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Note

Purification of allergens by high-performance liquid chromatography

V*. Purification of cat and dog epithelial allergens

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Among allergic patients, allergy to domestic animal epithelium comes after allergy to pollens, mites and fungus in frequency. There is as much as 5% of the population of the U.S.A. allergic to domestic animals, and allergy is an important occupational illness among veterinarians and people working in close contact with them.

Some studies have been made to define and purify allergens of animal origin, notably from cats and dogs¹⁻⁴. We have demonstrated that high-performance liquid chromatography (HPLC) has the advantages of speed, recovery and sensitivity over more conventional chromatographic techniques for the purification of allergens from crude allergenic extracts⁵⁻⁸. In connection with solid-phase radioimmunoassay (SPRIA), HPLC allow allergens from dog and cat hair and skin scrapings to be purified in 15-min runs.

EXPERIMENTAL

Allergenic materials

Cat and dog hair and skin scrapings were purchased from Allergon (Sweden). Urine samples were obtained from a local veterinary clinic. Albumin (fraction V) and immunoglobulin G were obtained from Sigma (St. Louis, MO, U.S.A.).

Allergen extraction

Unwashed hair and skin scrapings cut into short lengths were immersed in phosphate-buffered saline (PBS) containing 50 µg/ml of phenylmethylsulphonyl fluoride (PMSF) (Sigma) and stirred for 1 h in an ice-bath. After sieving to remove hair, the extract was centrifuged at 4°C for 15 min at 12000 g and the supernatant was filtered through a Millipore filter (pore size 0.45 µm). Urine was filtered through a Millipore filter before use.

* For Part IV, see ref. 8.

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Protein determinations were carried out by the method of Lowry *et al.*⁹.

HPLC

HPLC was performed as described in previous papers⁵⁻⁸.

SPRIA

This was carried out in accordance with the method detailed in ref. 5.

Polyacrylamide slab gel electrophoresis

Electrophoresis was performed according with the method of Laemmli¹⁰. The separating gel consisted of 12.5% of polyacrylamide and the stacking gel contained 4.5% of polyacrylamide. The gels were stained with 0.25% Coomassie Brilliant Blue G-250 in 45% methanol-10% acetic acid for 1 h and destained with methanol-acetic acid-water (7.5:5:87.5).

RESULTS

Cat hair and skin allergens

Size-exclusion chromatography of an epithelial cat extract is illustrated in Fig. 1. A high proportion of the UV-absorbing material is located in the low-molecular-weight region. This is also true for dog epithelia (see below), showing that not many proteins are extracted by aqueous buffers from hair and skin scrapings.

RIA performed on solid phase-attached separated fractions demonstrates (Fig. 1, bottom) that the allergens are located in fractions 7-9. These fractions contain proteins of molecular weight 60000-25000, as expected for cat albumin and for antigen 4 (as designated by Lowenstein¹¹). Nevertheless, albumin seems to be the most important allergic component in this sample, although other workers consider it to be a minor allergen³.

Dog allergens

According to Lowenstein¹¹, eleven allergens can be identified by crossed radioimmuno-electrophoresis. Important allergens are dog albumin and immunoglobulin G; Vanto *et al.*¹² have shown that dog serum albumin inhibits 15-30% of the total activity of hair and epithelium extracts.

The HPLC analysis of the dog hair and skin scraping extracts showed a complex composition (Fig. 2). The elution position of the fractions more active in SPRIA (fractions 7-8) corresponds to that of dog serum albumin. γ-Globulin, which elutes from the I-125 HPLC column in fraction 6, seems to have a lower activity than albumin. Commercial albumin and γ-globulin tested in SPRIA show clear but lower activity than epithelial extracts (results not shown). Nevertheless, the complex distribution of the biological activity shows, in accordance with previous reports¹¹⁻¹², that dog hair and skin contains other molecules with specific IgE-binding activity.

Dog and cat urine

Animal urine is another biological fluid that has been shown to be allergenic. HPLC separation of two individual samples of cat and dog urine are shown in Fig. 3. As expected, more UV-absorbing material in the low-molecular-weight range was

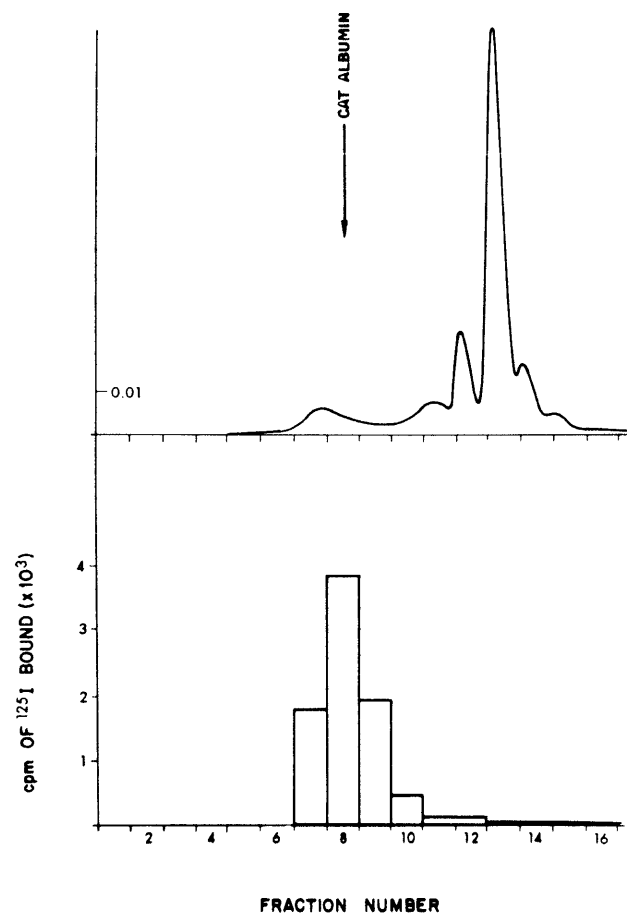


Fig. 1. Purification of cat hair and skin allergens by HPLC. A Protein Pak 125 column (Waters Assoc.) was used with PBS as eluent. Bottom: SPRIA of the separated fractions. A pool of five human allergic sera, class 3 RAST against cat epithelium, was employed. The elution position of commercial albumin is indicated by an arrow.

detected than in hair extracts. Nevertheless, when both samples were analysed by SPRIA against a pool of active human sera (class 3 in RAST) almost no activity could be found.

Only a small IgE-binding capacity can be attributed to fraction 7 in the cat urine, in accordance with the presence of a small peak. In fact, cat albumin is present in urine, as demonstrated by Lowenstein¹¹.

Purity of the selected allergens

The purity of the allergens separated by HPLC was demonstrated by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) (Fig. 4). When

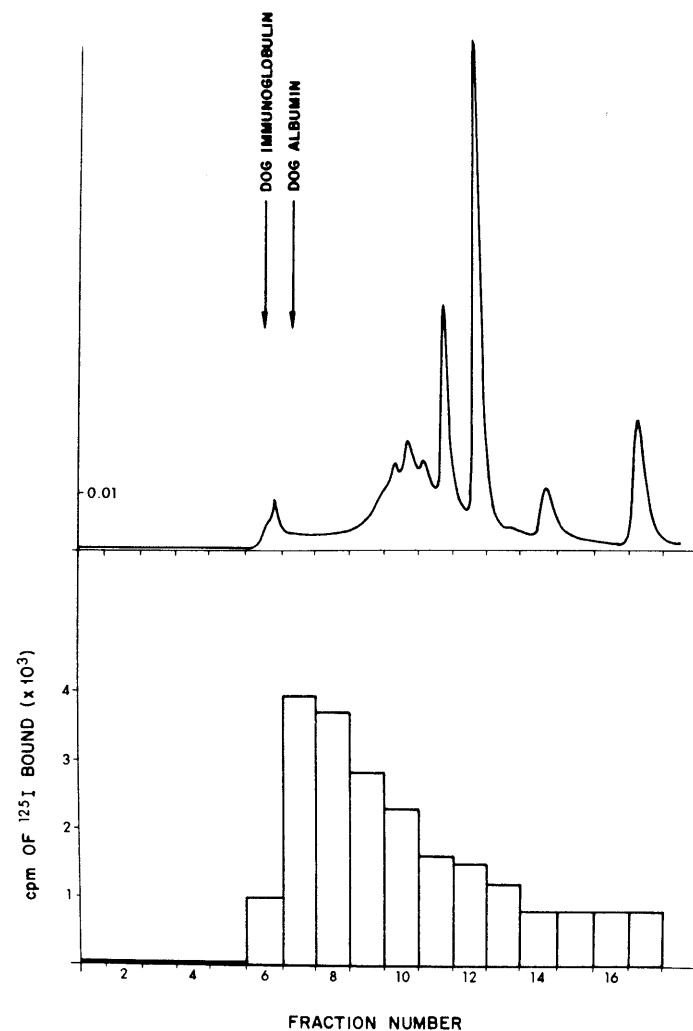


Fig. 2. HPLC of crude extract of dog hair and skin scraping. Bottom: SPRIA of separated fractions using a pool of class 3 RAST sera against dog epithelium and rabbit anti-IgE labelled with ^{125}I . The elution positions of commercial albumin and γ -globulin are indicated by arrows.

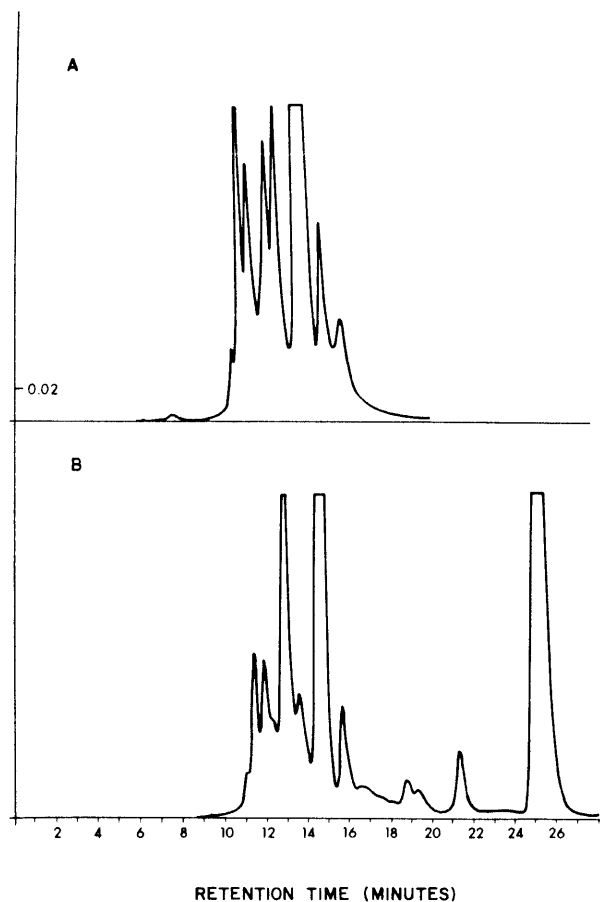


Fig. 3. Separation by HPLC of the components of (A) cat and (B) dog urine. Injections of 20 μ l of Millipore-filtered urine into a Protein Pak 125 column were made.

fractions 7 (dog) and 8 (cat), showing maximal activity in SPRIA, were run in SDS-PAGE, two bands of molecular weight *ca.* 67 000 were found. This is the molecular weight of bovine serum albumin, used as a molecular weight marker.

When the activity of the HPLC-purified albumin allergens were compared by RAST inhibition with an *in vivo* standardized extract, after dialysis against distilled water and lyophilization, we found that 2.4 ng for cat and 0.4 ng for dog were needed to reach 1 Biological Unit (an allergen solution contains 1000 Biological Units when it gives a mean wheal reaction of the same size as 0.1% histamine hydrochloride in the skin prick test). Hence the dog seems more allergenic than the cat on a weight basis.

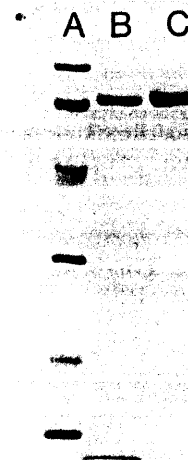


Fig. 4. SDS-PAGE of cat and dog allergens purified by HPLC. Lane A, molecular weight markers: phosphorylase *b* (MW 94 000), bovine serum albumin (67 000), ovalbumin (43 000), carbonic anhydrase (30 000), soybean trypsin inhibitor (20 100) and α -lactalbumin (14 400). Lane B, cat purified allergen (fraction 8 of Fig. 1). Lane C, dog purified allergen (fraction 7 of Fig. 2).

DISCUSSION

In animal allergenic species, it seems that relevant allergens are found in several biological sources: serum, saliva, urine and dander (epithelium). Serum proteins are found in the saliva of domestic animals, possibly the most potent source of allergens¹³. Saliva is not currently used as a source of allergens for therapeutic purposes because of the difficulty of obtaining it in large amounts.

Commercial suppliers of allergens, *e.g.*, Allergon, provide the allergenic extracts with a mixture of hair and skin scrapings. This is the reason that induced us to study and purify the allergens present in crude extracts of hair and skin scrapings from dogs and cats, a material currently used as a source for preparing extracts for hyposensitization.

Our results indicate that dog and cat albumin is a very important allergen in hair and skin. This agrees with results obtained by Calam *et al.*⁴, who also used HPLC to study epithelial allergens. We think that albumin may be present in hair and dermis because the animal applies saliva to the hair by licking. In the dog, other active components are present in the lower molecular weight region of the HPLC trace.

Hence the use of the Protein Pak 125 column is also useful for the study and purification of allergens originating from domestic animals. This has important eco-

nomic implications for the manufacture of animal extracts for the practice of clinical allergy.

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