

RAST in the diagnosis of hypersensitivity to dog and cat allergens

A comparison of different extract preparations with clinical history, skin test and provocation tests

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Summary

A comparison was made of clinical signs and provocation tests with the RAST for allergy to dog in eight and cat in twenty patients. Twenty-two had high IgE levels and all gave positive reactions to provocation tests. A commercially available dog epithelium extract gave more positive RASTs than five other dog allergen preparations, and there were no positive reactions to dog serum. The RAST was negative in patients with a low degree of sensitivity. All twenty patients allergic to cat gave positive intracutaneous test reactions and in the RAST the cat epithelial allergen showed 90% agreement with the *in vivo* findings.

Introduction

Patients with allergy to dog and cat frequently show different hypersensitivity symptoms such as asthma, rhinitis, tracheobronchitis, conjunctivitis, urticaria, angio-oedema, pruritus or eczema. In most cases the symptoms are due to an immediate type of allergic reaction with specific IgE antibodies to antigens in animal epithelia, dander, dandruff and serum. Some of these allergens seem to be in common for both cat and dog (Ohman *et al.*, 1976a). Since the introduction of the radioallergosorbent test (RAST) (Wide, Bennich & Johansson, 1967) it has been possible to measure specific IgE levels in serum. However, it has been reported that IgE antibodies cannot always be detected by RAST in patients with verified immediate hypersensitivity to dog or cat (Ahlstedt *et al.*, 1974; Havnen *et al.*, 1974; Wüthrich & Kopper, 1975; Eriksson, Ahlstedt & Belin, 1976; Kleinhans, 1977).

The present study was designed to compare the clinical significance of history, *in vivo* tests and measurement of specific IgE in serum using different dog allergen preparations in the RAST system in patients with a clinically immediate hypersensitivity

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to dog but not to cat. Similarly, atopics with symptoms suggesting hypersensitivity to cat and not to dog were also studied.

Materials and methods

Patients

Twenty-eight outpatients, seven males and twenty-one females, with asthma, rhinitis, conjunctivitis, contact urticaria and itching after exposure to dog or cat were studied. All had one or other of atopic disease. None had been hyposensitized to either dog or cat. Eight had a clinical history strongly suggestive of specific allergy to dog and not to cat, and the remaining twenty had a clear-cut history of allergy to cat and not to dog. A careful clinical history was recorded for each patient, including type, severity and duration of symptoms, together with present and past contact with the animals. The age range was between 12 and 59 years, with a mean value of 26 years.

An additional ten atopic patients with no symptoms on exposure to dog or cat were included as controls.

Skin test

All patients had prick tests, and where negative intracutaneous tests (0.02 to 0.03 ml) using commercially available aqueous dog and cat allergen preparations (Stallergènes, Licence Hollister-Stier Lab, test concentration 1:10³ w/v). The tests were graded from 0 (negative) to 4+ (strongly positive) in comparison with responses to control saline (with 0.4% phenol) and to the positive control test with histamine hydrochloride (0.1 mg/ml). The intracutaneous test is considered to be 100 times more sensitive than the prick test. The test reactions were graded as shown in Table 1. In a few cases scratch tests were done with the hairs of the patient's own dogs.

Table 1. Grades of skin test reactions

Score of skin test reaction	Intracutaneous test	Prick test
0	0	
1	±	
2	+	
3	2+	±
4	3+	+
5	4+	2+
6		3+

Provocation tests

The provocation tests were made with the same extracts as the skin tests, with increasing concentrations (1:10³, 1:10², 1:40 and 1:20 w/v solutions; stock concentration 1:10 w/v). Saline with 0.4% phenol was used as control. Conjunctival, nasal or bronchial provocation tests were chosen according to the affected organ.

Bronchial provocation tests (BPT). These were carried out according to the method

of Debelic (1968), described in detail elsewhere (Wüthrich, 1970). The results were assessed by the forced expiratory volume (FEV_1), using the Vitalograph (Buckingham Ltd). A decrease of at least 20% was regarded as positive.

Nasal provocation tests (NPT). These were made by placing a cotton-tipped applicator with aqueous allergen solution on the nasal mucosa. The NPT was considered positive, if itching with sneezing and rhinorrhea, possibly with conjunctivitis, occurred (Wüthrich, Much & Kopper, 1977). Tests with the control solution made first were all negative.

Conjunctival provocation tests (CPT). A drop of the aqueous extract was placed into the lower conjunctiva of the left eye. The test was considered positive if the conjunctiva became intensely red with tears (Fuchs, 1970). The right eye was tested first without reactions to the control solution.

In vitro tests

Blood was drawn and frozen serum stored at the time of the skin test procedure, but before the provocation tests. All sera were tested for specific IgE by Phadebas RAST® (Pharmacia Diagnostics AB, Uppsala, Sweden) using commercial dog epithelium and cat epithelium discs. In addition, four experimental discs (obtained from Dr Lars Yman, Department of Research, Pharmacia Diagnostics AB, Uppsala, Sweden), coupled with a preparation of dog dander in two different concentrations (A and B), a purified dog dander (purified through a Sephadex column) and dog serum albumin, respectively, were used. Dog dander A, B and purified dog dander were derived from the same raw material. All discs were run simultaneously and determinations were performed according to instructions in duplicate. RAST class 0 indicates no detectable specific IgE antibodies in the serum, classes 1–4 represent increasing concentrations of IgE antibodies.

Total IgE. These levels in all sera were determined by the Phadebas® IgE PRIST (paper radioimmunosorbent test) (Pharmacia Diagnostics AB, Uppsala, Sweden).

Results

Clinical symptoms

Specific hypersensitivity to dog or cat was shown in all cases by provocation tests. The clinical features are shown in Table 2.

All ten control patients had asthma, rhinitis, hay fever or urticaria. In all cases the causal allergens were pollens, moulds, house dust and animal allergens other than dog or cat.

Skin and provocation test results (Tables 2 and 3)

Among the patients allergic to dog, one had a doubtful intracutaneous test to dog, but the scratch test with his own dog hair was clearly positive. Despite the absence of clinical hypersensitivity to cat, three patients gave a positive and three doubtful skin test reactions. With the exception of one with asthma and an FEV_1 fall after inhalation of dog extract in a 1:10³ dilution, all the provocation tests were positive at 1:20 w/v, indicating a low degree of sensitivity to the dog allergens.

In the twenty patients allergic to cat, skin tests gave a clearly positive result. Only one cat-allergic patient gave a positive skin test result to dog, but the provocation test was negative.

Table 2. Clinical data, diagnosis, skin and provocation tests of patients allergic to dog

Patient number	Initials	Age (years)	Sex	Diagnosis	IgE (u/ml)	Score of skin tests		Provocation tests with dog extract (positive concentration w/v)		
						Cat	Dog	CPT	NPT	BPT
(1)	L.K.	21	F	A	99	0	2	n.d.	n.d.	+(1:10 ³)
(2)	M.S.	59	F	AR	24	2	1	n.d.	+(1:20)	n.d.
(3)	F.V.	43	F	A, AR	270	1	1*	n.d.	+(1:20)†	n.d.
(4)	K.S.	12	F	A	n.d.	2	2	n.d.	n.d.	+(1:20)†
(5)	N.M.	18	M	AR	110	0	2	n.d.	+(1:20)	n.d.
(6)	D.E.	20	F	A, AR	150	1	2	n.d.	n.d.	+(1:20)
(7)	D.C.	28	F	A, AR	410	2	2	n.d.	+(1:20)	n.d.
(8)	B.H.	27	M	AR	400	1	3*	n.d.	+(1:20)	n.d.

F = female; M = male; A = asthma; AR = allergic rhinitis; n.d. = not done.

* Scratch tests with own dog hair positive.

† Provocation tests with cat extract negative.

Table 3. Clinical data, diagnosis, skin and provocation tests in patients allergic to cat

Patient number	Initials	Age (years)	Sex	Diagnosis	IgE (u/ml)	Score of skin tests		Provocation tests with cat extract (positive concentration w/v)		
						Cat	Dog	CPT	NPT	BP
(1)	W.W.	31	M	CU, AD	6350	3	0	Neg.	Neg.	n.d.*
(2)	R.A.	33	F	AC	550	3	0	+(1:10 ²)	n.d.	n.d.
(3)	T.M.	30	F	A	1270	3	2	n.d.	n.d.	+(1:20)
(4)	S.R.	25	F	AR	160	4	0	n.d.	+(1:20)	n.d.
(5)	R.P.	29	M	AR, CA	n.d.	3	0	+(1:20)	+(1:20)	n.d.
(6)	K.C.	13	F	A	1370	2	0	n.d.	n.d.	+(1:20)
(7)	J.M.	20	F	AC	20	4	0	+(1:10 ²)	n.d.	n.d.
(8)	M.J.	31	F	A	320	3	1	n.d.	n.d.	+(1:10 ²)
(9)	R.H.	14	F	AR	1060	4	0	n.d.	+(1:10 ²)	n.d.
(10)	V.P.	22	M	AR	250	3	0	n.d.	+(1:20)	n.d.
(11)	M.A.	41	F	AC	410	2	0	+(1:40)	n.d.	n.d.
(12)	H.F.	33	M	A, AR	47	2	0	n.d.	+(1:20)	n.d.
(13)	O.C.	20	F	A, AR	930	2	0	n.d.	+(1:20)†	n.d.
(14)	O.S.	14	F	CU	120	2	0	Neg.	Neg.	n.d.*
(15)	A.G.	32	F	A	580	3	0	n.d.	Neg.	+(1:20)
(16)	S.R.	29	F	A	240	3	0	n.d.	n.d.	+(1:20)
(17)	M.A.	13	F	A, AR	1000	3	0	n.d.	+(1:20)	n.d.
(18)	W.S.	17	F	A, AR	180	3	0	n.d.	+(1:20)	n.d.
(19)	S.D.	32	F	A	1150	3	0	n.d.	n.d.	+(1:20)
(20)	E.M.	30	M	A, AR	18	3	0	Neg.	+(1:20)	n.d.

F = female; M = male; A = asthma; AR = allergic rhinitis; AC = allergic conjunctivitis; CU = contact urticaria; AD = atopic dermatitis; n.d. = not done.

* After skin contact with cat there was itching and CU.

† Provocation tests with dog extract negative.

Total IgE (Tables 2 and 3)

Among the patients with sensitivity to dog, six out of eight indicated high total IgE values, i.e. more than 120 u/ml (Phadebas® IgE PRIST). The geometric mean value amounted to 150 u/ml. Sixteen patients out of twenty allergic to cat also had high total IgE levels with a geometric mean of 347 u/ml. Low circulating levels of total IgE were found in seven of the ten atopic controls.

Table 4. RAST results of dog allergic patients (RAST classes 0-4)

Patient number	Phadebas® dog epithelium	Dog dander			Dog serum	Phadebas® cat epithelium
		A	B	Purified		
(1)	2	2	0	0	0	0
(2)	0	0	0	0	0	0
(3)	2	2	2	0	0	0
(4)	0*	2	2	1	0	0
(5)	0	0	0	0	0	0
(6)	0	2	2	2	0	0
(7)	3	3	3	2	0	0
(8)	1	2	2	2	0	0

* RAST performed 4 months before was positive (class 2).

Table 5. RAST results of cat allergic patients (RAST classes 0-4)

Patient number	Phadebas® dog epithelium	Dog dander			Dog serum	Phadebas® cat epithelium
		A	B	Purified		
(1)	0	1	2	0	0	3
(2)	0	0	0	0	0	2
(3)	0	2	2	1	0	4
(4)	0	0	1	0	0	3
(5)	0	0	0	0	0	2
(6)	0	0	0	0	0	0
(7)	0	0	0	0	0	2
(8)	0	0	0	0	0	2
(9)	0	2	2	2	0	2
(10)	0	0	0	0	0	3
(11)	0	1	1	1	0	2
(12)	0	0	0	0	0	0
(13)	0	0	0	0	0	2
(14)	0	0	0	0	0	3
(15)	0	1	1	0	0	4
(16)	0	0	0	0	0	2
(17)	0	1	1	0	0	2
(18)	0	0	0	0	0	2
(19)	0	1	2	0	0	3
(20)	0	0	0	0	0	2

Table 6. Distribution of RAST results with different animal allergen discs

						Disc
	Phadebas® dog epithelium	Dog dander			Dog serum	Phadebas® cat epithelium
Patients		A	B	Purified		
Dog-allergic (<i>n</i> = 8)						
Positive	4	6	5	4	0	0
Negative	4	2	3	4	8	8
Percentage agreement*	50	75	62·5	50	0	100
Cat-allergic (<i>n</i> = 20)						
Positive	0	7	8	3	0	18
Negative	20	13	12	17	20	2
Percentage agreement*	100	65	60	85	100	50
Controls (<i>n</i> = 10)						
Positive	0	0	0	0	0	0
Negative	10	10	10	10	10	10
Percentage agreement*	100	100	100	100	100	100

Positive RAST results = classes 1-4; negative RAST results = class 0.

* Agreement with case history and provocation test results.

RAST results

RAST results, using five different dog allergen preparations and one cat allergen disc are shown in Tables 4, 5 and 6. Four of the eight patients sensitive to dog gave a positive RAST with both the commercial dog epithelium disc and the purified dog dander extract. In six and five cases respectively the experimental dog dander preparations (A and B) were positive. No positives were obtained to the dog serum. No patient with pure dog sensitivity had a positive RAST to the commercial cat epithelium disc.

With the different dog allergen preparations in the patients allergic to cat, three had a positive RAST to the experimental purified dog dander preparation, and five and six respectively to dog dander extracts A and B; but none to the dog epithelium and dog serum discs. Eighteen out of twenty patients gave a positive RAST to the commercial cat epithelium disc. Negative RASTs to all the different allergen discs were given by the controls.

Comparison of in vivo tests and RAST results

Figs 1 and 2 illustrate the correlation between skin tests, using dog and cat aqueous allergen extracts and RAST results, obtained with commercial dog and cat epithelium discs. In the cat allergics there is an excellent correlation of positive RASTs in 90% to cat and negative is in 95% to dog. In the smaller group of dog-allergic patients the correlation is less clear, with 50% for dog and 62.5% for cat, with a higher degree of sensitivity for the skin test but a higher specificity for the RAST. A good correlation of 88% was found between provocation tests and RAST results for cat and a poor correlation of 50% for dog (Fig. 3). Conflicting negative RAST results were only found among the patients with a low degree of sensitivity, i.e. where provocation tests were positive only to the strongest allergen concentration (1:20 w/v).

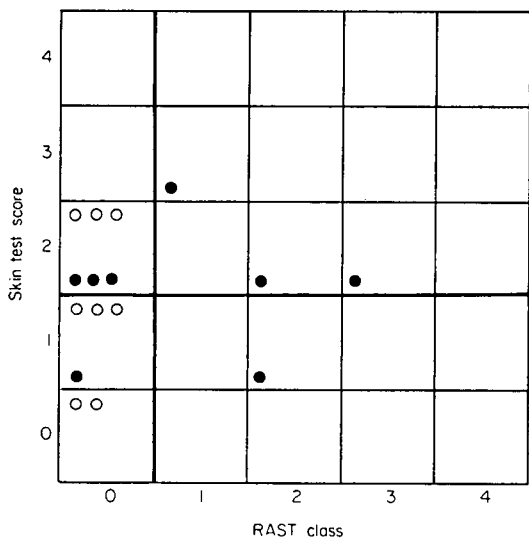


Fig. 1. Correlation between skin tests and RAST results with dog and cat allergens in dog-allergic patients ($n = 8$). (●) Dog epithelium: agreement four out of eight (50%); (○) cat epithelium: agreement five out of eight (62.5%).

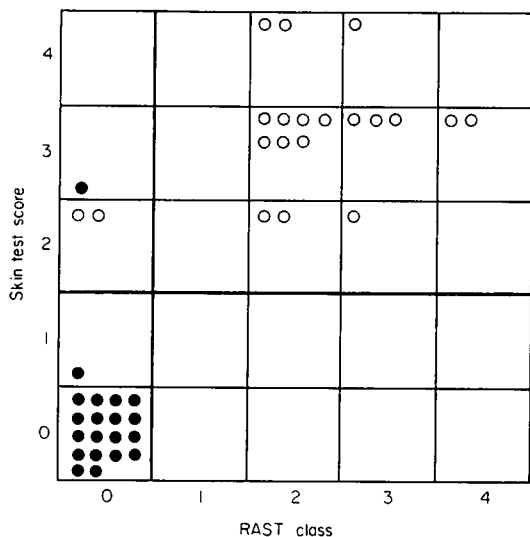


Fig. 2. Correlation between skin tests and RAST results with dog and cat allergens in cat-allergic patients ($n = 20$). (●) Dog epithelium: agreement nineteen out of twenty (95%); (○) cat epithelium: agreement eighteen out of twenty (90%).

Discussion

It is known that different relevant animal allergens can be derived from dandruff, dander, epithelia, saliva, serum proteins or urine (Ohman, Lowell & Bloch, 1973; Yman, Brandt & Ponterius, 1973; Stokes & Turner, 1975; Koers, Young & Berrens, 1977; Newman-Taylor, Longbottom & Pepys, 1977). Serum proteins of different

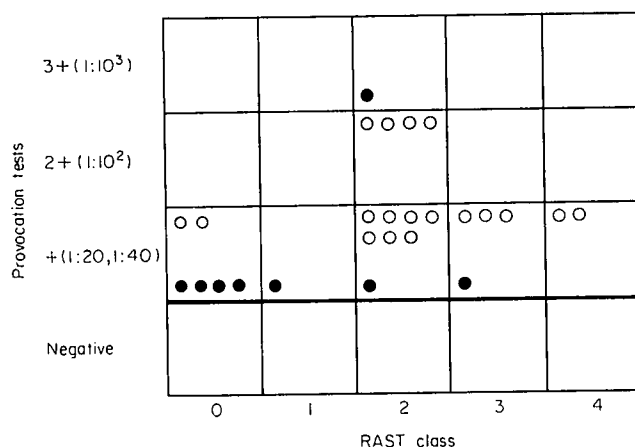


Fig. 3. Correlation between provocation tests and RAST results in cat- and dog-allergic patients. (●) Dog allergen: agreement four out of eight (50%); (○) cat allergen: agreement sixteen out of eighteen (88%). The allergen concentrations (w/v) are shown for the provocation tests.

animals show immunological cross-reactions (Brandt, Ponterius & Yman, 1973; Ohman *et al.*, 1976a) and some allergens in epithelia are common and cross-react while others are specific (Fagerberg & Wide, 1970; Ohman *et al.*, 1976b). Specific breed differences may also exist (Hooker, 1944; Voorhorst, 1965).

The present study was designed to find the most useful dog allergen preparation (Brandt & Yman, 1976) for the RAST to detect specific IgE in patients with suspected allergy to dog, and with no cross-reactions to cat antigens. None of the preparation tested seems to be ideal for a good correlation and high specificity in *in vivo* tests and the RAST. When using the commercial dog epithelium extract, the degree of specificity seems excellent, but the correlation with *in vivo* tests and clinical history is only moderate. The preparations of dog dander showed a higher correlation but also gave positive reactions in patients allergic to cat only. None of the patients, sensitive to dog or cat, could be identified by RAST with dog serum. These results are in contradiction to those of some investigators (Yman *et al.*, 1973), but are in agreement with the recent studies of others (Brehm *et al.*, 1975; Ohman *et al.* 1976a) and Leegaard & Roth (1977). Taken together the commercial dog and cat epithelium discs gave in this selected patient group a 78.5% correlation with the case history and provocation tests. We found in an unselected group of thirty-five asthmatics, given bronchial provocation tests with dog or cat allergens, an overall agreement of 71.5% (Wüthrich & Kopper, 1978). The correlation between *in vivo* test and RAST results is influenced by different factors, i.e. the source and composition of extracts, methods of preparation, potency of individual extracts and the degree of sensitivity of the patients. In patients with low sensitivity, only mast cell-bound specific IgE may be present in the target organ or in local secretions (Huggins & Brostoff, 1975). The time of the blood examination in relation to clinical exposure and symptom manifestations can also influence the RAST. For instance, patient No. 4 (dog-allergic) with negative RAST at the time of this study had had a clearly positive RAST result (class 2) a few months earlier. On the other hand, skin tests may lack specificity due to irritants in allergen extracts as shown here by three dog-allergic patients who gave positive skin tests to cat allergens and one cat allergic patient with a positive skin test to the dog extract.

In conclusion, the Phadebas® cat allergen disc in use at present is excellent for specificity and sensitivity in cat allergic patients. It seems that none of the other dog allergen preparations could achieve more reliable results than the commercial dog epithelium preparation.

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