

Therapeutic and prophylactic effects of PUVA photochemotherapy on atopic dermatitis-like lesions in NC/Nga mice

Hiroko Miyauchi-Hashimoto, Hiroyuki Okamoto, Akira Sugihara, Takeshi Horio

Department of Dermatology, Kansai Medical University, Osaka, Japan

Background: Psoralens and ultraviolet A radiation (PUVA) photochemotherapy has been used for severe cases of atopic dermatitis (AD). To understand the mechanisms of action is important for the choice of treatments. AD-like lesions can be induced experimentally in NC/Nga mice.

Objectives: To evaluate clinically and histologically the therapeutic and prophylactic effects of PUVA on AD-like dermatitis using NC/Nga mice.

Methods: PUVA therapy was performed with intraperitoneal injection of 4 mg/kg of 8-methoxypsoralen (8-MOP) and 4 J/cm²-UVA irradiation before and after development of AD-like lesions in NC/Nga mice which had been maintained in a conventional room (Conv-NC/Nga mice). Clinical skin conditions were evaluated periodically by a clinical severity score defined. Lesions were histologically examined in haematoxylin–eosin or toluidine blue-stained sections.

Plasma levels of total IgE were measured at various time points.

Results: In Conv-NC/Nga mice infested with mite, AD-like lesions started to develop at 8 week of age and thereafter increased in severity score. PUVA therapy at lower dose than minimal phototoxic dose suppressed the development of dermatitis and was also therapeutically effective against established lesions. Proliferation of dermal mast cells in AD-like lesions was suppressed, but IgE hyperproduction was not changed after PUVA.

Conclusions: These observations suggest that PUVA photochemotherapy reveals not only therapeutic but also prophylactic effects on human AD.

Key words: atopic dermatitis; mast cell; murine model; NC/Nga mice; PUVA.

Atopic dermatitis (AD) is one of the most common skin diseases, which is characterized by a chronic and relapsing inflammatory dermatitis with immunological disturbance such as hyperproduction of total and specific IgE (1).

Patients with AD usually have a personal or familial history of other atopic diseases (2–4). It is also well known that the onset of AD is influenced by environmental factors as suggested by epidemiological studies and elevated levels of specific IgE against many kinds of inhaled, ingested, and/or epicutaneously absorbed allergens. Furthermore, impairment of skin barrier function has been implicated in the pathogenesis of AD (5–8). Thus, AD is a multifactorial disease, and its pathogenesis and aetiology have not been clearly understood. A development of suitable animal models is useful to explore the pathogenesis and treatment of the disease.

NC/Nga mice were established as an inbred strain from Japanese fancy mice (9). Matsuda et al. demonstrated that NC/Nga mice, which had been raised in conventional circumstances, spontaneously developed AD-like skin lesions with marked elevation in plasma levels of total IgE. Barrier functions of the skin are also impaired in the mice similarly to the skin of AD patients (10). In contrast, NC/Nga mice grown in specific pathogen-free (SPF) circumstances showed neither dermatitis nor IgE hyperproduction (11). NC/Nga mice seem to be an appropriate model of AD, since the mice can develop chronic, eczematous skin changes based on the genetic background and environmental factors.

The first line of treatment modality for AD includes topical corticosteroids and/or immunosuppressants such as tacrolimus and oral antihistamines. Patients with widespread, severe AD, especially with unsatisfactory responses to topical therapy, are treated with

the second line therapies. Photochemotherapy with 8-methoxypsoralen (8-MOP) and UVA radiation (psoralens and ultraviolet A radiation (PUVA)) has been indicated in patients with severe AD resulting in improvement of their quality of life (12–15). In this paper, we describe the therapeutic and prophylactic effects of PUVA treatment on dermatitis in NC/Nga mice and discuss mechanisms of the action of the photochemotherapy.

Materials and methods

Animals

Male NC/Nga mice with brown hairs were purchased from Shizuoka Laboratory Animal Centre, Ltd. (Hamamatsu, Japan). The mice were given a standard diet and water *ad libitum*. Some mice were kept under SPF conditions (SPF-NC/Nga mice). The other mice were maintained in a conventional room (Conv-NC/Nga mice). Each experimental panel consisted of five to 10 mice. Mouse experiments were approved by the Kansai Medical University Subcommittee on Research Animal Care.

Photochemotherapy

Minimal phototoxic dose of PUVA treatment was determined in NC/Nga mice. Mice were irradiated with 2, 4, 6 or 8 J/cm² of UVA through a 4 mm thick pane of glass 2 h after intraperitoneal injection of 4 mg/kg body weight of 8-MOP (Taisho Pharmaceutical, Tokyo, Japan). The UVA source was a bank of 14 fluorescent black lights (FL32S. BL; Toshiba Medical Supply, Tokyo, Japan) with an emission spectrum of 300–430 nm peaking at 352 nm. The irradiance of UVA was measured by radiometer (UVR-305/365D(II), Toshiba Medical Supply). Ear thickness was measured with a dial thickness gauge (Peacock, Tokyo, Japan) immediately before and 48 h after irradiation. PUVA treatment was performed at a lower dose than minimal phototoxic dose three times a week for 4 weeks. Another group of mice was exposed to UVA without 8-MOP administration.

Evaluation of severity of dermatitis

Clinical skin conditions on the ears, head, nape and back were macroscopically examined once a week. A total clinical severity score for skin lesions was defined as the sum of the individual score grades as 0 (none), 1 (mild), 2 (moderate), 3 (severe) for each of five signs and symptoms (itch, erythema/ haemorrhage, oedema, excoriation/erosion, and scaling/dryness) as previously described (11, 16). Itching was evaluated by scratching behaviour observed during 2 min.

Histopathological examinations

Biopsy specimens were taken from the nape skin, in which the most prominent lesions usually appear, processed by the conventional method and stained with haematoxylin–eosin (HE) or toluidine blue.

Plasma IgE levels

Blood was collected periodically from NC/Nga mice before and after PUVA treatment. Plasma samples were obtained by centrifugation and stored at –20 °C until use. Total IgE levels were measured by a sandwich murine IgE ELISA kit (Yamasa Syoyu, Chiba, Japan).

Statistical analysis

Data were expressed as the mean ± SEM. Statistical significances were assessed by Student's *t*-test and *P* < 0.05 was taken as the level of significance.

Results

Skin changes

In contrast to the previous reports, neither SPF-NC/Nga nor Conv-NC/Nga mice developed abnormal skin manifestations throughout the observation period in our laboratory. Fur mites may play an important role in the development of dermatitis in NC/Nga mice (17). Then, ten 4-week-old NC/Nga mice were kept together with three fur mite (*Myocoptes musculus*)-infested NC/Nga mice in one cage in conventional rooms. All of these mice started to scratch the face, neck, and ears from the age of 8 weeks, gradually developing scaling, erythema, erosion, haemorrhage, and hair loss especially on the head, nape, and back. At the age of 14 week, total severity scores reached 7–15 points.

Effects of PUVA on established dermatitis

Exposure to 6 and 8 J/cm² of UVA 2 h after the administration of 8-MOP induced increases in ear thickness by 3.0×10^{-2} and 5.2×10^{-2} mm, respectively. Ear thickness was not changed after UVA exposure at 4 J/cm². Therefore, 4 J/cm² UVA was applied for PUVA treatment. Conv-NC/Nga mice at the age of 14 week with fully developed skin lesions received PUVA, UVA without 8-MOP or no treatment for 4 week. Non-treated and UVA-irradiated mice showed persistent, severe dermatitis, and scratching behaviour during the treatment. In contrast, PUVA-treated mice exhibited significant improvement of dermatitis in 1 week after the initiation of the treatment, and revealed only slight symptoms throughout the observation period (Fig. 1). The

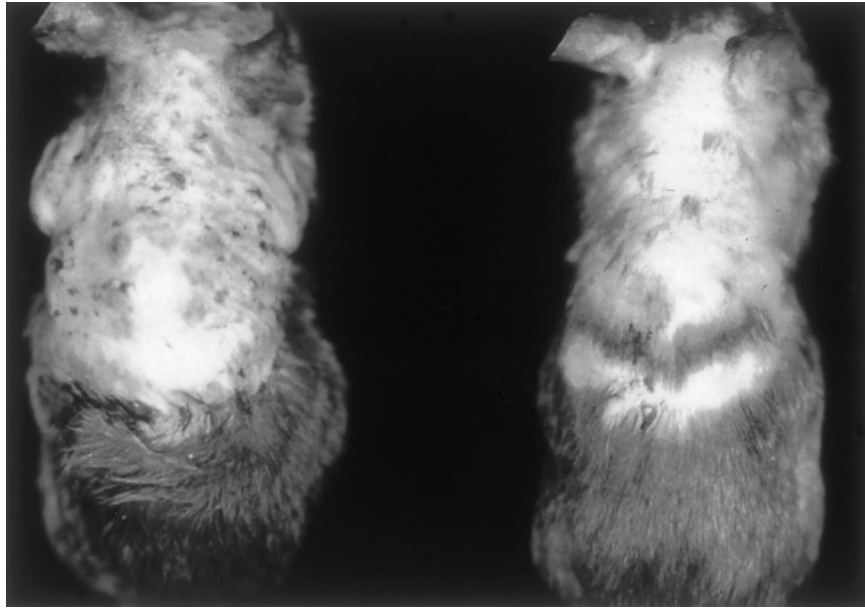


Fig. 1. Conv-NC/Nga mouse without treatment showed severe dermatitis (left). Mouse that had been treated with PUVA for 4 weeks exhibited significant improvement of dermatitis (right).

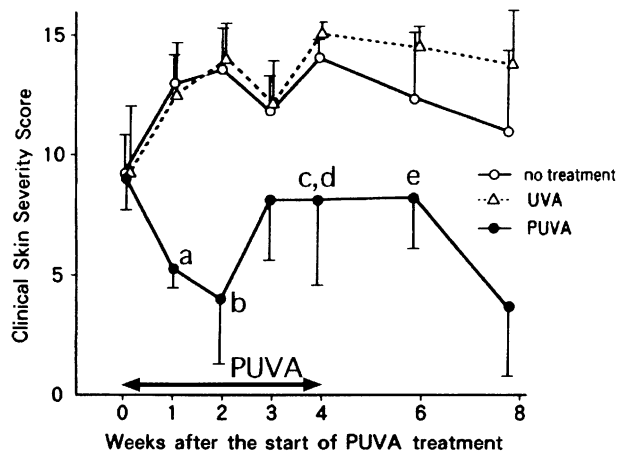


Fig. 2. The effect of psoralens and ultraviolet A radiation (PUVA) therapy on clinical severity scores of established dermatitis. Significant *P*-values: (a) $P = 0.0048$, PUVA vs. UVA and no treatment. (b) $P = 0.0485$, PUVA vs. UVA and no treatment. (c) $P = 0.0006$, PUVA vs. no treatment. (d) $P < 0.0001$, PUVA vs. UVA. (e) $P = 0.006$, PUVA vs. UVA and no treatment.

severity score at the start of treatments was 9.2 ± 1.7 . In non-treated and UVA-irradiated mice, the score increased gradually during 2 week, and was kept more than 11 for 6 week thereafter (Fig. 2). On the other hand, the score in PUVA-treated mice decreased rapidly during the first week and reached a minimum in 2 week. Although, the score in PUVA-treated mice once increased, significant suppression was observed

compared with non- and UVA-treated mice even after discontinuance of treatments for 4 week. Infesting mites were found in PUVA-treated mice as well as non-treated mice during the observation period.

Histological changes

The histological examinations of the dermatitis at the initiation of treatments revealed hyperkeratosis, thickening of the epidermis (acanthosis) and infiltrations of mononuclear cells, eosinophils and mast cells. The dermal infiltration of inflammatory cells, especially mast cells, increased in non- and UVA-treated mice at the 2 week after the treatment. At 4 week, these findings became more prominent. On the other hand, no acanthosis of the epidermis and only slight dermal infiltration of inflammatory cells were found in PUVA-treated mice at 4 week.

Marked infiltration of mast cells into the dermis was the characteristic feature of fully developed skin lesions. The number of mast cells in toluidine blue-stained sections increased to more than twice at 2 and 4 week compared with that at the start of treatment in non- and UVA-treated mice (Fig. 3). However, the number of mast cells remained unchanged in PUVA-treated mice during 4-week treatment. The ratio of the number of degranulated mast cells to that of total mast cells was more than 40% throughout the treatment period in non- and UVA-treated mice, while decreased to 14.1% in PUVA-treated mice by 4 week of PUVA treatment.

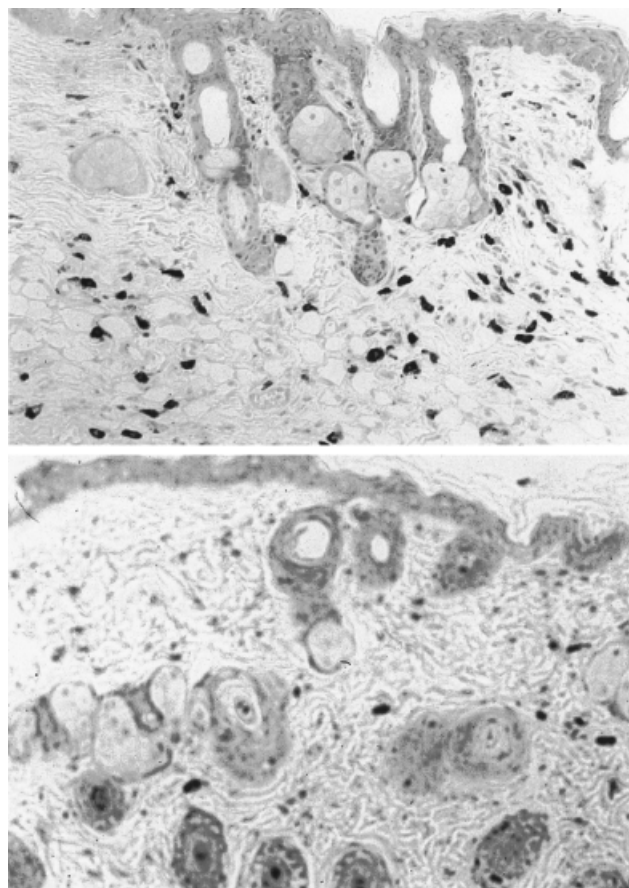


Fig. 3. Dermal mast cells in atopic dermatitis (AD)-like lesions of Conv-NC/ Nga mice. Proliferation of mast cells (top) was suppressed by psoralens and ultraviolet A radiation (PUVA) treatment (bottom).

Plasma IgE

Plasma levels of total IgE in Conv-NC/Nga mice were low at the age of 6 week, and greatly increased in association with development of dermatitis. Skin lesions were improved after PUVA treatments, but IgE levels in the treated mice were not altered keeping similar levels to those in non-treated and UVA-irradiated mice with severe dermatitis (Fig. 4).

Prophylactic effects of PUVA

Conv-NC/Nga mice at the age of 7 week, which had not developed dermatitis, received PUVA treatment, UVA irradiation without 8-MOP administration or no treatment for 6 week. Non- and UVA-treated mice showed scratching behaviour and mild to moderate dermatitis in 2 week, at the age of 9 week, and then to severe dermatitis, such as oedematous erythema, crusted papules, erosions, and haemorrhage at the age of 12 week (Fig. 5). In contrast, such changes were significantly weaker in PUVA-treated mice at the time. The severity scores in non- and UVA-treated

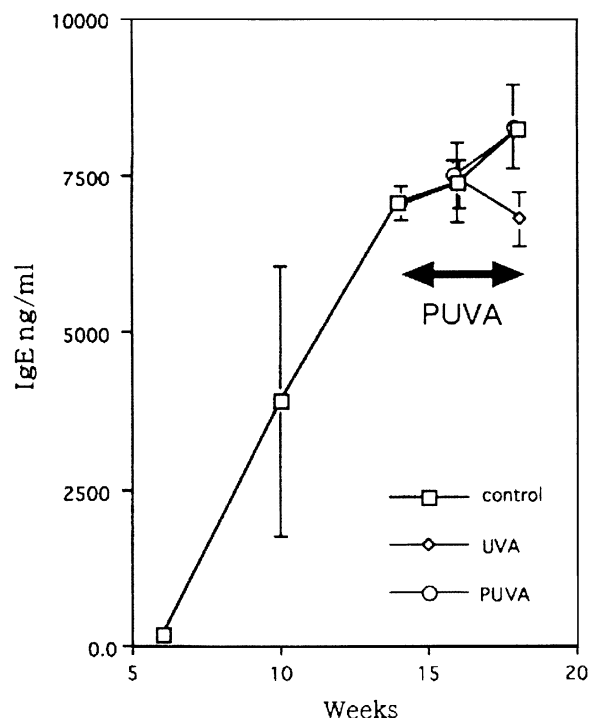


Fig. 4. Increased level of plasma IgE was not altered after psoralens and ultraviolet A radiation (PUVA) treatment.

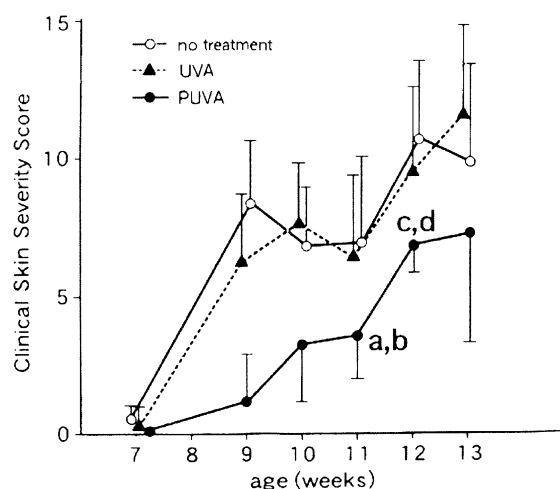


Fig. 5. The prophylactic effect of psoralens and ultraviolet A radiation (PUVA) therapy on clinical severity scores of dermatitis. Significant *P*-values: (a) $P = 0.0182$, PUVA vs. no treatment. (b) $P = 0.0111$, PUVA vs. UVA. (c) $P = 0.0023$, PUVA vs. no treatment. (d) $P = 0.0010$, PUVA vs. UVA.

mice reached more than 10 at the age of 13 week, while, in PUVA-treated mice, the score increased more slowly and did not reach 10.

Discussion

It seems to be well-established that NC/Nga mice is an animal model of AD, since they develop chronic dermatitis associated with increase of total IgE, when kept in air-uncontrolled conventional circumstances. Although, hypersensitivity to some agents in conventional rooms is suggested to induce dermatitis in NC/Nga mice (11), the responsible factor has not been identified. Therefore, AD-like lesions are not necessarily induced in the mice that are raised in any conventional condition. In our animal laboratory, the dermatitis developed in Conv-NC/Nga mice when infested with mites, as reported by Morita et al. (17). Therapeutic effects of tacrolimus (FK506) ointment, corticosteroid ointment (18), and transforming growth factor (TGF)- β 1 (16) have been studied using NC/Nga mice. It is not easy to examine suppressive effects of treatments on the development of new lesions in patients with AD. Conv-NC/Nga mice develop AD-like skin lesions from 8 week of age. Since onset time of the dermatitis can be expected, prophylactic effects of therapeutic modalities on AD-like lesions can be examined using Conv-NC/Nga mice. The present study demonstrated that photochemotherapy with PUVA at lower dose than minimal phototoxic dose reveals not only therapeutic effect on established AD-like lesions but also prophylactic effects on the development of the dermatitis. As previously reported (11, 19, 20), IgE hyperproduction was associated with the development of AD-like lesions in Conv-NC/Nga mice. Treatments with tacrolimus ointment (18) or TGF- β 1 (16) reduced the plasma IgE level correlated with improvement of the skin changes. Based on these findings, possible involvement of IgE hyperproduction has been suggested in the pathogenesis of AD-like lesions in Conv-NC/Nga mice as well as in patients with AD. In contrast, IgE levels were not altered in PUVA-treated mice in the present study, nevertheless the dermatitis was significantly improved. This is consistent with our clinical experiences in patients with AD treated with PUVA photochemotherapy (15). The role of IgE in AD has not been elucidated. Although, it is generally believed that IgE antibody may play a certain role in AD, there are several clinical evidences that IgE-mediated hypersensitivity is not fundamental in the aetiology of AD (21). In the present study, plasma IgE levels were not measured in the experiment of prophylactic effects of PUVA. Therefore, it is not clear whatever PUVA therapy in advance can suppress the IgE hyperproduction.

The increased number of granulated and degranulated mast cells in the dermis also a characteristic,

common feature of AD-like lesion in Conv-NC/Nga mice and AD in human patients. The present study showed that the dermal mast cells increased in number correlated with the severity score of the dermatitis in non-treated and UVA-irradiated Conv-NC/Nga mice. On the other hand, PUVA treatment inhibited the proliferation of mast cells and decreased the ratio of degranulated mast cells to total mast cells. Previously we have indicated that PUVA treatment suppressed mast cell degranulation and release of histamine, and thereby inhibited the development of skin oedema because of histamine liberators, compound 48/80 or concanavalin A (22, 23). Kolde et al. (24) showed a decrease in mast cell numbers and lowered histamine levels in the subepidermal space in the PUVA-treated lesions of *Urticaria pigmentosa*, cutaneous mastocytosis. Therefore, PUVA therapy may be useful for the treatment of mast cell-mediated skin diseases or for the relief of pruritus. In addition, suppressive effects of PUVA treatment on other immune competent cells, including Langerhans cells (25, 26) and T lymphocytes (27, 28), may be involved in the improvement of the dermatitis. Infested fur mites were not affected by PUVA treatment in the present experiments. However, the photochemotherapy can inhibit the bacterial colonization (29), and superantigen production (30), which may exacerbate AD lesions.

In conclusion, we confirmed that NC/Nga mice is a suitable murine model for study on AD. The present study demonstrated that systemic PUVA photochemotherapy revealed therapeutic and prophylactic effect on AD-like lesions developed in mite-infested Conv-NC/Nga mice. This implies that a maintenance therapy with PUVA after improvement of AD can induce long-lasting remission of the disease. Proliferation of dermal mast cells associated with the development of dermatitis was suppressed by PUVA therapy, while IgE hyperproduction was not altered after the treatment.

Acknowledgement

This work was supported in part by grant no.11670854 from the Ministry of Education, Science and Culture of Japan.

References

1. Cooper KD. Atopic dermatitis: recent trends in pathogenesis and therapy. *J Invest Dermatol* 1994; **102**: 128–137.
2. Uehara M, Kimura C. Descendant family history of atopic dermatitis. *Acta Derm Venereol (Stockh)* 1993; **73**: 62–63.

3. Larsen FS. Atopic dermatitis: a genetic-epidemiologic study in a population-based twin sample. *J Am Acad Dermatol* 1993; **28**: 719–723.
4. Diepgen TL, Blettner M. Analysis of familial aggregation of atopic eczema and other atopic disease by odds ratio regression models. *J Invest Dermatol* 1996; **106**: 977–981.
5. Werner Y. Transepidermal water loss in dry skin and clinically normal skin in patients with atopic dermatitis. *Acta Derm Venereol (Stockh)* 1986; **65**: 102–105.
6. Abe T, Ohkido M, Yamamoto K. Studies on skin surface barrier functions – skin surface lipids transepidermal water loss in atopic skin during childhood. *J Dermatol* 1978; **5**: 223–229.
7. Thune P. Evaluation of the hydration and the water-holding capacity in atopic skin and so-called dry skin. *Acta Derm Venereol (Stockh)* 1989; **144 (Suppl.)**: 133–135.
8. Berardesca E, Fideki D, Borroni G, et al. In vivo hydration and water retention capacity of stratum corneum in clinically uninvolved skin in atopic and psoriatic patients. *Acta Derm Venereol (Stockh)* 1990; **70**: 400–404.
9. Kondo K, Nagami T, Teramoto S. Differences in haematopoietic death among inbred strains of mice. In: Bond P, Sugawara S, eds. Comparative cellular and species radiosensitivity. Tokyo: Igakushoin, 1964; 20.
10. Aioi A, Tonogaito H, Suto H, et al. Impairment of skin barrier function in NC/Nga Tnd mice as a possible model for atopic dermatitis. *Br J Dermatol* 2001; **144**: 12–18.
11. Matsuda H, Watanabe N, Geba GP, et al. Development of atopic dermatitis-like skin lesion with IgE hyperproduction in NC/Nga mice. *Int Immunol* 1997; **9**: 461–466.
12. Morison WL, Parrish JA, Fitzpatrick TB. Oral psoralen photochemotherapy of atopic eczema. *Br J Dermatol* 2001; **144**: 12–18.
13. Atherson DJ, Carabott, Glover MT, Hawk JLM. The role of psoralen photochemotherapy (PUVA) in the treatment of severe atopic eczema in adolescents. *Br J Dermatol* 1988; **118**: 791–795.
14. Sheehan MP, Atherton DJ, Norris P, Hawk J. Oral psoralen photochemotherapy in severe childhood atopic eczema: an update. *Br J Dermatol* 1988; **118**: 791–795.
15. Uetsu N, Horio T. Treatment of persistent severe atopic dermatitis in 113 Japanese patients with oral psoralen photochemotherapy. *J Dermatol* 2003; **30**: 450–457.
16. Sumiyoshi K, Nakao A, Ushio H, et al. Transforming growth factor- β 1 suppresses atopic dermatitis-like lesions in NC/Nga mice. *Clin Exp Allergy* 2002; **31**: 309–314.
17. 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.
18. 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.
19. Suto H, Matsuda H, Mitsuishi K, et al. NC/Nga mice: a mouse model for atopic dermatitis. *Int Arch Allergy Immunol* 1999; **120 (Suppl. 1)**: 70–75.
20. Matsumoto M, Ra C, Kawamoto K, et al. IgE hyperproduction through enhanced tyrosine phosphorylation of Janus kinase 3 in NC/Nga mice, a model for human atopic dermatitis. *J Immunol* 1999; **162**: 1056–1063.
21. Holden CA, Parish WE. Atopic dermatitis. In: Champion RH, Burton JL, Burns DA, et al., eds. *Textbook of Dermatology*, 6th edn, Vol. 1. Oxford: Blackwell Scientific Publications, 1998; 681–708.
22. Danno K, Toda K, Horio T. The effect of 8-methoxy psoralen plus long-wave ultraviolet (PUVA) radiation on mast cells: PUVA suppresses degranulation of mouse skin mast cells induced by compound 48/80 or concanavain A. *J Invest Dermatol* 1985; **85**: 110–114.
23. Toda T, Danno K, Tachibana T, Horio T. Effect of 8-methoxypsoralen plus long-wave ultraviolet (PUVA) radiation on mast cells. II. In vitro PUVA inhibits degranulation of rat peritoneal mast cells induced by compound 48/80. *J Invest Dermatol* 1986; **87**: 113–116.
24. Kolde G, Frosch PJ, Czarnetzki BM. Response of cutaneous mast cells to PUVA in patients with *Urticaria pigmentosa*: histomorphometric, ultrastructural, and biochemical investigations. *J Invest Dermatol* 1984; **83**: 175–178.
25. Okamoto H, Horio T. The effect of 8-methoxypsoralen and long-wave ultraviolet light on Langerhans cell. *J Invest Dermatol* 1981; **77**: 345–346.
26. Horio T, Okamoto H. The mechanisms of inhibitory effect of 8-methoxypsoralen and long-wave ultraviolet light on experimental contact sensitization. *J Invest Dermatol* 1982; **78**: 402–405.
27. Okamoto H, Takigawa M, Horio T. Alteration of lymphocyte functions by 8-methoxypsoralen and long wave ultraviolet radiation. I. Suppressive effect of PUVA on T lymphocyte migration in vitro. *J Invest Dermatol* 1985; **84**: 203–205.
28. Okamoto H, Horio T, Maeda M. Alteration of lymphocyte functions by 8-methoxypsoralen and long wave ultraviolet radiation. II. The effect of in vivo PUVA on IL-2 production. *J Invest Dermatol* 1987; **89**: 24–26.
29. Yoshimura M, Namura S, Akamatsu H, Horio T. Antimicrobial effects of phototherapy and photochemotherapy in vivo and vitro. *Br J Dermatol* 1996; **135**: 528–532.
30. Yoshimura M, Namura S, Akamatsu H, Namura S, Horio T. Suppressive effect of ultraviolet (UVB and PUVA) radiation on superantigen production by *Staphylococcus aureus*. *J Dermatol Sci* 1999; **19**: 31–36.

Accepted for publication 7 February 2005

Corresponding author:

Takeshi Horio
 Department of Dermatology
 Kansai Medical University
 Fumizono
 10-15 Moriguchi
 Osaka 570-8507
 Japan
 Tel: +81 6 6992 1001
 Fax: +81 6 6996 3299
 e-mail: horio@takii.kmu.ac.jp