

Animal dander allergy

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Summary

In a group of twenty-three atopic patients skin tests were performed with the dander allergens of horse, cat and guinea-pig, and with house dust and Timothy pollen allergens. A good agreement was observed between positive skin reactions and the results of RAST with these various allergens. In a number of cases, positive skin reactions were not related to the clinical histories. Likewise, positive RAST scores in several instances proved clinically meaningless. In patients with history-related skin reactions better correlation with RAST was observed. In such patients, skin reactions with photo-inactivated allergens remained strongly positive.

Introduction

Previous investigations based on skin tests in patients with suspected animal dander allergy have led to the conclusion that weal and flare reactions to epithelium extracts of different species are often unrelated to the patient's clinical history of known antigenic contacts (Berrens, Hénocq & Koers, 1974). To explain these results, it was proposed that two mechanisms underlie skin reactivity, viz. an allergen-specific antibody-mediated reaction and a non-allergen-specific activation of a common mediator system. The present paper describes the results of experiments designed to verify this point.

As a working hypothesis, it was assumed that the specific mechanism might be equated with the well-described allergen-induced reaction mediated by IgE antibody. With regard to the possible contribution of a non-specific mechanism, both native and photo-inactivated allergens were considered for intradermal testing. The latter have been shown to be deprived of their skin-reactive potency in a large percentage of atopic subjects and to have lost their property of inactivating human haemolytic complement *in vitro* (Berrens, Hénocq & Radermecker, 1973).

Several authors have sought correlations between clinical parameters for reaginic allergy and serum IgE concentrations (RIST values) and/or the level of allergen-specific IgE antibodies (RAST values). On the subject of animal dander sensitivity,

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Fagerberg & Wide (1970) reported a 97% positive correlation between the clinical diagnosis and RAST with extracts of mixed dog epithelium. Intracutaneous tests and RAST agreed in 85% of the cases with mixed dog dander extracts and in 65% with dander allergens of individual breeds. Foucard (1973) examined animal dander allergy developing in children with asthmatoïd bronchitis and found 86% agreement between strongly positive skin tests and high RAST values. However, no more than 20% and 42% correlation was found between high RAST scores and weak to moderate skin test responses, respectively. Studies by Apold *et al.* (1974) and Havnen *et al.* (1974) have shown less correlation between the results of RAST, provocation tests and skin tests. They emphasized that the reliability of skin tests and RAST is allergen-dependent. Besides possible variation in the nature and quality of the different allergenic extracts (Aas & Lundkvist, 1973), discrepancies between RAST, skin tests or other provocation tests may also be understood by assuming that there is more than one mechanism contributing to skin reactions. The relative preponderance of these may be allergen-dependent.

The non-specific action of atopic allergens has been attributed to common structural sites of lysine-sugar bonding among distinct allergens (Berrens, 1971). The selective destruction of these sites by u.v. irradiation leaves the carrier antigen sufficiently intact to be recognized by specific antibody in the skin or bronchi of particular patients (Berrens *et al.*, 1973). Photo-inactivated allergens of this kind were employed in the present work to investigate further the hypothesis of two mechanisms in atopic allergy.

Materials and methods

The epithelial allergenic extracts of horse, guinea-pig and cat were prepared as described previously (Berrens *et al.*, 1974). A highly purified house dust allergen preparation, 74E, was isolated from pooled vacuum-cleaner dust according to published procedures (Berrens & Young, 1961). The grass pollen preparation was an unfractionated dialysed extract of ether-defatted pollen grains of Timothy grass (*Phleum pratense*, harvest 1971, donated by Bencard Laboratories). Photo-inactivated allergens were prepared from the active parent by u.v. irradiation. The inactivation process was monitored by measuring the fall of human complement-inactivating power (Berrens *et al.*, 1973). The irradiated products were recovered by lyophilization.

Blood samples were obtained by venous puncture; after clotting in glass, the blood sera were separated and stored in 0.5 ml aliquots in polypropylene tubes at -70°C until use.

Total serum IgE was assessed by radioimmunoassay (RIST), using the reagents provided with the Phadebas[®] test kit. Allergen-specific IgE was determined by the RAST technique, using the allergenic preparations described above coupled to Sepharose[®] beads according to the procedure of Aalberse (1974). Results were expressed as percentage radioactivity bound from a standard dose of ^{125}I -labelled anti-IgE per tube. Values at over 2% binding were considered positive.

Skin tests were performed by the intradermal route, using 0.05 ml per injection. All dander extracts were tested at 20 $\mu\text{g}/\text{ml}$; the test concentrations for house dust 74E and for Timothy pollen extracts were 2 $\mu\text{g}/\text{ml}$ and 1 $\mu\text{g}/\text{ml}$, respectively. The skin reactions were scored under a visual evaluation system as before (Berrens & Young, 1961; Maat-Bleeker, 1971).

Results

In a group of twenty-three atopic patients positive by skin test to animal dander allergens, total serum IgE levels were determined together with RAST values for specific IgE antibody against the allergens of horse, guinea-pig and cat dander, house dust and Timothy pollen. The results are shown collectively in Table 1. Breakdown of the analyses in relation to the skin tests and the clinical histories provided the following results.

Table 1. Total and specific IgE in the serum of twenty-three atopic patients with positive intradermal reactions to unmodified animal dander extracts. Skin reactions, graded according to intensity from 0 to 4, are shown in parentheses

Patient No.	Total IgE (iu/ml)	RAST (% binding)				
		Horse	Guinea-pig	Cat	Dust	Pollen
1	n.d.	21 (3)	3 (0)	42 (4)	38 (3)	52 (3)
2	960	6 (1)	2 (1)	22 (3)	28 (3)	0 (0)
3	2000	39 (3)	4 (0)	11 (1)	34 (3)	0 (0)
4	n.d.	0 (0)	3 (0)	10 (1)	15 (1)	4 (0)
5	115	1 (0)	1 (0)	46 (2)	32 (2)	1 (2)
6	1020	20 (2)	0 (0)	1 (0)	17 (2)	54 (4)
7	1800	39 (3)	10 (2)	56 (3)	52 (2)	0 (0)
8	3800	12 (2)	2 (0)	25 (2)	35 (2)	35 (2)
9	660	1 (2)	3 (0)	2 (3)	12 (3)	0 (0)
10	1180	24 (2)	57 (3)	19 (3)	44 (2)	9 (3)
11	820	12 (2)	45 (3)	5 (3)	35 (1)	6 (3)
12	440	12 (2)	0 (0)	0 (1)	12 (0)	54 (3)
13	970	1 (2)	0 (0)	2 (2)	15 (2)	16 (2)
14	147	4 (2)	23 (3)	1 (0)	19 (1)	0 (0)
15	430	20 (2)	0 (0)	1 (0)	20 (2)	52 (3)
16	285	0 (0)	0 (1)	0 (0)	2 (2)	0 (0)
17	1150	7 (1)	0 (0)	0 (0)	18 (0)	55 (4)
18	430	1 (2)	4 (2)	4 (0)	29 (2)	5 (0)
19	255	11 (1)	0 (0)	1 (0)	16 (0)	48 (3)
20	850	1 (2)	43 (4)	2 (2)	39 (0)	0 (0)
21	n.d.	0 (0)	0 (2)	0 (0)	1 (0)	0 (0)
22	n.d.	4 (0)	1 (0)	11 (1)	17 (3)	3 (1)
23	235	3 (1)	0 (0)	2 (2)	7 (0)	33 (3)

n.d. = Not done.

Total IgE in relation to the diagnosis

Total serum IgE levels were determined in nineteen patients reacting by skin test to animal epithelial extracts (Table 1). The mean level (923 iu/ml) was raised as compared to the mean for non-atopic subjects (100–200 iu/ml). There was a wide range in the individual IgE concentrations, extending downwards into the normal region (Table 1). In confirmation of earlier reports (Juhlin *et al.*, 1969; Johansson, 1967) it was observed that the diagnosis of atopic dermatitis and extrinsic asthma was often associated with high RIST values, while rhinitis occurred in even distribution in both the high-level and low-level groups.

On the basis of the personal histories, the group of nineteen patients was divided

Table 2. Mean RIST and RAST values in a group of nineteen patients arranged according to skin reactions related (R) or not-related (NR) to the clinical history. Mean $\pm$ s.d. indicated, except in series of less than five observations

Allergen	Related mean IgE (iu/ml)	Mean RAST (%binding)	Non-related mean IgE (iu/ml)	Mean RAST (%binding)
Timothy pollen	604 $\pm$ 388	37 $\pm$ 22	2490	16
Guinea-pig	685 $\pm$ 401	29 $\pm$ 23	1015	4
Cat	1338 $\pm$ 1194	21 $\pm$ 19	823	7
Horse	1607	30	1026 $\pm$ 985	11 $\pm$ 8
House dust	1383 $\pm$ 1063	27 $\pm$ 15	434 $\pm$ 310	24 $\pm$ 9

into those with history-related (R) and non-history-related skin reactions (NR). Table 2 shows that the highest RIST values in the R-group were observed in the sera of patients sensitive to house dust and horse dander, whereas in the NR-group, the highest total IgE values occurred in patients with skin reactions to grass pollen or guinea-pig dander, although natural exposure to these allergens apparently caused no complaints.

RIST in relation to RAST

Specific IgE antibody as evaluated by RAST was found twice as often in patients with RIST values >800 iu/ml than in patients with total IgE below this value (twenty-five out of fifty against eleven out of forty-five positive RASTs, see Tables 1 and 2). A diagnosis of house dust allergy combined with other allergies was prominent in the high RIST-RAST group. Five patients with serum IgE values of ≥ 1000 iu/ml had three or more concomitant allergies. By contrast, specific animal dander and grass pollen allergy occurred singly even in the group with low RIST values. These results confirm those of Wide, Bennich & Johansson (1967), who reported that multiple allergies and the number of corresponding positive RASTs are frequently associated with high RIST values. Elevated RIST values need not necessarily accompany pronounced monospecific allergies, e.g. to grass pollen.

RIST, RAST and skin reactions in relation to the history

Table 2 shows the mean RAST values in groups of patients chosen on the basis of the history-related or non-related skin reactions. Depending on the allergen, the trend was an association of only moderately elevated RIST values with significant RAST scores in the R-group, especially with the pollen, guinea-pig and horse dander allergens. Conversely, in the NR-group, markedly increased RIST values were associated with low-grade RAST scores, in particular with horse dander and house dust allergens.

As shown by the overall results in Table 1, there appeared to be a good agreement between the results of RAST scores and intradermal testing. A statistically significant positive correlation was observed with the Timothy pollen allergens (Spearman rank correlation coefficient 0.84, $P < 0.005$). The collected results in Fig. 1a and b demonstrate that the intensity of the skin test reactions correlated significantly with the RAST score with animal dander allergens in the R-group of patients, but not in the NR-group.

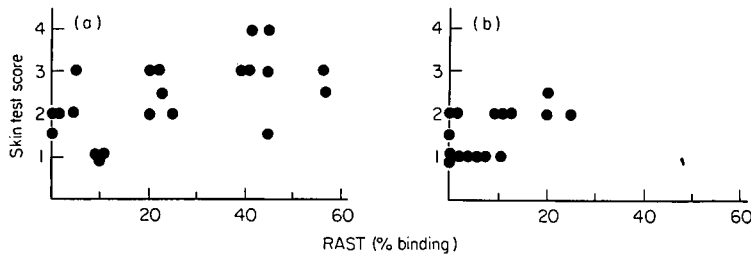


Fig. 1. Scatter diagrams of relationship between skin test score, intensity grade 0 to 4, and RAST to animal dander allergens in: (a) twenty-one cases of positive skin reactions related to the personal history (Spearman rank correlation coefficient $r = 0.53$, $P < 0.05$); and (b) sixteen observed positive skin reactions not related to the personal history ($r = 0.46$, $0.05 < P < 0.1$).

However, these results and those of others indicate that many skin reactions have no clinical importance. In many cases there is no evidence of actual antigen exposure apparently having caused sensitization. Moreover, actual antigenic contact quite often causes no clinical complaints (Berrens *et al.*, 1974; Rudolph *et al.*, 1975; Schultze-Werninghaus *et al.*, 1976). Table 3 demonstrates that the skin reactions to horse

Table 3. Statistical relationship between personal history of allergy and skin reactions to animal dander allergens. P -values calculated by Fischer test

History	Horse $P > 0.20$				Cat $P < 0.05$				Guinea-pig $P < 0.05$			
	Skin reaction				Skin reaction				Skin reaction			
	-	+	-	+	-	+	-	+	-	+	-	+
	4	14	18	5	5	7	12	11	12	3	15	8
+	1	4	5	5	0	11	11	5	2	6	8	9
	5	18			5	18			14	9		

dander did not correlate significantly with the personal histories in the present group of patients. Because of the agreement between RAST and skin reactions previously mentioned the authors therefore examined whether positive RASTs might be clinically meaningless.

As demonstrated by the statistical results collected in Table 4, there was no relationship between the results of RASTs and the patients' personal histories as regards allergy to horse and guinea-pig dander. Correlation was better with the allergens of cat and pollen, though in one RAST-positive case (Table 1, no. 8) there was no evidence of past or present complaints of pollen hay fever. With the dander allergens, 'false-positive' RAST results unrelated to the clinical history were often observed, especially with the horse dander allergens. False-positive RASTs to house dust allergen were also found, especially in the group of patients with skin reactions unrelated to their history. These results, and the distribution in R- and NR-groups of patients, are listed in Table 5.

From the results so far it appears that the RAST technique *in vitro* provides results comparing well with the skin test *in vivo*. As shown in Table 5, this implies that positive RASTs, not related to the patient's clinical history, are to be expected in

Table 4. Statistical relationship between personal history to animal dander allergens and RAST results with these allergens and the patient's sera. *P* values by Fisher test

History	Horse <i>P</i> > 0.20 RAST				Cat <i>P</i> < 0.05 RAST				Guinea-pig <i>P</i> > 0.20 RAST			
	—	—	+	—	—	—	+	—	—	—	+	—
	—	7	11	18	—	9	3	12	—	8	7	15
	+	1	4	5	+	2	9	11	+	3	5	8
		8	15			11	12			11	12	

Table 5. Distribution of positive skin reactions with native and photo-inactivated (u.v.) allergens together with RAST results in patients with skin tests related to their clinical history (R) and in a non-related group (NR)

Allergen	R-group					
	Contacts Symptoms		Pos. skin reaction		RAST	
			Native	u.v.	≥ 2% < 20% binding	≥ 20% binding
Grass pollen	23	11	10	n.d.	4	7
Guinea-pig	14	8	6	4	1	4
Cat	17	11	11	4	6	5
Horse	8	5	4	4	1	4
House dust	23	11	11	8	4	7

	NR-group					
	No contacts symptoms		Native	u.v.	≥ 2% < 20% binding	≥ 20% binding
Grass pollen	0	12	3	n.d.	2	1
Guinea-pig	9	9	3	0	7	0
Cat	6	6	4	0	4	0
Horse	15	15	14	1	8	2
House dust	0	12	6	0	7	4

approximately the same proportion as false-positive skin reactions. The RAST data for horse dander allergen in this respect (Table 5) correspond with our skin test data.

On considering a possible cause of non-related skin reactions, the suggestion has been forwarded that a second—unspecific—mechanism might underlie skin reactions. This mechanism was thought to be primed by common sites of lysine-sugar conjugation among different allergens. Removal of these sites by controlled u.v. irradiation consequently provides allergen-derivatives which might be expected to cause fewer non-related skin reactions (Berrens *et al.*, 1973).

Skin tests performed with u.v.-modified allergens in the present group of twenty-three patients did reduce the number of false-positive reactions in the NR-group to only one (horse).

In sharp contrast, however, the reactions to photo-inactivated allergens in the R-group of patients remained positive or even displayed increased intensity in 36–80% of these cases of highly specific allergy, depending on the kind of allergen. The results with the native and photo-inactivated specimen of each allergen have been listed in Table 5.

As carried out by other investigators (Apold *et al.*, 1974; Havnen *et al.*, 1974) the results of skin test reactions and the RAST score were compared. Arbitrarily, the RAST values were divided into a medium score group of 2–20% binding, and a high score group over 20% binding of added radioactivity. As demonstrated by the numerical data listed in Table 5, a good agreement was thus obtained with the high RAST scores and the results of intradermal tests with photo-inactivated allergens in the R-group of patients. It must be pointed out that in this particular group of patients the personal history was already highly convincing for a specific allergy to one of the animal dander allergens (e.g. cat, or guinea-pig). However, in the NR-group of patients, RAST scores over 20 were still found six times (to house dust or horse dander), although the skin reactions to photo-inactivated horse dander remained positive in only one case.

Intradermal skin tests with photo-inactivated allergens in our experience proved more valuable for diagnosing highly specific history-related allergies than skin tests with the native allergens *in vivo*, or RAST tests *in vitro*. More recent evidence in this respect indicates that highly specific allergies, especially to cat and guinea-pig dander, can easily be detected with the photo-inactivated allergens, even in those cases where the corresponding RASTs with anti-IgE are negative. Presumably, these may be immediate-type allergies mediated by an antibody class other than IgE (Bryant, Burns & Lazarus, 1975; Parish, 1970).

Discussion

In confirmation of earlier results, skin test reactions with animal dander extracts, and also with house dust and occasionally grass pollen allergens, are often unrelated to the patient's personal history of antigenic contacts or complaints on exposure. With IgE as an additional parameter, the present study shows that high total IgE values and low RAST scores are often observed in the NR-group of patients, particularly with house dust and horse dander allergens causing the positive skin reactions. In the R-group of patients, high RAST scores to the skin test positive allergens (i.e. grass pollen, guinea-pig and horse dander) were often associated with low or only moderately elevated total IgE values.

As was to be expected from the recorded overall agreement between RAST and skin tests, confirmed here, positive RAST results not related to the patient's history did occur. In this connection it is of note that other investigators have also observed that the mere possession of allergen-specific IgE need not imply clinical allergy to that allergen (Hoffman, 1975).

It would appear that such results are compatible with a dual action of allergens and the existence of at least two mechanisms underlying skin reactions and clinical allergy (Berrens, 1971, 1976). The present results with horse dander allergen are particularly telling in this respect. This atopic allergen has the characteristic properties of an atopen, i.e. the non-specific activation of mediating systems in NR-patients. It

also exhibits the properties of an allergen or antigen, i.e. the induction of, and combination with, specific IgE antibody in R-patients (Berrens, 1976). Discordance between clinical allergy and the results of skin tests or RASTs to dog, cow and horse dander allergen has also been noted by other investigators (Öhman & Johansson, 1974; Rudolph *et al.*, 1975; Schultze-Werninghaus *et al.*, 1976). In the present study it remains unexplained why positive RASTs to horse dander allergen not only occurred in the R-group of patients, but were also frequently observed in the NR-group.

A relationship was noted between parameters for horse dander and grass pollen allergy, both *in vivo* and *in vitro*. When separated into R- and NR-groups, the R-reactions to pollen (ten times) were accompanied by seven NR-skin reactions to horse dander. Conversely, the six counted R-reactions to horse dander allergen were in no instance related to NR-pollen reactions, excluding the possibility of cross-contamination of the extracts (Frankland, 1975). Further studies are intended to clarify the phenomenon of non-specific co-reactivity to horse dander allergen in hay fever patients in contrast to the reverse situation.

For detecting highly specific allergies, RAST proved no better than direct intradermal testing. In the cases of highly convincing personal histories, where impressive isolated skin reactivity was associated with high RAST scores, the authors have come to prefer skin tests with photo-inactivated allergens. As indicated in the Results section, the use of these derivatives drastically reduces the proportion of NR-reactions and convincingly aids in the detection of R-reactions. Furthermore, photo-inactivated allergens for skin testing may detect specific allergies in those cases where the anti-IgE RAST is negative, i.e. where the immediate type hypersensitivity reaction is apparently mediated by a different class of immunoglobulin.

The diagnosis of allergy and its causes may require a combination of quite different *in vivo* and *in vitro* techniques to define the precise mechanism underlying the disease in individual cases. Apart from skin and provocation tests with both native and photo-inactivated allergens (Hénocq, Garcelon & Berrens, 1973), RAST tests with both anti-IgE and possibly anti-IgG (Bryant *et al.*, 1975; Devey & Panzani, 1975), other parameters may be helpful in screening and guiding the individual patients. In patients with unspecific hyper-reactivity, complement-sensitivity *in vitro* may be an important factor (Berrens, 1975). In patients with highly specific allergies, parameters for evaluation may include simple data on HLA-type (Marsh *et al.*, 1975) or blood groups. A subsequent paper will describe the prevalence of A-type blood groups in specific grass pollen allergy.

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