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Delayed Type Hypersensitivity in Atopic Dermatitis

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Three different aspects of delayed type hypersensitivity in atopic dermatitis (A.D.) were studied. (*a*) Intradermal testing demonstrated that positive reactions to bacterial vaccines were distinctly lower in patients with A.D. (*b*) Patch testing in patients with A.D. compared to tests in patients with anal eczema showed a striking difference in results concerning the substances to which positive reactions were found. (*c*) Patients with A.D. ($n=18$), atopics without A.D. ($n=10$), patients with contact dermatitis ($n=10$), and normal controls ($n=10$), were patch tested with various human dander (H.D.) fractions after stripping the stratum corneum with skin tape. Only patients with A.D. showed positive reactions, the maximum response being at 24–48 hours. There was no difference in reaction pattern between partially purified H.D. subfractions. Histopathological examination revealed an eczema-reaction. Patch testing within the same patient groups with purified house dust mite allergen P_1 demonstrated similar results. Although this finding argues against a specific role of H.D. allergen in A.D. it may at least be concluded that H.D. preparations have the capacity to provoke immediate as well as delayed type skin reactions. *Key words: Human dander allergen; Atopic dermatitis; Delayed type reaction.*

MATERIALS AND METHODS

Patients: The following groups were investigated: A.D. (aged 18–42 years; $n=18$), atopics (asthma, rhinitis) without A.D. ($n=10$), patients with contact dermatitis ($n=10$) and normal controls ($n=10$). The contact dermatitis group and the normal controls had no history of atopy. All subjects gave their informed consent.

Allergens. H.D. allergen was prepared from acetone-washed human scalp scales and purified, and five subfractions were obtained and coded A through F, as earlier described (11). Purified house dust mite allergen P_1 was prepared by the method of Chapman et al. (12). The purified H.D. fraction E and mite fraction P_1 were coupled to cyanogen bromide activated cellulose discs according to Ceska et al. (13) for specific IgE determination. Radiolabelled anti-(Dε 2) IgE antibody was purchased from Pharmacia Diagnostics. Total IgE was determined by the Radio Immunosorbent Test (Pharmacia reagents).

Skin tests. All subjects were tested with H.D. fraction A at 20 μg/ml (0.05 ml intradermally). Skin reactions were read after 20 min and after 48 hours. All patients and controls were patch tested with aqueous H.D. fraction A (2 mg/ml) and control saline after stripping (15 times) of the stratum corneum with skin tape. In a separate series of experiments 10 out of 18 patients with A.D. and the 10 normal controls were patch tested with the following subfractions of H.D.: fraction C (2 mg/ml), D, E and F (1 mg/ml), and with the purified house dust mite allergen P_1 (1 mg/ml).

The patch tests were evaluated after 20 min, 24 h, 48 h and 72 h. The reactions were quantified as: + (erythema) and ++ (papular erythema).

Histopathology. Punch biopsies were taken from positive patch test sites after 24 h, 48 h and 72 h. Biopsy specimens were prepared for routine histological examination (formol-sublimate fixed, paraffin embedded and hematotoxylin-eosin stained).

RESULTS

Three different aspects of delayed type hypersensitivity in atopic dermatitis were investigated.

1. *Delayed type reactions to bacterial vaccines* were determined in two groups of patients: (*a*) patients with asthma and/or rhinitis, (*b*) patients with asthma and/or rhinitis and atopic dermatitis. The results are presented in Table I. From this table it is apparent that in the group of patients with asthma and/or rhinitis and atopic dermatitis the positive reactions are distinctly fewer than in the group of patients with asthma and/or rhinitis only. A similar conclusion was earlier made by Elliott et al. and Rajka (1, 2, 3).

2. *Contact allergy in atopic dermatitis*. Reports in the literature differ as to whether there is an increased incidence of allergic contact dermatitis in patients with atopic dermatitis.

No definite conclusions can be drawn from these conflicting results, which may partly be due to differences in the selection of patients for patch-testing and in the composition of the patch-test series. Therefore we decided to compare the results of patch-tests with a standard series in two selected groups of patients: (*a*) patients with atopic dermatitis and (*b*) patients with anal eczema. The results are presented in Table II. This table shows that there seems to be no great difference between the results of the patch-tests in these two groups of patients. If, however, not the total numbers of positive reactions are compared, but the substances to which positive reactions were found, great differences are found between the two groups of patients. This can be seen in Table III. There is a striking difference in results of patch-tests in these two groups of patients concerning the substances to which positive reactions were found.

3. *Reactions to human dander allergen*. All patients with A.D. and, with the exception of two, all atopics without A.D. showed positive reactions 20 min after intradermal challenge with unfractionated dialysed H.D.-extract, fraction A. The patients with contact dermatitis and the normal controls did not react. The reactions after 48 h were all negative. Only the A.D. group had a positive patch test reaction to the H.D. fraction A. Macroscopic reactions were seen in 17 out of 18 patients 48 h after testing and varied from erythema to confluent papular erythema. Patch tests with the same extract in atopics without A.D.

Table I. *Reactions to bacterial vaccines*

Results of tests with bacterial vaccines in 70 patients

Number of patients	Diagnosis	Reactions after 24 h (number of positive reactions)		
		Staph. aureus	Haem. infl.	Pneumococ.
35	Asthma/rhinitis	54	81	51
35	Asthma/rhinitis, atopic dermatitis	27	55	38

Table II. *Patch-tests with ICDRG standard list*

Patch-test results in 99 patients with anal eczema and atopic dermatitis respectively

Diagnosis	Total number of pat.	Number of positive reactions
Anal eczema	49 pat.	24 posit. reactions in 19 pat.
Atopic dermatitis	50 pat.	31 posit. reactions in 19 pat.

and in normal controls were all negative. Saline elicited no reaction in any group. Patch testing with the partially purified H.D. fractions C, D, E and F produced identical results as observed with fraction A, especially after 24–48 hours. A total number of 50 tests with the H.D. fractions A-E were performed in 10 patients with A.D., resulting in 42 positive reactions after 48 hours (Table IV).

These patients were also patch tested with purified D. pteronyssinus allergen P_1. After 24–48 hours a positive reaction was seen in every patient, almost similar in rate and

Table III. *Patch-tests with ICDRG standard list*

Patch-test results in 99 patients with anal eczema and atopic dermatitis respectively

Substances	Number of positive reactions	
	Anal eczema	Atopic dermatitis
Fragrance mix, wood tar, balsam of Peru, colofonium	12	3
Metal salts	1	21
Others	11	7
Total	24	31

Table IV. *Patch test results in patients with atopic dermatitis after challenge with human dander allergen (A–F) and house dust mite allergen (P1)*

Allergen fraction	Number of patients	Patch test result	Reading time			
			20 min	24 h	48 h	72 h
Human dander		++	3	7	5	2
A	$n=10$	+	5	2	5	6
		−	2	1	0	2
		++	5	8	5	
C	$n=10$	+	3	0	2	
		−	2	2	3	
		++	0	6	2	
D	$n=10$	+	4	2	6	
		−	6	2	2	
		++	1	6	2	
E	$n=10$	+	2	2	7	
		−	7	2	1	
		++	2	6	6	
F	$n=10$	+	4	2	2	
		−	4	2	2	
Number of patch tests	50	Total number of positive patch tests	29	41	42	
House dust mite						
		++	7	9	7	
P1	$n=10$	+	3	1	3	
		−	0	0	0	

appearance to the reaction pattern with the HD. allergens. The normal controls did not respond, neither to the H.D. fractions, nor to the P_1 allergen.

Histopathological examination of positive patch tests induced by H.D. showed mild spongiosis and a moderate, mainly perivascular lymphohistiocytic infiltrate, consistent with eczema. There was no correlation between the total IgE level, the specific IgE level against H.D. and the results of the patch tests with H.D. fraction A.

DISCUSSION

Delayed type reactions to human dander allergen which normally cause immediate allergic reactions, do seem to occur almost exclusively in patients with atopic dermatitis (A.D.). In 1982 Mitchell et al. (4) described delayed type reactions after patch testing with extracts of house dust mite, grass pollen and animal dander in a group of A.D. patients. On this basis, they proposed an important etiological role for these allergens in the pathogenesis of A.D. However, since these are inhalant allergens, the question arises if the quantity of these allergens which contacts the skin in vivo is sufficient to cause any effect in A.D. In this respect the human dander (H.D.) allergen seems a more natural allergen candidate in A.D. Storm van Leeuwen (5) was the first to describe a high incidence of positive immediate type reactions to H.D. allergen in atopic individuals. This was confirmed in A.D. by Keller (6), while Kopecka et al. (7) even suggested A.D. to be the result of an auto-allergic reaction to H.D.

Since the immediate type allergic reaction to the H.D. allergen does not concur with the clinical and histopathological features of A.D., there is so far no general consensus on the role of H.D.-allergens in the pathogenesis of A.D. Yet, Simon (8), Nexmand (9) and Uehara & Ofuji (10) have also described delayed type allergic reactions in A.D. patients after patch testing with H.D. allergen. The clinical and histopathological picture of these reactions was consistent with that of A.D.

The results of our experiments confirm earlier observations by Simon (8). Nexmand (9) and Uehara & Ofuji (10). Patch tests with H.D. allergen in A.D. patients produced reactions after 24–48 h; the other groups were negative. In particular, the group of patients with rhinitis and/or asthma, but without A.D., and with immediate type allergic reactions to H.D. allergen did not show a delayed type reaction after patch testing with the same allergenic preparation.

There was virtually no difference in the reactivity of the different H.D. subfractions 24–48 h after patch testing. Since very little is known about the biochemical nature of H.D. extracts or the component fractions, it is difficult to draw definite conclusions from these findings at the present stage. Berrens (11) has described a partially purified H.D. fraction E as being the most potent product in the immediate type allergic reaction in atopic patients. The potency of fraction E on a weight basis, proved to be about 100 times higher than that of fraction A. If the component in H.D. extract causing the immediate reaction were identical to the one provoking the delayed reaction, one could expect a difference in the reaction pattern between the A and the E fractions after patch testing. Although dose–response curves were not evaluated in the present study, the lack of different in reaction pattern after testing the H.D. fractions A–E suggests that the chemical structure involved in the delayed type reaction, occurs equally in all fractions. The identical reaction pattern to the P_1 allergen might implicate a cross-reactivity with the H.D. delayed type allergen. At the present time, the type and kinetics of the reaction pattern shown here do not allow a definite conclusion with respect to the role of atopic allergens in the pathogenesis of A.D. The clinical and histopathological observations so far certainly do not rule out an underlying unspecific hypersensitivity.

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