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RESEARCH PAPER

Anti-IL-31 receptor antibody is shown to be a potential therapeutic option for treating itch and dermatitis in mice

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BACKGROUND AND PURPOSE

IL-31, which is described as a pruritogenic cytokine, is linked to the itching that is associated with allergic and non-allergic eczema, but the precise pruritogenic mechanism of IL-31 and its potential as a therapeutic target for atopic dermatitis (AD) have not been determined.

EXPERIMENTAL APPROACH

We investigated the effects of existing drugs on the scratching behaviour induced by an i.v. injection of IL-31 to clarify whether IL-31 induced pruritus indirectly. In addition, we studied the effects of an anti-IL-31 receptor α subunit (anti-IL-31 receptor α) neutralizing antibody on chronic pruritus-inducing dermatitis in an AD-like model to determine whether IL-31 not only induces scratching behaviour, but is also the causative factor in an AD phenotype.

KEY RESULTS

The scratching behaviour induced by an i.v. injection of IL-31 was inhibited by pretreatment with an anti-IL-31 receptor α-neutralizing antibody. In contrast, it was not inhibited significantly by a non-sedative antihistamine (terfenadine), immunosuppressants (dexamethasone and tacrolimus), or a μ-opioid receptor antagonist (naloxone). The anti-IL-31 receptor α-neutralizing antibody reduced the ear swelling and dermatitis score in a chronic pruritus-inducing AD-like model. Moreover, treatment with the anti-IL-31 receptor α-neutralizing antibody showed therapeutic effects on the dermatitis even if it was injected after the disease had developed.

CONCLUSIONS AND IMPLICATIONS

Anti-IL-31 receptor α is a potential novel therapeutic approach for escaping from the itch–scratch cycle and also a treatment for dermatitis in AD.

Abbreviations

Ab, antibody; AD, atopic dermatitis; CS, contact sensitivity; *OSMR*, oncostatin M receptor; PiCl, picryl chloride; Th2, T helper 2

Table of Links

TARGETS	LIGANDS
IL-31 receptor	IL-31
Oncostatin-M receptor	Histamine
H receptor	Naloxone
μ-opioid receptor	Dexamethasone
	Terfenadine
	Codeine
	Tacrolimus

This Table lists key protein targets and ligands in this document, which are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Pawson *et al.*, 2014) and are permanently archived in the Concise Guide to PHARMACOLOGY 2013/14 (Alexander *et al.*, 2013a,b).

Introduction

Atopic dermatitis (AD) is a chronic, highly pruritic and inflammatory skin disease characterized by disease exacerbations and remission. In AD, a major diagnostic criterion that impairs the patient's quality of life is persistent pruritus (Williams *et al.*, 1994; Hanifin, 1999), which is also a cause of a more severe phenotype known as the itch–scratch cycle. In the itch–scratch cycle the strong actions of scratching facilitate both the susceptibility to increased itching and the exacerbation of skin lesions in patients with AD (Koblenzer, 1999; Stander and Steinhoff, 2002). Therefore, to escape from this vicious cycle by reducing the itching is crucial for preventing this aggravation of skin lesions and for improving the quality of life of patients with AD (Finlay, 2001; Greaves and Khalifa, 2004).

The mediators that induce chronic scratching behaviour have not been fully characterized, and remedies for persistent pruritus have not been developed. Histamine induces an acute itching sensation in humans (Handwerker *et al.*, 1991; Baron *et al.*, 2001), but histamine H_1 receptor antagonists generally do not have enough of an inhibitory effect on the itching and scratching of patients with AD, and, therefore, histamine is not considered to be a major pruritogen in AD (Wahlgren, 1991; Klein and Clark, 1999). Other factors, including neuropeptides, proteases, kinins and cytokines, induce itching and may also be considered as important mediators in the chronic itching sensation (Konishi *et al.*, 2002; Stander and Steinhoff, 2002; Novak *et al.*, 2003; Steinhoff *et al.*, 2006). Of these mediators, human and murine data have recently suggested IL-31 as a pruritogenic cytokine (Dillon *et al.*, 2004; Neis *et al.*, 2006; Paus *et al.*, 2006; Sonkoly *et al.*, 2006). IL-31 is produced mainly by T helper 2 (Th2) cells or by mast cells in response to antimicrobial peptides (Dillon *et al.*, 2004; Niyonsaba *et al.*, 2010) and is expressed more in lesional AD skin than non-lesional skin (Sonkoly *et al.*, 2006), and also in the skin-homing cutaneous lymphocyte antigen$^+$ T-cells (Bilsborough *et al.*, 2006). Serum IL-31 levels also increase in AD and there is a significant correlation with disease severity (Raap *et al.*, 2008; Ezzat *et al.*, 2011). Consistent with the human data, IL-31 transgenic mice developed spontaneous pruritus and skin lesions, which are hallmarks of AD (Dillon *et al.*, 2004), and in the NC/Nga mice, which spontaneously develop AD, IL-31 levels correlate with scratching behaviour (Takaoka *et al.*, 2005; 2006).

Recently, it has been reported that a single dose of IL-31 causes itch in experiments using mice, dogs and humans (Arai *et al.*, 2013; Gonzales *et al.*, 2013; Hawro *et al.*, 2014). These reports show a delay in the onset of IL-31-induced itch, but otherwise the pruritogenic mechanism of IL-31 remains largely undefined. Several factors, which either act directly by binding to the pruriceptors or indirectly by releasing other substances, can trigger or exacerbate the itching sensation by activating the sensory nerve fibres or modulating their activities (Metz *et al.*, 2011). The receptor for IL-31, which is a heterodimer of IL-31 receptor α subunit and the oncostatin M receptor (OSMR), is reported to be higher in the dorsal root ganglia of the sensory neurons (Sonkoly *et al.*, 2006) and in the primary afferent fibres of the spinal cord and dermis, which are said to be involved in the sensation of itch (Bando *et al.*, 2006). Therefore, IL-31 may activate pruritus-mediating nerve fibres directly. However, the IL-31 receptor α is also expressed in epidermal keratinocytes, eosinophils, mast cells, and activated monocytes and macrophages (Dillon *et al.*, 2004; Yamaoka *et al.*, 2009; Cheung *et al.*, 2010; Kasraie *et al.*, 2010), and IL-31 is attributed with a wide range of biological functions, such as the regulation of immune responses, cell proliferation and so on (Zhang *et al.*, 2008; Cornelissen *et al.*, 2012). Therefore, it is also possible that IL-31 may exert its pruritic effect indirectly in some way. The very recent reports that IL-31 does not immediately induce an itch response also suggest that IL-31 induced the scratching behaviour in an indirect fashion (Arai *et al.*, 2013; Hawro *et al.*, 2014). To investigate the pruritogenic mechanism of IL-31, we sequentially examined the scratching behaviour induced by IL-31 after i.v. injection, and also investigated the effects on that itching of some existing drugs for which antipruritic effects have been reported in *in vivo* models of atopic itching. Histamine is a major mediator that is released from mast cells and provokes the itching sensation (Handwerker *et al.*, 1991), and antihistamines are a popular treatment option in chronic pruritus. Terfenadine, a histamine H_1 receptor antagonist that inhibits the degranulation of mast cells, could inhibit the

release of histamine (Okayama *et al.*, 1994). Glucocorticoids and the calcineurin inhibitors, tacrolimus and pimecrolimus, also have therapeutic effects for patients with AD (Hanifin and Tofte, 1999; Assmann *et al.*, 2001), and there are a few reports of their antipruritic effects in *in vivo* models of atopic itching (Takano *et al.*, 2003; 2004). The calcineurin inhibitors interrupt cytokine gene expression, which leads to the down-regulation of T-cell activity and also inhibit the degranulation of mast cells (Sengoku *et al.*, 2000) and neuropeptide production (Kim *et al.*, 2009). As mast cell-independent mechanisms of itch, it is known that opiates such as codeine can cause pruritus, and opioid receptors have been found in the epidermis and on cutaneous sensory nerve fibres that are potentially responsible for triggering pruritus. Opioid-induced itch is mediated by activation of μ-opioid receptors, and μ-opioid receptor antagonists suppress the itch sensation in humans (Bernstein *et al.*, 1982; Monroe, 1989) and scratching behaviour in mice (Inagaki *et al.*, 2003; Takano *et al.*, 2004).

IL-31 is linked to itch that is associated with allergic and non-allergic eczema, but it is still not clear whether blocking IL-31 has therapeutic potential in the pathogenesis of AD. Indeed, a recent report has shown that, although the application of anti-IL-31 antibodies in NC/Nga mice reduces scratching behaviour, it has no effect on the development of dermatitis (Grimstad *et al.*, 2009). We used two dermatitis models to investigate whether blocking IL-31 can prevent the aggravation of skin lesions and whether the effect of an anti-IL-31 receptor α-neutralizing antibody (Ab) is produced by interrupting the itch–scratch cycle or preventing the onset of disease. The models used were the contact sensitivity (CS) reaction model, which is used to assess antigen-specific and T-cell-dependent immune response, but in which continuous pruritus is not induced (Xu *et al.*, 1996), and a chronic AD-like model that induces steady scratching behaviour and dermatitis, including haemorrhage, oedema and crust formation (Inoue *et al.*, 2002; Yamashita *et al.*, 2005; 2007).

In the present study, to clarify the pruritogenic mechanisms of IL-31, we investigated the scratching behaviour induced by a single i.v. injection of IL-31 in BALB/c mice and observed that the antipruritic effects of existing drugs used clinically to treat AD and other pruritic diseases do not include ameliorating IL-31-induced scratching behaviour. In addition, we observed that anti-IL-31 receptor α-neutralizing Ab reduced the ear swelling and dermatitis score in a chronic AD model. Furthermore, we showed that this antibody had therapeutic effects even if it was injected after the disease had been formed. Our finding suggests that IL-31 receptor α could be a novel therapeutic target for the treatment of itch and dermatitis in AD.

Methods

Animals

Female 5- or 6-week-old BALB/c mice were purchased from Charles River Japan Inc. (Kanagawa, Japan) and were used at the age of 6–10 weeks. The mice were kept in a room with a 12 h light/12 h dark cycle (lights on 07:00–19:00 h), and the room temperature and humidity were set in the range of 20–26°C and 35–75% respectively. The animals were provided with food and tap water *ad libitum*. All animal care and experiments were performed in Chugai's laboratory in accordance with the Guidelines for the Care and Use of Laboratory Animals at Chugai Pharmaceutical Co., Ltd., and the protocol was approved by the Institutional Animal Care and Use Committee at the institution. All studies involving animals are reported in accordance with the ARRIVE guidelines for reporting experiments involving animals (Kilkenny *et al.*, 2010; McGrath *et al.*, 2010).

Preparation of mouse IL-31

Recombinant mouse IL-31 was prepared at Chugai's Laboratory. Briefly, we established a stably transformed CHO cell line that constitutively expresses mouse IL-31. The mouse IL-31 was purified to homogeneity by sequential chromatography using a hydroxyapatite column, a Q-Sepharose/FF anion-exchange column and a Superdex75 gel filtration column. The activity of purified mouse IL-31 was checked by assessing the IL-31-dependent growth of Ba/F3 cells transfected with mouse IL-31 receptor α and mouse *OSMR* genes.

Reproduction of anti-mouse IL-31 receptor α monoclonal Ab, BM095

A recombinant anti-mouse IL-31 receptor α monoclonal IgG Ab (BM095) was also prepared in Chugai's laboratory. Briefly, anti-mouse IL-31 receptor α scFvs were isolated from phage display libraries of human Ab, and then a potent scFv clone was identified on the basis of the neutralizing activity against mouse IL-31-dependent proliferation of the Ba/F3 transfectants mentioned earlier. The variable regions of the light and heavy chains of the scFv clone were respectively ligated to the constant regions of mouse λ chain and mouse IgG2a to construct their expression vectors. The vectors were then co-transfected into CHO cells, and a stable cell line that secretes BM095 was established. BM095 was purified from its culture supernatant by protein A column and cation exchange chromatography.

Evaluation of BM095 neutralizing activity

The neutralizing activity of purified BM095 was evaluated using Ba/F3 transfectants mentioned earlier. Cells (6×10^3 cells per well), mouse IL-31 (2 ng·mL^{-1}), and each concentration of BM095 were incubated together for 2 days in a 96-well flat-bottomed plate. Cell growth was evaluated by measuring the absorbance using Cell Counting Kit-8 (Dojindo Laboratories, Kumamoto, Japan), with increase in absorbance taken to be an indicator of cell growth. Using cell growth as the indicator, inhibition of IL-31-dependent responses by BM095 was evaluated.

Evaluation of scratching behaviour

Scratching behaviour was measured using the MicroAct system (Neuroscience, Inc., Tokyo, Japan), which detects the behaviour automatically and analyses it objectively (Inagaki *et al.*, 2002; 2003). Briefly, under ether inhalation anaesthesia, a ring-type coated magnet (1 mm diameter, 3 mm long; SCT-MAGSP-TF, Neuroscience, Inc.) was surgically implanted under the skin on the dorsal side of both hind paws of each mouse 3 days before the test. Heart rate and body temperature were consistently monitored to detect the depth

of anaesthesia. Immediately after the operation, Lepetan (Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan) was administered s.c. at a rate of 1 μg per mouse in 100 μL for pain relief. After the mouse had awakened from the anaesthesia, the animal was returned to its cage. To measure scratching behaviour, each mouse was placed in an observation chamber surrounded by a round coil, and the current induced in the coil by movement of the magnets was amplified and recorded. Characteristic signals were identified as scratching behaviour using the following parameters: threshold, 0.05 V; event gap, 0.10 s; minimum duration, 0.30 s; maximum frequency, 20.0 Hz; minimum frequency, 5.0 Hz. The mice were acclimatized to the system device for 2 h before the i.v. injection of IL-31 or vehicle (PBS containing 0.5% mouse serum). The measurement of scratching behaviour started immediately after the IL-31 injection was given at around 15:00 h and was recorded continuously for 12 h. The number of scratching bouts in a 1 h period and in the total 12 h was counted.

To allocate mice to groups, we measured the scratching behaviour without injection. Individual mice with a very high or very low number of scratching bouts or with a high or low number of scratching movements were excluded from the study, and the remaining mice were allocated to groups so that there were no differences between groups with respect to basal number of scratching bouts or total number of scratching movements during the test.

Drug administration

The drugs were administered 1 h before the i.v. injection of 10 μg IL-31. Dexamethasone, tacrolimus and terfenadine were injected i.p., and BM095 or naloxone was injected i.v. or s.c. respectively. The doses of the drugs were as follows: dexamethasone (3 mg·kg^{-1}), tacrolimus (0.1 mg·kg^{-1}), terfenadine (30 mg·kg^{-1}), naloxone (10 mg·kg^{-1}) and BM095 (100–350 mg·kg^{-1}). These doses were based on those used to show antipruritic effects in other mouse pruritus models (Takano *et al.*, 2004; Yamashita *et al.*, 2007). The dose of BM095 was deemed necessary to block the *in vitro* neutralizing activity of 10 μg IL-31. The doses of these drugs had no sedative effects. In the comparison group, the vehicles of these drugs were injected instead of the drugs, and a mouse IgG against keyhole limpet haemocyanin, prepared in Chugai's Laboratory, was used as a control Ab.

Effect of anti-IL-31 receptor α Ab (BM095) on CS reaction

Mice were sensitized and challenged with picryl chloride (PiCl; Nacalai Tesque, Kyoto, Japan) as described previously (Fei *et al.*, 2005). Briefly, BALB/c mice were sensitized on day 0 with 50 μL of 7% PiCl solution (ethanol/acetone; 3:1 v v^{-1}) on their shaved ventral skins, and 5 days later, the ears were challenged with 20 μL of 1% PiCl solution (acetone/olive oil; 1:4 v v^{-1}). BM095 or vehicle was injected i.v. at 10 mg·kg^{-1} on days –1 and 4. Ear thickness was measured using a calibrated dial thickness gauge (Mitutoyo Corp., Kanagawa, Japan) before the PiCl challenge and at 24 and 48 h after the challenge and the degree of ear swelling of the PiCl-exposed ear determined by comparison with that of the vehicle-treated contralateral ear. Unsensitized animals were also challenged and used as negative controls to evaluate disease formation versus sensitized and challenged mice (positive control).

Effect of anti-IL-31 receptor α Ab (BM095) on dermatitis in the chronic AD model

Mice were sensitized and repeatedly challenged at the same site on the skin with PiCl, as described previously (Inoue *et al.*, 2002). Briefly, mice were sensitized by a single epicutaneous application of 20 μL of 0.5% PiCl solution (acetone/olive oil; 1:4 v v^{-1}) to the right ear 8 days before the first challenge (day 0), and then 20 μL of 0.25% PiCl solution (acetone/olive oil; 1:4 v v^{-1}) was repeatedly applied to the sensitized right ear at 2 day intervals for 6 weeks until day 44. To assess the preventive effect, BM095 was injected i.p. at 10 mg·kg^{-1} weekly from 1 day before PiCl sensitization (day –9). To evaluate the therapeutic effect, BM095 was injected weekly from day 20. In a disease control group, vehicle was injected weekly from day –9.

Ear thickness and dermatitis score were determined before PiCl application to evaluate the effect of BM095 on the severity of skin lesions. The thickness of the right ear was measured using a calibrated dial thickness gauge and the dermatitis score was determined by the development of haemorrhage (0 = *no symptoms*, 1 = *symptoms*), crust formation/desiccation (0 = *no symptoms or mild*, 1 = *moderate or severe*) and oedema (0 = <0.6 mm, 1 = ≥0.6 mm) through the observation period from day 0. In addition, the severity of the scab on day 42 was scored (–, no observed; +, mild; ++, moderate; +++, severe). The animals were killed 4 h after PiCl application on day 44 by exsanguination from the abdominal artery under deep anaesthesia and the right ear was collected for histopathological evaluation. The ears were cut longitudinally and fixed in 10% formalin. The tissues were embedded in paraffin, sectioned at approximately 5 μm and stained with haematoxylin and eosin. Serum IgE, IgG1 and IgG2b levels at day 44 were measured by ELISA.

Statistics

Statistical analysis of the scratching time-course was carried out using multiplicity adjustment of timewise comparisons designed for longitudinal measurement, and differences in scratching counts during the 12 h observation period were evaluated by Dunnett's multiple comparison test. Effects of BM095 on ear swelling and serum immunoglobulin (IgE, IgG1 and IgG2b) levels were evaluated using Student's *t*-test and the effect of BM095 on the dermatitis score was evaluated using Wilcoxon's test. These statistics were analysed using the software SAS System for Windows, Release 8.02 (SAS Institute Japan, Tokyo, Japan).

Reagents

Dexamethasone, terfenadine and naloxone hydrochloride were obtained from Sigma-Aldrich (Taufkirchen, Germany). Tacrolimus (Prograf®) was purchased from Astellas Pharma Inc. (Tokyo, Japan). Dexamethasone, tacrolimus and terfenadine were suspended in PBS containing 5% polyoxyethylene sorbitan monooleate, and naloxone hydrochloride was dissolved in saline.

Results

Characteristics of a single i.v. injection of IL-31

In our initial experiment, an i.v. injection of 10 μg IL-31 elicited significant scratching in BALB/c mice. The responses

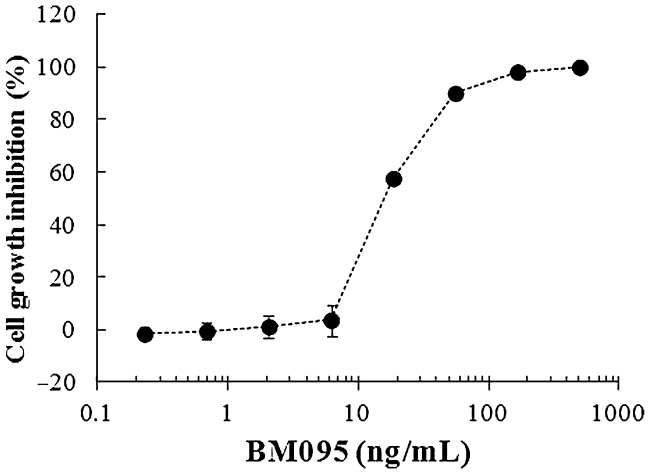

Figure 1

Inhibition of IL-31 response by anti-IL-31 receptor α Ab. The neutralizing activity of purified anti-IL-31 receptor α Ab (BM095) was evaluated using Ba/F3 cells transfected with mouse IL-31 receptor α and mouse *OSMR* genes. Using cell growth as the indicator, % inhibition of responses to 2 ng·mL^{-1} IL-31 by BM095 was evaluated (mean ± SD).

peaked 4–6 h after IL-31 treatment (Figure 2A and Supporting Information Fig. S2). The IL-31-induced response was dose-dependent and a similar response was observed with the s.c. injection of IL-31 (data not shown).

Inhibition of IL-31 response by BM095

BM095, an anti-mouse IL-31 receptor α-neutralizing Ab, inhibited the mouse IL-31-induced growth of Ba/F3 cells transfected with mouse IL-31 receptor α and mouse *OSMR* genes *in vitro*, in a concentration-dependent manner (Figure 1).

Moreover, the scratching behaviour induced by 10 μg IL-31 was inhibited by pretreatment with BM095 significantly and dose-dependently (Figure 2A, 2B, and Supporting Information Fig. S1).

Effects of existing drugs on scratching behaviour induced by i.v. injection of IL-31

The non-sedative antihistamine, terfenadine, did not inhibit the IL-31-induced scratching behaviour at a peritoneal dose of 30 mg·kg^{-1} (Figure 3 and Supporting Information Fig. S2). Therefore, histamine is not really involved in IL-31-induced pruritus.

Similarly, dexamethasone, tacrolimus and a μ-opioid receptor antagonist (naloxone) did not suppress scratching behaviour significantly either at any specific hour or in the total scratching counts over 12 h after the IL-31 injection (Figure 3 and Supporting Information Fig. S2). These results suggest that the IL-31-induced scratching behaviour is not inhibited by the antipruritic effects of existing drugs used clinically to treat AD and other pruritic disease.

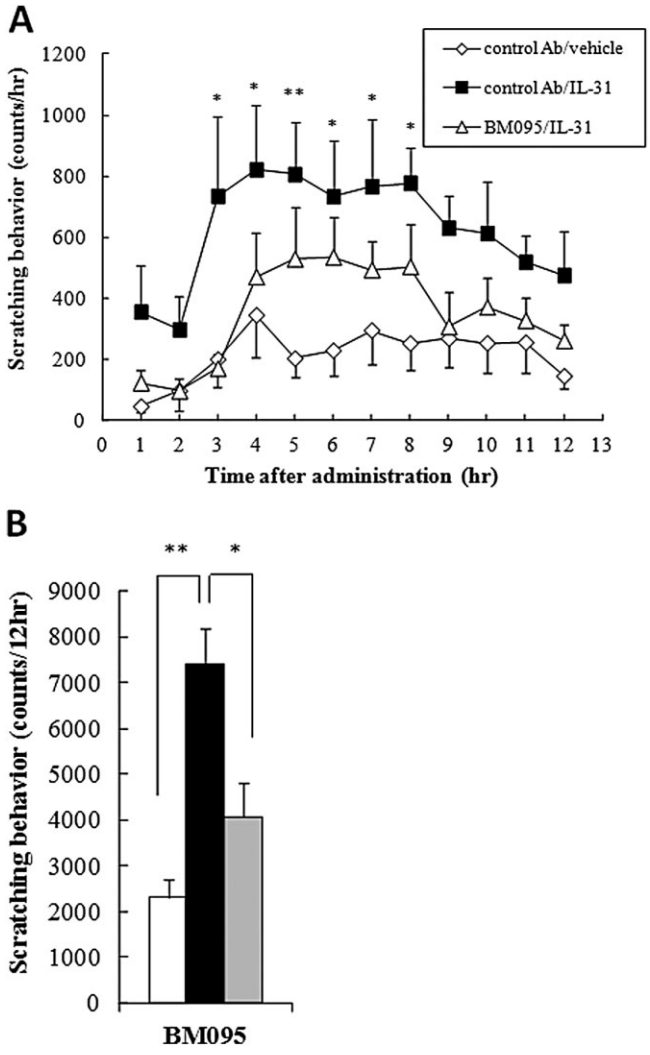

Figure 2

Scratching behaviour induced in BALB/c mice by IL-31. (A) Mice were i.v. injected with 10 μg IL-31 or vehicle after treatment with 300 mg·kg^{-1} control Ab or BM095. Scratching behaviour was recorded and analysed for 12 h after the injection, and the number of scratching bouts in each hour was counted. Each point represents the mean ± SEM of seven to eight mice. *P < 0.05, **P < 0.01 versus the vehicle group at the corresponding time point. (B) Scratching bouts were counted for 12 h in mice injected with IL-31 (black column) or vehicle (open column) after treatment with control Ab or BM095 (grey column). Each column represents the mean ± SEM of seven to eight mice. *P < 0.05, **P < 0.01 versus the non-agent group (control Ab/IL-31).

Effects of anti-IL-31 receptor α Ab on dermatitis in an acute CS model and a chronic AD model

The ear swelling response increased with the applications of PiCl following sensitization (positive control) compared with unsensitized mice (negative control) (Figure 4). In the CS model, BM095 did not have any effect on ear swelling at 24 and 48 h after the challenge, even if BM095 was injected i.v. at 10 mg·kg^{-1} 1 day before sensitization and challenge.

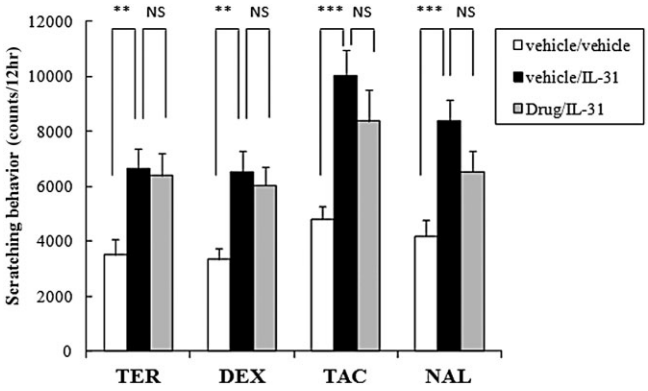

Figure 3

Effects of antihistamine, dexamethasone (DEX), tacrolimus (TAC) and naloxone (NAL) on the scratching behaviour induced by IL-31. Terfenadine (TER) at 30 mg·kg^{-1}, DEX at 3 mg·kg^{-1} and TAC at 0.1 mg·kg^{-1} were injected i.p., and NAL at 10 mg·kg^{-1} s.c. Vehicle or agent was administered 1 h before the IL-31 injection, or a vehicle injection only was given. The total number of scratching bouts was counted for 12 h. Each column represents the mean ± SEM of seven to eight mice. $^{**}P < 0.01$, $^{***}P < 0.001$ versus the untreated group (vehicle/IL-31). NS, not significant.

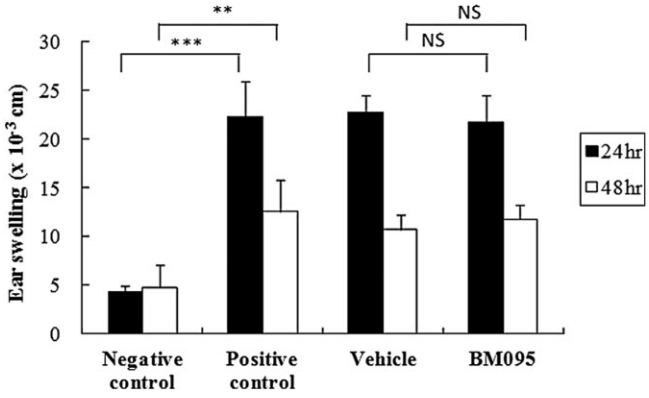

Figure 4

Effect of an anti-IL-31 receptor α Ab on the CS reaction produced by hapten-sensitization and challenge. The degree of ear swelling was determined by ear thickness of the sensitized ear compared with that of the vehicle-treated ear at 24 or 48 h after the challenge. Each column represents the mean ± SEM of five or six mice. $^{**}P < 0.01$, $^{***}P < 0.001$ at the corresponding time point. NS, not significant.

In the chronic AD model, ears that were given the repeated challenge with PiCl gradually grew thicker throughout the observation period (Figure 5A). In addition, severe dermatitis with oedema, erosion, scarring and haemorrhage was observed (Figure 5B, 5C). The BM095 treatment significantly inhibited the ear thickening and dermatitis scores from day 16 (Figure 5A, 5B). The crust formation on day 42 in the group pretreated with BM095 was significantly lower than that in the disease control group (Figure 5C). In addition, other than its preventive effects, BM095 showed therapeutic effects on the ear thickening and dermatitis scores, even if it was injected after disease had been formed (Figure 5A, 5B). Histopathologically, erosions and ulcers of the epidermis, scabs, inflammatory cell infiltration in the dermis and subcutis, and thickening of the epidermis were observed on day 44. The severity of the erosions and ulcers as well as scabs was lower in the preventive treatment group, but there was no difference in the severity of inflammatory cell infiltration or thickening of the epidermis (Figure 5D). And also, BM095 did not have significant effects on the levels of serum IgE (9.9 ± 1.7 μg·mL^{-1} vs. 10.8 ± 2.2 μg·mL^{-1} in control), IgG1 (3.4 ± 0.5 mg·mL^{-1} vs. 5.5 ± 1.1 mg·mL^{-1} in control), and IgG2a (119.4 ± 18.6 μg·mL^{-1} vs. 173.2 ± 31.0 μg·mL^{-1} in control) on day 44.

Discussion and conclusions

In this study, we demonstrated that IL-31-induced scratching behaviour was not inhibited by the antipruritic effects of existing drugs used clinically to treat AD and other pruritic diseases, suggesting that the itching induction by IL-31 might be caused by a different pruritogenic mechanism. A possible scenario is that IL-31 could up-regulate other potentially pruritogenic substances that are not affected by the existing drugs. As explained in the Introduction, IL-31 may directly activate pruritus-mediating nerve fibres in some way. However, an IL-31-induced phenotype eased 6–9 days after the last IL-31 injection (Dillon *et al.*, 2004), and we also found that the scratching elicited by an i.v. injection of IL-31 peaked at 4–6 h after treatment and had almost subsided after 10 h. This finding matches recent reports that IL-31 does not induce immediate itch, but late-onset itch after a skin challenge (Arai *et al.*, 2013; Hawro *et al.*, 2014). This suggests a novel mechanism other than direct activation of pruritus-mediating nerve fibres. For example, IL-31 is an important regulator of keratinocyte differentiation (Cornelissen *et al.*, 2012), and hence IL-31 may contribute to aggravating the AD pathology and persistent itching by qualitatively changing the skin barrier. In addition, as Hawro *et al.* mentioned, IL-31 may exert its pruritic effect indirectly via keratinocytes and subsequently released secondary mediators, such as VEGF (Hawro *et al.*, 2014). Alternatively, IL-31 may be involved in reducing the stimulus threshold of neuroreceptors, such as the capsaicin receptor.

As mentioned earlier, a study by Grimstad *et al.* found that applying anti-IL-31 antibodies to the NC/Nga mice reduced scratching behaviour, but had no effect on the development of dermatitis (Grimstad *et al.*, 2009). Therefore, to clarify whether IL-31 blockade has therapeutic potential in the pathogenesis of AD, we chose an AD-like model that is a reproducible and onset-controllable model. This chronic AD-like model results in a shift in the time-course from a typical delayed-type hypersensitivity reaction to an immediate-type response followed by a late-type reaction, a finding often observed in AD lesions. The antigen-specific development of these early-type responses is associated with elevated serum levels of antigen-specific IgE, and results in shifts in the local cytokine pattern from a Th1-type to a Th2-type profile (Kitagaki *et al.*, 1995; 1997). In addition, steady scratching behaviour is shown continuously (Yamashita *et al.*, 2005; 2007; Kido *et al.*, 2010). As most of these findings are also observed in patients with AD, this

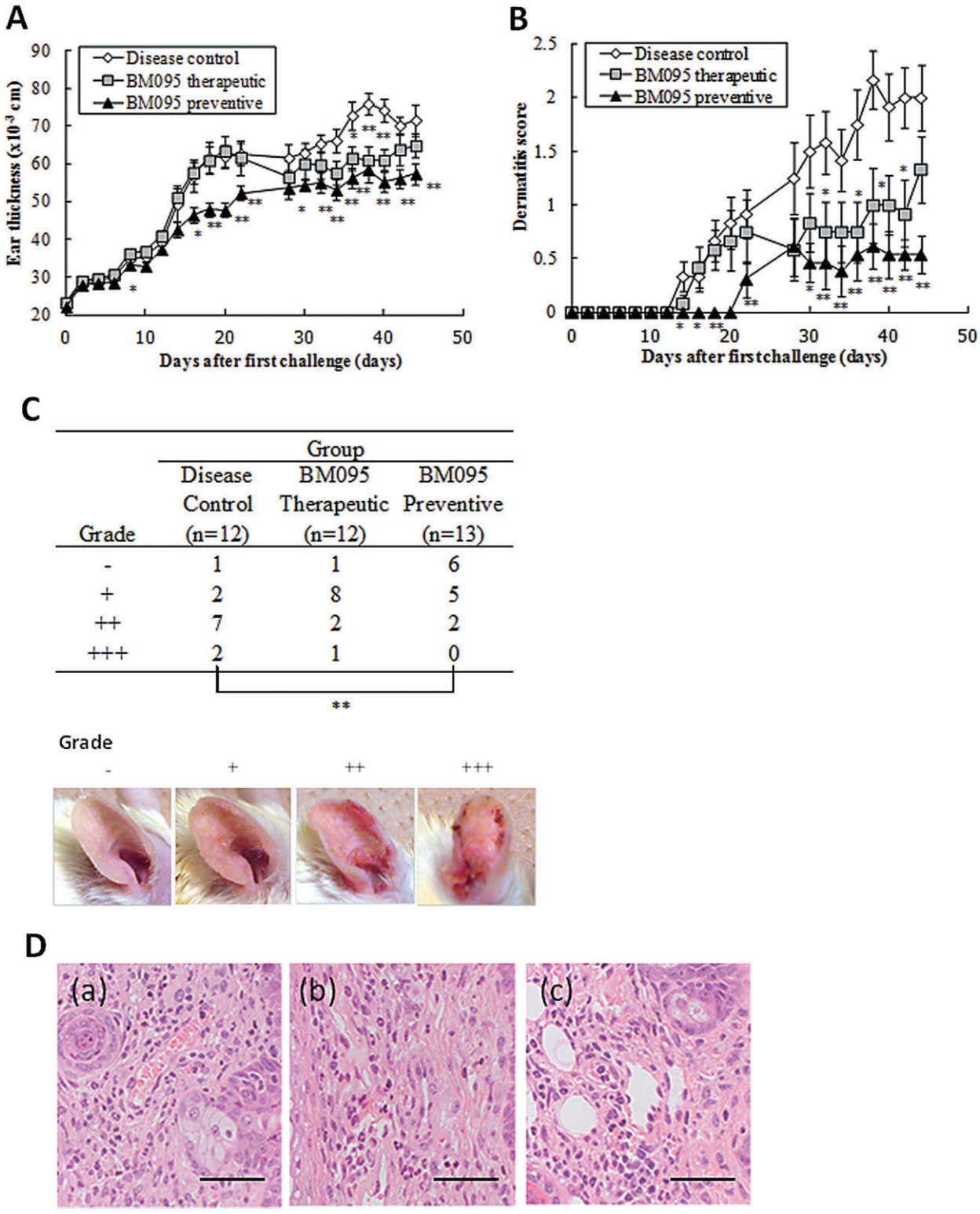

Figure 5

Exacerbation of dermatitis symptoms was inhibited by anti-IL-31 receptor α Ab in the chronic AD-like model. Ear thickness (A) and dermatitis score (B) measured for six weeks show the preventive effect of BM095 injected i.p. at 10 mg·kg^{-1} weekly from day –9 and the therapeutic effect of BM095 injected weekly from day 20. In the disease control, vehicle was injected weekly from day –9. Each symbol with a vertical bar represents the mean ± SEM of 12 or 13 mice. (C) The severity of the scab on day 42 was scored (–: none observed, +: mild, ++: moderate, +++: severe). Photographs show the appearance of the ear skin for each grade. *$P < 0.05$, **$P < 0.01$ versus the disease control group at the corresponding time point. (D) Histological appearance of the treated ear, 4 h after PiCl application on day 44. Images show inflammatory cell infiltration in the dermis of representative ear tissues from the disease control (a), BM095 therapeutic (b) and BM095 preventive group (c). The tissues were stained with haematoxylin and eosin . Bar = 50 μm.

mouse model appears to mimic many, if not all, events occurring in patients with AD. In this model, BM095 reduced the development of dermatitis, and furthermore, showed therapeutic effects on ear swelling and dermatitis, even when it was injected after disease onset. Our finding suggests a novel therapeutic potential of anti-IL-31 receptor α for treatment of itch and dermatitis in AD.

In the acute CS reaction model, BM095 did not have any effect on the ear swelling induced by PiCl-sensitization and challenge. These results show that BM095 prevented and treated the development of dermatitis in the chronic AD-like model not by preventing an antigen-specific or T-cell-dependent immune response, but possibly by blocking of the itch–scratch cycle. Actually, BM095 did not have effects on the severity of inflammatory cell infiltration in the histopathological evaluation, and on the serum IgE, IgG1 and IgG2a levels even though BM095 ameliorated the ear surface changes, epidermal thickening and crust formation. Our findings are also consistent with the recent report that the expression of IL-31 mRNA increased in conventional NC/Nga mice with long-lasting scratching behaviour, but was unchanged in 2,4,6-trinitrochlorobenzene-treated NC/Nga mice without long-lasting scratching behaviour (Takaoka *et al.*, 2006).

In the model induced by multiple application of PiCl, it is difficult to evaluate chronic scratching behaviour sequentially, because mice show increased scratching behaviour immediately after sensitization with PiCl or solvent on their ears, which is an effect of non-specific stimuli as shown in reported results (Yamashita *et al.*, 2005). Therefore, we tested the effects of BM095 on scratching behaviour in a mite antigen-induced AD model, in which AD is induced by i.d. administration of mite antigen. BM095 inhibited the total number of scratching movements in this model (unpublished observations).

In conclusion, our findings demonstrated that IL-31 receptor α could be a novel therapeutic target for the treatment of itch and dermatitis in AD, and that anti-IL-31 receptor α could be a treatment option for patients in whom existing treatments provide inadequate control of pruritus.

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Author contributions

Conception and design were done by K. K., M. H., H. K. and N. Y. Development of methodology was done by K. K. and H. A. Acquisition of data was done by K. K., E. F. and S. O. Analysis and interpretation of data were done by K. K. E. F. and S. O. Writing, review and/or revision of the paper were done by K. K., E. F. and N. Y. Study supervision was done by M. H., H. K. and N. Y.

Conflict of interest

As noted in Affiliations, some authors are employees of Chugai Pharmaceutical Co., Ltd.

References

Alexander SPH, Benson HE, Faccenda E, Pawson AJ, Sharman JL, Spedding M *et al.* (2013a). The Concise Guide to PHARMACOLOGY 2013/14: G protein-coupled receptors. Br J Pharmacol 170: 1459–1581.

Alexander SPH, Benson HE, Faccenda E, Pawson AJ, Sharman JL, Spedding M *et al.* (2013b). The Concise Guide to PHARMACOLOGY 2013/14: Catalytic receptors. Br J Pharmacol 170: 1676–1705.

Arai I, Tsuji M, Takeda H, Akiyama N, Saito S (2013). A single dose of interleukin-31 (IL-31) causes continuous itch-associated scratching behaviour in mice. Exp Dermatol 22: 669–671.

Assmann T, Homey B, Ruzicka T (2001). Topical tacrolimus for the treatment of inflammatory skin diseases. Expert Opin Pharmacother 2: 1167–1175.

Bando T, Morikawa Y, Komori T, Senba E (2006). Complete overlap of interleukin-31 receptor A and oncostatin M receptor beta in the adult dorsal root ganglia with distinct developmental expression patterns. Neuroscience 142: 1263–1271.

Baron R, Schwarz K, Kleinert A, Schattschneider J, Wasner G (2001). Histamine-induced itch converts into pain in neuropathic hyperalgesia. Neuroreport 12: 3475–3478.

Bernstein JE, Swift RM, Soltani K, Lorincz AL (1982). Antipruritic effect of an opiate antagonist, naloxone hydrochloride. J Invest Dermatol 78: 82–83.

Bilsborough J, Leung DY, Maurer M, Howell M, Boguniewicz M, Yao L *et al.* (2006). IL-31 is associated with cutaneous lymphocyte antigen-positive skin homing T cells in patients with atopic dermatitis. J Allergy Clin Immunol 117: 418–425.

Cheung PF, Wong CK, Ho AW, Hu S, Chen DP, Lam CW (2010). Activation of human eosinophils and epidermal keratinocytes by Th2 cytokine IL-31: implication for the immunopathogenesis of atopic dermatitis. Int Immunol 22: 453–467.

Cornelissen C, Marquardt Y, Czaja K, Wenzel J, Frank J, Luscher-Firzlaff J *et al.* (2012). IL-31 regulates differentiation and filaggrin expression in human organotypic skin models. J Allergy Clin Immunol 129: 426–433, 433, e421–e428.

Dillon SR, Sprecher C, Hammond A, Bilsborough J, Rosenfeld-Franklin M, Presnell SR *et al.* (2004). Interleukin 31, a cytokine produced by activated T cells, induces dermatitis in mice. Nat Immunol 5: 752–760.

Ezzat MH, Hasan ZE, Shaheen KY (2011). Serum measurement of interleukin-31 (IL-31) in paediatric atopic dermatitis: elevated levels correlate with severity scoring. J Eur Acad Dermatol Venereol 25: 334–339.

Fei M, Wu X, Xu Q (2005). Astilbin inhibits contact hypersensitivity through negative cytokine regulation distinct from cyclosporin A. J Allergy Clin Immunol 116: 1350–1356.

Finlay AY (2001). Quality of life in atopic dermatitis. J Am Acad Dermatol 45 (1 Suppl.): S64–S66.

Gonzales AJ, Humphrey WR, Messamore JE, Fleck TJ, Fici GJ, Shelly JA *et al.* (2013). Interleukin-31: its role in canine pruritus and naturally occurring canine atopic dermatitis. Vet Dermatol 24: 48–53, e11–e42.

Greaves MW, Khalifa N (2004). Itch: more than skin deep. Int Arch Allergy Immunol 135: 166–172.

Grimstad O, Sawanobori Y, Vestergaard C, Bilsborough J, Olsen UB, Gronhoj-Larsen C *et al.* (2009). Anti-interleukin-31-antibodies ameliorate scratching behaviour in NC/Nga mice: a model of atopic dermatitis. Exp Dermatol 18: 35–43.

Handwerker HO, Forster C, Kirchhoff C (1991). Discharge patterns of human C-fibers induced by itching and burning stimuli. J Neurophysiol 66: 307–315.

Hanifin JM (1999). Diagnostic criteria for atopic dermatitis: consider the context. Arch Dermatol 135: 1551.

Hanifin JM, Tofte SJ (1999). Update on therapy of atopic dermatitis. J Allergy Clin Immunol 104 (3 Pt 2): S123–S125.

Hawro T, Saluja R, Weller K, Altrichter S, Metz M, Maurer M (2014). Interleukin-31 does not induce immediate itch in atopic dermatitis patients and healthy controls after skin challenge. Allergy 69: 113–117.

Inagaki N, Igeta K, Kim JF, Nagao M, Shiraishi N, Nakamura N *et al.* (2002). Involvement of unique mechanisms in the induction of scratching behavior in BALB/c mice by compound 48/80. Eur J Pharmacol 448: 175–183.

Inagaki N, Igeta K, Shiraishi N, Kim JF, Nagao M, Nakamura N *et al.* (2003). Evaluation and characterization of mouse scratching behavior by a new apparatus, MicroAct. Skin Pharmacol Appl Skin Physiol 16: 165–175.

Inoue Y, Isobe M, Shiohara T, Goto Y, Hayashi H (2002). Protective and curative effects of topically applied CX-659S, a novel diaminouracil derivative, on chronic picryl chloride-induced contact hypersensitivity responses. Br J Dermatol 147: 675–682.

Kasraie S, Niebuhr M, Werfel T (2010). Interleukin (IL)-31 induces pro-inflammatory cytokines in human monocytes and macrophages following stimulation with staphylococcal exotoxins. Allergy 65: 712–721.

Kido M, Takeuchi S, Esaki H, Hayashida S, Furue M (2010). Scratching behavior does not necessarily correlate with epidermal nerve fiber sprouting or inflammatory cell infiltration. J Dermatol Sci 58: 130–135.

Kilkenny C, Browne W, Cuthill IC, Emerson M, Altman DG (2010). Animal research: reporting *in vivo* experiments: the ARRIVE guidelines. Br J Pharmacol 160: 1577–1579.

Kim HO, Lee CH, Ahn HK, Park CW (2009). Effects of tacrolimus ointment on the expression of substance P, nerve growth factor, and neurotrophin-3 in atopic dermatitis. Int J Dermatol 48: 431–438.

Kitagaki H, Fujisawa S, Watanabe K, Hayakawa K, Shiohara T (1995). Immediate-type hypersensitivity response followed by a late reaction is induced by repeated epicutaneous application of contact sensitizing agents in mice. J Invest Dermatol 105: 749–755.

Kitagaki H, Ono N, Hayakawa K, Kitazawa T, Watanabe K, Shiohara T (1997). Repeated elicitation of contact hypersensitivity induces a shift in cutaneous cytokine milieu from a T helper cell type 1 to a T helper cell type 2 profile. J Immunol 159: 2484–2491.

Klein PA, Clark RA (1999). An evidence-based review of the efficacy of antihistamines in relieving pruritus in atopic dermatitis. Arch Dermatol 135: 1522–1525.

Koblenzer CS (1999). Itching and the atopic skin. J Allergy Clin Immunol 104 (3 Pt 2): S109–S113.

Konishi H, Tsutsui H, Murakami T, Yumikura-Futatsugi S, Yamanaka K, Tanaka M *et al.* (2002). IL-18 contributes to the spontaneous development of atopic dermatitis-like inflammatory skin lesion independently of IgE/stat6 under specific pathogen-free conditions. Proc Natl Acad Sci U S A 99: 11340–11345.

McGrath J, Drummond G, McLachlan E, Kilkenny C, Wainwright C (2010). Guidelines for reporting experiments involving animals: the ARRIVE guidelines. Br J Pharmacol 160: 1573–1576.

Metz M, Grundmann S, Stander S (2011). Pruritus: an overview of current concepts. Vet Dermatol 22: 121–131.

Monroe EW (1989). Efficacy and safety of nalmefene in patients with severe pruritus caused by chronic urticaria and atopic dermatitis. J Am Acad Dermatol 21: 135–136.

Neis MM, Peters B, Dreuw A, Wenzel J, Bieber T, Mauch C *et al.* (2006). 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.

Niyonsaba F, Ushio H, Hara M, Yokoi H, Tominaga M, Takamori K *et al.* (2010). 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.

Novak N, Bieber T, Leung DY (2003). Immune mechanisms leading to atopic dermatitis. J Allergy Clin Immunol 112 (6 Suppl.): S128–S139.

Okayama Y, Benyon RC, Lowman MA, Church MK (1994). In vitro effects of H1-antihistamines on histamine and PGD2 release from mast cells of human lung, tonsil, and skin. Allergy 49: 246–253.

Paus R, Schmelz M, Biro T, Steinhoff M (2006). Frontiers in pruritus research: scratching the brain for more effective itch therapy. J Clin Invest 116: 1174–1186.

Pawson AJ, Sharman JL, Benson HE, Faccenda E, Alexander SP, Buneman OP *et al.* (2014). The IUPHAR/BPS Guide to PHARMACOLOGY: an expert-driven knowledgebase of drug targets and their ligands. Nucl Acids Res 42 (Database Issue): D1098–106.

Raap U, Wichmann K, Bruder M, Stander S, Wedi B, Kapp A *et al.* (2008). Correlation of IL-31 serum levels with severity of atopic dermatitis. J Allergy Clin Immunol 122: 421–423.

Sengoku T, Kishi S, Sakuma S, Ohkubo Y, Goto T (2000). FK506 inhibition of histamine release and cytokine production by mast cells and basophils. Int J Immunopharmacol 22: 189–201.

Sonkoly E, Muller A, Lauerma AI, Pivarcsi A, Soto H, Kemeny L *et al.* (2006). IL-31: a new link between T cells and pruritus in atopic skin inflammation. J Allergy Clin Immunol 117: 411–417.

Stander S, Steinhoff M (2002). Pathophysiology of pruritus in atopic dermatitis: an overview. Exp Dermatol 11: 12–24.

Steinhoff M, Bienenstock J, Schmelz M, Maurer M, Wei E, Biro T (2006). Neurophysiological, neuroimmunological, and neuroendocrine basis of pruritus. J Invest Dermatol 126: 1705–1718.

Takano N, Arai I, Kurachi M (2003). Analysis of the spontaneous scratching behavior by NC/Nga mice: a possible approach to evaluate antipruritics for subjects with atopic dermatitis. Eur J Pharmacol 471: 223–228.

Takano N, Arai I, Hashimoto Y, Kurachi M (2004). Evaluation of antipruritic effects of several agents on scratching behavior by NC/Nga mice. Eur J Pharmacol 495: 159–165.

Takaoka A, Arai I, Sugimoto M, Yamaguchi A, Tanaka M, Nakaike S (2005). Expression of IL-31 gene transcripts in NC/Nga mice with atopic dermatitis. Eur J Pharmacol 516: 180–181.

Takaoka A, Arai I, Sugimoto M, Honma Y, Futaki N, Nakamura A *et al*. (2006). Involvement of IL-31 on scratching behavior in NC/Nga mice with atopic-like dermatitis. Exp Dermatol 15: 161–167.

Wahlgren CF (1991). Itch and atopic dermatitis: clinical and experimental studies. Acta Derm Venereol Suppl (Stockh) 165: 1–53.

Williams HC, Burney PG, Pembroke AC, Hay RJ (1994). The U.K. working party's diagnostic criteria for atopic dermatitis. III. Independent hospital validation. Br J Dermatol 131: 406–416.

Xu H, DiIulio NA, Fairchild RL (1996). T cell populations primed by hapten sensitization in contact sensitivity are distinguished by polarized patterns of cytokine production: interferon gamma-producing (Tc1) effector CD8+ T cells and interleukin (Il) 4/Il-10-producing (Th2) negative regulatory CD4+ T cells. J Exp Med 183: 1001–1012.

Yamaoka K, Okayama Y, Kaminuma O, Katayama K, Mori A, Tatsumi H *et al*. (2009). Proteomic Approach to FcepsilonRI aggregation-initiated signal transduction cascade in human mast cells. Int Arch Allergy Immunol 149 (Suppl. 1): 73–76.

Yamashita H, Michibata Y, Mizukami H, Ogihara Y, Morita A, Nose M (2005). Dermal mast cells play a central role in the incidence of scratching behavior in mice induced by multiple application of the hapten, 2,4,6-trinitrochlorobenzene. Exp Dermatol 14: 438–444.

Yamashita H, Makino T, Mizukami H, Nose M (2007). Pharmacological characterization of a chronic pruritus model induced by multiple application of 2,4,6-trinitrochlorobenzene in NC mice. Eur J Pharmacol 563: 233–239.

Zhang Q, Putheti P, Zhou Q, Liu Q, Gao W (2008). Structures and biological functions of IL-31 and IL-31 receptors. Cytokine Growth Factor Rev 19: 347–356.

Supporting information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

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Figure S1 Scratching behaviour induced by IL-31 was inhibited dose-dependently by the anti-IL-31 receptor α Ab. Scratching bouts were counted for 12 h in mice injected with vehicle only or injected with IL-31 after treatment with 100 or 350 mg·kg^{-1} BM095 or vehicle. Each column represents the mean ± SEM of seven to eight mice. $^{**}P < 0.01$ versus the untreated group (vehicle/IL-31). NS, not significant.

Figure S2 Effects of antihistamine, dexamethasone, tacrolimus and naloxone on the scratching behaviour induced by IL-31. The number of scratching bouts in each hour for Figure 3 is shown. Vehicle or agent was administered 1 h before the IL-31 injection, or vehicle only was injected. Each point represents the mean ± SEM of seven to eight mice. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ versus the vehicle group at the corresponding time point.