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ORIGINAL ARTICLE

A Novel Atopic Dermatitis Model Induced by Topical Application with *Dermatophagoides Farinae* Extract in NC/Nga Mice

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ABSTRACT

Background: Atopic dermatitis is a chronically relapsing inflammatory skin disease. Animal models induced by relevant allergens play a very important role in the elucidation of the disease. The patients with atopic dermatitis are highly sensitized with mite allergens such as *Dermatophagoides farinae* (Df). Therefore, in the present study, we tried to develop a novel model for atopic dermatitis by repeated application with Df extract ointment.

Methods: Df extract ointment was repeatedly applied to the back of NC/Nga mice together with barrier disruption. Atopic dermatitis-like skin lesions were evaluated by dermatitis scores, skin histology and immunological parameters. The effect of corticosteroid and calcineurin inhibitor was also examined.

Results: Repeated application of Df extract ointment caused rapid increase in dermatitis scores. Clinical (skin dryness, erythema, edema and erosion) and histological symptoms (dermal and epidermal thickening, hyperkeratosis, parakeratosis and inflammatory cell infiltration) in this model were very similar to those in human atopic dermatitis. Serum total and Df-specific IgE levels were elevated in this model compared with normal mice, and draining lymph node cells isolated from the mice that exhibited dermatitis produced significant amounts of interleukin-5, interleukin-13 and interferon-γ after re-stimulation with Df. Furthermore, current first-line drugs for the treatment of human atopic dermatitis, corticosteroid and tacrolimus ointments, were effective against the clinical and histological symptoms in this model.

Conclusions: These results suggest that the model we have established is useful for not only elucidating the pathogenesis of atopic dermatitis but also for evaluating therapeutic agents.

KEY WORDS

animal model, atopic dermatitis, *Dermatophagoides farinae*, house dust mite, NC/Nga mice

INTRODUCTION

Atopic dermatitis is a chronically relapsing inflammatory skin disease accompanied by severe itching. It is a major public health problem worldwide occurring at rates of 10–20% in children and 1–3% in adults.[1] Prevalence of atopic dermatitis has increased in recent years, but the pathophysiology of this condition is only partly understood. In addition, although corticosteroids and calcineurin inhibitors are used in the treatment of atopic dermatitis,[2] there remains a need for topical anti-inflammatory drugs without side effects.

Recently, several models have been developed for atopic dermatitis in mice: a spontaneously developed dermatitis model in NC/Nga and its sub-strain mice under non-specific pathogen free (SPF) conditions,[3-5] a hapten-induced dermatitis model in NC/Nga mice under SPF conditions,[6] and a dermatitis model in HR-1 mice using a diet low in magnesium and zinc.[7]

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These models fulfill some of the features of atopic dermatitis, but differ from human atopic dermatitis in their pathogenesis. For example, spontaneous dermatitis in NC mice is thought to be induced by *Myocoptes musculinus* and *Myobia musculi*, which infest rodents, but not humans.[4,5] The triggers of hapten-induced dermatitis and dermatitis in HR-1 mice are also not relevant.

Patients with atopic dermatitis are highly sensitized to house dust mite (HDM) allergens based on the positive results from radioallergosorbent, scratch and patch tests.[8-10] A major species of HDM in Japan is *Dermatophagoides farinae* (Df), and its body and feces are well known as major environmental allergens.[11] Thus, we considered Df extract to be relevant for the induction of dermatitis, and examined the characteristic features of Df extract-induced dermatitis in NC/Nga mice. Previously, Matsuoka *et al.*[12] reported that Df extract has the ability to induce atopic dermatitis-like skin lesion in mice when applied as a Df extract suspension. However, the onset of dermatitis in their model was relatively slow. We considered the reason for the slow onset to be the rapid disappearance of allergens from the back of mice and hypothesized that Df extract ointment would remain longer than Df extract suspension. This led us to use Df extract ointment to achieve a rapid onset of the skin lesion. Repeated application with Df extract ointment together with barrier disruption could induce atopic dermatitis-like skin lesions in NC/Nga mice. The dermatitis caused by Df extract ointment was observed to be significant 2 weeks after the first application. Clinical, immunological and histological features of this model were similar to those of human atopic dermatitis. We also confirmed the efficacy of corticosteroid and tacrolimus ointments against this model. These results indicate that the Df extract ointment-induced dermatitis model in NC/Nga mice is useful for elucidating the pathogenesis of atopic dermatitis and also for evaluating therapeutic agents.

METHODS

MICE

Female NC/Nga mice were purchased from Charles River Japan (Yokohama, Japan). These mice were all housed under conditions of controlled temperature (20–26℃), humidity (30–70%) and lighting (lights on from 08:00 to 20:00). Food and tap water were provided *ad libitum*. All mice were 10–15 weeks of age. The animal experiments in this study were approved by the Animal Care and Use Committee of Shionogi Research Laboratories.

DRUGS

Betamethasone dipropionate ointment, 0.064%, (Rinderon®-DP, Shionogi, Osaka, Japan) and tacrolimus ointment, 0.1%, (Protopic®, Fujisawa, Osaka, Japan) were used in this study.

PREPARATION OF DF EXTRACT OINTMENT

Df were cultured, then the mite bodies were separated from their feces and culture media by flotation on saturated water solution of sodium chloride.[13] The Df body (Dfb) extract was prepared according to the method of Yamaguchi *et al.*.[14] In brief, floating mites were pooled and their bodies were homogeneously suspended in phosphate-buffered saline using an agate mortar and pestle. The suspension was stirred overnight, followed by centrifugation. The supernatant was dialyzed and lyophilized. The freeze-dried extracts were mixed with hydrophilic petrolactum (Maruishi, Osaka, Japan) at a concentration of 5 mg Dfb extract/1 *g* hydrophilic petrolatum, referred to as Dfb ointment.

INDUCTION OF DERMATITIS

Mice were anesthetized with ether and their hair on the upper back was shaved with a clipper and a shaver. Elicitation was performed by topical application of 100 mg Dfb ointment or ointment base (hydrophilic petrolatum) on the shaved dorsal skin and both surfaces of each ear. Barrier disruption was achieved by 150 μL of 4% sodium dodecyl sulfate treatment on the shaved dorsal skin and both surfaces of each ear 3 hours before the Dfb ointment application. These procedures were repeated twice a week. Mice were assigned to one of four groups at the beginning of the experiment: Group A, barrier disruption and Dfb; Group B, Dfb ointment without barrier disruption; Group C, barrier disruption and ointment base; and Group D, ointment base without barrier disruption. Following the last antigen challenge on day 25 after the first elicitation, these mice were sacrificed on day 28, and immunological and histological changes were investigated.

EVALUATION OF SKIN LESION

The severity of dermatitis was evaluated once a week, just before each elicitation. The development of 1) erythema/hemorrhage, 2) scarring/dryness, 3) edema, 4) excoriation/erosion was scored as 0 (none), 1 (mild), 2 (moderate) and 3 (severe). The sum of the individual scores was taken as the dermatitis score.

MEASUREMENT OF SERUM TOTAL IgE AND SPECIFIC IgE TO Dfb

Sera were collected by drawing blood from the heart under ether anesthesia. The concentration of total IgE was measured using a mouse IgE EIA kit (Yamasa, Tokyo, Japan), according to the manufacturer's protocol. Specific IgE to Dfb was evaluated using passive cutaneous anaphylaxis (PCA).[15] Briefly, female Sprague-Dawley rats (Charles River Japan, Yokohama, Japan), which were used as recipients, were sensitized with intradermal injection of 50 μL of serially diluted sera on shaved dorsal skin. They were

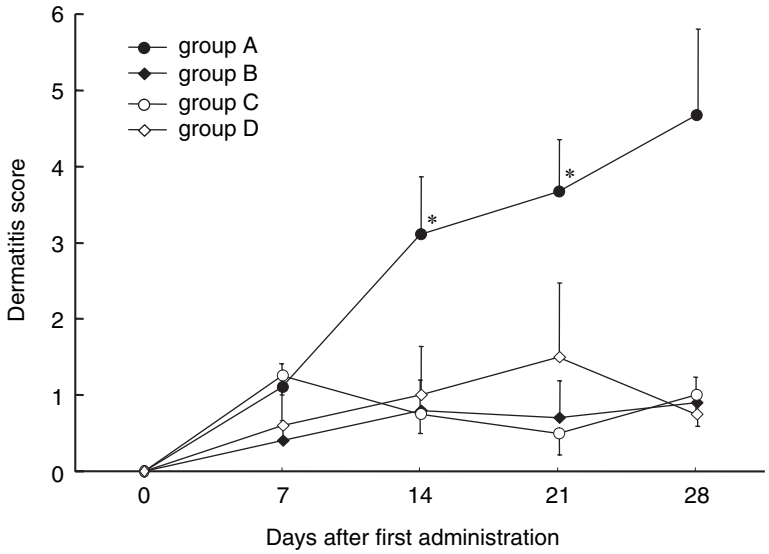

Fig. 1 Development of atopic dermatitis-like skin lesions after Df application in NC/Nga mice. Elicitation was performed by topical application of 100 mg Dfb ointment or ointment base (hydrophilic petrolatum) on the shaved dorsal skin and both surfaces of each ear. Barrier disruption was achieved by 150 μL of 4% sodium dodecyl sulfate treatment on the shaved dorsal skin and both surfaces of each ear 3 hours before the Dfb ointment application. These procedures were repeated twice a week. Mice were assigned to one of four groups: Group A, barrier disruption and Dfb ointment (closed circles); Group B, Dfb ointment (closed triangles); Group C, barrier disruption and ointment base (open circles); and Group D, ointment base (open triangles). Each data represents the mean ± S.E. of 5 − 8 mice. * $p < 0.05$ when compared with each control group (Wilcoxon test).

challenged 24 hours later by intravenous injection of 0.5 ml saline containing 1 mg Dfb and 5 mg Evans blue. Thirty minutes later, the animals were sacrificed by exsanguination under deep anesthesia. The pigmented area was measured from the inner side of the skin. An area more than 5 mm in diameter was regarded as a positive reaction, and the antibody titer was determined.

MEASUREMENT OF CYTOKINE LEVELS BY AXILLARY LYMPH NODE CELLS

Axillary lymph nodes were isolated, and a single cell suspension was prepared. The lymph node cells (5×10^6 cells) were re-stimulated with 10 μg/ml of Dfb in a 24-well flat-bottom microplate at 37℃ for 24 hours. After the incubation period, the culture supernatants were collected. The amount of interleukin (IL)-5, IL-13, IL-4 and interferon (IFN)-γ in the culture supernatant was determined using a murine ELISA kit (Genzyme, Minneapolis, Minnesota, USA).

HISTOLOGICAL OBSERVATION OF THE SKIN

Portions of the dorsal skin were fixed with 10% neutral formalin, embedded in paraffin and sectioned at 4 μm. Sections were stained with hematoxylin-eosin. In addition, for the detection of mast cells and eosinophils, sections were stained with toluidine blue and direct first scarlet, respectively. The number of mast cells was counted from the epidermis to the panniculus adiposus under a microscope with an eyepiece squared micrometer at a magnification of ×400.

EFFECT OF TACROLIMUS OINTMENT AND CORTICOSTEROID OINTMENT ON ESTABLISHED DERMATITIS

NC/Nga mice having demonstrated dermatitis with a score of over 3 for 2 weeks were used. Each ointment at a weight of 100 mg, was applied to the shaved dorsal skin and both surfaces of each ear twice a week. The dermatitis was scored on day 0, 1, 4, 5, 7, 8, 11, 12 and 13 after the start of each treatment. On day 13, skin from the upper back was excised and fixed with 10% neutral formalin, and the sections were stained as described above.

STATISTICAL ANALYSIS

Data were expressed as the means ± S.E. The statistical significance of differences was assessed by Student's *t* -test or Dunnet's test except for the dermatitis score, which was assessed by the Wilcoxon test or

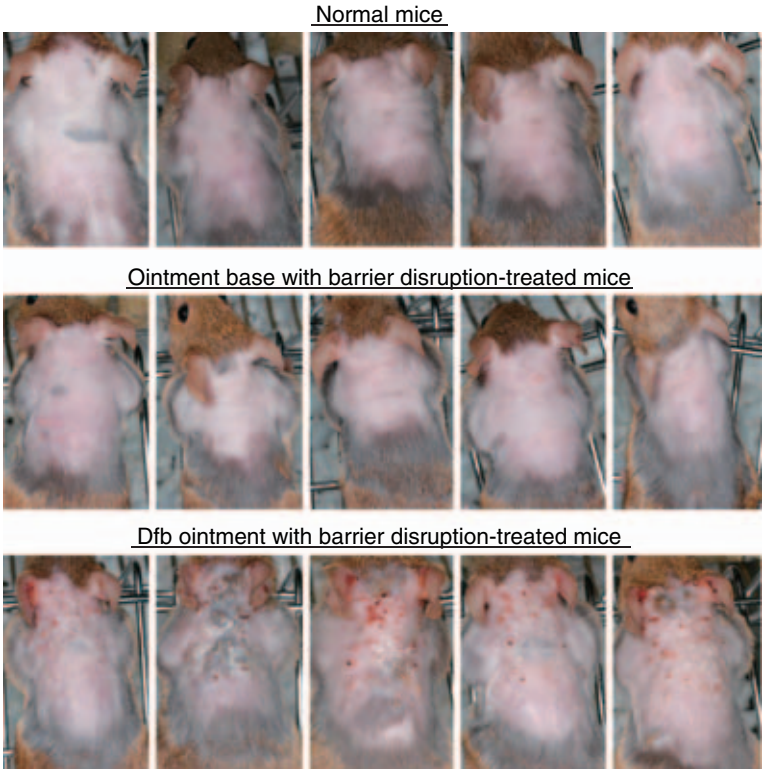

Fig. 2 Macroscopic features of atopic dermatitis-like skin lesions in NC/Nga mice on day 28. Normal mice, ointment base with barrier disruption-treated mice and Dfb ointment with barrier disruption-treated mice.

Steel's test. P-values less than 0.05 were considered to be statistically significant.

RESULTS

DEVELOPMENT OF ATOPIC DERMATITIS-LIKE SKIN LESION

To establish the Dfb-induced dermatitis model in NC/Nga mice, we repeatedly applied Dfb ointment to the dorsal skin. As shown in Figure 1, the dermatitis scores of Group A (barrier disruption + Dfb ointment) increased rapidly and became significant 2 weeks after the first elicitation. However, in Group B (Dfb ointment without barrier disruption), skin lesions were minimally observed. After the repeated application of Dfb ointment, skin dryness occurred first, followed by mild erythema, hemorrhage and edema. Finally, the skin became thick and severe erythema, hemorrhage, edema, scarring, erosion and excoriation were observed (Fig. 2). The application of Dfb ointment after barrier disruption once a week did not cause significant dermatitis (data not shown). In Group A (barrier disruption + Dfb ointment), scratching behavior of the back and ears with hind paws was also observed (data not shown). These symptoms could not be detected in Group C (barrier disruption + ointment base) and D (ointment base without barrier disruption). Therefore, we decided that the procedure used for Group A, *i.e.* barrier disruption and Dfb ointment twice a week, was optimal for the induction of dermatitis in NC/Nga mice.

HISTOLOGICAL EVALUATION OF THE LESIONAL SKIN

In a comparison of histological changes of the dorsal skin on day 28 after the first elicitation with Dfb ointment or ointment base with barrier disruption, it was found that lesional skin showed a significant thickening of the dermis and epidermis, hyperkeratosis, and parakeratosis in the Dfb ointment-treated group (Figs. 3A, B). In addition, the epidermis deepened into the upper dermis. An increased number of dermal inflammatory cells including mast cells (Figs. 3C, D) and eosinophils (Figs. 3E, F) were also observed. The number of total and degranulated mast cells in the Dfb ointment-treated group was much higher than those in the ointment base-treated group and normal mice (Fig. 4). Most of the mast cells in the upper dermis were degranulated.

SERUM TOTAL IgE AND SPECIFIC IgE TO Dfb

Serum levels of total IgE and specific IgE to Dfb in NC/Nga mice on day 28 after the first elicitation were

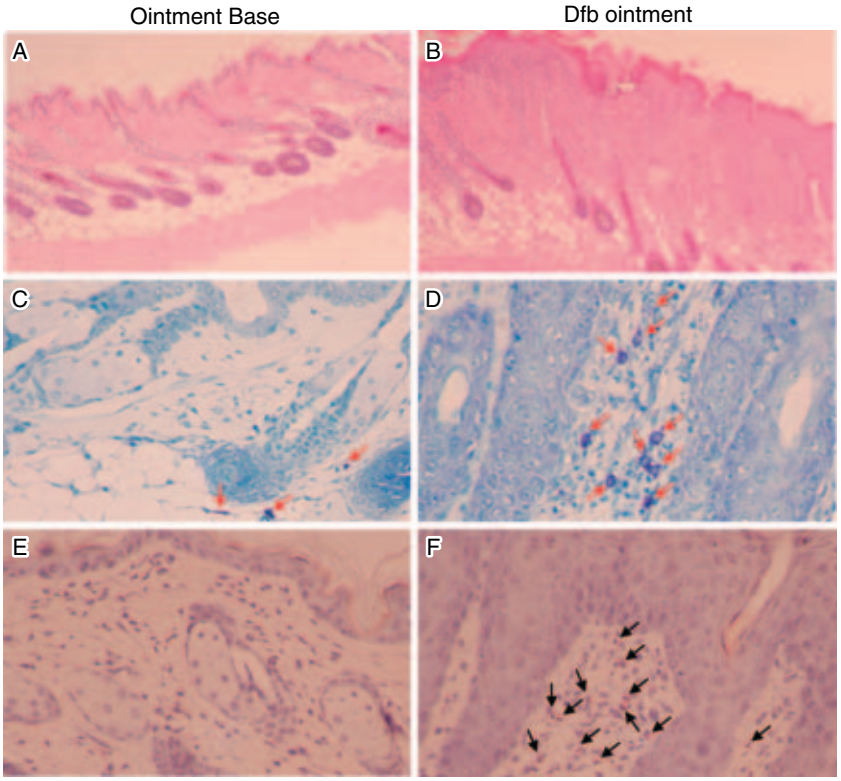

Fig. 3 Histological changes of the dorsal skin after Df application in NC/Nga mice. The dorsal skin of ointment base with barrier disruption-treated mice (**A, C, E**) and Dfb ointment with barrier disruption-treated mice (**B, D, F**) were extirpated on day 28 and stained with hematoxylin-eosin (**A, B**), toluidine blue (**C, D**) and direct first scarlet (**E, F**). **A** and **B** are of the original magnification ×100, **C**−**F** of the original magnification ×400.

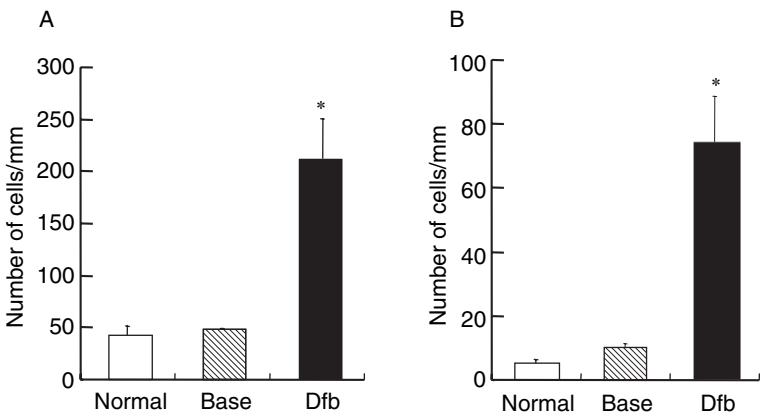

Fig. 4 The number of total (**A**) and degranulated (**B**) mast cells in normal, ointment base with barrier disruption-treated (Base) and Dfb ointment with barrier disruption-treated (Dfb) mice. Mast cells were counted from the epidermis to the panniculus adiposus under a microscope with an eyepiece squared micrometer at a magnification of ×400. Each data represents the mean ± S.E. of 5−8 mice. * $p < 0.05$ when compared with ointment base with barrier disruption-treated group (Student's *t* test).

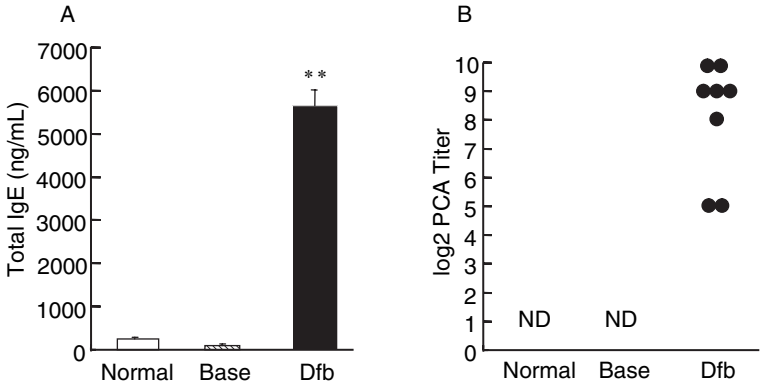

Fig. 5 Serum levels of total IgE (**A**) and specific IgE to Dfb (**B**) in normal, ointment base with barrier disruption-treated (Base) and Dfb ointment with barrier disruption-treated (Dfb) mice on day 28. Total IgE data represents the mean ± S.E. of 5 − 8 mice. * $p < 0.05$ when compared with ointment base with barrier disruption-treated group (Student's *t* test).

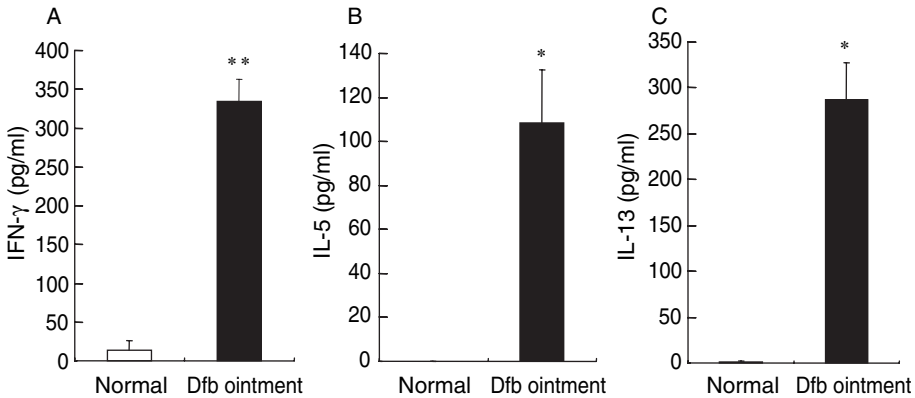

Fig. 6 Cytokine levels produced by axillary lymph node cells in normal and Dfb ointment with barrier disruption-treated mice. IFN-γ, IL-5 and IL-13 levels were examined in the culture supernatants after 24 hours of re-stimulation of lymph node cells with Dfb. Each data represent the mean ± S.E. of 5 mice. * $p < 0.05$ when compared with normal mice (Student's *t* test).

examined. The total IgE levels of mice in the Dfb ointment-treated group were significantly elevated compared with the normal and ointment base-treated groups (Fig. 5A). Specific IgE to Dfb were highly induced in the Dfb ointment-treated group, but not detected in the normal and ointment base-treated groups (Fig. 5B).

CYTOKINE PRODUCTION IN AXILLARY LYMPH NODE CELLS

We examined IL-5, IL-13, IL-4 and IFN-γ production of axillary lymph node cells obtained from the Dfb ointment-treated and normal mice after 48-hour incubation with Dfb. Lymph node cells from the Dfb ointment-treated mice showed marked elevation of IL-5, IL-13 and IFN-γ levels compared with those from normal mice (Fig. 6). IL-4 in the culture supernatant of lymph node cells from both the Dfb ointment-treated and normal mice were below the detection limit (data not shown).

EFFECT OF TACROLIMUS OINTMENT AND CORTICOSTEROID OINTMENT ON ESTABLISHED DERMATITIS

NC/Nga mice having demonstrated dermatitis with scores of over 3 for 2 weeks were used. As shown in the non-treated group, the dermatitis score did not decrease for at least two weeks even after application of barrier disruption and Dfb ointment was stopped. In the betamethasone dipropionate ointment-and tacrolimus ointment-treated groups, the dermatitis scores clearly decreased immediately after drug application (day 1), and significant suppression was observed from day 1 to day 13 in both groups compared

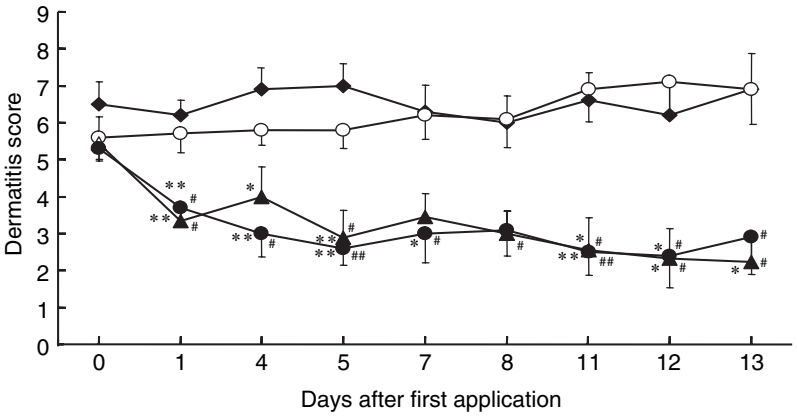

Fig. 7 Effect of betamethasone dipropionate ointment and tacrolimus ointment on established dermatitis model. NC/Nga mice having demonstrated dermatitis with scores over 3 for two weeks were used. Ointment base (open circles), betamethasone dipropionate ointment (closed circles) and tacrolimus ointment (closed triangles) at a weight of 100 mg were applied to shaved dorsal skin and both surfaces of each ear twice a week. No medication was applied in the Non-treated group (closed diamonds). Each data represents the mean ± S.E. of 9–10 mice. $^{*}p < 0.05$, $^{**}p < 0.01$ when compared with non-treated group. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ when compared with the ointment base-treated group (Steel's test).

with ointment base-treated group (Fig. 7).

Histologically, in the groups treated with betamethasone dipropionate ointment and tacrolimus ointment, epidermal thickening and infiltration of inflammatory cells in the dermis were very slight compared with those in the non-treated and ointment base-treated groups (Fig. 8). Betamethasone dipropionate ointment-treated mice showed significant reduction of the number of total and degranulated mast cells in the skin (Fig. 9).

DISCUSSION

In the present study, we demonstrated that repeated application with Df extract ointment induced atopic dermatitis-like skin lesions in NC/Nga mice. Interestingly, the dermatitis score remained high for at least 2 weeks after application of Dfb ointment was stopped. This is very convenient for evaluation of the efficacy of topically applied drugs, because there would be no question about the possibility for an interaction between the drug and the antigen. Indeed, we found that corticosteroid and tacrolimus ointments that are used for the treatment of human atopic dermatitis exhibited significant suppressive effects on the skin lesions. These findings strongly suggest that this model can be useful for not only elucidating the pathogenesis of atopic dermatitis but also for the evaluating novel therapeutic agents.

Atopic dermatitis is a chronically relapsing inflammatory skin disease and a major public health problem worldwide. Patients with atopic dermatitis are highly sensitized to HDM allergens. Thus, HDM allergens are considered to be common environmental allergens causing atopic dermatitis. As Df is a major species of HDM in Japan,[11] it would be logical to use Df to develop an atopic dermatitis model as a relevant antigen.

Repeated application with Df extract ointment could induce atopic dermatitis-like skin lesions in NC/Nga mice. Clinical symptoms appearing in this model began with skin dryness, followed by mild erythema, hemorrhage and edema. Finally, the skin became thick and severe erythema, hemorrhage, edema, scarring, erosion and excoriation were observed. Histologically, the lesional skin showed significant thickening of the dermis and epidermis, hyperkeratosis and parakeratosis. These changes were associated with an increased number of dermal inflammatory cells containing mast cells, eosinophils and lymphocytes. Both clinical and histological symptoms in our model were very similar to those in human atopic dermatitis.[16,17] Scratching behavior, one of the most important symptoms in atopic dermatitis, was also observed in our model. Df extract-induced dermatitis in mice was previously reported by Matusoka *et al*..[12] They used Df extract suspension to induce dermatitis, but its onset was relatively slow. In our preliminary experiment, repeated application with Df extract suspension revealed only minimal induction of dermatitis (data not shown). We hypothesized that the slow onset or failure to induce dermatitis was due to the quick disappearance of allergen from the back of mice and that Df extract ointment would remain longer than Df extract suspension.

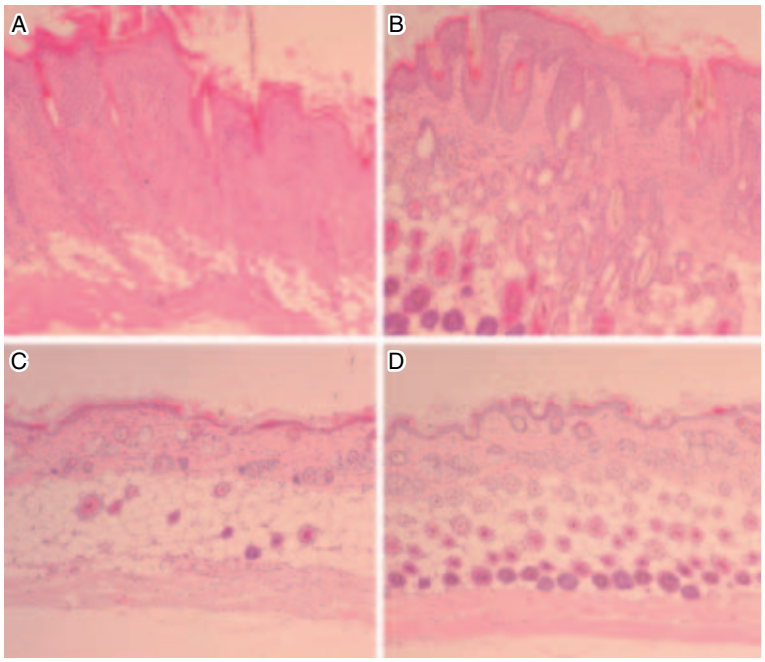

Fig. 8 Histological changes of the dorsal skin in the group treated with betamethasone dipropionate ointment and tacrolimus ointment. The dorsal skin of non-treated mice (**A**), ointment base-treated mice (**B**), betamethasone dipropionate ointment-treated mice (**C**) and tacrolimus ointment-treated mice (**D**) were stained with hematoxylin-eosin. Original magnification ×100 for all photos.

Therefore, to achieve a rapid onset of the skin lesion, we decided to use Df extract ointment to induce dermatitis. As expected, Df extract ointment-induced atopic dermatitis-like skin lesion displayed a rapid onset and a chronically sustained course.

Previous studies on atopic dermatitis suggest that skin lesions accompanied by topical eosinophilia and systemic IgE elevation are associated with Th2 type cytokine (IL-4, IL-5 and IL-13) expression. Indeed, predominant expression of Th2 cytokines were confirmed in both acute and chronic lesions of atopic dermatitis compared with the uninvolved skin of patients with atopic dermatitis or normal subjects.[18,19] However, recent studies have demonstrated that the expression of IFN-γ was more predominant than that of IL-4 in chronic skin lesions in atopic dermatitis, while the levels of IL-5 and IL-13 still remained high.[20] Therefore, the theory that activation of Th2 cells in the acute phase of atopic dermatitis, followed by both Th1 and Th2 activation leads to chronic dermatitis is currently considered to be plausible. In the present study, draining lymph node cells isolated from mice that exhibit chronic skin lesions produced significant amounts of IL-5, IL-13 and IFN-γ after re-stimulation with Df *in vitro*. Moreover, levels of serum total and Df-specific IgE were elevated in mice exhibiting dermatitis compared with normal mice. These results suggest that both Th1 and Th2 cells are involved in the development of chronic dermatitis in our model as well as in human atopic dermatitis. The expression of Th1 and Th2 cytokines in skin lesions needs to be clarified in the future.

Our dermatitis model established in the present study offers the advantage of allowing evaluation of topically applied drugs, because the established skin lesion in our model lasted for at least 2 weeks without further topical application of allergen. In patients with atopic dermatitis, itch-associated scratching damages the skin and increases the inflammation, which in turn increases the itching further.[21,22] We observed scratching behavior of mice at the sites of antigen application in our model, and the established skin lesions were improved by cutting off hind toenails of mice (data not shown). Therefore, the continuous scratching behavior would maintain the dermatitis without the further antigen application. Next, we investigated whether our model is available to evaluate the efficacy of drugs currently used for the treatment of human atopic dermatitis. The first-line drugs for atopic dermatitis are corticosteroids.[23] In addition, tacrolimus ointment, launched in 1999 in Japan, is particularly useful for skin lesions on the neck and face.[24] Tacrolimus ointment has been found to exhibit potent inhibitory effects on spontaneously developed dermatitis in NC/Nga mice, while corticosteroid ointment has only marginal effects.[25] Therefore, we ex-

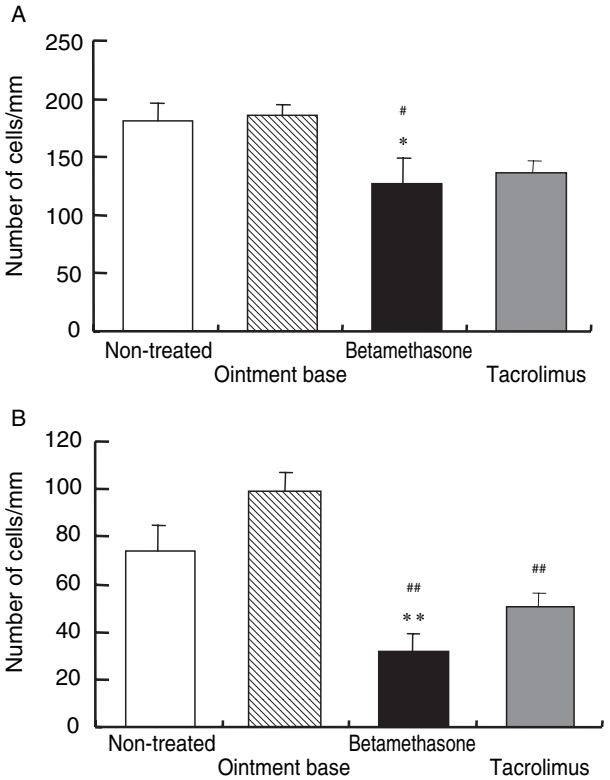

Fig. 9 Effect of betamethasone dipropionate ointment and tacrolimus ointment on total (**A**) and degranulated mast cells (**B**) in the skin of established dermatitis model. Mast cells were counted from epidermis to panniculus adiposus under a microscope with an eyepiece squared micrometer at a magnification of ×400. Each data represents the mean ± S.E. of 9–10 mice. $^{*}p < 0.05$, $^{**}p < 0.01$ when compared with the non-treated group. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ when compared with the ointment base-treated group (Dunnet's test).

amined the effect of betamethasone dipropionate ointment, a steroid ointment, and tacrolimus ointment on established dermatitis in our model. These ointments applied twice a week rapidly and clearly reduced dermatitis scores, and the inhibitory effects lasted through out the study. The efficacy of these ointments on dermatitis scores was accompanied by significant improvement of histological changes such as epidermal thickening and infiltration of inflammatory cells.

In conclusion, we developed a novel animal model for atopic dermatitis by repeated application with Df extract ointment in NC/Nga mice. All of the clinical, histological, and immunological features in this model were similar to the events observed in patients with atopic dermatitis. Furthermore, we confirmed the efficacy of corticosteroid and tacrolimus ointments against this model. Therefore, it is suggested that our model is useful for not only eludicating the pathogenesis of atopic dermatitis but also for evaluating therapeutic agents.

REFERENCES

1. Schultz-Larsen F, Hanifin JM. Epidemiology of atopic dermatitis. *Immunol. Allergy Clin. North Am.* 2002;**22**:1-24.
2. Furue M, Terao H, Moroi Y *et al*. Dosage and adverse effects of topical tacrolimus and steroids in daily management of atopic dermatitis. *J. Dermatol.* 2004;**31**:277-283.
3. Matsuda H, Watanabe N, Gregory PG *et al*. Development of atopic dermatitis-like skin lesion with hyperproduction in NC/Nga mice. *Int. Immunol.* 1996;**9**:461-466.
4. Morita E, Kaneko S, Hiragun T *et al*. Fur mites induce dermatitis associated with IgE hyperproduction in an inbred strain of mice, NC/Kuj. *J. Dermatol. Sci.* 1999;**19**:37-43.
5. Iijima OT, Takeda H, Komatsu Y, Matsumiya T, Takahashi H. Atopic dermatitis in NC/Jic mice associated with Myobia musculi infestation. *Comp. Med.* 2000;**50**:225-228.
6. Okada M, Hirasawa Y, Yoshijima K *et al*. Effect of clobetasol propionate, a corticosteroid on hapten-induced dermatitis in SPF NC/Nga mice. *Pharmacometrics* 2000;**59**:135-139.
7. Makiura M, Akamatsu H, Akita H *et al*. Atopic dermatitis-like symptoms in HR-1 hairless mice fed a diet low in magnesium and zinc. *J. Int. Med. Res.* 2004;**32**:392-399.
8. Holm L, van Hage-Hamsten M, Ohman S, Scheynius A. Sensitization to allergens of house-dust mite in adults with atopic dermatitis in a cold temperate region. *Allergy* 1999;**54**:708-715.
9. Sanda T, Yasue T, Oohashi M, Yasue A. Effectiveness of house dust-mite allergen avoidance through clean room therapy in patients with atopic dermatitis. *J. Allergy Clin. Immunol.* 1992;**89**:653-657.
10. Katoh N, Hirano S, Suehiro M, Matsuda K, Kishimoto S. The characteristics of patients with atopic dermatitis demonstrating a positive reaction in a scratch test after 48 hours against house dust mite antigen. *J. Dermatol.* 2004;**31**:720-726.
11. Matsuoka H, Maeda N, Atsuta Y, Ando K, Chinzei Y. Seasonal fluctuations of Dermatophagoides mite population in house dust. *Jpn. J. Med. Sci. Biol.* 1995;**48**:103-115.
12. Matsuoka H, Maki N, Yoshida S *et al*. A mouse model of the atopic eczema/dermatitis syndrome by repeated application of a crude extract of house-dust mite Dermatophagoides farinae. *Allergy* 2003;**58**:139-145.
13. Sasa M, Miyamoto J, Shinohara S, Suzuki H, Katsuhata A. Studies on mass culture and isolation of Dermatophagoides farinae and some other mites associated with house dust and stored food. *Jpn. J. Exp. Med.* 1970;**40**:367-382.
14. Yamaguchi T, Ono K, Shigeta S *et al*. Differences in response of patient's blood cells with bronchial asthma to diverse house dust mite allergens by histamine release assay. *Jpn. J. Allergol.* 1993;**42**:1754-1763.
15. Mota I, Wong D. Homologous and heterologous passive cutaneous anaphylactic activity of mouse antisera during the course of immunization. *Life Sci.* 1969;**8**:813-820.
16. Leung DYM, Bieber T. Atopic dermatitis. *Lancet* 2003;**361**:151-160.
17. Kang K, Stevens SR. Pathophysiology of atopic dermatitis. *Clin. Dermatol.* 2003;**21**:116-121.
18. Hamid Q, Boguniewicz M, Leung DYM. Diffential in situ cytokine gene expression in acute versus chronic atopic dermatitis. *J. Clin. Invest.* 1994;**94**:870-876.
19. Ohmen JD, Hanifin JM, Nickoloff BJ *et al*. Overexpres-

sion of IL-10 in atopic dermatitis. Contrasting cytokine patterns with delayed-type hypersensitivity reactions. *J. Immunol.* 1995;**154**:1956-1963.
20. Klunker S, Trautmann A, Akdis M *et al*. A second step of chemotaxis after transendothelial migration: keratinocytes undergoing apoptosis release IFN-gamma-inducible protein 10, monokine induced by IFN-gamma, and IFN-gamma-inducible alpha-chemoattractant for T cell chemotaxis toward epidermis in atopic dermatitis. *J. Immunol.* 2003;**171**:1078-1084.
21. Leung DY, Boguniewicz M, Howell MD *et al*. New insights into atopic dermatitis. *J. Clin. Invest.* 2004;**113**:651-657.
22. Wahlgren CF. Itch and atopic dermatitis: an overview. *J. Dermatol.* 1999;**26**:770-779.
23. Ellis C, Luger T, Abeck D *et al*. International Consensus Conference on Atopic Dermatitis II (ICCAD II): clinical update and current treatment strategies. *Br. J. Dermatol.* 2003;**63**:3-10.
24. Katoh N, Hirano S, Yasuno H, Kishimoto S. Effects of tacrolimus ointment on facial eruption, itch, and scratch in patients with atopic dermatitis. *J. Dermatol.* 2004;**31**: 194-199.
25. Hiroi J, Sengoku T, Morita K *et al*. Effect of tacrolimus hydrate (FK506) ointment on spontaneous dermatitis in NC/Nga mice. *Jpn. J. Pharmacol.* 1998;**76**:175-183.