Full Paper

Effects of Anti-Nerve Growth Factor Antibody on Symptoms in the NC/Nga Mouse, an Atopic Dermatitis Model

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Abstract. Nerve growth factor (NGF) is an important substance in the skin, where it can modulate nerve maintenance and repair. However, the direct link between NGF and pruritic disease such as atopic dermatitis is not yet fully understood. To determine whether NGF plays a major role in atopic dermatitis and in the development or maintenance of skin lesions, we performed a study using NC/Nga mice and compared mice with and without skin lesions. Our examinations of the NC/Nga mice sought to detect nerve fibers in the epidermis, measured serum and skin NGF content, and observed skin NGF by immunohistochemistry staining. We also examined the effects of anti-NGF antibody on dermatitis symptoms in NC/Nga mice. In these mice, nerve fibers were significantly increased in the epidermis of lesioned skin, and the NGF content of the serum and skin was significantly elevated. Anti-NGF antibodies significantly inhibited the development and proliferation of skin lesions and epidermal innervation and significantly inhibited any growth in scratching but did not ameliorate scratching already developed. Our findings suggest that NGF plays important roles in the pathogenesis of atopic dermatitis-like skin lesions and that inhibiting the physiological effects of NGF or suppressing increased NGF production may prevent or even moderate the symptoms of atopic dermatitis.

Keywords: nerve growth factor (NGF), atopic dermatitis, NC/Nga mouse, anti-NGF antibody

Introduction

As a characteristic symptom of various forms of dermatosis, particularly atopic dermatitis, itching constitutes a major diagnostic (1, 2). In a process known as the Itch-Scratch cycle, scratching tends to lead to increased itching and aggravated skin lesions in patients with atopic dermatitis (3, 4). Therefore, the most effective strategy would be to prevent this aggravation of skin lesions and improve quality of life for patients with atopic dermatitis by reducing itching and scratching (5).

Cutaneous nerve fibers are present at higher densities in the epidermis of lesional skin of atopic dermatitis (6, 7). This increase in nerve fibers in the epidermis is partly responsible for intense itching sensations in the lesional skin of patients with atopic dermatitis. It is now generally accepted that nerve growth factor (NGF),

which is released from keratinocytes in the skin, is one of the major mediators that determine skin innervation density (8–10). NGF is an important substance in the skin environment, where it modulates nerve maintenance and repair (11, 12). However, the nature of the relationship between NGF and pruritic conditions such as atopic dermatitis still remain not fully understood. A recent study has reported that NGF is overexpressed in prurigo nodularis, hypothesizing that NGF and its receptors contribute to the neurohyperplasia of the disease (13). NGF protein levels are increased in psoriatic skin compared to non-lesional and normal skin (14), and psoriatic keratinocytes express higher amounts of NGF than normal keratinocytes (15). Patients with atopic dermatitis also showed significant gains in NGF plasma levels compared to controls, and a strong correlation was noted between plasma NGF and severity of symptoms (16). These findings suggest that NGF may play an important role in the pathogenesis of atopic

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dermatitis, possibly regulating the development of atopic dermatitis lesions. Few reports have addressed the role of NGF in the pathogenesis, development, and maintenance of atopic dermatitis *in vivo*.

NC/Nga mice were originally established as an inbred strain of Japanese fancy mice. Under conventional conditions, NC/Nga mice develop spontaneous skin lesions with diagnostic characteristics of high concentrations of total immunoglobulin E in plasma and invasion of inflammatory cells into the skin lesions (17, 18). NC/Nga mice with severe skin lesions frequently scratch their face, ears, and rostral part of the back with their hind paws (19). Since all of these traits are similar to those observed in patients with atopic dermatitis, NC/Nga mice are considered a suitable model for human atopic dermatitis. To examine the hypothesis that NGF plays important roles in the pathogenesis, development, and maintenance of atopic dermatitis skin lesions, we used NC/Nga mice in this study.

Materials and Methods

Animals

Male NC/Nga mice purchased from SLC Japan (Shizuoka) were housed under conditions of controlled temperature ($23 \pm 3^\circ\text{C}$), humidity ($55 \pm 15\%$), and lighting (lights on from 7:00 to 19:00). Food and tap water were provided *ad libitum*. All studies reported here were reviewed by the Taisho Pharmaceutical Animal Care Committee and met the Japanese Experimental Animal Research Association Standards, as defined in the Guidelines for Animal Experiments (1987).

Murine studies have shown that age affects NGF levels (20, 21). In the present study, each experiment was investigated with mice of the same age to reduce the influence of age. The results for each group were then evaluated and compared.

Materials

An anti-NGF antibody (3.1 mg/mL, developed in rabbits using 2.5S NGF purified from mouse submaxillary glands; Sigma, St. Louis, MO, USA) was diluted with physiological saline. Rabbit polyclonal IgG (used as a control antibody, Sigma) was dissolved in physiological saline at a concentration of 3.0 mg/mL and used to assess effects on atopic dermatitis model mice. Tacrolimus (Prograf®; purchased from Fujisawa, Osaka) was dissolved in ethanol and applied to the rostral part of the backs of mice.

Comparison of some parameters between NC/Nga mice with and without dermatitis

The amount of nerve fiber in the skin and serum and

skin NGF content were compared for NC/Nga mice with severe dermatitis (skin-lesioned) and NC/Nga mice without dermatitis (non-lesioned). Mice of the two groups were used at 22 weeks of age.

Immunohistochemistry and nerve fiber amount analysis in the skin

The rostral back skins of each mouse were extracted and fixed in 10% formalin solution and then cut perpendicular to the dermal-epidermal surface in 20- μm -thick sections. The skin sections were incubated in blocking solution for 30 min [1% bovine serum albumin (Sigma) in physiological saline]. The sections were incubated with polyclonal rabbit antibodies against PGP9.5 (dilution 1:400; UltraClone Limited, Isle of Wight, UK) overnight at 4°C and washed with PBS and then were incubated with Alexa Fluor goat 488 anti-rabbit IgG (10 $\mu\text{g}/\text{mL}$; Molecular Probes, Eugene, Oregon, USA) for one hour at room temperature away from light. Immunofluorescent sections were observed at a magnification of $\times 200$ by conventional fluorescence microscopy (Carl Zeiss, Jena, Germany). The area of immunoreactive nerve fibers in the epidermis (fluorescent stain areas) was randomly quantified into ten fields per mouse by imaging software (IPLab; Solution Systems, Chiba).

Measurement of NGF amounts in serum or skin of NC/Nga mice

Serum and skin NGF content was measured in enzyme-linked immunosorbent assays (Chemicon International, Temecula, CA, USA) that recognize human and murine NGF. Mouse blood was centrifuged to acquire serum for the NGF assay. The rostral back skin was weighed and stored at -80°C until the assay. A summary of the procedure for extracting NGF from the skin was previously given (22). The tissue was homogenized in an extraction buffer containing 0.4 M NaCl, 0.5% bovine serum albumin, 10 mM EDTA (Wako, Osaka), 0.05% Tween (Kanto Kagaku, Tokyo), 20 $\mu\text{L}/\text{mL}$ aprotinin, 0.1 mM benzethonium chloride, and 0.1% phenylmethylsulfonylfluoride (Sigma) in phosphate buffer. Homogenization was carried out for 1 min, and the homogenized tissue was centrifuged for 30 min at $14,000 \times g$. The extracted supernatant was assayed for NGF.

Immunohistochemistry staining for NGF in skin of NC/Nga mice

The rostral back skins of each mouse were extracted and fixed in 10% formalin. The fixed samples were embedded in paraffin and 3- μm sections prepared. After deparaffinization with xylene and graded ethanol solu-

tions, these sections were pre-incubated in 1% bovine serum albumin-phosphate buffered saline (PBS) for 30 min at room temperature and rinsed with PBS and then incubated overnight with anti-mouse NGF antibodies (Alomone Labs, Jerusalem, Israel) at a dilution of 1:30 for 4°C. After washing with PBS, these sections were incubated with 10 µg/mL biotinylated goat anti-rabbit IgG (Vector Laboratories Inc., Barlingame, CA, USA) for 1 h at room temperature. The sections were immersed in methanol-hydrogen peroxide solution for 30 min to quench endogenous peroxidase activity and washed with PBS and then were incubated for 1 h with avidin-biotinylated peroxidase complex (Vector Laboratories Inc.) Peroxidase activity was visualized with an AEC chromogen kit (Sigma) and examined by microscope (Carl Zeiss).

Evaluation of inhibitory effects of anti-NGF antibody on established dermatitis and scratching behavior

Skin-lesioned NC/Nga mice were used at 22 weeks of age. Anti-NGF or control antibodies (300 µg) were administered intraperitoneally twice a week. Tacrolimus (0.1%) was applied at a volume of 200 µL to the rostral part of the backs of mice five times a week. The dermatitis scores for three parts (face, ear, and rostral back) were assessed once a week based on the following criteria: no lesion, 0; minor hair loss or wound without bleeding, 1; wound bleeding in parts, 2; broad area of serious wounds, 3. The scratching behavior of NC/Nga mice was measured as previously reported (23, 24) at least 24 h following the last injection of each antibody. For measurements, a small magnet (1 mm in diameter, 3-mm-long) was implanted subcutaneously into both hind paws of each mouse following ether anesthetization at least 6 h before measurement of scratching. The mouse was placed in an observation chamber (11 cm in diameter, 18-cm-high) surrounded by a round coil. The electric current induced in the coil by the movement of magnets implanted to the hind paws was amplified and recorded. The number of scratching behaviors was measured using a new device, MicroAct (Neuroscience, Tokyo), which automatically detects and evaluates the scratching behavior of mice (25, 26). The MicroAct Parameters for detecting waves were threshold: 0.1 V, event gap: 0.2 s, minimum duration: 1.5 s, maximum frequency: 20 Hz, and minimum frequency: 2 Hz.

Evaluation of inhibitory effects of anti-NGF antibody on development of dermatitis and scratching behavior

Non-lesioned NC/Nga mice were used at six weeks of age. To induce stable dermatitis and scratching behavior, NC/Nga mice were kept together with skin-lesioned mice for one week. The administration schedule,

dermatitis scoring, scratching behavior measurement, and immunohistochemistry of the rostral back skin were as described above.

Data analysis

Experimental values are given as means ± S.E.M. Statistical comparisons were made with Student's unpaired *t*-test to compare groups with or without dermatitis, Dunnett's multiple test for comparison with the control antibody administered group, and Student's paired *t*-test for comparison with pre-administration. A *P* < 0.05 value was considered statistically significant.

Results

Immunohistochemical staining of nerve fibers in the skin

Immunofluorescence methods showed PGP9.5-positive nerve fibers in the rostral back skin of NC/Nga mice. In the skin of non-lesioned NC/Nga mice, nerve fibers were observed in the dermis, but relatively few nerve fibers were observed in the epidermis (Fig. 1A). In the lesioned skin of the mice, nerve fibers were observed at many epidermal sites, and distinct acanthosis and hyperkeratosis were observed (Fig. 1B). Nerve fiber counts in the epidermis of skin-lesioned NC/Nga mice increased significantly compared to findings for non-lesioned NC/Nga mice (Fig. 1C). The sections in which the primary antibody was omitted showed no immunoreactivities (data not shown).

NGF contents in serum and skin, and immunohistochemistry staining for NGF in skin

Compared to non-lesioned NC/Nga mice, serum NGF content was increased significantly in skin-lesioned NC/Nga mice (Fig. 2A). The NGF content of the rostral back skin was also higher in skin-lesioned NC/Nga mice (Fig. 2B). Following immunohistochemistry staining, skin-lesioned NC/Nga mice exhibited distinct acanthosis and increased levels of NGF in keratinocytes in the skin, compared to non-lesioned NC/Nga mice (Fig. 3: A and B).

Effects of an anti-NGF antibody on established symptoms

In mice administered the control antibody, dermatitis scores remained relatively constant during the study. In mice administered the anti-NGF antibody, dermatitis scores improved significantly from week 2, compared to pre-administration, and at week 4, were comparable to a group administered the control antibody (Fig. 4A). Repeated administration of anti-NGF antibody also decreased the scratching behaviors, albeit not to a statistically significant extent (*P* = 0.054 vs control, Fig. 4B).

Fig. 1. Immunofluorescence of PGP9.5 in skin of NC/Nga mice. A: Non-lesioned NC/Nga mice, B: skin-lesioned NC/Nga mice, C: quantification of PGP9.5 immunoreactivity in skin of NC/Nga mice. The rostral back skin of each mouse was extracted, fixed in 10% formalin solution, and then cut perpendicular to the dermal-epidermal surface in 20-μm-thick sections. After blocking with 1% bovine serum albumin for 30 min, the sections with a polyclonal rabbit antibody against PGP9.5 (dilution 1:400) were incubated overnight at 4°C, while sections with Alexa Fluor goat 488 anti-rabbit IgG were incubated for 1 h at room temperature away from light. These sections were observed at a magnification of $\times 200$ with a conventional immunofluorescence microscope. Arrows indicate immunoreactive nerve fibers. Scale bar: 50 μm. The area of immunoreactive nerve fibers in the epidermis (fluorescent stain areas) was quantified by imaging software (IPLab). Values are the mean $\pm$ S.E.M. for four mice. ** $P < 0.01$, when compared to non-lesioned NC/Nga mice (Student's *t*-test).

In the case of the control antibody, nerve fibers were observed in many sites of the epidermis, and distinct acanthosis and hyperkeratosis were observed (Fig. 5A). On the other hand, few nerve fibers were observed in the epidermis, and acanthosis and hyperkeratosis were markedly improved in the mice administered anti-NGF antibodies (Fig. 5B). The number of nerve fibers in the epidermis declined significantly with repeated administration of anti-NGF antibodies (Fig. 5D). Tacrolimus inhibited the established dermatitis, scratching behavior, and epidermal nerve fiber counts (Figs. 4A, 4B, 5C, 5D). The anti-NGF antibodies and tacrolimus did not alter serum NGF concentrations in rostral back skin or its NGF content (data not shown).

Effects of an anti-NGF antibody on development of symptoms

In mice administered the control antibody, skin dermatitis appeared and increased gradually from study commencement. The development of dermatitis was significantly inhibited from week 5 in mice given the anti-NGF antibody or tacrolimus (Fig. 6A). Scratching behavior increased significantly for two weeks in all groups and then increased gradually until week 8 in mice administered the control antibody. Administering anti-NGF antibody or tacrolimus significantly inhibited these trends (Fig. 6B). Paralleling observations from the study

of effects of anti-NGF antibody on established symptoms in NC/Nga mice, nerve fibers were observed in many epidermal sites, and distinct acanthosis and hyperkeratosis were observed in mice administered the control antibody (Fig. 7A). On the other hand, relatively few nerve fibers were observed in the epidermis, and mice administered the anti-NGF antibody (Fig. 7B) and tacrolimus (Fig. 7C) showed marked improvements in acanthosis and hyperkeratosis. Repeated administration of anti-NGF antibody and tacrolimus significantly reduced the number of nerve fibers in the epidermis (Fig. 7D). Serum NGF concentrations and NGF content in the rostral back skin were not altered by anti-NGF antibody or tacrolimus administration (data not shown).

No differences were noted in body weight (control: 27.4 ± 0.7 , anti-NGF: 27.9 ± 0.7 g) or general symptoms between groups administered the control antibody and the anti-NGF antibody. We also found no difference in reaction latency with respect to antinociceptive effects between these two groups in the tail-pinch test (control: 3.4 ± 0.6 , anti-NGF: 3.2 ± 0.9 s).

Discussion

Skin-lesioned NC/Nga mice frequently scratch lesioned areas using their hind paws (19), a behavior believed to be elicited by cutaneous itching sensations

Fig. 2. NGF concentrations in serum or back skin of NC/Nga mice. The serum (A) and skin (B) NGF content of skin-lesioned or non-lesioned NC/Nga mice were measured with an enzyme-linked immunosorbent assay kit, as described in Materials and Methods. Values are the mean $\pm$ S.E.M. for 5–8 mice. * $P < 0.05$, ** $P < 0.01$, when compared to non-lesioned NC/Nga mice (Student's *t*-test).

Fig. 3. Immunohistochemistry staining for NGF. A: Non-lesioned NC/Nga mice, B: skin-lesioned NC/Nga mice. The rostral back skin of each mouse was extracted and fixed in 10% formalin. The fixed samples were embedded in paraffin and 3- μ m sections prepared. After deparaffinization with xylene and graded ethanol solutions, these sections were pre-incubated in 1% bovine serum albumin in phosphate-buffered saline (PBS) for 30 min at room temperature and rinsed with PBS and then incubated with anti-mouse NGF antibody at a dilution of 1:30 for 4°C overnight. After washing with PBS, these sections were incubated with 10 μ g/ml biotinylated goat anti-rabbit IgG for 1 h at room temperature. The sections were immersed in methanol-hydrogen peroxide solution for 30 min to quench endogenous peroxidase activity and with avidin-biotinylated peroxidase complex for 1 h. The peroxidase activity was visualized with an AEC chromogen kit and examined under a microscope. Arrows indicate the NGF-positive area. Scale bar: 50 μ m.

(27). In this study, PGP9.5-positive nerve fibers increased significantly in the epidermis of skin-lesioned NC/Nga mice compared to findings for non-lesioned NC/Nga mice. These results are thought to be similar to that seen in humans with atopic dermatitis. Moreover, the NGF content in the serum was found to be significantly higher in skin-lesioned NC/Nga mice than in non-lesioned NC/Nga mice, as reported in a study on humans (16). In lesioned skin, enzyme-linked immunosorbent assay or immunohistochemistry staining revealed higher NGF levels compared to normal skin. NGF was first identified as the main neurotrophic factor controlling the survival, development, differentiation, and function of sympathetic and sensory neurons (28). These findings suggest that the overproduction of NGF sends nerve fibers into the epidermis, intensifies itching sensation, and increases scratching behavior, thereby

contributing to maintenance of skin lesions.

To determine whether NGF plays a major important role in the pathogenesis development and maintenance of atopic dermatitis-like skin lesions, we investigated the effects of anti-NGF antibodies on symptoms *in vivo*. In the examination evaluating the effects of repeated administration of anti-NGF antibodies on established symptoms in NC/Nga mice, dermatitis scores and nerve fibers in epidermis declined significantly. The study evaluating the effects of repeated administration of anti-NGF antibodies on the development of symptoms used NC/Nga mice at six weeks of age. To induce stable symptoms, NC/Nga mice in this experiment were kept together with skin-lesioned mice. Our preliminary experiments had shown that the desirable age at the start of the study for inducing stable symptoms was 5–7 weeks. Dermatitis scores and the development of

Fig. 4. Effects of anti-NGF antibody and tacrolimus on established dermatitis and increased scratching behavior of NC/Nga mice. Anti-NGF antibodies (closed circles) or control antibodies (open circles) were injected intraperitoneally twice a week, and tacrolimus (0.1%, closed lozenges) applied to the rostral part of the back five times a week. The dermatitis scores (A) for the face, ear, and rostral back were assessed once a week based on the following criteria: no lesion, 0; minor hair loss or wound without bleeding, 1; wound bleeding in parts, 2; broad area of serious wounds, 3. The number of scratching behaviors (B) was measured once every two weeks using Micro-Act, which automatically detects and evaluates the scratching behavior of mice. Values are the mean and S.E.M. for eight mice. [#]*P*<0.05, ^{##}*P*<0.01, compared to pre-administration (Student's paired *t*-test); ^{**}*P*<0.01, compared to the group administered the control antibody (Dunnett's test).

Fig. 5. Effects of anti-NGF antibody and tacrolimus on neural outgrowth in epidermis of NC/Nga mice with established skin lesions. Control antibodies (A) or anti-NGF antibodies (B) were injected intraperitoneally twice a week and tacrolimus (0.1%, C) applied to the rostral part of the back five times a week. Fluorescent staining of the rostral back skin was performed as described for Fig. 1. Arrows indicate immunoreactive nerve fibers. Scale bar: 50 μm . The area of immunoreactive nerve fibers in the epidermis (fluorescent stain areas) was quantified by imaging software (IPLab). Values are the mean $\pm$ S.E.M. for four mice. ^{**}*P*<0.01, when compared to the group administered the control antibody (Dunnett's test).

Fig. 6. Effects of anti-NGF antibody and tacrolimus on development of dermatitis and scratching behavior in NC/Nga mice. To stabilize the incidence of dermatitis and scratching behavior, non-lesioned NC/Nga mice were kept together with skin-lesioned mice for one week before use in this experiment. Anti-NGF antibodies (closed circles) or control antibodies (open circles) were injected into the NC/Nga mice intraperitoneally twice a week, and tacrolimus (0.1%, closed lozenges) was applied to the rostral part of the back five times a week. The dermatitis scores (A) for the face, ear, and rostral back were assessed once a week, and the number of scratching behaviors (B) was measured once every two weeks, as for Fig. 3. Values are the mean and S.E.M. for eight mice. * $P < 0.05$, ** $P < 0.01$, when compared to the group administered the control antibody (Dunnett's test).

Fig. 7. Effects of anti-NGF antibody and tacrolimus on neural outgrowth in epidermis of NC/Nga mice. To stabilize the incidence of dermatitis and scratching behavior, non-lesioned NC/Nga mice were kept together with skin-lesioned mice for one week before use in this experiment. Control antibodies (A) or anti-NGF antibodies (B) was injected into the NC/Nga mice intraperitoneally twice a week, and tacrolimus (0.1%, C) was applied to the rostral part of the back five times a week. Immunofluorescent staining of rostral back skin was performed as described for Fig. 1. Arrows indicate immunoreactive nerve fibers. Scale bar: 50 μm . The area of immunoreactive nerve fibers in the epidermis (fluorescent stain areas) was quantified by imaging software (IPLab). Values are the mean $\pm$ S.E.M. for eight mice. ** $P < 0.01$, when compared to the group administered the control antibody (Dunnett's test).

scratching behavior and epidermal innervation in NC/Nga mice were significantly inhibited by anti-NGF antibody administration. On the other hand, administration of anti-NGF antibodies did not alter serum NGF concentrations or NGF content in the rostral back skin in either experiment (data not shown). Anti-NGF antibodies against 2.5 S NGF can block the effects of NGF in vitro (29) and in vivo (30). In the skin, NGF synthesized and released by keratinocytes (31–33) plays an important role in the survival and development of the peripheral nervous system (12). The study results suggest that anti-NGF antibodies block the effects of NGF on the periphery of the nervous system and suppress epidermal innervation, dermatitis, and scratching behavior. There is also growing evidence that NGF has biological effects on immune cells, such as mast cells (34), B cells (35), T cells (36), neutrophils (37), eosinophils (38), and basophils (39). Anti-NGF antibodies may suppress these symptoms in NC/Nga mice by blocking response to these immune cells.

Since NGF affects both the peripheral and central nervous systems, there is some concern that long-term systemic administration of anti-NGF antibody may lead to adverse effects. However, no differences were noted between the groups administered the control antibody and the anti-NGF antibody with respect to body weight, general symptoms, and antinociceptive response, suggesting repeated anti-NGF antibody administration does not lead to adverse effects.

The powerful inhibition of nerve growth by anti-NGF antibody failed to completely suppress scratching behavior. Other mediators, such as substance P, are believed to play important roles in the symptoms observed in NC/Nga mice and humans. Substance P is reported to play an important role in the pathogenesis of human atopic dermatitis (16, 40, 41), and some authors have suggested using in vivo models (42, 43).

Tacrolimus, an immunosuppressant, has therapeutic effects on atopic dermatitis (44–46) and effects on animal models of atopic dermatitis (47, 48). Topical application of tacrolimus, in the typical clinical manner, was used as a positive control in this study. In the present study, tacrolimus exerted curative and preventive effects on the dermatitis, scratching behavior, and epidermal innervation of NC/Nga mice. Tacrolimus has various effects capable of inhibiting production of cytokines such as interleukin-2 and interferon- γ from inflammatory cells (49–51). However, no reports indicate that tacrolimus affects the production or results of NGF. Serum NGF concentrations and NGF content in rostral back skin were not altered by the anti-NGF antibody (data not shown). Tacrolimus is believed to suppress dermatitis and scratching behavior through its anti-

inflammatory effects, thereby inhibiting epidermal innervation and thickening.

NGF mediates its effects by binding to two classes of transmembrane receptors, a high affinity receptor of approximately 140 kDa (TrkA) and a low affinity receptor of approximately 75 kDa (p75) (52, 53). The binding of NGF to the TrkA receptor is responsible for the transmission of NGF signals for mitogenesis and differentiation (54, 55). K252a, a selective inhibitor of TrkA tyrosin kinase activity, can inhibit NGF-induced neuritic outgrowth in PC12 cells (56, 57). The inhibition of cellular effects of NGF by K252a is mediated by the blocking of trk proto-oncogene tyrosine phosphorylation and kinase activities (58). The role of the p75 in these processes is less clear, but it may function as an accessory protein that modifies ligand-binding affinity (59) and recent evidence has implicated its role in the apoptotic cell death cascade (60–62). TrkA and p75 are expressed not only in neurons, but in keratinocytes (32, 63). NGF stimulates TrkA phosphorylation in human keratinocytes, and functions as a survival factor for keratinocytes through TrkA (32, 64, 65). Although p75 mRNA and protein in human keratinocytes are increased during their exponential growth phase (63), K252a, but not anti-p75, inhibits NGF-induced keratinocyte proliferation strongly (32), suggesting that TrkA is the functional NGF receptor in neurons and keratinocytes. Many investigations concerning the role of NGF in the pathology of psoriasis have been reported. Keratinocytes in lesional and non-lesional psoriatic tissue have been observed to express high levels of NGF (15, 66), and NGF receptors markedly upregulate in cutaneous nerves of psoriatic lesions (67, 68). Moreover, evidence from an in vivo study suggests that NGF and its high-affinity receptor play major roles in the pathogenesis of psoriasis (69). In atopic dermatitis, the roles played in pathogenesis by the two types of NGF receptors remain unclear. We currently plan to proceed with further studies involving immunohistochemistry staining for NGF receptors and K252a treatment effects on the atopic dermatitis model to clarify the functions of NGF receptors in this disease.

In conclusion, our results confirm that nerve fibers are significantly increased in the epidermis of lesioned skin and that NGF content in serum and skin are significantly elevated in skin-lesioned mice. Our results also indicate that repeated administration of an anti-NGF antibody may help heal skin lesions and decrease innervation into the epidermis and scratching behavior in an atopic dermatitis animal model, NC/Nga mice. We suggest that NGF plays important roles in the pathogenesis, development, and maintenance of atopic dermatitis-like skin lesions. The inhibition of the physiological effects

of NGF or the suppression of increased NGF production can prevent and even ameliorate the symptoms of atopic dermatitis.

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