

Identification and Clinical Significance of Allergenic Molecules of Cat Origin

Part of the DAS 76* Study

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Freeze-dried extracts from cat dander and the corresponding rabbit antibodies were used for establishing the CIE reference pattern for cat dander extracts. Anti-Cat Ag 1 and anti-cat albumin were used for identification of the corresponding antigens. CRIE on sera from selected groups of American and Danish cat-allergic patients demonstrated antigen-specific IgE binding to 10 of 15 cat dander antigens (Cat Ag 1 being the major allergen). Only minor differences were found between the two groups. Four of these allergens were serum proteins. Variable amounts of many of the 10 allergens were measured by QIE in saliva, serum, urine and three cat pelt extracts. However, extremely wide ranges for content of the serum allergens and the non-serum allergens were found. This was exemplified by an albumin/cat Ag 1 ratio between 1 and 400, smallest in cat dander. Immunoabsorption using anti-cat dander, anti-cat albumin and anti-Cat Ag 1 indicated that the anti-cat dander, anti-cat albumin, and the anti-Cat Ag 1 absorbed approximately 90 %, 25 %, and 56 %, respectively, of the dander RAST activity, and 87 %, 11 %, and 45 %, respectively, of the saliva RAST activity, confirming the major importance of Ag 1. It is concluded that cat allergenic extracts should contain only modest amounts of serum albumin and other serum-derived antigens and that any relevant standardization must include quantification of at least Cat Ag 1 and cat albumin.

Key words: cat allergenic molecules; source materials; CRIE; QIE; standardization.

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Among animals giving rise to type 1 allergic reactions, cat seems to be one of the most important and it has been shown that in house dust, cat allergens (Ag) as well as mite allergens are the sources which most often give rise to "house dust allergy" (1, 18, 19). In a series of investigations, Ohman and coworkers studied

patients' reactions to cat pelt extracts (16). From this material a highly allergenic fraction named Cat Ag 1 containing approximately 10 % (w/w) Cat Ag 1 molecules was prepared and characterized (14, 15). This material was used in a clinical trial, versus placebo, on asthmatic cat-allergic patients. The study, although performed on few patients, revealed a significantly better effect of the specific immunotherapy with the fraction containing cat Ag 1 than the effect obtained with placebo (21). Furthermore, the presence of Cat Ag 1 molecules in various fluids and tissues of cat origin was studied (18).

Independently, identification of allergenic molecules from an extract of cat hair and dander was included in the Danish Allergen

* DAS 76 (Danish Allergen Standardization 1976) was a multicentre study comprising the following allergy clinics and laboratories: Allergologisk Laboratorium (ALK), Copenhagen, Frederiksborg Amts Centralsygehus, Hillerød, Esbjerg Sygehus, Københavns Amts Centralsygehus, Lyngby, Holbaek Sygehus, Middelfart Sygehus, Nykøbing Falster Sygehus, Proteinlaboratoriet, Copenhagen, Rigshospitalet, Copenhagen, Ringkøbing Sygehus, Thisted Sygehus, Viborg Sygehus.

Standardization programme - DAS 76. One major allergen was detected in this extract as well as several other IgE-binding molecules (13). The presence of these allergens in urine, serum and saliva was also investigated (13). Finally, a preliminary study showed that the major allergen was immunochemically identical to Cat Ag 1 (13).

The aim of this study is to identify important allergenic molecules of cat origin. Since the natural source of cat allergenic material is hair and dander, this material is used as the basis in this study, but possible co-existence of the allergenic molecules from dander in other types of allergenic extracts like urine, saliva and serum will also be investigated. The allergen identification study will be performed as two independent studies using sera from selected American and Danish patients. The relative allergic significance of Cat Ag 1 and albumin in the various cat allergen sources will be studied by immunoabsorption techniques. The relative concentrations of Cat Ag 1, cat albumin and other identified allergens in a number of cat allergen extracts and biological fluids will be estimated and discussed in relation to standardization of cat allergenic extracts.

MATERIAL AND METHODS

Patients

A total of 17 American cat-allergic patients were selected according to the criteria given elsewhere (21). A total of 12 Danish cat-allergic patients were selected according to the DAS 76 criteria. In order to be eligible the patients should have had type 1 allergy for at least 5 years; obtain two scores in one of the following tests, or a sum of at least four scores in the four tests: case history, provocation test, skin test, and radioallergosorbent test (RAST) (for details, see ref. 9). A pool of sera from 1000 normal individuals was used as normal serum. In the RASTs included in the immunoabsorption experiments (see below), a pool of the 12 sera of the Danish cat allergics was used.

Antigens

Cat hair and dander was a mixture obtained from several breeds of *Felis domesticus* from the Royal Veterinary School, Copenhagen, Denmark. The samples were delivered to our laboratory and stored in closed containers at -20°C until used.

Dialysed, freeze-dried extract of this material denoted Cat 0580 was specially prepared by K. Danielsen for the Joint Steering Committee for Allergen Standardization Course held at the Protein Laboratory in August 1980. The procedure was slightly modified from that previously described for grass pollens (11). Briefly, the method consisted of extraction of hair and dander material (1:5, w/v) at 5°C for 20 h with a buffer solution (0.125 M NH_4HCO_3 and 0.015 M NaN_3 , adjusted to pH 7.5), filtration, centrifugation ($50,000 \times g$, 1 h, 0°C), dialysis (3×24 h, 5°C , using Union Carbide (Wisking) dialysis tubing with limiting pore size corresponding to approximate molecular weight < 5000), and freeze-drying. Solutions were freshly prepared in 0.15 M sodium chloride/glycerol (50 %, v/v) for use in quantitative immuno-electrophoresis (QIE) technique. Analysis of Cat 0580 by rocket immunoelectrophoresis (RIE) showed a content of 8.6 μg Cat Ag 1 and 13 μg cat albumin/mg freeze-dried material using Cat Ag 1 (EAP II) "new standard" and purified cat albumin as a reference (17). A similarly prepared cat dander extract - denoted Cat 0475 and used in the original DAS 76 study - was used for some identity studies.

Cat extract 160 was prepared from cat pelts; the ratio (w/w) between Cat Ag 1 (new standard) and cat albumin was 0.039 (private communication with Dr John Ohman). The extract was dissolved in 0.15 M NaCl/glycerol (50 %, v/v) and stored at -20°C . Analysis of Cat extract 160 by RIE showed a content of 5.6 μg Cat Ag 1 (new standard) and 270 μg cat albumin/mg freeze-dried material.

Cat extract 206 was obtained from cat pelts and prepared as described elsewhere (2). The final extract was dissolved in 0.15 M NaCl/glycerol (50 %, v/v) and stored at -20°C . Analysis of Cat extract 206 by RIE showed a

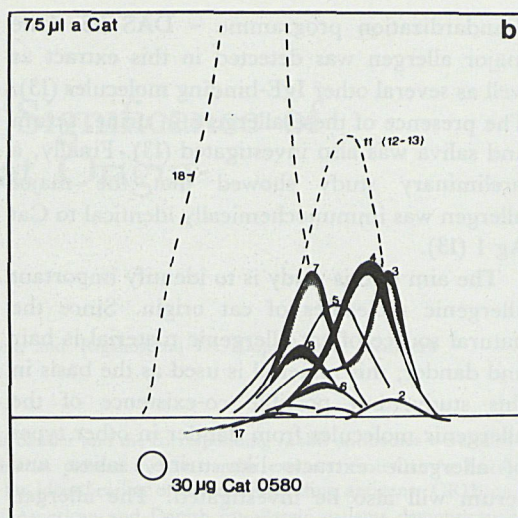
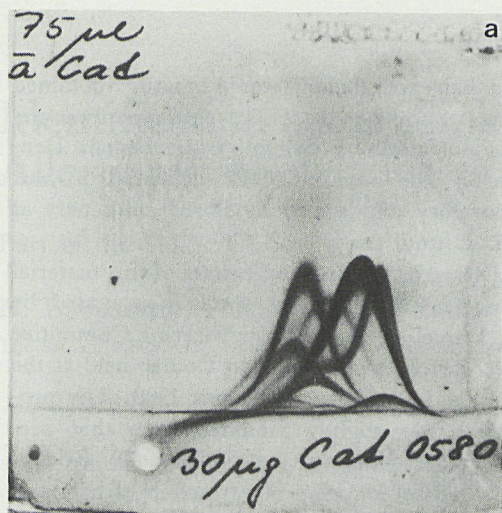


Fig. 1a. Crossed immunoelectrophoretic (CIE) analysis of the reference cat dander extract, Cat 0580, against anti-cat antibodies. CIE was performed in 1% agarose gel containing 0.073 M TRIS, 0.024 M barbital, 0.0006 M calcium lactate, and 0.0003 M sodium azide (pH 8.6, 25°C) at 15°C using 10 V/cm for 30 min in the first dimension and 2 V/cm for 15 h in the second dimension (12). Anodes to the right: first dimension, and at the top: second dimension. Amounts and types of antigen and antibody are indicated in the figure.

Fig. 1b is a drawing of the reference precipitation pattern with classification numbers for the precipitates and corresponding antigens.

content of 0.22 µg Cat Ag 1 (new standard) and 81 µg cat albumin/mg freeze-dried material.

Cat extract 149 was prepared from cat pelts. The ratio (w/w) between Cat Ag 1 (new standard) and cat albumin was 0.012 (private communication with Dr John Ohman). The extract was dissolved in 0.15 M NaCl/glycerol (50%, v/v) and stored at -20°C. Analysis of Cat extract 149 by RIE showed a content of 1 µg Cat Ag 1 (new standard) and 71 µg cat albumin/mg freeze-dried material.

Protein determination by means of Lowry (10) using bovine serum albumin as a reference revealed our working solutions for Cat 0580, 160, 206, and 149 to be 3.1, 4.5, 2.4, and 3.6 mg protein/ml respectively.

Saliva was prepared by Brighton and co-workers (5). A specific pathogen-free cat was anaesthetised with 600 µl of 1% Sernylan (Bio-Centric, Labs, Missouri, USA) and saliva collected from the mouth with a Pasteur pipette. The procedure was repeated on different days until 25 ml of saliva had been collected. The

pool of saliva was dialysed against 0.02 M ammonium bicarbonate and lyophilised in 1 ml aliquots. The material was kindly supplied by A. Ford, National Institute of Biological Standards and Control, London, UK.

Cat urine was obtained from the Royal Veterinary School, Copenhagen, Denmark and the urine proteins were prepared by ammonium sulphate precipitation according to (20).

Cat serum was obtained from the Royal Veterinary School, Copenhagen, Denmark.

Antigen standards. Cat Ag 1 (EAP II) and purified albumin was obtained from Dr John Ohman.

Antibodies

Anti-cat hair and dander antibodies were raised by immunizing three rabbits with the DAS cat hair and dander extract described in (13). The immunization and subsequent isolation of the immunoglobulins were performed according to

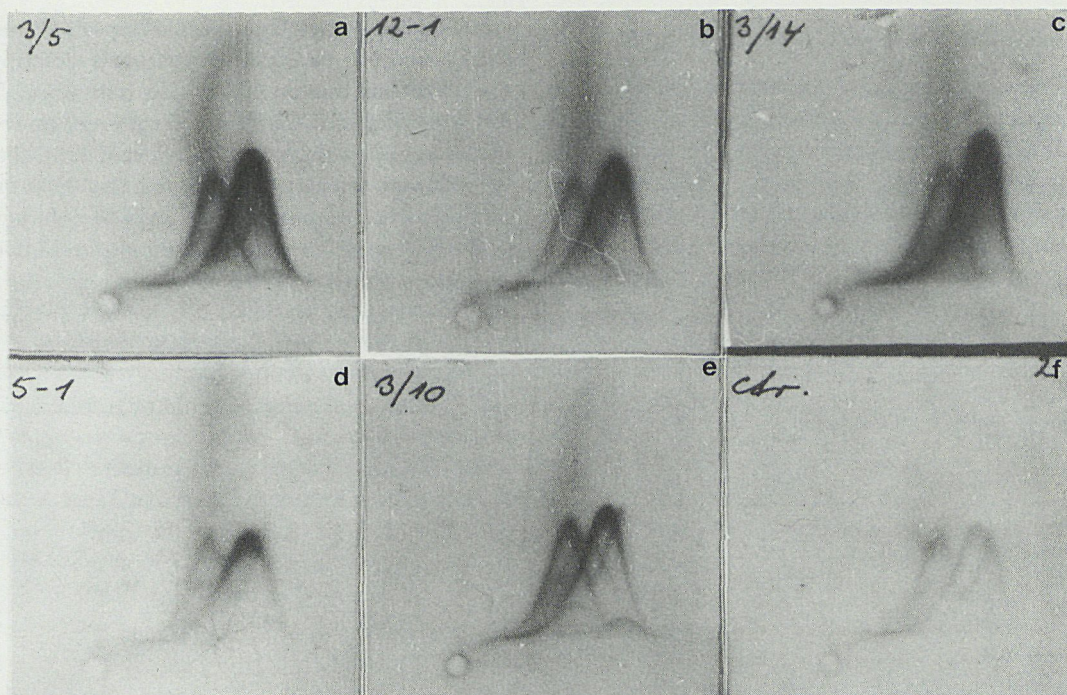


Fig. 2. CRIE performed by using the sera of five different cat-allergic patients (identification indicated) and a control serum (CTR) from a pool of 1000 normal individuals. The autoradiography was carried out for 7 days. The corresponding Coomassie Blue-stained CIE reference pattern is shown in Fig. 1.

Harboe & Ingild (7). During this isolation the concentration of the immunoglobulins was increased about five-fold.

Sheep anti-Cat Ag 1 (Sh 1388) as described in (2) and anti-cat albumin (Ra 7-7) were kindly supplied by Dr John Ohman.

Antibodies against extracts of pollens from false oat were prepared as described in (12).

The antibodies were stored at 4°C in a solution containing 0.015 M sodium azide and 1% (v/v) approtinin (NOVO, Mainz, FRG) to avoid proteolytic degradation of the antigens in the second dimension gel immunoelectrophoresis (12).

Quantitative immunoelectrophoresis

QIE method (2), including rocket-immunoelectrophoresis (RIE), crossed- (CIE) crossed-line- (CLIE), crossed with intermediate gel, and crossed radio-immunoelectrophoresis (CRIE), were performed as described recently

(12) and as specified in the figures.

In CRIE the autoradiography was performed from day 0 to day 3, from day 3 to day 4, and from day 4 to day 11, corresponding to 3, 1, and 7 days respectively. The degree of radiostaining of individual precipitates was evaluated by visual comparison of CRIE autoradiographies (140 nCi ^{125}I -anti-IgE) with autoradiographies of equal-sized Mancini rings with two-fold serial dilutions of ^{125}I -anti-IgE (23). For each of the nine Mancini rings a score number N_x was calculated from the equation:

$$N_x = \frac{256}{D}$$

where D is the dilution factor for ^{125}I -anti-IgE. For each CRIE autoradiography the score N_x of the individual precipitates was evaluated independently by at least two persons. The scores were corrected for the radiostaining obtained by using a pool of sera from normal individuals. Examples of CRIE are shown in Fig. 2.

Immunoabsorption experiments

These were performed in order to determine the relative allergenic significance of Cat Ag 1 and albumin as compared with cat hair and dander (0580) and saliva. Antibodies (3 ml of anti-Cat, anti-Cat Ag 1, anti-Cat albumin and anti-false oat) were coupled to cyanogen bromide-acti-

vated Sepharose 4B (Pharmacia, Uppsala, Sweden) according to the manufacturer's description. Identical immunoabsorption columns (3.5 ml) were prepared, equilibrated with sodium citrated buffer (ionic strength 0.05, pH 6.8). To the columns were applied 0.5 mg Cat 0580 or cat saliva and consecutive, stepwise elution performed with 3.5 ml of sodium citrate buffer

Table 1

IgE-binding to individual antigens of cat dander. CRIE-scores for 17 American and 12 Danish cat-allergics

Pt. ID	Antigen Nos.**									
	2 (1.19)	3 = Cat Alb* (1.09)	4 = Cat Ag1 (1)	5 (0.86)	6 (0.79)	7* (0.69)	8 (0.74)	11 (0.91)	17* (0.52)	18 (0.48)
3-1			16					4		
4-1			48					4		
5-1			48			48	4	16	48	
6-1			48				4	16		
7-1	4		112			48	4	16	48	
8-1			48							
9-1		16	16							
10-1			48							
11-1		48	48				8	8		
12-1	4		112		4	16	8	16	16	
13-1			48					8	16	
14-1	4	16	48			16	8	16	16	
15-1	4	16	48		16	16		8		
16-1	8		48				8	4		
17-1	4		48							
18-1	8		48		16	48	8	8	16	
19-1	8		16				8			
Σ CRIE scores	44	96	848	0	36	192	60	124	160	0
2/4	4		112		32	48		16		8
2/9				16						
2/15			48							
2/31	16		240				4	8		
2/33	8	48	16		4	112		32	16	
2/37			16							
3/17	4		112		4		8	8	16	
3/5	4		240		4	48	8	32		
3/6			16				8			
3/10	32		48	32	8	16	32	16		
3/14	8		112			16	16	8		
3/15			16				8			
Σ CRIE scores	76	48	976	48	52	240	84	120	32	8

* The CRIE-scores were reduced with 16, which were the scores obtained using the control pool from 1000 normal individuals for Ags 3, 4, 7, and 17.

** Migration velocity relative to cat Ag 1 in 1% (w/v) agarose gel in parenthesis.

(ionic strength 0.05, pH 6.8) and 3.5 ml sodium citrate buffer/sodium chloride (ionic strength 0.5, pH 6.8). Fractions (0.5 ml) were collected, adjusted to pH 7.5–8.5 and two activated paper discs added according to Ceska et al. (6) and radioallergosorbent test (RAST) (22) was subsequently performed using a pool of sera from the Danish cat allergics. When applying cat dander or cat saliva, the percentage of non-column bound activity eluted from the anti-Cat column relative to that from the anti-false oat column indicates the capability of the anti-Cat to bind allergens from cat hair and dander or from saliva. The percentages of column-bound activity eluted from the anti-Cat 1 and anti-Cat albumin columns relative to that eluted from the anti-Cat column give the significances of Cat Ag 1 and albumin relative to Cat 0580 and to saliva.

RESULTS

Antigens identified in extracts of cat hair and dander

Fig. 1a shows the CIE reference precipitation pattern obtained with Cat 0580 against anti-Cat extract. Appropriate separation of the antigens was obtained by varying the time for electrophoresis in the first dimension, and reasonable antigen/antibody balance was attained by varying the proportion of the reactants. By fixing and staining the first dimension gel it was confirmed that all proteins/staining components were included in the anodical portion of the gel transferred to the second dimension gel. Very weak precipitates were detected either by increasing the amounts of antigen and antibody and over-exposing a negative of the resultant stained plate, or after performance of CRIE, and these precipitates are indicated in the drawing (Fig. 1b) by dashed lines. The ability of the rabbit antibodies used to precipitate all potential allergens included in extract of cat hair and dander was controlled by means of immunoabsorption technique (see below).

The analyses revealed a total of 15 antigens (Fig. 1b) which all moved towards the anode.

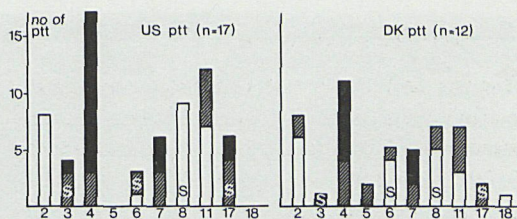


Fig. 3. Distribution frequency of IgE antibody responses to individual allergens obtained by CRIE. The allergens are numbered according to the CIE reference pattern (Fig. 1). Ag 3 is equal to cat albumin and Ag 4 equal to Cat Ag 1. CRIE scores (□, 1–10; ▨, 11–40; ■, 41–256) to individual allergens of cat dander were obtained by analysing the sera from 29 cat-allergic patients. The data are corrected for non-specific IgE binding(s). For determination of CRIE scores, see text.

The electrophoretic mobilities of most of the antigens relative to Ag 4 are given in Table 1.

CRIE experiments

Fig. 2 shows representative allergoprints using sera from two American and three Danish cat-allergic patients and a negative control. Rabbit Ig-precipitated Ag 4 binds IgE antibodies from all the patients. The control plate, comprising the pool of sera from Danish normal individuals, shows only a faint radiostaining to precipitated Ag 3, Ag 4, Ag 7 and Ag 17 corresponding to 16 CRIE scores. The five allergoprints differ both qualitatively and quantitatively in their binding of IgE to the various cat antigens present in the CIE reference precipitation pattern.

The binding of IgE from all patients towards the individual antigens precipitated was semi-quantified. The corrected CRIE scores for each of the 10 antigens towards which we observed IgE binding are given in Table 1. Ag 4 exhibited specific IgE binding with all patients' sera except one. Other antigens (e.g. 2, 8, and 11) showed specific IgE binding in more than half of the patient sera and less than half of the allergic individuals responded to Ags 3, 5, 6, 7, 17 and 18.

Figs. 3a and b are allergograms illustrating the distribution of IgE binding towards the various antigens. Ranking the allergens according to their total CRIE scores (cf. Table 1)

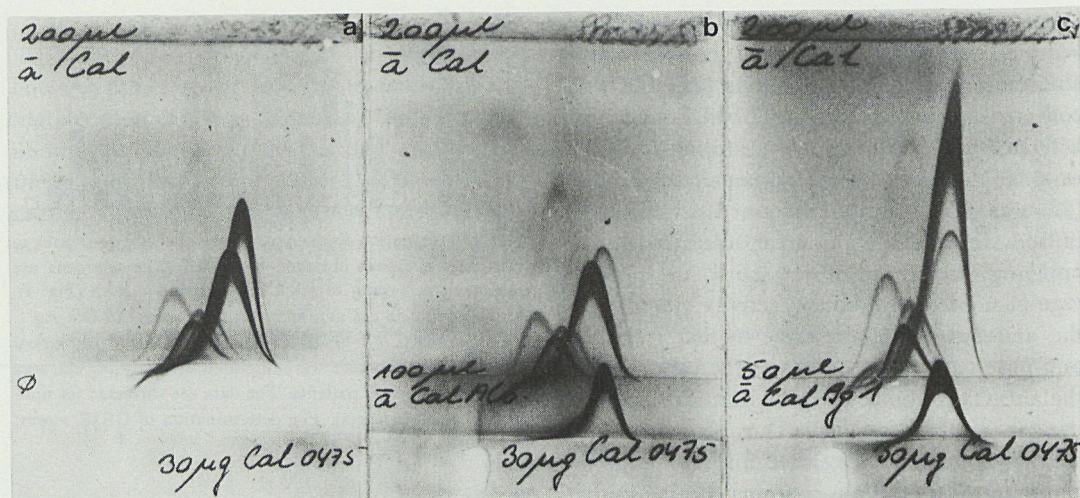


Fig. 4. Immunochemical identification of cat albumin and Cat Ag 1 in cat dander extract. CIE analysis of Cat 0476 against anti-cat antibodies with anti-cat albumin (b) or anti-Cat Ag 1 (c) included in the intermediate gel. Comparison of b and c with a identifies those precipitates in a that correspond to cat albumin and Cat Ag 1. The simultaneous increase of the cat albumin precipitate in c, together with the precipitation of Cat Ag 1 in the intermediate gel, is due to the partial identity between cat albumin and the sheep albumin contained in the anti-Cat Ag 1 serum.

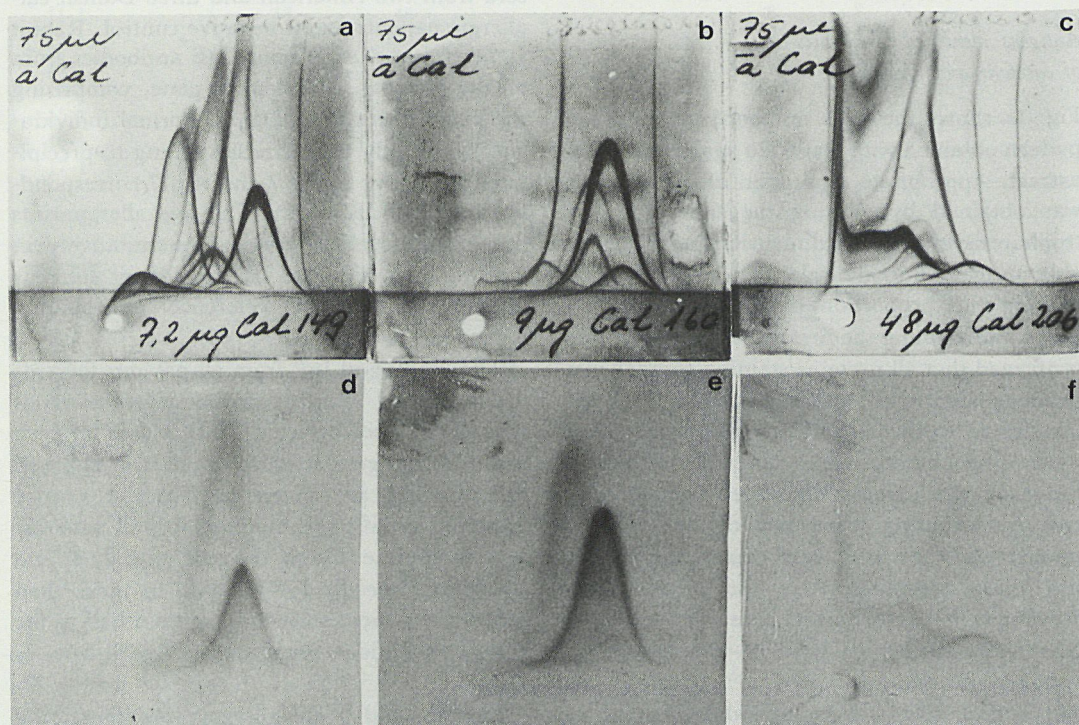


Fig. 5. CIE (upper part) and CRIE (lower part) analyses of three different cat extracts. A pool of 12 sera from cat-allergics was used in CRIE. Amounts and types of antigens and antibodies are indicated in the figures. Experimental conditions for CIE as described in legend to Fig. 1.

produced a significant correlation between the American and Danish data ($r = 0.64$, $P < 0.05$). The summed CRIE response towards the serum antigens (568 scores) was much lower than that of the other antigens (2676 scores).

Identification of cat allergenic molecules

In Fig. 4, CIE with intermediate gel demonstrates that Ag 4 is identical to Cat Ag 1 and Ag 3 is identical to Cat albumin. As judged from the serum origin, the electrophoresis mobility and morphology, Ag 17 might well be cat immunoglobulin.

Relative quantities of identified cat allergens in other cat allergenic extracts

Fig. 5 shows the results of CIE and CRIE analyses using the pool of sera from cat allergic patients on three different cat extracts. Quite different CIE precipitation patterns appeared, and when compared with the corresponding CRIE analyses it is revealed that the amount of IgE binding antigens differs significantly from extract to extract. By performance of addition-CIE (examples shown in Fig. 6) some of the

antigens in the three cat extracts were quantified relative to antigens from Cat 0580. Quantification of Cat Ag 1 and Cat albumin was also performed using RIE, including mono-specific antibodies in the gel (see Material and Methods). Table 2 gives the relative quantities of identified allergens including Cat Ag 1 and albumin relative to the same antigens in Cat 0580 in the extracts studied. The ratio between Cat Ag 1 and cat albumin was 60–70 times less for Cat 149 and 160 and 300 times for Cat 206 than for the cat dander reference.

Relative quantities of identified allergens in saliva, serum and urine

CLIE experiments were performed incorporating cat saliva, serum, and urine in the line gels. Presence was revealed either directly by fused line and crossed precipitates or indirectly by absence of *in situ* absorbed allergens. Some experiments are shown in Fig. 7. Cat saliva contained all the dander allergens except Ags 6, 11, 17 and 18; cat serum contained Ags 3, 6, 8 and 17; and cat urine contained only Ags 2, 3 and 8. Quantification of Cat Ag 1, cat albumin and other identified allergens relative to Cat

Table 2

Relative quantities by means of QIE of verified cat allergenic molecules in various preparations defining Cat 0580 as a standard, and normalizing the cat albumin content

Ag No.	Cat 0580 def. std.	Cat 206	Cat 149	Cat 160	Cat saliva	Cat serum	Cat urine
2	100	3.3	0.46	3.67	820	–	17.7
Cat Alb = 3	100	100	100	100	100	100	100
Cat Ag1 = 4	100	0.32	1.44	1.48	68	–	– ²
5	100	–	0.12	0.27	5152	–	–
6	100	0.19	–	0.51	–	–	–
7	100	–	0.62	–	488	–	–
8	100	–	0.21	0.59	1578	16.1	23.0
11	100	Trace	Trace	Trace?	–	–	–
17	100	Trace?	Trace	–	–	133.6	–
18	100	–	–	–	–	–	–
Cat Ag 1 ¹ (weight %)	67	0.27	1.41	2.08 (RIE)			
Cat Alb		0.21	0.95	1.00 (QIE)			

¹ The ratio between the absolute amounts of Cat Ag 1 and cat albumin (see Material and Methods).

² Another urine preparation contained traces of Cat Ag 1.

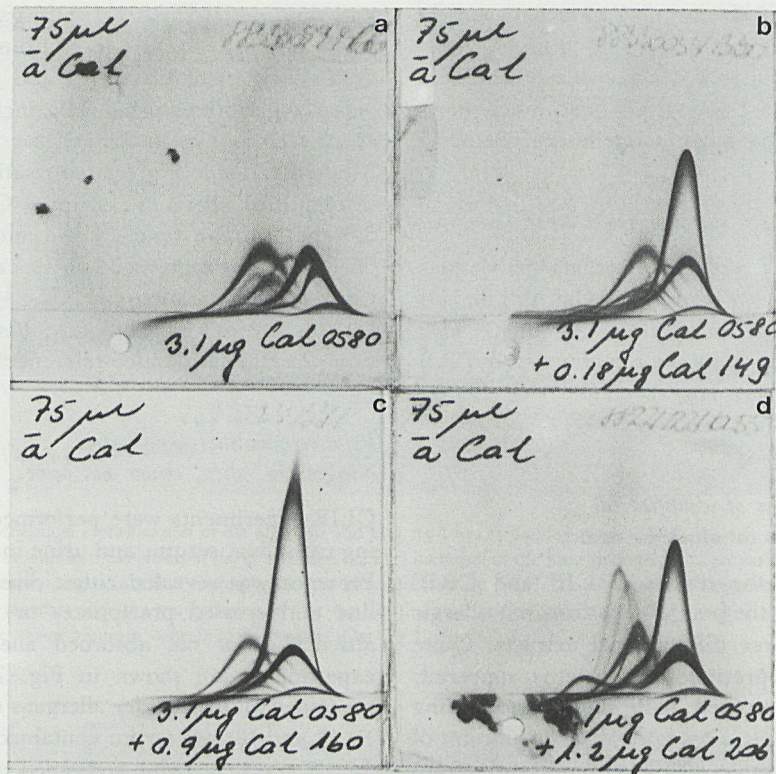


Fig. 6. CIE analyses of Cat 0580 and various amounts of other cat preparations added. Amounts and types of antigens and antibodies are indicated in the figures. The arrows indicate the precipitates corresponding to cat albumin. Comparisons of b, c and d with a show the increase of some precipitates – in particular cat albumin – indicating a content of these antigens in the preparations applied besides Cat 0580.

0580 was further performed using addition-CIE (see Fig. 6 and Table 2). The ratio between Cat Ag 1 and cat albumin was found to be within the same range for the cat hair and dander reference extract and saliva.

The allergenic significance of Cat Ag 1 and cat albumin in cat hair and dander and saliva

The results of direct RAST performed with fractions eluted from columns of immobilized anti-Cat Ag 1, anti-cat albumin, anti-cat dander extract, and anti-grass pollen extract are shown in Table 3. The allergenic activity of the anti-grass column was defined as 100 %. The results reveal that approximately 10 % of the total allergenic activity from cat dander and saliva was not bound by the rabbit antibodies; that approximately half of the activity in dander and

saliva was due to Cat Ag 1, and that 10–25 % was due to cat albumin.

DISCUSSION

Crossed immunoelectrophoretic analysis revealed that the cat dander extract 0580 is composed of 15 antigens, among which 12 were easily

Table 3
Non-absorbed RAST activity

Applied	Immobilized antibodies against			
	Cat dander	Cat Ag 1	Cat albumin	False oat
Cat dander	10	44	75	100*
Cat saliva	13	55	89	100*

* The integrated RAST activity eluted was defined as 100 %.

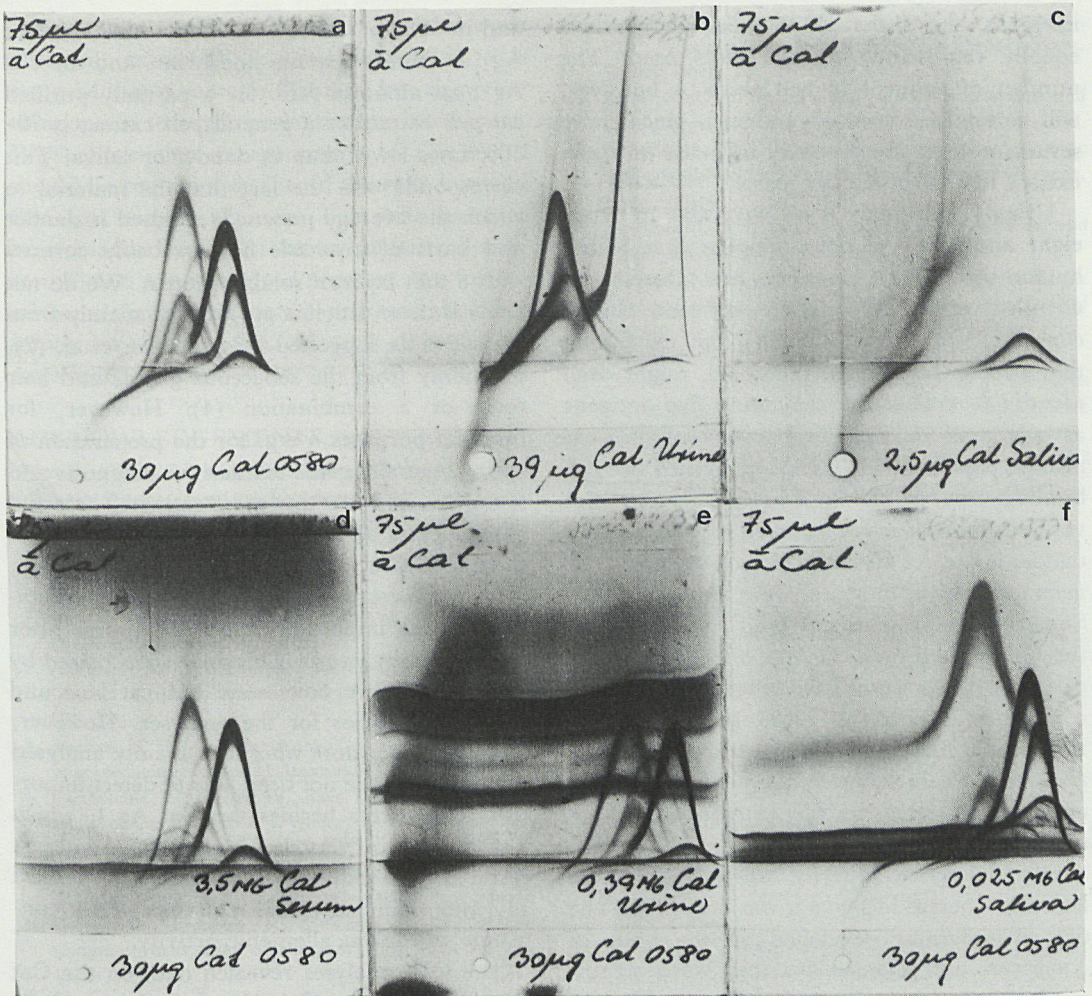


Fig. 7. Identification of cat allergens in preparations of cat urine, cat saliva and cat serum using CIE and CLIE techniques. The amounts and types of antigens and antibodies are indicated in the figures. a, b, and c are CIE analyses of Cat 0580, cat urine, and cat saliva, respectively. d, e, and f are *in situ* adsorption of antigens common for Cat 0580 and the serum urine and saliva antigens incorporated in the line gel. Elevated (fused-line and -crossed precipitates) or removed precipitates indicate antigens included in both Cat 0580 and the various fluids.

visible using standard experimental conditions. Previous studies using similar preparations and identical antibodies detected a total of 18 antigens (13). Using the same methodology, Anderson & Baer were able to detect eight antigens in a cat pelt extract (Cat 206) using anti-cat pelt antibodies (2). We have also analysed pelt extracts including Cat 206 and 149 and found at least 10 antigens using our anti-cat hair and dander antibodies. In the partly purified Cat 160 only six antigens were visible.

It is likely that the 15 components do not represent all the antigens of dander that we were able to extract. There may still be antigens present in so low amounts that the immunized rabbits make little or no antibodies. However, when we measured the capability of the antibodies to bind the allergenic activity (RAST) of the dander extract, only 10 % could not be accounted for. This control experiment will, of course, depend on two factors: 1) that the IgE serum pool represents IgEs against all potential

allergens, and 2) that all potential allergens are equally represented on the discs used. The number of antigens in pelt extracts, however, will certainly exceed 15 antigens, since more serum proteins are probably included in a pelt extract than in a dander extract.

Using CRIE analysis we were able to detect eight and 10 cat dander antigens that bound human IgE from American and Danish cat allergics, respectively. These antigens can be considered to be cat allergens, but expanding the number of patients studied might well identify several of the remaining five antigens as allergens in small subgroups of the cat allergic population. We semi-quantified the IgE binding towards the individual allergens and verified that the major allergen of cat hair and dander is Cat Ag 1 (13, 15). However, other antigens not present in serum were also important. Some serum proteins did bind IgE, but from the CRIE data only 20 % of the IgE binding capacity (the accumulated allergenic activity) could be ascribed to those serum proteins represented in the dander extract. This was apparently mainly due to the albumin, as was also demonstrated in the immunoabsorption experiments where approximately 25 % of the dander allergenic activity was associated to albumin whereas in saliva it was only 10 %. The two allergograms developed on the basis of American and Danish patients were significantly similar in spite of the low number of patients studied. The accumulated allergenic activity of Cat Ag 1 relative to the allergenic activity in dander (Cat 0580) was 68 %, which should be compared with immunoabsorption data giving 55 %. From these data we assume that although 10–25 % are due to serum proteins and 50–67 % are due to Cat Ag 1, a still significant amount of cat allergenicity is due to other dander molecules represented by Ags 2, 5, 7, 11 and 18, and also by Cat Ags 6, 8 and 17 which, however, are also present in serum. Thus from the present and several other studies (2, 13, 15) it is evident that the major contribution to the allergenic activity of cat origin is Cat Ag 1.

We found the highest ratio between Cat Ag 1 and cat albumin in dander extract and in saliva,

and in similar ratios. Contrary to that, no Cat Ag 1 is found in serum and urine, and the Cat Ag 1/cat albumin ratio for a partially purified cat pelt extract or a general pelt extract is 70–300 times lower than in dander or saliva. This corresponds with the fact that the material to which the average patient is exposed is dander and parts of insoluble hair probably covered with a thin layer of soluble protein. We do not know if these proteins are derived mainly from the saliva as suggested by Anderson et al. (2), or mainly from the sebaceous glands and hair roots or a combination (4). However, for practical purposes – e.g. for the preparation of optimal cat allergenic extracts for diagnosis and therapy – we know that these extracted hair and dander parts as well as saliva have the highest content of Cat Ag 1/weight unit. Furthermore, they also have the broadest known representation of other important cat allergens, present or not present in serum. This analysis is biased by the fact that we have used anti-cat hair and dander antibodies for the analyses. However, several investigators who have mainly analysed pelt extracts have not been able to detect important constituents besides the Cat Ag 1, which was present in very low amounts in cat pelt extracts. This indicates that we have not missed any important individual molecules which contribute to the cat allergenic activity.

The QIE analyses revealed data on the Cat Ag 1/cat albumin which did not match our RIE data. However, in spite of the wide variety of the various antigens in the pelt extract relative to the dander extract, those data which could be compared with the more accurate (12) RIE-derived data in all cases fit within a factor of 1.25–2. Some discrepancies were also found between the RIE analyses performed by Ohman et al. and ours although we used the same standards. In particular, the result for Cat 160 deviated and we have no explanation except that deterioration may occur during transport. On the other hand, we have recently been involved in another project quantifying Cat Ag 1 and cat albumin and similar data to ours were obtained from R. Aalberse & D. G. Marsh using independent methods and reagents (results not yet published).

From this study we conclude that for a cat allergenic extract to be clinically relevant it is essential that it contains Cat Ag 1 but also other components to which patients quite often react. We believe that measurement of Cat Ag 1 should be required, as should the ratio between Cat Ag 1 and cat albumin. This ratio seems to be a marker for the relative content of non-serum- and serum-derived proteins. We further advocate that the presence of at least some other important allergens (i.e. Ags 2, 7, 11 and 17) is required.

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