IL-33 Signaling in Sensory Neurons Promotes Dry Skin Itch

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Abstract

**Background:** Chronic pruritus, or itch, is common and debilitating, but the neuro-immune mechanisms that drive chronic itch are only starting to be elucidated. Recent studies demonstrate that the IL-33 receptor (IL-33R) is expressed by sensory neurons. However, whether sensory neuron-restricted activity of IL-33 is necessary for chronic itch remains poorly understood.

**Objectives:** We sought to determine if IL-33 signaling in sensory neurons is critical for the development of chronic itch in two divergent pruritic disease models.

**Methods:** Plasma levels of IL-33 were assessed in patients with atopic dermatitis (AD) and chronic pruritus of unknown origin (CPUO). Mice were generated to conditionally delete IL-33R from sensory neurons. The contribution of neuronal IL-33R signaling to chronic itch development was tested in mouse models that recapitulate key pathologic features of AD and CPUO, respectively.

**Results:** IL-33 was elevated in both AD and CPUO as well as their respective mouse models. While neuron-restricted IL-33R signaling was dispensable for itch in AD-like disease, it was required for the development of dry skin itch in a mouse model that mirrors key aspects of CPUO pathology.

**Conclusion:** These data highlight how IL-33 may be a predominant mediator of itch in certain contexts, depending on the tissue microenvironment. Further, this study provides insight for future therapeutic strategies targeting the IL-33 pathway for chronic itch.
Capsule Summary

IL-33 signaling in sensory neurons drives chronic itch in dry skin with minimal inflammation and is dispensable in AD-like disease. These findings provide insight on anti-IL-33 mAb therapies currently in phase 2 clinical trials.

Keywords

Atopic dermatitis; chronic pruritus of unknown origin; dry skin; IL-33; itch; neuroimmunology; pruriceptor; pruritogen

Introduction

Chronic pruritus, or itch, is a debilitating, often intractable condition that causes a reduction in quality of life similar to chronic pain and has a lifetime prevalence of up to 20%. Recent studies have identified that various cytokines can function as itch-inducing factors, or pruritogens, at the neuro-immune interface, and there is mounting interest in harnessing the therapeutic potential of blocking these interactions.

Epithelial cell-derived IL-33 is a potent amplifier of type 2 immune responses and is increasingly implicated in itch, although the mechanisms remain unclear. It has recently been demonstrated that the IL-33 receptor (IL-33R) is expressed in the dorsal root ganglia (DRG) and that IL-33 can directly activate sensory neurons. However, whether IL-33R expression in sensory neurons is specifically required for the development of chronic itch, and in what disease setting, remains poorly defined.

Results and Discussion

IL-33 acts as an ‘alarmin’ by being rapidly released from damaged epithelial cells to initiate type 2 inflammation. In addition to immune cells, IL-33R is also expressed by sensory neurons. We confirmed expression of IL-33R (ST2, Il1rl1) in mouse DRG (Fig 1, A) and, using calcium imaging, found that IL-33 activated 2.1% of DRG neurons (Fig 1, B and C). Further, we found that 52% and 63% of IL-33-responsive mouse DRG neurons also responded to histamine and the TRPV1-agonist capsaicin, respectively (Fig 1, D). Similarly, we found IL-33R was expressed by human DRG (Fig 1, E) and 6.6% of human DRG
neurons were responsive to IL-33 (Fig 1, F and G). Of these neurons, 60% also responded to capsaicin (Fig 1, H). Together these findings suggest that IL-33 can directly activate sensory neurons.

A recent study demonstrated that IL-33R knockdown within the DRG compartment attenuates itch in allergic contact dermatitis. While these findings suggest that neuronal IL-33R signaling may be a critically important itch pathway, the DRG contains a diversity of other cell types. The expression of IL-33R in the DRG, beyond sensory neurons, has yet to be fully assessed. To address this, we analyzed a single cell RNA-seq dataset of naïve mouse DRG (Fig 1, I). We found that Il1rl1 was indeed expressed by another cell type: DRG macrophages (Fig 1, J). Similarly, analysis of other neuronally expressed itch-associated cytokine receptors, such as Il4ra, revealed expression across numerous cell types (Fig 1, K). Taken together, these data underscore that targeted, lineage-specific approaches are likely required to determine the precise contribution of a distinct cell type to itch development. Therefore, the consequence of disrupting IL-33R signaling specifically in sensory neurons remains unknown.

We generated mice in which loxP sites were inserted into the Il1rl1 gene locus (IL-33Rflox mice) (Fig 2, A) and crossed these mice onto the SNS-Cre mouse line, generating mice that conditionally lack IL-33R in sensory neurons (IL-33RΔneuron mice). We confirmed the selective loss of Il1rl1 in sensory neurons, and not immune cells, isolated from IL-33RΔneuron mice (Fig 2, B). These mice exhibited normal motor function (Fig 2, C), thermal pain behavior (Fig 2, D), and acute itch response to the classical pruritogens histamine (Fig 2, E), chloroquine (Fig 2, F), and serotonin (Fig 2, G), indicating the mice have no gross developmental motor or sensory abnormalities.

Advances in our understanding of the mechanisms underlying chronic itch have largely drawn from studying inflammatory skin disorders such as atopic dermatitis (AD). AD presents with pruritic skin lesions driven by type 2 inflammation. Given the ability of IL-33 to promote type 2 inflammation, there is considerable interest in the therapeutic potential of anti-IL-33 monoclonal antibodies (mAbs) in AD. Several studies have found elevated levels of IL-33 in the skin and blood of patients with AD. In support, we found that patients with moderate-to-severe AD (N = 11, 5.17 ± 1.37) had increased IL-33 in their plasma compared to healthy control (HC) subjects (N = 11, 3.93 ± 1.20) (Fig 3, A and B, Table E1). We next utilized a model of AD-like disease, where mice are treated with MC903 (Fig E1, A). MC903-treated wild-type (WT) mice developed robust AD-like skin inflammation (Fig 3, C). Indeed, analyzing our previously published RNA-seq dataset, we found increased expression of Il33, along with transcripts for a number of other pruritogens, in the skin of MC903-treated WT mice compared to controls (Fig 3, D). However, while it is well-known that IL-33 is dysregulated in both human and murine AD-associated inflammation, whether IL-33 directly engages the sensory nervous system to elicit itch remains unclear. When we induced AD-like disease in IL-33RΔneuron mice, there were no notable differences in clinical or histopathological presentation (Fig 3, E), ear thickness (Fig 3, F), or scratching bouts (Fig 3, G) compared to littermate (LM) controls. Thus, our findings suggest that neuronal IL-33R is dispensable for AD-like disease.
In AD-like skin, many putative pruritogens are upregulated (Fig 3, D) and may override the contribution of IL-33 to itch. Thus, we next sought to test whether neuron-restricted IL-33R may play a more important role in itch that arises in the absence of robust skin inflammation. Chronic pruritus of unknown origin (CPUO) accounts for 10–40% of all chronic itch cases, is poorly understood, and lacks effective therapies.\(^1\),\(^14\) While patients with AD present with scaly, raised rashes (Fig 4, A), chronic itch in CPUO develops in the absence of overt cutaneous inflammation. Additionally, CPUO disproportionately occurs in aged individuals.\(^14\) A key pathogenic factor of CPUO is skin barrier dysfunction, which frequently manifests as dry skin (Fig 4, B). The histopathology of CPUO often resembles control skin (Fig 4, C), while AD lesional skin exhibits a number of characteristic inflammatory features including irregular epidermal hyperplasia and robust dermal inflammatory infiltrate (Fig 4, D). In contrast, CPUO pruritic skin generally exhibits a relatively normal epidermis and mild dermal infiltrate (Fig 4, E). We found that patients with CPUO (N = 8, 6.22 ± 2.54) had significantly higher levels of IL-33 compared to HCs (N = 11, 3.93 ± 1.20) (Fig 4, F and G, Table E1). Thus, we hypothesized that IL-33 may be a key factor in itch physiology associated with CPUO.

To examine the role of IL-33 in a disease model that recapitulates key pathological features of CPUO, we utilized the acetone/ether plus water (AEW) mouse model (Fig E1, B). This model is characterized by the development of dry skin (Fig 5, A) and other pathogenic changes that mimic aged skin.\(^15\),\(^16\) We have previously utilized the AEW mouse model to identify novel therapeutic approaches that led to proof-of-concept studies in CPUO.\(^8\) In contrast to other mouse models of chronic itch, AEW-elicited itch develops in the absence of notable cutaneous inflammation, similar to CPUO. Indeed, the frequency of cutaneous immune cells (Fig 5, B), including mast cells (Fig 5, C) and group 2 innate lymphoid cells (ILC2s) (Fig 5, D), were comparable between WT mice that were treated with AEW and water-only controls, despite significantly increased itch behavior in AEW-treated mice (Fig 5, E). Notably, \(I\!l\!3\!3\) was elevated in the skin of AEW-treated mice compared to controls (Fig 5, F).\(^17\) Taken together, our findings demonstrate that AEW-induced itch is associated with IL-33 dysregulation and minimal cutaneous inflammation, similar to CPUO.

It was recently reported that global deficiency of IL-33 or IL-33R results in decreased AEW-induced itch.\(^18\) However, how IL-33 drives the development of dry skin itch is poorly understood. Indeed, whether IL-33 can promote itch through a mechanism independent of canonical immune circuits remains unknown. Mast cells, and more recently basophils, have been implicated as key mediators of itch.\(^19\)–\(^21\) To test if these cell types contribute to dry skin itch, we employed MasTRECK mice, which allow for diphtheria toxin (DT)-mediated depletion of mast cells and basophils (Fig E2). However, AEW-induced scratching bouts were comparable between DT-treated LM control and MasTRECK mice (Fig 5, G). IL-33 also potently activates both ILC2s and T cells to modulate the skin immune responses.\(^4\) However, we found no difference in itch between AEW-treated lymphocyte-deficient \(R\!a\!g\!2\!/I\!l\!2\!g\!r\!−/−\) mice and controls (Fig 5, H). Finally, we generated mice that conditionally lack IL-33R in immune cells by crossing the IL-33R\(^{\text{flox}}\) mice with the Vav\(^{\text{Cre}}\) line (IL-33R\(^{\Delta\text{immune}}\)). Following AEW-treatment, there was no difference in the number of scratching bouts between LM control and IL-33R\(^{\Delta\text{immune}}\) mice (Fig 5, I). Collectively, these data suggest that immune cells are largely dispensable for the induction of dry skin

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itch and instead implicate sensory neurons as the potential primary target of IL-33 for itch development.

To test the hypothesis that neuronal IL-33R regulates dry skin itch, we utilized the IL-33RΔneuron mice. Strikingly, AEW-treated IL-33RΔneuron mice demonstrated significantly attenuated itch behavior compared to LM controls (Fig 5, J). Despite the requirement of neuronal IL-33R for dry skin itch, IL-33 alone was not sufficient to induce robust acute itch responses (Fig 5, K), similar to prior reports. This led us to hypothesize that IL-33 may instead sensitize sensory neurons. Indeed, it has been shown that AEW-treated mice exhibit enhanced responsiveness to exogenous pruritogens like chloroquine (CQ). However, the mechanisms underlying these observations are not well understood. Using calcium imaging, in a proof-of-concept experiment, we found that IL-33 treatment of DRG neurons increased the number of cells responding to CQ (Fig 5, L and M). Thus, although CQ is not a native endogenous pruritogen in dry skin, these studies represent one example by which IL-33 may amplify responses to pruritogens in order to promote chronic itch. Future studies will be required to determine the precise molecular mechanisms by which IL-33 may enhance itch in this manner.

Our findings suggest that neuron-restricted IL-33R signaling is a critical regulator of itch that arises in the setting of dry skin, independent of immune cells. Furthermore, our findings are consistent with prior studies demonstrating that IL-33 may be dispensable for the development of AD-like disease. Together, these findings may help explain why anti-IL-33 mAbs (e.g. etokimab) have failed to meet their primary endpoints or have been discontinued following recent phase 2 clinical trials in AD (NCT03533751, NCT03736967).

In contrast, IL-33 may be an important therapeutic target in dry skin itch and CPUO.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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**Abbreviations**

| Abbreviation | Definition |
|--------------|------------|
| Ab           | antibody   |
| AD           | atopic dermatitis |
| AEW          | acetone/ether plus water |
| bp           | base pair |
| Cap          | Capsaicin |
| CPUO         | chronic pruritus of unknown origin |
| CQ           | chloroquine |
| DRG          | dorsal root ganglia |
| DT           | diphtheria toxin |
| Epi          | epidermis |
| EtOH         | ethanol |
| HC           | healthy control |
| His          | histamine |
| i.d.         | intradermal |
| ILC2s        | group 2 innate lymphoid cells |
| IL-33R       | IL-33 receptor |
| i.p.         | intraperitoneal |
| KCl          | potassium chloride |
| LM           | littermate |
| loxP         | locus of X-over P1 |
| mAb          | monoclonal antibody |
| MACS         | magnetic-activated cell sorting |
| MasTRECK     | mast cell-specific enhancer-mediated toxin receptor-mediated conditional cell knockout |
| NS           | no significance |
| rh           | recombinant human |

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rm recombinant mouse
RNA-seq RNA sequencing
SC stratum corneum
Veh Vehicle
WT wild-type

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Key Message

• IL-33 is elevated in two divergent pruritic disease conditions and their respective models
• Signaling of the IL-33 receptor in sensory neurons is necessary for dry skin itch, but not itch associated with atopic dermatitis-like disease
Figure 1: Mouse and human DRG express IL-33R.

(A) Gel of *Il1rl1* RT-PCR product from dorsal root ganglia (DRG) isolated from one wild-type (WT) mouse. Representative of three mice. (B) Representative calcium imaging trace of mouse DRG neuron in response to vehicle (Veh), recombinant mouse (rm)IL-33, capsaicin (Cap), and potassium chloride (KCl). (C) Percent of rmIL-33-, histamine (His)-, and Cap-responsive DRG neurons out of all KCl-responsive DRG neurons. (D) Venn diagrams of overlapping responses between IL-33-responsive (IL-33⁺) and Cap-responsive (Cap⁺) or His-responsive (His⁺) neurons. (B-D) n > 900 neurons from at least 4 WT mice (6 combined experiments). (E) Gel of *IL1RL1* RT-PCR product from DRG isolated from one human donor. Representative of three donors. *Ladder has been previously published in Oetjen et al.*

(F) Representative calcium imaging trace of human DRG neuron in response to Veh, recombinant human (rh)IL-33, Cap, and KCl. (G) Percent of rhIL-33- and Cap-responsive DRG neurons out of all KCl-responsive DRG neurons. (H) Venn diagrams of overlapping responses between IL-33⁺ and Cap⁺ neurons. (F-H) N > 200 neurons from 2 human subjects.
(2 combined experiments). (I) t-SNE plot of single cell RNA-seq of mouse DRG colored by cell populations. Violin plots of (J) Il1rl1 and (K) Il4ra gene expression. Full dataset in Avraham et al.7
Figure 2: Generation of IL-33R conditional deletion mice

(A) Map of Il1rl1 conditional knock-out allele. blue triangles, loxP sites; gray boxes, exons; red box, conditionally deleted region. (B) Expression of Il1rl1 in lymph node-derived immune cells (left) and MACS-sorted sensory neurons (right) from littermate (LM) control and IL-33RΔneuron mice by RT-qPCR. n > 3 mice/group. (C-G) Assessment of (C) motor activity (rotarod), (D) thermal pain behavior (hot plate), and acute itch behavior following intradermal injection (i.d.) of (E) histamine, (F) chloroquine, and (G) serotonin in LM control and IL-33RΔneuron mice. (C-G) n > 4 mice/group (E-G), 2 combined experiments. Not significant (NS), *p<0.05 by unpaired, two-tailed t test.
Figure 3: IL-33R signaling in sensory neurons is dispensable for chronic itch in AD-like disease. (A) Schematic of the measurement of IL-33 in the plasma of 11 healthy control (HC) subjects and 11 patients with atopic dermatitis (AD) by Luminex multiplex ELISA. (B) Amount of IL-33 in the plasma of HC subjects and patients with AD. (C) Representative clinical images and H&E sections of ear skin from ethanol (EtOH)- or MC903-treated WT mice (day 12). Scale bar is 50 μm. (D) Heatmap and hierarchical clustering of significantly differentially expressed genes in the ear skin of EtOH- or MC903-treated WT mice (day 12). The most differentially expressed genes (1,300 genes) are displayed (based on the t statistic value). n = 4 mice/group. Full dataset in Oetjen et al. (E) Representative clinical images and H&E sections of MC903-treated LM control and IL-33RΔneuron mice (day 12). Scale bar is 20 μm. (F) Percent change in ear thickness and (G) number of scratching
bouts from MC903-treated LM control and IL-33R<sup>Δneuron</sup> mice over time (days). n = 13–18 mice/group (2–3 combined experiments). (B) *p<0.05 by unpaired, two-tailed t test. (F-G) NS by multiple t test using Holm-Sidak method.
Figure 4: IL-33 is elevated in CPUO.
Representative clinical images from a patient with (A) AD and (B) chronic pruritus of unknown origin (CPUO). Black boxes indicate zoomed-in view of skin. Representative H&E skin sections from (C) control, (D) patient with AD, and (E) patient with CPUO. Bracket, stratum corneum (SC); brace, epidermis (Epi); black arrow, spongiosis; gray arrow, vascular dilatation; white arrow, dermal perivascular immune infiltrate. Scale bar represents 100 μm. (F) Schematic of the measurement of IL-33 in the plasma of 11 HC subjects (same subjects as in Figure 1) and 8 patients with CPUO by Luminex multiplex ELISA. (G) Amount of IL-33 in the plasma of HC subjects and patients with CPUO. *p<0.05 by unpaired, two-tailed t test.
Figure 5: Dry skin itch is dependent on IL-33 signaling in sensory neurons.

(A) Representative clinical images of skin from water- or AEW-treated WT mice (day 5). Frequency of (B) immune cells (C) mast cells and (D) group 2 innate lymphoid cells (ILC2s) in the skin of water- or AEW-treated WT mice (day 5). n = 6–8 mice/group (2 combined experiments). (E) Number of scratching bouts from water- or AEW-treated WT mice (day 5). n = 5 mice/group (representative of 3 experiments). (F) Expression of IL33 by RT-qPCR in water- or AEW-treated skin of WT mice (day 4). n = 5–7 mice/group (2 combined experiments). Number of scratching bouts from AEW-treated (G) LM control and MastTRECK mice, (H) control (Cont) and Rag2/Il2rg−/− mice, (I) LM control and IL-33R immune mice, and (J) LM control and IL-33R neuron mice (day 5). (G-J) n = 9–18 mice/group (2 combined experiments). (K) Number of scratching bouts following i.d.
injection of Veh or rmIL-33 in WT mice. n = 6–8 mice/group (2 combined experiments).

(L) Representative calcium traces of mouse DRG neurons responding to chloroquine (CQ) after exposure to Veh or rmIL-33. Each trace represents one neuron. (M) Percent of CQ-responsive neurons out of all KCl-responsive neurons following exposure to Veh or rmIL-33. n = >400 neurons from 3 mice (2 combined experiments). (B–J, M) NS, *p<0.05, **p<0.01, ****p<0.0001 by unpaired, two-tailed t test. (K) NS by one-way ANOVA with multiple comparisons.