

IMMUNOLOGY

p38 α signaling in Langerhans cells promotes the development of IL-17–producing T cells and psoriasiform skin inflammation

Tingting Zheng,^{1*} Weiheng Zhao,^{1*} Hongjin Li,^{1*†} Shuxiu Xiao,¹ Ran Hu,¹ Miaomiao Han,² Heng Liu,³ Ye qiang Liu,⁴ Kinya Otsu,^{5,6} Xinguang Liu,³ Gonghua Huang^{1‡}

Dendritic cells (DCs) contribute to psoriasis pathogenesis. In a mouse model of imiquimod-induced psoriasiform skin inflammation, we found that p38 α activity in Langerhans cells (LCs), a skin-resident subset of DCs, promoted the generation of T cells that produce IL-17, a proinflammatory cytokine that is implicated in autoimmune disease. Deletion of p38 α in LCs, but not in other skin or circulating DC subsets or T cells, decreased T cell–mediated psoriasiform skin inflammation in mice. The activity of p38 α in LCs specifically promoted IL-17 production from $\gamma\delta$ and CD4⁺ T cells by increasing the abundance of IL-23 and IL-6, two cytokines that stimulate IL-17 secretion. Inhibition of p38 activity through either pharmacological inhibition or genetic deletion also reduced the severity of established psoriasiform skin inflammation. Together, our findings indicate a critical role for p38 α signaling in LCs in promoting inflammatory responses in the skin and suggest that targeting p38 α signaling in LCs may offer an effective therapeutic approach to treat psoriasis.

INTRODUCTION

Psoriasis is a common chronic inflammatory skin disease characterized by epidermal hyperplasia, erythematous plaque formation, and the inflammatory cell infiltration in the dermis and epidermis (1, 2). Psoriasis is traditionally regarded as a local skin disease, but it is also associated with many systemic inflammatory diseases, such as diabetes and cardiovascular disease (3). The etiology and pathogenesis of psoriasis are not fully understood, and accumulating evidence indicates that immune dysfunction plays pivotal roles in disease development (3, 4). Psoriasis was initially classified as a disease mediated by the T helper type 1 (T_H1) cell response; however, cytokines released by interleukin-17 (IL-17)–producing T cells such as IL-17, tumor necrosis factor- α (TNF α), IL-23, and IL-22 appear to be critical for the development of psoriasis (3, 5–7). Many of these cytokines are increased in psoriasis skin lesions (2, 8), and many psoriasis patients have been effectively treated by using a new generation of drugs that selectively target TNF α , IL-23, and IL-17 (9). Although these cytokine antagonists provide higher efficacy, they can cause adverse side effects, such as increased risk of serious infection, cardiovascular disorders, and cancer development in psoriasis patients (10). The TNF α antagonist paradoxically induces new onset psoriasis or aggravates preexisting quiescent psoriatic disease by an unknown mechanism (11). Moreover, these antagonist treatments are costly, and the responses are variable between different

psoriasis patients (10). Thus, therapeutic responses could be improved by characterization of the upstream cellular and molecular mechanisms that regulate generation of IL-17–producing T cells during psoriasis pathogenesis.

Dendritic cells (DCs) are the key sentinels of the immune system that bridge innate and adaptive immunity (12) and are critical for the development of psoriasis (13). DCs are a highly heterogeneous population, and different tissues have different DC subsets with different functions (14). Although much effort has been made to identify the precise taxonomy for skin DCs, the current classification for skin DC subsets is still somewhat complicated. There are at least three subsets of DCs in the steady-state human and mouse skin: epidermal Langerhans cells (LCs), dermal myeloid DCs (dDCs), and plasmacytoid DCs (pDCs) (15, 16). According to their surface expression of langerin, mouse dDCs can be further divided into langerin⁺ dDCs and langerin[–] dDCs (17). Under steady state, langerin⁺ dDCs are recruited from the blood to the dermis as well as capture tissue antigens and present these antigens to naïve T cells in the draining lymph nodes (DLNs) (17).

In an imiquimod (IMQ)–induced psoriasis-like dermatitis (18), IL-23 produced by either langerin[–] dDCs or LCs drives psoriatic plaque formation in mice (19, 20). Moreover, in an IL-23–induced psoriasis-like mouse skin inflammation (21), monocyte-derived inflammatory LCs and dDCs mediate the disease pathogenesis (22). DC-specific deletion of ABIN-1 (A20 binding and inhibitor of nuclear factor κ B 1) restricts Toll-like receptor–induced IL-23 production and protects mice from IMQ-induced psoriasis (23). IL-36 mediates the DC-keratinocyte (KC) cross-talk in an IMQ-induced psoriasiform dermatitis by regulating the IL-23/IL-17/IL-22 pathway, but the in vivo cellular source of IL-36 in psoriasis pathogenesis still needs to be clarified (24). In addition, retinoic acid–inducible gene I (RIG-1)–antiviral signaling drives endogenous IL-23 production in DCs to further promote psoriasis-like disease (25). Thus, despite strong evidence implicating DCs in psoriasis, the intracellular signaling pathways that regulate proinflammatory cytokines in psoriasis pathogenesis remains to be established.

Increased activity of p38 α mitogen-activated protein kinase, a central regulator of inflammatory responses, is associated with

¹Hongqiao International Institute of Medicine, Shanghai Tongren Hospital/Faculty of Basic Medicine, Shanghai Institute of Immunology, Key Laboratory of Cell Differentiation and Apoptosis of Chinese Ministry of Education, Shanghai Jiao Tong University School of Medicine, Shanghai 200025, China. ²Xin Hua Hospital affiliated to Shanghai Jiao Tong University School of Medicine, Shanghai 200092, China. ³Guangdong Provincial Key Laboratory of Medical Molecular Diagnostics, Guangdong Medical University, Dongguan 523808, China. ⁴Shanghai Skin Disease Hospital, Tongji University, Shanghai 200443, China. ⁵Department of Cardiovascular Medicine, Graduate School of Medicine, Osaka University, Osaka 565-0871, Japan. ⁶Cardiovascular Division, King's College London, London WC2R 2LS, UK.

*These authors contributed equally to this work.

†Present address: Department of Dermatology, Yueyang Hospital of Integrated Traditional Chinese and Western Medicine, Shanghai University of Traditional Chinese Medicine, Shanghai 200437, China.

‡Corresponding author. Email: gonghua.huang@shsmu.edu.cn

susceptibility to psoriasis in humans (26) and is characteristic of psoriatic skin lesions (27). p38 activity is also increased in KCs stimulated by stress stimuli, such as cytokines and ultraviolet irradiation (28). These findings indicate that targeting p38 could be a promising strategy to treat psoriasis (29). Unfortunately, p38 inhibitors have not shown efficacy in the treatment of psoriasis, and many have been withdrawn from clinical trials because of adverse side effects (28). Thus, it would be useful to define the cell types in which p38 is active in psoriasis.

Using a combination of genetic and molecular approaches, we report here that p38 α deletion in LCs, but not in other DC subsets or T cells, reduced IMQ-induced psoriasiform skin inflammation. Mice with p38 α -deficient LCs produced much less IL-23 and IL-6 in response to IMQ treatment. Consequently, when challenged with IMQ, mice with a DC-specific deficiency in p38 α showed reduced IL-17 production from $\gamma\delta$ and CD4⁺ T cells. p38 α signaling in LCs specifically promoted IL-17 production in $\gamma\delta$ and CD4⁺ T cells through secretion of IL-23 and/or IL-6. However, p38 α signaling in LCs was dispensable for the generation of interferon- γ (IFN γ)-, IL-4-, and Foxp3-expressing T cells. Intradermal injection of IL-23 restored the skin inflammation and proinflammatory cytokine expression in mice lacking p38 α in DCs. In contrast, IL-6 injection partially restored skin inflammation and proinflammatory cytokine expression. Inhibition of p38 activity also reduced the severity of an established psoriasiform skin inflammation. Thus, this study links p38 α signaling in LCs and IL-23 or IL-6 with IL-17 production from $\gamma\delta$ and CD4⁺ T cells, and it provides cellular and molecular mechanisms by which p38 α regulates susceptibility to psoriasis.

RESULTS

Deletion of p38 α in DCs ameliorates IMQ-induced psoriasiform skin disease in mice

In the IMQ-induced mouse psoriasiform skin disease model (18), p38 activity in CD45⁺ leukocytes was higher in IMQ-treated mouse skin than in control cream-treated mouse skin (Fig. 1A). Because p38 α

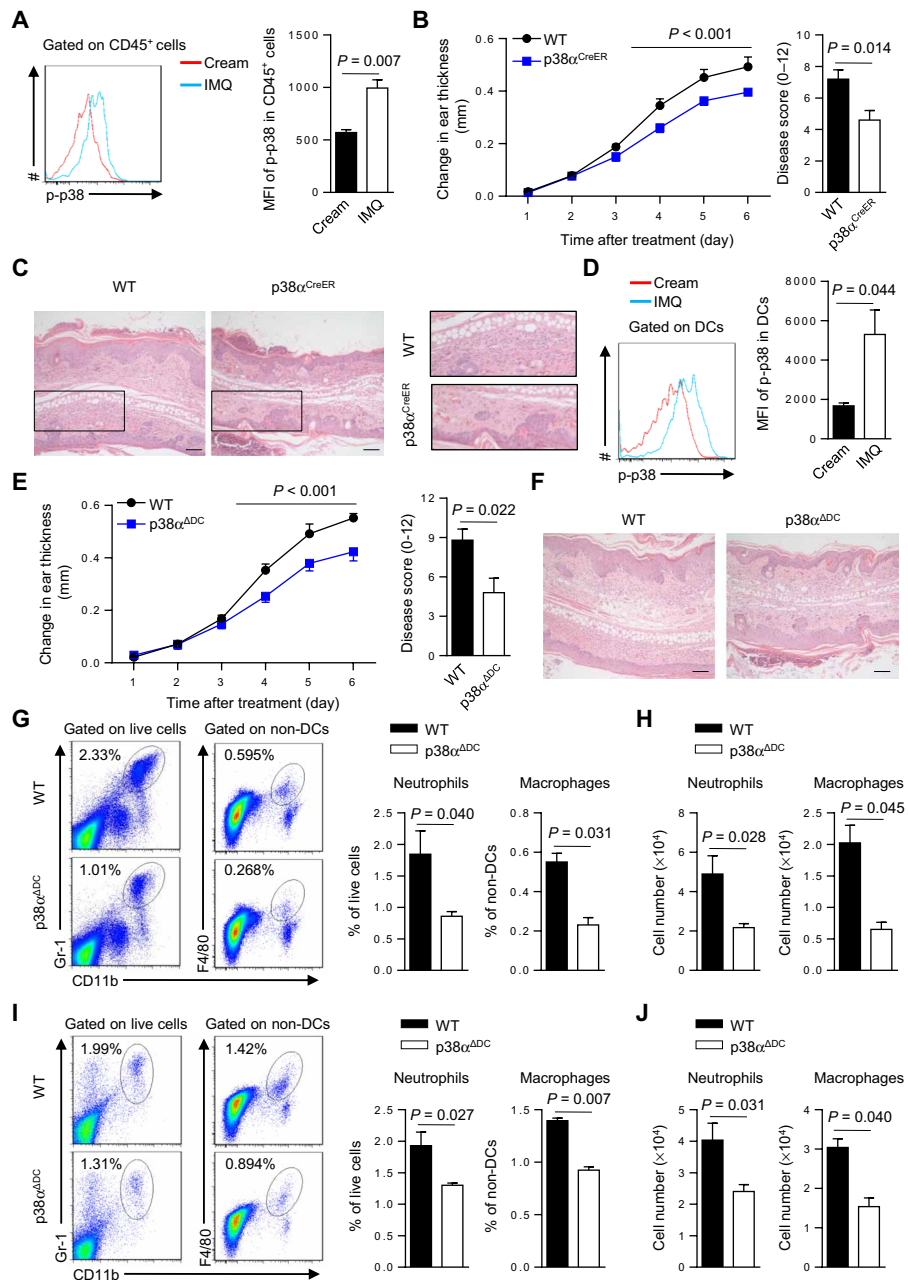


Fig. 1. Deletion of p38 α in DCs reduces IMQ-induced skin inflammation in mice. (A) Wild-type (WT) mice were topically treated with imiquimod (IMQ)-containing or control cream for two consecutive days, and the phosphorylation (p) of p38 in skin CD45⁺ cells was analyzed by flow cytometry ($n = 6$ mice per group). MFI, mean fluorescence intensity. (B and C) Tamoxifen-pretreated WT and p38 α ^{CreER} mice were topically treated with IMQ for six consecutive days. Change in ear thickness (left) and disease severity score (right) were recorded (B) ($n = 5$ mice per group). Histopathological changes in skin tissue of WT (left) and p38 α ^{CreER} (middle) mice were examined by hematoxylin and eosin (H&E) staining ($n = 3$ mice per group), and the marked area was magnified (right) (C). Scale bars, 200 μ m. (D) p-p38 in skin dendritic cells (DCs) was analyzed by flow cytometry in WT mice topically treated with control or IMQ-containing cream for two consecutive days ($n = 6$ mice per group). (E to J) WT and p38 α ^{ADC} mice were treated with IMQ for six consecutive days. Change in ear thickness (left) and disease severity score (right) (E) ($n = 5$ mice per group), representative images of H&E staining of skin sections ($n = 3$ mice per group) (F), the percentages (G) and cell numbers (H) of neutrophils and macrophages in the epidermis ($n = 4$ mice per group), and the percentages (I) and cell numbers (J) of neutrophils and macrophages in the dermis ($n = 4$ mice per group). Scale bars, 200 μ m. Two-sided Student's t tests [right panels of (A), (B), (D), and (E)]; (G) to (J)] and two-way analysis of variance (ANOVA) [left panels of (B) and (E)] were performed, and data are means \pm SEM. Data are representative of three (A to D) or four (E to J) independent experiments.

is the dominant p38 isoform that is expressed in immune cells (30), we generated *Mapk14^{fl/fl}Rosa26-Cre-ER^{T2}* mice [referred to as “p38^{CreER}” mice here, as described in our previous study (30)] to determine the role of p38 in psoriasis pathogenesis and detected efficient deletion of p38 α in skin tissue (fig. S1A). We explored the development of IMQ-induced psoriasiform skin inflammation in wild-type (WT) and p38^{CreER} mice, which were pretreated with tamoxifen to acutely delete p38 α in skin tissue. We found that p38^{CreER} mice had reduced psoriatic symptom severity than WT mice, including ear swelling, epidermal hyperplasia, and skin inflammatory cell infiltration (Fig. 1, B and C), suggesting that p38 α signaling could promote the development of IMQ-induced psoriasiform skin inflammation.

Consistent with the role of DCs in psoriasis development (13), IMQ-treated mouse skin had an increased percentage and number of DCs than control cream-treated skin (fig. S1B). In addition, p38 activity was higher in IMQ-treated skin DCs (Fig. 1D), suggesting that p38 α signaling in DCs might play a pivotal role in the immune mechanisms during psoriasis development. To delineate the specific role of DC-intrinsic p38 α signaling in psoriasis pathogenesis, we generated *Mapk14^{fl/fl}CD11c-Cre* mice [referred to as “p38^{ADC}” mice here (30)]. We observed efficient deletion of p38 α in skin DCs (fig. S1C), which did not affect the percentages or activation status of DCs in either the epidermis or dermis (fig. S2, A and B). When treated with IMQ-containing cream, p38^{ADC} mice had much less ear swelling and reduced composite psoriasis score than WT mice (Fig. 1E). Histological analysis showed that the skin of p38^{ADC} mice had less epidermal hyperplasia and inflammation (Fig. 1F). Flow cytometry analysis showed that the infiltration of neutrophils and macrophages in the skin was increased after IMQ treatment (fig. S3, A and B). However, infiltration of neutrophils and macrophages into the epidermis and dermis was reduced in p38^{ADC} mice (Fig. 1, G to J). Notably, the percentages and cell numbers of LCs, CD4⁺ T cells, and $\gamma\delta$ T cells, which are key producers of IL-17 during psoriasis, were comparable in the epidermis of IMQ-treated WT and p38^{ADC} mice (fig. S3, C and D). Furthermore, there were no significant differences in neutrophil or macrophage numbers in the spleens of IMQ-treated WT and p38^{ADC} mice (fig. S3, E and F). Collectively, these findings implicate a key role for DC-mediated p38 α signaling in IMQ-induced psoriatic skin disease.

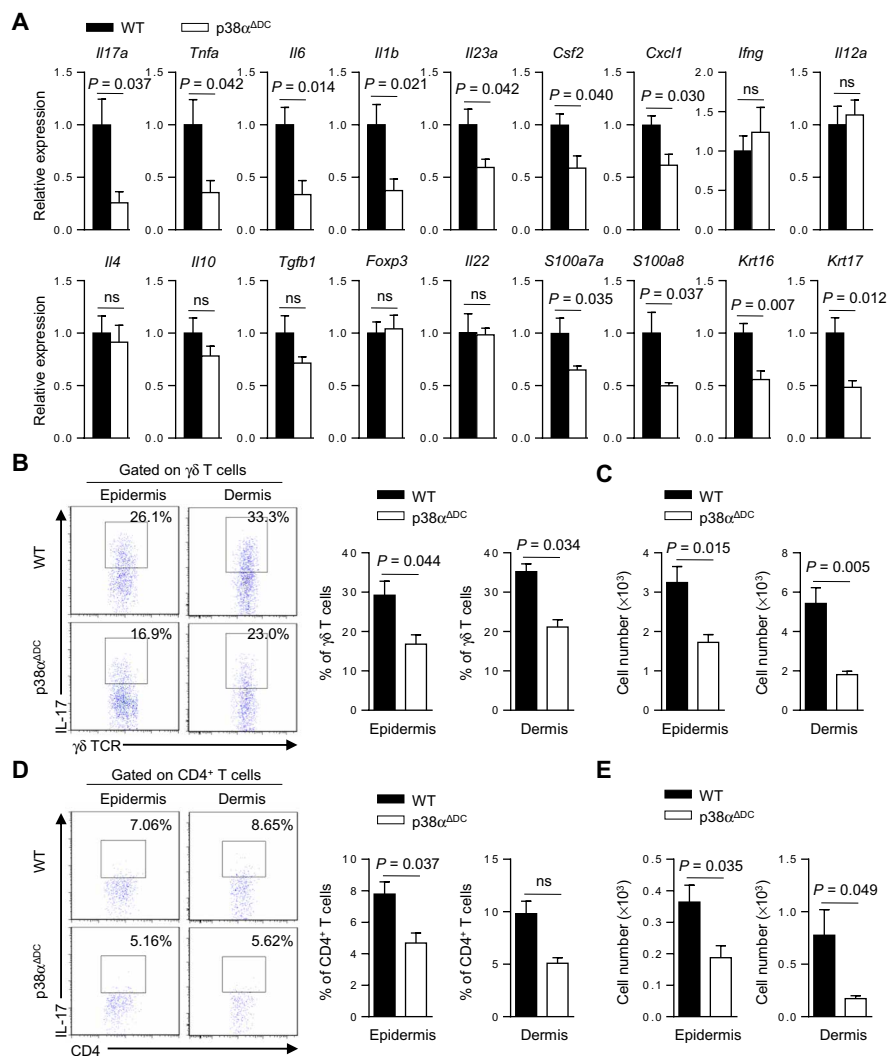


Fig. 2. p38 α activity in DCs is required for the generation of IL-17-producing T cell in vivo. WT and p38^{ADC} mice were topically treated with IMQ for six consecutive days. (A) Relative mRNA expression of inflammation-related genes in skin tissue was examined ($n = 5$ mice per group). ns, not significant. (B and C) The percentages (B) and cell numbers (C) of interleukin-17-positive (IL-17⁺) $\gamma\delta$ T cells in the epidermis and dermis ($n = 6$ mice per group). $\gamma\delta$ TCR, $\gamma\delta$ T cell receptor. (D and E) The percentages (D) and cell numbers (E) of IL-17⁺ CD4⁺ T cells in the epidermis and dermis ($n = 6$ mice per group). Two-sided Student's *t* tests were performed, and data are means \pm SEM. Data are representative of three independent experiments.

p38 α activity in DCs regulates the generation of IL-17-producing T cells in the skin

Consistent with the critical role of IL-17-producing T cell-mediated inflammatory responses in psoriatic disease pathogenesis (3), the skin tissues from IMQ-treated p38^{ADC} mice had lower expression of various related cytokine and chemokine mRNAs, such as *Il17a*, *Tnfa*, *Il6*, *Il1b*, *Il23a*, *Csf2*, and *Cxcl1* (Fig. 2A). However, expression of *Ifng*, *Il12a*, *Il4*, *Il10*, *Tgfb1*, and *Foxp3* was similar to that in WT mice (Fig. 2A). Although IL-22 plays an essential role in inflammatory skin disorders, including psoriasis (31–34), the expression of *Il22* mRNA was comparable between WT and p38^{ADC} mice (Fig. 2A). Moreover, certain genes encoding antimicrobial peptides such as *S100a7a* and *S100a8*, and genes responsible for KC proliferation including *Krt16* and *Krt17*, were also significantly decreased in p38^{ADC} mice (Fig. 2A

and fig. S4A). Consistent with the mRNA expression data, enzyme-linked immunosorbent assay (ELISA) showed that IL-17, TNF α , IL-1 β , and IL-6 production was lower in skin tissue from IMQ-treated p38 $\alpha^{\Delta DC}$ mice (fig. S4B). We found by flow cytometry that CD45 $^{+}$ cells, especially $\gamma\delta$ T cells, were a major source of IL-17 both in the epidermis and dermis (fig. S5, A and B), as previously demonstrated (5). The expansion of IL-17-producing $\gamma\delta$ T cells was greater than that of $\alpha\beta$ T cells in both the epidermis and dermis of WT mice (fig. S5, C and D), and the extent of this expansion was decreased in p38 $\alpha^{\Delta DC}$ mice (Fig. 2, B to E). The decreased IL-17 production from CD4 $^{+}$ and $\gamma\delta$ T cells was also observed in the DLNs of p38 $\alpha^{\Delta DC}$ mice, whereas the frequencies of IFN γ^{+} and Foxp3 $^{+}$ CD4 $^{+}$ T cells were similar in the two groups of mice (fig. S5, E and F). WT and p38 $\alpha^{\Delta DC}$ mice had comparable Ki-67 and active caspase-3 staining in both $\gamma\delta$ T cells and CD4 $^{+}$ T cells upon IMQ treatment (fig. S6, A and B), indicating that p38 α deficiency in DCs did not affect T cell proliferation and survival. These results demonstrate that p38 α signaling in DCs is specifically important for the generation of IL-17-producing T cells in mouse skin upon IMQ treatment.

p38 α activity in LCs is important for the generation of IL-17-producing T cells and the pathogenesis of psoriasis

We next sought to identify the DC subsets in mouse skin in which p38 α signaling is important for psoriasiform skin inflammation. For this, we transplanted bone marrow (BM) cells of WT or p38 $\alpha^{\Delta DC}$ mice into x-ray-irradiated WT or p38 $\alpha^{\Delta DC}$ mice to generate WT \rightarrow WT, WT \rightarrow p38 $\alpha^{\Delta DC}$, p38 $\alpha^{\Delta DC}$ \rightarrow WT, and p38 $\alpha^{\Delta DC}$ \rightarrow p38 $\alpha^{\Delta DC}$ chimeras. Two months after transplantation, chimeras were treated with IMQ to induce psoriasiform skin disease. Compared with IMQ-treated WT \rightarrow WT chimeras, WT \rightarrow p38 $\alpha^{\Delta DC}$ chimeras showed decreased ear thickness and composite psoriasis scores (Fig. 3A and fig. S7A). Histological analysis showed that the skin of WT \rightarrow p38 $\alpha^{\Delta DC}$ chimeras had substantially less epidermal hyperplasia and inflammation (Fig. 3B). Flow cytometry analysis showed that WT \rightarrow p38 $\alpha^{\Delta DC}$ chimeras had diminished infiltration of neutrophils in the epidermis and dermis (Fig. 3C and fig. S7B). Intracellular staining also showed lower IL-17 production from $\gamma\delta$ and CD4 $^{+}$ T cells in the DLNs of WT \rightarrow p38 $\alpha^{\Delta DC}$ chimeras than did those from WT \rightarrow WT chimeras (Fig. 3D and fig. S7C). Moreover, skin samples from WT \rightarrow p38 $\alpha^{\Delta DC}$ chimeras exhibited lower

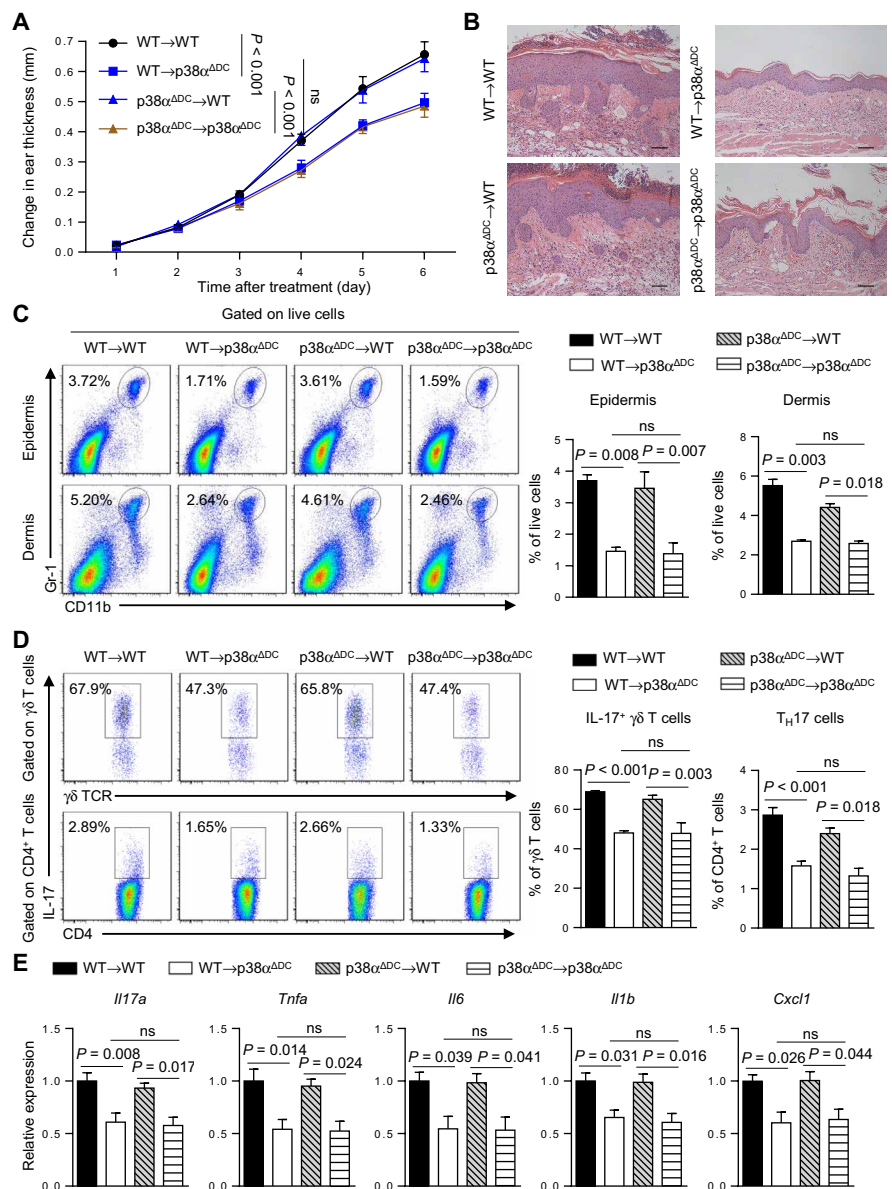


Fig. 3. p38 α signaling in LCs is important for the development of IMQ-induced skin inflammation. Bone marrow (BM) cells of WT or p38 $\alpha^{\Delta DC}$ mice were transplanted into x-ray-irradiated WT or p38 $\alpha^{\Delta DC}$ mice, respectively, to make the WT \rightarrow WT, WT \rightarrow p38 $\alpha^{\Delta DC}$, p38 $\alpha^{\Delta DC}$ \rightarrow WT, and p38 $\alpha^{\Delta DC}$ \rightarrow p38 $\alpha^{\Delta DC}$ chimeras. The chimeras were topically treated with IMQ for six consecutive days. (A) Change in ear thickness ($n = 6$ mice per group). (B) Representative images of H&E staining of skin section ($n = 3$ mice per group). Scale bars, 200 μ m. (C) The percentages of neutrophils in the epidermis and dermis ($n = 6$ mice per group). (D) The percentages of IL-17 $^{+}$ $\gamma\delta$ T cells and IL-17 $^{+}$ CD4 $^{+}$ T cells in the draining lymph nodes (DLNs) ($n = 6$ mice per group). (E) The relative expression of inflammation-related genes in skin tissue ($n = 6$ mice per group). Two-way ANOVA with Bonferroni post tests (A) and one-way ANOVA with Bonferroni post tests (C to E) were performed, and data are means \pm SEM. Data are representative of two independent experiments.

expression of *Il17a*, *Tnfa*, *Il6*, *Il1b*, and *Cxcl1* mRNA expression than WT \rightarrow WT chimeras (Fig. 3E). Because LCs are resistant to x-ray irradiation (35) and both WT \rightarrow WT and WT \rightarrow p38 $\alpha^{\Delta DC}$ chimeras contained the same dDC and circulating DC subsets but different LCs, these results indicate that p38 α signaling in LCs is important for IMQ-induced psoriasiform skin inflammation.

When transplanting WT or p38 $\alpha^{\Delta DC}$ BM cells into x-ray-irradiated WT mice, we found that both IMQ-treated WT \rightarrow WT and p38 $\alpha^{\Delta DC}$ \rightarrow WT chimeras had comparable ear thickness and composite psoriasis score

(Fig. 3A and fig. S7A), histological changes (Fig. 3B), as well as similar infiltration of neutrophils into the epidermis and dermis (Fig. 3C and fig. S7B). The IL-17 production from $\gamma\delta$ and $CD4^+$ T cells in the DLNs was comparable (Fig. 3D and fig. S7C). Moreover, mRNA expression of *Il17a*, *Tnfa*, *Il6*, *Il1b*, and *Cxcl1* was comparable between WT \rightarrow WT and p38 α^{ADC} \rightarrow WT chimeras (Fig. 3E). In addition, we found that WT \rightarrow p38 α^{ADC} and p38 α^{ADC} \rightarrow p38 α^{ADC} chimeras had comparable skin inflammation and cytokine production upon IMQ treatment (Fig. 3, A to E, and fig. S7, A to C). Thus, our results clearly show that p38 α activity in host radioresistant LCs, but not in dDCs, is important for the generation of IL-17-producing T cells and the pathogenesis of psoriasis.

Signaling through p38 α in LCs promotes IL-17-producing T cell generation and psoriasis pathogenesis by regulating the expression of IL-23 and IL-6

To assess whether p38 α signaling in LCs stimulates T cells to produce IL-17, we cultured LCs from WT and p38 α^{ADC} mice with $\gamma\delta$ T cells in the presence of R848 for 48 hours. $\gamma\delta$ T cells cocultured with p38 α^{ADC} LCs produced significantly less IL-17 than did those cocultured with WT LCs (Fig. 4A). To determine whether p38 α mediates LC-T cell cross-talk by driving the lineage differentiation of antigen-specific naïve precursors, we cultured naïve $CD4^+$ transgenic T cells specific for ovalbumin (OT-II) together with LCs isolated from WT and p38 α^{ADC} mice in the presence of cognate antigen and R848 for 5 days. Fewer T cells cocultured with p38 α^{ADC} LCs developed into IL-17 $^+$ cells than did those cocultured with WT LCs (Fig. 4B). This effect was associated with lower *Il17a* mRNA expression in T cells activated by R848-pulsed p38 α^{ADC} LCs, but comparable mRNA expression of *Ifng*, *Il4*, and *Foxp3* (fig. S8A). These results indicate that p38 α in LCs stimulates IL-17 production in T cells in vitro.

Next, we explored the molecular mechanisms by which p38 α acted in LCs to promote IL-17-producing T cell development. WT and p38 α^{ADC} mice expressed comparable costimulatory molecules, such as CD40, CD80, and CD86 in LCs upon IMQ treatment (fig. S8B). To determine whether p38 α signaling in LCs regulates

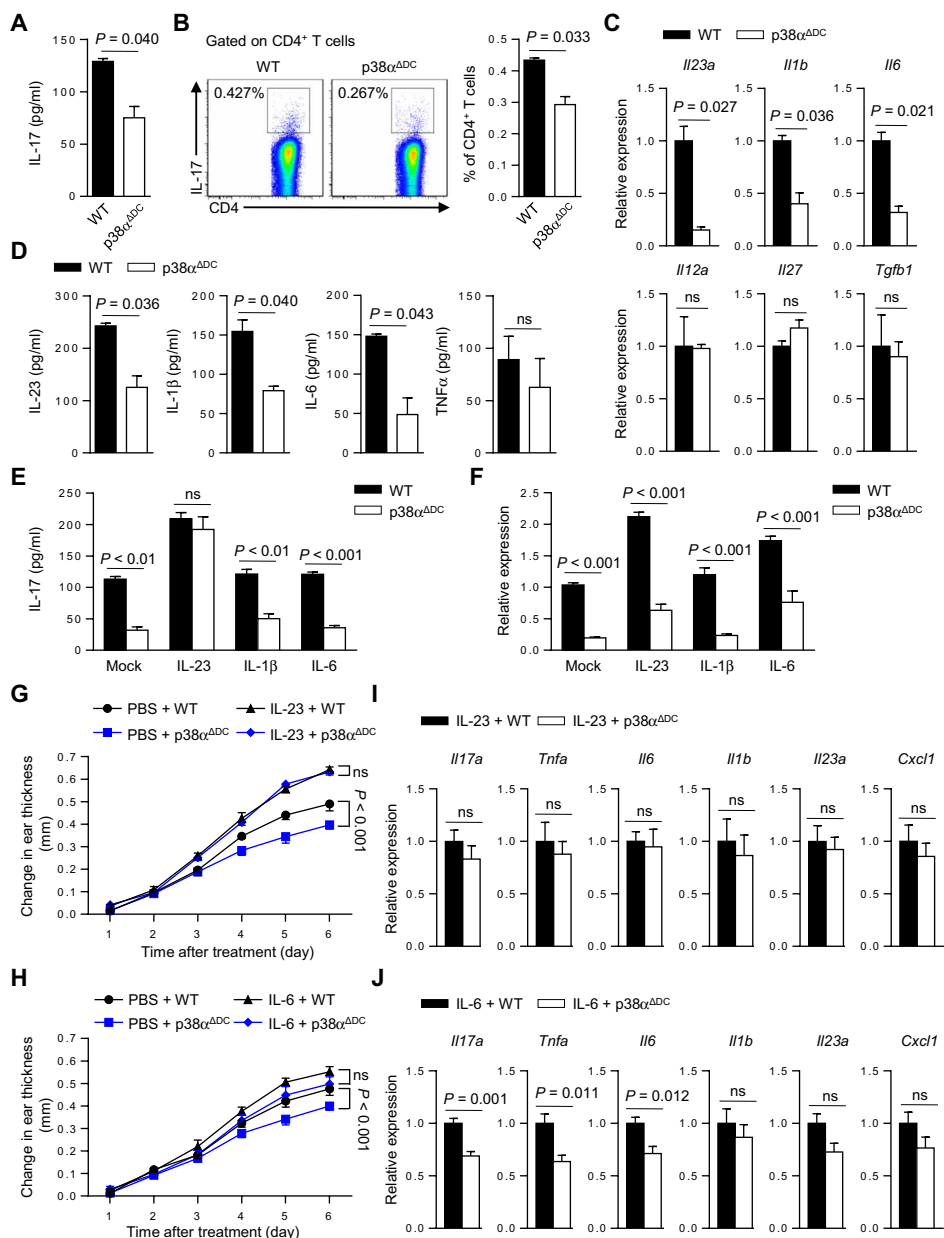


Fig. 4. Signaling by p38 α in LCs controls IL-17-producing T cell generation and skin inflammation by regulating the expression of IL-6 and IL-23. (A) IL-17 production in the supernatant of $\gamma\delta$ T cells cocultured with R848-stimulated WT and p38 α^{ADC} Langerhans cells (LCs) for 48 hours ($n = 3$ biological replicates). (B) The differentiation of T_H17 cells in $CD4^+$ T cells activated with R848-pulsed WT and p38 α^{ADC} LCs for 5 days ($n = 3$ biological replicates). (C and D) Cytokine expression from WT and p38 α^{ADC} LCs stimulated with R848 for 5 (C) and 24 hours (D) ($n = 3$ biological replicates). (E) IL-17 production from $\gamma\delta$ T cells cocultured with WT and p38 α^{ADC} LCs in the presence or absence of IL-23, IL-1 β , or IL-6 ($n = 3$ biological replicates). (F) Relative mRNA expression of *Il17* in $CD4^+$ T cells cocultured with WT and p38 α^{ADC} LCs in the presence or absence of IL-23, IL-1 β , or IL-6 ($n = 3$ biological replicates). (G) Change in ear thickness of IMQ-treated WT and p38 α^{ADC} mice subcutaneously injected with IL-23 or control phosphate-buffered saline (PBS) ($n = 5$ to 6 mice per group). (H) Change in ear thickness of IMQ-treated WT and p38 α^{ADC} mice subcutaneously injected with IL-6 or control PBS ($n = 5$ to 8 mice per group). (I and J) Inflammation-related gene expression of IMQ-treated WT and p38 α^{ADC} mice subcutaneously injected with IL-23 (I) or IL-6 (J) ($n = 5$ mice per group). Two-sided Student's t tests (A to D and I and J) and two-way ANOVA with Bonferroni post tests (E to H) were performed, and data are means \pm SEM. Data are representative of five (A and B), three (C and D and G to J), or four (E and F) independent experiments. Cells used in (A) to (F) were isolated from four to six mice per group.

the expression of cytokines that biases IL-17-producing T cell development, we stimulated LCs from WT and $p38\alpha^{\Delta DC}$ mice with R848 for either 5 or 24 hours. Among the cytokines that potentiate IL-17-producing T cell differentiation, the expression of IL-6, IL-1 β , and IL-23 in LCs was lower in $p38\alpha^{\Delta DC}$ mice than that in WT mice at both mRNA and protein levels (Fig. 4, C and D, and fig. S8C), but the mRNA levels of *Il12a*, *Il27*, and *Tgfb1* and the protein level of TNF α were comparable in LCs from WT and $p38\alpha^{\Delta DC}$ mice (Fig. 4, C and D).

To identify the cytokine lost in $p38\alpha^{\Delta DC}$ LCs that was responsible for reduced T cell IL-17 production, we added back recombinant IL-23, IL-1 β , or IL-6 to LC- $\gamma\delta$ T cell cocultures. The addition of IL-23 to $p38\alpha^{\Delta DC}$ LC- $\gamma\delta$ T cell cocultures completely restored the defective IL-17 production from $\gamma\delta$ T cells, whereas the addition of IL-1 β or IL-6 partially or did not restore the defective IL-17 production (Fig. 4E). In LC-CD4 $^+$ T cell cocultures, we found that addition of IL-23 or IL-6, but not IL-1 β , partially restored *Il17* expression in CD4 $^+$ T cells activated by $p38\alpha^{\Delta DC}$ LCs (Fig. 4F). Collectively, these data showed that $p38\alpha$ signaling orchestrated a program for LC-dependent IL-17-producing T cell differentiation.

We sought to further assess the functional importance of $p38\alpha$ -dependent cytokine production in WT and $p38\alpha^{\Delta DC}$ mice treated with IMQ to induce psoriasisiform inflammation. Intradermal injection of IL-23 and IL-6, but not IL-1 β , aggravated the severity of IMQ-induced psoriasisiform disease (Fig. 4, G and H, and fig. S9A). Injection of either IL-23 or IL-6, but not IL-1 β , restored ear swelling in $p38\alpha^{\Delta DC}$ mice (Fig. 4, G and H, and fig. S9A). In addition, IL-23 injection completely restored expression of *Il17a*, *Tnfa*, *Il6*, *Il1b*, *Il23a*, and *Cxcl1* in IMQ-treated $p38\alpha^{\Delta DC}$ mice. In contrast, IL-6 and IL-1 β restored expression of some cytokines (Fig. 4, I and J, and fig. S9B). Together, these results showed that $p38\alpha$ mediated the effect on IL-17-producing T cell development and psoriasis pathogenesis through distinct cytokines.

We explored the role of $p38\alpha$ signaling in different DC subsets on cytokine production and IL-17-producing T cell development with R848-stimulated dDCs from WT and $p38\alpha^{\Delta DC}$ mice. We found that compared with WT dDCs, $p38\alpha^{\Delta DC}$ dDCs secreted less IL-6 but comparable IL-23 and IL-1 β (fig. S10A). Next, we cocultured WT and $p38\alpha^{\Delta DC}$ dDCs with $\gamma\delta$ T or naive CD4 $^+$ T cells and found that CD4 $^+$ T cells activated by $p38\alpha^{\Delta DC}$ dDCs secreted less IL-17, whereas $\gamma\delta$ T cells activated by both WT and $p38\alpha^{\Delta DC}$ dDCs expressed comparable levels of IL-17 (fig. S10, B and C). Because $\gamma\delta$ T cells are the major IL-17-producing cells that are critical in IMQ-induced psoriasis (5), $p38\alpha$ signaling in different DC subsets instructs IL-17-

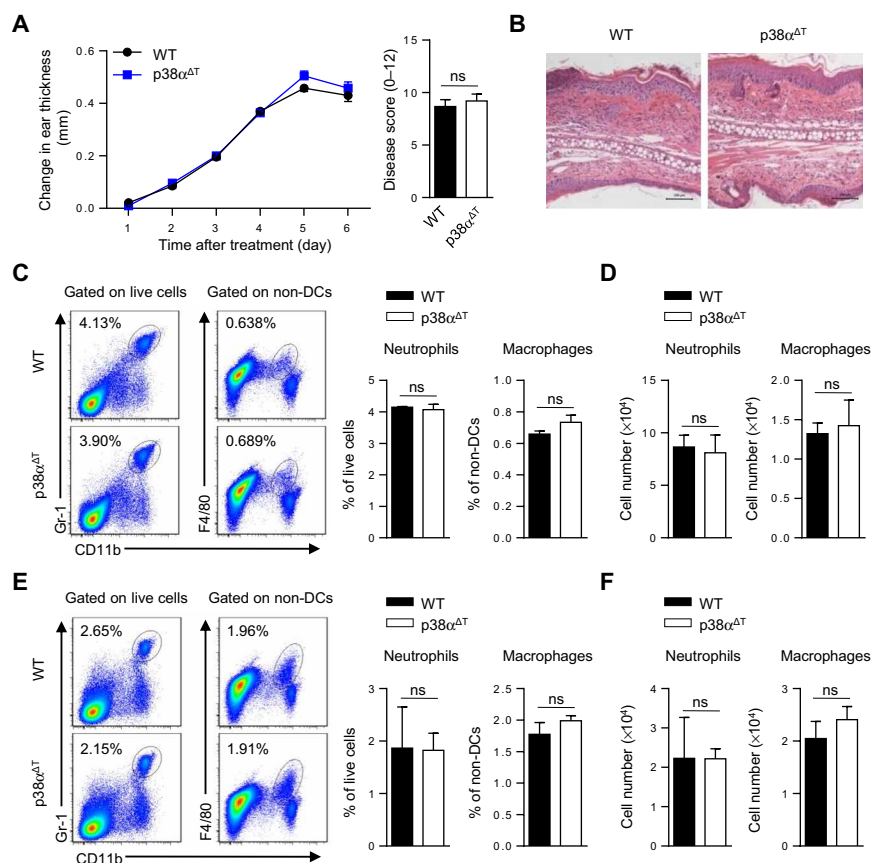


Fig. 5. $p38\alpha$ MAPK in T cells is dispensable for the induction of psoriasisiform inflammation. WT and $p38\alpha^{\Delta T}$ mice were topically treated with IMQ cream for six consecutive days. (A) Change in ear thickness (left) and disease severity score (right) ($n = 5$ to 6 mice per group). (B) Representative images of H&E staining in skin section ($n = 3$ mice per group). Scale bars, 200 μ m. (C and D) The percentages (C) and cell numbers (D) of neutrophils and macrophages in the epidermis ($n = 5$ to 6 mice per group). (E and F) The percentages (E) and cell numbers (F) of neutrophils and macrophages in the dermis ($n = 5$ to 6 mice per group). Two-way ANOVA [left panel of (A)] and two-sided Student's *t* tests [right panel of (A); (C) to (F)] were performed, and data are means \pm SEM. Data are representative of three independent experiments.

producing T cell generation and promotes psoriasis pathogenesis through the regulation of IL-23 expression.

IMQ-induced psoriasisiform skin disease does not require $p38\alpha$ in T cells

Although $p38\alpha$ signaling in T cells is not required for T_H17 cell differentiation (30), T cell-intrinsic $p38\alpha$ could potentially influence psoriasis pathogenesis by other mechanisms. To evaluate the potential role for T cell-dependent $p38\alpha$ activation in psoriasis, we generated *Mapk14^{fl/fl}CD4-Cre* mice [referred to as " $p38\alpha^{\Delta T}$ mice" here (30)], which efficiently ablates $p38\alpha$ from T cells (fig. S11A). T cell-specific deletion of $p38\alpha$ did not influence the severity or onset of psoriasis as indicated by comparable disease scores, pathological changes, and immune cell recruitment between IMQ-treated WT and $p38\alpha^{\Delta T}$ mice (Fig. 5, A to F). Moreover, genetic abrogation of $p38\alpha$ in T cells did not appreciably affect IL-17 production by $\gamma\delta$ T cells or the expression of *Il17a*, *Tnfa*, *Il6*, *Il1b*, *Il23a*, and *Cxcl1* in the skin (fig. S11, B and C). Thus, our results demonstrate that $p38\alpha$ signaling in T cells does not affect IL-17-producing T cell generation or psoriasis development.

Inhibition of p38 ameliorates psoriasiform skin disease

To evaluate p38 α as a potential therapeutic target for the treatment of psoriasis, we examined whether inhibition of p38 activity can alleviate psoriasiform inflammation. When the p38 inhibitor SB203580 was injected into mice that had been previously treated with IMQ, we found that these mice had markedly diminished ear swelling and lower psoriasis disease scores than vehicle-treated mice (Fig. 6A). Histological analysis showed that the skin of SB203580-treated mice had substantially less epidermal hyperplasia and inflammation than vehicle-treated mice (Fig. 6B). The infiltration of neutrophils and macrophages into the epidermis and dermis was also reduced in SB203580-treated mice (Fig. 6, C to F). Moreover, the skin tissues

from SB203580-treated mice had lower expression of *Il17a*, *Tnfa*, *Il6*, *Il1b*, *Il23a*, and *Cxcl1* compared with vehicle-treated mice (Fig. 6G). However, the expression of *Il10* and *Tgfb1* in skin tissue was not affected by SB203580 treatment (Fig. 6G). IL-17 production by CD4⁺ and $\gamma\delta$ T cells was also decreased in the DLNs of SB203580-treated mice, whereas the frequencies of IFN γ ⁺ and Foxp3⁺ CD4⁺ T cells were similar between the two groups (Fig. S12, A and B). Because a secondary approach to evaluate whether ablation of p38 signaling can ameliorate established psoriatic disease, we administered tamoxifen to WT and p38 α ^{CreER} mice to acutely ablate p38 α expression on days 5 to 8 post-IMQ exposure. Consistent with our SB203580 treatment results, we observed substantial reductions in ear swelling, composite psoriasis score, neutrophil infiltration, the production of IL-17 from CD4⁺ and $\gamma\delta$ T cells, and the frequency of IFN γ ⁺ CD4⁺ T cells in the DLNs in tamoxifen-treated p38 α ^{CreER} mice (fig. S13, A to C). The frequency of Foxp3⁺ CD4⁺ T cells was similar in the DLNs from tamoxifen-treated WT and p38 α ^{CreER} mice (fig. S13C). These results collectively suggest that inhibition of p38 could offer an approach to treat psoriasis and other inflammatory skin diseases.

DISCUSSION

Although mounting evidence demonstrates essential roles for IL-17-producing T cell-mediated inflammation and DC-T cell cross-talk in the pathogenesis of psoriasis, DC-specific signaling pathways that regulate IL-17-producing T cell responses in the skin still remain poorly defined. Here, we report that p38 α signaling in LCs, but not in other DC subsets or T cells, centrally regulated IL-17 production by CD4⁺ and $\gamma\delta$ T cells in IMQ-induced psoriasis model, whereas leaving IFN γ -, IL-4-, and Foxp3-expressing T cell generation unaffected. p38 α activity in LCs differentially promoted IL-17 production from $\gamma\delta$ and CD4⁺ T cells by secreting IL-23 and IL-6, respectively. Our findings highlight a crucial role for an axis involving p38 α , IL-23, IL-6, and IL-17-producing T cells in psoriasis and suggest that targeting p38 α signaling in LCs may provide an attractive treatment for inflammatory skin disease.

The success of IL-17 blockade in the treatment of psoriasis patients underscores the central role of this cytokine in the pathophysiology of psoriasis (9). IL-17 can be secreted by multiple cell types, including CD4⁺ (T_H17), CD8⁺ (Tc17), $\gamma\delta$ ⁺ T cells, innate lymphocytes, and neutrophils (36). The cytokines IL-1 β , IL-6, IL-23, and TGF β (transforming growth factor- β) contribute

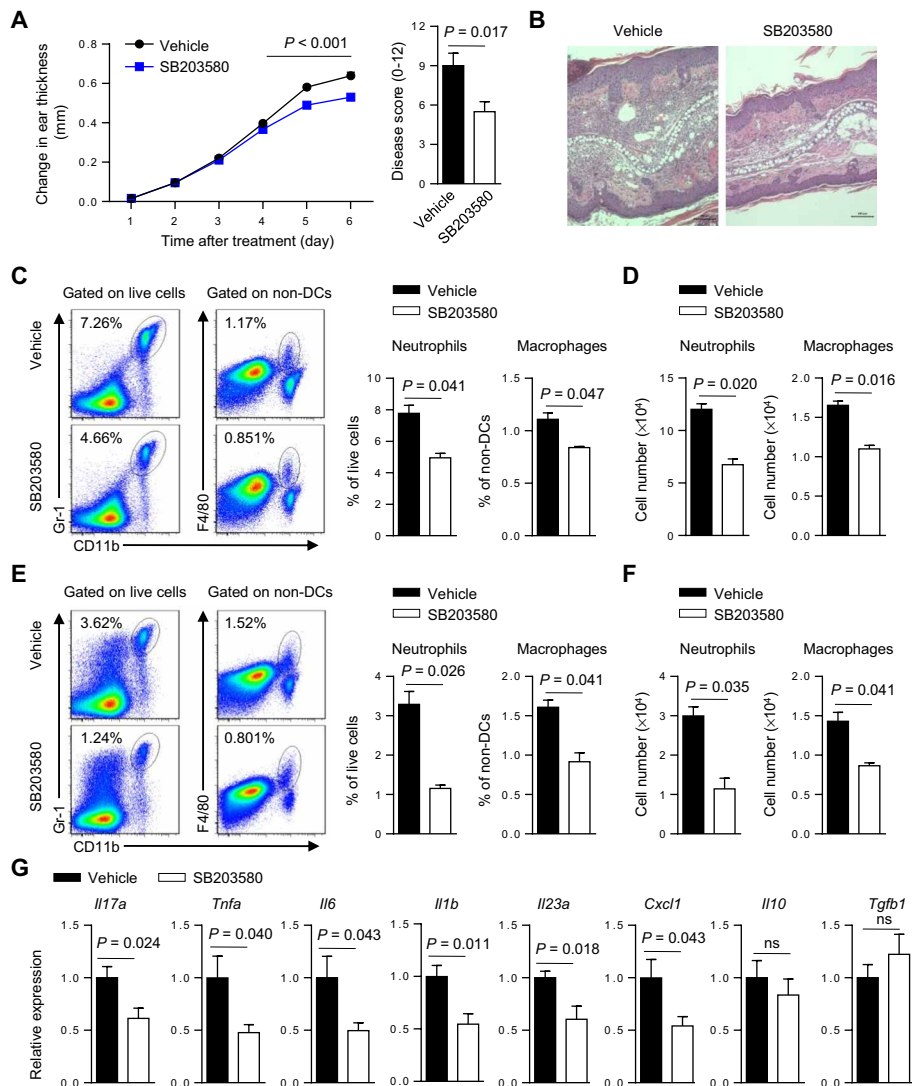


Fig. 6. Inhibition of p38 activity reduces disease severity in mice with established skin inflammation. WT mice were topically treated with IMQ for six consecutive days and received either the p38 inhibitor SB203580 or control vehicle daily by intraperitoneal injection from day 3. (A) Change in ear thickness (left) and disease severity score (right) ($n = 6$ mice per group). (B) Representative images of H&E staining in skin section ($n = 3$ mice per group). Scale bars, 200 μ m. (C and D) The percentages (C) and cell numbers (D) of neutrophils and macrophages in the epidermis ($n = 6$ mice per group). (E and F) The percentages (E) and cell numbers (F) of neutrophils and macrophages in the dermis ($n = 6$ mice per group). (G) Relative expression of inflammation-related genes in skin tissue ($n = 6$ mice per group). Two-way ANOVA [left panel of (A)] and two-sided Student's t tests [right panel of (A); (C) to (G)] were performed, and data are means \pm SEM. Data are representative of three independent experiments.

to the differentiation of T_H17 cells (37–39), but the importance of these individual cytokines in directing T_H17 cell development in vivo still remains controversial and may vary depending on the disease models, environmental factors, or target organs (40–42). Our previous work establishes that p38 α signaling in splenic DCs stimulates T_H17 cell differentiation through the production of IL-6, IL-27, and CD86 expression but is dispensable for IL-1 β and IL-23 production (30). In contrast, we showed in the current study that p38 α signaling in LCs regulated T_H17 cell differentiation independently of *Il27* and CD86 expression (Fig. 4C and fig. S8B). Furthermore, we discovered that IL-23 production downstream of p38 α signaling contributed to LC-mediated regulation of IL-17 production by $\gamma\delta$ T cells. However, p38 α was not required for IL-1 β and IL-23 production by skin dDCs, which was similar with our previous findings in splenic DCs (30). Thus, the discrepant regulation of specific cytokines by the same intracellular signaling pathway in splenic DCs or dDCs compared to skin LCs further highlights the functional heterogeneity of DCs. Further identification of the molecular pathways that underpin the cell-specific regulation inflammatory responses by p38 α is needed to unlock the full clinical potential of DC-targeted therapeutics.

DCs bridge innate and adaptive immunity by capturing antigens and migrating into lymph nodes to initiate protective immune responses (12). Although DC numbers are increased in psoriasis lesions, the results from different groups on the roles of DCs in psoriasis pathogenesis are still controversial because of the usages of different triggers, genetic models, and experimental time points (13, 19, 20, 22, 43–48). Our BM chimera results demonstrated that p38 α signaling in LCs, but not in other dDCs or inflammatory DCs, was crucial for IL-17-producing T cell development and disease pathogenesis. These results provided genetic evidence supporting the idea of a key proinflammatory function of LCs in psoriasis and that p38 α signaling could differentially regulate these effects in distinct cell types.

Because p38 plays an important role in the regulation of numerous proinflammatory responses and disease models (26, 49), p38 α has been extensively investigated for the treatment of inflammatory diseases. Although the advance of p38 inhibitors into clinical trials has been halted, further research into the relevant disease mechanisms could improve the clinical development of p38 inhibitors (50). Our results showing that inhibition of p38 α activity after the onset of disease reduced psoriasis disease progression suggest that p38 inhibitors could be effective for IL-17-mediated diseases. Moreover, our results imply that selectively targeting p38 α inhibitors to LCs ameliorated psoriasis symptoms, suggesting that new drug-delivery vehicles that target p38 α inhibitors to specific tissues or cell types could be a promising strategy for avoiding undesirable side effects (51). Local delivery of a p38 inhibitor to the lung reduces inflammation and decreases adverse effects by minimizing exposure of the nontarget organs to the drug (52). Given that current cytokine antagonist biologic treatments are expensive and have considerable adverse side effects (10), targeted delivery of small-molecule p38 inhibitors might provide a potential opportunity to improve psoriasis treatment. Therefore, understanding p38 α -dependent regulation of DC functions and subsequent T cell responses might be further exploited for innovative immune therapies.

MATERIALS AND METHODS

Animals

p38 α^{fllox} and CD11c Cre mice have been described previously (30, 53, 54). Rosa26-Cre-ER^{T2} and CD45.1⁺ mice were provided by B. Su (Shanghai

Jiao Tong University School of Medicine, China). CD4-Cre mice were provided by H. Wang (Shanghai Jiao Tong University School of Medicine, China). OT-II mice were purchased from The Jackson Laboratory. C57BL/6 mice were purchased from Shanghai SLAC Laboratory Animal Co., Ltd (Shanghai, China). All mice were backcrossed to C57BL/6 background for at least eight generations. Age- and sex-matched mice at 6 to 10 weeks of age were used for all experiments. WT or Cre⁺ littermate control mice were used where relevant. No adverse effects due to Cre expression itself were observed in vitro and in vivo in these studies. All mice were kept in specific pathogen-free conditions in the Animal Resource Center at Shanghai Jiao Tong University School of Medicine. Animal protocols were approved by Institutional Animal Care and Use Committee of Shanghai Jiao Tong University School of Medicine.

BM chimeras

For BM chimeric experiments, BM cells from WT or p38 α^{ADC} mice were intravenously transferred into lethally irradiated either WT or p38 α^{ADC} mice (5×10^6 BM cells per recipient), respectively. Recipient mice were treated with oral antibiotics for 2 weeks after transplantation.

In vivo tamoxifen treatment

WT and p38 α^{CreER} mice were intraperitoneally injected with 2 mg of tamoxifen (Sigma-Aldrich) per mouse for three consecutive days and then rested for 7 days before experiments.

IMQ-induced mouse psoriasiform skin disease model

A cream containing 5% IMQ (3M Pharmaceuticals or MedShine) providing a dose of 25 mg, or control Vaseline (Fagron), was topically applied to the ear of each mouse daily for six consecutive days. Ear thickness was measured daily using a micrometer, and skin inflammation was scored on day 6 on the basis of the extent and severity of erythema, scaling, and thickening according to the clinical psoriasis area and severity index as previously described (18). Briefly, the score was determined as follows: 0 = none, 1 = slight, 2 = moderate, 3 = marked, and 4 = very marked. Erythema, scaling, and thickening were scored independently, and the cumulative score served as the disease severity score (scale, 0 to 12). In some experiments, IMQ-treated mice were intradermally injected with recombinant IL-23 (R&D Systems), IL-6 (BD Biosciences), or IL-1 β (R&D Systems) at a dose of 50 ng in 20 μ l of phosphate-buffered saline (PBS) per ear on days 0, 2, and 4. Control mice were injected with the same volume of PBS with 0.1% bovine serum albumin (w/v) on the same schedule.

Pharmacological inhibition of p38

IMQ-treated WT mice were intraperitoneally administered with p38 inhibitor SB203580 (Merck Calbiochem) at a dose of 0.75 mg/kg body weight from days 3 to 5. Liquid SB203580 dissolved in dimethyl sulfoxide (DMSO; MP Biomedicals) was diluted with PBS for injection into mice. The same volume of DMSO was diluted with PBS for injection into control mice.

Skin cell preparation

Mouse ear skin samples were collected and split into dorsal and ventral halves, and then the subcutaneous fat tissue was carefully scraped off and ears were floated split side down for 40 min at 37°C on the surface of 0.5% trypsin (w/v) (Gibco). The dermis was separated from the epidermis. Each sheet was cut into small pieces and placed into digestion solution containing collagenase IV [1.5 mg/ml (for dermis) or 1 mg/ml (for epidermis); Gibco]. Digestion was performed

for 90 min (for dermis) or 80 min (for epidermis) at 37°C with brief mixing. After the digestion, the solution was mixed thoroughly and filtered through a nylon filter to obtain single-cell suspension.

Cell purification, cultures, and DC cytokine assays

Epidermal LCs, $\gamma\delta$ T cells, and naïve CD4⁺ T cells were enriched with microbeads (Miltenyi Biotec) and sorted with a BD FACSAria III sorter. For LC- $\gamma\delta$ T cell coculture, LCs from naïve WT and p38 α ^{ADC} mice and $\gamma\delta$ T cells from WT mice were mixed in the presence of R848 (1 μ g/ml) (InvivoGen). After 48 hours, culture supernatants were collected for ELISA measurements. For LC-CD4⁺ T cell coculture, LCs from either naïve or IMQ-treated WT and p38 α ^{ADC} mice and naïve CD4⁺ T cells from OT-II mice were mixed in the presence of ovalbumin peptide (10 μ g/ml) (OVA₃₂₃₋₃₃₉) and R848 (1 μ g/ml). After 5 days, live T cells were harvested and stimulated with PMA (phorbol 12-myristate 13-acetate; Sigma-Aldrich) and ionomycin (Sigma-Aldrich) in the presence of protein transport inhibitor (BD Biosciences) for 5 hours for intracellular cytokine staining, or with plated-bound α -CD3 (2C11; Bio X Cell) for 5 hours to measure mRNA expression. In some experiments, recombinant IL-23 (20 ng/ml) (R&D Systems), IL-1 β (R&D Systems), or IL-6 (BD Biosciences) cytokines were added to the coculture systems. For DC cytokine assays, LCs from naïve or IMQ-treated WT and p38 α ^{ADC} mice were stimulated with R848 (1 μ g/ml) either for 5 hours before RNA analysis or for 24 hours before ELISA measurement.

Flow cytometry

For analysis of surface markers, cells were stained in PBS containing 2% (v/v) fetal bovine serum with anti-CD45 (30-F11), anti-CD11b (M1/70), anti-Gr-1 (RB6-8C5), anti-F4/80 (BM8), anti-CD11c (N418), anti-MHC-II (M5/114.15.2), anti-EpCAM (G8.8), anti- $\gamma\delta$ TCR (eBioGL3), anti-CD3 (17A2), anti-CD4 (RM4-5), anti-CD8 α (53-6.7), anti-TCR β (H57-597), anti-CD40 (1C10), anti-CD80 (16-10A1), anti-CD86 (GL1), and 7-AAD (all from eBioscience). For intracellular staining with anti-IL-17 (eBio17B7), anti-IFN γ (XMG 1.2e), anti-IL-23 (fc23cpg), anti-IL-1 β (NJTEN3), and anti-IL-6 (MP5-20F3) (all from eBioscience), cells were stimulated with PMA and ionomycin or R848 in the presence of protein transport inhibitor for 5 hours before being stained according to the manufacturer's instructions (BD Biosciences). For intracellular phosphorylation assays, cells were stained with anti-phospho-p38 (28B10, Cell Signaling Technology) according to the manufacturer's instructions (BD Biosciences). Staining with anti-Foxp3 (FJK-16S, eBioscience) and anti-Ki-67 (SolA15, eBioscience) were done according to the manufacturer's instructions (eBioscience). For cell apoptosis analysis, cells were stained with Active Caspase-3 Apoptosis Kit (BD Biosciences). Flow cytometry data were acquired on BD FACSCanto II or BD LSRFortessa X-20 and were analyzed with FlowJo software (Tree Star).

Histopathological analysis

Formalin-preserved mouse ear sections were embedded in paraffin according to standard techniques. Longitudinal sections (6 μ m thick) were stained with hematoxylin and eosin and analyzed by microscopic examination.

Protein and RNA analyses

For cytokine detection in skin tissue, 45-mg skin tissue was weighted and homogenized in 0.5-ml ice-cold CelLytic MT Cell Lysis reagent (Sigma-Aldrich). Concentrations of IL-17, TNF α , IL-23, IL-6, and IL-1 β in homogenized or culture supernatants were measured by

ELISA according to the manufacturer's instructions (eBioscience). Total RNA of skin tissue and cells was isolated using the TRIzol reagent (Invitrogen) and RNeasy Mini Kit (Qiagen), respectively. Reverse transcription was performed via PrimeScript RT Master Mix (Takara) according to the manufacturer's instructions. Quantitative polymerase chain reaction (PCR) was carried out with SYBR Green PCR Master Mix (Applied Biosystems) in a Vii7 Real-Time PCR system (Applied Biosystems). Relative mRNA levels were determined with hypoxanthine-guanine phosphoribosyltransferase (HPRT) as a reference gene. The following primers sequences were used: *Hprt*, TCAGTCAACGGGGACATAAA (forward) and GGGGCTGTACTGCT-TAACCAG (reverse); *Il17a*, TCAGCGTGTCCAAACTGAG (forward) and CGCCAAGGGAGTTAAAGACTT (reverse); *Tnfa*, CAGCGG-TGTCCTATGTCTC (forward) and CGATCACCCGAAAGTTCAGTAG (reverse); *Il6*, CTGCAAGAGACTTCCATCCAG (forward) and AGTGGTAT AGACAGGTATGTTGG (reverse); *Il1b*, GCAACTGTTCT-GAACTCAACT (forward) and ATCTTTTGGGGTCCGTCACCT (reverse); *Il23a*, GCCCGTATCCAGTGTGA (forward) and GCTGCCACTGCTGACTAG (reverse); *Csf2*, GGCCCTGGAAGCATGTAGAGG (forward) and GGAGAAGCTCGTTAGAGACGACTT (reverse); *Cxcl1*, TGCACCCAAACCGAAGTCAT (forward) and TTGTCAGAAGCCAGCGTTCAC (reverse); *Ifng*, GCCACGGCA-CAGTCATTGA (forward) and TGCTGATGGCCTGATTGTCTT (reverse); *Il12a*, CAATCACGCTACCTCCTCTTTT (forward) and CAGCAGTGCAGGAATAATGTTTC (reverse); *Il4*, GGTCTCAAC-CCCCAGCTAGT (forward) and GCCGATGATCTCTCTCAAGT-GAT (reverse); *Il10*, CTTACTGACTGGCATGAGGATCA (forward) and GCAGCTCTAGGAGCATGTGG (reverse); *Tgfb1*, CTCCCGT-GGCTTCTAGTGC (forward) and GCCTTAGTTTGGACAGGATCTG; *Foxp3*, CACCATGCCACCCTTATCCG (forward) and CATCG-TAAACCAATGGTAGA (reverse); *Il22*, ATGAGTTTTTCCTTAT-GGGGAC (forward) and GCTGGAAGTTTGGACACCTCAA (reverse); *S100a7a*, TGCTCTTGGATAGTGTGCCTC (forward) and GCTCTGTGATGTAGTATGGCTG (reverse); *S100a8*, TGTCCT-CAGTTTGTGCAGAATATAAA (forward) and TCACCATCGCAAG-GAACTCC (reverse); *Krt16*, GGTGGCTCTAACAGTGATCT (forward) and TGCATACAGTATCTGCCTTTGG (reverse); *Krt17*, ACCATCCGCCAGTTTACCTC (forward) and CTACCCAGGC-CACTAGCTGA (reverse); *Il27*, CTGTTGCTGCTACCCTTGCTT (forward) and CACTCCTGGCAATCGAGATTC (reverse); and *Mapk14*, GAGGTGCCCCGAACGATAC (forward) and TGGCGT-GAATGATGGACT (reverse).

Statistical analysis

The data were analyzed with GraphPad Prism 5 or SPSS 17.0 and are means \pm SEM. Analysis of variance (ANOVA) with Bonferroni post test was used for multiple comparisons, and Student's *t* test was used when two conditions were compared. *P* values were indicated, and *P* < 0.05 was considered significant. Two-sided Student's *t* tests and one-way or two-way ANOVA was performed. ns indicates no significance. Error bars represent SEM.

SUPPLEMENTARY MATERIALS

www.sciencesignaling.org/cgi/content/full/11/521/eaao1685/DC1

Fig. S1. p38 α deletion in mouse skin tissue and DCs.

Fig. S2. Normal DC development and activation status in the epidermis and dermis of p38 α ^{ADC} mice.

Fig. S3. Cell infiltration analysis in the skin and spleen of WT and p38 α ^{ADC} mice upon IMQ treatment.

Fig. S4. Gene expression in KCs and cytokine production in skin tissue of WT and p38 α ^{ADC} mice upon IMQ treatment.

Fig. S5. Decreased IL-17 production from $\gamma\delta$ and CD4⁺ T cells in the skin and DLNs of p38^{ADC} mice.
 Fig. S6. The proliferation and apoptosis of T cells in IMQ-treated WT and p38^{ADC} mice.
 Fig. S7. p38 α activity in LCs is important for psoriasiform skin inflammation.
 Fig. S8. LC p38 α -mediated T_H17 cell differentiation and IL-23, IL-1 β , and IL-6 expression.
 Fig. S9. The effect of p38 α signaling in DCs on skin inflammation is IL-1 β -independent.
 Fig. S10. p38 α in dDCs affects IL-17 production from CD4⁺ T cells but not $\gamma\delta$ T cells.
 Fig. S11. p38 α activity in T cells does not contribute to the IMQ-induced psoriasiform skin inflammation.
 Fig. S12. Decreased IL-17 production from $\gamma\delta$ and CD4⁺ T cells in the DLNs upon SB203580 treatment.
 Fig. S13. Acute deletion of p38 α reduces the severity of an ongoing psoriasiform skin inflammation.

REFERENCES AND NOTES

- M. A. Lowes, A. M. Bowcock, J. G. Krueger, Pathogenesis and therapy of psoriasis. *Nature* **445**, 866–873 (2007).
- F. O. Nestle, D. H. Kaplan, J. Barker, Psoriasis. *N. Engl. J. Med.* **361**, 496–509 (2009).
- M. A. Lowes, M. Suárez-Fariñas, J. G. Krueger, Immunology of psoriasis. *Annu. Rev. Immunol.* **32**, 227–255 (2014).
- J. L. Harden, J. G. Krueger, A. M. Bowcock, The immunogenetics of Psoriasis: A comprehensive review. *J. Autoimmun.* **64**, 66–73 (2015).
- Y. Cai, X. Shen, C. Ding, C. Qi, K. Li, X. Li, V. R. Jala, H.-g. Zhang, T. Wang, J. Zheng, J. Yan, Pivotal role of dermal IL-17-producing $\gamma\delta$ T cells in skin inflammation. *Immunity* **35**, 596–610 (2011).
- S. Pantelyushin, S. Haak, B. Ingold, P. Kulig, F. L. Heppner, A. A. Navarin, B. Becher, Roryt+ innate lymphocytes and $\gamma\delta$ T cells initiate psoriasiform plaque formation in mice. *J. Clin. Invest.* **122**, 2252–2256 (2012).
- P. Morel, J.-P. Revillard, J.-F. Nicolas, J. Wijdenes, H. Rizova, J. Thivolet, Anti-CD4 monoclonal antibody therapy in severe psoriasis. *J. Autoimmun.* **5**, 465–477 (1992).
- D. A. Martin, J. E. Towne, G. Kricorian, P. Klekotka, J. E. Gudjonsson, J. G. Krueger, C. B. Russell, The emerging role of IL-17 in the pathogenesis of psoriasis: Preclinical and clinical findings. *J. Invest. Dermatol.* **133**, 17–26 (2013).
- J. Kim, J. G. Krueger, Highly effective new treatments for psoriasis target the IL-23/type 17 T cell autoimmune axis. *Annu. Rev. Med.* **68**, 255–269 (2016).
- Y. Deng, C. Chang, Q. Lu, The inflammatory response in psoriasis: A comprehensive review. *Clin Rev Allergy Immunol* **50**, 377–389 (2016).
- K. E. Kip, J. M. Swoger, L. M. Grandinetti, A. M. Barrie III, J. B. Greer, M. D. Regueiro, Tumor necrosis factor α antagonist-associated psoriasis in inflammatory diseases: An analysis of the FDA adverse event reporting system. *Inflamm. Bowel Dis.* **19**, 1164–1172 (2013).
- A. Iwasaki, R. Medzhitov, Regulation of adaptive immunity by the innate immune system. *Science* **327**, 291–295 (2010).
- E. Glitzner, A. Korosec, P. M. Brunner, B. Drobits, N. Amberg, H. B. Schonhaler, T. Kopp, E. F. Wagner, G. Stingl, M. Holcmann, M. Sibilia, Specific roles for dendritic cell subsets during initiation and progression of psoriasis. *EMBO Mol. Med.* **6**, 1312–1327 (2014).
- T. L. Murphy, G. E. Grajalas-Reyes, X. Wu, R. Tussiwand, C. G. Briseño, A. Iwata, N. M. Kretzer, V. Durai, K. M. Murphy, Transcriptional control of dendritic cell development. *Annu. Rev. Immunol.* **34**, 93–119 (2016).
- L. C. Zaba, J. G. Krueger, M. A. Lowes, Resident and “inflammatory” dendritic cells in human skin. *J. Invest. Dermatol.* **129**, 302–308 (2009).
- B. Malissen, S. Tamoutounour, S. Henri, The origins and functions of dendritic cells and macrophages in the skin. *Nat. Rev. Immunol.* **14**, 417–428 (2014).
- F. Ginhoux, M. P. Collin, M. Bogunovic, M. Abel, M. Leboeuf, J. Helft, J. Ochando, A. Kissenpfennig, B. Malissen, M. Grisotto, H. Snoeck, G. Randolph, M. Merad, Blood-derived dermal langerin⁺ dendritic cells survey the skin in the steady state. *J. Exp. Med.* **204**, 3133–3146 (2007).
- L. van der Fits, S. Mourits, J. S. A. Voerman, M. Kant, L. Boon, J. D. Laman, F. Cornelissen, A.-M. Mus, E. Florencia, E. P. Prens, E. Lubberts, Imiquimod-induced psoriasis-like skin inflammation in mice is mediated via the IL-23/IL-17 axis. *J. Immunol.* **182**, 5836–5845 (2009).
- C. Wohn, J. L. Ober-Blöbaum, S. Haak, S. Pantelyushin, C. Cheong, S. P. Zahner, S. Onderwater, M. Kant, H. Weighardt, B. Holzmann, B. Reizis, B. Becher, E. P. Prens, B. E. Clausen, Langerin^{neg} conventional dendritic cells produce IL-23 to drive psoriatic plaque formation in mice. *Proc. Natl. Acad. Sci. U.S.A.* **110**, 10723–10728 (2013).
- R. Yoshiki, K. Kabashima, T. Honda, S. Nakamizo, Y. Sawada, K. Sugita, H. Yoshioka, S. Ohmori, B. Malissen, Y. Tokura, M. Nakamura, IL-23 from Langerhans cells is required for the development of imiquimod-induced psoriasis-like dermatitis by induction of IL-17A-producing $\gamma\delta$ T cells. *J. Invest. Dermatol.* **134**, 1912–1921 (2014).
- J. R. Chan, W. Blumenschein, E. Murphy, C. Diveu, M. Wiekowski, S. Abbondanzo, L. Lucian, R. Geissler, S. Brodie, A. B. Kimball, D. M. Gorman, K. Smith, R. de Waal Malefyt, R. A. Kastelein, T. K. McClanahan, E. P. Bowman, IL-23 stimulates epidermal hyperplasia via TNF and IL-20R2-dependent mechanisms with implications for psoriasis pathogenesis. *J. Exp. Med.* **203**, 2577–2587 (2006).
- T. P. Singh, H. H. Zhang, I. Borek, P. Wolf, M. N. Hedrick, S. P. Singh, B. L. Kelsall, B. E. Clausen, J. M. Farber, Monocyte-derived inflammatory Langerhans cells and dermal dendritic cells mediate psoriasis-like inflammation. *Nat. Commun.* **7**, 13581 (2016).
- J. A. Callahan, G. E. Hammer, A. Agelides, B. H. Duong, S. Oshima, J. North, R. Advincula, N. Shifrin, H.-A. Truong, J. Paw, J. Barrera, A. DeFranco, M. D. Rosenblum, B. A. Malynn, A. Ma, Cutting edge: ABIN-1 protects against psoriasis by restricting MyD88 signals in dendritic cells. *J. Immunol.* **191**, 535–539 (2013).
- L. Tortola, E. Rosenwald, B. Abel, H. Blumberg, M. Schäfer, A. J. Coyle, J.-C. Renaud, S. Werner, J. Kisielow, M. Kopf, Psoriasiform dermatitis is driven by IL-36-mediated DC-keratinocyte crosstalk. *J. Clin. Invest.* **122**, 3965–3976 (2012).
- H. Zhu, F. Lou, Q. Yin, Y. Gao, Y. Sun, J. Bai, Z. Xu, Z. Liu, W. Cai, F. Ke, L. Zhang, H. Zhou, H. Wang, G. Wang, X. Chen, H. Zhang, Z. Wang, F. Ginhoux, C. Lu, B. Su, H. Wang, RIG-I antiviral signaling drives interleukin-23 production and psoriasis-like skin disease. *EMBO Mol. Med.* **9**, 589–604 (2017).
- A. Mavropoulos, E. I. Rigopoulou, C. Liascos, D. P. Bogdanos, L. I. Sakkas, The role of p38 MAPK in the aetiopathogenesis of psoriasis and psoriatic arthritis. *Clin. Dev. Immunol.* **2013**, 569751 (2013).
- C. Johansen, K. Kragballe, M. Westergaard, J. Henningsen, K. Kristiansen, L. Iversen, The mitogen-activated protein kinases p38 and ERK1/2 are increased in lesional psoriatic skin. *Br. J. Dermatol.* **152**, 37–42 (2005).
- K. Mihara, G. R. Elliott, A. M. Boots, R. L. Nelissen, Inhibition of p38 kinase suppresses the development of psoriasis-like lesions in a human skin transplant model of psoriasis. *Br. J. Dermatol.* **167**, 455–457 (2012).
- L. Soegaard-Madsen, C. Johansen, L. Iversen, K. Kragballe, Adalimumab therapy rapidly inhibits p38 mitogen-activated protein kinase activity in lesional psoriatic skin preceding clinical improvement. *Br. J. Dermatol.* **162**, 1216–1223 (2010).
- G. Huang, Y. Wang, P. Vogel, T.-D. Kanneganti, K. Otsu, H. Chi, Signaling via the kinase p38 α programs dendritic cells to drive T_H17 differentiation and autoimmune inflammation. *Nat. Immunol.* **13**, 152–161 (2012).
- L. A. Zenewicz, R. A. Flavell, Recent advances in IL-22 biology. *Int. Immunol.* **23**, 159–163 (2011).
- H. Fujita, The role of IL-22 and Th22 cells in human skin diseases. *J. Dermatol. Sci.* **72**, 3–8 (2013).
- A. B. Van Belle, M. de Heusch, M. M. Lemaire, E. Hendrickx, G. Warnier, K. Dunussi-Joannopoulos, L. A. Fouser, J.-C. Renaud, L. Dumoutier, IL-22 is required for imiquimod-induced psoriasiform skin inflammation in mice. *J. Immunol.* **188**, 462–469 (2012).
- H.-L. Ma, S. Liang, J. Li, L. Napierata, T. Brown, S. Benoit, M. Senices, D. Gill, K. Dunussi-Joannopoulos, M. Collins, C. Nickerson-Nutter, L. A. Fouser, D. A. Young, IL-22 is required for Th17 cell-mediated pathology in a mouse model of psoriasis-like skin inflammation. *J. Clin. Invest.* **118**, 597–607 (2008).
- M. Merad, P. Hoffmann, E. Ranheim, S. Slaymaker, M. G. Manz, S. A. Lira, I. Charo, D. N. Cook, L. L. Weissman, S. Strober, E. G. Engleman, Depletion of host Langerhans cells before transplantation of donor alloreactive T cells prevents skin graft-versus-host disease. *Nat. Med.* **10**, 510–517 (2004).
- D. J. Cua, C. M. Tato, Innate IL-17-producing cells: The sentinels of the immune system. *Nat. Rev. Immunol.* **10**, 479–489 (2010).
- G. Huang, Y. Wang, H. Chi, Regulation of T_H17 cell differentiation by innate immune signals. *Cell. Mol. Immunol.* **9**, 287–295 (2012).
- T. Korn, E. Bettelli, M. Oukka, V. K. Kuchroo, IL-17 and Th17 cells. *Annu. Rev. Immunol.* **27**, 485–517 (2009).
- C. Dong, Genetic controls of Th17 cell differentiation and plasticity. *Exp. Mol. Med.* **43**, 1–6 (2011).
- W. Hu, T. D. Troutman, R. Edukulla, C. Pasare, Priming microenvironments dictate cytokine requirements for T helper 17 cell lineage commitment. *Immunity* **35**, 1010–1022 (2011).
- P. R. Burkett, G. Meyer zu Horste, V. K. Kuchroo, Pouring fuel on the fire: Th17 cells, the environment, and autoimmunity. *J. Clin. Invest.* **125**, 2211–2219 (2015).
- D. R. Littman, A. Y. Rudensky, Th17 and regulatory T cells in mediating and restraining inflammation. *Cell* **140**, 845–858 (2010).
- S. P. Jariwala, The role of dendritic cells in the immunopathogenesis of psoriasis. *Arch. Dermatol. Res.* **299**, 359–366 (2007).
- T.-G. Kim, D. S. Kim, H.-P. Kim, M.-G. Lee, The pathophysiological role of dendritic cell subsets in psoriasis. *BMB Rep.* **47**, 60–68 (2014).
- C. Xiao, Z. Zhu, S. Sun, J. Gao, M. Fu, Y. Liu, G. Wang, X. Yao, W. Li, Activation of Langerhans cells promotes the inflammation in imiquimod-induced psoriasis-like dermatitis. *J. Dermatol. Sci.* **85**, 170–177 (2016).

46. E. Martini, M. Wiken, S. Cheuk, I. Gallias Serezal, F. Baharom, M. Stähle, A. Smed-Sörensen, L. Eidsmo, Dynamic changes in resident and infiltrating epidermal dendritic cells in active and resolved psoriasis. *J. Invest. Dermatol.* **137**, 865–873 (2017).
47. F. O. Nestle, C. Conrad, A. Tun-Kyi, B. Homey, M. Gombert, O. Boyman, G. Burg, Y.-J. Liu, M. Gilliet, Plasmacytoid predendritic cells initiate psoriasis through interferon- α production. *J. Exp. Med.* **202**, 135–143 (2005).
48. C. Albanesi, C. Scarponi, S. Pallotta, R. Daniele, D. Bosisio, S. Madonna, P. Fortugno, S. Gonzalvo-Feo, J.-D. Franssen, M. Parmentier, O. De Pità, G. Girolomoni, S. Sozzani, Chemerin expression marks early psoriatic skin lesions and correlates with plasmacytoid dendritic cell recruitment. *J. Exp. Med.* **206**, 249–258 (2009).
49. J. Han, J. D. Lee, L. Bibbs, R. J. Ulevitch, A MAP kinase targeted by endotoxin and hyperosmolarity in mammalian cells. *Science* **265**, 808–811 (1994).
50. S. Bühler, S. A. Laufer, p38 MAPK inhibitors: A patent review (2012–2013). *Expert Opin. Ther. Pat.* **24**, 535–554 (2014).
51. J. C. Sy, G. Seshadri, S. C. Yang, M. Brown, T. Oh, S. Dikalov, N. Murthy, M. E. Davis, Sustained release of a p38 inhibitor from non-inflammatory microspheres inhibits cardiac dysfunction. *Nat. Mater.* **7**, 863–868 (2008).
52. P. Chopra, V. Kanoje, A. Semwal, A. Ray, Therapeutic potential of inhaled p38 mitogen-activated protein kinase inhibitors for inflammatory pulmonary diseases. *Expert Opin. Investig. Drugs* **17**, 1411–1425 (2008).
53. K. Nishida, O. Yamaguchi, S. Hirotsu, S. Hikoso, Y. Higuchi, T. Watanabe, T. Takeda, S. Osuka, T. Morita, G. Kondoh, Y. Uno, K. Kashiwase, M. Taniike, A. Nakai, Y. Matsumura, J.-i. Miyazaki, T. Sudo, K. Hongo, Y. Kusakari, S. Kurihara, K. R. Chien, J. Takeda, M. Hori, K. Otsu, p38 α mitogen-activated protein kinase plays a critical role in cardiomyocyte survival but not in cardiac hypertrophic growth in response to pressure overload. *Mol. Cell. Biol.* **24**, 10611–10620 (2004).
54. M. L. Caton, M. R. Smith-Raska, B. Reizis, Notch–RBP–J signaling controls the homeostasis of CD8⁺ dendritic cells in the spleen. *J. Exp. Med.* **204**, 1653–1664 (2007).

Acknowledgments: We thank J. R. Lukens (University of Virginia, USA) for critical reading and editing of the manuscript and B. Su (Shanghai Jiao Tong University School of Medicine, China) for providing the insightful suggestion. We also thank B. Wang (Shanghai Jiao Tong University School of Medicine, China) for confirming the appropriate statistical tests used in our study.

Funding: This work was supported by the National Natural Science Foundation of China (31670897, 81471528, and 91642104 to G.H. and 81671399 to X.L.), the Ministry of Science and Technology of China (973 Basic Science Project 2014CB541803 to G.H.), the Program for Professor of Special Appointment (Eastern Scholar) at Shanghai Institutions of Higher Learning (to G.H.), the Shanghai Municipal Commission of Health and Family Planning (20154Y0120 to R.H. and 20164Y0127 to T.Z.), and the Shanghai Sailing Program (17YF1416600 to T.Z.). **Author contributions:** T.Z., W.Z., and H. Li designed and performed the in vivo and cellular experiments and contributed to manuscript writing. S.X. and M.H. contributed to gene expression analyses and molecular experiments. H. Liu contributed to chimera experiments. R.H. contributed to animal colony management. Y.L. contributed to histopathology analysis. K.O. contributed mouse models. X.L. provided reagents and contributed to chimera experiments. G.H. designed experiments, analyzed the data, wrote the manuscript, and provided overall direction. **Competing interests:** The authors declare that they have no competing interests. **Data and materials availability:** All data needed to evaluate the conclusions in the paper are present in the paper or the Supplementary Materials.

Submitted 21 June 2017

Accepted 13 February 2018

Published 13 March 2018

10.1126/scisignal.aao1685

Citation: T. Zheng, W. Zhao, H. Li, S. Xiao, R. Hu, M. Han, H. Liu, Y. Liu, K. Otsu, X. Liu, G. Huang, p38 α signaling in Langerhans cells promotes the development of IL-17–producing T cells and psoriasiform skin inflammation. *Sci. Signal.* **11**, eaao1685 (2018).

p38 α signaling in Langerhans cells promotes the development of IL-17–producing T cells and psoriasiform skin inflammation

Tingting Zheng, Weiheng Zhao, Hongjin Li, Shuxiu Xiao, Ran Hu, Miaomiao Han, Heng Liu, Yejiang Liu, Kinya Otsu, Xinguang Liu and Gonghua Huang

Sci. Signal. **11** (521), eaao1685.
DOI: 10.1126/scisignal.aao1685

p38 α signaling in psoriasis

Psoriasis is an autoimmune skin condition that is linked to the proinflammatory cytokines IL-23, which triggers epidermal hyperplasia, and IL-17, which is produced by T cells in the skin. Zheng *et al.* found that p38 α signaling specifically in skin-resident dendritic cells known as Langerhans cells was important for the pathogenesis of psoriasis in a mouse model of the disease. p38 α signaling in Langerhans cells stimulated the production of IL-23, which is critical for the development of IL-17–producing T cells that are implicated in the disease. Genetic deletion or pharmacological inhibition of p38 α reduced skin inflammation in mice with established psoriatic disease. Together, these data identify an important cellular source of pathogenic IL-23 and suggest that p38 α in skin-resident Langerhans cells could be targeted to treat psoriasis.

ARTICLE TOOLS

<http://stke.sciencemag.org/content/11/521/eaao1685>

SUPPLEMENTARY MATERIALS

<http://stke.sciencemag.org/content/suppl/2018/03/09/11.521.eaao1685.DC1>

RELATED CONTENT

<http://stke.sciencemag.org/content/sigtrans/10/467/eaaf8823.full>
<http://stke.sciencemag.org/content/sigtrans/9/447/ra95.full>
<http://stm.sciencemag.org/content/scitransmed/9/411/eaan2514.full>
<http://stke.sciencemag.org/content/sigtrans/11/525/eaao3428.full>
<http://stke.sciencemag.org/content/sigtrans/11/551/eaau0727.full>
<http://stke.sciencemag.org/content/sigtrans/12/602/eaao3829.full>

REFERENCES

This article cites 54 articles, 14 of which you can access for free
<http://stke.sciencemag.org/content/11/521/eaao1685#BIBL>

PERMISSIONS

<http://www.sciencemag.org/help/reprints-and-permissions>

Use of this article is subject to the [Terms of Service](#)

Science Signaling (ISSN 1937-9145) is published by the American Association for the Advancement of Science, 1200 New York Avenue NW, Washington, DC 20005. The title *Science Signaling* is a registered trademark of AAAS.

Copyright © 2018 The Authors, some rights reserved; exclusive licensee American Association for the Advancement of Science. No claim to original U.S. Government Works