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Monoclonal antibody based radioimmunoassay for the quantitation of the main cat allergen (*Fel d* I or Cat-1)

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A two-site solid-phase radioimmunoassay has been developed for the quantitation of the main cat allergen, *Fel d* I or cat allergen 1. The assay is based on two different monoclonal antibodies which recognize different epitopes on the *Fel d* I molecule; one antibody (C5/24) was immobilized on the solid phase and the other (C5/8) was labeled with ^{125}I , being the allergen molecule sandwiched between them. The assay is specific for the *Fel d* I molecule and sensitive enough to detect as little as 0.25 ng/ml of allergen. The *Fel d* I RIA was compared with a radial immunodiffusion technique for the determination of allergen levels in several cat extracts and a good quantitative correlation was found. The same good correlation was found when the results obtained with the *Fel d* I RIA were compared with the determination of the total allergenic activity by RAST inhibition. The results indicate that the MAb RIA could be very useful in the standardization of allergenic extracts from feline origin.

Key words: Cat main allergen; Monoclonal antibody; Radioimmunoassay, solid-phase

Introduction

Extracts of naturally occurring allergenic materials, used in patients for diagnosis and immunotherapy, are a complex mixture of molecules of which only a few have allergenic activity. Therefore, the control or standardization of allergenically active materials is an urgent need. Different *in vitro* methods have been devised for quantitation of allergens in crude extracts (Lich-

tenstein et al., 1966; Ceska et al., 1972; Weeke, 1973; Weeke and Lowenstein, 1973). These techniques are based on the use of serum or basophils from allergic patients or on the use of polyclonal antisera and are difficult to standardize.

The use of MAb has become a powerful tool for purification and characterization of allergenic structures as described for allergens of *Dermatophagoides* mites (Chapman et al., 1984; Heymann et al., 1986; Ley et al., 1986), *Parietaria judaica* pollen (Corbi et al., 1985b), *Ambrosia* pollen (Olson and Klapper, 1986) and grass pollen (Esch and Klapper, 1987). These MAb have been used to develop specific immunoassays for quantitation of individual allergens in crude extracts (Krilis et al., 1983; Corbi et al., 1985a; Chapman et al., 1987). These assays are highly sensitive and simple, fast and suitable for testing large numbers of samples.

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Abbreviations: BSA, bovine serum albumin; MAb, monoclonal antibody(ies); PBS, phosphate-buffered saline; RAST, radioallergosorbent test; RIA, radioimmunoassay; RID, radial immunodiffusion; SDS-PAGE, polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate.

Recently, MAb against the main cat allergen, that is, cat allergen 1 or *Felis domesticus* I (*Fel d* I), have been produced (Aalberse et al., 1987; Duffort et al., 1988) and preliminary studies have reported the usefulness of a monoclonal immunoassay for measuring the presence of *Fel d* I in dust and air of patients' homes (Brown et al., 1987). In the present paper, we describe a two-site solid-phase RIA for the quantitation of *Fel d* I. The results show that the assay is specific and sensitive and gives a good correlation with allergenic activity, as determined by RAST inhibition, and with *Fel d* I content measured by RID.

Materials and methods

Extracts

The extracts used in the present work were obtained from Alergia e Inmunología Abelló S.A. The raw materials used in the production of the cat extracts (Table III) were: skinscraping (extracts 1, 5, and 12), hair plus skinscraping (2 and 6), dander (3, 4, 8, 9, 10 and 11) and whole skin (extract 7). Cat albumin and cat IgG were purified from cat serum by classical biochemical techniques.

Purification of monoclonal antibodies and ^{125}I -labelling

The MAb anti-*Fel d* I C5/8 and C5/24 were obtained as previously described (Duffort et al., 1988). Ascites tumors were produced by injecting 5×10^6 hybridoma cells into pristane-immunosuppressed BALB/c mice. After 2 weeks growth, ascites fluids were collected and centrifuged at $20\,000 \times g$ for 30 min at 4°C . The MAb C5/8 and C5/24 were purified from the respective fluid by hydroxylapatite chromatography (Stanker et al., 1985). The purified preparations only exhibited heavy and light chains when analyzed by SDS-PAGE (Laemmli, 1970). The purified MAb were radioiodinated to a specific activity of $15 \times 10^6 \text{ cpm}/\mu\text{g}$ according to Fraker and Speck (1978).

Purification of *Fel d* I

An immunosorbent column was prepared by coupling 10 mg of the purified C5/8 MAb to 3 ml of CNBr-activated Sepharose 4B (Pharmacia,

Uppsala, Sweden). Before use, the column was washed with 3 M KSCN in PBS, and equilibrated with PBS. Then 5 ml (5 mg/ml) of cat dander extract (number 3, Table III) were passed through the column. After washing with PBS and PBS-Tween 0.1%, the bound allergen was eluted with 3 M KSCN in PBS and dialyzed against PBS. The protein content, as measured by Lowry's method (Lowry et al., 1951), was 0.4 mg ($\sim 1.5\%$ of the initial protein), and the purification of the allergen was confirmed by SDS-PAGE and crossed immunoelectrophoresis. The allergenic activity of the preparation was assessed by RAST inhibition and immunoblotting after SDS-PAGE (Duffort et al., 1987).

Fel d I radioimmunoassay

Quantitation of *Fel d* I was achieved by a two-site solid-phase RIA as follows: PVC wells (Titertek immunoassay plate, Flow Laboratories) were coated with $50 \mu\text{l}$ /well of C5/24 at $10 \mu\text{g}/\text{ml}$ in PBS for 1 h at 37°C or overnight at 4°C . Wells were incubated for 15 min with 1% BSA in PBS to block the remaining adsorptive sites, and then they were incubated for 2 h at room temperature with $50 \mu\text{l}$ of samples diluted in 0.2% BSA in PBS. After four washes with the same buffer, the wells were incubated for 2 h at room temperature with ^{125}I -C5/8 (about 80 000 cpm in a final volume of $50 \mu\text{l}$ /well). Finally, the wells were again washed and the radioactivity bound was counted in a gamma-counter. Samples were assayed in duplicate at several dilutions. The *Fel d* I content, in mass units, was obtained from a control curve using different dilutions (range 100–0.25 ng/ml) of the affinity-purified allergen.

Radial immunodiffusion

The *Fel d* I content was also measured by RID as indicated (assay for cat allergen 1, methods of the laboratory of Allergenic Products, FDA). Reference reagents were generously supplied by Dr. Baer (FDA, Bethesda, MD). The *Fel d* I content, determined by RID, was expressed as units of allergen/ml.

RAST inhibition

Allergenic activity was determined by RAST inhibition according to the procedure reported by

Yman et al. (1975). Paper discs were activated with CNBr and coupled with cat dander extract as described by Ceska et al. (1972). A pool of human sera from 30 patients, which contained specific IgE to cat dander extract (RAST class 3 or 4), was used throughout the experiments. These patients had a positive skin prick test to cat dander extract and clinical history of hypersensitivity to cat. The anti-human IgE MAb used throughout the experiments was Hamlet- ^{125}I (Alergia e Inmunología Abelló S.A.).

In order to compare allergenic activities, a RAST UNIT was defined as the amount of protein producing 50% inhibition.

Results and discussion

Table I shows a comparison of the combinations of anti-*Fel d* I MAb in the two-site solid-phase RIA. When the ^{125}I -labeled MAb was the same as that on the solid phase there was no binding of radioactivity. However, that was not the case when the MAb on the solid phase was different to that used as second antibody. The results indicate that C5/8 and C5/24 MAb probably recognize two different non-overlapping epitopes on the *Fel d* I molecule, and consequently it was possible to use them in a two-site RIA to quantitate the *Fel d* I allergen. The best results were obtained with C5/24 MAb immobilized on the solid support and C5/8 MAb, labeled with

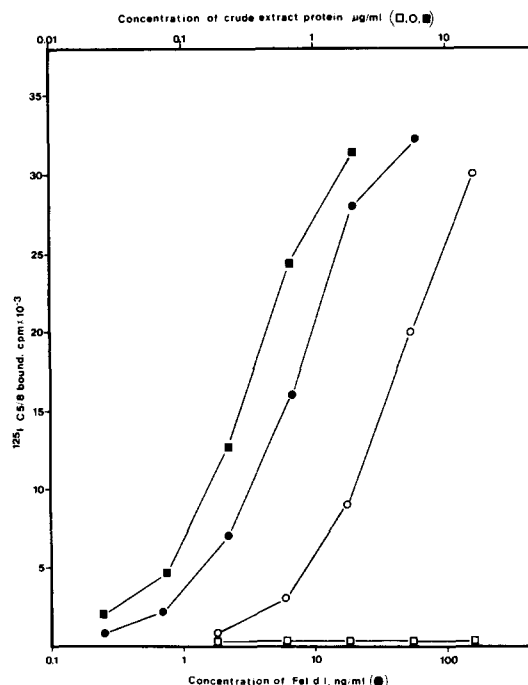


Fig. 1. Dose response in the *Fel d* I RIA of different extracts and purified *Fel d* I. Non-specific binding using 1 mg/ml BSA in PBS (310 cpm) has been subtracted. ●, affinity-purified *Fel d* I. ■, cat dander extract (no. 3, Table III). ○, cat skinscraping extract (no. 1, Table III). □, *D. farinae* extract.

^{125}I , as second antibody, and for this reason this particular combination was used hereafter.

Fig. 1 shows the binding curve of the affinity-purified *Fel d* I preparation in the two-site solid-phase RIA. It is observed that, using this preparation as standard, it is possible to quantify the *Fel d* I content in the range from 60 to 0.2 ng/ml with an intra-assay coefficient of variation of about 4% in the middle zone of the curve. The same figure shows the binding curves for two cat extracts of different origin: one is an extract from cat dander and the other is from cat skinscraping. It can be seen that the binding curves are parallel to that obtained with the purified *Fel d* I preparation, suggesting that the allergen had not been antigenically altered during the purification process and that the assay is measuring the same molecule in the different preparations. The only difference found in the binding curves is that there was a shift on the x-axis according to the different allergen content (see Table III).

TABLE I

COMPARISON OF THE DIFFERENT COMBINATIONS OF THE ANTI-*Fel d* I MAb IN THE SOLID-PHASE RIA

MAb bound to the solid phase ^a	cpm bound of ^{125}I -labeled MAb ^b	
	C5/8	C5/24
C5/8	257	21 428
C5/24	30 445	395

^a PVC wells were coated with 50 μl (10 $\mu\text{g}/\text{ml}$) of the MAb indicated. After quenching with 1% BSA in PBS, the wells were incubated for 2 h with 50 μl of cat dander extract at 10 $\mu\text{g}/\text{ml}$ as source of *Fel d* I.

^b After washing, the wells were incubated for 2 h with 80 000 cpm of the appropriate ^{125}I -MAb, washed again and the radioactivity bound was determined.

TABLE II
SPECIFICITY OF THE RIA FOR *Fel d* I QUANTITATION

Sample ^a	cpm bound of ¹²⁵ I-C5/8
<i>Parietaria judaica</i> 1 mg/ml	290
<i>Lolium perenne</i> 1 mg/ml	275
<i>Alternaria alternata</i> 1 mg/ml	165
<i>Dermatophagoides farinae</i> 1 mg/ml	207
Dog dander 1 mg/ml	4125
Cat albumin 0.2 mg/ml	435
Cat IgG 0.2 mg/ml	375
Cat dander 10 µg/ml	27365

^a PVC wells were coated with the C5/24 MAb as indicated in the materials and methods section and then they were incubated with the samples indicated. After washing, the wells were incubated for 2 h with 80000 cpm of ¹²⁵I-C5/8 and the radioactivity bound was determined.

The specificity of the assay is shown in Table II. There was no significant binding of ¹²⁵I-C5/8 to wells incubated with different allergenic extracts known to lack *Fel d* I. In the case of dog dander, there was a significant binding of the labeled anti-*Fel d* I MAb, which corresponds to a *Fel d* I content of about 0.0001% of the total protein (~10000 times lower than the *Fel d* I content in cat dander). This low content could correspond to a contamination of the dog raw material or to a small cross-reactivity between the *Fel d* I molecule and a dog dander component(s). In this sense, several authors have described the existence of cross-reactivity between cat and dog allergenic extracts (Viander et al., 1983). Table II also shows that there was no significant binding of ¹²⁵I-C5/8 to wells incubated with purified cat albumin or cat IgG. On the other hand, the binding curves in the *Fel d* I RIA for a cat dander and a cat skinscraping extracts, which had a very different albumin content, were parallel (Fig. 1) and showed a shift on the x-axis in agreement with the different *Fel d* I content, as determined by other techniques (Table III). These results indicate that cat albumin and cat IgG do not interfere in the *Fel d* I RIA which is very important because in some cat pelt extracts the albumin content can be as much as 100 times the *Fel d* I content (Lowenstein et al., 1985).

The *Fel d* I concentration was measured, using the MAb RIA, in several samples (Table III).

TABLE III
COMPARISON OF *Fel d* I RIA WITH RAST ACTIVITY AND *Fel d* I CONTENT BY RID

Extract	Total protein concentration (mg/ml) ^a	<i>Fel d</i> I content (µg/ml) ^b	<i>Fel d</i> I content (U/ml) ^c	Allergenic activity (RAST U/ml) ^d
1	5.0	12.5	5.0	565
2	4.0	41.0	16.0	1727
3	1.0	23.5	10.0	1240
4	3.7	45.0	22.0	2386
5	1.3	6.5	3.0	393
6	1.4	4.6	3.2	455
7	0.26	1.7	1.8	63
8	0.7	9.5	3.7	251
9	3.5	47.0	33.0	1884
10	1.7	36.5	19.0	1790
11	1.9	40.0	17.0	1209
12	0.3	1.7	0.9	392

^a Protein concentration was determined by Lowry's method.

^b *Fel d* I content determined by MAb RIA. The values shown are the mean of three independent experiments.

^c *Fel d* I content determined by RID. The values shown are the mean of three independent experiments.

^d Total allergenic activity determined by RAST inhibition. The values shown are the mean of four independent experiments.

Extracts prepared from cat dander had a mean content of about 2% of the total protein, while in extracts prepared from cat pelts or skinscraping this content was considerably less, in agreement with previous data (Lowenstein et al., 1985). Table III also shows for comparison purposes the *Fel d* I content, as determined by RID, and the total allergenic activity of the samples, determined by RAST inhibition.

The results of the MAb RIA and RID technique were compared by linear regression analysis (Fig. 2). A good correlation was obtained ($r = 0.944$, $P = 4 \times 10^{-6}$), suggesting that both techniques were measuring the same molecule. The results of the RID assay were expressed in U/ml (Table III, Fig. 2) where one unit has been claimed to be approximately equal to 2 µg of *Fel d* I (Ohman and Sundin, 1987). This is in good agreement with the calculated equivalency (1 U *Fel d* I = 1.9 µg) from the regression line (Fig. 2), indicating that there was a good quantitative correlation between the *Fel d* I RIA and the RID assay.

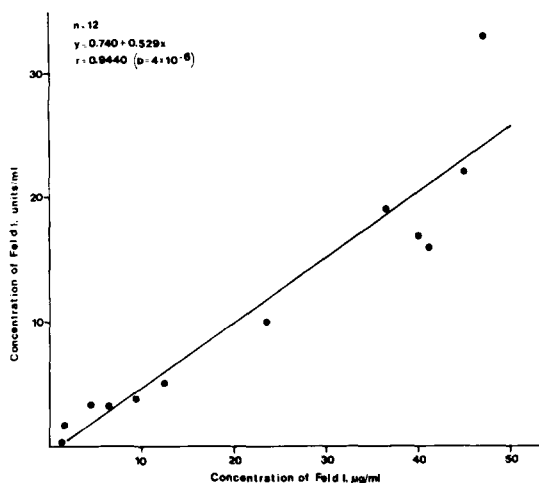


Fig. 2. Correlation between the *Fel d I* RIA and radial immunodiffusion. The concentration of *Fel d I* in 12 different extracts was determined by RIA (abscissa) and radial immunodiffusion (ordinate) and the results were analyzed by linear regression.

Finally, Fig. 3 shows the comparison of the results of the *Fel d I* RIA and RAST inhibition. A good correlation can be observed between these two assays ($r = 0.9445$, $P = 4 \times 10^{-6}$). This result was not surprising because *Fel d I* is the most important cat allergen (Ohman et al., 1974;

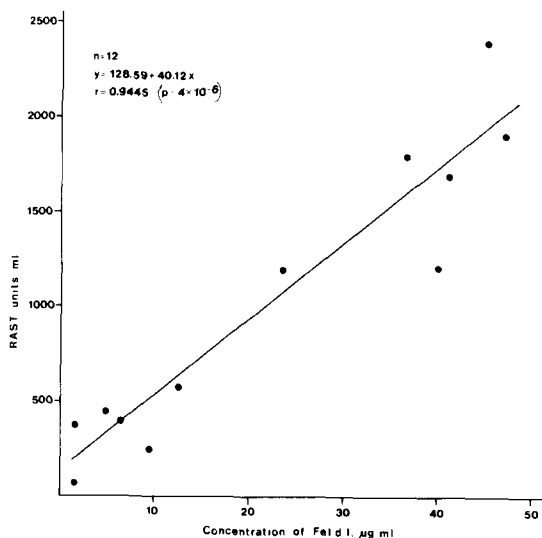


Fig. 3. Correlation between the allergenic activity (RAST U/ml) of 12 different extracts and their *Fel d I* content ($\mu\text{g/ml}$) measured by the MAb RIA. Results were compared by linear regression analysis.

Anderson and Baer, 1981) and more than 90% of the cat sensitive patients have specific IgE against the *Fel d I* molecule (Lowenstein et al., 1985; Duffort et al., 1988). Similarly, good correlations between the determination of a major allergen by a RIA based on MAb and RAST inhibition have been demonstrated in other systems (Corbi et al., 1985a; Chapman et al., 1987), indicating that such assays can also be useful for evaluation of the total activity of allergenic extracts. In this way, time consuming incubations, as well as human serum variations can be avoided.

In conclusion, we have developed a solid-phase RIA for quantitation of the main cat allergen based on specific MAb and affinity-purified allergen. The assay is simple, specific and sensitive and could be a useful tool in the standardization of allergenic extracts.

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