

Interleukin-31 does not induce immediate itch in atopic dermatitis patients and healthy controls after skin challenge

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Abstract

Background: The most intriguing function attributed to interleukin-31 (IL-31) is its ability to induce pruritus in pathologic conditions, such as atopic dermatitis (AD). As of today, this feature of IL-31 was tested *in vivo* only in animal models. **Methods:** Ten patients with AD and 10 healthy controls were challenged with IL-31 and NaCl (negative control) by skin prick testing. Twenty additional healthy controls were subjected to skin prick testing with histamine. Itch and local inflammatory responses of the skin were assessed for up to 72 h.

Results: All of the histamine-challenged subjects developed immediate pruritus (i.e. within the first 5 min). In contrast, only one IL-31- and two of the NaCl-challenged subjects reported immediate itch at the provocation site (short lasting, for 2–6 min). Nine subjects (five patients with AD) reported late itch responses to IL-31 challenges with a mean delay of 143 min. No subject reported late itch responses to histamine or NaCl testing. There was no significant difference in IL-31-induced itch start time, duration and intensity between patients with AD and healthy volunteers.

Conclusion: IL-31 does not induce immediate itch responses in humans. The late onset of IL-31-induced itch supports the notion that IL-31 exerts its pruritic effect indirectly via keratinocytes and secondary mediators, rather than through its receptors on cutaneous nerves.

Interleukin-31 (IL-31), an IL-6 family cytokine, was discovered, characterized and extensively investigated during the last decade. IL-31 binds to a heterodimeric receptor, consisting of the IL-31 R alpha (IL-31 RA) and oncostatin M receptor beta (OSMR), subsequently signalling through the Janus kinase (Jak)/signal transducer and activator of transcription (STAT) pathway, the phosphoinositide-3-kinase/Akt (PI3K/AKT) pathway and the mitogen-activated protein kinase (MAPK) pathway (1–3). IL-31 is attributed with a wide range of biological functions such as the regulation of immune responses, the induction of chemokines, signal proteins, proinflammatory cytokines, the regulation of cell proliferation and, most prominently, the induction of itch (2, 4).

IL-31's important role as an itch inducer is supported by observations: (i) IL-31-expression is increased in the lesional skin of patients with itchy skin disorders including atopic dermatitis (AD; 1), (ii) its receptors are localized in dorsal

root ganglia in humans (5) and in dorsal root ganglia and cutaneous nerve fibres in mice (6), (iii) scratching behaviour is increased in transgenic mice overexpressing IL-31, in mice injected with IL-31 (1) and, as shown very recently, in dogs injected with IL-31 (7). However, there are no *in vivo* data available on the induction of itch by IL-31 in humans.

Itch is the most prominent symptom of AD, and IL-31 has been claimed to be critical for itch in AD. IL-31 is expressed in activated CD4⁺ T cells in TH2-skewed conditions (1). Skin homing (CLA⁺) circulating T cells were shown to produce IL-31 after activation, and T cells in the epidermis of patients with AD express IL-31 mRNA (8). Lesional and uninvolved skin of patients with AD reportedly exhibit up-regulated mRNA expression of IL-31 as compared to healthy controls and patients with psoriasis (5). Also, in lesional skin of patients with AD and patients with allergic contact dermatitis, IL-31 mRNA is up-regulated as compared to patients

with psoriasis and healthy controls (9). Analyses on the protein level confirmed elevated expression of IL-31 in infiltrating inflammatory cells in AD lesions, as compared to perilesional AD and lesional psoriasis, Sezary syndrome, mycosis fungoides and alopecia areata samples (10). Exposure of the skin of patients with AD to *staphylococcal* superantigen has been demonstrated to induce IL-31 mRNA expression *in vivo*, and the exposure of peripheral blood mononuclear cells (PBMCs) to *staphylococcal* superantigens *in vitro* leads to IL-31 expression in both healthy controls and patients with AD (patients with AD > healthy controls; 5). In adults, serum IL-31 levels have been shown to be higher in patients with AD as compared to healthy controls and correlate with disease severity, regardless of AD type (extrinsic *vs* intrinsic; 11). Similarly, serum IL-31 levels were also reported to be significantly higher in children with AD than in healthy controls and correlated with disease severity (12). Taken together, the reports implicate IL-31 as a major driver of itch and disease severity in patients with AD. But as of now, there is no direct evidence that IL-31 induces itch in humans. The present study was performed to assess the role of IL-31 in the induction of itch in patients with AD and healthy subjects.

Materials and methods

Characterization of the subjects and skin challenge

The study was approved by the Ethics Committee of the Charité – Universitätsmedizin Berlin, No. EA4/063/12, and informed consent was obtained from all participants.

In this study, all tested substances were applied by skin prick testing, which results in minimal local tissue trauma, triggers less unspecific reactions as compared to intracutaneous injections (13) and allows for substance deposition around the dermo-epidermal junction, close to cutaneous itch receptors and IL-31-receptor-expressing keratinocytes.

IL-31 was first prick-tested on the volar surface of the forearm in four subjects (physicians, coauthors of the paper) with concentrations starting from 1 µg/ml up to 1 mg/ml, increasing logarithmically: 1 and 10 µg/ml did not result in itch or visible skin reactions; 100 µg/ml, in two of four subjects, and 1 mg/ml, in all subjects, induced long-lasting skin erythema (visible up to 48 h after skin challenge). Subsequently, 10 healthy subjects with a negative history of atopy (seven women, mean age ± SD: 31.2 ± 4.9 years) and 10 patients with AD (eight women, mean age ± SD: 30.7 ± 6.3 years) were challenged with IL-31 (1 mg/ml, volar forearm) and 0.9% NaCl (negative control, contralateral volar forearm) by skin prick testing. 50% of subjects received IL-31 solution on the right side, and the remaining 50% on the left side, in a randomized and blinded order. In patients with AD, only unaffected skin of the forearm was challenged. Four of the patients with AD exhibited allergic rhinitis, and three of them also had allergic asthma. The severity of AD was assessed using scoring AD (SCORAD) on the first test day, before skin challenge. The mean SCORAD value ± SD of patients with AD was 27.1 ± 13.1. four patients with AD

exhibited white dermographism; the other six patients with AD and all healthy controls showed red dermographism.

In 20 additional healthy, nonatopic subjects (15 women, mean age ± SD: 34.0 ± 8.1 years), histamine (10 mg/ml; histamine dihydrochloride; ALK-Abello, Horsholm, Denmark) was applied by skin prick testing (forearm, 50% on the left side). Skin challenges were always performed between 9.00 and 11.00 a.m. Study subjects did not use any topical steroids at their forearms, systemic immunosuppressive treatment within the last 30 days preceding test procedures or antihistamines within the last 7 days preceding the skin tests.

Assessment procedures

Itch intensity was assessed using a 10-cm visual analogue scale (VAS). Subjects were asked to evaluate itch intensity every minute over the first 60 min after challenge and to record the intensity and duration of every itch episode at the challenge site within the next 72 h. Skin erythema intensity was measured using a Mexameter (Courage & Khazaka, Cologne, Germany). The measurement principle is based on the absorption capacity of a monochromatic light of wavelength corresponding to the spectral absorption peak of haemoglobin, reflecting haemoglobin content and, in consequence, blood perfusion of the skin. Results are expressed as arbitrary units (AU) and may range from 0 to 999. For the analyses, erythema increment was applied, resulting from subtraction of the initial erythema value from the value at each subsequently analysed time point. Skin erythema was assessed before, 20 and 60 min, 3, 6, 24, 48 and 72 h after challenge. Skin erythema area was measured with a ruler and recorded at the same time points.

Statistical analysis

Statistical analyses were performed using Statistica 7.0 (StatSoft Inc., Tulsa, OK, USA), and graphs were prepared using Prism software (GraphPad, San Diego, CA, USA). Three-way analysis of variance (ANOVA) for repeated measurements was employed, with time and challenging substance predefined as a within-subjects factor and health status (AD *vs* healthy controls) predefined as a between-subjects factor. Post-test with Bonferroni's adjustment was employed when appropriate. Normality of distribution of variables was tested with Kolmogorov-Smirnov test, sphericity was confirmed with a Mauchly's test, and homogeneity of variance was tested with Levene's test. Statistical significance was set at $P < 0.05$.

Results

Interleukin 31 does not induce immediate itch responses

All 20 histamine-challenged subjects reported immediate itch responses (i.e. starting within 5 min after challenge), disappearing within 26 min in all of them. IL-31 challenge did not induce immediate itch in any of the 20 subjects except for one patient with AD, who reported mild pruritus for 3 min

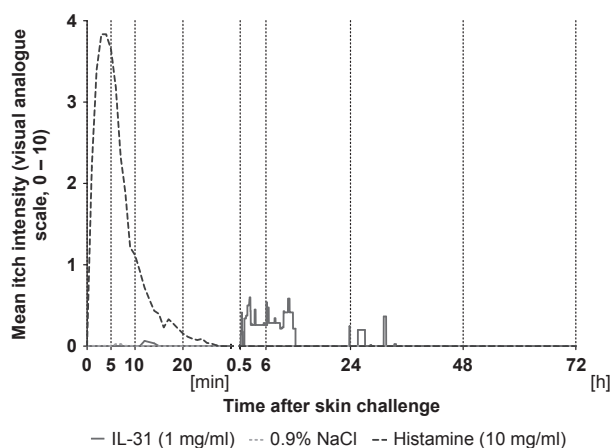


Figure 1 IL-31 does not induce itch in humans in the first 30 min after application.

within the first 30 min after IL-31 application. Two subjects (one patient with AD) reported immediate mild pruritus (lasting for 2 and 6 min, respectively) after NaCl skin prick testing (Fig. 1).

IL-31 challenge can induce late itch responses

Late itch responses, starting at minute 142.6 on average, were found in nine of 20 subjects (five patients with AD) challenged with IL-31 (Fig. 1). In contrast, no subject reported itch later than 30 min after skin prick testing with NaCl or histamine. There was no difference in the onset of itch between patients with AD and healthy volunteers. There was also no significant difference in the duration or maximum intensity of IL-31-induced itch between patients with AD and healthy volunteers (Fig. 2A,B) or in the area under the curve for itch intensity in the observation period (up to 72 h after challenge) between patients with AD and healthy volunteers (data not shown).

IL-31 skin challenge results in skin erythema

IL-31 challenge was found to induce strong and long-lasting erythema in almost all subjects, increasing in size and intensity over the first 3 h after application (Fig. 3A,B). Twenty minutes after skin challenge with IL-31, erythema was present in 10 subjects (five patients with AD); 1 h after skin challenge, erythema was present in 14 subjects (six patients with AD); and by 3 h after skin challenge, all but two patients with AD and all healthy controls had developed erythema. The two patients with AD had white dermographism and did not develop any erythema over the whole observation period (72 h). Forty-eight hours after skin challenge with IL-31, four subjects (all healthy volunteers) still showed skin erythema, and 72 h after challenge, this had resolved in all of them. Erythema intensity was significantly more intensive in IL-31- than in NaCl-challenged sites (ANOVA; $P < 0.001$), but there was no difference in IL-31-induced erythema between patients with AD and healthy controls (Fig. 3A). The size of

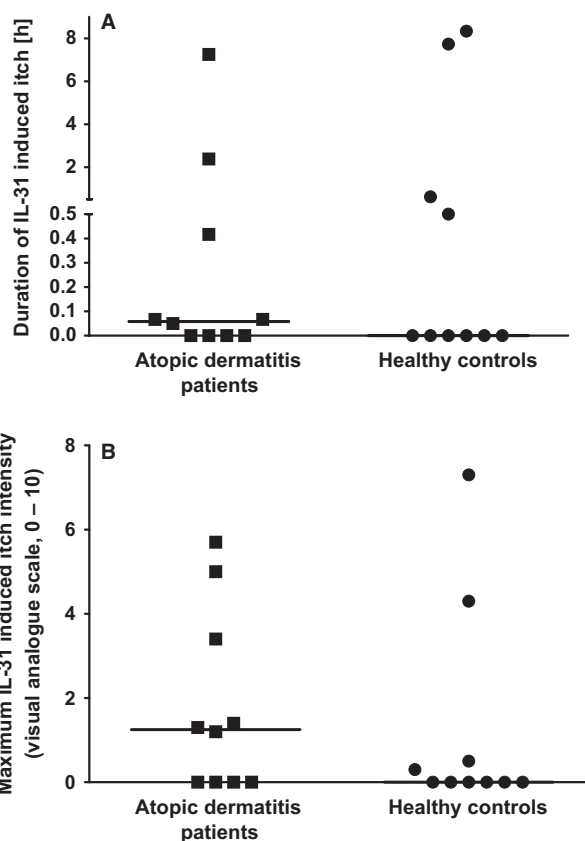


Figure 2 There are no differences in the characteristics of IL-31-induced itch between patients with atopic dermatitis and healthy controls. Median values are shown as horizontal lines. (A) Itch duration. (B) Maximum itch intensity.

erythema after IL-31 provocation was somewhat more pronounced in healthy controls as compared to patients with AD, showing a trend towards statistical significance (ANOVA; $P = 0.068$; Fig. 3B).

Discussion

Our results show that IL-31 does not induce immediate itch responses in humans. In contrast, and very different from responses to histamine, IL-31 induced inconsistently delayed and mild itch, with no major differences observed in patients with AD and healthy controls. Surprisingly, IL-31 resulted in strong and long-lasting skin erythema in the majority of subjects challenged, with weaker responses in patients with AD than in healthy controls. The observed lack of immediate itch and the delay in pruritus responses argue against an immediate induction of itch by IL-31 through its own receptors on skin neuronal endings (3, 14).

The only published data on experimentally IL-31-induced itch derive from animal experiments in mice and dogs and confirm the ability of IL-31 to induce delayed itch responses. Dillon and coworkers reported that mice administered with murine IL-31 through continuous, subcutaneous infusion

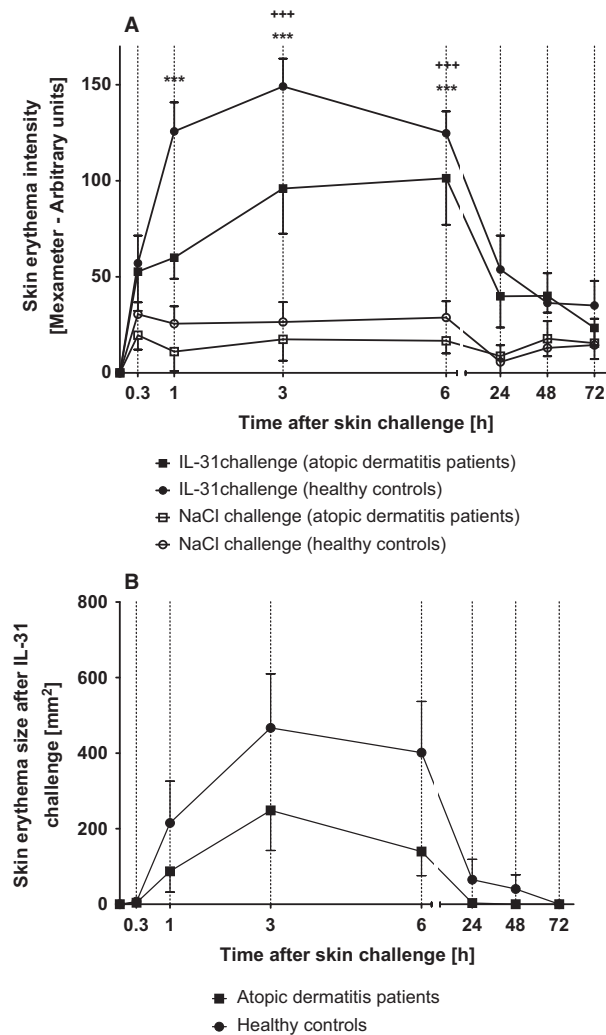


Figure 3 Kinetic of skin erythema in patients with atopic dermatitis (AD; $n = 10$) and healthy controls ($n = 10$). *** $P < 0.001$, difference between IL-31 and 0.9% NaCl skin challenge in healthy controls; ††† $P < 0.001$, difference between IL-31 and 0.9% NaCl skin challenge in patients with AD, post-test with Bonferroni's adjustment. (A) Erythema intensity after IL-31 and 0.9% NaCl skin challenge. (B) Erythema size after IL-31 skin challenge.

develop scratch behaviour not until the third day of infusion (1). Very recently, Gonzales et al. (7) reported pruritus-related responses (scratching, licking, chewing, scooting, head shaking and body rubbing) in beagle dogs receiving canine IL-31 (intravenous, subcutaneous and intradermal). However, the observation period in this study started 30 min after IL-31 challenge, so no data on the immediate itch response are available. One limitation of animal models of itch is that they deliver only indirect data such as scratching behaviour, and these may be influenced also by other factors such as anxiety and stress.

Effector cells for IL-31 in the skin include keratinocytes. Both IL-31 RA and OSMR components of a functional

IL-31 receptor complex are constitutively expressed on human keratinocytes, which respond to IL-31 stimulation by releasing chemokines. This suggests that they are involved in the recruitment of polymorphonuclear cells, monocytes and T cells, sustaining and promoting local inflammation (1, 8, 10). Interestingly, Kasraie et al. (3) showed recently that IL-31 stimulation of human keratinocytes can also induce the secretion of vascular endothelial growth factor (VEGF), as was previously proven also for human bronchial epithelial cells (15). We have proposed, very recently, a role of VEGF in pruritus, demonstrating dramatic improvement of itch after treatment with the monoclonal VEGF antibody bevacizumab, and increased serum and skin levels of VEGF in prurigo (16). Taken together, these results strengthen the hypothesis that IL-31 exerts its pruritic effects indirectly via keratinocytes and subsequently released secondary mediators, such as VEGF.

Itch intensity, in contrast to erythema responses, was not different between IL-31-challenged AD patients and healthy controls, suggesting that uninvolved skin of patients with AD is not more sensitive to IL-31, as compared to the skin of healthy controls. This observation is in line with results on the expression of IL-31 receptors by Neis and coworkers, showing that the expression of IL-31 RA mRNA in biopsy specimens taken from the lesional skin of AD, allergic contact dermatitis, psoriasis patients or from healthy controls was not different, and by Nobbe and coworkers who showed recently that IL-31 RA and OSMR proteins are expressed constitutively on human keratinocytes and that their expression is not different between AD lesional and perilesional skin and lesional skin specimens taken from patients with many different dermatologic conditions (9, 10). Moreover, Nobbe and coworkers showed that IL-31 is overexpressed in the lesional skin of patients with AD as compared to the uninvolved AD skin and the lesional skin in many dermatologic disorders (10). Our study does not allow us to answer the question whether or not IL-31 plays a role in the pathogenesis including the induction of itch in AD. If it does, then this appears to be due to the overexpression of IL-31 in AD skin rather than due to increased sensitivity of AD skin to IL-31.

Skin erythema reflects local vasodilatation, and the mechanisms leading to vasodilatation upon IL-31 challenge remain to be elucidated. One possible explanation is that IL-31 itself or the mediators released in response to IL-31 bind to peripheral sensory nerves causing neurogenic inflammation. Again, the time-delayed appearance of local erythema argues in favour of a role of secondary mediators, released in response to IL-31. Another possible explanation is an IL-31-induced release of mediators from keratinocytes, which then act on endothelial cells. One such mediator could be VEGF, which stimulates the production of nitric oxide by endothelial cells and leads to vasodilatation (17, 18). Decreased erythema responses in patients with AD as compared to healthy controls were previously described also for IL-2 (19), histamine, compound 48/80 (20) and substance P (21) application and are likely to result from cutaneous pallor and vascular abnormalities in AD. The fact that the only two patients with AD

who did not develop erythema after IL-31 challenge exhibited white dermographism supports this notion.

It must be noted that in the current study an exogenous IL-31 was used, which might result in different tissue concentrations, distribution and release kinetics as compared to endogenous IL-31. A further limitation is that only uninvolved skin of patients with AD was challenged. In inflammatory skin, a reaction to IL-31 could be different because of possible interplay with other mediators of inflammation or different expression of IL-31 receptors in effector cells.

Taken together, IL-31 skin challenges in humans inconsistently induce mild and delayed itch sensations that are not different between patients with AD and healthy volunteers. This suggests that, in humans, the role of IL-31 in itch induction may be minor and limited to the release of pruritogenic mediators from keratinocytes.

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Conflicts of interest

The authors state no relevant conflicts of interest.

Author contributions

TH designed the study, performed statistical analyses and drafted the manuscript. MMe designed the study, contributed to interpretation of the data and revised the manuscript. MMA designed the study, contributed to interpretation of the data, revised the manuscript and finally approved the version to be published. All authors collaborated in the retrieval of data and revising the manuscript.