

Detection of specific IgE antibodies towards cat flea (*Ctenocephalides felis felis*) in patients with suspected cat allergy

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Cat flea sensitivity is considered one of the most important skin diseases in cats and dogs. Cat fleas, however, are also a growing allergen problem for humans. Cat flea-specific IgE antibodies were studied in serum samples from 70 patients with suspected cat allergy, using RAST-based techniques and the nitrocellulose immunoblotting method. Results from RAST studies, using cat and cat flea as allergosorbents, showed that 46% of the patients were RAST positive against both cat and cat flea. 9% of the patients were RAST positive only against the cat flea. The nitrocellulose immunoblotting experiments were in agreement with the RAST results showing specific IgE to cat flea. The results indicate that some cat-allergic patients have specific IgE both towards cat and cat flea but also that some of the patients with suspected cat allergy might have specific IgE towards the cat flea and not the cat. RAST-inhibition and immunoblotting experiments also indicate that the allergen composition of cat flea extract differs from that of cat extract, even if common allergens have been detected, leading to cross-reactivity in some sera.

Key words: cat allergy; cat flea allergy; *Ctenocephalides felis felis*; IgE; immunoblotting; RAST.

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The cat is the primary host of the cat flea (*Ctenocephalides felis felis*) but the insect is also common on dogs. The indoor climate of modern buildings has caused a northward extension of the cat flea's habitat in Europe and the US. (5, 6, 7, 9, 12). Cat flea sensitivity is considered one of the most important skin diseases in cats and dogs. Cat fleas, however, are also a growing problem for humans (2, 3, 4). Feingold & Benjamin (2) noticed reactions on the skin of human beings bitten by the cat flea and were of the opinion that this was caused by an active allergen present in the oral secretions of these fleas. Joseph (4) stated that *Ctenocephalides felis orientis* could induce hypersensitivity in humans. A severe case of cat flea bite dermatitis in a woman was published by Hunter et al. (3).

This study was undertaken to investigate sera from patients with suspected cat allergy for specific IgE antibodies towards cat flea and to study the allergen composition.

MATERIAL AND METHODS

Allergens

Cat fleas (*Ctenocephalides felis felis*) and cat epithelium (skin scraping) were supplied by Allergon AB, Engelholm, Sweden. Cat fleas, not contaminated with cat allergen, were obtained by taking cat flea larvae from live cats and growing them for 4 weeks on a medium containing no cat or dog proteins. After moulting, the adult cat fleas were separated from the medium.

The allergens used for preparation of allergosorbent were extracted (1/10 w/v) in 0.1 M phosphate buffer pH 7.4 for 16-18 h at +4°C. Particulate matter was removed by centrifugation at 19,000 rpm for 30 min at +4°C. The allergens used for the immunoblotting investigations were extracted in 0.125 M NH₄HCO₃ for 16-18 h at +4°C, centrifuged, filtered and lyophilised.

Patient sera

Serum samples were obtained from 70 patients where cat allergy was suggested by one or more of the following criteria: positive skinprick test, positive history, asthma or rhinoconjunctivitis to cat. Thirty of the patients were from the south and middle of Sweden and the remainder from the US. Seven sera were chosen from the patient panel for further investigation. Four were RAST-positive only to cat flea and three RAST-positive to both cat and cat flea.

RAST measurements

IgE antibodies in the sera were measured, using the Phadebas RAST® (Pharmacia AB, Uppsala) standard procedure in duplicate with allergosorbents, cat epithelium, cat fleas and fresh culturing medium. The results were expressed in Phadebas RAST classes according to manufacturer's instructions. RAST classes 1–4 are considered positive.

RAST inhibition

The RAST-inhibition experiments were performed essentially as previously described by Yman et al. (13), using 30 µl of serial dilutions of cat epithelium extract 1:20 (w/v) and cat flea extract 1:10 (w/v), respectively. The allergen extract was incubated with 20 µl patient serum for 2 h at room temperature in triplicate. The results were expressed as % inhibition compared to a negative control (0.1 M phosphate buffer pH 7.4).

Nitrocellulose immunoblotting

The immunoblotting experiments were carried out as previously described by Bengtsson et al. (1). The electrophoretic separation was carried out essentially by the procedure of Neville (8), with the SDS-g PAGE gel molded in BIORAD, Protein Dual Slab Cell/16 cm equipment, according to operating instructions. The separated antigens were transferred electrophoretically to a nitrocellulose membrane (Transblot nitrocellulose membrane 0.45 µm, BIORAD), using BIORAD transblot cell. The blotting procedure was carried

out according to TransBlotcell operating instructions. After completed transfer, the unoccupied binding sites on the nitrocellulose membrane were blocked by placing them in phosphate buffer pH 7.4 containing 0.5 % Tween 20 at 37°C for 1 h. The blotted gel and reference gel lane with non-blotted sample were stained with Coomassie Brilliant Blue R250 in 30 % methanol and 7 % Hac. The nitrocellulose membrane was cut into strips, each 0.5 cm wide, and incubated overnight on a shaker (200 rpm) at room temperature with 200–500 µl patients' serum diluted up to a total volume of 1 ml in phosphate-buffer pH 7.4.

After washing twice in saline with 0.5 % Tween 20, the strips were incubated with 1 ml ¹²⁵I-anti-IgE (Phadebas RAST®) or enzyme labelled anti-IgE (Phadezym RAST®) on a shaker for 4 h.

After three washes in saline with 0.5 % Tween 20 and twice in saline, the strips were dried. The isotope-labelled strips were taped on to glass plates, put on X-ray film (Du Pont Cronex-4) and exposed in a Kodak X-omatic cassette equipped with a Du Pont cronex intensifying screen for 20 h at 70°C (11). The enzyme-labelled strips were immersed in a chromogenic substrate solution for 20 s and then put on a filter paper prewetted with chromogenic substrate for 20–30 min at 25°C. The chromogenic substrate used was a mixture of 1.5 ml 2-naphtylbeta-D-galactopyranoside monohydrate (3.3 mg/ml) in methanol and 1.0 ml Fast Blue B (200 mg/ml) phosphate buffered saline containing 200 µl MgCl₂ · 6H₂O/ml, diluted to 10 ml with the same phosphate buffered saline (10). Non-specific binding of anti-IgE was checked with a negative control serum from non-allergic healthy donors.

RESULTS

Demonstration of cat flea IgE using Phadebas RAST

The scores from the RAST measurements of the serum samples, using epithelium and cat flea allergens, are presented in Fig. 1.

Thirty-two patients (46 %) were positive to both cat and cat flea allergens, while six of the 70 patients (9 %) were positive *only* to the cat flea allergen, being negative to cat epithelium. Twenty

Similar results were seen for serum 6 with the ability of the cat extract to inhibit specific cat IgE to 98.5 %, but also to affect the cat flea IgE to some degree, and for the cat flea extract to inhibit cat flea IgE to 96.5 % and also inhibit cat IgE to 96.8 %.

Cat flea IgE specificities detected by immunoblotting

The nitrocellulose immunoblotting technique was used to study the cat and cat flea IgE specificities of four of the patient sera. Cat and cat flea nitrocellulose prints were incubated with four patient sera, respectively, and detected with ¹²⁵I-labelled anti-IgE.

According to Fig. 2, serum No. 1, RAST-positive only to cat flea, shows staining of a broad spectrum of specific IgE towards the cat flea but no staining at all on the cat print. Serum No. 2, RAST-positive to both cat flea and cat, shows IgE specificities to both the cat and cat flea prints. The two IgE specificity patterns are, however, completely different, with cat-specific IgE towards components of lower molecular weight (8000–20,000 d) and cat flea IgE binding compo-

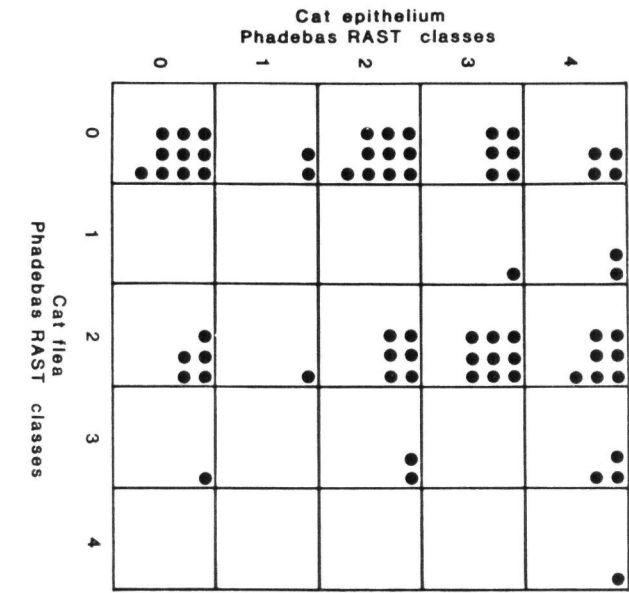


Fig. 1. Cat epithelium and cat flea RAST scores of the 70 patient serum samples, given as Phadebas RAST® classes 0–4.

-two patients (31 %) were positive to cat epithelium and negative to cat flea. Negative scores for both cat epithelium and cat flea were found in 10 patients (14 %). All patients were negative to the cat flea medium.

Cat flea IgE specificity detected by RAST inhibition

The specificity of the IgE towards cat flea allergen was studied using RAST inhibition. The cat flea extract was found to inhibit more than 95 % of the IgE antibodies to cat flea in serum from four patients RAST-positive only to cat flea (Table 1, sera 1–4). Cat allergen did not inhibit cat flea-specific IgE in these sera. The ability of both cat flea extract and cat extract to inhibit two sera (sera 5 and 6) with specific IgE towards both cat flea and cat, was also studied. The specific cat flea IgE and the specific cat IgE were inhibited to 98.6 % and to 98.8 % using cat flea extract (allergosorbent cat flea disc) and cat extract (allergosorbent cat epithelium disc), respectively in serum 5. When inhibiting the specific cat flea IgE with a cat extract and using the cat flea disc as allergosorbent, the specific cat flea IgE was not affected significantly, but when inhibiting the specific cat IgE with a cat flea extract, using cat epithelium disc as allergosorbent, the specific cat IgE was inhibited to 89 % in serum 5.

Table 1

Per cent RAST inhibition of four sera RAST-positive only to cat flea, using cat flea and cat extracts as inhibitors and cat flea discs as allergosorbent

	Cat flea RAST score PRU/ml	Inhibitor	
		Cat flea	Cat
Serum 1	9.0	>97.6	8.8
Serum 2	16.7	>97.0	0
Serum 3	9.7	>98.1	0
Serum 4	10.6	>99.1	0

Per cent RAST inhibition of two sera RAST-positive to both cat flea and cat, using cat flea and cat extracts as inhibitors and cat flea and cat discs as allergosorbents

	RAST score PRU/ml		Cat flea inhibitor		Cat inhibitor	
	Cat flea	Cat	Cat flea disc	Cat disc	Cat flea disc	Cat disc
Serum 5	15.5	10.0	>98.6	15.5	89.8	>98.8
Serum 6	3.3	15.7	>96.5	48.4	96.8	>98.5

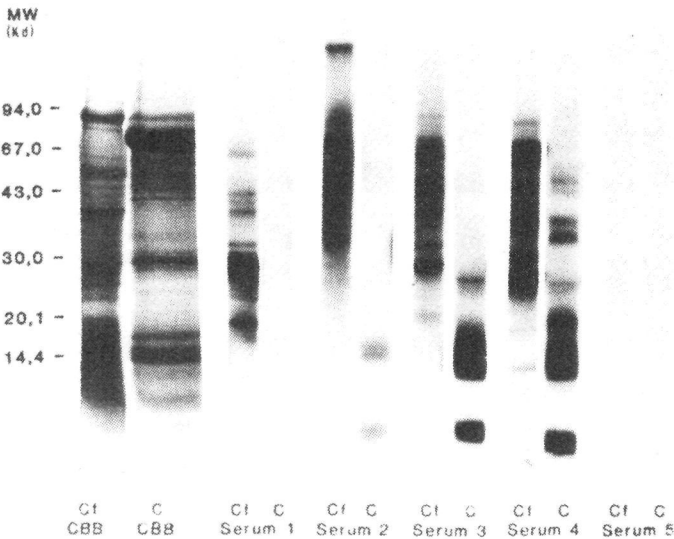


Fig. 2. Nitrocellulose prints with cat (c) and cat flea (cf) allergens from immunoblotting experiments, using 4 different serum samples. Strip No. 5 is a negative control serum. CBB = Coomassie Brilliant Blue stained SDS-g PAGE patterns of cat flea (cf) and cat (c).

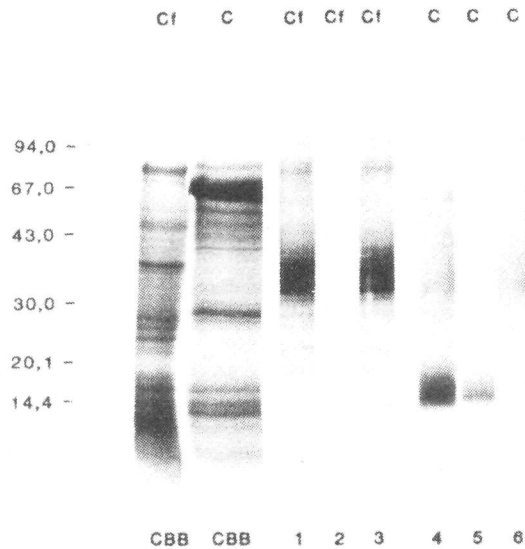


Fig. 3. An inhibition immunoblotting experiment, using a serum RAST-positive to both cat flea and cat. CBB = Coomassie Brilliant Blue stained SDS-g PAGE patterns of cat flea (cf) and cat (c). Three cat flea prints (cf) were incubated with the serum not inhibited (strip 1), inhibited with cat flea extract (strip 2) and inhibited with cat extract (strip 3). Three cat prints (c) were incubated with the serum not inhibited (strip 4), inhibited with cat flea extract (strip 5) and cat extract (strip 6).

nents in a molecular weight area of 25,000–70,000 d. Sera Nos. 3 and 4, also RAST-positive to both cat and cat flea, show the same type of

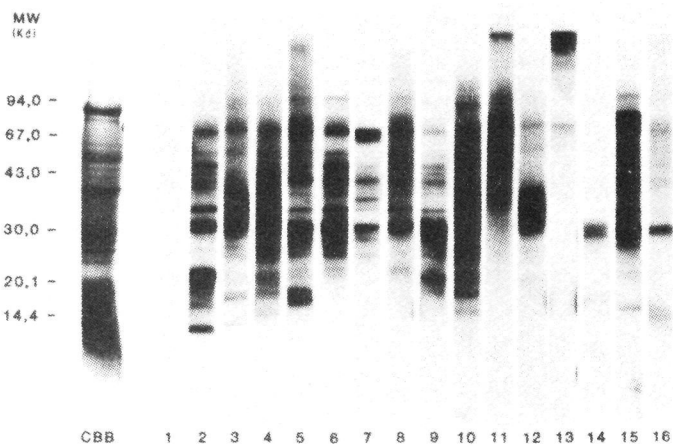


Fig. 4. Screening of IgE specificity patterns in a panel of cat flea RAST-positive patient sera using nitrocellulose prints with cat flea allergens from immunoblotting experiments. CBB = Coomassie Brilliant Blue SDS-g PAGE patterns of the cat flea extract.

differences between cat and cat flea IgE specificities as serum No. 2.

An inhibition immunoblotting experiment was also performed, using serum No. 5 from RAST-inhibition experiments, RAST-positive both to cat flea and cat, detected with enzyme-labelled anti-IgE and a chromogenic substrate. According to Fig. 3, three cat flea strips were incubated with the serum, not inhibited (strip 1), inhibited with cat flea extract (strip 2), and inhibited with cat extract (strip 3). Three cat strips were also incubated with the serum, not inhibited (strip 4), inhibited with cat flea extract (strip 5), and inhibited with cat extract (strip 6). As can be seen from strips 1–3 (Fig. 3), the cat flea extract, but not the cat extract, was able to completely inhibit cat flea-specific IgE in the serum. A cat extract was also able to inhibit cat-specific IgE (strips 4 and 6). However, it seems that also the cat flea extract has the ability to inhibit cat-specific IgE to some degree (strip 5), which is in accordance with the RAST inhibition results.

Screening of IgE specificity patterns in a panel of cat flea RAST-positive patient sera

Fig. 4 demonstrates a screening of IgE specificity patterns in a panel of 15 cat flea RAST-positive patient sera, using the nitrocellulose immunoblotting technique. The IgE response, measured as the

degree of visible radiostaining, ranges from IgE towards a few cat flea components (strips 13 and 14) to very broad spectrum sera with IgE towards many cat flea components (strips 2, 3, 4, 5, 10). Most of the patient sera have quite heavy radiostaining towards components in the MW area of 30,000–70,000 d. Twelve of the 15 patient sera have specific IgE towards a component with a molecular weight of approximately 30,000 d, indicating that this component is an important cat flea allergen.

DISCUSSION

Using RAST, RAST-inhibition and immunoblotting techniques, we have demonstrated that the cat flea can act as an allergen in human beings.

Nine percent of a panel of 70 patients with suspected cat allergy had specific IgE not to cat but to cat flea. Many of the patients studied were positive to both cat and cat flea.

Specificity of IgE to cat flea allergen was also shown in RAST-inhibition experiments, where cat flea extract inhibited more than 95 % of the IgE antibodies to cat flea in four different patient sera. Cat allergen extract was not able to inhibit the cat flea-specific IgE in these sera. RAST inhibition and immunoblotting experiments indicate a difference in the allergen composition of the cat flea and cat. However, in some sera cat IgE was inhibited with cat flea extract, indicating the existence of common allergens, leading to cross-reactions in some sera. This could be the result of contamination with cat allergen, despite the fact that the cat flea was cultured on separate medium.

A study of cat flea IgE specificities in 15 patient sera revealed a high degree of variability in IgE specificity patterns between the patients, ranging from broad spectrum specificities to IgE against a few cat flea components. The heaviest radiostaining in most patient sera was located in the range 30,000–70,000 d. Most of these patient sera have specific IgE towards a component with a molecular weight of approximately 30,000 d, indicating that this component is an important cat flea allergen.

Further investigation will be needed to show the clinical importance of cat flea allergy.

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