

# Identification of dander-specific and serum-specific allergens in cat dandruff extract

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*Cat dandruff extract was characterized by crossed immunoelectrophoresis (CIE) and crossed radioimmuno-electrophoresis (CRIE) using sera from 27 cat-sensitive individuals. By applying a CIE/CRIE system with intermediate gel, the content of dander-specific and serum-specific allergens was established. Thirteen serum-related and eight dander-related antigens were identified in cat dandruff. Of these, eight antigens were visibly radiostained in CRIE. Five allergens were serum-specific and of these allergens cat albumin was the most important (intermediate allergen). Three allergens were dander-specific and of these three only cat allergen I fulfilled the criteria for a major allergen.*

## INTRODUCTION

Allergy to cat allergens is a frequent and important problem among people with household domestic animals in various parts of the world. The diagnosis of cat allergy is mainly made by clinical history, skin testing, provocation tests, and RAST. Cat allergen extracts prepared for diagnosis and treatment are produced from different source materials: whole pelt, epithelium (skin scrapings), hair, dander, saliva, serum, urine, or mixtures of these.<sup>1-7</sup> Of these source materials, cat hair and cat epithelium are believed to give the most complete allergen extracts to be used for diagnosis and treatment.

The quality of cat allergen extracts varies with the nature of source materials, the processing of source materials, and the extraction conditions used.<sup>5,6</sup> Different biochemical and immunochemical methods have been used to characterize cat allergen extracts.<sup>8-15</sup> By using CIE/CRIE, a more detailed analysis of the antigenic/allergenic composition of cat extracts has been possible.<sup>4,12,13</sup>

A clinically important allergen (cat allergen I) was identified by using skin testing and RAST.<sup>1,9</sup> This cat allergen was also shown to be present at different concentrations in various source materials, but was

From Pharmacia Diagnostics AB, Uppsala, Sweden.

most abundant in saliva.<sup>16</sup> Based upon the content of cat allergen I component, cat allergen extracts have been standardized to achieve batch-to-batch consistency.

In this study, we report a detailed CIE/CRIE analysis of the antigenic and allergenic composition of cat dandruff (dander-specific and serum-specific allergens). This cat dandruff extract was further used to study the IgE responses of a clinically well documented patient panel by CRIE and RAST analysis.

## MATERIALS AND METHODS

### Cat Dander Extract

Cat dander was prepared from cat dandruff (lot no 20480) obtained from Allergon AB, Sweden. Extraction was performed at a wt/vol ratio of 1:20 in 0.125 M ammonium bicarbonate buffer, pH 7.8, for 18 hours and continuous stirring while in a cold room (4 to 8°C). The extract solution was cleared by centrifugation at 14,000 *g* followed by filtration and subsequent lyophilization.

### Rabbit and Goat Antisera

Rabbit antisera to cat dander proteins were raised in rabbits (New Zealand White) by multiple intracutaneous (monthly) injections of a crude cat dander extract (200 µg protein/injection) in Freund's complete adjuvant.<sup>17</sup> The antiserum used was a pool of several bleedings of three to six rabbits. Antiserum to

cat serum proteins (raised in goat) was bought from Miles Scientific, Naperville, IL, USA (lot no G212).

### Patient Sera

Patient serum was obtained from 27 patients with a clinical history of cat allergy. None of the patients had been subjected to immunotherapy.

### RAST

RAST analyses were performed on the individual patient serum samples with commercial Phadebas RAST\* kits (el, cat epithelium discs), Pharmacia Diagnostics AB, Uppsala. The RAST results are given as Phadebas RAST Units/mL (PRU/mL).

### Protein Determination

The total protein content in the cat dander extract was determined by amino acid analysis.<sup>18</sup>

### CIE/CRIE Analysis

Crossed immunoelectrophoresis (CIE) was performed as previously described on 5 × 7-cm Gel Bond Film (Pharmacia Fine Chemicals).<sup>19</sup> Samples of 5 µL crude cat source material (11 mg dw/mL) was applied for the first dimension electrophoresis at 10 V/cm for 40 minutes. The first dimension gel with the separated proteins was then transferred to the 5 × 7-cm plastic film and an antibody-containing gel was cast with 4 µL/cm<sup>2</sup> of goat antiserum to cat serum proteins in the intermediate gel and 10 µL/cm<sup>2</sup> of rabbit antiserum to cat dander compo-

nents in the upper gel. The second dimension electrophoresis was performed at 2 V/cm perpendicular to the first for 16 hours. Cathodic migration was examined in separate experiments by casting an agarose gel with antibodies on the cathodic side of the first dimension gel slab. Identification of individual components was achieved using tandem CIE.<sup>20</sup>

Crossed radioimmuno-electrophoresis was performed substantially as previously described.<sup>19</sup> The washed CIE plates were incubated with 0.4 mL of the patients' sera (diluted to 7 mL) for 18 hours at room temperature. After four washings, the CIE-plates were incubated with [<sup>125</sup>I]anti-IgE solution (diluted to 72,000 cpm/mL) for 18 hours at room temperature. The plates were again washed four times, dried, and

placed on x-ray film for rapid exposure at -70 °C in a cassette equipped with an x-ray intensifying screen (Du Pont Lighting Plus). The CRIE reference system designed by Aukrust and Aas<sup>21</sup> was used to classify the degree of radiostaining (specific IgE binding).

## RESULTS

A high resolution crossed immunoelectrophoresis system was developed to examine the antigenic content in cat dander. Figure 1A demonstrates the CIE patterns of cat dander extract against both rabbit anti-cat dander and goat anti-cat serum after protein staining with Coomassie Brilliant Blue. By using a CIE system with an intermediate gel containing antibodies to cat serum proteins and an upper gel containing antibodies against cat dander antigens, it was possible to

discriminate directly between cat dander-specific antigens and cat serum-specific antigens. The serum-specific antigens precipitated in the intermediate gel, while the dander-specific antigens passed the intermediate gel and precipitated in the upper gel containing rabbit antibodies against cat dander antigens. No visible protein-stained antigens moved towards the cathode under the experimental conditions applied. This indicates that the resolved cat antigens were mostly acidic in nature. The protein-stained CIE pattern of the cat dander extract shows more serum-related protein components than dander-related components. Figure 1B is a schematic drawing of the CIE reference pattern of cat dander extract. Twenty-one antigens were identified in the cat dander extract studied, of which 13 were serum-related and eight were dander-related components. The individual antigens were arbitrarily numbered from the anode to the cathode. The constructed cat CIE reference pattern was established after performing several experiments by varying the ratio of cat antigen/rabbit antisera and goat antisera. One immunoprecipitate illustrated by a dotted line (Ag19) in the cat reference pattern was only detected after CRIE analysis. By tandem, CIE Ag 2 was identified as cat allergen I, Ag 3 as cat albumin, and Ag 14 as cat immunoglobulin G.

Specific IgE binding in 27 individual patient sera with clinically documented cat allergy was studied by CRIE analysis and RAST (Table 1). The individual patient's IgE binding was expressed in a semi-quantitative system<sup>21</sup> based on radiostaining of an x-ray film after exposure for different time periods (one hour to 30 hours) that corresponded to CRIE class A (dense radiostaining) and to CRIE class G (weak radiostaining). Five serum-specific allergens and three dander-specific allergens could be identified in the patient panel studied (Table

Table 1. Specific IgE Binding Against Cat Dander Components Measured by Radiostaining in CRIE Analysis\*

| PATIENT | AGE | SEX | PRU | Ag 1 | Ag 2 | Ag 3 | Ag 4 | Ag 5 | Ag 6 | Ag 7 | Ag 8 | Ag 9 | Ag 10 | Ag 11 | Ag 12 | Ag 13 | Ag 14 | Ag 15 | Ag 16 | Ag 17 | Ag 18 | Ag 19 | Ag 20 | Ag 21 |
|---------|-----|-----|-----|------|------|------|------|------|------|------|------|------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| 1       | 17  | F   | 1.2 | +    | +    | +    | +    | +    | +    | +    | +    | +    | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     |
| 2       | 17  | F   | 1.2 | +    | +    | +    | +    | +    | +    | +    | +    | +    | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     |
| 3       | 17  | F   | 1.2 | +    | +    | +    | +    | +    | +    | +    | +    | +    | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     |
| 4       | 17  | F   | 1.2 | +    | +    | +    | +    | +    | +    | +    | +    | +    | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     |
| 5       | 17  | F   | 1.2 | +    | +    | +    | +    | +    | +    | +    | +    | +    | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     |
| 6       | 17  | F   | 1.2 | +    | +    | +    | +    | +    | +    | +    | +    | +    | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     |
| 7       | 17  | F   | 1.2 | +    | +    | +    | +    | +    | +    | +    | +    | +    | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     |
| 8       | 17  | F   | 1.2 | +    | +    | +    | +    | +    | +    | +    | +    | +    | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     |
| 9       | 17  | F   | 1.2 | +    | +    | +    | +    | +    | +    | +    | +    | +    | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     |
| 10      | 17  | F   | 1.2 | +    | +    | +    | +    | +    | +    | +    | +    | +    | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     |
| 11      | 17  | F   | 1.2 | +    | +    | +    | +    | +    | +    | +    | +    | +    | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     |
| 12      | 17  | F   | 1.2 | +    | +    | +    | +    | +    | +    | +    | +    | +    | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     |
| 13      | 17  | F   | 1.2 | +    | +    | +    | +    | +    | +    | +    | +    | +    | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     |
| 14      | 17  | F   | 1.2 | +    | +    | +    | +    | +    | +    | +    | +    | +    | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     |
| 15      | 17  | F   | 1.2 | +    | +    | +    | +    | +    | +    | +    | +    | +    | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     |
| 16      | 17  | F   | 1.2 | +    | +    | +    | +    | +    | +    | +    | +    | +    | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     |
| 17      | 17  | F   | 1.2 | +    | +    | +    | +    | +    | +    | +    | +    | +    | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     |
| 18      | 17  | F   | 1.2 | +    | +    | +    | +    | +    | +    | +    | +    | +    | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     |
| 19      | 17  | F   | 1.2 | +    | +    | +    | +    | +    | +    | +    | +    | +    | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     |
| 20      | 17  | F   | 1.2 | +    | +    | +    | +    | +    | +    | +    | +    | +    | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     |
| 21      | 17  | F   | 1.2 | +    | +    | +    | +    | +    | +    | +    | +    | +    | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     |
| 22      | 17  | F   | 1.2 | +    | +    | +    | +    | +    | +    | +    | +    | +    | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     |
| 23      | 17  | F   | 1.2 | +    | +    | +    | +    | +    | +    | +    | +    | +    | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     |
| 24      | 17  | F   | 1.2 | +    | +    | +    | +    | +    | +    | +    | +    | +    | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     |
| 25      | 17  | F   | 1.2 | +    | +    | +    | +    | +    | +    | +    | +    | +    | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     |
| 26      | 17  | F   | 1.2 | +    | +    | +    | +    | +    | +    | +    | +    | +    | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     |
| 27      | 17  | F   | 1.2 | +    | +    | +    | +    | +    | +    | +    | +    | +    | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     |

\* Different amounts of bound specific IgE are shown as hatched squares, symbolizing the different CRIE classes A-G (class A = high degree of radiostaining and class G = low degree of radiostaining).

1). The individual patients exhibited different degrees of visible radiostaining against the individual antigens.

Figure 2 demonstrates some representative CRIE patterns of patient sera from Table 1. Patient "a" (upper left hand panel) shows a high degree of specific IgE binding against both dander-related and serum-related components, while patient "b" (upper right hand panel) shows weak response to cat serum-related antigens and strong IgE binding to cat allergen I. On the other hand, patient "c" (lower left hand panel) exhibits strong IgE binding to cat albumin and weak specific IgE binding to cat dander components. Patient "d" (lower right hand panel) gave only radiostaining towards cat dander components and especially to cat allergen I.

By combining the intensity of radiostaining and the frequency of radiostaining of the studied patient panel, allergens may be grouped as major, intermediate, and minor allergens.<sup>19,21</sup> Ag 2 (cat allergen I) fulfills the criteria of a major allergen: 26/27 patients showed specific IgE

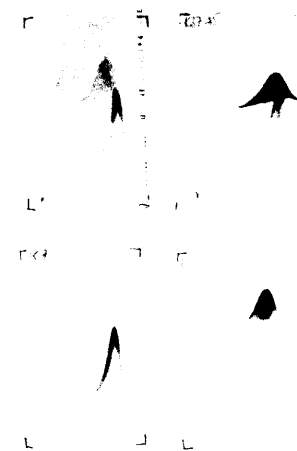


Figure 2. X-ray films from CRIE experiments, demonstrating the radiostaining pattern of four different patient sera. The number of visible radiostained precipitates and the intensity indicate various degree of IgE-binding. Exposure corresponding to CRIE class G. (See "Results" for explanation of panels.)

binding. Ag 3 (cat albumin) and Ag 19 constitute intermediate allergens as 17/27 and 14/27 showed variable degrees of specific IgE binding. As antigen nos 7, 10, 12, 14, and 18 did not fulfill the criteria for major or intermediate allergens, they are thus classified as minor allergens.

RAST data of the 27 subjects are also included in Table 1. High PRU values are obtained for patients showing strong CRIE-radiostaining towards major/intermediate allergens or moderate radiostaining against several allergens. Low PRU values are characteristic of patients exhibiting specific IgE binding towards a limited number of allergens. RAST (e1 discs) giving the total specific IgE binding showed comparable sensitivity and specificity to CRIE analysis of the individual patient sera.

## DISCUSSION

This study was undertaken to char-

acterize a cat dander extract with respect to the content of dander-related and serum-related allergens. The origin of the cat antigens responsible for allergic reactivity has been extensively studied. The distribution in tissues and fluids of "cat allergen I" was extensively studied by Brown and coworkers.<sup>16</sup> Guerin and Hewitt<sup>7</sup> and Anderson and Baer<sup>12</sup> compared the content of cat allergens in extracts from cat fur, pelt, saliva, serum, and urine, respectively. Recently Bartholome et al<sup>23</sup> have also demonstrated the presence of cat allergen I in mucous salivary glands and hair roots.

The main source of the cat allergen I appears to be saliva, which indicates that it originates from some excretory gland. Significant amounts of cat allergen I could also be recovered from surface washes of living cats.<sup>25</sup>

Antigens derived from cat dander are acidic in nature as they migrate towards the anode of CIE analysis (Figs 1 and 2). By using the system with intermediate gel it was possible to differentiate between the source of antigens: 13 antigens were serum-related and eight antigens were radiostained in CRIE analysis of a selected patient panel showing their function as allergens (five serum-specific and three dander-specific). Löwenstein<sup>4</sup> studied an extract of cat hair and dander by CRIE. They found 18 antigens of which 12 bound specifically to IgE. But, only one antigen fulfilled the criteria for a major allergen according to their definition. Based upon our studies it is most likely that some of these IgE-binding components are partially identical (multiple antigens). On the other hand, Anderson and Baer,<sup>12</sup> using a cat pelt extract, found eight antigens in the CIE analysis and four of these also showed specific IgE binding in CRIE analysis.

A recently reported CIE/CRIE study by Sacchi et al<sup>13</sup> using cat saliva as antigen and antibodies raised against saliva resulted in 16

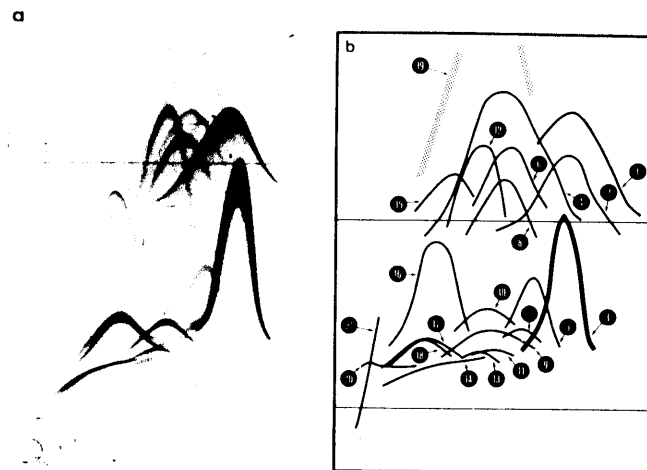


Figure 1. (Left hand panel) Crossed immunoelectrophoresis of cat dander extract against rabbit anti-cat dander and goat anti-cat serum after protein staining with Coomassie Brilliant Blue. Five microliters cat extract was analysed in agarose electrophoresis, followed by electrophoresis perpendicular to the first dimension in agarose gel containing antibodies (Upper gel: rabbit antisera against cat dander proteins, 10  $\mu$ L/cm<sup>2</sup> and intermediate gel: goat antisera against cat serum proteins, 4  $\mu$ L/cm<sup>2</sup>). Anode to the right in the first dimension electrophoresis and at top in second dimension electrophoresis. (Right hand panel) Schematic representation of the immune precipitates (Fig. 1A), arbitrary numbered from the anode. Visible protein-stained precipitates—precipitates only visible after radiostaining.

identified antigens of which some also showed specific IgE binding. Due to the cat source material used, many different quantities of antigens/allergens have been found, which indicates the necessity of using well-defined source material.

By using a CIE/CRIE system with intermediate gel we have been able to divide the cat antigens into serum-related and dander-related components without any prior separation. The CRIE analysis (Table 1) demonstrates that all patients exhibit specific IgE binding against dander components supporting previous findings. Furthermore, this study shows convincingly that the major cat allergen I is a dander-related component. According to our classification system,<sup>21</sup> another dander component (Ag 19) and Ag I (cat albumin) fulfilled the criteria for intermediate allergens.

Cat albumin has not always been recognized as an allergen. It is present in cat saliva and cat dandruff and is obviously not a major allergen: 17 out of 27 patients have significant levels of IgE towards cat albumin. The serum proteins from cat are present in saliva and dandruff to varying degrees. Sacchi and coworkers<sup>13</sup> showed that at least six serum proteins could be detected in saliva by CIE using intermediate gel. The origin of those serum proteins is probably both saliva and excretion from the vascular layers during cat dander formation.

The present investigation shows that cat dandruff source material can give a cat allergen extract with a balanced content of dander-specific and serum-specific allergens. Such a standardized allergen preparation might be a superior preparation for accurate diagnosis of cat allergy and for treatment because patients with cat allergy react to dander-specific allergens as well as serum-specific allergens.

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