

IgE antibody to cat allergens in an allergic population

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Clinical cat sensitivity and IgE antibody to cat allergens were studied in 200 consecutive patients who presented themselves for an allergy evaluation. Fifty-nine percent of the patients had no evidence of allergy to cats (group 1). Twenty-three percent of the patients had positive histories of allergy to cats and a positive prick test to cat epithelial extracts (group 2). The remaining 18% had either positive prick tests or positive histories alone (groups 3 and 4). The group of patients with strong evidence of allergy to cats (group 2) was on an average younger and had a higher incidence of asthma than did group 1 patients. Significant binding of IgE antibody to various insolubilized allergen preparations was defined as that binding which exceeded the 95% confidence interval for group 1 patients. Significant binding of IgE antibody to insolubilized cat allergen 1 was present in 65%, 15%, and 10% of groups 2, 3, and 4 patients, respectively. Significant binding of IgE antibody to insolubilized cat pelt extract was present in 28%, 11%, and 0% of group 2, 3, and 4 patients, respectively. Data for IgE antibody binding to insolubilized cat serum and cat albumin revealed no significant difference between the groups. It is concluded that cat allergen 1 is an important allergen in patients with strong evidence of allergy to cats.

The measurement of serum IgE antibody, it is claimed, represents a useful diagnostic tool to supplement or supplant more established procedures such as skin testing or bronchial challenge.¹⁻³ Previous studies have shown a correlation between IgE antibody levels and the results of skin testing and bronchial challenge in a number of different allergen systems.^{4, 5}

The measurement of IgE antibody as well as other diagnostic procedures in allergy require a reliable source of allergen.⁶⁻⁸ Most allergen preparations including those derived from animal danders have remained poorly standardized.^{9, 10} Recently, a single allergen (cat allergen 1) has been isolated from cat pelt extract. Cat allergen 1 is a major contributor to the allergenic activity of cat pelt extract as well as other cat epithelial extracts.^{11, 12}

In the present report, IgE antibody levels to cat allergen 1 and several other cat allergen preparations

were measured in a population of patients who presented themselves for an allergy evaluation. Patients with allergy to cats were defined by a careful history and skin testing with standardized extracts. The diagnostic usefulness of IgE antibody levels was thereby assessed.

METHODS

Patient population

Two hundred consecutive patients who presented themselves for an allergy evaluation were selected. The only requirement for inclusion into this study was that each patient receive a complete allergic history and be skin tested with allergenic extracts. Such a procedure is carried out in over 90% of the patients who present themselves in our offices. The history included a detailed environmental survey of factors which the patient felt to be related to symptoms of asthma, rhinoconjunctivitis, or urticaria. Asthma was defined as a history of episodic wheezing or shortness of breath. A few patients complained primarily of cough. These patients were given the diagnosis of "bronchitis" if further evidence of reversible airways obstruction could not be obtained. The skin testing included prick tests carried out as previously described¹² with the use of 1:20 w/v extracts of timothy and ragweed pollens and a 1:10 w/v extract of cat dander (Hollister-Stier Laboratories, Yeadon, Pa.). In addition, a cat pelt extract prepared in this laboratory containing 2.0 mg/ml total solid and 0.043 mg/ml cat allergen 1 was used.¹² A positive prick test was defined as that

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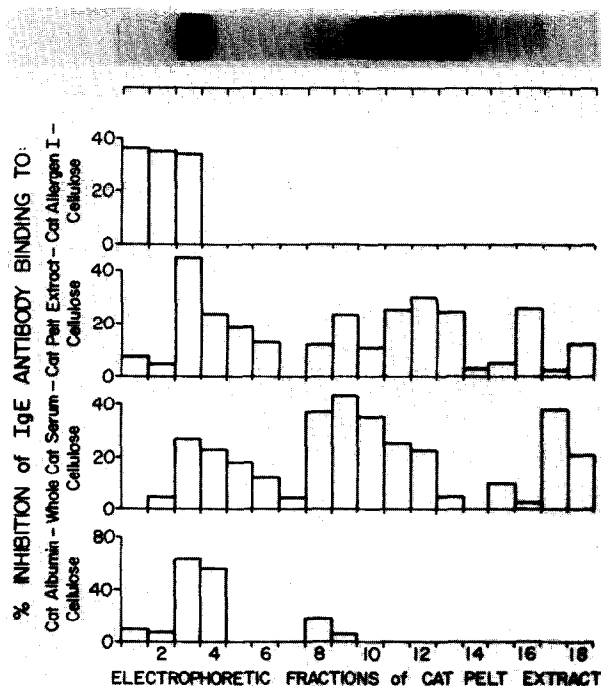


FIG. 1. Inhibition of binding of IgE antibodies in the positive control serum to insolubilized allergens. Soluble allergen in 18 fractions of crude cat pelt extract prepared by electrophoresis in acrylamide gel was used to inhibit binding of IgE antibody to each of the insolubilized allergen preparations.

TABLE I. Binding of IgE antibody in the positive control serum

Insolubilized allergen	Positive control serum	Normal human serum
Cat pelt extract-cellulose	1,449*	280
Cat allergen 1-cellulose	1,615	260
Cat serum-cellulose	1,195	213
Cat albumin-cellulose	1,695	319

*Serum, 50 μ l, was added to 0.5 mg of insolubilized allergen. The IgE antibody binding to the absorbents was then measured as indicated in the section on methods; numbers represent cpm.

producing a flare of greater than or equal to 10 mm in average diameter. Serum was obtained from each patient at the time of initial evaluation for measurement of IgE antibody.

Without knowledge of the IgE antibody levels, the patients were divided into 4 groups. These groups were defined as follows:

Group 1 included patients with no history of allergic symptoms on exposure to cats and negative prick test reactions to commercial cat dander extract and cat pelt extract.

Group 2 included patients with positive histories of allergic symptoms on exposure to cats and positive prick test reactions to one or both of the cat epithelial extracts. Also included in this group were patients who were not initially aware of cat allergy but who had significant exposure to cats

and had positive prick test reactions to one or both cat extracts. Most of these latter patients were constantly exposed to cats and experienced improvement in symptoms when the cat was removed from the environment.

Group 3 included patients with no history of allergic symptoms on exposure to cats but who had positive prick test reactions to one or both cat extracts. Included in this group were some patients who could not remember significant exposure to cats.

Group 4 included patients with positive histories of allergic symptoms on exposure to cats and negative prick test reactions to both cat extracts.

Allergen-containing preparations

Cat pelt extract was prepared from lyophilized defatted cat pelts.¹⁴ Cat allergen 1 was isolated from cat pelt extract as previously described.¹² Pooled cat serum was obtained from cats anesthetized with pentobarbital. Cat albumin was isolated from cat serum as previously described.¹¹

The radioallergosorbent test

The radioallergosorbent test was performed as previously described.¹¹ Cat pelt extract, cat allergen 1, or cat albumin (1 mg) was coupled to 100 mg of cyanogen bromide-activated cellulose particles. Cat serum, 25 μ l, was also coupled to 100 mg of activated cellulose. Patient serum, 50 μ l, was diluted with an equal volume of assay buffer and added to plastic test tubes containing 0.5 mg of cellulose linked to one of the above allergen preparations. After rotation of the tubes for 12 hr at room temperature, the cellulose particles were washed 3 times with borate-buffered (0.005 M) saline (0.15 M), pH 8.0 (BBS), and approximately 10 ng of ¹²⁵I-labeled anti- ϵ antibody with an activity of 50,000 to 100,000 counts per minute (cpm) were added to each tube. The tubes were again rotated at room temperature for 12 hr and the cellulose particles washed 3 times with BBS. The radioactivity bound to the cellulose particles was measured in a crystal scintillation spectrometer. All tests were carried out in duplicate. The variation in results between duplicate tests was less than 5%.

IgE antibody levels in patient sera were expressed as the percent of a positive control serum according to the following formula:

$$\text{IgE antibody level} = \frac{\text{cpm bound with patient serum}}{\text{cpm bound with positive control serum}} \times 100.$$

The positive control serum was obtained from a patient selected because of intense allergy to cats. This serum contained IgE antibody which bound to cat pelt extract, cat allergen 1, cat serum, and cat albumin (Table I) but not to an insolubilized ragweed fraction. The positive control serum was included each time the radioallergosorbent test was carried out.

In order to characterize the insolubilized allergens, an experiment was carried out in which soluble electrophoretic fractions of cat pelt extract were used to inhibit IgE antibody binding to each of the 4 insolubilized allergens. By the

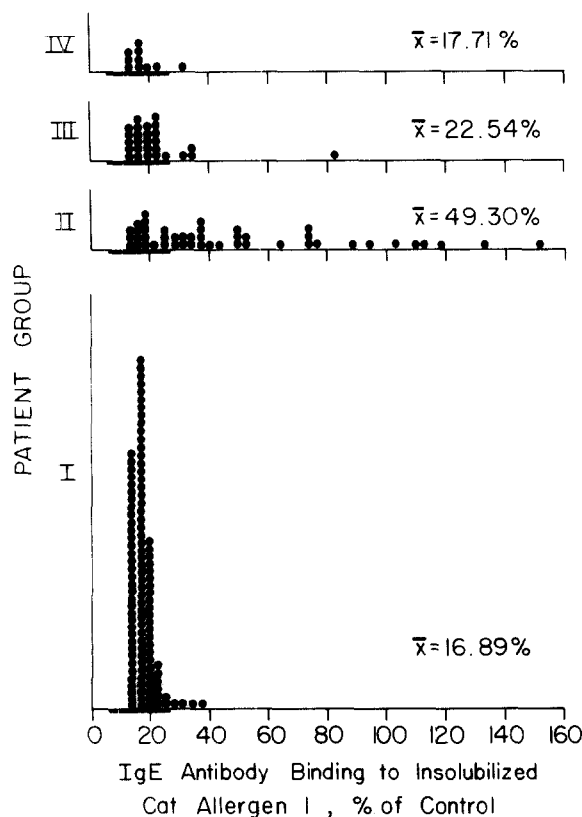


FIG. 2. Binding of IgE antibody to insolubilized cat allergen 1 in the 4 patient groups. The *darkened portion* of the horizontal axes represents the 95% confidence interval for group 1 patients.

use of such a technique it was possible to demonstrate differences in the allergen content of the insolubilized allergens.

Crude cat pelt extract (1.08 mg) was subjected to electrophoresis in 7% acrylamide gel as previously described.¹¹ One gel was sliced into 5-mm fractions and each fraction was eluted with 5 ml of phosphate-buffered (0.01 M) saline (0.15 M) (PBS). For purposes of visual identification, a second gel column that had been prepared under identical conditions was stained with Coomassie blue. Each fraction, 100 μ l, was added to 0.5 mg of the various insolubilized allergen preparations followed by the addition of the positive control serum. Soluble allergen present in the fractions inhibited binding of IgE antibody to insolubilized allergen. The resulting cpm bound were compared with a test in which soluble allergen had not been added. The percent inhibition of IgE antibody binding was calculated as previously described.¹²

RESULTS

Properties of insolubilized allergen: Inhibition of IgE binding by fractions of crude cat pelt extract

Fig. 1 shows the results of an inhibition experiment in which fractions of crude cat pelt extract obtained by electrophoresis in polyacrylamide gel were used to

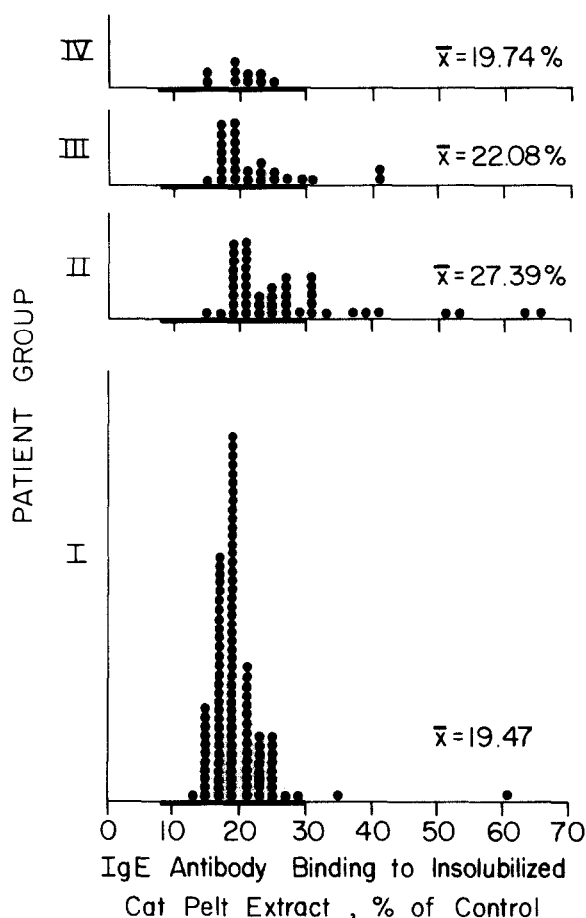


FIG. 3. Binding of IgE antibody to insolubilized cat pelt extract in the 4 patient groups. The *darkened portion* of the horizontal axes represents the 95% confidence interval for group 1 patients.

inhibit binding of IgE antibody to the insolubilized allergens. A stained acrylamide gel is illustrated in the top portion of the figure. Fractions 3 and 4 contained protein with the mobility of albumin. In the *upper panel*, fractions of cat pelt extract were used to inhibit binding of IgE antibody to insolubilized cat allergen 1. The bulk of inhibitory activity was localized to that portion of the gel that contained protein of prealbumin and albumin mobility. It has previously been shown that cat allergen 1 has prealbumin mobility in 7% acrylamide gel.¹³ In the *upper middle panel*, the same fractions of cat pelt extract were used to inhibit binding of IgE antibody to insolubilized crude cat pelt extract. A different pattern of inhibition is seen. Although fractions 1 and 2 contain cat allergen 1, they did not inhibit significantly. Nevertheless, a number of other peaks of inhibitory activity were seen. It is likely that cat allergen 1 is poorly represented on the insolubilized cat pelt extract particles. In the *lower middle panel*, fractions of crude cat pelt extract were used to inhibit the binding of IgE

TABLE II. Patient characteristics

Patient group*	No. of patients	Male/female†	Mean age (range)	Diagnosis‡			
				Asthma	Rhinitis only	Urticaria	Other‡
1. No history of allergy to cats; no skin reactivity to cat extracts	117	50/67	35 (11-79)	37	67	7	4
2. Positive history of allergy to cats; skin reactivity to cat extracts	46	14/32	24 (13-53)	26	20	0	0
3. No history of allergy to cats; skin reactivity to cat extracts	27	16/11	30 (12-74)	7	16	3	1
4. Positive history of allergy to cats; no skin reactivity to cat extracts	10	2/8	33 (18-69)	5	4	0	1

* See section on methods for a more detailed description of the groups.

† No. of patients.

‡ This includes 1 patient with a nonurticarial skin rash, 1 patient with headaches, and 4 patients with bronchitis.

antibody to insolubilized cat serum. Inhibitory activity was seen in fractions that contain protein of albumin mobility and also in a number of fractions of slower mobility. The ability of crude pelt extract to inhibit binding to cat serum indicates that crude cat pelt extract contains a number of components that are possibly derived from cat serum. In the lower panel the same fractions were used to inhibit binding to insolubilized cat albumin. The bulk of inhibitory activity is present in fractions 3 and 4 which contain protein of albumin mobility. This provides evidence that a portion of the IgE antibody in the control serum is directed specifically against albumin.

In summary, the above results suggest that insolubilized cat allergen 1 and cat albumin can be used to measure specific IgE antibody to these components. Furthermore, insolubilized crude cat pelt extract and cat serum measure IgE antibody to a number of additional allergenic components that are distinct from cat allergen 1 and cat albumin.

Patient characteristics

Characteristics of the 200 patients are summarized in Table II. There were 46 patients with positive histories of allergy to cats and positive prick tests to cat pelt extract, cat dander extract, or both (group 2). In comparison with the 117 patients (group 1) without histories of allergy to cats and without prick test reactions to cat extracts, the patients in group 2 were younger (mean age, 24 vs 35) and had a significantly higher percentage with asthma (56% vs 32%). The difference in percent with asthma between groups 1 and 2 was significant to the 0.01 level with the use of the χ^2 test.¹⁴ Twenty-seven patients had positive prick test reactions to one or both cat epithelial extracts but had negative histories of cat allergy

(group 3). It is possible that a number of these patients would develop allergic symptoms if they were significantly exposed to cats. Ten patients felt that they were allergic to cats but had negative prick test reactions to both of the cat epithelial extracts (group 4). It is possible that some of these patients would have had positive prick test reactions if more concentrated extracts were used.

IgE antibody binding to cat allergens

Fig. 2 shows IgE antibody binding to insolubilized cat allergen 1 in the 4 patient groups. In the 117 patients without any evidence of allergy to cats (group 1), the binding averaged $16.89\% \pm 8.34\%$ (mean $\pm$ 2 SD). It is probable that this result does not reflect the binding of specific IgE antibody but instead reflects the nonspecific binding of IgE immunoglobulin in the test sera and/or the nonspecific binding of ¹²⁵I-labeled anti- ϵ antibody to the cellulose particles. In the patients with strong evidence of allergy to cats (group 2) IgE antibody binding to cat allergen 1 averaged $49.30\% \pm 72.36\%$. This value was significantly different than that obtained with group 1 ($p < 0.01$, t test). Thirty of the 46 patients in this group had values above the 95% confidence interval of group 1. The 27 patients with positive prick test reactions but negative histories (group 3) had a mean binding to cat allergen 1 of $22.54\% \pm 13.40\%$, which was slightly but significantly higher than the mean obtained with group 1 ($p < 0.01$). Only 4 of the 10 patients in group 3 exceeded the 95% confidence interval of group 1. The mean binding in patients with positive histories but negative prick test reactions (Group IV) was not significantly different than that in group 1.

Fig. 3 shows IgE antibody binding to insolubilized

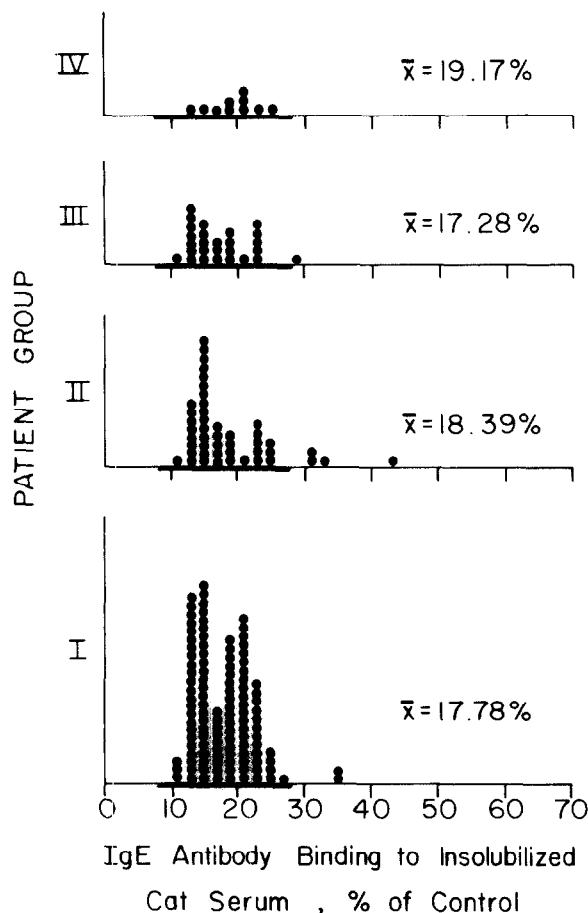


FIG. 4. IgE antibody binding to insolubilized cat serum in the 4 patient groups. The *darkened portion* of the horizontal axes represents the 95% confidence interval for group 1 patients.

cat pelt extract. The mean value for group 2 ($27.39\% \pm 22.72\%$) was significantly greater than the value for group 1 ($19.59\% \pm 9.84\%$, $p < 0.01$). However, only 13 of the 46 patients in group 2 exceeded the 95% confidence interval of group 1. Twelve of these 13 patients also had significantly increased binding to insolubilized cat allergen 1. Therefore, IgE antibody binding to insolubilized crude cat pelt extract was associated with clinical sensitivity less often than IgE antibody binding to insolubilized cat allergen 1. The IgE antibody binding in groups 3 and 4 did not differ significantly from the binding in group 1.

Fig. 4 shows IgE antibody binding to insolubilized cat serum. There was no significant difference in binding among the 4 patient groups. Values for all sera were less than 45% of control, which indicates that the IgE antibody in control serum was unusual in its ability to bind to components in cat serum.

Fig. 5 shows IgE antibody binding to insolubilized

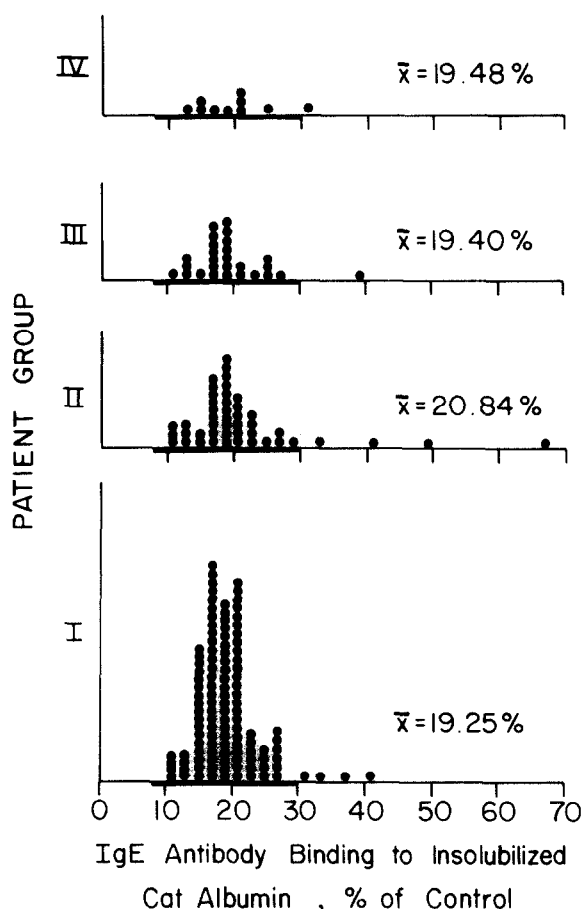


FIG. 5. IgE antibody binding to insolubilized cat albumin in the 4 patient groups. The *darkened portion* of the horizontal axes represents the 95% confidence interval for group 1 patients.

cat albumin. As with cat serum there was no significant difference in binding among the 4 patient groups. It thus appears that IgE antibody binding to insolubilized cat serum and cat albumin is not associated with clinical sensitivity in this patient population. Rare cat-sensitive patients may, however, have a high degree of binding to these preparations as exemplified by the positive control serum.

DISCUSSION

In this study 200 consecutive patients who presented themselves for an allergy evaluation were categorized as cat-sensitive or not cat-sensitive by the use of careful history and skin testing. Those with a doubtful diagnosis (i.e., positive history and negative skin tests to cat epithelial extracts or vice versa) were grouped separately. After patients were so categorized, an attempt was made to correlate these clinically defined categories with the results of IgE antibody measurements.

IgE antibody binding to the insolubilized allergen preparations (in counts per minute) was expressed as a percent of the cpm bound with a positive control serum. Results so expressed are less subject to day-to-day changes in test conditions.¹⁵

Evaluation of the clinical usefulness of IgE antibody levels requires a reliable determination of what is a significantly positive result. The dividing line between negative and positive results has not been well defined and has been related either to a negative control serum¹⁶ or a standard curve with the use of a positive control serum.⁶ In the present study, a significantly positive result could be defined as that which fell above the 95% confidence interval of patients with negative histories of cat allergy and negative skin reactivity to cat epithelial extracts (group 1). The results in group 1 patients had the appearance of a normal distribution and may represent the nonspecific binding of labeled anti- ϵ antibody to the solid-phase particles.

With the use of the above definition of significance, IgE antibody in serum of cat-sensitive patients (group 2) bound most often to insolubilized cat allergen 1 and less often to insolubilized cat pelt extract, cat serum, and cat albumin. Even among group 2 sera, however, 35% did not bind significantly to cat allergen 1. There are at least three explanations for the apparent lack of IgE antibody to cat allergen 1 in these cat-sensitive patients. First, the radioallergosorbent test as used in our laboratory may not be sensitive enough in every case to measure clinically significant amounts of IgE antibody to cat allergen 1. Second, relevant IgE antibody may be present only on cell surfaces and not in serum. Third, allergens other than cat allergen 1 may be important in these patients. We were not able to identify more than one patient who had IgE antibody to crude cat pelt extract, cat serum, or cat albumin who did not also have IgE antibody to cat allergen 1. There may be as yet unidentified cat allergens that were not represented on any of the particles used in this study.

Sera from patients in groups 3 and 4 who lacked evidence usually considered requisite for a diagnosis of clinical sensitivity to cats seldom had significant IgE antibody to cat allergen 1 or to any of the other insolubilized allergens. This indicates that the presence of measurable IgE antibody is highly specific for clinical cat sensitivity. The high specificity of the radioallergosorbent test has been observed by others who have tried to correlate in vitro results with clinical disease.^{1, 17}

It is clear from our results that some cat-sensitive patients have IgE antibody that binds to allergenic components in cat pelt extract or cat serum that are

distinct from cat allergen 1 (Figs. 3, 4, and 5). Some properties of cat serum allergens have been described.^{18, 19} Only a small minority of cat-sensitive patients in this study had IgE antibody to cat serum components (Figs. 4 and 5). Therefore, the presence of IgE antibody to cat serum components including cat albumin is very poorly correlated with clinical sensitivity. The present study confirms the importance of cat allergen 1 as a major allergen in cat sensitivity.

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