

Atopic Dermatitis-Like Skin Lesions Induced by Topical Application of Mite Antigens in NC/Nga Mice

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Key Words

Allergy · Animal model · Mite · Th2 response · Dermatitis

Abstract

Background: Atopic dermatitis (AD) is a chronic relapsing inflammation usually observed in patients with an individual or a familial history of atopic diseases, precipitated by environmental factors including mite antigens (Ag). However, the exact etiology of AD is unclear. To further explore the pathogenesis and treatment of AD, a suitable animal model is necessary. In this study, we developed a new animal model of AD induced by mite Ag in NC/Nga mice. **Methods:** We injected the extracts of mite Ag intradermally at the ventral side of the ear of SPF NC/Nga mice on days 0, 2, 4, 7, 9, 11, 14 and 16, and measured the clinical symptoms and the ear thickness. On day 18, we collected blood and submandibular lymph nodes (LN) of the immunized ear to perform a histochemical analysis, and to measure the plasma immunoglobulins and cytokines. **Results:** The NC/Nga mice immunized with mite Ag suffered from AD-like skin lesions including erythema followed by edema, excoriation and scaling. The histological and immunohistochemical ex-

aminations of the affected skin showed epidermal hyperplasia with hyperkeratosis, severe infiltration of CD4⁺ T lymphocytes, eosinophils and macrophages, and degranulation of mast cells. The total plasma IgE level was markedly elevated in mite Ag-treated mice. LN cells of mice immunized with mite Ag synthesized IgE in an Ag-dependent manner and secreted interleukin-4 (IL-4) and IL-5 but not interferon- γ . **Conclusions:** NC/Nga mice treated with mite Ag manifest clinical and immunological aspects similar to patients with AD, suggesting that this model is suitable for exploring the pathogenesis of human AD.

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Introduction

Atopic dermatitis (AD) is one of the most common skin diseases in patients with a personal or a family history of atopic disorders [1], and its prevalence is still increasing. Patients with AD frequently show elevated immunoglobulin E (IgE) levels against many kinds of allergens [2]. Epidemiological studies [3, 4] suggest that environmental factors including mite antigens (Ag) [5, 6],

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air pollution [7] and mental stress might contribute to the onset or progression of AD [8]. Mite Ag have been focused on because of their high potency as allergens. More than 90% of patients with AD are positive for the IgE radioallergosorbent test (IgE-RAST) against mite Ag [9], and clinical symptoms of AD improved after mites had been removed [10, 11]. *Dermatophagoides pteronyssinus* (Dp), a representative allergen of human AD, is involved in the pathogenesis of AD [12]. Although mite Ag are major allergens in human AD, there is no suitable animal model for investigating the importance of mite Ag in AD.

It is known that T helper (Th) cells can be grouped into two functionally distinct subsets, Th1 and Th2, characterized by the different effector cytokines that they secrete. Th1 cells produce IL-2 and IFN- γ , whereas Th2 cells secrete IL-4, IL-5, IL-10 and IL-13 which enhance humoral immunity. Overexpression of IL-4 and IL-5 was observed in the affected skin sites of AD [13, 14], and IFN- γ production by T cells of the patients with AD was lower than that by T cells of healthy donors [15–17], suggesting the polarization of Th2 in patients with AD. Although Th2 seems to contribute to the pathogenesis of AD, the differentiation process of Th2 in patients with AD is not completely understood. Therefore, developing an animal model showing a Th2-dominant immune reaction would help to clarify the pathogenesis of AD.

Matsuda and his colleagues [18–21] proposed that NC/Nga mice might become an excellent animal model for human AD. NC/Nga mice were established as an inbred strain in 1957 [22, 23] and have the following biological characteristics: liver and kidney esterase like a DBA/2 strain, high susceptibility to X-irradiation and high susceptibility to anaphylactic shock. NC/Nga mice raised in air-uncontrolled conventional circumstances spontaneously suffer from AD-like skin lesions with a marked elevation in plasma levels of total IgE, whereas NC/Nga maintained in specific pathogen-free (SPF) conditions do not show any clinical symptoms. However, a variety of Ag can be involved in the development of AD-like skin lesions in NC/Nga, which makes it difficult to analyze the pathogenesis of AD-like skin lesions. To further investigate the pathophysiology of AD, a specific Ag-induced model is necessary.

Environmental factors are thought to trigger the skin lesions of AD since NC/Nga mice grown in SPF circumstances showed neither clinical signs nor IgE hyperproduction. The mice maintained under conventional conditions are often infected with fur mites, and the infection can provoke dermatitis in NC/Nga mice [24]. However, it has not been clearly understood whether mite Ag causes

AD-like skin lesion in NC/Nga mice without other environmental factors. Excluding the involvement of environmental factors is significant in order to analyze the pathogenesis of AD-like dermatitis in NC/Nga mice, and SPF conditions are preferable for analyzing the pathogenicity of mite Ag.

In this study, a local injection of Dp, a major allergen, could induce AD-like skin lesions in NC/Nga mice in SPF conditions. Lymphocytes in lymph nodes (LN) of Dp-injected mice produced IL-4 and IL-5, but not IFN- γ , and produced anti-Dp-specific IgG and IgE in response to Dp. These results demonstrated that Dp could evoke an Ag-specific Th2 immune response in NC/Nga mice and suggest that Dp-injected NC/Nga mice are a useful model for investigating the pathogenesis of AD.

Material and Methods

Mice

Six-week-old SPF NC/Nga or BALB/c female mice were purchased from Charles River Japan (Osaka, Japan) or Fujisawa Technical Services (Osaka, Japan), and maintained in SPF conditions for more than 1 week before use. All conditions related to keeping and handling the animals were approved by the Japanese Society of Laboratory Animals and implemented according to the National Institutes of Health (NIH) Guidelines for the Care and Use of Laboratory Animals.

Cytokines and Antibodies

Monoclonal antibodies (Abs) for mouse CD4 (PharMingen; San Diego, Calif., USA) and mouse F4/80 (Serotec, Oxford, UK) were used for immunohistochemistry. Fluorescein isothiocyanate (FITC)-conjugated monoclonal Abs (mAbs) for mouse T cell receptor (TCR) $\alpha\beta$, CD4, CD8 α , CD69, Mac-1, B220 and IFN- γ , and phycoerythrin (PE)-conjugated mAb for mouse IL-4 were purchased from PharMingen. Recombinant IL-6 was purchased from PharMingen. For enzyme-linked immunosorbent assay (ELISA), mAbs for mouse IL-2 (JES6-1A12), IL-4 (BVD4-1D11), IL-5 (TRFK5) and IFN- γ (R4-6A2) as capture Abs, and biotin-conjugated mAbs for IL-2 (JES6-5H4), IL-4 (BVD6-24G2), IL-5 (TRFK4) and IFN- γ (XMG1.2) as detection Abs were also purchased from PharMingen.

Repeat Application of Mite Ag

NC/Nga mice were injected intradermally with saline or 5 μ g of Dp extract (LSL, Japan) dissolved in saline on the ventral side of their right ears on days 0, 2, 4, 7, 9, 11, 14 and 16. The ear thickness of the ear injected with Ag was measured with an ear thickness gauge (OZAKI MFG, Osaka, Japan) 24 h after each intradermal injection. On day 18, blood was collected and the plasma sample was stored at -20°C until quantitative analysis for Igs.

Cytokines and Igs Production by LN Cells

Submandibular LN were removed in sterile conditions from Dp-injected and age-matched nontreated mice on day 18, and single-cell suspensions were prepared in RPMI 1640 (Life Technologies; Grand

Island, N.Y., USA) supplemented with 10% FCS (Hyclone; Logan, Utah, USA), 10^{-4} M 2-mercaptoethanol, 50 U/ml penicillin and 50 µg/ml streptomycin. Cells were incubated in 24-well culture plates (Sumitomo Bakelite; Tokyo, Japan) at a concentration of 5×10^6 cells/ml with 10 µg/ml of Dp, 1 µg/ml of concanavalin A (Con A; WAKO Chemical Corp., Osaka, Japan) or 100 U/ml of recombinant IL-6 at 37°C under 5% CO₂. The culture supernatants were collected after 24 h and 7 days to determine cytokine and Ig production, respectively.

ELISA

IgG titer in plasma specific to Dp were measured by ELISA using Dp and anti-mouse IgG mAb. Briefly, 96-well immunoplates (Sumitomo Bakelite) were coated with 50 µl of Dp (50 µg/ml) or saline (PBS) for negative control and incubated for 1 h at 37°C. After washing 3 times with PBS containing 0.05% Tween 20, the plates were blocked with PBS supplemented with 0.5% BSA for 30 min at room temperature. After washing, 100 µl of standards and samples were added to the wells and incubated for 1 h at room temperature. Then 50 µl of peroxidase-conjugated anti-mouse IgG (Zymed; San Francisco, Calif., USA) was added and they were further incubated for 30 min at room temperature. The plates were washed and substrate (*o*-phenylenediamine tablet; Sigma, St. Louis, Mo., USA) and H₂O₂ mixed according to the manufacturer's instructions were added. The absorbance at 492 nm wavelength was measured which SPECTRA MAX 250 (WAKO, Osaka, Japan) after stopping the reaction with H₂SO₄. Total IgE levels were measured with sandwich ELISA using two kinds of rat anti-mouse IgE mAb. Immunoplates (Sumitomo Bakelite) were coated with 50 µl of mAb to mouse IgE (10 µg/ml; YAMASA, Chiba, Japan) and incubated for 1 h at 37°C. Plates were washed and blocked. Collected samples or standard mouse IgE (YAMASA) were added to the wells and incubated for 1 h at room temperature. After washing, 50 µl biotinylated mAb for mouse IgE (YAMASA) was added to the wells and incubated for 1 h at room temperature. Two hundred microliters of peroxidase-conjugated streptavidin (Zymed) was added and incubated for 1 h at 37°C. After washing, *o*-phenylenediamine was added and the reaction was stopped with the addition of H₂SO₄. Then the absorbance at 492 nm wavelength was measured. Concentrations of IL-2, IL-4, IL-5 and IFN-γ in culture supernatants were also measured with sandwich ELISA similar to that of total IgE. Immunoplates (Nunc Roskilde, Denmark) and a TMB Peroxidase EIA Kit (Bio-Rad Laboratories, Hercules, Calif., USA) as a substrate were provided and the absorbance at 450 nm wavelength was measured after stopping the reaction with H₂SO₄. The sensitivities of IL-2, IL-4, IL-5 and IFN-γ assays were 3.125, 3.906, 3.906 and 50 pg/ml, respectively.

Flow-Cytometric Analysis

To examine the cell populations in LN, flow-cytometric analyses were performed. Freshly isolated LN cells were incubated with mAbs for TCRαβ, CD4, CD8α, CD69, Mac-1 or B220 for 30 min at 4°C and analyzed by fluorescence-activated cell sorter (FACScan; Becton Dickinson, Bedford, Mass., USA). To examine the populations of IL-4 and IFN-γ producing cells in submandibular LN, cell preparations were incubated with Dp (10 µg/ml) for 19 h and treated with CytoStain™ Kits (PharMingen) for an additional 5 h. Then the cells were harvested and stained with FITC-conjugated anti-mouse IFN-γ and PE-conjugated anti-mouse IL-4 Ab, and analyzed.

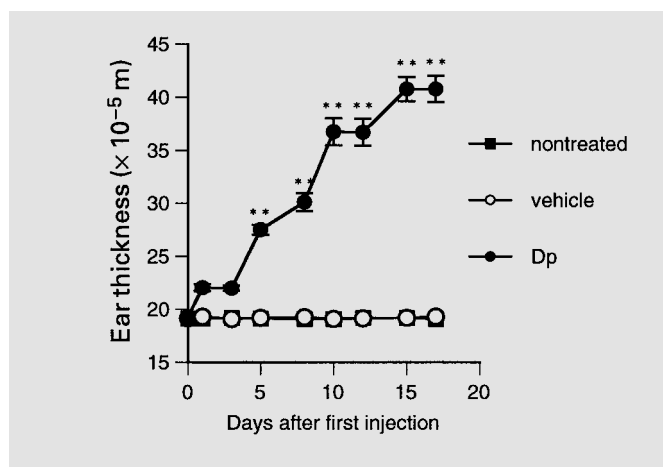


Fig. 1. Ear thickness and clinical features of the ear of mice injected with Dp or vehicle and age-matched nontreated mice. NC/Nga mice were injected with Dp (●) or vehicle (○) on day 0, 2, 4, 7, 9, 11, 14 and 16. Ear thickness was evaluated by a thickness gauge 24 h after each injection. That of age-matched nontreated mice (■) was evaluated at the same time when Dp and vehicle (saline-injected) mice were done. Each symbol represents the mean ± SE of 10 mice. ** $p < 0.01$, when compared with the vehicle group.

Histological Analysis

Right ears of Dp-injected and nontreated mice were removed 2 days after the last injection of Dp, and one part of the ear was fixed in 10% phosphate-buffered formalin (pH 7.2), embedded in paraffin, cut in 3 µm, and stained with hematoxylin and eosin or toluidine blue (pH 4.0). The other part of the ear was embedded in OCT compound (Miles; Elkhart, Ind., USA) and snap-frozen in dry-ice ethanol for immunohistochemistry. Frozen sections cut in 5 µm were fixed in cold acetone. After blockage of endogenous peroxidase, mAb for mouse CD4 or F4/80 was applied overnight at 4°C, and then goat anti-rat IgG conjugated with peroxidase (Jackson, West Grove, Pa., USA) was overlayed. The enzyme reaction was visualized with 3-amino-9-ethylcarbazole (Aldrich Chemical Co., Milwaukee, Wisc., USA).

Statistical Analysis

Data were expressed as mean ± SE. Dunnet's multiple comparison was performed for statistical analysis and $p < 0.05$ was taken as the level of significance.

Results

Development of AD-Like Skin Lesions

Since Dp is one of the major allergens involved in the pathogenesis of human AD, we examined whether mite Ag could induce AD-like disease in NC/Nga mice raised in SPF conditions. We injected the extracts of Dp intradermally at the ventral site of the ear of SPF NC/Nga mice

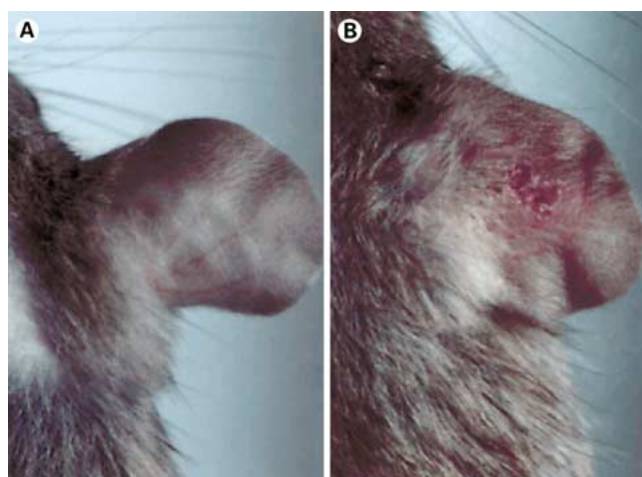


Fig. 2. Clinical feature of the ears of NC/Nga mice on day 18. Erythema, edema, excoriation and scaling were noticed in the ear injected with Dp (**B**), but not with vehicle (**A**).

and observed ear thickness and the clinical skin severity 24 h after every application of Ag. From day 5–8, the ear thickness was definitely increased (fig. 1). The ear thickness and the severity of the skin lesions were increased with further Ag injections until day 18. Erythema, edema, excoriation and scaling were observed at the dorsal site of the ears (fig. 2). Itching was also observed. The mice injected with vehicle showed neither skin lesions nor ear thickening throughout the application (fig. 1). Neither increased ear thickness nor skin lesions were observed in BALB/c mice, in which Dp had been used in the same way as in NC/Nga mice (data not shown).

Table 1. Numbers of eosinophils and mast cells in dorsal skin of vehicle and Dp-injected NC/Nga mice

Skin	Number of cells/5 fields				T cells CD4+
	eosinophils	mast cells			
		granulated	mildly degranulated	severely degranulated	
Nontreated	0	36.3±3.6	0	0	3.8±0.7
Dp	252±21	18.8±2.8	24.0±3.0	43.1±3.9	60.0±6.4

The numbers of eosinophils, the degranulation of mast cells, and CD4+ cells in dorsal skin of vehicle and Dp-injected NC/Nga mice. Hematoxylin and eosin staining for the calculation of eosinophils, toluidine blue staining for mast cells and immunostaining for CD4+ cells were done as described in Materials and Methods. Cells between epithelium and panniculus carnosus were counted under a microscope at a magnification of × 400 and were expressed as the total number of cells in 5 fields. Each value was obtained from 6 mice per group (mean ± SE).

Histopathological Analysis of the Skin Lesion

The skin lesions were examined histopathologically on day 18. Epidermal hyperplasia with areas of parakeratosis and severe infiltrations of eosinophils and a small number of mononuclear cells in the dermis of ears injected with Dp were found (fig. 3B, table 1). We also observed an increased number of mast cells (table 1) and most of the mast cells were degranulated (fig. 3C, D) in the skin lesions but not in the nontreated ear. A great number of CD4+ T lymphocytes and F4/80+ macrophages were identified in the dermis of the ear injected with Dp by immunohistochemical staining, whereas neither cell was found in the ear of nontreated mice (fig. 3F, H, table 1).

Anti-Dp-Specific IgG Titer and Total IgE Levels in Plasma

IgG and IgE are considered to be associated with symptoms of AD, and plasma levels of anti-Dp-specific IgG and total IgE in NC/Nga mice on day 18 were quantitated with ELISAs. The total IgE level in Dp-injected mice was higher than those in nontreated mice (fig. 4B). Anti-Dp IgG were detected in Dp-injected mice, but not in nontreated mice (fig. 4A).

In vitro Ig Production by Dp

At first, we investigated the cell number and cell population of drainage LN and spleen of Dp-injected NC/Nga mice, and compared them with those of nontreated mice. The cell number of submandibular LN of Dp-injected mice was dramatically elevated when compared to those of nontreated mice (fig. 5A). However, the splenic cell number of Dp-injected mice was the same as that of non-

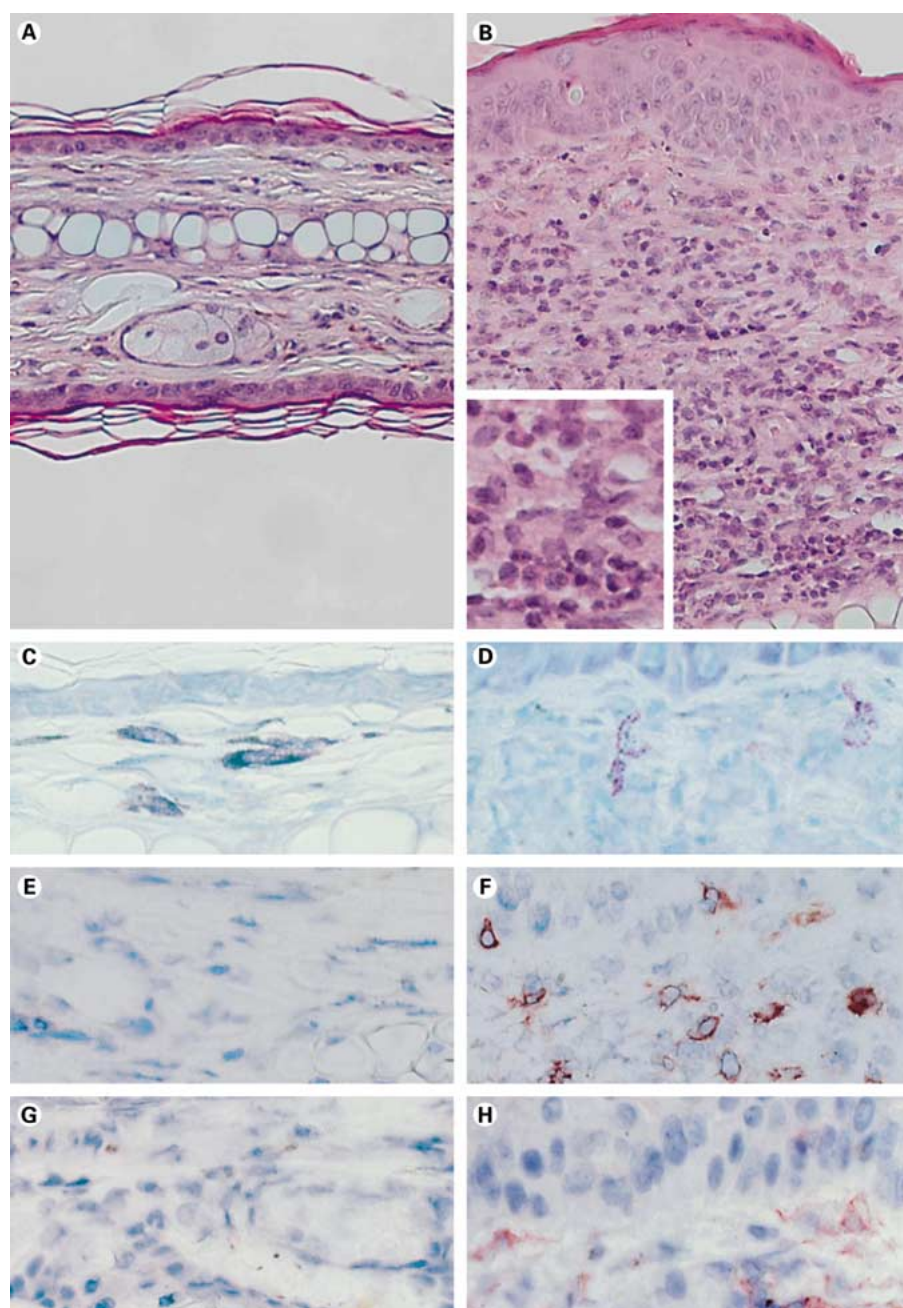


Fig. 3. Histological analysis of the non-treated and Dp-injected ears on day 18. Hematoxylin and eosin staining preparation demonstrated that hyperplasia of the epidermis and infiltration of eosinophils (insert) in the dermis were pronounced in Dp-injected mice (**B**), but not in nontreated mice (**A**). It was shown in toluidine blue-staining preparation that mast cells in Dp-injected mice (**D**) were degranulated, whereas those in nontreated mice (**C**) were not. Immunostaining for CD4 of nontreated (**E**) and Dp-injected mice (**F**), and for F4/80 of nontreated (**G**) and Dp-injected mice (**H**) demonstrated that infiltrations of CD4+ T lymphocytes and F4/80+ macrophages were observed in Dp-injected mice.

treated mice (fig. 5B). To determine the population that had proliferated in response to Dp, freshly isolated LN cells were analyzed with a flow cytometry. B220+ cells and CD69+ T cells in LN of Dp-injected mice were increased (table 2).

Next, to confirm the contribution of Dp in this model, the response of drainage LN cells of Dp-injected mice to Dp was analyzed and compared with those of nontreated

mice. LN cells of Dp-injected and nontreated mice were cultured with Dp or IL-6, and anti-Dp-specific IgG titer and IgE concentration in the culture supernatant were examined. When LN cells were incubated with Dp, LN cells of Dp-injected mice produced anti-Dp-specific IgG and IgE (fig. 6), whereas those of nontreated mice could produce neither Igs. Splenic cells did not synthesize Igs when stimulated with Dp (data not shown).

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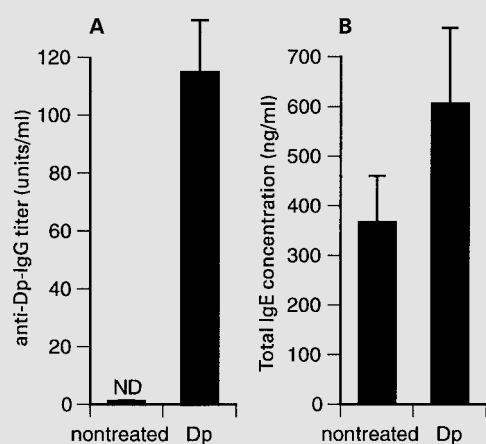


Fig. 4. Plasma levels of anti-Dp-specific IgG and total IgE. Blood was collected on day 18 from the retroorbital plexus and diluted 4 times with saline, and plasma samples were obtained. Anti-Dp-specific IgG titer (**A**) and total IgE level (**B**) were measured with ELISA as described in Materials and Methods. Each point represents the mean $\pm$ SE of 7 mice. ND = Not detected.

Fig. 5. Cell number of submandibular LN and spleen of nontreated and Dp-injected mice. LN and spleens of nontreated and Dp-injected mice were collected and homogenated to make a single-cell suspension. The cell numbers of freshly isolated LN cells (**A**) or splenic cells (**B**) were counted with 0.25% trypan blue solution. These data represent six separate experiments.

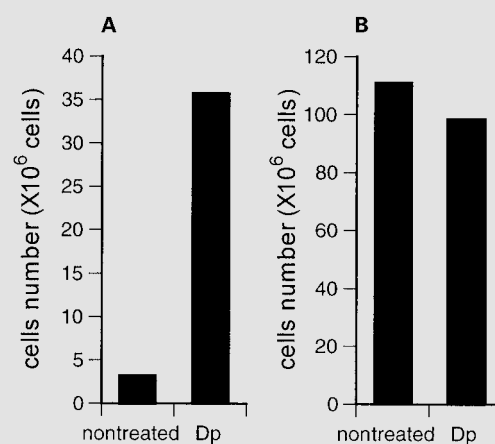
Fig. 6. In vitro Ig production by LN cells in response to Dp. 5×10^6 cells isolated from submandibular LN of nontreated and Dp-injected mice were incubated in 24-well culture plates with Dp (10 μ g/ml) or IL-6 (100 U/ml) for 7 days. Anti-Dp-specific IgG titer (**A**) and total IgE level (**B**) in the culture supernatants were measured with ELISA. Ig production by LN cells of nontreated mice ($\square$) and Dp-injected mice ($\blacksquare$) is shown. These data represent six separate experiments performed in duplicate. ND = Not detected.

Table 2. Population in LN cells (%)

	TCR $\alpha\beta$	CD4	CD8 α	CD69	Mac-1	B220
Nontreated	60.95	38.82	18.39	10.73	8.83	35.28
Dp	45.53	29.81	15.77	18.21	0.77	55.16

FACS analysis in LN cells. LN cells were freshly obtained on day 18. The cells were stained with either FITC-conjugated mAbs for mouse TCR $\alpha\beta$, CD4, CD8 α , CD69, Mac-1 or B220. The cell preparations were analyzed by a flow cytometer. The percentage of positive cells was calculated from the control. The data represent three experiments.

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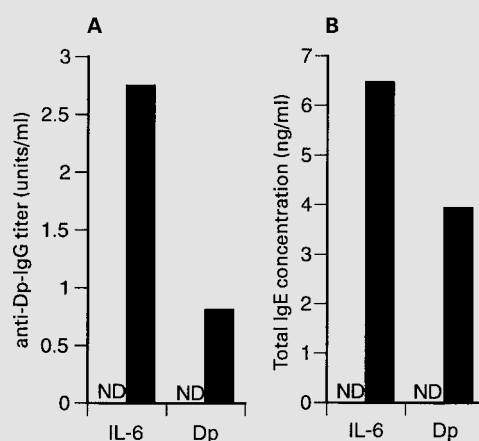


Table 3. Th1/Th2 population in LN (%)

Cell type	Nontreated	Dp-injected
IL-4- IFN- γ -	98.84 $\pm$ 0.68	90.1 $\pm$ 4.26
IL-4+ IFN- γ -	0.77 $\pm$ 0.64	8.38 $\pm$ 3.14
IL-4- IFN- γ +	0.14 $\pm$ 0.03	0.50 $\pm$ 0.30
IL-4+ IFN- γ +	0.19 $\pm$ 0.11	1.02 $\pm$ 0.81

Values represent mean $\pm$ SE. LN cells were isolated from Dp-injected or nontreated mice on day 18, and stimulated with Dp (10 μ g/ml) for 19 h. Then cells were harvested and stained with FITC-conjugated anti-mouse IFN- γ mAb and PE-conjugated anti-mouse IL-4 mAb, and analyzed by FACScan.

Th2 Responses in LN Cells of Dp-Injected Mice

The elevation of the plasma IgE level is induced by Th2 differentiation. Therefore, we investigated the population of IL-4-producing cells in LN (table 3). Submandibular LN cells were incubated with Dp for 19 h, and intracellular IL-4 and IFN- γ were investigated with FACScan analysis. The IL-4+ IFN- γ - Th2 population was greater than the IL-4- IFN- γ + Th1 population in LN cells of Dp-injected mice. The population of both Th1 and Th2 was small in LN of nontreated mice in response to Dp.

Next, IL-2, IL-4, IL-5 and IFN- γ synthesis by LN cells of Dp-injected or nontreated mice was examined (fig. 7). When LN cells of nontreated mice were incubated with Dp for 24 h, IL-4, IL-5 or IFN- γ in the culture supernatant were below the detection level of ELISA. IL-4 and IL-5 in the culture supernatant were below the detection level of ELISA. IL-4 and IL-5 in the culture supernatant of LN cells isolated from Dp-injected mice were detected but not IFN- γ , despite there being a detectable level of IFN- γ in the culture supernatant of LN cells of Dp-injected mice stimulated with Con A. Only IL-2 was detectable in the culture supernatant of LN cells isolated from nontreated mice stimulated with Con A.

Discussion

The mice injected with Dp manifested erythema, edema, excoriation and scaling only in the injected sites and also showed an increased level of plasma IgE. Histopathologically, the degranulation of mast cells, and infiltration of eosinophils, CD4+ T cells and macrophages to dermis were noticed in the skin lesion. All these findings are typical symptoms observed in patients with AD, indicating the relationship of IgE, mast cells, eosinophils, CD4+ T cells and macrophages to the pathogenesis of AD-like skin lesions. Thus, this model has the same characteristics of human AD and is useful for clarifying the pathogenesis of AD.

In this study, we have demonstrated that intradermal injections of mite Ag extracts, a major Ag participating in the development of AD, could induce AD-like skin lesions in NC/Nga mice. Matsuda and his colleagues [18–21] demonstrated that NC/Nga mice suffered AD-like skin lesions when they were maintained in air-uncontrolled conventional circumstances, and they suggest that several environmental factors may be involved in the pathogenesis of the dermatitis. In patients with AD, mite Ag is one of the major environmental factors, and it is important to induce AD-like skin lesions by mite Ag for

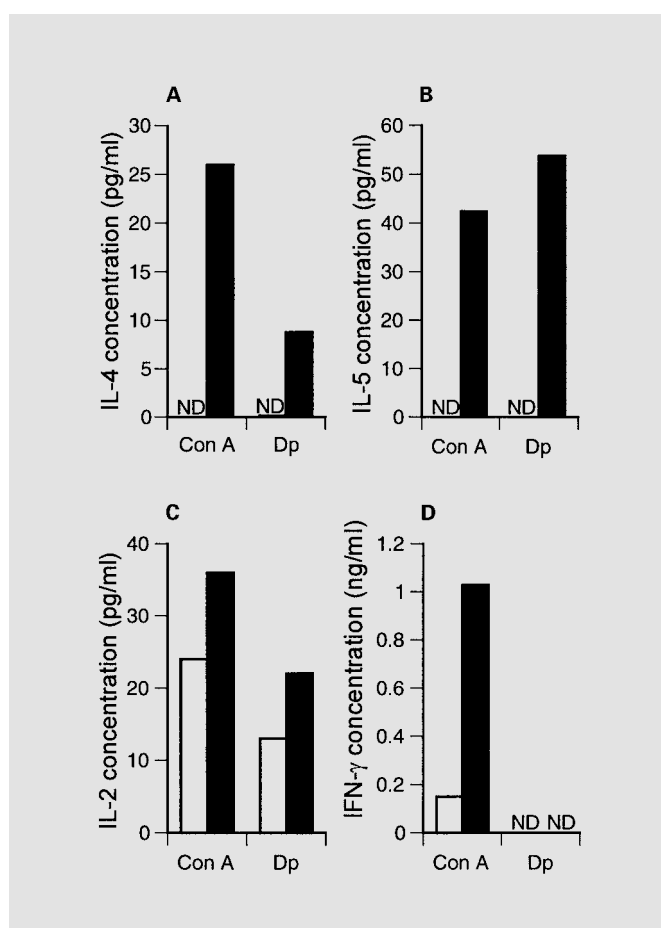


Fig. 7. Cytokine production by LN cells in response to Dp. LN cells isolated from mice injected with Dp (■) or vehicle (□) were stimulated with Dp (10 μ g/ml) or Con A (1 μ g/ml) for 24 h. Concentrations of IL-4 (A), IL-5 (B), IL-2 (C) and IFN- γ (D) in culture supernatants were measured with ELISA as described in Materials and Methods. These data represent three separate experiments performed in duplicate. ND = Not detected.

investigating the significance of mite Ag to human AD. We induced AD-like dermatitis in NC/Nga mice only with mite Ag extracts without other environmental factors, and this model might show further information relevant for the pathogenesis of human AD.

Our data indicate that mite Ag induce Th2 reactions in NC/Nga mice. The percentage of IL-4+ IFN- γ - cells in the LN cells stimulated with Dp increased and the cells generated IL-4 and IL-5, but not IFN- γ , suggesting Ag-specific IgG and IgE production and the infiltration of eosinophils. Mite Ag are reported to induce Th2 cytokines and responses [25–27]. IL-4 is the principal factor for IgE synthesis, and IL-5 is also the key factor for the activation/

proliferation of eosinophils [14, 28, 29]. Th2 responses are often noticed in patients with AD, and we can observe Th2 responses in this model which are the same as in patients with AD [27, 30, 31]. Therefore a further investigation of this model will provide us with useful information about the mechanism of human AD.

We have demonstrated that mite Ag without adjuvants can induce AD-like dermatitis in SPF NC/Nga mice. Immunization without adjuvants does not usually provoke a strong immune reaction [32–36]. In this study, mite Ag without adjuvants generated skin lesions and a Th2 immune response in NC/Nga mice. Mite Ag could not induce severe clinical symptoms in BALB/c, suggesting that NC/Nga mice might have a genetic background making them susceptible to skin lesions in response to mite Ag like patients with AD. Recent studies have demonstrated the complex interrelationship of genetic and environmental factors that contribute to the development of human AD [37]. We demonstrated that mite Ag, which

is a major environmental factor in patients with AD, could induce AD-like dermatitis in NC/Nga mice which might have some genetic background. This model might show the relationship between mite Ag and genetic background in AD-like dermatitis.

In conclusion, we have succeeded in inducing AD-like skin lesions and Th2 responses in SPF NC/Nga mice with mite Ag alone. This model is very similar to human AD, suggesting that it is suitable for clarifying the pathogenesis of AD.

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