

Mechanisms of allergy

Escherichia coli expression and purification of recombinant dog albumin, a cross-reactive animal allergen

Budhi Pandjaitan, PhD,^a Ines Swoboda, PhD,^a Franziska Brandejsky-Pichler, VMD,^b
Helmut Rumpold, MD,^a Rudolf Valenta, MD,^c and Susanne Spitzauer, MD^a Vienna, Austria

Background: Animal hair-dander represents an important source of indoor allergens. Diagnosis and therapy of animal allergy would benefit from the availability of defined recombinant allergens. They may be preferred to animal-derived proteins, particularly for in vivo application in patients.

Objective: The purpose of this study was to express and purify recombinant dog albumin, an important cross-reactive animal allergen.

Methods: Complementary (c)DNA sequences coding for dog albumin were obtained by reverse transcription and subsequent PCR amplification from dog liver RNA. Dog albumin-encoding cDNA sequences were inserted into phage λ gt11, and IgE-reactive phage clones were isolated by immunoscreening with serum IgE from a patient with dog allergy. Dog albumin was expressed as IgE-reactive recombinant protein in *Escherichia coli* and purified by inclusion body preparation, resolubilization, and diethylaminoethyl cellulose chromatography. Cross-reactivity of dog albumin with cat and human albumin was examined by immunoblot, as well as ELISA inhibition experiments.

Results: A cDNA sequence coding for complete dog albumin was obtained by reverse transcription and subsequent PCR amplification from dog liver. The cDNA and deduced amino acid sequence of dog albumin was highly homologous to the sequences of albumins from animals to human subjects, thus explaining the extensive cross-reactivities among albumins. Recombinant dog albumin was expressed in *E coli* and purified. It reacted with serum IgE from patients allergic to dog albumin and a monoclonal anti-human albumin antibody. Immunologic competition experiments performed with serum IgE from patients allergic to dog albumin and a mouse monoclonal antihuman albumin antibody indicated the presence of similar epitopes on dog, cat, and human albumin.

Conclusion: Recombinant dog albumin may be used for diagnostic purposes to identify patients who are cross-sensitized to many animal species and perhaps for specific immunotherapy

of sensitized individuals. (J Allergy Clin Immunol 2000;105:279-85.)

Key words: Animal allergy, recombinant allergen, dog albumin, cross-reactivity

Contact with animal hair or dander allergens frequently leads to perennial type I allergies.¹ Up to 30% of atopic individuals experience type I allergic symptoms on exposure to cat allergen, dog allergen, or both,² and it has been reported that cat and dog allergy are often associated.^{3,4} The major allergens from cat and dog (Fel d 1, Can d 1, and Can d 2) have been characterized extensively by protein and immunochemical techniques and have been expressed as recombinant allergens.^{5,6}

Immunologic studies of cross-reactive allergens in cat and dog hair or dander identified serum albumins as frequently detected cross-reactive allergens.^{7,8} Albumins represent the major component (approximately 40 mg/mL) in the circulatory system of animals and human subjects. They represent nonglycosylated proteins of approximately 65 to 69 kd, the sequence and 3-dimensional structure of which are highly conserved throughout evolution.⁹ Structural analysis of albumin demonstrated that this protein has a high α -helical content and an unusually large number of disulfide bonds, with the latter being a feature that is thought to contribute to the stability of plasma proteins in the circulation.¹⁰⁻¹² The fact that a major component of serum can act as an allergen is unusual for at least 2 reasons. First, it is surprising that a serum protein has the potential to sensitize atopic individuals and to act primarily as a respiratory allergen. Second, it is remarkable that albumins from animals (dog, cat, horse, pig, and cattle) that are very similar in sequence, structure, and function to human serum albumin can be recognized by the immune system as allergens instead of tolerance being induced.

Nevertheless, albumin was recognized as a potent antigen more than 60 years ago,¹³ and cross-reactivity of IgE antibodies from patients allergic to albumins with albumins from cat, horse, pig, and dog has been reported.¹⁴⁻¹⁶ We previously identified dog albumin, Can d 3, as a cross-reactive allergen for up to 35% of patients allergic to dogs.¹⁷ The importance of dog albumin as allergen was supported by several findings. It was demonstrated that dog albumin contained many of the IgE epitopes present on albumins from other animals and

From ^athe Institute of Medical and Chemical Laboratory Diagnostics, AKH;

^bthe University of Veterinary Medicine, Department of Internal Medicine; and ^cthe Institute of General and Experimental Pathology, AKH, University of Vienna, Vienna, Austria.

Supported by the "Hochschuljubiläumsstiftung" of the University of Vienna and by grants P13-383-MED and F0506 of the Austrian Science Fund.

Received for publication Jan 28, 1999; revised Sept 8, 1999; accepted for publication Sept 9, 1999.

Reprint requests: Rudolf Valenta, MD, Molecular Immunopathology Group, Institute of General and Experimental Pathology, Vienna General Hospital, University of Vienna, Währinger Gürtel 18-20, A-1090 Vienna, Austria.

Copyright © 2000 by Mosby, Inc.

0091-6749/2000 \$12.00 + 0 1/1/103053

thus represents a cross-reactive allergen. Moreover, dog albumin bound up to 90% of dog-specific IgE antibodies and induced specific and dose-dependent histamine release from basophils of sensitized patients.¹⁷

By using dog liver RNA as a source, dog albumin-encoding complementary (c)DNA sequences were obtained by reverse transcription and subsequent PCR amplification. cDNA sequences coding for dog albumin were inserted into phage λ gt11. IgE-reactive phage clones were selected with serum IgE from patients allergic to dogs. The primary sequence of a complete, IgE-reactive dog albumin cDNA clone was analyzed and aligned with sequences from cat and human serum albumin. To achieve expression of the recombinant allergen, the dog albumin cDNA sequence was inserted into plasmid pET-17b and transformed into *Escherichia coli*. Recombinant dog albumin was purified and tested for its ability to bind IgE antibodies from patients allergic to dogs and a monoclonal antihuman serum albumin antibody. Sequence features of dog albumin are related to immunologic cross-reactivities, and the application of recombinant dog albumin for basic allergy research, diagnosis, and treatment are discussed.

METHODS

Bacterial strains, plasmids, antibodies, and sera

Competent cells from *E coli* BL21(DE3) and plasmid pET-17b were purchased from Novagen (Novagen, Madison, Wis). A mouse monoclonal antihuman serum albumin antibody (IgG₁; mouse ascites fluid clone HSA-11) was purchased from Sigma (Sigma, St Louis, Mo). The mouse monoclonal anti-Bet v 1 antibody (IgG₁) Bip 1¹⁸ was used for control purposes. Sera were obtained from patients allergic to dogs that contained IgE antibodies directed against natural dog albumin. Serum from a nonatopic individual was used for control purposes. All patients were characterized by case history, skin prick testing, and determination of specific IgE antibodies by CAP-RAST measurements (Pharmacia, Uppsala, Sweden).

Isolation of a cDNA sequence coding for dog albumin

RNA was isolated from dog liver as described.¹⁹ Two-microgram aliquots of dog liver RNA were reverse transcribed by using a cDNA synthesis kit (Amersham, Buckinghamshire, UK) with 0.5 μ g of an oligo-dT primer containing an *EcoRI* restriction site in its 5' end. PCR was carried out with a PCR-Core kit (Perkin Elmer Applied Biosystems, Foster City, Calif) by using reverse-transcribed cDNA as a template and the following 5'-albumin encoding oligonucleotide primer (MWG, Ebersberg, Germany): 5'-G AGA GAG AGA GAA TTC GAT ACA TAC AAG AGT GAA ATT GCT CA-3' containing an internal *EcoRI* restriction site (underlined).

Amplified dog albumin-encoding cDNA was digested with *EcoRI*, ligated into λ gt11 *EcoRI*-cut arms (Pharmacia Biotech), and packaged in vitro by using an in vitro packaging kit (Pharmacia Biotech). Recombinant IgE-binding phage clones were detected with serum IgE as described.¹⁷

Amplification of phage DNA, subcloning into plasmid pET-17b, and DNA sequencing

Dog albumin-encoding cDNA was amplified from IgE-reactive phage clones by using a PCR-Core kit (Perkin Elmer Applied Biosystems) and the following synthetic oligonucleotide primers (MWG-Biotech, Ebersberg, Germany): 5'-G GAA TTC CAT ATG

GAT ACA TAC AAG AGT GAG ATT GCT-3' (5' end of dog albumin) and 5'-CCG GAA TTC TCA TCA GAC TAA GGC AGC TTG AGC AGC-3' (3' end of dog albumin). The primers contained an *EcoRI* site (underlined) and an *NdeI* site (italics), respectively, to allow in-frame insertion of the dog albumin-encoding cDNA into plasmid pET-17b. PCR products were digested with *NdeI* and *EcoRI*, gel purified, and subcloned into the *NdeI/EcoRI* restriction sites of pET-17b.²⁰

The DNA sequence of these constructs was determined by sequencing²¹ with a T7 polymerase sequencing kit (Pharmacia, Uppsala, Sweden), ³⁵S-deoxycytidine 5'-triphosphate (NEN, Stevenage, UK) and sequence-specific oligonucleotide primers (MWG-Biotech).

E coli expression and purification of recombinant dog albumin

To achieve expression of recombinant dog albumin plasmid pET-17b containing the dog albumin cDNA was transformed into *E coli* BL21(DE3). Transformed *E coli* cells were grown in LB medium containing 100 mg/L ampicillin at 37°C to an OD (600 nm) of 0.4. Protein expression was induced by addition of isopropyl- β -D-thiogalactosidase to a final concentration of 0.5 mmol/L, and cells were grown for 4 hours. *E coli* cells were harvested by centrifugation at 4500 rpm (Hettich Rotanta TRC) at 4°C for 10 minutes. Total *E coli* extracts for SDS-PAGE were obtained by resuspending cells in sample buffer²² and boiling for 5 minutes.

Recombinant dog albumin accumulated in the insoluble inclusion body fraction of the bacteria and was purified by inclusion body preparation, solubilization, and diethylaminoethyl cellulose chromatography, as described.²³ In brief, cells were lysed in 25 mmol/L imidazole (pH 7.4) and 0.1% vol/vol Triton X-100 containing lysozyme (50 μ g/g cells). The homogenate was treated with deoxyribonuclease I (100 μ g/g cells) for 2 hours at room temperature. After centrifugation (14,000g for 30 minutes at 4°C) the pellet was resuspended in buffer P (20 mmol/L TRIS [pH 8.0], 200 mmol/L NaCl, 1% desoxycholate, and 1 mmol/L β -mercaptoethanol). The homogenate was centrifuged again, and the inclusion body pellet was washed 4 times with 4-fold diluted buffer P and finally with 10 mmol/L TRIS (pH 8.0) and 3% vol/vol isopropanol. Inclusion bodies were then solubilized with 8 mol/L urea, 10 mmol/L TRIS (pH 8.0), 1 mmol/L EDTA, and 5 mmol/L β -mercaptoethanol and dialyzed against 5 mmol/L TRIS (pH 7.4). The dialysate was centrifuged (14,000g for 30 minutes at 4°C), and the supernatant was fractionated by diethylaminoethyl cellulose chromatography. Fractions containing pure recombinant dog albumin were pooled and stored at -20°C until use.

SDS-PAGE and immunoblot analysis of expressed recombinant dog albumin

The protein contents of extracts were compared by using SDS-PAGE²² and Coomassie brilliant blue (Coomassie Brilliant Blue R-250; Bio Rad Laboratories, Richmond, Calif) staining.²⁴ Molecular weights were determined by using prestained protein standards (Amersham). Aliquots containing approximately 80 μ g/cm total *E coli* BL21(DE3) protein extract, 0.5 μ g/cm purified recombinant dog albumin, or 5 μ g/cm natural dog, cat, or human albumin (Sigma) were loaded onto 12% SDS-PAGE and blotted onto nitrocellulose membranes (Nitrocell, Pharmacia Biotech), as previously described.²⁵ Immunoblot and immunoblot inhibition experiments with mouse mAbs and patient sera were performed as previously described.^{17,18}

ELISA and quantitative inhibition experiments

Ninety-six-well ELISA plates (Nunc Maxisorb, Roskilde, Denmark) were coated with natural dog albumin dissolved in PBS (4

μg/mL) and blocked with PBS containing 0.05% vol/vol Tween 20 and 0.5% wt/vol gelatine. Patient sera (diluted 1:10) or the α-human serum albumin mAb (diluted 1:50,000) were preincubated with 10 μg of cat albumin, human albumin, and human transferrin (control). Preadsorbed sera/antibodies were exposed to ELISA plate-bound dog albumin, and bound human IgE or mouse antibodies were detected as described.^{7,18}

Complete cDNA sequences coding for dog serum albumin were obtained by reverse transcription and subsequent PCR amplification from dog liver RNA. DNA sequence analysis of one IgE-reactive dog albumin-expressing clone shows that the coding region consists of an open-reading frame of 1752 bp, which codes for a polypeptide of 584 amino acids (Fig 1). A molecular weight of 65.5 kd and an isoelectric point of 5.2 were calculated on the basis of the deduced amino acid sequence of dog albumin. Sequence analysis of the deduced dog albumin amino acid sequence and comparison with other sequences of the albumin superfamily²⁶

shows that the dog albumin sequence closely resembles common sequence features of albumins. Dog albumin has a low content of tryptophan (ie, one residue) but is rich in leucines and cysteines. The 35 cysteines that are responsible for the formation of 17 disulfide bridges are located in positions that are highly conserved among albumins (Fig 2).⁹ The alignment of the dog albumin amino acid sequence with the sequences from cat¹⁴ and human serum albumins²⁷ revealed a high degree of sequence identity (86% and 79%, respectively). The 3 signature motifs of the serum albumin family^{26,28,29} are highly conserved in the dog albumin sequence (Fig 1, *underlined sequences*). The prediction of the secondary structure elements of dog albumin on the basis of its deduced amino acid sequence indicates that the protein consists mainly of α -helix elements, which is in good agreement with results obtained from the spectroscopical and x-ray analyses of albumins from other species.⁹ Of note, sites with predicted high antigenic index (N- as well as C-terminus and several portions within the dog albumin sequence) appeared to be less conserved among dog, cat, and human albumin. Several amino acids in the N- and C-terminal portions of dog albumin were different from those in the cat and human albumin sequences.

dog	1	DTYKSEIAHRYNDLGEEHFRGLVLVAFSQYLQCCPFEDHVKLAKEVTEFAKACAAEESGA	60
cat		EAHQ-----F-----VN-----G-V-DQ-A-	
human		DAHK--V---FK-----N-KA---I--A-----VN-----T-V-D--AE	
dog	61	NCDKSLHTLFGDKLCTVASLRDKYGDMAECCEKQEPDRNECFLAHKDDNPGFPPLVAPEP	120
cat		--E---E-L-----E-----K--E-----Q-----GQ--T--A	
human		-----T--ET--E-----A---G-----Q-----NL-R--R--V	
dog	121	DRLCAAFQDNEQLFLGKYLYEIAARRHPYFYAPELLYYAQQYKGVFAECCQAADKAACLG	180
cat		-AM-T--HE--R-----EE-----T--E-----T--	
human		-VM-T--H---ET--K-----FF-KR--AA-T-----L-	
dog	181	KTEALREKVLSSAKERFKCASLQKFGDRAFKAWSVARLSQRFPPKADFAEISKVVDLTK	240
cat		-VD-----A-----L-----E-----K---E-----L---A-	
human		-LDE--DEGKA---Q-L-----E-----A-----E--V--L-----	
dog	241	VHKECCHGDLLECADDRADLAKYMCENQDSISTKLKECCDKPVLEKSQCLAEVERDELPG	300
cat		I-----I-----G-----H-IS-----A	
human		--T-----I-----S-----E--L---H-I---N--M--A	
dog	301	DLPSLAADFVEDKEVCKNYQEAKDAFLGTFLYEYSRRHPEYSVSLLLRLAKEYEATLEKC	360
cat		---P--V-----V-----	
human		-----S-D---A---V--M---A---D--V-----T--T-----	
dog	361	<u>CATDDPPTCYAKVLDEFKPLVDEPQNLVKTNCLEFEKLGEYGFQNALLVRYTKKAPQVST</u>	420
cat		-----A---H-F-----E--H-----V-----	
human		--AA--HE-----F-----E-----I-Q-----EQ---K-----V-E---	
dog	421	PTLIVEVSRKLGKVGTKCKKPESEMSCADDFLSVVLNRLCVLHEKTPVSEKVTCKCCSES	480
cat		-----S-----S---TH--A--L---E-Y-----T--	
human		-----N-----S---H--AK--P--E-Y-----Q-----D-----T--	
dog	481	LVNRRPCFSGLEVDETYVPKEFNAETFTTFHADLCTLPEAEKQVKKQTALVELLKHKPKAT	540
cat		-----A-Q-----S-----I--S-----	
human		-----A-----I---S-K-R-I-----V-----	
dog	541	DEQLKTVMGDFGAFVEKCCAAENKEGCFSEEGPKLVAAAQAALV	584
cat		E-----S--D-----D--A--A-----A.	
human		K---A--D--A-----K-DD--T--A---K-----S---GL	

FIG 2. Similarity of the dog albumin amino acid sequence with the cat and human albumin sequences. The deduced amino acid sequence of dog albumin is displayed in the top line. Identical amino acids in the cat and human sequences are printed as *dashes*, points indicate lacking amino acids. The 3 amino acid signature motifs of the albumin superfamily are *underlined*.

(Fig 2). In this context it has been reported that amino acid exchanges caused by mutations appeared to have little effect on the solubility of albumin and its function as serum protein.⁹

Expression, purification, and antibody reactivity of recombinant dog albumin

Expression of the dog albumin-encoding cDNA in bacteria yielded low levels of recombinant protein (<5%) in total bacterial extracts (Fig 3, *lanes 2*). The recombinant protein was found primarily in the insoluble inclusion body fraction (Fig 3, *lanes 3*) and could be solubilized in urea. Recombinant dog albumin remained soluble after dialysis to 50 mmol/L TRIS (pH 7.4; Fig 3,

lanes 4) and could be purified by diethylaminoethyl cellulose chromatography (Fig 3, *lanes 5*). Serum IgE from a patient allergic to dogs reacted with a 65-kd band in total bacterial extracts containing recombinant dog albumin (Fig 3, *IgE lane 2*) but not with extracts from nonrecombinant bacteria (Fig 3, *IgE lane 1*). IgE antibodies reacted with recombinant dog albumin in the inclusion body fraction (Fig 3, *IgE lane 3*), the dialysate (Fig 3, *IgE lane 4*) and with the purified recombinant protein (Fig 3, *IgE lane 5*). The low-molecular-weight IgE-reactive bands in protein extracts from recombinant bacteria most likely represent degradation products of recombinant dog albumin. A monoclonal anti-human serum albumin antibody, but not an isotype-matched control anti-

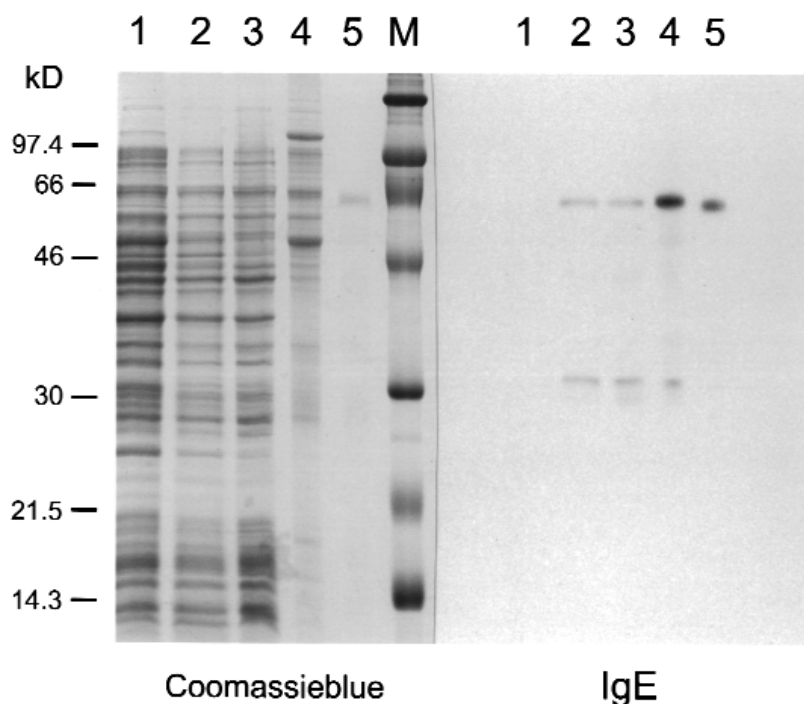


FIG 3. Purification and IgE-binding capacity of recombinant dog albumin. Coomassie-stained gel (*Coomassie blue*) and IgE immunoblot (*IgE*) containing total protein extract from *E coli* transformed with empty plasmid pET-17b (*lanes 1*), total protein extract from *E coli* expressing recombinant dog albumin (*lanes 2*), inclusion body fraction (*lanes 3*), soluble dialysate before application to diethylaminoethyl column (*lanes 4*), and purified recombinant dog albumin (*lanes 5*) are shown. Lane *M* contains a molecular weight marker. Molecular weights are indicated at the left margin.

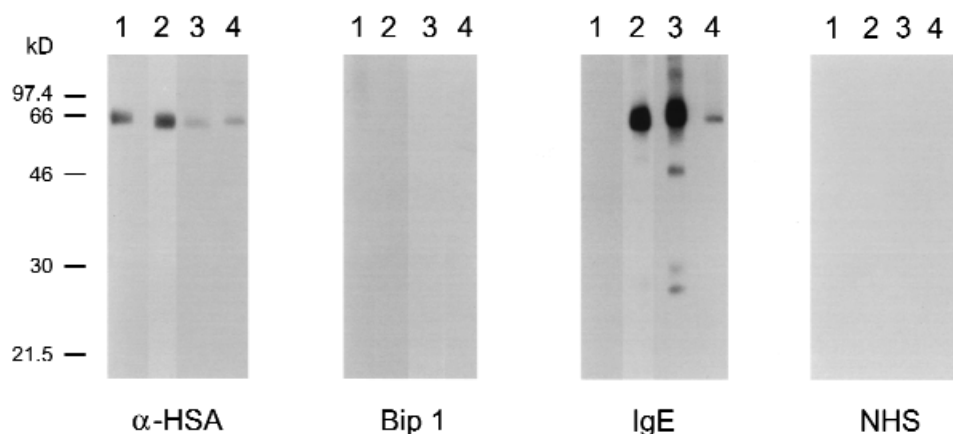


FIG 4. Antibody-binding capacity of natural albumins and recombinant dog albumin. Nitrocellulose-blotted natural albumins from human subjects (*lanes 1*), cats (*lanes 2*), and dogs (*lanes 3*), as well as recombinant dog albumin (*lanes 4*), were exposed to a mouse monoclonal antihuman albumin antibody (α -HSA), serum IgE from a patient allergic to dogs (*IgE*), and, for control purposes, to an isotype-matched mAb (*Bip 1*), as well as to serum from a nonatopic individual (*NHS*). Molecular weights are indicated at the left margin.

body, reacted with human, cat, natural, and recombinant dog albumin (Fig 4, α -HSA and *Bip 1*). Human serum IgE cross-reacted with cat and natural and recombinant dog albumin, but not with human albumin, presumably because of the high concentrations of human albumin present in human serum (Fig 4, *IgE*). Serum from a nonatopic person did not react with any of the purified proteins (Fig 4, *NHS*).

Immunoblot and ELISA inhibition experiments indicate common epitopes on dog, cat, and human albumin

Preincubation of sera from 5 patients allergic to animals containing IgE antialbumin antibodies with cat albumin (Fig 5, *lanes 3*), but not with transferrin (Fig 5, *lanes 1*), led to a varying inhibition of IgE binding to

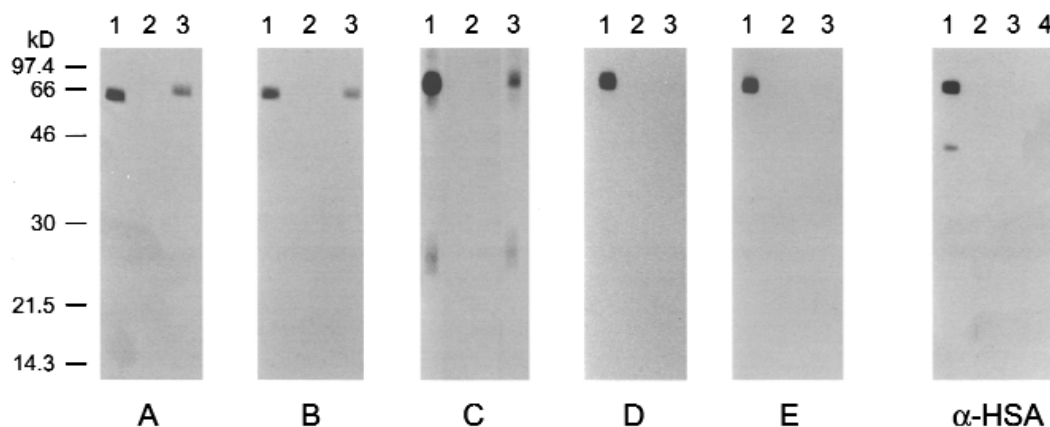


FIG 5. Immunoblot inhibition experiments: dog, cat, and human albumin share epitopes. Nitrocellulose-blotted dog albumin was exposed to sera from patients allergic to animals (**A-E**) containing dog albumin-specific IgE antibodies and to a monoclonal anti-human albumin antibody (α -HSA). Sera and the mAb were preincubated with human serum transferrin (lanes 1), dog albumin (lanes 2), cat albumin (lanes 3), and human albumin (lane 4). Molecular weights are indicated at the left margin.

nitrocellulose-blotted dog albumin (Fig 5, A-E). Preincubation of sera with dog albumin (Fig 5, A-E lanes 2) completely inhibited IgE binding to blotted dog albumin. Preincubation of a monoclonal anti-human albumin antibody with cat, dog, or human albumin, but not with transferrin, abolished its reactivity to blotted dog albumin (Fig 5, α -HSA). Preincubation of sera from 10 albumin-reactive patients with cat albumin led to a substantial inhibition of IgE binding to dog albumin, ranging from 16% to 72% (mean percentage of inhibition, 52%) in 9 of 10 individuals. Preincubation of the mouse monoclonal anti-human albumin antibody with cat and human albumin strongly reduced mouse IgG binding to dog albumin (cat albumin, 80%; human albumin, 87%).

DISCUSSION

We have isolated a cDNA coding for complete dog albumin, an important cross-reactive animal allergen. Dog albumin reacts with serum IgE from approximately 30% of patients allergic to animals and therefore represents an intermediate allergen.⁷ On the basis of the fact that recombinant dog albumin represents the third cloned dog allergen, we propose to designate it Can d 3.

The dog albumin amino acid sequence was deduced from the cDNA sequence and showed a sequence identity of 86% to cat albumin and 79% to human albumin. The high degree of sequence homologies among albumins thus explains the extensive cross-reactivity of patient IgE antibodies with albumins from many species.^{7,30}

A protocol for the purification of recombinant dog albumin was established, which is based on the preparation of inclusion bodies, solubilization, and diethylaminoethyl chromatography. Purified recombinant dog albumin exhibited a comparable capacity to bind serum IgE antibodies as natural dog albumin and shared epitopes with cat and human albumin. We suggest the use of recombinant dog albumin for various purposes. First,

recombinant dog albumin may be used for basic immunologic research to investigate the interaction of T cells, antibodies, and effector as well as inducer cells with this allergen. On the basis of the complete amino acid sequence of dog albumin, overlapping peptides can be synthesized to determine T-cell epitopes. In parallel it will be possible to determine the major IgE epitopes of dog albumin by gene fragmentation and expression techniques. Second, purified recombinant dog albumin can be used for *in vitro*, as well as *in vivo*, diagnosis of a group of individuals allergic to animals who are at risk to exhibit allergic reactions to albumins from various animals. Although the *in vitro* diagnosis might be also achieved by using dog albumin from natural sources, we believe that *in vivo* testing of patients should be done preferentially with a defined recombinant protein preparation that is unlikely to contain infectious contaminations. Third, recombinant dog albumin or hypoallergenic versions of this molecule may be used for specific immunotherapy. Strategies for the generation of hypoallergenic dog albumin derivatives may originate from detailed epitope-mapping studies³¹ supplemented by structural considerations based on the recently obtained 3-dimensional structure of human serum albumin.¹² The high degree of sequence and possibly structural similarity of dog and human albumin is of note for several reasons. It has been demonstrated that patients with severe atopy display IgE reactivity not only to exogenous but also to endogenous proteins (autoallergens).³²⁻³⁴ Although we have so far not found IgE antibodies that cross-reacted between dog and human albumin, it is possible that cross-reactive T-cell clones can be found that may be involved in the maintenance of chronic inflammatory processes in patients allergic to albumin. In light of our finding that a mouse monoclonal antihuman albumin antibody cross-reacted with dog albumin, it will be also interesting to find out whether the lack of detectable IgE cross-reactivity is due to absorption of autoreactive

IgE antialbumin antibodies by the huge excess of human albumin in serum. It is equally possible that dog albumin-specific IgE antibodies react primarily with continuous epitopes that are different between dog and human albumin and therefore fail to cross-react with human serum albumin. There is indeed evidence that IgE antibodies of patients allergic to albumin bind to continuous epitopes^{35,36} and react preferentially with denatured albumin.^{15,37-39}

In conclusion, we believe that the availability of the dog albumin sequence and recombinant dog albumin will contribute to the analysis of interactions of the humoral and cellular immune responses with this allergen. Recombinant dog albumin and genetically engineered hypoallergenic variants thereof may be useful tools for allergy diagnosis and immunotherapy, respectively.

REFERENCES

- Haahela T, Jaakonmaki I. Relationship of allergen-specific IgE antibodies, skin prick tests and allergic disorders in unselected adolescents. *Allergy* 1981;36:251-6.
- Schou C. Defining allergens of mammalian origin. *Clin Exp Allergy* 1993;23:7-14.
- Viander M, Valovirta E, Vanto T, Koivikko A. Crossreactivity of cat and dog allergen extracts. *Int Arch Allergy Appl Immunol* 1983;71:252-60.
- Boutin Y, Hebert H, Vrancken ER, Mouraud W. Allergenicity and cross-reactivity of cat and dog allergenic extracts. *Clin Allergy* 1988;18:287-93.
- Morgenstern JP, Griffith IJ, Brauer AW, Rogers BL, Bond JF, Chapman MD, et al. Amino acid sequence of Fel d 1, the major allergen of the domestic cat: protein sequence analysis and cDNA cloning. *Proc Natl Acad Sci USA* 1991;88:9690-4.
- Konieczny A, Morgenstern JP, Bizinkauskas CB, Lilley CH, Brauer AW, Bond JF, et al. The major dog allergens, Can f 1 and Can f 2, are salivary lipocalin proteins: cloning and immunological characterization of the recombinant forms. *Immunology* 1997;92:577-86.
- Spitzauer S, Pandjaitan B, Söregi G, Mühl S, Ebner C, Kraft D, et al. IgE cross-reactivities against albumins in patients allergic to animals. *J Allergy Clin Immunol* 1995;96:951-9.
- Spitzauer S, Pandjaitan B, Mühl S, Ebner C, Kraft D, Valenta R, et al. Major cat and dog allergens share IgE epitopes. *J Allergy Clin Immunol* 1997;99:100-6.
- Peters T. All about albumin. San Diego: Academic Press; 1996.
- He XM, Carter DC. Atomic structure and chemistry of human serum albumin. *Nature* 1992;358:209-15.
- Ho JX, Holowachuk EW, Norton EJ, Twigg PD, Carter DC. X-ray and primary structure of horse serum albumin (*Equus caballus*) at 0.27-nm resolution. *Eur J Biochem* 1993;215:205-12.
- Curry S, Mandelkow H, Brick P, Franks N. Crystal structure of human serum albumin complexed with fatty acid reveals an asymmetric distribution of binding sites. *Nat Struct Biol* 1998;5:827-35.
- Heidelberger M. The molecular composition of specific immunoprecipitates from rabbit sera. *J Am Chem Soc* 1938;60:212-44.
- Hilger C, Grigoni F, Hentges F. Sequence of the gene encoding cat (*Felis domesticus*) serum albumin. *Gene* 1996;169:295-6.
- Goubran Botros H, Gregoire C, Rabillon J, David B, Dandeu JP. Cross-antigenicity of horse serum albumin with dog and cat albumins: study of three short peptides with significant inhibitory activity towards specific human IgE and IgG antibodies. *Immunology* 1996;88:340-7.
- Hilger C, Kohnen M, Grigioni F, Lehnert C, Hentges F. Allergic cross-reactions between cat and pig serum albumin. Study at the protein and DNA levels. *Allergy* 1997;52:179-87.
- Spitzauer S, Schweiger C, Sperr WR, Pandjaitan B, Valent P, Mühl S, et al. Molecular characterization of dog albumin as a cross-reactive allergen. *J Allergy Clin Immunol* 1994;93:614-27.
- Laffer S, Vangelista L, Steinberger P, Kraft D, Pastore A, Valenta R. Molecular characterization of Bip 1, a monoclonal antibody that modulates IgE binding to birch pollen allergen, Bet v 1. *J Immunol* 1996;157:4943-62.
- Davis LG, Dibner MD, Battey JF. Basic methods in molecular biology. New York: Elsevier; 1986.
- Sambrook J, Fritsch EF, Maniatis T. Molecular cloning: a laboratory manual. Cold Spring Harbor: Cold Spring Harbor Laboratory Press; 1989.
- Sanger F, Nicklen S, Coulson AR. DNA sequencing with chain terminating inhibitors. *Proc Natl Acad Sci* 1977;74:5463-8.
- Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 1970;227:680-5.
- Vrtala S, Susani M, Sperr WR, Valent P, Laffer S, Dolecek C, et al. Immunologic characterization of purified recombinant timothy grass pollen (*Phleum pratense*) allergens (Phl p 1, Phl p 2, Phl p 5). *J Allergy Clin Immunol* 1996;97:781-7.
- Bradford MM. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 1976;72:248-54.
- Towbin H, Staehelin T, Gordon J. Electrophoretic transfers of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc Natl Acad Sci* 1979;76:4350-4.
- Haefliger DN, Moskaitis JE, Schoenberg DR, Wahli W. Amphibian albumins as members of the albumin, alpha-fetoprotein, vitamin D-binding protein multigene family. *J Mol Evol* 1989;29:344-54.
- Dugaiczky A, Law SW, Dennison OE. Nucleotide sequence and the encoded amino acids of human serum albumin mRNA. *Proc Natl Acad Sci USA* 1982;79:71-5.
- Schoentgen F, Metz-Boutique MH, Jolles J, Constans J, Jolles P. Complete amino acid sequence of human vitamin D-binding protein (group-specific component): evidence of a three-fold internal homology as in serum albumin and alpha-fetoprotein. *Biochim Biophys Acta* 1986;871:189-98.
- Schoentgen F, Metz-Boutique MH, Jolles J, Constans J, Jolles P. Homology between the human vitamin D-binding protein (group-specific component), alpha-fetoprotein and serum albumin. *FEBS Lett* 1985;185:47-50.
- Wuthrich B, Guerin B, Hewitt B. Cross-allergenicity between extracts of hair from different dog breeds and cat fur. *Clin Allergy* 1985;15:87-93.
- Vrtala S, Ball T, Spitzauer S, Pandjaitan B, Suphioglu C, Knox B, et al. Immunization with purified natural and recombinant allergens induces mouse IgG1 antibodies that recognize similar epitopes as human IgE, and inhibit the human IgE-allergen interaction and allergen-induced basophil degranulation. *J Immunol* 1998;160:6137-44.
- Valenta R, Maurer D, Steiner R, Seiberler S, Sperr WR, Valent P, et al. Immunoglobulin E response to human proteins in atopic patients. *J Invest Dermatol* 1996;107:203-8.
- Natter S, Seiberler S, Hufnagl P, Binder BR, Hirschl AM, Ring J, et al. Isolation of cDNA clones coding for IgE autoantigens with serum IgE from atopic dermatitis patients. *FASEB J* 1998;12:1559-69.
- Valenta R, Natter S, Seiberler S, Wichlas S, Maurer D, Hess M, et al. Molecular characterization of an autoallergen, Hom s 1, identified by serum IgE from atopic dermatitis patients. *J Invest Dermatol* 1998;107:203-8.
- Morel GA, Yarmush DM, Colton CK, Benjamin DC, Yarmush ML. Monoclonal antibodies to bovine serum albumin: affinity and specificity determinations. *Mol Immunol* 1988;25:7-15.
- Benjamin DC, Herr JC, Sutherland WM, Woodward MP, DeCourcy K, Condon TP. A unique epitope on human serum albumin recognized by monoclonal antibody HSA-1: a probe for identification of the human origin of blood or tissue. *Hybridoma* 1987;6:183-90.
- Peters JH, Goetzel E. Recovery of greater antigenic reactivity in conformationally altered albumin. *J Biol Chem* 1969;244:2068-74.
- Goetzel EJ, Peters JH. Immunologic alterations in bovine serum albumin resulting from partial or complete reduction and alkylation. *J Immunol* 1972;108:785-99.
- Restani P, Fiocchi A, Velona T, Giovannini M, Galli CL. Effects of structural modifications on IgE binding properties of serum albumins. *Int Arch Allergy Immunol* 1998;117:113-9.