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.)

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**Abbreviations used**

*Fel d* I: *Felis domesticus* I (cat 1)

BSA: Bovine serum albumin

PBS: Phosphate-buffered saline

PBS-AT: PBS containing BSA and Tween 20

sp*: Solid phase containing a variable amount of *Fel d* I from the *Felidae* extracts

sp**: Solid phase containing an equivalent amount of *Fel d* I from the *Felidae* extracts

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This finding makes the cat model very convenient for the 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-
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 1-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, an elephant keeper, and an allergy for lions.

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.

**MATERIAL AND METHODS**

**Allergens**

Cat-dander extract was obtained from ALK (Copenhagen, Denmark) (Diephuis Laboratory, Groningen, The Netherlands). Fel d 1 was affinity-purified with monoclonal antibody CLB-Fd-la, as previously described. 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'Brien (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 1, 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 1 (CLB-Fd-1a and CLB-Fd-1b) and two polyclonal antisera (rabbit 73246 and 73248). These antibodies were previously described. 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. Histamine content in the incubated samples was measured with an automated fluorometric method.

**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 Na₂SO₄, 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 1, and Fel d 1 Sepharose, 1.8 units of affinity-purified Fel d 1.
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 μl of Felidae extract for all eight animals; these allergosorbents are coded “cat sp,” 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 allergosorbents are coded “cat spw.”

**IgE RAST**

The RAST experiments were performed with 250 μl of the allergosorbent cat dander, Fel d I and cat spw (in a concentration of 2 mg/ml) and 50 μl 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 μl of the allergosorbents cat dander, Fel d I, and cat spw were incubated with 20 μl 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*).

<table>
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<tr>
<th>Patient</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
<th>G</th>
<th>H</th>
<th>I</th>
<th>J</th>
<th>K</th>
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<td></td>
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<td></td>
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<td>51.8</td>
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<td>63.3</td>
<td>39.4</td>
<td>40.7</td>
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<td>1.4</td>
<td>1.4</td>
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<td>8.3</td>
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<tr>
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<td>1.5</td>
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<tr>
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<td>17.9</td>
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<td>Snow leopard</td>
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</table>

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.

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 dI equivalent determination

We used affinity-purified Fel dI, coupled to CNBr-activated Sepharose and cross-reactive monoclonal antibody CLB-Fd-1b as the reference system for the Fel dI equivalent determination in the Felidae species. Two hundred fifty microliters of several twofold dilutions of the Fel dI 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 dI concentration was calculated as Fel dI equivalent units per gram of hair extracted. One unit (Food and Drug Administration reference) is approximately 42 μg of Fel dI.

Inhibition of the IgE and IgG4 RAST

One hundred microliters of appropriately diluted serum were 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 d1), affinity-purified Fel d1 (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 d1, and cat sp*, after which normal RAST procedures followed.

As a control, the same inhibition study was performed with an irrelevant monoclonal RAST for the same cat dander extract.

RESULTS

The results of the leukocyte mediator release of L.4 and F are listed in Table III. In both patients, allergen-specific mediator release, calculated as the ratio of Fel dI, was significantly increased in the IgE RAST.

IgG4 RAST

All cat extracts evoked high levels of IgG4 antibodies in none of the patients. In the zoo extracts, the IgG4 RAST was not performed due to insufficient antigen availability.

Monoclonal antibodies

The results of the monoclonal antibody inhibition studies are presented in Table IV. In all serum samples, no significant inhibition was observed. In monoclonal antibody inhibition studies, the same dilutions were used as for the IgE and IgG4 RAST. The monoclonal antibodies were preincubated with an equal volume of inhibitory factor. The extracts used were cat-dander extract (concentration 100,000 ALK SQ units per milliliter, i.e., 5.2 Food and Drug Administration units of Fel d1), affinity-purified Fel d1 (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 d1, and cat sp*, after which normal RAST procedures followed.
TABLE III. *Fel d 1* equivalent concentrations of four *Felidae* species with purified *Fel d 1* and cross-reactive monoclonal antibody CLB-Fd-1b as reference system

<table>
<thead>
<tr>
<th>Allergen source</th>
<th>Units <em>Fel d 1</em>/gr hair extracted</th>
</tr>
</thead>
<tbody>
<tr>
<td>Siberian tiger</td>
<td>690.0</td>
</tr>
<tr>
<td>Lion</td>
<td>46.0</td>
</tr>
<tr>
<td>Snow leopard</td>
<td>36.0</td>
</tr>
<tr>
<td>Serval</td>
<td>5.6</td>
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</tbody>
</table>

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 1* or whole cat-dander extract.

IgE RAST on *Felidae* Sepharose cat sp

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

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

*Fel d 1* equivalent determination and RAST with equivalent *Fel d 1* quantities

The *Fel d 1* equivalent concentrations (units of *Fel d 1* per gram of hair extracted), calculated with affinity-purified *Fel d 1*, 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 1* amounts, 1.8 equivalent units to 100 mg of activated Sepharose; these allergosorbents are coded “cat sp.” The response of the cross-reactive monoclonal antibody CLB-Fd-1b was as expected in the case of tiger and lion allergosorbent cat sp, that is, similar to the response to *Fel d 1* Sepharose (Fig. 3, A). The response toward the leopard and serval allergosorbent cat sp, 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 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-
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 (allergosorbent cat sp₅₀) with cat-dander extract, purified Fel d 1, 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 1 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 1, 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).
**DISCUSSION**

**Evidence for a Fel d 1-like structure in the Felidae species**

In the cat-dander system, a single protein ("major allergen"), Fel d 1, dominates the IgE response in cat-allergic patients. 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 1-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 1, Siberian tiger extract, and lion extract, respectively, as inhibitors. Data are expressed as percentage inhibition of the cat-dander RAST, Fel d 1 RAST, Siberian tiger RAST, and lion RAST (cat sp9), respectively. B-C, IgE and IgG4 RAST-inhibition assay of cat-allergic patient C, with cat-dander extract, purified Fel d 1, Siberian tiger extract, and lion extract, respectively, as inhibitors. Data are expressed as percentage inhibition of the cat-dander RAST, Fel d 1 RAST, Siberian tiger RAST, and lion RAST (cat sp9), respectively. D-E, IgE and IgG4 RAST-inhibition assay of cat-allergic patient E, with cat-dander extract, purified Fel d 1, Siberian tiger extract, and lion extract, respectively, as inhibitors. Data are expressed as percentage inhibition of the cat-dander RAST, Fel d 1 RAST, Siberian tiger RAST, and lion RAST (cat sp9), 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 1 Sepharose. For this purpose, we have developed a "Fel d 1 equivalent determination" with cross-reactive monoclonal CLB-Fd-1b and affinity-purified Fel d 1 Sepharose as reference system. After the above-described estimation of the Fel d 1 content of the Felidae extracts, we have coupled an equal amount of Fel d 1 from the cat-dander extract, the siberian tiger extract, and the Felidae Sepharose. This procedure allows a more accurate assessment of the IgE responses in the RAST inhibition assay. As indicated in Fig. 3, IgE antibodies in the Felidae Sepharoses cross-react to a lesser extent, from cat Felid Fel d 1, indicating that Fel d 1 is not the only human allergen. Positive results were obtained with the affinity-purified Fel d 1 and the individual variant found, which is a common allergen among Felid Fel d 1 patients.

The relation between IgG4 and IgE RAST inhibition

It is often assumed that an IgE response is always accompanied by an IgG4 response. This is true for the Felidae species, but not for domestic cat Fel d 1. Alberse et al. found that IgG4 antibodies in the Felidae Sepharose cross-react with several animal allergens, but that these responses are not related to the IgE response with Fel d 1.

The relation between cat-dander RAST and Fel d 1 and its possible role in the allergic response is still under investigation.
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. 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 demonstrated 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 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 mimick 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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REFERENCES


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