IMMUNOLOGY

p38α signaling in Langerhans cells promotes the development of IL-17–producing T cells and psoriasiform skin inflammation

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Dendritic cells (DCs) contribute to psoriasis pathogenesis. In a mouse model of imiquimod-induced psoriasiform skin inflammation, we found that p38α 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 p38α 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 p38α in LCs specifically promoted IL-17 production from γδ 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 p38α signaling in LCs in promoting inflammatory responses in the skin and suggest that targeting p38α 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 (TNFα), 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 TNFα, 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 TNFα 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 κB 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 p38α mitogen-activated protein kinase, a central regulator of inflammatory responses, is associated with

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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 p38α deletion in LCs, but not in other DC subsets or T cells, reduced IMQ-induced psoriasiform skin inflammation. Mice with p38α-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 p38α showed reduced IL-17 production from γδ and $CD4^+$ T cells. p38α signaling in LCs specifically promoted IL-17 production in γδ and $CD4^+$ T cells through secretion of IL-23 and/or IL-6. However, p38α signaling in LCs was dispensable for the generation of interferon-γ (IFNγ)–, IL-4–, and Foxp3-expressing T cells. Intradermal injection of IL-23 restored the skin inflammation and proinflammatory cytokine expression in mice lacking p38α 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 p38α signaling in LCs and IL-23 or IL-6 with IL-17 production from γδ and $CD4^+$ T cells, and it provides cellular and molecular mechanisms by which p38α regulates susceptibility to psoriasis.

RESULTS

Deletion of p38α 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 p38α

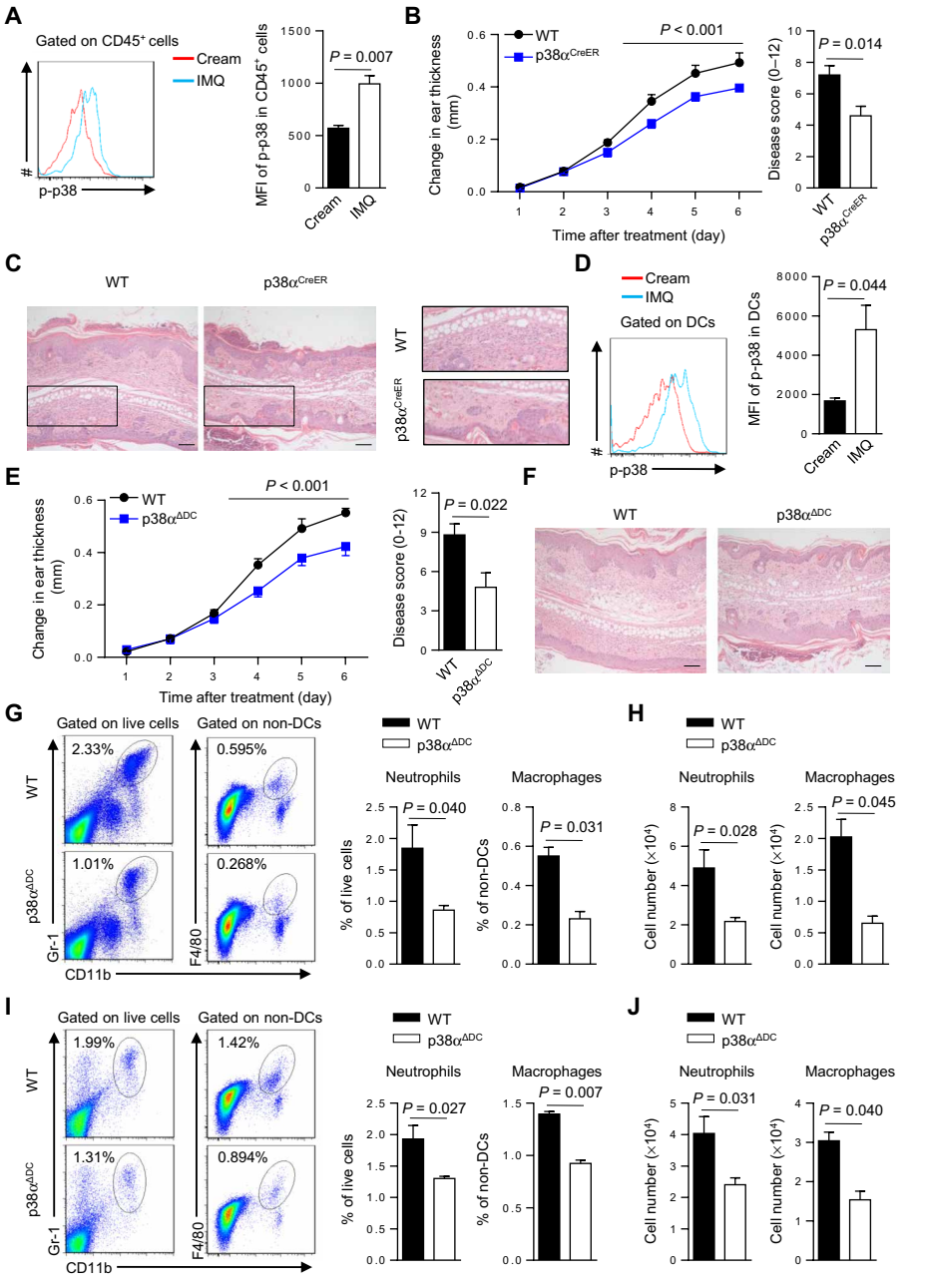

Fig. 1. Deletion of p38α in DCs reduces IMQ-induced skin inflammation in mice. (**A**) Wild-type (WT) mice were topically treated with imiquimod (IMQ)–containing or control cream for two consecutive days, and the phosphorylation (p) of p38 in skin $CD45^+$ cells was analyzed by flow cytometry ($n = 6$ mice per group). MFI, mean fluorescence intensity. (**B** and **C**) Tamoxifen-pretreated WT and $p38\alpha^{CreER}$ 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 $p38\alpha^{CreER}$ (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 μm. (**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 $p38\alpha^{\Delta DC}$ 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 μm. 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 ± 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 "p38α^{CreER} mice" here, as described in our previous study (*30*)] to determine the role of p38 in psoriasis pathogenesis and detected efficient deletion of p38α in skin tissue (fig. S1A). We explored the development of IMQ-induced psoriasiform skin inflammation in wild-type (WT) and p38α^{CreER} mice, which were pretreated with tamoxifen to acutely delete p38α in skin tissue. We found that p38α^{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 p38α 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 p38α signaling in DCs might play a pivotal role in the immune mechanisms during psoriasis development. To delineate the specific role of DC-intrinsic p38α signaling in psoriasis pathogenesis, we generated *Mapk14*$^{fl/fl}$CD11c-Cre mice [referred to as "p38α$^{\Delta DC}$ mice" here (*30*)]. We observed efficient deletion of p38α 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, p38α$^{\Delta DC}$ mice had much less ear swelling and reduced composite psoriasis score than WT mice (Fig. 1E). Histological analysis showed that the skin of p38α$^{\Delta DC}$ 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 p38α$^{\Delta DC}$ mice (Fig. 1, G to J). Notably, the percentages and cell numbers of LCs, CD4$^+$ T cells, and γδ T cells, which are key producers of IL-17 during psoriasis, were comparable in the epidermis of IMQ-treated WT and p38α$^{\Delta DC}$ 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 p38α$^{\Delta DC}$ mice (fig. S3, E and F). Collectively, these findings implicate a key role for DC-mediated p38α signaling in IMQ-induced psoriatic skin disease.

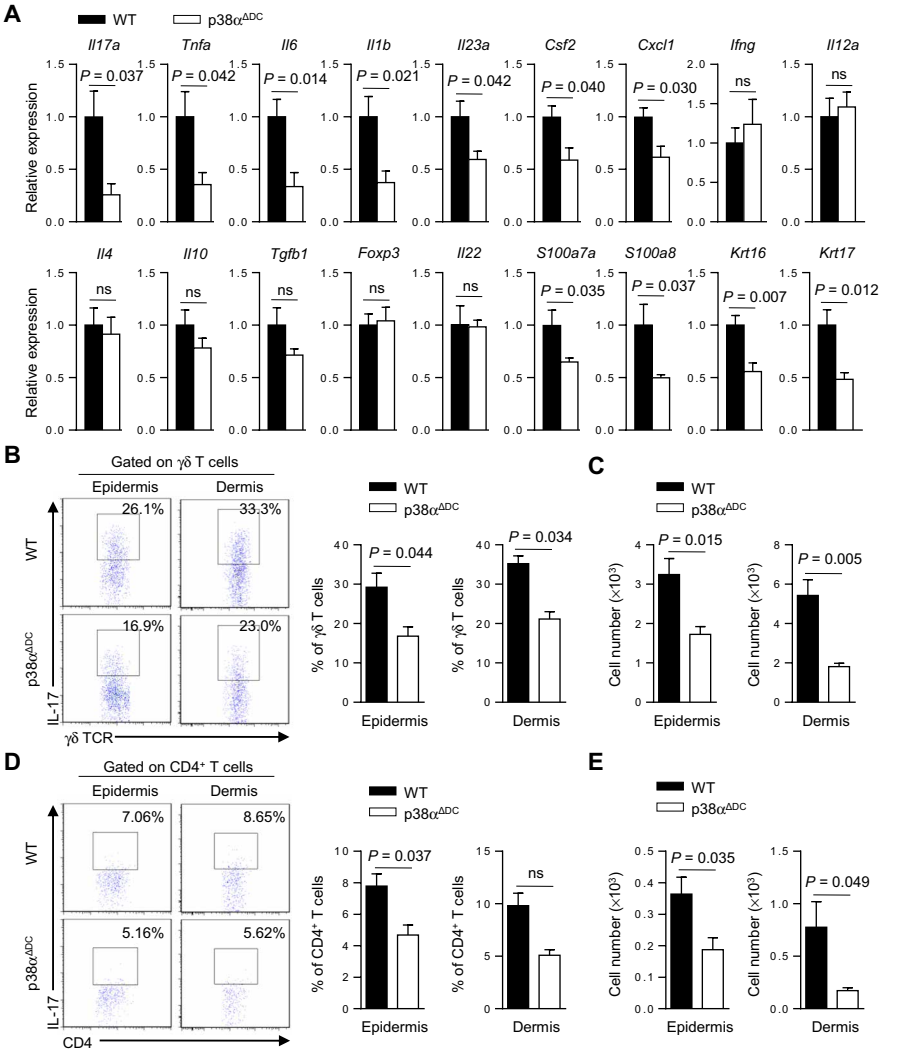

Fig. 2. p38α activity in DCs is required for the generation of IL-17–producing T cell in vivo. WT and p38α$^{\Delta DC}$ mice were topically treated with IMQ for six consecutive days. (**A**) Relative mRNA expression of inflammation-related genes in skin tissue was examined (n = 5 mice per group). ns, not significant. (**B** and **C**) The percentages (B) and cell numbers (C) of interleukin-17–positive (IL-17$^+$) γδ T cells in the epidermis and dermis (n = 6 mice per group). γδTCR, γδ 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 ± SEM. Data are representative of three independent experiments.

p38α 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 p38α$^{\Delta DC}$ 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 p38α$^{\Delta DC}$ 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 p38α$^{\Delta DC}$ mice (Fig. 2A

and fig. S4A). Consistent with the mRNA expression data, enzyme-linked immunosorbent assay (ELISA) showed that IL-17, TNFα, IL-1β, and IL-6 production was lower in skin tissue from IMQ-treated p38α$^{\Delta DC}$ mice (fig. S4B). We found by flow cytometry that CD45$^+$ cells, especially γδ 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 γδ T cells was greater than that of αβ 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 p38α$^{\Delta DC}$ mice (Fig. 2, B to E). The decreased IL-17 production from CD4$^+$ and γδ T cells was also observed in the DLNs of p38α$^{\Delta DC}$ mice, whereas the frequencies of IFNγ$^+$ and Foxp3$^+$ CD4$^+$ T cells were similar in the two groups of mice (fig. S5, E and F). WT and p38α$^{\Delta DC}$ mice had comparable Ki-67 and active caspase-3 staining in both γδ T cells and CD4$^+$ T cells upon IMQ treatment (fig. S6, A and B), indicating that p38α deficiency in DCs did not affect T cell proliferation and survival. These results demonstrate that p38α signaling in DCs is specifically important for the generation of IL-17–producing T cells in mouse skin upon IMQ treatment.

p38α 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 p38α signaling is important for psoriasiform skin inflammation. For this, we transplanted bone marrow (BM) cells of WT or p38α$^{\Delta DC}$ mice into x-ray–irradiated WT or p38α$^{\Delta DC}$ mice to generate WT→WT, WT→p38α$^{\Delta DC}$, p38α$^{\Delta DC}$→WT, and p38α$^{\Delta DC}$→p38α$^{\Delta DC}$ chimeras. Two months after transplantation, chimeras were treated with IMQ to induce psoriasiform skin disease. Compared with IMQ-treated WT→WT chimeras, WT→p38α$^{\Delta DC}$ chimeras showed decreased ear thickness and composite psoriasis scores (Fig. 3A and fig. S7A). Histological analysis showed that the skin of WT→p38α$^{\Delta DC}$ chimeras had substantially less epidermal hyperplasia and inflammation (Fig. 3B). Flow cytometry analysis showed that WT→p38α$^{\Delta DC}$ chimeras had diminished infiltration of neutrophils into the epidermis and dermis (Fig. 3C and fig. S7B). Intracellular staining also showed lower IL-17 production from γδ and CD4$^+$ T cells in the DLNs of WT→p38α$^{\Delta DC}$ chimeras than did those from WT→WT chimeras (Fig. 3D and fig. S7C). Moreover, skin samples from WT→p38α$^{\Delta DC}$ chimeras exhibited lower expression of *Il17a*, *Tnfa*, *Il6*, *Il1b*, and *Cxcl1* mRNA expression than WT→WT chimeras (Fig. 3E). Because LCs are resistant to x-ray irradiation (*35*) and both WT→WT and WT→p38α$^{\Delta DC}$ chimeras contained the same dDC and circulating DC subsets but different LCs, these results indicate that p38α signaling in LCs is important for IMQ-induced psoriasiform skin inflammation.

When transplanting WT or p38α$^{\Delta DC}$ BM cells into x-ray–irradiated WT mice, we found that both IMQ-treated WT→WT and p38α$^{\Delta DC}$→WT chimeras had comparable ear thickness and composite psoriasis score

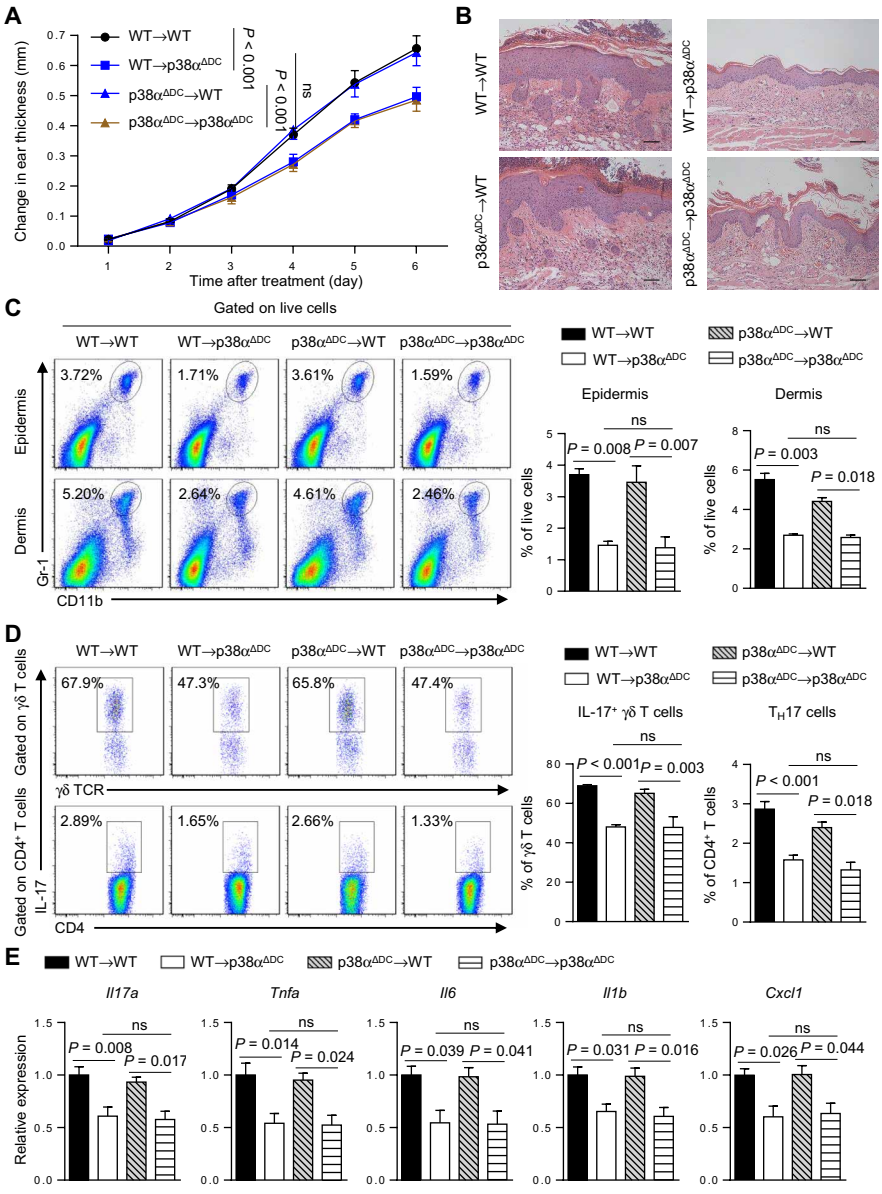

Fig. 3. p38α signaling in LCs is important for the development of IMQ-induced skin inflammation. Bone marrow (BM) cells of WT or p38α$^{\Delta DC}$ mice were transplanted into x-ray–irradiated WT or p38α$^{\Delta DC}$ mice, respectively, to make the WT→WT, WT→p38α$^{\Delta DC}$, p38α$^{\Delta DC}$→WT, and p38α$^{\Delta DC}$→p38α$^{\Delta DC}$ 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 μm. (**C**) The percentages of neutrophils in the epidermis and dermis (n = 6 mice per group). (**D**) The percentages of IL-17$^+$ γδ 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 ± SEM. Data are representative of two independent experiments.

(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 γδ 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→WT and $p38\alpha^{\Delta DC}$→WT chimeras (Fig. 3E). In addition, we found that WT→$p38\alpha^{\Delta DC}$ and $p38\alpha^{\Delta DC}$→$p38\alpha^{\Delta DC}$ 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 p38α 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 p38α 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 p38α signaling in LCs stimulates T cells to produce IL-17, we cultured LCs from WT and $p38\alpha^{\Delta DC}$ mice with γδ T cells in the presence of R848 for 48 hours. γδ T cells cocultured with $p38\alpha^{\Delta DC}$ LCs produced significantly less IL-17 than did those cocultured with WT LCs (Fig. 4A). To determine whether p38α 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 $p38\alpha^{\Delta DC}$ mice in the presence of cognate antigen and R848 for 5 days. Fewer T cells cocultured with $p38\alpha^{\Delta DC}$ LCs developed into $IL\text{-}17^+$ 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 $p38\alpha^{\Delta DC}$ LCs, but comparable mRNA expression of *Ifng*, *Il4*, and *Foxp3* (fig. S8A). These results indicate that p38α in LCs stimulates IL-17 production in T cells in vitro.

Next, we explored the molecular mechanisms by which p38α acted in LCs to promote IL-17–producing T cell development. WT and $p38\alpha^{\Delta DC}$ mice expressed comparable costimulatory molecules, such as CD40, CD80, and CD86 in LCs upon IMQ treatment (fig. S8B). To determine whether p38α signaling in LCs regulates

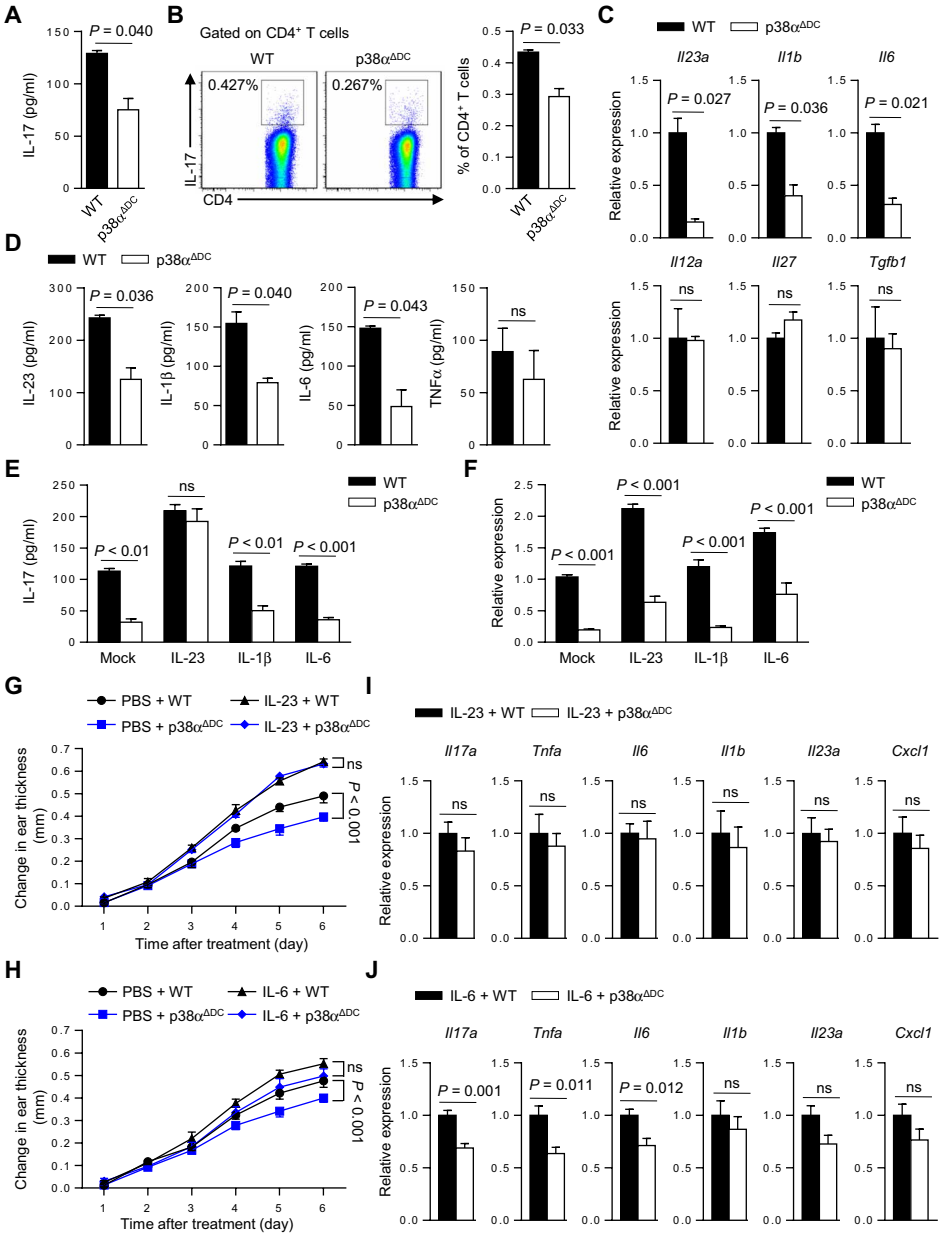

Fig. 4. Signaling by p38α in LCs controls IL-17–producing T cell generation and skin inflammation by regulating the expression of IL-6 and IL-23. (**A**) IL-17 production in the supernatant of γδ T cells cocultured with R848-stimulated WT and $p38\alpha^{\Delta DC}$ Langerhans cells (LCs) for 48 hours ($n = 3$ biological replicates). (**B**) The differentiation of T_H17 cells in $CD4^+$ T cells activated with R848-pulsed WT and $p38\alpha^{\Delta DC}$ LCs for 5 days ($n = 3$ biological replicates). (**C** and **D**) Cytokine expression from WT and $p38\alpha^{\Delta DC}$ LCs stimulated with R848 for 5 (C) and 24 hours (D) ($n = 3$ biological replicates). (**E**) IL-17 production from γδ T cells cocultured with WT and $p38\alpha^{\Delta DC}$ LCs in the presence or absence of IL-23, IL-1β, or IL-6 ($n = 3$ biological replicates). (**F**) Relative mRNA expression of *Il17* in $CD4^+$ T cells cocultured with WT and $p38\alpha^{\Delta DC}$ LCs in the presence or absence of IL-23, IL-1β, or IL-6 ($n = 3$ biological replicates). (**G**) Change in ear thickness of IMQ-treated WT and $p38\alpha^{\Delta DC}$ 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 $p38\alpha^{\Delta DC}$ 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 $p38\alpha^{\Delta DC}$ 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 ± 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 p38α$^{\Delta DC}$ 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-1β, and IL-23 in LCs was lower in p38α$^{\Delta DC}$ 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 TNFα were comparable in LCs from WT and p38α$^{\Delta DC}$ mice (Fig. 4, C and D).

To identify the cytokine lost in p38α$^{\Delta DC}$ LCs that was responsible for reduced T cell IL-17 production, we added back recombinant IL-23, IL-1β, or IL-6 to LC–γδ T cell cocultures. The addition of IL-23 to p38α$^{\Delta DC}$ LC–γδ T cell cocultures completely restored the defective IL-17 production from γδ T cells, whereas the addition of IL-1β 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-1β, partially restored *Il17* expression in CD4$^+$ T cells activated by p38α$^{\Delta DC}$ LCs (Fig. 4F). Collectively, these data showed that p38α signaling orchestrated a program for LC-dependent IL-17–producing T cell differentiation.

We sought to further assess the functional importance of p38α-dependent cytokine production in WT and p38α$^{\Delta DC}$ mice treated with IMQ to induce psoriasiform inflammation. Intradermal injection of IL-23 and IL-6, but not IL-1β, aggravated the severity of IMQ-induced psoriasiform disease (Fig. 4, G and H, and fig. S9A). Injection of either IL-23 or IL-6, but not IL-1β, restored ear swelling in p38α$^{\Delta DC}$ 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 p38α$^{\Delta DC}$ mice. In contrast, IL-6 and IL-1β restored expression of some cytokines (Fig. 4, I and J, and fig. S9B). Together, these results showed that p38α mediated the effect on IL-17–producing T cell development and psoriasis pathogenesis through distinct cytokines.

We explored the role of p38α signaling in different DC subsets on cytokine production and IL-17–producing T cell development with R848-stimulated dDCs from WT and p38α$^{\Delta DC}$ mice. We found that compared with WT dDCs, p38α$^{\Delta DC}$ dDCs secreted less IL-6 but comparable IL-23 and IL-1β (fig. S10A). Next, we cocultured WT and p38α$^{\Delta DC}$ dDCs with γδ T or naïve CD4$^+$ T cells and found that CD4$^+$ T cells activated by p38α$^{\Delta DC}$ dDCs secreted less IL-17, whereas γδ T cells activated by both WT and p38α$^{\Delta DC}$ dDCs expressed comparable levels of IL-17 (fig. S10, B and C). Because γδ T cells are the major IL-17–producing cells that are critical in IMQ-induced psoriasis (*5*), p38α signaling in different DC subsets instructs IL-17–producing T cell generation and promotes psoriasis pathogenesis through the regulation of IL-23 expression.

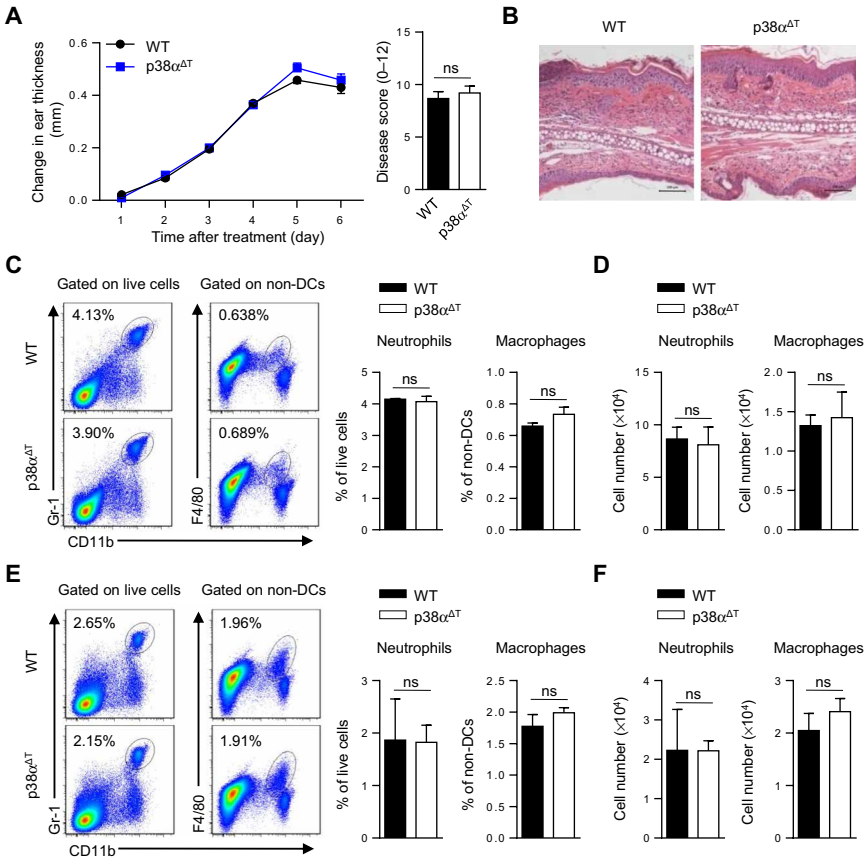

Fig. 5. p38α MAPK in T cells is dispensable for the induction of psoriasiform inflammation. WT and p38α$^{\Delta T}$ mice were topically treated with IMQ cream for six consecutive days. (**A**) Change in ear thickness (left) and disease severity score (right) (n = 5 to 6 mice per group). (**B**) Representative images of H&E staining in skin section (n = 3 mice per group). Scale bars, 200 μm. (**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 ± SEM. Data are representative of three independent experiments.

IMQ-induced psoriasiform skin disease does not require p38α in T cells

Although p38α signaling in T cells is not required for T_H17 cell differentiation (*30*), T cell–intrinsic p38α could potentially influence psoriasis pathogenesis by other mechanisms. To evaluate the potential role for T cell–dependent p38α activation in psoriasis, we generated *Mapk14*$^{fl/fl}$CD4-Cre mice [referred to as "p38α$^{\Delta T}$ mice" here (*30*)], which efficiently ablates p38α from T cells (fig. S11A). T cell–specific deletion of p38α 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 p38α$^{\Delta T}$ mice (Fig. 5, A to F). Moreover, genetic abrogation of p38α in T cells did not appreciably affect IL-17 production by γδ 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 p38α 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 p38α 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 C*xcl1* 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 γδ T cells was also decreased in the DLNs of SB203580-treated mice, whereas the frequencies of $IFN\gamma^+$ 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 $p38\alpha^{CreER}$ mice to acutely ablate p38α expression on days 5 to 8 post-IMQ exposure. Consistent with our SB2034580 treatment results, we observed substantial reductions in ear swelling, composite psoriasis score, neutrophil infiltration, the production of IL-17 from $CD4^+$ and γδ T cells, and the frequency of $IFN\gamma^+$ $CD4^+$ T cells in the DLNs in tamoxifen-treated $p38\alpha^{CreER}$ mice (fig. S13, A to C). The frequency of $Foxp3^+$ $CD4^+$ T cells was similar in the DLNs from tamoxifen-treated WT and $p38\alpha^{CreER}$ mice (fig. S13C). These results collectively suggest that inhibition of p38 could offer an approach to treat psoriasis and other inflammatory skin diseases.

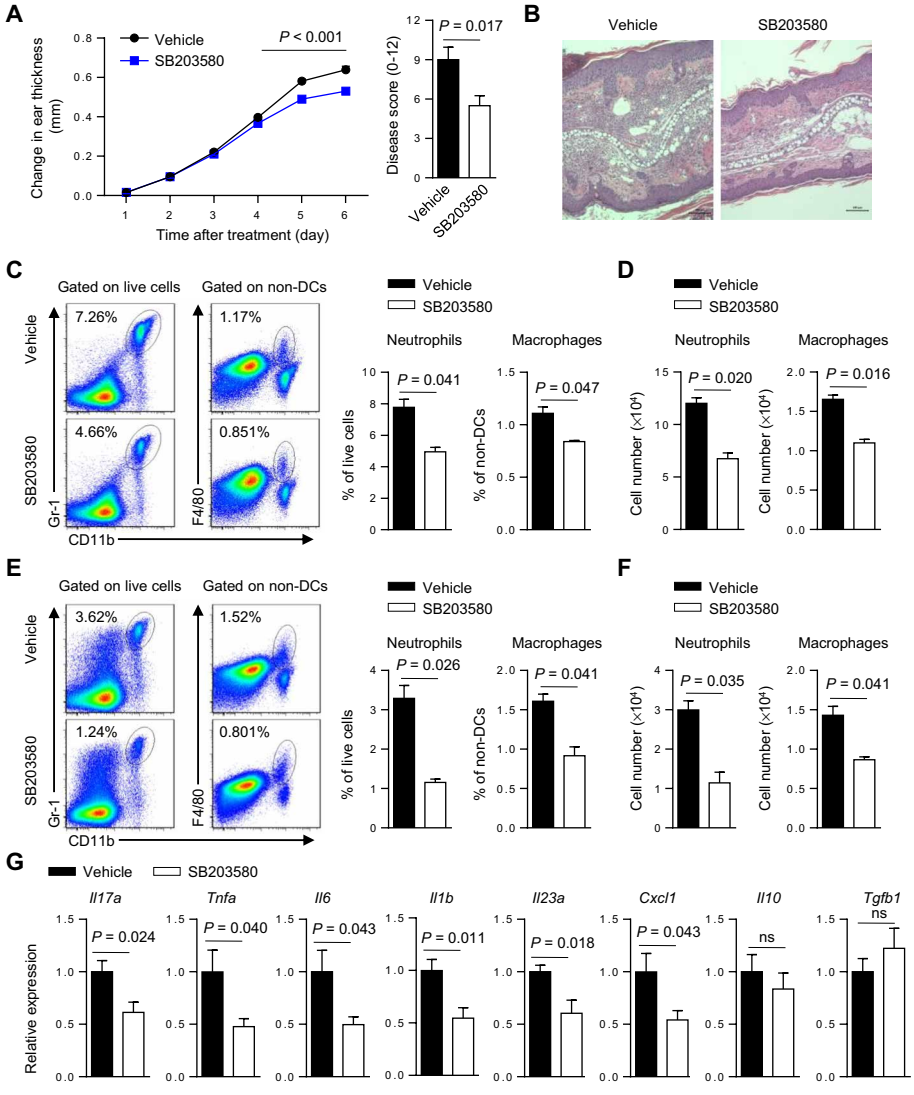

Fig. 6. Inhibition of p38 activity reduces disease severity in mice with established skin inflammation. WT mice were topically treated with IMQ for six consecutive days and received either the p38 inhibitor SB203580 or control vehicle daily by intraperitoneal injection from day 3. (**A**) Change in ear 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 μm. (**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 ± SEM. Data are representative of three independent experiments.

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 p38α signaling in LCs, but not in other DC subsets or T cells, centrally regulated IL-17 production by $CD4^+$ and γδ T cells in IMQ-induced psoriasis model, whereas leaving IFNγ-, IL-4–, and Foxp3-expressing T cell generation unaffected. p38α activity in LCs differentially promoted IL-17 production from γδ and $CD4^+$ T cells by secreting IL-23 and IL-6, respectively. Our findings highlight a crucial role for an axis involving p38α, IL-23, IL-6, and IL-17–producing T cells in psoriasis and suggest that targeting p38α 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-1β, IL-6, IL-23, and TGFβ (transforming growth factor–β) contribute

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 p38α 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-1β and IL-23 production (*30*). In contrast, we showed in the current study that p38α 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 p38α signaling contributed to LC-mediated regulation of IL-17 production by γδ T cells. However, p38α was not required for IL-1β 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 p38α 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 p38α 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 p38α 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*), p38α 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 p38α 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 p38α inhibitors to LCs ameliorated psoriasis symptoms, suggesting that new drug-delivery vehicles that target p38α 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 p38α-dependent regulation of DC functions and subsequent T cell responses might be further exploited for innovative immune therapies.

MATERIALS AND METHODS

Animals

$p38\alpha^{flox}$ 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 $p38\alpha^{\Delta DC}$ mice were intravenously transferred into lethally irradiated either WT or $p38\alpha^{\Delta DC}$ 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 $p38\alpha^{CreER}$ 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-1β (R&D Systems) at a dose of 50 ng in 20 μl 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 administrated 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, γδ T cells, and naïve $CD4^+$ T cells were enriched with microbeads (Miltenyi Biotec) and sorted with a BD FACSAria III sorter. For LC–γδ T cell coculture, LCs from naïve WT and $p38\alpha^{\Delta DC}$ mice and γδ T cells from WT mice were mixed in the presence of R848 (1 μg/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 $p38\alpha^{\Delta DC}$ mice and naïve $CD4^+$ T cells from OT-II mice were mixed in the presence of ovalbumin peptide (10 μg/ml) ($OVA_{323–339}$) and R848 (1 μg/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-1β (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 $p38\alpha^{\Delta DC}$ mice were stimulated with R848 (1 μg/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-γδTCR (eBioGL3), anti-CD3 (17A2), anti-CD4 (RM4-5), anti-CD8α (53-6.7), anti-TCRβ (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-IFNγ (XMG 1.2e), anti–IL-23 (fc23cpg), anti–IL-1β (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 μm 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, TNFα, IL-23, IL-6, and IL-1β 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*, TCAGTCAACGGGGGACATAAA (forward) and GGGGCTGTACTGCTTAACCAG (reverse); *Il17a*, TCAGCGTGTCCAAACACTGAG (forward) and CGCCAAGGGAGTTAAAGACTT (reverse); *Tnfa*, CAGGCGGTGCCTATGTCTC (forward) and CGATCACCCCGAAGTTCAGTAG (reverse); *Il6*, CTGCAAGAGACTTCCATCCAG (forward) and AGTGGTAT AGACAGGTATGTTGG (reverse); *Il1b*, GCAACTGTTCCTGAACTCAACT (forward) and ATCTTTTGGGGTCCGTCAACT (reverse); *Il23a*, GCCCCGTATCCAGTGTGA (forward) and GCTGCCACTGCTGACTAG (reverse); *Csf2*, GGCCTTGGAAGCATGTAGAGG (forward) and GGAGAACTCGTTAGAGACGACTT (reverse); *Cxcl1*, TGCACCCAAACCGAAGTCAT (forward) and TTGTCAGAAGCCAGCGTTCAC (reverse); *Ifng*, GCCACGGCACAGTCATTGA (forward) and TGCTGATGGCCTGATTGTCTT (reverse); *Il12a*, CAATCACGCTACCTCCTCTTTT (forward) and CAGCAGTGCAGGAATAATGTTTC (reverse); *Il4*, GGTCTCAACCCCCAGCTAGT (forward) and GCCGATGATCTCTCTCAAGTGAT (reverse); *Il10*, CTTACTGACTGGCATGAGGATCA (forward) and GCAGCTCTAGGAGCATGTGG (reverse); *Tgfb1*, CTCCCGTGGCTTCTAGTGC (forward) and GCCTTAGTTTGGACAGGATCTG; *Foxp3*, CACCTATGCCACCCTTATCCG (forward) and CATGCGTAAACCAATGGTAGA (reverse); *Il22*, ATGAGTTTTTCCTTATGGGGAC (forward) and GCTGGAAGTTTGGACACCTCAA (reverse); *S100a7a*, TGCTCTTGGATAGTGTGCCTC (forward) and GCTCTGTGATGTAGTATGGCTG (reverse); *S100a8*, TGTCCTCAGTTTGTGCAGAATATAAA (forward) and TCACCATCGCAAGGAACTCC (reverse); *Krt16*, GGTGGCCTCTAACAGTGATCT (forward) and TGCATACAGTATCTGCCTTTGG (reverse); *Krt17*, ACCATCCGCCAGTTTACCTC (forward) and CTACCCAGGCCACTAGCTGA (reverse); *Il27*, CTGTTGCTGCTACCCTTGCTT (forward) and CACTCCTGGCAATCGAGATTC (reverse); and *Mapk14*, GAGGTGCCCGAACGATAC (forward) and TGGCGTGAATGATGGACT (reverse).

Statistical analysis

The data were analyzed with GraphPad Prism 5 or SPSS 17.0 and are means ± 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. p38α deletion in mouse skin tissue and DCs.

Fig. S2. Normal DC development and activation status in the epidermis and dermis of $p38\alpha^{\Delta DC}$ mice.

Fig. S3. Cell infiltration analysis in the skin and spleen of WT and $p38\alpha^{\Delta DC}$ mice upon IMQ treatment.

Fig. S4. Gene expression in KCs and cytokine production in skin tissue of WT and $p38\alpha^{\Delta DC}$ mice upon IMQ treatment.

Fig. S5. Decreased IL-17 production from γδ and $CD4^+$ T cells in the skin and DLNs of $p38\alpha^{\Delta DC}$ mice.
Fig. S6. The proliferation and apoptosis of T cells in IMQ-treated WT and $p38\alpha^{\Delta DC}$ mice.
Fig. S7. p38α activity in LCs is important for psoriasiform skin inflammation.
Fig. S8. LC p38α-mediated T_H17 cell differentiation and IL-23, IL-1β, and IL-6 expression.
Fig. S9. The effect of p38α signaling in DCs on skin inflammation is IL-1β–independent.
Fig. S10. p38α in dDCs affects IL-17 production from $CD4^+$ T cells but not γδ T cells.
Fig. S11. p38α activity in T cells does not contribute to the IMQ-induced psoriasiform skin inflammation.
Fig. S12. Decreased IL-17 production from γδ and $CD4^+$ T cells in the DLNs upon SB203580 treatment.
Fig. S13. Acute deletion of p38α reduces the severity of an ongoing psoriasiform skin inflammation.

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p38α signaling in Langerhans cells promotes the development of IL-17–producing T cells and psoriasiform skin inflammation

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p38α 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 p38α 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.