

Neuronal Mechanisms of Psoriatic Itch: Role of IL-17R/ERK/TRPV4 Signaling Pathway



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Journal of Investigative Dermatology (2025) 145, 2753–2762; doi:10.1016/j.jid.2025.03.037

Itch represents a major disease burden of psoriasis. Despite recent clinical studies showing the effectiveness of IL-17– and IL-17R–blocking antibodies in alleviating psoriatic itch, significant questions remain unanswered. Specifically, the crucial cellular site of action and the impacted signaling pathway of IL-17/IL-17R in psoriatic itch are elusive. Itch sensation relies on dorsal root ganglion (DRG) sensory neurons that transmit pruriceptive signals from the periphery to the CNS. IL-17RA and IL-17RC, 2 cognate receptors for IL-17, are expressed in DRG neurons. In this study, we demonstrated that IL-17RA and IL-17RC are upregulated in DRG neurons in a mouse model of psoriasis induced by imiquimod. Notably, conditional knockout of *Il17ra* or *Il17rc* in sensory neurons potently attenuated psoriasis-like itch. Furthermore, our in vitro assay with cultured neurons and in vivo experiment with animal model of psoriasis demonstrated that IL-17RA and IL-17RC upregulate the pruritic ion channel TRPV4 in DRG neurons through the extracellular signal–regulated kinase (ERK) signaling pathway. Specific deletion of *Trpv4* or suppression of phosphorylation of ERK in DRG neurons mitigated psoriasis-like itch. These findings suggest that the IL-17R/ERK/TRPV4 signaling pathway in sensory neurons plays a significant role in psoriatic itch.

Keywords: Dorsal root ganglion sensory neurons, IL-17, Itch, Psoriasis, TRPV4

INTRODUCTION

Psoriasis is an autoimmune-mediated, T helper 1/T helper 17–dominant systemic inflammatory skin disease. The majority of patients with psoriasis suffer from itch, which negatively impacts their quality of life (Elewski et al, 2019; Yosipovitch et al, 2000). Clinical studies have demonstrated that itch intensity may not correlate with the overall severity of psoriatic skin lesions (Elewski et al, 2019; Roblin et al, 2014; Yosipovitch et al, 2000). In addition, patients have reported itch on both lesional and nonlesional psoriatic skin (Szepietowski et al, 2002). Itching and scratching can result in Koebner phenomena (Ji and Liu, 2019), with formation of new psoriatic plaques, further exacerbating psoriatic

symptoms. Therefore, deconstructing the mechanisms of psoriatic itch is an eminent question toward improved management of psoriasis.

A notable advancement in the psoriasis field is the discovery that the IL-17/IL-17R signaling plays a central role in disease pathogenesis (Vidal et al, 2021). The IL-17 family consists of 6 members (IL-17A–F), with IL-17A being a key member that signals through a heterodimeric receptor complex IL-17RA/IL-17RC. IL-17A– or IL-17RA–blocking antibodies have been recently advanced into clinical trials for treating psoriatic itch (Gottlieb et al, 2018; Kimball et al, 2018; Yosipovitch et al, 2019). Despite effectiveness, these biologics do not fully relieve itch and do not work for all patients. To improve IL-17–/IL-17R–targeting interventions and identify new therapeutic targets, it is crucial to know where and how IL-17/IL-17R signaling works in psoriatic itch. Unfortunately, our knowledge of its underlying mechanisms is limited. Itch is a process in which sensory neurons in the dorsal root ganglion (DRG) detects itch signals through their peripheral projections that extend to the periphery and relay these signals to CNS (Lay and Dong, 2020). IL-17RA and IL-17RC are expressed in DRG neurons (Enamorado et al, 2023; Luo et al, 2021, 2019; Segond von Banchet et al, 2013). However, whether sensory neuron–IL-17RA/IL-17RC drives psoriatic itch is unclear. In addition, detection of pruriceptive cues by sensory neurons relies on specific transduction machinery. Key components of it belong to ion channels (Moore et al, 2018; Shirolkar and Mishra, 2022). Yet, it remains unidentified whether they are involved in IL-17/IL-17R–driven psoriatic itch.

Emerging studies have suggested significant contributions of TRPV4, a multimodally activated nonselective cation ion

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Abbreviations: cKO, conditional knockout; dn-MEK, dominant-negative mitogen-activated protein kinase; DRG, dorsal root ganglion; IMQ, imiquimod; MEK, mitogen-activated protein kinase kinase; pERK, phosphorylated extracellular signal–regulated kinase; WT, wild-type

Received 28 October 2024; revised 11 March 2025; accepted 28 March 2025; accepted manuscript published online 17 April 2025; corrected proof published online 10 May 2025

channel, to acute and chronic itch (Zhang et al, 2021). TRPV4-knockout mice have been reported to show reduced scratching behaviors evoked by histaminergic (histamine and compound 48/80), partial histaminergic (serotonin), and nonhistaminergic (chloroquine) pruritogens (Akiyama et al, 2016; Chen et al, 2016; Kim et al, 2016). TRPV4 was detected in rodent and human primary sensory neurons (Chen et al, 2013; Liedtke and Friedman, 2003; Suttle et al, 2023). Employing conditional knockout (cKO) mice, we recently uncovered that TRPV4 in sensory neurons contributes to scratching behaviors evoked by histamine, compound 48/80, and serotonin (Zhang et al, 2022). In addition, TRPV4 in sensory neurons was reported to be essential for chronic itch associated with dry skin but not with allergic contact dermatitis in mouse models (Lee and Shim, 2021; Zhang et al, 2022). These findings allude to a potential role of sensory neuron-TRPV4 in certain forms of chronic itch. Interestingly, in a mouse model of psoriasis induced by imiquimod (IMQ), *Trpv4* mRNA levels in DRGs were elevated, whereas the gene expression of other itch-related ion channels, including *Trpv1*, *Trpa1*, *Trpm8*, *MrgprA3*, *MrgprC11*, and *MrgprD*, was either reduced or unchanged (Sakai et al, 2016), highlighting a possible contribution of sensory neuron TRPV4 to psoriatic itch. Furthermore, a previous study of cultured DRG neurons demonstrated that TRPV4 expression is increased upon stimulation of IL-17A (Segond von Banchet et al, 2013).

Against this background, we interrogated whether the IL-17R signaling in sensory neurons drives psoriasis-like itch through TRPV4. Specifically, we sought to determine whether IL-17A and IL-17RC in sensory neurons are essential for psoriasis-like itch, whether they promote psoriasis-like itch by regulating

TRPV4 expression, and how they regulate TRPV4 expression under the condition of psoriasis-like skin inflammation.

RESULTS

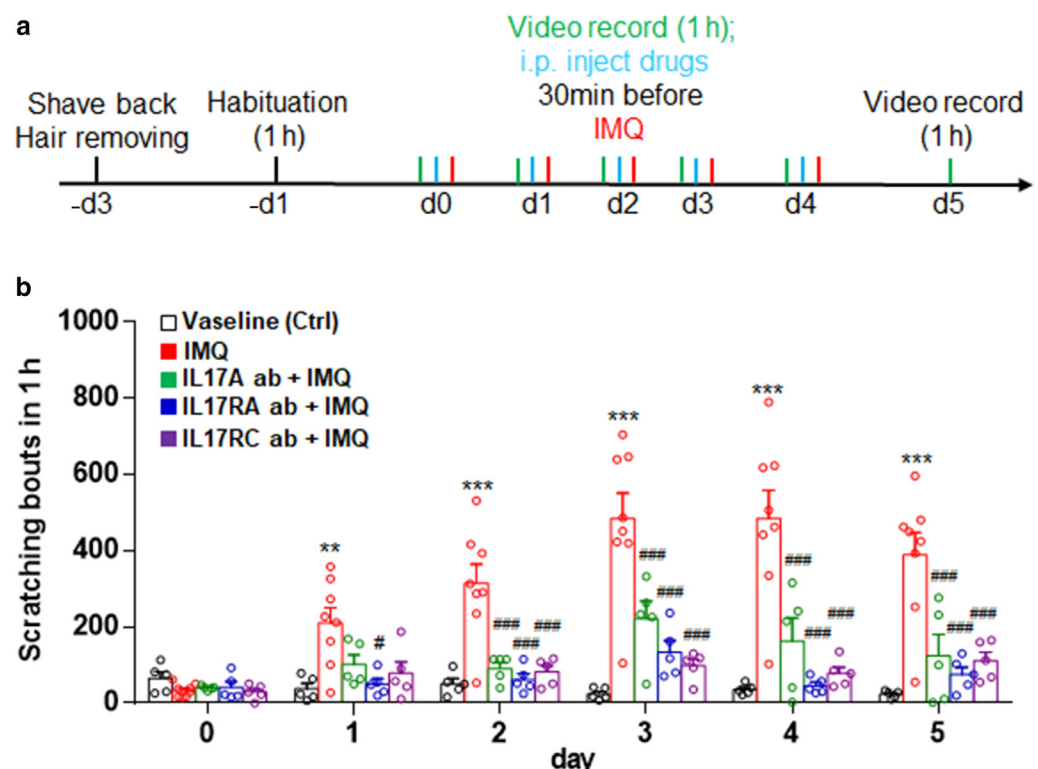
Systemic blockade of IL-17 or IL-17R reduces psoriasis-like itch

Consistent with prior research (Sakai et al, 2016), topical application of IMQ induced robust scratching behaviors in mice from day 1 to 5 (Figure 1). To evaluate the involvement of IL-17/IL-17R signaling in psoriasis-like itch, IL-17A α -, IL-17RA α -, or IL-17RC α -blocking antibodies at 2 mg/kg were intraperitoneally administered 30 minutes before daily IMQ treatment. Behavioral analysis showed that systemic blockade of IL-17A, IL-17RA, or IL-17RC robustly attenuated itch evoked by IMQ (Figure 1). These reverse-translational data underscore the pivotal role of IL-17/IL-17R signaling in the development of psoriasis-like itch.

Characterization of IL-17R-expressing DRG sensory neurons in mice with psoriasis-like skin inflammation

Previous studies have revealed the presence of IL-17RA and IL-17RC in DRG neurons (Enamorado et al, 2023; Luo et al, 2021, 2019; Segond von Banchet et al, 2013). In this study, we characterized IL-17RA α - or IL-17RC α -expressing DRG neurons in mice treated with IMQ. We found that these 2 receptors are located in CGRP-labeled (peptidergic) and P2X3-labeled (nonpeptidergic) neurons as well as in peripherin-labeled (small sized) neurons (Figure 2a–d), in line with a previous report (Luo et al, 2019). Further analysis of size distribution demonstrated that IL-17RA α - or IL-17RC α -expressing DRG neurons are predominantly small to medium (Figure 2e and f). It has been documented that TRPV1- or TRPA1-expressing sensory neurons are involved in

Figure 1. i.p. injection of IL-17A α -, IL-17RA α -, or IL-17RC α -blocking antibodies (2 mg/kg) suppresses psoriasis-like itch induced by IMQ. (a) The experimental design and timeline. (b) Quantitative analysis of scratching behaviors. $^{**}P < .01$ and $^{***}P < .001$ versus Vaseline (ctrl) and $^{\#}P < .05$ and $^{##}P < .001$ versus IMQ, with 2-way repeated-measures ANOVA with Bonferroni posthoc test. $n = 8$ mice for IMQ group and 5 for other groups. ab, antibody; ctrl, control; h, hour; IMQ, imiquimod; i.p., intraperitoneal.



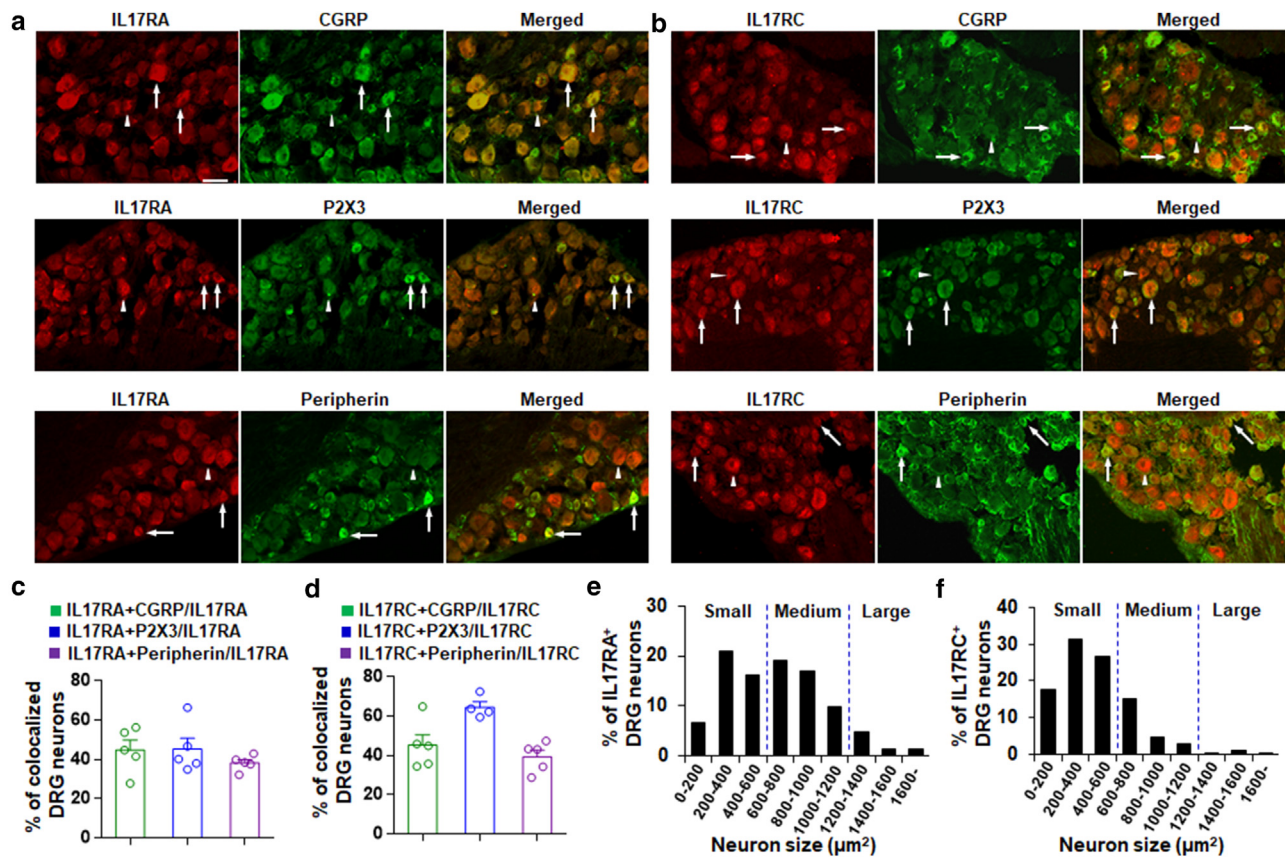


Figure 2. Characterization of IL-17RA- or IL-17RC-expressing DRG neurons in mice treated with IMQ. (a, b) Double-labeling images and (c, d) quantitative analysis showed colocalization of IL-17RA or IL-17RC with CGRP, P2X3, and peripherin in DRG neurons in mice treated with IMQ (day 5). Arrows in images indicate colocalized neurons, and arrowheads indicate IL-17R-positive but marker-negative neurons. $n = 4-5$ mice per group. (e, f) Size frequency distribution analysis showed that IL-17RA and IL-17RC are predominantly expressed in small-medium-sized DRG neurons. $n = 442$ and 461 for IL-17RA- and IL-17RC-positive neurons, respectively, from 4 mice were analyzed. Bar = 50 μm . DRG, dorsal root ganglion; IMQ, imiquimod.

itch. We also found that IL-17RA and IL-17RC coexpress with these 2 ion channels in DRG neurons (Supplementary Figure S1). This documented pattern of expression suggests that IL-17RA and IL-17RC are expressed in pruriceptive neurons.

IL-17R expression in DRG sensory neurons is upregulated after psoriasis-like skin inflammation, and cKO of sensory neuron-*Il17r* attenuates psoriasis-like itch

To determine whether sensory neurons serve as the key cellular site for IL-17/IL-17R signaling in psoriasis-like itch, we first intrathecally injected IL-17A to test whether it elicits scratching under normal condition. Interestingly, injection of IL-17A at 2, 20, and 200 ng/ μl (5 μl) did not significantly evoke scratching behaviors in naïve mice (Figure 3a). Using the cheek model (Shimada and LaMotte, 2008), we observed that intradermal injection of IL-17A at 200 ng/ μl (5 μl) into the cheek failed to evoke itch or pain behaviors (Figure 3a and b). These results, together with a prior observation that no itch was induced by intradermal injection of IL-17A into back skin (Pavlenko et al, 2020), suggest that IL-17A by itself does not function as a pruritogen.

We were then prompted to shed more light into how IL-17/IL-17R signaling promotes itch under psoriatic condition. Interestingly, we found that IL-17RA and IL-17RC are

upregulated in DRG neurons after IMQ treatment (Figure 3d–g), suggesting that sensory neuron-IL17ra and IL17rc cKO possibly drives psoriasis-like itch. We then employed mice with sensory neuron-derived *Il17ra* and *Il17rc* cKO to test this possibility. Targeted deletion of *Il17ra* or *Il17rc* in cKO mice was validated by their marked reduction of expression in DRG neurons (Figure 3h–k). IMQ-induced scratching behaviors were significantly reduced in *Il17ra* or *Il17rc* cKO mice (Figure 3l), suggesting a critical involvement of sensory neuron-IL17-RA and IL-17RC in psoriasis-like itch.

IL-17/IL-17R signaling upregulates TRPV4 expression in DRG neurons through extracellular signal-regulated kinase under the condition of psoriasis-like skin inflammation

In view of previous studies showing that (i) IL-17A stimulation upregulates TRPV4 in cultured DRG neurons isolated from naïve mice (Segond von Banchet et al, 2013) and (ii) *Trpv4* mRNA in DRGs is increased in mice after IMQ treatment (Sakai et al, 2016), we asked whether IL-17/IL-17R signaling increases TRPV4 expression under psoriasis-like skin inflammation condition. First, we found that TRPV4 is highly colocalized with IL-17RA and IL-17RC in DRG neurons of wild-type (WT) control mice, with ~70% of IL-17RA and ~65% of IL-17RC immunoreactive neurons expressing

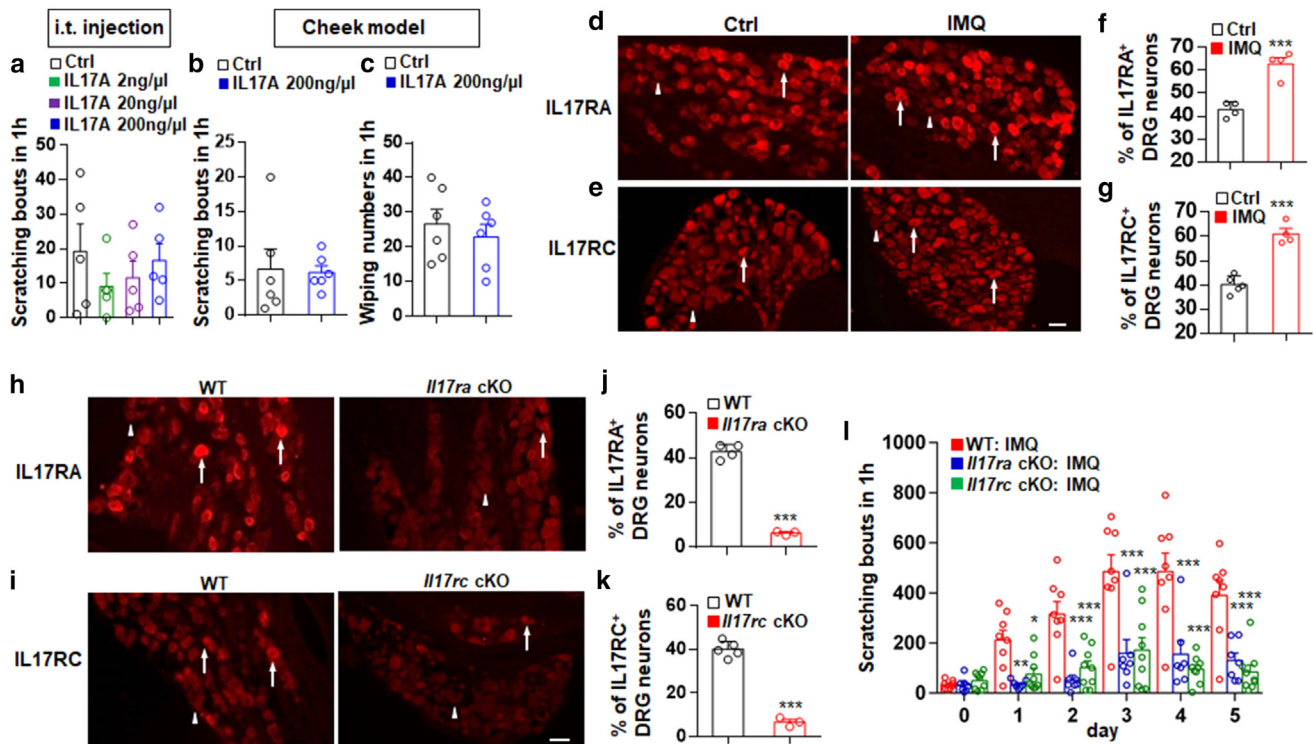


Figure 3. IL-17RA and IL-17RC expression in DRG neurons is upregulated after IMQ treatment, and cKO of *Il17ra* or *Il17rc* in DRG neurons reduces psoriasis-like itch induced by IMQ. (a) i.t. injection of IL-17A at 2, 20, 200 ng/μl (5 μl) did not cause itch in naïve mice. One-way ANOVA with Bonferroni posthoc test was performed; $n = 4-5$ mice per group. (b, c) i.d. injection of IL-17A at 200 ng/μl (5 μl) into mouse cheek did not cause scratching (b) itch or wiping (c) pain behaviors in naïve mice. Student's *t*-test was performed; $n = 6$ mice per group. (d, e) Representative images and (f, g) quantitative analysis showed that IL-17RA and IL-17RC expression in DRG neurons was significantly upregulated after IMQ (day 5). $***P < .001$, with Student's *t*-test; $n = 4-5$ mice per group. (h, i) Representative images and (j, k) quantitative analysis showed a large reduction of IL-17RA and IL-17RC expression in DRG neurons of *Il17ra*- or *Il17rc*-cKO mice after tamoxifen induction. $***P < .001$ versus WT, with Student's *t*-test; $n = 3-5$ mice per group. (l) cKO of sensory neuron *Il17ra* or *Il17rc* significantly suppressed psoriatic itch. $*P < .05$, $**P < .01$, and $***P < .001$ versus WT: IMQ, with 2-way repeated-measures ANOVA with Bonferroni posthoc test; $n = 6-9$ mice per group. Arrows and arrowheads in images represent positive and negative DRG neurons, respectively. Ctrl in a–c represents normal saline, and that in d–g represents Vaseline. Bar = 50 μm. cKO, conditional knockout; Ctrl, control; DRG, dorsal root ganglion; i.d., intradermal; IMQ, imiquimod; i.t., intrathecal; WT, wild type.

TRPV4 (Figure 4a and b). Second, we found that IMQ-induced upregulation of TRPV4 in DRG neurons is nearly reversed in *Il17ra* or *Il17rc* cKO mice (Figure 4c and d).

We proceeded to determine the signaling pathway through which IL-17R regulates TRPV4. We focused on extracellular signal-regulated kinase (ERK) signaling because it acts as a key signaling pathway involved in various pathological events driven by IL-17/IL-17R (Amatya et al, 2017). First, using cultured DRG neurons isolated from WT naïve mice, we found that inhibition of mitogen activated protein kinase kinase (MEK), an upstream regulator of ERK, with U0126 suppressed the enhanced expression of TRPV4 by IL-17A treatment (Figure 4e). Second, we utilized dominant-negative mitogen-activated protein kinase kinase (dn-MEK) mice, in which MEK function is suppressed specifically in neurons, to assess the regulatory role of ERK in TRPV4 expression in DRG neurons after psoriasis-like skin inflammation. Our results demonstrated that IMQ-induced upregulation of TRPV4 in DRG neurons is significantly suppressed in dn-MEK mice (Figure 4c and d).

Building upon the finding of TRPV4 upregulation by IL-17R through ERK signaling, we next asked whether phosphorylated ERK (pERK) levels in DRG neurons are elevated after

IMQ treatment and, specifically, whether any such elevation depends on IL-17R. Immunostaining demonstrated an increased expression of pERK in DRG neurons after IMQ application. Indicative of the key role of IL-17R signaling, the increase of pERK was significantly reduced by cKO of *Il17ra* or *Il17rc* in DRG neurons (Figure 4f and g).

TRPV1 and TRPA1 are known to be involved in acute and chronic itch (Moore et al, 2018; Shiolkar and Mishra, 2022). We also examined whether IL-17R regulates their expressions in DRG neurons after psoriasis-like skin inflammation. First, TRPV1 and TRPA1 coexpressed with IL-17RA or IL-17RC, with the percentage of their coexpressing neurons remaining unchanged after IMQ treatment (Supplementary Figure S1). Second, in line with previous studies (Kodji et al, 2019; Sakai et al, 2016), TRPV1 and TRPA1 expressions in DRG neurons were not significantly altered after IMQ treatment. Finally, their expressions in DRG neurons were not changed in *Il17ra* or *Il17rc* cKO mice after IMQ (Supplementary Figure S2). These data suggest that the IL-17R signaling-driven psoriasis-like itch does not involve the regulation of expression of TRPV1 and TRPA1 in DRG neurons.

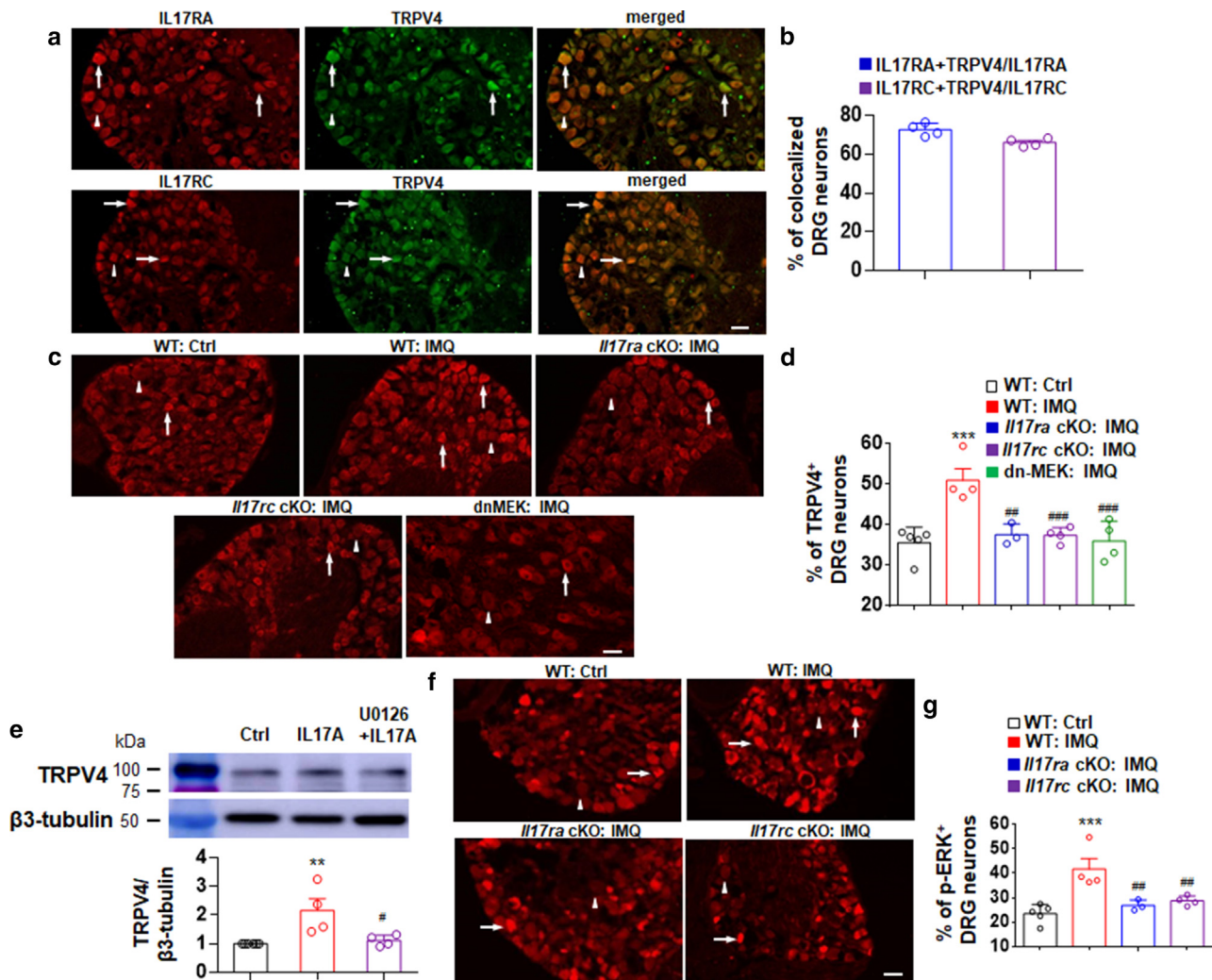


Figure 4. IL-17RA and IL-17RC upregulate TRPV4 expression in DRG neurons through ERK signaling under psoriasis-like skin inflammation condition. (a) Representative images and (b) quantitative analysis showed colocalization of IL-17RA + TRPV4 or IL-17RC + TRPV4 in DRG neurons; $n = 4$ mice per group. (c) Representative images and (d) quantitative analysis showed that IMQ-induced upregulation of TRPV4 in DRG neurons is significantly reduced in *Il17ra*-cKO, *Il17rc*-cKO, or dn-MEK mice (day 5 IMQ). *** $P < .001$ versus WT: Ctrl (Vaseline) and # $P < .01$ and ### $P < .001$ versus WT: IMQ, with 1-way ANOVA with Bonferroni posthoc test; $n = 3-5$ mice per group. (e) Western blot showed that stimulation of cultured DRG neurons with IL-17A (100 ng/ml) upregulates TRPV4, which is almost reversed by the MEK selective inhibitor U0126 (10 μ M). ** $P < .01$ versus ctrl (medium) and # $P < .05$ versus IL-17A, with 1-way ANOVA with Bonferroni posthoc test; $n = 4-5$ mice per group. (f) Representative images and (g) quantitative analysis showed that IMQ-induced upregulation of p-ERK in DRG neurons is significantly reduced in *Il17ra*- or *Il17rc*-cKO mice (day 5 IMQ). *** $P < .001$ versus WT: Ctrl (Vaseline) and # $P < .01$ versus WT: IMQ, with 1-way ANOVA with Bonferroni posthoc test; $n = 3-5$ mice per group. Arrows and arrowheads in images represent positive and negative DRG neurons, respectively. Ctrl in c, d, f, and g represents Vaseline, and that in e represents normal saline. Bar = 50 μ m. cKO, conditional knockout; Ctrl, control; dn-MEK, dominant-negative mitogen activated protein kinase kinase; DRG, dorsal root ganglion; ERK, extracellular signal-regulated kinase; IMQ, imiquimod; MEK, mitogen activated protein kinase kinase; p-ERK, phosphorylated extracellular signal-regulated kinase; WT, wild type.

cKO of sensory neuron-*Trpv4* or specific suppression of neuronal ERK attenuates psoriasis-like itch

We then employed *Trpv4* cKO and dn-MEK mice to determine the functional contribution of sensory neuron TRPV4 and ERK to psoriasis-like itch. Targeted deletion of *Trpv4* from DRG neurons of cKO mice was confirmed in our previous studies by observing a marked reduction of TRPV4 expression in DRG neurons with immunostaining (reduced TRPV4 immunoreactive neurons: 27.7% for WT mice vs 6.5% for *Trpv4*-cKO mice) (Chen et al, 2021) and calcium ion imaging assay (reduced DRG neurons in response to the TRPV4 selective agonist 4 α -phorbol 12,13-didecanoate: 34.9% for WT vs 6.2% for *Trpv4*-cKO mice)

(Zhang et al, 2022). In dn-MEK mice, an α -tubulin promoter element that confers neuronal-specific expression of the transgene was used to drive the expression of a hemagglutinin-tagged K97M dominant-negative form of MEK (Shalin et al, 2004). Using immunostaining, we have previously confirmed hemagglutinin presence in sensory neurons of dn-MEK mice (Chen et al, 2013). Behavioral analysis showed that *Trpv4*-cKO mice exhibit a notable decrease in psoriasis-like itch (Figure 5a), highlighting a pronounced pruritic role of sensory neuron-TRPV4 in psoriasis-like skin inflammatory condition. We also found that dn-MEK mice displayed significant reduction of psoriasis-like itch (Figure 5b).

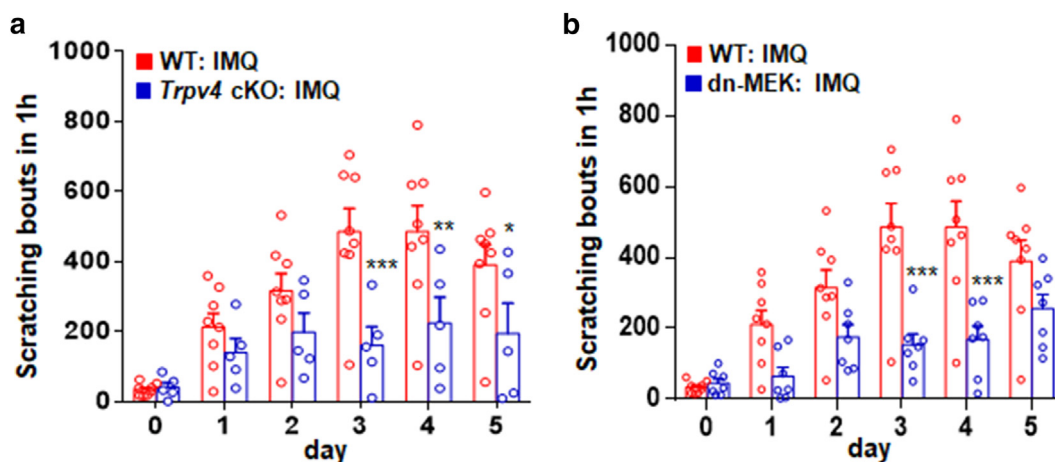


Figure 5. Psoriasis-like itch induced by IMQ is significantly reduced in *Trpv4*-cKO and dn-MEK mice. (a) *Trpv4*-cKO and (b) dn-MEK mice. * $P < .05$, ** $P < .01$, and *** $P < .001$ versus WT: IMQ, with 2-way repeated-measures ANOVA with Bonferroni posthoc test. $n = 5$ –8 mice per group. cKO, conditional knockout; dn-MEK, dominant-negative mitogen activated protein kinase; IMQ, imiquimod; WT, wild type.

Psoriasis-like skin inflammation remains unchanged in *Il17r*-cKO, *Trpv4*-cKO, and dn-MEK mice

Finally, we asked whether sensory neuron IL-17R, TRPV4, or ERK has any impact on psoriasiform skin inflammation. Surprisingly, we observed that there was no difference in general appearance of skin inflammation (eg, erythema, scaling, and thickening) induced by IMQ between *Il17ra*-cKO, *Il17rc*-cKO, *Trpv4*-cKO, or dn-MEK mice and WT mice (Figure 6a). Quantitative assessment of H&E staining in skin also revealed that the increase of epidermal thickness and dermal inflammatory cells infiltration, both psoriasis hallmarks, evoked by IMQ was not reduced in *Il17ra*-cKO, *Il17rc*-cKO, *Trpv4*-cKO, or dn-MEK mice (Figure 6b–g). These data suggest that the IL-17R/ERK/TRPV4 signaling in sensory neurons are not involved in the overall development of psoriasis-like skin inflammation.

DISCUSSION

Patients with psoriasis report itch on both lesional and non-lesional skin, and those who achieve clearance of skin inflammation may continue to experience itch (Szepietowski et al, 2002). This suggests that sensitization of the peripheral and/or CNS might be developed during chronic psoriatic itch. Therefore, it is important to deconstruct the neuronal mechanisms of psoriatic itch for developing more effective treatments. In this study, using a well-established mouse model of psoriasis-like itch induced by IMQ, we found that IL-17RA and IL-17RC in DRG sensory neurons are upregulated. Leveraging the power of genetically engineered mice, we showed that cKO of *Il17ra* or *Il17rc* in sensory neurons largely alleviates psoriasis-like itch. We further demonstrated that IL-17RA and IL-17RC upregulate TRPV4, a pruritic ion channel, in sensory neurons through ERK. cKO of *Trpv4* and specific suppression of ERK activation in sensory neurons resulted in reduced psoriasis-like itch. Collectively, our findings suggest that the IL-17R → MEK/ERK → TRPV4 signaling pathway in DRG neurons is significantly involved in psoriatic itch.

The IL-17/IL-17R signaling is a key player for psoriasis pathogenesis (Gottlieb et al, 2018; Kimball et al, 2018;

Leonardi et al, 2012; Mease et al, 2016; Yosipovitch et al, 2019). The pathophysiology is understood as dermal T helper 17 T cells secreting IL-17, which binds to IL-17R on keratinocytes, triggering the production of cytokines, chemokines, and antimicrobial peptides that recruit additional immune cells to the site of inflammation. This recruitment further amplifies the inflammatory response, creating a cycle that perpetuates skin inflammation (Brembilla et al, 2018; Zhou et al, 2022). In contrast to the wealth of knowledge about the mechanisms of IL-17/IL-17R signaling in the pathogenesis of psoriatic skin inflammation, we know very little about where and how it drives psoriatic itch. Using a mouse model of psoriasis, we found that IL-17RA and IL-17RC are upregulated in sensory neurons. Genetic loss-of-function approach demonstrated that cKO of sensory neuron *Il17ra* and *Il17rc* potentially attenuates psoriasis-like itch, pointing to skin-innervating DRG sensory neurons as key cellular site, with IL-17R identified as a key pruritic signaling in these neurons. Our findings potentially open an opportunity for facilitating improved IL-17R-based itch therapies. For example, it may be beneficial to consider selective inhibition of IL-17R signaling in DRG neurons as add-on treatment approach for patients with itch as dominant symptom.

Emerging evidence suggests the pivotal involvement of ion channels in sensory neurons in acute and chronic itch (LaMotte et al, 2014; Xie and Hu, 2018; Zhang et al, 2021). Notably, among a number of itch-related ion channels examined, TRPV4 stood out as the only one to be upregulated in DRGs in the IMQ model of psoriasis (Sakai et al, 2016). On the basis of this finding, along with the previous observation that IL-17 stimulation upregulates TRPV4 in cultured DRG neurons (Segond von Banchet et al, 2013), we interrogated whether sensory neuron IL-17/IL-17R signaling drives psoriasis-like itch through TRPV4. Indeed, our experiments with cKO mice demonstrated that upregulation of TRPV4 in DRG neurons after psoriasis-like skin inflammation is dependent on IL-17R. Furthermore, specific deletion of *Trpv4* from sensory neurons attenuated psoriasis-like itch. Interestingly, De Logu et al (2023) recently reported that proinflammatory cytokines, including IL-17A, enhances the

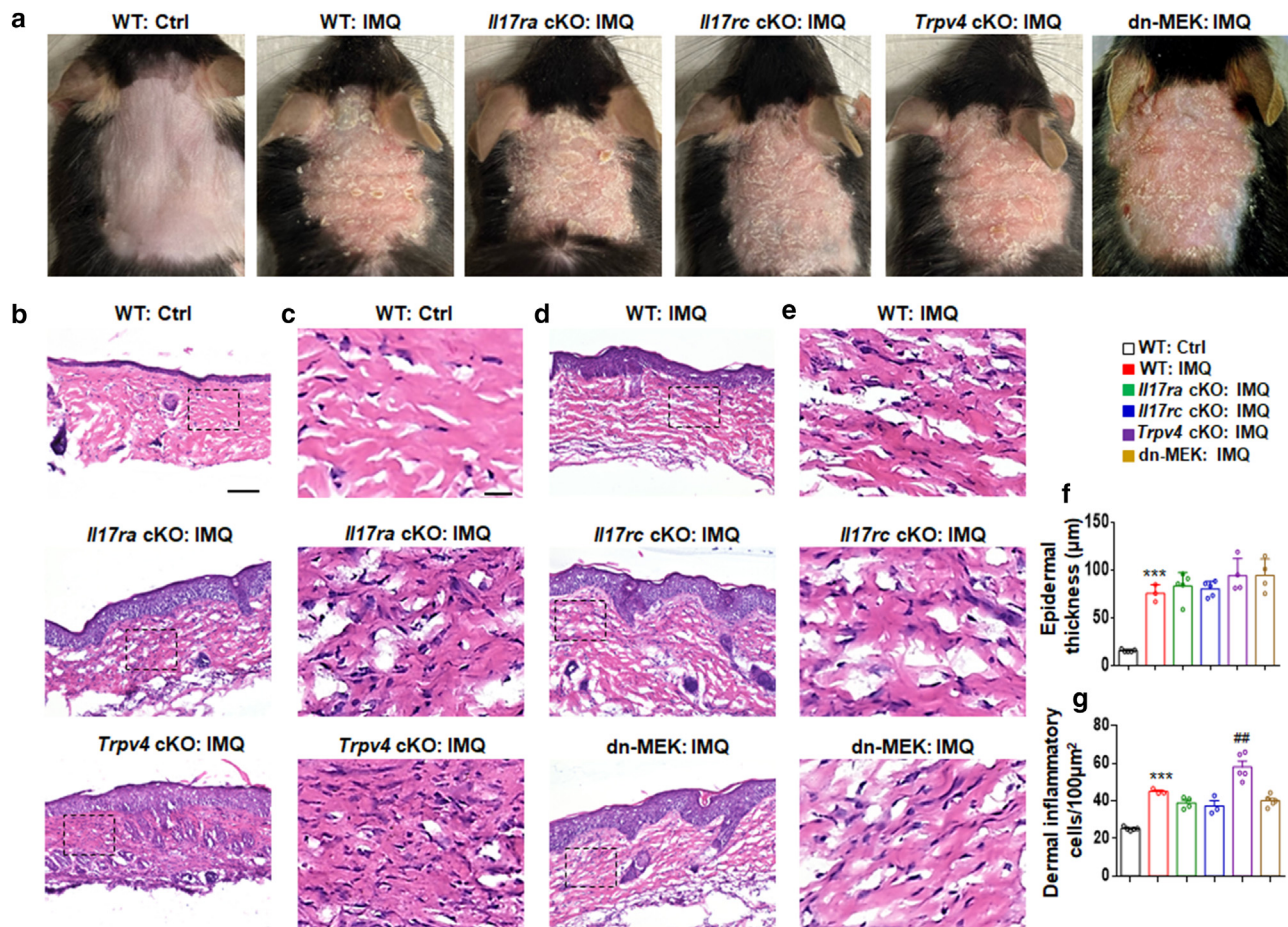


Figure 6. Psoriasis-like skin inflammation remains unchanged in *Il17ra*-cKO, *Il17rc*-cKO, *Trpv4*-cKO, and dn-MEK mice. (a) Representative photographs of dorsal neck skin showed that there were no notable differences in general appearance of psoriasis-like inflammation between *Il17ra*-cKO, *Il17rc*-cKO, *Trpv4*-cKO, or dn-MEK and WT mice (day 5 IMQ). (b–e) Representative images and (f, g) quantitative analysis of H&E staining showed that there are no significant differences in (b, d, f) epidermal thickness and (c, e, g) dermal inflammatory cell (blue–purple nucleus in the dermis) infiltration except that (g) an increase in inflammatory cells infiltration is observed in *Trpv4*-cKO mice (day 5 IMQ). (c, e) Enlarged higher-magnified images from the boxed areas of (b) and (d) show dermal inflammatory cells (with blue–purple nucleus) with a clearer view. *** $P < .001$ versus WT Ctrl and ## $P < .01$ versus WT: IMQ, with 1-way ANOVA with Bonferroni posthoc test; $n = 3–5$ mice per group. Ctrl represents Vaseline. Bar in (b) = 100 μm; bar in (c) = 400 μm. cKO, conditional knockout; Ctrl, control; dn-MEK, dominant-negative mitogen activated protein kinase; IMQ, imiquimod; WT, wild type.

expression of miR-203b-3p from skin keratinocytes. Once secreted from keratinocytes, miR-203b-3p induces psoriasis-like itch by activating TRPV4 in sensory neurons by direct binding to 5-HT2B (De Logu et al, 2023). Together, these findings imply that increased expression of TRPV4 in sensory neurons by the IL-17/IL-17R signaling promotes psoriatic itch.

ERK activation in the spinal cord neurons contributes to acute itch evoked by histamine and chronic itch in mouse models of allergic contact dermatitis, atopic dermatitis, dry skin, and psoriasis (Huang et al, 2018; Jiang et al, 2015; Zhang et al, 2014; Zhao et al, 2013). Notably, RNA-sequencing analysis of mouse spinal cord demonstrated that ERK is the most significantly upregulated gene cluster among 2933 genes after psoriasis-like skin inflammation (Liu et al, 2021). In this study, we found that ERK can also be activated in DRG neurons after psoriasis-like skin inflammation in an IL-17R-dependent manner, and importantly, specific suppression of ERK activation in these neurons reduces psoriasis-like itch. Our in vitro and in vivo studies

demonstrated that inhibiting ERK suppresses the increased expression of TRPV4 in DRG neurons in psoriasis-like skin inflammation. These findings imply that ERK, as a downstream target of sensory neuron IL-17R, contributes to psoriatic itch by upregulating TRPV4.

Similar to cytokines such as IL-33, IL-4, and oncostatin M, which do not act as classic pruritogens but facilitate chronic itch in skin disorders (Liu et al, 2016; Oetjen et al, 2017; Trier et al, 2022; Tseng and Hoon, 2021), we found that IL-17 does not directly cause scratching, but the sensory neuron IL-17/IL-17R signaling promotes psoriasis-like itch. On the basis of our data, we speculate that IL-17/IL-17R signaling may sensitize sensory neuron through increased ERK phosphorylation and TRPV4 expression in DRG neurons, which potentially contributes to the development of psoriasis-like itch. In support of this concept, for example, it has been shown that pERK upregulation in DRG neurons is necessary and sufficient for maintaining peripheral sensitization, resulting in heightened excitability of DRG neurons in response to pruritogens and increased scratching evoked by

pruritogens (Zhao et al, 2013). In addition, De Logu et al (2023) reported that miR-203b-3p, released from keratinocytes, can induce psoriasis-like itch by activating TRPV4 in sensory neurons through direct binding to the 5-HTR2B/TRPV4 complex. We speculate that increased TRPV4 expression in sensory neurons may enhance their responses to pruritogenic signals, including miR-203b-3p, thereby facilitating psoriatic itch.

TRPV1 and TRPA1 have been recognized as important mediators for chronic itch (Moore et al, 2018; Shirolkar and Mishra, 2022). However, their roles in psoriasis and psoriatic itch are complex, and existing evidence is inconsistent. For instance, studies have shown an increased expression of both channels in psoriatic skin (Jena et al, 2019; Nattkemper et al, 2018; Zhou et al, 2019), and knockout of *Trpv1* or *Trpa1* attenuated psoriasis-like skin inflammation (Zhou et al, 2019, 2018). In contrast, Kemény et al (2018) reported that the inflammation is exacerbated in mice lacking *Trpa1*. In addition, knockout or inhibition of TRPV1 and TRPA1 did not affect psoriasis-like itch in the model of IMQ (Kemény et al, 2018; Kodji et al, 2019), whereas another study showed that psoriasis-like itch is enhanced in *Trpv1*- or *Trpa1*-knockout mice in the same model (Follansbee et al, 2019). In this study, we observed that TRPV1 and TRPA1 expressions in DRG are not altered after psoriasis-like skin inflammation. Furthermore, their expression remained unchanged in *Il17r*-cKO mice. These data suggest that the IL-17/IL-17R signaling-driven psoriatic itch may not involve the regulation of TRPV1 and TRPA1 in DRG neurons. IL-17/IL-17R signaling not only contributes to itch but also to pain (Luo et al, 2021, 2019). Interestingly, on the other hand, we recently found that IL-17 induces mechanical pain through the activation of sensory neuron TRPV1 (Luo et al, 2021). These findings indicate distinct mechanisms for the IL-17/IL-17R signaling in pain versus itch. In addition, we support the concept that the causes for psoriatic itch are multifactorial. Most likely, it involves multiple mediators, cells, and neural circuits that work together in a cocontributory manner. For example, other transient receptor potential channels such as TRPV3 have been implicated in psoriatic itch (Nattkemper et al, 2023; Yoshioka et al, 2009; Zhao et al, 2020), and whether they cocontribute to the IL-17/IL-17R signaling-driven psoriatic itch warrants further investigation.

Despite psoriatic itch being significantly reduced, psoriasisform skin inflammation remained unchanged in neuronal *Il17r*-cKO, *Trpv4*-cKO, or dn-MEK mice. This finding reinforces the clinical insight that the mechanisms underlying itch and skin inflammation are at least partially uncoupled in psoriasis (Roblin et al, 2014). Previous studies have demonstrated that systemic inhibition of IL-17R or global knockout of *Trpv4* alleviate psoriasis-like skin inflammation (Lebwohl et al, 2015; Vandeghinste et al, 2018). Our observation of unchanged inflammatory characteristics in sensory neuron *Il17r*- or *Trpv4*-cKO mice highlights the importance of non-neuronal skin cells in psoriasis-like skin inflammation. In support of this, a recent study showed that selective deletion of IL-17RA in keratinocytes results in a marked reduction of psoriasis dermatitis (Moos et al, 2019). Moreover, TRPV4 expression was elevated in epidermal keratinocytes of patients with psoriasis and mice with psoriasis-like skin

inflammation (Amalia et al, 2023; Liang et al, 2024; Yan et al, 2021), and subcutaneous injection of the TRPV4 selective inhibitor HC-067047 reduced psoriasis dermatitis (Amalia et al, 2023).

In summary, using mouse lines engineered to specifically target sensory neuron IL-17R, TRPV4, and ERK, we elucidated, to our knowledge, a previously unreported neuronal mechanism underlying psoriasis-like itch. Our findings provide a deeper insight into the pathogenesis of psoriasis-like itch. In addition, it may facilitate the development of improved interventions that target neuronal IL-17R and open doors to develop new therapeutics aimed at inhibiting sensory neuron TRPV4 and ERK. Although our data suggest that the IL-17R/ERK/TRPV4 signaling pathway drives psoriasis-like itch through a targeted neuronal mechanism, the role of non-neuronal cells, such as skin cells, and their interactions with sensory neurons should not be excluded. For instance, the study by De Logu et al (2023) mentioned earlier supports the contribution of keratinocyte IL-17R to psoriasis-like itch. Given that keratinocyte or macrophage TRPV4 contributes to chronic itch associated with dry skin, liver disease, or allergic contact dermatitis (Chen et al, 2021; Luo et al, 2018), it would be interesting to study whether TRPV4 in these non-neuronal cells is involved in psoriatic itch.

MATERIALS AND METHODS

All animal-related work was approved by Duke University Institutional Animal Care and Use Committee in compliance with National Institutes of Health guidelines. Detailed information for materials and experimental procedures are provided in [Supplementary Materials and Methods](#).

Statistical analysis

All data are expressed as the mean $\pm$ SEM. Student *t*-test, 1-way ANOVA test, or 2-way repeated-measures ANOVA with Bonferroni posthoc test were used for group comparisons. $P < .05$ was considered statistically significant.

DATA AVAILABILITY STATEMENT

Materials and raw data generated from this study are available on request from the corresponding author. No large datasets were generated or analyzed during this study.

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CONFLICT OF INTEREST

The authors state no conflict of interest.

ACKNOWLEDGMENTS

This work was supported by Duke/Duke-National University of Singapore Collaboration Pilot grant (to YC and RSPL) and National Institutes of Health grants (R01DE032676 to YC and R01AR073858 to JYZ). We thank Garrett Scapellato and Yingai J. Jin for their technical support. We also thank Clara Sandu for the schematic drawing of graphic abstract.

AUTHOR CONTRIBUTIONS

Conceptualization: QJZ, QZ, JYZ, WL, YC; Funding Acquisition: YC, RSPL, JYZ; Investigation: QJZ, MJ, FCD, QZ, PW, HT, EC, YC; Methodology: QJZ, HT, JYZ, YC; Supervision: YC; Writing - Original Draft Preparation: YC; Writing - Review and Editing: QJZ, MJ, FCD, JYZ, RSPL, WL, YC

SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at www.jidonline.org, and at <https://doi.org/10.1016/j.jid.2025.03.037>.

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SUPPLEMENTARY MATERIALS AND METHODS

Animals

Mice with sensory neuron–derived *Il17ra* or *Il17rc* conditional knockout were generated by mating *Il17ra^{fl/fl}* (stock number 031000) or *Il17rc^{fl/fl}* (number 031002) with Adv-iCre^{ERT2} (number 032027) mice, which were purchased from the Jackson Laboratory. Adv-iCre recombinase is observed specifically in ~90% of dorsal root ganglion (DRG) neurons (Lau et al, 2011). Deletion of *Il17ra* or *Il17rc* from sensory neurons was induced through daily intraperitoneal injection of tamoxifen (75 mg/kg, Sigma-Aldrich, St. Louis, MO) for 5 days. Given that TRPV4 is predominantly expressed in nociceptors (Chen et al, 2013), mice with sensory neuron–derived *Trpv4* conditional knockout were generated by crossing *Trpv4^{fl/fl}* with *Nav1.8-Cre* mice as described in our recent study (Chen et al, 2021). The *Nav1.8-Cre* mice enable gene recombination commencing at birth selectively in nociceptive sensory neurons. Mice with mutant dominant-negative MAPK/extracellular signal–regulated kinase kinase (from our laboratory [Chen et al, 2013], originally obtained from Robert W. Gereau's laboratory [Karim et al, 2006]) in which MAPK/extracellular signal–regulated kinase kinase's function is suppressed exclusively in neurons were also used. In mice with dominant-negative MAPK/extracellular signal–regulated kinase kinase, a 1.1 kb α -tubulin promoter element that confers neuronal-specific expression of the transgene was used to drive the expression of hemagglutinin-tagged K97M dominant negative form of MAPK/extracellular signal–regulated kinase kinase (Karim et al, 2006). Given that the prevalence of psoriasis is almost equal between sexes, that psoriasis severity appears greater in males than in females, and that psoriatic itch is highly present in both sexes (Bahali et al, 2017; Goldburg et al, 2022), we used male *Il17ra*-conditional knockout, *Il17rc*-conditional knockout, *Trpv4*-conditional knockout, dominant-negative MAPK/extracellular signal–regulated kinase kinase, and wild type mice (genetic background: C57bl/6 for all mouse lines, aged 2–3.5 months) for this study. Mice were housed in climate-controlled rooms on a 12/12-hour light/dark cycle with water and diet available ad libitum. Animal protocols were approved by Duke University Institutional Animal Care and Use Committee in compliance with National Institutes of Health guidelines.

Animal model of psoriasis, scratching behavior assessment, and pharmacological treatment

Mice were shaved at the dorsal neck, followed by application of hair removal cream (Nair, Ewing, NJ). Three days later, mice were then topically daily treated with 62.5 mg Aldara cream (5% imiquimod, 3M Pharmaceuticals, Maplewood, MN) on the skin (2 × 1.5 cm) for 5 days. Control mice received Vaseline. Mice were allowed to acclimate to Plexiglas chambers for 1 hour 1 day before testing. Scratching behavior was video recorded for 1 hour. One scratch bout is defined as a lifting of the hindlimb, rapid back-and-forth hindpaw motions toward and contacting the treated area, and ending with licking/biting of the toes or placement of the hindpaw on the floor. Behaviors were analyzed by observers in a blinded manner.

To determine the effect of systemic inhibition of IL-17/IL-17R signaling on psoriasis-like itch, blocking antibodies against IL-17A (Bio X Cell, Lebanon, NH), IL-17RA (R&D Systems, Minneapolis, MN), or IL-17RC (R&D Systems) at 2 mg/kg were intraperitoneal injected 30 minutes before daily imiquimod treatment for 5 days. To examine whether IL-17A acts as a pruritogen, following our previously described method (Yeo et al, 2021; Zhang et al, 2018), intrathecal injection of IL-17A at doses of 2, 20, and 200 ng/5 μ l was performed in naïve wild type mice using a 30 G needle connected to a 10 μ l Hamilton-syringe. We also intradermally injected IL-17A at 200 ng/5 μ l into the cheek of naïve wild type mice. Behaviors were recorded for 1 hour right after injection. Scratching behaviors were quantified as described earlier. For the cheek injection, wiping behavior, an indicative of spontaneous nociceptive pain, was also evaluated by counting the numbers of the wiping to the injected site with ipsilateral forelimb, following the method previously described (Shimada and LaMotte, 2008). All drugs were dissolved in normal saline, and control mice received normal saline.

Immunohistochemistry and quantitative analysis

Cervical DRGs were dissected and sectioned at 12 μ m. Sections were blocked with 5% normal donkey serum (Jackson-ImmunoResearch, West Grove, PA) and incubated overnight with primary antibodies for single or double staining: rabbit anti-IL-17RA (1:800, Invitrogen, Carlsbad, CA), rabbit anti-IL-17RC (1:300, Biorbyt LLC, Cambridge, United Kingdom), rabbit anti-TRPV4 (1:4000, Novus Biologicals, Littleton, Canada), sheep anti-TRPV4 (1:500, Lifespan Biosciences, Lynnwood, WA), rabbit antiphosphorylated extracellular signal–regulated kinase (1:100, Cell Signaling Technology, Danvers, MA), goat anti-CGRP (1:3000, Abcam, Waltham, MA), guinea pig anti-P2X3 (1:1000, Sigma-Aldrich), mouse antiperipherin (1:50, Santa Cruz Biotechnology, Dallas, TX), guinea pig anti-TRPV1 (1:800, Invitrogen, Waltham, MA), sheep anti-TRPA1 (1:300, LifeSpan-Biosciences, Seattle, WA), and rabbit anti-TRPA1 (1:500, Aviva System Biology, San Diego, CA). Sections were subsequently incubated with secondary antibodies (AlexaFluor-594 and -488; 1:600; Invitrogen). Images were captured using Keyence BZ-X710 microscope, and 4–6 sections/DRGs were analyzed. For single staining, the percentage of positive neurons relative to the total number of DRG neurons was analyzed. For double staining, the percentage of colocalized neurons relative to the total number of IL-17R–positive neurons was analyzed. To analyze the size frequency distribution of IL-17R–positive DRG neurons, the cross-sectional area was measured by ImageJ and defined as small (<600 μ m²), medium (600–1200 μ m²), and large (>1200 μ m²), following our previous studies (Chen and Sommer, 2007, 2006; Chen et al, 2013).

DRG neurons culture

DRGs from wild type were dissected and enzymatically digested with 1 mg/ml collagenase (Worthington, Lakewood, NJ) and 5 mg/ml Dispase (Invitrogen) for 1 hour, followed by trituration. The resulting cell suspension was filtered through a 70- μ m cell strainer (BD-Biosciences) to remove debris. Neurons were cultured in DH10 medium (Invitrogen) with 10% fetal bovine serum (Sigma-Aldrich), 100 U/ml penicillin,

100 µg/ml streptomycin (Gibco, Waltham, MA), and 50 ng/ml nerve GF (United States Biological, Salem, MA) on coverslips coated with poly-D-lysine and laminin (Invitrogen) and incubated with 5% carbon dioxide at 37 °C.

Western blot of cultured DRG neurons

To assess whether IL-17A increases TRPV4 expression in DRG neurons, IL-17A at 100 ng/ml was applied to the cultured neurons for 24 hours. To determine whether IL-17A induced TRPV4 upregulation through extracellular signal-regulated kinase signaling pathway, neurons were pretreated with the MEK, MAPK/extracellular signal-regulated kinase kinase selective inhibitor U0126 30 minutes before IL-17A stimulation. Cultured neurons were lysed in radioimmunoprecipitation assay (Sigma-Aldrich) buffer, and proteins were separated by 4–15% polyacrylamide gel (Bio-Rad Laboratories, Hercules, CA) and then transferred to polyvinylidene difluoride membranes (Thermo Fisher Scientific, Waltham, MA). The membranes were blocked with 5% BSA (Sigma-Aldrich) in Tris buffered saline with Tween and incubated overnight at 4 °C with rabbit anti-TRPV4 (Novus Biologicals, 1:1000). Subsequently, they were incubated for 2 hours with the secondary antibody (anti-rabbit peroxidase conjugated, 1:4000, Jackson ImmunoResearch) and developed using ECL chemiluminescence substrate (GE-Healthcare, Chicago, IL). β3-Tubulin (rabbit anti-β3-tubulin, Santa Cruz Biotechnology, 1:1000) was used as a loading control specifically for neurons. Immunoblotting bands were quantified using ImageJ software (National Institutes of Health).

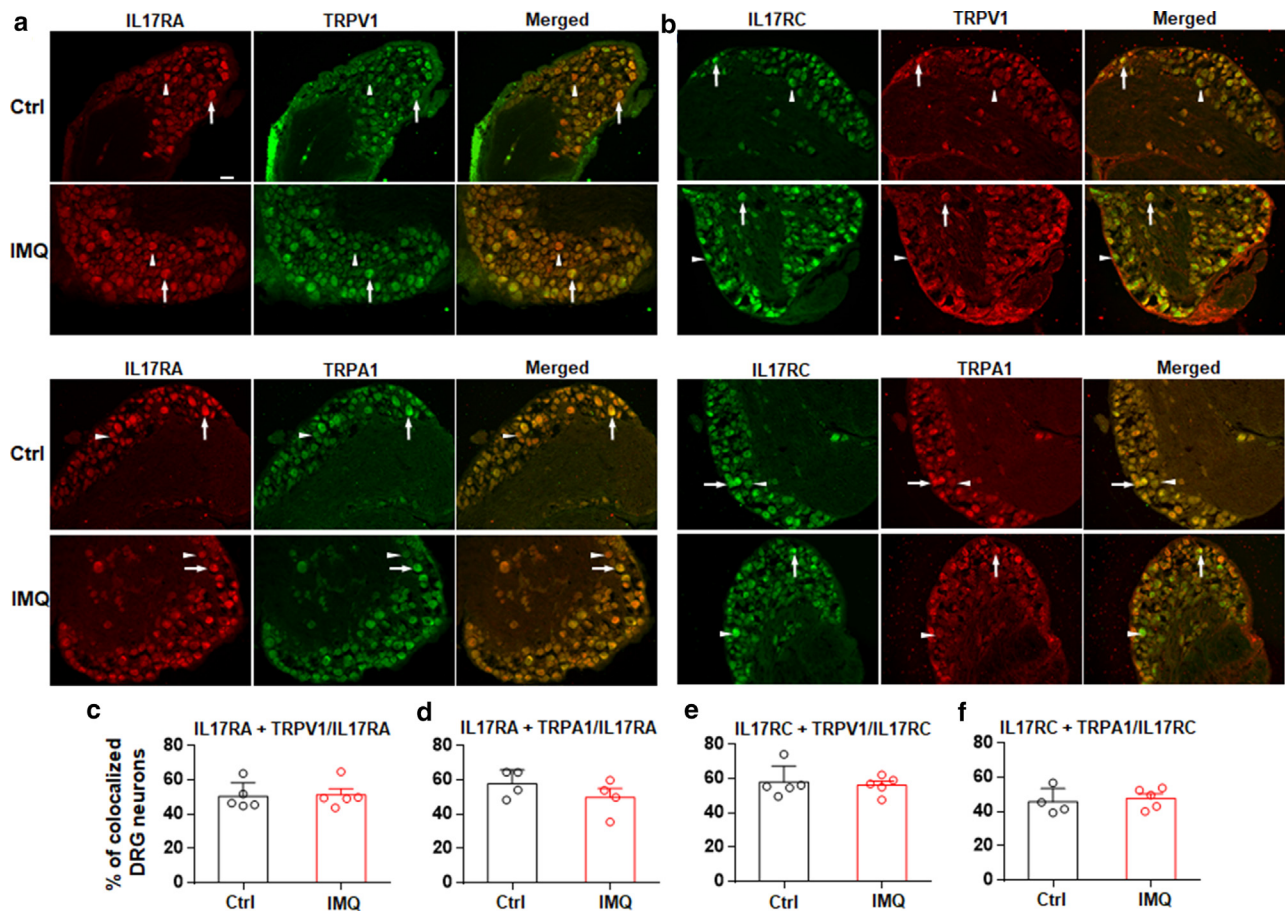
Skin pathological analysis

Dorsal neck skin was dissected and fixed in 4% paraformaldehyde and embedded with optimal cutting temperature (Tissue-Tek, Torrance, CA). Skin sections at 8 µm were stained with H&E (Sigma-Aldrich). Epidermal thickness was measured with ImageJ. To quantitatively analyze the inflammatory infiltrating cells in the dermis, 3 areas for each section

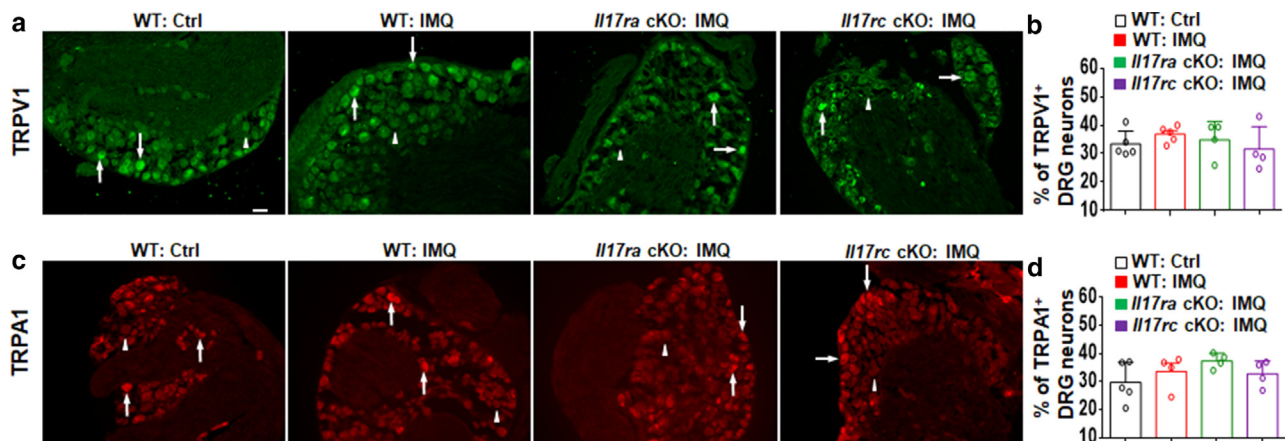
were randomly selected, and the number of cells were manually counted. Four to 5 sections per mouse were used. All images were acquired using Keyence BZ-X710 microscope.

SUPPLEMENTARY REFERENCES

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Supplementary Figure S1. TRPV1 or TRPA1 expression in IL-17R-positive DRG neurons is not significantly changed after IMQ treatment. (a, b) Representative images and (c–f) quantitative analysis showed that the percentage of the coexpression of TRPV1 or TRPA1 with IL-17R relative to the total number of IL-17R-positive neurons between control- and IMQ-treated (day 5) mice are comparable. Arrows in images indicate colocalized neurons, and arrowheads indicate IL-17R-positive but marker-negative neurons. Student's *t*-test was performed; *n* = 4–5 mice per group. Ctrl represents Vaseline. Bar = 50 μ m. Ctrl, control; DRG, dorsal root ganglion; IMQ, imiquimod.



Supplementary Figure S2. TRPV1 and TRPA1 expression in DRG neurons is comparable between WT and *Il17r*-cKO mice after IMQ treatment. (a, c) Representative images and (b, d) quantitative analysis showed the comparable percentage of TRPV1- or TRPA1-positive neurons relative to the total number of DRG neurons between WT control, WT IMQ, and *Il17r*-cKO IMQ mice. Arrows and arrowheads in images indicate TRPV1- or TRPA1-positive and -negative neurons, respectively. Student's *t*-test was performed; *n* = 4–5 mice per group. Ctrl represents Vaseline. Bar = 50 μ m. cKO, conditional knockout; Ctrl, control; DRG, dorsal root ganglion; IMQ, imiquimod; WT, wild type.