

Allergens of mammalian origin. V.

Properties of extracts derived from the domestic cat

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Summary

Eight commercial cat dander extracts and two pelt extracts derived from mongrel and Siamese cats were compared. Cat allergen 1 and cat albumin were measured by radial immunodiffusion. Allergenic activity was evaluated by prick test and a modified radioallergosorbent test. In the latter, the dilution of each extract that produced 50% inhibition of binding of IgE antibodies to insolubilized cat allergen 1 (RAST 1) and insolubilized cat serum (RAST 2) was determined. The total non-dialysable solid content of the extracts did not correlate with any other parameter. Cat allergen 1 content determined by radial immunodiffusion correlated with average prick test results in ten cat-sensitive subjects and with RAST 1 activity. Cat albumin content correlated weakly with RAST 2 activity but not with any other measure of allergenic activity. Absorption of each extract with the γ -globulin fraction of rabbit antiserum to cat allergen 1 significantly reduced prick test reactivity and RAST 1 activity, but not RAST 2 activity. These results indicate that cat allergen 1 is an important allergen in cat dander extracts and its measurement may be used to standardize the allergenic activity of such extracts.

Introduction

Human allergic reactions to the domestic cat are commonly encountered. Until recently the allergens responsible for such reactions were poorly defined. The properties of one important allergen, cat allergen 1, derived from extracts of cat pelt have recently been described (Ohman, Lowell & Bloch, 1974). The contribution of cat allergen 1 to the allergenic activity of diverse cat extracts remained to be determined.

In this report immunochemical and allergenic properties of two extracts of cat pelt prepared in this laboratory and eight commercial extracts of cat epithelia are compared. On the basis of these comparisons it appears that cat allergen 1 is a major contributor to the allergenic activity of such extracts and that it may be used to standardize their allergen content.

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Methods

Extracts

Cat epithelial extracts (1:10 w/v) were purchased from eight different commercial sources in the United States. Cat pelt extract (1:10 w/v) was prepared as previously described from twenty lyophilised and defatted mongrel cat pelts (Ohman, Lowell & Bloch, 1973). In addition, an extract was similarly prepared from two Siamese cat pelts.

Purified antigens

Cat allergen 1 was prepared from cat pelt extract by sequential fractionation on DE52 cellulose and Sephadex gel chromatography columns (Ohman *et al.*, 1974). Allergen was identified in the effluent of the columns by gel diffusion against antiserum to cat allergen 1 (Ohman *et al.*, 1974). We have slightly modified the purification procedure previously described. In brief, DE52 cellulose was equilibrated with 0.01 M phosphate buffer, pH 7.5 and poured into columns. Cat pelt extract that had been dialysed against this buffer was applied to the columns. Following elution of the first peak of protein, a continuous ionic gradient was established in the eluting solution by gradually adding 0.4 M NaCl in phosphate buffer (0.01 M, pH 7.5) to a reservoir containing the starting buffer. Cat allergen 1 was detected in eluate that also contained cat albumin. These fractions of the eluate were pooled, concentrated by lyophilization, and applied to Sephadex G-75 columns. The proteins applied to the columns were resolved into major (peak) and minor (trailing shoulder) fractions. Cat allergen 1 was identified in the minor fractions; these were combined, concentrated and reappplied to Sephadex G-75 columns. Elution resulted in a single protein peak containing cat allergen 1. Cat albumin was isolated from pooled cat serum as previously described (Ohman *et al.*, 1974).

Antisera

The preparation of antiserum to cat albumin has previously been described (Ohman *et al.*, 1974).

A new antiserum to cat allergen 1 was prepared by immunizing rabbits with 1 mg of cat allergen 1 prepared as described in the previous section and emulsified in complete Freund's adjuvant. Antiserum was collected after 30 days. This antiserum was tested by immunoelectrophoresis against crude cat pelt extract; a single precipitin line was observed. In gel diffusion, this antiserum and the previously described absorbed antiserum to cat allergen 1 were reacted in adjacent wells against crude cat pelt extract; a single precipitin line of identity was observed.

Radial immunodiffusion for quantitation of cat allergen 1 and cat albumin

Radial immunodiffusion in agarose gel (MCI Biomedical, Rockland, Maine) was carried out using the technique described by Mancini, Carbonera & Heremans (1965). Four millilitres of antiserum was mixed with 20 ml of 1% agarose in phosphate buffered (0.01 M, pH 7.5) saline (0.15 M) (PBS) at 56°C and poured onto lantern slides (83 × 102 mm). A series of wells measuring 3 mm in diameter were cut into the agarose layer after it had hardened and 20 µl of antigen-containing solution was placed in the wells. After 48 hr, the diameter of the precipitin ring was measured. Dilutions of a standard antigen solution were included on each slide. A linear relationship was

observed between the log of the diameter of the precipitin ring and the log concentration of antigen. The lowest concentration of cat allergen 1 and cat albumin that could be estimated by this technique was approximately 15 µg protein/ml.

Because of the low concentration of cat allergen 1 and cat albumin in some of the extracts, each extract was dialysed against 0.05 M ammonium bicarbonate, lyophilized and reconstituted in a tenth or a fiftieth of the original volume. Concentrated extract was applied to the wells. Results are reported as cat allergen 1 or cat albumin content in unconcentrated (1:10 w/v) extract.

Measurement of allergenic activity using a modified radioallergosorbent test

The radioallergosorbent test was performed as previously described (Ohman *et al.*, 1976). One milligram of cat allergen 1 or 25 µl of cat serum was coupled to 100 mg of cyanogen bromide-activated cellulose particles. Fifty microlitres of patient serum was diluted with an equal volume of assay buffer and added to plastic test tubes containing 0.25 mg of cat allergen 1 or cat serum linked to cellulose. After rotation of the tubes for 12 hr at room temperature, the cellulose particles were washed three times with borate-buffered (0.005 M) saline (0.15 M), pH 8.0 (BBS) and approximately 10 ng of ¹²⁵I-labelled anti-ε antibody with an activity of 50,000–100,000 ct/min were added to each tube. The tubes were again rotated at room temperature for 12 hr and the cellulose particles washed three times with BBS. The radioactivity bound to the cellulose was measured in a crystal scintillation spectrometer.

In order to determine allergenic activity in the extracts, an inhibition procedure was carried out as previously described (Ohman *et al.*, 1976). Each 1:10 (w/v) extract was dialysed against PBS and diluted to twice the original volume. Serial four-fold dilutions of the extracts were prepared and 100 µl of each diluted solution were added to duplicate test tubes containing insolubilized cat allergen 1 or insolubilized cat serum. Patient serum was then added to the test tubes. Two sera from cat sensitive patients were selected for these studies on the basis of their content of IgE antibody to cat allergen 1 (patient A) and whole cat serum (patient B). Following the initial incubation, the cellulose particles were reacted with ¹²⁵I-labelled anti-ε antibody; the amount of radioactivity (ct/min) bound to the cellulose particles was compared to that of a control in which soluble extract had not been added. In all tests the amount of radioactivity bound non-specifically was determined by the addition of serum from a non-allergic subject to the cellulose particles followed by the addition of ¹²⁵I-labelled anti-ε antibody. Variation in results between duplicate tests was less than 5%.

Percentage inhibition was calculated according to the following formula:

$$\text{Percentage inhibition} = 100 - \frac{\left[\left(\frac{\text{ct/min bound}}{\text{in presence of inhibitor}} \right) - \left(\frac{\text{ct/min bound}}{\text{non-specifically}} \right) \right]}{\left[\left(\frac{\text{ct/min bound}}{\text{in absence of inhibitor}} \right) - \left(\frac{\text{ct/min bound}}{\text{non-specifically}} \right) \right]} \times 100$$

Figure 1 shows the extent to which dilutions of four of the extracts inhibited the binding of IgE antibodies in serum from patient A to insolubilized cat allergen 1. The dilution of each extract that produced 50% inhibition was estimated. Similarly, the dilution of each extract that produced 50% inhibition of binding of IgE antibodies in serum from patient B to insolubilized cat serum was estimated. This procedure permitted a direct comparison of the various extracts for allergenic activity.

Skin testing

Prick tests were performed as previously described using extracts prepared in 50% glycerol (Ohman *et al.*, 1973). Informed consent was obtained from each volunteer subject before testing. The average diameter of the flare in mm was noted after 15 min. Reactions of less than 5 mm in diameter were disregarded.

In order to compare skin reactivity of the extracts, prick tests were performed in ten subjects with a history of allergy to cats; a 1:2, 1:10, or 1:50 dilution of crude undialysed extract was tested. The dilution chosen for testing in each subject was based on the intensity of previous prick test reactions to cat dander extracts. Subjects with the most intense responses were prick tested with a 1:50 dilution of each extract; subjects with less intense responses were prick tested with a 1:10 or 1:2 dilution of

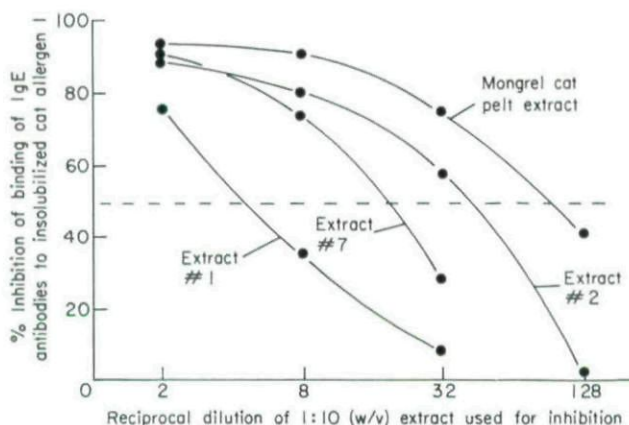


Fig. 1. Measurement of allergenic activity of cat extracts using the modified radioallergosorbent test. The reciprocal dilution of extract that produced 50% inhibition of binding of IgE antibodies (from serum of patient A) to insolubilized cat allergen 1 was determined.

each extract. The diameter of the flare of each subjects' maximum reaction was taken as 100%; other skin test reactions were expressed as a percentage of the maximum reaction. For each extract under study, the results obtained in ten subjects were combined and the mean determined.

Immunoabsorbent columns

The γ -globulin fraction (Sober & Peterson, 1958) of antiserum to cat allergen 1 and normal rabbit serum were coupled to cyanogen bromide-activated Sepharose 4B (Pharmacia Fine Chemicals, Piscataway, New Jersey) as previously described (Axen, Porath & Ernback, 1967); the resulting conjugates were poured into columns with a bed volume of 10 ml. Approximately 20 mg of protein was coupled per ml of Sepharose 4B. The various cat extracts were applied to the columns which were then eluted with PBS. The recovered protein was pooled and concentrated by lyophilization. The columns were washed with PBS, followed by 10% acetic acid. After further washing with PBS, the columns were reused.

Two immunoabsorbent columns prepared with either the γ -globulin fraction of antiserum to cat allergen 1 or normal rabbit serum (each of which had been used ten times) were tested as follows: 2 ml of cat pelt extract containing 128 μ g of cat allergen 1

and 700 µg of cat albumin was applied to the top of each column. Following elution of the void volume (5 ml), 20 ml of eluate was collected, dialysed against 0.05 M ammonium bicarbonate, and concentrated by lyophilization. Eluate from the column consisting of normal rabbit γ-globulin linked to Sepharose 4B yielded 43 µg of cat allergen 1 and 450 µg of cat albumin. Concentrated eluate from the column consisting of the γ-globulin fraction of antiserum to cat allergen 1 linked to Sepharose 4B yielded <1.8 µg of cat allergen 1 and 450 µg of cat albumin.

Results

Allergenic activity of cat epithelial extracts

Table 1 summarizes the allergenic activity of each extract as measured by prick testing and by the modified radioallergosorbent test. It is apparent that all of the extracts were capable of eliciting a positive skin test reaction in subjects with a history of allergy to cats. In addition, all of the extracts were shown to inhibit the binding of IgE antibodies to insolubilized cat allergen 1 and to insolubilized cat serum. The dilution of extract that produced 50% inhibition of binding varied considerably.

Solid and antigen content of cat epithelial extracts

There was wide variation in the total non-dialysable solid content, cat allergen 1 and cat albumin content in the ten cat epithelial extracts (Table 2). There was no apparent

Table 1. Allergenic activity of extracts derived from the domestic cat

Extract	Prick test reactions (%)*	Reciprocal dilution of extract that produced 50% inhibition of binding of human IgE antibodies to	
		Cat allergen 1	Whole cat serum
Commercial cat epithelial extract			
1	23	6	30
2	55	41	8
3	81	68	200
4	63	60	800
5	46	32	6
6	61	66	170
7	41	11	50
8	59	39	25
Mongrel cat pelt extract	77	90	260
Siamese cat pelt extract	65	32	80

* The diameter of the flare of each subjects' maximum reaction was taken as 100%; other skin test reactions were expressed as a percentage of the maximum reaction. For each extract under study, the results obtained in ten subjects with a history of allergy to cats were combined and the mean determined.

Table 2. Content of extracts derived from the domestic cat

Extract 1:10 (w/v)	Non-dialysable solid* ($\mu\text{g/ml}$)	Cat allergen 1 ($\mu\text{g/ml}$)	Cat albumin ($\mu\text{g/ml}$)
Commercial cat epithelial extract			
1	80	1	4
2	140	8	1
3	780	25	26
4	1,220	21	28
5	5,260	13	3
6	440	19	16
7	460	5	22
8	240	15	4
Mongrel cat pelt extract	650	33	18
Siamese cat pelt extract	180	14	4

* Five millilitres of each extract (1:10 w/v) were dialysed against 6 l of 0.05 M ammonium bicarbonate for 3 days with one change of dialysing solution. The dialysate was lyophilized and the dried powder weighed.

correlation between any of these measurements in eight of the extracts; commercial extracts 1 and 2 had low values for all three measurements.

Table 3 summarizes the statistical relationship between the measurements that were made on each of the extracts. There was a significant correlation between prick test reactions to the crude extracts and the reciprocal dilution of extract that

Table 3. Relationships between various properties of extracts derived from the domestic cat, correlation coefficients

Parameter measured*						
	A	B	C	D	E	F
A	—	0.868‡	0.381	-0.110	0.895‡	0.443
B	0.868‡	—	0.498	-0.033	0.956‡	0.479
C	0.381	0.498	—	-0.025	0.497	0.708†
D	-0.110	-0.033	-0.025	—	0.048	-0.118
E	0.895‡	0.956‡	0.497	0.048	—	0.520
F	0.443	0.479	0.708†	-0.118	0.520	—

* A, Prick test reactions (%); B, reciprocal dilution of extract that produced 50% inhibition of binding of IgE antibodies to insolubilized cat allergen 1; C, reciprocal dilution of extract that produced 50% inhibition of binding of IgE antibodies to insolubilized cat serum; D, non-dialysable solid content ($\mu\text{g/ml}$); E, cat allergen 1 content ($\mu\text{g/ml}$); F, cat albumin content ($\mu\text{g/ml}$).

† $P < 0.05$; ‡ $P < 0.01$.

produced 50% inhibition of binding of IgE antibodies to insolubilized cat allergen 1 (RAST 1) ($r=0.868$, $P<0.01$). There was no correlation between prick test reactions and the reciprocal dilution of extract that produced 50% inhibition of binding of IgE antibodies to insolubilized cat serum (RAST 2). The total non-dialysable solid content of the extracts did not correlate with any other parameter. Cat allergen 1 content exhibited a high degree of correlation with two measures of allergenic activity; prick test reactions ($r=0.895$, $P<0.01$) and RAST 1 activity ($r=0.956$, $P<0.01$). The cat

Table 4. Effect of absorption of cat extracts with antiserum to cat allergen 1

Extract	Percentage inhibition of binding of IgE antibodies to insolubilized			
	Cat allergen 1			Whole cat serum
	Patient A	Patient B	Patient C	Patient B
Commercial cat dander extracts				
(1) Absorbed*	0	3	0	28
Control†	6	0	0	36
(2) Absorbed	9	6	7	—
Control	57	47	40	—
(3) Absorbed	26	2	3	69
Control	73	75	62	70
(4) Absorbed	0	2	8	95
Control	69	73	56	82
(5) Absorbed	5	10	7	42
Control	66	62	53	48
(6) Absorbed	0	5	1	68
Control	70	74	54	78
(7) Absorbed	6	9	5	—
Control	38	46	30	—
(8) Absorbed	4	31	6	—
Control	62	65	53	—
Mongrel cat pelt extract				
Absorbed	0	3	0	68
Control	75	66	63	82
Siamese cat pelt extract				
Absorbed	51	37	23	—
Control	67	60	47	—

* Extract was passed through an immunoabsorbent column consisting of the γ -globulin fraction of antiserum to cat allergen 1 coupled to Sepharose 4B as described in Methods.

† As a control, extract was passed through an immunoabsorbent column consisting of normal rabbit γ -globulin coupled to Sepharose 4B as described in Methods.

Table 5. Effect of absorption of cat extracts with antiserum to cat allergen 1

Extract	Prick test reactions, mean diameter of the flare (mm)					
	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6
Commercial cat dander extract						
(1) Absorbed*	0	0	0	18	0	9
Control*	0	0	0	25	0	11
(2) Absorbed	0	0	0	22	6	10
Control	11	18	20	27	5	30
(3) Absorbed	7	6	15	22	13	20
Control	14	17	30	25	18	25
(4) Absorbed	0	0	0	19	0	14
Control	18	10	28	19	18	20
(5) Absorbed	0	0	0	20	0	—
Control	10	6	25	20	13	—
(6) Absorbed	0	0	20	22	0	18
Control	15	9	20	20	18	19
(7) Absorbed	0	0	20	20	0	0
Control	5	6	20	15	10	5
(8) Absorbed	11	0	15	18	0	20
Control	14	8	20	25	15	21
Mongrel cat pelt extract						
Absorbed	0	0	0	20	0	15
Control	10	18	26	19	22	21
Siamese cat pelt extract						
Absorbed	10	0	0	20	0	10
Control	10	7	0	19	13	15

* See footnotes to Table 4.

albumin content of the extracts correlated only with RAST 2 activity ($r=0.708$, $P<0.05$), and not with prick test reactions or RAST 1 activity.

Effect of absorption of cat extracts with antiserum to cat allergen 1

Of each extract 0.5 ml was applied to an immunoabsorbent column made with the γ -globulin fraction of antiserum to cat allergen 1 and to a column made with normal rabbit γ -globulin. The effluent from each column was collected, concentrated by lyophilization, and resuspended in 0.5 ml of PBS. Effluent from the column containing Sepharose-bound antibody to cat allergen 1 was compared to effluent from the column containing Sepharose-bound normal rabbit γ -globulin.

For assay of allergenic activity by the modified radioallergosorbent test, an aliquot of the effluent was diluted 1:4 and 100 μ l of this solution was tested for its ability to inhibit binding of human IgE antibodies to insolubilized cat allergen 1. Three sera, from cat sensitive patients A, B, and C, were used. The results are sum-

marized in Table 4. Most of the extracts that were absorbed with normal rabbit γ -globulin produced greater than 40% inhibition. Extract No. 1 produced little or no inhibition probably due to the low levels of allergen in the starting material. Almost all of the extracts that were absorbed with antiserum to cat allergen 1 produced little or no inhibition with the exception of Siamese cat pelt extract which inhibited from 23 to 51%.

The absorbed extracts were also tested for their ability to inhibit the binding of IgE antibodies in serum of patient B to insolubilized cat serum; absorption of the extracts did not significantly affect their ability to inhibit this system.

For assay of allergenic activity by the prick test, the eluate from the immunoabsorbent columns was diluted 1:8. Prick tests were performed in six subjects with known sensitivity to cats (Table 5). Extracts that were absorbed with normal rabbit γ -globulin produced a mean flare reaction of 15 mm. The same extracts, absorbed with the antiserum to cat allergen 1, produced a mean reaction of 8 mm. In several patients (No. 4 and 6) absorption of many of the extracts with antiserum to cat allergen 1 did not reduce or eliminate the prick test reactions. These findings suggest that these two patients may have been primarily sensitive to allergens other than cat allergen 1. Absorption of the Siamese cat pelt extract with antiserum to cat allergen 1 had a variable effect on the prick test reactions to this extract.

Discussion

This report compares allergenic and immunochemical properties of commercial cat epithelial extracts and two extracts of cat pelt prepared in this laboratory. All of the extracts were shown to contain allergenic activity in varying amounts. Allergenic activity was measured by an *in vivo* and an *in vitro* method. In the former, average prick test reactions to each extract in ten randomly selected cat sensitive subjects were calculated. In the latter, a modified radioallergosorbent test was used; dilutions of extract were used to inhibit binding of IgE antibodies to insolubilized cat allergen 1 (RAST 1) and to insolubilized whole cat serum (RAST 2). It is probable that RAST 1 measures only the presence of cat allergen 1 in the extracts (Table 4). Cat allergen 1 has previously been isolated from cat pelts and some of its properties have been described (Ohman *et al.*, 1974). It has previously been shown that RAST 2 measures the presence of one or more serum components that have a higher molecular weight and a slower electrophoretic mobility than cat allergen 1 (Ohman *et al.*, 1976).

Cat allergen 1 and cat albumin were measured quantitatively in each extract by radial immunodiffusion. Cat albumin was selected for measurement because it has been implicated as an allergen by other investigators (Brandt, Ponterius & Yman, 1973), although our previous studies suggested that it is not a major allergen (Ohman *et al.*, 1976).

Two parameters of allergenic activity, prick test reactions and RAST 1 activity, correlated strongly with each other (Table 3). Both parameters also correlated individually with cat allergen 1 content. RAST 2 activity, which measures cat serum allergens, correlated with cat albumin content but did not correlate with any other parameter. It has previously been shown that only a minority of cat sensitive subjects have serum IgE antibody that binds significantly to insolubilized whole cat serum (Ohman *et al.*, 1976).

Absorption of commercial cat dander extracts and mongrel cat pelt extract with antiserum to cat allergen 1 significantly reduced RAST 1 activity and also reduced skin

reactivity to most of the extracts in four out of six subjects. The effect of absorption on the allergenic activity of commercial extract No. 1 could not be assessed because control levels of allergenic activity in this extract were minimal (Tables 4 and 5). Absorption of the Siamese cat pelt extract with antiserum to cat allergen 1 reduced RAST 1 activity to a minimal extent (Table 4). The explanation for this observation is uncertain but may indicate that cat allergen 1 present in Siamese cat pelt extract binds less firmly to rabbit antibody than cat allergen 1 present in the other extracts.

Previous studies have shown considerable heterogeneity in allergens derived from the domestic cat (Brandt *et al.*, 1973; Holford-Strevens, 1973; Ohman *et al.*, 1973, 1974; Stokes & Turner, 1975). Some of these allergens are derived from cat serum (Brandt, *et al.*, 1973; Ohman *et al.*, 1976). Others, such as cat allergen 1, are found predominantly in the skin of the animal (Ohman *et al.*, 1974). The present report provides strong evidence that cat allergen 1 is of major importance for most cat sensitive subjects.

Studies from Scandinavia have shown a wide variation in the allergenic potency of commercial cat epithelial extracts (Brandt *et al.*, 1973; Varga & Ceska, 1972). The present study suggests that the measurement of a single allergen, cat allergen 1, may be used to standardize the allergenic activity cat epithelial extracts.

Acknowledgments

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