

Establishment of Allergic Dermatitis in NC/Nga Mice as a Model for Severe Atopic Dermatitis

Xiu Kun GAO, Nobuaki NAKAMURA, Kazutoshi FUSEDA, Hiroyuki TANAKA, Naoki INAGAKI, and Hiroichi NAGAI*

Department of Pharmacology, Gifu Pharmaceutical University, 5-6-1 Mitahorahigashi, Gifu 502-8585, Japan.

Received April 19, 2004; accepted June 4, 2004

Mite antigen has been suggested to play important roles in the onset and/or development of atopic dermatitis, and mite antigen-induced dermatitis models appear beneficial for the basic study of atopic dermatitis. In the present study therefore, we attempted to establish an allergic dermatitis model in mice using *Dermatophagoides farinae* crude extract as an antigen. Mite antigen solution at a concentration of 1 or 10 mg/ml was painted 5 times repeatedly at an interval of 7 d onto the ear of NC/Nga or BALB/c mice with or without simultaneous tape-stripping. Apparent biphasic ear swelling was observed after the 4th and 5th antigen applications in both strains of mice treated with 10 mg/ml of antigen solution. Thickening of the epidermis, fibrosis of the dermis, and the accumulation of inflammatory cells were also observed after the 5th application. The inflammatory changes were more evident in NC/Nga mice than in BALB/c mice and potentiated by tape-stripping. The ear swelling was accompanied by increased serum IgE, increased expression of interleukin-4 mRNA and decreased expression of interferon- γ mRNA in cervical lymph nodes and ears. These results indicate that ear swelling caused by repeated mite antigen application with simultaneous tape-stripping has a Th2-dominant background and that the inflammatory responses are expressed more potently in NC/Nga mice than in BALB/c mice. The dermatitis caused by mite antigen in NC/Nga mice appears to be a useful model for the basic study of atopic dermatitis.

Key words atopic dermatitis; mite antigen; NC/Nga mouse; IgE; Th1/Th2 balance; tape-stripping

Atopic dermatitis is a complex eczematous skin disease accompanied by severe itching and episodes are frequently repeated.^{1,2)} In most cases, onset of the disease is observed in infants and is considered to be dependent on both genetic and environmental factors. Elevated serum IgE levels are also a characteristic feature in many patients. The itching is the most important problem, and scratching worsens the dermatitis. Although patients with atopic dermatitis, especially adult patients with severe symptoms, have been increasing recently, the pathogenesis of the disease has yet to be elucidated.

To understand the onset and development of a disease, appropriate animal models are essential. For atopic dermatitis, some artificially-induced and naturally occurring animal models have been reported. In artificially induced models, passively sensitized animals with specific IgE are exposed to a corresponding allergen^{3,4)} or intact animals are exposed to an allergen repeatedly to induce dermatitis.^{5–8)} In contrast, NC/Nga mice naturally develop an interesting form of dermatitis with some features of atopic dermatitis.^{9–16)} An inbred mouse strain, NC/Nga, was established in 1955 and those mice exhibit dermatitis on the face and neck from the age of 2–3 months. Furthermore, anaphylactic shock can be induced easily, and anemia, glomerulonephritis, and systemic lupus erythematosus-like symptoms are observed in aged mice.^{9–11)} In 1997, Matsuda *et al.* reevaluated the dermatitis as a model for atopic dermatitis.¹²⁾ The dermatitis is developed in mice kept under conventional conditions but not in specific pathogen-free conditions. Under the same conditions, BALB/c mice do not develop this form of dermatitis, indicating that genetic factors are also involved.¹³⁾ Although the dermatitis in NC/Nga mice appears to be triggered by some environmental factor(s), the mechanisms for the trigger have not yet been defined.

We have studied allergic dermatitis models using repeated

topical application of a hapten, 2,4-dinitrofluorobenzene, in BALB/c mice.^{17,18)} The induced dermatitis is accompanied by elevated serum IgE levels, increased expression of Th2 cytokine mRNA, eosinophil accumulation in the lesions and frequent scratching behavior, which are characteristic features of atopic dermatitis. On the other hand, mite antigen has been suggested to play important roles in the onset and/or development of atopic dermatitis^{19–21)} and breakdown of the epidermal barrier system due to inflammation and scratching is an important worsening factor for dermatitis. In the present study therefore, we attempted to establish a severe atopic dermatitis model by repeated topical application of mite antigen coupled with tape-stripping in NC/Nga mice.

MATERIALS AND METHODS

Mice Female NC/Nga and BALB/c mice, 6 weeks of age, were obtained from Japan SLC (Hamamatsu, Japan) and maintained for 2 weeks before the start of experiments. They were housed in an air-conditioned animal room with a temperature of $22 \pm 1^\circ\text{C}$ and a humidity of $60 \pm 5\%$, and fed a laboratory diet and water *ad libitum*. Experiments were undertaken following the Guidelines for the Care and Use of Experimental Animals of the Japanese Association for Laboratory Animal Science (1987).

Mite Antigen *Dermatophagoides farinae* crude extract (mite antigen, lyophilized, Torii, Tokyo, Japan) was used as an antigen.²²⁾ Mite antigen was dissolved in phosphate-buffered saline (PBS) containing 0.5% Tween 20.

Induction of Dermatitis in the Mouse Ear Both surfaces of mouse ear lobes were stripped 3 times using a surgical tape (W129, Nichiban, Tokyo, Japan). One hour after the tape-stripping, 25 μl of 1 or 10 mg/ml mite antigen solution was painted onto each surface of both ear lobes. Tape-stripping and mite antigen painting were repeated 5 times at an

* To whom correspondence should be addressed. e-mail: nagai@gifu-pu.ac.jp

interval of 7 d, and ear thickness was measured using a dial thickness gauge (R12-1A, Ozaki, Tokyo, Japan) immediately before each tape-stripping, and 1, 4, and 24 h after each mite antigen application. In control mice, vehicle was painted instead of mite antigen solution. Results are expressed as increased ear thickness after subtracting the value obtained before the first tape-stripping from each value.

Cervical lymph nodes and ears were removed for the evaluation of cytokine mRNA expression 4 h after the 5th antigen application. Blood samples and ears were obtained 24 h after the 5th antigen application and used for the measurement of serum IgE levels and histologic observation, respectively.

Measurement of Serum IgE Total serum IgE levels were measured using the enzyme-linked immunosorbent assay. In brief, 100 μ l of 5 μ g/ml rat anti-mouse IgE heavy chain (Serotec, Oxford, U.K.) in PBS was placed in each well of an immunoplate (Nunc Immunoplate I, 96-well, Nalge Nunc International, Rochester, NY, U.S.A.) and the plate was stored overnight at 4 °C. After washing the wells with PBS containing 0.1% Tween 20 (washing buffer) 5 times, 200 μ l of PBS containing 1% bovine serum albumin (BSA-PBS) was placed in each well. After 1 h at room temperature, the wells were washed 5 times with washing buffer and 100 μ l of serum samples diluted 30-fold with BSA-PBS were placed in the wells. After incubation for 1 h at room temperature, wells were washed 5 times with washing buffer and then 100 μ l of peroxide-labeled polyclonal anti-mouse IgE goat IgG antibody (Nordic Immunological Laboratory, Tilburg, Netherlands) diluted 5000-fold with washing buffer was added to the wells and the plate was stored for 1 h at room temperature. After washing 5 times with washing buffer, the enzyme reaction was initiated by adding 100 μ l of substrate solution (containing citric acid 0.1 M, Na₂HPO₄ 0.2 M, *o*-phenylene diamine, and H₂O₂) and the plate was kept for 30 min at room temperature in a dark place. The reaction was terminated by adding 50 μ l of 2 M H₂SO₄ into each well, and absorbance at 492 nm was measured immediately using an immunoreader (Titertek Multiscan MCC/340, Dainippon, Osaka, Japan). A standard curve was prepared using monoclonal anti-DNP IgE (Sigma, St. Louis, MO, U.S.A.) diluted with BSA-PBS.

Detection of Cytokine mRNA The expression of interleukin-4 (IL-4) and interferon- γ (IFN- γ) mRNA in cervical lymph nodes and ears was examined. Excised lymph nodes and ears were homogenized in Isogen (Nippon Gene, Tokyo, Japan) using an HG 30 Homogenizer (Hitachi, Tokyo, Japan). One milliliter of homogenate was mixed vigorously with 200 μ l of chloroform (Nacalai Tesque, Kyoto, Japan), and centrifuged at 13000 rpm for 15 min at 4 °C. The aqueous phase was separated and RNA in the phase was precipitated by mixing with 0.5 ml of 2-propanol (Nacalai Tesque). The precipitate was washed with 75% ethanol (Nacalai Tesque), dried, and then dissolved in diethyl pyrocarbonate (DEPC)-treated water (Nacalai Tesque). The total RNA content was calculated based on the absorbance at 260 nm and the quality was confirmed by electrophoresis.

The reverse transcriptase-polymerase chain reaction was employed for the detection of mRNA. A mixture of 11 μ l containing RNA 1 μ g, DEPC-treated water, and random primer (Gibco BRL, Grand Island, NY, U.S.A.) was heated at 70 °C for 10 min, and then mixed with 4 μ l of 5 $\times$ First Strand Buffer (Gibco), 1 μ l of 10 mM deoxynucleoside triphosphate

Table 1. Primers Employed for Detecting Cytokine mRNA

Primer (Product size)	Sequence
β -Actin (245 bp)	
Sense	5' GTG GGC CGC TAG GCA CCA 3'
Antisense	5' CGG TTG GCC TTA GGG TTC AGG GGG G 3'
IL-4 (279 bp)	
Sense	5' ACG GAG ATG GAT GTG CCA AAC GTC 3'
Antisense	5' CGA GTA ATC CAT TTG CAT GAT GC 3'
IFN- γ (405 bp)	
Sense	5' TAC TGC CAC GGC ACA GTC ATT GAA 3'
Antisense	5' GCA GCG ACT CCT TTT CCG CTT CCT 3'

(dNTP, Gibco), and 2 μ l of 0.1 M dithiothreitol (Gibco). After 5 min at 25 °C, 1 μ l of reverse transcriptase (Superscript II, Gibco) was added and reverse transcription at 25 °C for 10 min, 42 °C for 50 min, and then 70 °C for 15 min, was performed on Trio-Thermoblock (Biometra, Goettingen, Germany). Then cDNA at a volume of 1 μ l was mixed with Tris-HCl 100 mM (pH 8.3), KCl 500 mM, MgCl₂ 1.5 mM, 0.01% gelatin, dNTP 10 mM, 5 units/ml ampliTaQ DNA polymerase (TaKaRa Taq, Takara, Kyoto, Japan), and primers 1 μ M (Stratagene, La Jolla, CA, U.S.A., Table 1) and the polymerase chain reaction (denaturation, 94 °C for 1.5 min; annealing, 62 °C for 1.5 min; extension, 72 °C for 1.5 min, 35 cycles) was performed on Trio-Thermoblock. Products were electrophoresed on 2% agarose gel containing ethidium bromide. The bands were recorded with a Polaroid camera (Polaroid 665 film, Nippon Polaroid, Tokyo, Japan) and densitometrically scanned for semiquantitative evaluation. Results were normalized by β -actin expression.

Histopathologic Specimens Excised ear lobes were fixed with formaldehyde, embedded in paraffin, and thin sections were made. The skin sections were stained with hematoxylin and eosin.

Statistics The results of ear swelling and serum IgE levels are expressed as mean $\pm$ S.E.M. Data from two groups were statistically evaluated using Student's or Welch's *t*-test based on the variance of data examined with the *F*-test. Data from three groups were examined using nonparametric or parametric Dunnett's multiple-comparison test after confirming the variance of data using Bartlett's test. When the *p* value was less than 0.05, the difference was considered to be significant.

RESULTS

Ear Swelling Caused by Mite Antigen Application

Mite antigen solution at a concentration of 1 or 10 mg/ml was painted onto the ear lobes 1 h after tape-stripping, and the procedure was repeated 5 times at intervals of 7 d.

Changes in ear thickness are indicated in Fig. 1. In NC/Nga mice, apparent biphasic ear swelling occurred after the 4th and 5th applications of 10 mg/ml of antigen solution. Tape-stripping potentiated the swelling response. When 1 mg/ml of antigen solution was applied, weak swelling was induced after the 5th application. Similarly, in BALB/c mice apparent ear swelling was observed after the 4th and 5th applications of 10 mg/ml of antigen solution. However, the swelling in BALB/c mice was weaker than that in NC/Nga

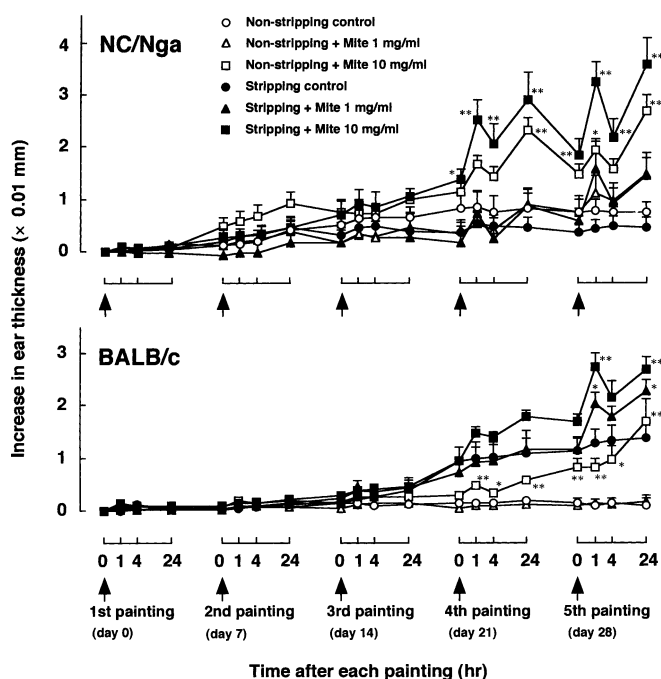


Fig. 1. Change in Ear Thickness Caused by Repeated Painting with Mite Antigen Solution in NC/Nga and BALB/c Mice

Mite antigen solution at a concentration of 1 or 10 mg/ml was painted 5 times weekly onto the mouse ear with or without previous tape-stripping. Ear thickness was measured immediately before, and 1, 4, and 24 h after each antigen painting. Each value represents the mean $\pm$ S.E.M. of 5–8 mice. * p < 0.05, ** p < 0.01.

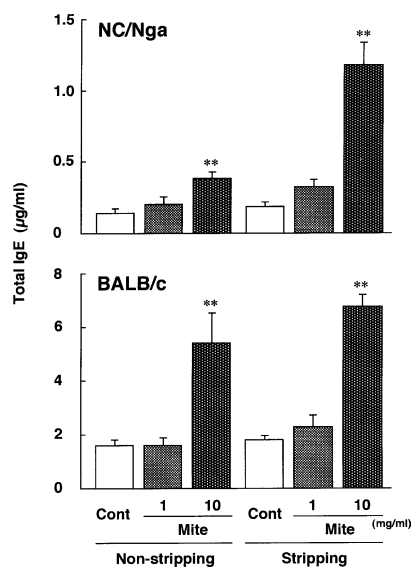


Fig. 2. Total Serum IgE Levels in NC/Nga and BALB/c Mice Treated Repeatedly with Mite Antigen

Mite antigen solution at a concentration of 1 or 10 mg/ml was painted 5 times weekly onto the mouse ear with or without previous tape-stripping. Serum samples were obtained 24 h after the 5th antigen painting and serum IgE was quantified by enzyme-linked immunosorbent assay. Each value indicates the mean $\pm$ S.E.M. of 5–8 mice. ** p < 0.01.

mice. In tape-stripped BALB/c mice, ear swelling was induced after the 5th antigen application, although ear thickness increased gradually in control mice.

Serum IgE Total IgE levels in sera obtained after the 5th antigen application are shown in Fig. 2. Repeated application of mite antigen solution at a concentration of 10 mg/ml caused a significant elevation of serum IgE levels in both

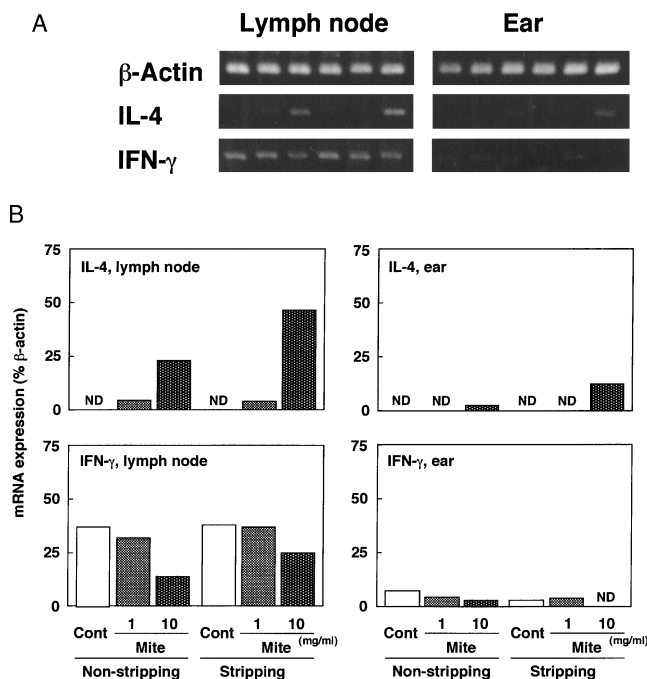


Fig. 3. Expression of IL-4 and IFN- γ mRNA in the Cervical Lymph Nodes and Ears of NC/Nga Mice Treated Repeatedly with Mite Antigen

Mite antigen solution at a concentration of 1 or 10 mg/ml was painted 5 times weekly onto the mouse ear with or without previous tape-stripping. Cervical lymph nodes and ears were obtained 4 h after the 5th antigen painting. Expression of mRNA was examined using the reverse transcriptase-polymerase chain reaction. Resulting electrophoretic bands (A) were semiquantitatively evaluated using NIH Image software (B). Experiments with a similar design were repeated at least 2 times and a representative one is indicated. ND, not detected.

NC/Nga and BALB/c mice. The IgE levels in BALB/c mice were higher than those in NC/Nga mice. Although in BALB/c mice tape-stripping did not affect the elevation, it apparently potentiated the elevation in NC/Nga mice.

Cytokine mRNA Expression IL-4 and IFN- γ mRNA expression in cervical lymph nodes and ears 4 h after the 5th antigen application was examined. The results in NC/Nga mice are indicated in Fig. 3. Repeated mite antigen application resulted in an increase in IL-4 mRNA expression and a decrease in IFN- γ mRNA expression in both cervical lymph nodes and ears. The change was apparent in mice treated with 10 mg/ml of mite antigen solution and was potentiated by tape-stripping except for the case of IFN- γ mRNA expression in cervical lymph nodes. The results in cervical lymph nodes in BALB/c mice are shown in Fig. 4. Mite antigen application caused an increase in IL-4 mRNA expression and the change was potentiated by tape-stripping. Although a significant change in IFN- γ mRNA expression with mite antigen application was not observed in mice without tape-stripping, a decrease occurred in tape-stripped mice. In contrast, IL-4 mRNA expression was not induced in the ears of BALB/c mice by mite antigen application. Furthermore, IFN- γ mRNA expression was also undetectable in BALB/c mouse ears (data not shown).

Histopathologic Observation Histopathologic specimens were prepared using mouse ear lobes separated 24 h after the 5th antigen application. Histologic images of NC/Nga mice are shown in Fig. 5. In the ear lobes treated with 1 mg/ml of mite antigen solution (Figs. 5B, E), the ap-

pearance was almost comparable to that of vehicle-treated control ear lobes (Figs. 5A, D) and histologic changes were minimal. In contrast, potent inflammatory changes, such as thickening of the epidermis, fibrosis in the dermis, and accumulation of inflammatory cells such as lymphocytes, eosinophils and neutrophils were observed in the ear lobes treated with 10 mg/ml of mite antigen solution (Figs. 5C, F). Tape-stripping slightly potentiated the inflammation. Histologic images of BALB/c mice are shown in Fig. 6. In mice without tape-stripping, inflammatory changes were not induced even in mice treated with 10 mg/ml of mite antigen solution (Fig. 6B). In contrast, in tape-stripped mice, repeated application of 10 mg/ml of mite antigen solution caused inflammatory changes similar to those in NC/Nga mice, but the

magnitude was less potent than that in NC/Nga mice.

DISCUSSION

In atopic dermatitis, elevated serum IgE is observed in about 80% of patients,^{23,24} and blood eosinophil counts are also elevated. Activated eosinophils are accumulated and deposition of eosinophil cationic protein released from the activated eosinophils is demonstrated in skin lesions. It is well established that Th2 cytokines play important roles in the onset and development of atopic dermatitis. CD4⁺ helper T lymphocytes are divided into two groups, Th1 and Th2, based on the cytokines they secrete.^{25,26} T lymphocytes responsible for delayed-type skin reactions that secrete IL-2 and IFN- γ belong to the Th1 subset, and those that secrete IL-4 and IL-5 responsible for IgE production and eosinophil activation, respectively, are the Th2 subset. The activation and function of both subsets of T lymphocytes are regulated

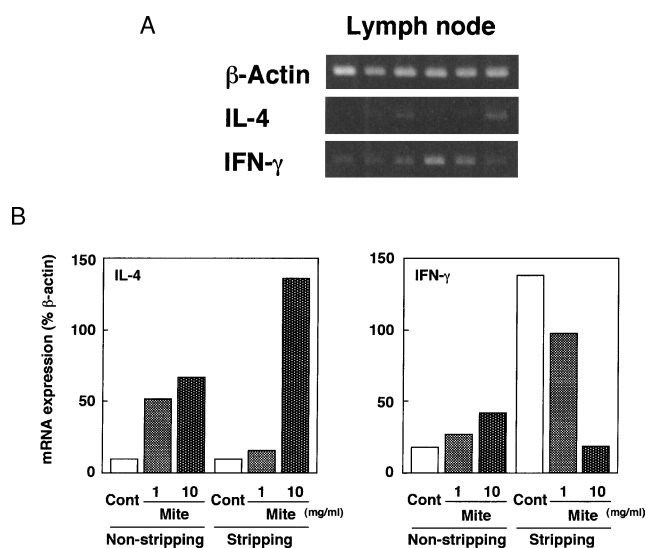


Fig. 4. Expression of IL-4 and IFN- γ mRNA in the Cervical Lymph Nodes of BALB/c Mice Treated Repeatedly with Mite Antigen

Mite antigen solution at a concentration of 1 or 10 mg/ml was painted 5 times weekly onto the mouse ear with or without previous tape-stripping. Cervical lymph nodes were obtained 4 h after the 5th antigen painting. Expression of mRNA was examined using the reverse transcriptase-polymerase chain reaction. Resulting electrophoretic bands (A) were semiquantitatively evaluated using NIH Image software (B). Experiments with a similar design were repeated at least 2 times and a representative one is indicated.

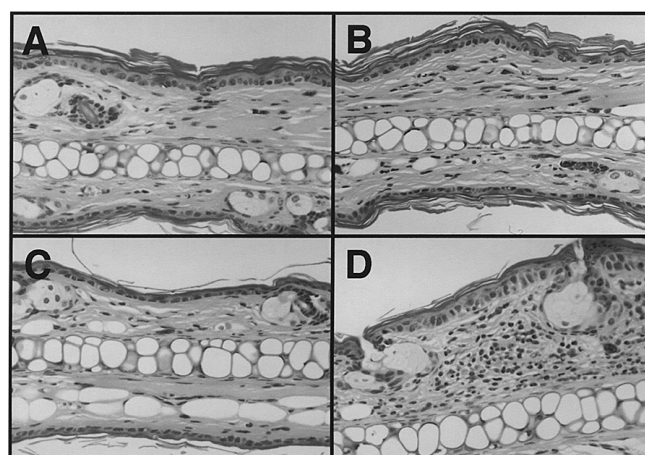


Fig. 6. Histopathologic Images of a BALB/c Mouse Ear Treated Repeatedly with Mite Antigen

Mite antigen solution at a concentration of 10 mg/ml was painted 5 times weekly onto the mouse ear with or without previous tape-stripping. The mouse ear was excised 24 h after the 5th antigen painting. Tissue sections were stained with hematoxylin-eosin. A and B, without tape-stripping; C and D, tape-stripped; A and C, control; B and D, mite antigen 10 mg/ml.

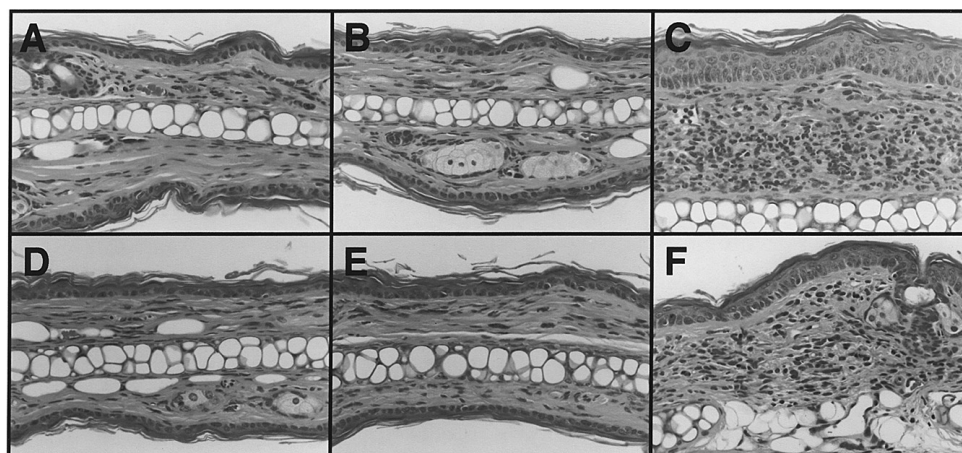


Fig. 5. Histopathologic Images of an NC/Nga Mouse Ear Treated Repeatedly with Mite Antigen

Mite antigen solution at a concentration of 1 or 10 mg/ml was painted 5 times weekly onto the mouse ear with or without previous tape-stripping. The mouse ear was excised 24 h after the 5th antigen painting. Tissue sections were stained with hematoxylin-eosin. A—C, without tape-stripping; D—F, tape-stripped; A and D, control; B and E, mite antigen 1 mg/ml; C and F, mite antigen 10 mg/ml.

mutually through the cytokines they secrete, and the balance of both subsets is considered to be skewed to Th2 in atopic diseases including atopic dermatitis.^{27–29)} In the chronic phase of atopic dermatitis, mRNA expression for Th1 cytokines such as IFN- γ is also detected and an increase in cells expressing IL-12 mRNA is observed.^{30,31)} T lymphocyte accumulation is also demonstrated. Furthermore, itching is an important and characteristic symptom of atopic dermatitis.

In the present study, we induced an allergic dermatitis in NC/Nga mice by repeated epicutaneous application of mite antigen associated with tape-stripping. The dermatitis was accompanied by elevated serum IgE levels, accumulation of inflammatory cells including eosinophils in the lesions, and a Th1/Th2 balance skewed to Th2. Therefore the dermatitis possesses some features observed in atopic dermatitis.

Repeated mite antigen application caused an increase in IL-4 mRNA expression and a decrease in IFN- γ mRNA expression in cervical lymph nodes and ear lobes in NC/Nga mice. The expression of IL-4 mRNA was low in the cervical lymph nodes and undetectable in the ear lobes of control mice. In contrast, IFN- γ mRNA expression was detectable in control mice. Similar cytokine mRNA expression levels were observed in the cervical lymph nodes of BALB/c mice. Therefore repeated mite antigen application seems to produce a Th1/Th2 balance skewed to Th2. The Th2-dominant state thus may contribute to the increased IgE production, eosinophil accumulation, and induction of ear swelling.

An elevation of serum IgE levels was caused by repeated mite antigen application in both NC/Nga and BALB/c mice. Although IL-4 mRNA expression was apparently induced in cervical lymph nodes of both strains of mice, it was faint in the ears of NC/Nga mice and undetectable in those of BALB/c mice (data not shown). Therefore IgE production appears to be dependent on IL-4 induced in lymph nodes.¹⁸⁾ Once IgE is produced and mast cells are sensitized with specific IgE, an IgE-dependent immediate cutaneous reaction could be induced. Mite antigen application caused a biphasic cutaneous reaction after the 4th and 5th applications and the first-phase reaction peaked at around 1 h after the challenge, suggesting that the first phase of the response is mediated by IgE. On the other hand, hapten application also caused a biphasic cutaneous reaction in mice systemically sensitized with IgE.^{3,4,32)} Both phases of the hapten-induced cutaneous reaction are solely dependent on IgE sensitization, indicating that the second-phase response observed in mice treated with mite antigen could be induced IgE dependently. Furthermore, repeated mite antigen application shifted the Th1/Th2 balance toward a Th2-dominant state. Therefore IgE may also contribute, at least in part, to the second phase of the biphasic reaction caused by mite antigen application.

Repeated application of mite antigen solution at a concentration of 10 mg/ml caused ear swelling in both NC/Nga and BALB/c mice independent of tape-stripping. In NC/Nga mice, however, the swelling and inflammatory changes were more potent than those in BALB/c mice, although the serum IgE levels were lower in NC/Nga mice than in BALB/c mice. Therefore NC/Nga mice may be sensitive to mite antigen applied epicutaneously in the development of dermatitis. It was suggested that NC/Nga mice have some genetic factors that facilitate the induction of dermatitis. The factors seem to be

independent of the genetic factors that facilitate the IgE production.¹³⁾ Furthermore, Aioi *et al.* reported that the skin barrier function in NC/Nga mice is impaired.¹⁶⁾ The impaired skin barrier in NC/Nga mice may facilitate the penetration of mite antigen through the skin. These characteristics may contribute to the potent dermatitis observed in NC/Nga mice. On the other hand, removal of the outer surface of the epidermis by tape-stripping may also facilitate the penetration of mite antigen. Tape-stripping potentiated the swelling and inflammatory and immunologic changes. Tape-stripping seems to facilitate the shift of the Th1/Th2 balance toward the Th2-dominant state induced by mite antigen, as reported by Kondo *et al.*³³⁾

In conclusion, repeated mite antigen application coupled with tape-stripping produces a dermatitis with a Th2-dominant background. The dermatitis in NC/Nga mice reflects some features of atopic dermatitis and appears to be useful for the basic study of atopic dermatitis.

REFERENCES

- 1) Furue M., Furukawa F., Hide M., Takehara K., *Jpn. J. Dermatol.*, **114**, 135–142 (2004).
- 2) Wahlgren C. F., *Allergy*, **47**, 65–67 (1992).
- 3) Ray M. C., Tharp M. D., Sullivan T. J., Tigelaar R. E., *J. Immunol.*, **131**, 1096–1102 (1983).
- 4) Katayama I., Tanei R., Yokozeki H., Nishioka K., Dohi Y., *Int. Arch. Allergy Appl. Immunol.*, **93**, 148–154 (1990).
- 5) Thomas W. R., Asherson G. L., Watkins M. C., *J. Exp. Med.*, **144**, 1386–1390 (1976).
- 6) Kitagaki H., Fujisawa S., Watanabe K., Hayakawa K., Shiohara T., *J. Invest. Dermatol.*, **105**, 749–755 (1995).
- 7) Kitagaki H., Kimishima M., Teraki Y., Hayakawa J., Fujisawa S., Shiohara T., *J. Immunol.*, **163**, 1265–1273 (1999).
- 8) Natsuaki M., Yano N., Yamaya K., Kitano Y., *Contact Dermatitis*, **43**, 267–272 (2000).
- 9) Kondo K., Nagami T., Teramoto S., “Comparative Cellular and Species Radiosensitivity,” ed. by Bondo P. V., Sugahara T., Igakushoin, Tokyo, 1969, pp. 20–29.
- 10) Kudo K., Ando F., Ohashi M., Asai J., *Nippon Hifuka Gakkai Zasshi*, **95**, 1473–1480 (1985).
- 11) Koizumi T., Hayakawa J., *Jikken Dobutsu*, **35**, 159–163 (1986).
- 12) Matsuda H., Watanabe N., Geba G. P., Spert J., Tsudzuki M., Hiroi J., Matsumoto M., Ushio H., Saito S., Askenase P. W., Ra C., *Int. Immunol.*, **9**, 461–466 (1997).
- 13) Tsudzuki M., Watanabe N., Wada A., Nakane Y., Hiroi J., Matsuda H., *Immunogenetics*, **47**, 88–90 (1997).
- 14) Matsumoto M., Ra C., Kawamoto K., Sato H., Itakura A., Sawada J., Ushio H., Suto H., Mitsuishi K., Hikasa Y., Matsuda H., *J. Immunol.*, **162**, 1056–1063 (1999).
- 15) Vestergaard C., Yoneyama H., Murai M., Nakamura K., Tamaki K., Terashima Y., Imai T., Yoshie O., Irimura T., Mizutani H., Matsushima K., *J. Clin. Invest.*, **104**, 1097–1105 (1999).
- 16) Aioi A., Tonogaito H., Suto H., Hamada K., Ra C., Ogawa H., Maibach H., Matsuda H., *Br. J. Dermatol.*, **144**, 12–18 (2001).
- 17) Nagai H., Hiyama H., Matsuo A., Ueda Y., Inagaki N., Kawada K., *J. Pharmacol. Exp. Ther.*, **283**, 321–327 (1997).
- 18) Nagai H., Matsuo A., Hiyama H., Inagaki N., Kawada K., *J. Allergy Clin. Immunol.*, **100**, S39–44 (1997).
- 19) Elliston W. L., Heise E. A., Huntley C. C., *Arch. Dermatol.*, **118**, 26–29 (1982).
- 20) Norris P. G., Schofield O., Camp R. D., *Br. J. Dermatol.*, **118**, 435–440 (1988).
- 21) Beck H. I., Korsgaard J., *Br. J. Dermatol.*, **120**, 245–251 (1989).
- 22) Koda A., Inagaki N., Tsuruoka N., Daikioku M., Nagai H., Yagi A., Nishioka I., *J. Pharmacobio-Dyn.*, **10**, 104–111 (1987).
- 23) Hoffman D. R., Yamamoto F. Y., Geller B., Haddad Z., *J. Allergy Clin. Immunol.*, **55**, 256–267 (1975).
- 24) Sampson H. A., Albergo R., *J. Allergy Clin. Immunol.*, **74**, 26–33

- (1984).
- 25) Mosmann T. R., Cherwinski H., Bond M. W., Giedlin M. A., Coffman R. L., *J. Immunol.*, **136**, 2348—2357 (1986).
- 26) Mosmann T. R., Coffman R. L., *Annu. Rev. Immunol.*, **7**, 145—173 (1989).
- 27) Wierenga E. A., Snoek M., Bos J. D., Jansen H. M., Kapsenberg M. L., *Eur. J. Immunol.*, **20**, 1519—1526 (1990).
- 28) van der Heijden F. L., Wierenga E. A., Bos J. D., Kapsenberg M. L., *J. Invest. Dermatol.*, **97**, 389—394 (1991).
- 29) Kay A. B., Ying S., Varney V., Gaga M., Durham S. R., Moqbel R., Wardlaw A. J., Hamid Q., *J. Exp. Med.*, **173**, 775—778 (1991).
- 30) Gerwe M., Gyufko K., Schopf E., Krutmann J., *Lancet*, **343**, 25—26 (1994).
- 31) Hamid Q., Maseer T., Minshall E. M., Song Y. L., Boguniewicz M., Leung D. Y., *J. Allergy Clin. Immunol.*, **98**, 225—231 (1996).
- 32) Nagai H., Sakurai T., Inagaki N., Mori H., *Biol. Pharm. Bull.*, **18**, 239—245 (1995).
- 33) Kondo H., Ichikawa Y., Imokawa G., *Eur. J. Immunol.*, **28**, 769—779 (1998).