

Immunotherapy with cat- and dog-dander extracts

II. In vivo and in vitro immunologic effects observed in a 1-year double-blind placebo study

G. Hedlin, M.D.,* V. Graff-Lonnevig, M.D.,** H. Heilborn, M.D.,***
G. Lilja, M.D.,** K. Norrlind, M.D.,*** K-O Pegelow, M.D.,*
B. Sundin, M.D.,* and H. Löwenstein, Ph.D.**** Huddinge, Sweden

The effects of immunotherapy on the skin prick test, allergen-specific IgE, and IgG in 39 patients (19 adults and 20 children), treated with partially purified cat- or dog-dander extracts or placebo for 1 year, were studied by use of a double-blind protocol. IgG levels were measured by three different assays: IgG RAST, IgG4 RAST, and Staph A IgG1, 2, and 4. The skin prick test reaction decreased continuously in the allergen-treated patients, the decrease being the first sign of an immunologic effect of the therapy. Allergen-specific IgE levels increased during the first 9 months in both children and adults. The RAST activity during the last 3 months continued to rise for the children, whereas it declined for the adults. IgG levels measured by all three methods demonstrated an increase in the allergen-treated patients and no increase in the placebo-treated patients. The children developed higher values on IgG RAST and IgG4 RAST than the adults. IgG RAST correlated negatively with IgE levels in the cat allergen-treated group. No correlation between skin prick test results, IgE levels, and IgG levels was found, nor was there any correlation between these parameters and the patients' own subjective evaluation or the allergen bronchial challenge test. In summary, the expected change in skin prick test reaction and allergen-specific IgE and IgG levels was found. The children tended to be more immunologically active than the adults. (J ALLERGY CLIN IMMUNOL 77:488-96, 1986.)

The beneficial effects of immunotherapy with partially purified extracts have been convincingly demonstrated in studies of pollen-allergic patients.¹⁻⁵ Successful hyposensitization has also been reported with extracts of *Dermatophagoides pteronyssinus* in both adults⁶ and children.⁷ Furthermore, immunotherapy of insect-hypersensitive patients with insect venom has been demonstrated to elicit protection against sting reactions.⁸ Previous studies on immunotherapy with crude animal-dander extracts have yielded doubtful results.^{9, 10} In a study by Taylor et al.¹¹ with partially purified cat-dander extract, a reduction in skin reactivity and bronchial sensitivity was reported. In an article in this issue of the JOURNAL, page 478 we have reported the clinical results of a double-blind placebo study on cat and dog allergen-sensitive children with

Abbreviations used

HEP:	Histamine equivalent prick
BPT:	Bronchial provocation test
PC ₂₀ :	Provocation concentration of allergen or histamine causing a rise to a 20% decrease in peak expiratory flow rate
SPT:	Skin prick test
SI:	Skin index
Al:	Aluminum hydroxide
Staph A:	Protein A from <i>Staphylococcus aureus</i>

asthma and adults treated with a partially purified cat- or dog-dander extract (Alutard SQ) during a period of 12 months. A significant decrease in bronchial sensitivity was observed after immunotherapy, and most patients reported an increased tolerance to cat/dog exposure. The observed responses in IgE and IgG levels to the individual allergenic molecules, together with an analysis of the level of cat- and dog-derived material in dust from patients' homes will be presented in a subsequent paper.¹²

The immunologic responses to hyposensitization as

From the *Huddinge, **Danderyd, and ***Sachs University Hospitals, Stockholm, Sweden, and the ****Protein Laboratory, University of Copenhagen, Copenhagen, Denmark.

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Reprint requests: Gunilla Hedlin, M.D., Department of Pediatrics, Huddinge University Hospital, S-141 86 Huddinge, Sweden.

measured by skin tests and by allergen-specific IgE and IgG levels have also been reported in most of the above mentioned studies on immunotherapy with pollen, venom, or house dust-mite extracts. A decrease in skin reactivity has been observed^{3, 4, 13, 14} in treated pollen-allergic patients, and an initial rise in specific IgE levels followed by a decrease has been reported in several studies.^{2, 15, 16, 17} The effects of the pollen season, however, make comparisons difficult. With house dust mite-allergic patients, immunotherapy has led to divergent results with respect to the specific IgE response. D'Souza et al.⁶ found an increase in IgE levels in half of their patients, whereas Turner et al.¹⁸ found no significant change in specific IgE levels. In venom-treated patients both increasing and decreasing venom-specific IgE titers have been reported.¹⁹ The IgE response has not been reported in the previous studies on animal-dander immunotherapy. Levels of allergen-specific antibodies of the IgG type generally increase during successful immunotherapy.²⁰ A correlation between the rise in IgG level and the clinical response has been claimed^{21, 22} but has been questioned in other studies.^{18, 23} In a study of venom immunotherapy by Golden et al.,²⁴ an allergen-specific IgG level above 5 µg/ml is claimed to be protective for venom-allergic patients.

The present article describes the effect of immunotherapy with a partially purified cat- or dog-dander extract on skin reactivity and in vitro parameters during a 12-month treatment period. Allergen-specific IgE (RAST) and IgG levels are reported, the latter having been measured by three different methods.

MATERIAL AND METHODS

Allergen extracts

Standardized, partially purified allergenic extracts of cat dander and dog dander were obtained from ALK, Copenhagen, Denmark. A 50% (v/v) glycerol solution (Soluprick SQ) was used for SPT, and a water-soluble form (Aquagen SQ) of the same material was used for all the in vitro tests. Methods of standardization, as well as a detailed antigenic analysis of the extracts, are reported in accompanying articles pages 478 and 497.

Patients

The 41 patients, 21 adults (18.1 to 47.2 years of age) and 20 children (8.0 to 16.1 years of age), participating in this study, have been described in an article page 478. All had a history of bronchial asthma after exposure to cats and/or dogs. Twenty-three patients were treated with active cat- or dog-dander extracts. Eighteen patients were treated with placebo and then were divided into two groups, the cat-allergic placebo-treated and dog-allergic placebo-treated groups, respectively. One patient in each group did not complete the treatment and was considered as a dropout.

SPTs

SPTs were performed on all the patients before the start of immunotherapy and again after 3, 6, and 12 months of continuous immunotherapy. The procedure on each occasion included the use of histamine hydrochloride (1 mg/ml) as positive control and the diluent as negative control. Increasing allergen concentrations of 0.1, 0.3, 1, 3, and 10 HEP were used for SPTs. The HEP potency is a measure of the biological activity of the extract.²⁵ The SPT was made on the volar side of the forearm with an ordinary blood lancet placed at an angle of approximately 60 degrees to the skin. The lancet tip, inside the allergen drop just entering the epidermis, was tipped slightly and withdrawn. After 5 minutes the drops were wiped off. After a further 10 minutes, each wheal was outlined with a ballpoint pen, and the outline was transferred to squared millimeter paper with cello tape. A wheal of 2 mm or more was regarded as a positive reaction to the allergen.

Each SPT result is presented in the form of an SI, which was calculated by multiplying the largest diameter of the wheal by the largest diameter perpendicular to this and then dividing this product by the corresponding product of diameters for the histamine wheal. The tests were performed singly with the skin titration procedure with both cat- and dog-dander extracts each time. The same trained nurses performed all the tests made throughout the study.

Specific IgE levels

Serum-specific IgE levels were measured by Al RAST as recently described.¹⁵ The allergen extracts (0.2 ml), cat SQ units (0.4 mg/ml) and dog SQ units (0.9 mg/ml), were dissolved in 2 ml of coupling buffer (Tris HCl 0.1 mol/L, pH 7.5) with 1% (w/v) Tween 20 and 0.015 mol/L of sodium azide and absorbed to 50 µl of Alhydrogel (Superfos; Vedbaek, Denmark) containing 2% (w/v) Al(OH)₃ (equivalent to 1.3% [w/v] Al₂O₃). The suspensions were shaken gently for 15 minutes at room temperature and then were centrifuged at 3000 rpm for 15 minutes. The supernatant was withdrawn cautiously, leaving about 0.2 ml above the precipitates. This washing procedure was repeated four times with 2.5 ml of coupling buffer. After washing, the precipitates were redissolved in 5 ml of coupling buffer and were now ready for use. Aliquots of 0.1 ml of the well-mixed suspension were distributed in small plastic tubes, and 0.05 ml of serum sample was applied and allowed to react for 24 hours at room temperature. Coupling buffer (2.5 ml) was added, and the tubes were centrifuged at 3000 rpm for 3 minutes followed by suction, leaving 0.2 ml of free supernatant. This last procedure was repeated three times. After the fourth washing procedure, all tubes were turned upside down for a few seconds in order to decant the free supernatant. Fifty micrograms of ¹²⁵I-labeled anti-IgE (Pharmacia Diagnostics AB, Uppsala, Sweden) was added to the tubes. After 24 hours at room temperature, 2.5 ml of coupling buffer was added. The mixture was centrifuged and washed as described previously. After the fourth washing procedure, the last 0.2 ml of free supernatant above the precipitates was retained. Finally, the

TABLE I. Skin index before treatment and after 3, 6, and 12 months of treatment in the four respective groups (mean $\pm$ SD)

Allergen-treated patients		Placebo-treated patients	
Cat-allergic SI (15) (3 HEP)	Dog-allergic SI (7) (3 HEP)	Cat-allergic SI (12) (3 HEP)	Dog-allergic SI (5) (3 HEP)
Before treatment			
1.8	1.2	1.8	2.5
± 0.6	± 0.6	± 1.0	± 2.2
After treatment			
3 mo 1.2*	1.2	1.7	2.6
± 0.8	± 0.6	± 1.5	± 1.4
6 mo 1.0 [†] , [‡]	1.0	2.2	1.1
± 0.3	± 0.7	± 1.2	± 0.3
12 mo 0.8 [§] , [‡]	0.6*	4.3	3.8
± 0.4	± 0.3	± 5.5	± 3.6

Number of patients in parentheses.

*Difference after 3 months of treatment, $p < 0.05$.

[†]Difference after 6 months of treatment, $p < 0.01$.

[‡]Difference from placebo group, $p < 0.05$.

[§]Difference after 12 months of treatment, $p < 0.001$.

amount of specifically labeled anti-IgE bound to the Al-hydrogel-adsorbed allergen was counted (5 minutes). The result was expressed as a percentage of the total added radioactivity.

All analyses were performed in duplicate, and the mean values were used. The errors in the analyses for the individual allergen extracts range from 10% to 20% relative deviation.

Specific IgG levels

The isolation of allergen-specific immunoglobulins was performed as follows: Sera (0.05 ml) from the patients were applied on columns (0.2 ml) packed with cat or dog SQ units of extract coupled to Sepharose 4B and equilibrated with buffer 1 (sodium citrate and sodium chloride, ionic strength, 0.5, pH 6.8). The columns were washed with 5-column volumes of buffer 1 after which desorption of allergen-specific immunoglobulins was achieved by washing with 5 volumes of buffer 2 (sodium citrate and sodium chloride, ionic strength, 0.5, pH 2.7). The desorbed immunoglobulin samples were adjusted to pH 7.6, diluted with 1% (w/v) bovine serum albumin to a total volume of 2.0 ml, and stored at -20°C before being analysed.

IgG RAST was performed as for IgE RAST²⁶ with cat or dog SQ units of extract coupled to paper discs (coupling conditions, 1 mg/ml) as allergosorbent. Bound allergen-specific IgG was detected by use of Fc-specific rabbit antihuman IgG (DAKO, Copenhagen, Denmark), which was immunoabsorbed and desorbed exactly as described for anti-IgE,²⁷ and was finally labeled by use of the chloramine-T method.²⁸

IgG4 RAST was performed as IgG RAST except with ¹²⁵I-anti-IgG4 instead of ¹²⁵I-anti-IgG (Fc). Sheep antihuman IgG4 was supplied as described recently from the Central Laboratory of the Netherlands Red Cross Transfusion

Service, Amsterdam, Holland.²⁹ The antibodies were radiolabeled as described for anti-IgG (Fc) and used for detection.

Staph A IgG1, 2, and 4 test was performed as described elsewhere^{30, 31} except with paper discs instead of Sepharose 4B as the solid support for cat or dog SQ units of extracts. Coupling conditions were as above. Detection of bound cat or dog allergen-specific IgG1, 2, and 4 was performed with ¹²⁵I-Staph A (batch NEX 146 L) obtained from New England Nuclear, Boston, Mass.

The results were listed, by all three methods, as percentage of binding relative to amount added of labeled materials.

Statistics

Student's *t* test for paired and unpaired observations and linear regression analysis were used when they were applicable (SPT and RAST). The comparisons within the same group of patients at different times were performed by means of Wilcoxon matched-paired signed-rank test (IgG RASTs); comparisons between matched groups of patients at the same time were performed by means of Mann-Whitney statistics and/or Student's *t* test for unpaired observations; comparisons between different assays were performed by means of the Spearman rank correlation test. All stochastic probabilities <0.05 were considered significant.

RESULTS

SPTs

All patients demonstrated an initially positive SPT (SI > 0.05) to both the 3 HEP and 10 HEP extracts. There was no significant change in the size of the histamine wheal in any of the allergen-treated groups during the study. A decrease in the histamine wheal

was, however, observed in the cat placebo-treated group after 12 months ($p < 0.05$).

A continuous decrease in the wheal reaction in the allergen SPTs was observed throughout the study for both allergen-treated groups. The two lowest concentrations (0.1 and 0.3 HEP) generally elicited wheal reactions that were too small to be measured. Mean values for SI obtained with the 3 HEP concentration during the study are presented in Table I for the allergen- and placebo-treated groups. A significant decrease in SI was observed after only 3 months of treatment in the cat-allergic group ($p < 0.05$) with the 10 HEP cat-allergen extract (data not presented). When the 3 HEP SI in this group, after 6 and 12 months of treatment, was compared with the initial SI, the decrease was highly significant ($p < 0.01$ and $p < 0.001$, respectively) (Table I). Decreases in wheal sizes were observed also in the dog-allergic group. After 12 months of treatment, SI obtained by the 3 HEP extract had decreased significantly ($p < 0.05$) (Table I).

There was no decrease in SI among the patients treated with placebo during the study. In the cat-allergic placebo-treated group, there was an increase in SI during the last 6 months of the study ($p < 0.05$) (Table I) that is probably explicable on the basis of the decrease in the size of the histamine wheal. When a comparison was made between the allergen-treated and placebo-treated cat-allergic patient groups, SI was not significantly different before the start of immunotherapy in the two groups. After 6 and 12 months of treatment, the SI had decreased in the cat allergen-treated group relative to the placebo-treated group ($p < 0.05$ for 1, 3, and 10 HEP) (Table I).

When the SI obtained with dog-allergen extracts in the allergen-treated cat-allergic group were considered and vice versa for the allergen-treated dog-allergic group, no changes in SI with 1, 3, or 10 HEP extracts were observed in these respective groups. No differences in the SPT reactions in children relative to adults were observed in either the allergen- or placebo-treated groups.

Specific IgE (RAST)

The RAST results expressed as a percentage of the total radioactivity were comparable in the allergen-treated and placebo-treated cat- and dog-allergic patients, respectively, before therapy was started. Thirty-seven patients were RAST positive, and two of the adults were RAST negative. The allergen-treated children, however, had higher RAST values than the allergen-treated adults ($p < 0.05$). This difference persisted after the 12-month study period.

The allergen-treated cat-allergic children demonstrated an increase in specific IgE RAST activity after

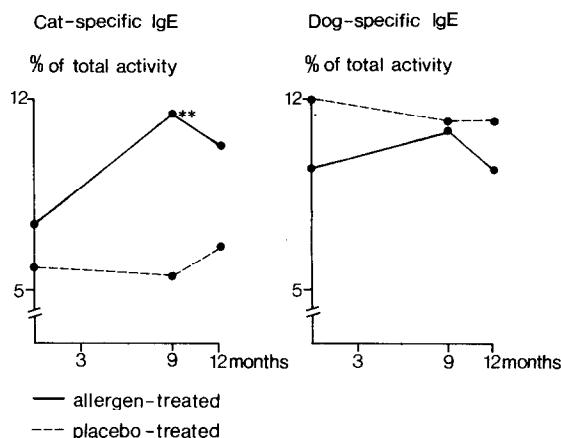


FIG. 1. Changes in mean allergen-specific IgE. The results are listed in percent of total activity (^{125}I -anti-IgE) added in the radioimmunoassay. Asterisks indicate differences from the RAST activity before treatment (* $p < 0.05$, ** $p < 0.01$); —, allergen-treated; ---, placebo-treated.

12 months of treatment ($p < 0.05$), whereas an increase was observed after only 9 months in the cat-allergic adults ($p < 0.01$). The latter group then demonstrated decreased activity with a significant change again after 12 months ($p < 0.05$), although the RAST activity at that time was no longer significantly different from the initial activity. The mean specific IgE response for cat-allergic adults and children in the allergen- and placebo-treated groups is illustrated in Fig. 1 (left panel). There was no change in the dog RAST activity in either of the groups treated with cat-allergen extracts.

In the allergen-treated dog-allergic patients, there was no significant change in dog RAST activity after 12 months of treatment either in the children or in the adults (Fig. 1, right panel), nor was there any change in the cat RAST activity.

In the placebo-treated patients there was no change in either the cat or dog RAST values.

Specific IgG levels

Allergen-specific IgG antibodies against the antigens present in cat or dog SQ extracts were determined by three independent assays performed on isolated antigen-specific immunoglobulins. The concentrations of these antibodies before the start of hyposensitization were determined by rockets and found to be within the range 4 to 51.2 $\mu\text{g/ml}$ (mean 14.4 $\mu\text{g/ml}$) of total IgG for children and adults when concentrations were estimated relative to total IgG in a pool of sera from 1000 normal individuals.³²

The changes in IgG levels for allergen- and placebo-treated cat- and dog-allergic groups are illustrated in Fig. 2, A and B. After 12 months of treatment,

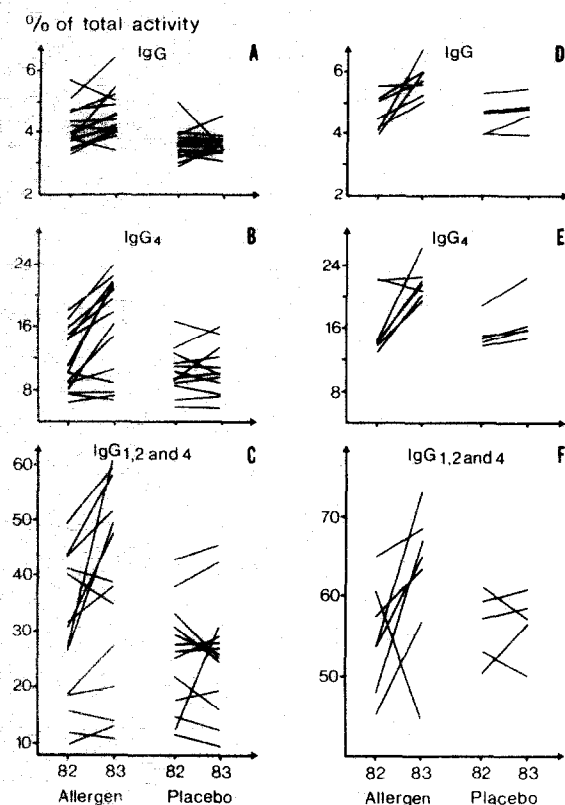


FIG. 2. Changes in allergen-specific IgG determined by three independent methods. **A** and **D**, IgG RAST; **D** and **E**, IgG4 RAST; **C** and **F**, Staph A method. The corresponding results for the individual patients before and after 1 year of treatment are indicated. The results are listed in percent of total activity (^{125} I-anti-IgG, ^{125} I-anti-IgG4, and ^{125} I Staph A) added in the radioimmunoassays.

statistically significant differences were found between the allergen- and placebo-treated groups (cat, $p < 0.05$; dog, $p < 0.01$). However, the pretreatment levels of cat allergen-specific IgG differed slightly in the groups. The levels of specific IgGs after 12 months of treatment demonstrated statistically significant differences from the pretreatment values in both the allergen-treated cat-allergic group ($p < 0.01$) and the dog-allergic group ($p < 0.05$) but not in the placebo-treated groups.

The IgG4 data varied similarly (Fig. 2, *B* and *E*). After 12 months of treatment, statistically significant differences (cat, $p < 0.05$; dog, $p < 0.05$) were found for the matched allergen- and placebo-treated patient groups. By comparison with the pretreatment values, both the cat allergen- and dog-allergen-treated patient groups demonstrated statistically significant increases in the IgG4 values ($p < 0.01$ and $p < 0.05$, respectively).

The IgG data for IgG1, 2, and 4 obtained by the Staph A method (Fig. 2, *C* and *F*) revealed few dis-

tinct results. Only the differences between the pretreatment and 12-month levels for the cat-allergic group were statistically significant ($p < 0.05$).

Treating the cat and dog allergen-treated groups statistically as one group, significant differences were found for the matched allergen- and placebo-treated patients after 12 months of treatment in terms of IgG RAST and IgG4 RAST measurements (IgG, $p < 0.001$; IgG4, $p < 0.01$). Statistically significant increases relative to the pretreatment values were found with all three assays (IgG, $p < 0.001$; IgG4, $p < 0.001$; and IgG1, 2, and 4, $p < 0.05$).

We also performed statistical analyses on the IgG responses for cat allergen-treated children and adults alone as well as on the entire group of both cat allergen- and dog allergen-treated children and adults. For the cat allergen-treated children and adults, significant differences after 12 months of treatment were found between the matched allergen- and placebo-treated groups in one of the three tests with the children (IgG, $p < 0.05$) and in two of the three tests with the adults (IgG, $p < 0.01$; IgG4, $p < 0.05$). Relative to the pretreatment values, statistically significant increases were found for the children in all three tests (IgG, $p < 0.05$; IgG4, $p < 0.05$; IgG1, 2, and 4, $p < 0.05$) and for the adults in one test (IgG4, $p < 0.05$).

Statistically significant differences were also found for the matched allergen- and placebo-treated cat- and dog-allergic children after 12 months of treatment (IgG, $p < 0.01$; IgG4, $p < 0.05$; IgG1, 2, and 4, $p < 0.05$) but not for the adults. Relative to the pretreatment values, statistically significant increases were found for the children in all tests (IgG, $p < 0.01$; IgG4, $p < 0.01$; IgG1, 2, and 4, $p < 0.01$) and for the adults in two tests (IgG, $p < 0.05$; IgG4, $p < 0.01$).

No differences in IgG, IgG4, and IgG1, 2, and 4 levels between children and adults were observed before immunotherapy was started. After 12 months of treatment, the children in the allergen-treated groups had higher levels of IgG and IgG4 than the allergen-treated adults ($p < 0.05$ for both). However, there was no significant difference in IgG1, 2, and 4 levels between the children and the adults.

Comparison of the IgG assays

A comparison of the results of the three IgG assays within the entire group of patients, i.e., all allergen- and placebo-treated cat- and dog-allergic patients, revealed significant correlations between IgG and IgG4 levels ($r = 0.49$; $p < 0.01$), IgG and IgG1, 2, and 4 levels ($r = 0.49$; $p < 0.01$), and IgG4 and IgG1, 2, and 4 levels ($r = 0.53$; $p < 0.001$).

In the cat allergen-treated group, the best correlation was found between IgG4 and IgG1, 2, and 4 levels ($r = 0.74$; $p < 0.001$), whereas in the dog allergen-treated group, the levels of IgG and IgG1, 2, and 4 demonstrated a very good correlation ($r = 0.93$; $p < 0.001$).

Correlations

The changes in specific IgE levels correlated negatively with the changes in IgG levels when the results for both allergen-treated groups were analyzed after the treatment period ($r = 0.55$; $p < 0.01$). There was no significant correlation between the changes in the results of bronchial allergen-challenge tests and the SPT results for the allergen-treated groups, and there was no correlation between these *in vivo* parameters and the changes in IgG, IgG4, IgG1, 2, and 4 or allergen-specific IgE levels.

DISCUSSION

As in most previous investigations, a steady decrease in skin reactivity to the allergen extracts was observed during the treatment. This effect has been noted previously, mostly with pollen allergen-treated patients^{3, 4, 13, 14, 33} but also in two controlled studies by Taylor et al.¹¹ and by Ohman et al.³⁴ of cat-allergen hyposensitization. In the present study the decrease in skin reactivity was an early sign of the immunologic effect of the therapy, a significant decrease in SI being observed after only 3 months of treatment. Skin reactivity continued to decrease during the following 9 months but relatively less so than during the previous 3 months. No significant correlation between the decrease in SI and the patients' subjective evaluation after 1 year was found. Nor was there any correlation with the changes in allergen-specific IgE levels. If, however, the mean change in SI after 1 year of treatment was compared for those patients who felt a subjective improvement in their cat/dog allergy and those who felt no subjective improvement, there was a significantly greater decrease of SI in the former group. This difference indicates a clinical relevance of the change in SI. No predictive information could be obtained from the SPTs after 3 months of treatment, since the individual variations were large.

In the cat-allergic placebo-treated patient group, the mean size of the histamine wheal decreased during the treatment period. This significant change, which was partly caused by small histamine wheals in two patients, contributed to an increase of mean SI (Table I), a fact that could have affected the comparison of the SI for the allergen- and placebo-treated cat-allergic groups. However, the increased SI in the placebo-treated patients could also be attributed to an immu-

nologic effect caused by the histamine-containing placebo extracts. It has been suggested that histamine influences the immune process, e.g., by reducing the suppressor cell activity and thereby increasing the immune response.³⁵

The initial rise in allergen-specific IgE levels during immunotherapy reported by others^{2, 15-17, 36} was also confirmed in this study. The children started with higher allergen-specific IgE levels than the adults. The age group of children participating in this study is, however, known to have higher total IgE levels than adults,³⁷ a fact that could explain this difference. Another difference between the children and adults was the persistent rise in allergen-specific IgE levels among the children also during the months 9 to 12 of therapy. A comparable finding was made by Nordvall et al.¹⁶ with a timothy pollen-treated group of children. They observed no suppression of IgE synthesis attributable to the immunotherapy in treated children relative to a group of untreated children. This is an indication of the increased tendency for development of IgE antibodies in children that could be the reason for the persistent rise in IgE levels for the children throughout the study. There was, however, no sign of any effects of the higher level of allergen-specific IgE on the SPT results or on the allergen bronchial provocation results for the children, since no differences in SPT and BPT were noted between children and adults. The adults demonstrated a decrease in allergen-specific IgE levels during the last part of the study, such as has been observed in pollen- and venom-treated adults.^{15, 17, 19, 38, 39} There was no significant correlation between the initial RAST score and the subjective improvement reported by the patients. Neither was there any correlation between the change in RAST and the change in skin reactivity or the PC₂₀ of the bronchial provocation tests. This is in keeping with other studies that have also failed to demonstrate any predictive importance of the RAST value or any correlations between the allergen-specific IgE levels and clinical improvement.^{17, 40, 41} A negative correlation was found between the change in IgE RAST and IgG RAST in the allergen-treated groups. Other studies have pointed out such a relation between the level of IgE and IgG.^{15, 17, 41, 42}

Østerballe et al.,¹⁵ Gleich et al.,⁴¹ and Weyer et al.⁴² found a positive correlation between the rise in IgE and IgG levels during preseasonal hyposensitization with pollen extracts. In the latter two studies,^{41, 42} the IgE-IgG level correlation persisted after 1 year of treatment. Lichtenstein et al.,¹⁷ on the other hand, found a negative correlation between the rise in IgG and the fall in IgE that is comparable to our finding. The immunologic effects of the pollen season make com-

parisons to our study difficult. In neither mite nor cat hyposensitization studies have correlations between IgE and IgG levels been reported.^{18, 34} The relationship between IgE and IgG levels might depend on the duration of the immunotherapy, since the most frequently reported effects are an initial rise in IgE level simultaneously with a rise in IgG level. The IgE level then decreases, whereas the IgG level continues to rise to a plateau value that is then maintained during continuous therapy. Thus, one probable explanation of the inverse correlation between IgE and IgG levels in our study is that during the first year of treatment IgG levels are still rising, whereas IgE levels have started to fall.

The change in IgG levels was measured after 12 months of treatment, and the mean rise measured by IgG RAST, IgG4 RAST, and IgG Staph A was significant in the allergen-treated groups, whereas the placebo-treated groups demonstrated no significant changes in either of the IgG assays. As measured by IgG RAST, the initial level of IgG was higher in the allergen-treated children than in the placebo-treated children, which is probably a coincidental finding, since the IgG levels were comparable before treatment in the allergen- and placebo-treated adults and in both children and adults when the other two IgG assays were used. Thus, there is no convincing evidence of a higher basic IgG level in children than in adults. However, after 12 months of immunotherapy, the IgG RAST and IgG4 RAST activity was higher for the children than for the adults. Two explanations could be proposed. First, children may be immunologically more active than adults, which would be in keeping with our finding of a persistent rise in IgE levels during therapy. To our knowledge no such comparison between children and adults undergoing immunotherapy has previously been made. Second, the different ways of reaching the maintenance dose could have influenced the IgG response. Norman⁴³ (1980) reported that a clustered-dose regimen induced a greater IgG response than a slower weekly dose regimen in insect venom-treated patients. The children in our study were all started on the therapy by rush hyposensitization (accompanying article page 478) and may thus have responded by providing a higher IgG level initially. Furthermore, the different IgG levels are most probably independent of the allergen dosage administered, since the cumulative dose was similar in children and adults.

The IgG response to cat- and dog-dander allergen immunotherapy was comparable to the IgG response measured in pollen,^{15, 17, 21} insect venom,^{19, 24} and cat-allergen immunotherapy studies,^{23, 34} although comparisons are difficult, mainly because many different

IgG assays were used and because the results are not presented in comparable figures. The IgG response in seasonal and perennial allergic patients is not comparable. Insect venom-treated patients are not always atopic patients and could thus demonstrate a partly different immunologic response to hyposensitization. In these patients the protective effect is easy to measure that is not the case in animal- and mite-allergic patients. The cat pelt hyposensitization study by Ohman et al.²³ demonstrated an IgG response that is partly dose-dependent and demonstrated clinically beneficial effects of the therapy. No attempt was made, however, to correlate the clinical improvement to the level of IgG. In the mite hyposensitization studies by Turner et al.¹⁸ and D'Souza et al.,⁶ increases in specific IgG levels were noted, but only in the latter study was there any relation to clinical improvement.

Most pollen-allergy studies have failed to correlate IgG levels to clinical improvement as measured by patients' subjective evaluation and/or nasal/bronchial provocation tests.^{14-17, 43, 44} This was confirmed in our study in which no correlation was found between the change in IgG levels and the patients' subjective evaluation or sensitivity in allergen bronchial provocation tests. However, if the allergen-treated patients who felt improvement were compared with respect to their IgG or IgG4 levels with those patients with no subjective improvement, there were significantly higher mean IgG and IgG4 levels in the former group ($p < 0.01$ for both). There is thus a relation between IgG levels and clinical effects, although the individual differences make it impossible to find a "protective" IgG level as has been postulated by Golden et al.²⁴ for insect venom-treated patients. In our study there was no correlation between initial IgG level and the rise in IgG levels during treatment, i.e., the initial IgG level appears to have no predictive value just as the initial IgE level also failed to be predictive of the effects of the therapy.

In conclusion, 12 months of immunotherapy with partially purified cat- and dog-dander extracts resulted in a significant rise in IgG levels measured by three different assays. There was an initial increase in allergen-specific IgE levels, and this was followed by a decrease during the last 3 months in the adults, whereas there was a persistent rise in IgE levels in the children. The SPT reaction decreased in all patients during the period of therapy. The only observed relationship between the changes in IgG and IgE levels and SPT was a significant inverse correlation between the changes in IgG RAST and IgE RAST. No correlation was found between either the allergen bronchial provocation test results or the patients' subjective

evaluation and any of the following parameters, IgG and IgE levels or SPT.

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