

IgE cross-reactivities against albumins in patients allergic to animals

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Background: Type I allergic symptoms and severe asthma in particular are frequently caused by animal hair/dander proteins, among which albumins are possible cross-sensitizing allergenic components.

Methods: The significance and degree of IgE-cross-reactivities against various albumins were studied in a representative number ($n = 200$) of patients allergic to animals with hair/dander extracts, purified albumins from different animals, and a recombinant dog albumin fragment expressed in lysogenic *Escherichia coli* Y1089 and purified as a β -galactosidase fusion protein.

Results: Despite a high degree of sequence homology among different albumins, a remarkable variability of IgE cross-reactivities was observed, indicating that some patients were sensitized preferentially against certain albumins. Most of the patients allergic to albumins, however, reacted to dog, cat, and horse albumin, which also bound a high percentage of albumin-specific IgE.

Conclusion: The purified recombinant dog albumin fragment, representing 265 amino acids of the mature protein, bound IgE from all 15 patients allergic to albumin tested suggesting its potential usefulness for diagnosis and perhaps therapy. (*J ALLERGY CLIN IMMUNOL* 1995;96:951-9.)

Key words: Animal hair/dander allergy, albumin, IgE-cross-reactivity, recombinant dog albumin fragments, diagnosis.

IgE-mediated hypersensitivities against animal danders, particularly to cat and dog allergens, are of clinical importance because of an increased incidence of asthma and severe atopic reactions in patients allergic to animals.^{1,2} Up to 30% of allergic patients are sensitized against animal hair/dander proteins.³ A number of allergenic components present in animal hair/dander extracts were characterized by protein and immunochemical techniques, which led to the identification of major allergens of cat, dog, horse, guinea pig, and other

rodents.⁴⁻⁹ Most of the major allergens described, especially those from cats and dogs, are derived from epithelium.³ However, many investigators have observed IgE reactivities against serum proteins present in hair/dander extracts.^{10,11} In previous studies it was demonstrated that among serum-derived allergens, dog albumin represents an important allergen for up to 35% of patients allergic to dogs. The relevance of dog albumin as an allergenic component was further substantiated by complementary DNA cloning techniques, and it was demonstrated that a remarkably high proportion of dog hair/dander-specific IgE in patients allergic to dog albumin is directed against dog albumin.¹²

In this study 200 patients allergic to animal hair/dander were tested for IgE reactivity with hair/dander extracts. Approximately 30% of the patients tested reacted with albumins from different animals. In spite of the high degree of structural similarities of different albumins, a remarkable variability of IgE reactivities was observed. However, 85% of patients allergic to albumin

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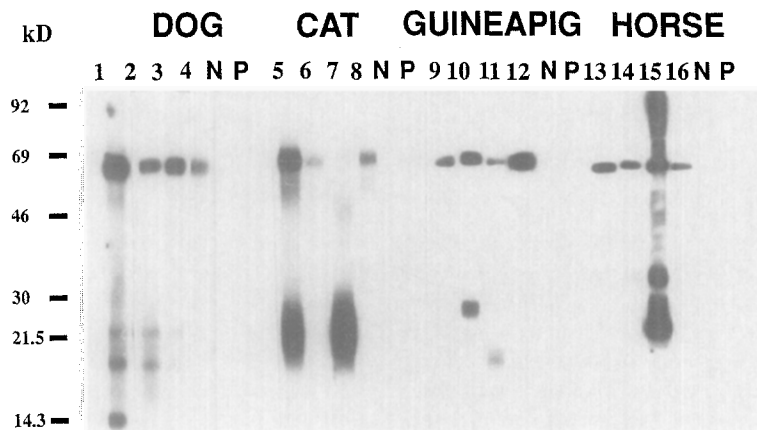
HAIR / DANDER EXTRACTS FROM:

FIG. 1. IgE reactivity of patients allergic to animal hair/dander with nitrocellulose-blotted hair/dander protein extracts. Aqueous extracts prepared from dog, cat, guinea pig, and horse hair/dander were tested for IgE binding with sera from patients allergic to animals.¹⁻¹⁶ N represents serum from a nonallergic individual and in P buffer without serum added.

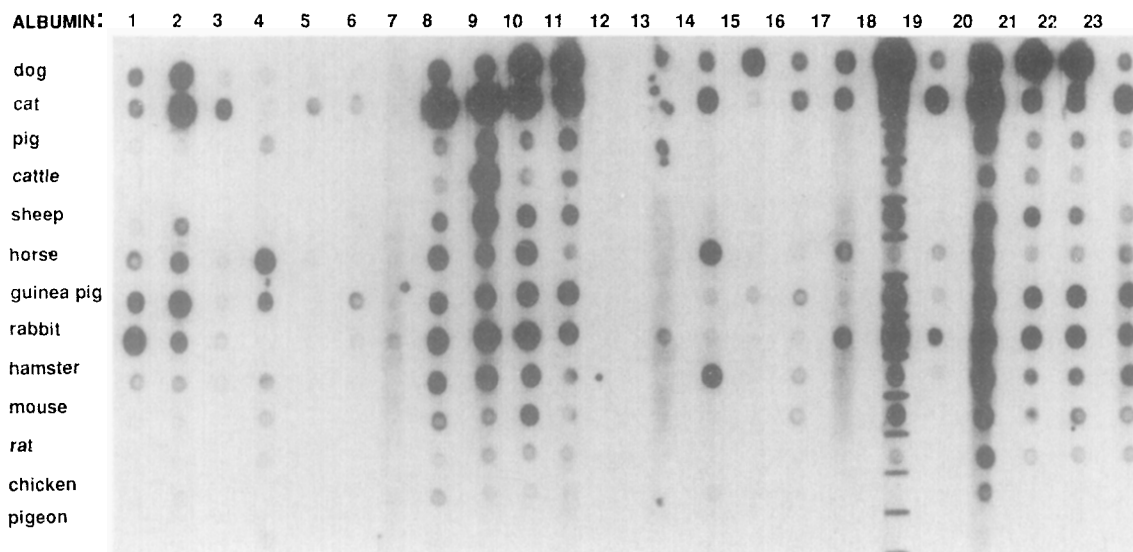


FIG. 2. Testing of patients allergic to animals for IgE reactivity with dot-blotted albumins from different species. Patients described in Table I were tested with albumins from dog, cat, pig, sheep, cattle, guinea pig, hamster, rabbit, horse, chicken, and pigeon.

showed IgE reactivity against cat and dog albumin, which when tested in ELISA inhibitions bound a high proportion of albumin-specific IgE. A recombinant dog albumin fragment making up a major epitopic region of dog albumin was successfully used in ELISA assays to allow a diagnosis in patients allergic to albumin.

METHODS

Characterization of allergic patients

Sera from 200 patients allergic to animals were selected according to case history (allergic symptoms upon

contact with animals), positive RAST (RAST class >2), or positive skin prick test responses. All patients were tested for IgE reactivity with nitrocellulose-blotted hair/dander extracts. Thirty percent ($n = 60$) displayed IgE reactivity to albumins. These sera were further tested in dot-blot experiments with albumins from different species. Of the 60 patients with albumin-positive sera, eight patients with a broad reactivity to different albumins were chosen for ELISA-inhibition studies. Fifteen patients with IgE reactivity to dog albumin were selected for ELISA studies with the recombinant dog albumin fragment. Sera from 22 healthy individuals with a negative case history of any type I allergy, and negative skin

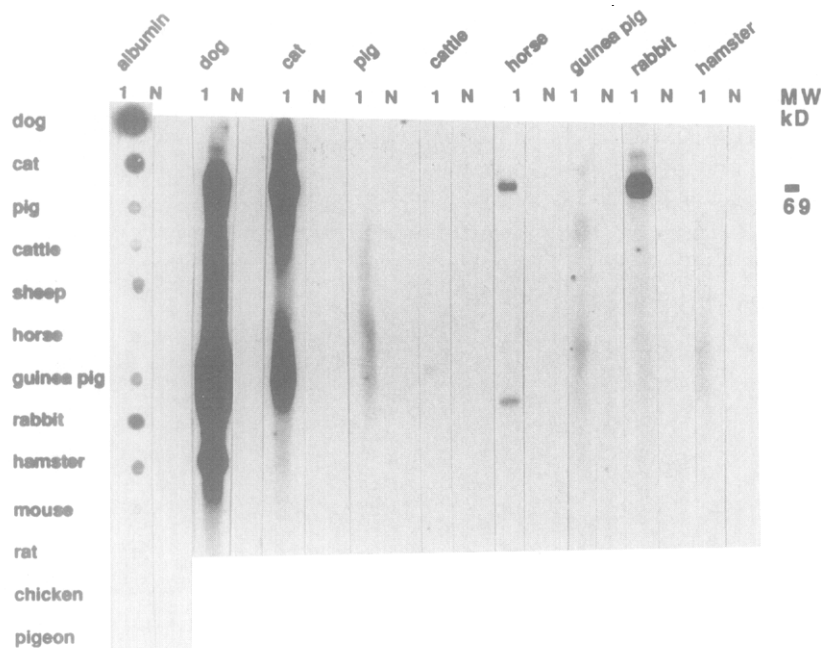


FIG. 3. Testing of different SQ extracts for presence of albumin. Serum from a patient allergic to albumin with IgE binding to dog, cat, pig, cattle, sheep, horse, guinea pig, rabbit, hamster, mouse, and rat albumin (lane 1) was tested for IgE reactivity with nitrocellulose blotted SQ-extracts from dog, cat, pig, cattle, horse, guinea pig, rabbit, and hamster. The position of albumin at 69 kD is indicated. Serum from a nonallergic individual (N) was used as control.

prick test response, and RAST results to cat, dog, guinea pig, and horse allergens were used as negative control sera.

Natural allergen extracts

Powdered hair/dander from cat, dog, guinea pig, and horse were purchased from Allergon, AB (Engelholm, Sweden) and extracted as previously described.¹³ The protein concentration of the hair/dander extracts was determined by the Peterson modification of the Lowry method.¹⁴ Dog, cat, pig, cattle, sheep, horse, guinea pig, rabbit, hamster, mouse, rat, chicken, and pigeon serum albumins were purchased (Sigma, St. Louis, Mo.) and dissolved in 0.9% NaCl at a concentration of 1 mg/ml. Dog, cat, pig, cattle, horse, guinea pig, rabbit, and hamster-SQ extracts (ALK Copenhagen, Denmark) were analyzed for the presence of albumin.

Expression of a recombinant dog albumin fragment as β -galactosidase fusion protein: Purification of the recombinant dog albumin fragment

Lysogenic *E. coli* Y1089 were infected with recombinant phage capable to express a dog albumin fragment, which was described previously.¹² *E. coli* were grown and induced to express the dog albumin β -galactosidase fusion protein as described.¹⁵ *E. coli* pellets were resuspended in 30 ml buffer A (100 mmol/L Tris, HCL pH 7.4,

10 mmol/L ethylenediamine tetraacetic acid, 1 mmol/L Phenyl-Methyl-Sulfonyl Fluorid), disrupted with an Ultraturrax (Ika, Marburg, Germany) on ice. Lysates were then centrifuged for 20 minutes at 15000g to separate insoluble particles. Proteins were precipitated overnight at 4° C with 75% (wt/vol) ammonium sulphate. Precipitated proteins were centrifuged for 30 minutes at 4° C at 5000g, and the pellet was resuspended in 10 ml ice cold buffer A and diluted fourfold with buffer B (50 mmol/L Tris, HCL pH 7.3).

A β -galactosidase affinity matrix (Promega, Madison, Wis.) was washed with buffer B. The sample was passed twice over the column, which was subsequently washed with 20 ml buffer C (50 mmol/L Tris, HCL pH 7.3, 0.2% NP-40). The bound dog albumin β -galactosidase fusion protein was eluted with 10 ml buffer D (0.1 mol/L NaHCO₃/Na₂HCO₃, pH 10.8) and 10 ml buffer E (50 mmol/L Tris, HCL pH 7.3, 150 mmol/L NaCl). The eluat¹ was then dialyzed two times overnight at 4° C against distilled water, lyophilized, dissolved in distilled water at a concentration of 0.1 mg/ml, and stored at -20° C until use.

Sodium dodecylsulfate-polyacrylamide gel electrophoresis, immunoblotting, and dot blots

Sodium dodecylsulfate-polyacrylamide gel electrophoresis and IgE immunoblotting were performed as

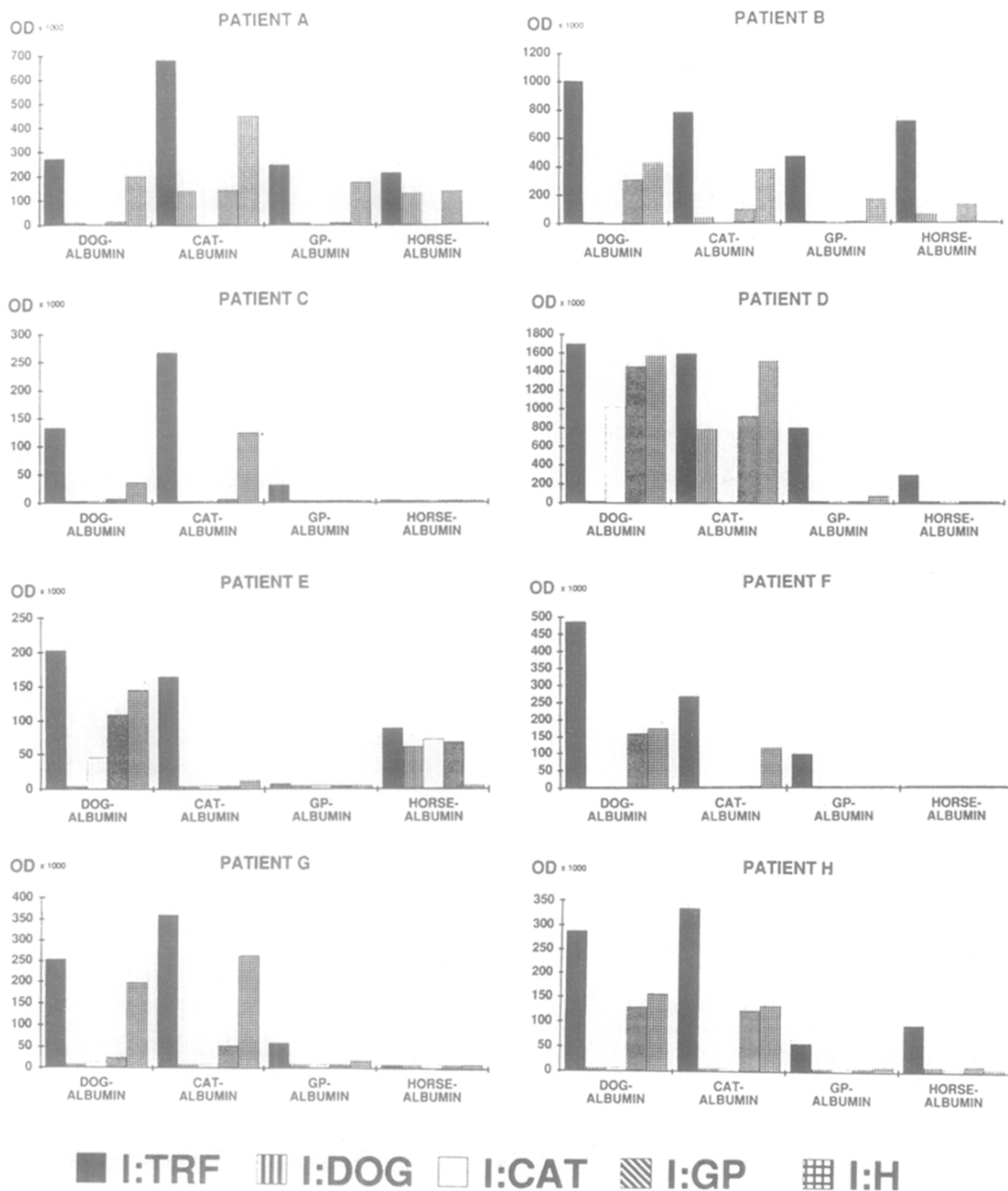


FIG. 4. IgE-binding capacity of albumins from dog, cat, guinea pig, and horse. Sera from eight patients were tested by ELISA for IgE reactivity to these four albumins after preincubation with transferrin (*TRF*, control), dog albumin (*DOG*), cat albumin (*CAT*), guinea pig albumin (*GP*), and horse albumin (*H*), respectively. The y-axis displays extinctions measured as optical density (*OD*) at 405 nm. All values represent means of duplicate determinations.

previously described.^{16,17} Dog, cat, pig, cattle, horse, guinea pig, rabbit, and hamster SQ skin prick extracts were analyzed for the presence of albumin by separating 1 mg of each protein extract (according to the manufacturer's advice) on a preparative sodium dodecylsulfate

polyacrylamide gel electrophoresis (1 mg/15 cm gel) and subsequent immunoblotting. To measure IgE reactivity of allergic patients to non-denatured albumins, dot blots were done. In brief, 3 µg albumin from various species were dot-blotted on nitrocellulose strips. Detection of

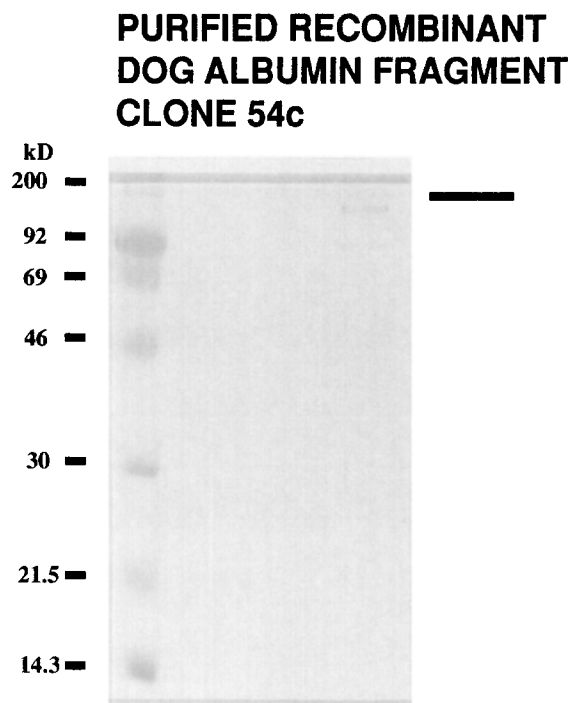


FIG. 5. Purification of recombinant β -galactosidase-fused dog albumin fragment c54. Coomassie-stained gel illustrating purity of β -galactosidase-fused c54 dog albumin fragment. Recombinant fusion protein was purified with anti- β galactosidase antibodies.

specific IgE against albumins was done as for immunoblots with the exception that the buffer used to incubate the nitrocellulose strips and to dilute sera contained no bovine serum albumin. Autoradiography was performed at -70°C for 72 to 96 hours, with Lanex R Fast intensifying screens (Kodak, Heidelberg, Germany) and Kodak ORTHO G x-ray films (Kodak, Heidelberg, Germany).

ELISA experiments

Ninety-six well ELISA plates (COSTAR, Cambridge, U.K.) were coated with 100 μl /well recombinant dog albumin fragment (Clone 54c) or natural dog, cat, guinea pig, or horse albumin dissolved in phosphate-buffered saline solution (2 $\mu\text{g}/\text{ml}$) overnight at 4°C . Wells were washed twice with 200 μl buffer A (phosphate-buffered saline solution, 0.05%, Tween 20) subsequently blocked for 2 hours at room temperature with 200 μl buffer B (phosphate-buffered saline solution 0.05%, Tween 20, 0.5% human serum albumin) and incubated with 100 μl of patients' sera diluted 1:10 (eight sera from nonallergic individuals were included as controls on each plate) in buffer B overnight at 4°C . Wells were then washed five times with 200 μl buffer A and incubated overnight at 4°C with 100 μl of mouse antihuman IgE (Pharmingen, San Diego, Calif.) diluted 1:1000 in buffer B. After washing as described previously, wells were incubated for 30 minutes at 37°C and for 30 minutes at 4°C with 100 μl peroxidase-labeled sheep antimouse IgG di-

luted 1:2000 in buffer B. After washing, 2,2'-azino-di-[3-ethyl-benzthiazolin-sulfonate (6)] was added (Amersham, Buckinghamshire, U.K.), and extinctions were determined at 405 nm with a microplate ELISA reader (Dynatech, Denkendorf, Germany).

ELISA inhibition experiments

To compare the amounts of IgE directed against different albumins in eight patients allergic to albumin, ELISA inhibition experiments were performed. The 1:10 diluted sera were preincubated with 10 μg dog, cat, guinea pig, horse albumin, and human transferrin (control). The percentage of remaining IgE-binding to various albumins was then determined by ELISA. For control purposes sera from eight nonallergic individuals were included in these experiments.

RESULTS

Determination of the percentage of patients allergic to animals with IgE reactivity against albumin

Sera from 200 patients allergic to animals were tested for IgE reactivity with nitrocellulose-blotted hair/dander extracts from dog, cat, guinea pig, and horse. IgE reactivity with albumin was found in 30% of the patients. Fig. 1 shows the typical IgE reaction pattern of 16 representative patients allergic to animals tested against different hair/

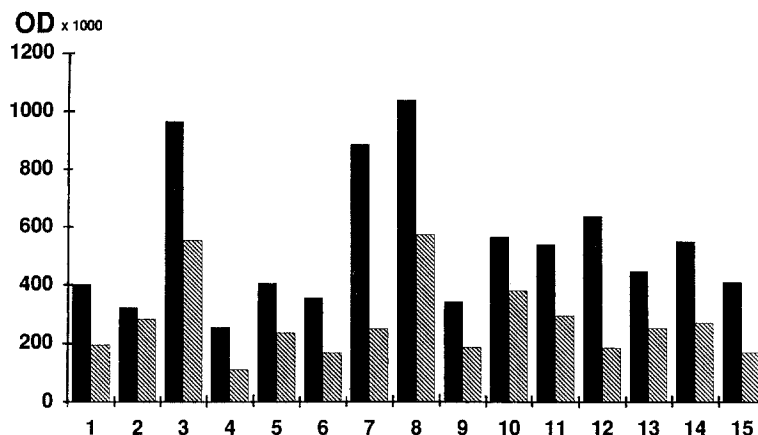


FIG. 6. Percentage of dog albumin-specific IgE directed against recombinant c54 dog albumin fragment. IgE binding of 15 patients allergic to albumin against natural dog albumin and recombinant dog albumin fragment c54 was compared by ELISA. Closed bar represents IgE binding to dog albumin, whereas cross-hatched bar reflects IgE binding to c54 fragment. The y-axis displays extinctions measured as optical density (OD) at 405 nm. All values represent means of duplicate determinations.

dander extracts. IgE reactivity against albumin at 69 kd was usually rather intense. Only a few patients such as patient 7 displayed a very weak reactivity to albumin. No IgE reactivity was observed when serum from a nonallergic individual was used (Fig. 1, lane N).

Variability of patients' IgE reactivity with various albumins

Sera from 60 patients allergic to albumin were tested with dot-blotted albumins from dog, cat, pig, cattle, sheep, horse, guinea pig, rabbit, hamster, mouse, rat, chicken, and pigeon. The results for 23 patients are shown in Fig. 2. Eighty-five percent of the patients had IgE antibodies directed against dog albumin, cat albumin, or both. Seventy-five percent showed IgE reactivity with albumin from guinea pig, and 60% reacted with rabbit and hamster albumin. Horse albumin represented a target for IgE in 70% of the patients, 50% reacted with pig, sheep, mouse, and rat albumin, and 20% of the patients detected bovine and chicken serum albumin. One patient reacted with pigeon serum albumin. In addition to a different percentage of sera reactive with certain albumins, the intensity of IgE reactivity with various albumins was differently pronounced. High titers of specific IgE were directed against dog, cat, horse, guinea pig, and rabbit albumin. Most of the patients showed IgE binding to a broad spectrum of different albumins, whereas a few patients displayed a highly selective IgE reactivity against albumins from only one, two,

or three different species (Fig. 2, patients 5, 6, and 12).

Testing of different skin prick test solutions for the presence of albumin

The serum of a patient with IgE reactivity to albumins from dog, cat, pig, cattle, sheep, horse, guinea pig, rabbit, hamster, mouse, and rat (Fig. 3, lane 1) was tested with SQ extracts from dog, cat, pig, cattle, horse, guinea pig, rabbit, and hamster. Albumin was detected in dog, cat, horse, and rabbit SQ extracts, whereas no albumin was found in pig, cattle, guinea pig, and hamster SQ extracts.

Quantitation of IgE directed against common epitopes of various albumins by ELISA

To quantitate the amount of IgE against different albumins, ELISA assays were done on serum samples of eight representative patients who were allergic to animals. Fig. 4 shows that dog and cat albumin bound most of the albumin-specific IgE. Less IgE binding was observed to horse and guinea pig albumin. The titers of IgE directed against different albumins in relation to each other were determined by competitive ELISA assays. Serum samples were preincubated with different albumins or transferrin (control) before binding to other albumins. The ELISA inhibition in Fig. 4 shows that dog and cat albumin most efficiently inhibited IgE binding to

TABLE 1. Characterization of patients allergic to animals who were tested with dot-blotted albumins in Fig. 2.

Patients			Skin prick test							Specific IgE RAST	Total IgE (kU/L)
No.	Clinical symptoms with:		Dog	Cat	Pig	Cattle	Horse	Guinea pig	Rabbit		
1	s, rh	Cat, guinea pig	ND	ND	ND	ND	ND	ND	ND	e1 (2), e6 (4)	660
2	as	Cat, horse	++	+	-	-	++	-	+	e1 (5), e3 (3)	336
3	rh	Cat, dog, horse, cow	++	++	+	++	++	-	-	e1 (4), e3 (3), e4 (1), e5 (3)	163
4	as, rh	Horse	+	+-	-	+	++	-	++	e3 (5)	ND
5	as, rh	Cat, dog	++	-	++	+	++	-	-	e1 (4), e3 (4), e5 (3)	322
6	as	Cat	-	+	-	-	-	+	-	e1 (4)	180
7	de	Cat	++	++	-	+	+	+	+	e1 (5)	1937
8	rh	Cat	-	-	-	-	-	-	-	e1 (4)	1042
9	as	Cat	-	-	-	-	-	-	-	e1 (6)	>2000
10	as	Cat	++	+-	-	+	++	-	-	e1 (6)	559
11	as	Cat	-	++	-	-	++	-	-	e1 (4)	91
12	as	Cat, dog	++	++	-	+	+	+-	+	e1 (4), e5 (4)	500
13	as	Dog	ND	ND	ND	ND	ND	ND	ND	e5 (4)	ND
14	as, rh	Cat, dog	+	+	+	-	+	-	+	e1 (4), e5 (4)	ND
15	as	Dog	++	++	-	ND	++	++	++	e5 (4)	>2000
16	as, rh	Cat, dog	+	-	+	+	+	-	-	e1 (5), e5 (5)	560
17	as	Cat, dog	++	+	-	+	++	+	-	e1 (5), e5 (5)	755
18	as	Cat, dog	++	++	ND	ND	ND	ND	ND	e1 (4), e5 (5)	927
19	as, rh	Cat, dog	++	++	+	++	++	++	++	e1 (4), e5 (4)	446
20	rh	Cat, dog	++	++	-	-	-	-	-	e1 (4), e5 (4)	ND
21	co	Dog	++	++	++	++	++	+	-	e5 (4)	174
22	co	Dog	++	++	+	++	++	+	-	e5 (4)	180
23	as	Cat, dog	+	+-	-	-	-	++	-	e1 (5), e5 (4)	965

as, Asthma bronchiale; de, dermatitis; rh, rhinitis; co, conjunctivitis; e1, cat epithelium; e3, horse epithelium; e4, cow epithelium; e5, dog epithelium; e6, guinea-pig epithelium; ND, not done.

other albumins. Although albumins share a high degree of sequence homology, dog albumin poorly inhibited IgE binding to cat albumin in patient D, and dog and cat albumin weakly inhibited IgE binding to horse albumin in patients A and E. In general, strong inhibition of IgE binding to all albumins was obtained on preincubation of serum samples with either dog or cat albumin, which also seemed to contain most of the relevant IgE epitopes.

Purification of a recombinant dog albumin fragment fused to β -galactosidase: IgE-binding capacity of the purified recombinant dog albumin fragment

A recombinant dog albumin fragment comprising 265 amino acids of dog albumin was expressed as β -galactosidase fusion protein on infection of lysogenic *E. coli* Y1089. The β -galactosidase fusion protein was purified to almost complete homogeneity by affinity to sepharose-coupled anti- β galac-

tosidase antibodies. Fig. 5 shows that a highly pure fusion allergen was obtained that migrates between 97 kD –200 kD because of the fused β -galactosidase portion. As tested by immunoblotting the additional bands migrating below the fusion protein represent cleavage products that coeluted with the purified protein. The purified recombinant dog albumin fragment could be coupled to ELISA plates for the measurement of specific IgE titers.

IgE binding of the sera of 15 patients allergic to dog albumin and to the recombinant dog albumin fragment was determined by ELISA and compared with the amount of IgE binding directed against complete natural dog albumin. Fig. 6 shows that all 15 patients allergic to albumin displayed IgE reactivity with natural dog albumin and the recombinant dog albumin fragment. The percentage of IgE against the recombinant fragment was approximately 50% of the dog albumin-specific IgE in most of the patients tested.

DISCUSSION

Type I allergy against animal hair/dander proteins is a frequent cause of severe asthma in sensitized patients.¹⁸ Some of the animal hair/dander allergens have been reported to occur exclusively in certain species, whereas others were found in hair/dander extracts prepared from different animals.³ In a recent study we characterized a partial complementary DNA coding for an IgE-binding fragment of dog albumin.¹² The deduced amino acid sequence of the dog albumin fragment showed a high degree of sequence homology (80% average sequence identity) with albumins from different species, and experiments indicated that these albumins are immunologically closely related and might be responsible for IgE cross-reactivities among patients allergic to animals.

In this study a representative number of 200 patients allergic to animals was tested for IgE reactivity with albumins from different animals. Thirty percent of the patients displayed IgE binding to various albumins, which therefore can be designated as intermediate allergens. Although albumins from various species are highly homologous, a remarkable difference in intensity of IgE reactivities with various albumins was observed. When 60 patients allergic to albumin were tested with dot-blotted albumins, most reacted with dog, cat, horse, and guinea pig albumin. Dog, cat, horse, and guinea pig albumins also bound a high percentage of IgE as measured by ELISA. When different albumins were then tested in competitive ELISA assays, it could again be demonstrated that dog and cat albumin bound most of the IgE antibodies directed against common albumin epitopes. However, as was already observed in the dot-blot experiments, some IgE epitopes appeared to be specific for certain albumins.

Despite the variability of IgE reactivities with different albumins, dog albumin and cat albumin reacted with serum IgE from most patients allergic to albumin. A bacterially expressed fragment of dog albumin bound IgE of all ($n = 15$) patients allergic to dog albumin. The percentage of dog albumin-specific IgE bound by the recombinant dog albumin fragment was in the range of 50%.

In conclusion, IgE antibodies of patients allergic to animals frequently reacted with albumins from different species. Although a remarkable difference in intensity of IgE reactivity to albumins from different animals was found, dog and cat albumin were cross-reactive with serum IgE from most patients. When the skin test reactivities (Table I)

are compared with the dot blots (Fig. 2), it can be seen that several patients who reacted with dot-blotted albumins had negative skin test results. The most likely explanation for this observation would be the absence of albumin from different batches of test solutions. In fact, Fig. 3 shows that several skin prick solutions did not contain albumin.

The study of albumins as allergenic components is considered to be of particular interest because albumins apparently represent important cross-reactive allergens for a considerable proportion of patients allergic to animal hair/dander and bind a high percentage of IgE in sensitized patients. In spite of a high degree of sequence homology of different albumins and overall cross-reactivity of allergic patients, a significant variability of IgE reactivities was observed indicating the presence of IgE epitopes, which are specific for certain albumins. The high IgE-binding capacity of dog and cat albumin and a recombinant dog albumin fragment might encourage the use of recombinant dog or cat albumin fragments for diagnosis and perhaps immunotherapy in patients allergic to albumin. Although albumins represent proteins that can be easily isolated from natural sources, we believe that it is useful to develop recombinant albumin fragments under controlled conditions to exclude problems that might arise from the application of animal products to man.

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