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IL-6R/STAT3-signaling in keratinocytes rather than T cells induces psoriasis-like dermatitis in mice

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Abstract

STAT3 is important for psoriasis pathogenesis as STAT3-signaling downstream of IL-6, IL-21, IL-22 and IL-23 contributes to Th17 cell development and keratinocyte STAT3 expression in transgenic mice (K14-Stat3C mice) develop psoriasis-like dermatitis. Herein, the relative contribution of STAT3-signaling in keratinocytes versus T cells was evaluated in the imiquimod model of psoriasis-like dermatitis. Mice with STAT3 inducible deletion in keratinocytes (K5-STAT3$^{-/-}$ mice) had decreased psoriasis-like dermatitis and epidermal phosphorylation of STAT3 (pSTAT3) compared with wt mice, whereas mice with constitutive deletion of STAT3 in all T cells were like wt mice. Interestingly, mice with keratinocyte-inducible deletion of IL-6 receptor alpha had similar findings as K5-STAT3$^{-/-}$ mice, identifying IL-6/IL-6R as a predominant upstream signal for keratinocyte-STAT3-induced psoriasis-like dermatitis. Moreover, psoriasis-like dermatitis inversely associated with type 1 immune gene products, especially CXCL10, whereas CXCL10 limited psoriasis-like dermatitis, suggesting that keratinocyte-STAT3 signaling promoted psoriasis-like dermatitis by restricting downstream CXCL10 expression. Finally, treatment of mice with the pan-JAK inhibitor, tofacitinib reduced psoriasis-like dermatitis and epidermal

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AUTHOR CONTRIBUTIONS

Conceptualization: NKA, LSM, AR; Investigation: AR, SN, MA, DD, CY, YW, NO, GP, SL, RO, HL, RM, CD, MM, SC, NKA; Formal Analysis: AR, SN, MA, DD, CY, GP, NKA, LSM; Funding Acquisition: NKA, LSM; Writing – original draft: NKA, LSM; Writing – review and editing: NKA, LSM.

CONFLICTS OF INTEREST

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pSTAT3 expression. Taken together, STAT3-signaling in keratinocytes rather than T cells was a more important determinant for psoriasis-like dermatitis in a mechanism that involved upstream keratinocyte IL-6R-signaling and downstream inhibition of type 1 immunity-associated CXCL10 responses.

INTRODUCTION

Psoriasis is a chronic inflammatory skin disease that affects ~2-3% of the population (Armstrong and Read, 2020, Greb et al., 2016). The signal transducer and activator of transcription 3 (STAT3) signaling molecule has been linked to the pathogenesis of psoriasis as STAT3-signaling is downstream of IL-6, IL-21, IL-22 and IL-23, which contribute to Th17 cell development and function (Burkett et al., 2015, Greb et al., 2016, van der Fits et al., 2009). However, transgenic mice with constitutive keratinocyte STAT3 expression (K5-Stat3C mice) develop spontaneous psoriasis-like dermatitis (Sano et al., 2005). Although STAT3 might have differential functions in Th17 and keratinocytes responses, the relative contribution and mechanism of STAT3 signaling in keratinocytes versus T cells in psoriasis pathogenesis has not been directly evaluated.

Therefore, in this study we evaluated the role of STAT3-intrinsic signaling in keratinocytes and T cells by comparing mice with keratinocyte-inducible deletion of STAT3 (K5-STAT3$^{-/-}$ mice) and mice with T cell-intrinsic deletion of STAT3 (Lck-STAT3$^{-/-}$ mice) in the imiquimod mouse model of psoriasis-like dermatitis (Swindell et al., 2017, van der Fits et al., 2009). Since many cytokines signal via STAT3 and STAT3 regulates many downstream immune responses, we further evaluated specific upstream and downstream immune responses that contributed to STAT3-mediated psoriasis-like dermatitis. Finally, we tested the therapeutic potential of the pan-JAK inhibitor tofacitinib in reducing psoriasis-like dermatitis and whether it impacted keratinocyte STAT3 responses.

RESULTS

STAT3 signaling in keratinocytes rather than T cells induces psoriasis-like dermatitis

To model psoriasis-like dermatitis in mice, imiquimod was applied to the ears of C57BL/6 wild-type (wt) and two strains of cre/lox mice: (1) Keratin 5 (K5)-cre^{ERT2} mice crossed with a STAT3$^{fl/fl}$ mouse strain to generate tamoxifen inducible STAT3 deletion specifically in keratinocytes (K5-STAT3$^{-/-}$ mice) and (2) Lck-cre mice crossed with a STAT3$^{fl/fl}$ mouse strain to generate constitutive deletion of STAT3 in all T cells (Lck-STAT3$^{-/-}$ mice). For inducible deletion of STAT3 in keratinocytes in mouse ears (inducible deletion was necessary because STAT3 constitutive deletion is embryonic lethal (Takeda et al., 1997)), the ears of the K5-STAT3$^{-/-}$ mice were treated topically with 10 μl of tamoxifen (10 mg/mL) for 5 consecutive days. After an additional 2 days of no treatment, topical imiquimod was then applied to the mouse ears daily for an additional 5 days, as previously described (van der Fits et al., 2009). Tamoxifen-induced STAT3 deletion in K5-STAT3$^{-/-}$ mice was confirmed by Q-PCR for the excised floxed STAT3 gene (see Methods).

In the imiquimod model, K5-STAT3$^{-/-}$ mice (tamoxifen-treated) with STAT3 deletion in keratinocytes had significantly reduced ear thickness, epidermal thickness and phosphorylated STAT3 (pSTAT3) compared with tamoxifen-treated wt mice (Fig. 1a-d). In contrast, Lck-STAT3$^{-/-}$ mice with constitutive STAT3 deletion in all T cells had no significant differences in ear thickness, epidermal thickness and pSTAT3 compared with wt mice (Fig. 1e-h). Since Lck-cre mediated deletion is inefficient in adult γδ T cells (Fiala et al., 2019) and γδ T cells are important for imiquimod-induced psoriasis-like dermatitis (Pantelyushin et al., 2012), we also performed the imiquimod model in mice with Cre expression under the tamoxifen-inducible TCRδ-cre^{ER} promoter crossed with STAT3$^{fl/fl}$ mice (TCRδ-STAT3$^{-/-}$ mice), which have improved deletion efficiency in γδ T cells (Zhang et al., 2015). Similar to Lck-STAT3$^{-/-}$ mice, we found that TCRδ-STAT3$^{-/-}$ mice had no differences in ear and epidermal thickness compared to wt mice (Supplementary Fig. S1a-c). Therefore, STAT3-signaling in keratinocytes rather than T cells was a more important determinant for inducing psoriasis-like dermatitis.

STAT3 signaling in keratinocytes regulates lymph node CD4$^+$ and γδ T cell subset numbers

Given the reported roles of CD4$^+$ T cells (*i.e.*, Th1, Th17 and Th22 cells) and γδ T cells in contributing to the psoriasis-like dermatitis in the imiquimod model (Cai et al., 2011, Di Cesare et al., 2009, Ishizaki et al., 2011, Pantelyushin et al., 2012, Skepner et al., 2014), we determined whether keratinocyte-intrinsic STAT3 signaling regulated the numbers of these T cell subsets in the ear-draining lymph nodes (dLNs). In tamoxifen-treated K5-STAT3$^{-/-}$ and wt mice, T cells were isolated from the dLNs on day 5 of the imiquimod treatment and were stimulated *ex vivo* with PMA/ionomycin prior to performing intracellular flow cytometry. The numbers of IL-17A$^+$ CD4$^+$ T cells (Th17), IL-22$^+$ CD4$^+$ T cells (Th22), IL-17$^+$ γδ T cells, and IL-22$^+$ γδ T cells but not IFNγ$^+$ CD4$^+$ T cells (Th1) were reduced, whereas IFNγ$^+$ CD8$^+$ T cells (Tc1) were increased in the dLNs of K5-STAT3$^{-/-}$ mice compared with wt mice (Fig. 2a,b). Furthermore, we determined whether T cell-intrinsic STAT3 signaling regulated the numbers of T cell subsets in the dLNs by comparing Lck-STAT3$^{-/-}$ with wt mice and found there was no significant difference in the numbers of T cell subsets examined (Supplementary Fig. S1d).

Keratinocyte STAT3 signaling promotes IL-23 expression in Langerhans cells

Since keratinocyte STAT3 activation is reported to induce IL-23 production in Langerhans cells (LCs) (Nakajima et al., 2019) and LCs are required for the induction of IL-17-producing γδ T cells in the imiquimod model (Yoshiki et al., 2014), we performed immunofluorescence co-staining for IL-23 and Langerin on day 1 skin from K5-STAT3$^{-/-}$ and wt mice. In K5-STAT3$^{-/-}$ mice, there was a significant reduction in the total number of IL-23+ cells compared to wt mice, with IL-23+ cells localized to the dermis (Supplementary Fig. S2a,b). Furthermore, despite a similar number of LCs in the skin of K5-STAT3$^{-/-}$ and wt mice (Supplementary Fig. S2c), there was a significant reduction in the number of IL-23+ LCs in K5-STAT3$^{-/-}$ mice compared with wt mice (Supplementary Fig. S2d).

IL-6R signaling contributes to keratinocyte-STAT3-mediated psoriasis-like dermatitis

IL-6 receptor alpha (IL-6R) is expressed by proliferating keratinocytes (Wang et al., 2004, Yoshizaki et al., 1990). Also, IL-6/IL-6R signaling through STAT3 contributes to

the differentiation of naïve T cells to Th17 cells (Zhou et al., 2007) and is implicated in psoriasis pathogenesis (Blauvelt, 2017). Therefore, we hypothesized that keratinocyte IL-6R-signaling was the predominant STAT3 signal that contributed to the findings between wt and K5-STAT3$^{-/-}$ mice (Fig. 1a-d). To test this, the imiquimod mouse model was performed in K14-cre^{ERT2} mice crossed with IL-6R$^{fl/fl}$ mice that resulted in tamoxifen inducible IL-6R deletion specifically in keratinocytes (K14-IL-6R$^{-/-}$ mice). K14-IL-6R$^{-/-}$ mice had significantly reduced ear thickness, epidermal thickness and pSTAT3 expression compared with wt mice (Fig. 2c-f). Furthermore, K14-IL-6R$^{-/-}$ mice had reduced Th17, Th22, and IL-17^{+} and IL-22^{+} γδ T cells but not Tc1 and Th1 cells in the dLNs compared with wt mice (Fig. 2g). To determine whether IL-23 was involved in the keratinocyte STAT3-mediated psoriasis-like dermatitis, we performed rIL-23 i.d. injections in the ears of K5-STAT3$^{-/-}$ and wt mice, as described (Gauld et al., 2018). We found that K5-STAT3$^{-/-}$ mice had comparable ear thickness and slightly elevated epidermal thickness as wt mice (Supplementary Fig. S3a-c). Therefore, since the findings in K14-IL-6R$^{-/-}$ mice closely resembled those of K5-STAT3$^{-/-}$ mice, IL-6R-signaling was likely the predominant keratinocyte-intrinsic STAT3 signal that contributed to psoriasis-like dermatitis.

Keratinocyte overexpression of STAT3 promoted psoriasis-like dermatitis without impacting T cell subsets in dLNs

Next, we examined the mechanism by which overexpression of STAT3 in keratinocytes in K14-Stat3C transgenic mice contributed to imiquimod induced psoriasis-like dermatitis. In the imiquimod model, K14-Stat3C transgenic mice had increased ear thickness, epidermal thickness and pSTAT3 immunofluorescence compared with wt mice (Fig. 3a-d). However, there were no significant differences in the numbers of Th1, Th17, Th22 cells and IL-17^{+} or IL-22^{+} γδ T cells in the dLNs of K14-Stat3C transgenic mice and wt mice (Fig. 3e). Therefore, constitutive overexpression of STAT3 in keratinocytes in K14-Stat3C transgenic mice was sufficient to promote psoriasis-like dermatitis in the absence of any changes in the numbers of T cell subsets in the dLNs.

Keratinocyte STAT3-signaling restricts type 1 response gene expression

Next, we evaluated how STAT3-signaling in keratinocytes impacted the skin transcriptome of wt mice, K5-STAT3$^{-/-}$ mice (with inducible STAT3 deletion in keratinocytes), and K14-Stat3C mice (with transgenic STAT3 expression in keratinocytes) by performing Q-PCR on the ear skin on day 5 of imiquimod treatment. In K5-STAT3$^{-/-}$ mice, there was significantly increased expression of transcripts involved in type 1/IFNγ immunity (*e.g.*, *CD8a*, *Cxcl10*, *Cxcl11*, *IL-12a*, and *Nos2*) whereas there were no differences in transcripts involved in type 3/IL-17 immunity (e.g., *Il1a*, *Il1b*, *Il6*, *Il17a*, and *Tgbf1*) compared with wt mice (Fig. 4a,b). To determine whether differences occurred at earlier time points, we performed Q-PCR on the ear skin on day 1 and day 3 of imiquimod treatment in K5-STAT3$^{-/-}$ and wt mice. On day 1 of imiquimod treatment, there were no differences in transcripts involved in either type 1 or type 3 immunity between K5-STAT3$^{-/-}$ and wt mice (Supplementary Fig. S4a,b). However, on day 3 there was markedly increased expression of *Cxcl10* and *Cxcl11* expression and a significant decrease in *Il6* expression in K5-STAT3$^{-/-}$ mice compared to wt mice (Supplementary Fig. S4c,d). Conversely, K14-Stat3C mice had significantly decreased expression of transcripts involved in type 1

immunity, especially *Cxcl10* and *IL12b* but not *Cxcl11*, with no differences in expression of transcripts involved in type 3 immunity compared with wt mice (Fig. 4c,d). To confirm whether the increased *Cxcl10* transcripts also resulted in increased CXCL10 protein in K5-STAT3$^{-/-}$ mice, immunohistochemistry for CXCL10 protein expression was performed and there was increased CXCL10 protein expression in K5-STAT3$^{-/-}$ mice compared with wt mice (Fig. 4e). In contrast, K14-IL-6R$^{-/-}$ mice had similar expression of CXCL10 in the skin compared with wt mice (Supplementary Fig. S5a,b). Taken together, keratinocyte STAT3-signaling-induced psoriasis-like dermatitis inversely correlated with expression of transcripts involved in type 1 immunity, especially CXCL10.

Given these findings, we hypothesized that CXCL10 functions to restrict keratinocyte STAT3-signaling-induced psoriasis-like dermatitis. To test this in the imiquimod model, wt mice were treated with an anti-CXCR3 antibody, thereby blocking CXCL10 binding to its receptor CXCR3. Anti-CXCR3 treatment resulted in a statistically significant increase in psoriasis-like dermatitis compared with isotype control antibody treatment (Fig. 4f). Furthermore, anti-CXCR3 treated mice had reduced Tc1 and Th17 cells, but increased IL-17^{+} γδ T cells in the dLNs compared with isotype control mice (Fig. 4g). Therefore, CXCL10 was likely an important factor that counter-regulated keratinocyte STAT3-signaling-induced psoriasis-like dermatitis.

JAK inhibition inhibits keratinocyte STAT3 activation and alleviates psoriasis-like dermatitis

To therapeutically target keratinocyte-STAT3 signaling by inhibiting IL-6/IL-6R activity as well as other factors that signal via STAT3 that contributed to imiquimod-induced psoriasis-like dermatitis, mice were treated with the pan-JAK inhibitor tofacitinib. Tofacitinib inhibits JAK1, JAK2 and JAK3, blocking cytokines that signal via the common γ chain (γc) (*e.g.*, IL-2, IL-4, IL-7, IL-15 and IL-21) as well as non-γc cytokines, including IFN-γ and IL-6 and to a lesser extent IL-12 and IL-23 (Ghoreschi et al., 2011). Wt mice were orally administered tofacitinib during the imiquimod model and this systemic treatment resulted in reduced ear thickness, epidermal thickness and pSTAT3 activation (Fig. 5a-d). Thus, although tofacitinib is not specific for keratinocyte IL-6R/STAT3 inhibition, these data provide the proof-of-concept for using JAK inhibition to therapeutically target keratinocyte-induced psoriasis-like dermatitis.

DISCUSSION

Recent findings have implicated Th17 cells as a major cellular mediator in psoriasis pathogenesis (Blauvelt and Chiricozzi, 2018, Di Cesare et al., 2009, Greb et al., 2016). IL-6, IL-21, IL-22 and IL-23, which all signal via STAT3, contribute to the differentiation and maintenance of Th17 cell development and function (Burkett et al., 2015, Greb et al., 2016, van der Fits et al., 2009), implicating T cell-intrinsic STAT3-signaling in Th17 biology in psoriasis. However, in addition to the role of STAT3 in the Th17 cells, transgenic mice with constitutive keratinocyte STAT3 expression (K5-Stat3C mice) develop spontaneous psoriasis-like dermatitis (Sano et al., 2005). Since STAT3 could contribute to the pathogenesis of psoriasis through differential roles on Th17 cells and keratinocytes, we

used cre/lox mice to study potential functional roles of STAT3 and the mechanisms involved. Unexpectedly, we found that K5-STAT3$^{-/-}$ mice had markedly decreased psoriasis-like dermatitis in the imiquimod model of psoriasis. In contrast, Lck-STAT3$^{-/-}$ mice had no difference in the degree of psoriasis-like dermatitis. These findings uncovered that STAT3-signaling by keratinocytes was a more important determinant than STAT3-signaling by T cells for inducing of psoriasis-like dermatitis. This result provides several important insights into the pathogenesis of psoriasis.

First, we found an important contribution of STAT3-signaling in keratinocytes during psoriasis-like dermatitis, which resembles previously identified roles of STAT3 in keratinocyte biology. For example, STAT3 regulates keratinocyte proliferation (Chan et al., 2004) and is activated in the keratinocytes of psoriatic patients (Sano et al., 2005). The role of keratinocyte-intrinsic STAT3-signaling was demonstrated in the markedly decreased epidermal thickness along with diminished epidermal pSTAT3 immunofluorescence in imiquimod-treated K5-STAT3$^{-/-}$ mice compared with wt mice. Conversely, in the K14-Stat3C mice with transgenic expression of STAT3 in keratinocytes, there was increased epidermal thickness along with enhanced epidermal pSTAT3 immunofluorescence in the imiquimod model. In accordance with a previous report (Nakajima et al., 2019), we discovered that keratinocyte STAT3 signaling promoted IL-23 production in LCs upon imiquimod treatment. This may be a potential mechanism whereby keratinocyte STAT3 signaling induces psoriasis-like dermatitis and downstream T cell responses, as LCs are required for the induction of IL-17-producing γδ T cells and psoriasis-like dermatitis in the imiquimod model (Yoshiki et al., 2014). These findings provide key evidence that STAT3-signaling in keratinocytes plays an important role in the pathogenesis of psoriasis-like dermatitis.

Second, an unexpected finding was that T cell-intrinsic STAT3-signaling was dispensable for skin inflammation, despite the known role of STAT3 in Th17 development and the importance of IL-17 producing T cells in the imiquimod psoriasis-like dermatitis model (Burkett et al., 2015, van der Fits et al., 2009). Potential explanations include a role for STAT3-independent IL-17 production, which was reported in PLZF-expressing innate αβ+ T cells (e.g., iNKT, CD4−/CD8+, and CD4−/CD8− T cells) (St Leger et al., 2018), the involvement of dermal Vγ6^{+} γδ T cells, which produce IL-17 in a STAT3-independent manner (Cai et al., 2019), or a compensatory role for ILCs (Pantelyushin et al., 2012) or IL-17-producing PMNs and eosinophils in the absence of T cell-intrinsic STAT3 signaling (Guerra et al., 2017, Kim et al., 2018, Lin et al., 2011, Sumida et al., 2014). It could also be that the imiquimod mouse model of psoriasis dermatitis is less dependent on Th17 responses than human psoriasis (Swindell et al., 2017). Nonetheless, our results suggest that keratinocyte-intrinsic STAT3-signaling contributed to enhancing psoriasis-like dermatitis and mediating epidermal thickening.

Third, we found that K14-IL-6R$^{-/-}$ mice with tamoxifen inducible deletion of IL-6R specifically in keratinocytes had findings that closely resembled those of K5-STAT3$^{-/-}$ mice in the imiquimod model. However, this mechanism did not involve IL-23 signaling upstream of keratinocyte STAT3, as K5-STAT3$^{-/-}$ mice had similar psoriasis-like dermatitis as wt mice when injected with rIL-23 at the time points measured. This result suggests

that IL-6/IL-6R-signaling, but not IL-23 was the predominant mechanism by which STAT3-signaling in keratinocytes contributes to psoriasis-like dermatitis and epidermal thickening. In addition, we found that K14-IL-6R$^{-/-}$ mice had decreased Th17, Th22 as well as IL-17^{+} γδ T cells, and IL-22^{+} γδ T cells in the dLNs during the imiquimod model. This finding links STAT3 signaling in keratinocytes to impacting the numbers of T cell subsets that are relevant for inducing psoriasis-like dermatitis. Moreover, the finding that keratinocyte IL-6R and STAT3 expression directly impacts the development of IL-17^{+} and IL-22^{+} T cells not only has important implications for psoriasis but provides additional explanations for why dominant negative *STAT3 or IL6ST* mutations in Hyper-IgE syndrome patients or humans with loss-of-function mutations in *IL6R* have impaired Th17 and Th22 responses (Béziat et al., 2020, Holland et al., 2007, Ma et al., 2008, Milner et al., 2008, Minegishi et al., 2007, Spencer et al., 2019). However, IL-6 blockade is either ineffective for psoriasis or can induce new-onset psoriasis-like disease (Blauvelt, 2017), suggesting that IL-6R-independent keratinocyte STAT3 activation may be essential for human psoriasis. Moreover, the aberrant transgenic expression of STAT3 in K14-Stat3C mice did not impact the number of T cell subsets in the draining lymph nodes, despite these mice having enhanced epidermal thickness in the imiquimod model. Given that keratinocyte STAT3-signaling could affect the numbers and function of T cells in the inflamed skin itself or through other mechanisms, these possibilities will be evaluated in our future work.

In addition, we found that the expression of keratinocyte STAT3 had an inverse correlation with type 1 immune transcripts and specifically with CXCL10. This result could have been due to enhanced STAT1-signaling which induces type 1 interferon responses. This might have especially been the case because IL-6 signals via both STAT3 and STAT1 (Hirahara et al., 2015) and in the absence of keratinocyte STAT3 in the K5-STAT3$^{-/-}$ mice, IL-6 might have led to enhanced keratinocyte STAT1-signaling. Indeed, we found that K5-STAT3$^{-/-}$ mice had exaggerated type 1 gene expression (e.g., *Il12a and Cxcl10*) and Tc1 cells, potentially involving a mechanism whereby IL-6R preferentially signals through STAT1 in the absence of STAT3 in keratinocytes, especially since K14-IL-6R$^{-/-}$ mice did not have exaggerated *Cxcl10* expression and Tc1 cells. Moreover, we had found that there were functional consequences to the inverse correlation between keratinocyte STAT3 expression and CXCL10 as wt mice treated with an anti-CXCR3 blocking antibody had exaggerated psoriasis-like dermatitis and IL-17-producing γδ T cells, suggesting that CXCL10 restricted keratinocyte-intrinsic STAT3-induced psoriasis-like dermatitis through a mechanism that involves a reduction in IL-17^{+} γδ T cells. These results recapitulate findings in mouse models of experimental autoimmune encephalomyelitis (EAE) whereby CXCL10 neutralization increased disease severity (Narumi et al., 2002), and CXCR3-deficient mice had higher CNS infiltrating Th17 cells compared to wt mice (Chung and Liao, 2016, Liu et al., 2006, Müller et al., 2007). However, K14-IL-6R$^{-/-}$ mice had reduced psoriasis-like dermatitis despite similar *Cxcl10* expression as wt mice, suggesting that mechanisms in addition to CXCL10 regulate keratinocyte IL-6R/STAT3-mediated psoriasis-like dermatitis. Despite similar efficacy of anti-IL-12/23p40 and anti-IL-23p19 treatment for psoriasis (Levin and Gottlieb, 2014), our results regarding the keratinocyte STAT3-mediated counter-regulation between CXCL10 and IL-17-producing γδ T cells may be more clinically relevant to the emerging role of JAK inhibitors (which also inhibit STATs)

in psoriasis (Kvist-Hansen et al., 2020, Solimani et al., 2019). Moreover, JAK inhibitors with increased selectivity are in development or in clinical trials (e.g., selective JAK1 inhibitors: Abrocitinib, Itacitinib, and Upadacitinib) (Kvist-Hansen et al., 2020, Solimani et al., 2019), which may reveal counter-regulatory immune mechanisms. Together, these results provide a potential mechanism for keratinocyte STAT3 signaling to promote IL-17-producing T cells in psoriasis-like dermatitis in mice. However, whether other potential mechanisms exist for keratinocyte STAT3 activation in the development of T cell responses will be the focus of future work.

Finally, to determine whether keratinocyte-intrinsic STAT3 signaling could be therapeutically targeted, mice were treated with the pan-JAK inhibitor tofacitinib, which reduced skin inflammation and epidermal pSTAT3 immunofluorescence. Similarly, tofacitinib treatment in psoriasis patients had attenuated disease scores and epidermal pSTAT3 by immunohistochemistry (Krueger et al., 2016). In another report, a topical STAT3 inhibitor STA-21 was applied to the skin of psoriasis patients that resulted in improvement of the skin inflammation and applied to the skin of TPA-treated K5-Stat3C mice that caused significantly reduced psoriasis-like dermatitis and epidermal pSTAT3 (Miyoshi et al., 2011). Whether a topical STAT3 inhibitor had a similar effect in wt and K5-STAT3$^{-/-}$ mice in the imiquimod model will be the subject of our future work. Nonetheless, our findings with tofacitinib provide the proof-of-concept that inhibition of keratinocyte STAT3-signaling could be a viable therapeutic target to alleviate skin inflammation in psoriasis.

There were several limitations. First, the imiquimod model does not replicate all aspects of human psoriasis (Hawkes et al., 2017, Swindell et al., 2017), limiting the translatability of the findings. Thus, future experiments in other mouse models of psoriasis-like dermatitis and especially in human psoriasis patients are warranted to confirm these results. Second, the use of tamoxifen to inducibly delete STAT3 or IL-6R expression in the K5-STAT3$^{-/-}$ and K14-IL-6R$^{-/-}$ mice, respectively, likely influenced some of the immune mechanisms involved in the imiquimod-induced skin inflammation (such as neutrophil function (Corriden et al., 2015)). To control for these immune effects of tamoxifen, we also treated the control wt comparison groups with tamoxifen in this study. Furthermore, deletion efficiency in γδ T cells in Lck-cre and TCRδ-cre^{ER} mice is ~20% and ~60%, respectively (Fiala et al., 2019, Zhang et al., 2015), leaving the possibility that residual STAT3 activity in γδ T cells could have played a role in the psoriasis-like dermatitis. Finally, the anti-CXCR3 mAb blocks not only CXCL10, but CXCL9 and CXCL11 signaling. Although the wt mouse strain used in this study does not have functional CXCL11 (Sierro et al., 2007), we cannot rule out a role for CXCL9 in the imiquimod model.

In summary, our findings identified keratinocyte-intrinsic STAT3-signaling as an essential mechanism for mediating psoriasis-like dermatitis, which was more relevant than T cell-intrinsic STAT3-signaling. In addition, the mechanism of keratinocyte-STAT3-induced psoriasis-like dermatitis involved upstream IL-6/IL-6R-signaling in keratinocytes and downstream inhibition of type 1 immunity-associated gene products, especially CXCL10. This keratinocyte-intrinsic immune pathway that was uncovered has implications in psoriasis pathogenesis and could represent a therapeutic target.

MATERIALS AND METHODS

Mice

K5-cre^{ERT2}×STAT3$^{fl/fl}$ (K5-STAT3$^{-/-}$), K14-cre^{ERT2}×IL-6Rα$^{fl/fl}$ (K14-IL-6R$^{-/-}$), Lck-cre×STAT3$^{fl/fl}$ (Lck-STAT3$^{-/-}$), and TCRδ-cre^{ER}×STAT3$^{fl/fl}$ (TCRδ-STAT3$^{-/-}$) mice on a C57Bl/6J background were generated and wildtype C57Bl/6J (wt) mice were purchased from Jackson Laboratories (Bar Harbor, ME). K14-Stat3C mice on a C57Bl/6J background were a generous gift from Dr. Qing-Sheng Mi (Henry Ford Health System). All mouse strains were bred and maintained under the same specific pathogen-free conditions, with air isolated cages at an American Association for the Accreditation of Laboratory Animal Care (AAALAC)-accredited animal facility at Johns Hopkins University and handled according to procedures described in the Guide for the Care and Use of Laboratory Animals as well as Johns Hopkins University's policies and procedures as set forth in the Johns Hopkins University Animal Care and Use Training Manual, and all animal experiments were approved by the Johns Hopkins University Animal Care and Use Committee. Gender-and age-matched 6-8 week old mice were used for each experiment.

Imiquimod mouse model

To induce skin inflammation, mice were anesthetized with 2% isoflurane and 70 μl (62.5 mg) of imiquimod (5% Taro Pharmaceuticals, Hawthorne, NY) was applied to both sides of a mouse ear daily for 5 days. Prior to imiquimod application, ear thickness was measured with a manual caliper (Peacock (0.01-10mm). A day after the last application, full thickness ear skin was excised with a 6 mm punch biopsy (Acuderm, Fort Lauderdale, FL) and bisected for histological and qPCR analysis. Draining lymph nodes (dLNs) were harvested for FACs analysis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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DATA AVAILABILITY STATEMENT

The authors confirm that the data supporting the findings of this study are available within the article and its supplementary text and are available free of charge to all researchers wherever possible and with minimal reuse restrictions.

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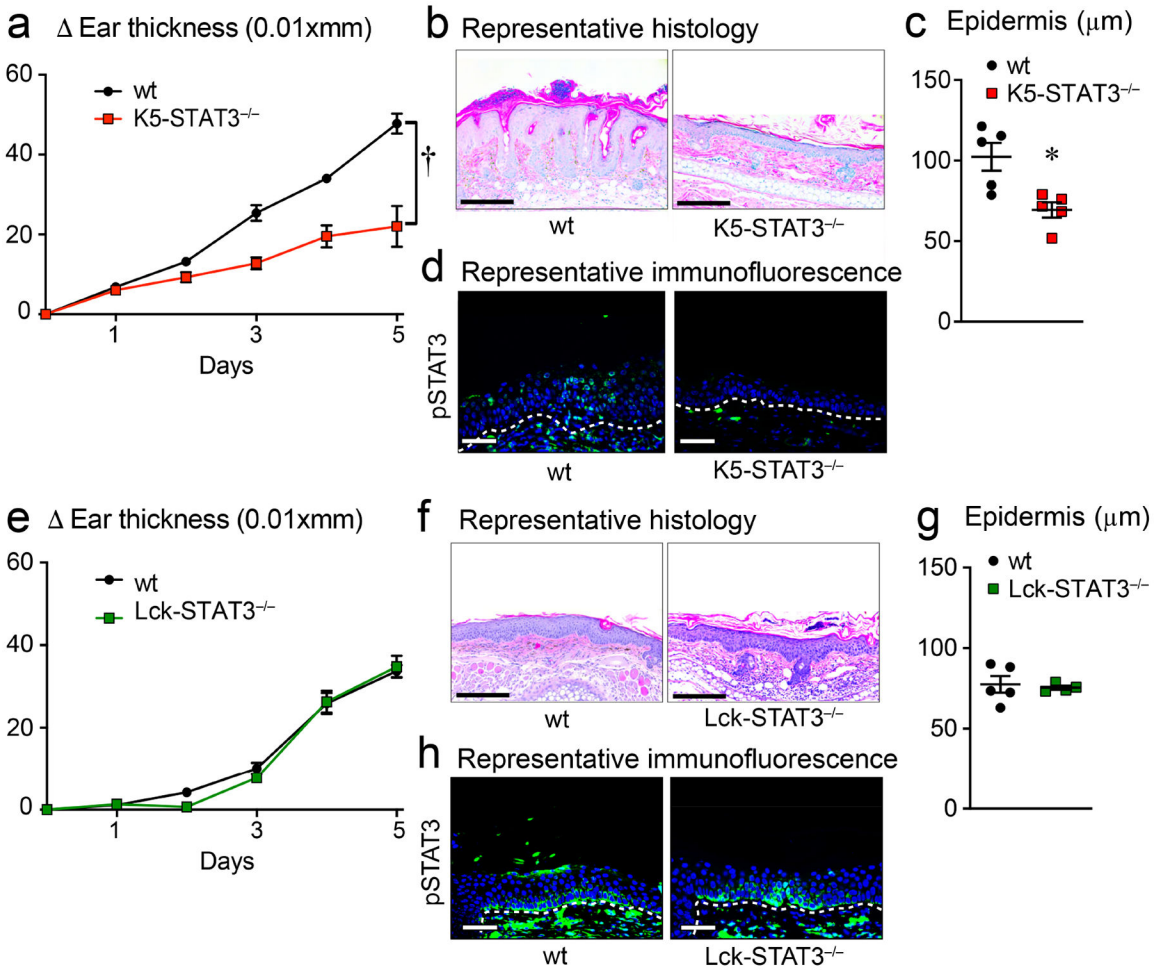

Figure 1. Keratinocyte STAT3 regulates imiquimod induced skin inflammation.

(a-d) Wt and K5-STAT3$^{-/-}$ mice ears were pretreated with tamoxifen and given 5 daily imiquimod applications. (a) Mean ± SEM ear thickness measurements (0.01 x mm) (n = 4-5). (b) Representative day 5 skin histology (hematoxylin and eosin stain). Scale bar = 200 μm. (c) Mean ± SEM epidermal thickness (μm) (n = 5). (d) Representative skin immunofluorescence with anti-pSTAT3 mAb (green) and DAPI (blue). Dashed line = dermoepidermal junction. Scale bar = 50 μm. (e-g) wt and Lck-STAT3$^{-/-}$ mouse ears were given 5 daily imiquimod applications. (e) Mean ± SEM ear thickness measurements (0.01 x mm) (n = 4-5). (f) Representative day 5 skin histology (hematoxylin and eosin stain). Scale bar = 200 μm. (g) Mean ± SEM epidermal thickness (μm) (n = 4-5). (h) Representative skin immunofluorescence with anti-pSTAT3 mAb (green) and DAPI (blue). Dashed line = dermoepidermal junction. Scale bar = 50 μm. *P < .05, and †P < .01, wt versus K5-STAT3$^{-/-}$ or wt versus Lck-STAT3$^{-/-}$ as calculated by 2-way ANOVA (a,e) or 2-tailed Student t test (c,g). Results are representative of at least 2 independent experiments.

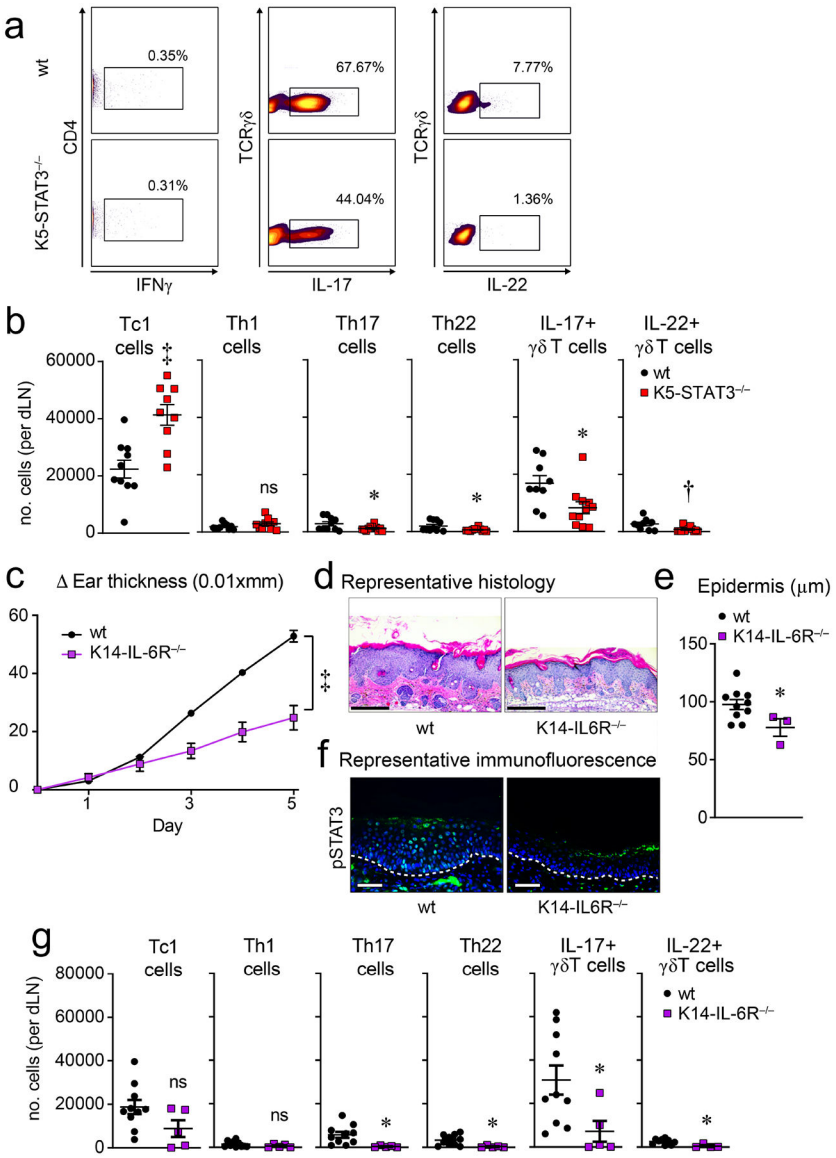

Figure 2. Keratinocyte IL-6 receptor controls T cell responses and skin inflammation.

(a,b) Wt and K5-STAT3$^{-/-}$ day 5 dLN cells were stimulated and analyzed by flow cytometry. (a) Representative flow plots for cytokine producing T cells. (b) Mean ± SEM number of cytokine producing T cells from dLNs (n = 9-11). (c-g) Wt and K14-IL-6R$^{-/-}$ mice ears were pretreated with tamoxifen and given imiquimod for 5 days. (c) Mean ± SEM ear thickness measurements (0.01 x mm) (n = 8-13). (d) Representative skin histology (hematoxylin and eosin stain). Scale bar = 200 μm. (e) Mean ± SEM epidermal thickness (μm) (n = 3-10). (f) Representative skin immunofluorescence with anti-pSTAT3 mAb (green) and DAPI (blue). Dashed line = dermoepidermal junction. Scale bar = 50 μm. (g) Mean ± SEM number of cytokine producing T cells from dLNs (n = 5-10). ns = not significant, *P < .05, †P < .01, and ‡P < .001, wt versus K5-STAT3$^{-/-}$ or wt versus K14-IL-6R$^{-/-}$ as calculated by 2-way ANOVA (c) or 2-tailed Student t test (b,e,f). Results are representative of at least 2 independent experiments.

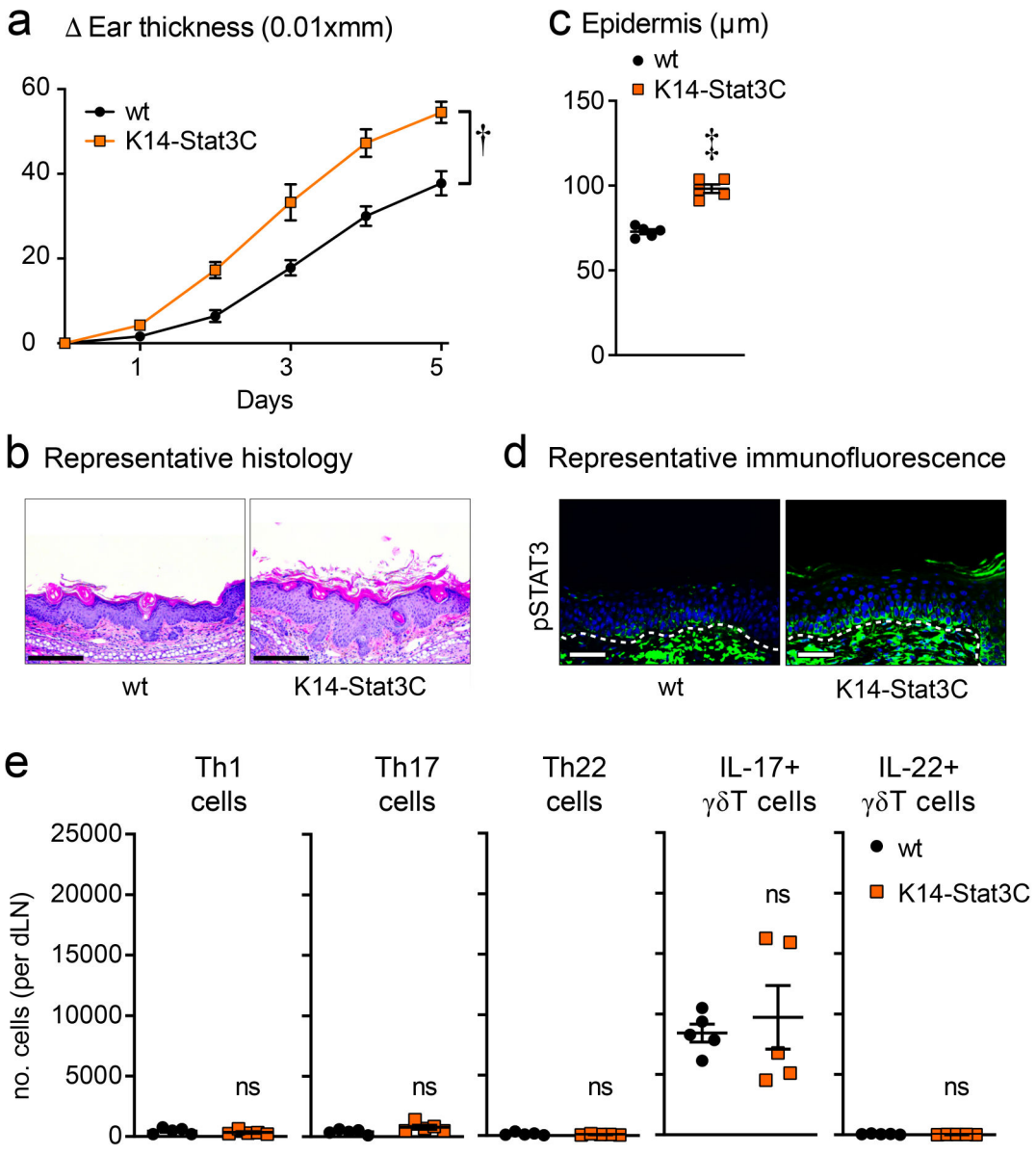

Figure 3. Constitutive STAT3 expression in keratinocytes promotes skin inflammation.

Wt and K14-Stat3C mice ears were given 5 daily imiquimod applications. (a) Mean ± SEM ear thickness measurements (0.01 x mm) (n = 4-5). (b) Representative skin histology (hematoxylin and eosin stain). Scale bar = 200 μm. (c) Mean ± SEM epidermal thickness (μm) (n = 5). (d) Representative skin immunofluorescence with anti-pSTAT3 mAb (green) and DAPI (blue). Dashed line = dermoepidermal junction. Scale bar = 50 μm. (e) Mean ± SEM number of cytokine producing T cells from dLNs (n = 5). ns = not significant, †P < .01, and ‡P < .001, wt versus K14-Stat3C as calculated by 2-way ANOVA (a) or 2-tailed Student t test (c,d). Results are representative of at least 2 independent experiments.

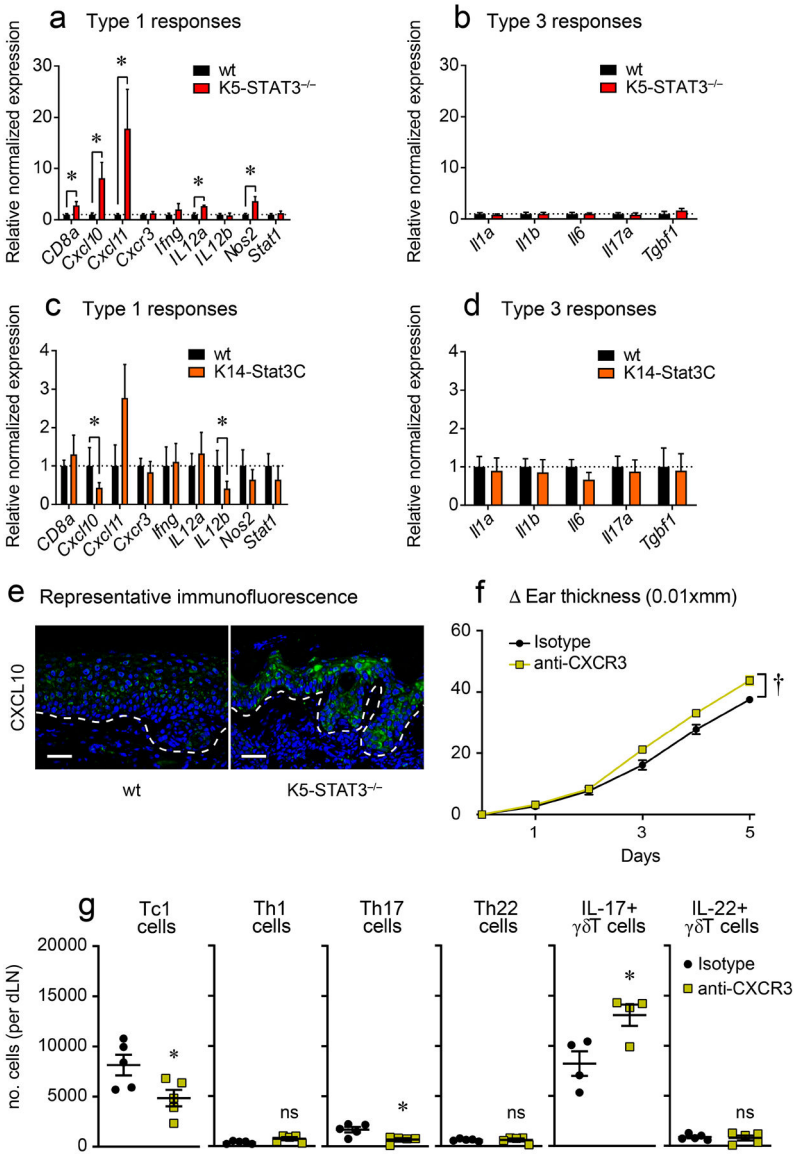

Figure 4. Keratinocyte STAT3 controls type 1 cytokine expression.

Wt, K5-STAT3$^{-/-}$, and K14-Stat3C ears were treated with imiquimod for 5 days. Mean ± SEM relative expression of (a) type 1 and (b) type 3 response genes between wt and K5-STAT3$^{-/-}$ mice ears (n = 4), or relative expression of (c) type 1 and (d) type 3 response genes between wt and K14-Stat3C mice ears (n = 4-5). Data were normalized to *Hrpt1* expression. (e) Representative skin immunofluorescence with anti-CXCL10 pAb (green) and DAPI (blue). Dashed line = dermoepidermal junction. Scale bar = 50 μm. (f,g) Wt mice were i.p. injected with isotype control or anti-CXCR3 mAb during imiquimod treatment. (f) Mean ± SEM ear thickness measurements (0.01 x mm) (n = 9-10). (g) Mean ± SEM number of cytokine producing T cells from dLNs (n = 4-5). ns = not significant, *P < .05, and †P < .01, wt versus K5-STAT3$^{-/-}$ or wt versus K14-Stat3C as calculated by 2-tailed Student t test (a-d), or isotype control versus anti-CXCR3 as calculated by 2-way ANOVA (f) or 2-tailed Student t test (g). Results are representative of at least 2 independent experiments.

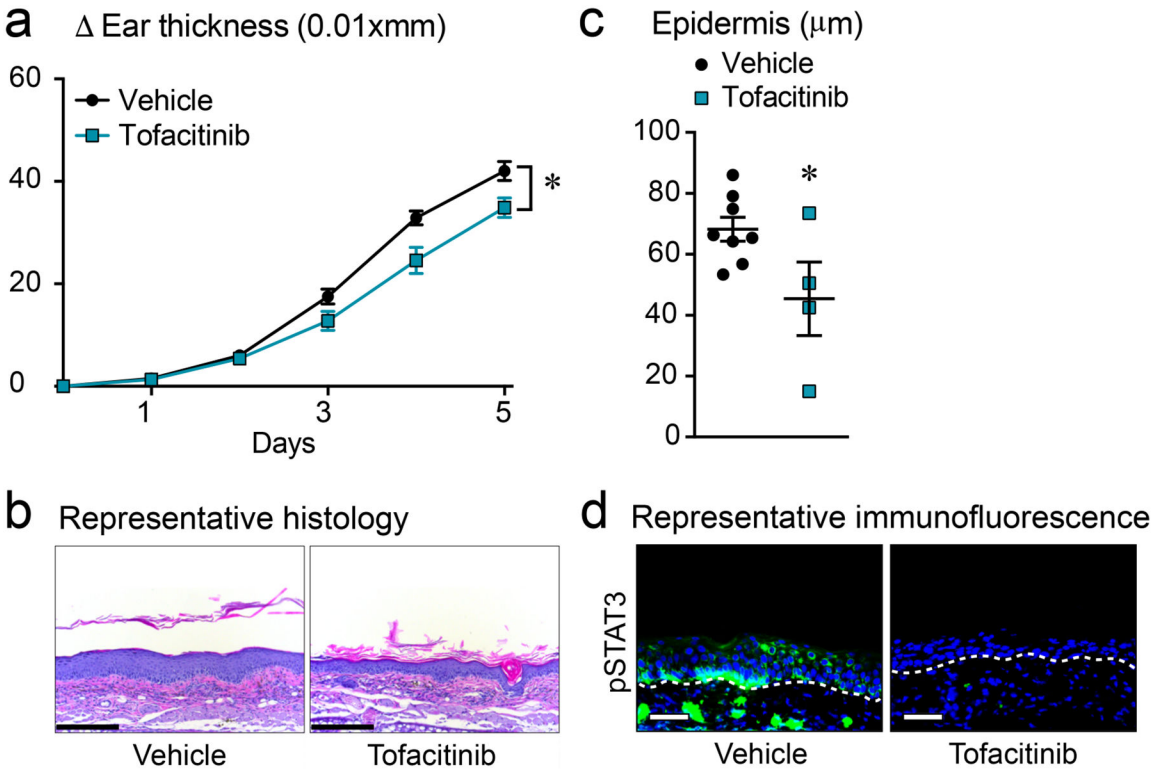

Figure 5. The JAK/STAT inhibitor, Tofacitinib, reduces imiquimod mediated skin inflammation.

Wt mice were treated twice daily with vehicle or Tofacitinib by oral gavage during imiquimod application. (a) Mean ± SEM ear thickness measurements (0.01 x mm) (*n* = 8-9). (b) Representative skin histology (hematoxylin and eosin stain). Scale bar = 200 μm. (c) Mean ± SEM epidermal thickness (μm) (*n* = 4-8). (d) Representative skin immunofluorescence with anti-pSTAT3 mAb (green) and DAPI (blue). Dashed line = dermoepidermal junction. Scale bar = 50 μm. ns = not significant, **P* < .05, and ‡*P* < .001, vehicle versus Tofacitinib as calculated by 2-way ANOVA (a) or 2-tailed Student t test (c). Results are representative of at least 2 independent experiments.