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Rapid communication

Expression of IL-31 gene transcripts in NC/Nga mice with atopic dermatitis

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Abstract

To search for the pruritogen of atopic dermatitis, a characteristic symptom in atopic dermatitis patients, we examined interleukin-31 (IL-31) mRNA expression in NC/Nga mice as an animal model of atopic dermatitis. The expression of IL-31 mRNA in the skin of NC/Nga mice with scratching behavior was significantly higher than that in NC/Nga mice without scratching behavior. Our findings suggest that IL-31 may participate in the cause of itch sensation and promote scratching behavior in NC/Nga mice with atopic dermatitis.

Keywords: IL-31; Scratching; NC/Nga mice

NC/Nga mice develop dermatitis when the mice were raised under conventional conditions, but not so under specific pathogen-free (SPF) conditions (Suto et al., 1999). Furthermore, skin-lesioned NC/Nga mice frequently scratch their body using their hind paws. As all of these features are similar to events in atopic dermatitis patients, the NC/Nga mice may be a suitable model of human atopic dermatitis. We reported that scratching behavior (over 1.5 s duration) correlates with symptom of dermatitis (Takano et al., 2003), and dexamethasone and taclolims suppressed it, but not so antihistaminergics (Takano et al., 2004; Hashimoto et al., 2004a), which is consistent with human data (Klein and Clark, 1999); hence, histamine is not considered to be a major pruritogen in NC/Nga mice.

Recently, Dillon et al. (2004) reported that transgenic mice overexpressing IL-31 showed increased scratch behavior and developed severe dermatitis. They also evaluated the increase in IL-31 receptor A mRNA expression in asthmatic model, but it remained to be evaluated if IL-31 is involved in the development of disease in a naturally occurring dermatitis model. To determine if IL-31 plays a pivotal role in promoting itch sensation in an animal model of atopic dermatitis, we examined the IL-31 mRNA expression in NC/Nga mice and found that IL-31 mRNA were significantly increased in NC/Nga mice expressing itch-associated scratch behavior.

Seven-week-old male NC/Nga mice ($N=5$) under SPF condition were purchased from Charles River Japan (Kanagawa, Japan). To induce stable scratching behavior in a short period, NC/Nga mice were housed in the same cage with 15-week-old skin-lesioned NC/Nga mice, under conventional condition for 2 weeks. We referred these scratch-induced mice as CNV-NC/Nga mice and NC/Nga mice housed without skin-lesioned NC/Nga mice as SPF-NC/Nga mice. At the end of the 2 weeks, spontaneous scratching behavior by SPF-and CNV-NC/Nga mice was measured for 24 h (15:00–15:00 h) as reported earlier (Takano et al., 2003). Scratching counts of CNV-NC/Nga mice were significantly higher than that of SPF-NC/Nga mice (414.4±58.9 and 8.2±3.6 counts/24 h, Fig. 1A) and then, and eczematous lesions and obvious histopathological changes in the tissue sections were not observed.

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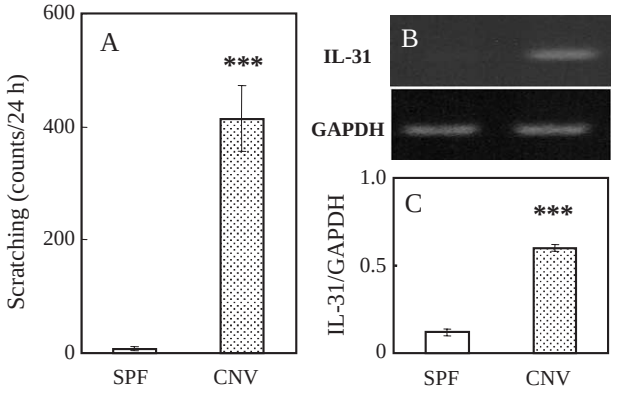

Fig. 1. Scratching counts and IL-31 mRNA expression in the skin of NC/Nga mice. (A) Total scratchings for 24 h of SPF-NC/Nga (SPF) ($N=5$) and CNV-NC/Nga mice (CNV) ($N=5$). ***$P<0.001$ when compared with scratching counts of SPF-NC/Nga (Student's t-test). (B) Typical pattern of RT-PCR analysis of IL-31 and GAPDH mRNA expression in the skin of NC/Nga mice. Sizes of the PCR products were 327 bp (IL-31) and 452 bp (GAPDH). (C) The mean ratio of IL-31/GAPDH mRNA band intensity of NC/Nga mice. ***$P<0.001$ when compared with values of SPF-NC/Nga (Student's t-test).

The total RNA was isolated from the dorsal skin of each mouse using Trizol (Invitrogen, Carlsbad, USA). cDNA was synthesized using both SuperScript III First-Strand Synthesis System (Invitrogen, Carlsbad, USA) for reverse transcription (RT) and Takara Ex Taq (Takara, Tokyo, Japan) for polymerase chain reaction (PCR). PCR amplification was carried out on cDNA equivalent to 100 ng of starting mRNA, using primers for IL-31 (forward: 5′-TCGGTCATCATAGCACATCTGGAG-3′ and reverse: 5′-GCACAGTCCCTTTGGAGTTAAGTC-3′), glyceroaldehyde 3-phosphate dehydrogenase (GAPDH) (forward: 5′-ACCACAGTCCAT GCCATCAC-3′ and reverse: 5′-TCCACCACCCTGTTGCTGTA-3′) synthesized at Qiagen (Tokyo, Japan). cDNA was heated for 2 min at 94 °C, and then amplified by 33 cycles at IL-31 or 18 cycles at GAPDH (94 °C for 30 s, 58 °C for 30 s, 72 °C for 1 min) followed by 10 min of extension at 72 °C. The PCR products were electrophoresed on 1.5% agarose gels, stained with ethidium bromide and visualized by UV light. Values were normalized to GAPDH. IL-31 transcript was clearly detected in CNV-NC/Nga mice, but not so in SPF-NC/Nga mice (Fig. 1B). The mean ratio of IL-31/GAPDH mRNA band intensity of CNV- was significantly higher than that of SPF-NC/Nga mice (0.60±0.02 and 0.12±0.02, Fig. 1C).

We found that IL-31 is expressed in the skin of an animal model of atopic dermatitis. Within 2 weeks, CNV-NC/Nga mice showed an increased number of scratching behavior, while dermatitis was not observed in this period, and it developed after 4 weeks (Hashimoto et al., 2004b). Therefore, the conditions of the skin in CNV-NC/Nga mice in this experiment likely mimic the very early stage of dermatitis. Our data that IL-31 expression was very clearly up-regulated in CNV-NC/Nga mice is consistent with Dillon's report, and it is interesting that increase in IL-31 expression precede the appearance of dermatitis. This suggests that IL-31-induced excessive scratching behavior might be the important factor leading to dermatitis in NC/Nga mice. Further investigation determines if IL-31 directly or indirectly induces itch and/or inflammation. Indeed, IL-31 may be one important factor related to itch sensation and promoting scratch behavior in NC/Nga mice with atopic dermatitis.

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