

# Involvement of Leukotriene B<sub>4</sub> Released from Keratinocytes in Itch-associated Response to Intradermal Interleukin-31 in Mice

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**A recent study suggests that interleukin-31 (IL-31) exerts its effect via indirect mechanisms rather than through direct stimulation of cutaneous nerves. However, the underlying peripheral mechanisms of IL-31-induced itch in the skin remain unclear. Therefore, the present study investigated the peripheral mechanisms underlying IL-31-induced itch in mice. IL-31-induced itch-related response was inhibited by anti-allergic drugs (tranilast and azelastine), but not by an H<sub>1</sub> histamine receptor antagonist (terfenadine). Furthermore, a 5-lipoxygenase inhibitor (zileuton), but not a cyclooxygenase inhibitor (indomethacin), and a leukotriene B<sub>4</sub> (LTB<sub>4</sub>) receptor antagonist (CMHVA) attenuated the action of IL-31. IL-31 receptor-immunoreactivity was observed in the epidermis and primary sensory neurones. IL-31 receptor mRNA was expressed in mouse keratinocytes and dorsal root ganglia neurones. IL-31 increased the production of LTB<sub>4</sub> in mouse keratinocytes. These results suggest that IL-31 elicits itch not only through direct action on primary sensory neurones, but also by inducing LTB<sub>4</sub> production in keratinocytes.**

**Key words:** itch; leukotriene B<sub>4</sub>; interleukin-31; keratinocytes; scratching; dorsal root ganglia.

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**I**itch (or pruritus), an unpleasant skin sensation that evokes the desire to scratch, is the most common symptom of dermatitis (e.g. atopic dermatitis) and certain systemic disorders (e.g. cholestasis). Histamine from the mast cells has been thought to play an essential role in itch. H<sub>1</sub> histamine receptor antagonists are the drugs of first choice for the treatment of itch. However, many severe pruritic diseases, except acute urticaria, respond poorly to H<sub>1</sub> histamine receptor antagonists (1–3). Therefore, the underlying mechanisms and mediators of itch in most pruritic diseases are unclear.

Interleukin-31 (IL-31), a cytokine released from T cells (especially, CD4<sup>+</sup>-Th2 cells), signals through a receptor complex comprising IL-31RA and oncostatin M receptor β (OSMRβ) (4, 5). IL-31 plays a major role in the induction of chronic inflammation in diseases such as dermatitis, allergic rhinitis and asthma (6). In

particular, the expression of IL-31 mRNA is elevated in the skin of patients with atopic dermatitis (7, 8) as well as in animal models of atopic dermatitis (9) and contact dermatitis (10). IL-31 induces skin inflammation (5) and scratching, an itch-associated response (11). However, in animal models of atopic dermatitis, the administration of anti-IL-31 antibody inhibited spontaneous scratching, but not dermatitis (12). These findings suggest that IL-31 is a mediator of itch.

The peripheral mechanisms of IL-31-induced pruritus are not completely clear. A recent study showed that IL-31 increases the intracellular Ca<sup>2+</sup> ion concentration in the primary cultures of mouse dorsal root ganglia (DRG) neurones, suggesting that IL-31 directly activates primary afferents and induces itch sensation (13). However, IL-31 receptor complex is expressed not only in DRG neurones, but also in several immune cells (e.g. activated monocytes, macrophages, eosinophils, and basophils) and keratinocytes (4). A recent study showed that IL-31 exerts its pruritic effect indirectly via keratinocytes and secondary mediators rather than through the stimulation of its receptors on cutaneous nerves (14). In addition, our previous studies on the peripheral mechanisms of itch have shown the important role of keratinocytes and their itch mediators, including leukotriene B<sub>4</sub> (LTB<sub>4</sub>) (15–18). Therefore, this study investigated the involvement of LTB<sub>4</sub> in peripheral mechanism of IL-31-induced itch.

## MATERIALS AND METHODS

### Animals

Male ICR mice aged 4–11 weeks were used in this study. The mice were purchased from Japan SLC (Shizuoka, Japan). For one part of the experiment, neonatal mice were used for the isolation of cutaneous keratinocytes. Neonatal mice were obtained from late pregnant mice purchased from Japan SLC (Shizuoka). They were housed in a room with controlled temperature (21–23°C), humidity (45–65%), and light (on from 07.00h to 19.00h). Food and water were freely available. All experimental procedures involving animals were approved by the Committee for Animal Experiments at the University of Toyama and conducted in accordance with the guidelines of the Japanese Pharmacological Society.

### Drugs

Recombinant mouse IL-31 was prepared in Chugai Pharmaceutical Co., Ltd (Shizuoka, Japan). Briefly, IL-31 was purified from the supernatant of mouse IL-31-transformed Chinese hamster ovary cells using hydroxyapatite column (Bio-Rad Laboratories, Inc., Hercules, CA, USA), anion-exchange column (GE healthcare, Chicago, IL, USA), and gel filtration column (GE healthcare). IL-31 was dis-

solved in phosphate buffered-saline (PBS) and administered intradermally. Naltrexone hydrochloride (Sigma-Aldrich, St Louis, MO, USA) (18–20) was dissolved in physiological saline and administered subcutaneously 15 min before IL-31 injection. Terfenadine (16, 18, 20) and tranilast (Sigma-Aldrich) (21) were dissolved in 0.5% sodium carboxymethyl cellulose and administered orally 30 and 60 min before IL-31 injection, respectively. Azelastine hydrochloride (Sigma-Aldrich) (16, 18, 22) was dissolved in tap water and administered orally 30 min before IL-31 injection. Indomethacin (Sigma-Aldrich) (15, 18), zileuton (Ono Pharmaceutical Co. Ltd, Osaka, Japan) (15, 18, 23), and CMHVA (5-[2-(2-carboxyethyl)-3-[6-(4-methoxyphenyl)-5E-hexenyl]oxyphenoxy] valeric acid) (Ono Pharmaceutical Co. Ltd) (15, 16, 18, 24, 25)) were dissolved in 0.5% sodium carboxymethyl cellulose and administered orally 30, 60, and 60 min before IL-31 injection, respectively.

#### Behavioural tests

The mouse hair was clipped over the rostral part of the back the day before the experiment. For acclimation, the mice were housed individually in the observation cage (an acrylic cage composing 4 cells (13×9×35 cm)) for at least 1 h. Immediately after the intradermal injection, the mice were put back into the same cells, and their behaviour was videotaped for 1 h by personnel kept out of the observation room. Scratching by the hind paws at the injection site was observed during video playback (26). The mice stretched either the left or the right hind paw, leaned their head towards the paws, rapidly scratched themselves, and brought their toenails to their mouth. A series of these movements was counted as one bout of scratching (15).

#### Immunohistochemistry

Under anaesthesia with pentobarbital (80 mg/kg, intraperitoneal), the mice were transcardially perfused with PBS and then 4% paraformaldehyde (PFA). The rostral back skin was isolated and post-fixed with 4% PFA at 4°C overnight. The skin was immersed in 30% sucrose solution at 4°C for at least 2 days. The tissue was embedded in Tissue-Tek® optimum cutting temperature (OCT) compound (Sakura Finetek Co., Ltd, Tokyo) and stored at –80°C until use. The frozen samples were sectioned at 20 µm with a cryostat (Leica, Wetzlar, Germany). After being washed with PBS 3 times, the sections were treated with 0.3% Triton X-100 in PBS and then with 0.25% foetal bovine serum to block non-specific immunoglobulin binding. The sections were incubated with goat anti-IL-31 receptor A (IL-31RA) antibody (AF2107; 1:500, R&D Systems, Inc., Minneapolis, MN, USA) and rabbit anti-protein gene product 9.5 antibody (RA95101; 1:2000, Ultracell Ltd, Isle of Wight, UK) at 4°C overnight. After washing, the preparations were incubated with Alexa Fluor 594-conjugated anti-rabbit IgG and Alexa Fluor 488-conjugated anti-goat IgG antibodies (Life Technologies, Carlsbad, CA, USA) for 1 h at room temperature. The fluorescence signals were observed using a confocal laser-scanning microscope (Bio-Rad Laboratories Inc., Hercules, USA).

#### Primary cultures of murine keratinocytes and dorsal ganglion neurones

The skin from neonatal mice was removed and treated with 0.05% collagenase A (Roche Diagnostics GmbH, Mannheim, Germany) dissolved in serum-free MCDB 153 medium (Sigma-Aldrich) containing 0.67% 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (Dojindo, Kumamoto, Japan), 0.12% sodium bicarbonate (Wako Pure Chemical Industries Ltd, Osaka, Japan), 0.01% penicillin G (Meiji Seika Pharma Co., Ltd, Tokyo, Japan), and 0.006% kanamycin (Wako Pure Chemical Industries Ltd) at 4°C overnight. The epidermal sheets were peeled gently from the underlying

dermis. The keratinocytes were dissociated by gently shaking the serum-free MCDB 153 medium containing the epidermal sheet. After washing with keratinocyte growth medium (CELLnTEC Advanced Cell Systems AG, Bern, Switzerland), the keratinocytes were cultured in collagen-coated 24-well or 6-cm-diameter plates.

The bilateral DRGs at C1–T13 levels were removed from the 6-week-old mice. DRG was treated with 0.25% collagenase (Wako Pure Chemical Industries Ltd) in serum-free Dulbecco's Modified Eagle's medium (DMEM) containing antibiotics (100 units/ml penicillin and 100 µg/ml streptomycin) at 37°C for 1 h and was shaken gently for dissociation. The dissociated cells were treated with 0.25% trypsin-EDTA at 37°C for 15 min and then the cells were washed with DMEM containing antibiotics. The cells suspended in DMEM containing antibiotics, 10% foetal bovine serum, and 50 µM cytosine β-D-arabinofuranoside (Sigma-Aldrich), were cultured in poly-D-lysine-coated 6-cm-diameter plates.

#### Reverse transcription and PCR

The total RNA from the primary cultures of murine keratinocytes and DRG neurones was extracted by using GeneElute Mammalian Total RNA miniprep kit (Sigma-Aldrich). The total RNA (1 µg) was reverse transcribed into the cDNA using oligo (dT)<sub>16</sub> primer and Reverscript III® (Wako Pure Chemical Industries Ltd). PCR was performed as described previously (16). The primer sequences used were as follows: IL-31RA (sense) 5'-tgctgctatggcatgaagtc-3'; IL-31RA (antisense) 5'-tcaagtgccaacaagacagc-3', OSMRβ (sense) 5'-taaaccaggcgctccgttac-3'; OSMRβ (antisense) 5'-aaggtttccacggtagtg-3', glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (sense) 5'-ccaaggtcatccatgacaac-3'; GAPDH (antisense) 5'-ttactcttgaggcccatgt-3'. The reaction product was separated on 2% agarose gel and stained with ethidium bromide.

#### Measurement of LTB<sub>4</sub>

To measure LTB<sub>4</sub> production in keratinocytes, the culture medium (200 µl) was collected (removed) from the primary cultures of murine keratinocytes 10 min after IL-31 administration and assayed for LTB<sub>4</sub> with an enzyme immunoassay (EIA) kit (Cayman Chemical, Ann Arbor, MI, USA). Zileuton and the vehicle (dimethyl sulphoxide, final concentration 0.001%) were treated 60 min before IL-31 application. Anti-IL-31RA antibody (Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA) and non-specific IgG (Sigma-Aldrich) were treated 30 min before IL-31 application. The remaining keratinocytes were treated with 1% Triton X-100 and used for protein determination by using a protein assay kit (Bio-Rad Laboratories, Inc.). The amount of LTB<sub>4</sub> was normalized to the amount of protein.

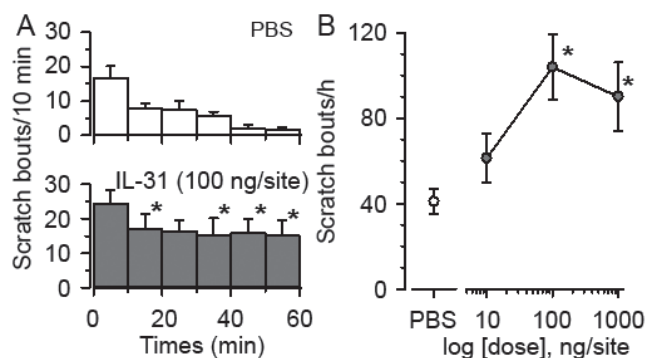
#### Data processing

Data are presented as means ± standard error of the mean (SEM). The statistical significance was analysed using the Student's *t*-test (2 groups), one-way analysis of variance (ANOVA), or 2-way repeated measures-ANOVA followed by a *post hoc* Holm–Šidák test (3 or more groups). A *p*-value <0.05 was considered statistically significant.

## RESULTS

### Scratching behaviour induced by IL-31

An intradermal injection of IL-31 (100 ng/site), but not vehicle, into the rostral back elicited hind-paw scratching directed towards the injection site (**Fig. 1A**). This effect peaked during the first 10 min. Compared with the

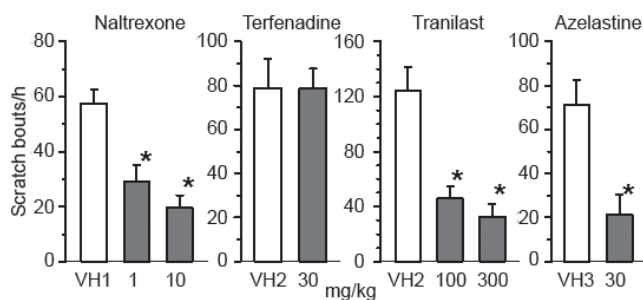


**Fig. 1. Scratching induced after the intradermal injection of interleukin (IL)-31 in mice.** (A) The time course of scratching after vehicle (upper panel) and IL-31 (lower panel, 100 ng/site) injection. (B) The dose-response curve for the scratch-inducing effects of IL-31. The mice were given an intradermal injection of IL-31 or vehicle (PBS). Data are presented as mean and standard error of the mean (SEM) ( $n=8-16$ ). \* $p<0.05$  compared with that of PBS (Holm-Šidák test). PBS: phosphate buffered-saline.

treatment of vehicle (PBS) control, IL-31 (100 ng/site)-induced scratching increased significantly from 10 min after the injection, and the significant effects continued for at least 50 min (Fig. 1A). The dose-response curve for IL-31 was bell-shaped (Fig. 1B).

#### Effects of various drugs on IL-31-induced scratching

The effects of systemic pretreatment on the scratching induced by an intradermal injection of IL-31 (100 ng/site) were examined by using several agents. The  $\mu$ -opioid receptor antagonist naltrexone hydrochloride (1 and 10 mg/kg) significantly inhibited IL-31-induced scratching (Fig. 2). The  $H_1$  histamine receptor antagonist, terfenadine (30 mg/kg), did not exhibit any effect on scratching (Fig. 2). The anti-allergic agents tranilast (100 and 300 mg/kg; Fig. 2) and azelastine hydrochloride (30 mg/kg; Fig. 2) inhibit IL-31-induced scratching. The 5-lipoxygenase inhibitor, zileuton (30 and 100 mg/kg), inhibited IL-31-induced scratching, whereas the cyclooxygenase



**Fig. 2. Effects of various drugs on interleukin (IL)-31-induced scratching in mice.** IL-31 (100 ng/site) was injected intradermally. Naltrexone hydrochloride (naltrexone) was injected subcutaneously 15 min before IL-31 injection. Terfenadine, tranilast, or azelastine hydrochloride (azelastine) was administered orally 30, 60 or 30 min, respectively before IL-31 injection. Vehicle (VH)1 (saline), VH2 (0.5% sodium carboxymethyl cellulose) and VH3 (tap water) denote the vehicle administered. Data are presented as mean and standard error of the mean (SEM) ( $n=5-8$ ). \* $p<0.05$  when compared with that of VH (Student's  $t$ -test or Holm-Šidák test).

inhibitor indomethacin (10 mg/kg) did not (Fig. 3). The  $LTB_4$  receptor antagonist CMHVA (10, 30 and 100 mg/kg) also attenuated IL-31-induced scratching (Fig. 3). Naltrexone hydrochloride (10 mg/kg), tranilast (30 mg/kg), azelastine hydrochloride (30 mg/kg), zileuton (100 mg/kg, p.o.), and CMHVA (100 mg/kg) did not affect the spontaneous locomotor activity, suggesting that these drugs at the dosage used do not have sedative effects (Fig. S1<sup>1</sup>).

*Distribution of IL-31 receptor A in the mouse skin, and the expression of IL-31 receptor mRNA in the mouse skin, dorsal root ganglion (DRG), and primary cultures of DRG neurones and keratinocytes*

IL-31RA-immunoreactivity was mainly localized in the epidermis and PGP-9.5-immunoreactive primary afferents (Fig. 4A).

The RT-PCR revealed the substantial expression of IL-31RA and OSMR $\beta$  mRNA in the DRG and skin (Fig. 4B). In addition, the primary cultures of DRG neurones and keratinocytes also expressed both IL-31RA and OSMR $\beta$  mRNA (Fig. 4B).

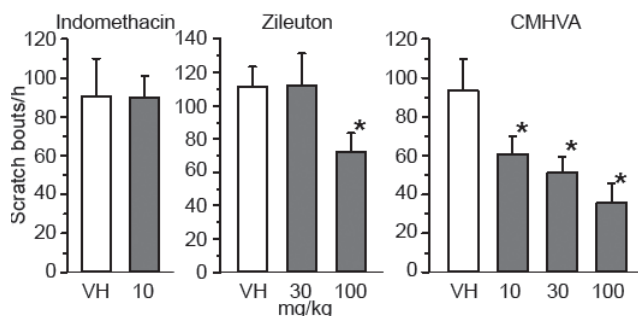
#### IL-31-induced production of $LTB_4$ in primary cultures of mouse keratinocytes

A bath-application of IL-31 (0.2 and 2  $\mu$ g/ml) to the primary cultures of mouse keratinocytes significantly increased the production of  $LTB_4$  (Fig. 5A). The effect of IL-31 (2  $\mu$ g/ml) was almost abolished by the 5-lipoxygenase inhibitor zileuton (10  $\mu$ M; Fig. 5A). In addition, anti-IL-31RA antibody (10  $\mu$ g/ml) significantly inhibited IL-31-induced  $LTB_4$  production (Fig. 5B).

## DISCUSSION

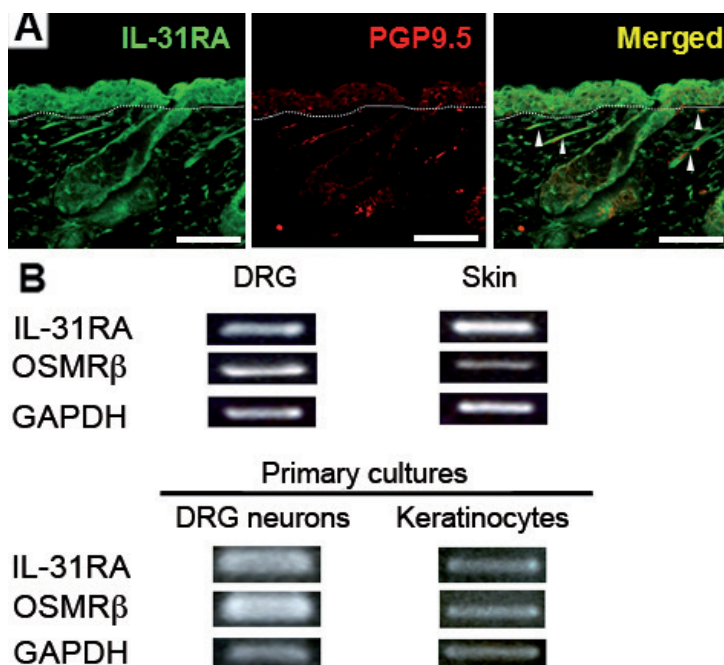
This study aimed to demonstrate peripheral mechanisms of IL-31-induced itch and found that IL-31-induced itch

<sup>1</sup><https://www.medicaljournals.se/acta/content/abstract/10.2340/00015555-2697>



**Fig. 3. Effects of arachidonic acid metabolism-related drugs on interleukin (IL)-31-induced scratching in mice.** IL-31 (100 ng/site) was injected intradermally. Indomethacin, zileuton, or CMHVA was administered orally 30, 60, or 60 min, respectively before IL-31 injection. Vehicle (VH) (0.5% sodium carboxymethyl cellulose) denotes the vehicle administered. Data are presented as mean and SEM ( $n=5-8$ ). \* $p<0.05$  compared with that of VH (Holm-Šidák test).





**Fig. 4. Distribution of interleukin-31 receptor A (IL-31RA) in the mouse skin and the expression of IL-31 receptors mRNA.** (A) The typical examples of the distribution of IL-31 receptor A (IL-31RA) and primary afferent neurones in the mouse skin. IL-31RA (green) and PGP-9.5 (red, a marker of neurone) were immunostained in the rostral back skin. Arrowheads indicate primary sensory neurones, and the dotted lines indicates the boundary between the dermis and epidermis. Scale bar = 100  $\mu$ m. (B) The expression of IL-31 receptor (IL-31RA and OSMR $\beta$ ) mRNA in the mouse skin, dorsal root ganglion (DRG), and primary cultures of DRG neurones and keratinocytes. IL-31RA, OSMR $\beta$ , and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) mRNAs were determined using RT-PCR.

through the production of LTB<sub>4</sub> in epidermal keratinocytes.

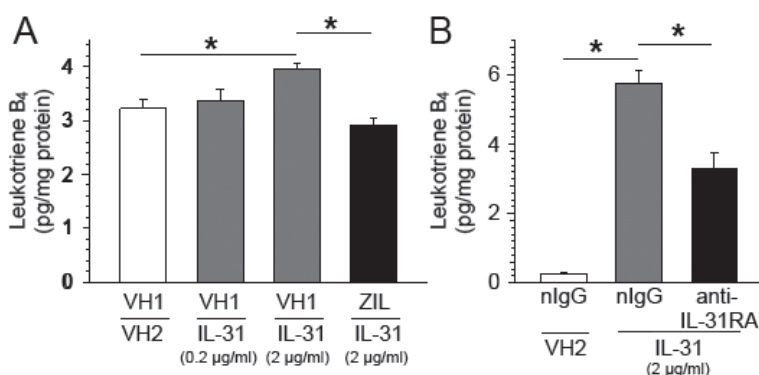
An intradermal injection of IL-31 elicited scratching at the injection site. Next, it was examined whether scratching in mice was an itch-related behaviour. The scratching was inhibited by the  $\mu$ -opioid receptor antagonist naltrexone.  $\mu$ -Opioid receptor antagonists inhibit the scratching induced by several pruritogens (e.g. substance P, sphingosylphosphorylcholine, serotonin, gastrin-releasing peptide, and  $\alpha$ -melanocyte-stimulating hormone) (18–20, 27, 28), dermatoses (e.g. skin allergy, atopic dermatitis and xerosis) in rodents (29–31), and itching/

scratching in humans with pruritic diseases (e.g. cholestasis, urticaria and atopic dermatitis) (32, 33).  $\mu$ -Opioid receptor antagonists suppress itch-associated behaviour without inhibiting the pain-related behaviour (34, 35). The opioid antagonists may inhibit itching/scratching by acting on the  $\mu$ -opioid receptors in the central nervous system, especially in the lower brain-stem (36–39). Thus, it can be suggested that IL-31-induced scratching is an itch-associated response in mice.

It has been reported that IL-31 acts directly on primary afferent neurones (13). In this study, although an intradermal administration of IL-31 elicited scratching with the effect peaking during the first 10-min period, the significant effect of IL-31 continued for another 50 min after the attainment of peak effect, suggesting that IL-31 affects not only directly, but also indirectly, primary afferent neurones.

The H<sub>1</sub> histamine receptor antagonist terfenadine (30 mg/kg) inhibited histamine-induced scratching in mice (30). However, the same dosage of terfenadine did not affect IL-31-induced scratching, suggesting that histamine may not play an important role in scratching. However, anti-allergic drugs (tranilast and azelastine) attenuated IL-31-induced scratching. Tranilast has an inhibitory action on the production of LTB<sub>4</sub> (40). In addition to H<sub>1</sub> histamine receptor antagonistic activity, azelastine has an inhibitory effect on LTB<sub>4</sub> production and LTB<sub>4</sub> receptor antagonistic activity (22). The IL-31-induced scratching was inhibited by 5-lipoxygenase inhibitor zileuton and LTB<sub>4</sub> receptor antagonist CMHVA. However, the cyclooxygenase inhibitor indomethacin did not show any effect on scratching. An intradermal injection of LTB<sub>4</sub>, but not prostaglandin E<sub>2</sub>, elicits scratching in mice (41). Taken together, these findings suggest that LTB<sub>4</sub> is involved in IL-31-induced scratching.

5-Lipoxygenase is a key enzyme involved in the production of LTB<sub>4</sub> (42). In the skin, 5-lipoxygenase is



**Fig. 5. Interleukin (IL)-31-induced leukotriene B<sub>4</sub> (LTB<sub>4</sub>) production in the primary cultures of keratinocytes.** (A) IL-31-induced production of LTB<sub>4</sub> and effect of 5-lipoxygenase inhibitor zileuton on LTB<sub>4</sub> production. Zileuton (10  $\mu$ M) or the vehicle (VH1) was treated 60 min before IL-31 or the vehicle (VH2) application. The concentration of LTB<sub>4</sub> in the tested medium was determined 10 min after IL-31 application. Data are presented as mean and standard error of the mean (SEM) ( $n = 18$ ). \* $p < 0.05$  compared with that of VH1+VH2 (Holm–Šidák test). (B) Effect of anti-IL-31RA on IL-31-induced LTB<sub>4</sub> production. Anti-IL-31RA antibody (anti-31RA, 10  $\mu$ g/ml) or non-specific immunoglobulin G (IgG) (nIgG, 10  $\mu$ g/ml) were treated 30 min before IL-31 (2  $\mu$ g/ml) or VH2. The concentration of LTB<sub>4</sub> in the tested medium was determined 10 min after IL-31 application. Data are presented as mean and SEM ( $n = 6$ ). \* $p < 0.05$  compared with that of nIgG+VH2 (Holm–Šidák test).

expressed mainly in the keratinocytes (43) and mast cells (44). IL-31 receptors are expressed in the keratinocytes, but not in the mast cells (4, 6, 45; Fig. 4 and Fig. S2A<sup>1</sup>). In addition, an intradermal injection of IL-31 elicited scratching in mast cell-deficient mice and the normal littermates (Fig. S2B<sup>1</sup>). Therefore, it is suggested that the mast cells are not involved in IL-31-induced LTB<sub>4</sub> production. Our previous report showed that an intradermal injection of proteinase-activated receptor-2 (PAR2) agonist SLIGRL-NH<sub>2</sub> (peptidergic agonist) induced LTB<sub>4</sub> production (46). PAR2 is mainly expressed in the keratinocytes in the skin (25). These findings suggest that intradermal pruritogens including IL-31 act on the epidermal keratinocytes. In this study, IL-31 induced LTB<sub>4</sub> production in the primary cultures of mouse keratinocytes via IL-31 receptor. Thus, it can be suggested that cutaneous keratinocytes are the main LTB<sub>4</sub> producing cells. The mechanisms underlying IL-31-induced LTB<sub>4</sub> production in keratinocytes remain unclear. IL-31 activates 3 signalling pathways: Jak/STAT pathway, PI3K/AKT pathway, and MAPK pathway (47). However, whether these pathways are involved in the production of LTB<sub>4</sub> remain unknown. IL-31 also increases the intracellular Ca<sup>2+</sup> concentration in keratinocytes (48). The increase in intracellular free Ca<sup>2+</sup> ions activates phospholipase A<sub>2</sub>, a key enzyme for the production of arachidonic acid (49). Arachidonic acid, a substrate of 5-lipoxygenase, is involved in the production of LTB<sub>4</sub>. Thus, as a possible mechanism, IL-31-induced Ca<sup>2+</sup> influx is involved in the production of LTB<sub>4</sub>.

LTB<sub>4</sub> directly activates DRG neurones and induces scratching (41, 50). The LTB<sub>4</sub> receptors, BLT1 and BLT2, have high and low binding affinities for LTB<sub>4</sub>, respectively (51). The LTB<sub>4</sub> receptor antagonist CMHVA has a similar affinity for both receptors (51). The DRG neurones and skin in the normal mice expressed BLT1 receptor mRNA, but not BLT2 receptors (50). Most (81%) of the BLT1-immunoreactive DRG neurones are transient receptor potential vanilloid 1 (TRPV1, a marker of unmyelinated C-fibre neurones) (50). Therefore, it is suggested that BLT1 receptor is involved in LTB<sub>4</sub>-mediated IL31-induced scratching. A recent study showed that BLT2 antagonist, but not BLT1 receptor antagonist, inhibits LTB<sub>4</sub>-induced scratching (52). However, our previous study has been shown that an intradermal injection of BLT2 receptor agonist (12(S)-hydroxy-(5Z, 8E, 10E)-heptadecatrienoic acid and CAY10538) did not induce scratching in mice (data not shown). Therefore, further study on the role of BLT2 in scratching will be necessary.

In this study, IL-31 receptors were expressed in the DRG neurones/primary afferents, suggesting that IL-31 directly activates the primary afferents. Cevikbas et al. showed that IL-31 acts on IL-31RA(+)/TRPV1(+) neurones (13). In addition, IL-31-induced scratching is attenuated in TRPV1-deficient mice (13). Our previous

reports have shown that TRPV1-deficiency or -desensitization in primary sensory neurones abolish the scratching induced by pruritogens (e.g. substance P and serotonin) (27, 28) and allergy (e.g. mosquito allergy) (53). Taken together, TRPV1-positive sensory neurones play an important role in the itch signalling induced by IL-31 and LTB<sub>4</sub>. IL-31 acts not only on IL-31RA(+)/TRPV1(+) neurones, but also on IL-31RA(+)/transient receptor channel potential cation channel ankyrin subtype 1 (TRPA1)(+) neurones (13). Fernandes et al. (52) showed that LTB<sub>4</sub>-induced scratching is attenuated in both TRPV1- and TRPA1-deficient mice. Therefore, it is suggested that TRPA1-positive sensory neurones also play an important role in IL-31- and LTB<sub>4</sub>-induced scratching.

In conclusion, IL-31 elicits itch not only through direct action on primary sensory neurones, but also by inducing LTB<sub>4</sub> production in keratinocytes.

## ACKNOWLEDGEMENTS

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