

Lowell Memorial Article

Francis Cabot Lowell, one of the leading allergists of his time, died just over two years ago at the age of seventy. A group of his former fellows have established a series of memorial articles in this JOURNAL with the approval of the editorial board of the JOURNAL and the Committee on Awards, Memorials and Commemorative Lectureships of the American Academy of Allergy. This is appropriate, since he had served on the editorial board for eighteen years and was editor of the JOURNAL from 1957 to 1963.

The article by Ohman, Marsh, and Goldman, the first of this series, is particularly fitting because Jack Ohman and Maury Goldman are former fellows. Most allergists will remember Frank Lowell for the first well-controlled studies showing the efficacy of immunotherapy. He believed that immunotherapy with animal dander was clinically important. This article represents an extension of these interests.

Antibody responses following immunotherapy with cat pelt extract

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IgG antibody responses to a major allergen, cat allergen 1 (cat 1), and to a minimally allergenic protein, cat albumin (alb), were studied in patients who had received 4 to 11 mo of immunotherapy with three concentrations of crude cat pelt extract. A double-antibody radioimmunoassay utilizing ^{125}I -labeled antigen was used to measure IgG and IgE antibody. The high-dose group (HDG) ($n = 5$), which was composed of the active-treatment group of a previously reported double-blind study,¹ received a mean cumulative dose of 3440 μg cat 1 and 10,660 μg alb. The intermediate-dose group (IDG) ($n = 6$) and low-dose group (LDG) ($n = 5$) received 70 and 0.930 μg of cat 1 and 217 and 2.86 μg of alb. IgE antibody to cat 1 before treatment was measurable in most patients. IgE antibody to alb was either not measurable or of a low level. Mean (geometric) before-and-after IgG levels (U/ml) to cat 1 in the HDG, IDG, and LDG were 420 and 7794, 158 and 1388, and 191 and 1365, respectively. Mean before-and-after IgG levels (U/ml) to alb in the HDG, IDG, and LDG were 72 and 348, 1.9 and 4584, and 0.4 and 508, respectively. Two patients (HDG) had no IgG antibody to alb after treatment. Another patient (HDG) had 82,364 U/ml IgG antibody to alb and precipitating antibody by gel diffusion. Very intense IgG antibody responses to both cat 1 and alb were seen over a wide range of immunizing doses. (J ALLERGY CLIN IMMUNOL 69:319, 1982.)

In our previously published double blind-study,¹ we reported strong evidence that the allergic response to cat antigens were ameliorated after immunotherapy with dialyzed crude cat pelt extract. It was shown that cumulative doses of 16 to 45 mg of cat antigen containing 2 to 5 mg of the major allergen, cat 1, produced significant decreases in bronchial sensitivity to the crude extract in the active-treatment group. Skin-test reactivity to the crude extract also decreased after active treatment. This study provided strong evidence that immunotherapy is effective in extrinsic asthma.

This article describes in vitro responses in this same group of patients and compares them with the re-

sponses of patients who received lower doses of the crude extract. Antibody responses to cat 1 and to a minimally allergenic protein, alb, were studied. Alb was selected because it is a defined antigen that is present in high concentration in most treatment extracts, including the one used in the present study. Alb is allergenic in a minority of cat-sensitive individuals.

A rise in IgG antibody to allergen during the course of immunotherapy has in the past been considered a correlate of clinical response. This article, which compares IgG antibody responses over a wide range of immunizing doses in a defined group of patients, raises some question as to the relevance of absolute levels of IgG antibody in protecting against allergic pulmonary responses.

MATERIALS AND METHODS

Patient selection

Patients were selected for inclusion into three treatment groups: HDG, IDG, and LDG. The HDG consisted of the five patients in the active-treatment group of a previously reported double-blind study of immunotherapy in cat-induced asthma.¹ These patients were selected on the basis of (1) a history of cough, wheeze, and shortness of breath after exposure to cats, (2) a positive skin test to the treat-

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Abbreviations used

cat 1:	Cat allergen 1
alb:	Cat albumin
HDG:	High-dose group
IDG:	Intermediate-dose group
LDG:	Low-dose group
BSA:	Bovine serum albumin
BBS:	Borate-buffered saline

ment preparation (crude cat pelt extract) containing 20 $\mu\text{g}/\text{ml}$ cat 1, and (3) positive bronchial-challenge response to the same preparation. Several months prior to the start of this double-blind study, it was decided to collect serum specimens from two additional groups of patients who were starting immunotherapy with lower doses of the same cat pelt extract. The patients in the IDG and LDG were selected on the basis of a history of the symptoms of asthma and/or rhinoconjunctivitis on exposure to cats and a positive prick-test reaction to the treatment preparation at a concentration of 20 $\mu\text{g}/\text{ml}$ cat 1. As with the HDG, the patients in the IDG and LDG had not received previous immunotherapy with cat epithelial extracts. Prick-test reactions were performed and graded as previously described,² with the additional requirement that a control reaction with the diluent be negative.

Controls consisted of five patients who had been randomly assigned to the placebo group of the double-blind study (control 1). Additional controls consisted of 10 patients who met the criteria of the IDG and LDG. Six of these patients were being treated with extracts of pollen, dust, and/or mold and had one or more blood samples drawn over a 5- to 12-mo period (control 2).

Treatment preparations and immunotherapy schedules

A single large batch of cat pelt extract was prepared as previously described and was aliquoted in freeze-dried form.² The concentrations of cat 1 and alb in the treatment preparation were determined by Mancini radial immunodiffusion as previously described.³ The most concentrated material for use in immunotherapy in the HDG, IDG, and LDG contained 1600, 400, and 40 $\mu\text{g}/\text{ml}$ cat 1, respectively. The content of alb in each of these preparations was 4960, 1204, and 124 $\mu\text{g}/\text{ml}$, respectively.

Prior to starting immunotherapy in a given patient, 1:10, 1:100, and 1:1000 dilutions were made of the most concentrated treatment preparation. The starting dose was 0.1 ml of the 1:1000 dilution given subcutaneously. At weekly intervals the dose was doubled until 0.4 ml of the most concentrated material was administered. This dose was repeated at least three times.

Antigens

Purified cat 1 and alb were prepared as previously described.³ Both of these antigens migrated as a single protein band on electrophoresis in 7% acrylamide gel. It should be

noted that the weight estimate for cat 1 is only approximate. All previous publications^{1, 3} as well as the present study have made use of a single standard preparation of cat 1 for measuring this antigen.

Assay of serum IgE and IgG antibodies

IgG antibodies to cat 1 and alb were measured by a highly sensitive double-antibody radioimmunoassay procedure similar to that previously described for measurement of IgG antibody toward pollen antigens.⁴ This assay employed cat 1 and alb radiolabeled with ¹²⁵I by the chloramine-T procedure. Excess ¹²⁵I was removed by gel filtration and dialysis, and the radiolabeled antigens were stored at -70°C in small aliquots in 5% BSA in BBS at pH 8.3 (BSA-BBS). Titration of the antigens against serial dilutions of a standard serum pool (from patients who had been extensively treated with cat pelt extract) showed that the optimal concentration for each antigen was 12.5 ng/ml, using dilutions of the standard serum of 1/40 to 1/5120. Under such conditions the antigens were in excess, and sigmoidal antigen-binding curves were obtained.

In brief, the assay was performed as follows: radiolabeled antigen (100 μl at 12.5 ng/ml in 5% BSA-BBS) was allowed to incubate with patient's diluted serum (100 μl) at 23°C for 4 hr. Serum dilutions were chosen in the range of 1/10 to 1/2000 so that the resultant antibody level gave a degree of antigen binding falling on the linear portion of the antigen-binding curve of the standard serum. Dilutions of patients' sera, standard serum, and a control serum up to 1/40 were prepared in BBS. In the case of dilutions greater than this, a 1/40 dilution of the control serum (from a nonallergic subject with no detectable IgG antibody) was used as the diluent to maintain an adequate amount of total IgG for subsequent immunoprecipitation. Goat anti-human IgG (Fc-specific) was then added in a slight excess in order to precipitate all the IgG, including the radiolabeled antigen bound to specific IgG antibody. After incubation for 18 to 20 hr at 4°C , the resultant precipitates were washed and counted. All analyses were performed in duplicate, and radioactivity values (cpm) having coefficients of variation $>10\%$ were redetermined. After subtraction of the "control counts" from the nonallergic serum, antibody levels in the patients' sera were computed from the antigen-binding curve of the standard serum pool.

Several different sera from pretreatment and posttreatment samples from the three treatment groups were analyzed at different serum dilutions. The resultant values for antibody content toward cat 1 and alb (after multiplication by the dilution factors) showed no significant differences. This result indicates that antibody avidity is not a factor in computing antibody levels in the patients' sera.

Attempts were made to estimate the absolute concentrations of anti-cat 1 and anti-alb antibody in the standard serum according to the procedure of Mulligan et al.⁵ However, since no clear-cut plateau regions in the binding curves were obtained, estimated nanogram values can only be regarded as approximate and are cited in "units," where $1\text{ U} \approx 1\text{ ng}$.

Serum IgE antibody levels against cat 1 and alb were also

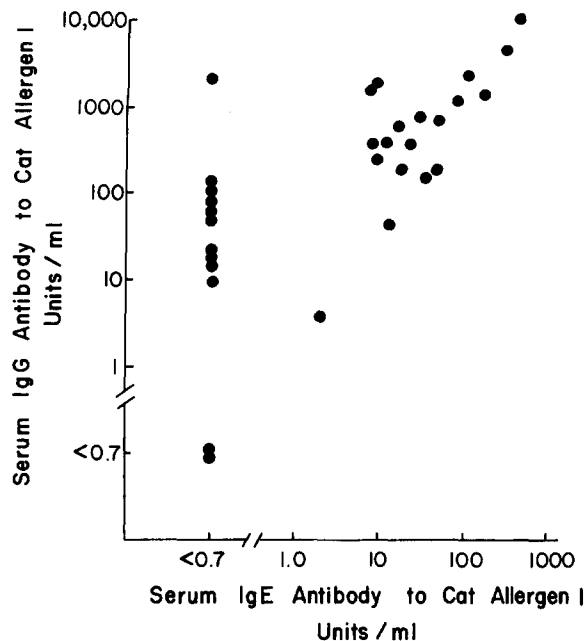


FIG. 1. Relationship between IgE and IgG antibody levels to cat 1 before treatment in all patient groups. A positive correlation was observed ($r = 0.706$). Log values were used in the calculation of the correlation coefficient. Values that were <0.7 U/ml were assigned a value of 0.07 U/ml for this calculation.

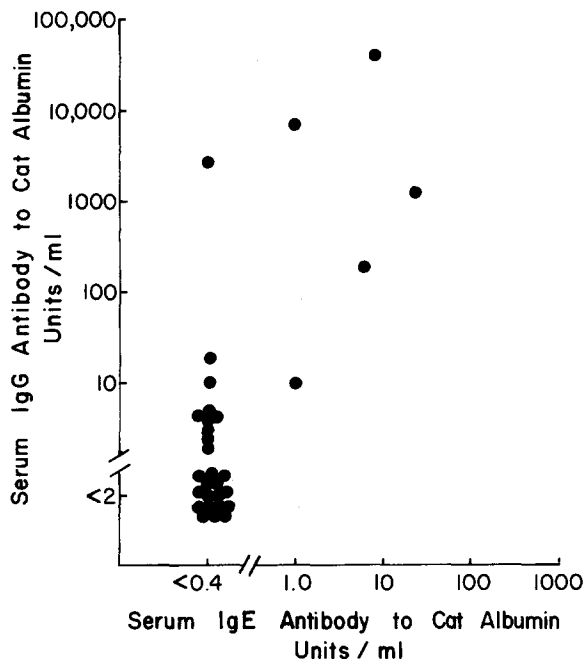


FIG. 2. Relationship between IgE and IgG antibody levels to alb before treatment in all patient groups. A positive correlation was observed ($r = 0.740$). Log values were used in the calculation of the correlation coefficient. Values that were <2 and <0.4 U/ml were assigned values of 0.2 and 0.04 U/ml, respectively.

TABLE I. Patient characteristics

Group	Proportion of males (%)	Mean age (range)	No. with asthma
HDG (n = 5)	0	27 (20-33)	5
IDG (n = 6)	17	22 (16-29)	4
LDG (n = 5)	20	23 (18-33)	4
Control 1* (n = 5)	20	30 (21-43)	5
Control 2† (n = 10)	50	23 (9-53)	7

*Matched placebo groups selected at the same time as the HDG.

†Controls selected at the same time as the IDG and LDG.

estimated by an antigen-binding assay. This assay was similar to the IgG assay, except that goat anti-human IgE (Fc-specific) was used as a second antibody.⁶ All sera were run at dilutions of $1/2$ because of the low levels of IgE antibody. To obtain an adequate amount of precipitate to enable successful washing to be performed, it was necessary to add a small amount of an IgE myeloma serum ($10 \mu\text{l}$ of a $1/10$ dilution of serum from patient P. S.), together with a slight excess of anti-IgE in the second part of the assay. Levels of IgE antibodies were estimated by reference to the IgG standard curves for cat 1 and alb, respectively. Since the resultant IgE and IgG curves for each antigen were parallel, concentrations of IgE antibody could be expressed in the same units as IgG antibody.

RESULTS

Patient characteristics

Patient characteristics are summarized in Table I for the three treatment groups and for the two control groups. There was a predominance of female subjects in all groups except for the control 2 group, in which the sex ratio was equal. Mean ages for the five groups ranged from 22 to 30 yr. The presence of asthma was required for inclusion in the HDG and control 1 group. Asthma was present in 71% of patients in the remaining groups.

Comparison of IgE and IgG antibody levels to cat 1 and to alb before treatment

Fig. 1 illustrates the relationship between IgE and IgG antibody to cat 1 before treatment in all the patients. A positive correlation ($r = 0.706$) was observed. The majority of patients (19/31) had measurable IgE antibody to cat 1 and an even higher proportion (29/31) had IgG antibody to cat 1.

Fig. 2 illustrates the relationship between IgE and IgG antibody to alb before treatment in all of the

TABLE II. Mean cumulative dose of cat 1 and alb in cat pelt extract*

Group	Cat 1 (μg of protein)	Alb (μg of protein)
LDG	0.93 (0.36-1.48)	2.86 (1.12-4.56)
IDG	70.00 (21.12-110.80)	217.12 (65.60-343.60)
HDG	3,440 (1,700-4,700)	10,660 (5,270-14,570)

*Data presented as arithmetic mean, with range in parentheses.

patients; a positive correlation ($r = 0.740$) was seen. In contrast to the results with cat 1, a smaller proportion of patients had IgE antibody binding to alb (6/31), and when it was present it was of a low level. Fifteen of the 31 patients had IgG antibodies to alb.

High levels of IgG antibody to an antigen can significantly inhibit measurement of IgE antibody in this system by competing with IgE for binding sites on the labeled antigen. Our experience in comparing IgE and IgG measurements on posttreatment sera would indicate that IgG levels below 5000 U/ml are unlikely to inhibit IgE antibody binding to any appreciable degree. It is apparent from the data summarized in Figs. 1 and 2 that high levels of IgG antibody to cat 1 or alb did not prevent measurement of IgE antibody. It is possible that those patients with IgG antibody to cat 1 or alb greater than 5000 U/ml had actual IgE levels higher than those measured.

IgG antibody level to cat allergen and cat albumin in control patients

IgG antibody levels to cat 1 are summarized in Fig. 3. Measurable IgG binding was seen in all but one of the patients. There was no significant trend in the level of IgG binding in those patients who had more than one measurement over the study interval. Three of the five patients in the control 1 group had consistent IgG binding above 1000 U/ml. These data suggest that environmental exposure results in appreciable IgG antibody responses, which in some cases approach posttreatment values (see below). We do not have detailed histories of the extent of cat exposure in these patients; therefore, a correlation of level of IgG antibody with extent of exposure to cats is not possible.

IgG antibody levels to alb are summarized in Fig. 4. With the exception of two patients in the control 1 group, levels were in general below 10 U/ml. These data suggest that for most patients, either there is much less environmental exposure to alb or alb is a weaker antigen than cat 1.

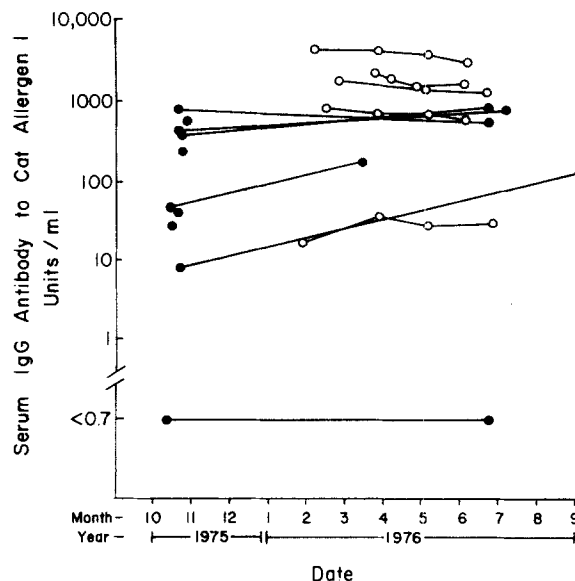


FIG. 3. IgG antibody levels to cat 1 in control patients. Open circles, control 1 group; closed circles, control 2 group.

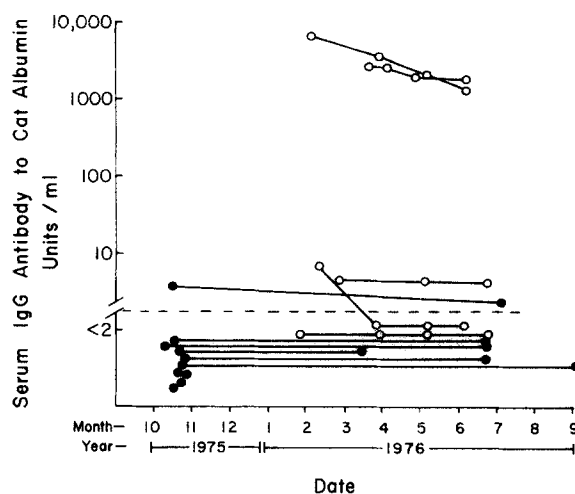


FIG. 4. IgG antibody levels to alb in control patients. Open circles, control 1 group; closed circles, control 2 group.

Serum IgG antibody levels to cat 1 and alb in treated patients

The mean cumulative doses of cat 1 and alb in the treatment groups are given in Table II. There was roughly a 50- to 80-fold difference between groups in cumulative dose. Because the same preparation was used for each group, there was a fixed ratio of cat 1 to alb of 0.33. As is true with many cat pelt or epithelial extracts, there was more alb than cat 1 in this preparation.

IgG antibody responses to cat 1 in the three treatment groups are summarized in Fig. 5. Even in the

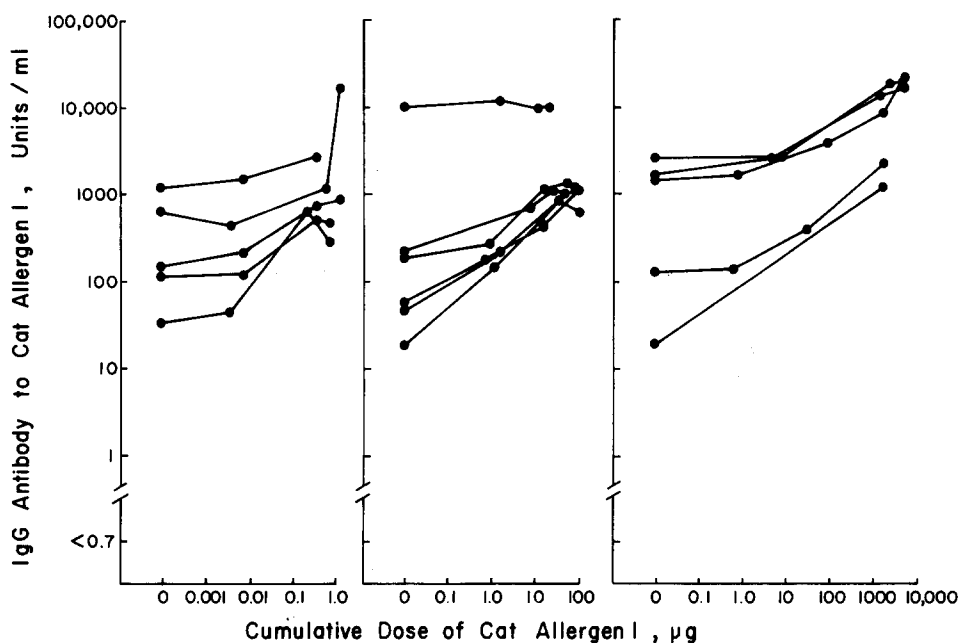


FIG. 5. IgG antibody levels to cat 1 in response to treatment in the LDG (left panel), IDG (middle panel), and HDG (right panel).

LDG, which received only a mean cumulative dose of $0.93 \mu\text{g}$ of cat 1, there was a substantial increase in IgG binding with treatment. Mean (geometric) IgG antibody levels before and after treatment were 191 and 1365 U/ml, respectively. The IDG, which received a mean cumulative dose of $70.0 \mu\text{g}$ of cat 1, had mean (geometric) IgG antibody levels before and after treatment of 158 and 1388 U/ml, respectively. The HDG, with a mean cumulative dose of $3440 \mu\text{g}$ of cat 1, had mean (geometric) IgG antibody levels before and after treatment of 420 and 7794 U/ml, respectively. Even though the numbers in the group are small, it appears that differences in IgG antibody response between the groups are small compared with the large differences in cumulative dose that the three groups received.

IgG antibody responses to alb in the three treatment groups are summarized in Fig. 6. Mean (geometric) values of anti-alb antibody in the LDG, IDG, and HDG before and after therapy were 0.4 and 508, 1.9 and 4584, and 42 and 348 U/ml, respectively. Two participants in the HDG had pretreatment levels of IgG antibody above 1000 U/ml. Two additional patients in the HDG who had low levels of anti-alb antibody before treatment failed to respond to high doses of immunizing antigen. The low posttreatment mean for the HDG reflects these two patients who did not respond. The serum with the highest posttreatment level of antibody to alb (82,364 U/ml) had detectable precipitating antibody when tested by gel diffusion in 1% agar against purified alb in a concen-

tration of $50 \mu\text{g/ml}$. All other sera in the same three treatment groups did not have detectable precipitating antibody.

In contrast to the results obtained with cat 1, low pretreatment levels of antibody to alb were seen with vigorous responses to cumulative doses of alb below $10 \mu\text{g}$ in the LDG. Two patients were apparently incapable of responding to high immunizing doses of alb.

DISCUSSION

This article describes IgG antibody responses to a major allergen, cat 1, and a minimally allergenic protein, alb, in patients undergoing immunotherapy with a crude extract of cat pelt. Pretreatment IgE antibody levels were also obtained. Because of the interfering effects of high levels of IgG antibody, we could not reliably estimate IgE responses after immunotherapy. The double-antibody radioimmunoassay procedure used in this study to measure IgE antibody is particularly susceptible to this inhibiting effect; this assay was selected because it requires only microgram quantities of purified allergen.

It should be noted that the undiluted extract administered to the LDG contained $40 \mu\text{g/ml}$ cat 1. This is a higher concentration than is present in many commercial 1:10 w/v extracts of cat epithelium.³ Therefore the LDG did not receive low-dose therapy in the sense that this term is usually used in the field of clinical allergy.

Patients in the HDG were selected in a different manner than patients in the IDG and LDG, as noted in

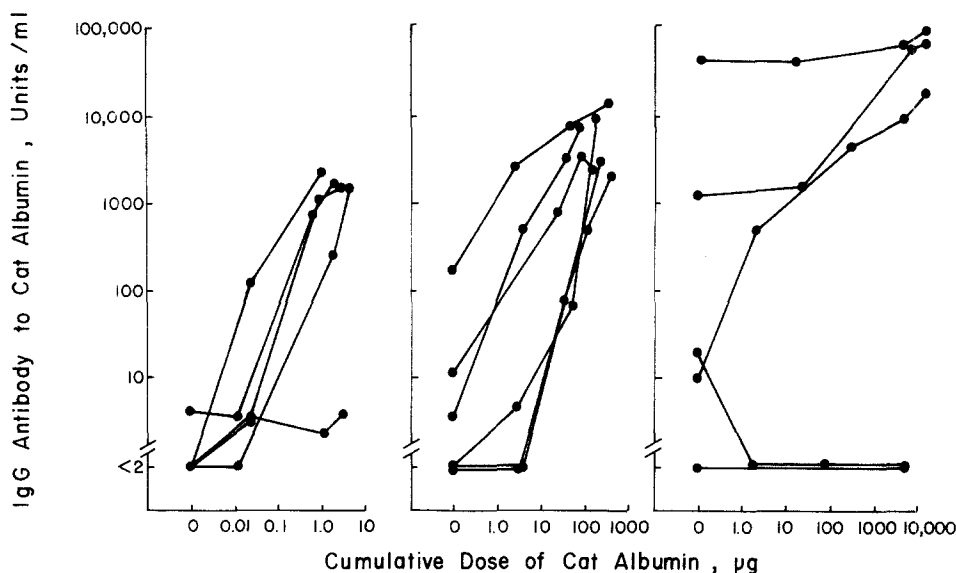


FIG. 6. IgG antibody responses to alb in response to treatment in the LDG (left panel), IDG (middle panel), and HDG (right panel).

the Materials and Methods section. We believe that a comparison of these groups is valid because the age range and baseline antibody levels in the three groups were generally similar. Even though the presence of asthma was not required for inclusion in the IDG and LDG, the majority of patients in these groups had a diagnosis of asthma. The duration of therapy could have an effect on the magnitude of the IgG antibody responses observed. However, the total duration of therapy in the three groups was similar, i.e., 4 to 11 mo.

The majority of patients (19 of 31) showed significant IgE antibody binding to cat 1, whereas only a minority (6 of 31) showed IgE binding to alb. These results are similar to those obtained in a previous study of IgE antibody to cat allergens in an allergic population.⁷ Patients with undetectable IgE antibody to cat 1 and alb did not have high levels of IgG antibody to explain these negative results. Unfortunately, at the time this study was begun we did not have highly purified cat 1 for skin testing purposes; correlation between *in vitro* and *in vivo* testing was therefore not possible. Some of the patients with undetectable IgE antibody to both cat 1 and alb might have been primarily reactive to as yet undefined allergens. We^{7, 8} and others⁹ have shown that a minority of cat-sensitive subjects react to allergens with a molecular weight of 70,000 and above. At least some of these allergens are present in cat serum and crossreact with allergens in dog serum.⁸

This study shows significant differences in baseline level of IgG antibody to cat 1 and alb. All patients had measurable levels of IgG antibody to cat 1 before treatment. In the majority of patients this level was

above 100 U/ml. In contrast, the majority of patients had undetectable baseline levels of IgG antibody to alb. These results suggest that ambient exposure to cats regularly results in the production of substantial quantities of IgG antibody to cat 1. In spite of this, the patients in the HDG all had positive bronchial-challenge responses before treatment to small doses of the crude extract containing less than 50 µg of cat 1.¹

After treatment, there was a vigorous increase in IgG antibody to both cat 1 and alb over a wide range of cumulative doses. These results suggest that both cat 1 and alb are potent antigens and that ambient exposure to alb is often not sufficient to engender detectable IgG antibody.

The intensity of the responses to alb and the detection of precipitating quantities of antibody in one patient are of some concern. Alb is probably not a significant allergen in most patients, and hence a vigorous immune response to this antigen is probably not beneficial in any way. The induction of immune complex disease remains a potential danger of immunotherapy.

There were approximately 50- to 80-fold differences in cumulative dose of extract between each of the treatment groups. The increase in IgG antibody to both cat 1 and alb were of a lower degree. There was an approximately 4000-fold difference in cumulative dose between the LDG and the HDG. There was, however, only a slightly more than twofold difference in posttreatment IgG antibody between these groups. It would be pertinent to know whether similar dose-response relationships also pertain to parameters of clinical response.

In the HDG, approximately 20-fold increases in

IgG antibody to cat 1 over the treatment interval were associated with mean decreases in bronchial sensitivity of approximately 50-fold. The high level of IgG antibody to cat allergen before treatment in patients who had a high degree of bronchial sensitivity to aerosolized cat allergen raises questions as to the relevance of absolute levels of IgG antibody in protecting against allergic pulmonary responses.

These observations are, of course, complicated by the fact that the crude extract used for challenge contained other potential allergens. There is strong evidence, however, that cat 1 is the major allergen.^{3, 10, 11} Further studies of immunotherapy in asthma should be directed at establishing dose-response relationships more clearly with respect to immunologic parameters as well as to clinical response. The use of relevant purified antigens would more clearly establish these relationships.

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REFERENCES

1. Taylor WW, Ohman JL Jr, Lowell FC: Double-blind trial of immunotherapy in cat-induced asthma. *J ALLERGY CLIN IMMUNOL* **61**:283, 1978.
2. Ohman JL Jr, Lowell FC, Bloch KL: Allergens of mammalian origin: characterization of allergen extracted from cat pelts. *J ALLERGY CLIN IMMUNOL* **52**:231, 1973.
3. Ohman JL Jr, Lowell FC, Bloch KL et al: Allergens of mammalian origin. V. Properties of extracts derived from domestic cat. *Clin Allergy* **6**:419, 1976.
4. Black PL, Marsh DG, Jarrett E et al: Family studies of association between HLA and specific immune responses to highly purified pollen allergens. *Immunogenetics* **3**:349, 1976.
5. Mulligan JJ, Osler AG, Rodriguez E: Weight estimates of rabbit anti-human serum albumin based on antigen-binding capacity. *J Immunol* **96**:324, 1966.
6. Platts-Mill TAE, Snajdr M, Ishizaka K et al: Measurement of IgE antibody by an antigen binding assay: correlation with PK activity and IgG and IgA antibodies to allergens. *J Immunol* **120**:1201, 1978.
7. Ohman JL Jr, Kendall S, Lowell FC: IgE antibody to cat allergens in an allergic population. *J ALLERGY CLIN IMMUNOL* **60**:317, 1977.
8. Ohman JL Jr, Bloch KJ, Kendall S et al: Allergens of mammalian origin. IV. Evidence from common allergens in cat and dog serum. *J ALLERGY CLIN IMMUNOL* **57**:560, 1976.
9. Holdford-Strevens V: Allergenic activity of cat and dog skin fractions obtained by Sephadex gel filtration. *Clin Allergy* **6**:225, 1973.
10. Ohman JL Jr, Lowell FC, Bloch KL: Allergens of mammalian origin. III. Properties of a major feline allergen. *J Immunol* **113**:1668, 1974.
11. Anderson MC, Baer H: Allergenic active components of cat allergen extracts. *J Immunol* **127**:972, 1981.