

Major cat and dog allergens share IgE epitopes

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Background: Patients allergic to cats and dogs frequently display IgE reactivity against allergens from different animals, suggesting a cross-sensitization to common allergenic determinants. Although albumins have been recognized as relevant cross-reactive allergens, little is known regarding cross-reactive epitopes of the major cat and dog allergens.

Objective: In this study, sera from patients allergic to cats and/or dogs were used to investigate the presence of common IgE epitopes among the major cat and dog allergens.

Methods: The IgE reactivity profile of 109 patients who were allergic to allergens from several species of animals was determined with nitrocellulose-blotted cat and dog allergens. Sera from patients who were strongly allergic to the major cat and dog allergens were tested for the presence of cross-reactive IgE antibodies by one-dimensional and two-dimensional immunoblot inhibition experiments and by quantitative measurements obtained with the CAP-FEIA system (Pharmacia).

Results: Sixty-eight of 109 patients with animal allergy showed IgE reactivity to cat allergens and dog allergens. Sera from patients with both cat and dog allergy detected allergens of similar molecular weight in nitrocellulose-blotted cat and dog hair/dander extracts. Common, as well as species-restricted, IgE epitopes of the major cat and dog allergens could be demonstrated by IgE inhibition studies.

Conclusion: Shared IgE epitopes of the major cat and dog allergens may provide an explanation for the clinical observation that allergies to cats and dogs are frequently associated. (*J Allergy Clin Immunol* 1997;99:100-6.)

Key words: Cat allergy, dog allergy, immunoblotting, IgE-immunoblot inhibition, IgE-cross-reactivity

Animal danders represent an important source of inhalant allergens.^{1,2} Almost 30% of atopic individuals display type I allergic symptoms on exposure to cat and/or dog allergens,^{3,4} and it has been reported that cat and dog allergy are frequently associated.⁵⁻⁸

Major cat and dog allergens can be found in hair/dander extracts and saliva and are hence considered to be epithelial allergens.⁹⁻¹⁸ The major cat allergen, Fel d 1, has been characterized extensively by protein and immunochemical techniques

Abbreviation used

SDS-PAGE: Sodium dodecylsulfate-polyacrylamide gel electrophoresis

and was recently expressed as a recombinant allergen.¹⁹ Fel d 1 represents an approximately 36 kd dimer, which is composed of two 17 kd subunits. Each subunit is assembled by two different chains (chain 1 and 2), of which chain 1 shows sequence homology to uteroglobin.²⁰ Less information is available regarding the major dog allergens. Sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis and immunoblotting have revealed two IgE-binding components in dog hair/dander extracts with molecular masses of 23 kd and 19 kd.²¹ These components have been designated Can d 1 and Can d 2, although no primary sequence information has as yet been published.⁴ Little information is available regard-

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ing the presence of cross-reactive IgE epitopes in animal epithelial allergens, although albumins have been recently identified as relevant cross-reactive allergens.^{5, 22, 23} Albumins occur at high concentrations in animal hair/dander extracts and represent important cross-reactive allergens for approximately 30% of patients with animal allergy. Sera from patients who are sensitized against albumins contain a high percentage of albumin-specific IgE.^{24, 25} The presence of common IgE epitopes on cat and dog albumins in part explains allergic symptoms in individuals allergic to dogs and cats as a result of immunologic cross-reactivities. This study addresses the question of whether common IgE epitopes can also be found among the major cat and dog allergens by using sera from patients allergic to cats and/or dogs for one- and two-dimensional immunoblot inhibition experiments.

METHODS

Characterization of allergic patients

Highly reactive sera from 109 patients with positive case histories, positive skin test responses, and positive RAST results (RAST class >3) with cat and/or dog allergens were investigated. Sera from 22 healthy individuals with negative case histories of any Type I allergy, negative skin prick test responses, and negative RAST results to cat and/or dog allergens were used as negative controls.

Natural allergen extracts

Powdered hair/dander from cats and dogs were purchased from Allergon, AB (Engelholm, Sweden) and extracted as previously described.²² The protein concentration of each extract batch was estimated by SDS-PAGE and Coomassie Brilliant Blue (BioRad, Richmond, Calif.) staining²⁶ and adjusted to a final concentration of approximately 1.5 mg/ml.

SDS-PAGE, isoelectric focusing, and two-dimensional electrophoresis

Proteins were separated by SDS-PAGE according to the method of Laemmli²⁷ and were transferred to nitrocellulose as described by Towbin et al.²⁸ Approximately 200 µg/cm of cat or dog extract was separated by using a 12% preparative gel. Molecular weights were estimated with prestained protein standards (Amersham, Buckinghamshire, U.K.). Two-dimensional electrophoresis was performed on the ISO-DALT electrophoresis system of Anderson and Anderson²⁹ (Electro Nucleonics, Inc., Oak Ridge, Tenn.) as described.³⁰

Immunoblot

Nitrocellulose sheets containing extracts separated by two-dimensional electrophoresis or nitrocellulose strips

(0.5 cm) cut from preparative sheets were saturated for 30 minutes with buffer A (50 mmol/L Na₂HPO₄, pH 7.4, 0.5% Tween 20 [vol/vol], 0.05% [wt/vol] NaN₃) and subsequently incubated with patients' serum, diluted 1:10 in buffer A at 4°C overnight. Strips were washed three times for 20 minutes with buffer A, and bound IgE was detected by using iodine 125-labeled anti-human IgE antibodies (Pharmacia, Uppsala, Sweden) as described.²¹ Autoradiography was performed at -70°C for 72 to 96 hours, by using Lanex R FAST intensifying screens and Kodak ORTHO G x-ray films (Kodak, Heidelberg, Germany).

Qualitative IgE inhibition experiments

Patients with IgE reactivity to the major cat and dog allergens were selected by immunoblot experiments. Sera were diluted 1:10 in buffer A and preincubated overnight at 4°C with approximately 30 µg/ml dog hair/dander extract, 30 µg/ml cat hair/dander extract, or 30 µg/ml human transferrin (control). Preadsorbed sera were then used to detect nitrocellulose-blotted cat or dog allergens as described above.

Quantitative IgE inhibition experiments

Sera from five patients allergic to cats and dogs but without IgE against albumins (as determined by immunoblot experiments) were diluted 1:2 and preincubated with human transferrin (100 µg/ml) (negative control), cat hair/dander extract (100 µg/ml), or dog hair/dander extract (100 µg/ml) overnight at 4°C. Quantitative determinations of IgE specific for cat (e1) and dog (e5) extracts were done in the Pharmacia CAP FEIA system.

RESULTS

Patients allergic to cats and dogs display IgE reactivity to components of similar molecular weight present in cat and dog hair dander extracts

The IgE reactivity profile with cat and dog hair/dander extracts was determined for 109 sera from patients allergic to cats and/or dogs by immunoblotting. Sixty-eight patients reacted with both nitrocellulose-blotted cat and dog hair/dander extracts. Twenty-nine of 109 patients reacted exclusively with cat allergens, and 12 patients showed IgE binding to dog allergens only. Eighty-nine percent of patients with cat allergy reacted with Fel d 1, 71% and 74% of patients with dog allergy reacted with 19 kd and 23 kd components, respectively, presumably representing Can d 1 and Can d 2. Twenty-eight patients reacted with cat and dog albumin. Sixty-five patients who displayed IgE reactivity to the major cat allergen Fel d 1 at 18 kd in cat extract had IgE that recognized the major dog allergens, Can d 1 and Can d 2, at comparable molecular weights in dog extract.

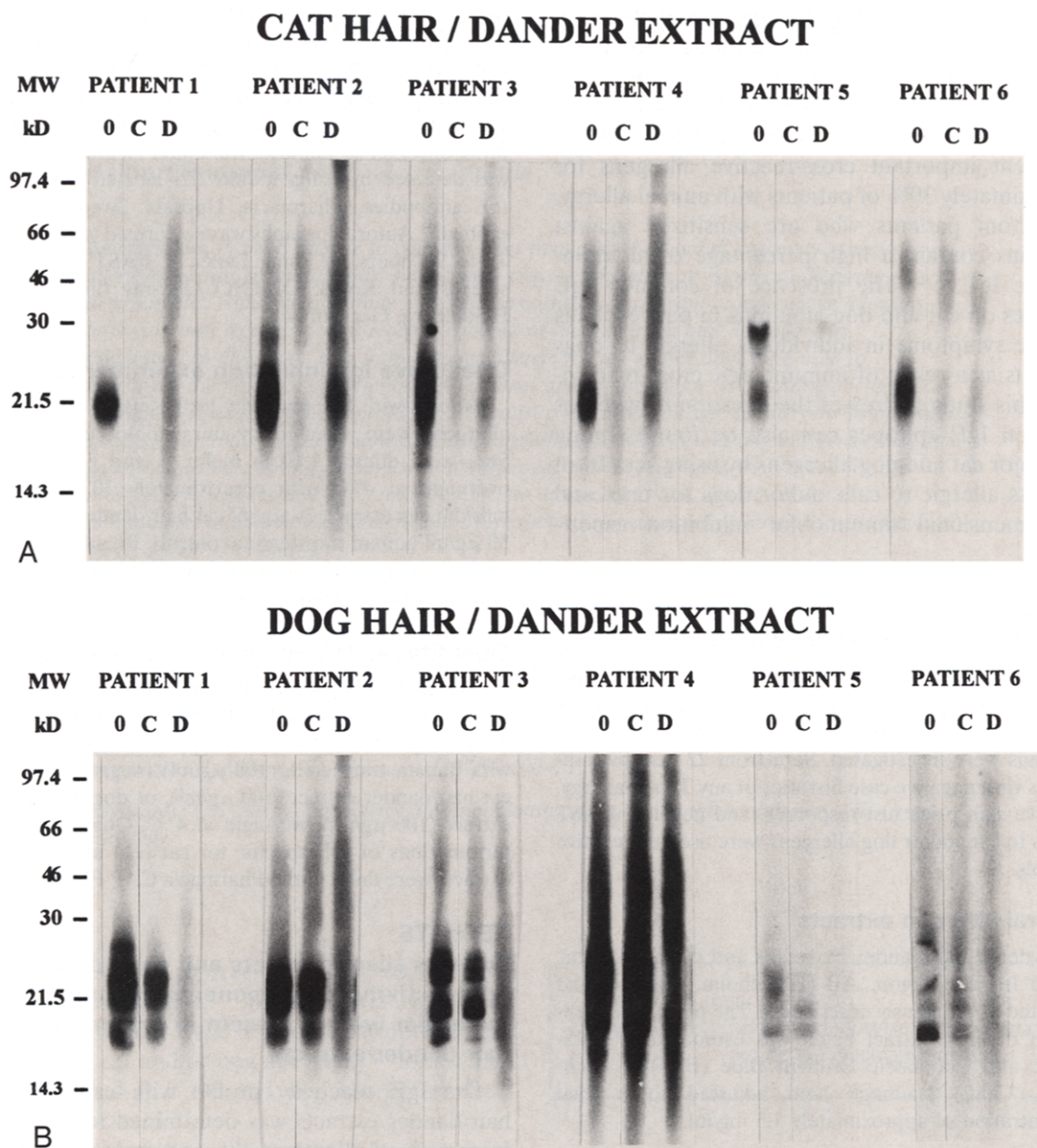


FIG. 1. Common IgE-reactive epitopes of the major cat and dog allergens revealed by IgE-immunoblot inhibitions. Cat hair/dander extracts (**A**) and dog hair/dander extracts (**B**) were separated by SDS-PAGE. Sera from six patients with IgE reactivity to cat and dog hair/dander allergens were preincubated with transferrin (*lane 0*), cat hair/dander proteins (*lane c*), or dog hair/dander proteins (*lane d*) before incubation with the nitrocellulose-blotted extracts. MW, Molecular weight.

Immunoblot inhibition

The presence of common IgE-reactive epitopes in the major cat and dog allergens was investigated by IgE-immunoblot inhibition experiments by use of sera from 17 patients. Fig. 1 shows the results obtained in six representative patients. Preincubation of sera with dog hair/dander extract substan-

tially reduced IgE binding to cat hair/dander proteins in 14 of 17 patients. Preincubation with cat hair/dander extract significantly reduced IgE binding to dog hair/dander proteins in 11 of 17 patients. The control inhibition performed with transferrin did not lead to a reduction of IgE binding to either dog or cat allergens. As an additional control

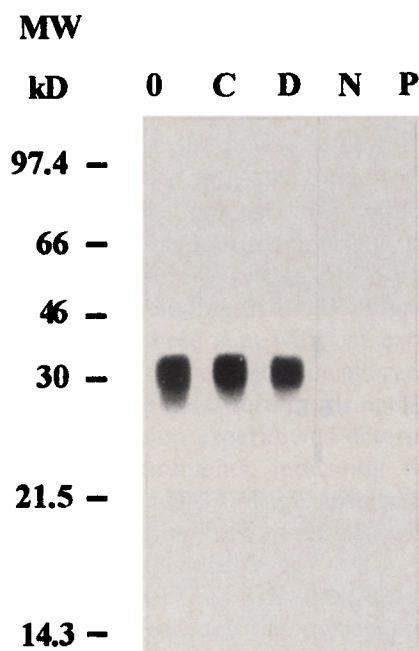


FIG. 2. Preincubation of serum of a patient allergic to timothy grass pollen with cat or dog extracts does not affect IgE binding to nitrocellulose-blotted timothy grass pollen extract. The serum was preincubated with transferrin (lane 0), cat (lane C), or dog extract (lane D). Lane N shows the results after incubation with serum from a nonallergic individual. Lane P represents the buffer control. MW, Molecular weight.

experiment, it was demonstrated that preincubation with cat or dog extract did not lead to non-specific inhibition of IgE binding to timothy grass pollen allergens by using serum from an individual allergic to grass pollen (Fig. 2).

To further compare the cross-reactive components, cat and dog extracts were separated on the same gel. Fig. 3 shows that preincubation of the serum from a patient allergic to cats and dogs with cat or dog extract leads to a significant inhibition of IgE binding to the major cat and dog allergens at approximately 21 kD (apparent molecular weight determined by SDS-PAGE) in both extracts. Preincubation of two sera from patients with cat and dog allergy with cat extract significantly inhibited IgE binding to at least three spots at isoelectric point 6 migrating at 23 kD in two-dimensional separated dog allergen extracts (Fig. 4).

Quantitative IgE inhibition

Sera from five patients allergic to both cat and dog hair/dander without IgE against albumin were preadsorbed with cat or dog hair/dander extract to determine the level of IgE-mediated cross-reactiv-

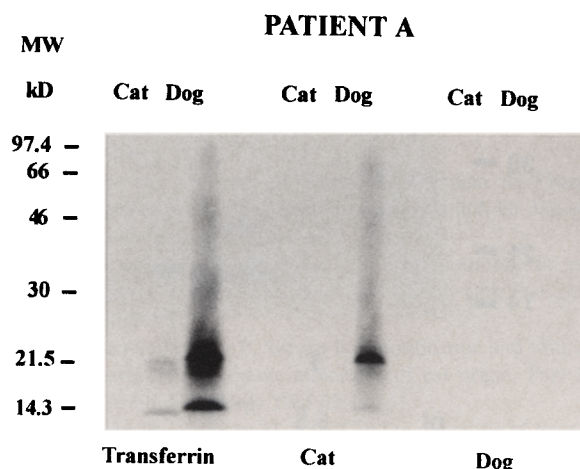


FIG. 3. Proteins from cat and dog extract were separated on the same gel and transferred to nitrocellulose. Sheets containing cat and dog extracts were then incubated with serum from a patient with dog and cat allergy, which had been preincubated with transferrin, cat extract, or dog extract. Bound IgE was then detected with ^{125}I -labeled anti-human IgE antibodies. MW, Molecular weight.

ity against the major allergens in the Pharmacia CAP FEIA system. On average, dog extract inhibited 86.6% of IgE binding to CAP-bound cat allergens (e1), whereas cat extract was able to inhibit 57% of IgE reactivity to CAP-bound dog allergens (e5) (Table I).

DISCUSSION

Patients allergic to animals frequently display IgE reactivity to hair/dander proteins from different animals.¹⁻⁴ Earlier studies based on RAST inhibition assays have indicated that sera from such patients contain cross-reacting IgE antibodies.⁵⁻⁸ More recent studies have identified albumins from different animals as relevant cross-reactive animal allergens.²⁵ The great sequence and structural similarities among albumins constitute the molecular basis for the observed immunologic relationship among mammalian albumins.²⁴ Although the presence of albumin in different animal hair/dander extracts can explain the occurrence of allergic symptoms in patients on contact with various animals, little is known regarding the presence of additional cross-reactive IgE-binding epitopes among other hair/dander allergens.

In this study the presence of cross-reactive IgE-binding epitopes of the major cat and dog allergens was studied by IgE inhibition experiments. Allergies to cats and dogs are common in industrialized countries in which up to 30% of atopic individuals experience symptoms of allergy on contact with

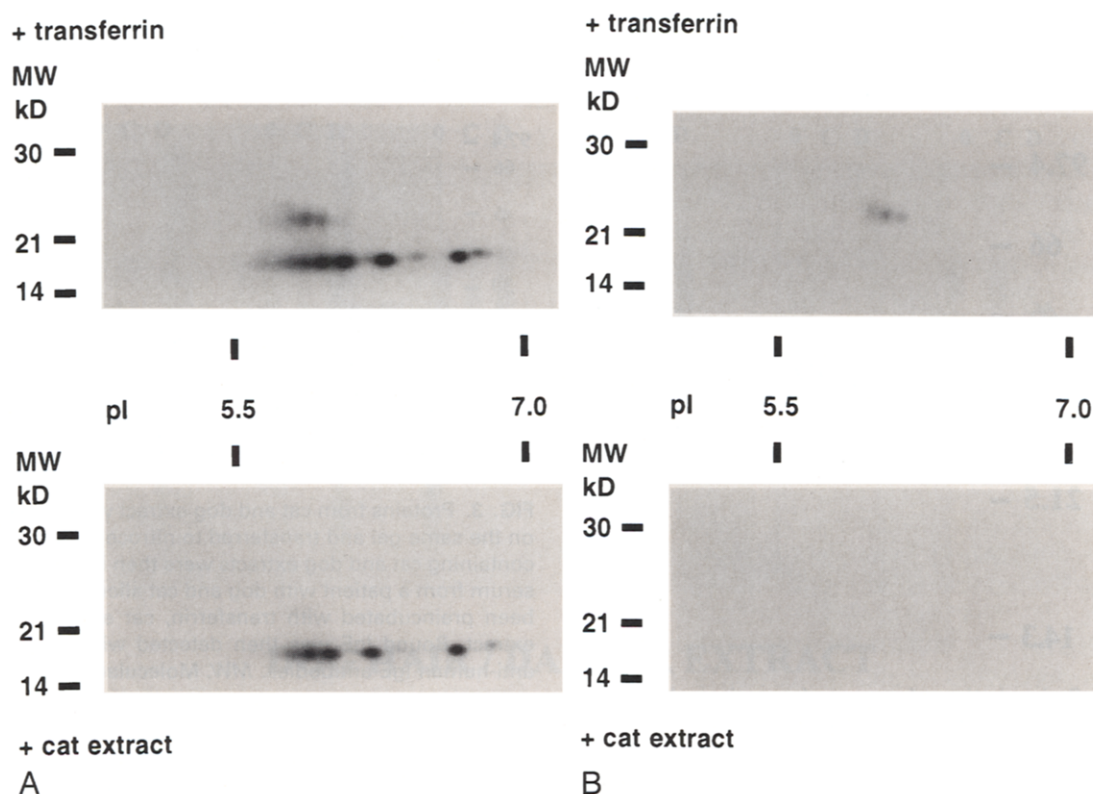


FIG. 4. Two-dimensional immunoblot inhibition. **A and B,** Sera from two individuals allergic to both cats and dogs were preincubated with transferrin (negative control) or with cat extract. Preadsorbed sera were used to detect two-dimensional separated dog allergens. *MW*, Molecular weight; *pl*, isoelectric point.

TABLE I. Quantitative IgE inhibition

Patient	Remaining IgE reactivity (kUA/L [%]) to					
	Cat (e1) extract			Dog (e5) extract		
	TRF	Sera preincubated with cat extract	dog extract	TRF	Sera preincubated with cat extract	dog extract
AS	59.6 (100)	0.7 (1,2)	3.7 (6,2)	44.9 (100)	8.9 (19,8)	1.2 (2,7)
AR	75.2 (100)	2.5 (3,3)	22.2 (29,5)	79.1 (100)	49.2 (62,2)	7.1 (9,0)
PS	19.6 (100)	0.4 (2,0)	2.9 (14,8)	20.9 (100)	9.2 (44,1)	0.5 (2,4)
GG	44.8 (100)	0.5 (1,1)	4.3 (9,6)	20.6 (100)	8.1 (39,3)	1.5 (7,3)
SS	21.8 (100)	1.5 (6,9)	1.5 (6,9)	86.8 (100)	43.5 (50,1)	3.7 (4,3)

Sera from five patients allergic to dogs and cats were preincubated with transferrin (TRF), cat extract, or dog extract before IgE specific for cat (e1) and dog (e5) was measured in the CAP-FEIA system.

kUA/L, Kilo units antigen per liter; *TRF*, transferrin.

cats and dogs. Sixty-eight of the 109 sera from individuals allergic to animals investigated in this study showed IgE reactivity to dog and cat allergens. Immunoblotting revealed IgE-binding components of similar molecular weight in dog and cat extracts. Among the patients allergic to both animals, 98% of the sera reacted with an 18 kd band in cat extracts, corresponding to the two dissoci-

ated subunits of the major cat allergen Fel d 1, and all of these sera bound to proteins migrating between 19 and 23 kd in dog hair/dander extracts, corresponding to the proposed major dog allergens Can d 1 and Can d 2.

To demonstrate the presence of common IgE-reactive epitopes in the major cat and dog allergens, IgE immunoblot inhibitions were performed.

The IgE immunoblot inhibition technique was chosen as a qualitative technique to visualize the reduction of IgE binding to components of defined molecular weight. Preadsorption of sera with cat and dog extracts significantly reduced IgE binding to the major cat and dog allergens, respectively, thus indicating the presence of common IgE epitopes. When sera from patients with preferential dog allergy were investigated, dog extracts inhibited the IgE binding to Fel d 1 significantly. Preincubation of these sera with cat extracts reduced the binding to the major dog allergens much less (Fig. 1). These data were confirmed by quantitative IgE inhibition experiments determined by CAP-FEIA measurements, indicating that substantial amounts (>50%) of IgE specific for allergens other than albumin cross-reacted with major cat and dog allergens (Table I).

However, the lack of IgE cross-reactivity in several patients indicates the presence of additional noncross-reactive epitopes among the major cat and dog allergens. It may therefore be proposed that certain patients were sensitized initially against allergens from one species, thus forming IgE antibodies against common and species-restricted epitopes. The IgE antibodies induced against common determinants may then lead to symptoms of allergy on contact with related allergens from other species. This hypothesis would not exclude the possibility that certain patients were also co-sensitized against related allergens from several other species.

The presence of common IgE-reactive epitopes among the major cat and dog allergens explains why many patients with animal allergies react to cat and dog hair/dander extracts, and this finding may also have implications for diagnosis and therapy of allergies to animals. The limited degree of cross-reactivity indicates, however, that for diagnosis and perhaps therapy the major cat and dog allergens cannot completely replace each other.

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