

High prevalence of sensitization to cat allergen among Japanese children with asthma, living without cats

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Summary

Background Cat allergy is common among children with asthma. Many cat-allergic patients in Japan and elsewhere do not keep cats, but nonetheless become sensitized through environmental exposure to cat allergen.

Objective To assess the frequency of cat allergy and cat-specific immunoglobulin E (IgE) and immunoglobulin G (IgG) antibody responses in young Japanese patients with asthma in relation to self-reported cat exposure and Fel d 1 levels in dust samples.

Methods Cat dander-specific IgE antibody was measured in sera from asthma patients using the CAP system. IgE and IgG antibody to Fel d 1 was measured by antigen binding radioimmunoassay and by chimeric enzyme immunoassay. Fel d 1 levels in dust samples from a subset of patients' homes were measured by monoclonal antibody-based enzyme immunoassay.

Results Cat-specific IgE (CAP class ≥ 2) was found in sera from 70% of 44 patients who kept cats and 34% of 394 patients who had never kept cats. The prevalence of sensitization increased progressively to age 6 years (40%: positive), and then increased gradually to age 16 years (approximately 60%: positive) in patients who had never kept cats. There was an excellent correlation between cat CAP values and IgE levels to Fel d 1. The absolute amount of IgE antibody to Fel d 1 ranged from 0.01 to 15.6% of total IgE. Most patients who did not keep cats were exposed to Fel d 1 levels ranging from 0.07–8 $\mu\text{g/g}$ dust.

Conclusions Sensitization to cat allergen is common among young asthmatic patients in Japan, even among patients who do not keep cats. Use of CAP and the chimeric enzyme-linked immunosorbent assay allows accurate diagnosis of cat allergy and quantification of specific IgE antibody levels.

Keywords: bronchial asthma, cat allergen, environmental allergen, IgE antibody

Clinical and Experimental Allergy, Vol. 29, pp. 754–761. Submitted 11 March 1998; revised 13 May 1998; accepted 23 June 1998.

Introduction

Cats are common pets in many countries, including Japan. Cat allergen is one of the most important indoor allergens, second only to dust mite as a cause of asthma among Japanese children. Exposure to cat allergen causes sensitization in 15 to 60% of atopic individuals and is responsible for acute asthma attacks, as well as prolonged bronchial hyperresponsiveness [1–4].

The development of monoclonal antibodies to cat allergen, Fel d 1, has facilitated studies on cat allergen exposure [5,6]. Fel d 1 is a 38-kDa glycoprotein homodimer composed of two 17-kDa subunits each with two peptide chains [7]. Although Fel d 1 is found in dander, hair and saliva, it is mainly produced in skin sebaceous glands [8–11]. Fel d 1 differs from the dust mite allergen Der p 1 in aerodynamic particle size and distribution [12,13]. There is a large amount of Fel d 1 in the dust from homes with cats (up to 3000 $\mu\text{g/g}$ dust) and several studies have reported that Fel d 1 is also found in homes without cats [3,4,14,15]. However, it is not known whether the Fel d 1 levels found

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in houses without cats are sufficient to cause allergic sensitization.

In this study, we analysed immunoglobulin (IgE) antibody responses in a large cohort of over 400 young Japanese patients with asthma who completed a questionnaire on environmental allergen exposure. A surprising finding was that 34% of patients were sensitized to cats, but did not keep them. Antibody responses were analysed according to age and IgE antibody to Fel d 1 was quantified by antigen binding radioimmunoassay (RIA) and a chimeric enzyme-linked immunosorbent assay (ELISA). The results show that IgE antibody responses to Fel d 1 can develop over the first few years of life, even among patients who do not keep cats in their homes.

Materials and methods

Subjects

Five hundred and four asthma patients out of a total number of 15 000 patients who visited the outpatient clinic at Department of Pediatrics, Doai Memorial Hospital, Tokyo, between 1 December 1993 and 31 May 1994, were enrolled in the study. Children and their parents who visited the clinic were invited to complete a questionnaire asking if they kept cats or dogs in their homes and whether there was a history of allergic symptoms associated with contact with these animals. Diagnosis of asthma was based on symptoms of repetitive wheezing and coughing and responsiveness to inhaled β -agonist. Some of the patients who visited the clinic were over 15 years old and had visited our clinic since childhood. Sera were assayed for IgE antibodies to cat dander, dog epithelium, *Dermatophagoides farinae* and pollen of *Cryptomeria japonica* (Japanese cedar) by quantitative fluoroimmunoassay using the CAP system (Pharmacia Diagnostics, Uppsala, Sweden) [16–18]. Selected sera were assayed for IgE antibody to mite ($n = 402$), Japanese cedar ($n = 419$), cat dander ($n = 438$) or dog epithelium ($n = 414$).

One hundred and 46 sera with a cat CAP class ≥ 1 were used to compare CAP with assays for specific IgE antibodies (Ab) to Fel d 1. Fifteen additional sera from patients with IgE to cat (CAP class ≥ 1) were also included in these assay, making a total of 161 sera in all. The 15 sera were from the patients who participated in a follow-up study of environmental exposure.

Environmental exposure to cat allergen

In a follow-up study, dust samples were randomly obtained from 50 homes of patients with asthma living in Tokyo, from November 1994 to August 1995. In 37 of these homes, cats had never been kept, but in 6/37 homes cats were kept

in the same building. In seven other homes, cats had been kept in the past and in six homes cats were kept during this study.

Dust was collected from each area (approximately 4 m^2) of the floor with a vacuum cleaner (HA-15, Panasonic, Osaka, Japan) for 5 min. The collected dust was sieved through a 0.3 mm mesh screen to obtain fine dust, then extracted in PBS-T (0.2% w/v) for 2 h at room temperature, with constant rotation, as described previously [19]. Supernatants were obtained after centrifugation (10 min at 2000 r.p.m.) and stored at -20°C prior to assay.

Fel d 1 was measured by two site ELISA [6] using mouse monoclonal antibodies specific for two different epitopes on Fel d 1 (monoclonal antibody [MoAb] 6F9 for capture and MoAb 3E4 for detection). The level of Fel d 1 was quantified in Fel d 1 U/mL using a reference (E3). The detection limit was $0.07 \mu\text{g}$ Fel d 1/g fine dust. One FDA unit Fel d 1 is approximately $4 \mu\text{g}$ Fel d 1 protein [6].

Purification of Fel d 1 and ^{125}I labelling

Fel d 1 was purified from house dust extract by immunoaffinity chromatography using the MoAb Fd1a coupled to Sepharose, followed by size exclusion chromatography (Superdex TM75 FPLC), as described previously [5,6]. Twenty-five micrograms of Fel d 1 were labelled with $0.5 \text{ mCi } ^{125}\text{I}$ (IMS 30, Amersham International, Amersham, UK) by the chloramine T technique (specific activity, $11.6 \mu\text{Ci}/\mu\text{g}$).

Measurement of total IgE

Total serum IgE antibody was measured with a two-site ELISA, modified from a previously described RIA [2]. Microtitre plates were coated with monoclonal anti-IgE (CIA/E/4.15, kindly provided by Dr Andrew Saxon, University of California, Los Angeles, CA, USA), samples were applied, and the bound IgE was detected with streptavidin-peroxidase and biotinylated affinity purified antihuman IgE (Kirkegaard and Perry Labs, Gaithersburg, MD, USA). The results were expressed as International Units IgE per millilitre using NIH human serum IgE standard (A-699-001-500; 900 IU/mL).

Antigen binding radioimmunoassays

Antigen binding RIA [20,21] were used to measure IgG and IgE ab to Fel d 1. Sera diluted 1:2 and 1:10 for IgE, and 1:12.5 and 1:50 for IgG, were incubated with ^{125}I -labelled Fel d 1 (approximately 100 000 c.p.m. added) for 4 h at room temperature. Fel d 1-specific antibodies were precipitated overnight at 4°C using monospecific sheep antihuman IgG or sheep antihuman IgE serum. The precipitates were washed three times in borate-buffered saline, pH 8.0, and

the residual radioactivity was counted in a γ -counter. Each assay was quantified by using doubling dilutions of a reference serum (from patient V.L.) to form an IgG control curve. Results for both IgG and IgE antibody were interpolated from the control curve and expressed as arbitrary units of binding activity/mL. When assaying high dilutions of sera, (>1:100), carrier immunoglobulins (either 0.1 mL of a 1:1000 dilution of normal human serum) were added to form precipitates. All the assays for IgE Ab required the addition of 0.1 mL of 1:200 IgE myeloma serum from patients P.S. (kindly provided by Dr Ishizaka).

'Chimeric' ELISA for *Fel d 1*-specific IgE

Microtitre plates were coated with 1 μ g/well of anti *Fel d 1* MoAb 6F9 (50% ammonium sulphate fraction of ascites), overnight at 4 °C. The plate was washed, blocked with 1% BSA-PBS-T, and incubated for 1 h with 0.1 mL 1:100 dilution of cat extract (Bayer, Spokane, WA, USA) as a source of *Fel d 1*. After washing, sera were added at 1:2 and 1:10 dilution and incubated for 2 h. Biotinylated goat anti-IgE (1:4000) was added to detect bound IgE and following incubation with streptavidin-peroxidase the reaction was developed with 1 mmol 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (Sigma, St Louis, MO, USA).

Quantification of IgE Ab to *Fel d 1* using a chimeric mouse/human IgE Ab to Der p 2

The ELISA for IgE antibody to *Fel d 1* was quantified using a chimeric mouse Fab/human Fc epsilon antibody. This antibody was engineered from the V_H domains and light chains of a murine monoclonal antibody to Der p 2 (clone 2B12) and the heavy chain domains of IgE. The development of this chimeric antibody (2B12-IgE) was recently described in detail [22–24]. Dilutions of 2B12-IgE from 0.07 to 140 ng/mL (2.4 ng/mL = 1 IU/mL) were used to form a control curve in an ELISA using anti-Der p 2 capture MoAb (α DpX) and *Dermatophagoides pteronyssinus* extract (10 000 AU/mL, Bayer Co., Spokane, WA, USA) as a source of Der p 2. The extract was diluted to 200 ng/mL Der p 2 for use in the ELISA. The bound chimeric 2B12-IgE was detected with biotinylated anti-IgE and streptavidin-peroxidase, as described above. For measuring IgE Ab to *Fel d 1*, ELISA plates were coated with anti-*Fel d 1* MoAb 6F9 followed by diluted cat allergen extract containing 20 mU/mL *Fel d 1*. Sera were added and bound IgE antibody was detected as above. Control curves using IgE antibody to *Fel d 1* in sera from cat-allergic patients were parallel to those obtained using 2B12-IgE antibody binding to MoAb presented Der p 2. Thus absolute values (ng or IU/mL) IgE antibody to *Fel d 1* were extrapolated directly from the 2B12-IgE standard curve using a preparation of 2B12-IgE

which contained 6.8 μ g/mL IgE compared with the NIH IgE reference (A-699-001-500).

Statistical analysis

Prevalence of IgE-specific sensitization to cats and dogs in the patients with pets was compared by the chi-square test. The Mann-Whitney test was used to assess differences between *Fel d 1* levels in each group. *P*-values <0.05 were considered to be significant. IgE antibodies and IgG antibodies to *Fel d 1* were compared by linear regression analysis.

Results

Study population

The ages of the 504 patients recruited for this study ranged from 8 months to 34 years (median 9 years 7 months). There were 323 males and 181 females. All patients were diagnosed with asthma. Some patients also had symptoms of atopic dermatitis (63%), allergic rhinitis (54%) or allergic conjunctivitis (21%). The prevalence of atopic dermatitis tended to be higher than the other conditions in children less than 6 years. The presence or absence of pets was assessed by direct questioning by the physician. Cats and dogs had been kept in 49 (10%) and 63 (13%) of 504 homes, respectively. The patients who had kept cats reported nasal, ocular or respiratory symptoms (16 to 29%), induced by contact with cats. The patients who had never kept cats also reported similar symptoms (6 to 10%).

Sensitization rate to cat dander in CAP system

The prevalence of IgE antibody to mite, Japanese cedar, cat and dog allergen among Japanese children and young adults with asthma was compared in relation to age. The prevalence of IgE antibody to *D. farinae* (CAP class ≥ 2) was the highest of the four allergens in each age group (Fig. 1). Over 60% of asthmatic children were sensitized to *D. farinae* by age 2 years and this figure progressively increased to more than 90% by age 10 years. Most of the patients (62%) had a CAP class of 4–6 by age 10 years, confirming the importance of mite allergens as a cause of asthma in Japan.

After mite, Japanese cedar pollen and cat allergen were the most important causes of sensitization, with the prevalence of sensitization to cat being slightly lower than that to cedar pollen (Fig. 1). The time course of sensitization to both these allergens was later than to dust mite. By age 6 years, approximately 40% of patients had IgE antibody to cat and this increased to 60% by age 16 years. Overall, 70% (31/44) of patients who kept cats, and 34% of those who did

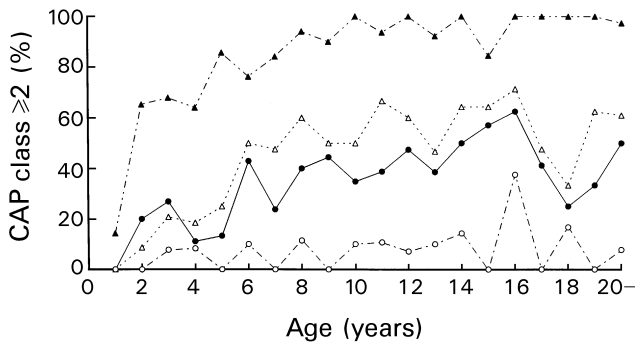


Fig. 1. Prevalence of immunoglobulin E (IgE) antibodies to inhaled allergens analysed according to age. Sera were analysed for IgE antibody to mite (*D. farinae*) ($n=402$) (▲), Japanese cedar ($n=419$) (Δ), cat ($n=394$) (●) and dog ($n=358$) (○) using the CAP system. The number of patients in each age group ranged from 6 to 36. Sera assayed for IgE antibody to cat or dog were obtained from patients living without cats or dogs, respectively.

not report keeping cats, had a CAP class ≥ 2 to cat dander. In contrast, the prevalence of sensitization to dog dander (as assessed by CAP) was generally only 5 to 10% and did not change significantly with age.

IgE and IgG antibodies to Fel d 1

To further analyse sensitization to cat allergen among this group of asthma patients, we compared IgE responses to cat in the CAP system with IgE responses to Fel d 1 measured by antigen binding RIA or a newly developed chimeric ELISA [22–24]. There was an excellent quantitative correlation between the antigen binding RIA and CAP (Fig. 2a; $r=0.88$, $P<0.001$). The CAP system appeared to be more sensitive than the RIA and 37% of patients had positive IgE antibody to cat by CAP, but were negative in RIA. The chimeric ELISA was more sensitive than the RIA and also showed an excellent correlation with CAP (Fig. 2b; $r=0.90$, $P<0.001$). Only 8/161 sera (5%) had a significant CAP score and showed undetectable IgE Ab to Fel d 1 by chimeric ELISA. Those results suggested that the chimeric ELISA for IgE Ab to Fel d 1 had comparable sensitivity to the IgE CAP system. The chimeric ELISA correlated with the antigen binding RIA for IgE antibody to Fel d 1, but was more sensitive (Fig. 2c).

We also compared IgE and IgG Ab responses to Fel d 1 by antigen binding RIA. The results showed a modest but statistically significant correlation (Fig. 2d; $r=0.59$, $P<0.001$). Some sera (approximately 13%) contained high levels of IgE anti-Fel d 1 (>5 U/mL) and had high IgG:IgE antibody ratios (from 10:1 to >100 :1). In one serum, IgE antibody to Fel d 1 could not be measured because the IgG antibody levels were so high (10 400 U/mL).

IgE Ab to Fel d 1 as a percentage of total IgE

The chimeric ELISA was quantified in absolute units of IgE antibody by reference to the 2B12-IgE chimeric anti Der p 2 control antibody. This made it possible to directly compare IgE antibody to Fel d 1 as a percentage of total IgE. The results showed that IgE anti-Fel d 1 could account for 0.01 to 15.6% of total IgE and that there was a significant inverse correlation between these values (Fig. 3).

Comparisons of the sensitivity of CAP, antigen binding RIA and chimeric ELISA are summarized on Table 1. The results show that Fel d 1 is an excellent marker of sensitization to cats and suggest that the chimeric ELISA is an excellent method for measuring specific IgE antibody to Fel d 1.

Fel d 1 levels in homes

To confirm exposure to cat allergen, Fel d 1 was measured in dust from patients' houses as part of a follow-up study (Fig. 4). The levels of Fel d 1 in the floor dust of six homes with cats ranged from 7–2450 $\mu\text{g/g}$ fine dust, whereas the levels in seven homes where cats had been kept in the past, but were no longer present, were significantly lower (0.7–8 $\mu\text{g/g}$, $P<0.001$). Thirty-seven homes without cats were divided into two groups based on the presence or absence of cats in the same building. When cats were kept in the same building, Fel d 1 was detected in 5/6 homes at levels up to 6 $\mu\text{g/g}$ fine dust. When there was no cat in the building, Fel d 1 was detected in 24/31 homes at levels up to 1.5 $\mu\text{g/g}$ fine dust. The Fel d 1 levels of the homes with cats in the same building were higher than those in the homes without cats ($P<0.05$).

Discussion

The prevalence of sensitization to cat dander in Japanese patients living without cats progressively increased as the patients grew older, up to the age of 16 years. These patients must have been sensitized by a small amount of cat allergen in their homes or in other places. There are no data regarding the age of sensitization to cats in asthma patients living without cats. In this study, we found that the prevalence of sensitization to cat reached 40% after the age of 6 years. In Japan, children enter a primary school at age 6 years and most of them attend a kindergarten or day nursery before primary school. Small amounts of Fel d 1 have been found in the dust of daycare centres in Europe [25,26]. Fel d 1 has also been detected in dust in school classrooms from various sources such as chairs, tables and floors [27–30]. These allergens are presumed to be present because of passive transport by individuals who have been in contact with cats, for example, through friends or relatives who kept cats in their homes. Custovic *et al.* [31] also showed that

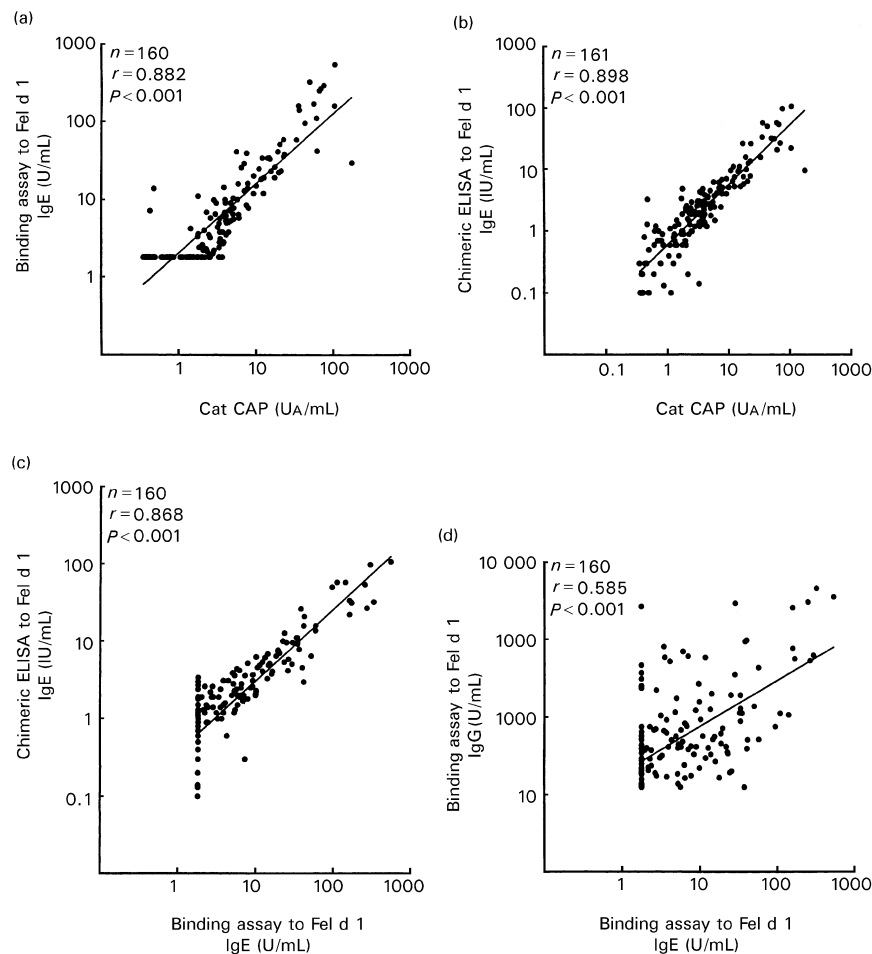


Fig. 2. Correlations between assays for IgE and IgG antibodies to cat allergen. IgE antibodies to cat allergen measured using the CAP system were compared by linear regression analysis with IgE antigen binding radioimmunoassay (RIA) for (a) Fel d 1 or (b) chimeric enzyme-linked immunosorbent assay (ELISA). Also compared were correlations between the (c) RIA and ELISA and (d) IgG and IgE antibody to Fel d 1 measured by RIA.

Fel d 1 levels could be significantly higher in public spaces than in private homes. Fel d 1 of levels of $>8 \mu\text{g/g}$ were found in 79% of the upholstered seats or furniture samples in public buildings or public transport in the UK.

Several previous studies have documented the presence of cat allergen in homes without cats [3,14,32]. The levels of Fel d 1 in homes without cats in our follow-up study (0.07–6 $\mu\text{g/g}$) are consistent with those reported previously. Most patients living with cats are exposed to more than 8 $\mu\text{g/g}$ Fel d 1 and this level has been proposed as a tentative threshold for exposure leading to sensitization. Our data suggest that approximately one-third of children become sensitized when exposed to Fel d 1 levels of 1–8 $\mu\text{g/g}$, which are common in homes without cats. A much lower incidence of sensitization to cat (13%) has recently been reported among Swedish children exposed to $<1 \mu\text{g/g}$ Fel d 1 in their homes [33]. Similarly, in the German Multicentre Atopy Study [34], cumulative rates of sensitization to Fel d 1 among children with a family history of atopy were 5 to 6% in the first 3 years of life if Fel d 1 exposure exceeded $>0.2 \mu\text{g/g}$ dust. Taken together with our data, these results provide some

evidence for a dose response between sensitization to Fel d 1 and levels of environmental exposure.

Some of the patients in Japan lived in apartments. Fel d 1 is known to persist for several months after the removal of the cats from apartments [35]. A significant proportion of Fel d 1 is also carried on small particles ($<5 \mu\text{m}$ diameter) in the air [12,36]. These results suggest that Fel d 1 in apartments without cats could be derived from previous cat occupancy or could have entered from adjacent apartments. It is also possible that visitors from homes with cats passively transferred the allergen.

In contrast to the results with cat, there was a low prevalence of sensitization to dog allergen in this group of Japanese patients with asthma. Overall, only 41/414 sera (10%) contained IgE antibody detectable by CAP (class 2–6). A recent European study reported a high level of cosensitization to both cat and dog allergens and suggested that these allergens shared IgE epitopes [37]. These results appear to represent concomitant sensitization to cats and dogs. In our study, more than half of the patients (54%) with IgE antibody to cats (CAP class 2–6) (as well as IgE

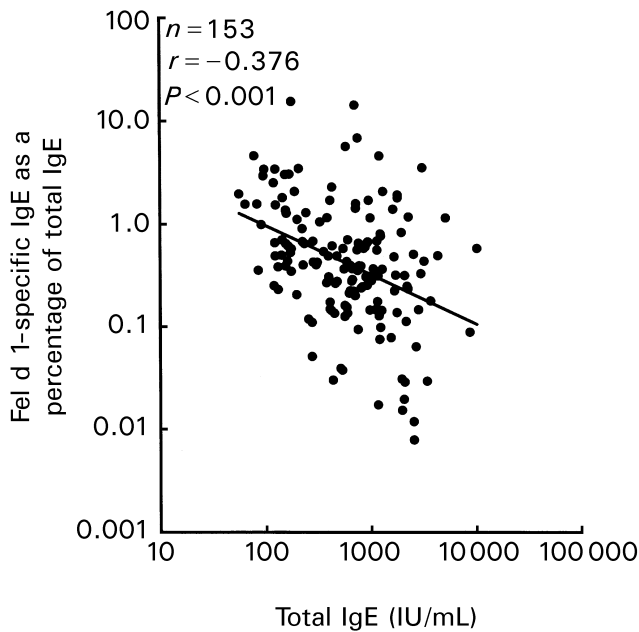


Fig. 3. Relationship between levels of specific IgE antibody to Fel d 1 and total IgE. IgE antibody to Fel d 1 was measured by chimeric enzyme-linked immunosorbent assay and the results expressed as a percentage of total IgE. There was a significant inverse correlation between the percentage of Fel d 1-specific IgE antibody and total IgE levels.

antibody to Fel d 1) had no detectable IgE Abs to dogs. Thus, we have been unable to confirm extensive cross-reactivity between cat and dog allergens among Japanese asthma patients.

We examined Fel d 1-specific antibody to investigate the role of Fel d 1 in the immunological response to cat allergen.

There was a good correlation between cat dander-specific IgE in cat-CAP and Fel d 1-specific IgE antibody. Furthermore, 95% of CAP cat positive sera (class ≥ 1) had Fel d 1-specific IgE detected by chimeric ELISA. Many of the methods used for assaying IgE antibodies to allergens are quantified in arbitrary units and do not provide specific levels of IgE antibody. Because the chimeric ELISA assay allows the absolute amount of anti-Fel d 1 IgE antibody to be calculated in international units, we could compare Fel d 1 by specific IgE with total IgE in the same unit system. Fel d 1-specific IgE ranged from 0.01 to 15.6% of total IgE. This is in keeping with a previous report that Der p 1-specific IgE could account for 0.1 to 27% of the total IgE [21]. The lower limit of allergen-specific IgE that we were able to detect suggests that the chimeric ELISA assay is more sensitive than RIA.

There was a significant quantitative correlation between IgG and IgE antibody to Fel d 1 and, as in previous studies using RIA, most sera from allergic patients contained IgG anti-Fel d 1 antibody, whereas those from nonallergic donors did not. This is in contrast to studies using ELISA, where IgG anti-Fel d 1 has been reported in sera from allergic and nonallergic individuals [38]. Certainly, the ratio of IgG : IgE antibody to Fel d 1 in some sera was often very high (> 100:1). We also found one serum in which IgE anti-Fel d 1 was not measurable because the IgG antibody level was too high. This result is in keeping with a recent report that high levels of IgG antibody (2–4 $\mu\text{g}/\text{mL}$) induced by natural allergen exposure can inhibit immediate skin test reactivity to Fel d 1 [39]. The high levels of IgG antibody also provide a further marker of cat allergen exposure among Japanese patients with asthma.

In conclusion, approximately one-third of Japanese asthmatic children who had never kept cats became sensitized to

Table 1. Comparison of IgE and IgG antibodies to cat allergen using different assays.

Assay	Antigen-binding RIA		Chimeric ELISA
	IgE Fel d 1	IgG Fel d 1	IgE Fel d 1
Detection limit	2 U/mL*	12.5 U/mL*	0.1 IU/mL†
<i>CAP positive sera (class ≥ 1)</i>			
<i>n</i>	160	161	161
Positive sera (%)	63	84	95
Range	0–330	0–10460	0–108
<i>CAP negative sera (class = 0)</i>			
<i>n</i>	50	50	50
Positive sera (%)	0	14	32
Range	<2	0–100	0–25

* Arbitrary unit using patient serum which has a high concentration of IgG ab to Fel d 1.

† International unit of IgE.

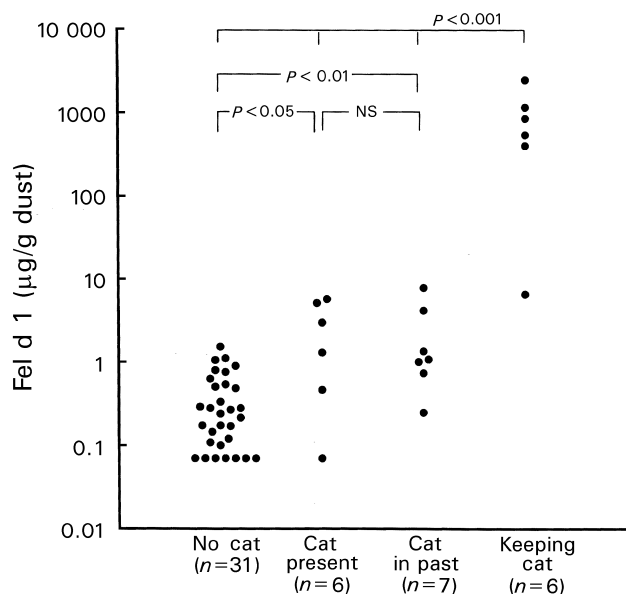


Fig. 4. Levels of Fel d 1 in dust samples collected from 50 homes

cat allergen in the early years of life. By age 6 years, approximately 40% of these patients, who were exposed to low levels of Fel d 1 in their homes, developed IgE antibody to Fel d 1. Fel d 1 was an excellent marker for cat sensitization, and use of CAP or the chimeric ELISA allowed accurate diagnosis of cat allergy.

Acknowledgements

We thank Dr Masahiro Sakaguchi for his technical advice on the measurement of Fel d 1 in Japanese homes in the early stages of this study. This work was supported in part by National Institutes of Health Grants AI 32557 and AI 34607.

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