

Molecular characterization of dog albumin as a cross-reactive allergen

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Abstract

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Indoor allergens comprise a group of allergenic proteins that are commonly derived from house dust mite and cat and dog dander. In addition to the two major dog allergens (molecular weights: 19 and 23 kd), dog albumin represents an important allergen for up to 35% of patients who are allergic to dogs. In IgE immunoblot inhibition studies and histamine release tests it has been demonstrated that patients who react to dog albumin exhibit IgE reactivity with purified albumins from cat, mouse, chicken, and rat. The proportion of dog-specific IgE directed against dog albumin was determined for patients allergic to dog albumin, and it ranges from 70% to 90%. By IgE immunoscreening of a lambda gt11 expression library from a dog salivary gland, we identified a number of reactive complementary DNA clones. All patients with IgE reactivity against natural dog albumin displayed IgE reactivity to the β -galactosidase fusion protein encoded by clone 54c, which was therefore assumed to contain major IgE epitopes of dog albumin. The deduced amino acid sequence of clone 54c was compared with the Swiss-Prot library, and significant sequence homologies were found with albumins from different species (human: 82.6%, pig: 81.8%, cattle: 77.3%, sheep: 78.8%, mouse: 75.8%, and rat: 76.2%). Several

other IgE-positive clones hybridized with oligonucleotides that were prepared according to this sequence. Partial complementary DNA coding for dog albumin fragments may be considered a useful tool for further characterization of major IgE epitopes of dog albumin. (J ALLERGY CLIN IMMUNOL 1994;93:614-27.)

cDNA Complementary DNA

SDS-PAGE Sodium dodecylsulfate-polyacrylamide gel electrophoresis

Key words: [Dog allergens](#), [albumin](#), [cDNA cloning](#), [IgE](#)

Type I allergy to indoor allergens ¹ is caused by proteins derived from mites, molds, and animals ² such as cats and dogs. Dog allergens have been detected in protein extracts prepared from dander, hair, and saliva by immunochemical techniques, ³⁻⁷ and a panel of relevant allergens has already been described. ⁸⁻¹⁰ Most patients who are allergic to dogs display IgE reactivity to two major allergens with molecular weights ranging from 19 kd to 23 kd. ^{7, 11-14} Although both allergens show molecular weights similar to that of the major cat allergen *Fel d I*, ^{15, 16} no clear immunologic relationship has yet been described, which provides an explanation for the clinical observation that cat and dog allergy, as well as allergies to other animal dander and hair components, are frequently associated. ¹⁷⁻¹⁹ In a previous study we tested different dog tissues for the presence of IgE-binding proteins. ⁷ Most allergens are detectable in dog hair and dander extracts, whereas protein extracts prepared from dog liver, serum, salivary glands, and keratinocytes contain less IgE-binding components. However, all extracts tested contain an IgE-binding protein with a molecular weight of 69 kd, representing a target for IgE antibodies in 35% of patients allergic to dogs. Previous studies ^{7, 20} and IgE inhibition studies suggest that this protein is dog albumin. ⁷

In this study we demonstrated that up to 90% of dog-specific IgE is directed against dog albumin in sensitized patients. In IgE inhibition experiments and histamine release tests performed with purified albumins from dog, rat, mouse, and chicken we identified albumins as cross-reactive indoor allergens.

Complementary DNA (cDNA) coding for dog albumin fragments was isolated from a dog salivary gland expression cDNA library with IgE from patients with dog allergy.

Different cDNAs coded for different portions of dog albumin, as determined by oligonucleotide hybridization and DNA sequencing. Recombinant dog albumin fragments were expressed as IgE-binding proteins in *Escherichia coli*.

METHODS

Characterization of allergic patients' sera

Sera from 110 patients were selected for this study according to case history, positive skin prick test results and positive RAST results, as described by Jarolim et al. ²¹ All patients showed reactivity to other allergens (e.g., tree pollen, grass pollen, mite, cat and horse dander, guinea pig epithelia). Sera from 22 healthy persons with a negative case history of any type I allergy and negative skin prick test and RAST results to dog allergens were pooled (normal human serum pool) and used as a negative control.

Protein extracts

Powdered dog hair and dander from five different breeds (Alsatian, Boxer, Collie, Dachshund, and Poodle) were obtained from Allergon, AB (Engelholm, Sweden) and extracted according to the method of Ipsen and Loewenstein ²² with the following modifications: 10% wt/vol powdered dog hair and dander were extracted in 10 mmol potassium hydrogen phosphate, 20 mg/L polyvinylpyrrolidone, 2 mmol ethylene diaminetetraacetic acid, 10 mmol sodium diethylthiocarbonate, and 3 mmol sodium azide (pH 7) under continuous shaking for 20 hours at 4° C. Particles were removed by centrifugation (40.000 *g* for 1 hour at 4° C). The supernatant was dialyzed against 10 mmol potassium hydrogen phosphate (pH 7), lyophilized, and stored at –20° C. Before use, extracts were dissolved in distilled water and diluted 3:1 in a fourfold concentrated sample buffer. ²³ The protein concentration of all extracts had been determined by the Peterson modification ²⁴ of the Lowry method.

Sodium dodecylsulfate–polyacrylamide gel electrophoresis

Samples were diluted in a fourfold Laemmli sample buffer and heated at 95° C for 10 minutes. Extracts were applied in a concentration of 200 to 300 µg/cm gel. Sodium dodecylsulfate–polyacrylamide gel electrophoresis (SDS-PAGE) was performed on a vertical slab gel apparatus (Hoefer, San Francisco, Calif.). Twelve percent homogenous gels with 5% stacking gels were used. Electrophoresis was done according to the method of Laemmli. ²³ Molecular weights were estimated with the use of prestained protein standards (14.3 to 200 kd) (Amersham, Buckinghamshire, U.K.).

IgE immunoblots and IgE inhibitions

Proteins separated by SDS-PAGE were transferred to nitrocellulose as described by Towbin et al. ²⁵ Nitrocellulose sheets were cut into 0.5 cm strips and incubated for 30 minutes at room temperature in buffer A containing: 50 mmol/L sodium phosphate (pH 7.5), 0.5% Tween 20, 0.5% bovine serum albumin, and 0.05% sodium azide. Strips were then incubated overnight at 4° C with patients' sera, diluted 1:10 in buffer A. Strips were washed three times for 20 minutes in buffer A. Bound IgE was detected by iodine 125–labeled rabbit anti-human IgE (Pharmacia, Uppsala, Sweden) diluted 1:10 in buffer A and incubated overnight at 4° C. Strips were washed three times for 20 minutes. Control strips were processed with either a normal human serum pool or buffer, respectively. Autoradiography was performed at –70° C for 48 to 72 hours, with Lanex R FAST intensifying screens (Kodak, Heidelberg, Germany) and Kodak ORTHO G x-ray films (Kodak, Heidelberg, Germany).

IgE inhibition studies with immunoblotted dog allergens were performed as described by Valenta et al. ²⁶ Briefly, patients' sera were diluted 1:10 and preincubated with either 10

µg/ml of purified dog albumin (Biogenesis, Bournemouth, U.K.) or 10 µg/ml timothy grass pollen proteins overnight at 4° C. Immunoblots were then performed as described above. IgE inhibition studies with native, nondenatured dog allergens were carried out to determine the proportion of dog hair/dander-specific IgE directed against dog albumin. Serial serum dilutions of seven representative patients were tested with 3 µg dot-blotted dog hair/dander extract and 3 µg dot-blotted dog albumin to determine dilutions in which allergens were in excess of IgE antibodies. One milliliter of each serum diluted 1:40 or 1:80 in buffer A, respectively, was preincubated with 6 µg dog hair/dander extract, 6 µg dog albumin, or 6 µg of timothy grass pollen proteins. Preabsorbed sera were then incubated with 3 µg dot-blotted dog hair/dander extract, and bound IgE was detected with ¹²⁵I-labeled rabbit anti-human IgE (Pharmacia), as described for the immunoblots. Dot blots were then autoradiographed and subjected to densitometry (ELSCRIPT 400 AT/SM, Hirschmann, Unterhaching, Germany). All determinations were done in duplicate, and the albumin-induced inhibition of IgE binding to dog/hair dander extracts is shown in percentages that reflect mean values (■Table I).

TABLE I. Percentage of dog allergen-specific IgE directed against dog albumin in different patients

Patient No.	<u>IgE reactivity with</u>			<u>Percent inhibition induced with</u>		
	19 kd	23 kd	69 kd	Dog albumin	Hair/dander extract	Timothy extract
1	+/-	-	+	100	100	0
2	+/-	-	+	100	100	0
3	-	-	+	97	100	0
4	-	+	+	91	100	0
5	+	-	+	90	100	0
6	+	+	+	74	100	0
7	+	+	-	0	100	0

IgE screening of a dog salivary gland cDNA library

A lambda gt11 cDNA library prepared from dog salivary glands was purchased from Clontech (Palo Alto, Calif.), *E. coli* Y1090 were infected with 200,000 phages and plated on Lurea broth medium plates containing 100 µg/ml Ampicillin. Each plate, containing approximately 20,000 phages, was incubated at 43° C until plaques were visible. The expression of the βgalactosidase fusion proteins was induced by overlay with nitrocellulose filters (Schleicher, Schuell, Dassel, Germany), which had been soaked in 10 mmol/L isopropyl-β-D-thiogalactoside at 37° C. IgE-binding proteins were detected as described for IgE immunoblots.

Isolation of phage DNA, subcloning, and DNA sequencing

Recombinant lambda gt11 phages containing cDNAs coding for IgE-binding proteins were recloned, and phage DNA was prepared. ²⁷ Fragments of the restriction endonucleases, KpnI and Sac I, containing the cDNA inserts together with two flanking portions of lambda gt11 DNA, were ligated into the plasmid pUC 18, and transformed into *E. coli* XL 1 blue.

Recombinant *E. coli* were identified by restriction analysis of the plasmids. With the use of lambda gt11 forward- and reversed-sequencing primers, approximately 250 bases of the DNA sequences of clones 25-I and 48cII were determined by the chain termination method with sulfur 35-labeled deoxycytidine triphosphate. ²⁸ In the case of clone 54 c, the complete cDNA sequence of the insert was determined with the use of additional internal sequencing primers (Biomedica, Vienna, Austria). The cDNA sequences were then compared with the European Molecular Biology Laboratory databases in order to identify homologous sequences.

Expression of dog albumin fragments as recombinant fusion proteins

Lysogenic *E. coli* Y 1089 infected with the recombinant phage were grown at 32° C to an optical density of 600 of 0.5. The temperature of the cultures was rapidly increased to 42° C, and *E. coli* were grown at 42° C for 20 minutes. Expression of recombinant allergen- β -galactosidase fusion proteins was then induced by addition of isopropyl- β -D-thiogalactoside to a final concentration of 10 mmol/L and further growth at 37° C for 1 hour.

E. coli cells were harvested by centrifugation and washed two times with 0.9% sodium chloride. Pellets were stored at -20° C and boiled in sample buffer before SDS-PAGE.

Testing of recombinant dog albumin fragments with serum IgE from patients allergic to dogs

Proteins from *E. coli* Y1089 infected with recombinant phage or nonrecombinant lambda gt11 phage were separated by 7.5% SDS-PAGE ²³ and blotted onto nitrocellulose. ²⁵ Nitrocellulose strips were incubated with patients' sera diluted 1:10 in buffer A overnight. Bound IgE was detected with a ¹²⁵I-labeled rabbit anti-human IgE (Pharmacia), as described above.

Synthetic oligonucleotides complementary to different portions of the dog albumin cDNA

The cDNA sequence of clone 54c coding for a dog albumin fragment was aligned with the cDNA sequence of human serum albumin. Three oligonucleotides designated A, B, and C were synthesized complementary to the dog and human serum albumin messenger RNA. Oligonucleotide A covers the 5' end of dog albumin, which aligns with nucleotides 1243 to 1266 of the cDNA coding for human serum albumin. ²⁹ Oligonucleotide B aligns with nucleotides 1372 to 1401 of human serum albumin, and oligonucleotide C corresponds to nucleotides 1481 to 1504 of human serum albumin. The positions of the oligonucleotides derived from clone 54c are underlined in ■Fig. 1. ■Figs. 2A, ■2B, and ■2C show a comparison of albumin amino acid sequences from human beings, pigs, cattle, sheep, mice, and rats.

FIG. 1. Complementary DNA and deduced amino acid sequence of clone 54c.

CTT	TCA	TCT	GCC	AAG	GAG	AGA	TTC	AAG	TGT	GCC	AGC	CTC	CAA	AAA	
Leu	Ser	Ser	Ala	Lys	Glu	Arg	Phe	Lys	Cys	Ala	Ser	Leu	Gln	Lys	
TTC	GGA	GAT	AGA	GCC	TTT	AAA	GCC	TGG	TCA	GTA	GCT	CGC	CTG	AGC	
Phe	Gly	Asp	Arg	Ala	Phe	Lys	Ala	Trp	Ser	Val	Ala	Arg	Leu	Ser	
CAG	CGA	TTT	CCC	AAA	GCT	GAC	TTT	GCA	GAG	ATC	TCC	AAG	GTG	GTG	
Gln	Arg	Phe	Pro	Lys	Ala	Asp	Phe	Ala	Glu	Ile	Ser	Lys	Val	Val	
ACA	GAT	CTT	ACC	AAA	GTC	CAC	AAG	GAA	TGC	TGC	CAT	GGT	GAC	CTG	
Thr	Asp	Leu	Thr	Lys	Val	His	Lys	Glu	Cys	Cys	His	Gly	Asp	Leu	
CTG	GAG	TGT	GCA	GAT	GAC	AGG	GCG	GAT	CTT	GCC	AAG	TAT	ATG	TGT	
Leu	Glu	Cys	Ala	Asp	Asp	Arg	Ala	Asp	Leu	Ala	Lys	Tyr	Met	Cys	
GAA	AAT	CAA	GAT	TCA	ATC	TCC	ACT	AAA	CTG	AAG	GAA	TGC	TGT	GAT	
Glu	Asn	Gln	Asp	Ser	Ile	Ser	Thr	Lys	Leu	Lys	Glu	Cys	Cys	Asp	
AAG	CCT	GTG	TTG	GAA	AAA	TCC	CAG	TGT	CTT	GCT	GAG	GTG	GAA	AGA	
Lys	Pro	Val	Leu	Glu	Lys	Ser	Gln	Cys	Leu	Ala	Glu	Val	Glu	Arg	
GAT	GAG	TTA	CCT	GGT	GAC	CTG	CCC	TCA	TTA	GCT	GCT	GAT	TTT	GTT	
Asp	Glu	Leu	Pro	Gly	Asp	Leu	Pro	Ser	Leu	Ala	Ala	Asp	Phe	Val	
GAA	GAT	AAG	GAG	GTT	TGC	AAA	AAC	TAT	CAG	GAG	GCA	AAG	GAT	GTG	
Glu	Asp	Lys	Glu	Val	Cys	Lys	Asn	Tyr	Gln	Glu	Ala	Lys	Asp	Val	
TTC	CTG	GGC	ACG	TTT	TTG	TAT	GAA	TAC	TCA	AGA	AGG	CAT	CCA	GAG	
Phe	Leu	Gly	Thr	Phe	Leu	Tyr	Glu	Tyr	Ser	Arg	Arg	His	Pro	Glu	
TAC	TCT	GTC	TCA	TTG	CTT	TTG	AGA	CTC	GCC	AAG	GAA	TAT	GAA	GCC	
Tyr	Ser	Val	Ser	Leu	Leu	Leu	Arg	Leu	Ala	Lys	Glu	Tyr	Glu	Ala	
ACA	CTA	GAG	AAA	TGC	TGT	GCC	ACC	GAT	GAT	<u>CCT</u>	<u>CCT</u>	<u>ACA</u>	<u>TGC</u>	<u>TAT</u>	Oligo A
Thr	Leu	Glu	Lys	Cys	Cys	Ala	Thr	Asp	Asp	Pro	Pro	Thr	Cys	Tyr	
<u>GCC</u>	<u>AAA</u>	<u>GTG</u>	CTT	GAT	GAA	TTT	AAA	CCT	CTT	GTG	GAT	GAG	CCT	CAG	
Ala	Lys	Val	Leu	Asp	Glu	Phe	Lys	Pro	Leu	Val	Asp	Glu	Pro	Gln	
AAT	TTA	GTC	AAA	ACA	AAC	TGT	GAA	CTT	TTT	GAA	AAA	CTT	GGA	GAG	
Asn	Leu	Val	Lys	Thr	Asn	Cys	Glu	Leu	Phe	Glu	Lys	Leu	Gly	Glu	
TAT	GGC	TTC	CAA	AAT	GCG	CTC	TTA	<u>GTT</u>	<u>CGT</u>	<u>TAC</u>	<u>ACC</u>	<u>AAG</u>	<u>AAA</u>	<u>GCA</u>	Oligo B
Tyr	Gly	Phe	Gln	Asn	Ala	Leu	Leu	Val	Arg	Tyr	Thr	Lys	Lys	Ala	
<u>CCC</u>	<u>CAA</u>	<u>GTG</u>	TCA	ACT	CCA	ACT	CTC	GTC	GTG	GAG	GTC	TCA	AGA	AAA	
Pro	Gln	Val	Ser	Thr	Pro	Thr	Leu	Val	Val	Glu	Val	Ser	Arg	Lys	
CTA	GGA	AAA	GTG	GGC	ACC	AAA	TGT	TGT	AAG	AAA	CCT	GAA	TCA	GAG	
Leu	Gly	Lys	Val	Gly	Thr	Lys	Cys	Cys	Lys	Lys	Pro	Glu	Ser	Glu	
<u>AGA</u>	<u>ATG</u>	<u>TCC</u>	<u>TGT</u>	<u>GCT</u>	<u>GAT</u>	<u>GAC</u>	<u>TTT</u>	<u>CTG</u>	<u>TCC</u>						Oligo C
Arg	Met	Ser	Cys	Ala	Asp	Asp	Phe	Leu	Ser						

FIG. 2A. Alignment of the deduced amino acid sequence of clone 54c with albumin sequences. The deduced amino acid sequence of clone 54c coding for a dog albumin fragment is compared with albumin sequences; human (82.6%), pig (81.8%), cattle (77.3%), sheep (78.8%), mouse (75.8%), and rat (76.2%). *Vertical bars* represent identical amino acids.

COMPARISON OF ALBUMIN AMINO ACID SEQUENCES

human	RDEGKASSAKQRLKCASLQKFGERAFAKAWAVARLSQRFPKA	250
pig	REKVLTSAAKQRLKCASIQKFGERAFAKAWSLARLSQRFPKA	
dog	LSSAKERFKCASLQKFGDRAFAKAWSVARLSQRFPKA	
cattle	REKVLTSARQRLRCASIQKFGERALKAWSVARLSQKFPKA	
sheep	REKVLASSARQRLRCASIQKFGERALKAWSVARLSQKFPKA	
mouse	KEKALVSSVRQRMKSSMQKFGERAFAKAWAVARLSQTFPNA	
rat	KEKALVA AVRQRMKSSMQRFGERAFKAWAVARMSQRFPNA	
human	EFAEVSKLVTDLT KVHTECCHGDLLECADDRADLAKYICEN	
pig	DFTEISKIVTDLAKVHKECCHGDLLECADDRADLAKYICEN	
dog	DFAEISKVVTDLTKVHKECCHGDLLECADDRADLAKYMCEN	
cattle	EFVEVTKLVTDLT KVHKECCHGDLLECADDRADLAKYICDN	
sheep	DFTDVTKI VTDLT KVHKECCHGDLLECADDRADLAKYICDH	
mouse	DFAEITKLATDLTKVNKECCHGDLLECADDRAELAKYMCEN	
rat	EFAEITKLATDVT KINKECCHGDLLECADDRAELAKYMCEN	
human	QDSISSKLKECCEKPLLEKSHCIAEVENDEMPADLP SLAAD	
pig	QDTISTKLKECCDKPLLEKSHCIAEAKRDELPADLNPLEHD	
dog	QDSISTKLKECCDKPVLEKSQCLAEVERDELPGDLPSLAAD	
cattle	QDTISSKLKECKDPCLLEKSHCIAEVEKDAIPEDLPPLTAD	
sheep	QDALSSKLKECCDKPVLEKSHCIAEVDKDAVPENLPPLTAD	
mouse	QATISSKLQTCCDKPLLKKAHCLSEVEHDTMPADLP AIAAD	
rat	QATISSKLQACCDKPV LQKSQCLAETEHDNIPADLP SIAAD	

A

FIG. 2B. See legend to FIG. 2A.

human	FVESKDVCKNYAEAKDVFLGMFLYEYARRHPDYSVVLRL
pig	FVEDKEVCKNYKEAKDVFLGTFLYEYSRRHPDYSVSLRL
dog	FVEDKEVCKNYQEAKDVFLGTFLYEYSRRHPEYSVSLRL
cattle	FAEDKDVCKNYQEAKDAFLGSFLYEYSRRHPEYAVSVLRL
sheep	FAEDKEVCKNYQEAKDVFLGSFLYEYSRRHPEYAVSVLRL
mouse	FVEDQEVCKNYAEAKDVFLGTFLYEYSRRHPDYSVSLRL
rat	FVEDKEVCKNYAEAKDVFLGTFLYEYSRRHPDYSVSLRL
human	AKTYETTLEKCCAAADPHECYAKVFDEFKPLVEEPQNLIKQ
pig	AKIYEATLEDCCAKEDPPACYATVFDKFQPLVDEPKNLIKQ
dog	AKEYEATLEKCCATDDPPTCYAKVLDEFKPLVDEPQNLVKT
cattle	AKEYEATLEECCKADDPHACYTSVFDKLKHLVDEPQNLIKQ
sheep	AKEYEATLEDCCAKEDPHACYATVFDKLKHLVDEPQNLIKQ
mouse	AKKYEATLEKCCAEANPPACYGTVLAEFQPLVEEPKNLVKT
rat	AKKYEATLEKCCAEGDPPACYGTVLAEFQPLVEEPKNLVKT
human	NCELFEQLGEYKFQNALLVRYTKKVPQVSTPTL.VEVSRL
pig	NCELFEKLGEYGFQNALIVRYTKKVPQVSTPTL.VEVARKL
dog	NCELFEKLGEYGFQNALLVRYTKKAPQVSTPTLVVEVSRKL
cattle	NCDQFEKLGEYGFQNALIVRYTRKVPQVSTPTL.VEVSRL
sheep	NCELFEKHGEYGFQNALIVRYTRKAPQVSTPTL.VEISRL
mouse	NCDLYEKLGEYGFQNALIVRYTQKAPQVSTPTL.VEAARNL
rat	NCELYEKLGEYGFQNAVLVRYTQKAPQVSTPTL.VEAARNL

B

FIG. 2C. See legend to FIG. 2A.

human	GKVGSKCKHPEAKRMPCAEDYLS	478
pig	GLVGSRCCKRPEEERLSCAEDYLS	
dog	GKVGTKCCKKPESERMSCADDFLS	
cattle	GKVGTRCCTKPESERMPCTEDYLS	
sheep	GKVGTKCCAKPESERMPCTEDYLS	
mouse	GRVGTKCCTLPEDQRLPCVEDYLS	
rat	GRVGTKCCTLPEAQRLPCVEDYLS	C

Testing of recombinant phage with serum IgE from a patient allergic to dog albumin

Confluent lawns of *E. coli* Y1090 were infected in a dot procedure with a recombinant phage expressing IgE-binding proteins, the major birch pollen allergen *Bet v I*, [30](#) and nonrecombinant lambda gt11 phages. Plates were incubated at 43° C until lysed spots were visible. Expression of β -galactosidase fusion proteins was induced with nitrocellulose filters soaked in 10 mmol/L isopropyl- β -D-thiogalactoside, as described above. Serum IgE from a patient with IgE reactivity to dog albumin was used to detect recombinant phage containing albumin-encoding cDNAs as described for IgE immunoblots.

Hybridization of recombinant dog allergens with synthetic oligonucleotides

Confluent lawns of *E. coli* Y1090 were infected in a dot procedure with a recombinant phage and a control phage. Phages were incubated overnight at 43° C. Nitrocellulose filters containing plaque lifts were prehybridized as described by Sambrook et al. [27](#) One set of filters containing an identical set of plaque lifts was hybridized with phosphorous 32–labeled oligonucleotides A, B, and C. The oligonucleotides had been 5′ labeled with ³²P- γ -adenosine triphosphate (NEN, Stevenage, U.K.) and polynucleotide kinase (Boehringer Mannheim, Germany).

Labeled oligonucleotides were separated from unincorporated nucleotides with nick columns (Pharmacia). Hybridization was carried out as described by Sambrook et al., [27](#) and blots were autoradiographed with Kodak X-OMAT films and intensifying screens at –70° C.

Histamine release from patients' basophils with purified albumins

Blood basophils from patients allergic to dog albumin and healthy individuals were prepared from heparinized blood after informed consent was obtained from the patients. A dextran sedimentation procedure was used for isolation of granulocytes. [31](#) Granulocytes were incubated with various concentrations of purified albumins from dog, rat, mouse, and

chicken or anti-IgE monoclonal antibody E-124-2-8 as positive control. Histamine release was measured by radioimmunoassay in the cell-free supernatants (Immunotech, Marseille, France).

RESULTS

Determination of the percentage of patients allergic to dog albumin

FIG. 3A. IgE reactivity of selected patients allergic to albumin with dog hair/dander extract. IgE Immunoblot with dog hair/danDer proteins that were blotted to nitro cellulose. Six patients with IgE reactivity to dog albumin had been selected and were compared with a human serum pool prepared from nonallergic patients (N). P represents the buffer control.

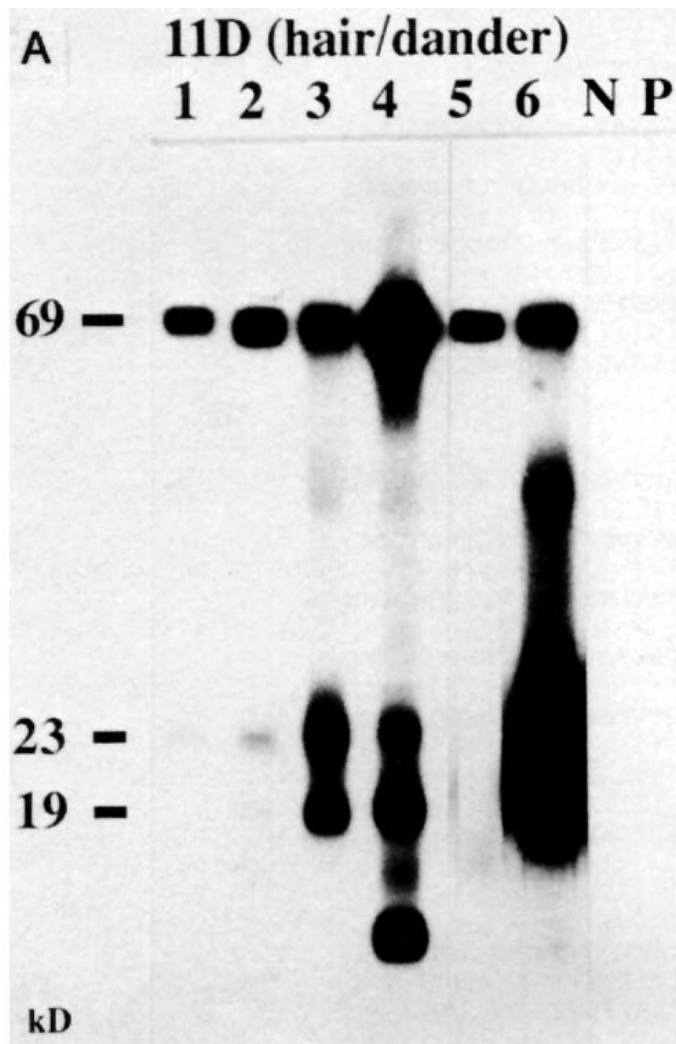
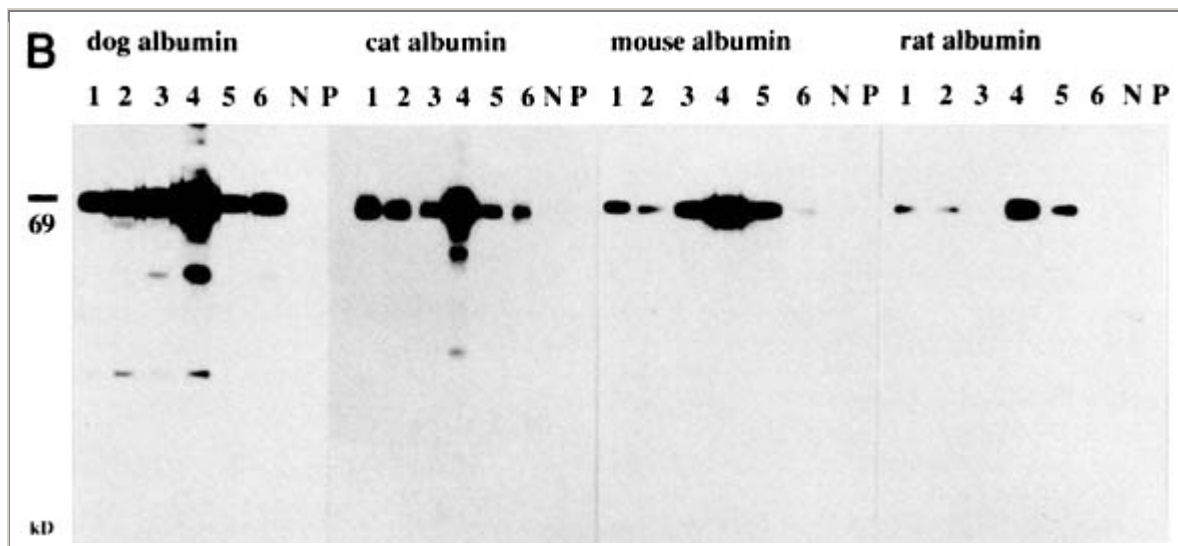


FIG. 3B. IgE reactivity of patients allergic to dogs with different albumins. The same group of individuals (patient 1 to 6) as in Fig. 3A was tested for IgE reactivity to nitrocellulose-blotted purified albumins from dog, cat, mouse, and rat. NP represents a serum pool from nonallergic patients and P represents the buffer control. IgE binding to the various albumins is differently pronounced.



One hundred ten allergic patients to dogs were tested for reactivity with dog hair/dander extracts by IgE immunoblotting (data not shown). Ninety-six percent of the patients reacted to the major dog allergens *Can f* I [7, 13](#) at a molecular weight of 23 kd and/or *Can f* II [7, 14](#) at a molecular weight of 19 kd. Most of the patients showed IgE reactivity to both allergens, and only a few patients showed IgE binding to either one or the other of these allergens. Dog albumin at 69 kd bound serum IgE in 35% of the tested patients and may therefore be considered to be an intermediate allergen.

Percentage of IgE directed against dog albumin

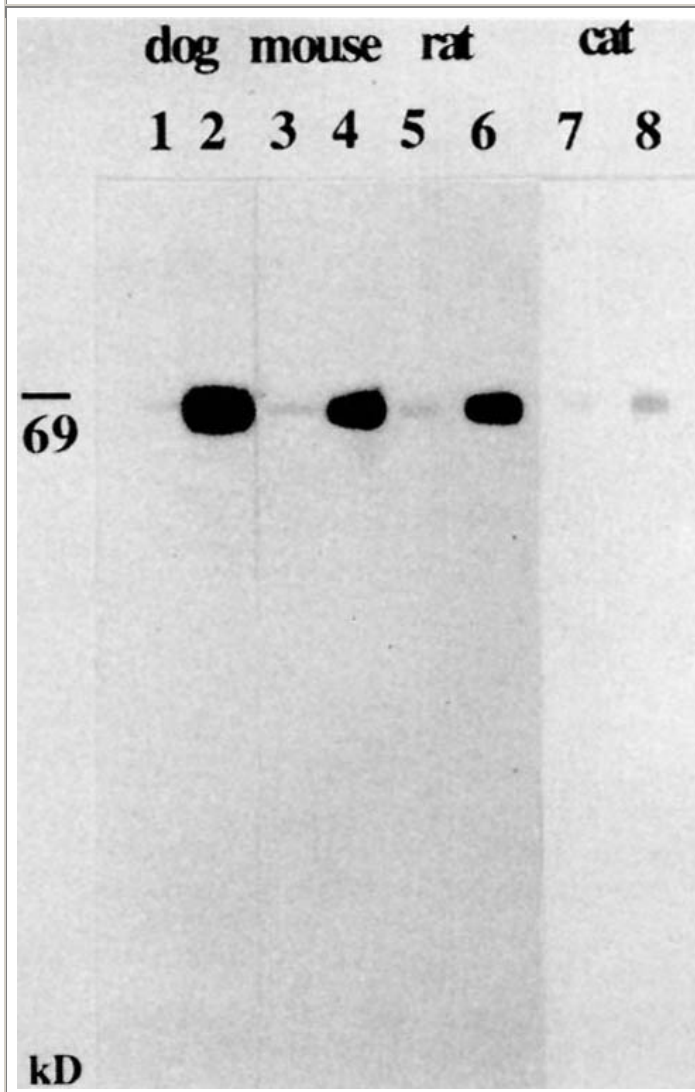
■ Table I shows the reduction of IgE binding to dog hair/dander extracts obtained by preincubation of sera with dog albumin or dog hair/dander extract in a panel of representative individuals. Patients with strong IgE reactivity to dog albumin on immunoblots exhibit inhibition of IgE binding ranging from 70% to 100%. In patients without IgE reactivity to immunoblotted dog albumin, no reduction is observed after preincubation with dog albumin. Preincubation with dog hair/dander extract leads to 100% inhibition, whereas timothy grass pollen extract does not affect IgE binding to dog allergens. Nonallergic individuals have no IgE antibodies directed against dot-blotted dog allergens.

Cross-reactivity of patients' serum IgE with different albumins

To corroborate earlier observations that albumin may represent a cross-reactive allergen in hair and dander of different animals, [7, 17-19](#) purified albumins from dog, cat, mouse, and rat were tested with serum IgE from patients allergic to dog albumin. Six patients with IgE reactivity at 69 kd on nitrocellulose-blotted dog hair/dander extracts (■ Fig. 3A) were tested with the purified albumins (■ Fig. 3B). A serum pool prepared from nonallergic individuals was included as a negative control. ■ Fig. 3B shows that with the exception of patients 3 and 6, who did not react to rat albumin, all patients cross-reacted with the different albumins. The intensity of IgE reactivity with the various albumins varied considerably, suggesting that some of the IgE epitopes of dog, cat, mouse, and rat albumin may be different. To further test for common IgE epitopes of dog, cat, mouse, and rat albumin, IgE immunoblot-inhibition experiments were done. Serum from a patient allergic to dog albumin was diluted 1:10 in buffer A and preincubated either with dog albumin (■ Fig. 4,

lanes 1, 3, 5, and 7) or with timothy grass (*Phleum pratense*) pollen extract (lanes 2, 4, 6, and 8) before incubation with nitrocellulose-blotted dog (lanes 1 and 2), mouse (lanes 3 and 4), rat (lanes 5 and 6), and cat (lanes 7 and 8) albumins. ■Fig. 4 shows that preincubation with dog albumin diminishes or at least substantially reduces the IgE binding to other mammalian albumins when compared with preincubation with timothy grass pollen extract.

FIG. 4. Inhibition of IgE binding to different albumins with dog albumin. Purified albumins from dog, mouse, rat, and cat were transferred to nitrocellulose. Strips were incubated with serum from a patient allergic to dog albumin. In lanes 1, 3, 5, and 7 the serum was preincubated with purified dog albumin and in lanes 2, 4, 6, and 8 the serum was preincubated with timothy grass pollen extracts. IgE binding to dog albumin and the other mammalian albumins was significantly reduced on preincubation with dog albumin.

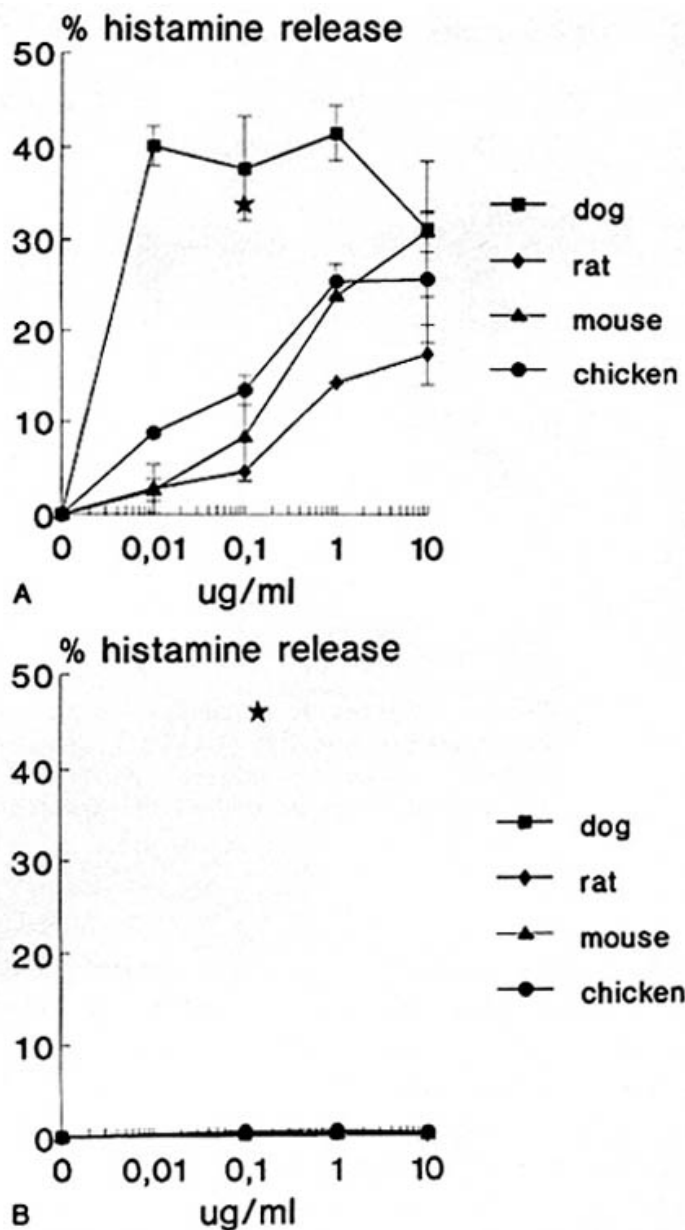


Histamine release from patients' basophils with different albumins

■Fig. 5 shows the dose dependency of the histamine release obtained from blood basophils from a patient allergic to dog albumin (■Fig. 5, A) and a nonallergic individual (■

Fig. 5, B). Both individuals showed specific and dose-dependent histamine release on stimulation with monoclonal anti-IgE antibody E-124-2-8, whereas only in the case of a patient allergic to dog albumin was a significant and dose-dependent histamine release observed with dog, rat, mouse, and chicken albumin. The histamine release was most pronounced with dog albumin and less pronounced with rat, mouse, and chicken albumin. IgE antibodies directed against dog albumin not only cross-reacted with albumins from other species but also elicited histamine release from patients' basophils.

FIG. 5. Dose dependency of the histamine release from basophils of a patient allergic to albumin (A) and a control subject without albumin-specific IgE (B). Granulocytes of the donors (A, B) were incubated with various concentrations of albumin from dog, rat, mouse, chicken, buffer, or monoclonal anti-IgE antibody. Histamine release in the cell-free supernatants was determined by radioimmunoassay. Percentage of total histamine is displayed and reflects means of triplicate determinations. The standard deviation is displayed for each value with the exception of the anti-IgE control, where the maximum release is indicated with an asterisk.



Isolation and characterization of cDNAs coding for dog albumin fragments from a dog salivary gland cDNA library with allergic patients' IgE

From a dog salivary gland expression cDNA library, several IgE-binding clones were isolated with serum IgE prepared from a pool of patients allergic to dogs. Recombinant phage was isolated, and the cDNAs were subcloned into plasmid pUC 18. The cDNA insert of clone 54c was determined with lambda gt11 forward-sequencing primers, lambda gt11 reversed-sequencing primers, and additional internal oligonucleotide primers. The cDNA sequence and deduced amino acid sequence of clone 54c is displayed in Fig. 1. The deduced amino acid sequence was compared with the Swiss Prot database, and an overall sequence identity in the range of 80% with albumins from human beings, pig, cattle, sheep, mouse, and rat was found, indicating that this clone codes for dog albumin. Because the library was constructed by random priming, no poly A⁺ tail was found. The clone expresses an internal dog albumin fragment of 265 amino acids. Loss of DNA as a result of internal restriction sites in the insert can be excluded because the sequenced insert was obtained by a KpnI/SacI digest and contains lambda gt11 flanking portions.

The recombinant phage obtained by IgE screening were then probed with serum IgE from a patient allergic to dog albumin with additional specificity for the major birch pollen allergen, *Bet v I*, [30](#) in a plaque assay (Fig. 6, A and B). Nonrecombinant lambda gt11 phage was included as a negative control, and a phage containing the cDNA coding for the major birch pollen allergen, *Bet v I*, was used as a positive control. With the exception of three clones 3-I, 41-I, and the nonrecombinant phage (nKo), 24 clones and the *Bet v I*-specific clone bound serum IgE from this patient. Clones 3-I and 41-I may therefore code for different dog allergens or may contain albumin epitopes that are not targets for the patients' IgE. To further characterize the isolated clones, synthetic oligonucleotides (Fig. 7), specific for different portions of dog albumin, were used for plaque hybridization. Fig. 8, A and B shows the results of the plaque hybridization procedure. Most of the clones hybridized with all three oligonucleotides, the nonrecombinant lambda gt11 phages (nKo) were negative with all three oligonucleotides, and the positive control clone 54c (pKo) hybridized with all three oligonucleotides. Clones 41-I and 25cIII hybridized exclusively with oligonucleotide C and therefore expressed a C-terminal portion of dog albumin, whereas clone 25-I hybridized only with oligonucleotides A and B and therefore contained an N-terminal portion of dog albumin.

FIG. 6. Testing of recombinant phage with serum IgE from a patient allergic to dog albumin. **A**, Nitrocellulose filters I and II containing plaque lifts of phage, which had been induced to produce recombinant proteins, were tested with serum IgE from a dog albumin-allergic patient who also displays IgE reactivity to the major birch pollen allergen, *Bet v I*. **B**, Recombinant phages, which had been isolated from a dog salivary gland cDNA library in filters I and II, are designated with various numbers: *nKo* represents nonrecombinant lambda gt11 phage and *Bet v I* represents the phage expressing the major birch pollen allergen *Bet v I* as a positive control.

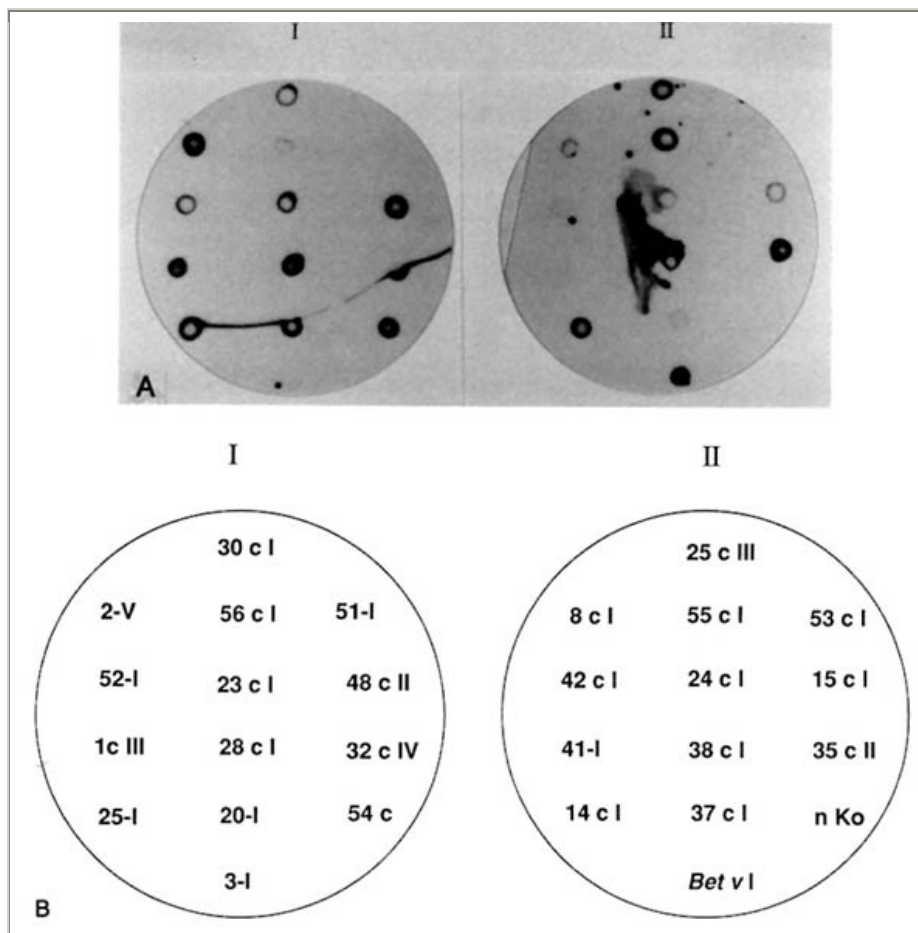


FIG. 7. Sequences of oligonucleotides complementary to different portions of the dog albumin cDNA.

Oligo A

5' CAC TTT GGC ATA GCA TGT AGG AGG 3'

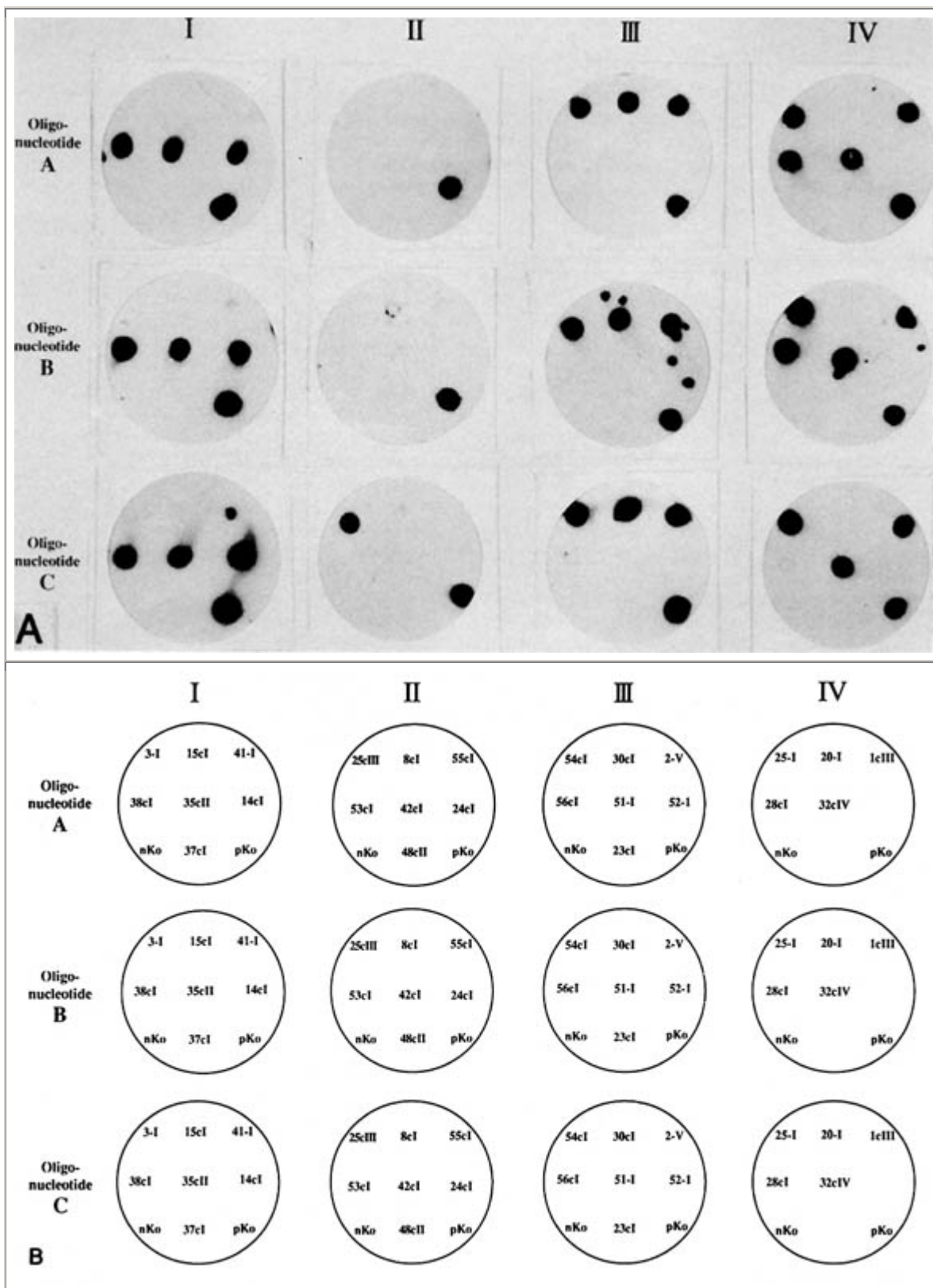
Oligo B

5' CAC TTG GGG TGC TTT CTT GGT GTA ACG AAC 3'

Oligo C

5' GAA AGT CAT CAG CAC AGG ACA TTC 3'

FIG. 8. Hybridization of albumin-specific oligonucleotides with cDNA clones coding for dog allergens. **A**, Plaque lifts of phages, which had been isolated with serum IgE from patients allergic to dogs, nonrecombinant lambda gt11 phage (*nKo*), and phages that contain cDNA from clone 54c coding for dog albumin (*pKo*) were hybridized with synthetic oligonucleotides complementary to different portions of the albumin cDNA (**A**). **B**, Localization of the clones on the nitrocellulose filters.

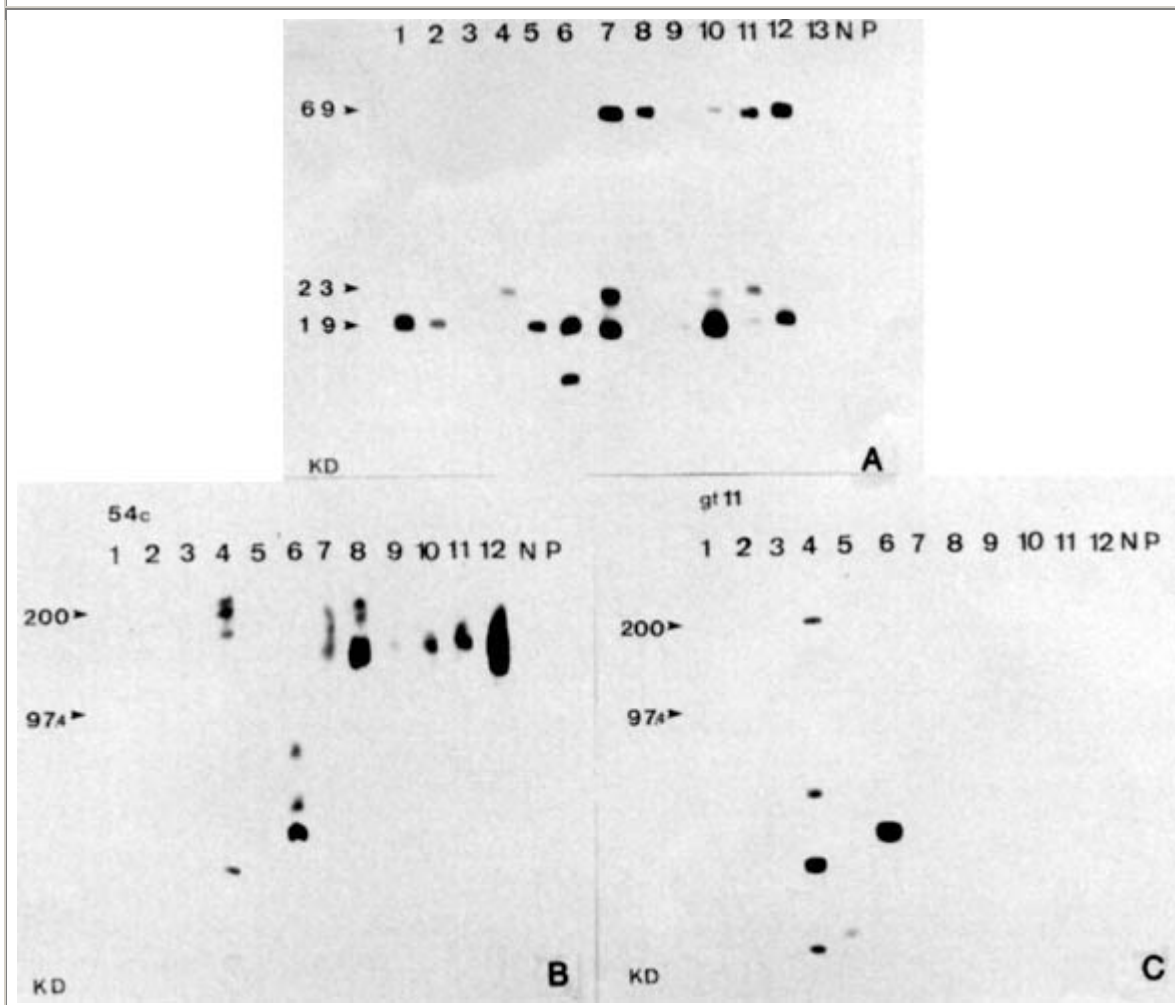


Comparison of patients' IgE binding to natural dog albumin and to dog albumin fragments expressed in *E. coli*

■ Fig. 9 compares the IgE binding to natural dog albumin and recombinant dog albumin fragments. Sera from 11 patients allergic to dogs, two patients allergic to pollen without dog allergy (patients 3 and 13), a nonallergic individual (N), and the buffer (P) without

addition of serum were tested for IgE binding to dog hair/dander extract (■Fig. 9, A), to the dog albumin fragment of clone 54 c expressed as β -galactosidase fusion protein in *E. coli* (■Fig. 9, B), and to proteins from *E. coli* infected with a nonrecombinant lambda gt11 phage (■Fig. 9, C). All patients (7, 8, 10, 11, and 12) with IgE reactivity against native dog albumin at 69 kd (■Fig. 9, A) exhibited binding of their IgE to the recombinant dog albumin fragment expressed as a 150 kd β -galactosidase fusion protein (■Fig. 9, B) but did not exhibit reactivity to *E. coli* proteins in this molecular weight range (■Fig. 9, C). Patients 4 and 6 showed reactivity to proteins from *E. coli* but not to the recombinant dog albumin fragment.

FIG. 9. Comparison of the IgE binding to natural dog hair/dander proteins and to recombinant albumin fragments. All patients with IgE binding to dog albumin in natural dog hair/dander extracts (A) show comparable reactivity with a recombinant dog albumin fragment expressed in *E. coli* as β -galactosidase fusion protein (B) at around 150 kd. No IgE binding is observed with protein extracts prepared from lysogenic *E. coli* Y1089 infected with nonrecombinant lambda gt11 phage in the 150 kd region-negative control (C).



DISCUSSION

Type I allergies to mammalian proteins, in particular to dog and cat allergens, are frequently related. [17-19](#) Although the major dog allergens *Can f* I [7, 13](#) and *Can f* II [7, 14](#) and

the major cat allergen *Fel d I* [15](#), [16](#) show similar molecular weights, these allergens are neither immunologically nor structurally related. However, albumin was identified as a relevant IgE-binding structure in dog and cat hair/dander extracts. [7](#), [12](#), [20](#) About 35% of patients allergic to dogs display IgE reactivity to dog albumin, and in individual patients a considerable proportion (70% to 100%) of dog-specific IgE is directed against dog albumin. According to case histories, all patients with reactivity to dog albumin show clinical symptoms on contact with cat and/or other animal hair or dander. To determine whether there is a molecular basis for this clinical observation, IgE immunoblot-inhibition experiments were performed, demonstrating that IgE antibodies directed against dog albumin cross-react with albumins from cat, mouse, and rat. In parallel, histamine release was inducible from basophils derived from patients allergic to dog albumin on incubation with albumins from rat, mouse, and chicken, indicating that albumins from different species represent functional cross-reactive allergens. To further characterize dog albumin, recombinant techniques were applied. With serum IgE from patients allergic to dog albumin, a number of cDNAs coding for dog albumin were isolated from a lambda gt11 expression library prepared from dog salivary glands. The cDNA sequence of clone 54c was determined and significant sequence identities, with about 80% homology, were found with albumins from human beings, pig, cattle, sheep, mouse, and rat. The cDNA of clone 54c codes for an internal fragment of dog albumin, which contains major IgE epitopes of dog albumin ([Fig. 9](#)). To further characterize IgE-positive clones and to obtain information about the distribution of IgE epitopes, oligonucleotides were synthesized complementary to different regions of the dog albumin cDNA sequence of clone 54c and used for plaque hybridization. Several clones coding for dog albumin fragments were incomplete either at the 5' end or at the 3' end. This indicates that a number of epitopes derived from dog albumin may be involved in IgE binding. As demonstrated in [Fig. 9](#), all tested patients with IgE reactivity against natural dog albumin displayed IgE binding to a recombinant dog albumin epitope, which was expressed as a β -galactosidase fusion protein. Partial epitope clones, used as a starting material for further detailed analysis of dog albumin IgE epitopes, may help to develop IgE-binding peptides, which do not lead to release of mediators from mast cells and basophils.

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Major cat and dog allergens share IgE epitopes

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