IL-31 is crucial for induction of pruritus, but not inflammation, in contact hypersensitivity

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IL-31, which is a member of the IL-6 family of cytokines, is produced mainly by activated CD4+ T cells, in particular activated Th2 cells, suggesting a contribution to development of type-2 immune responses. IL-31 was reported to be increased in specimens from patients with atopic dermatitis, and IL-31-transgenic mice develop atopic dermatitis-like skin inflammation, which is involved in the pathogenesis of atopic dermatitis. However, the role of IL-31 in development of contact dermatitis/contact hypersensitivity (CHS), which is mediated by hapten-specific T cells, including Th2 cells, is not fully understood. Therefore, we investigated this using IL-31-deficient (Il31−/−) mice, which we newly generated. We demonstrated that the mice showed normal migration and maturation of skin dendritic cells and induction of hapten-specific T cells in the sensitization phase of FITC-induced CHS, and normal induction of local inflammation in the elicitation phase of FITC- and DNFB-induced CHS. On the other hand, those mice showed reduced scratching frequency and duration during FITC- and/or DNFB-induced CHS. Our findings suggest that IL-31 is responsible for pruritus, but not induction of local skin inflammation, during CHS induced by FITC and DNFB.
IL-31 is not essential for skin DC function or hapten-specific LN cell response in the sensitization phase of CHS. Treatment of mice that developed atopic dermatitis-like skin inflammation with anti-IL-31 or anti-IL-31RA neutralizing Ab resulted in attenuation of scratching behavior, but not the severity of skin inflammation\(^{10,19}\). On the other hand, the role of IL-31 in the development of contact dermatitis/contact hypersensitivity (CHS) is not well understood. CHS is a T-cell-mediated cutaneous allergic response\(^{21}\). Several studies using type-2-cytokine-deficient mice showed that type-2 cytokines are important for the development of CHS (reviewed in\(^{25}\)), suggesting that IL-31 may be involved in induction of CHS. IL31ra\(^{−/−}\) mice showed enhanced type-2 immune responses due to hyperresponsiveness to OSM by increased formation of OSM receptors (the complex of gp130 and OSMR) rather than due to failure to form IL-31 receptors (the complex of IL-31RA and OSMR)\(^{25}\). Accordingly, we deemed that murine strain to be unsuitable for examining the role of IL-31 in CHS, and we instead used IL-31-deficient (IL31ra\(^{−/−}\)) mice, which we newly generated.

Results

Generation of IL31ra\(^{−/−}\) mice. IL31ra\(^{−/−}\) mice were reported to have not only loss of IL-31 function but also elevated responsiveness to OSM\(^{13}\), suggesting that it is necessary to elucidate the exact roles of IL-31 in vivo by using IL31ra\(^{−/−}\) mice rather than IL31ra\(^{+/−}\) mice. Therefore, we newly generated IL31ra\(^{−/−}\) mice by replacing IL31 genes with a cassette consisting of IRES-EGFP and a neomycin resistance gene, flanked by loxp sequences (Fig. 1a). IL31\(^{−/−}\) mice were born at the expected Mendelian ratio, fertile, and did not show any gross phenotypic abnormalities under specific-pathogen-free housing conditions (data not shown). Expression of IL31 mRNA was below the limit of detection by qPCR in the lung and in PMA-ionomycin-stimulated spleen cells of IL31ra\(^{−/−}\) mice (Fig. 1b and data not shown). No apparent abnormalities were found in the proportions of immune cells in the thymus, LNs, spleen and/or bone marrow between wild-type and IL31ra\(^{−/−}\) mice (Fig. 1c, and data not shown).

IL-31 is involved in pruritus, but not inflammation, during CHS. We next investigated whether IL-31 is involved in the pathogenesis of FITC-induced CHS. The degree of skin inflammation was assessed by measuring the thickness of the ear skin after challenge with FITC. The ear thickness was comparable between wild-type and IL31ra\(^{−/−}\) mice at 24 hours after challenge with FITC or vehicle (Fig. 4b). We also used ELISA to examine the levels of FITC-specific IgG subsets and total IgE in sera from naïve and FITC-treated mice. After FITC challenge, the levels of FITC-specific IgG1, IgG2b and IgG2c and total IgE in sera from IL31ra\(^{−/−}\) mice increased similarly to those from wild-type mice (Fig. 4c). On the other hand, the scratching frequency and duration were significantly decreased in IL31ra\(^{−/−}\) mice compared with wild-type mice at 24 hours after FITC challenge (Fig. 5). Similar phenotypes were also observed for IL31\(^{−/−}\) mice during DNF-induced CHS. Although the ear thickness was slightly, but not significantly, increased in IL31\(^{−/−}\) mice compared with wild-type mice at the indicated time points after the initial epicutaneous application of DNF (Fig. 6a), the scratching frequency and duration were significantly decreased in IL31\(^{−/−}\) mice compared with wild-type mice at 24 hours after DNF challenge (Fig. 6b). GFP\(^{+}\) cells were hardly detected in LNs from IL31\(^{−/−}\) mice during DNF-induced CHS (data not shown). We found that a part of F4/80\(^{+}\) and CD11c\(^{+}\) cells, but not CD3\(^{+}\) cells, Gr1\(^{+}\) cells and tryptase\(^{+}\) cells (data not shown), were producers of IL-31 in skin from wild-type mice, but not IL31ra\(^{−/−}\) mice, at 24 h after DNF challenge (Fig. 7). The signals in keratinocytes from wild-type mice were due to non-specific binding of the anti-IL-31 Ab, since they were detected even in IL31ra\(^{−/−}\) mice (Fig. 7). These observations suggest that IL-31 is important for induction of pruritus, but not inflammation, in CHS induced by FITC and DNF.
Discussion

IL-31 is preferentially produced by Th2 cells in Th subsets, and excessive IL-31 production in IL-31 Tg mice resulted in development of dermatitis due to enhancement of type–2 immune responses. By contrast, Il31ra−/− mice showed enhanced type–2 immune responses during nematode infection, suggesting that IL-31R signaling has a suppressive role in type–2 immune responses. Regarding the apparent discrepancies in phenotype between IL-31 Tg mice and Il31ra−/− mice, the latter were reported to be hyperresponsive to OSM due to increased formation of OSM receptors (gp130 and OSMR) caused by the lack of IL-31R (IL-31RA and OSMR), resulting

Figure 1. Generation of Il31−/− mice. (a) IL-31 gene targeting strategy. The region containing a part of exon 2 and exon 3 of Il31 was replaced with a cassette consisting of IRES-EGFP and a neomycin resistance gene (Neo'), flanked by loxP sequences. (b) Expression of Il31 mRNA in lung tissue from wild-type (n = 8) and Il31−/− (n = 8) mice by qPCR. The data show the mean ± SEM. (c) Leukocyte profiles in the spleen from wild-type and Il31−/− mice (n = 3). The percentages of CD19+CD3− and CD19−CD3+ cells and of Gr1−Siglec F− and Gr1−Siglec F+ cells in 7-AAD-negative spleen cells, and the percentages of CD8+CD4− and CD8−CD4+ cells in 7-AAD-negative CD3+ spleen cells are shown. Representative flow cytometry data are shown.
in OSM-mediated suppression of type–2 immune responses. Therefore, to investigate the precise role of IL-31 in vivo, we newly generated Il31−/− mice for the present study. Using those mice, we demonstrated for the first time that IL-31 is important for induction of pruritus, but not inflammation, in CHS induced by FITC and DNFB. IL-31 is considered to be involved in the pathogenesis of type–2 cytokine-associated allergic disorders such as rhinitis, asthma and dermatitis. In support of that notion, the levels of IL-31 were increased in patients with allergic rhinitis, asthma and atopic dermatitis. DNFB- and/or FITC-induced CHS was attenuated in Il4−/− mice and/or st56−/− mice, suggesting that type-2 cytokines are important for induction of DNFB- and/or FITC-induced CHS. In addition, the levels of IL-31 were also elevated in patients with contact dermatitis. These observations suggest that IL-31 may be involved in induction of type-2 cytokine-associated CHS. However, we demonstrated that Il31−/− mice showed normal migration and maturation of skin DCs and induction of hapten-specific T cells in the sensitization phase of FITC-induced CHS, and normal induction of local

**Figure 2.** Normal skin DC function in Il31−/− mice. (a) The proportion of FITC-positive cells among 7-AAD-negative MHC class II hi CD11c+ cells in draining LN from wild-type (n = 4) and Il31−/− (n = 4) mice 24 hours after sensitization with FITC or vehicle alone was determined by flow cytometry. Representative flow cytometric data are shown. Shaded areas = LN obtained from the vehicle-treated left ear (lower number [%]), and solid lines = LN obtained from the FITC-treated right ear (upper number [%]). The data show the mean ± SEM. (b) The expression levels of CD86, CD40 and OX40L on 7-AAD-negative MHC class II hi CD11c+ FITC-positive cells in draining LN from wild-type and Il31−/− mice 24 hours after sensitization with FITC or vehicle alone were determined by flow cytometry. Representative flow cytometric data are shown for wild-type (n = 6) and Il31−/− (n = 4) mice. Shaded areas = isotype-matched control Ig staining, and solid lines = specific mAb staining.
inflammation in the elicitation phase of FITC- and DNFB-induced CHS, indicating that IL-31 is not essential for induction of skin inflammation during FITC- or DNFB-induced CHS.

As a unique function of IL-31, it is known to be involved in itching via signal activation of IL-31 receptors on sensory nerve cells\(^5,27\). Indeed, IL-31 Tg mice show severe scratching behavior accompanied by exfoliation of epidermis\(^1\). Administration of anti-IL-31 or anti-IL-31RA neutralizing Ab resulted in attenuation of scratching behavior, but not the severity of skin inflammation, in mice that developed atopic dermatitis-like skin inflammation\(^18,19\). Consistent with this, we showed that \(IL31^{-/-}\) mice had reduced scratching frequency and duration in FITC- and/or DNFB-induced CHS, indicating that IL-31 is responsible for pruritus in the setting. However, the underlying mechanism of this remains unclear and should be further investigated in future studies.

In summary, we demonstrated here that IL-31 is involved in pruritus reactions without affecting induction of local skin inflammation in CHS.

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**Figure 3.** Normal hapten-specific LN cell responses in \(IL31^{-/-}\) mice. (a) LN cells from FITC-sensitized wild-type (n = 5) and \(IL31^{-/-}\) (n = 5) mice were cultured in the presence and absence of 40 μg/ml FITC for 48 and 72 hours. The levels of IFN-γ, IL-4 and IL-17 in the culture supernatants were determined by ELISA. The data show the mean ± SEM.
Methods

Mice. C57BL/6N wild-type mice were obtained from Japan SLC, Inc. (Hamamatsu, Japan). Il31−/− mice on the C57BL/6N background were generated as follows. The Il31 gene was disrupted by replacement of the region from a part of exon 2 to a part of the intron behind exon 3 with a cassette consisting of IRES-EGFP and a neomycin resistance gene, flanked by loxP sequences (Fig. 1a). Homologous regions were amplified by PCR using the following primers:

- 5′-CAACCTATTCCTAGTTCCCTCACC-3′
- 5′-AAAGCAGCACCAGGGTAGGCTTCG-3′

To generate a 6-kb fragment, and
- 5′-TGAATGAGGGGAACAGAAAATTACC-3′
- 5′-TGATGAATAATGATATTCCTTACC-3′

To generate a 2-kb fragment. The targeting vector was electroporated into C57BL/6N ES cells (EGR-101, kindly provided by Dr. Masaru Okabe, Osaka University). Male chimeric mice were obtained from three distinct targeted clones and mated with C57BL/6N female mice. Genotyping of Il31−/− mice was performed by PCR using the following primers:

IL-31 is not essential for skin inflammation during FITC-induced CHS. (a) Wild-type (n = 47) and Il31−/− (n = 41) mice were epicutaneously sensitized and challenged with FITC. The ear skin thickness of the mice was measured at the indicated time points after challenge with FITC or vehicle alone. The data show the mean ± SEM. (b) Sections of ear skin from wild-type and Il31−/− mice at 24 hours after challenge with FITC or vehicle alone were stained with hematoxylin and eosin. Representative data are shown. Scale bar = 50 μm. (c) Ninety-six hours after challenge with FITC, sera were collected from wild-type (n = 5 [naive], and n = 21 [FITC]) and Il31−/− (n = 5 [naive], and n = 15 [FITC]) mice. The levels of FITC-specific IgG1, IgG2a and IgG2c and total IgE in the sera were determined by ELISA. The data show the mean ± SEM.
common 1 (5′-GACAACCCCTTATATATTGCCTGG-3′), WT 1 (5′-GCTTCCATAGCTGGCTTTATATCG-3′) and IL-31 KO (5′-CGACACCGGCCCTTTATATCGC-3′). The common 1 and WT 1 primers were used for detection of wild-type alleles (~550 bp), and the common and IL-31 KO primers were used for detection of mutant alleles (~390 bp). For generation of Il31 egfp/egfp mice, a plasmid carrying Cre cDNA (pCAG-Cre, kindly provided by Dr. Jun-ichi Miyazaki, Osaka University) was injected into fertilized eggs from Il31−/− mice to deplete the neomycin resistance gene, and it was flanked by loxP sequences. The eggs were then transferred into pseudopregnant female mice. Genotyping of Il31 egfp/egfp mice was performed by PCR using the following primers: common 2 (5′-TGGGAAGATACATGAAACCAATCC-3′), WT 2 (5′-CACTGGCTGCTCTTCCAGAGGACC-3′) and Neor (5′-GACGTGCTACTTCCATTTGTCACG-3′). The common and WT primers were used for detection of wild-type alleles (~493 bp), and the common and Neor primers were used for detection of mutant alleles (no band). All mice were housed in a specific-pathogen-free environment at The Institute of Medical Science, The University of Tokyo.

The animal protocol for experiments was approved by the Institutional Review Board of the Institute (A11-28 and A14-10), and all experiments were conducted according to the ethical and safety guidelines of the Institute.

**Quantitative PCR.** Total RNA was prepared from lungs using Sepasol (Nacalai Tesque, Inc.) and treated with DNase (TURBO DNA-free-kit; Thermo Fisher Scientific Inc., MA). cDNA was synthesized from the isolated RNA by RT-PCR (PrimeScript RT Reagent kit; TAKARA BIO Inc., Shiga, Japan). Quantitative real-time PCR was performed with SYBER Premix Ex Taq (TAKARA BIO Inc.) or SYBER Premix DimerEraser (TAKARA BIO Inc.) using a CFX384™ Touch Real-time PCR Detection System (BioRad Laboratories, Inc., Hercules, CA). Relative gene expression was determined against HPRT gene expression. The following PCR primers were designed: forward primer 5′-ATACAGCTGCCGTGTTTCAG-3′ and reverse primer 5′-AGCCCATCTTTATCAGCCAAGAAC-3′ for Il31 mRNA; and forward primer 5′-GGCCAGACTTTGTTGGATTTG-3′ and reverse primer 5′-CGCTCATCTTGGGATATTG-3′ for Hprt mRNA.

**Flow cytometry.** Spleen cells were incubated with anti-mouse CD16/CD32 mAb (2.4G2; BD Biosciences, San Jose, CA) in FACS buffer (HBSS containing 2% FCS) for FcR blocking for 15 min on ice, and then incubated with BV421-conjugated anti-mouse CD3 mAb (145-2C11; Biolegend, San Diego, CA), APC-conjugated anti-mouse CD8 mAb (53-6.7; Biolegend), PE-conjugated anti-mouse CD4 (GK1.5 Biolegend) or Siggle F (ES50-2440; BD Biosciences) mAb, PE-Cy7-conjugated anti-mouse CD19 mAb (6D5; Biolegend) or Gr1 (RB6-8C5; Affymetrix eBioscience, San Diego, CA) mAb for 20 min on ice. The expression profile of each cell surface marker on 7-aminoactinomycin D (7-AAD; Sigma-Aldrich Co. LLC, St. Louis, MO)-negative cells was analyzed on a
MACSQuant Analyser (Miltenyi Biotec, Bergisch Gladbach, Germany) with MACSQuantify Software (Miltenyi Biotec) and FlowJo software (Tree Star Inc., Ashland, OR).

**Skin DC migration.** Skin DC migration was determined as described elsewhere. Briefly, mice were epicutaneously treated with 40 µl of 0.5% (w/v) FITC isomer I (Sigma-Aldrich Co. LLC) in a 1:1 mixture of acetone (Wako Pure Chemical Industries, Ltd., Osaka, Japan) and dibutyl phthalate (Sigma-Aldrich Co. LLC) (20 µl to each surface of the left ear) and the vehicle alone (20 µl to each surface of the right ear). Twenty-four hours later, submaxillary lymph nodes (LNs) were separately collected from both the FITC-treated left and vehicle-treated right ears. After incubation with anti-mouse CD16/CD32 mAb (2.4G2, BD Biosciences) for 15 minutes on ice, LN cells were stained with PE-conjugated anti-mouse CD11c mAb (HL3, BD Biosciences) and APC-conjugated anti-mouse MHC Class II (I-A/I-E) mAb (M5/114.15.2, Affymetrix eBioscience) for 20 minutes on ice. The percentage of FITC-positive cells among 7-AAD-negative MHC Class II hi CD11c + cells was determined using a FACS Verse (BD Biosciences), and data analyses were performed using FlowJo software (Tree Star Inc.).

**Skin DC maturation.** A shaved area on the back and both surfaces of both ears of mice were painted with 150 µl and 20 µl of 0.5% (w/v) FITC isomer I, respectively. Twenty-four hours later, inguinal, axillary, and submaxillary LNs were harvested and pooled. LN single-cell suspensions were prepared and incubated with anti-mouse CD16/CD32 mAb (2.4G2, BD Biosciences) for 15 minutes on ice, LN cells were stained with PE-conjugated anti-mouse CD11c mAb (HL3, BD Biosciences) and APC-conjugated anti-mouse MHC Class II (I-A/I-E) mAb (M5/114.15.2, Affymetrix eBioscience) for 20 minutes on ice. The percentage of FITC-positive cells among 7-AAD-negative MHC Class II hi CD11c + cells was determined using a FACS Verse (BD Biosciences), and data analyses were performed using FlowJo software (Tree Star Inc.).

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**Figure 6.** IL-31 is important for pruritus, but not skin inflammation, during DNFB-induced CHS. (a) Wild-type (n = 12) and Il31−/− (n = 12) mice were epicutaneously sensitized and challenged with DNFB. The ear skin thickness of the mice was measured at the indicated time points after challenge with DNFB or vehicle alone. The data show the mean + SEM. (b) Scratching behavior (number of times and duration) in wild-type (WT; n = 8–13) and Il31−/− (n = 8–12) mice was assessed at 24 and 48 hours after challenge with DNFB or vehicle alone. The data show the mean + SEM. *p < 0.05 and **p < 0.01.
Induction of contact hypersensitivity (CHS). CHS was induced with FITC and DNFB as described elsewhere. Briefly, 2 days after shaving the back hair with electrical clippers, the back skin was treated with 150 µl of 1.0% FITC isomer I in a 1:1 mixture of acetone and dibutyl phthalate or 25 µl of 0.5% DNFB (SIGMA) in a 4:1 mixture of acetone and olive oil. Five days later, the respective mice were challenged with 40 µl of 0.5% FITC isomer I or 0.2% DNFB (each surface of the left ear) and 40 µl of the vehicle alone (each surface of the right ear). The ear thickness was measured before and after FITC challenge using dial thickness gauges (Ozaki MFG. Co., Ltd., Tokyo, Japan).

Measurement of levels of serum immunoglobulins. Sera were collected from mice 4 days after FITC challenge during FITC-induced CHS. The levels of FITC-specific IgGs in the sera were determined by ELISA as described elsewhere. In brief, 96-well flat-bottom plates (Thermo Fisher Scientific Inc.) were coated with 20 µg/ml FITC-conjugated OVA at 4 °C overnight. After the wells were blocked with PBS containing 1% FBS, optimally diluted serum samples (IgG1 = 1/100; IgG2b = 1/100; IgG2c = 1/10) were placed in the wells, and the plates were incubated at room temperature for 2 hours. After washing, HRP-conjugated anti-mouse IgG1, IgG2b or IgG2c mAb (Bethyl Laboratories, Inc., TX) was added, followed by incubation at room temperature for 1 hour. For enzymatic reaction, TMB substrate (Nacalai Tesque Inc.) was used as a substrate. The reaction was stopped by addition of 0.2 M H2SO4, and then the absorbance (450 nm) was measured using a VersaMax (Molecular Devices, LLC, Sunnyvale, CA). Data show the absorbance value at 450 nm. The levels of total IgE in sera were determined using an ELISA kit (Bethyl Laboratories, Inc.) in accordance with the manufacturer’s instructions.

FITC-specific LN cell responses. FITC-specific LN cell responses were determined as described elsewhere. Briefly, mice were epicutaneously treated with 1.0% FITC isomer I in a 1:1 mixture of acetone and
dibutyl phthalate on both the left and right ears (20 µl on one surface of each ear). Six days later, submaxillary LNs were collected. The LN cells were cultured in the presence and absence of 40 µg/ml FITC at 37 °C for 72 hours. The levels of cytokines in each culture supernatant were determined with mouse IFN-γ, IL-4 and IL-17A ELISA kits obtained from Biolegend (San Diego, CA) or Peprotech (Rocky Hill, NJ).

Assessment of scratching behaviour. SCLABA-Real (NOVELTEC) was used to assess scratching behavior (frequency and duration) at 24 and 48 hours after challenge with FITC or DNFB during FITC- or DNFB-induced CHS.

Histology. Twenty-four hours after FITC or vehicle challenge, the ear skins were harvested, fixed in Carnoy’s fluid and embedded in paraffin. Sections were prepared and stained with hematoxylin-eosin.

Immunohistochemistry. Twenty-four hours after epicutaneous challenge with DNFB as described above, the ear skin was frozen in OCT compound (Sakura Finetek, Tokyo, Japan). Sections (4-µm thick) were prepared using a cryostat (Leica) and dried on aminosilane-coated slide glasses (Matsunami Glass, Osaka, Japan). The sections were fixed in 4% paraformaldehyde at room temperature for 10 min and incubated with Blocking One HistO (Nacalai Tesque, Inc.) and Avidin/Biotin Blocking Kit (Vector Laboratories, CA) at room temperature for 10 min. They were then incubated with primary Abs, rabbit anti-mouse CD3 Ab (ab5690; Abcam plc.), rat anti-mouse CD11c Ab (ab33484; Abcam plc.), rat anti-mouse F4/80 (MCA497G; Bio-Rad), biotin-conjugated goat anti-mouse IL-31 Ab (BAF3028; R&D Systems), rat anti-mouse Ly-6G/-6C Ab (ab2557; Abcam plc.) or rabbit anti-mouse tryptase Ab (ab134932; Abcam plc.), at 4 °C overnight. After washing, the sections were incubated with secondary Abs/reagent, Alexa fluor® 594-conjugated donkey anti-rabbit Ab (ab150064; Abcam plc.), Alexa fluor® 594-conjugated goat anti-mouse IL-31 Ab (BAF3028; R&D Systems), rat anti-mouse Ly-6G/-6C Ab (ab2557; Abcam plc.) or rabbit anti-mouse tryptase Ab (ab134932; Abcam plc.), at 4 °C overnight. After washing, the sections were incubated with secondary Abs/reagent, Alexa fluor® 594-conjugated donkey anti-rabbit Ab (ab150064; Abcam plc.), Alexa fluor® 594-conjugated donkey anti-rat Ab (ab150156; Abcam plc.), Alexa fluor® 594-conjugated goat anti-hamster Ab (ab21113; Thermo Fisher Scientific), or Alexa fluor® 488-conjugated streptavidin (S11223; Thermo Fisher Scientific), at 4 °C for 60 min. Nuclei were stained with DAPI (R37606; Thermo Fisher Scientific). Images were acquired using a fluorescent microscope (BZX-700, KEYENCE, Tokyo, Japan) and analyzed with a software (BZ-analysis application, KEYENCE).

Statistics. A two-tailed Mann-Whitney U test was performed for statistical significance using Graphpad Prism software (Graphpad Prism). P values of less than 0.05 were considered statistically significant.

References
1. Dillon, S. R. et al. Interleukin 31, a cytokine produced by activated T cells, induces dermatitis in mice. Nat Immunol 5, 752–760, https://doi.org/10.1038/ni1084 (2004).
2. Ishi, T. et al. Pivotal role of mast cells in pruritogenesis in patients with myeloproliferative disorders. Blood 113, 5942–5950, https://doi.org/10.1182/blood-2008-09-179416 (2009).
3. Niyonsaba, F. et al. Antimicrobial peptides human beta-defensins and cathelicidin LL-37 induce the secretion of a pruritogenic cytokine IL-31 by human mast cells. J Immunol 184, 3526–3534, https://doi.org/10.4049/jimmunol.0900712 (2010).
4. Cornelissen, C. et al. Ultraviolet B radiation and reactive oxygen species modulate interleukin-31 expression in T lymphocytes, monocytes and dendritic cells. Br J Dermatol 165, 966–975, https://doi.org/10.1111/j.1365-2133.2011.10487.x (2011).
5. Kabenhorst, A. & Hartmann, K. Interleukin 31: a novel diagnostic marker of allergic diseases. Curr Allergy Asthma Rep 14, 423, https://doi.org/10.1007/s11882-014-0423-y (2014).
6. Hermanns, H. M. & Oncostatin M. and interleukin-31: Cytokines, receptors, signal transduction and physiology. Cytokine Growth Factor Rev 26, 545–558, https://doi.org/10.1016/j.cytogfr.2015.07.006 (2015).
7. Horejs-Hoeck, J. et al. DC-stimulating cytokine IL-31 induces pro-inflammatory mediators upon IL-31 treatment. J Immunol 188, 5319–5326, https://doi.org/10.4049/jimmunol.1101044 (2012).
8. Dambacher, J. et al. Interleukin 31 mediates MAP kinase and STAT1/3 activation in intestinal epithelial cells and its expression is upregulated in inflammatory bowel disease. Gut 56, 1257–1265, https://doi.org/10.1136/gut.2006.118679 (2007).
9. Ip, W. K. et al. Interleukin-31 induces cytokine and chemokine production from human bronchial epithelial cells through activation of mitogen-activated protein kinase signalling pathways: implications for the allergic response. Immunology 122, 532–541, https://doi.org/10.1111/j.1365-2567.2007.02668.x (2007).
10. Karsaei, S., Niebuhr, M. & Werfel, T. IL-31 induces pro-inflammatory cytokines in human monocytes and macrophages following stimulation with staphylococcal exotoxins. Allergy 65, 712–721, https://doi.org/10.1111/j.1398-9995.2009.02255.x (2010).
11. Baumann, R. et al. The release of IL-31 and IL-13 after nasal allergen challenge and their relation to nasal symptoms. Clin Transl Allergy 2, 13, https://doi.org/10.1186/2045-7022-2-13 (2012).
12. Lei, Z. et al. SCF and IL-31 rather than IL-17 and RANK are potential indicators in patients with allergic asthma. Allergy 63, 327–333, https://doi.org/10.1111/j.1398-9995.2007.01566.x (2008).
13. Bilsborough, J., Mudri, S., Chadwick, E., Harder, B. & Dillon, S. R. IL-31 receptor (IL-31RA) knockout mice exhibit elevated responsiveness to oncostatin M. J Immunol 185, 6023–6030, https://doi.org/10.4049/jimmunol.0902769 (2010).
14. Sonkoly, E. et al. IL-31: a new link between T cells and pruritus in atopic skin inflammation. J Allergy Clin Immunol 117, 411–417, https://doi.org/10.1016/j.jaci.2005.10.033 (2006).
15. Raap, U. et al. Correlation of IL-31 serum levels with severity of atopic dermatitis. J Allergy Clin Immunol 122, 421–423, https://doi.org/10.1016/j.jaci.2008.05.047 (2008).
16. Neis, M. M. et al. Enhanced expression levels of IL-31 correlate with IL-4 and IL-13 in atopic and allergic contact dermatitis. J Allergy Clin Immunol 118, 930–937, https://doi.org/10.1016/j.jaci.2006.07.015 (2006).
17. Takaoka, A. et al. Expression of IL-31 gene transcripts in NC/Nga mice with atopic dermatitis. Eur J Pharmacol 516, 180–181, https://doi.org/10.1016/j.euphar.2005.04.040 (2005).
18. Grimstad, O. et al. Anti-interleukin-31 antibodies ameliorate scratching behaviour in NC/Nga mice: a model of atopic dermatitis. Exp Dermatol 18, 35–43, https://doi.org/10.1111/j.1600-0625.2008.00766.x (2009).
19. Kasutani, K. et al. Anti-IL-31 receptor antibody is shown to be a potential therapeutic option for treating itch and dermatitis in mice. Br J Pharmacol 171, 5049–5058, https://doi.org/10.1111/bjhp.12733 (2014).
20. Ruzicka, T. et al. Anti-Interleukin-31 Receptor A Antibody for Atopic Dermatitis. N Engl J Med 376, 826–835, https://doi.org/10.1056/NEJMoa1606490 (2017).
21. Kaplan, D. H., Igartu, B. Z. & Gaspari, A. A. Early immune events in the induction of allergic contact dermatitis. Nat Rev Immunol 12, 114–124, https://doi.org/10.1038/ni3150 (2012).
22. Oboki, K., Ohno, T., Saito, H. & Nakae, S. Th17 and allergy. *Allergol Int* **57**, 121–134, https://doi.org/10.2332/allergolint.R-07-160 (2008).
23. Perrigoue, J. G. *et al.* IL-31-IL-31R interactions negatively regulate type 2 inflammation in the lung. *J Exp Med* **204**, 481–487, https://doi.org/10.1084/jem.20061791 (2007).
24. Weigmann, B. *et al.* Diminished contact hypersensitivity response in IL-4 deficient mice at a late phase of the elicitation reaction. *Scand J Immunol* **45**, 308–314 (1997).
25. Traidl, C., Jugert, F., Krieg, T., Merk, H. & Hunzelmann, N. Inhibition of allergic contact dermatitis to DNCB but not to oxazolone in interleukin-4-deficient mice. *J Invest Dermatol* **112**, 476–482, https://doi.org/10.1046/j.1523-1747.1999.00550.x (1999).
26. Yokozeki, H. *et al.* Signal transducer and activator of transcription 6 is essential in the induction of contact hypersensitivity. *J Exp Med* **191**, 995–1004 (2000).
27. Cevikbas, F. *et al.* A sensory neuron-expressed IL-31 receptor mediates T helper cell-dependent itch: Involvement of TRPV1 and TRPA1. *J Allergy Clin Immunol* **133**, 448–460, https://doi.org/10.1016/j.jaci.2013.10.048 (2014).
28. Suto, H. *et al.* Mast cell-associated TNF promotes dendritic cell migration. *J Immunol* **176**, 4102–4112 (2006).
29. Oboki, K. *et al.* IL-33 is a crucial amplifier of innate rather than acquired immunity. *Proc Natl Acad Sci USA* **107**, 18581–18586, https://doi.org/10.1073/pnas.1003059107 (2010).

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**Author Contributions**

A.T. and S.N. designed the experiments. A.T., K. Sato, S.Y., T.N., T.S., T.Y., K.A. and H.M. performed the experiments. A.N., S.Y., K. Sudo and S.N. generated the *Il31−/−* mice. K. Matsuda and H.M. assessed the scratching behavior. K. Matsumoto, J.K. and K.O. provided resources. A.T. and S.N. wrote the manuscript. All authors reviewed the article.

**Additional Information**

**Competing Interests:** The authors declare no competing interests.

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