

The Characteristics of Patients with Atopic Dermatitis Demonstrating a Positive Reaction in a Scratch Test after 48 Hours against House Dust Mite Antigen

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Abstract

The clinical characteristics of patients in whom an IgE-mediated reaction against house dust mite (HDM) antigens that contribute to the pathogenesis of atopic dermatitis (AD) remain unclear. This study attempted to elucidate the characteristics of patients who exhibit a positive reaction 48 hr or later against HDM in scratch tests. The reactions after epicutaneous application of the allergen to skin with prior scratching were observed for one week in sixteen AD patients showing positive immediate-type hypersensitivity reactions for HDM. Fifty percent of the patients demonstrated positive reactions at 48 hours after epicutaneous application of HDM. Significantly higher values were demonstrated in the group positive for HDM after 48 h in serum total IgE, specific IgE for Der f 1, and lactate dehydrogenase, peripheral eosinophil counts, eruption score, and the area of eruption than in the group negative for HDM after 48 h. Domestic exposure to Der f 1 was also higher in the group positive for HDM after 48 h than in the negative group. These results indicate that the patients in whom the HDM-induced reaction continuing more than 48 h and contributing to their real eczematous eruptions are characterized by considerably increased levels of specific IgE for HDM antigens, high disease activity in AD, and increased exposure to domestic HDM.

Abbreviations: AD: atopic dermatitis, HDM: house dust mite, LC: Langerhans cells, FcεRI: high affinity receptor for IgE, DTH: delayed type hypersensitivity reaction

Key words: house dust mite; delayed type hypersensitivity reaction; the high affinity receptor for IgE

Introduction

Atopic dermatitis (AD) is a chronic inflammatory skin disease frequently seen in individuals with a genetic predisposition to developing IgE-mediated hypersensitivity reactions against food- and aero-antigens

(1–3). Elevated serum levels of house dust mite (HDM)-specific IgE are often observed in patients with AD (4). Clinically, certain patients with AD experience exacerbation of their eczema after encounters with HDM allergen (5). Improvement in AD following HDM avoidance has also been reported (6). In addition, the enhanced antigen presenting capacity of HDM antigens to T cells by Langerhans cells (LC) from AD patients (7, 8) *via* the high affinity receptor for IgE (FcεRI) (9–11) has established the concept that an IgE-mediated delayed type hypersensitivity reaction (DTH) against HDM antigens contributes to the pathogenesis of AD.

On the other hand, Jürgens et al. demon-

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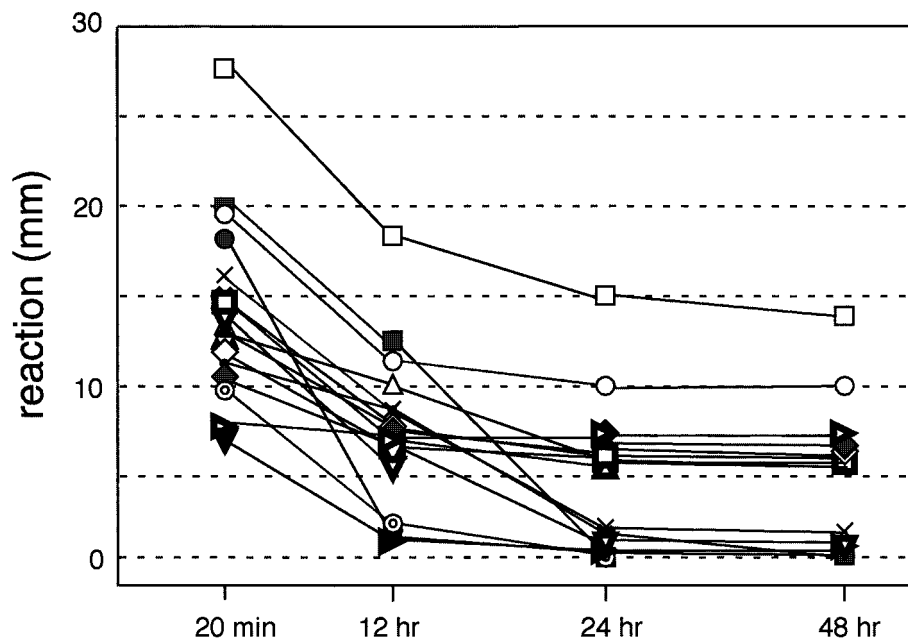


Fig. 1. Reaction to the epicutaneous application of Der f antigen with prior scratching

strated that IgE-mediated activation of LC was not observed in all patients with AD (11). More importantly, the eruptions do not exacerbate after HDM exposure in every AD patient who has elevated serum levels of HDM-specific IgE and/or who exhibits a positive skin test response against HDM antigens. Although many groups have studied the clinical characteristics of patients who demonstrate DTH for HDM antigens in atopy patch tests response, the results are variable. Thus the role of HDM in provoking or maintaining AD has been the cause of both controversy and uncertainty (12–16).

We consider that the application of HDM antigen after scratching resembles the real situation in AD more than an atopy patch test (17). In this study, we therefore observed the reaction against HDM after epicutaneous application of the HDM allergen to skin with prior scratching for up to one week in patients who showed positive immediate-type hypersensitivity reactions for HDM. The positive reactions in some patients lasted up to one week. The clinical

characteristics of patients in whom not only positive immediate-type reactions but also positive reactions lasting longer than 48 h induced by scratch testing with HDM antigens are discussed.

Subjects and Methods

Patients

Sixteen patients with AD (aged 18–38 years, median 26; male, 7 patients) diagnosed according to the criteria of Hanifin and Rajka (18) and the positive immediate-type prick test and positive RAST scores (<class 2) for HDM were randomly selected from in-patients at our hospital. No systemic steroids, immune suppressive agents, nor ultraviolet therapy were administered. Topical steroids on the testing site were discontinued at least seven days before testing. Oral antihistamines were also discontinued at least two days before testing. On the day of testing, the eruption score and area were estimated, peripheral blood was obtained, and the serum was stored at -80°C . After the one week of observation, treatment with oral antihistamines and topical steroids (0.1% aclometasone dipropionate) was started and continued after dis-

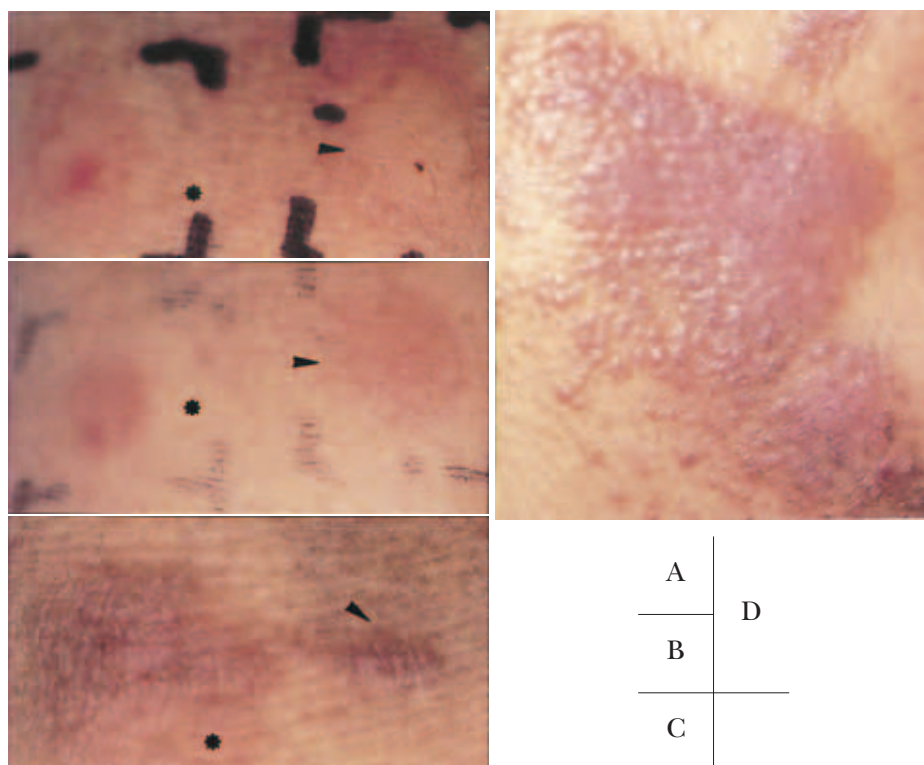


Fig. 2. Positive reaction to the epicutaneous application of Der f and *Candida albicans*. Representative results in a patient. A: 20 min, B: 24 hours, and C: 168 hours after testing. Asterisk: reaction against Der f. Arrowhead: reaction against *Candida albicans*. D: The real eruption in this patient

charge from the hospital. The clinical activity of their skin lesions was established with our scoring system as reported previously (18). All participating subjects or their parents gave their informed consent.

Allergens and Scratch Tests

Commercially available aqueous preparations of *Dermatophagoides farinae* (Der f, 120 $\mu\text{g mL}^{-1}$) and *Candida albicans* (60 $\mu\text{g mL}^{-1}$) containing 50% glycerin were purchased from Torii Pharmaceuticals (Tokyo, Japan). The scratch test was performed as follows: one shallow, five millimeter long superficial epidermal cut was made with a 27 gauge needle and 20 μL of allergen solution was dropped on it. The reaction of a wheal after 20 min and infiltrated erythema or papules at 48 h were regarded as positive when its length vertical to the scratch mark was more than 5 mm and over 2-fold when compared with that of the negative control (distilled water containing 50%

glycerin). Histamine dihydrochloride, 10 mg mL^{-1} , was used as a positive control. After 20 minutes, and 1, 12, 24, and 48 hours the reactions were evaluated. When positive reactions were detected 48 hours after allergen exposure, the reactions were observed every day up to 7 days.

IgE Measurements

Total IgE for sera from the patients with AD was measured using a fluoroimmunoassay (CAP system; Pharmacia, Uppsala, Sweden) according to the manufacturers' instructions. Specific IgE for Der f 1 and *Candida albicans* was also measured using the CAP system (Pharmacia). Sera were first assayed undiluted, and, when necessary, assays were repeated with the sera diluted to enable quantitative measurement of specific IgE antibodies.

Dust Collection and HDM-ELISA

Table 1. Clinical characteristics of the patients who demonstrated positive cutaneous reaction after 48 h against HDM

	IgE for Der f 1 (UA mL ⁻¹)	IgE for <i>Candida albicans</i> (UA mL ⁻¹)	Eosinophil (μL ⁻¹)	LDH (IU L ⁻¹)	IgE (U mL ⁻¹)	Eruption area (%)	Eruption score
positive-48h	781±445	16± 6	1,407±672	632±153	25,665±16,659	77±20	66±24
negative-48h	65± 39	37±30	720±300	414±113	8,978± 9,331	56±15	48±16
P value	0.0012	0.6434	0.0436	0.0342	0.0206	0.0278	0.0021

The patients were subdivided into two groups: positive ($n=8$, positive-48h) and negative ($n=8$, negative-48h) reactions after 48h against Der f 1. Serum IgE specific for Der f 1 and *Candida albicans*, IgE Peripheral eosinophil counts, serum levels of lactate dehydrogenase (LDH) and total IgE, eruption score, and eruption area were compared between the two groups using unpaired Student *t* tests. Reference values: serum IgE, <380 U mL⁻¹; serum LDH, 234–471 IU mL⁻¹; peripheral eosinophil counts, <420 μL⁻¹.

Dust samples were collected from the mattresses of the patients' beds by vacuuming an area of 600 cm² for 120 seconds. The dust was weighed, and then Der f 1 was extracted and quantified using a commercially available ELISA kit (LCD laboratory, Osaka, Japan).

Statistical analysis Data are expressed as the mean ± SD unless otherwise indicated. Differences between the two groups (patients with AD and positive results after 48 hours and patients with AD and negative results after 48 hours) were compared by the paired and unpaired Student *t* tests, when not otherwise specified. The Pearson's correlation coefficient was analyzed between the diameter of scratch test reaction with HDM and serum anti-Der f 1 IgE titers. All statistical analyses were assessed using StatView Ver. 5 (SAS Institute Inc. NC) on a Macintosh™ computer. A *P* value of less than 0.05 was regarded as significant.

Results

Reactions Induced by Scratch Testing with HDM Antigens

Since all the patients revealed less than 3 mm reactions with the negative control reagent, a reaction over 6 mm long was regarded as positive. As shown in Figure 1, positive wheal reactions against HDM were observed in all participants. A positive late-

phase reaction determined after 12 hours of testing was observed in 11 patients. Positive reactions against *Candida albicans* were also observed in 12 patients, and their degrees were comparable to those against HDM (data not shown). Fifty percent of the patients demonstrated positive reactions at 48 hours after epicutaneous application of HDM antigens. Their positive reactions lasted 129 ± 55 hours and, in one patient, up to 7 days after testing. Representative reactions are shown in Figure 2.

Clinical Characteristics of DTH-Positive Patients

In order to determine the clinical backgrounds of the patients whose positive reactions for HDM antigens lasted more than 48 hours, the patients were subdivided into two groups: patients with positive results after 48 hours (referred to as positive-48h) and patients with negative results after 48 hours (referred to as negative-48h). No significant differences in the reactions against HDM after 20 min or 12 hours were observed between the positive-48h group and the negative-48h groups (data not shown).

As shown in Table 1, serum levels of specific IgE for Der f 1 were significantly higher in the positive-48h group than the negative-48h one, while those for *Candida albicans*

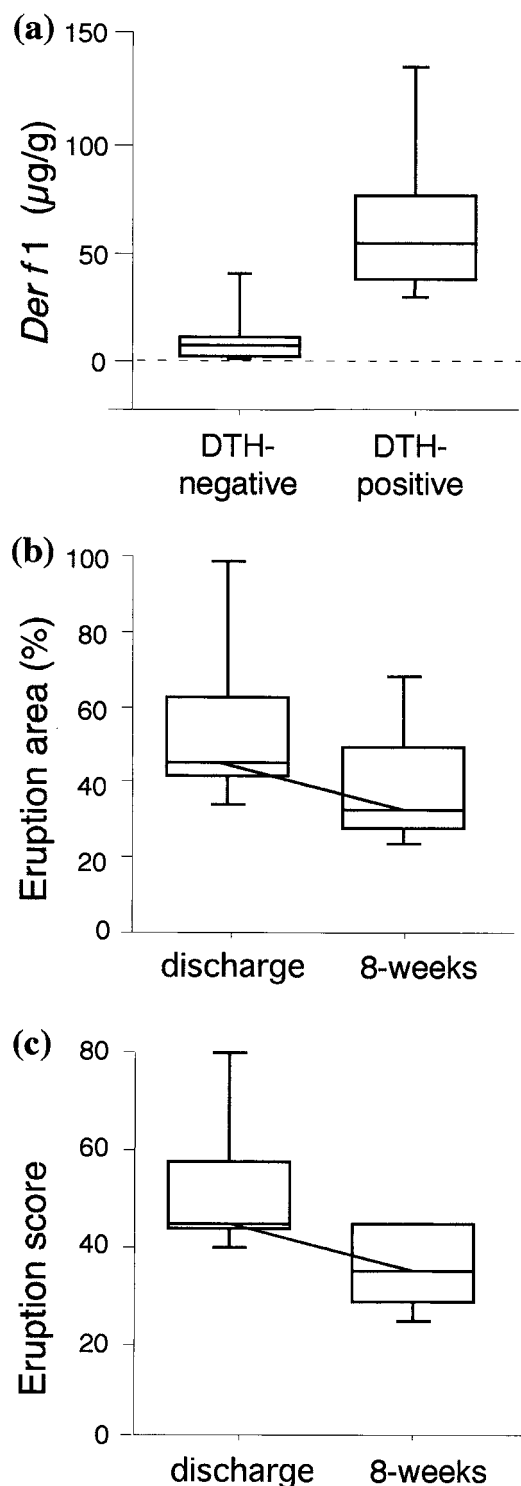


Fig. 3. Der f 1 exposure. A: Domestic exposure to Der f 1 in patients with positive-48h and negative-48h, the transition of the eruption area (B) and the eruption score (C) between the day of discharge and eight weeks after discharge

were not significantly different between the two groups. The correlation efficiency between the diameter of scratch test reaction with HDM and serum anti-Der f 1 IgE titers was not significant at 20 min ($R=0.015$, $P=0.96$), while that after 48 h was statistically significant ($R=0.609$, $P=0.014$). Significant differences were shown between the patients in the positive-48h group and negative-48h group in peripheral eosinophil counts, serum levels of lactate dehydrogenase, eruption score, and the area of eruption. Serum total IgE levels were also significantly higher in the positive-48h group than in the negative-48h group.

HDM Exposure

As shown in Figure 3a, the individual Der f 1 concentration in the patient's mattress dust was significantly higher in the positive-48h groups than in the negative-48h group ($P=0.0088$). After discharge, the patients who exhibited positive-48h for HDM were instructed to use a bed covering system (Microguard®, Teijin Co.Ltd, Osaka, Japan), which is water vapour permeable but HDM allergen impermeable, to reduce HDM. The Der f 1 concentration in their mattress dust was reduced below one μg/g eight weeks after discharge. Significant decreases between the day of discharge and eight weeks after discharge were observed in the eruption area ($P=0.0293$; Fig. 3b) and the score ($P=0.0401$; Fig. 3c).

Discussion

The mechanisms by which HDM contribute to the pathogenesis of the eruption in AD have been considered to be as follows (20): wheal reaction due to immediate type hypersensitivity against HDM, an erythematous reaction appearing as a late phase response, and eczematization by scratching these eruptions known as "itch-scratch cycle". In this study, most of the patients revealed an equal degree of positive immediate and late phase reactions for HDM and *Candida albicans*. However, only the positive reaction for HDM continued more than 48

hours. This result suggests that other mechanisms in addition to those mentioned previously play a role in the pathogenesis of AD. IgE-mediated DTH against mite antigens is the most probable mechanism which contributes to this reaction, although the possibility that the late phase reaction lasted for 48 h should be taken into consideration.

In IgE-mediated DTH, aeroantigens are internalized into LC after engagement of FcεRI on these cells by combining with antigen specific IgE and efficiently presented to antigen-specific T cells (21). Aggregation of FcεRI on LC by multivalent antigens requires that the two receptors be sufficiently close. Therefore, the closer FcεRI are expressed on LC, the more easily they are cross-linked by the antigen that is followed by activation of LC and antigen specific T cells (11). There have been many reports that the expression of FcεRI on the cell surface is positively regulated by extracellular IgE as a FcεRI ligand and stabilizer in mast cells, basophils, and LC (22–24). In our observation, patients with high values of serum total IgE and, more importantly, who exhibited high levels of HDM-specific IgE, demonstrated HDM-induced reactions after 48 h or later. These results suggest that a high concentration of antigen-specific IgE in the peripheral blood and the close expression of FcεRI bound with the antigen specific IgE are important for FcεRI-IgE-mediated antigen presentation by LC. The values of HDM-specific IgE which showed positive-48h against HDM in this study were considerably high (RAST: 781 ± 445 UA mL⁻¹). The sensitivity of FcεRI-mediated signals may be lower in LC than in mast cells and basophils, probably because LC lack the signal amplifier beta chain of FcεRI (9, 10). Taken together, higher HDM-specific IgE in the serum and on the surface of LC are required for the HDM-induced long-lasting reaction when compared with HDM-induced immediate type hypersensitivity reaction by mast cells and basophils, and the values are at least not indicated by the criteria “higher than class 2”.

In this study, a positive HDM-induced reaction was observed after 48 h or later in patients with high disease activity and frequent exposure to HDM. Both the epidermal barrier dysfunction associated with aggravation of AD and the elevated exposure to domestic HDM facilitate the engagement of FcεRI on mast cells with HDM-specific IgE in the HDM-sensitized patients. Histamine secreted by activated mast cells induces the maturation of dendritic cells which polarize naïve CD4⁺ T cells toward Th2 cells (25). The exacerbation of AD is indeed correlated with the enhanced activity of Th2 cells (19). Th2-type cytokines such as IL-4 upregulate the production of IgE by B cells, creating the positive feedback loop of the IgE-FcεRI-mediated hypersensitivity reaction (23–25). Moreover, barrier dysfunction leads to up-regulation of the antigen presenting function by LC and elevated proinflammatory cytokine production by keratinocytes, both of which enhance DTH (26, 27). It is therefore suggested that the barrier dysfunction associated with the exacerbation of AD and increased HDM exposure contributes not only to the immediate type hypersensitivity reaction but also to IgE-mediated DTH by activated LC.

We conclude that patients in whom HDM-induced cutaneous reaction contributes to their real eczematous eruptions are characterized by considerably increased levels of specific IgE for HDM antigens, high disease activity in AD, and increased exposure to domestic HDM.

Acknowledgments

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References

- 1) Leung DYM: Atopic dermatitis: The skin as a window into the pathogenesis of chronic allergic disease, *J Allergy Clin Immunol*, **96**: 302–319, 1995.
- 2) Leung DYM: Pathogenesis of atopic dermatitis, *J Allergy Clin Immunol*, **104**: s99–s108, 1999.
- 3) Cooper KD: Atopic dermatitis: Recent trends in pathogenesis and therapy, *J Invest Dermatol*, **102**:

- 128–137, 1994.
- 4) Platts-Mills TAE, Mitchell EB, Rowntree S, et al: The role of dust mite allergens in atopic dermatitis, *Clin Exp Dermatol*, **8**: 233–247, 1983.
- 5) Tupker RA, de Monchy JGR, Coenraads P-J: House-dust mite hypersensitivity, eczema, and other nonpulmonary manifestations of allergy, *Allergy*, **53** (suppl 48): 92–96, 1998.
- 6) Tan BB, Weald D, Strickland I, Friedmann PS: Double-blind controlled trial of effect of house-dust-mite allergen avoidance on atopic dermatitis, *Lancet*, **347**: 15–18, 1996.
- 7) Bruijnzeel-Koomen C, van Wicker D, Toonstra J, Bruijnzeel P: The presence of IgE molecules on epidermal Langerhans cells from patients with atopic dermatitis, *Arch Dermatol Res*, **278**: 199–205, 1986.
- 8) Mudde GC, van Reijssen FC, Boland GJ, de Gast GC, Bruijnzeel PL, Bruijnzeel-Koomen CA: Allergen presentation by epidermal Langerhans' cells from patients with atopic dermatitis is mediated by IgE, *Immunol*, **69**: 335–341, 1990.
- 9) Bieber T, de la Salle H, Wollenberg A, et al: Human epidermal Langerhans cells express the high affinity receptor for immunoglobulin E (FcεRI), *J Exp Med*, **175**: 1285–1290, 1992.
- 10) Wang B, Rieger A, Kilgus O, et al: Epidermal Langerhans cells from normal human skin bind monomeric IgE via FcεRI, *J Exp Med*, **175**: 1353–1365, 1992.
- 11) Jürgens ML, Wollenberg A, Hanau D, de la Salle H, Bieber T: Activation of human epidermal Langerhans cells by engagement of the high affinity receptor for IgE, FcεRI, *J Immunol*, **155**: 5184–5189, 1995.
- 12) Gutgesell C, Seubert A, Junghans V, Neumann C: Inverse correlation of domestic exposure to *Dermatophgoides pteronyssinus* antigen patch test reactivity in patients with atopic dermatitis, *Clin Exp Allergy*, **29**: 920–925, 1999.
- 13) Wananukul S, Huiprasert P, Pongprasit P: Eczematous skin reaction from patch testing with aeroallergens in atopic children with and without atopic dermatitis, *Pediatr Dermatol*, **10**: 209–213, 1993.
- 14) Langeveld-Wildschut EG, Thepen T, Bihari IC, et al: Evaluation of variables influencing the outcome of the atopy patch test, *J Allergy Clin Immunol*, **96**: 66–73, 1995.
- 15) Langeveld-Wildschut EG, Bruijnzeel PL, Mudde GC, et al: Clinical and immunologic variables in skin of patients with atopic eczema and either positive or negative atopy patch test reactions, *J Allergy Clin Immunol*, **105**: 1008–1016, 2000.
- 16) Friedman PS: The role of dust mite antigen sensitization and atopic dermatitis, *Clin Exp Allergy*, **29**: 869–872, 1999.
- 17) Norris PG, Schofield O, Camp RDR: A study of the role of house dust mite in atopic dermatitis, *Br J Dermatol*, **118**: 435–440, 1988.
- 18) Hanifin JM, Rajka G: Diagnostic features of atopic dermatitis, *Acta Derm Venereol (Stockh)*, **92** (suppl): 44–47, 1980.
- 19) Katoh N, Hirano S, Suehiro M, et al: Soluble CD30 is more relevant to disease activity of atopic dermatitis than soluble CD26, *Clin Exp Immunol*, **121**: 1–7, 2000.
- 20) Holden CA, Parish WE: Atopic dermatitis, in Champion RH, Burton LJ, Burns DA, Breathnach SM (eds): *Textbook of Dermatology*, 6th ed, Vol. 1, Blackwell Science Ltd, Oxford, 1998, pp 681–708.
- 21) Bieber T: Fc epsilon RI on human epidermal Langerhans cells: An old receptor with new structure and functions, *Int Arch Allergy Immunol*, **113**: 30–34, 1997.
- 22) Wollenberg A, Kraft S, Hanau D, Bieber T: Immunomorphological and ultrastructural characterization of Langerhans cells and a novel, inflammatory dendritic epidermal cell (IDEC) population in lesional skin of atopic eczema, *J Invest Dermatol*, **106**: 446–453, 1996.
- 23) Yamaguchi M, Sayama K, Yano K, et al: IgE enhances Fcε receptor I expression and IgE-dependent release of histamine and lipid mediators from human umbilical cord blood-derived mast cells: Synergistic effect of IL-4 and IgE on human mast cell Fcε receptor I expression and mediator release, *J Exp Med*, **185**: 663–672, 1997.
- 24) Lantz CS, Yamaguchi M, Oettgen HC, et al: IgE regulates mouse basophil FcεRI expression *in vivo*, *J Immunol*, **158**: 2517–2521, 1997.
- 25) Mazzoni A, Young HA, Spitzer JH, Visintin A, Segal DM: Histamine regulates cytokine production in maturing dendritic cells, resulting in altered T cell polarization, *J Clin Invest*, **108**: 1865–1873, 2001.
- 26) Katoh N, Hirano S, Kishimoto S, Yasuno H: Acute cutaneous barrier perturbation induces maturation of Langerhans' cells in hairless mice, *Acta Derm Venereol (Stockh)*, **77**: 365–369, 1997.
- 27) Wood LC, Jackson SM, Elias PM, Grunfeld C, Feingold KR: Cutaneous barrier perturbation stimulates cytokine production in the epidermis of mice, *J Clin Invest*, **90**: 482–487, 1992.