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Lymphocyte Transformation Test for House Dust Mite in Atopic Dermatitis:

Relationship between Mite Antigens for Type I and Type IV Allergy

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Using a crude extract obtained from *Dermatophagoides farinae*, and its four fractions (I, II, III and IV) partially purified by high-speed gel filtration chromatography, scratch tests and lymphocyte transformation tests (LTTs) were performed on 37 patients with atopic dermatitis. Crude mite antigen provoked positive scratch test reactions in 25 (68%), and positive LTT reactions in 19 (51%) of the 37 patients examined. Mite antigen fractions I, II, III and IV induced a positive LTT reaction in 19, 22, 17 and 7 patients, respectively. Of the 65 positive LTT reactions, 25 accompanied a positive scratch test to the antigen fraction which provoked the positive LTT reaction, but 40 accompanied a negative scratch test. These findings suggest that house dust mite antigen fractions for type IV allergy are different from those for type I allergy in a considerable proportion of patients with atopic dermatitis. *Key words: Dermatophagoides farinae; Antigen fractionation; Cell-mediated immunity; Scratch test.*

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INTRODUCTION

The pathomechanism of atopic dermatitis (AD) is still unknown. The histological and immunohistological features of the disease correspond roughly to those of type IV allergic reaction (1–3), indicating that type IV allergy is implicated in the pathomechanism of AD.

On the other hand, patients with AD often show a positive type I and type IV allergic skin reaction to environmental antigens, such as house dust mite. Consequently some authors assert that both type I and type IV allergy to house dust mite are involved in the etiology of the disorder. Mitchell et al. (4) and others (5–7) reported that patch tests with house dust mites could induce eczematous lesions in patients with AD but only in those who also showed a positive immediate skin reaction to the same allergen. However, house dust mites contain a large number of antigenic substances (8, 9). It is therefore not clear whether house dust mite antigen(s) for type I allergy and those for type IV allergy in patients with AD are identical, or not.

To settle this question, we performed scratch tests and lymphocyte transformation tests (LTTs) with partially purified mite antigen fractions in patients with AD.

MATERIALS AND METHODS

Patiens

A total of 37 patients with AD, 22 males and 15 females, were selected for this study. Their ages ranged from 8 to 48 years (mean, 21 years). The diagnosis of AD was made according to the criteria of Hanifin & Rajka (10). Most patients had been treated with topical corticosteroids. Oral corticosteroids were withheld for at least a month before the present study started.

Ten healthy volunteers served as controls. They had neither a personal history nor a family history of atopic diseases.

The study details were fully discussed with each patient, and informed consent was obtained.

Fractionation of mite antigen extract

One gram of *Dermatophagoides farinae* was suspended in 10 ml of phosphate-buffered saline, and stirred overnight at 4°C. The supernatant solution was separated from the residue by centrifugation at 11,000 *g* for 20 min, and dialysed three times against distilled water, using dialyser tubing with a pore size cut-off of molecular weight (MW) 3,500. The supernatant was concentrated to a volume of about 1 ml with a concentrator. The concentrated supernatant was designated crude mite extract.

High-speed gel filtration chromatography of the crude extract was carried out on a column of porous silica-based aqueous gels (TSK-GEL G2000SW, Tosoh Mfg Co) (11). According to the eluting order, the extract was divided into four fractions (fractions I to IV) (Fig. 1). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis was carried out according to Laemmli's method (12). We used the gels containing 10% acrylamide. Fraction I produced many protein bands of MW 30,000 or more. Fraction II had a major protein band of MW 18,000. Fraction III contained mainly a protein band of MW 29,000. Fraction IV was eluted after the total available volume of the column, and scarcely contained any substances that were stainable with Coomassie Brilliant Blue R, indicating that they consisted of substances which interacted with the silica-based aqueous gels (Fig. 2).

The crude extract and each fraction of solution were sterilized by passing them through a polymer filter (0.22 μm, Millex-GV filter, Millipore). These test solutions were drawn into sterilized plastic syringes and frozen in aliquots at −70°C until used.

The measurement of protein volume was conducted using Lowry's method (13).

Scratch tests with house dust mite antigen fractions

Scratch test solutions containing 0.3–0.5 mg of proteins per ml of physiological saline were prepared from the crude mite antigen extract and each mite antigen fraction from the gel chromatography.

Macroscopically normal skin from flexor forearm of all the patients was used for the skin tests. One drop each of test solution and physiological saline were applied by the scratch technique. Readings were made after 20 min. A wheal reaction 3 mm greater than that elicited by the saline was interpreted as positive.

Lymphocyte transformation tests with the crude mite extract and each fraction

Venous blood (10 ml) was collected aseptically, and mononuclear cells were isolated with the Ficoll-Hypaque method. The cells were resuspended at a concentration of 2×10^6 lymphocytes per ml of a culture

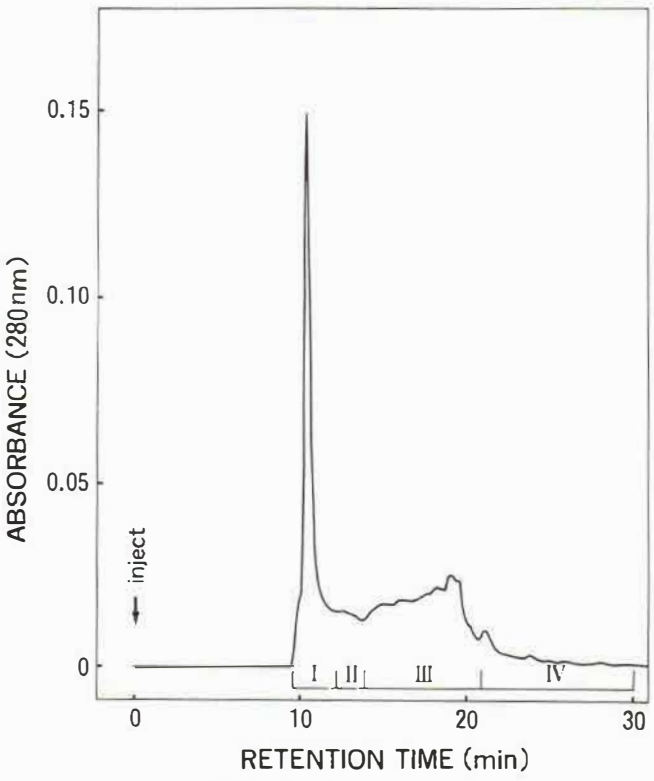

Fig. 1. High-speed gel filtration chromatography of the house dust mite extract. (*I:* Fraction I; *II:* Fraction II; *III:* Fraction III; *IV:* Fraction IV).

medium which was developed at Roswell Park Memorial Institute (RPMI 1640), supplemented with 20% human AB serum, L-glutamine (300 mg/l), and kanamycin (80 mg/l). The solutions were divided into a series of culture tubes (2 ml tube). The crude mite extract or each fraction was added separately to test cultures. Cultures were incubated for 7 days, at 37°C with 5% CO_2. Control cultures were incubated with saline instead of mite antigen. An hour prior to harvesting the cultures, 10 μmol bromodeoxyuridine (BrdU) was added (14).

Preliminarily we performed lymphocyte transformation tests on the crude mite extract and on each fraction. The optimal concentration was found to be around 50 μg/ml for crude extract and around 30 μg/ml for each fraction. In the present study, therefore, we used the crude extract at concentration of 50 μg/ml and each fraction at 30 μg/ml. All cultures were performed in triplicate.

After completing the cultures, the cells were fixed in Farmer's fluid (a mixture of methanol and acetic acid; 3:1) for 30 min. After centrifugation, the cells were again mixed with 0.1 ml of Farmer's fluid, for 1 min. The cells were spread on pre-chilled microscope slides, using the flame dry method.

Immunohistochemical staining

Using monoclonal antibody to BrdU, immunohistochemical staining of the cultured cells was performed (15, 16). Briefly, after denaturing the cellular DNA, the cells were incubated with monoclonal anti-BrdU antibody (Becton Dickinson Co), then with biotin-conjugated horse anti-mouse antibody, and finally with the avidin-biotin-peroxidase complex. Immunoreactants were stained with diaminobenzidine (DAB) and hydrogen peroxide. Hematoxylin was used as the counterstain.

It has been confirmed that the proportion of cells stained by the monoclonal anti-BrdU antibody method is almost identical with the tritiated thymidine labelling index (17–19).

Estimation of lymphocyte transformation tests

A total of 1,000 cells were examined for DAB-positive reaction, using a microscope (×100) and the double-blind method. Data were expressed as follows:

$$\text{Stimulation index (SI)} = \frac{\text{Number of DAB-positive cells in cultures with antigen}}{\text{Number of DAB-positive cells in cultures without antigen}}$$

A preliminary study showed that in non-atopic subjects, the SI value of the crude mite antigen extract was less than 180%. In the present study, therefore, the lymphocyte transformation test was judged to be positive when the SI value exceeded the average SI plus twofold standard deviations in control subjects (20).

RESULTS

Scratch tests with the crude mite extract and each fraction

The results of scratch tests using the crude mite extract and each fraction are shown in Table I. The mite extract provoked positive reactions in 68% (25/37) of patients with AD. Fraction III induced positive reactions in 65% (24/37) of the patients. Thus, the incidence of positive reactions to fraction III was almost the same as that to crude mite extract. Fractions I and II provoked positive responses in 11% (4/37) and 38% (14/37) of the patients, respectively. Fraction IV consistently revealed negative responses in the patients examined.

Lymphocyte transformation tests with the crude mite extract and each fraction

The results of lymphocyte transformation tests (LTTs) to the crude mite extract and each fraction are shown in Fig. 3. The crude extract provoked positive LTT reactions in 51% (19/37) of the patients with AD. The proportions of positive LTT reactions to fractions I, II, III and IV were 51% (19/37), 59% (22/37), 46% (17/37) and 19% (7/37), respectively.

Relationship between lymphocyte transformation tests and scratch tests to the crude mite extract

Of the 19 patients with AD who showed a positive LTT to the crude mite extract, 12 patients had a positive scratch test to the crude extract (Table II). The remaining 7 patients had a negative scratch test reaction to the extract.

Table I. *Scratch tests with the crude house dust mite extract and each fraction in patients with atopic dermatitis*

Mite antigen fraction	Molecular weight	Scratch test		% of positive reaction
		Positive	Negative	
Crude extract		25	12	68%
Fraction I	>30,000	4	33	11%
Fraction II	18,000	14	23	38%
Fraction III	29,000	24	13	65%
Fraction IV	<10,000	0	37	0%

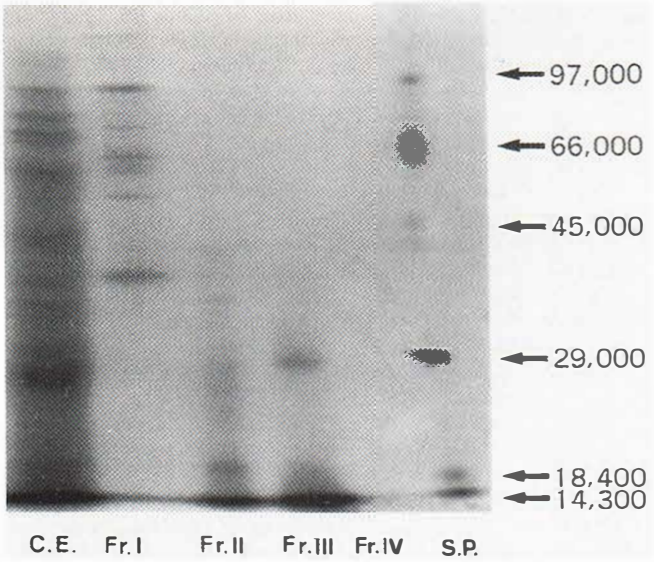

Fig. 2. Analysis of house dust mite proteins by 10% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (*C.E.*: crude extract, *S.P.*: standard proteins with molecular weights indicated).

Relationship between lymphocyte transformation tests and scratch tests to mite antigen fractions

Fractions I, II, III and IV provoked a positive LTT reaction in 19, 22, 17 and 7 patients with AD, respectively. Thus, a total of 65 positive LTTs were obtained, of which 25 accompanied a positive scratch test to the antigen fraction which provoked the positive LTT reaction. However, the remaining 40 positive LTT reactions were accompanied by a negative scratch test to the antigen fraction that induced the positive LTT response (Table III).

Table II. *Scratch tests using crude house dust mite extract in patients with atopic dermatitis who showed a positive lymphocyte transformation test (LTT) reaction to the crude extract*

	No. of patients	Scratch test reaction to the crude extract	
		Positive	Negative
Patients with AD who showed positive LTT reaction to crude mite extract	19	12	7

DISCUSSION

The present results have demonstrated that scratch tests with house dust mite were positive in approximately 70% of patients with atopic dermatitis, and that lymphocyte transformation tests (LTTs) with the mite were positive in about 50% of these patients. These proportions are substantially in agreement with the findings of previous reports (20–22).

When patients with a positive LTT to the crude mite extract were examined, many of them simultaneously showed a positive scratch test to the crude extract, though some showed a negative scratch test. Reitamo et al. (23) also observed a group of patients with AD who showed a positive patch test result

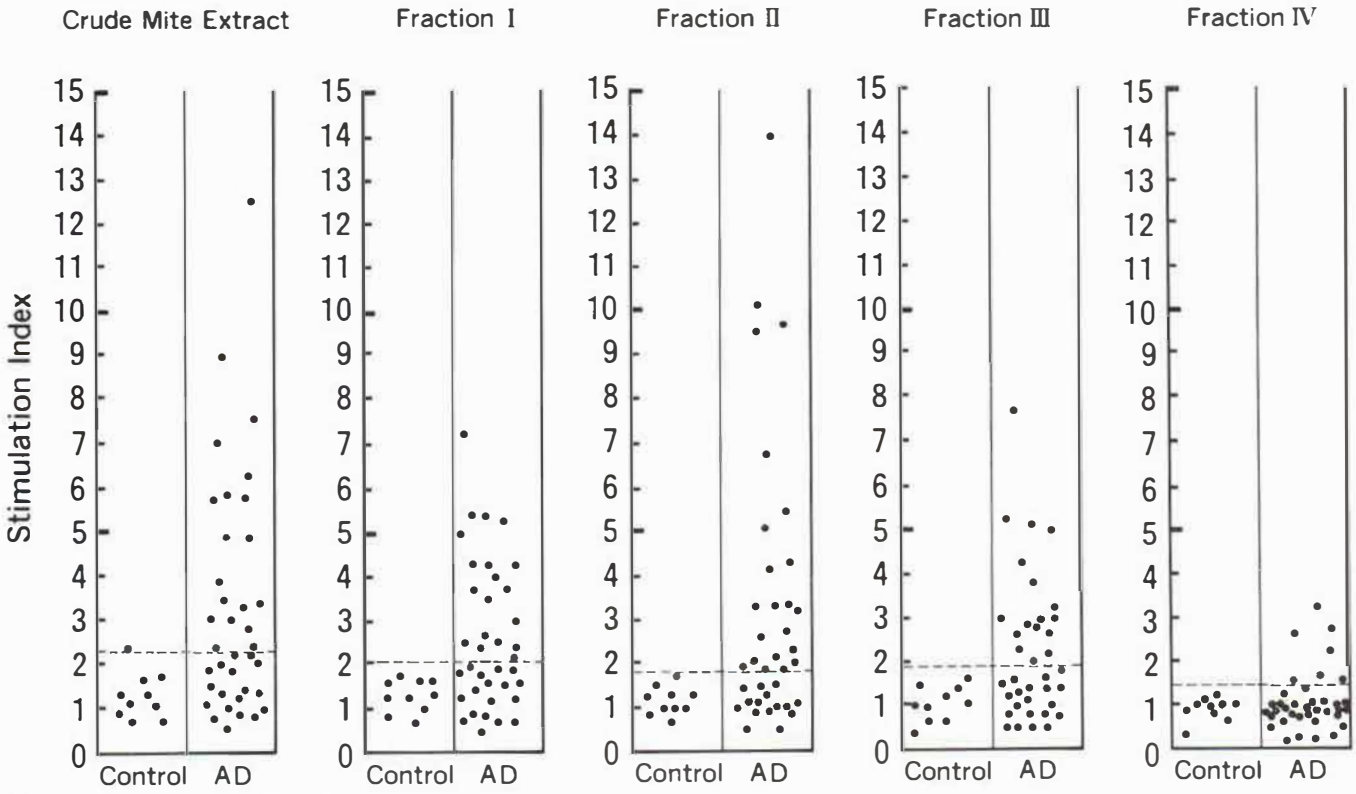

Fig. 3. Stimulation index of lymphocyte transformation tests to the crude mite extract and each fraction. ------, mean + 2 S.D. for 10 control subjects.

Table III. *Scratch tests with mite antigen fractions in patients with atopic dermatitis who showed a positive lymphocyte transformation test (LTT) reaction to the mite fractions*

	No of AD patients with positive LTT reaction	Scratch tests in AD patients with positive LTT reaction	
		Positive	Negative
Fraction I	19	2	17
Fraction II	22	9	13
Fraction III	17	14	3
Fraction IV	7	0	7
Total	65	25	40

and a negative scratch test to house dust mite. Thus, it seems likely that some patients with AD show type IV allergy alone to house dust mite. On the other hand, Bruynzeel-Koomen et al. (6) stated that only patients with an immediate-type skin reaction to house dust mite showed a positive patch test reaction to the mite. However, it should be noted that they examined only a group of patients with AD who showed an immediate-type skin reaction to the mite. It has been confirmed that 30–40% of patients with AD do not show an immediate-type skin reaction to the mite (24).

By dividing house dust mite antigens into four fractions (I, II, III and IV), the present study demonstrated that fractions II (MW 18,000) and III (MW 29,000) contained the main antigens for positive scratch test reactions in patients with AD. These observation are consistent with previous reports that major mite antigens are present in protein fractions of MW 8,000–20,000 and 24,000–29,000 (9, 25, 26).

In contrast, house dust mite antigens for positive LTT response were not restricted to the fraction II and III. Some patients with AD showed a positive LTT to fractions I and IV which usually elicited a negative scratch test reaction. These findings indicate that in patients with AD, a positive reaction is induced by a wider range of house dust mite antigens in the LTT than in the scratch test.

The present study further demonstrated that in a considerable number of patients with AD a specific house dust mite antigen fraction provoked a positive LTT reaction and a negative scratch test. Thus, it is evident that in at least some patients with atopic dermatitis, type I allergic reaction to a house dust mite antigen is not a prerequisite for type IV allergic reaction to the mite antigen.

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