

expressed. These findings suggest that lipid metabolism is affected in Dsk5 sebocytes, possibly involving a deregulation of *Scds*.

Conclusions

Our data indicate that the EGFR is an important regulator of SG size, cell number and lipid output. Cell transplantation and lineage tracing studies revealed that the SG is fuelled by multiple stem cell populations (22,23). Committed cells entering the SG initially locate at the gland periphery and proliferate for some time, before accumulating large amounts of lipids to finally disintegrate and release their content via holocrine secretion (24,25). Proliferating sebocytes express high levels of c-myc (20), and increased c-myc expression results in larger and cell-richer SGs (17–19). Our finding of increased c-myc expression in the skin of Dsk5 mice suggests that this transcription factor mediates the proliferative actions of the EGFR in sebocytes. As a consequence of the increased cell number, Dsk5 SGs become enlarged and synthesize higher amounts of lipids. The finding of deregulated expression of *Scds*, critical lipid synthesis enzymes during late sebocyte differentiation (21), indicates that the lipid metabolism is affected in the enlarged Dsk5 SGs.

The apparent discrepancy to the reported EGFR-mediated inhibition of sebaceous lipogenesis *in vitro* (7,8) can be explained by two non-mutually exclusive hypotheses. Possibly, lipid synthesis is indeed reduced at the single cell level in our model, but the increased number of cells/gland results in larger amounts of sebum. More probably, as EGFR expression is downregulated in mature sebocytes *in vivo* (5,6), EGFR activation will have less influence on lipogenesis in our model compared to the *in vitro* culture systems, in which sebocytes undergo only partial differentiation (9).

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Author contribution

M.D. performed the research and interpreted the data; M.H.A. and E.W. contributed essential tools; M.R.S. designed the study, interpreted the data, and wrote the manuscript.

Conflict of interests

The authors state no conflict of interests.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Data S1. Materials and methods.

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Letter to the Editor

A single dose of interleukin-31 (IL-31) causes continuous itch-associated scratching behaviour in mice

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Abstract: We investigated the effects of a single dose of mouse interleukin-31 (IL-31) on scratching behaviour in comparison with spontaneous skin-lesion- or serotonin (5-HT)-induced scratching behaviour in NC/Nga and BALB/c mice. Intradermal (i.d.) injection of IL-31 caused a gradual increase in long-lasting

scratching (LLS, over 1.5 s) about 3 h after administration followed by a gradual decrease for over 24 h after administration. I.d. injection of IL-31 significantly increased the total LLS counts/24 h but not short-lasting scratching (SLS, 0.3–1.5 s). In skin-lesioned NC/Nga mice, the LLS but not SLS counts were

significantly higher than those in non-skin-lesioned NC/Nga mice. We also investigated 5-HT-induced scratching in BALB/c mice, SLS but not LLS increased immediately after the injection and then decreased to baseline after at 20 min. These results suggest that IL-31 may participate in the sensation of itching and

promote scratching behaviour in skin-lesioned NC/Nga mice, an animal model of atopic dermatitis (AD).

Key words: IL-31 – itch – pruritogen – scratch – serotonin

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Background

As itching is a characteristic symptom of various forms of dermatitis, especially AD, it constitutes a major diagnostic criterion for patients with these diseases (1,2). The factors that induce itching and promote scratching have not yet been clearly identified. IL-31 is a recently discovered T-cell cytokine that, when overexpressed in mice, results in pruritus and dermatitis similar to AD (3). Consequently, we suggested that IL-31 may be one of the most important factors in inducing itching and promoting scratching in NC/Nga mice. It has been shown that cutaneous IL-31 mRNA is significantly increased in NC/Nga mice and that these changes coincide with increases in scratching counts (4,5). While IL-31-induced itching and scratching are important factors in the development of dermatitis, the mechanisms that underlie these phenomena are poorly understood.

Questions addressed

We previously designed new methods to study the pattern of spontaneous scratching in NC/Nga mice (6). NC/Nga mice, an animal model of AD, exhibit chronic itching and spontaneously develop skin lesions under conventional conditions (skin-lesioned NC/Nga mice), but not under SPF conditions (7), and trimming of their toenails inhibits the development of dermatitis and has a therapeutic effect (8). Thus, scratching is the most important factor that promotes skin lesions in NC/Nga mice. 5-HT elicits dose-dependent scratching behaviour in several strains of mice (9,10). In this study, the effect of a single dose of IL-31 on scratching behaviour was examined in NC/Nga or BALB/c mice and compared with the scratching behaviour of 5-HT-treated BALB/c and skin-lesioned NC/Nga mice.

Experimental design

Mouse IL-31, cDNA spanning amino acid 24–163, was cloned in frame with pET30A (Novagen, Darmstadt, Germany), and the construct was transformed in BL-21 cells (Novagen). After induction with isopropyl- β -D-thiogalactopyranoside, IL-31 protein was purified under denaturation conditions by nickel-chelating sepharose (Qiagen, Benelux B.V., Netherlands), dialysed in phosphate buffer solution (11). Scratching behaviour was measured as we described previously (12). All data are presented as the mean $\pm$ SEM. Statistical significance was analysed using the parametric Student's *t*-test. *P* values of less than 0.05 were considered statistically significant.

Results

The acute injection of IL-31 (1.0 μ g/site, i.d.) increased LLS (Fig. 1a,e) but not SLS (Fig. 1b,f) from 3 and 2 h after injection, especially at night time, over 24 h after injections in both mice. The total counts for 0.3 to 3.0 μ g/site of IL-31 were significantly higher than that in the saline group (Fig. 1c,g). No significant changes were observed in SLS in both mice (Fig. 1d,h). The LLS of skin-lesioned NC/Nga mice was significantly higher than that in NC/Nga mice (Fig. 2a,b), but no significant changes were observed in SLS (Fig. 2c,d). The LLS induced by 5-HT (0.25 μ g/

site, i.d.) in BALB/c mice tended to increase immediately after injection, but only temporary (Fig. 2e,f). In contrast, the SLS induced by 5-HT significantly increased immediately after injection, but ageing only temporary (Fig. 2g,h).

Conclusion

A single i.d. injection of IL-31 (0.3, 1.0 and 3.0 μ g/site) continuously increased LLS, but not SLS, in NC/Nga and BALB/c mice. There was no difference on sensitivity of IL-31-induced scratching behaviour between NC/Nga and BALB/c mice. We measured spontaneous scratching behaviours of various durations in several strains mice including skin-lesioned NC/Nga mice. There was marked difference in LLS behaviour in skin-lesioned NC/Nga mice (6). Scratching for a duration of 0.3–1.5 s duration (SLS) is

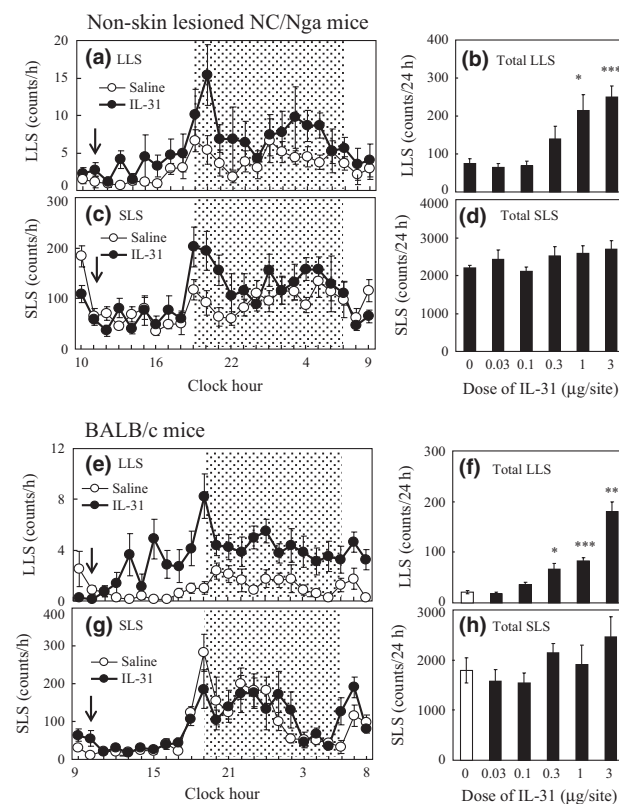


Figure 1. Effect of a single dose of IL-31 on scratching behaviour in NC/Nga and BALB/c mice. LLS; long-lasting scratching (over 1.5 s) induced by IL-31 (1.0 μ g/site, i.d.) was measured for 24 h in NC/Nga mice and BALB/c mice (a, e). Total LLS counts for 24 h after the injection of various doses of IL-31 (0.03–3.0 μ g/site, i.d.) in NC/Nga and BALB/c mice (b, f). SLS; short-lasting scratching (0.3–1.5 s) induced by IL-31 (1.0 μ g/site, i.d.) was measured for 24 h in NC/Nga and BALB/c mice (c, g). Total SLS counts for 24 h after the injection of various doses of IL-31 (0.03–3.0 μ g/site, i.d.) in NC/Nga and BALB/c mice (d, h). An arrow-shaped mark indicates administration point. The lateral axis indicates the clock hour, and the shaded area represents the night time (7:00 pm to 7:00 am). **P* < 0.05, ****P* < 0.01 and *****P* < 0.001 compared with saline injection mice (Student's *t*-test). All data are shown as the mean $\pm$ SE (*N* = 8).

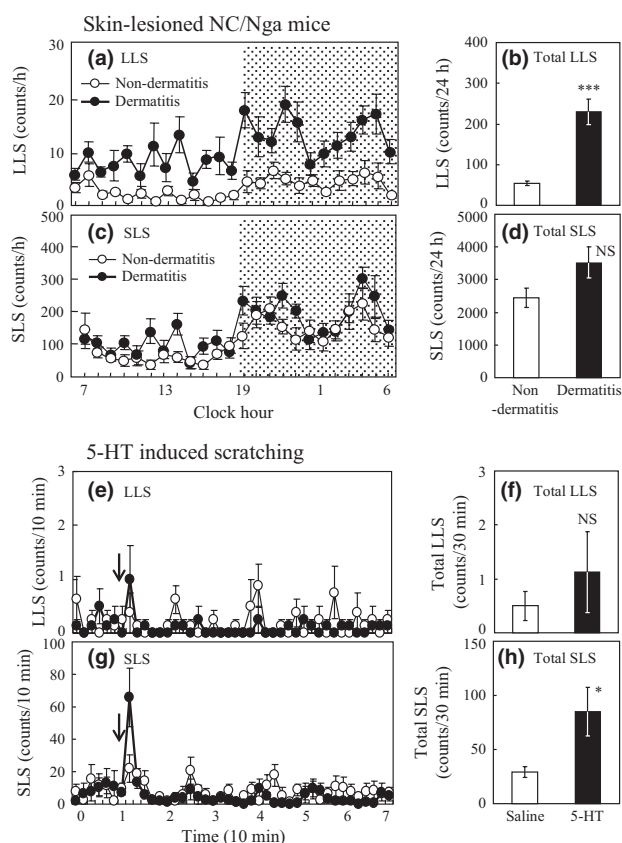


Figure 2. The scratching behaviour in skin-lesioned NC/Nga and 5-HT-treated BALB/c mice. LLS; long-lasting scratching (over 1.5 s) in skin-lesioned NC/Nga and 5-HT-treated BALB/c mice were measured for 24 h (a, e). Total LLS counts for 24 h in skin-lesioned NC/Nga and 5-HT-treated BALB/c mice for 0.5 h (b, f). SLS; short-lasting scratching (0.3–1.5 s) in skin-lesioned NC/Nga mice and 5-HT-treated BALB/c mice were measured for 24 h (c, g). Total SLS counts in skin-lesioned NC/Nga mice and 5-HT-treated BALB/c mice (d, h). The lateral axis indicates the clock hour, and the shaded area represents the night time (7:00 pm to 7:00 am) (a, c). An arrow-shaped mark indicates administration point (e, g). * $P < 0.05$ compared with saline injection mice (Student's *t*-test). All data are shown as the mean $\pm$ SE ($N = 8$).

seen in several strains mice, similar to locomotor activity, and thus, SLS is considered to be a general feature of behaviour and does not contribute to the development of dermatitis. On the other hand, as Protopip and prostaglandin D₂ agonist specifically inhibits LLS counts and dermatitis, so that LLS closely partici-

pates in the dermatitis in skin-lesioned NC/Nga mice (13–15). These results indicate that LLS is a characteristic behaviour seen only in mice with symptoms of dermatitis. As reported previously, LLS can be induced in NC/Nga mice by housing them with skin-lesioned NC/Nga mice (16) and other strains of mice (17). These data suggest direct stimulation of the skin surface of mice by mite (18). This direct stimulation may induce the expression of some endogenous agents under the skin which have strong pruritic activity. IL-31 is a novel cytokine that is involved in pruritic skin diseases including atopic dermatitis (3). We have demonstrated that cutaneous IL-31 gene transcripts were significantly increased in skin-lesioned NC/Nga mice, and these changes coincided with increases in scratching counts (4,5). In addition, anti-IL-31 antibody reduces scratching behaviour in NC/Nga mice with atopiclike dermatitis (19). Serum IL-31 was previously shown to be overexpressed in patient with AD (20). Moreover, serum IL-31 protein and tissue IL-31 mRNA levels are associated with severe pruritus in humans (21,22). This suggests that IL-31 is one of the most important factors in inducing itching and promoting scratching in mice and humans. Many endogenous chemicals are locally pruritogenic when injected into the skin (23). However, the main pruritogen in AD is not yet clear. A single intradermal injection of histamine (12), 5-HT (24), compound 48/80 (25), substance P (26) or PAR2 (24) elicited SLS, but not LLS, behaviour that began during the initial 5-min observation period and persisted for at least 30 min. On the other hand, IL-31 elicited LLS, but not SLS, behaviour that began gradually about 2 h after intradermal injection and persisted over 24 h. This phenomenon is completely different from those seen with other locally injected pruritogens directly via their specific receptors. IL-31 might indirectly like as via activation/sensitization of TRPV1 stimulate sensory neurons of the skin to initiate itch. These findings suggest that IL-31 is a main pruritogen in skin-lesioned NC/Nga mice, as the scratching behaviour induced by the injection of IL-31 closely resembles that seen in skin-lesioned NC/Nga mice.

Author contribution

I Arai performed the research and wrote the manuscript. N Akiyama contributed essential reagents. M Tsuji, H Takeda and S Saito designed the research study.

Conflict of interests

The authors have declared no conflicting interests.

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