

Itch-associated scratching contributes to the development of dermatitis and hyperimmunoglobulinaemia E in NC/Nga mice

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Abstract: Atopic dermatitis (AD) is related to immunoglobulin E (IgE) production, and a type-1 and type-2 helper T cell (Th1/Th2) imbalance has been hypothesized as the aetiology. While itching and scratching are important factors in the development of dermatitis, the mechanisms underlying these phenomena are poorly understood. We investigated the relationship between scratching, transepidermal water loss (TEWL), signs of dermatitis and serum Ig levels in NC/Nga mice, a model of AD. We also sensitized specific pathogen-free (SPF)-NC/Nga mice and BALB/c mice to mite antigen to determine the effects of IgE overproduction on scratching and investigated the involvement of mast cells and T/B cells in the induction of scratching using WBB6F1-W/W^v mice and C.B.17/Icr-scid mice. Under conventional conditions, the scratch counts increased, followed by increases in TEWL and the inflammation score in

NC/Nga mice that were not kept under SPF conditions. However, no change was observed in scratching, TEWL, or signs of dermatitis in mite antigen-sensitized SPF-NC/Nga and BALB/c mice, although the serum total IgE, IgG₁ and IgG_{2a} levels increased. The scratch count increased significantly in both the WBB6F1-W/W^v mice and C.B.17/Icr-scid mice when they were co-housed with skin-lesioned NC/Nga mice, raised under conventional conditions. These results show that IgE overproduction results from itch-associated scratching-induced dermatitis in NC/Nga mice.

Key words: atopic dermatitis – immunoglobulin E – NC/Nga mouse – scratching behaviour

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Introduction

Atopic dermatitis (AD) is one of the most common skin diseases accompanied by severe itching in children (1). Itching and scratching are important factors in the development of AD (2), because lesions on the back only develop in areas that the patient can reach to scratch (3), and scratching aggravates the skin lesions of patients with AD (2). It is well known that more than 80% of patients with AD have an increased serum IgE level (4) and that this increase is correlated with the clinical symptoms (5). However, some patients with severe AD do not have an elevated IgE level (6). The increased serum IgE level is closely related to the production of type-2 helper T-cell (Th2) cytokines (7), and the overproduction of IgE and Th2 cytokines can result in allergic disorders. However, recent clinical studies have revealed that the immunological aspects of the skin lesions differ considerably according to the clinical stage of AD (8).

NC/Nga mice, an animal model of AD with chronic itching, spontaneously develop skin lesions with IgE overproduction, Th2 expression and invasion of the skin by inflammatory cells under conventional conditions, but not under SPF conditions (9). IgE overproduction and Th2-dominant states are widely thought to play important roles in the development of dermatitis. Recent studies have revealed that the presence of IgE and Th2 cells is indispensable to the development of dermatitis in NC/Nga mice (10,11). There are reports that NC/Nga mice raised under conventional conditions (CNV-NC/Nga mice) with skin lesions frequently scratch their face, ears and anterior part of their back with their hind paws (12), but that cutting off their toenails inhib-

its the development of dermatitis and has a therapeutic effect (13). Thus, scratching is the most important factor that promotes skin lesions in CNV-NC/Nga mice but the relationship between the elevation of serum IgE and the development of dermatitis in CNV-NC/Nga mice remains unclear.

Dermatophagoides species, including *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*, have the highest population among house dust mite, and mite antigens have been suggested to play important roles in the onset and/or development of AD. CNV-NC/Nga mice with skin lesions were infected with fur mites such as *Mycopetes musculus* (14) and *Myobia muscili* (15), which are specific for mice and rats, and close relationships have been noted among mite infection, serum IgE levels and the severity of dermatitis. Exposure to *Dermatophagoides farinae* mite antigen causes an elevation of serum IgE levels and a shift in the Th1/Th2 balance to a Th2-dominant state in both SPF-NC/Nga and BALB/c mice (16,17), and the dermatitis in NC/Nga mice appears to be triggered by environmental factors, including mite, although the mechanisms by which they trigger dermatitis have never been identified. In this study, we investigated the relationship between scratching, the cutaneous barrier, sign of dermatitis and the serum total IgE, IgG₁ and IgG_{2a} levels in NC/Nga mice.

Materials and methods

Animals

Male SPF- and CNV-NC/Nga mice at 4–13 weeks of age and BALB/c, WBB6F1-W/W^v mice (W/W^v mice, mast cell-deficient mice) and WBB6F1-^{+/+} mice (normal littermates) at 6 weeks of age were purchased from SLC Japan (Shizuoka, Japan). Male

C.B.17/Icr-scid (Scid mouse, T/B cell-deficient mice) and C.B.17/Icr-^{+/+} mice (normal littermates) at 6 weeks of age were purchased from CLEA Japan (Tokyo, Japan). The animals were housed under conditions of controlled temperature ($23 \pm 3^\circ\text{C}$), humidity ($50 \pm 20\%$) and lighting (lights on from 7:00 am to 7:00 pm). All the animals were given free access to food and tap water. All the experiments reported in this article were reviewed by the Institutional Animal Care and Use Committee of Taisho Pharmaceutical Co., LTD. and were in accordance with the Guidelines for Proper Conduct of Animal Experiments (Science Council of Japan, 2006).

Measurement of scratching counts

As itching elicits a strong desire to scratch, measuring scratching is a useful method of evaluating itching (18). Prolonged scratching (over 1.5 s) by NC/Nga mice is correlated with signs of dermatitis (19) and is inhibited by dexamethasone and tacrolimus, but not by antihistaminergics (20,21), consistent with clinical data (22). Scratching was measured as we described previously (23). The number of scratches was automatically detected and objectively evaluated using MicroAct (Neuroscience, Tokyo, Japan). The analysis parameters for detecting the waves were as follows: threshold, 0.1 V; event gap, 0.2 s; minimum duration, 1.5 s; maximum frequency, 20 Hz; and minimum frequency, 2 Hz.

Measurement of transepidermal water loss (TEWL)

The disruption of the cutaneous barrier is characterized by TEWL, which is an indicator of the water evaporation rate from the skin surface and reflects the integrity of the stratum corneum water barrier (24). The TEWL level was measured quantitatively using a Tewameter[®] (TM300; Courage & Khazawa, Köln, Germany) on the anterior portion of the animal's back after shaving the back with an electric razor and was compared with the scratch count and the severity of the dermatitis.

Evaluation of inflammation score

Dermatitis was evaluated by assigning an inflammation score. Inflammation of the face, ears and the anterior part of the body was scored using the following scale: 0, none; 1, mild; 2, moderate; and 3, severe. This scoring was based on the severity of erythema/haemorrhage, oedema, excoriation/erosion and scaling/dryness. The total score (minimum 0 and maximum 12) for the four symptoms in each mouse was regarded as the score for that mouse (9).

Measurement of serum total IgE, IgG₁ and IgG_{2a}

As the serum IgE levels are correlated with the severity of the signs of dermatitis in most patients with AD (5) and the Th2 immune response is dominant, the serum total IgE, IgG₁ (Th2-associated) and IgG_{2a} (Th1-associated) levels were measured and compared with the scratch counts, the inflammation score and the TEWL value. Under isoflurane anaesthesia, blood samples were collected from the femoral vein and serum samples were obtained by centrifugation (800 g, 30 min, 4°C) and stored at -80°C until further use. The serum total IgE concentrations were measured using a Mouse IgE ELISA Quantitation kit (Bethyl Laboratories, Montgomery, TX, USA) and the serum IgG₁ and IgG_{2a} concentrations were measured using mAb pairs (A85-3 and A85-1 for IgG₁, R11-89 and R19-15 for IgG_{2a}; BD Biosciences, Franklin Lakes, NJ, USA) according to the manufacturer's instructions.

Sensitization of mice to mite antigen

As the sensitization of the mice shifted the Th1/Th2 balance towards the Th2-dominant state (16), the effect of sensitizing

SPF-NC/Nga and BALB/c mice to mite antigen was evaluated. *Dermatophagoides farinae* crude extract (LSL, Tokyo, Japan) was used as the antigen, and the back of the neck of 6-week-old SPF-NC/Nga and BALB/c mice was subcutaneously injected with a 0.25 mg dose of mite antigen/2 mg aluminium hydroxide gel/0.25 ml phosphate buffer, three times at 7-day intervals. The scratch counts, TEWL value and dermatitis inflammation score were measured 7 days after the final injection, a blood sample was collected, and the serum Ig levels were measured as described previously.

Scratching induction

Scratching was induced in SPF-NC/Nga mice by co-housing them with skin-lesioned CNV-NC/Nga mice (25,26) for 24 h or by painting mite antigen [0.1 mg *Dermatophagoides farinae* (LSL)/0.1 ml Vaseline] once on the back of the neck (27). The scratch count was measured as described earlier and compared between the two protocols. To investigate the involvement of mast cell and T/B cell in scratching, scratching was induced in W/W^v and Scid mice by co-housing them with skin-lesioned CNV-NC/Nga mice, and the scratch counts were determined as described earlier.

Histological study

Hair on the back of the neck of SPF-NC/Nga and CNV-NC/Nga mice at 4 and 13 weeks of age was shaved off using an electric razor. The block skin was removed and fixed in 10% buffered formalin solution. A block of forehead skin was removed and embedded in paraffin using conventional methods. Sections 3–4 μm thick were stained with haematoxylin–eosin (H.E.) or toluidine blue (T.B.). To determine the existence of T cells in the skin in each NC/Nga mice group, unstained paraffin sections were used for an immunohistochemical study. The de-waxed sections were boiled in 0.01-M citrate buffer solution at pH 6.0 (00-5000; Zymed Laboratories Inc., San Francisco, CA, USA) for 30 min for antigen retrieval and allowed to cool to room temperature. After the blocking of non-specific binding with 1% bovine serum albumin-phosphate-buffered saline, these sections were incubated with rabbit anti-CD3 polyclonal antibody (A0452, diluted $\times 1200$, 0.5 $\mu\text{g}/\text{ml}$; DAKO, Glostrup, Denmark) overnight at 4°C and processed for examination using the avidin–biotin peroxidase method.

Statistical analysis

The dermatitis score were reported as the median quartile deviation; other parameters were reported as the mean $\pm$ SEM. The non-parametric Wilcoxon test was used to statistically analyse the inflammation scores, and the parametric Student's *t*-test with Bonferroni correction was used to analyse the other value. *P*-values of <0.05 were considered statistically significant.

Results

Time course changes in scratch count, TEWL value, inflammation score and serum Ig levels in NC/Nga mice

The scratch count, TEWL value, inflammation score and serum total IgE, IgG₁, and IgG_{2a} levels in SPF- and CNV-NC/Nga mice were compared at 4, 7, 10 and 13 weeks of age. The scratch counts, TEWL value and inflammation score for CNV-NC/Nga mice were significantly higher than those for SPF-NC/Nga mice from 4, 7 and 10 weeks of age onwards and were markedly higher at 13 weeks of age (Fig. 1a,b,c). There were no changes in the

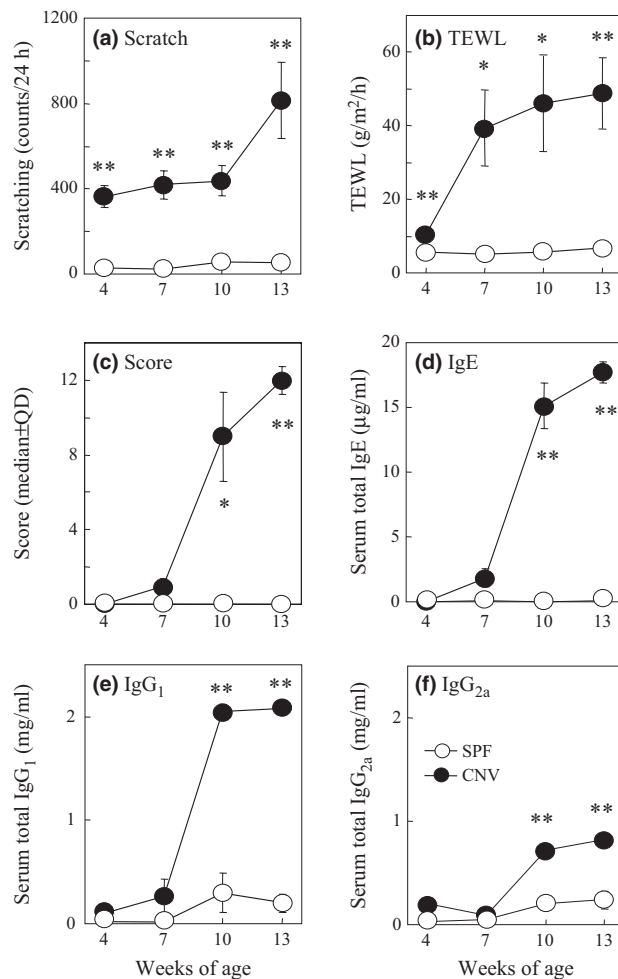


Figure 1. Time course changes in scratch counts, transepidermal water loss (TEWL) value, inflammation scores and serum Ig levels in NC/Nga mice. Scratch counts; spontaneous long-lasting scratching (over 1.5 s) by NC/Nga mice was measured the following day for 24 h (a), TEWL value; transepidermal water loss was measured using a skin-evaporative water recorder (b), dermatitis score; this scoring was based on the severity of erythema/haemorrhage, oedema, excoriation/erosion and scaling/dryness (c), serum IgE levels: a sign of Th2 immunity, (d), serum IgG₁ level; a sign of Th2 immunity (e), serum IgG_{2a} level; a sign of Th1 immunity (f) in specific pathogen-free (SPF)- and CNV-NC/Nga mice at 4, 7, 10 and 13 weeks of age were measured as described in the text. The values are means $\pm$ SEM from six mice. * P < 0.05 and ** P < 0.01 compared with SPF-NC/Nga mice of the same age (Student's *t*-test with Bonferroni correction). Dermatitis score represents the median $\pm$ quartile deviation from six mice. * P < 0.05 and ** P < 0.01 compared with SPF-NC/Nga mice of the same age (non-parametric Wilcoxon test).

scratch count, TEWL value or inflammation score in the SPF-NC/Nga mice during the experimental period.

The serum total IgE level of the CNV-NC/Nga mice at 4, 7, 10 and 13 weeks of age was significantly higher than that for SPF-NC/Nga mice from 10 and 13 weeks of age (Fig. 1d, *P*-values were 0.112, 0.125, below 0.001 and below 0.001, respectively), and there was no change in the total IgE level in the SPF-NC/Nga mice. The serum IgG₁ level of the CNV-NC/Nga mice at 4, 7, 10 and 13 weeks of age was significantly higher than that for SPF-NC/Nga mice from 10 and 13 weeks of age (Fig. 1e, *P*-values were 0.363, 0.598, below 0.001 and below 0.001, respectively), and there

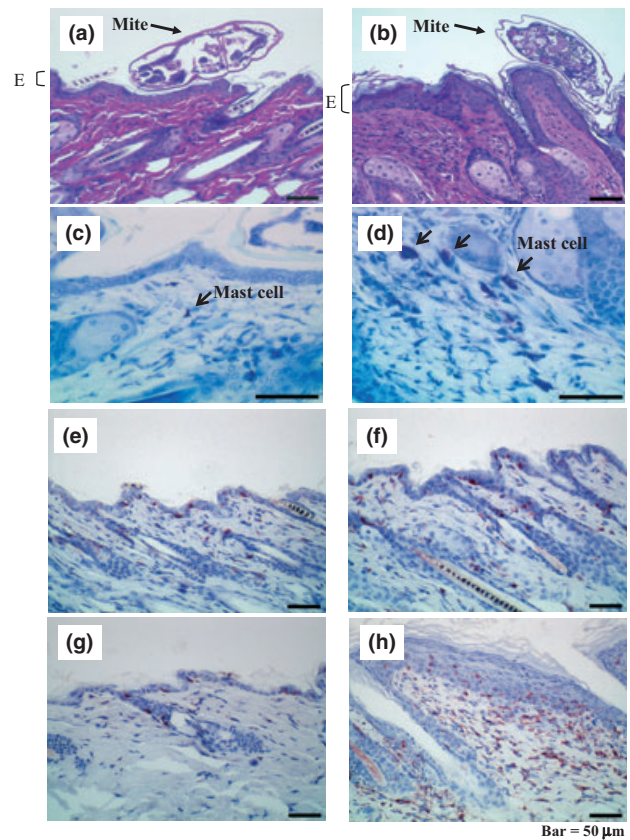


Figure 2. Histological comparison of CNV-NC/Nga mice skin. The mice were treated as described in Fig. 1. Paraffin sections of skin from the back of CNV-NC/Nga mice at 4 weeks of age (a and c) and 13 weeks of age (b and d) were stained with hematoxylin and eosin (a and b) or toluidine blue (c and d) as described in the text. E, epidermis. Detection of CD3 positive cells by avidin-biotin method described in materials and methods. Specific pathogen-free (SPF)-NC/Nga mice at 4 weeks (e) and 13 weeks (f) and 13 weeks (g) of age and CNV-NC/Nga mice at 4 weeks (h) of age, respectively. Bar = 50 μ m.

were almost no change in the IgG₁ level in the SPF-NC/Nga mice. The serum IgG_{2a} level of the CNV-NC/Nga mice at 4, 7, 10 and 13 weeks of age was significantly higher than that for SPF-NC/Nga mice from 10 and 13 weeks of age (Fig. 1f, *P*-values were 0.200, 0.454, below 0.001 and below 0.001, respectively), and there were almost no change in the IgG_{2a} level in the SPF-NC/Nga mice.

Histological comparison of the skin of NC/Nga mice

The histological features of the skin were compared in H.E.- and T.B.-stained specimens from CNV-NC/Nga mice at 4 weeks of age (itchy and no inflammation) and 13 weeks of age (itchy and inflammation), and changes in the histological features were compared with the scratching and inflammation score. Mites were observed in the upper epidermis at both ages (Fig. 2b). There were no obvious histological changes in CNV-NC/Nga mice at 4 weeks of age (Fig. 2a,c). By contrast, marked hyperplasia, thickening of the epidermis (Fig. 2b) and infiltrations of hypertrophic mast cell were observed in CNV-NC/Nga mice at 13 weeks of age, compared with observations at 4 weeks of age (Fig. 2d). Moreover, we studied the infiltrations of T cells in the skin of itchy NC/Nga mice at 4 weeks of age using CD3 immunohistochemical

study, but CD3-positive cells were found dispersed throughout the epidermis and not in the dermis; no differences between CNV-NC/Nga (itchy) and SPF-NC/Nga (non-itchy) mice were observed (Fig. 2e,f). Thus, no infiltrations of T cells in the skin of the itchy CNV-NC/Nga mice were apparent at 4 weeks of age. In contrast, CD3-positive cells were distinctly observed in the epidermis and dermis of CNV-NC/Nga mice at 13 weeks of age, compared with similarly aged SPF-NC/Nga mice (Fig. 2g,h).

Scratch count, TEWL, inflammation score and serum total Ig levels in mite antigen-sensitized SPF-NC/Nga and BALB/c mice

The effects of the sensitization of SPF-NC/Nga and BALB/c mice to mite antigen on the scratch count, TEWL, inflammation score and serum total Ig levels were examined. Sensitization to mite antigen had no effect on the scratch count, TEWL value and inflammation score of either strain of mice (Fig. 3a,b,c). The serum total IgE level of the sensitized mice was significantly higher than that for control of both strains mice (Fig. 3d, *P*-values were 0.001 and below 0.001). The serum IgG₁ level of the sensitized mice was significantly higher than that for control of both strains mice (Fig. 3e, *P*-values were 0.006 and below 0.001). The serum IgG_{2a} level of the sensitized SPF-NC/Nga mice was lower than that for control both strains mice. Sensitization to mite antigen significantly decreased the IgG_{2a} level of BALB/c mice in comparison with the control group (Fig. 3f, *P*-values were 0.051 and below 0.001).

Comparison of scratch-induction after 24 h of co-housing with skin-lesioned CNV-NC/Nga mice or mite antigen application

As reported previously, itch-associated scratching is inducible in SPF-NC/Nga mice by co-housing them with skin-lesioned CNV-NC/Nga mice (25), but sensitization to mite antigen induced no scratching (Fig. 3a). There is a report that mite antigen application causes dermatitis and increases serum IgE and IgG₁ production in SPF-NC/Nga mice (27). The scratch count of SPF-NC/Nga mice co-housed with skin-lesioned CNV-NC/Nga mice for 24 h (the co-housing treatment group) or those subjected to mite antigen (the mite antigen-treated group) was measured. In the co-housing treatment group, no obvious change was observed on the first day. On the second day, the scratch count was significantly increased (12.8 ± 2.0 counts/2 h at 7:00 p.m., Figure S1a), compared with the 2.0 ± 1.0 counts/2 h in the not co-housing group. The scratch count in the co-housing treatment group gradually increased and remained at a higher level (Figure S1a), whereas no change was observed in the mite antigen-treated group during the experimental period (Figure S1b).

Scratching induced in W/W^v and Scid mice

Co-housing with skin-lesioned CNV-NC/Nga mice significantly increased the scratching counts, compared with the day 0 value, in both WBB6F1-^{+/+} and W/W^v mice (Fig. 4a), but no significant differences were observed at each observation point between the WBB6F1-^{+/+} and W/W^v mice (*P*-values were 0.319, 0.763, 0.412 and 0.068, respectively). Co-housing with skin-lesioned CNV-NC/Nga mice significantly increased the scratching counts, compared with the day 0 value in both C.B.17/Icr-^{+/+} and Scid mice (Fig. 4b), but no significant differences were observed at each observation point between the C.B.17/Icr-^{+/+} and Scid mice (*P*-values were 0.179, 0.484, 0.397 and 0.463, respectively). The

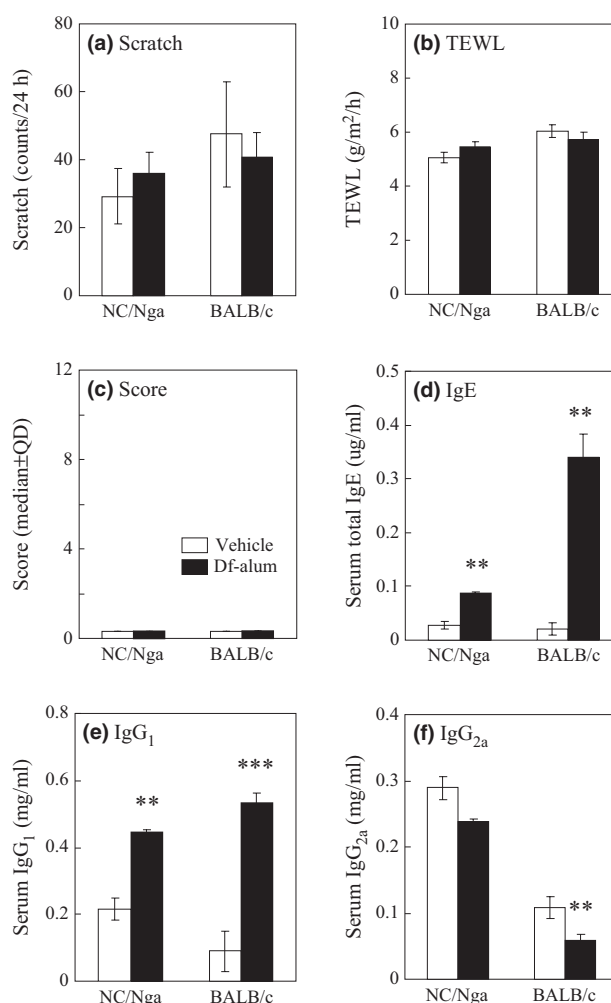


Figure 3. Scratch counts, transepidermal water loss (TEWL) value, inflammation scores and serum Ig levels of mite antigen-sensitized specific pathogen-free (SPF)-NC/Nga and BALB/c mice. The back of the neck of SPF-NC/Nga and BALB/c mice was subcutaneously injected with 0.25 mg mite antigen/2 mg aluminium hydroxide gel/0.25 ml phosphate-buffered solution, three times, at 7-day interval. Seven days after the final injection, scratch counts; spontaneous long-lasting scratching (over 1.5 s) by NC/Nga mice was measured the following day for 24 h (a), TEWL value; transepidermal water loss was measured using a skin-evaporative water recorder (b), dermatitis score; this scoring was based on the severity of erythema/haemorrhage, oedema, excoriation/erosion and scaling/dryness (c), serum IgE levels; a sign of Th2 immunity (d), serum IgG₁ level; a sign of Th2 immunity (e), serum IgG_{2a} level; a sign of Th1 immunity (f) were measured as described in the text. The values are means $\pm$ SEM from six mice. ***P* < 0.01 and ****P* < 0.001 compared with each strain non-sensitized mice (Student's *t*-test). Dermatitis score represents the median $\pm$ quartile deviation from six mice.

scratch counts were significantly increased in the W/W^v and Scid mice after 14 days of co-housing with skin-lesioned CNV-NC/Nga mice, similar to the results observed in other strains of mice (e.g. BALB/c, ICR and C3H/HeN), but these counts were less than half the number observed in NC/Nga mice (26).

Discussion

The skin is a large and complex organ that provides a protective interface between the organism and its environment. The skin barrier prevents the penetration of foreign matter into the skin and excessive moisture loss from the body. By damaging this barrier, infectious agents can easily penetrate the body, triggering inflam-

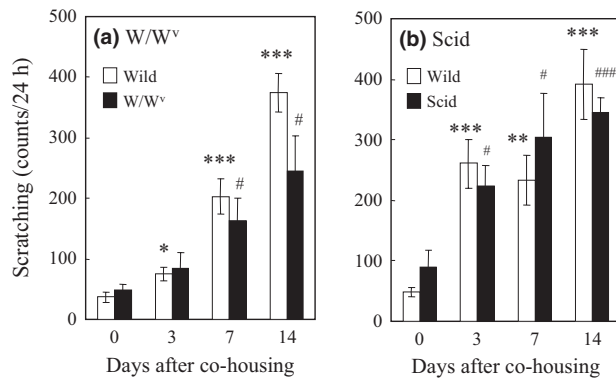


Figure 4. Scratch count in mast cell-deficient mice and T/B cell-deficient mice. WBB6F1-W/W^v mice; male W/W^v mice, mast cell-deficient mice and WBB6F1-^{+/+} mice (normal littermates) were housing them with skin-lesioned CNV-NC/Nga mice for 0, 3, 7 and 14 days (a), male C.B.17/lcr-scid mice; T/B cell-deficient mice and C.B.17/lcr-^{+/+} mice (normal littermates) were housing them with skin-lesioned CNV-NC/Nga mice for 0, 3, 7 and 14 days (b), and scratch (long-lasting) counts were measured as described in the text. * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$, when compared with the day 0 value of the wild type of W/W^v or Scid mice. # $P < 0.05$ and ### $P < 0.001$, when compared with the day 0 value of W/W^v or Scid mice. NS, no significance at each day point, compared between wild-type and cell-deficient mice.

mation as an immune response to defend against the foreign matter. Th2 cell-derived cytokine induced IgE production by B cells, and allergen-induced IgG₁ play an important role in the manifestation of allergic disease (28), while interferon- γ secreted by Th1 cells is crucial for the production of IgG_{2a}. Once IgE is produced and the mast cells are activated, an IgE-dependent immediate reaction is induced, explaining how the scratching-induced disruption of the skin barrier led NC/Nga mice to develop dermatitis and produce Th2-dominant serum Ig.

The histological findings of the skin showed mite infection in NC/Nga mice at 4 weeks of age (itchy and no dermatitis) and 13 weeks of age (itchy and dermatitis), but severe inflammation (hyperplasia and thickening of epidermis and the infiltration of hypertrophic mast cells and T cells) was only observed at 13 weeks of ages. The biological functions of these cells appear to include a role in innate immunity, involvement in host defense mechanisms against parasitic infestations, and the modulation of the immune system. Thus, these findings may indicate histopathological reactions caused by the onset of dermatitis. The sensitization of mice to mite antigen had no effect on the scratch counts, TEWL value, or the inflammation score, but the serum total IgE and IgG₁ levels increased. Th1 and Th2 cytokine released by activated T cells play important roles in the pathology of AD in human (29) and NC/Nga mice (30). Mite antigen causes IgE overproduction and a shift to a Th2-dominant state in mice (16,17). Moreover, we examined the sensitization of SPF-NC/Nga mice to other mite antigen, such as *Dermatophagoides pteronyssinus* and *Tyrophagus putrescentiae*, but the results were the same as those for *Dermatophagoides farinae* (data not shown). As reported previously, NC/Nga mice are more sensitive to mite antigen than BALB/c mice (26), and the serum total IgE levels of mite-sensitized SPF-NC/Nga mice were lower than those in mite-sensitized BALB/c mice. Whereas the serum total IgE level in skin-lesioned CNV-NC/Nga mice was higher than that in mite-sensitized SPF-NC/Nga mice, other factor may play an important role in

the elevation of the serum IgE level in CNV-NC/Nga mice. These results indicate that mite antigen induces allergic dermatitis by inducing strong systemic Th2-skewed immune responses (31), but the presence of IgE is not indispensable to the induction of scratching in NC/Nga mice (10,11).

Since scratching was observed in CNV-NC/Nga mice at 4 weeks of age even though, mast cell accumulation was not apparent (Fig. 2c) and mast cell involvement in scratching was assessed in W/W^v mice. The involvement of T/B cell in scratching was assessed in Scid mice. Scratching was induced in SPF-NC/Nga, W/W^v and Scid mice, AD is characterized by serum IgE elevation, and mast cell infiltration can develop mast cell and T cell independently (32). As infection is one of the most thoroughly studied triggers of AD (33), we examined the involvement of skin contact and blood infection in scratching. The method used for the skin contact procedure was to rub the back of a skin-lesioned CNV-NC/Nga mouse against the back of an SPF-NC/Nga mouse five times within a few seconds. As a result, the scratch count increased significantly in SPF-NC/Nga mice at 2 months after skin contact with the skin-lesioned CNV-NC/Nga mice, but the adaptive transfer of serum isolated from skin-lesioned CNV-NC/Nga mice did not induce scratching in SPF-NC/Nga mice (data not shown). These results indicate that skin contact, but not blood infection, play a critical role in the development of scratching. Mite antigen-treated SPF-NC/Nga mice did not increase their scratching behaviour, but several strains of mice (e.g. SPF-NC/Nga, BALB/c, ICR, C3H/HeN, W/W^v and Scid mice) increased their scratching behaviour after only a few days of co-housing with skin-lesioned NC/Nga mice. Based on these data, we suggest that direct stimulation by living mites infecting the skin of one mouse to the skin surface of another mouse may induce scratching. Direct stimulation may result in the expression of some kind of endogenous agent under the skin that may have a strong pruritic activity.

The factors that induce itching and promote scratching have never been clearly identified. Many endogenous chemicals, e.g. amines, proteases, growth factors, neuropeptides, opioids and cytokines, are locally pruritogenic when injected into the skin (34). Histamine is well known to cause itching in humans. These effects are mediated through at least four histamine receptors: H₁–H₄. Recently, an H₄ receptor antagonist was shown to inhibit hapten-induced scratching behaviour in BALB/c and NMRI mice (35). We examined the effects of H₁, H₂ and H_{3/4} receptor antagonists on the scratching behaviour in NC/Nga mice, but these antagonists did not inhibit the behaviour (21). These results suggested that the scratching behaviour in NC/Nga mice is not mediated by histamine and that the mechanism may differ from that of hapten-induced scratching behaviour. Tominaga et al. reported that neurite density and the expression levels of nerve growth factors (NGF) were markedly increased in the epidermis of CNV-NC/Nga mice, compared with those in SPF-NC/Nga mice (36). Furthermore, anti-NGF antibodies significantly inhibited the development of dermatitis and scratching (37). These results suggest that NGF is an important factor in the induction of itching and promotes scratching in NC/Nga mice. Moreover, interleukin-31 (IL-31) is a novel cytokine involved in pruritic skin diseases, including atopic dermatitis (38). We have demonstrated that IL-31 gene transcripts were

significantly increased in CNV-NC/Nga mice and that these changes coincided with increases in the scratching counts (39). Furthermore, anti-IL-31 antibody reduces scratching behaviour in NC/Nga mice with atopic-like dermatitis (40). Consequently, we suggested that IL-31 may be one of the most important factors in inducing itching and promoting scratching in NC/Nga mice. As painful stimuli evoke itching in patients with chronic pruritus (41), nociceptive sensations may lead to increased scratching by NC/Nga mice. More studies are needed to clarify the mechanisms involved in the development of itching and dermatitis in NC/Nga mice. In our previous study, an increase in scratching proceeded an increase in the TEWL value, the development of dermatitis and an increase in the serum IgE levels in NC/Nga mice, which are deficient of protective immune responses (42). It is well known that house dust mites are the most important allergens associated with respiratory allergies as well as with AD. Other environmental factors may stimulate IgE production

triggering an 'allergic march' and thus may contribute to the manifestation of allergic asthma and eczema in children predisposed to allergy.

In conclusion, this study demonstrated that IgE overproduction results from itch-associated scratching-induced dermatitis in NC/Nga mice. AD has long been suspected of being a T-cell-mediated disease associated with a hyperactivated Th2 immune abnormality. We suggest that the presence of IgE is not indispensable to the induction of itching and dermatitis in NC/Nga mice.

Authorship

Y. Hashimoto conducted the research and wrote the paper; A. Takaoka, M. Sugimoto, Y. Honma and N. Futaki conducted the research and analysed the data; T. Sakurai carried out the histological study, and I. Arai designed the research study and wrote the paper.

Conflict of interests

The authors declare that they have no conflict of interests to disclose.

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Supporting Information

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

Figure S1. Comparison of scratch count between housing with skin-lesioned CNV-NC/Nga mice and mite antigen application.

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