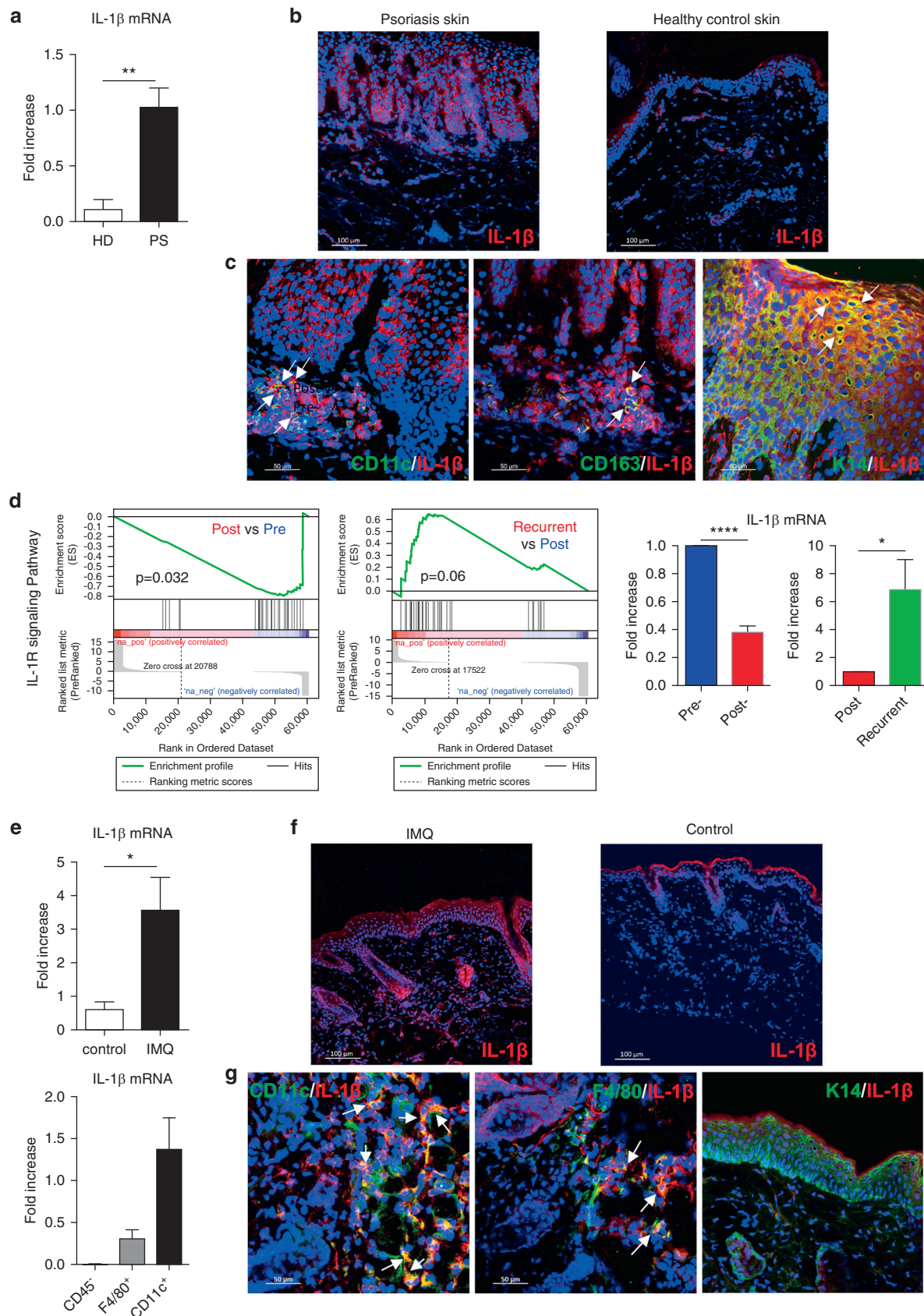


Figure 1. IL-1 β expression was significantly increased in both murine and human psoriatic lesions. (a) IL-1 β mRNA levels from skin biopsy samples collected from psoriasis patients (PS, n = 5) and healthy donors (HD, n = 4). (b) Frozen sections were stained with IL-1 β mAb (red) and DAPI (blue). Scale bar = 100 μ m. (c) Frozen sections from psoriatic lesional skin were stained with IL-1 β mAb (red); human CD11c mAb (green), human CD163 mAb (green), keratin 14 mAb (green); and DAPI (blue). Scale bar = 50 μ m. (d) Gene set enrichment analysis identifies significant transcriptional down-regulation of the IL-1R signaling

Because our studies showed that IL-1R on other cells, such as radio-resistant cells, is also critical in skin inflammation, particularly for epidermal thickness (Figure 3), we examined whether IL-1 β functions on other cells leading to such effect. Keratinocytes have been shown to produce IL-1 β . IL-1 β also functions on keratinocytes in an autocrine fashion (Kupper et al., 1988). IL-1 β stimulated primary murine keratinocytes to produce chemokines (Cai et al., 2011) including CCL20 (Figure 5a). IL-1 β -induced CCL-20 production in mouse keratinocytes was dependent on the STAT3 and NF- κ B pathway but independent of the MAPK and mTOR pathways. CD27 can be used as a functional marker to differentiate IL-17-producing versus IFN- γ -producing $\gamma\delta$ T cells (Ribot et al., 2009). IL-17-producing $\gamma\delta$ T cells do not express CD27 and constitutively express CCR6, which is the receptor for chemokine CCL20. In contrast, IFN- γ -producing $\gamma\delta$ T cells express CD27 but not CCR6. As shown in Figure 5b, supernatants from IL-1 β -stimulated keratinocytes chemoattracted CD27⁺ but not CD27⁺ $\gamma\delta$ T cells. We also noticed that IL-1 β slightly stimulated mouse keratinocytes for proliferation (Figure 5c). To examine whether human IL-1 β has similar functions, we stimulated human keratinocytes with IL-1 β . Similarly, IL-1 β stimulated human keratinocytes to produce chemokines, including CCL20 (Figure 5d). This was also associated with the STAT3 and NF- κ B pathway. Further chemotaxis assay showed that supernatants from IL-1 β -stimulated keratinocytes chemoattracted CCR6⁺ T cells (Figure 5e). However, IL-1 β did not enhance human keratinocyte proliferation (Figure 5f). Taken together, these data suggest that IL-1 β can stimulate keratinocytes to secrete chemokines that chemoattract IL-17 capable of producing CCR6⁺ T cells.

Skin commensals endow IL-1 β production in health and psoriasis

Having shown the critical roles of IL-1 β in skin inflammation, one question that remains unanswered is how IL-1 β secretion is regulated in the skin. We found that the mRNA and protein levels of IL-1 β were significantly lower in germ-free (GF) mice than in specific pathogen-free mice (Figure 6a). In contrast, IL-23 mRNA expression level was not affected (data not shown). Correspondingly, dermal $\gamma\delta$ T cells and IL-17-producing $\gamma\delta$ T cells in the skin were significantly lower in GF mice (Figure 6b). Because IL-23-induced dermal $\gamma\delta$ T-cell IL-17 production is dependent on IL-1 β , we stimulated dermal $\gamma\delta$ T cells with IL-23 and found that dermal $\gamma\delta$ T-cell IL-17 production from GF mice was significantly lower than from specific pathogen-free mice (see Supplementary Figure S3a online). Addition of exogenous IL-1 β did not fully rescue IL-23-induced IL-17 production from dermal $\gamma\delta$ T cells in GF mice. Notably, dermal $\gamma\delta$ T cells from GF mice showed lower IL-1R expression level than specific pathogen-free mice (see Supplementary Figure S3b).

These data suggest that skin commensals play a critical role in skin immune homeostasis, particularly for dermal $\gamma\delta$ T17 cells via an endogenous IL-1 β production.

Previous studies have shown that psoriatic skin has substantially altered skin commensals (Alekseyenko et al., 2013; Gao et al., 2008). In our studies, swabs from psoriatic lesional skin and skin of healthy (control) individuals were collected. Using high-throughput 16S rRNA gene sequencing, we assayed the cutaneous bacterial communities and characterized these samples. As shown in Figure 6c, skin microbiota from psoriasis patients had altered bacterial abundance at the phylum distribution level. Shannon index decreased from control to lesion, suggesting that healthy skin has higher alpha diversity compared with lesions (Figure 6c). The five most common phyla (Proteobacteria, Firmicutes, Actinobacteria, Bacteroidetes and Deinococcus-Thermus), with relative abundance more than 5%, were shown. Comparison of the relative abundance of Actinobacteria, Firmicutes, and Deinococcus-Thermus showed significant difference between control and lesional skin tissues.

To test the hypothesis that altered skin commensals lead to increased IL-1 β production, thus expanding IL-17-producing dermal $\gamma\delta$ T cells, *Corynebacterium pseudodiphtheriticum* was applied to mice. Indeed, the IL-1 β protein level was increased in these mice (Figure 6d). Correspondingly, dermal $\gamma\delta$ T17 cells were also significantly increased in *C. pseudodiphtheriticum*-associated mice (Figure 6e). Thus, skin commensals contribute to both skin immune homeostasis and dysregulated immune responses such as psoriasis.

DISCUSSION

The IL-1 β –IL-1R signaling pathway has been shown previously to play critical roles in psoriasis pathogenesis (Lowes et al., 2014). However, many questions remain unanswered. Despite elevated IL-1 β mRNA expression levels in psoriatic skin, it is not conclusive whether IL-1 β protein expression is also elevated. In addition, it is not fully understood how IL-1 β secretion is regulated in normal skin and psoriatic skin. Here, we show that IL-1 β mRNA expression level is significantly elevated in human psoriatic lesional skin and also in IMQ-treated mouse skin, which is consistent with previous studies (Debets et al., 1997; Dombrowski et al., 2011). In addition, we show that the IL-1 β –IL-1R signaling pathway is associated with disease progression and treatment response. This finding is important and suggests that this pathway may serve not only as targets for psoriasis treatment but also as biomarkers for disease progression and treatment response. Furthermore, elevated IL-1 β protein is observed in psoriatic lesional skin as assessed by immunofluorescent staining. Biologically active IL-1 β protein expression requires

pathway in psoriasis patients initially effectively treated with glucocorticoid, whereas up-regulation occurred in patients with recurrent disease after stopping treatment (National Center for Biotechnology Information Gene Expression Omnibus with the accession number GSE114729). The mRNA expression of IL-1 β by real-time PCR analysis was calculated using pretreatment or posttreatment level as the base level. (e) C57BL/6 mice received daily topical application with IMQ or vehicle control for 3–5 days. IL-1 β mRNA levels from IMQ-treated or vehicle control mouse skin were measured. CD11c⁺ cells, F4/80⁺ cells, and CD45⁺ cells were sorted from 3 days of IMQ-treated mouse skin, and IL-1 β mRNA level was measured. (f) Skin frozen sections from IMQ- or vehicle control-treated mice were stained with IL-1 β mAb (red) and DAPI (blue). Scale bar = 100 μ m. (g) Skin frozen sections from IMQ-treated mice were stained with IL-1 β mAb (red); mouse CD11c mAb (green), mouse F4/80 mAb (green), or keratin 14 mAb (green); and DAPI (blue). Scale bar = 50 μ m. * P < 0.05, ** P < 0.01, *** P < 0.001. IL-1R, IL-1 receptor; IMQ, imiquimod; mAb, monoclonal antibody.

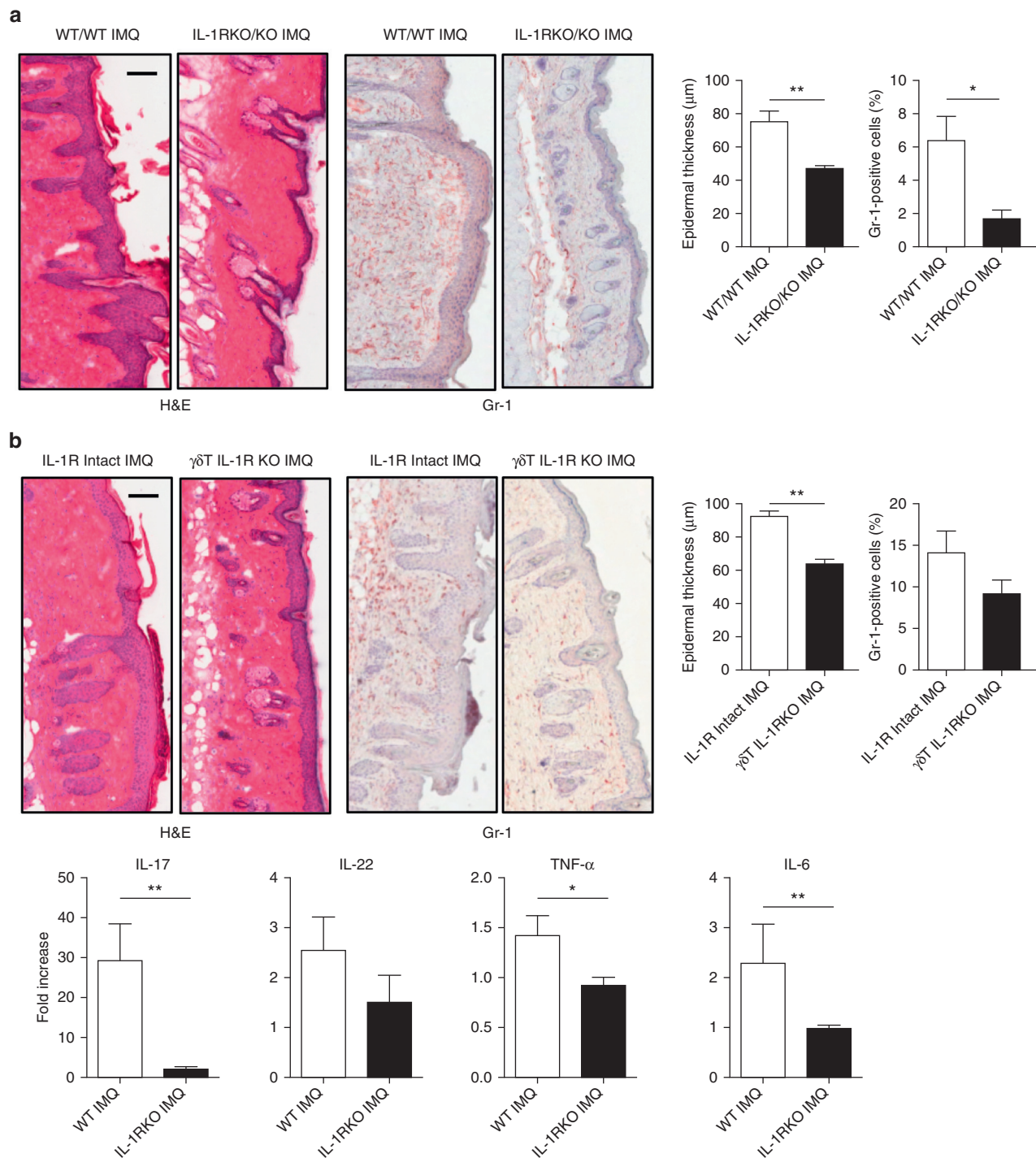


Figure 2. IL-1R signaling in $\gamma\delta$ T cells is essential to induce skin inflammation. (a) Reconstituted WT or IL-1R-KO chimeric mice ($n = 3$) were treated daily for 5 days with IMQ or vehicle control. Representative H&E-stained sections and frozen sections stained with Gr-1 are shown. Gr-1-positive cells are brown. Skin tissues were also stained with CD45 and Gr-1 and assessed by flow cytometry. Epidermal thickness and percentage of CD45⁺Gr-1⁺ cells were measured. Scale bar = 100 μm . Data are representative of two independent experiments with similar results. (b) Reconstituted mice ($n = 3$) with WT $\gamma\delta$ T cells or IL-1R deficiency in $\gamma\delta$ T cells were treated daily for 5 days with IMQ or vehicle control. Representative H&E-stained sections and frozen sections stained with Gr-1 are shown. Gr-1 positive cells are brown. Skin tissues were also stained with CD45 and Gr-1 and assessed by flow cytometry. Epidermal thickness and percentage of CD45⁺Gr-1⁺ cells were measured. Scale bar = 100 μm . Data are representative of two independent experiments with similar results. The mRNA levels of IL-17, IL-22, TNF- α , and IL-6 were measured by real-time PCR analysis. * $P < 0.05$, ** $P < 0.01$. H&E, hematoxylin and eosin; IL-1R, IL-1 receptor type 1; IMQ, imiquimod; KO, knockout; WT, wild type.

proteolytic activation of pro-IL-1 β by protease caspase-1 (Lopez-Castejon and Brough, 2011). Previous studies showed that normal human keratinocytes produce but do not

process pro-IL-1 β to be a mature functional IL-1 β (Mizutani et al., 1991). However, caspase-1 activity is significantly increased in psoriatic skin (Dombrowski et al., 2011),

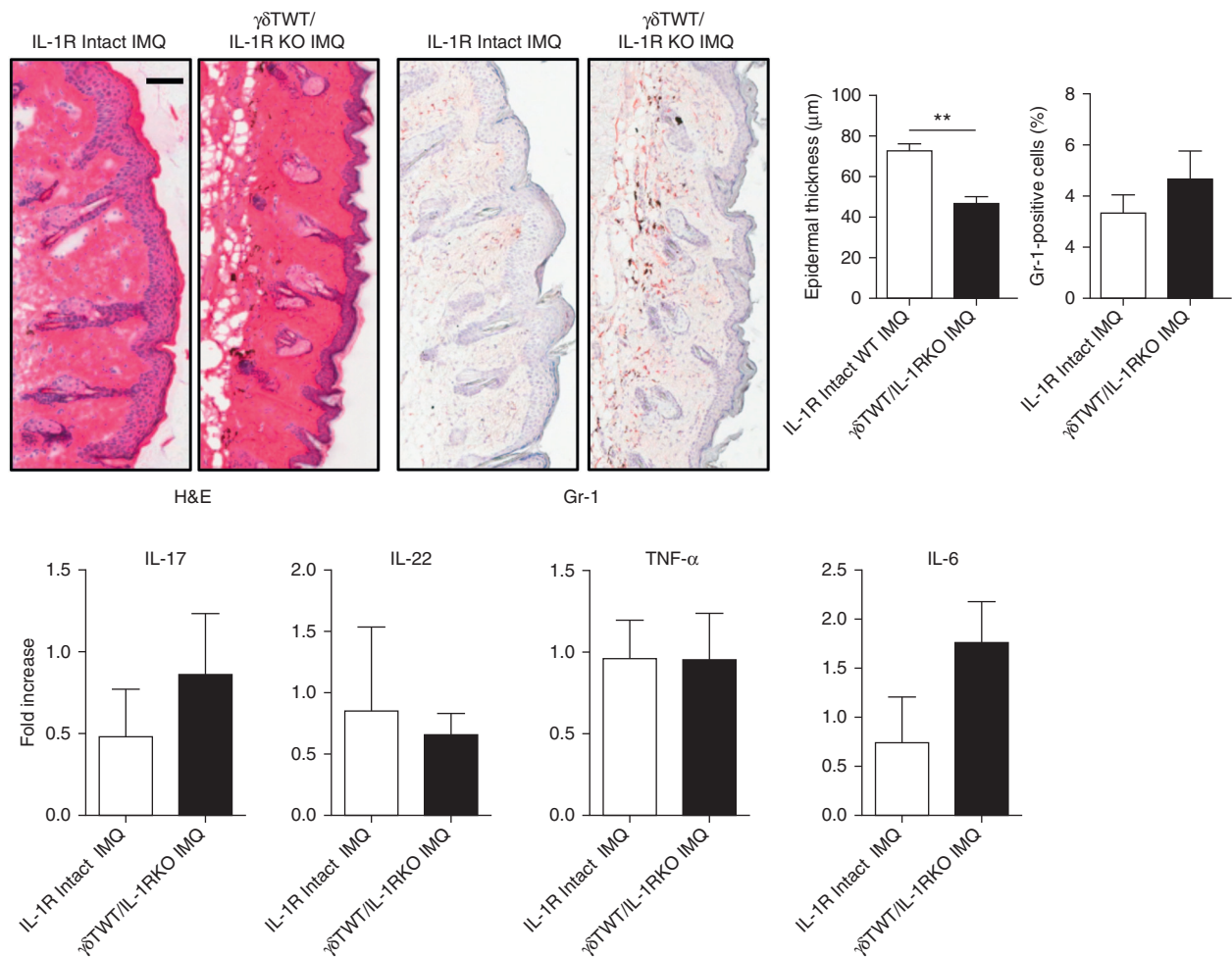


Figure 3. IL-1R signaling in other cells is important to induce full-fledged skin inflammation. Reconstituted mice ($n = 3$) with WT $\gamma\delta$ T cells and IL-1R intact or deficiency in other cells were treated daily for 5 days with IMQ or vehicle control. Representative H&E-stained sections and frozen sections stained with Gr-1 are shown. Gr-1 positive cells are brown. Skin tissues were also stained with CD45 and Gr-1 assessed by flow cytometry. Epidermal thickness and percentage of CD45⁺Gr-1⁺ cells were measured at day 5. Scale bar = 100 μ m. Data are representative of two independent experiments with similar results. The mRNA levels of IL-17, IL-22, TNF- α , and IL-6 were measured by quantitative PCR analysis. * $P < 0.05$. H&E, hematoxylin and eosin; IL-1R, IL-1 receptor type 1; IMQ, imiquimod; KO, knockout; WT, wild type.

suggesting that pro-IL-1 β could be processed to secrete mature IL-1 β , at least in an inflammatory condition. In our study, we used IL-1 β antibody, which recognizes the mature form of IL-1 β (17 kDa) and showed that IL-1 β protein expression is elevated in human psoriatic skin as well as in IMQ-treated mouse skin. These data suggest that under inflammatory conditions, pro-IL-1 β can be processed to become an active, functional IL-1 β . We also analyze the cellular origins of IL-1 β in the skin. In human psoriatic lesional skin, keratinocytes are the major IL-1 β producer as shown by immunofluorescent staining. Macrophages and DCs also produce IL-1 β . In contrast, macrophages and DCs in IMQ-treated skin are the major IL-1 β producers. Keratinocytes do not secrete appreciable levels of IL-1 β . These findings highlight the differences between mouse models and human disease.

One critical question related to IL-1 β production is how IL-1 β is regulated. Previous studies have shown that cytosolic DNA fragments in psoriatic skin stimulate inflammasome AIM2 activation in keratinocytes, leading to increased IL-1 β production (Dombrowski et al., 2011). In our study,

we observed a low but detectable level of IL-1 β in normal human skin and naïve mouse skin. Cytosolic DNA fragments could not be detected in healthy skin (Dombrowski et al., 2011). We thus hypothesize that skin commensals may be related to endogenous IL-1 β production. Indeed, GF mice have significantly lower IL-1 β mRNA and protein expression levels compared with specific pathogen-free mice. This is also associated with lower frequency of dermal $\gamma\delta$ T cells and IL-17 production in GF mice. These data suggest that skin commensals are important to maintain skin immune homeostasis, which plays a critical role in local immunity and inflammation (Naik et al., 2012). These results also support the idea that IL-1 signaling is diminished in the absence of commensals as shown by lower IL-1R expression level on dermal $\gamma\delta$ T cells from GF mice. Furthermore, it implies that resident commensals may provide a protective role in skin infection. Indeed, deficiency of IL-1R leads to severe cutaneous vaccinia virus infection (Tian et al., 2017). This notion is also supported by a recent study showing that an ocular commensal induces IL-17-producing $\gamma\delta$ T cells, thus protecting against corneal

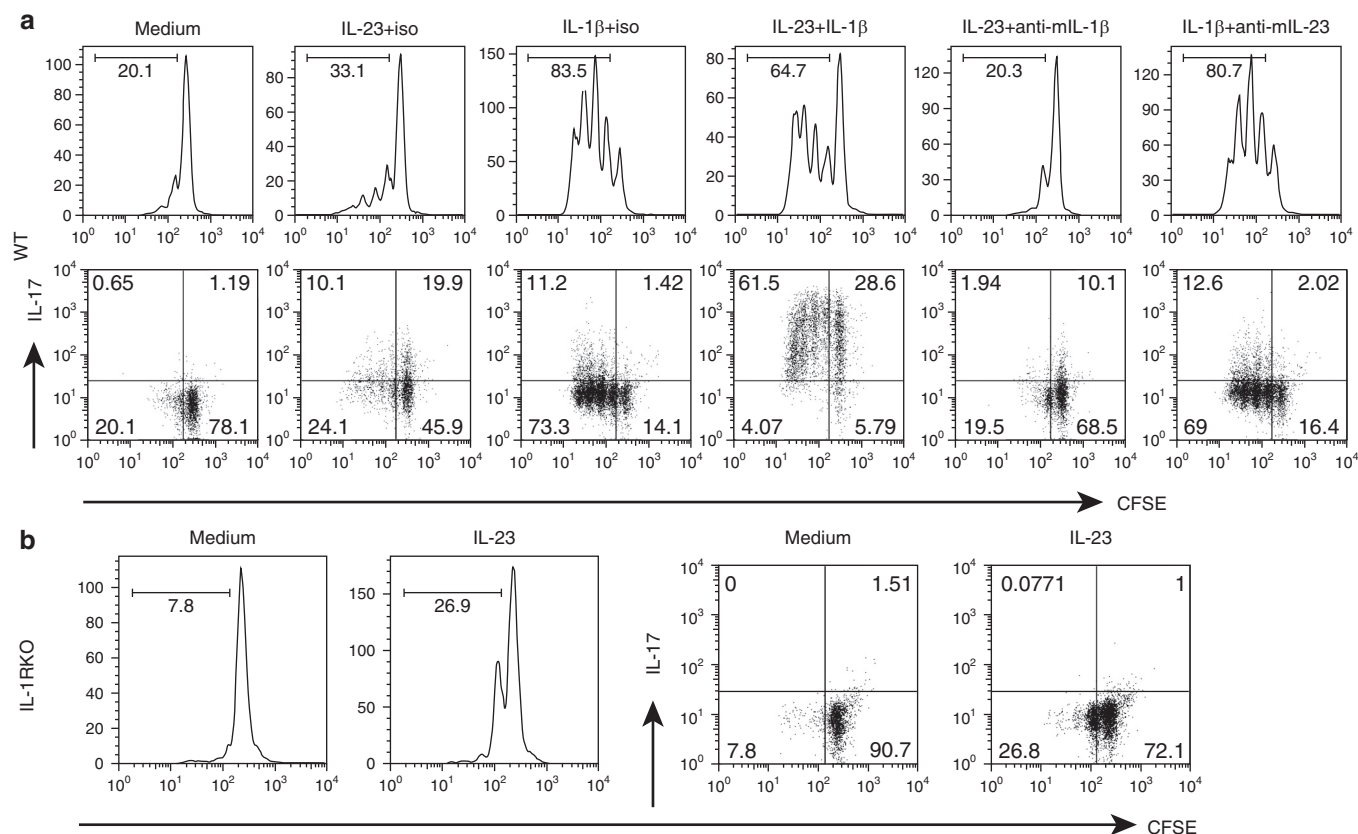


Figure 4. IL-1R signaling is essential in dermal $\gamma\delta$ T cell proliferation and IL-17 production. (a) Whole skin cell suspensions from C57BL/6 WT mice were labeled with CFSE and then stimulated with IL-23, IL-1 β , and IL-23 plus IL-1 β for 3 days. CFSE dilution and intracellular IL-17 production by dermal $\gamma\delta$ T cells were determined by flow cytometry. Flow plots gated on CD3^{int} $\gamma\delta$ TCR^{int} cells are representative of at least three independent experiments with similar results. (b) Whole skin cell suspensions from IL-1R-KO mice labeled with CFSE were stimulated with IL-23 for 3 days. CFSE dilution and intracellular IL-17 production by dermal $\gamma\delta$ T cells were determined by flow cytometry. Flow plots gated on CD3^{int} $\gamma\delta$ TCR^{int} cells are representative of at least three independent experiments with similar results. CFSE, carboxyfluorescein succinimidyl ester; IL-1R, IL-1 receptor type 1; KO, knockout; WT, wild type.

infection (St Leger et al., 2017). These data lead us to examine whether altered bacterial commensals in psoriatic skin are associated with increased IL-1 β and IL-17 production in patients with psoriasis. In our study, Actinobacteria is the most common phylum in psoriasis samples, but it is Firmicutes in control individuals, which is different from a previous study (Gao et al., 2008). The differences may be related to the different sampling and skin conditions or different ethnic groups. Nevertheless, *Corynebacterium* species, which belongs to the Actinobacteria phylum, is significantly elevated at the genus level in psoriatic lesional skin (Alekseyenko et al., 2013). Application of *C. pseudodiphtheriticum* in mouse skin increases IL-1 β production along with increased dermal $\gamma\delta$ T17 cells. Increased IL-17 production may also stimulate keratinocytes to secrete more IL-1 β (Cho et al., 2012), thus establishing an amplification loop of inflammatory responses. It will be interesting to determine whether increased *Corynebacterium* colonization in healthy normal skin also leads to elevated IL-1 β production, thus leading to priming of IL-17-producing cells in humans.

IL-1 β is known to be critical in inducing Th17 and $\gamma\delta$ T17 cell differentiation and effector function. Indeed, we show that IL-1 β stimulates dermal $\gamma\delta$ T cell for proliferation and synergizes with IL-23 for IL-17 production in mice. IL-

23-induced IL-17 production is dependent on IL-1R signaling, suggesting a critical role of IL-1R signaling in dermal $\gamma\delta$ T cell activation. Our study shows that IL-1 signaling on $\gamma\delta$ T cells is important in IMQ-induced epidermal hyperplasia. It is worth noting that although the IMQ-induced skin inflammation model has been widely used in preclinical psoriasis studies, there are many limitations with this model such as untended consequences of topical treatment and limited aspects of human psoriasis (Hawkes et al., 2017). It thus needs to be determined whether IL-1R signaling is critical in activating human dermal IL-17-producing cells. In addition, it has been shown previously that dermal $\gamma\delta$ T cells are the major cellular source of IL-17 in IMQ-induced skin inflammation (Cai et al., 2011). In contrast, both $\alpha\beta$ and $\gamma\delta$ T cells produce IL-17 in psoriatic lesional skin (Cai et al., 2011; Matos et al., 2017), and $\alpha\beta$ T cells are considered to play a predominant role in disease initiation (Matos et al., 2017).

We show that IL-1 β stimulates epidermal keratinocytes to secrete chemokines. Of interest, chemokine CCL20 is induced upon IL-1 β stimulation. This is in both mouse and human primary keratinocytes. Notably, CCL20 is significantly increased in lesional psoriatic skin (Homey et al., 2000; Liu et al., 2010). CCR6 is expressed on mouse dermal $\gamma\delta$ T17 cells (Cai et al., 2011, 2014) and human V δ 1

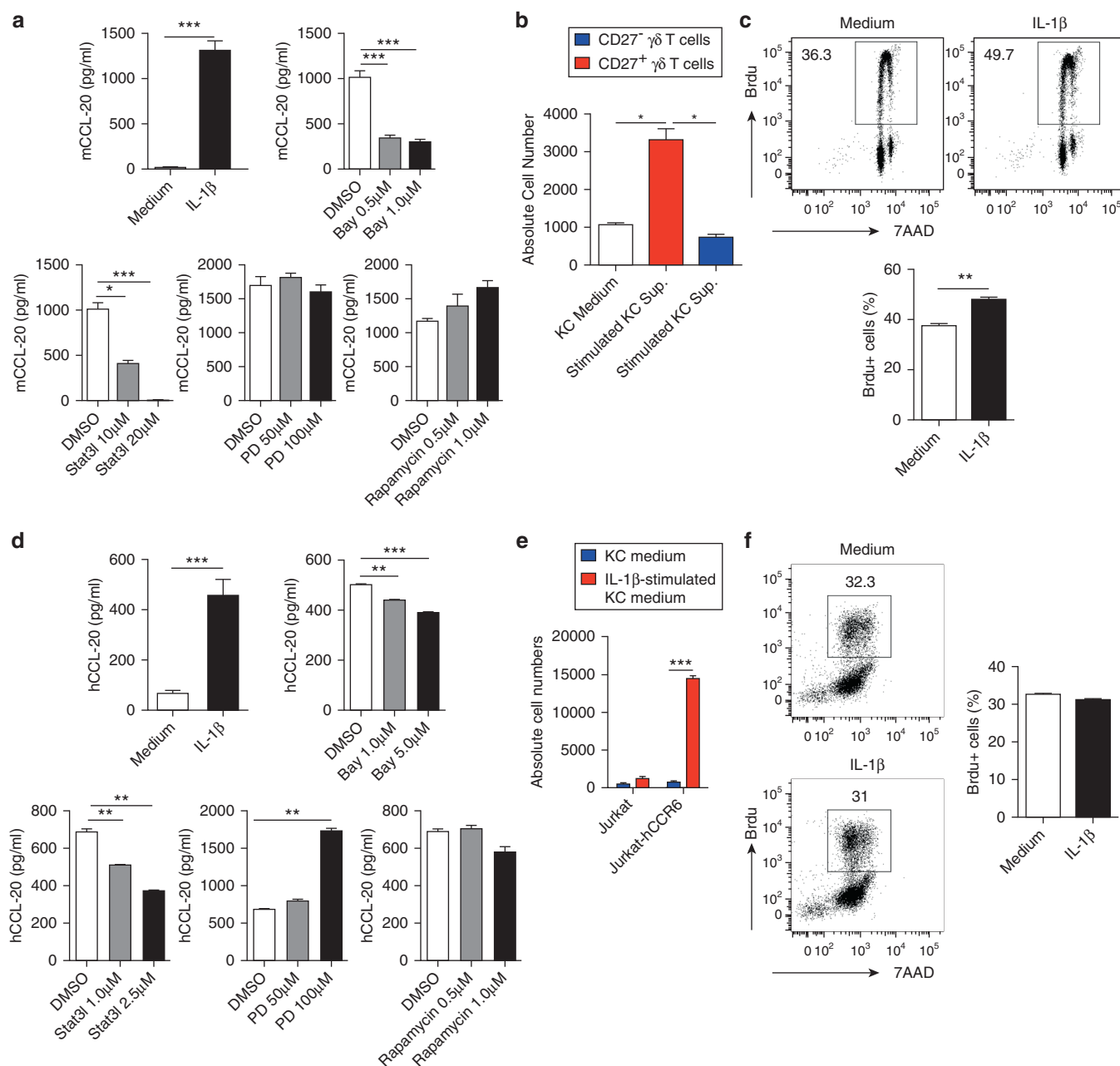


Figure 5. IL-1 β stimulates keratinocytes (KCs) to secrete chemokines, which are capable of chemoattracting $\gamma\delta$ T17 cells. (a) Primary mouse KCs were stimulated with IL-1 β in the absence or presence of signaling pathway inhibitors at indicated concentrations. CCL20 level was measured by ELISA. (b) Sorted CD27⁻/CD27⁺ $\gamma\delta$ T cells were added on the top of insert and incubated with supernatants from mouse KCs stimulated with or without IL-1 β . Cells were harvested and quantified by flow cytometry. (c) Primary mouse KCs were stimulated with IL-1 β for 24 hours. BrdU was added in the culture for the last 2.5 hours. Cells were stained with anti-BrdU Ab and 7-AAD and analyzed by flow cytometry. Representative dot plots and summarized BrdU⁺ cells are shown. (d) Primary human KCs were stimulated with IL-1 β in the absence or presence of signaling pathway inhibitors. Human CCL20 level was measured by ELISA. (e) Cultured Jurkat or Jurkat-hCCR6 cells were added on the top of insert and incubated with supernatants from human KCs stimulated with or without IL-1 β . Cells were harvested and quantified by flow cytometry. (f) Primary human KCs were cultured and stimulated with IL-1 β for 24 hours. BrdU was added in the culture for the last 2.5 hours. Cells were stained with anti-BrdU Ab and 7-AAD and analyzed by flow cytometry. Representative dot plots and summarized BrdU⁺ cells are shown. * P < 0.05, ** P < 0.01, *** P < 0.001. 7-AAD, 7-aminoactinomycin D; Ab, antibody; M, mol/L.

and V δ 2 $\gamma\delta$ T17 cells, as well as circulating V δ 2 $\gamma\delta$ T17 cells with a skin-homing CLA⁺ phenotype (Caccamo et al., 2011; Laggner et al., 2011; Wu et al., 2014). We observe that supernatants from IL-1 β -stimulated keratinocytes preferentially chemoattract CD27⁻ IL-17 capable of producing $\gamma\delta$ T cells. These findings suggest that IL-1 β may induce an

amplified inflammatory cascade through recruiting $\gamma\delta$ T17 from the periphery.

In summary, we show that IL-1 β –IL-1R signaling is essential to regulating skin immunosurveillance and inflammation. Endogenous low levels of IL-1 β stimulated by skin commensals maintains $\gamma\delta$ T17 homeostasis in mouse skin,

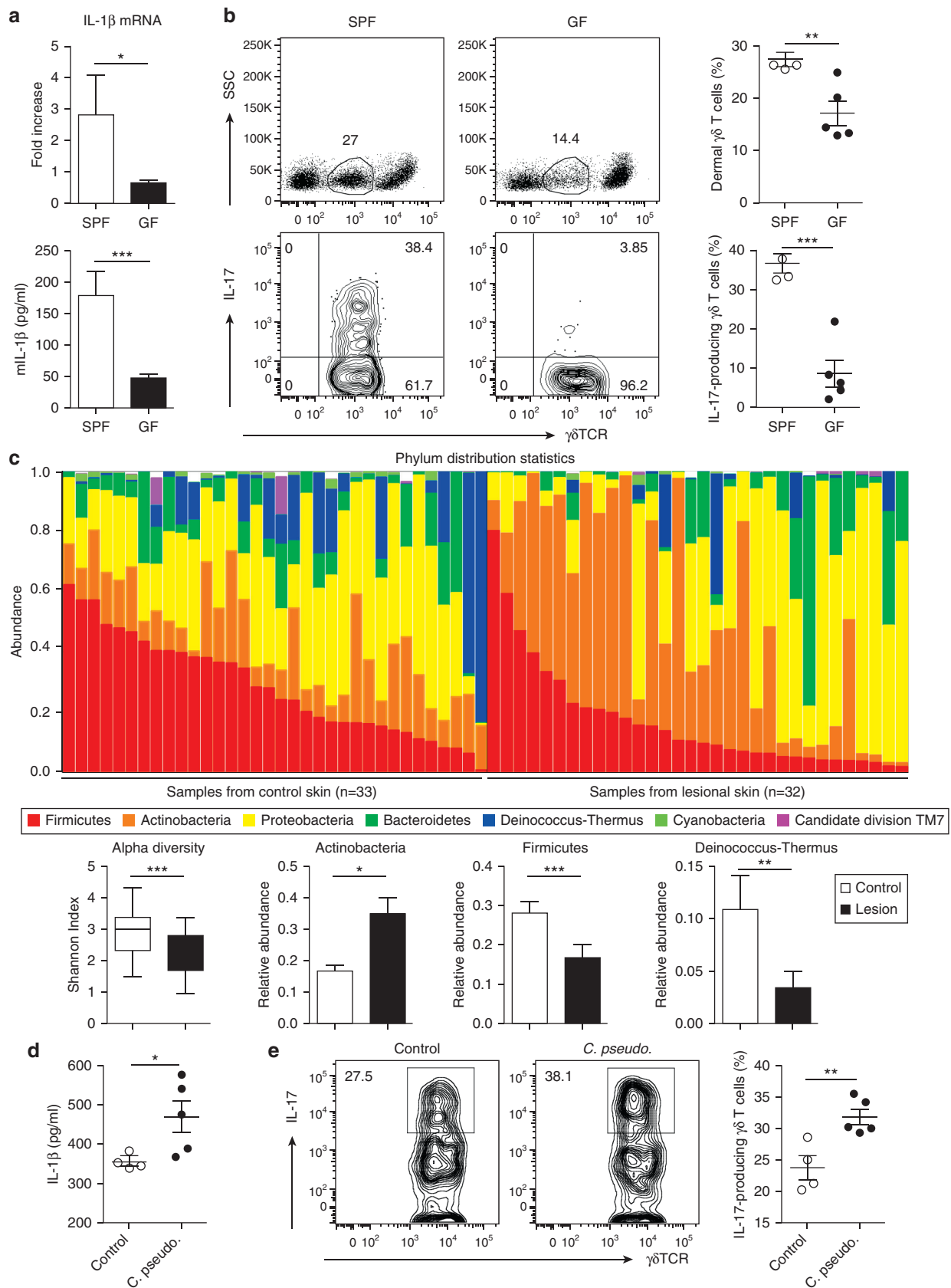


Figure 6. Skin commensals or altered skin microbiota contribute to IL-1 β -mediated dermal $\gamma\delta$ T17 expansion. (a) The mRNA and protein levels of IL-1 β in C57BL/6 SPF mice versus GF mice. (b) The frequency of dermal $\gamma\delta$ T cells and $\gamma\delta$ T17 cells in C57BL/6 SPF and GF mice are shown. Flow plots gated on CD3 $^{+}$ cells (top) or CD3 int $\gamma\delta$ TCR int cells (bottom) are representative of two independent experiments with similar results. (c) Skin swabs from psoriasis patients and healthy individuals were sequenced for skin microbiota analysis. Bacterial abundance at the phylum level, the Shannon index, and relative

whereas microbiota dysbiosis up-regulates IL-1 β , leading to expanded $\gamma\delta$ T17 cells, which may prime skin for the induction of inflammation. IL-1 β can directly activate $\gamma\delta$ T17 cells in mice and also stimulate keratinocytes for chemokine secretion, which chemoattracts more IL-17 capable of producing cells from the periphery, thus establishing an amplified inflammatory response, leading to skin inflammation such as psoriasis pathogenesis.

MATERIALS AND METHODS

Mice

C57BL/6 wild-type, *TCR $\delta^{-/-}$* , and *Il1r1 $^{-/-}$* mice were purchased from the Jackson Laboratory (Bar Harbor, ME). GF mice were kindly provided by Yang-xin Fu (University of Chicago, Chicago, IL and UT Southwestern, Dallas, TX). All animals were housed and treated in accordance with institutional guidelines and approved by the Institutional Animal Care and Use Committee at the University of Louisville, Louisville, KY.

Human participants

See the [Supplementary Materials and Methods](#) online.

Skin tissue preparation and cell stimulation

Whole skin cells from mouse back skin were prepared as previously described (Cai et al., 2011). The detailed methods are described in the [Supplementary Materials and Methods](#).

Flow cytometry analysis and intracellular cytokine staining

See the [Supplementary Materials and Methods](#).

T-cell chemotaxis assay

See the [Supplementary Materials and Methods](#).

Generation of neonatal thymocytes/bone marrow chimeric mice

The neonatal thymocytes/bone marrow chimeras were generated as previously reported (Cai et al., 2014). Briefly, recipient mice were lethally irradiated with 950 cGy and then were intravenously transferred with $5\text{--}10 \times 10^6$ thymocytes from neonatal mice 0–48 hours after birth. After 24 hours, the recipient mice received $5\text{--}10 \times 10^6$ bone marrow cells. All chimeric mice were allowed to reconstitute for at least 8 weeks before use in experiments.

Establishment of psoriasis-like mouse models

IMQ-induced psoriasis-like mouse model was established as previously described (Cai et al., 2014). The detailed methods are described in the [Supplementary Materials and Methods](#).

Topical association of bacteria

C. pseudodiphtheriticum (10700; ATCC, Manassas, VA) were cultured for 18 hours in brain heart infusion broth at 37 °C. Bacteria were enumerated by assessing colony-forming units using traditional bacteriology techniques and by measuring optical density at 600 nm using a spectrophotometer. For topical association of bacteria, each mouse was associated with bacteria by applying bacterial suspension (approximately 1×10^9 colony-forming units/ml, 0.5 ml for two ears). Application of bacterial suspension was repeated every other day a total of four times. Mice were sacrificed at 2 weeks.

Skin histology, immunohistochemical staining, and immunofluorescence staining

See the [Supplementary Materials and Methods](#).

Measurement of mouse IL-1 β , mCCL-20, and hCCL-20 by ELISA

See the [Supplementary Materials and Methods](#).

Statistical analysis

All quantitative data are shown as mean \pm standard error of the mean unless otherwise indicated. All samples were compared using two-tailed, unpaired Student *t* test. A *P*-value less than 0.05 was considered significant. Statistical analysis was performed with GraphPad Prism software (GraphPad, La Jolla, CA).

CONFLICT OF INTEREST

All authors declared no conflict of interest in this study.

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SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at www.jidonline.org, and at <https://doi.org/10.1016/j.jid.2018.07.025>.

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← abundances of Actinobacteria, Firmicutes, and *Deinococcus-Thermus* between lesional skin and healthy control skin are shown. (d) The IL-1 β protein levels from C57BL/6 mice associated with or without *Corynebacterium pseudodiphtheriticum* were measured by ELISA. (e). Representative dot plots and summarized dermal $\gamma\delta$ T17 cells in mice associated with or without *C. pseudodiphtheriticum* are shown. Flow plots gated on CD3^{int} $\gamma\delta$ TCR^{int} cells are representative of two independent experiments with similar results. **P* < 0.05, ***P* < 0.01, ****P* < 0.001. C. pseudo., *Corynebacterium pseudodiphtheriticum*; GF, germ-free; SPF, specific pathogen-free.

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