Acta Derm Venereol (Stockh) 1991; 71: 301–305

Patch Tests with House Dust Mite Antigens in Atopic Dermatitis Patients: Methodological Problems

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Patch tests with house dust mite allergens were performed in 21 atopic dermatitis patients with a positive prick test and RAST for house dust mite. Variables in methodology of patch testing, i.e. allergen concentration, application time, and intensity of tape stripping, were studied. Tests were performed with *Dermatophagoides pteronyssinus* solutions containing 20×, 100× and 500× the prick test concentration and purified HDM antigen: 10 and 50 µg/ml P1Ag solution. The series was applied on 8× or 15× tape-stripped and clinically normal skin on the back during 24 and 48 h. Non-specific reactions due to tape stripping, fixation tape or patch test occlusion were frequently observed: after 15× tape-stripping in 3/7 (24-h application) and 6/7 (48-h application) patients, after 8× tape-stripping in 2/19 (24-h application) and 8/19 (48-h application) patients.

Reactions clinically assessed as specific occurred in 6/21 (29%) atopic dermatitis patients, 4/6 occurring in the 10 patients with a serum IgE > 1000 kU/l. High allergen concentrations and 48 h of application increased the number of patients with specific reactions. If 15× tape-stripping had been omitted, 2/3 patients tested in this manner and showing specific test reactions would have been negative.

Further conclusions regarding the value and the preferable method of patch testing with atopic allergens require an in vitro control test. *Key words: Atopic Dermatitis; House dust mite; Patch tests.*

(Accepted December 17, 1990).

Acta Derm Venereol (Stockh) 1991; 71: 301–305.

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Recently several studies have demonstrated positive and supposedly specific reactions to epicutaneous patch tests with house dust mite (HDM) antigens in atopic dermatitis (AD) patients (1–9). In these studies the methods varied markedly. Different allergens were used: *Dermatophagoides pteronyssinus* (1, 2, 5, 9), *Dermatophagoides farinae* (3–6) and P1-antigen (7, 8). Different allergen concentrations were used: 1× (9), 100× (1), 500× (2) and 1000× (3) the prick test concentration. Application times ranged from 20 min to 8 days. The skin was pretreated by tape-stripping (1), abrasion with a scalpel (7, 8) or scratching (4). In other studies, normal skin was tested (2, 3, 5, 6, 9).

The results varied according to the characteristics of the population studied. Most AD patients tested had a positive RAST and prick test for HDM. Some patients had concomitant asthma or rhinitis (2, 6, 7). The proportion of AD patients with a positive HDM prick test showing a positive HDM patch test varied between 22% and 100%. A positive patch test was also found in a few AD patients with a negative prick test (2, 3, 5) and in some patients with atopic respiratory disease only (8). None of the healthy controls proved positive (1, 2, 5, 8).

The purpose of this study was to evaluate the methodology of patch testing with HDM in AD patients, focusing on the following variables: allergen concentration, application time, and pretreatment of the skin by tape-stripping. Furthermore attention was given to the serum IgE level and the presence of atopic respiratory disease (ARD).

MATERIAL AND METHODS

Patients

Twenty-one patients with AD (median age 24 yrs, range 18–58 yrs; 10 men, 11 women) and positive (≥ 2+) prick tests with HDM solutions from both laboratories mentioned further on and a positive RAST for anti-HDM IgE entered the study, 11 patients with a serum IgE level < 1000 kU/l and 10 patients with a serum IgE level > 1000 kU/l. The diagnosis of AD was established according to Hanifin's criteria (10).

Antigens

Patch tests were performed with: [1] *Dermatophagoides pteronyssinus* solutions containing 20× (600 BU/ml = 12.000 SQ-U/ml), 100× (3000 BU/ml = 60.000 SQ-U/ml) and 500× (15.000 BU/ml = 300.000 SQ-U/ml) the standard prick test concentration (30 BU/ml = 600 SQ-U/ml) pro-

Table I. *Patch tests with 24 h or 48 h application of house dust mite allergens on 8× (n = 19) or 15× (n = 7) tape-stripped skin of atopic dermatitis patients: reactions clinically assessed as non-specific due to tape-stripping, fixation tape, or occlusion.*

	Tape-stripping	Fixation tape	Occlusion	Total
Tape-stripping: 8×				
24 h application	2/19	0/19	1/19	2/19
48 h application	4/19	2/19	4/19	8/19
Tape-stripping: 15×				
24 h application	3/7	0/7	0/7	3/7
48 h application	5/7	4/7	0/7	6/7

vided by Diephuis Laboratorium (DL), Groningen, The Netherlands, a subsidiary of Allergologisk Laboratorium, Horsholm, Denmark; [2] *Dermatophagoides pteronyssinus* solutions containing 20× (2000 AU/ml), 100× (10000 AU/ml) and 500× (50000 AU/ml) the standard prick test concentration (100 AU/ml) provided by HAL Allergen Laboratorium (HAL), Haarlem, The Netherlands; [3] 10 and 50 μg/ml P1-Aq, a purified HDM antigen prepared according to the method of Chapman et al. (Allergology Laboratory, Department of Medicine, University Hospital, Groningen) (8), in DL solution fluid. Control patch tests with the solution fluids of DL and HAL were added. The DL solution fluid consisted of phosphate-buffered saline solution (pH = 7.4) with human serum albumin 0.03% g/v as stabilizer and phenol 0.5% g/v as preservative. The HAL solution fluid consisted of a buffer salt solution (pH 6.8–6.9) containing 6-aminohexa-acetic acid 1.31% g/v, dipotassium hydrogenphosphate 0.4% g/v, potassium dihydrogenphosphate 0.34% g/v, potassium chloride 0.05% g/v and phenol 0.05% g/v.

Patch tests

We used silver patches consisting of a square Whatman filtration paper 1 × 1 cm attached to the polyethylene-coated side of a round piece of aluminium foil (van der Bend BV, Hellevoetsluis, the Netherlands) and fixation with hypoallergenic tape. Of all solutions mentioned above, 0.03 ml was applied on the filtration paper. One blank patch was added, making a series of 11 patches. The series as applied on tape-stripped and clinically normal skin on the back for 24 and 48 h. The series were read at 24, 48, 72 and 92 h. Tape-stripping, i.e. removal of the upper corneal layer, was performed with Transelasta adhesive plastic tape always using new strips of tape for each stripping application followed by immediate removal. Patients were tape-stripped 15× (n = 7; median age 38 yrs; range 24–58 yrs) or 8× (n = 19; median age 24 yrs; range 18–58 yrs). Of the 15× tape-stripped patients, 5/7 were also tested after 8× tape-stripping.

Reading of patch tests

The non-specific reactions were classified as tape-stripping, fixation tape or occlusion reactions. Tape-stripping reactions were characterized by an erythematous band at the site of tape-stripping including control patch tests. Fixation tape reactions were erythematous reactions at the site of the fixation tape. Occlusion reactions were characterized by erythematous, often slightly elevated lesions at the site of the polyethylene-coated aluminium foil excluding the site of the filtration paper. Toxic reactions at the border of the filtration paper, papulopustular and follicular reactions were also classified as non-specific. Reactions clinically assessed as specific were characterized by a reaction at the site of the filtration paper often extending beyond that site and absence of reactions at the control patch tests. Scoring was performed according to the standard reading procedure: − = no reaction, + = erythema, ++ = erythema and induration/papules, +++ = vesicles/exsudation.

RESULTS

Non-specific reactions were found in a majority of patients pretreated with 15× tape-stripping (Table I). In 5/7 15× tape-stripped patients in whom the tests were repeated on 8× tape-stripped skin, the number of patients with tape-stripping reactions decreased from 4/5 to 1/5. Prolonged application (48 h versus 24 h) resulted in an increase of non-specific reactions. The abundance of non-specific reactions hampered the detection of potentially specific reactions.

Patch test reactions clinically assessed as specific were found in 6/21 (29%) AD patients (Table II), more so in patients with serum IgE ≥ 1000 kU/l (4/10) than in patients with serum IgE < 1000 kU/l (2/11). All 6 AD patients with reactions clinically assessed as specific had a history of concomitant ARD. The median serum IgE in patients with a positive history of ARD was elevated (n = 12; 2800 kU/l) compared with the median serum IgE in the group without ARD (n = 7; 375 kU/l). In 3/6 patients (nos. 1–3) with patch test reactions clinically assessed as specific we were confident that the clinical assessment was correct. The specificity of these tests was supported by the fact that in 2 subjects (nos. 1, 3) the specific reactions were reproduced.

In 2/3 patients (nos. 1, 2) with multiple specific patch test reactions these were seen only on 15× tape-stripped skin. The proportion of patients with supposedly specific reactions was greater when tested on 15× tape-stripped skin (3/7 = 43%) compared with the results on 8× tape-stripped (5/19 = 26%) or normal skin (5/21 = 23%). Patients 1* and

Table II. *Patch tests with house dust mite allergens: reactions clinically assessed as specific in 6/21 AD patients tested.*

IgE pat.	Tape		DL solution**			HAL solution**			P1Ag (μg/ml)		Serum IgE (kU/l)
	Appl. time	Strip. freq.	20×	100×	500×	20×	100×	500×	10	50	
IgE < 1000 kU/l											
1	48h	15×	−	−	++	nd	nd	nd	++	++	177
1	24h	15×	−	−	−	nd	nd	nd	−	−	
1	24/48h	0×	−	−	−	nd	nd	nd	−	−	
1*	48h	8×	+++	++	+++	−	−	+++	+++	+++	
1*	48h	0×	−	−	−	−	−	+++	−	+++	
1*	24h	0×, 8×	−	−	−	−	−	−	−	−	
2	48h	15×	++	++	++	nd	nd	nd	++	++	343
2	24h	15×	−	++	++	nd	nd	nd	++	++	
2	24/48h	0×	−	−	−	nd	nd	nd	−	−	
IgE > 1000 kU/l											
3	48h	15×	?	?	?	nd	nd	nd	?	?	9692
3	24h	15×	++	++	++	nd	nd	nd	++	+++	
3	48h	0×	++	++	++	nd	nd	nd	++	++	
3	24h	0×	−	−	−	nd	nd	nd	−	−	
3*	48h	8×	+++	+++	+++	+++	+++	++	−	++	
3*	24h	8×	+	+	++	++	++	++	−	++	
3*	48h	0×	−	−	−	+++	++	++	−	−	
3*	24h	0×	−	−	++	−	−	++	−	−	
4	48h	8×	−	−	−	−	−	−	−	−	1485
4	24h	8×	−	−	−	−	−	++	−	−	
4	48h	0×	−	−	−	−	−	++	−	−	
4	24h	0×	−	−	−	−	−	++	−	−	
5	48h	8×	−	−	−	−	−	−	−	++	3586
5	24h	8×	−	−	−	−	−	−	−	−	
5	24/48h	0×	−	−	−	−	−	−	−	−	
6	48h	8×	++	−	−	−	−	−	−	−	3710
6	48h	0×	−	−	++	−	−	−	−	−	
6	24h	0×, 8×	−	−	−	−	−	−	−	−	

nd = not done.
? = fixation tape and tape stripping reactions hampered reading.
* The patch tests were repeated in patients 1 and 3.
** Diephuis laboratory (DL) and Haarlems Allergen Laboratory (HAL) test solutions containing 20×, 100× and 500× the standard prick test concentration.

3* showed more supposedly specific reactions on 8× tape-stripped skin than on normal skin.

Application for 48 h compared with 24 h resulted in 4 more patients with supposedly specific reactions (nos. 1, 1*, 5, 6). After 48 h (but not 24 h) application, specific reactions were shown by 3 patients (nos. 1*, 3, 6) on normal skin, one patient (no. 1) on 15× and one patient (no. 5) on 8× tape-stripped skin.

Higher antigen concentrations appeared to result in more patients with specific reactions. Table II shows that patch test reactions with one of the three solutions would have been absent in 5 patients (nos 1, 1*, 3*, 4, 5) if the highest concentration had not been used, especially so in patients with few reactions (nos 1, 4, 5). The DL, HAL and P1Ag solutions did not appear to be superior to each other in eliciting HDM-specific patch test reactions.

DISCUSSION

Evidence is accumulating that epicutaneous patch tests with atopic allergens in AD patients can elicit

reactions due to a specific cellular response. IgE-bearing Langerhans' cells (5, 11–13), which can also be HDM-Ag positive (5), allergen specific T-lymphocytes in both peripheral blood and skin (13–18), eosinophilic granulocytes (1, 4, 8) and possibly mast cells (13, 19) appear to participate in this reaction. In functional studies, the T-lymphocytes in the peripheral blood of atopic patients have been found to produce an increased amount of interleukin-4 (IL-4) and a decreased amount of gamma-interferon when stimulated with phytohaemagglutinin or HDM-Ag, compared with controls (17, 20). Furthermore, hyperresponsiveness to IL-4 of T-lymphocytes of AD patients and an increased T-lymphocyte proliferative response to IgE-positive epidermal Langerhans' cells of AD patients has been observed, compared with normal non-atopic controls and AD patients with IgE-negative epidermal Langerhans' cells (13, 21). The magnitude of the HDM-specific T-lymphocyte response may correlate with the HDM-specific humoral IgE response (14, 15). A reliable and practical in vitro parameter using this abnormal cell-mediated atopic allergen specific response is not yet available.

The histological data as regards the presence of eosinophilic granulocytes in positive patch test reactions are not consistent. Some authors mention their presence (1, 4, 8), others do not (3, 7). Studies correlating the presence of HDM-specific T-lymphocytes in the skin with the presence of eosinophilic granulocytes have not been reported yet.

Our data suggest that patch test reactions assessed as specific were more frequently found in patients with a serum IgE level > 1000 kU/l. Most patients with specific patch test reactions reported in the literature had elevated serum IgE levels (1, 4–8). A serum IgE level > 1000 kU/l correlated with a positive history of ARD in our studies, as in other studies, but an elevated serum IgE level is also correlated with the severity of AD (22). The presence of positive skin prick and RAST tests to HDM may correlate more to a personal and family history of ARD than to the severity of the AD (23).

One would expect a positive correlation between the severity of AD and the probability of developing non-specific and possibly also specific patch test reactions. Assessing this severity, both in the subjective history and by clinical examination at the moment of patch-testing, non-specific reactions (but not specific reactions) were found more frequently in patients from our study with a maximum percentage of skin involved with erythematous dermatitis above the geometric mean. This difference was not statistically significant (data not shown). Supposedly specific reactions were found in 4 patients (nos. 1, 3, 4, 6) with (very) severe AD and 2 patients (2, 5) with mild AD.

Regarding the optimal method of obtaining specific patch test reactions with HDM, our data favour: 1) the use of the highest allergen concentrations, i.e. 500× the standard concentration used for intracutaneous testing and/or 50 μg/ml P1Ag; 2) 48 h application time; 3) rigorous tape-stripping (15×). These conclusions remain valid when only the 2 patients in whom the positive patch tests were reproducible are evaluated. We did not find data justifying preferential use of any one of the three test solutions. Our data cannot definitely exclude that patch testing on 8× tape-stripped skin yields more patients with reactions assessed as specific, compared with patch testing on normal skin. Using 48 h application and 15× tape-stripping, one can expect non-specific reactions, as were present in abundance in our study. Moreover, in patients with a large proportion of erythematous skin at the moment of testing, one can expect even more non-specific reactions hampering the clinical assessment of the specific reactions. Regarding the reliability of patch tests clinically assessed as specific and the need for stripping, no definite conclusions can be drawn because of the lack of an in vitro control test.

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