

Detection of allergens in a cat pelt extract

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Allergenic components of cat pelt extract fractionated by SDS-polyacrylamide gel electrophoresis (PAGE) and transferred to nitrocellulose membranes were identified using sera from 15 allergic patients who showed positive skin test and RAST to cat extract. Two components showed the highest IgE-binding frequency; 14 kD (being recognized by 86% of the sera examined) and 29 kD (73% of the sera tested). Both components were purified by electroelution and subjected to RAST inhibition assays. The 29 kD fraction was able to bind specific IgE to *D. pteronyssinus* and inhibited a RAST assay of 29 kD component (Der p I) purified from a *D. pteronyssinus* extract. These results could explain the finding of positive skin test with some commercial cat allergen solutions in patients with *D. pteronyssinus* allergy.

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The importance of antigens of mammalian origin in allergic disease has long been recognized. Domestic cat (*Felis domesticus*) appears to be the source of a potent allergen causing allergic diseases. Immediate hypersensitivity to inhaled allergen produced by the domestic cat has been demonstrated in 20 to 30% of patients with asthma (16, 18). Patients with cat asthma are numerous and globally distributed, and the cause and effect relationship between cat exposure and asthma is, usually, readily demonstrable.

A major allergen in cat extracts has been purified and characterized by Ohman et al. (10). This allergen, Fel d I (12), formerly known as cat-1, was originally purified from cat pelt extracts, although it is known that the main source of Fel d I is saliva (1, 2, 4). Fel d I has been used as an immunological marker to monitor changes in humoral immune response after desensitization treatment and to assess environmental cat antigen exposure (14, 15, 21).

Cat extracts include other allergens, as has been demonstrated by Anderson (1) and Lowenstein (11) using a CRIE procedure.

This study was performed in order to investigate the presence of allergens in a cat pelt extract, as well as to isolate the main allergens using an electroelution procedure. We also investigate the possibility of cross-reactivity between cat and dust mite (*D. pteronyssinus*) extracts.

Material and methods

Sera samples were obtained from 15 patients with asthma and/or rhinitis provoked by cat exposure. All of them demonstrated specific IgE to cat pelt using a RAST assay (Pharmacia).

The cat pelt extract was obtained from Bencard

(Betchworth, UK). A *Dermatophagoides pteronyssinus* extract was obtained through Abello (Madrid, Spain). Both materials were supplied as freeze-dried powder. Protein determinations were carried out using the Bio-Rad protein assay.

SDS-PAGE and electrotransfer to nitrocellulose

Electrophoresis (9) was performed in 12% acrylamide separating gel in 0.375 M Tris (Bio-Rad) pH 8.8 containing 0.1% SDS. Samples were made up in 0.01 M Tris buffer containing 2% SDS and 5% 2-mercaptoethanol.

Gel slices were stained for protein with Coomassie brilliant blue R-250. Standard low molecular weight markers (Bio-Rad) were used for calibration.

Before transfer, the unstained portions of the gel were washed for 5 min in 0.025 M Tris, 0.192 M glycine, 20% methanol, (pH 8.3). Proteins were transferred overnight in the same buffer, at 0.1 amps in an electrotransfer tank (Bio-Rad). After the transfer the nitrocellulose sheet (Bio-Rad) was blocked for 1 h at room temperature with 3% BSA (Sigma) and 0.1% Tween-20 (Bio-Rad) in PBS, and was cut into strips to enable incubation with different antisera.

Probing of nitrocellulose blots and autoradiography

Blocked 5 mm-wide nitrocellulose strip transfers were incubated overnight with sera, washed extensively for 30 min with 0.1% Tween in PBS, and incubated for 3 h with [¹²⁵I] anti-IgE (Pharmacia) 120 000 cpm/strip in 0.1% Tween-20-BSA buffer. After washing with 0.1% Tween-20 in PBS, strips were air-dried, wrapped in plastic film, and loaded

into an X-ray film cassette for autoradiography. The cassette was stored at -70°C for 2 days. Sera from subjects allergic to pollens but not to cat dander were used as controls.

Electroelution

Unstained portions of the gel corresponding to the 29 kD region and 14 kD region were electroeluted in 50 mM Tris, 0.01% SDS at 100 mA for 16 h. The portions of the gel were introduced in dialysis membrane bags (MW cutoff 3500) (Spectra/por. Spectrum Med. Ind. Los Angeles USA). The electroeluted samples were dialysed in 50 mM $\text{NH}_4\text{CO}_3\text{H}$ for 48 h before using.

RAST inhibition

Crossreactivity between cat and mite extracts was assessed by RAST inhibition. Briefly, different concentrations of cat extract (0.2, 2, 20 and 200 $\mu\text{g}/\text{ml}$) were incubated with a pool of sera from *D. pteronyssinus*-sensitive patients for 24 h at room temperature. A *D. pteronyssinus* disc (Pharmacia) was added to each tube and incubated for 3 h. The discs were then washed with 0.05% Tween PBS and 50 μl of [^{125}I]-labelled anti-IgE added. After a further incubation of 3 h, the discs were washed again and the bound radioactivity counted in a gamma counter. The results are expressed as percentage inhibition.

In another assay, we used several concentrations (0.2 to 200 $\mu\text{g}/\text{ml}$) of the 29 kD electroeluted samples from the cat extract, and the discs used (nitrocellulose discs, 0.5×0.5 mm) had been adsorbed with the 29 kD protein electroeluted from an SDS-PAGE from *D. pteronyssinus* extract.

Results

Detection of IgE binding-material in cat extract

Results from autoradiographies are shown in Fig. 1. The components that bind IgE antibodies both strongly and with higher frequency were those with a relative mobility around 14 kD (93%), 29 kD (73%), 39 kD (40%) and 34 kD (26%). Some sera recognized a high molecular weight material (97 kD). Although most sera identified several bands in the extract, there were some of them which recognized only few protein-bands (sera i, m and o).

Electroelution

The components with higher immunoreactivities (14 kD and 29 kD) were electroeluted from unstained SDS-PAGE. Purity from the electroelution as assessed by subsequent SDS-PAGE is shown in Fig. 2. The 30 kD band appearing after the 14 kD isolation is likely to be due to partial dimerization.

RAST inhibition assays

A RAST from *D. pteronyssinus* was, surprisingly, inhibited by the cat pelt extract (Fig. 3). This finding led us to investigate which fraction from the cat pelt extract could be responsible for the inhibition. As shown in Fig. 4, the 29 kD band from the cat extract inhibited the RAST from a 29 kD fraction obtained from a *D. pteronyssinus* extract. In another assay (Fig. 5), the *D. pteronyssinus* extract did not inhibit the RAST from the 14 kD fraction obtained from the cat extract.

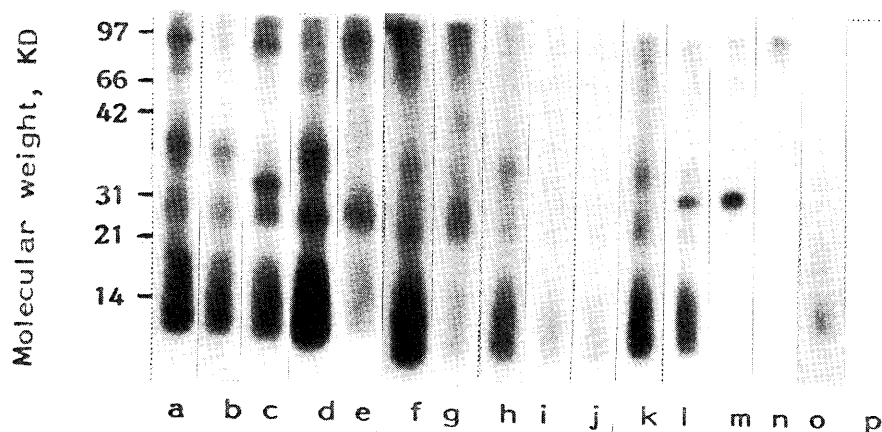


Fig. 1. Autoradiographs showing cat extract proteins recognized by specific IgE from different patients' sera samples. Lanes a-o: patients with cat allergy. p.: control serum.

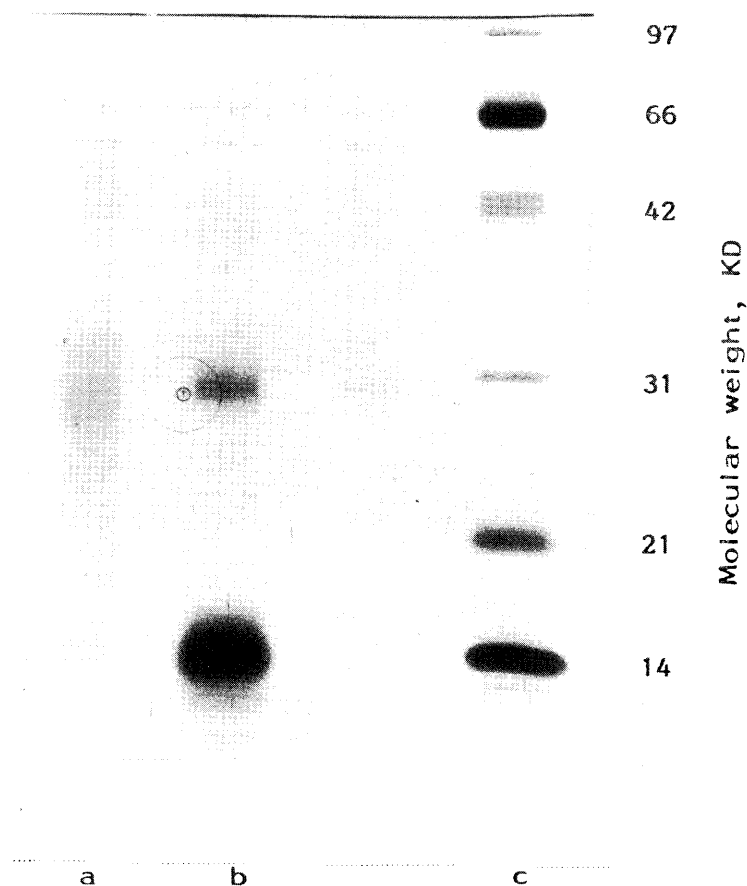


Fig. 2. Electroelution-purified fractions from cat extract as assessed by SDS-PAGE. Lanes a and b, 29 and 14 kD materials respectively. c: molecular weight markers.

Discussion

The identification of allergens in an extract by means of SDS-PAGE and immunoblotting is a widely used technique (17, 20). In this study, this procedure reveals several proteins with allergenic activity in a cat pelt extract. It is noteworthy that: 1) most sera recognize proteins along the whole range of molecular weights, 2) most sera identify several components in the extract. Similar results are found in other allergens when the blotting system is used (6, 19).

There are two important allergens: the 14 kD fraction (identified by 93% of the positive sera), and the 29 kD component (73% of the positive sera). Both seem to be the major allergens of the cat extract.

The 14 kD protein is in the molecular weight range of the Fel d I, and could represent the same antigen. Leitermann & Ohman (10) obtained a molecular weight of 18 kD for the cat-1 allergen using an SDS-PAGE. However, Duffort et al. (5) found that the

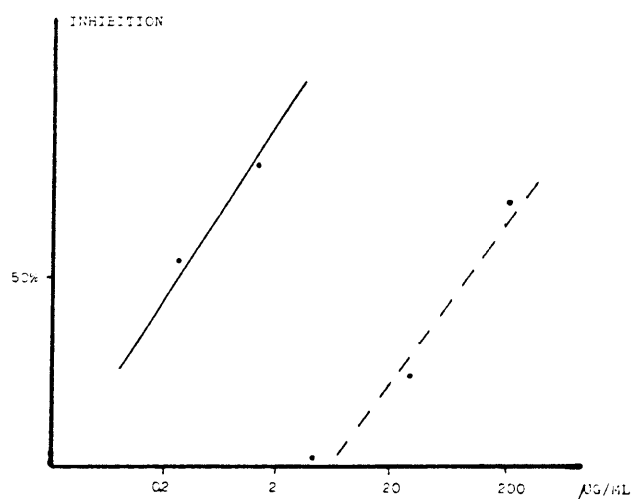


Fig. 3. Inhibition of binding of IgE antibodies to *D. pteronyssinus* coated discs with *D. pteronyssinus* extract (—) and cat extract (-----).

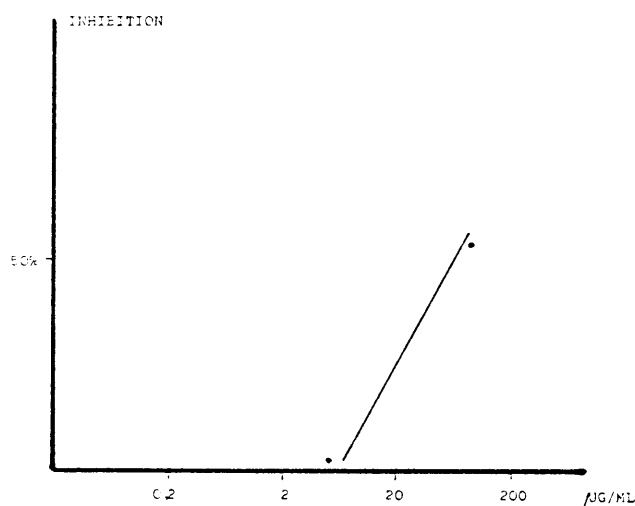


Fig. 4. Inhibition of binding of IgE antibodies to the *D. pteronyssinus* 29 kD component-coated discs, with the 29 kD component from the cat extract (—).

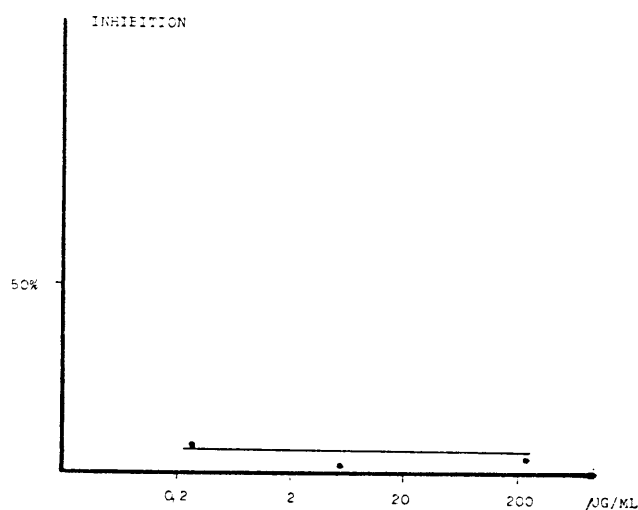


Fig. 5. Inhibition of binding of IgE antibodies to the cat extract 14 kD component-coated discs, with the *D. pteronyssinus* extract (—).

18 kD protein (Fel d I), under reducing conditions renders 2 subunits (14 kD and 4 kD). This is in contrast to the results from Leitermann who showed no modification of the original molecular weight from the Fel d I after its reduction.

Another point which deserves attention, is the demonstration of other proteins with allergenic activities, apart from Fel d I, and the presence of a pair of sera which do not recognize Fel d I but other allergens.

This fact shows the usefulness of getting an extract that includes all the allergens, for both diagnostic and therapeutic applications. This has already

been stressed by other authors studying cat allergens (by means of CRIE) (1, 11).

The electroelution procedure is a common biochemical technique, used in methodology involving DNA and RNA, and it has been claimed as a useful tool in protein isolation (3). In spite of this, it has been rarely used in allergen standardization. Meier-Davis et al. (13) described the purification of a glycoprotein, with a molecular weight of 66 kD in an *Alternaria* extract, by electroelution. In our study, it has been shown as an easy and useful single step technique, to isolate the main allergens in the cat extract.

The unexpectedly high incidence of positive skin prick tests with Bencard cat allergen solution, and the finding of positive skin prick tests to cat (Bencard) in patients who, on clinical history and skin test with Abello allergens, were sensitive to dust mite but not to cat, led us to investigate the existence of proteins in the Bencard cat extract that could be identified by specific IgE to *D. pteronyssinus*.

We restricted the study to the Bencard cat allergen solution because this was the only extract we found to give a high false positive incidence rate on skin testing. This was not the case with a biologically standardized extract (Biological units, Abello).

An SDS-PAGE, immunoblotting, and autoradiography from *D. pteronyssinus* extract (data not shown) demonstrated an important allergen in a 29 kD component. This is in agreement with the molecular weight described for the major allergen Der P I under reducing conditions (7). In a first step, we demonstrated a strong inhibition of the *D. pteronyssinus* extract with the cat extract (Fig. 3). Recently, Kemeny et al. (8) reported the same finding with the same cat extract, pointing to a contamination of the cat extract with mite components. In a second step, we found that the 29 kD fraction from the cat extract was able to inhibit the RAST of the 29 kD component from *D. pteronyssinus* (Fig. 4). So, the 29 kD fraction in the cat extract could be responsible for the positive skin tests obtained in patients with allergy to *D. pteronyssinus*.

In spite of these results, the 14 kD component, as has been discussed previously, appears to be the Fel d I protein, and not a *D. pteronyssinus* protein, for instance Der P II, is of similar size. This fact is supported by the absence of inhibition in the 14 kD RAST with *D. pteronyssinus* extract. Another point arguing against a *D. pteronyssinus* source of the 14 kD component is that sera from patients showing a positive RAST to cat, but not to *D. pteronyssinus*, recognized this 14 kD band on immunoblotting.

We planned initially to do skin tests with the electroeluted 29 kD component in patients allergic to *D. pteronyssinus*, in order to investigate by other

methods its allergenic activity. This was rejected for ethical reasons, owing to the decision to avoid using laboratory obtained material for human experimentation.

The finding of a fraction in a cat extract that could be responsible for false positive skin tests in allergic patients to *D. pteronyssinus* must be taken into account in clinical practice when using this cat extract for diagnostic purposes.

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