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Purified Der p1 and p2 Patch Tests in Patients with Atopic Dermatitis: Evidence for Both Allergenicity and Proteolytic Irritancy

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Atopic dermatitis has many similarities with allergic contact dermatitis. Previous studies have revealed delayed-type allergic reactions indicating specific cell-mediated immune reactions in subgroups of patients. It has recently been recognized that purified house dust mite major allergens, Der p1 and Der p2 from *Dermatophagoides pteronyssinus*, exhibit a proteolytic enzyme activity similar to papain and maybe serine proteases (e.g. trypsin), respectively. This opens the possibility that house dust mites apart from an allergic epitope could elicit irritant reactions in atopic skin. We examined cutaneous reactivity to the purified proteins of house dust mite antigens, Der p1 and Der p2, in 36 consecutive patients with atopic dermatitis. We also patch-tested with trypsin and papain, in order to see if these proteolytic enzymes could induce irritant reactions. Twelve patients had type I allergy to Der p1 and two of these had type IV reactivity to *D. pteronyssinus* extract. Positive reactions were observed in another four patients, but they had also irritant reactions to papain and trypsin, indicating that the enzymatic activity may have elicited the reactions. The cutaneous reactivity was not linked to total serum IgE, but the patients with specific allergic patch tests had type I reactions to *D. pteronyssinus* extract. Our observations indicate that allergic patch tests towards Der p1 and p2 are rare and that irritant reactions from *D. pteronyssinus* proteolytic activity may be a more common phenomenon when patch-testing atopic dermatitis patients with house dust mite antigen extract. **Key words:** Der p1; Der p2; atopic dermatitis.

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Atopic dermatitis has many features in common with contact dermatitis. Epicutaneous patch-testing in patients with atopic dermatitis has shown reactions to house dust mites (1–6), *Pityrosporum orbiculare* (7, 8), *Staphylococcus aureus* enterotoxins (9, 10), pollens (11, 12), and extracts of human dander (13). These antigens are more or less constantly present and could explain the chronic nature of atopic dermatitis. The interest in contact sensitivity as a major causative factor in atopic dermatitis is supported by the observation of IgE on Langerhans' cells in epidermis and on dendritic cells in the skin of atopic dermatitis patients (1, 14–18).

Sensitization with new antigens such as dinitrochlorobenzene, however, is decreased in patients with atopic dermatitis

(19) and recall reactions can be elicited, although apparently slightly reduced (20). Many irritant reactions have been observed when patch-testing atopic dermatitis patients (21).

Various preparations of house dust mite antigens show quite different reactivity in atopic patients (6). We therefore decided to perform a study on cutaneous reactivity to the purified house dust mite antigens, Der p1 and Der p2, in unselected patients with atopic dermatitis. We included the proteolytic enzymes trypsin and papain in the patch tests, because they may have an enzymatic activity similar to the purified antigens of house dust mite (22), perhaps enabling us to differentiate between specific immune reactions and skin irritancy.

PATIENTS AND METHODS

Patients

A total of 36 patients were included (21 women and 15 men, age range 15–53 years, mean 28 years), all suffering from moderate to severe atopic dermatitis of almost lifelong duration. Eight suffered also from asthma and seven from allergic rhinitis.

Allergological investigations

Prick tests were performed using the standard series from ALK-ABELLÓ (Hørsholm, Denmark). Histamine 10 mg/ml was used as the positive control and the tests were read after 15 min. The European standard epicutaneous patch-test series was from Hermal Chemie (Hamburg, Germany).

The house dust mite patch-test series included the major allergens from the house dust mite *Dermatophagoides pteronyssinus*, Der p1 and Der p2, which were purified as described elsewhere (23). The following concentrations were used: Der p1 (MW 25000) 5 × 10 HEP (4 µM) and 250 × 10 HEP (200 µM), Der p2 (MW 15000) 5 × 10 HEP (0.5 µM) and 50 × 10 HEP (5 µM). HEP means histamine equivalent of prick test and corresponds to the amount of allergen which is necessary for giving a type I reaction equivalent to histamine hydrochloride 10 mg/ml. The concentrations of these particular allergens were between 5 and 250 times higher than is used in the prick test.

The enzymatic activity of Der p1 was tested by radial enzyme diffusion at pH 7.3 into agarose gel containing 1 mg/ml of casein essentially as described (24). Purified Der p1 has a cysteine-protease activity, which is activated *in vitro* after the addition of 10 mM cysteine and 2 mM EDTA. *In vivo*, the enzyme activity is activated in the skin milieu. Der p1 was found to have 33% of the enzymatic activity of papain on a molar basis. Given the evidence that purified Der p1 has amino acid homology with papain, actinidin and cathepsin B and H and that Der p2 has homology with serine proteases such as trypsin and chymotrypsin (22), we also tested the patients with papain (Sigma P4762, MW 23000) 0.25% w/v and with trypsin 0.25% w/v (Sigma T2395, MW 23200), i.e. both enzymes were applied at a concentration of approximately 100 µM. The molar concentrations of enzymes and purified allergen molecules in the solutions applied were thus in the same range.

Table I. Results of prick and epicutaneous tests with house dust mite antigen

Pt.	IgE serum	Mite prick	Der p1 $\times$ 5 patch tests	Der p1 $\times$ 250	Der p2 $\times$ 5	Der p2 $\times$ 50	Trypsin	Papain
A	127	1+	0	0	3+	3+	0	0
B	182	2+	1+	2+	1+	1+	0	0
C	250	0	1+	1+	2+	0	ir	ir
D	292	2+	0	1+	0	0	0	ir
E	905	2+	1+	0	1+	1+	ir	ir
F	2910	2+	0	1+	0	0	ir	ir
G	n.d.	0	0	0	0	0	ir	0
H	n.d.	2+	0	0	0	0	ir	ir

Conclusions: Four patients with type IV reactions to *D. pteronyssinus* allergens. Two patients with type IV reactions to *D. pteronyssinus* allergens, without irritant reactions to trypsin and papain.

All allergens and enzymes were dissolved in Soluprick solution (ALK), sterilized by filtration and kept at 4°C.

The test series were placed on the back of the patients using Finnepore chambers. At the time of testing active eczema was not present on the back, but could be active elsewhere. Topical glucocorticosteroids were not used at the test sites during the preceding 3 days (minimum). Before patch-testing, tape-stripping was done 15 times on the test area. The tests were left for 48 h with ensuing reading at 48 and 72 h. Reactions were graded according to the recommendations from the International Contact Dermatitis Group.

RESULTS

Total serum IgE levels were above the normal range in 77% of the patients. Prick tests were negative in 17/36 (47%), 9/36 (25%) had one positive prick test, whereas 10/36 (29%) had two or more positive tests. Twelve patients (33%) had positive prick test against house dust mite antigen. The patients were asked not to take antihistamines 48 h prior to prick-testing and all the patients reacted positively to histamine hydrochloride 10 mg/ml.

Six patients had positive patch tests towards the purified *D. pteronyssinus* allergens (Table I). Of these six patients, four had a positive prick test against house dust mite antigen. However, four of the patients also had irritant patch-test reactions to papain and/or trypsin, suggesting that enzymatic activity rather than allergenicity elicited the reactions. The clinical appearance of the enzyme-induced patch-test responses was characterized primarily by erythema and infiltration, while the house dust mite-induced patch test reactions had erythema, infiltration and vesicles.

Table II. IL-8 mRNA in epidermal scrapings from one patient exhibiting both atopic eczema and positive patch test towards house dust mite antigen. The patient did not react to papain or trypsin

Location of scraping	IL-8 mRNA
Non-eczematous skin	0
Eczematous skin	30
Der p1 250 $\times$ (2+)	10
Der p1 5 $\times$ (1+)	10
Der p2 50 $\times$ (1+)	10
Papain	0

Results are expressed as a ratio between mRNA for IL-8 and GAPDH.

One patient had a single positive prick test to house dust mite and a 2+ patch test reaction towards Der p1 250 $\times$ (Table I). This patient did not show irritant reactions towards the enzymes. On this patient we performed epidermal scraping for quantitative PCR analysis of IL-8 mRNA expression (25). A strong upregulation of IL-8 was observed in eczematous, non-tested skin and in the patch test areas with a positive clinical reaction (Table II). The reactivity pattern of IL-8 was comparable to what is seen in other allergic patch test reactions.

Five of twenty-five patients tested had a positive type IV reaction towards an allergen from the standard patch test series. There was no correlation with the skin reactivity towards *D. pteronyssinus* allergens (results not shown).

DISCUSSION

In this series of 36 adult patients with moderate to severe atopic dermatitis of lifelong duration, who were not selected on the basis of being type I allergic to house dust mite, we observed that 2 of 36 had what we would call a true allergic type I and IV reactivity towards purified Der p1 and/or Der p2. In one of the patients we observed an IL-8 upregulation similar to what is found for allergic patch tests (25).

We saw positive reactions in another four patients, but observed at the same time an irritant reaction to papain and trypsin. Three of these patients had type I reactivity towards house dust mite. Thus, out of 12 patients with type I allergy to house dust mite, 2 (17%) had definite and 4 (34%) possible type IV skin reactivity. There was no correlation with total serum IgE or with type IV reactivity towards other allergens. None of the patients without type I allergy to Der p had type IV reactions.

Our results support previous observations of type IV allergy towards house dust mite. The incidence of contact allergy to Der p seems, however, to differ quite considerably, depending on the composition and concentration of the materials used for testing. This has recently been demonstrated in 313 patients with atopic dermatitis, where 54% reacted to lyophilized purified allergen (Bayropharm). Using extracts of whole body mites from different companies (Allergopharma-Bracco and Lofarma), 51% and 21% of the patients reacted with a positive reaction (6).

Patients with atopic dermatitis have increased sensitivity to irritants (26), and irritant reactions occur quite frequently when patch-testing atopic dermatitis patients. Lammintausta et al. (21) observed approximately 33% irritant reactions among 851 patch-tested patients and the reactivity did not dis-

appear when the allergen concentration was diluted to 50%. Also, van Voorst Vader et al. (5) observed irritant reactions in 29% of 21 patients with atopic dermatitis tested with house dust mite antigens. We observed that 6/36 patients (17%) (Table I) showed irritant reactions towards papain and trypsin.

Der p1 is a cystein proteinase in which the catalytic site is distinct from its allergenic epitopes (24). Recent studies *in vitro* using bronchial mucosa demonstrated that in 3 h Der p1 0.3 mg/ml doubled the flow of albumin through the mucosa cells (27).

The epidermal barrier in atopic dermatitis is not normal (26), and it is likely that house dust mites may function not only as an allergen but also as an irritant and upregulator of pro-inflammatory cytokines. A recent double-blind study on house dust mite antigen avoidance showed an improvement in clinical disease activity (28). This could include both aspects of allergenicity and irritancy.

Future studies on house dust mite allergy need to use well-defined antigens and also well-defined vehicle and patch-test procedures, perhaps including proteolytic enzymes, to distinguish between allergenicity and irritancy. However, avoidance of house dust mites seems beneficial—irrespective of this dichotomy (28).

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REFERENCES

1. Bruynzeel-Koomen CAFM, van Wichen DF, Toonstra J, Berrens L, Bruynzeel PLB. The presence of IgE molecules on epidermal Langerhans cells in patients with atopic dermatitis. *Arch Dermatol Res* 1986; 278: 199–205.
2. Gondo A, Saeki N, Tokuda Y. Challenge reactions in atopic dermatitis after percutaneous entry of mite antigen. *Br J Dermatol* 1986; 115: 485–493.
3. Norris PG, Schofield O, Camp RDR. A study of the role of house dust mite in atopic dermatitis. *Br J Dermatol* 1988; 118: 435–440.
4. Tanaka M, Aiba S, Matsumura N, Aoyama H, Tabata N, Sekita Y, Tagami H. IgE-mediated hypersensitivity and contact sensitivity to multiple environmental allergens in atopic dermatitis. *Arch Dermatol* 1994; 130: 1393–1401.
5. van Voorst Vader PC, Lier JG, Woest TE, Coenraads PJ, Nater JP. Patch tests with house dust mite antigens in atopic dermatitis patients: methodological problems. *Acta Derm Venereol (Stockh)* 1991; 71: 301–305.
6. Manzini BM, Motolese A, Doninni M, Seidenari S. Contact allergy to *Dermatophagoides* in atopic dermatitis patients and healthy subjects. *Contact Dermatitis* 1995; 33: 243–246.
7. Kieffer M, Bergbrant I-M, Faergemann J, Jemec GBE, Ottevanger V, Skov PS, Svejgaard E. Immune reactions to *pityrosporum* ovale in adult patients with atopic and seborrheic dermatitis. *J Am Acad Dermatol* 1990; 22: 739–742.
8. Rokugo M, Tagami H, Usuba Y, Tomita Y. Contact sensitivity to *Pityrosporum ovale* in patients with atopic dermatitis. *Arch Dermatol* 1990; 126: 627–632.
9. McFadden JP, Noble WC, Camp RDR. Superantigenic exotoxin-secreting potential of staphylococci isolated from atopic eczematous skin. *Br J Dermatol* 1993; 128: 631–632.
10. Strange P, Skov L, Lisby S, Nielsen PL, Baadsgaard O. Staphylococcal enterotoxin B applied on intact normal and intact atopic skin induces dermatitis. *Arch Dermatol* 1996; 132: 27–33.
11. Reitamo S, Visa K, Kähönen K, Käyhkö K, Stubbs S, Salo OP. Eczematous reactions in atopic patients caused by epicutaneous testing with inhalant allergens. *Br J Dermatol* 1986; 114: 303–309.
12. Bruynzeel-Koomen CAFM, Van Wichen DF, Spry CJF, Venge P, Bruynzeel PLB. Active participation of eosinophils in patch test reactions to inhalant allergens in patients with atopic dermatitis. *Br J Dermatol* 1988; 118: 229–238.
13. Sasaki K, Sugiura H, Uehara M. Patch test reactions to the acetone-soluble fraction of human dander in atopic dermatitis. *Dermatology* 1994; 189: 58–61.
14. Bieber T, Dannenberg B, Prinz JC, Rieber EP, Stolz W, Braun-Falco O, Ring J. Occurrence of IgE-bearing epidermal Langerhans cells in atopic eczema: a study of the time course of the lesions and with regard to the IgE serum level. *J Invest Dermatol* 1989; 92: 215–219.
15. Mudde GC, van Reijnsen FC, Boland GJ, de Gast GC, Bruynzeel PLB, Bruynzeel-Koomen CAFM. Allergen presentation by epidermal Langerhans cells from patients with atopic dermatitis is mediated by IgE. *Immunology* 1990; 69: 335–341.
16. Jürgens M, 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, FcεR1. *J Immunol* 1995; 155: 5184–5189.
17. 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* 1996; 106: 446–453.
18. Klubal R, Osterhoff B, Wang B, Kinet J-P, Maurer D, Stingl G. The high-affinity receptor for IgE is the predominant IgE-binding structure in lesional skin of atopic dermatitis patients. *J Invest Dermatol* 1997; 108: 336–342.
19. Rees J, Friedmann PS, Matthews JNS. Contact sensitivity to dinitrochlorobenzene is impaired in atopic subjects. *Arch Dermatol* 1990; 126: 1173–1175.
20. Cronin E, McFadden JP. Patients with atopic eczema do become sensitized to contact allergens. *Contact Dermatitis* 1993; 28: 225–228.
21. Lammintausta K, Kalimo K, Fagerlund V-L. Patch test reactions in atopic patients. *Contact Dermatitis* 1992; 26: 234–240.
22. Stewart GA, Thompson PJ, Simpson RJ. Protease antigens from house dust mite. *Lancet* 1989; ii: 154–155.
23. Lind P. Purification and partial characterization of two major allergens from the house dust mite *Dermatophagoides pteronyssinus*. *J Allergy Clin Immunol* 1985; 76: 753–761.
24. Löwenstein H, Ingild A. A micromethod for determination of proteolytic enzymes in the pH range of 2.8 to 4.8. Radial enzyme diffusion into skim milk containing agarose gel. *Analyt Biochem* 1986; 71: 204–208.
25. Paludan K, Thestrup-Pedersen K. Use of the polymerase chain reaction in quantification of interleukin 8 mRNA in minute epidermal samples. *J Invest Dermatol* 1992; 99: 830–835.
26. Tupker RA, Pinnagoda J, Coenraads PJ, Nater JP. Susceptibility to irritants: role of barrier function, skin dryness and history of atopic dermatitis. *Br J Dermatol* 1990; 123: 199–205.
27. Herbert CA, Stewart GA, Holgate ST, Thompson PJ, Robinson C. The proteolytic effect of mite allergen Der p1 augments bronchial mucosal permeability to albumin. (Abstract)
28. Tan BB, Weald D, Strickland I, Friedmann PS. Double-blind controlled trial of effect of housedust-mite allergen avoidance on atopic dermatitis. *Lancet* 1996; 347: 15–18.