

PATCH TESTING, TUBERCULIN TESTING AND SENSITIZATION WITH DINITROCHLOROBENZENE AND NITROSODIMETHYLANILINI OF PATIENTS WITH ATOPIC DERMATITIS

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Abstract. Cell-mediated immunity was studied in patients with atopic dermatitis. 113 patients were patch tested with ten contact allergens. The frequency of positive reactions to patch testing with "common contact allergens" was found to be lower in patients with "high IgE values" than in those with $\text{IgE} \leq 1000$ U/ml. A larger number of patients with severe dermatitis reacted negatively to PPD and were more difficult to sensitize with DNCB and NDMA as compared with the patients with mild dermatitis. The results of this investigation support the findings of earlier workers that patients with atopic dermatitis show disturbances in the cell-mediated immune system and these disturbances appear to be correlated to the degree of severity of the dermatitis.

Key words: Dermatitis, atopic; Cell-mediated immunity; IgE; Tuberculin test; Patch tests

The immunological mechanisms involved in atopic dermatitis (AD) are still obscure. Signs of hyporeactivity within the cell-mediated immune system have been observed in several studies of this condition. A number of workers (5, 11, 12) have reported that the frequency of positive reactions to tuberculin is low in cases of AD. Hyporeactivity has also been observed in lymphocyte transformation tests with PPD and herpes simplex antigen (6). Luckasen et al. (9) and Schöpf (13) have found reduced T-lymphocyte counts in patients with AD. In sensitization trials with Rhus antigen, Jones et al. (7) found the number of sensitized AD patients to be smaller than in healthy controls.

Opinions are still divided as to whether the frequency of contact allergy in patients with AD is normal (2, 4, 16), high (3) or low (7, 10, 14). The varying results of these investigations may be explained in different ways.

1) In the majority of trials with patch testing the authors used patients with different kinds of skin diseases as controls. The results therefore may be biased.

2) The frequency of contact allergy to substances used for topical application may be high in patients with AD as they often treat their dermatitis with these.

3) When patch testing patients with active dermatitis the frequency of irritant reactions can be high and they may be misinterpreted as allergic.

The present investigation was carried out to determine whether there was any difference between patients with extensive AD and patients in whom the condition involved only a small area of the body surface with respect to cell-mediated immunity and whether patients with IgE values >1000 U/ml differ from those with lower IgE values in this respect. The results of patch testing, tuberculin testing and sensitization trial with 2,4 dinitrochlorobenzene and *p*-nitrosodimethylanilini were used as an indication of the patients' delayed hypersensitivity.

MATERIAL AND METHODS

Patients

113 patients (51 men and 62 women) participated in the patch tests: their ages ranged from 15 to 62 years. They were divided into two groups according to the extent of the skin involvement: (1) "Severe dermatitis" = active dermatitis involving more than 1/3 of the body surface and (2) "Mild dermatitis" = active dermatitis involving less than 1/3 of the surface of the body. The mean ages of the patients in these two groups did not differ by more than 2 years.

The IgE serum levels were determined according to RIST (Commercial Method, Pharmacia, Sweden). In this investigation, IgE levels of more than 1000 U/ml are called "high IgE levels".

Table I. Correlation between the degree of severity of atopic dermatitis and serum IgE levels

	No. of patients tested	"Severe dermatitis"	
		No. of patients	%
IgE > 1 000 U/ml	39	23	59 ^a
IgE ≤ 1 000 U/ml	71	10	14 ^a

^a $p < 0.001$

None of the patients had been treated with corticosteroid tablets within the past 6 months. The tests and sensitization trials were carried out when the most acute symptoms had regressed.

Patch tests

All patients were patch tested. The AL-test (IMECO Astra Agency Co., Stockholm, Sweden) was used as the patch test unit and the allergens were applied to the skin of the upper back. The adhesive plaster was leucoplast (Beiersdorf & Co., Hamburg, West Germany) and dermicil (Johnson & Johnson, New Brunswick, N.Y., USA), the latter being used in patients who had a history of irritation of the skin by plaster. The patches were removed after 48 hours and the readings made 24 hours later. A reaction was judged positive only if a reddish, palpable infiltration was present.

Two batteries of substances were used in patch testing. Group I included the following six common contact allergens ("common contact allergens"):

- 1 potassium dichromate, 0.5% in petrolatum,
- 2 nickel sulphate, 5% in petrolatum,
- 3 mercaptobenzothiazole (MBT), 2% in petrolatum,
- 4 tetramethylthiuramdisulfide (TMTD), 2% in petrolatum,
- 5 balsam of Peru, 25% in petrolatum,
- 6 *p*-phenylenediamine (PPD), 1% in petrolatum.

Group II comprised four contact allergens ("allergens for topical application"):

- 1 parabenes (methyl-, ethyl-, propyl-, butyl-, benzyl-) 3% each in petrolatum,
- 2 vioform, 5% in petrolatum,
- 3 neomycin sulphate, 20% in petrolatum, and
- 4 wool alcohols, 30% in petrolatum.

As the allergens in group II are generally used for topical application it was assumed that patients with severe and recurrent AD are often exposed to them.

Tuberculin tests

The tuberculin tests were carried out on 92 patients. Those patients who had been revaccinated with BCG during the past 2 years were excluded. The tests consisted in intracutaneous injection of 0.1 ml PPD-tuberculin 2 T.U. (The State Serum Institute, Copenhagen, Denmark) into the ventral aspect of the forearm. The diameter of the induration was measured after 72 hours. The reaction was judged negative if the diameter of the induration was less than 6 mm.

Sensitization

Agreement to participate was signified by 85 patients. The allergens were reagent grade 1,3-dinitro-4-chlorobenzene (DNCB) and *p*-nitrosodimethylanilini (NDMA) from E. Merck AG, Darmstadt, Germany.

Table II. Results of patch tests in patients with atopic dermatitis

	"Severe dermatitis" 33 (29%)	"Mild dermatitis" 80 (71%)	IgE > 1 000 U/ml 39 (35%)	IgE ≤ 1 000 U/ml 71 (65%)
Group I (common contact allergens)				
Chromate	1	0	0	1
Nickel	1	1	0	2
Balsam of Peru	0	3	0	3
MBT	0	1	0	1
TMTD	0	3	0	3
P-phenylenediamine	1	0	0	1
Total	3 (9%)	8 (10%)	0 (0%) ^a	11 (15%) ^a
Group II (allergens for topical application)				
Parabenesters	0	0	0	0
Lanolin	1	1	1	1
Vioform	0	1	0	1
Neomycin	1	1	1	1
Total	2 (6%)	3 (3%)	2 (5%)	3 (4%)
Total group I + II	5 (15%)	11 (13%)	2 (5%)	14 (19%)

^a $p = 0.0074$ (Fischer's exact test).

Table III. Results of tuberculin skin tests in patients with atopic dermatitis

	No. of patients tested	Negative reactions	
		No. of patients	%
"Severe dermatitis"	32	19	59 ^a
"Mild dermatitis"	60	17	28 ^a
IgE > 1 000 U/ml	37	19	51
IgE ≤ 1 000 U/ml	54	17	32

^a $p < 0.01$.

DNCB (350 γ in acetone) and NDMA (350 γ in acetone) were applied to the skin of the back on circular occlusive patches with a diameter of 10 mm. The patches were removed after 48 hours. After an interval of 3–5 weeks the patients were patch tested with DNCB (50 γ in acetone) and NDMA (50 γ in acetone) according to the same technique as described above.

RESULTS

Table I shows that 59% of the patients with "high IgE values" had "severe dermatitis" as compared with 14% in the group with IgE ≤ 1 000 U/ml. The difference is statistically significant ($p < 0.001$).

The results of patch testing are given in Table II. There was no difference between the patients with "severe dermatitis" and "mild dermatitis" with respect to the frequency of positive reactions. However, the frequency of positive reactions to "common contact allergens" was higher in the patients with IgE values ≤ 1 000 U/ml than in the patients whose IgE values were more than 1 000 U/ml ($p = 0.0074$ Fischer's exact test).

Table III shows the results of the tuberculin tests. It is seen that the frequency of positive reactions was higher in the group "mild dermatitis" than in the group "severe dermatitis", the difference being

statistically significant ($p < 0.01$). The difference between the patients with IgE values of ≤ 1 000 U/ml and those who had IgE values of > 1 000 U/ml was smaller and not statistically significant. Table IV shows the results of the sensitization trials with DNCB and NDMA. It is seen that patients with "severe dermatitis" and patients with "high IgE levels" were more often refractory to sensitization with DNCB and NDMA than patients with "mild dermatitis" ($p < 0.01$) and IgE values ≤ 1 000 U/ml ($p < 0.05$) respectively.

DISCUSSION

This study has shown that the serum IgE values are correlated to the degree of severity of AD. This confirms the findings of earlier workers (1, 15, 17). Thus the IgE serum concentration in some way appears to be related to the development of AD. Recent investigations have shown that the T-cell function is impaired in AD (6, 8) and Luckasen et al. (9) and Schöpf (13) have found the number of T-lymphocytes to be reduced in patients with AD. Schöpf (13) also expressed the view that the number of T-lymphocytes which suppress the formation of IgE is diminished in patients with AD and this may explain the high IgE values in these cases. The impaired function of the T-lymphocytes may also explain the reduced reactivity of patients with AD to bacterial, fungal and viral antigens in skin tests, as has been demonstrated in several studies (11, 12).

The results of the tuberculin tests carried out in this investigation also showed that the frequency of positive reactions was lower in patients with extensive AD as compared with patients with mild AD. A reduced capacity for sensitization to contact allergens probably also reflects an impaired function of the T-cells. According to this investigation this

Table IV. Results of sensitization trials with DNCB and NDMA in patients with atopic dermatitis

	No. of patients tested	Sensitized to both DNCB and NDMA		Sensitized to either DNCB or NDMA		Not sensitized to either DNCB or NDMA	
		No. of patients	%	No. of patients	%	No. of patients	%
"Severe dermatitis"	25	8	32	5	20	12	48 ^a
"Mild dermatitis"	60	25	42	25	42	10	16 ^a
IgE > 1 000 U/ml	31	9	29	9	29	13	42 ^b
IgE ≤ 1 000 U/ml	52	23	44	20	38	9	18 ^b

^a $p < 0.01$ ^b $p < 0.05$.

impairment seems to be correlated to the severity of the disease.

The results of patch testing which have been reported in the literature are conflicting. Some of the possible reasons for this have been given in the introduction to this paper. In this investigation two types of contact allergens were chosen: Six common allergens to which any individual can be exposed and four allergens for topical application with which particularly patients with a severe dermatitis come in contact. In addition we correlated the results of the patch tests to the serum IgE determination and the degree of severity of the dermatitis. This correlation has so far not been investigated. A difference was found between patients with "high IgE values" and those who had IgE values ≤ 1000 U/ml with respect to their capacity for sensitization to "common contact allergens" but there was no difference in this respect between patients with "severe dermatitis" and patients with "mild dermatitis". A possible reason for this may be that patch testing was carried out on irritated skin or before all acute symptoms had regressed in the cases of extensive AD. This may have led to the inclusion of irritant reactions in the group with "severe dermatitis".

There was no tendency to any difference between the groups with respect to the allergens used for topical application. This suggests that repeated and prolonged exposure to these allergens compensates for the impaired capacity for sensitization of patients with high IgE levels and severe atopic dermatitis.

The sensitization trials with DNCB and NDMA showed that patients with "severe dermatitis" were more difficult to sensitize than patients with "mild dermatitis". We also found that the capacity for sensitization with these allergens was lower in the patient group with "high IgE levels" than in the group with $\text{IgE} \leq 1000$ U/ml. Since there was a good correlation between "severe dermatitis" and "high IgE levels" (Table I) it is not surprising that the results in these two groups are similar. It was not possible from this investigation to draw any conclusions as to whether this hyporeactivity is best correlated to IgE levels or to the extent of the dermatitis.

The results of this investigation support the observations made in previous studies that the cell-mediated immune system appears to be disturbed in patients with atopic dermatitis. This disturbance seems to be correlated to the degree of severity of the atopic dermatitis.

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