The pruritus- and T_H2-associated cytokine IL-31 promotes growth of sensory nerves

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Background: Pruritus is a cardinal symptom of atopic dermatitis, and an increased cutaneous sensory network is thought to contribute to pruritus. Although the immune cell–IL-31–neuron axis has been implicated in severe pruritus during atopic skin inflammation, IL-31's neuropoietic potential remains elusive.

Objective: We sought to analyze the IL-31–related transcriptome in sensory neurons and to investigate whether IL-31 promotes sensory nerve fiber outgrowth.

Methods: *In vitro* primary sensory neuron culture systems were subjected to whole-transcriptome sequencing, ingenuity pathway analysis, immunofluorescence, and nerve elongation, as well as branching assays after IL-31 stimulation. *In vivo* we investigated the cutaneous sensory neuronal network in wild-type, *Il31*-transgenic, and IL-31 pump–equipped mice.

Results: Transgenic *Il31* overexpression and subcutaneously delivered IL-31 induced an increase in the cutaneous nerve fiber density in lesional skin *in vivo*. Transcriptional profiling of IL-31–activated dorsal root ganglia neurons revealed enrichment for genes promoting nervous system development and neuronal outgrowth and negatively regulating cell death. Moreover, the growth cones of primary small-diameter dorsal root ganglia neurons showed abundant IL-31 receptor α expression. Indeed, IL-31 selectively promoted nerve fiber extension only in small-diameter neurons. Signal transducer and activator of transcription 3 phosphorylation mediated IL-31–induced neuronal outgrowth, and pharmacologic inhibition of signal transducer and activator of transcription 3 completely abolished this effect. In contrast, transient receptor potential cation channel vanilloid subtype 1 channels were dispensable for IL-31–induced neuronal sprouting.

Conclusions: The pruritus- and T_H2-associated novel cytokine IL-31 induces a distinct transcriptional program in sensory neurons, leading to nerve elongation and branching both *in vitro* and *in vivo*. This finding might help us understand the clinical observation that patients with atopic dermatitis experience increased sensitivity to minimal stimuli inducing sustained itch. (J Allergy Clin Immunol 2016;■■■:■■■-■■■.)

Key words: *IL-31, IL-31 receptor α, dorsal root ganglia, atopic dermatitis, nerve growth, cutaneous hyperinnervation*

In patients with atopic dermatitis (AD), a chronic T_H2-dominated inflammatory skin disease, pruritus is the cardinal symptom with the most significant adverse effect on patients' quality of life and high socioeconomic costs.[1,2] Physical and psychological stress responses in patients with AD[3] and T_H2 cytokine–related skin barrier defects[4] might promote the pruritus sensation. Development of chronic pruritus relies not only on increased availability of itch mediators but also most likely takes advantage of increased density of cutaneous neuronal networks with prolonged sensory nerve fibers extending into the epidermal compartment.[5-7] Moreover, the diameter of these fibers appears to be thicker in skin of patients with AD because of an increased number of axons on single nerve fibers.[8] Phenotypic characterization of cutaneous nerve fibers reveals an increased number of substance P–positive and/or calcitonin gene-related protein–positive nerve fibers in the skin of atopic subjects.[9-11] Several reports have proposed a role for neurotrophins, cytokines, or both in AD-associated cutaneous nerve growth.[11,12] However, the mechanism that controls sensory nerve fiber growth in patients with atopic skin inflammation and that might contribute to the typical pruritic hypersensitivity of patients with AD still remains elusive.

The novel atopy-associated cytokine IL-31 plays a crucial role in AD, asthma, allergic rhinitis, and mastocytosis.[13-15] IL-31 belongs to the IL-6 family of cytokines[16] and is mainly, but not exclusively, produced by activated T_H2 cells.[17,18] Transcription of the *Il31* gene in T_H2 and mast cells requires IL-4 signaling.[19] The IL-31 receptor subunits IL-31 receptor α (IL-31RA) and oncostatin M receptor β are coexpressed on sensory neurons,[20,21] and recent evidence indicates that IL-31 from skin-infiltrating

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Abbreviations used
AD: Atopic dermatitis
DRG: Dorsal root ganglia
ERK: Extracellular signal-regulated kinase
IL-31RA: IL-31 receptor α
IPA: Ingenuity pathway analysis
NGF: Nerve growth factor
PGP9.5: Protein gene product 9.5
PI3K: Phosphoinositide 3-kinase
Prph: Peripherin gene
RNA-Seq: RNA sequencing
STAT3: Signal transducer and activator of transcription 3
STRT: Single cell–tagged reverse transcription
Tg: Transgenic
TrkA: Tropomyosin receptor kinase A
TRPV1: Transient receptor potential cation channel vanilloid subtype 1

T_H2 lymphocytes can communicate with sensory neurons, thereby triggering the development of pruritus and skin lesions in mice.[17,18,22,23] Skin areas devoid of T-lymphocyte infiltration are not affected by IL-31 signaling. Indeed, in patients with AD, IL-31 provides a novel link connecting *Staphylococcus*-related T-cell activation and pruritus.[20]

We reported recently that IL-31–induced pruritus in mice requires functional ion channels, namely transient receptor potential cation channel vanilloid subtype 1 (TRPV1) and transient receptor potential A1, on cutaneous sensory neurons and that this process is uncoupled from mast cells.[18] Moreover, pharmacologic inhibition of extracellular signal-regulated kinase (ERK1/2) signaling hampers IL-31–mediated pruritus.[18] However, neither immunosuppressants nor μ-opioid receptor or a histamine H_1 antagonist alleviate pruritus elicited by exogenous IL-31.[24] In contrast, in mice with chronic atopy-like skin inflammation, itch-scratch cycles are significantly reduced by administration of neutralizing anti–IL-31 or anti–IL-31RA antibodies.[22,24] Intriguingly, a recent clinical phase I trial in patients with AD using a humanized mAb targeting IL-31RA demonstrated significant improvement of pruritus,[25] providing further evidence that IL-31 links T_H2-related inflammation to pruritus.

Although the T_H2 cell–IL-31–sensory neuron axis and its role in pruritus are now well established, the question of whether IL-31 is also involved in the increased density of sensory networks within the skin remains elusive.

METHODS

Mice and sample collection

Six to 8-week-old wild-type C57BL/6, *Il31ra*, and *Trpv1* knockout mice were kept under specific pathogen-free conditions. *Il31* transgenic (Tg) mice specifically overexpressing *Il31* under the Eμ-Lck promoter in lymphocytes[17] and control littermates were housed for up to 9 months under specific pathogen-free conditions until characteristic lesions developed spontaneously. For further details, see the Methods section in this article's Online Repository at www.jacionline.org.

Preparation and treatment of dorsal root ganglia neurons

Adult C57BL/6 mice and *Il31ra* knockout mice were killed and DRGs from the lumbar, thoracic, and cervical regions were removed to prepare sensory neurons from dissociated dorsal root ganglia (DRG) neurons. For further details, see the Methods section in this article's Online Repository.

Immunofluorescence and image analysis

OCT-embedded skin samples were cut in 20-μm-thick sections to analyze cutaneous innervation. For further details, see the Methods section in this article's Online Repository.

RNA sequencing and data analysis

The single cell–tagged reverse transcription (STRT) method was used[26] with minor modifications to measure transcription initiation at the 5′ end of polyA$^+$ transcripts starting from 10 ng of total RNA as template. For further details, see the Methods section in this article's Online Repository.

Western blotting

Proteins from IL-31–activated DRG neurons were harvested with Roti-Load buffer (Carl Roth, Karlsruhe, Germany) supplemented with 2-mercaptoethanol at the indicated time points and boiled for 10 minutes. For further details, see the Methods section in this article's Online Repository.

Quantitative real-time PCR

RNA was prepared from IL-31– and nerve growth factor (NGF)–activated DRG neurons with the RNeasy kit (Qiagen, Hilden, Germany) and reverse transcribed with SuperScript II (Invitrogen, Carlsbad, Calif), according to the manufacturer's instructions. For further details, see the Methods section in this article's Online Repository.

Statistical analysis

Results are expressed as means ± SEMs. At least 3 independent experiments were conducted ($n \geq 3$). Statistical analysis was performed with GraphPad Prism 5 software (GraphPad software, La Jolla, Calif). Significance was evaluated by using the paired *t* test, Mann-Whitney test, Wilcoxon matched-pairs signed-rank test, and 1-way ANOVA with *post hoc* Tukey, Newman-Keuls, or Dunnett tests. Significance was set at a P value of less than .05.

RESULTS

Transgenic overexpression of *Il31* results in increased cutaneous innervation

IL-31 is associated with AD and directly activates peripheral sensory neurons to induce pruritus.[18] Patients with AD with chronic pruritus show increased cutaneous innervation.[5] To unravel an additional role for IL-31 in cutaneous innervation, we took advantage of *Il31*Tg mice, which have an AD-like skin phenotype with severe pruritus spontaneously affecting the nape of the neck and ears (Fig 1, *A*, and see Fig E1 in this article's Online Repository at www.jacionline.org). First, we characterized the nerve fiber density in lesional and nonlesional skin from *Il31*Tg mice[17] and healthy skin from wild-type littermates (Fig 1, *B*) by using immunofluorescence to visualize protein gene product 9.5 (PGP9.5)$^+$ nerve fibers. Our results demonstrated that *Il31*Tg mice show a marked and significant increase in the cutaneous nerve fiber density in lesional skin (8.5 ± 2.7 PGP9.5$^+$ fibers, $P < .01$) compared with that in uninvolved or healthy skin (*Il31*Tg nonlesional: 0.75 ± 0.4 PGP9.5$^+$ fibers or healthy C57BL/6 wild-type: 0.42 ± 0.2 PGP9.5$^+$ fibers; Fig 1, *B* and *C*). Expression of the DRG neuron-specific transcript peripherin gene *(Prph)* is increased in the skin of *Il31*Tg mice (see Fig E2, *G*, in this article's Online Repository at