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

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Abstract

IL-1 signaling pathway has been shown to play a critical role in the pathogenesis of chronic, autoinflammatory 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 (IMQ)-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 which preferentially chemoattract peripheral CD27⁻ CCR6⁺IL-17 capable producing $\gamma\delta$ T cells ($\gamma\delta$ T17). Further studies reveal 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*, bacterial enriched in human psoriatic lesional skin has increased IL-1 β and dermal $\gamma\delta$ T17 cell expansion. Thus, IL-1 β -IL-1R signaling pathway may contribute to skin inflammation and psoriasis pathogenesis via the direct

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regulation of dermal IL-17-producing cells and stimulation of keratinocytes for amplifying inflammatory cascade.

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 revealed that cytokines such as TNF- α /IL-23/IL-17 play critical roles in psoriasis pathogenesis (Loves et al., 2014). Antibodies against TNF- α /IL-23/IL-17 have been approved for the treatment of patients with moderate-to-severe plaque psoriasis. 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 SNP array has identified IL1RL1 gene as one of the new susceptibility loci for psoriasis (Zuo et al., 2015). Further genetic studies reveal that polymorphisms in 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 (IL-1RN) (Jensen, 2010). IL-1 α has been shown to be essential in the induction of Munro's 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 (PASI) 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 (Loves et al., 2014). IL-1 β inhibitors have been tested in clinical trials for the treatment of psoriasis. Recently there was a case report demonstrating 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 production is regulated in healthy skin and psoriatic lesional skin and the exact cellular and molecular mechanisms by which the IL-1 β -IL-1R signaling pathway regulates skin inflammation have not been fully understood.

In the current 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 reveal 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 chemoattract IL-17-capable producing T cells. Notably, 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 as well as 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 compared to that in 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 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 (RNA-seq) to analyze lesional skin samples from patients initially effectively treated with glucocorticoid but disease recurred after stopping treatment. Gene-set-enrichment analysis (GSEA) revealed that IL-1R signaling pathway-related genes were downregulated in patients effectively treated with glucocorticoid whereas these genes were upregulated 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, 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 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-1 receptor type 1 (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 KO 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 only deficient in $\gamma\delta$ T cells to test this hypothesis. As additional controls to verify the chimeric mice, chimeric mice from IL-1R KO mice (Figure S1A) indeed showed a reduced erythema and scales (Figure S2A). Histopathologically, these mice had decreased epidermal thickness and neutrophil infiltration as compared to chimeric mice from WT mice (Figure 2A). We then used chimeric mice in which IL-1R is deficient in $\gamma\delta$ T cells (Figure S1B). Upon IMQ topical

treatment, these mice showed a reduced erythema and scales (Figure S2B). In addition, epidermal thickness from $\gamma\delta$ T IL-1R KO chimeric mice was significantly decreased compared to 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 (Figure S1C). Upon IMQ topical treatment, these mice showed a reduced erythema and scales (Figure S2C). Histopathologically, these mice also showed decreased epidermal thickness as compared to 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 as well as 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 KO mice (Figure 4A, B), suggesting that IL-1R signaling is required for IL-17 production in dermal $\gamma\delta$ T cells.

Since 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 KC for proliferation (Figure 5C). To examine whether human IL-1 β has similar function, 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 STAT3 and NF- κ B pathway. Further chemotaxis assay revealed 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 which chemoattract IL-17 capable-producing CCR6⁺ T cells.

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

Having demonstrated 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 as compared to those in specific-pathogen free (SPF) 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). Since 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 those from SPF mice (Figure S3A). 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 SPF mice (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 individuals (controls) 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 healthy skin has higher alpha diversity compared to 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, mice were applied with *Corynebacterium pseudodiphtheriticum*. 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 *Corynebacterium 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 (Lowe 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 proteolytic activation of pro-IL-1 β by protease caspase-1 (Lopez-Castejon and Brough, 2011). Previous studies demonstrate 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), 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 use IL-1 β antibody which recognizes mature form of IL-1 β (17 kDa) and demonstrate 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 revealed by immunofluorescent staining. Macrophages and DCs also produce IL-1 β . In contrast, macrophages and DCs in the IMQ-treated skin are the major IL-1 β producer. Keratinocytes do not secrete appreciable level 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 observe a low but detectable level of IL-1 β in normal human skin and naive 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 to SPF 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 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 while Firmicutes in controls, 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* which belongs to

Actinobacteria phylum is significantly elevated at the genus level in psoriatic lesional skin (Alekseyenko et al., 2013). Intriguingly, application of *Corynebacterium pseudodiphtheriticum* in mouse skin increases IL-1 β production along with increased dermal $\gamma\delta$ 17 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 human.

IL-1 β is known to be critical in inducing Th17 and $\gamma\delta$ 17 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. Interestingly, IL-23-induced IL-17 production is dependent on IL-1R signaling, suggesting 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 IMQ-induced skin inflammation model although has been widely used in preclinical psoriasis studies, there are many limitations with this model such as unintended 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 $\gamma\delta$ 17 cells (Cai et al., 2011, Cai et al., 2014) and human V δ 1 and V δ 2 $\gamma\delta$ 17 cells as well as circulating V δ 2 $\gamma\delta$ 17 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 $\gamma\delta$ 17 from the periphery.

In summary, we demonstrate that IL-1 β -IL-1R signaling is essential in regulating skin immunosurveillance and inflammation. Endogenous low level of IL-1 β stimulated by skin commensals maintains $\gamma\delta$ 17 homeostasis in mouse skin while microbiota dysbiosis upregulates IL-1 β leading to expanded $\gamma\delta$ 17 cells which may prime skin for the induction of inflammation. IL-1 β can directly activate $\gamma\delta$ 17 cells in mice and also stimulate keratinocytes for chemokine secretion which chemoattracts more IL-17 capable producing cells from the periphery, thus establishing an amplified inflammatory response, leading to skin inflammation such as psoriasis pathogenesis.

Material and Methods

Mice.

C57BL/6 WT, *TCR δ ^{-/-}*, and *Il1r1^{-/-}* mice were purchased from the Jackson Laboratory. GF mice were kindly provided by Dr. Yang-xin Fu (University of Chicago & UT Southwestern). All animals were housed and treated in accordance with institutional guidelines and approved by the IACUC at the University of Louisville.

Human Subjects:

See the supplemental material and methods.

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 supplemental material and methods.

Flow cytometry analysis and intracellular cytokine staining.

See the supplemental material and methods.

T cell chemotaxis assay.

See the supplemental material 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 $5-10 \times 10^6$ thymocytes from neonatal mice 0–48 hours after birth. After 24 hours, the recipient mice received $5-10 \times 10^6$ bone marrow (BM) cells. All chimeric mice were allowed to reconstitute for at least 8 weeks before use in experiments.

Establishment of psoriasis-like mouse models.

Imiquimod-induced psoriasis-like mouse model was established as previously described (Cai et al., 2014). The detailed methods are described in the supplemental material and methods.

Topical association of bacteria.

Corynebacterium pseudodiphtheriticum (ATCC 10700) 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 (OD) at 600nm using a spectrophotometer.

For topical association of bacteria, each mouse was associated with bacteria by applying bacterial suspension (approximately 1×10^9 c.f.u./ml, 0.5ml for two ears). Application of bacterial suspension was repeated every other day a total of four times. Mice were sacrificed at two weeks.

Skin histology, IHC staining, and Immunofluorescence staining.

See the supplemental material and methods.

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

See the supplemental material and methods.

Statistical analysis.

All quantitative data are shown as mean \pm s.e.m unless otherwise indicated. All samples were compared using two-tailed, unpaired Student's *T* test. A *P* value less than 0.05 was considered significant. Statistical analysis was performed with GraphPad Prism software.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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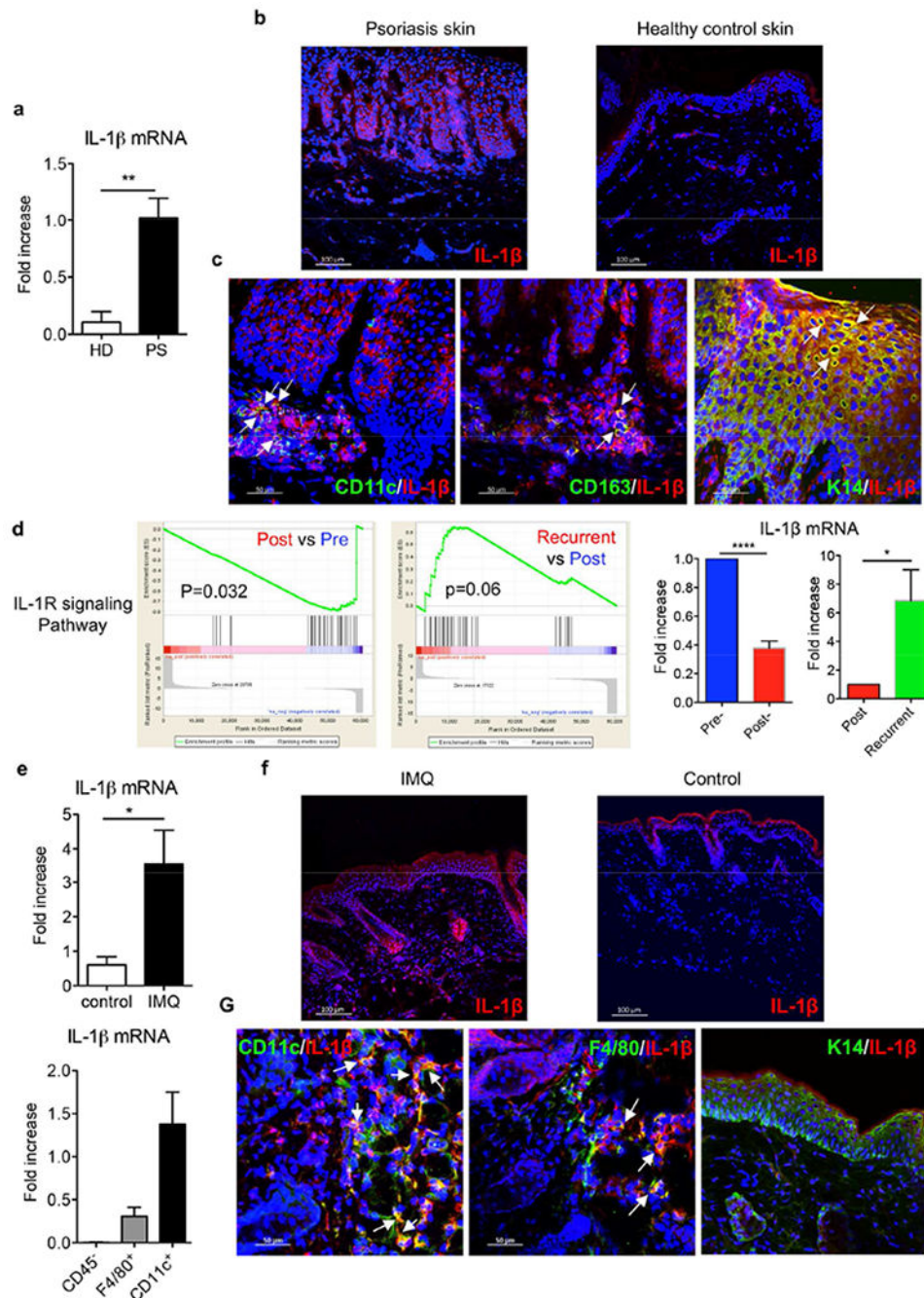


Figure 1. IL-1 β expression was significantly increased in both murine and human psoriatic lesions.

(a) IL-1 β mRNA levels from skin biopsies 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) and human CD11c mAb (green) or human CD163 mAb (green) or keratin 14 mAb (green), and DAPI (blue). Scale bar =50 μ m. (d) Gene set enrichment analysis identifies significant transcriptional downregulation of IL-1R signaling pathway in psoriasis

patients initially effectively treated with glucocorticoid while upregulation in recurrent patients after stopping treatment (NCBI GEO with the accession number GSE114729). The mRNA expression of IL-1 β by real-time PCR analysis was calculated using Pre- or Post-treatment level as 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) and mouse CD11 c mAb (green), or 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.

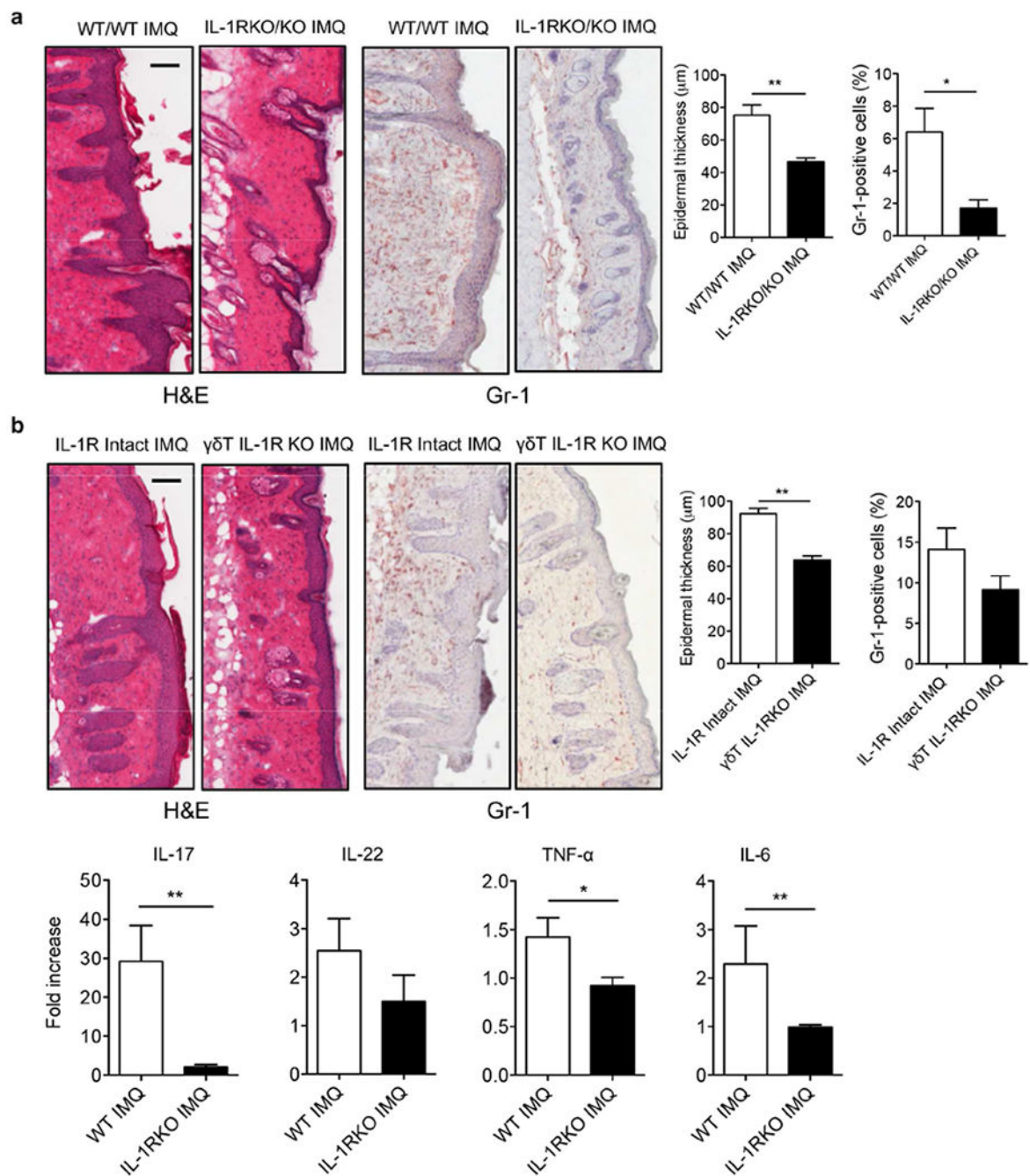


Figure 2. IL-1R signaling in $\gamma\delta$ T cells is essential to induce skin inflammation.

(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 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 assessed by flow cytometry. Epidermal thickness and percentage of CD45⁺Gr-1⁺ cells were measured. 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 real-time PCR analysis. *p< 0.05, **p< 0.01.

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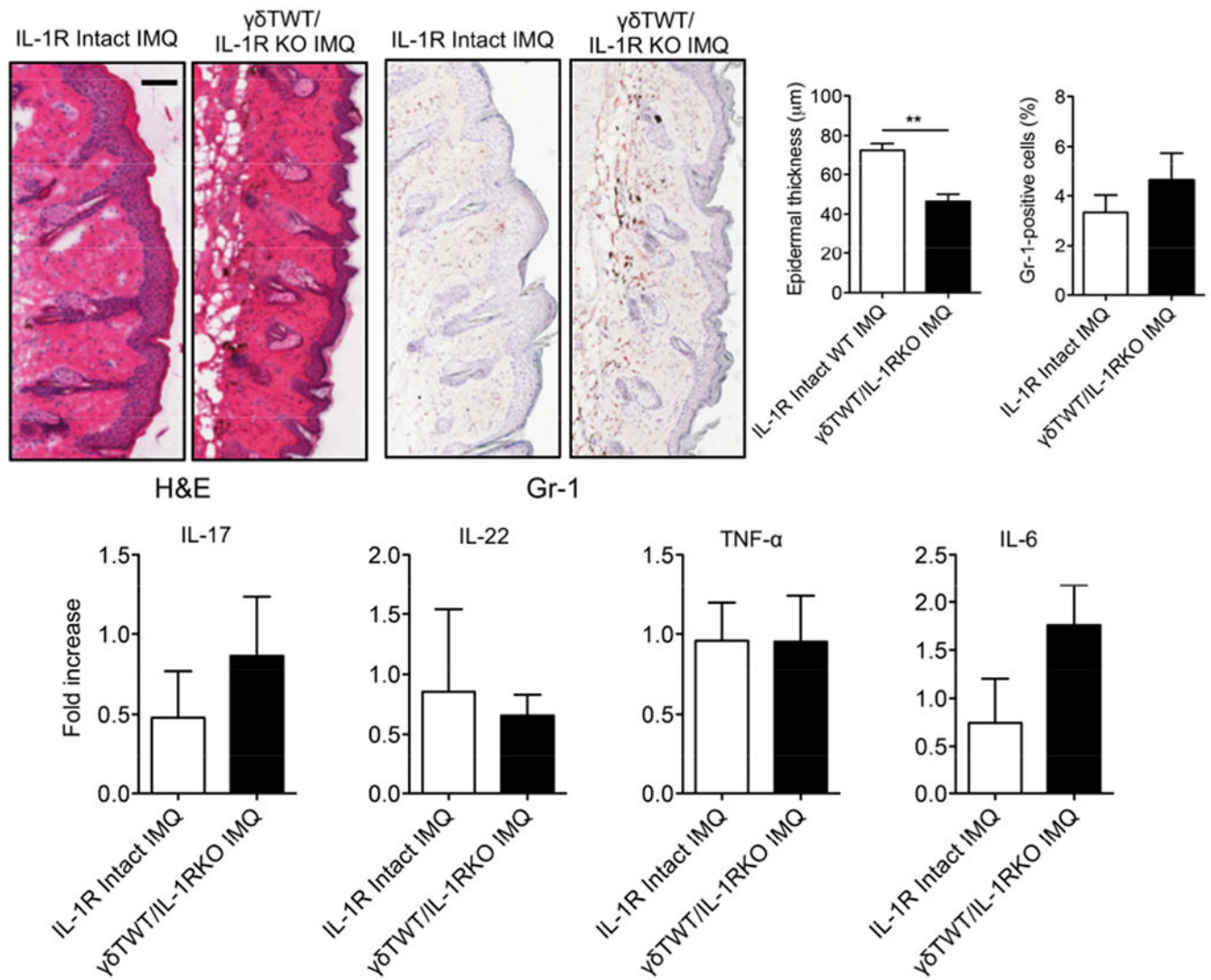


Figure 3. IL-1R signaling in other cells is important to induce full-fledged skin inflammation. Reconstituted mice ($n=3$) with WT $\gamma\delta$ T cells and IL-1R intact or deficiency in other cells were treated daily for 5 days with IMQ or vehicle control. Representative H&E-stained sections and frozen 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 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 qPCR analysis. * $p < 0.05$.

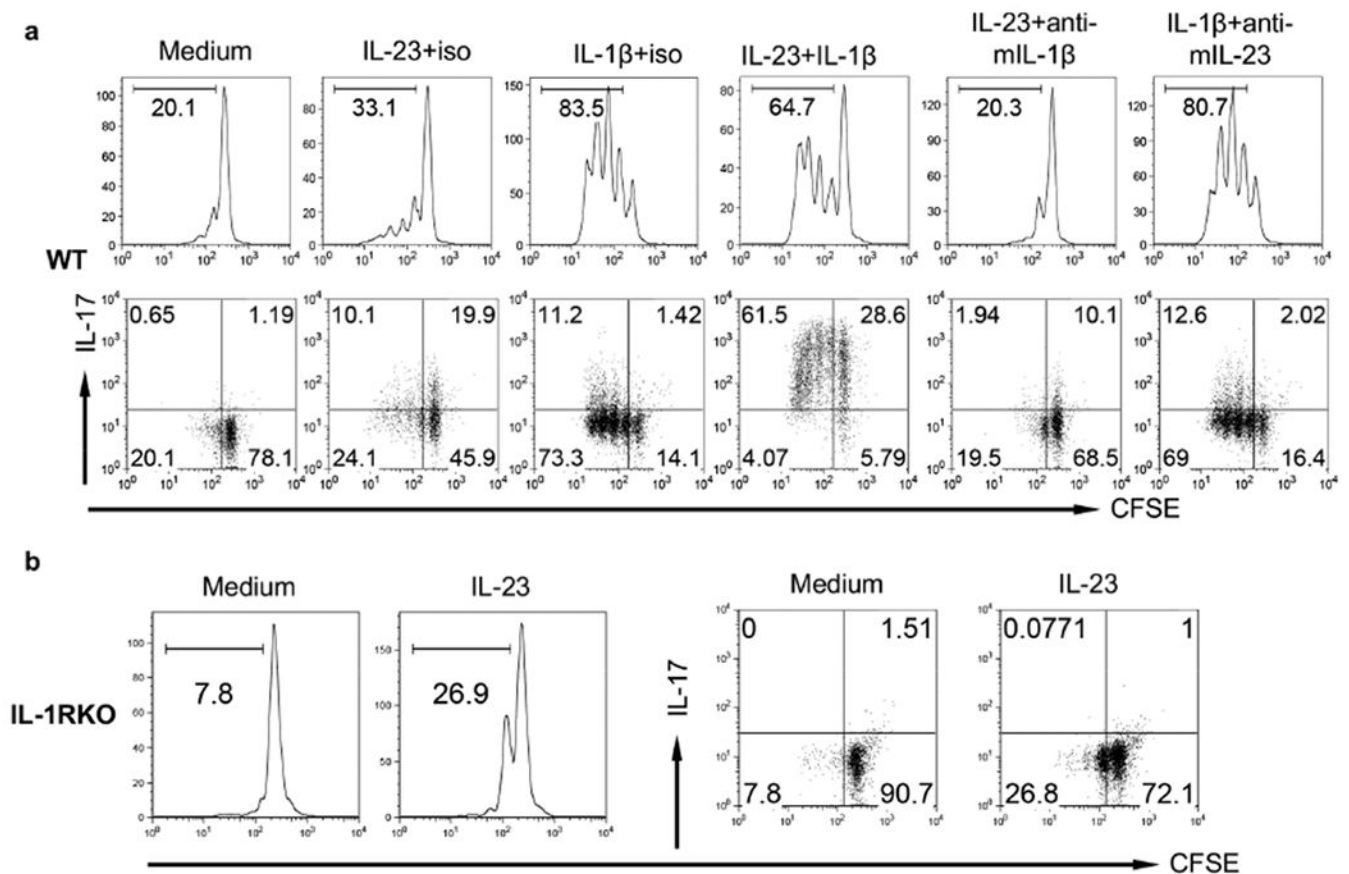


Figure 4. IL-1R signaling is essential in dermal $\gamma\delta$ T cell proliferation and IL-17 production.

(a) Whole skin cell suspensions from C57BL/6 WT mice were labeled with CFSE and then stimulated with IL-23, IL-1 β , 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.

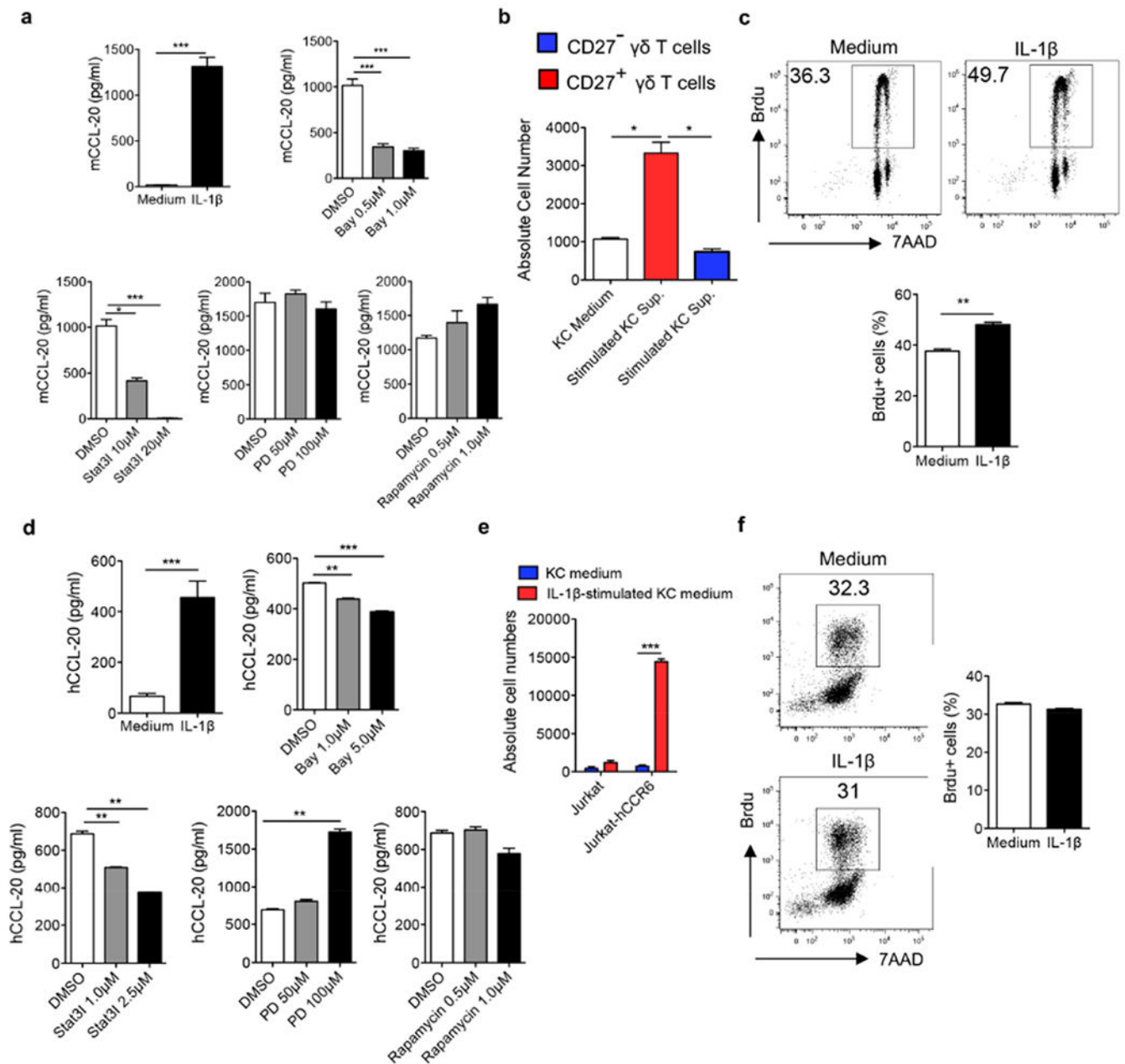


Figure 5. IL-1 β stimulates keratinocytes (KCs) to secrete chemokines, which are capable of chemoattracting $\gamma\delta$ T17 cells.

(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.

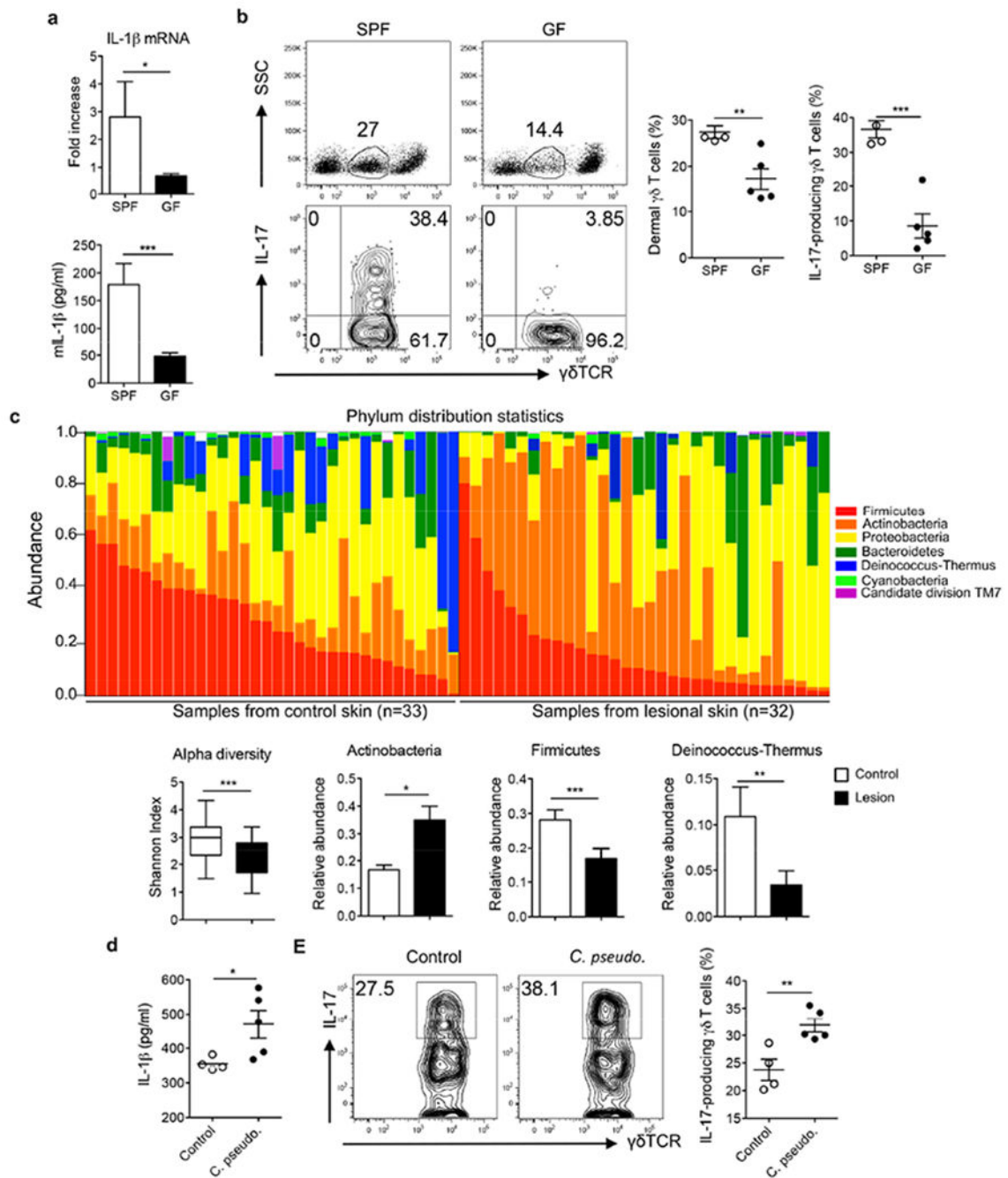


Figure 6. Skin commensals or altered skin microbiota contribute to IL-1 β -mediated dermal $\gamma\delta$ T17 expansion.

(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 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 *Corynebacterium 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.

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