Original articles

Immunotherapy in cat-induced asthma. Double-blind trial with evaluation of in vivo and in vitro responses

J. L. Ohman, Jr., M.D. Steven R. Findlay, M.D., and K. M. Leitermann, S.B.
Boston, Mass.

The efficacy of immunotherapy in cat-induced asthma was studied by use of a purified fraction of cat-pelt extract and a double-blind protocol. Nine active-treatment subjects who received a mean cumulative dose of cat allergen, 1 of 10.9 units, and eight placebo-treatment subjects completed the study. Active treatment resulted in significant reductions in bronchial sensitivity ($p < 0.05$) and prick test titer ($p < 0.01$). In addition, active treatment resulted in a significant delay in the onset of ocular ($p < 0.05$) and pulmonary ($p < 0.02$) symptoms on exposure to living cats. Significant increases in IgG antibody to cat allergen 1 ($p < 0.001$) and cat albumin ($p < 0.01$) also occurred with active treatment. There was no significant change in bronchial reactivity to methacholine or in the sensitivity of circulating basophils. These results confirm the validity of immunotherapy in allergic asthma where there is careful patient selection and well defined treatment preparations. (J ALLERGY CLIN IMMUNOL 74:230-9, 1984.)

The clinical effectiveness of immunotherapy in allergic asthma has not been well established. Previous studies have suggested that immunotherapy results in a reduction in bronchial sensitivity to allergen.[1-4] Immunotherapy has also been associated with improvement in the clinical severity of asthma[5-7]; other studies have failed to demonstrate clinical improvement.[3, 4, 8, 9] Bronchial provocation with the use of aerosolized aqueous extracts may not be a good model for naturally occurring asthma.

Well-designed studies of efficacy of immunotherapy in asthma require that the causative allergen be well delineated. This demonstration has not been clearly made in seasonal forms of asthma.[10, 11] Asthma occurring in an individual patient may have multiple causes.

Asthma caused by exposure to the domestic cat is a useful model for the study of immunotherapy because the causative agent can usually be well defined by the history. The major allergen produced by the cat, cat allergen 1, has been partially characterized and can be measured quantitatively in treatment preparations.[12, 13] A previous double-blind study of immunotherapy with cat-pelt extract has demonstrated that treatment resulted in a significant reduction in bronchial sensitivity to cat allergen.[14]

Abbreviations used

BU:	Breath units
PD_{20} $FEV_{1.0}$:	Provocation dose (in BU) that produces a 20% drop in the 1.0 sec forced expiratory volume
NS:	Not significant
PBS:	Phosphate-buffered saline

From the Allergy Laboratory, Veterans Administration Outpatient Clinic, and the Department of Medicine, Tufts University School of Medicine, Boston, Mass.

Supported in part by the Medical Research Service, Veterans Administration, U.S. Public Health Service grant P50 AI-18023-02, the Foundation for the Study of Asthma and Related Diseases, and Key Pharmaceuticals.
Received for publication Aug. 25, 1983.
Accepted for publication Feb. 15, 1984.

Reprint requests: John L. Ohman, Jr., M.D., VA Outpatient Clinic, 17 Court St., Boston, MA 02108.

In this study we have tested the efficacy of immunotherapy in cat-induced asthma with the use of a partially purified preparation of cat allergen 1 and a double-blind protocol. In vivo procedures to test efficacy included the skin test titer and bronchial challenge with cat allergen and methacholine. In addition, naturally occurring asthma was tested by exposure of subjects to cats. In vitro responses were also measured. These responses consisted of basophil histamine release and IgG antibody to cat allergen 1 and cat albumin.

METHODS

Subject selection and study design

Subjects were selected from respondees to a newspaper advertisement requesting volunteers for a study of the treatment of cat-induced asthma. After a brief telephone interview to screen for suitability, prospective subjects were observed in person. Selection criteria were as follows: (1) good general health with no need for daily asthma medication, (2) history of cough, wheeze, or shortness of breath on exposure to cat with such a reaction within the last 2 yr, (3) no previous history of immunotherapy with cat epithelial extracts, (4) $FEV_{1.0}$ of 70% of predicted or higher, (5) positive prick test reaction to the treatment preparation containing a cat allergen 1 concentration of 4 U/ml, and (6) positive bronchial challenge response to the treatment preparation containing a maximum inhaled cat allergen 1 concentration of 4 U/ml.

Before the start of immunotherapy, subjects were observed on three consecutive weeks. At the initial visit a history, physical examination, skin tests, baseline pulmonary function measurements, and a bronchial provocation with methacholine were obtained. Informed consent was obtained. One week later subjects underwent a cat exposure (described below). On the third week a bronchial provocation with cat allergen was performed. Subjects were categorized according to their skin test reactivity: group A had a prick test end point titer of $\leq 1:10$, and group B had a titer of $>1:10$. On the third week subjects' data were reviewed, and if all of the selection criteria were met, each subject was randomly and blindly assigned to either the active-treatment or placebo-treatment group. Group A and group B were randomly assigned separately. Assignment was performed by a technician who had no information about the subjects other than their names and study numbers; this technician individually prepared and numbered the treatment preparations. Immunotherapy was carried out for 16 wk (see protocol below). Final evaluations were carried out on three consecutive weeks starting 1 wk after the last immunotherapy injection.

Treatment preparations and standardization of cat allergen 1 content

The treatment preparation was a purified fraction of cat-pelt extract obtained by acetone precipitation followed by Sephadex G-75 (Pharmacia Fine Chemicals, Inc., Piscataway, N.J.) chromatography. Cat-pelt extract was prepared as previously described.[12] Acetone was added to the crude extract in order to make a concentration of 80% acetone. The resulting mixture was then stirred for 20 min at room temperature, centrifuged at $500 \times g$ for 20 min, and the resulting precipitates were washed with an equal volume of 80% acetone in water. After repeat centrifugation and decantation, the precipitate was dissolved in $^1/_{10}$ of the original volume of phosphate-buffered (0.01M, pH 8.0) saline (0.15M). Material insoluble in PBS was separated by centrifugation and discarded. The supernatant containing acetone-precipitated protein was applied to Sephadex G-75 columns that had been equilibrated in PBS. Gel filtration resulted in two main peaks of protein. The first or void peak contained proteins of 80,000 daltons or more. A second large peak with a molecular weight of 68,000 consisted predominantly of cat albumin. A fraction containing cat allergen 1 consisting of proteins of 15,000 to 45,000 daltons was collected, dialyzed against 0.05M ammonium bicarbonate (200 times volume), sterilized, and freeze-dried in aliquots. Each vial of the treatment preparation contained 6.3 units of cat allergen 1 and 160 mcg of albumin.

Previous publications[13, 14] have used a microgram designation for cat allergen 1. Because of uncertainty as to the accuracy of this weight estimate for cat allergen 1, the unit designation was adopted in collaboration with Dr. Harold Baer of the National Center for Drugs and Biologics. One microgram of cat allergen 1 referred to in our previous publications is equal to 0.04 unit.

Immunotherapy protocol

Active-treatment vials reconstituted in 50% glycerine to a concentration of 13 units of cat allergen 1 per milliliter (300 mcg/ml of cat albumin). Placebo-treatment vials that were identical in appearance were similarly prepared containing 0.6 mg/ml of histamine phosphate. A 1:10 and 1:100 dilution of each was prepared in normal saline that contained 0.05% human serum albumin. A technician who was not otherwise involved in the study prepared the vials and supplied them to the investigator labeled with the patients' names and numbers.

Immunotherapy was conducted as follows: an initial subcutaneous injection of 0.05 cc of a 1:100 dilution was administered and at weekly intervals the dose was doubled until the maximum tolerated dose was achieved or 0.3 cc of the undiluted preparation was administered. The largest dose was repeated on several successive weeks. If a systemic reaction or an uncomfortably large local reaction occurred during the course of immunotherapy, the dose for the next week was reduced by one fourth, and subsequent doses were cautiously increased. Immediately after the second cat exposures, the adequacy of the blinding was evaluated. Each subject was asked to fill out a form that indicated whether they believed that they had received the active treatment or the placebo treatment and the reasons for this opinion. The investigator who was involved in the skin testing, bronchial provocation, and cat exposures before and after immunotherapy was not involved in the immunother-

TABLE I. Subject characteristics

	Placebo-treatment group	Active-treatment group
Number	8	9
Male/female	0/8	2/7
Mean age (range)	30 (24 to 48)	26 (22 to 31)
Mean baseline $FEV_{1.0}$, % predicted (range)	85 (70 to 105)	85 (71 to 98)
Mean cumulative dose cat allergen 1, units (range)	—	10.9 (0.24 to 24.8)
Mean cumulative dose cat albumin, micrograms (range)	—	272 (6 to 621)

apy. During the assessment after immunotherapy, subjects were instructed not to discuss their immunotherapy with this investigator.

Skin testing

A vial of the active-treatment preparation was reconstituted immediately before testing at a concentration of 4 U/ml of cat allergen 1 in normal saline (0.15M NaCl) containing 0.5% human serum albumin. Six serial 1:10 dilutions were made. A droplet of each dilution was applied to the volar forearm. A 26-gauge needle was passed through the droplet, and the underlying skin was punctured with the tip of the needle. After 15 min the average diameter of the resulting wheal was measured and recorded. A plot of the wheal diameter versus the log reciprocal dilution was made. The reciprocal dilution that produced a 3-millimeter wheal was derived from the plot. A skin test titer of 10 would mean that a 1:10 dilution of the active-treatment preparation (containing 0.4 units of cat allergen 1 per milliliter) produced a 3-millimeter wheal.

Bronchial provocation with methacholine and cat allergen

Bronchial provocations were performed according to a standardized procedure.[15] Methacholine (Siegfried Limited, Zofingen, Switzerland) was prepared in a 25 mg/ml solution in normal saline (0.15M NaCl) (NS). Dilutions in normal saline containing 10, 2.5, 0.25, and 0.025 mg/ml were prepared. Bronchial provocations with allergen were conducted on the same day that the skin test titer was performed, and the same dilutions of the treatment preparation of cat allergen were used (see above). The initial dilution of methacholine used contained 0.025 mg/ml. The initial dilution of cat allergen used was tenfold more dilute than that dilution that elicited a 3-millimeter wheal on skin testing.

The $FEV_{1.0}$ was measured from a kymographic tracing obtained on a 6-liter recording spirometer (Warren E. Collins, Inc., Braintree, Mass.). One inhalation followed by five inhalation doses (via a breath activated dosimeter) of successively more concentrated solutions of methacholine or cat allergen were tested until the $FEV_{1.0}$ dropped $\geq 20\%$ compared with baseline values. Measurements of the $FEV_{1.0}$ were made 3 min after inhalations of methacholine and 10 min after inhalations of cat allergen. BU was defined as one inhalation (containing 0.05 cc) of a 1.0 mg/ml solution for methacholine or one inhalation of a 1:100 dilution (0.04 units of cat allergen 1 per milliliter) for cat allergen. The cumulative provocation dose in BU that resulted in a 20% drop of $FEV_{1.0}$ ($PD_{20}FEV_{1.0}$) was determined from a plot of the log cumulative BU versus the percent decrease in the $FEV_{1.0}$.[14]

Cat exposures

Cat exposures were conducted in an apartment in which two cats had been housed for at least 4 mo. Immediately before the exposure, the cats were placed in an open wire cage, and subjects were brought into the room and instructed to sit 5 feet away from the cage. Subjects were asked to record the severity of their symptoms before entering the apartment and at 5, 15, 30, 45, 60, and 90 min. Symptoms were subdivided into three categories: (1) eye, itchy or watery; (2) nose, stuffy or running and sneezing; and (3) lungs, cough, wheeze, shortness of breath, or chest tightness. At each time interval severity of symptoms was recorded according to the following symptom score: 0, no symptoms; 1, trivial or barely noticeable; 2, definitely present but not bothersome; 3, bothersome but not disabling or intolerable; and 4, disabling and/or intolerable.

In the absence of severe allergic symptoms, each subject stayed in the apartment for 90 min. If any symptom reached a grade of 4 or if the overall symptom complex was judged by the patient to be intolerable, the subject was instructed to leave the apartment regardless of the time.

Immediately after the second cat exposure (after immunotherapy), subjects were asked to compare the second cat exposure to the first cat exposure for the three symptom categories according to the following rating scale: (1) much more intense, (2) slightly more intense, (3) unchanged, (4) slightly less intense, and (5) much less intense.

Subjects were not given access to their first cat exposure report form so that their rating was entirely on the basis of memory of their intensity of symptoms. Peak flow rates were obtained at the above time intervals with a Wright peak flow meter (Armstrong Industries, Inc., Northbrook, Ill.). The best of three efforts was recorded.

Basophil histamine release

Basophil histamine release was performed as previously described[16] with cells obtained from venous heparinized blood after sedimentation in dextran. Cells were added in

TABLE II. Characteristics of active-treatment subjects who had reactions to Immunotherapy

Patient	Number of systemic reactions	Number of large local reactions	Baseline reciprocal skin test titer	Baseline PD_{20} $FEV_{1.0}$ (BU)	Baseline concentration of cat allergen 1 that caused 50% histamine release (units)	Cumulative immunotherapy dose of cat allergen 1 (units)
1	3	—	32	6.5	0.0015	6.8
2	3	—	10	7.7	0.0025	2.8
3	1	—	316	12.6	0.0002	24.8
4	3	—	178	1.0	0.0025	0.6
5	—	2	3162	0.7	0.0003	0.2
	Average value (range) of four active-treatment patients who had no reaction to immunotherapy		93 (10 to 215)	8.2 (2.5 to 21.2)	0.01 (0.00025 to 0.025)	15.6 (8.8 to 21.0)

duplicate to aliquots of affinity-purified cat allergen 1[17] that ranged in concentration from 10^{-7} to 10^{-3} U/ml. Total cellular histamine was determined by lysis of cell aliquots with 3% perchloric acid. The concentration of cat allergen 1 that resulted in 50% of maximum histamine release was calculated as previously described.[16] The maximum net percent histamine release was calculated as follows: maximum net % histamine release = (maximum histamine in test supernatants) minus (histamine in the supernatant of cells buffer) divided by (total histamine from lysed cells). All values were averages of duplicates. Spontaneous release (histamine in the supernatant of cells in buffer) never exceeded 5%. The extraction and analysis of histamine in supernatants was performed according to the automated fluorometric assay method described by Siraganian.[18]

Quantitative immunoelectrophoresis assay of cat allergen 1 and cat albumin

Cat allergen 1 and cat albumin were assayed by the quantitative immunoelectrophoresis method described by Laurell.[19] Briefly, antiserum was mixed with liquid 1% agarose in 56° C and poured to form a 2 mm gel layer on 183 by 102 mm glass plates. Plates were placed on a cooling platform and connected by paper wicks to electrophoresis buffer reservoirs (Multiphor, LKB Instruments, Bromma, Sweden). Samples to be tested and standards of known concentration of the protein to be measured were placed in individual wells at the cathode end of the gel. A voltage of approximately 10 V/cm was applied across the gel for 3 to 4 hr. Gels were dried and stained with Coomassie blue G-250 (LKB) according to the manufacturer's instructions. The log height of the resulting precipitin arc was directly proportional to the log concentration of antigen in the sample. Linear regression analysis was used to calculate the antigen concentration in unknown samples from the log heights of their precipitin arcs. The standard used in this assay was a crude pelt extract (149), stored in lyophilized aliquots containing 16 units of cat allergen 1 and 1240 mcg of cat albumin.

Measurement of IgG antibody to cat allergen 1 and cat albumin

Antibody to cat allergen 1 and cat albumin was measured with ELISA by use of 96-well microtiter plates (Immulon I, Dynatech, Alexandria, Va.). Each plastic well was incubated at 4° C with 0.2 ml of cat allergen 1 (0.8 U/ml) or cat albumin (4 mcg/ml) in 40 mM carbonate, pH 9.6, for 16 hr.[13, 17] Serum samples (stored at −20° C until testing) were diluted 1:10 in phosphate buffered (0.01M, pH 7.4) saline (0.15M) containing Tween-20 (0.05%), placed in washed, coated wells in triplicate (0.15 ml per well), and incubated for 90 min at room temperature. Sample wells were washed three times with PBS-Tween, and 0.15 ml of a solution of alkaline phosphatase-labeled affinity-purified anti-human IgG (gamma chain specific) (Sigma Chemical Co., St. Louis, Mo.) in PBS-Tween was added to each well and incubated overnight at 4° C. The wells were again washed three times in PBS-Tween and 0.15 ml of enzyme substrate (P-nitrophenyl phosphate, disodium [Sigma 104, Sigma Chemical Co.] in 25 mM carbonate, 2 mM $MgCl_2$, pH 9.5) was added to each well. The optical density of each well was read at 405 nm in a microeliza plate reader (Titertek Multiskan, Flow Laboratories, McLean, Va.).

Each microtiter plate included a positive high-titer standard serum as well as a negative control serum obtained from a nonatopic and cat allergen skin test–negative subject. The mean optical density reading obtained from the negative control serum was subtracted from the reading obtained in the other wells of a given plate. Results were then expressed as a percent of the optical density obtained with the positive control serum. In general, values more than 15% of control fell above the 95% confidence interval of repetitive measurements of the negative control serum.

Statistical analysis

Baseline measurements for the placebo-treatment and active-treatment groups were compared by use of the t test for continuous data and a nonparametric test[20] for noncontinuous data. Response to treatment within groups was ana-

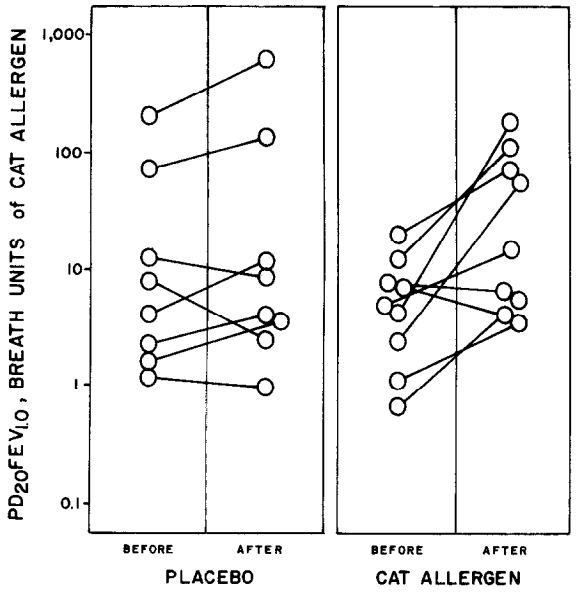


FIG. 1. Bronchial provocation with cat allergen. The geometric mean cumulative provocation dose of cat allergen in BU that resulted in a 20% drop in the $FEV_{1.0}$ ($PD_{20}FEV_{1.0}$, allergen) changed from 8.8 to 12.3 BU in the placebo-treatment group (NS) and from 4.27 to 20.7 BU in the active-treatment group ($p < 0.05$). A comparison of change from baseline between the placebo- and active-treatment groups revealed no significant difference.

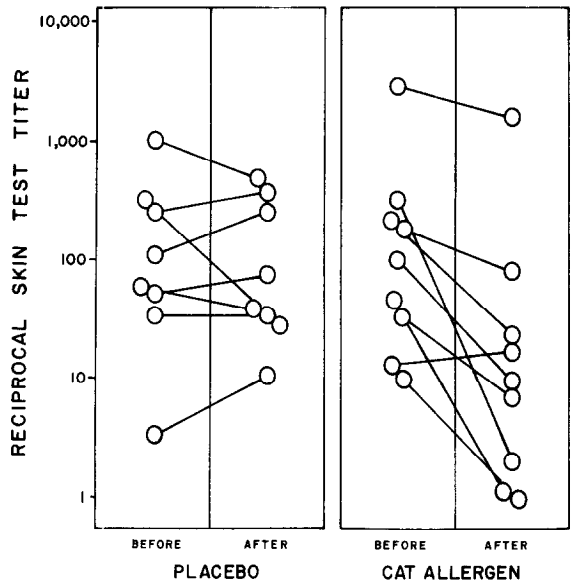


FIG. 2. Skin test responses. The geometric mean of reciprocal prick test titer changed from 82 to 76 in the placebo-treatment group (NS) and from 94 to 9.8 in the active-treatment group ($p < 0.01$). A comparison of change from baseline between the placebo- and active-treatment groups revealed a highly significant difference (< 0.01).

lyzed by either the paired t test for continuous data or the signed ranks test[20] for noncontinuous data. In addition, change from baseline was computed for each measurement; the response to active and placebo treatment was then compared directly by use of the t test or the signed ranks test.

RESULTS

Subject characteristics and immunotherapy side effects

Subject characteristics are summarized in Table I. Eight subjects received the placebo treatment and nine subjects received the active treatment. There was no significant difference between the groups with respect to age or baseline pulmonary function (t test). In addition there were no significant differences between the groups before treatment with respect to skin test titer, bronchial provocation with methacholine or allergen, time to first increase in symptoms on cat exposure (ocular, nasal, or pulmonary), IgG-antibody levels to cat allergen 1, or maximum histamine release to cat allergen 1. The subjects who received the active treatment received a mean cumulative dose of cat allergen 1 of 10.9 units with a range of 0.24 to 24.8 units.

Large local reactions severe enough to warrant modifications of the immunotherapy schedule occurred on three occasions in two patients who received the active treatment. Systemic reactions were observed on two occasions in one patient who received the placebo treatment and on 10 occasions in four patients who received the active treatment. In all instances these reactions were mild and responded rapidly to treatment. These reactions consisted of rhinoconjunctivitis, asthma, itching and swelling of the facial area, and hives. In all instances of systemic reactions, there was a reduction in subsequent doses as indicated in the methods. Table II summarizes baseline data in those active-treatment subjects who had reactions to immunotherapy. It is clear that skin test titer, bronchial reactivity, and sensitivity of white blood cells to allergen did not predict reliably those subjects who would have reactions to immunotherapy. In general, lower cumulative doses were achieved in those subjects who had reactions.

After immunotherapy, three of the eight subjects who received the placebo treatment believed that they had received the active treatment. Eight of the nine subjects in the active treatment group believed that they had received the active treatment. The difference between the active-treatment and placebo-treatment groups was significant ($p < 0.05$, chi-square analysis). The intensity of the local reaction or the occurrence of a systemic reaction were common reasons for judging that active treatment had been administered although four active-treatment and four placebo-treatment subjects based their decision at least par-

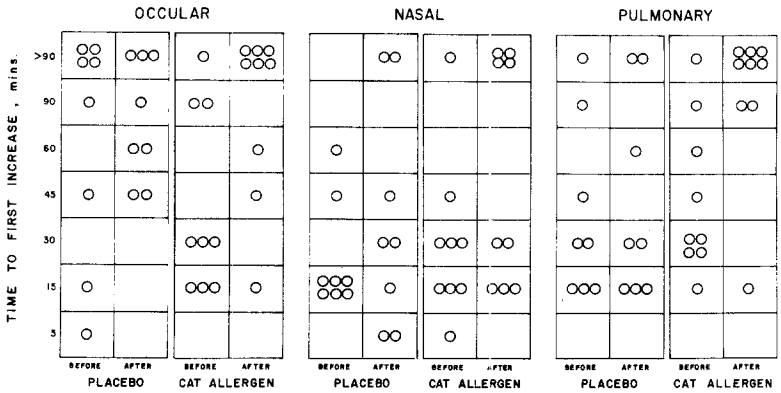


FIG. 3. Time to first increase in symptoms on exposure to cats. Subjects were requested to record a symptom score after 15-minute time intervals after entering a room with cats. The time to a first increase in symptoms was recorded for each patient before and after treatment. For ocular symptoms *(left panel)* there was a significant delay in the appearance of symptoms only with active treatment ($p < 0.05$). A comparison of change scores from baseline in the placebo- and active-treatment groups revealed no significant difference. There was no significant difference in time to a first increase in nasal symptoms before and after treatment in either the placebo- or active-treatment groups *(middle panel)*. A highly significant delay in pulmonary symptoms occurred only with active treatment ($p < 0.05$) *(right panel)*. In addition, a comparison of change scores from baseline in the placebo- and active-treatment groups revealed a significant difference ($p < 0.05$).

tially on changes in their tolerance of cats. It was therefore impossible to clearly distinguish adequacy of blinding from the therapeutic effect.

Bronchial provocation with cat allergen and with methacholine

Active treatment resulted in a significant decrease in bronchial sensitivity to cat allergen. The $PD_{20}FEV_{1.0}$ for allergen increased significantly in the active-treatment group ($p < 0.05$), but no significant change occurred in the placebo-treatment group. A direct comparison of change scores from baseline revealed no significant difference between the active-treatment and placebo-treatment groups (Fig. 1).

Responses to bronchial provocation with methacholine demonstrated no significant change in either the active-treatment or placebo-treatment groups (results not illustrated). For the active-treatment group the geometric means $PD_{20}FEV_{1.0}$ and ranges before and after treatment were 3.0 (0.27 to 30.3) and 4.7 (0.21 to 75.7) BU, respectively. For the placebo-treatment group before and after values were 1.7 (0.34 to 54.9) and 3.8 (0.24 to 17.7) BU, respectively.

Skin test responses

The reciprocal prick test titer decreased significantly with active treatment ($p < 0.01$) but not with placebo treatment (Fig. 2). A direct comparison of change scores from baseline revealed highly significant differences between the active-treatment and placebo-treatment groups (Fig. 2) ($p < 0.025$). The change in skin test titer correlated significantly with the cumulative treatment dose ($r = -0.73$, $p < 0.05$). No significant correlation was observed between the cumulative dose and other parameters of response such as the change in the $PD_{20}FEV_{1.0}$ or responses on cat exposure.

Cat exposures

The response to a controlled exposure to living cats was evaluated in several ways. The severity of symptoms varied with the duration of exposure; therefore it was difficult to develop a reliable index of severity of symptoms. The most useful way to evaluate cat exposures was to record the time of first increase in symptoms. For ocular symptoms there was a significant delay in symptoms in the active-treatment group (Fig. 3) ($p < 0.05$). A comparison of change in scores between the active-treatment and placebo-treatment groups revealed no significant difference. For nasal symptoms, no significant change occurred (Fig. 3). For pulmonary symptoms, a significant delay in symptoms occurred with active treatment (Fig. 3) ($p < 0.05$). In addition the change score difference between the active-treatment and placebo-treatment groups was significant ($p < 0.03$).

In general, peak flow measurements after cat exposure demonstrated small changes, but there was a strong suggestion of a beneficial response to active

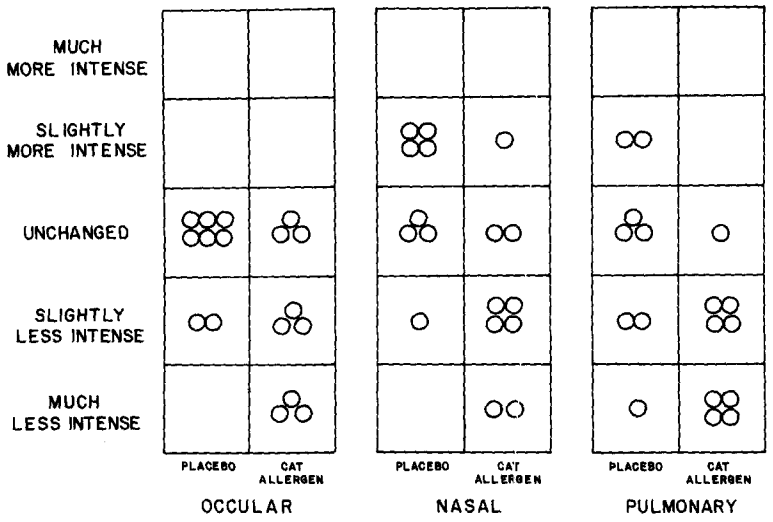


FIG. 4. Subjective evaluation of cat exposures. After the second cat exposure after the conclusion of treatment, subjects were asked to compare the second cat exposure with the first cat exposure with respect to ocular, nasal, and pulmonary symptoms. There was a significant difference between the placebo- and active-treatment subjects with respect to all three categories of symptoms ($p = 0.03$, 0.01, and 0.03, respectively).

treatment. There was no significant difference between the active-treatment subjects and placebo-treatment subjects with respect to mean baseline peak flow before the first cat exposure (97 ± 13% and 92 ± 13% predicted, respectively). Before treatment in the active-treatment group, there was a highly significant drop of 9% in the mean peak flow rate at the time of leaving the cat exposure room ($p < 0.001$, two-tailed paired t test) with before and after values and standard deviations of 453 ± 90 and 414 ± 80 L/min, respectively. In the placebo-treatment group there was a 3% drop with mean values of 375 ± 56 and 363 ± 75 L/min, respectively (not significant). After treatment there was no significant change in peak flow rate on cat exposure in either group with before and after values of 453 ± 83 and 448 ± 98 L/min in the active-treatment group and 371 ± 59 and 355 ± 73 in the placebo-treatment group. A comparison of peak flow rate change between the active-treatment and placebo-treatment groups revealed no significant differences.

After the conclusion of treatment and after the second cat exposure, subjects were asked to compare the second cat exposure with the first cat exposure with respect to ocular, nasal, and pulmonary symptoms. The results of their subjective evaluation are summarized in Fig. 4. Active-treatment subjects perceived a significant improvement in intensity of ocular, nasal, and pulmonary symptoms with treatment compared with placebo-treatment subjects ($p = 0.03$, 0.01, and 0.03, respectively).

Basophil histamine release

Maximum histamine release to cat allergen 1 did not change significantly with treatment in either the placebo- or active-treatment group (Fig. 5). Similarly, the concentration of cat allergen 1 that produced 50% maximal histamine release did not change significantly in either group (results not illustrated). For the active-treatment group the geometric means and ranges before and after treatment were 5.2×10^{-5} (8.0×10^{-6} to 1.0×10^{-3}) and 4.4×10^{-5} (6.0×10^{-6} to 8.0×10^{-4}) U/ml, respectively. For the placebo-treatment group, before and after values were 2.0×10^{-4} (1.2×10^{-5} to 1.2×10^{-2}) and 1.4×10^{-4} (1.2×10^{-5} to 4.0×10^{-2}) U/ml, respectively.

In order to evaluate basophil responsiveness to cat albumin, a single concentration of cat albumin was tested (10 mcg/ml). For the active-treatment group, the means and ranges of percent histamine release at this concentration before and after treatment were 9% (0% to 39%) and 25% (0% to 93%), respectively (not significant). For the placebo-treatment group before and after values were 34% (0% to 89%) and 36% (0% to 86%), respectively (not significant).

IgG-antibody response

IgG-antibody responses to cat allergen 1 are illustrated in Fig. 6. Mean IgG-antibody levels rose only in the active-treatment group from 35% to 91% of control. Of particular note is the wide scatter in baseline measurements of antibody in our patients (range 0% to 124%).

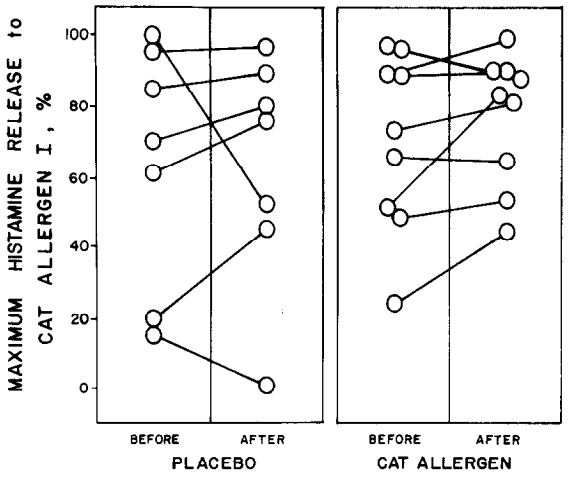


FIG. 5. Maximum basophil histamine release to cat allergen 1. Mean maximum histamine release changed from 66% to 62% in the placebo-treatment group (NS) and from 69% to 75% in the active-treatment group (NS).

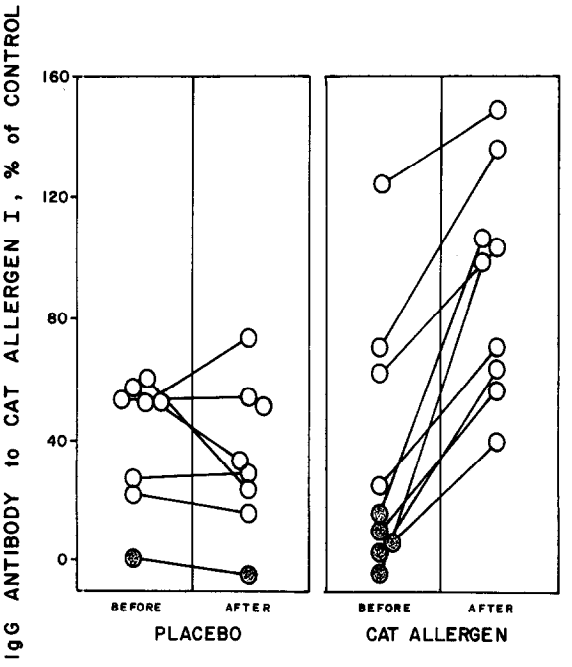


FIG. 6. IgG-antibody response to cat allergen 1. Mean IgG-antibody levels to cat allergen 1 changed from 41% to 35% of standard in the placebo-treatment group (NS) and from 35% to 91% in the active-treatment group ($p < 0.001$). A comparison of change from baseline between the placebo- and active-treatment groups revealed a highly significant difference ($p < 0.001$). *Shaded circles* represent values that fall within the 95% confidence interval of repetitive measurements of the normal control serum.

IgG antibody to cat albumin also rose significantly in the active-treatment group from 3% to 26% of control (Fig. 7). Two patients in the placebo-treatment group had levels of antibody before treatment that exceeded the posttreatment levels of the active treatment group.

DISCUSSION

Study of the efficacy of immunotherapy in allergic asthma is complicated by the highly variable natural history of the disease and by the fact that bronchial asthma has a number of nonallergic causes. Even in presumed allergic asthma, it is sometimes difficult to be sure of what allergens are causing the disease.[10, 11] An optimal study of efficacy of immunotherapy in allergic asthma should meet the following criteria: (1) selection of subjects whose disease is likely caused by allergens in the treatment preparation, (2) adequate dose of a standardized treatment preparation, (3) placebo control, (4) random assignment into treatment groups, (5) adequate indices of response, and (6) blinded evaluation of the response with adequate statistical analysis. The present study that was designed to meet these criteria clearly demonstrates that a brief 4-month course of immunotherapy results in the amelioration of allergic asthma.

This study has confirmed the results of a previous study,[14] demonstrating that immunotherapy with cat allergen results in a significant decrease in bronchial sensitivity as well as a significant decrease in skin test reactivity to cat allergen. The clinical relevance of this immunotherapy was also demonstrated by testing subjects' responses after exposure to living cats. There was a significant delay in the appearance of ocular and pulmonary but not nasal symptoms on cat exposure.

The cumulative dose of cat allergen 1 administered to the nine patients who received the active treatment ranged from 0.24 to 24.8 units. This is significantly lower than the cumulative dose of cat allergen 1 administered in the previous study.[14] Five active-treatment patients in that study received from 69 to 188 units over the same time interval. In this study we have used a unit designation for cat allergen 1 in collaboration with Dr. Harold Baer of the National Center for Drugs and Biologics. One microgram of cat allergen 1 referred to in our previous study[14] is equal to 0.04 units.

This study used a purified fraction of cat-pelt extract as the treatment preparation. Partial purification of the cat allergen was carried out because of the crude nature of the starting material (extracts of whole cat pelt) and because of the high albumin content of many such extracts.[21] The most concentrated material used for treatment in the present study contained 13 units of cat allergen 1 and 300 mcg/ml of albumin.

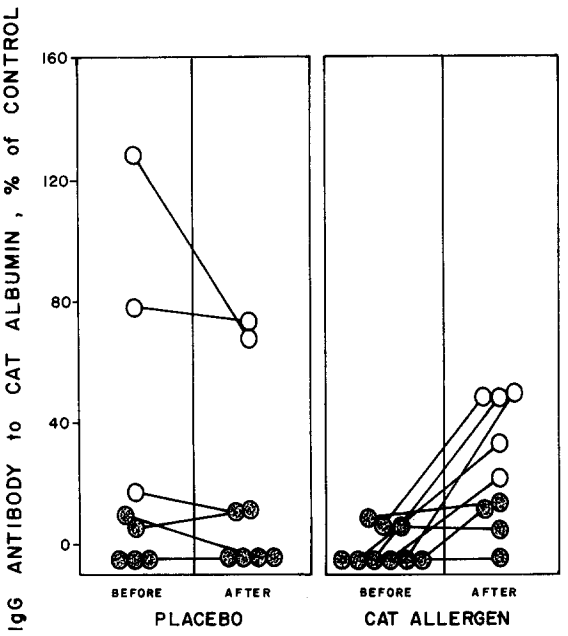


FIG. 7. IgG antibody to cat albumin. Mean IgG-antibody levels to cat albumin changed from 30% to 21% of standard in the placebo-treatment group (NS) and from 3% to 26% in the active-treatment group ($p < 0.01$). A comparison of change from baseline between the placebo- and active-treatment groups revealed a significant difference($p < 0.01$). *Shaded circles* represent values that fall within the 95% confidence interval of repetitive measurements of the normal control serum.

The most concentrated material used in the previous study[21] contained 64 units of cat allergen 1 and 4960 mcg/ml of albumin. The cumulative doses of cat allergen 1 administered in our studies would be difficult to achieve with commercial cat epithelial extracts. The cat allergen 1 content of eight randomly selected 1:10 w/v commercial cat epithelial extracts ranged from 0.04 to 1 U/ml.[13]

IgG antibody to both cat allergen 1 and cat albumin rose significantly with treatment (Figs. 6 and 7), and as we have observed before with a different assay system,[21] most patients had measurable levels of IgG antibody to cat allergen 1 before treatment. The rises that occurred with treatment were overshadowed by the scatter of baseline values. As we have observed before,[21] levels of IgG antibody to albumin are frequently unmeasurable before treatment. Immunotherapy resulted in a significant increase in IgG antibody to albumin, although the posttreatment levels of some subjects actually fell below the before-treatment levels of several other subjects (Fig. 7). It appears that IgG antibody to albumin increased less intensively in this study than in our previous study in which higher immunizing doses of albumin were used.[21]

This study failed to show a significant change in basophil sensitivity to cat allergen 1 with treatment. Neither maximum histamine release nor the concentration of cat allergen 1 that produced a 50% maximal histamine release changed significantly. Previous studies of pollen allergy have frequently demonstrated a decrease in basophil sensitivity after more prolonged immunotherapy regimens,[22–24] but this has not always been observed.[25] It is clear from the results of the present study that beneficial changes in at least some parameters of clinical response can occur in the absence of a significant change in the sensitivity of circulating basophils. The reduction in skin test and bronchial sensitivity suggests that mast cells behave differently than basophils in response to immunotherapy.

There was a high frequency of systemic reactions to the immunotherapy in the present study. Ten such reactions occurred in four patients who received the active treatment. A similar frequency was also observed in our previous study.[13] All reactions were mild and easily treated. We believe that it is likely that this is due to the intensive nature of the treatment schedule. We cannot rule out, however, that there is some peculiar property of cat allergens that is responsible for the higher apparent rate of such reactions. Future immunotherapy protocols with cat allergens should be modified to allow a more gradual increase in immunizing dose.

It might be expected that a high frequency of systemic and large local reactions would cause difficulty in the blinding of the study. Eight of nine subjects who received the active treatment and three of eight subjects who received the placebo treatment believed that they had received the active treatment; the two groups differed significantly ($p < 0.05$). As indicated in the results, the decision of four active-treatment and four placebo-treatment subjects was at least partially on the basis of an evaluation of their tolerance of cats. It was therefore impossible to clearly distinguish adequacy of blinding from the therapeutic effect. An evaluation of the adequacy of blinding in future studies would be more nearly accurate if exposure to cats could be eliminated until this evaluation was carried out.

A partial unblinding of the study would be expected to effect a subjective analysis (Fig. 4) more than objective measures. Stringent precautions were taken to exclude the investigator who carried out the clinical evaluations (skin testing and bronchial provocation) from a knowledge of the side effects of immunotherapy. A rigorous analysis of adequacy of blinding has seldom been carried out in previous studies of immunotherapy.

In summary, a double-blind evaluation of immuno-

therapy in cat-induced asthma was carried out in 17 carefully selected subjects. Four months of immunotherapy resulted in a significant reduction in cutaneous and bronchial sensitivity to cat allergen as well as a delay in the onset of asthmatic and ocular symptoms on exposure to living cats. This was associated with a significant increase in levels of IgG antibody to cat allergen 1 and less intense but significant increases in IgG antibody to albumin. These changes occurred in the absence of significant change in basophil sensitivity to cat allergen.

We wish to thank Marsha Williams for her technical assistance.

REFERENCES

1. Citron KM, Frankland AW, Sinclair JD: Inhalation tests of bronchial hypersensitivity in pollen asthma. Thorax 13:229, 1958
2. McAllen MK: Bronchial sensitivity testing in asthma. Thorax 16:30, 1961
3. Aas K: Hyposensitization in house dust allergy asthma. A double-blind controlled study with evaluation of the effect on bronchial sensitivity to house dust. Acta Paediatr Scand 60:264, 1971
4. Tuchinda M, Chai H: Effect of immunotherapy in chronic asthmatic children. J ALLERGY CLIN IMMUNOL 51:131, 1973
5. Bruun E: Control examination of the specificity of specific desensitization of asthma. Acta Allergol II:122, 1949
6. Johnstone DE, Crump L: Value of hyposensitization therapy for perennial bronchial asthma in children. Pediatrics 27:39, 1961
7. Warner JO, Soothill JF, Price JF, Hey EN: Controlled trial of hyposensitization to dermatophagoides pteronyssinus in children with asthma. Lancet 2:912, 1978
8. Hill DJ, Hosking CS, Shelton MJ, Turner MW: Failure of hyposensitization in treatment of children with grass pollen asthma. Br Med J 284:306, 1982
9. Forgacs P, Swan AV: A report from the Research Committee of the British Tuberculosis Association: treatment of house dust allergy. Br Med J 3:774, 1968
10. Bruce CA, Norman PS, Rosenthal RR, et al: The role of ragweed pollen in autumnal asthma. J ALLERGY CLIN IMMUNOL 59:449, 1977
11. Salvaggio J, Aukrust L: Mold-induced asthma. J ALLERGY CLIN IMMUNOL 68:327, 1981
12. Ohman JL Jr, Lowell FC, Bloch KJ: Allergens of mammalian origin: characterization of allergens extracted from cat pelts. J ALLERGY CLIN IMMUNOL 52:231, 1973
13. Ohman JL Jr, Lowell FC, Bloch KJ, Kendall S: Allergens of mammalian origin. V. Properties of extracts derived from the domestic cat. Clin Allergy 6:419, 1976
14. Taylor WW, Ohman JL Jr, Lowell FC: Double-blind trial of immunotherapy in cat induced asthma. J ALLERGY CLIN IMMUNOL 61:283, 1978
15. Chai H, Farr RS, Froehlich LA, Mathison DA, McLean JA, Rosenthal RR, Sheffer AL II, Spector SL, Townley RG: Standardization of bronchial inhalation challenge procedures. J ALLERGY CLIN IMMUNOL 56:323, 1975
16. Findlay SR, Dvorak AM, Kagey-Sobotka A, Lichtenstein LM: Hyperosmolar triggering of basophil histamine release in vitro. J Clin Invest 67:1604, 1981
17. Leitermann K, Ohman JL Jr: Affinity purification as a rapid and efficient means of allergen purification. J ALLERGY CLIN IMMUNOL 69:104, 1982 (Abst)
18. Siraganian RP: An automated continuous-flow system for the extraction and fluorometric analysis of histamine. Anal Biochem 57:383, 1974
19. Laurell CB: Quantitative estimation of proteins by electrophoresis in agarose gel containing antibodies. Anal Biochem 15:45, 1966
20. Wilcoxon FD: Biometrics Bul 1:80, 1945
21. Ohman JL Jr, Marsh DG, Goldman M: Antibody responses following immunotherapy with cat pelt extract. J ALLERGY CLIN IMMUNOL 69:319, 1982
22. Sadan N, Rhyne MB, Mellits ED, et al: Immunotherapy of pollinosis in children. Investigation of the immunologic basis of clinical improvement. N Engl J Med 280:623, 1969
23. Lichtenstein LM, Norman PS, Winkenwerder WL: A single year of immunotherapy for ragweed hay fever. Immunologic and clinical studies. Ann Intern Med 75:663, 1971
24. Irons JS, Pruzansky JJ, Patterson R, Zeiss CR: Studies of perennial ragweed immunotherapy. J ALLERGY CLIN IMMUNOL 59:190, 1977
25. Levy DA, Lichtenstein LM, Goldstein EO, Ishizaka K: Immunologic and cellular changes accompanying the therapy of pollen allergy. J Clin Invest 50:360, 1971