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The Cytokine TGF-β Induces Interleukin-31 Expression from Dermal Dendritic Cells to Activate Sensory Neurons and Stimulate Wound Itching

Highlights
- IL-31 expression is increased in skin wounds during the peak of itch response
- Il31−/− mice have less itch behavior during wound healing
- IL-31 increases calcium influx and Il31ra, Trpv1 expression in itch sensory neurons
- TGF-β1 induces expression of IL-31 in dermal conventional type 2 dendritic cells

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In Brief
Xu et al. find that TGF-β, produced as part of the wound healing process, induces expression of interleukin-31 by dermal conventional type 2 DCs, which, in turn, increases neuron sensitivity and provokes itching.

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The Cytokine TGF-β Induces Interleukin-31 Expression from Dermal Dendritic Cells to Activate Sensory Neurons and Stimulate Wound Itching

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SUMMARY

Cutaneous wound healing is associated with the unpleasant sensation of itching. Here we investigated the mechanisms underlying this type of itch, focusing on the contribution of soluble factors released during healing. We found high amounts of interleukin 31 (IL-31) in skin wound tissue during the peak of itch responses. Il31−/− mice lacked wound-induced itch responses. IL-31 was released by dermal conventional type 2 dendritic cells (cDC2s) recruited to wounds and increased itch sensory neuron sensitivity. Transfer of cDC2s isolated from late-stage wounds into healthy skin was sufficient to induce itching in a manner dependent on IL-31 expression. Addition of the cytokine TGF-β1, which promotes wound healing, to dermal DCs in vitro was sufficient to induce Il31 expression, and Tgfbr1f/f CD11c-Cre mice exhibited reduced scratching and decreased Il31 expression in wounds in vivo. Thus, cDC2s promote itching during skin wound healing via a TGF-β-IL-31 axis with implications for treatment of wound itching.

INTRODUCTION

Cutaneous wound healing is a highly coordinated process that includes production of an ordered and dynamic inflammatory response involving multiple growth factors and cytokines (Ashcroft et al., 1999; Keyes et al., 2016; Massague, 1999; Ross and Odland, 1968). For most wounds, itching is an unpleasant symptom that arises in the latter part of the healing process. Clinically, in patients with more extensive wounds (e.g., burns), there is more severe and persistent itching (Prasad et al., 2019; Parnell et al., 2012). Chemically induced itching is evoked by pruritogens activating primary afferent sensory neurons, which innervate the skin and have cell bodies in dorsal root ganglia (DRGs) and trigeminal ganglia. In mice, itch-inducing agents are detected by two nonoverlapping classes of sensory neurons that are thought, in part, to use the ion channel transient receptor potential cation channel subfamily V, member 1 (TRPV1) (Imamachi et al., 2009; Shim et al., 2007). One of these classes of neurons is marked by the transmitter natriuretic polypeptide B (NPPB) (Mishra and Hoon, 2013), which may convey pruritic signals to spinal cord circuits. The second class of neurons is defined by expression of Mas-related G-protein-coupled receptor a3 (Mrgrpa3; Han et al., 2013), which express several itch receptors, including Mrgrpr3 and Mrgrpc11. Activation of Nppb is sufficient to generate itch behavior (Huang et al., 2018) and is required for interleukin-31 (IL-31)-induced itching (Solinski et al., 2019b).

The interaction between the nervous system and the immune system has been suggested to be critically involved in normal hemostasis and pathogenic processes (Chavan et al., 2017). It has also become increasingly clear that neurological systems regulate immune responses through cell-cell contact (Tian et al., 2009) and/or release of soluble factors (Chavan et al., 2017). However, few studies have investigated the reverse direction; i.e., regulation of sensory neurons by the immune system. Specific inflammatory cytokines have been suggested to influence itching in skin (Bautista et al., 2014; Jin et al., 2009; Oetjen et al., 2017), suggesting that inflammation in the skin might play a role in development and pathogenesis of itching. However, the primary factor(s) and the underlying mechanisms of immune-mediated itching during wound healing remain elusive.

Here we utilized an experimental mouse model of wound healing to investigate the effects of immune responses on itching. We showed that IL-31, a prominent cytokine implicated previously in itching (Cevikbas et al., 2014; Dillon et al., 2004), was upregulated in skin wound tissue on the fifth day of healing, when itch
responses are maximal. We confirmed the itch-promoting effects of IL-31 on the skin and elucidated that IL-31 increased expression of the transduction channel TRPV1 and potentiated calcium influx in DRG neurons. Importantly, we also showed that the major cellular source of IL-31 production is dermal conventional type 2 dendritic cells (cDC2s).

**RESULTS**

Itch Responses and Changes in IL-31 Expression Follow Similar Time Courses during Wound Repair

To determine the progression of itch responses during skin wound healing, we monitored pruritus elicited by acute wound healing in humans and mice. For human subjects, we evaluated pruritus in 12 patients (diagnosed with a superficial cyst or benign tumor) using questionnaires for pruritus self-assessment prior to and every other day after surgery, employing a 10-cm visual analog scale (VAS; see STAR Methods for details). We observed that, in most patients, pruritus reached a peak on the fifth day after surgery (Figure 1A). Next we used an experimental mouse model of skin wound healing (Ashcroft et al., 2000; Figure S1) and found that, similar to humans, mouse scratching responses (Video S1) were maximal on the fifth day of healing (Figure 1B).

To investigate whether there are soluble factor(s) present in wounded skin produced by immune cells during wound healing, which might be responsible for itching, we collected tissue and sequenced RNA transcripts several times before and during wound healing. For an unbiased analysis, we examined RNA sequencing (RNA-seq) reads, exploring changes in expression of all genes and focusing on those whose expression changes over the course of the healing process. We hypothesized that the expression of factors contributing to itching would peak at about the same time as itch responses. Next we examined genes, based on published literature (Bautista et al., 2014; Nattkemper et al., 2018; Table S1), that might be involved in this process. Using these criteria, we uncovered IL-31 as a candidate for induction of itching after cutaneous incision (Figures 1C and 1D; Table S2). Confirming our RNA-seq analysis, real-time quantitative polymerase chain reaction (qPCR) and ELISA showed that there was increased expression of IL-31 throughout wound healing that peaked on day 5 after incision (Figure 1E; Figure S2A).

IL-31 Is Responsible for Itch Responses during Wound Healing

If IL-31 is responsible for itch responses during wound healing, then we predicted that administration of additional IL-31 should further potentiate these responses. To test this, we injected IL-31 (30 ng/site, intradermally [i.d.]) into wounds starting on day 4 (see STAR Methods for details). In line with our hypothesis, mice treated with additional IL-31 displayed a higher number of scratching bouts compared with untreated animals (Figure S2B). To further investigate the role of IL-31 in itching during wound healing, we used Il31−/− mice (Figure S2C). Corroborating a role of IL-31 in wound-induced itching, Il31−/− mice scratched much less than wild-type mice on day 5 of wound healing (Figure 1F). A similar phenomenon was observed when bone marrow from Il31−/− mice was transferred into irradiated C57BL/6 recipients (Figures S2D and S2E), indicating that hematopoietic cells, and possibly leukocytes, might be the main source of secreted IL-31.

IL-31 Increases Itch Sensory Neuron Sensitivity

Next, using RT-PCR, we compared the gene expression of molecules associated with signal transduction in DRGs that innervate the wound site between baseline and 5 days after incision. This comparison uncovered that expressions of Il31ra, Trpv1, and Nppb were upregulated during healing in sensory neurons, whereas expression of Trpa1 did not change significantly (Figures S3A–S3D). Because DRG neurons are a heterogeneous population, Trpv1-lineage reporter mice (Trpv1-tdTomato mice) were also used to enrich for transcripts present in itch sensory neurons (Mishra et al., 2011). qPCR showed that Il31ra, Trpv1, and Nppb (Figures 2A–2C) were upregulated in sorted DRGs of Trpv1-lineage mice (which innervate wounds on day 5 after incision). This result is consistent with IL-31 being involved in induction of increased itch sensitivity in sensory neurons. To further test this hypothesis, we treated mouse DRG sensory neurons in vitro with IL-31. We found that IL-31 treatment on its own was sufficient to induce upregulation of Trpv1, Il31ra, and Nppb expression (Figures S3E–S3G). In addition, IL-31 treatment for 24 h could potentiate capsaicin (50 nM)-stimulated calcium influx in cultured DRG neurons (Figures 2D, S3H, and S3I) and could slightly increase the number of capsaicin-responding neurons in functional assays (Figure S3J). These results suggest that IL-31 may increase the expression of key signal transduction molecules in sensory neurons and that it can sensitize these nerves.

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**Figure 1. Pruritus Elicited by Acute Wound Healing and IL-31 Is Important for Itch during Wound Healing**

(A) 12 patients with 1- to 3-cm sterile surgical wounds who experienced pruritus were surveyed over 7 days and asked to score the level of itching they felt on each day. One-way ANOVA was used for comparisons. Bars represent mean ± SEM. *p < 0.05.

(B) A mouse wound healing model was established by making 2 equidistant, 1 cm, full-thickness incisional wounds through the dorsal skin and left to heal. The number of scratching bouts per 30 min of observation were measured for 7 days following wounding. Data are from 3 independent experiments (n = 9) and were analyzed with a one-way ANOVA for comparisons. Bars represent means ± SEM. *p < 0.05.

(C and D) Mouse wound skin at different time points was harvested, followed by RNA-seq of the mRNAs. The Venn diagram (C) shows overlapping of the genes that were significantly changed (p < 0.01) on the fifth day versus other time points of wound healing and genes related to itching based on published literature. The heatmap (D) shows all the itch-associated genes which changed on the fifth day were compared with any other time points. For RNA-seq, 2 wound tissues from one animal were pooled, and 3 animals were used per time point.

(E) The expression of IL-31 in wounds was confirmed by qPCR. Data are from 3 independent duplicated experiments (n = 12) and were analyzed with a one-way ANOVA for comparisons. Bars represent means ± SEM. **p < 0.001.

(F) Itching behaviors of Il31−/− mice during wound healing were compared with wild-type mice. Data are pooled from 2 independent experiments (n = 6) and represented as mean ± SEM; two-way ANOVA was used. ***p < 0.0001.

See also Figure S2 and Tables S1 and S2 for more details.
Because IL-31, via the IL-31ra receptor, may induce changes in gene expression and neural activity through a Jak1-mediated pathway (Zhang et al., 2008), we investigated potential additional downstream effectors of IL-31 activation. To do this, we explored, in DRG neurons, which Stat molecules might be regulated by IL-31 treatment. These experiments revealed that IL-31 induces phosphorylation of Stat3 (Figure 2E). If Stat3 phosphorylation is required for potentiation of neuronal activity, then administration of a specific Stat3 inhibitor should attenuate IL-31-induced increases in calcium responses to capsaicin. Indeed, the Stat3 inhibitor S31-201 blocked IL-31-stimulated increases in calcium influx and the numbers of neurons responding to capsaicin (Figures S3K–S3M). Furthermore, S31-201 inhibited IL-31-induced upregulation of Trpv1 and Il31ra expression (Figures S3N and S3O), indicating that these IL-31-mediated effects are Stat3 dependent.

We wondered how IL-31 acts on itch sensory neurons to alter gene expression. To investigate this, we tested the effects of IL-31 on neurons at different time points. We uncovered that Stat3 phosphorylation occurred within 15 min after IL-31 treatment (Figure S3P), but the increase in Il31ra and Trpv1 expression was delayed and started between 1 and 3 h later (Figures S3Q and S3R). Furthermore, when we injected IL-31 (1 µg/site i.d.) into mutant (mut-)Stat3 mice, the same amount of initial scratching was observed in mut-Stat3 mice as in wild-type control mice. However, 8 h after the first IL-31 injection, when we gave a second IL-31 dose, scratching was increased markedly in wild-type mice but not in mut-Stat3 animals (Figure 2F). In addition, concordant with results from our experiments with a Stat3 inhibitor, Il31ra and Trpv1 gene expression in DRGs that innervate the injected area of skin also showed that IL-31 injection in vivo could not upregulate these two genes in the short term.
Figure 3. Dermal DCs Are a Key Source of IL-31 in Wounds

(A) Il31 gene expression was determined in CD45– cells, CD45+CD3+ cells, and CD45+CD3– cells sorted from fifth-day wounds tissue by qPCR. Data were from 2 independent experiments with 2 samples each time, and each sample was pooled from 4 wounds in 2 mice. Data were analyzed with one-way ANOVA for comparisons. Bars represent means ± SEM. ****p < 0.0001.

(B) Expression of IL-31 in wounds on the fifth day were tested in Rag1–/– mice and wild-type mice by real-time PCR. Data are from 3 independent experiments (n = 6), and two-way ANOVA was used for comparison. Bars represent means ± SEM.

(C) Scratching behaviors were observed in Rag1–/– mice before and on the fifth day of wound healing. Data are from 2 independent experiments (n = 5); Student’s t test was used. Bars represent means ± SEM.

(D) DCs (CD45+CD3–CD11c+MHC II+), macrophages (CD45+CD3–CD11c–CD11b+F4/80–), granulocytes (CD45+CD3–CD11c–CD11b–F4/80+), and mast cells (CD45+CD3–CD11c–CD11b–FceR1a+) were sorted from fifth-day wounds, and the expression of Il31 was determined by real-time PCR. Data were from 2 independent experiments with 2 samples each time, and each sample was pooled from 4 wounds in 2 mice. Data were analyzed with one-way ANOVA for comparisons. Bars represent means ± SEM. ****p < 0.0001.

(legend continued on next page)
term (within 1 h) but could increase expression over the long term (8 h) in wild-type mice (Figures S3S and S3T). Notably, this IL-31-induced upregulation did not occur in mut-Stat3 mice (Figures S3S and S3T). Furthermore, to investigate whether scratching during wound healing is associated with Nppb/Npr1, we injected an Nppb receptor agonist, Npr1 (JS-11, 5 mg/kg intraperitoneally [i.p.]) into mice on day 5 of wound healing (Solinski et al., 2019a). JS-11-treated mice exhibited attenuated scratching 15 min after injection (Figure S3U), indicating that the wound-associated itch is Nppb/Npr1 dependent. Taken together, our results suggest that IL-31 has short-term effects that are Stat3 independent and has longer-term effects on neuronal sensitivity, as evidenced by Stat3-dependent increased Il31ra and Trpv1 expression.

### Dendritic Cells Cause Itching during Wound Healing

We next investigated the source of IL-31 in the skin during wound healing. IL-31 has been classified as a T helper 2 (Th2) cytokine (Dillon et al., 2004); however, CD3+ T cells sorted from wound tissues on day 5 after incision expressed much lower Il31 than CD3− non-T cells within the CD45+ immune cell population (Figure 3A). Our analysis also revealed that CD45− non-immune cells expressed minimal Il31 mRNA (Figure 3A). Confirming these results, the expression of IL-31 detected in wounds from Rag1−/− mice was the same as in control mice (Figures 3B and S4A), indicating that T cells or B cells are not a major source of IL-31 in wounds. Again, in line with B and T cells having minimal involvement in wound itching, Rag1−/− mice exhibited similar wound-induced scratch behaviors as wild-type mice (Figure 3C). When we further fractionated CD3− non-T cells from wound skin, we found that dendritic cells (DCs) had higher expression of Il31 compared with macrophages, granulocytes, and mast cells (Figure 3D). Moreover, higher numbers of CD11c+ cells were found in day 5 wounds compared with normal skin (Figures 3E, 3F, and S4B).

If DCs are the major source of IL-31 in wounds, then depletion of DCs in the skin should alleviate itching during wound healing. To test this, we made a previously characterized DC depletion mouse model (Meredith et al., 2012) by transferring bone marrow from zDCDTTR mice, in which human diphtheria toxin receptor is expressed in cDCs, but not monocytes or other immune cell populations, into irradiated C57BL/6 hosts and injecting diphtheria toxin (DT) to specifically deplete cDCs (Figure S4C). These mice did not exhibit increased itch behavior during wound healing (Figure 3G), indicating that cDCs may be the main source of IL-31. Furthermore, Il31 expression in the wounds of these mice was also lower than in control animals (Figure S4E), but these mice displayed no change in wound closure and healing (Figure S4D). These results establish that DCs are the primary source of IL-31, which causes itching during wound healing.

cDC2s Are a Key Source of IL-31 in Wounds

To determine which subset of DCs express IL-31, we next sorted Langerhans cells (LCs), type 1 cDCs (cDC1s), and cDC2s, as well as macrophages and found that cDC2s expressed the highest level of Il31 of all of these types of cells (Figure 4A). We further used LangDTR mice, which express the human DT receptor (DTR) downstream of the internal stop codon of the Langerin gene, to deplete LCs (Figures S5A–S5C). We also used a well-characterized monocyte/macrophage depletion mouse model by crossing Lys2DTR mice and Csf1rDTR mice (Schreiber et al., 2013) (hereafter called MMDTR mice). In Csf1rDTR mice, a DTR preceded by a loxP-flanked transcriptional stop element under control of the Csf1r promoter, expression of Cre recombinase excises the stop element and allows transcription and translation of DTR in cells expressing Csf1r, so administering DT to MM−/− mice could deplete macrophages in the skin (Figures S5D–S5F). Itch behaviors during wound healing in these two models were observed. Both models have normal frequencies of cDCs in the skin (Figures S5A and S5D) and normal itch behavior on day 5 during wound healing. We also compared Il31 expression between dermal cDC1s and cDC2 by using CD103 and CD207 as markers to distinguish dermal cDC1s and cDC2s (Kashem et al., 2017) and found that dermal cDC2s indeed expressed higher levels of Il31 than cDC1 (Figures S5G and S5H). Moreover, Il31 mRNA expression in cDC2s from day 5 wounds was much higher than in cDC2s from normal skin (Figure 4B). The proportions (Figures 4C and 4D) and total numbers of cDC2s (Figure 4E) were also increased on day 5 after incision compared with naive skin.

These results suggest that cDC2s are the likely source of Il31, producing increased scratching during wound healing. To further validate this, we examined whether cDC2s are sufficient to elicit wound-like itching. We purified cDC2s from day 5 wounds or normal skin and injected them intra-dermally in uninjured recipient mice (1.5 × 104 cells per site). cDC2s from wound sites increased scratching in recipient mice 3 h after injection (Video S2) but cDC2s from normal skin did not (Figure 5A). In addition, consistent with these cDC2s evoking itching through activation of an IL-31 process, expression of Il31ra (Figure 5B), Trpv1 (Figure 5C), and Nppb (Figure 5D) was upregulated in DRGs of recipient mice that innervated the skin around cDC2 injection. To probe whether scratching evoked by dermal cDC2 administration elicits itching or nociceptive sensations, we turned to the cheek model (Shimada and LaMotte, 2008). In this model, scratch responses to intradermal injection of a substance into the face are interpreted to be caused by itching, whereas wipe responses are believed to be triggered by nociception (production of pain). Consistent with cDCs provoking itching, injection of cDC2s from day 5 of wound healing into the cheek caused prominent scratching compared with cDCs from naive mice (Figure 5E). In contrast, wiping responses were similar for cDCs from

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(E and F) The frequency (E) and numbers (F) of CD11c+ cells from day 5 wounds was calculated and compared with those from normal skin. Data were from 3 independent experiments (n = 5–6), analyzed with Student’s t test for comparisons. Bars represent means ± SEM. ***p < 0.0001.

(G) Itching behaviors during wound healing were observed in diphtheria toxin (DT)-treated (i.p., 500 ng/mouse for the first time and 100 ng/mouse on all subsequent days) zDCDTTR–to-C57BL/6 bone marrow chimera mice and were compared with DT-treated C57BL/6-to-C57BL/6 bone marrow chimera mice (WT [wild-type]). Data were pooled from 6 mice for each condition in two independent experiments and are represented as mean ± SEM. Two-way ANOVA was used for multiple comparisons. ***p < 0.001.

See also Figure S4 for more details.
naive and day 5 wounds (Figure 5F). Supporting this conclusion itch responses evoked by day 5 wound cDC2s from Il31−/− mice were similar to the behavior elicited by administration of cDCs from naive skin (Figure 5G). Taken together, these results suggest that cDC2 is the major source of IL-31 in wounds, which produces itching associated with wound repair.

TGF-β Increases IL-31 in Dermal cDC2s
Because cDC2s in healthy skin contain low amounts of IL-31, we wondered which cues in wounds might be involved in increasing its expression. The cytokines IL-1β, IL-6, IL-17a, transforming growth factor β1 (TGF-β1), and tumor necrosis factor alpha (TNF-α) have been reported to be important participants in

Figure 4. IL-31 in Wounds Was Mostly from Dermal cDC2s
(A) Macrophages (CD45+CD11c−CD64+CD11b+F4/80−), LCs (CD45+CD11c−CD64−CD11b−MHC II−), and dermal cDC2s (CD45+CD11c+CD64+CD11b+MHC II+), and dermal cDC1s (CD3+CD11c−CD64−CD11b−MHC II−) were sorted from fifth-day wounds, and Il31 expression was determined by real-time PCR. Data were from 2–3 independent experiments with 2 samples each time, and each sample was pooled from 4 wounds in 2 mice. Data were analyzed with one-way ANOVA for comparisons. Bars represent means ± SEM. ***p < 0.001.
(2) Il31 gene expression in dermal cDC2 from fifth-day wounds was compared with cDC2s from normal skin by real-time PCR. Data are from 3 independent experiments with 2 samples each time, and each sample was pooled from 4 wounds in 2 mice. Data were analyzed with Student’s t test for comparisons. Bars represent means ± SEM. ***p < 0.001.
(C–E) Flow cytomter analysis showed that the frequency (C and D) and total numbers of dermal cDC2s (CD45+CD11c+CD64+CD11b+MHC II+) (E) were increased in fifth-day wound skin tissues compared with normal skin. In (D) and (E), each circle represents one mouse. Data were from 3 independent experiments (n = 5–6), and Student’s t test was used. Bars represent means ± SEM. ***p < 0.0001.
Figure 6. TGF-β Increases IL-31 in Dermal cDC2

(A) IL-1β (10 ng/mL), IL-6 (50 ng/mL), IL-17a (10 ng/mL), TGF-β1 (2 ng/mL), and TNF-α (10 ng/mL) were used in vitro to treat cDC2s sorted from healthy skin individually, and IL31 expression in dermal cDC2s was determined 6 h after treatment. Data were from 3 independent experiments (n = 6), and each circle represents one culture well. Data were analyzed with one-way ANOVA for comparisons. Bars represent means ± SEM. ***p < 0.001.

(B and C) The expression of IL31 in TGF-β1-treated dermal cDC2s from Tgfbr1−/− mice (tamoxifen-treated Tgfbr1−/−Ert2-Cre mice) (B) and Smad3−/− mice (C) were determined and compared with WT dermal cDC2s. Data were from 2 independent experiments (n = 4–8). Each circle represents one culture well, and two-way ANOVA was used for comparisons. Bars represent means ± SEM. ****p < 0.0001.

(D and E) The wound healing model was set up in Tgfbr1−/−Cd11c-Cre (D) and Smad3−/− mice (E). Itching behaviors were observed in Tgfbr1−/−Cd11c-Cre mice and Smad3−/− mice and compared with WT controls. Data were from 3 independent experiments (n = 6–8) and represented as mean ± SEM. Two-way ANOVA was used for comparisons. ***p < 0.001.

(legend continued on next page)
coordinating wound repair (Barrientos et al., 2008; Mast and Schultz, 1996). Therefore, we tested the effects of these cytokines on induction of IL-31 expression in isolated dermal cDC2s in vitro. Unexpectedly, only TGF-β1 substantially increased IL31 expression in cDC2s from normal skin (Figure 6A). Consistent with TGF-β1 inducing IL-31 in dermal cDC2s, we found that TGF-β1 expression was raised between days 3 and 5 after incision (Figure S6A). Because Smad3 is downstream of canonical TGF-β signaling, we used a TGF-β receptor I (TGF-βRI) inhibitor (SB431542) or a selective Smad3 inhibitor (SIS3) under TGF-β1 culture conditions. Corroborating TGF-βRI and Smad3 as being part of the signal cascade for induction of IL31 expression, their inhibitors reduced induction of IL-31 transcription (Figure S6B). Further substantiating the contribution of TGF-β in induction of IL-31 expression, the increase in IL31 expression in response to TGF-β is absent in dermal cDC2s from TGF-βRI global knockout mice (Tgfb1−/− Ert2-Cre) (Figure 6B) and Smad3−/− mice (Figure 6C), and in vitro TGF-β1 treatment failed to increase IL31 expression in dermal cDC2s from these knockout mice (Figures 6B and 6C). To further investigate the role of TGF-βRI in DCs during wound healing, we crossed Cd11c-Cre+ mice with Tgfb1−/− mice to conditionally deplete TGF-βRI in DCs, whereas other types of cells were untouched. Histological staining showed that conditional depletion of TGF-βRI in DCs did not alter the speed of the wound healing process (Figure S6C). Like the Tgfb1−/− Ert2-Cre mice, TGF-β1 treatment also failed to increase IL31 expression in dermal cDC2s from Tgfb1−/− CD11c-Cre mice in vitro (Figure S6D). Compared with wild-type mice, Tgfb1−/− CD11c-Cre mice exhibited reduced scratching behaviors (Figure 6D) and a lower IL31 expression in wounds (Figure S6E) on day 5 after injury. Notably, expression of Il31ra (Figure S6F), Trpv1 (Figure S6G), and Nppb (Figure S6H) were also not upregulated in the DRGs that innervated wounds. Furthermore, we generated Tgfb1−/− Ly22-Cre mice, in which TGF-βRI is eliminated on macrophages but not on DCs. These mice displayed a similar healing process and itching behaviors as wild-type mice (Figures S6J and S6K). Moreover, although the wound healing processes were accelerated (Figure S6L), scratch- and itch-related gene expression in Smad3−/− mice also declined relative to control littermates at all time points (Figures 6E and S6M–S6O). Finally, intradermal administration, in recipient animals, of TGF-β1-pretreated dermal cDC2s increased itch behavior (1.5 ± 10^6 cells per site; Figure 6F). In contrast, TGF-β1-pretreated dermal cDC2s isolated from Tgfb1−/− Ert2-Cre mice (Figure 6G) and Tgfb1−/− CD11c-Cre mice (Figure S6E) failed to elicit itch responses. Moreover, even at a low dose of injection (5,000 cells per site), TGF-β1-pretreated dermal cDC2s could elicit itch responses (Figure S6P) and upregulate Il31ra gene expression in DRGs of recipient mice that innervated the skin injected with these cells, although no significant difference in Trpv1 gene expression could be found (Figure S6Q), whereas TGF-β1-treated dermal cDC2s from Tgfb1−/− Ert2-Cre mice failed to do so (Figures S6R and S6S). Together, these results demonstrate the crucial role of TGF-β signaling in IL-31 production in dermal cDC2s during wound healing.

**DISCUSSION**

In this study, we investigated the cellular and molecular mechanisms underlying itching during cutaneous wound healing, showing that TGF-β induces expression of IL-31 in dermal cDC2s and that this contributes to generation of scratch behavior through activation of sensory neurons. First we found a close association of the peak of itch responses and cutaneous IL-31 expression. Second, we showed that IL-31 has short-term effects evoking itching, which is Stat3 independent, and long-term effects on increasing the sensitivity of sensory neurons. Third, we established that dermal cDC2s are critical cellular sources of IL-31 during wound repair and that day 5 dermal cDC2s are sufficient to evoke itch responses in naive mice. Finally we demonstrate that IL-31 expression in cDC2s requires Smad-dependent TGF-β signaling. These findings provide the immunological and neurological underpinnings of the widely known sensation of itching that is experienced in the later stages of skin wound healing.

It is thought that many pruritogens evoke itching (Steinhoff et al., 2018; Trier and Kim, 2018), but the principle underlying the agents responsible for the itching induced during cutaneous repair have not been studied. Here we discovered that the cytokine IL-31, based on our unbiased RNA-seq analysis, qPCR, and protein analysis, was prominently expressed, peaking at approximately the same time when itching was most intense. In mice and humans, type 2 cytokine signaling contributes to itching (Oetjen et al., 2017), and IL-31 was initially described as a Th2 cytokine mainly related to dermatitis and some respiratory diseases (Bilsborough et al., 2010; Cevikbas et al., 2014; Dillon et al., 2004). Supporting this function of IL-31 in itching, anti-IL-31 receptor antibodies have been reported to relieve dermatitis in a mouse model and human patients (Kasutani et al., 2014; Ruzicka et al., 2017). Therefore, we propose that IL-31 is also a likely agent contributing to pruritus when the skin is recovering from injury, and a slow build-up of IL-31 causes the increasing pruriceptive sensation that builds during wound healing during this process. For this reason, IL-31 might be a good target for treatment of pruritis in more extensive body surface areas, such as with burns.

We wondered why IL-31 might be elevated during wound healing and considered that it might influence immune cell functions at the repair site and, therefore, be important in wound closure or other processes producing an intact barrier. This idea was not borne out because, in Il31−/− mice, we did not find significant changes in wound healing. This still does not answer what
IL-31 might be doing, but it means that therapeutic agents neutralizing IL-31 would not impair wound recovery. Future experiments might investigate the reasons for elevated IL-31 during wound repair. In addition, studies have shown that IL-31 has roles in nerve growth (Feld et al., 2016) and neuropeptide release (Meng et al., 2018); it is reasonable that wound itching occurs when nerve fibers are remodeling into the dermis/epidermis.

IL-31 has been reported previously to acutely induce itching via activation of IL-31Ra on TRPV1+ sensory neurons, which innervate the skin (Cevikbas et al., 2014), and to also evoke delayed itching (Arai et al., 2013; Hawro et al., 2014). Here we showed that, at least in part, IL-31 may increase sensory neuron activity by upregulation of expression of molecules involved in signaling reception (Il31Ra), signal transduction (Trpv1), and signal transmission (Nppb). These increases in expression are long-term effects and are Stat3 dependent. In turn, this suggests that Stat3 might be another potential therapeutic target for use in IL-31-dependent itching.

In the dermis, cDCs are conventional resident DCs and have critical roles in guarding the host against invading pathogens while limiting tissue damage. They are involved in some pathologies, including infectious and parasitic diseases transmitted through the skin (Clausen and Stoitzner, 2015; Valladeau and Saeland, 2005). cDCs are the most abundant type of DCs in the healthy mouse dermis (Malissen et al., 2014; Tamoutounour et al., 2013); upon migration to draining lymph nodes, they can trigger Th2 cell differentiation (Kitajima and Ziegler, 2013) and induce production of regulatory T cells (Guilliams et al., 2010). For these reasons, their numbers are substantially increased at sites of barrier disruption. It has been reported previously that nociceptive sensory neurons, by interacting with dermal cDCs, can regulate the IL-23/IL-17 pathway and control cutaneous immune responses in a model of psoriasis (Riol-Blanco et al., 2014). Here we focused on the reverse direction and investigated how immune cells affect the peripheral nervous system and behavior. Our results show that elevated amounts of IL-31 in wounds is predominantly generated by dermal cDC2s. Therefore, our results show that there is reciprocal signaling between cutaneous cDC2s and peripheral sensory neurons, highlighting the importance of the interaction between the immune and nervous systems in the skin.

TGF-β signaling is crucial during wound healing; it has a broad spectrum effects on almost every cell type, including keratinocytes, fibroblasts, DCs, and monocytes (Ashcroft et al., 1999). TGF-β1 is important for wound closure by facilitating fibroblast contraction in the collagen matrix (Meckmongkol et al., 2007) and development of hypertrophic and keloid scars (Colwell et al., 2005) and for inducing and sustaining activation of keloid fibroblasts (Wang et al., 2007). TGF-β1 directs differentiation of monocytes into LCs and is crucial for the cutaneous contingent of migratory DCs (Felker et al., 2010). Moreover, TGF-β may induce tolerogenic DCs, which can skew effector T cell immune response into cells of a predominantly anti-inflammatory Th2-like phenotype or may induce generation of regulatory T cells (Chen et al., 2003; Esebanmen and Langridge, 2017; van Duivenvoorde et al., 2006). In this study, we expand the list of TGF-β functions to wound healing-induced itching by demonstrating that TGF-β signaling increases IL-31 production in dermal cDC2s.

Here we provide evidence of an IL-31-mediated effect on the sensory nervous system and mammalian behavior. By identifying this neuro-immunologic pathway and determining key mechanistic steps involved in this process, we identify potential therapeutic targets for itching that occurs during wound healing and potentially other conditions that disrupt the skin barrier and cause itching.

Limitations of Study

Although our study shows that IL-31 is responsible for itch responses during wound healing, it remains an exciting question whether there is any other biological function of IL-31 in that period. Future experiments might also investigate the reasons for elevated IL-31 during wound repair; it would be insightful to focus on the roles of IL-31 in nerve growth and neuropeptide release. Our study could be improved by some direct visual evidence of IL-31 expression in the skin, which we were unable to provide because of the coronavirus disease 2019 (COVID-19) pandemic.

STAR METHODS

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SUPPLEMENTAL INFORMATION

Supplemental Information can be found online at https://doi.org/10.1016/j.immuni.2020.06.023.

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AUTHOR CONTRIBUTIONS

J.X. designed and performed experiments, analyzed data, and wrote the manuscript. P.Z., L.H., D.Z., N.L., W.J., and Y.H. designed and performed experiments. P.-Y.T. provided critical methods and analyzed RNA sequence data. F.W., O.L., N.G., J.Y., A.C., and S.W. provided critical scientific input. M.A.H. supervised the study and wrote the manuscript. W.C. conceived, initiated, and supervised the whole study; designed experiments; and wrote the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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# STAR METHODS

## KEY RESOURCES TABLE

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| **Antibodies**      |        |            |
| Anti-mouse CD45 Alexa Fluor 700 (30-F11) | eBioscience | Cat# 56-0451-82; RRID:AB_891454 |
| Anti-mouse CD3 PerCP-eFluor 710 (17A2) | eBioscience | Cat# 46-0032-82; RRID:AB_1834427 |
| Anti-mouse CD3 APC-eFluor 780 (17A2) | eBioscience | Cat# 47-0032-82; RRID:AB_1272181 |
| Anti-mouse CD11c PerCP/Cy5.5 (N418) | BioLegend | Cat# 117328; RRID:AB_2129641 |
| Anti-mouse CD11c FITC (N418) | eBioscience | Cat# 11-0114-82; RRID:AB_464940 |
| Anti-mouse CD11b APC (M1/70) | eBioscience | Cat# 17-0112-82; RRID:AB_469343 |
| Anti-mouse CD11b eFluor 450 (M1/70) | eBioscience | Cat# 48-0112-82; RRID:AB_1582236 |
| Anti-mouse CD326 APC (G8.8) | eBioscience | Cat# 17-5791-82; RRID:AB_2716944 |
| Anti-mouse CD326 APC-eFluor 780 (G8.8) | eBioscience | Cat# 47-5791-82; RRID:AB_2573986 |
| Anti-mouse CD64 PE-Cy7 (X54-5/7.1) | BioLegend | Cat# 139313; RRID:AB_2563903 |
| Anti-mouse CD64 APC (X54-5/7.1) | eBioscience | Cat# 17-0641-82; RRID:AB_2735010 |
| Anti-mouse CD64 PerCP-eFluor 710 (X54-5/7.1) | eBioscience | Cat# 46-0641-82; RRID:AB_2735016 |
| Anti-mouse F4/80 APC (BM8) | eBioscience | Cat# 17-4801-82; RRID:AB_2784648 |
| Anti-mouse F4/80 PE-eFluor 610 (BM8) | eBioscience | Cat# 61-4801-82; RRID:AB_2574612 |
| Anti-mouse MHC class II (I-A/I-E) PE (M5/114.15.2) | eBioscience | Cat# 12-5321-82; RRID:AB_465928 |
| Anti-mouse MHC class II (I-A/I-E) eFluor 450 (M5/114.15.2) | eBioscience | Cat# 48-5321-82; RRID:AB_1272204 |
| Anti-mouse CD103 PE (2E7) | eBioscience | Cat# 12-1031-82; RRID:AB_465799 |
| Anti-mouse CD207 PE (eBioL31) | eBioscience | Cat# 12-2075-82; RRID:AB_763452 |
| Anti-mouse CD117 (2B8) | eBioscience | Cat# 48-1171-82; RRID:AB_2574037 |
| Anti-mouse FcγR1 alpha PerCP-eFluor 710 (MAR-1) | eBioscience | Cat# 46-5898-82; RRID:AB_2573801 |
| Anti-mouse Stat1 | Cell Signaling | Cat# 9172; RRID:AB_2198300 |
| Anti-mouse pStat1 | Cell Signaling | Cat# 9171S; RRID:AB_331591 |
| Anti-mouse Stat3 | Cell Signaling | Cat# 9139; RRID:AB_331757 |
| Anti-mouse pStat3 | Cell Signaling | Cat# 4904S; RRID:AB_331269 |
| Anti-mouse pStat4 | Cell Signaling | Cat# 9131S; RRID:AB_331586 |
| Anti-mouse pStat4 | Cell Signaling | Cat# 9145S; RRID:AB_2491009 |
| Anti-mouse Stat5 | Cell Signaling | Cat# 9363; RRID:AB_2196923 |
| Anti-mouse pStat5 | Cell Signaling | Cat# 9351S; RRID:AB_235225 |
| Anti-mouse Gapdh | Cell Signaling | Cat# 5014S; RRID:AB_10693448 |
| Anti-mouse IL-31 | ABcam | Cat# ab102750; RRID:AB_10710722 |
| TGF-β1 Elisa kit | Promega | Cat# G7591; RRID:AB_2858628 |
| IL-31 Elisa kit | eBioscience | Cat# BMS6030; RRID:AB_2575853 |
| **Chemicals, Peptides, and Recombinant Proteins** | | |
| Recombinant mouse IL-1β | R&D System | Cat# 401-ML |
| Recombinant mouse IL-6 | R&D System | Cat# 206-IL |
| Recombinant mouse IL-17a | R&D System | Cat# 421-ML |
| Recombinant mouse IL-31 | R&D System | Cat# 3028-ML |
| Recombinant human TGF-β1 | R&D System | Cat# 240-B |
| Recombinant mouse TNF-α | R&D System | Cat# 410-MT |
| SB431542 | Selleckchem | Cat# S1067 |
| S31-201 | Sigma | Cat# SML0330 |
| SIS3 | Sigma | Cat# S0447 |
| Capsaicin | Sigma | Cat# M2028 |

(Continued on next page)
| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| poly-D-lysine       | Sigma  | Cat# A-003-M |
| Tamoxifen           | Sigma  | Cat# T5648  |
| Liberase DH         | Sigma  | Cat# LIBDH-RO |
| Papain              | Worthington | Cat# LS003127 |
| Collagenase type II | Worthington | Cat# LS004177 |
| Dispase type II     | Worthington | Cat# LS02104 |
| Fluo-4              | Thermo Fisher Scientific | Cat# F14201 |
| Pluronic F-127      | Thermo Fisher Scientific | Cat# P3000MP |
| JS-11               | Solinski et al., 2019a | N/A |

Critical Commercial Assays

| Critical Commercial Assays | SOURCE | IDENTIFIER |
|---------------------------|--------|------------|
| RNeasy Mini Kit           | QIAGEN | Cat# 74106 |
| RNeasy Micro Kit          | QIAGEN | Cat# 74004 |
| High-Capacity cDNA Reverse Transcription Kit | Applied Biosystems | Cat# 4368814 |
| TaqMan Gene Expression Master Mix | Applied Biosystems | Cat# 4369016 |
| TaqMan PreAmp Master Mix Kit | Applied Biosystems | Cat# 4488593 |

Deposited Data

| Deposited Data | SOURCE | IDENTIFIER |
|----------------|--------|------------|
| Murine cutaneous wounds tissue RNA sequence data | This Paper | GEO accession GSE128193 https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE128193 |

Biological information

Pruritus self-assessments of patients who were scheduled for minor skin superficial surgery were recorded before and every other day after surgery for 7 days, no human tissue sample was taken for this study

| Experimental Models: Organisms/Strains | SOURCE | IDENTIFIER |
|---------------------------------------|--------|------------|
| Mouse: C57BL/6                         | The Jackson Laboratory | Cat# 000664 |
| Mouse: Il31 <Cre>                      | Takamori et al., 2018 | N/A |
| Mouse: Trpv1-cre                       | The Jackson Laboratory | Cat# 017769 |
| Mouse: Rosa26<DTomato                  | The Jackson Laboratory | Cat# 007914 |
| Mouse: Trpv1-tdTomato                  | This paper | N/A |
| Mouse: mut-Stat3                       | The Jackson Laboratory | Cat# 027952 |
| Mouse: zDC<DTTR>                       | The Jackson Laboratory | Cat# 019506 |
| Mouse: Rag1 +/-                        | The Jackson Laboratory | Cat# 002216 |
| Mouse: Lang<DTTR>                      | The Jackson Laboratory | Cat# 016940 |
| Mouse: Csf1<DTTR>                      | The Jackson Laboratory | Cat# 024046 |
| Mouse: Csf1<DTTR> Lyz2-cre              | Schreiber et al., 2013 | N/A |
| Mouse: Tgfb1<Cre> Lyz2-cre              | Tu et al., 2018 | N/A |
| Mouse: Smad3<Cre>                      | Yang et al., 1999 | N/A |
| Mouse: Tgfb1<Cre Cd11c-cre              | This paper | N/A |
| Mouse: Tgfb1<Cre Lyz2-cre               | This paper | N/A |

Oligonucleotides

| Oligonucleotides | SOURCE | IDENTIFIER |
|------------------|--------|------------|
| TaqMan Hprt primer, Mm00446968_m1 | Applied Biosystems | N/A |
| TaqMan Gapdh primer, Mm99999915_g1 | Applied Biosystems | N/A |
| TaqMan Il31 primer, Mm01194496_m1 | Applied Biosystems | N/A |
| TaqMan Il31a primer, Mm01304494_m1 | Applied Biosystems | N/A |
| TaqMan Trpa1 primer, Mm01227437_m1 | Applied Biosystems | N/A |
| TaqMan Trpv1 primer, Mm01246302_m1 | Applied Biosystems | N/A |
| TaqMan Nppb primer, Mm01255770_g1 | Applied Biosystems | N/A |
RESOURCE AVAILABILITY

Lead Contact
Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, WanJun Chen (wchen@mail.nih.gov).

Materials Availability
This study did not generate new unique reagents

Data and Code Availability
Murine cutaneous wounds tissue RNA sequence data in this paper were deposited in GEO accession (GSE128193; https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE128193)

EXPERIMENTAL MODELS AND SUBJECT DETAILS

Human data of pruritus questionnaires
The questionnaires for pruritus self-assessment was approved by the Institutional Ethics Committee of Beijing Stomatological Hospital affiliated to Capital Medical University; informed consent was obtained from all participants. Pruritus self-assessments were recorded for subjects who were scheduled for minor skin superficial surgery (please see Method Details for details). 12 subjects were enrolled finally (3 women and 9 men, mean age ± SD: 53.3 ± 12.6 years). Pruritus intensity was assessed using a 10-cm visual analog scale (VAS). Subjects were asked to evaluate and record the pruritus intensity before and every other day after surgery within 7 days. No human tissue sample was taken for this study.

Mice
C57BL/6, Rag1−/− mice, Trpv1-Cre mice, Rosa26rtTomato mice, mut-Stat3 mice (Steward-Tharp et al., 2014), zDCDTR mice (Meredith et al., 2012), Csf1rDTR mice, LangDTR mice (Kissenpfennig et al., 2005) were obtained from The Jackson Laboratory. Il31−/− mice (Takamori et al., 2018) were obtained from Dr. Nakae’s Lab. Tgfb11/2Ert2-Cre, Smad3−/− (on a C57BL/6 background) were previously described and bred in our facility under specific pathogen-free conditions. Trpv11stTomato mice were generated in-house by crossing Trpv1-Cre mice with Rosa26rtTomato mice. Csf1rDTR Lys2-Cre mice (MMDTR mice) (Schreiber et al., 2013) were generated in-house by crossing Lys2-Cre mice with Csf1rDTR mice. Tgfb11/2 Cd11c-Cre+ mice were generated in-house by crossing Cd11c-Cre mice with Tgfb11/2 mice. Tgfb11/2 Lyz2-Cre+ were generated in-house by crossing Lyz2-Cre mice with Tgfb11/2 mice. Tgfb11/2 Ert2-Cre mice were treated with tamoxifen (1 µg/mouse) per day for 5 days to delete Tgfb. All mice used for experiments were aged 6-12 weeks, both male and female. All animal studies were performed according to National Institutes of Health (NIH) guidelines for use and care of live animals and approved by the Animal Care and Use Committees of National Institute of Dental and Craniofacial Research (NIDCR).

METHOD DETAILS

Enroll and exclusion criteria for human pruritus questionnaires
All subjects must fulfill all the following items: (1) a superficial surgery is necessary for the subject diagnosed with a superficial cyst or benign tumor; (2) the expected wound will be 1 cm to 3 cm long; (3) the subject has no active systemic disease for 1 year and have a negative history of skin diseases; (4) the surgical area has no inflammation within the recent 6 months and have no sign of infection; (5) subject must be informed of the investigational nature of this study and given written informed consent. Exclusion criteria: (1) Active, uncontrolled infections in surgical area or systemically; (2) pathology report shows as a malignant tumor or Kimura’s disease; (3) failed to follow up entirely.
Bone marrow chimeras and diphtheria toxin injection
C57BL/6 hosts were irradiated with 12Gy split into 3 doses and reconstituted by tail vein injection of 5 \times 10^6 bone marrow cells from WT, Il31^+/− or zDC^DTR donors. Mice were allowed to reconstitute for 6 weeks prior to use. Diphtheria toxin (DT) was purchased from Sigma-Aldrich. zDC^DTR bone marrow chimeras were injected i.p. with 20 ng DT per gram of body weight (500 ng/mouse) on the first day of DC depletion and with 4 ng DT per gram body weight (100 ng/mouse) on all subsequent days. C57BL/6, Lang^DTR, and MM^DTR mice received 4 ng DT per gram body weight (100 ng/mouse) at any time. Since we found that macrophage depletion at the beginning of incising would delay the wound healing or even fatal for mice, DT was injected (i.p., 100ng/mouse) to MM^DTR mice on the 3rd of wound healing and every day thereafter.

Murine wound healing
Mice were anesthetized, dorsum shaved, cleaned with alcohol, and 2 equidistant 1cm full-thickness incisional wounds were made through the skin and left to heal. Wounds tissue (0.5 cm around incision) and dorsal root ganglion (DRG) that innervate the wound were harvested at indicated days and bisected for histology, snap-frozen in for RNA analysis/protein extraction, or DMEM contained 10% fetal bovine serum on ice for flow cytometry. Wound sizes were measured and wound healing rates were estimated by the formula \( \frac{1}{3} \times \text{length} \times \text{width} - \left( \frac{\pi}{4} \times \text{length} \times \text{width} \right)^{\text{day}} - \left( \frac{\pi}{4} \times \text{length} \times \text{width} \right)^{\text{day}\_0} \times 100 \) (Cukjati et al., 2001; Lyman et al., 1970) and HE staining (Braiman-Wiksman et al., 2007).

IL-31 intradermal injection
For wound healing model, we intradermally injected IL-31 30 ng/site (by this dose IL-31 could not induce itch in naive mice 8 hours later; Arai et al., 2013) into wounds every 12 hours for 3 times from the fourth day after wounds were cut, and the behavior recordings were taken 8 hours after the last injection. For mut-Stat3 mice or wild-type control mice, we injected IL-31 1 μg/site on the dorsal area intradermally, and observe the itching behaviors 1 hour later; 8 hours after the 1st injection, another IL-31 injection (1 μg/site, i.d.) on the same spot, itching behaviors were recorded again 1 hour after the 2nd injection.

Pruriceptive behavior measurement
To measure the pruriceptive behaviors, mice were placed in clear plastic enclosures with an optical cannula, which could rotate to allow free movement of the mouse. Itch behavioral responses were videotaped during the experiment. The behavior experiments and analysis were done blinded. Scratch (by the hind leg) bouts around the wounds or dendritic cells injected area were counted for 30 or 60 minutes.

RNA-seq analysis
Two wounds tissue from one animal were pooled, three animals were used per time point. The skin was cleaned of muscle and fat tissue; total RNA was extracted using the RNeasy mini kits (QIAGEN), purified using Direct-zol RNA MiniPrep kit (Zymo Research). Two wounds tissue from one animal were pooled, three animals were used per time point. The skin was cleaned of muscle and fat tissue and cut into small pieces, incubated in Liberase DH (0.5 mg/ml, Sigma) for 90 min before finally shredding through 70-μm cell strainers (BD PharMingen). After isolation, cell suspensions were washed by 0.5% BSA in PBS and...
passed through 40 μm cell strainers (BD PharMingen), and cell populations were characterized by flow cytometry. Stained cells were analyzed on LSRFortessa (BD Biosciences) or separated by FACS Aria cell sorter (BD Biosciences). Data were analyzed with FlowJo software.

**Dermal conventional type 2 dendritic cells culture and injection**

Dermal conventional type 2 dendritic cells (cDC2) were sorted by FACS Aria cell sorter (Zombie-CD45+CD3-CD64-CD11c+CD326-CD11b+MHC II+) and cultured with complete medium (RPMI 1640 supplemented with 10% FBS, L-glutamine, penicillin/streptomycin, 2-mercaptoethanol) contained GM-CSF (20 ng/ml, Peprotech). For cDC2 injection, mice were dorsum shaved 1 day before injection, cDC2 were washed and suspended in HBSS+/+ RP free buffer and injected intradermally (5000 cells or 15000 cells in 20 μL per site), pruricceptive behaviors were observed before and after injections.

**Real-time PCR**

Total RNA was derived from cultured cells with RNeasy Mini kits (QIAGEN) or RNeasy Micro kits (QIAGEN), cDNA was synthesized using a High Capacity cDNA Reverse Transcription kit (Applied Biosystems). For sorted cells, a TaqMan PreAmp Master Mix Kit (Applied Biosystems) was used before quantitative real-time PCR. Quantitative real-time PCR was performed according to the protocol of TaqMan gene expression assay kits (Applied Biosystems). Results from leukocytes were normalized to the expression of Hprt mRNA, results from tissue were normalized to the expression of Gapdh mRNA.

**Antibodies and reagents**

The following fluorochrome-conjugated antibodies were used for flow cytometry surface staining: anti-mouse CD45 (30-F11, eBioscience), anti-mouse CD3 (17A3, eBioscience), anti-mouse CD11c (N418, eBioscience), anti-mouse CD11b (M1/70, eBioscience), anti-mouse MHC class II (I-A/I-E) (M5/114.15.2, eBioscience), anti-mouse F4/80 (BM8, eBioscience), anti-mouse CD64 (X54-5/7.1, eBioscience), anti-mouse CD117 (2B8, eBioscience), anti-mouse Fc epsilon Receptor 1 alpha (MAR-1, eBioscience), anti-mouse CD207 (eBioL31, eBioscience), anti-mouse CD326 (G8.8, eBioscience), anti-mouse CD103 (2E7, eBioscience). Dead cells were excluded from analysis using Zombie Yellow Fixable Viability Kit (Biolegend). Recombined proteins and chemicals: IL-1β (401-ML, R&D System), IL-6 (206-IL, R&D System), IL-17a (421-ML, R&D System), IL-31 (3028-ML, R&D System), TGF-β1 (240-B, R&D System), TNF-α (410-MT, R&D System), SB431542 (ALK5 inhibitor, Selleckchem), S3I-201 (Stat3 inhibitor, Selleckchem), and SIS3 (Smad3 inhibitor, Sigma) were used in cell culturing for different conditions. Anti-mouse Stat1 (#9172, Cell Signaling), anti-mouse pStat1 (#9171S, Cell Signaling), anti-mouse Stat3 (#9139, Cell Signaling), anti-mouse pStat3 (#9131S, Cell Signaling), anti-mouse Stat5 (#9363, Cell Signaling), anti-mouse pStat5 (#9351S, Cell Signaling), anti-mouse Gapdh (#5014S, Cell Signaling), anti-mouse IL-31 (ab102750, ABcam) were used for western blot. TGFβ1 Elisa kit (G7591, Promega) and IL-31 Elisa kit (BMS6030, eBioscience) were used for TGFβ1 or IL-31 protein determination.

**Statistical analysis**

Statistical analysis was performed using either unpaired two-tailed Student’s t tests or one-way ANOVA in GraphPad Prism.