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Detection of an allergen in dog dander that cross-reacts with the major cat allergen, Fel d 1

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Clinical and Experimental Allergy

Summary

Background A considerable proportion of animal-allergic patients are sensitized to both cat and dog allergens but knowledge about cross-reactive allergens in cat and dog dander is limited.

Objective To investigate whether dog dander contains an allergen that cross-reacts with the major cat allergen, Fel d 1.

Methods Recombinant Fel d 1 with the same immunological properties as natural Fel d 1 was used for quantitative (CAP) IgE competition experiments performed with sera obtained from cat-allergic patients ($n = 36$). A Fel d 1 cross-reactive dog allergen was characterized by one- and two-dimensional immunoblotting using rFel d 1 for IgE inhibition experiments and with monospecific, polyclonal rabbit anti-recombinant Fel d 1 antibodies.

Results In 25% of Fel d 1-reactive cat-allergic patients, more than 50% inhibition of IgE reactivity to dog allergens was achieved with recombinant Fel d 1. An Fel d 1 cross-reactive 20 kDa allergen with a pI of approximately 3.4 was detected in dander extracts of several different dog breeds.

Conclusion This is the first report demonstrating the presence of an Fel d 1-like allergen in dog dander extracts, which may be responsible for double positivity to cat and dog in serology. However, the clinical relevance of this cross-sensitization needs to be confirmed. These results are important for the diagnostic and therapeutic use of dog dander allergen extracts.

Keywords cat allergy, cross-reactivity, dog allergy, Fel d 1, major cat allergen, recombinant allergen

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Introduction

Type I allergic reactions to cats and dogs affect up to 30% of allergic patients [1, 2]. A frequent clinical association between cat and dog allergy has already been noted several decades ago [3]. The association between cat and dog allergy has raised the question of whether this phenomenon is due to co-sensitization to different allergenic molecules or whether cross-reactivity with allergens present in dog and cat dander may also play an important role. Evidence for the presence of cross-reactive allergens in cat and dog dander extracts comes from radioallergo-sorbant test and immunoblot inhibition studies [4–9]. By cDNA cloning techniques, the serum protein albumin

(Fel d 2, Can f 3) could be unambiguously identified as a cross-reactive allergen in cat and dog dander [10–13]. Furthermore, the molecular structures of the major cat allergen, Fel d 1 [14–20], other cat allergens (e.g. Fel d 3: cystatin [21]) and the major dog allergens, Can f 1 and Can f 2 [22], have been revealed. However, for none of the latter molecules has any evidence for cross-reactivity between cat and dog been provided so far.

Fel d 1, a 38 kDa acidic glycoprotein of unknown biological function, represents the major allergen of cat. It consists of two identical non-covalently linked 19 kDa heterodimers, which again are composed of two polypeptide chains anti-parallel linked to each other by three

disulphide bonds [18–19]. Chain 1, an 8 kDa polypeptide, comprises 70 aa and shows sequence homology with rabbit uteroglobulin and a secretory protein from human Clara cell [16]. The chain 2 is 10 kDa in size, comprises 92 aa and carries a glycosylation site at Asn33 [15, 18, 20]. Chains 1 and 2 are encoded on different genes [16], each consisting of three exons [17].

The two-chain composition makes it difficult to produce Fel d 1 as a recombinant protein, and several attempts have been made to express the allergen in *Escherichia coli* [23–26] and in baculovirus [27]. Recently, recombinant Fel d 1 (rFel d 1), expressed as a chain 2-chain 1 fusion protein, has been generated in *E. coli*. This recombinant Fel d 1 was able to mimic completely the immunological properties of the natural allergen [19].

In the present study, we used this rFel d 1 molecule to investigate whether an Fel d 1 cross-reactive allergen exists in dog dander. Sera from 36 allergic patients containing IgE antibodies to cat and dog dander extracts were used in quantitative CAP inhibition studies with rFel d 1. Furthermore, IgE immunoblot inhibition experiments were performed and a specific rabbit antiserum was raised against rFel d 1 to search for a cross-reactive allergen in dog dander extracts from several different dog breeds.

Materials and methods

Patients and sera

The diagnosis of IgE-mediated allergy was based on anamnesis, skin prick testing (Allergopharma, Reinbek, Germany and ALK-Abello, Frederiksberg, Denmark) and/or measurement of cat and dog dander-specific serum IgE antibodies (CAP-FEIA, Pharmacia Diagnostics, Uppsala, Sweden) (Table 1). The sensitization profile was determined using dot-blotted purified allergens rFel d 1, nFel d 2, nCan f 1 and nCan f 3. Non-allergic persons were specified as lacking any history of allergic symptoms and skin or IgE reactivity to common allergen extracts (Table 1).

Recombinant Fel d 1, Fel d 1-specific antibodies

Recombinant Fel d 1 was expressed as a hexahistidine-tagged chain 2-chain 1 fusion protein in *E. coli* BL21 (DE3) pLysS (Novagen, Madison, WI, USA) and purified to homogeneity as previously described [19].

Fel d 1-specific rabbit antibodies were obtained by immunizing a New Zealand white rabbit with purified rFel d 1 (200 µg/injection) using Freund's complete (first immunization) and incomplete (booster injections) adjuvant (Charles River, Kisslegg, Germany). The titre of rabbit Fel d 1-specific IgG was determined in ELISA assays, in which the rabbit antiserum was applied in the following dilutions: 1 : 1000, 1 : 5000, 1 : 10 000, 1 : 50 000 and

1 : 100 000 to Fel d 1-coated ELISA plates (0.5 µg/well). Bound rabbit IgG was detected with 1 : 1000 diluted HRP-conjugated donkey anti-rabbit Ig antibodies (Amersham Biosciences, Little Chalfont, UK). Rabbit anti-Fel d 1 antiserum detected ELISA plate-bound Fel d 1 up to a dilution of 1 : 100 000.

Quantitative immunoglobulin E inhibition experiment

Cat dander-, dog dander- and house dust mite-specific IgE antibody levels were quantified by CAP FEIA measurements after pre-incubation of sera ($n = 36$) with rFel d 1 (10 µg/100 µL serum) or with 10 µL phosphate-buffered saline with 0.5% v/v Tween 20 (PBST). The percentage inhibition of IgE binding was calculated as follows:

Percentage inhibition = $100 \times (\text{IgE}_{\text{PBST}} - \text{IgE}_{\text{Fel d 1}}) / \text{IgE}_{\text{PBST}}$, with IgE_{PBST} being the CAP result in kUA/L for the sera pre-incubated with PBST, and $\text{IgE}_{\text{Fel d 1}}$ being the CAP result for sera pre-incubated with rFel d 1.

Protein extracts, sodium dodecyl sulphate-polyacrylamide gel electrophoresis

Standard quality (SQ) cat and dog dander extracts were purchased from ALK-Scherax, (Hamburg, Germany). Dog dander from four different dogs (Kuvasz, Maltese Terrier, long-haired German shepherd dog and Dalmatian-Collie half breed) were collected and extracts were prepared as described [28].

Protein concentrations of extracts were determined by a BioRad DC Protein Assay and Coomassie Brilliant Blue (BioRad, Richmond, CA, USA) staining [29]. Approximately 100 µg protein/cm gel of each extract was separated under non-reducing conditions by SDS-PAGE [30] and blotted onto nitrocellulose [31].

Two-dimensional electrophoresis

Dog dander proteins were first separated according to their pI using 17 cm ReadyStrip immobilized pH gradient (IPG) Strips (pH 3–6) (BioRad). For this purpose, 100 µg lyophilized extract was dissolved in 250 µL rehydration buffer (8 M urea, 2% v/v Triton X-100, 10 mM dithioerythritol, 1 mM PMSF and 2% v/v IPG buffer, pH 3–10) (Amersham Pharmacia Biotech, Uppsala, Sweden), and applied to the strips overnight at room temperature during rehydration of the strips. Iso-electric focusing was performed with a Multiphor II Electrophoresis unit (Amersham Pharmacia Biotech) at 200 V for 5 h, 1000 V for 9 h, 2000 V for 5 h and 3500 V for 5 h. For separation of the proteins according to their molecular weights, strips were then equilibrated for 15 min in equilibration buffer (6 M urea, 50 mM Tris pH 8.8%, 30% v/v glycerol, 2% w/v SDS) containing 2% w/v dithioerythritol and for 15 min with an equilibration buffer containing 2.5% w/v

Table 1. Characterization of cat-allergic patients

Patient	Age	Sex	SPT wheel diameter (mm)				CAP result (kUA/L)		Symptoms upon contact with:		Dot blot results: IgE reactivity to			
			Cat	dog	Hist	buffer	e1	e5	cat	dog	Fel d1	Fel d2	Can f1	Can f3
1	36	f	pos	n.d.	pos	neg	48.2	29.4	R, C		+	—	+	—
2	38	f	pos	n.d.	pos	neg	47.8	7.58	R, C, As		+	—	—	—
3	22	f	pos	n.d.	pos	neg	> 100	> 100	AD		+	+	n.d.	+
4	40	f	6	0	7	0	> 100	14.4	AD, As		+	—	n.d.	—
5	35	m	pos	n.d.	pos	neg	43.8	15.8	R, C, As	R, C, As	+	—	—	—
6	27	m	pos	n.d.	pos	neg	40.0	23.5	AD		+	—	—	—
7	32	m	6	5	9	0	35.8	10.2	AD, R, C, As		+	—	n.d.	—
8	23	m	7	4	9	0	45.0	8.37	R, C, As		+	—	n.d.	—
9	27	f	pos	n.d.	pos	neg	36.1	7.59	R, C	R, C	+	—	—	—
10	25	m	pos	pos	pos	neg	17.3	2.34	AD, R, C, As		+	—	+	—
11	25	m	pos	n.d.	pos	neg	19.2	4.15	R, C, As		+	—	—	—
12	26	f	5	n.d.	4	0	16.9	2.57	R, C, As		+	—	n.d.	—
13	22	f	pos	n.d.	pos	neg	4.65	5.81	R, C, As	R, C, As	+	—	+	—
14	18	m	24	4	pos	neg	14.1	2.60	R, C, As		+	—	—	—
15	9	m	8	6	6	0	38.7	3.68	AD, As		+	—	—	—
16	15	m	8	5	6	0	16.1	2.40	R, C, As		+	—	+	—
17	14	m	5	3	6	0	13.7	2.45	R, C, As		+	—	—	—
18	11	m	n.d.	n.d.	n.d.	n.d.	> 100	14.0	AD, C, As		+	—	—	—
19	8	m	8	5	6	0	89.7	7.83	R, C, As		+	—	+	—
20	10	m	5	3	5	0	14.0	5.21	AD, R, C, As		+	—	—	—
21	12	m	8	4	pos	neg	4.84	1.20	AD, R, C, As		+	—	—	—
22	18	m	16	4	7	0	3.80	1.90	R, C, As		+	—	n.d.	—
23	18	m	6	5	5	0	2.55	1.07	R, C, As		+	—	—	—
24	15	m	5	3	5	0	2.27	0.86	R, C, As		+	—	—	—
25	13	m	8	0	6	0	2.00	3.21	R, C		+	—	—	—
26	16	m	18	4	6	0	2.06	1.21	R, As		+	—	—	—
27	18	m	5	3	6	0	1.56	1.56	R, C, As		+	—	—	—
28	17	m	6	5	6	0	0.81	0.94	R, C, As		+	—	+	—
29	14	m	5	6	8	0	> 100	15.9	R, C, As	R, C, As	+	+	+	—
30	6	m	11	6	8	0	51.5	16.8	R, C, As		+	—	+	—
31	10	m	4	4	5	0	40.1	4.65	R, C, As		+	—	+	—
32	9	m	6	3	8	0	15.3	4.46	AD, R, C, As		+	—	—	—
33	13	m	15	5	7	0	4.09	1.70	C, As		+	—	—	—
34	4	m	n.d.	n.d.	n.d.	0	4.30	1.24	AD, As		+	—	—	—
35	11	m	4	4	4	n.d.	22.4	94.0	AD, As	AD, As	+	+	+	+
36	18	f	8	3	5	0	2.72	1.25	R, C, As		+	—	—	—
Mean age	18													

SPT, skin prick test; hist, histamine; e1, cat dander extract; e5, dog dander extract; f, female; m, male; R, rhinitis; C, conjunctivitis; As, asthma; AD, atopic dermatitis; pos, positive; neg, negative; n.d. not done.

Grey boxes indicate those cat allergic patients whose IgE reactivity to dog dander extract was inhibited to more than 50 % by rFel d 1 in quantitative inhibition experiments (Table 2).

iodoacetamide and placed on top of a 12% PAGE. After separation by SDS-PAGE [30], proteins were blotted onto nitrocellulose membranes [31].

Immunoblotting, dot-blotting and immunoblot inhibitions

Purified natural Can f 1 was purchased from Indoor Biotechnologies Inc. (Charlottesville, VA, USA), purified natural Can f 3 and Fel d 2 were purchased from Sigma (St Louis, MO, USA). For dot-blot assays, purified allergens

rFel d 1, nFel d 2, nCan f 1 and nCan f 3 were dotted onto nitrocellulose (0.5 µg/dot).

Nitrocellulose-blotted cat or dog dander proteins and dot-blotted allergens were exposed to 1 : 10 diluted sera from allergic patients or from non-atopic control individuals [28]. Bound IgE antibodies were detected with ¹²⁵I-labelled anti-human IgE antibodies (Pharmacia Diagnostics) and visualized by autoradiography.

For IgE immunoblot inhibition experiments, patients' sera were pre-incubated with rFel d 1 (10 µg/1 mL serum

dilution) and for control purposes with PBS, cat dander SQ extract (70 µg/1 mL serum dilution), dog dander SQ extract (70 µg/1 mL serum dilution) or an immunologically unrelated allergen (major birch pollen allergen, rBet v 1; Biomay, Vienna, Austria) (10 µg/1 mL serum dilution) for 12 h at 4 °C. Thereafter, IgE immunoblotting was performed as described above.

Nitrocellulose-blotted cat dander and dog dander proteins were probed with the 1 : 1000 diluted rabbit anti-Fel d 1 antiserum, the corresponding pre-immune serum or buffer alone. For immunoblot inhibition experiments, the rabbit anti-Fel d 1 antiserum dilutions were pre-incubated with rFel d 1 (30 µg/mL) or rBet v 1 (30 µg/mL) as an immunologically unrelated protein for 12 h at 4 °C and then exposed to nitrocellulose-blotted SQ cat and dog dander extracts. Two-dimensionally separated, nitrocellulose-blotted dog dander proteins from the Kuvasz breed were also probed with a 1 : 1000 diluted rabbit anti-rFel d 1 antiserum or the corresponding pre-immune serum. Bound rabbit antibodies were detected with 1 : 1000 diluted ¹²⁵I-labelled donkey anti-rabbit whole antibody (Amersham Biosciences) [32].

Results

Quantitative CAP inhibition experiments demonstrate that rFel d 1 inhibits immunoglobulin E reactivity of certain Fel d 1-reactive cat-allergic patients to dog allergens

Table 1 summarizes the clinical symptoms and sensitizations of cat-allergic patients. Besides allergic symptoms upon cat contact, such as rhinitis, conjunctivitis, asthma and exacerbation of atopic dermatitis, regularly experienced by all patients ($n = 36$, mean age: 18 years), five patients also reported allergic symptoms upon dog contact. In skin prick tests (SPT) however, for 23 patients, a positive result was obtained with dog dander extract and for all patients with cat dander extract. IgE reactivity to Fel d 1 was detected in all patients and IgE reactivity to the dog allergen Can f 1 in 10 patients (#1, 10, 13, 16, 19, 28–31, 35). In addition, IgE reactivity to Fel d 2 (cat albumin) was found in three patients (#3, 29, 35) and to Can f 3 (dog albumin) in two patients (#3, 35).

Table 2 shows the quantitative inhibition of IgE reactivity to dog dander extract achieved by pre-adsorption of sera with rFel d 1. The patients (1–36) had a mean IgE level of 29.3 and 9.3 kUA/L specific to cat dander and dog dander allergens, respectively. An average inhibition of IgE binding to dog allergens of 41.0% was observed (Table 2). A greater than 50% inhibition of IgE binding to dog allergens after pre-adsorption of sera with rFel d 1 was observed for nine out of 36 (25%) Fel d 1-positive cat-allergic patients. The specificity of the IgE inhibition was demonstrated by a control experiment, resulting in almost no inhibition (9% or less) of IgE reactivity to mite

allergens after pre-adsorption of sera with rFel d 1. Statistical comparison of the percentages of inhibition as measured in the CAP inhibitions with the non-parametric Wilcoxon rank sum test for micro samples resulted in a significant P -value < 0.001 .

rFel d 1 inhibits immunoglobulin E reactivity to a 20 kDa dog allergen

IgE immunoblot inhibition experiments under non-reducing conditions were performed with sera from patients 1, 2 and 14–17 using nitrocellulose-blotted cat and dog dander extract and rFel d 1 as an inhibitor (Fig. 1).

Sera from the allergic patients but not serum from a non-allergic individual showed IgE binding to a 20 kDa moiety (Fig. 1) in nitrocellulose-blotted cat as well as dog dander extract. The IgE reactivity to this 20 kDa component in dog dander was completely inhibited by rFel d 1 in those sera for which a strong inhibition was already observed in the quantitative CAP inhibition experiments (patient 2: 86%, patient 14: 86%, patient 15: 73%, patient 16: 62%, patient 17: 61%, Table 2). A weak inhibition of IgE binding to dog dander extract was observed for serum from patient 1, which is in accordance with the result obtained in the quantitative inhibition experiment (patient 1: 7% inhibition, Table 2).

IgE binding to cat dander allergens was almost completely inhibited by rFel d 1 for patients 1 and 2 (Fig. 1). Complete inhibition of IgE binding to blotted dog dander allergens was demonstrated for sera pre-incubated with dog dander extract (Fig. 1, panel b, lane c). Likewise, complete inhibition of IgE binding to blotted cat dander allergens was observed for sera pre-incubated with cat dander extract (Fig. 1, panel a, lanes c), whereas no inhibition was found when the sera were pre-incubated with an immunologically unrelated allergen, the recombinant major birch pollen allergen rBet v 1 (Fig. 1, panels a and b, lanes d).

A rabbit anti-rFel d 1 antiserum reacts with an acidic 20 kDa allergen in dog dander extracts from different dog breeds

In order to further characterize the Fel d 1 cross-reactive allergen in dog dander, a monospecific polyclonal rabbit anti-rFel d 1 antiserum was used. The rabbit anti-rFel d 1 antiserum reacted with a 20 kDa allergen present in a commercial SQ dog dander extract as well as with a 20 kDa allergen present in dander extracts from Kuvasz, Maltese Terrier, long-haired German shepherd and Dalmatian-Collie half-breed dogs (Fig. 2, lanes c). The rabbit anti-rFel d 1 antiserum also reacted with Fel d 1 (20 kDa) in the SQ cat dander extract (lanes c), while buffer (lanes a) and the rabbit's pre-immune serum (lanes b) showed no reactivity with any of the tested extracts. For

Table 2. Quantitative inhibition of IgE reactivity to dog allergens with rFel d 1 in 36 cat allergic patients

Sera pre-incubated with → CAP → Patient	PBST Cat dander (e1) kUA/L	PBST Dog dander (e5) kUA/L	rFel d 1 Dog dander (e5) kUA/L	% Inhibition	PBST House dust mite (d1) kUA/L	rFel d 1 House dust mite (d1) kUA/L	% Inhibition
1	> 100	31.5	29.2	7	5.65	5.51	2
2	42.6	7.10	0.97	86	< 0.35	n.d.	–
3	79.9	17.0	12.1	29	< 0.35	n.d.	–
4	> 100	13.8	2.32	83	< 0.35	n.d.	–
5	45.5	16.3	11.7	28	< 0.35	n.d.	–
6	43.2	32.0	25.4	21	> 100	n.d.	–
7	34.0	9.63	6.44	33	12.2	n.d.	–
8	33.5	7.59	3.99	47	< 0.35	n.d.	–
9	32.6	6.55	4.11	37	< 0.35	n.d.	–
10	20.2	2.72	2.43	11	99.5	n.d.	–
11	19.0	4.31	2.72	37	16.7	n.d.	5
12	18.9	2.99	2.46	18	56.2	n.d.	–
13	3.92	6.31	5.35	15	0.36	n.d.	–
14	14.3	3.17	0.43	86	21	20.5	2
15	42.0	3.78	1.03	73	1.59	1.61	– 1
16	17.5	2.48	0.94	62	12.1	11.8	2
17	15.2	2.72	1.07	61	> 100	n.d.	–
18	71.2	12.1	12.0	1	68.1	66.0	3
19	65.9	6.53	1.39	79	< 0.35	n.d.	–
20	11.7	4.79	3.58	25	> 100	> 100	0
21	4.03	1.17	0.68	42	> 100	> 100	0
22	3.42	1.78	0.67	62	36.0	33.1	8
23	2.89	1.19	0.63	47	< 0.35	n.d.	–
24	2.30	0.85	0.45	47	31.1	33.3	– 7
25	2.11	3.21	3.07	4	28.3	30.2	– 7
26	1.72	1.11	0.64	42	62.9	64.1	– 2
27	1.63	1.67	1.17	30	47.3	50.0	– 6
28	0.93	1.16	0.73	37	12.9	11.7	9
29	> 100	21.6	14.4	33	1.83	1.73	5
30	44.0	19.3	17.5	9	< 0.35	n.d.	–
31	35.0	5.69	4.02	29	0.76	0.75	1
32	14.4	4.96	3.23	35	> 100	n.d.	–
33	4.72	1.89	1.39	26	96.7	n.d.	–
34	4.20	1.60	0.73	54	> 100	n.d.	–
35	18.3	74.5	58.8	21	0.88	n.d.	–
36	2.67	1.24	0.80	35	42.8	38.9	9
Mean (<i>n</i> = 36)	29.3	9.3		41.0	34.9		1.5

Cat allergen-specific IgE levels in kUA/L are displayed for each serum and as mean. Dog and house dust mite allergen-specific IgE reactivities are shown for both the sera pre-adsorbed with rFel d 1 (rFel d 1) and untreated (PBST). The percentage of inhibition of IgE reactivity to dog dander extract achieved by pre-incubation of sera with rFel d 1 are displayed. Inhibitions of more than 50% are indicated by grey boxes.

PBST, phosphate-buffered saline with 0.5% v/v Tween 20.

the other four dog dander extracts, the rabbit's pre-immune serum did not react with the cross-reactive 20 kDa allergen but exhibited reactivity to several other components of approximately 60, 50, 40, 30 and 10 kDa (Fig. 2, lanes b). Binding of rabbit anti-rFel d 1 antibodies to rFel d 1 in cat dander extract and to the 20 kDa protein in SQ dog dander extract could be inhibited by pre-incubation of the rabbit antiserum with rFel d 1 (Fig. 2, lanes d). Pre-incubation of the rabbit anti-Fel d 1 antiserum with the immunologically unrelated allergen rBet

v 1 did not affect IgG binding to nitrocellulose-bound cat or dog proteins (Fig. 2, lanes e).

In the 2-D-separated dog dander extracts from Kuvasz, a 20 kDa antigen with an isoelectric point (pI) of 3.4 was specifically detected by the rabbit anti-rFel d 1 antiserum (Fig. 3: I). Similar to 1-D immunoblotting experiments, we observed that the pre-immune serum reacted with low- and high-molecular-weight moieties other than the 20 kDa cross-reactive allergen (Fig. 3: P).

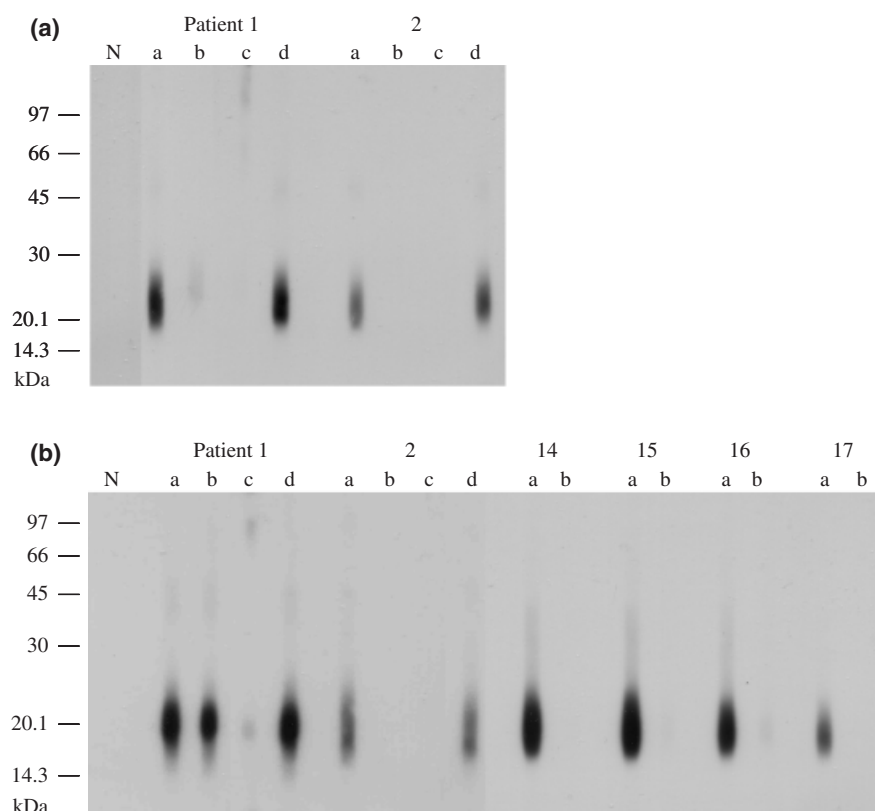


Fig. 1. Inhibition of IgE binding to nitrocellulose-blotted cat and dog allergens with rFel d 1. In a, sera from cat-allergic patients (patients 1 and 2) were pre-incubated with buffer (lanes a), rFel d 1 (lanes b), cat dander extract (lanes c) or rBet v 1 (lanes d) and then exposed to nitrocellulose-blotted cat dander extract. In b, sera from cat-allergic patients (patients 1, 2 and 14–17) were pre-incubated with buffer (lanes a), rFel d 1 (lanes b), dog dander extract (lanes c) or rBet v 1 (lanes d) and then exposed to nitrocellulose-blotted dog dander extract. Lanes N show serum from a non-allergic individual. Bound IgE antibodies were detected with ^{125}I -labelled anti-IgE and visualized by autoradiography. Molecular weights (kDa) are shown on the left margin.

Discussion

In this study, we provide evidence for the presence of an allergen in dog dander extracts that cross-reacts with the major allergen of cat, Fel d 1. Using defined molecular tools (i.e. purified recombinant Fel d 1, monospecific, polyclonal rabbit anti-rFel d 1 antibodies), we demonstrate that standardized commercial dog dander extracts as well as house-made extracts from several different dog breeds contained an Fel d 1-like allergen. This evidence for the presence of an Fel d 1-like cross-reactive dog allergen comes from several different experimental approaches. First, we demonstrate by quantitative IgE CAP inhibition experiments performed with sera from 36 Fel d 1-reactive cat-allergic individuals that pre-incubation of such sera with purified recombinant Fel d 1 can markedly inhibit IgE binding to dog dander allergens. For 9 out of 36 Fel d 1-reactive cat-allergic patients (25%), a more than 50% inhibition of IgE-reactivity to dog dander extract was achieved. This finding demonstrates that the Fel d 1-cross-reactive moiety in dog dander extracts has cross-reactive as well as species-specific (genuine cat-

derived) IgE epitopes or shows different affinity for the cross-reactive IgE. It also speaks against the possibility that the dog dander extract was contaminated with cat-derived Fel d 1, because in this case a similar inhibition of IgE reactivity would have been observed for each of the Fel d 1-reactive sera. The latter assumption is also supported by the finding that the degree of inhibition of IgE-reactivity to dog dander extract was not associated with the levels of Fel d 1-specific IgE antibodies in the sera. For example, patient 18 exhibited high titres of cat dander-specific IgE and reacted only with Fel d 1 but did not show cross-reactivity with the Fel d 1-like moiety in dog dander extract. In addition, we did not obtain inhibition of IgE reactivity to nitrocellulose-blotted dog dander extract in a serum reactive with Fel d 1, whereas IgE reactivity to blotted Fel d 1 was completely inhibited (patient 1; Fig. 1).

By qualitative techniques, we were able to further characterize the Fel d 1-like moiety in dog dander extracts. Using sera with cross-reactivity to the Fel d 1-like moiety for IgE immunoblot inhibition studies, we could demonstrate that pre-incubation of these sera with

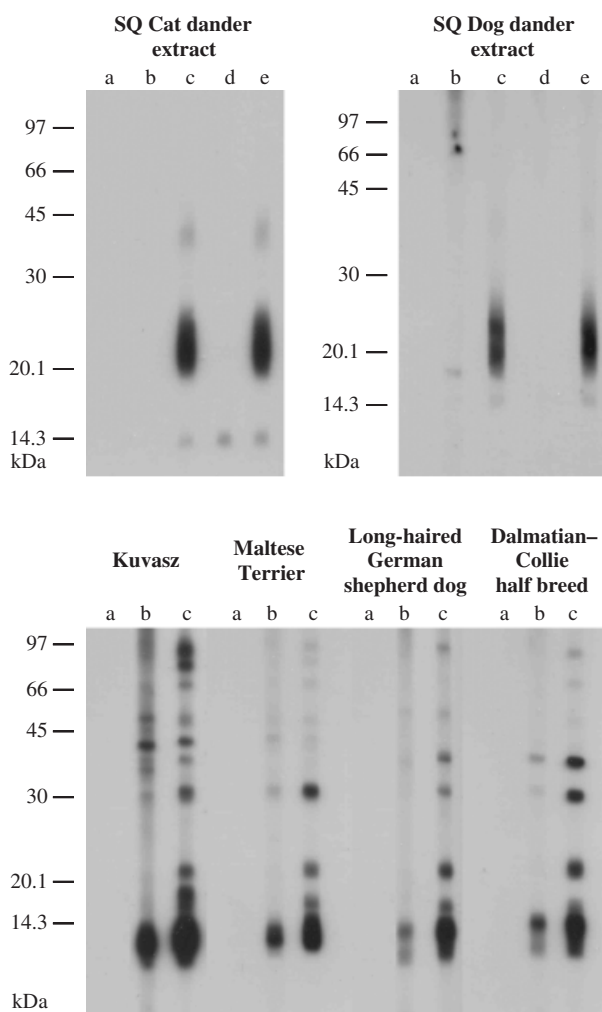


Fig. 2. Rabbit anti-rFel d 1 antibodies cross-react with an allergen in dog dander extracts. SQ cat and dog dander extracts and extracts from four different dog breeds were probed with rabbit anti-rFel d 1 antibodies (lanes c), the pre-immune serum (lanes b) or with buffer alone (lanes a). Rabbit anti-Fel d 1 antibodies were pre-incubated with rFel d 1 (lanes d) or rBet v 1 (lanes e) and then exposed to nitrocellulose-blotted SQ allergen extracts. Molecular weights (kDa) are indicated on the left margin.

rFel d 1 inhibited IgE reactivity to a 20 kDa component in dog dander extract.

In another approach, rFel d 1 was used to induce Fel d 1-specific polyclonal rabbit antibodies. These antibodies detected the Fel d 1-like protein in a commercially available dog dander extract as well as in dog dander extracts prepared from four different dog breeds. The recognition of different bands in SQ and in-house-made extracts (10 kDa moieties) may be rather due to different extraction procedures used for the preparation of the extracts than to unspecific reactivity of the antiserum. In fact, the specificity of the rabbit anti-Fel d 1 antibodies could be demonstrated in an immunoblot inhibition experiment showing that purified rFel d 1 inhibited its binding to nitrocellulose-blotted nFel d 1. The immunogen (rFel d 1) had inhibited IgE reactivity to the Fel d 1-like dog allergen, which is in accordance with the reactivity of the antiserum with this protein. This result does not necessarily contradict previous experiments carried out with an Fel d 1-specific mouse monoclonal antibody [33–34] in which no cross-reactivity was detected, because of the limited epitope specificity of a monoclonal antibody vs. the polyclonal nature of the antiserum made by us.

Besides the data from the quantitative IgE inhibition experiments, also other experiments carried out in this study speak against the possibility that the cross-reactivity observed in our experiments was caused by the contamination of dog allergen extracts with cat-derived Fel d 1. In fact, we were able to detect an Fel d 1-like antigen in extracts prepared from four individual dog breeds of which neither dog was exposed to cats.

We thus can conclude that dog dander extracts may contain an Fel d 1-like allergen with a molecular weight of 20 kDa that does not dissociate completely under reducing conditions and has an isoelectric point of 3.4. It represents a target for cross-reactive IgE antibodies present in the sera of 25% of animal-allergic patients. Our results thus

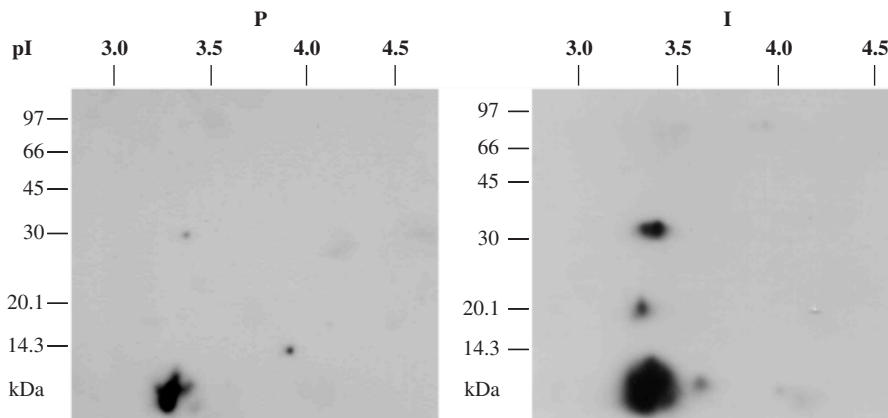


Fig. 3. Reactivity of a rabbit anti-rFel d 1 antiserum with 2D-separated dog allergens. Dog dander allergens (Kuvasz) that were 2D-separated under reducing conditions were probed with a rabbit anti-rFel d 1 antiserum (I) or the corresponding pre-immune serum (P). Molecular weights are shown on the left margins and the pI values on the top.

provide evidence that dog and cat dander, besides Can f 3/ Fel d 2 (albumin) [10–13] and perhaps Fel d 4 (lipocalin) [35], contain other cross-reactive antigens. The clinical importance of this cross-reactivity is not yet clear because none of the patients displaying IgE cross-reactivity to the Fel d 1-like dog allergens in our study presented clinical symptoms to dogs. Nevertheless, it will be possible to use rFel d 1 in IgE competition experiments to discriminate patients who exhibit cross-reactivity to cat and dog dander mediated exclusively by the Fel d 1-like dog allergen from patients who are co-sensitized to both allergen sources. We also believe that our observation that more than 20% of cat/dog allergic patients mount IgE antibodies directed against an Fel d 1 cross-reactive dog allergen may have implications for diagnosis and therapy of type I allergy to dogs.

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