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Evidence for a *Fel d* I-like molecule in the "big cats" (*Felidae* species)

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In this study, we investigated the cross-reactivity pattern of IgE and IgG4 antibodies to the major feline allergen, Fel d I. We studied the IgE and IgG4 response of 11 cat-allergic patients against Fel d I-like structures in eight members of the Felidae family: ocelot, puma, serval, siberian tiger, lion, jaguar, snow leopard, and caracal. Hair from these "big cats" was collected, extracted, and used in a RAST system and histamine-release test. By means of a RAST-inhibition assay with affinity-purified Fel d I from cat dander, it was established that, in the Felidae species, a Fel d I equivalent is present that reacts with IgE and IgG4 antibodies. We found that all patients had cross-reacting IgE antibodies to seven of the Felidae tested; no IgE antibodies reactive with the caracal were found. Eight of 10 patients with IgG4 antibodies directed to cat dander also had IgG4 antibodies directed to several Felidae species, including the caracal. However, the correlation between the IgE and the IgG4 antibody specificity was low, indicating that, in the case of Fel d I IgE and IgG4, antibodies do not necessarily have the same specificity. (J ALLERGY CLIN IMMUNOL 1990;86:107-16.)

Fel d I, a component in cat-dander extracts, was found to be responsible for the induction of the major part of IgE antibodies in cat-allergic patients.¹⁻⁴ This finding makes the cat model very convenient for the

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Abbreviations used

<i>Fel d</i> I:	<i>Felis domesticus</i> I (cat 1)
BSA:	Bovine serum albumin
PBS:	Phosphate-buffered saline
PBS-AT:	PBS containing BSA and Tween 20
sp _{var} :	Solid phase containing a variable amount of <i>Fel d</i> I from the <i>Felidae</i> extracts
sp _{eq} :	Solid phase containing an equivalent amount of <i>Fel d</i> I from the <i>Felidae</i> extracts

study of the human immune response directed to inhalant allergens.

In the present study, we investigated the reactivity of IgE and IgG4 antibodies of cat-allergic patients to variants of the cat allergen, obtained from phylogenetically related species. For this purpose, we collected hair from various *Felidae* species and investi-

TABLE I. *Familia Felidae* (cat family)

	Common name	Species name
Ocelot lineage	Ocelot	<i>Leopardus pardalus</i>
Domestic cat lineage	Cat	<i>Felis domesticus/catus</i>
Pantherine lineage		
Subgroup A	Puma	<i>Puma concolor</i>
	Serval	<i>Leptailurus serval</i>
Subgroup B	Siberian tiger	<i>Panthera tigris longipilis</i>
	Lion	<i>Panthera leo</i>
	Jaguar	<i>Panthera onca</i>
	Snow leopard	<i>Panthera uncia</i>
Subgroup C	Caracal	<i>Caracal caracal</i>

Phylogenetic position of the *Felidae* species tested.⁹

gated the antibodies of 11 cat-allergic patients to eight members of the cat family.

We used the technique of RAST and RAST inhibition to investigate whether a *Fel d* I-like substance is conserved throughout the *Felidae* species; these results might have implications for cat-allergic patients. Reviewing the literature, we found only a few publications concerning these exotic animal allergies, among others, a deer hunter,⁵ an elephant keeper,⁶ and an allergy for lions.⁷

Furthermore, we tested whether the IgG4 specificity of cat-allergic patients matched the observed IgE specificity; this information might indicate how close the IgE and IgG4 antibody responses are related.⁸

MATERIAL AND METHODS

Allergens

Cat-dander extract was obtained from ALK (Copenhagen, Denmark) (Diephuis Laboratory, Groningen, The Netherlands). *Fel d* I was affinity-purified with monoclonal antibody CLB-Fd-1a, as previously described.⁴ Hairs from the *Felidae* species were obtained from our local zoo, Natura Artis Magistra, Amsterdam, The Netherlands. The hairs were collected by brushing the animals at the time they were losing the winter fur. The hairs were extracted overnight at 5% (wt/vol) with a buffer containing 0.037 mol/L of NaH₂PO₄, 0.036 mol/L of Na₂HPO₄, 0.13 mol/L of NaCl, and 0.5% (wt/vol) of phenol at pH 6.8. After filtration, the extracts were either insolubilized by coupling them to solid phase to perform RAST tests or used in RAST-inhibition assays. The *Felidae* species from which we collected the hairs were grouped on the basis of the alleged phylogenetic relationships, as described by Collier and O'Brien⁹ (Table I).

Subjects

Blood samples for RAST analysis were obtained from 11 patients (average age, 31.6 years; range 18 to 61 years) with a positive history of cat allergy and a strong positive intracutaneous reaction with cat-dander extract. Eight of 11 patients did not respond to cat serum in a RAST. The three

patients with a positive RAST result with cat serum were not used in the RAST-inhibition assays or the histamine-release test. As control groups, we used sera from five mite-allergic patients with high IgE antibody titers directed to *Dermatophagoides pteronyssinus* (>20% bound anti-IgE with a total IgE ranging from 186 to 353 IU/ml of serum) and strongly positive skin reactions to the mite extract, five nonallergic individuals, and four employees of the zoo who worked intensively with the *Felidae* species. None of these control sera contained IgE antibodies directed to cat dander, *Fel d* I, or cat serum; the case history regarding cat allergy was also negative for these control groups.

Antibodies

We used two types of monoclonal antibodies directed to *Fel d* I (CLB-Fd-1a and CLB-Fd-1b) and two polyclonal antisera (rabbit 73246 and 73248). These antibodies were previously described.^{4, 10} The polyclonal antisera reacted weakly with cat-serum components (3.8% bound radioactivity when antisera were tested on cat-serum Sepharose).

Histamine release

The histamine release test was performed on two cat-allergic patients according to the procedure as described by Lichtenstein and Osler.¹¹ Histamine content in the incubated samples was measured with an automated fluorometric method.^{12, 13}

RAST

CNBr-activated Sepharose was obtained from Pharmacia Fine Chemicals (Uppsala, Sweden). Radioimmunoassay buffer (PBS-AT) contained PBS (0.01 mol/L of phosphate and 0.14 mol/L of NaCl), 0.3 mg/ml of BSA (Poviet, Amsterdam, The Netherlands), 10 mmol/L of ethylenediaminetetraacetic acid, 5 mmol/L of NaN₃, and 0.1% (vol/vol) of Tween 20 (Baker, Deventer, The Netherlands) at pH 7.4. We prepared four different batches of allergosorbents; for coupling to 100 mg of activated Sepharose, we used the following quantities of allergens: cat-dander Sepharose, 1,000,000 ALK SQ units of cat dander, containing approximately 52 units of *Fel d* I, and *Fel d* I Sepharose, 1.8 units of affinity-purified *Fel d* I.

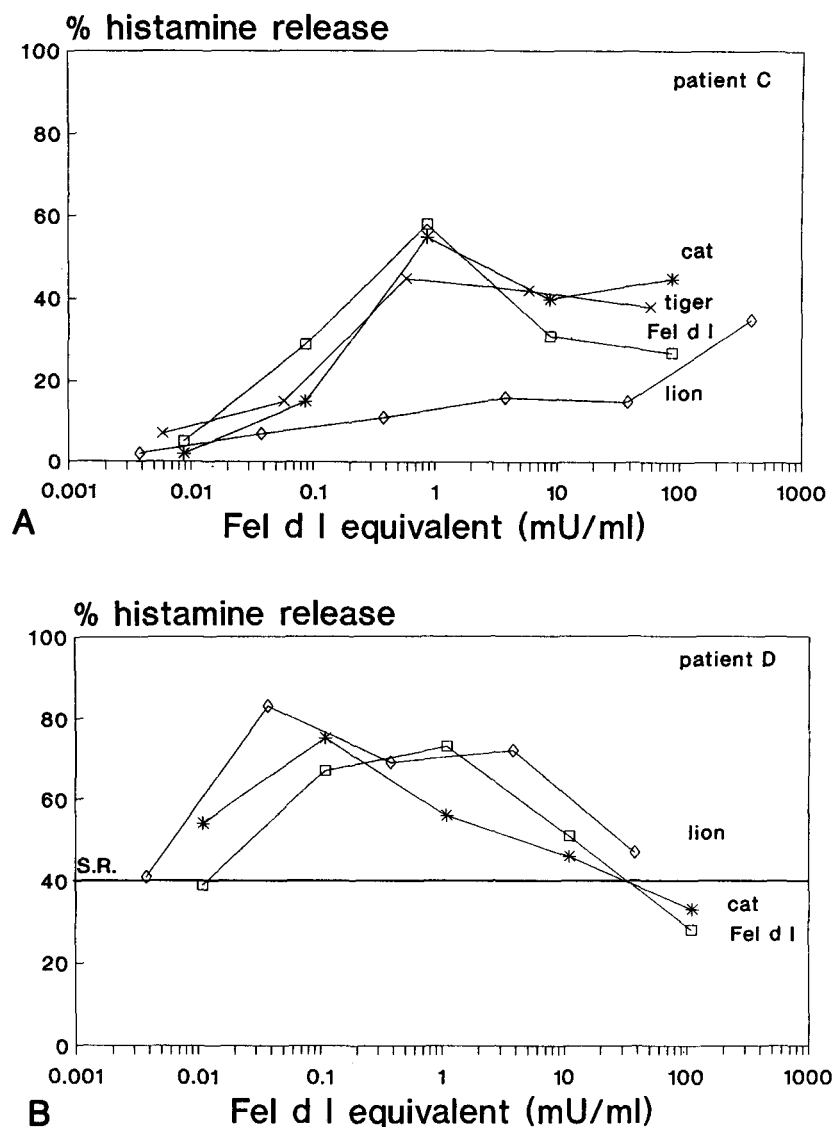


FIG. 1. Percent histamine release from basophil leukocytes in two cat-allergic patients **A**, Patient C. **B**, Patient D. The concentration of cat-dander extract is expressed as the dilution of an ALK cat-dander extract. The concentration of the purified *Fel d I* was adjusted on the basis of the *Fel d I* content of the cat-dander extract. The concentrations of the lion and tiger extracts are expressed as *Fel d I* equivalent milliunits per milliliter. Results are illustrated as percentage histamine released. S.R., spontaneous release.

Coupling efficiency of both cat dander and *Fel d I* was >90%. Two protocols for the preparation of *Felidae* Sepharose were used: (1) A constant volume of 100 μ l of *Felidae* extract for all eight animals; these allersorbents are coded "cat sp_{var}," and (2) the equivalent of 1.8 units of *Fel d I* from the extracts of siberian tiger, lion, serval, and snow leopard, as determined with the cross-reactive monoclonal antibody as described below. These allersorbents are coded "cat sp_{eq}."

IgE RAST

The RAST experiments were performed with 250 μ l of the allersorbent cat dander, *Fel d I* and cat sp_{var} (in a

concentration of 2 mg/ml) and 50 μ l of serum. After incubation overnight and washing with saline buffer, ¹²⁵I-labeled sheep anti-IgE was added, and another incubation overnight followed. The results are expressed as percentage-bound labeled anti-IgE after correction for nonspecific binding.¹⁴

IgG4 RAST

For the detection of IgG4 antibodies directed to the *Felidae* species, we used a modified RAST procedure; 250 μ l of the allersorbents cat dander, *Fel d I*, and cat sp_{var} were incubated with 20 μ l of serum. After washing and incubation with ¹²⁵I-labeled monoclonal anti-IgG4, the percentage of

TABLE II. Total IgE (international units per milliliter) and specific IgE and IgG4 response (percent bound radioactivity) of 11 cat-allergic patients directed to the eight members of the *Felidae* species tested (cat sp_{var})

	Patient										
	A	B	C	D	E	F	G	H	I	J	K
IgE											
<i>Fel d I</i>	69.8	45.0	62.9	48.9	20.6	51.8	6.3	28.6	17.8	12.8	0.1
Cat dander	72.7	43.4	63.3	39.4	40.7	56.6	4.2	47.4	23.1	45.9	3.7
Cat serum	0.9	0.2	1.4	1.4	1.4	29.0	0.1	49.6	1.7	66.9	0.1
Ocelot	34.5	9.2	20.9	16.5	10.5	26.4	0.0	12.3	0.2	34.1	0.2
Puma	17.2	0.8	22.1	5.3	7.3	26.0	0.1	5.9	0.8	6.8	1.2
Serval	31.8	12.9	15.7	20.1	12.2	19.8	1.1	3.9	2.3	5.8	0.1
Siberian tiger	35.7	16.3	37.6	23.1	4.6	31.8	1.3	18.9	2.7	19.7	0.3
Lion	37.4	13.3	43.4	22.2	7.8	34.5	1.0	13.9	2.5	14.2	0.3
Jaguar	15.0	0.2	14.3	6.9	4.0	17.4	0.3	9.9	0.2	9.9	0.0
Snow leopard	22.2	8.0	10.3	5.3	5.4	17.0	0.8	3.9	2.0	7.8	0.5
Caracal	1.0	0.0	0.9	2.0	-0.5	0.0	0.0	0.0	0.0	0.4	-0.4
Total IgE	3500	170	10000	3000	82	730	421	1000	78	306	25
IgG4											
<i>Fel d I</i>	5.4	7.2	26.6	7.5	11.2	27.3	-0.7	23.7	3.0	8.7	2.5
Cat dander	20.1	11.0	27.4	12.5	18.1	29.8	1.2	25.9	14.2	23.2	15.9
Cat serum	1.7	0.8	2.2	1.6	0.1	1.5	0.2	12.3	3.3	12.4	0.2
Ocelot	2.3	0.7	16.9	6.4	1.7	17.1	0.0	17.8	4.3	7.7	2.4
Puma	1.5	0.4	11.9	8.3	1.1	4.2	0.5	11.3	2.8	7.7	3.5
Serval	1.1	1.5	11.0	5.6	1.0	10.2	0.2	18.1	3.9	4.9	1.9
Siberian tiger	1.4	1.0	19.5	7.8	2.7	14.9	0.4	12.6	3.1	7.0	3.3
Lion	1.7	1.0	21.3	16.0	2.0	13.1	0.5	17.9	7.2	7.3	6.6
Jaguar	1.0	-0.3	5.6	6.8	-0.4	1.2	-0.3	6.3	0.1	3.2	2.2
Snow leopard	1.6	1.4	10.4	4.9	1.9	14.5	0.4	7.3	3.0	10.6	2.8
Caracal	1.0	0.3	2.4	4.4	0.9	1.3	-0.2	2.6	3.2	3.0	1.3

bound radioactivity was measured on the third day; >2% binding (after blank correction) was regarded as a positive result.¹⁵

Monoclonal antibodies

Allergen Sepharose was incubated overnight with 50 µl of 1:1000 diluted monoclonal antiserum, washed, and incubated with ¹²⁵I-labeled goat antimouse IgG, and percentage-bound radioactivity was measured the next day.¹⁶

Rabbit antisera

Allergen Sepharose was incubated overnight with 50 µl of 1:300 diluted polyclonal antiserum, washed, and incubated with ¹²⁵I-labeled sheep antirabbit IgG, and percentage-bound radioactivity was measured the next day.

Fel d I equivalent determination

We used affinity-purified *Fel d I*, coupled to CNBr-activated Sepharose and cross-reactive monoclonal antibody CLB-Fd-1b as the reference system for the *Fel d I* equivalent determination in the *Felidae* species. Two hundred fifty microliters of several twofold dilutions of the *Fel d I* Sepharose

were incubated overnight with 50 µl of a 1:1000 dilution of monoclonal antibody CLB-Fd-1b; after incubation and washing, ¹²⁵I-labeled goat antimouse antibodies were added. Binding of radioactivity thus achieved was compared with the results obtained with dilutions of *Felidae* Sepharose. The *Fel d I* concentration was calculated as *Fel d I* equivalent units per gram of hair extracted. One unit (Food and Drug Administration reference) is approximately 4.2 µg of *Fel d I*.

Inhibition of the IgE and IgG4 RAST

One hundred microliters of appropriately diluted serum was preincubated for 2 hours with an equal volume of inhibitor. The extracts used for inhibition were cat-dander extract (concentration 100,000 ALK SQ units per milliliter, i.e., 5.2 Food and Drug Administration units of *Fel d I*), affinity-purified *Fel d I* (0.25 U/ml), siberian tiger extract (5% wt/vol), and lion extract (5% wt/vol), respectively. Of these mixtures, 100 µl for the IgE RAST and 40 µl for the IgG4 RAST, respectively, were incubated overnight with 250 µl of allergen Sepharose cat dander, *Fel d I*, and cat sp_{eq}, after which normal RAST procedures followed.

As a control, the same inhibition study was performed

TABLE III. *Fel d I* equivalent concentrations of four *Felidae* species with purified *Fel d I* and cross-reactive monoclonal antibody CLB-Fd-1b as reference system

Allergen source	Units <i>Fel d I</i> /gr hair extracted
Siberian tiger	690.0
Lion	46.0
Snow leopard	36.0
Serval	5.6

with an irrelevant system. For this purpose, the *D. pteronyssinus* RAST of two mite-allergic patients was tested with the same concentrations of the *Felidae* extracts as inhibitors.

RESULTS

Histamine release

The results of the histamine release tests with the leukocytes of patients C and D are illustrated in Fig. 1, A and B, respectively. Despite the high spontaneous release of the leukocytes of patient D, it is clear that in both patients hair extracts of the lion and/or the siberian tiger are capable of inducing histamine release, comparable to the release induced by purified *Fel d I* or whole cat-dander extract.

IgE RAST on *Felidae* Sepharose cat sp_{var}

All cat-allergic patients had IgE antibodies directed to seven of the *Felidae* species tested; no IgE antibodies were found directed to the caracal (Table II). In none of the sera from the control groups or from the zoo employees were IgE antibodies to the *Felidae* extracts found.

IgG4 RAST on *Felidae* Sepharose cat sp_{var}

The IgG4 RAST was positive in eight of 11 cat-allergic patients for all *Felidae* hair extracts, including the caracal (Table II). In the control sera tested, we found no IgG4 antibodies directed to the *Felidae*. In one zoo employee we found a strong positive IgG4 RAST with several *Felidae* (see Fig. 3, C).

Monoclonal antibodies

The response of the two monoclonal antibodies to four members of the *Felidae* species (allergosorbents cat sp_{var}) is illustrated in Fig. 2. CLB-Fd-1a was not cross-reactive with the *Felidae*, whereas CLB-Fd-1b reacted with several cats.

Polyclonal antisera

Both rabbit 73246 and 73248 reacted with seven members of the *Felidae* species. No response (<2%

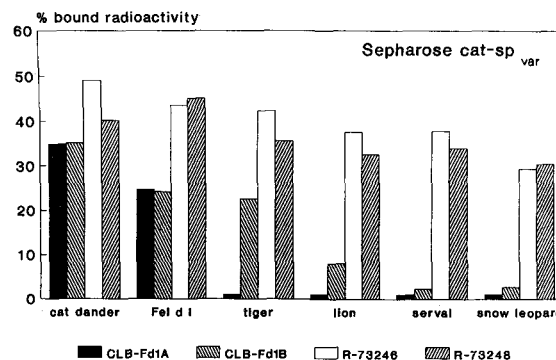


FIG. 2. Response of two monoclonal antibodies, CLB-Fd-1a and CLB-Fd-1b (1:1000 diluted in PBS-AT), and two rabbit antisera (R-73246 and R-73248, 1:300 diluted in PBS-AT) to the *Felidae* species. On the axis, the allergen Sepharose cat sp_{var}, on the ordinate, the percentage bound radioactivity (goat antimouse and sheep antirabbit, respectively).

bound radioactivity) was found toward the caracal. The response to siberian tiger, lion, serval, and snow leopard (allergosorbents cat sp_{var}) is illustrated in Fig. 2.

Fel d I equivalent determination and RAST with equivalent *Fel d I* quantities

The *Fel d I* equivalent concentrations (units of *Fel d I* per gram of hair extracted), calculated with affinity-purified *Fel d I*, coupled to Sepharose and cross-reactive monoclonal antibody CLB-Fd-1b as reference system, are summarized in Table III. Allergosorbents were prepared with equivalent *Fel d I* amounts, 1.8 equivalent units to 100 mg of activated Sepharose; these allergosorbents are coded "cat sp_{eq}." The response of the cross-reactive monoclonal antibody CLB-Fd-1b was as expected in the case of tiger and lion allergosorbent cat sp_{eq}, that is, similar to the response to *Fel d I* Sepharose (Fig. 3, A). The response toward the leopard and serval allergosorbent cat sp_{eq}, however, was less than expected.

Five sera from the above-mentioned cat-allergic patients were diluted in 6% BSA in such a way that the binding in the RAST for cat dander was the same for all five sera (i.e., for IgE, approximately 30% bound radioactivity; for IgG4, approximately 10% bound radioactivity). RAST results with the *Felidae* Sepharoses cat sp_{eq} are summarized in Fig. 3, B (IgE response) and Fig. 3, C (IgG4 response).

That the IgE response directed to the *Felidae* species (siberian tiger, lion, serval, and snow leopard) is related to the IgG4 response to these same extracts is illustrated in Fig. 4. However, marked individual vari-

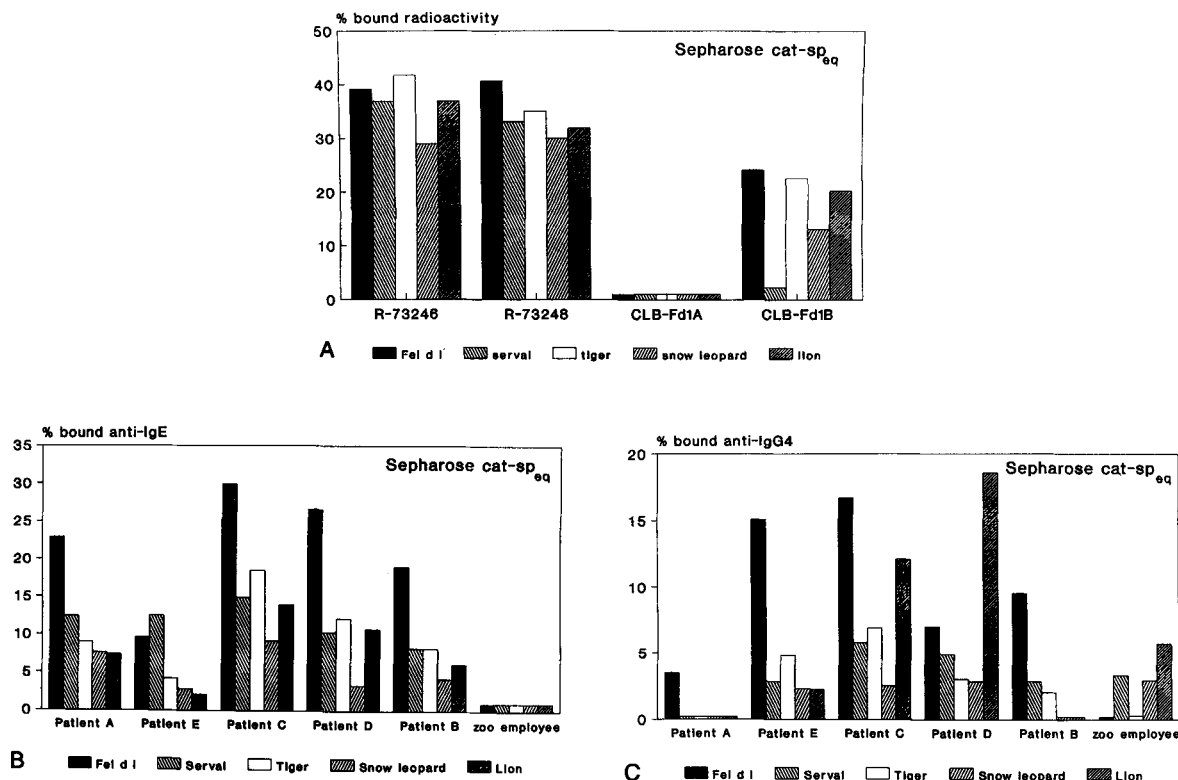


FIG. 3. **A**, Response of two monoclonal antibodies, CLB-Fd-1a and CLB-Fd-1b (1:1000 diluted in PBS-AT), and two rabbit antisera (R-73246 and R-73248, 1:300 diluted in PBS-AT) to the *Felidae* species. On the *axis*, the allergen Sepharose cat sp_{eq} , on the *ordinate*, the percentage bound radioactivity (goat antimouse and sheep antirabbit, respectively). **B**, *Felidae* RAST with the allergen Sepharose cat sp_{eq} of serval, siberian tiger, snow leopard, and lion extract, performed on five cat-allergic patients and a zoo employee. Results are illustrated as percentage bound anti-IgE. **C**, *Felidae* IgG4 RAST with the allergen Sepharose cat sp_{eq} with serval, siberian tiger, snow leopard, and lion extract, performed on five cat-allergic patients and a zoo employee. Results are illustrated as percentage bound anti-IgG4.

ation is found between the IgE and IgG4 response; notice, for example, the high IgG4/IgE ratio for lion in patient D (Fig. 4, C) and the low IgG4/IgE ratio for serval in patient E (Fig. 4, D).

RAST inhibition

The results of the RAST-inhibition assay for three patients are illustrated in Fig. 5, A, B, and D. These data are expressed as percentage inhibition of the *Felidae* RAST (allergosorbents cat sp_{eq}) with cat-dander extract, purified *Fel d I*, siberian tiger extract, and lion extract. In RAST-inhibition assay performed with two other cat-allergic patients, a comparable pattern was found. These results indicate that purified *Fel d I* was a strong inhibitor of the tiger RAST (range, 53% to 80%; average, 70% inhibition) and a moderate inhibitor of the lion RAST (range, 12% to 52%; average, 37% inhibition) for the patients tested. Furthermore, a distinct inhibition of the *Fel d I* RAST by the siberian tiger extract (range, 30% to 57%; average,

39% inhibition) was found, whereas lion extract appeared to be a weak inhibitor of the *Fel d I* RAST (range, 7% to 37%; average, 20% inhibition).

In the irrelevant *D. pteronyssinus* system, no inhibition by the *Felidae* extracts was found with the two mite-allergic patients; <4% inhibition was found with cat-dander extract, *Fel d I*, lion, or tiger extract.

IgG4 RAST inhibition

In two patients with IgG4 antibodies directed to the siberian tiger, we found a strong inhibition of the IgG4 RAST by affinity-purified *Fel d I* (73% and 81%), as can be observed in Fig. 5, C and E. No inhibition (<1%) was found in two patients with IgG4 antibodies directed to lion with purified *Fel d I* as inhibitor. The inhibition of the *Fel d I* IgG4 RAST in four patients with the siberian tiger extract was in the range of 28% to 45% (average, 36% inhibition). Lion extract was a weaker inhibitor of the *Fel d I* IgG4 RAST (range, 11% to 24%; average, 16% inhibition).

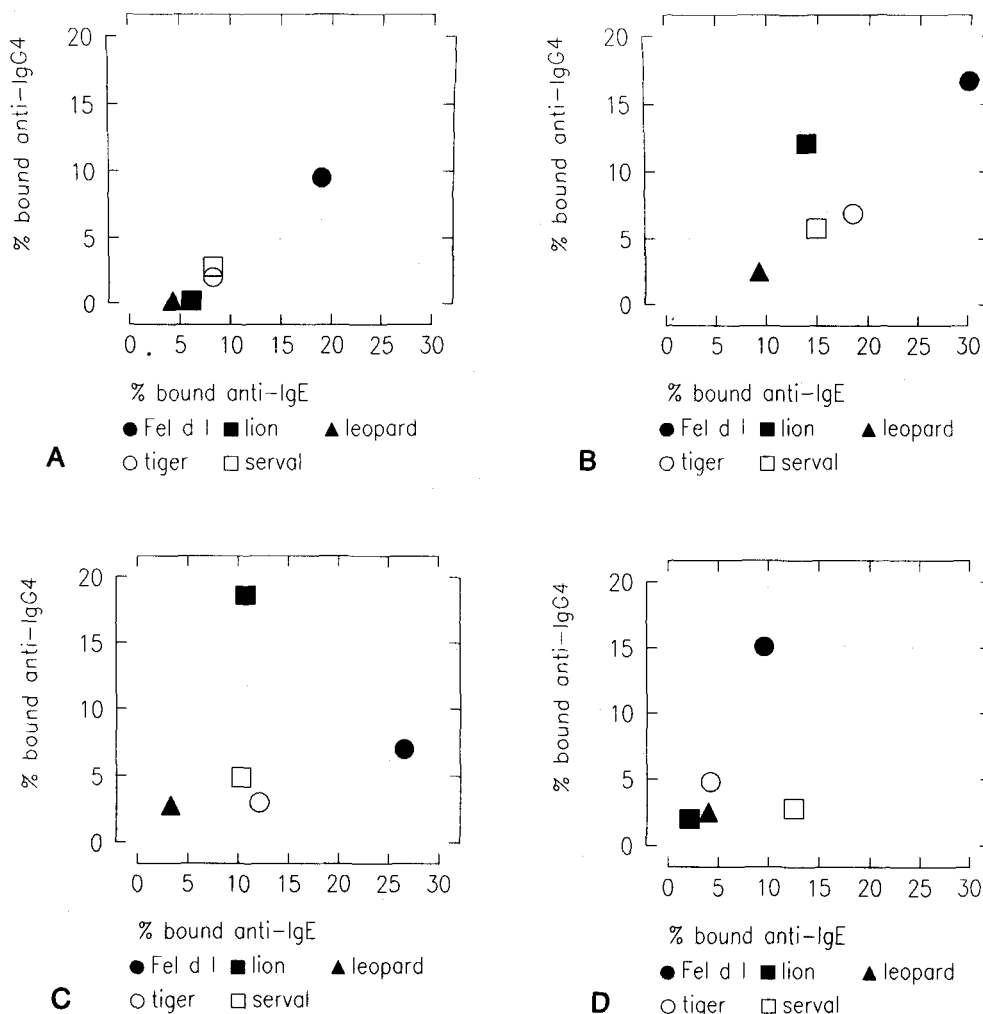


FIG. 4. Relation between the IgE response and the IgG4 response of four cat-allergic patients. On the *axis*, the IgE response (percentage bound anti-IgE), on the *ordinate*, the IgG4 response (percentage bound anti-IgG4), tested with allergen Sepharose cat sp_{eq}. **A**, Patient B. **B**, Patient C. **C**, Patient D. **D**, Patient E.

DISCUSSION

Evidence for a *Fel d I*-like structure in the *Felidae* species

In the cat-dander system, a single protein ("major allergen"), *Fel d I*, dominates the IgE response in cat-allergic patients.⁴ This fact is the reason why the cat-dander system may serve as a good model system for the study of the human IgE response. We collected hair from several *Felidae* species. We used the extracts as allergens in the histamine-release test and coupled the extracts to a solid phase to study whether a *Fel d I*-like structure was present. By means of the histamine-release test, we found clear evidence of biologic activity of a cross-reacting *Felidae* extract in the two cat-allergic patients tested. The second patient (D) avoided visits to a zoo or circus show, but because

of a marked nonspecific hyperreactivity, it was unclear whether complaints were really related to allergy. Patient C did not recall any visit to these rare animals in the past.

With the RAST, we found that IgE as well as IgG4 antibodies of 11 cat-allergic patients reacted to a variable degree with hair extracts from other *Felidae* species, ocelot, puma, serval, siberian tiger, lion, jaguar, and snow leopard. Neither the rabbit antibodies nor the IgE antibodies reacted with the caracal; some reactivity of IgG4 antibodies with the caracal was noticeable in the sera of four patients, indicating that antigenic compounds were present in our caracal hair extract. Presumably, the concentration and/or the cross-reactivity is low.

To study the IgE and IgG4 antibody specificity of

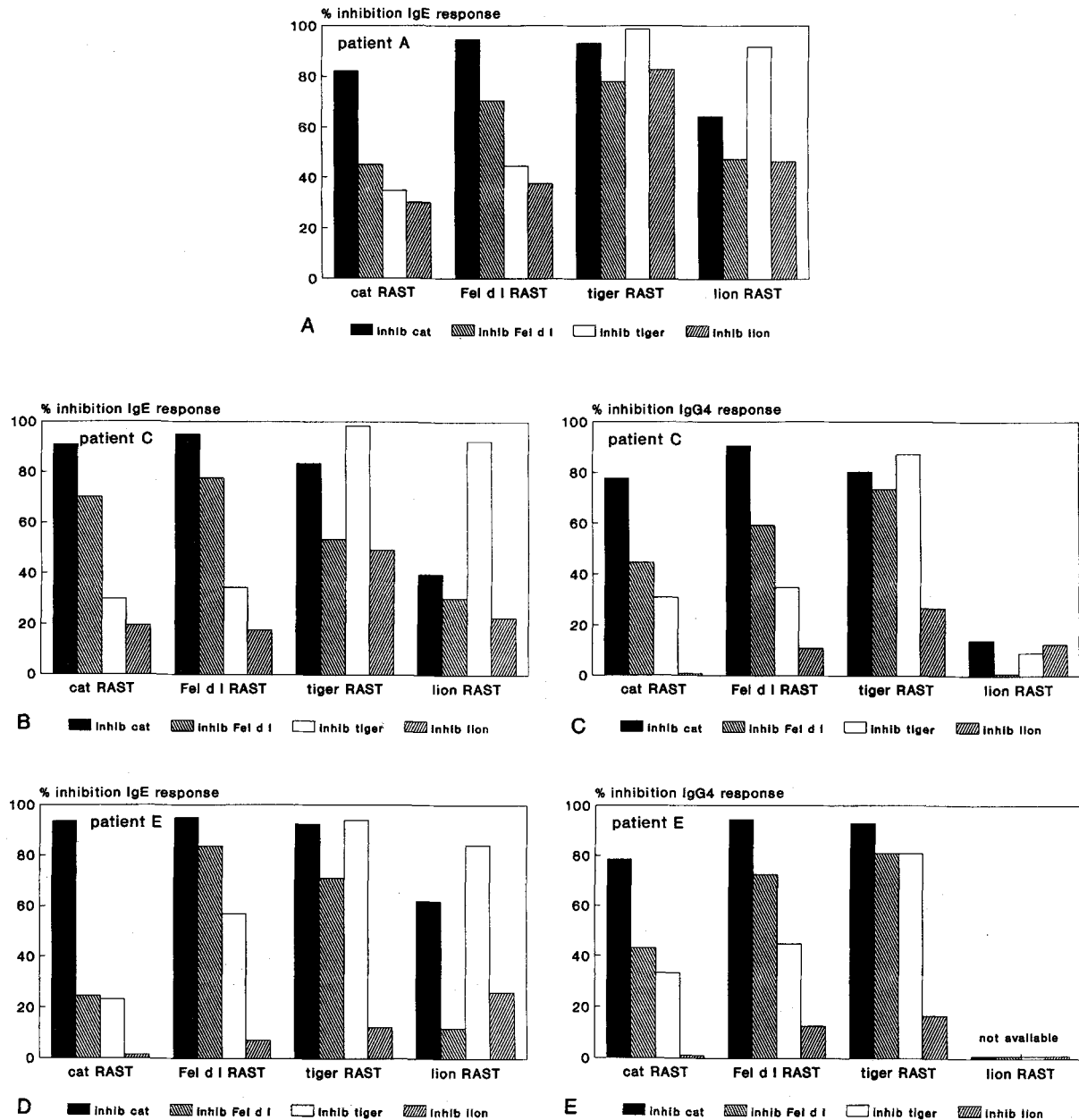


FIG. 5. A, IgE RAST-inhibition assay of cat-allergic patient A, with cat-dander extract, purified *Fel d I*, siberian tiger extract, and lion extract, respectively, as inhibitors. Data are expressed as percentage inhibition of the cat-dander RAST, *Fel d I* RAST, siberian tiger RAST, and lion RAST (cat sp_{eq}), respectively. B-C, IgE and IgG4 RAST-inhibition assay of cat-allergic patient C, with cat-dander extract, purified *Fel d I*, siberian tiger extract, and lion extract, respectively, as inhibitors. Data are expressed as percentage inhibition of the cat-dander RAST, *Fel d I* RAST, siberian tiger RAST, and lion RAST (cat sp_{eq}), respectively. D-E, IgE and IgG4 RAST-inhibition assay of cat-allergic patient E, with cat-dander extract, purified *Fel d I*, siberian tiger extract, and lion extract, respectively, as inhibitors. Data are expressed as percentage inhibition of the cat-dander RAST, *Fel d I* RAST, siberian tiger RAST, and lion RAST (cat sp_{eq}), respectively.

cat-allergic patients in more detail, it is useful to make the *Felidae* Sepharoses comparable with the cat-dander Sepharose and the affinity-purified *Fel d I* Sepharose. For this purpose, we have developed a "*Fel d I* equivalent determination" with cross-reactive

monoclonal CLB-Fd-1b and affinity-purified *Fel d I* Sepharose as reference system. After the above-described estimation of the *Fel d I* content of the *Felidae* extracts, we have coupled an equal amount of *Fel d I* from the cat-dander extract, the siberian

tiger extract, and lion extract to CNBr-activated Sepharose. This procedure might be biased by use of a single cross-reacting monoclonal antibody. The results might well have been different with polyclonal antibodies.

As indicated in Fig. 3, A, the response of the cross-reactive monoclonal antibody CLB-Fd-1b was approximately as expected, that is, a similar response in the case of cat dander, *Fel d I*, tiger, and lion; however, the response of the leopard allergosorbent was less than expected, whereas the response of the serval allergosorbent was very low. The equivalence determination obviously depends on the cross-reactivity of the antibody used. These data presented in Fig. 3 indicate that the cross-reactivity of the monoclonal antibody CLB-Fd-1b with serval is less than that of the polyclonal rabbit antisera (Fig. 3, A) or of the human IgE antibodies (Fig. 3, B).

Evidence for the existence of a *Fel d I*-like molecule in the *Felidae* species was provided by the RAST-inhibition assay. First, the *Fel d I* RAST was inhibited by extracts derived from the siberian tiger and, to a lesser extent, from the lion. Furthermore, it was demonstrated that the IgE antibodies directed to the siberian tiger and the lion were inhibited for >50% by preincubation of patient's serum with an excess of affinity-purified *Fel d I*. However, a marked interindividual variation of the inhibition by *Fel d I* was found, which indicated the presence of cross-reacting allergens other than *Fel d I*.

It is concluded that hair extracts obtained from "big cats" (*Familia Felidae*) do indeed contain a component similar to *Fel d I*. These *Fel d I* analogues cross-react both with IgE and IgG4 antibodies from cat-allergic patients, but to a variable degree.

The relation between the IgE and the IgG4 response

It is often assumed that the IgG4 response is linked to an IgE response. However, in our study we found an IgG4 response directed to several members of the *Felidae* species in a nonatopic zoo keeper who owned a domestic cat for more than 10 years. Furthermore, Aalberse et al.¹⁷ demonstrated the presence of IgG4 antibodies in nonatopic laboratory workers exposed to several animal allergens, providing evidence that chronicity of allergen exposure can evoke an IgG4 response without a concomitant IgE response.

The relation between the IgE and IgG4 response and its possible role in atopic individuals has been studied intensively in the past. Reviewing the literature, one may find specificity studies of IgE and IgG4 antibodies at different levels of antigenic complexity, total allergen extracts, individual components, and individual epitopes. Several studies have demon-

strated concomitant appearance of IgE and IgG4 antibodies directed to *total allergen extracts*, for example, to animal dander,¹⁸ honeybee venom,¹⁹ and the major allergen of grass pollen, *Lol p I*.²⁰ Evidence for a possible blocking role of IgG4 antibodies in a parasitic model was provided by Hussain and Ottesen²¹ who demonstrated that IgG4, directed against *single protein components*, demonstrated the same antigenic specificity as antiparasitic IgE. Stapel et al.²² investigated the specificities of IgE and IgG4 responses elicited by grass-pollen immunotherapy in a group of 11 pollen-allergic individuals, among others, by means of immunoblotting and found that IgG4 specificity did not completely match the observed IgE reactivity. This finding was confirmed by means of an allergen-binding assay, as described by Aalberse.²³

In this study, we investigated the relation of the IgE and the IgG4 response directed to one or a few *epitope(s)* of an allergen extract. We found no complete matching of the IgG4 and IgE specificity with respect to the response directed to the cross-reactive *Fel d I* equivalent of the *Felidae* species. We conclude that the IgG4 response directed to a member of the *Felidae* family can be predicted only to a limited extent by the IgE response to this animal. This means that, in the cat-dander system, IgG4 does not necessarily mimic IgE specificity.

To elucidate the possible protective role of IgG antibodies induced during immunotherapy with cat-dander extract, we are investigating the specificity of the IgE and IgG subclasses response directed to the *Felidae* species more extensively in a group of cat-allergic patients who received immunotherapy (manuscript in preparation).

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