Acta derm.-venereol. 49: 294–298, 1969

DELAYED SKIN REACTION TO HUMAN DANDER IN ATOPIC DERMATITIS

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Many investigators have noted that atopic dermatitis cannot be directly related to atopic reagins, and if allergic mechanisms need be involved in its pathogenesis, the allergy might be of the delayed type (9).

It is well known that many patients with atopic dermatitis have an immediate wheal type of sensitivity to human dander extract (1, 7, 22, 23). However, there are no reported studies concerning the incidence and characteristics of the delayed intradermal reactions to the extract.

The present study was carried out to determine (i) the incidence and the gross and histological characteristics of the delayed intradermal reactions to human dander extract and (ii) the delayed reactivities to bacterial and fungal antigens and the inflammatory responses to chemical agents, in patients with atopic dermatitis.

Materials and Methods

Selection of patients.—Patients with atopic dermatitis, bronchial asthma and nonatopic dermatoses were examined. In the asthmatic group, the patients who had active atopic dermatitis simultaneously were excluded. The nonatopic dermatoses consisted of contact dermatitis, acne vulgaris, dermatophytosis, psoriasis, vitiligo, and other noneczematous dermatoses.

Preparation of dander extract.—Dander from the scalp of healthy young men was washed in a large volume of acetone for 24 hours. The acetone washings were discarded, and the residue was dried at room temperature. Two grams of the dry powder were mixed in 20 ml of phosphate buffered saline (pH 8.0) and homogenized in a glass homogenizer. After the addition of 180 ml of the buffered saline, the homogenate was shaken at 4°C for 48 hours, and centrifuged at 12,000 rpm for 15 minutes. The supernatant was dialyzed against repeated changes of phosphate buffered saline (pH 7.2) at 4°C for 5 days. The dialyzed extract was filtered through Chamberland L3; thimerosal was added to make a final concentration of 0.01 %. Cultures of the extract on blood agar and in thioglycolate liquid medium were negative. Determination of total nitrogen of the extract by micro-Kjeldahl method gave 9 mg/100 ml. The extract was diluted with 15 volumes of sterile saline, stored in sterile vials at 4°C, and used for intracutaneous tests.

Preparation of other Test Substances

a) Staphylococcus albus.—*S. albus* was harvested on agar plates, washed three times with saline, mixed with 50 ml of saline and crushed by a French-presser. The mixture was adjusted to pH 8.0 by the addition of a few drops of 10 % NaOH, shaken at 4°C for 48 hours, and centrifuged at 10,000 rpm for 10 minutes. The supernatant was filtered through Chamberland L3, and thimerosal was added to make a final concentration of 0.01 %. The sterility of the extract was confirmed by cultures on blood agar and in thioglycolate liquid medium. The extract was diluted to contain 1 mg of total protein per ml.

b) Staphylococcus aureus.—An extract

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of *S. aureus* was prepared by the same procedure as in a).

c) Corynebacterium acnes.—*C. acnes* was cultured on agar plates by the ion steel method (Parker), and the extract was prepared by the same procedure as in a).

d) Alternaria.—An extract of alternaria was prepared by the method described in the paper by Fujisawa *et al.* (3).

e) Tuberculin.—Old tuberculin in a dilution of 1 : 2000 was prepared.

f) Croton oil.—0.01 % croton oil in saline containing polysorbate at the concentration of 0.1 mg/ml was prepared.

Skin test. 0.1 ml of each substance was injected intradermally. The test site was usually the upper portion of the back, but when the area was involved by eruptions, the flexor surfaces of the forearms were used. Readings were made 15 minutes and 48 hours after injection. The immediate reactions were graded by the diameter of the wheal and the delayed reactions by the diameter of the maculopapule.

Results

Table 1 shows the results of delayed reactions to human dander extract. The reactions consisted of bright or dark red maculopapules with various degrees of induration. As 49 out of 50 nonatopic controls developed maculopapules with diameters less than 5 mm, the reactions were graded as follows:

— =maculopapules with diameters less than 5 mm,
+ =diameters between 5–10 mm,
++ =diameters between 11–15 mm,
+++ =diameters larger than 15 mm.

The incidence of positive delayed reactions was significantly higher in the patients with atopic dermatitis than in the asthmatic patients or the nonatopic controls (chi-square test, $P<0.001$).

The relationship of delayed reactions to the age of the patients with atopic dermatitis is shown in table 2. Positive delayed reactions were produced in 56 % of the patients aged 1–5 years, 53 % of the patients aged 6–15 years, and 66 % of the patients over 15 years of age.

Table 1. *Delayed reaction to human dander*

	No. of cases	Reaction			
		—	+	++	+++
Atopic dermatitis	100	42	20	19	19
asthma	20	16	4	0	0
nonatopic	50	49	1	0	0

Table 2. *Delayed reaction to human dander and age of patients with atopic dermatitis*

Age of patients (years)	No of cases	Reaction			
		—	+	++	+++
1–5	32	14	10	5	3
6–15	38	18	5	8	7
16–40	30	10	5	6	9

Table 3. *Immediate reaction to human dander*

	No. of cases	Diameter (mm)		
		10—15	16—20	21—
Atopic dermatitis	100	49	20	31
asthma	20	8	8	4
nonatopic	50	40	5	5

Table 4. *Relationship of delayed and immediate reaction in patients with atopic dermatitis*

Wheal (mm)	Delayed reaction			
	—	+	++	+++
10–15	27	10	8	4
16–20	11	5	2	2
21–	5	6	7	13

Table 3 shows that intense immediate reactions to the extract were seen more frequently in the patients with atopic dermatitis and the asthmatic patients than in the nonatopic controls.

The relationship of delayed to immediate reactions in the patients with atopic dermatitis is presented in table 4. Although positive delayed reactions tended to be more common in patients who had initially developed intense immediate reactions, there were a considerable number of patients who developed weak immediate and strong

Table 5. *Delayed reaction to bacterial and fungal antigens*

Antigens	Patients	No. of cases	Reaction	
			negative	positive
Staphyloc. albus	Atopic dermatitis	10	8	2
	nonatopic	8	1	7
Staphyloc. aureus	Atopic dermatitis	15	8	7
	nonatopic	45	20	25
Corynebact. acnes	Atopic dermatitis	7	7	0
	nonatopic	19	6	13
Alternaria	Atopic dermatitis	20	17	3
	nonatopic	40	15	25
Tuberculin	Atopic dermatitis	42	15	27
	nonatopic	42	3	39

delayed reactions. In the patients with bronchial asthma, however, an intense immediate reaction was usually not followed by a positive delayed reaction (tables 1 and 3).

Histological characteristics of the delayed reactions

The reactions were examined histologically in four patients with atopic dermatitis. Slight hyperkeratosis and acanthosis were present. The dermis exhibited a moderate or intense perivascular infiltrate consisting largely of mononuclear cells and varying amounts of eosinophils. Neutrophils were scanty. No changes were seen in the walls of the blood vessels.

Intracutaneous tests with other antigens

All of the subjects were over 15 years of age. The results of delayed reactions to bacterial and fungal antigens are summarized in table 5. The reaction was considered positive if maculopapule of 10 mm or greater in horizontal diameter was present. Delayed reactions to staphylococcus albus, s. aureus, corynebacterium acnes, alternaria, and tuberculin were weaker, or at least not stronger, in the patients with atopic dermatitis than in the nonatopic controls.

Histology of tuberculin reaction in patients with atopic dermatitis

Five biopsies were performed on test sites which manifested an induration of 5 mm or greater at 48 hours. Slight hyperkeratosis and acanthosis were present. The dermis showed an intense inflammatory infiltrate which was composed largely of mononuclear cells and varying number of eosinophils. Two of the five specimens contained a moderate number of eosinophils and the other three contained a few scattered eosinophils.

Intracutaneous tests with chemical agents

Inflammatory responses to formalin and croton oil at 48 hours were almost the same in the patients with atopic dermatitis and the nonatopic controls (table 6).

Discussion

Delayed reactions to various antigens in patients with atopic dermatitis have been discussed by many investigators.

Ofuji *et al.* (12, 13, 14) reported that delayed reactions to S. albus, S. aureus, alternaria, aspergillus, penicillium, and tuberculin were weaker, or at least not stronger, in patients with atopic dermatitis than in controls. Gudjónsson *et al.* (5) showed that the tuberculin reaction was

Table 6. *Inflammatory responses to chemical agents*

Agents	Patients	No. of case	Reaction (mm)		
			0—5	6—10	11—
Formalin	Atopic dermatitis	18	1	17	0
	nonatopic	18	0	18	0
Croton oil	Atopic dermatitis	19	2	17	0
	nonatopic	18	1	17	0

positive in 90 % of Swedish children but was negative in 34.5 % of children with atopic dermatitis. Rajka (17, 18, 19) observed that delayed reactivity to staphylovaccine, streptococcal extract, tuberculin, PPD, various mold extracts, and mumps vaccine was low in patients with atopic dermatitis.

On the other hand, Palacios *et al.* (15) found no appreciable difference in delayed reactivity to bacterial, viral and fungal antigens between patients with atopic dermatitis and controls. Green *et al.* (4), experimenting with 12 common inhalant antigens, reported that atopic individuals exhibited no greater incidence of delayed reactions than nonatopic individuals.

In regard to the delayed reactions to human dander extract, Kopecka *et al.* (8) made passing reference to the occurrence of such reactions in patients with atopic dermatitis. The present study showed that the incidence of positive delayed reactions to human dander extract was significantly greater in patients with atopic dermatitis than in patients with bronchial asthma or nonatopic dermatoses, whereas delayed reactions to various bacterial and fungal antigens were weaker in patients with atopic dermatitis than in nonatopic controls.

Parish (16) noted that contamination of skin by bacteria or fungi might play a role in an intradermal reaction to skin material. However, as shown in this study and described by other investigators, delayed reactions to bacteria or fungi are generally weaker in patients with atopic dermatitis than in controls. This would eliminate, largely if not completely, the possibility that bacterial or fungal contamination is involved in the occurrence of positive delayed reactions to human dander extract in patients with atopic dermatitis.

Another possibility is that an increased nonspecific inflammatory reactivity might play a role in the development of positive delayed reactions to human dander extract. This would be also excluded from the results of intracutaneous tests with the chemical agents and the bacterial and fungal antigens, which indicate that nonspecific inflammatory reactivity is not enhanced in patients with atopic dermatitis.

In the asthmatic patients, the incidence of positive delayed reactions to human dander extract was significantly low, although the immediate reactivity to the extract was almost the same as in the patients with atopic dermatitis. In the dermatitis patients, however, positive delayed reactions to the extract were frequently observed even among those who developed weak immediate reactions. This would indicate that the delayed reaction to the extract is not related to the immediate atopic allergy.

Thus, it seems that the delayed reaction to human dander extract is specific to atopic dermatitis, and the gross and histological resemblance of the delayed reaction to tuberculin reaction would suggest that the delayed reaction to human dander extract might be a manifestation of delayed hypersensitivity.

Finally, Simon (21) demonstrated that a high percentage of children with atopic dermatitis gave positive eczematous patch tests to human dander. Although he suggested the etiological significance of human dander in the dermatitis, other investigators (11, 20) regarded the dander as a nonspecific prurigenic stimulus. However, there

are reports that a certain substance can cause both eczematous- and tuberculin-type hypersensitivity (2, 6, 10, 24), so that Simon's studies would deserve re-evaluation.

SUMMARY

Delayed skin reactivity to human dander extract in patients with atopic dermatitis was examined by intracutaneous testing. The incidence of positive delayed reactions to the extract prepared was significantly higher in patients with atopic dermatitis than in asthmatic patients or nonatopic controls.

Delayed reactions to bacterial and fungal antigens were, on the other hand, generally weaker in the patients with atopic dermatitis than in nonatopic controls, and inflammatory responses to chemical agents were the same in patients with atopic dermatitis and nonatopic controls.

It seems that the development of the delayed reactions to human dander extract is specific to atopic dermatitis and not related to atopic allergy.

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