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Immunohistochemical Studies on Dust Mite Antigen in Positive Reaction Site of Patch Test

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We demonstrated that eczematous reactions could be induced by patch testing with mite antigens in the majority of patients with atopic dermatitis (AD). By using immuno-double labelling technique, many mite antigen-bearing Langerhans cells were seen in the epidermis in the early stage of the patch testing. Twenty-four hours later, these cells were observed only in the deep dermis. Immunoelectron microscopically, it was found that the mite antigens were trapped by macrophages, which were apposing lymphocytes.

On the other hand, we observed that Langerhans cells and Leu 3a positive cells in the AD lesions carried IgE molecules. Furthermore, many IgE-positive dendritic cells bearing mite antigen were seen in the positive patch testing sites.

Taken together, IgE-mediated contact hypersensitivity to mite antigen may play an important role in the pathogenesis of AD. ***Key words: Mite antigen; IgE, Langerhans cells; T-lymphocytes.***

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Many patients with atopic dermatitis (AD) show an immediate reaction to mite allergen in skin tests. Moreover, their serum IgE levels are usually high, and IgE-RAST for mite antigens are frequently positive. We previously reported that serum levels of mite-specific IgG, IgG4, IgE and IgE immune complexes in patients with AD were significantly higher than those in healthy controls. However, the skin lesions of AD are characteristic of delayed-type hypersensitivity, differing from the immediate wheal reaction. To investigate if the two different immune reactions play a role in the pathogenesis of AD, patch testing with mite antigens, along with immunohistochemical and immunoelectron microscopic studies, were done.

MATERIALS AND METHODS

Patients and control subjects

The following groups were investigated: Twenty patients with AD (aged 2–25 years, mean 14.9 years; serum IgE level 20–12 306 U/ml, mean 1 858.9 U/ml) and twenty healthy volunteers having no history of atopy (aged 21–25 years, mean 22.5 years; serum IgE level 20–297 U/ml, mean 107.1 U/ml). All subjects gave their informed consent.

Antigens. Antigen solutions of Dermatophagoides pteronyssinus (DP) and D. farinae (DF) were prepared from full-grown mite cultures using the method of Miyamoto et al. (1). Before solutions were extracted, the source culture was defatted in anhydrous acetone and homogenized with phosphate buffered saline (0.005 M phosphate buffer pH 7.2 containing 0.15 M NaCl). After centrifugation at 15 000 g for 20 min, the supernatants were dialyzed against distilled water and freeze-dried. The antigens obtained from whole cultures of DP and DF were denoted DP-WCE and DF-WCE respectively. Mite-free culture medium extract (CME) was also prepared using the same procedure.

Patch tests. White petrolatum containing 0.1 % sodium lauryl sulphate (SLS) was used as the vehicle in accordance with the SLS provocative patch test described by Kligman (2). Patch testing with 0.1 % (w/w) DP-WCE and 0.1 % (w/w) DF-WCE, in the vehicle was performed with Finn Chambers on clinically normal skin of the back. On control sites, vehicle alone and vehicle containing 0.1 % (w/w) mite-free CME were applied. The test reactions were read after 48 h and evaluated according to the criteria of ICDRG.

Histopathology. Five biopsy specimens were obtained from positive patch test sites of 5 patients. Moreover, to investigate the time-course of the inflammatory reaction of the patch test site biopsies were taken after 1 h, 6 h, 24 h and 48 h. Furthermore, twelve biopsy specimens were obtained from active lesions of the other AD patients group, in order to compare the reaction with that of positive patch test sites. One half of each biopsy specimen was prepared for routine histological examination, while the remaining half was processed for immunohistochemical and immunoelectron microscopical studies as described below.

Antisera and affinity purified antibodies. Antisera to DP-WCE and DF-WCE were obtained by immunizing New Zealand white rabbits with both antigens using complete Freund's adjuvant.

Each antiserum was absorbed with CME. For specific purification, 10 mg of each extract was bound to Sepharose activated with CNBr. One hundred ml of anti-DP-WCE or anti-DF-WCE antisera was passed over an affinity column containing DP-WCE or DF-WCE immunosorbent. The bound antibody was eluted with 0.1 M glycine-HCl, pH 2.5, dialyzed with PBS, and stored at −20°C and labelled affinity purified anti-DP (1 mg/ml) and anti-DF (1 mg/ml) antibodies. By immunodiffusion, this anti-mite antibody showed several cross-reactive precipitation lines against DP-WCE, DF-WCE and house dust extract. But reactions to CME, whole culture extracts of Tyrophagus putrescetiae and Glycyphagus priva-

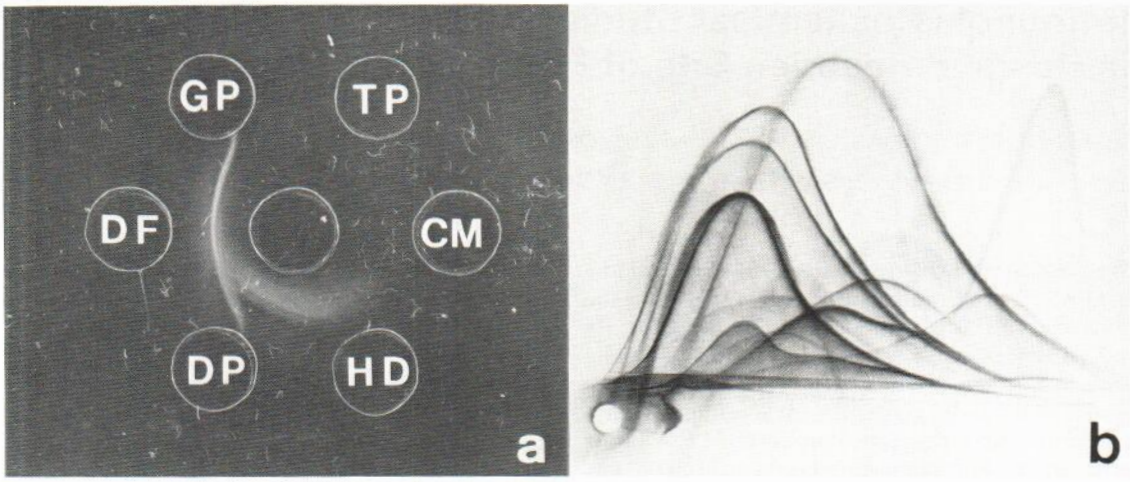

Fig. 1. Immunodiffusion and crossed immunoelectrophoresis analysis of anti-mite antibody (mixture of affinity purified rabbit anti-DP and anti-DF antibody). (*a*) Immunodiffusion against extracts of DF, DP, house dust (HD), mite-free culture medium (CM), Tyrophagus putrescetiae (TP) and Glycyphagus privatus (GP). The center well contained anti-mite antibody. (*b*) Crossed immunoelectrophoresis pattern of DF-WCE reacting with anti-mite antibody. 100 μg applied in well. Anodical antibody-containing gel with 5 μl/cm^2. Electrophoretic conditions: 1) dimension 10 v/cm, for 45 min. 2) dimension 2 v/cm for 16 h. Stained with Coomassie Brilliant Blue.

tus were not observed (Fig. 1 *a*). Crossed immunoelectrophoresis demonstrated up to 28 precipitates for the DF-WCE (Fig. 1 *b*) and 27 for the DP-WCE respectively.

Processing of skin biopsies. Biopsy specimens were fixed using 4% paraformaldehyde in 0.1 M phosphate buffer (PB), pH 7.4, for 6 h at 4°C for immuno-double labelling and immunoelectron microscopy. They were then washed in 10–20% sucrose in PBS overnight, snap-frozen and stored at −80°C. The 5 μm frozen sections were incubated with the anti-mite antibody (1:50 in PBS) for 30 min at 37°C, followed by FITC conjugated anti-rabbit IgG (TAGO, 1:50 in PBS) for 30 min at 37°C. Afterwards, the sections were treated with monoclonal OKT 6 or Leu 3a (1:100 in PBS) for 30 min at 37°C, followed by incubation with rhodamine conjugated anti-mouse IgG (TAGO, 1:100 in PBS) for 30 min at 37°C. The specimens were then examined under a Zeiss fluorescent microscope with an appropriate FITC and rhodamine filter setting.

RESULTS

Results of the patch tests are shown in Table I. Fourteen of twenty patients with AD showed positive reactions to DP-WCE and thirteen were positive to DF-WCE. In the mite RAST-positive AD group (a RAST score of 2 or more was regarded as positive), the positive reaction rate of patch test was higher than in the mite RAST negative patient group. No positive reactions were observed at the control sites of the patients or the healthy volunteers. The positive reaction sites showed edematous erythema with papules and vesicles. Histologically, acanthosis, spongiosis and perivascular lymphocytic infiltration was observed. These reactions resembled AD lesions clinically and histologically. By using immuno-double labelling technique, it was demonstrated that mite antigens were present in the epidermis and the dermis, mostly located to the OKT 6 positive cells (Langerhans cell, LC). And it was observed that mite antigens were distributed around a cluster of Leu 3a positive cells in the dermis of a positive test site (Fig. 2).

The immunoelectron microscopic study revealed that some macrophages in the dermis exhibited positive labelling with antimite antibody on the cytoplasmic membrane as well as positively labelled small

Table I. *Results of patch tests with mite antigen in patients with atopic dermatitis (n=20)*

	Total no. of patients	Patch test with DP-WCE	
		Positive	Negative
RAST positive for DP	14	13	1
RAST negative for DP	6	1	5
RAST positive for DF	13	12	1
RAST negative for DF	7	1	6

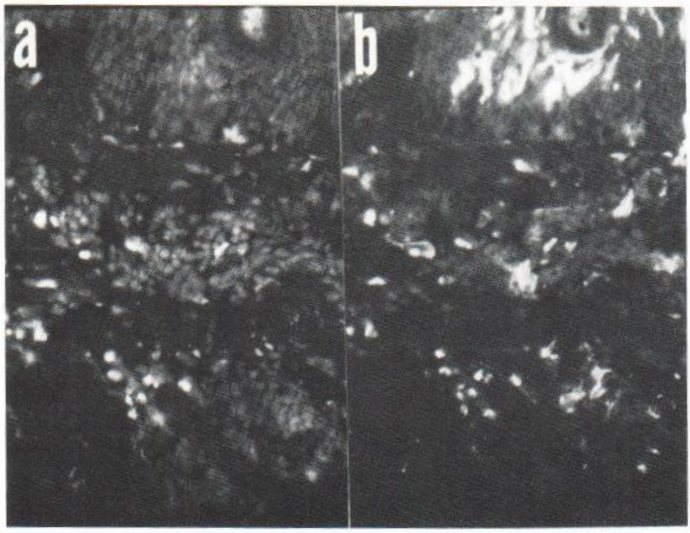

Fig. 2. The micrographs illustrate the immuno-double labelling findings. The biopsy was taken from a positive patch test site. Micrograph *a* reveals the presence of mite antigens by FITC immunofluorescence. Micrograph *b* shows rhodamine immunofluorescence of OKT 6 positive cells. It can be seen that many OKT 6 positive cells carry mite antigens.

phagocytosed particles in the cytoplasm (Fig. 3). These macrophages were often in apposition to lymphocytes.

Mite antigen bearing LCs were seen mainly in the epidermis from 1 to 6 h of the patch testing. After 24–48 h, many mite antigen-bearing LCs were observed in the dermis. Mite antigen bearing LCs were seen in the active lesions in 7 of 12 AD patients.

DISCUSSION

It has been reported by some authors (3–7) that inhalant allergens such as house dust mite could provoke delayed type skin reactions in patients with AD.

In this study, according to the modification of SLS provocative patch test by Kligman (2), an eczematous reaction could be induced by patch testing with mite antigen after 48 h in the majority of patients with AD. Mitchell et al. (4) and Gondo et al. (7) have succeeded in reproducing eczematous lesions in abraded skin of AD patients. Abrasion and SLS application may simulate naturally occurring conditions, scratching and sweating, in AD.

Our immunohistochemical study demonstrated that mite antigen invaded the skin and was trapped by LCs both in positive patch test sites and in the lesions of AD. And it can be hypothesized that LCs may trap mite antigens in the epidermis, migrate to the dermis, appose lymphocytes and present the antigen to these. Silberberg et al. (8) reported that after DNCB challenge in passiely sensitized guinea pigs, LCs in the epidermis decreased after 6 hrs and increased in the dermis, and apposition of mononuclear cells to LCs were seen mainly in the dermis at 3 or more h after challenge. Our results are compatible with theirs.

Recently, Bruynzeel-Koomen et al. (9) reported that IgE molecules were seen on epidermal LCs in AD lesions and this phenomenon seemed to be specific for patients with AD. We confirm this finding by use of the immuno-double labelling method. However, many IgE-carrying LCs were found not only in the epidermis but also in the dermis. Furthermore, IgE-

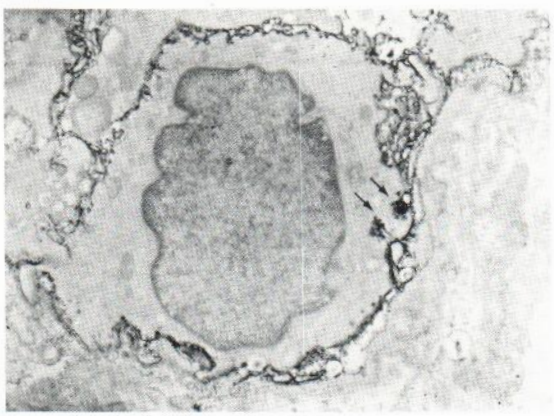

Fig. 3. Immunoelectron microscopic picture. The same biopsy specimen as shown in Fig. 1. A macrophage in the dermis of a patch-tested site carries the granular particles on the cell membrane. Some of the particles, which are mite antigens, are phagocytosed (arrow).

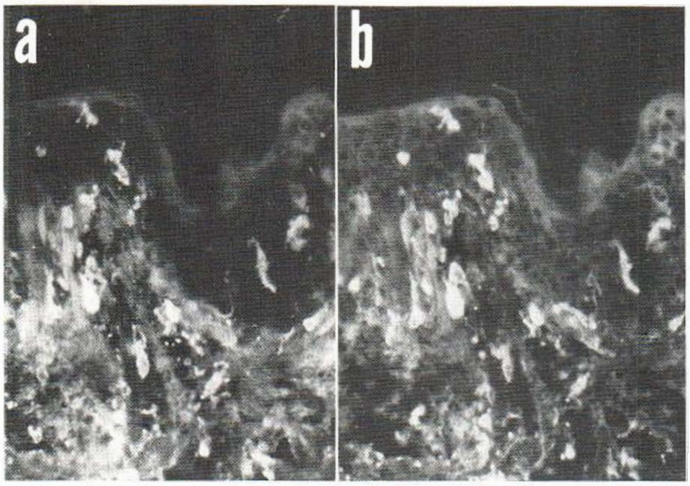

Fig. 4. Both micrographs illustrate the immuno-double labelling finding with IgE molecules and mite antigens. The specimen was taken from a positive reaction site of dust mite patch testing. Micrograph *a* shows FITC-immunofluorescense of IgE molecules. Micrograph *b* reveals rhodamine immunofluorescense of mite antigens. Many dendritic cells bearing both IgE molecules and mite antigens are observed.

carrying LCs were observed in the epidermis and dermis of the lesions of allergic contact dermatitis and other pruritic skin diseases, although less frequent than in AD. Recently, Lever et al. (10) demonstrated dermal IgE-bearing T lymphocytes in the dermis of AD lesions. The same findings were observed in the present study. Ishizaka et al. (11) reported that T lymphocytes bearing Fcε receptors could produce IgE binding factor and modulate the response to IgE. These IgE-carrying cells were also seen in the positive patch test sites and some of these cells were dendritic and carried mite antige (Fig. 4). It is still unclear whether antigen bind to IgE molecules on the surface of LCs. However, it may be hypothesized that IgE-mediated allergic contact sensitivity to mite allergen is playing an important role in the pathogenesis of AD.

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