

Short communication

IgE and IgG4 binding to the ocelot variant of the cat (*Felis domesticus*) major allergen *Fel d I*

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The cross-reactivity of human IgE and IgG4 antibodies of 22 cat-allergic patients with the ocelot variant of *Fel d I* was studied. Throughout the Felidae species, this allergen, found in cat saliva, dander, sebaceous glands, and lacrimal glands (1–3, 5) is conserved, as shown in radioallergosorbent test (RAST) inhibition assays with affinity-purified *Fel d I* and hair extracts from various members of the Felidae family (6). The Felidae species we used in these experiments originate from different lineages (4). Because of the phylogenetic distance among these species, they are useful tools to study the specificities of IgE and IgG4 responses. Other feline species, e.g., tiger and lion, were found to be so closely related as to be almost immunologically identical, and therefore less suited for this type of investigation.

Material and methods

Twenty-two cat-allergic patients with high levels of IgE and IgG4 antibodies against *Fel d I* were selected for this study. The panel included 13 women

and nine men (aged 17–56 years), most of whom had a positive history of cat allergy. The patients had never received immunotherapy, except for one person who had received cat immunotherapy more than 10 years before.

Hair from an ocelot was collected and extracted. Extracts were coupled to Sepharose, and IgE and IgG4 RAST experiments were performed. Sera were affinity-purified on *Fel d I*-Sepharose, to exclude antibody responses against other allergens such as feline albumin. The fraction of IgE and IgG4 antibodies against *Fel d I* cross-reactive with ocelot was calculated.

For isolation of *Fel d I*-specific antibodies against 5 ml of cat-allergic serum, 100 µl 0.5 M EDTA and 50 µl 10% Tween-20 were added, and the serum was incubated for 2 h with 10 mg Sepharose containing 1.2 U *Fel d I* (7). After washing of the Sepharose with PBS, *Fel d I*-specific antibodies were eluted with 1 ml 0.3 mg/ml BSA in 0.1 M glycine, pH 2.5. The eluate was neutralized immediately with 60 µl 1 M Tris, pH 9, and dialyzed against PBS.

Results and Discussion

In the ocelot RAST with 50 μ l affinity-purified antibody, binding percentages of ^{125} I-labeled anti-IgE and ^{125} I-labeled anti-IgG4 ranged from 5.0% to 45.9% and from 4.5% to 46.9%, respectively. From the IgE- and IgG4-binding curves on *Fel d* I-Sepharose and ocelot-Sepharose, the fractions of IgE and IgG4 antibodies against *Fel d* I reactive with ocelot (cross-reactivity) were calculated. The fractions of *Fel d* I-specific antibodies reactive with ocelot ranged from 0.25 to 0.91 and from 0.14 to 0.60 for IgE and IgG4, respectively. In Fig. 1, the fraction of *Fel d* I-specific IgE cross-reactive with ocelot is plotted against the fraction of cross-reactive IgG4. Cross-reactivity of IgE with *Fel d* I from ocelot was

significantly higher than cross-reactivity of IgG4 ($P < 0.001$; paired, two-tailed Student's *t*-test). Mean values of cross-reactivity were 0.66 and 0.37 for IgE and IgG4, respectively.

Our results show that IgE antibodies are more cross-reactive than IgG4 antibodies, at least in this particular model system. This is a demonstration of a difference in epitope-specificity between IgE and IgG4 antibodies.

Acknowledgments

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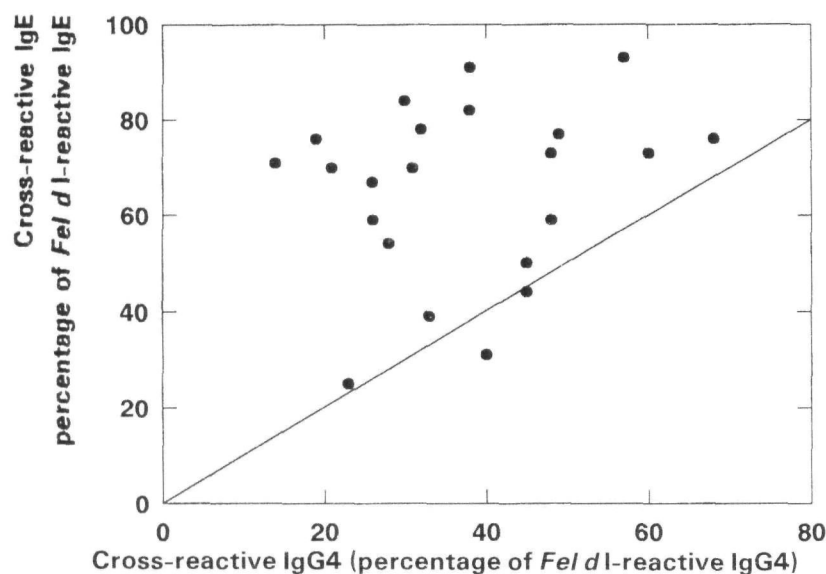


Fig. 1. Fractions of *Fel d* I-specific IgE and IgG4 antibodies cross-reactive with ocelot. Mean values of cross-reactivity were 0.66 and 0.37 for IgE and IgG4, respectively ($P < 0.001$; paired, two-tailed Student's *t*-test).

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