

Monoclonal antibody–standardized cat extract immunotherapy: Risk-benefit effects from a double-blind placebo study

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Abstract

Twenty-eight patients (14 in an active treatment group and 14 in a placebo group) with rhinoconjunctivitis and asthma caused by cat hypersensitivity took part in a placebo-controlled, randomized, double-blind study to evaluate the risk-benefit quotient of the maintenance dose corresponding to 13.2 µg *Fel d* I antigen. The cat extract was biologically standardized and quantified with monoclonal antibodies (100 biological units: 33 µg of *Fel d* I antigen, 650 µg of albumin, and 99 µg of *Fel d* Bd/K30 antigen). After 1 year of treatment, the systemic reactions to cat extract immunotherapy were mild and infrequent. Improvement was observed in the active treatment group in comparison with the placebo group in the medication-symptoms score ($p < 0.001$); in skin prick test ($p < 0.001$), conjunctival provocation test ($p < 0.001$), and allergen bronchoprovocation test ($p < 0.05$) results, and in nonspecific bronchial hyperreactivity measured with methacholine, which was not statistically significant. This study demonstrates the favorable risk-benefit quotient of the dose of 13.2 µg of *Fel d* I antigen, which may serve as a future reference in defining the optimum dose and appropriate proportion of antigens that should be administered in cat extract immunotherapy. (J ALLERGY CLIN IMMUNOL 1994;93:556-66.)

BU	Biological units
CPT	Conjunctival provocation test
ET	End-point titration
FEV₁	Forced expiratory volume in 1 second
MBT	Methacholine bronchoprovocation test
PC₂₀	Provocative concentration causing a 20% fall in FEV ₁
PLA	Parallel line assay
PSE	Patient's self-evaluation
SMS	Symptom-medication score
SPT	Skin prick test

Key words: [Immunotherapy](#), [monoclonal antibodies](#), [cat](#), [Fel d I antigen](#)

Domestic pets are a frequent cause of hypersensitivity in our society, ¹⁻³ and the cat is the one most commonly associated with this type of response. ⁴⁻⁶ Although animal avoidance is the first therapeutic alternative, it is not always possible. In addition, significant allergen levels have been measured in cat-free environments ⁷⁻⁹ because such allergens can be found in extraordinarily volatile particles of 2.5 µm in diameter. ¹⁰ Thus other therapeutic measures should be tried, of which immunotherapy is the best choice in some cases. Since the publication by Taylor et al. ¹¹ in 1978 of the first double-blind study with cat extract, which revealed a reduction in specific bronchial hyperreactivity, various studies, which also support the efficacy of immunotherapy with this extract, have appeared. ¹²⁻¹⁴ However, its clinical efficacy has yet to be fully defined because several of the studies did not include tabulation of the clinical improvement or because the results were inconclusive. ¹⁵

The identification, characterization, and purification of the allergens most commonly found in some extracts (including cat extract), ^{16, 17} the subsequent extraction of monoclonal antibodies to them, ¹⁸ and finally the use of these reagents in immunochemical techniques ¹⁹ has meant that it is now possible to define precisely the quantity of active substance (i.e., allergen) present in the extract being handled. In our opinion, this represents a new challenge in the standardization of allergenic extracts and in the field of clinical research on the specific immunotherapy for each patient with allergy. It enables us to begin to define the quantity of allergen that should be administered to ensure that immunotherapy is safe and effective because the immunotherapy maintenance dose with aeroallergens is as yet not fully characterized. The inefficacy of low doses has been demonstrated ²⁰; the maximum doses tolerated induce an unacceptable number of systemic reactions, ²¹ so that a dose, which balances efficacy with "optimum dose" safety, must be sought. ²²

The aim of this study was to evaluate the efficacy and tolerance over a 1-year period of a dose of 13.2 µg of *Fel d* I, 39.6 µg of *Fel d* BD/K30, and 260 µg of albumin in a lyophilized cat dander extract, which is biologically standardized and quantified with monoclonal antibodies. Immunotherapy efficacy was assessed by patient self-evaluation (PSE) and symptom-medication score (SMS) and by the skin prick test (SPT), conjunctival provocation test (CPT), methacholine bronchoprovocation test (MBT), and allergen bronchoprovocation test (ABT). In vitro results will be presented in a subsequent article.

METHODS

Patients

The study was performed with 28 patients, ranging in age from 15 to 65 years, who had rhinoconjunctivitis and asthma of more than 18 months' duration caused by exposure and who had positive skin test results and specific IgE to cats.

All of the subjects had taken environmental prophylactic measures recommended for these cases [23-25](#) for at least 12 months before the study. It was emphasized that these measures not only be carried out at home but also, whenever possible, at work, school, or other sites frequently visited (such as the homes of neighbors, friends, relatives, etc.). In all of the cases the patients eliminated the presence of cats in their homes. Nonetheless, the clinical symptoms of rhinoconjunctivitis and asthma persisted, satisfying the criteria for their inclusion in this study.

Patients who had previously received immunotherapy, those sensitized to other perennial allergens, and those in situations in which immunotherapy is contraindicated were excluded. [26](#) The patients' characteristics are shown in [Table I](#).

TABLE I. In vivo and in vitro parameters before treatment

	Active group	Placebo group
No. of patients	14	14
Sex (Male/Female)	3/11	3/11
Age (yr)*	23.5	28.6†
SPT <i>Fel d</i> I (mm ²)‡	60.05	46.07†
CPT (BU/mL)§	4	4†
MBT (mg/ml)‡	0.56	0.81†
ABT (BU/ml)‡	0.76	1.08†
Specific IgE (HRU/ml)‡	4.48	3.97†

HRU, Hamlet RAST unit.

*Arithmetic mean.

†No statistical difference.

‡Geometric mean.

§Median value.

The study was approved by our hospital's ethics committee, and the patients gave their consent for participation in the study.

The allergen extract

The allergen extract was obtained from Alergia e Inmunología (Abelló, S.A., Madrid, Spain) and prepared by extracting the raw material (cat dander supplied by Allergon AB Engelholm, Valinge, Sweden) for 1 hour at room temperature with 50 mmol/L of NH_4HCO_3 , pH 8, at a 5% (wt/vol) ratio. The resulting suspension was centrifuged at 27,000 *g* for 20 minutes; and the supernatant was extensively dialyzed against distilled water, sterilized by filtration through a 0.22 μm pore membrane, distributed into 2 ml aliquots, freeze-dried, and stored at 4° C until use.

The allergenic activity of the extract (in biological units [BU]/ml) was determined by RAST inhibition in relation to an internal reference previously calibrated in biological units by a skin prick test with a modified Brighton's method. [27](#), [28](#) The quantification of the major allergen (*Fel d* I) was done by a two-site monoclonal antibody–based radioimmunoassay, as indicated. [19](#) Monoclonal antibodies were also used to determine the amount of albumin and *Fel d* Bd 4.1/30K. [18](#) Total protein content of the extract was determined by Lowry's method. Each vial of freeze-dried extract contained 5.25 μg of protein (300 BU), 100 μg of *Fel d* I, and 2 mg of albumin.

The complete extract was used to obtain purified *Fel d* I with the use of previously described methods. [17](#), [19](#) The purified *Fel d* I and the complete extract were used to examine changes in skin reactivity with immunotherapy.

The diagnoses and treatments used in the study were prepared when needed by resuspending the freeze-dried extract with the corresponding solution. The placebo used was a succession of dilutions based on a 0.1 mg/ml histamine phosphate solution, which was colored with burned glucose so that its appearance was identical to that used in active treatment. Histamine phosphate was reconstituted with depot diluent (aluminum hydroxide) for the maintenance doses.

Study design

A placebo-controlled, randomized, double-blind study was carried out. All of the patients had asthma and had been matched for age, sex, level of specific IgE, SPT, CPT, cat extract, and methacholine provocative concentration causing a 20% fall in FEV_1 (PC_{20}). Patients, nurses, and physicians were unaware of whether placebo or active treatment injections were being administered. At the end of the study, just before the statistical analysis was performed, the randomization codes were revealed.

Each patient was evaluated before immunotherapy (time T0), on reaching maintenance dose (time T1), and 1 year after commencement of the immunotherapy (T2). Evaluation was done with SPT; blood samples and CPT for T0, T1, and T2; and MBT and ABT for T0 and T2.

Specific immunotherapy

The immunotherapy administration schedule for active or placebo treatment was identical

in both cases (■Table II) and carried out in the hospital. The initial dose was 0.004 µg of *Fel d* I, provided that it was less than the end-point titration (ET). Doses were administered every Monday and Thursday until a dose of 13.2 µg of *Fel d* I was reached, or if not possible, until the maximum doses tolerated were reached. Once a maintenance dose was reached, it was repeated monthly with the extract absorbed in aluminum hydroxide gel.

All patients remained under observation for 1 hour after administration of the treatment, and the local and systemic reactions were carefully checked.

TABLE II. Dose schedule for immunotherapy with cat extract and placebo preparation

Vial	Volume	Dose
I 0.05 BU/ml	0.25 ml	0.0125 BU
I 0.05 BU/ml	0.50 ml	0.0250 BU
I 0.05 BU/ml	0.75 ml	0.0375 BU
II 0.5 BU/ml	0.1 ml	0.05 BU
II 0.5 BU/ml	0.2 ml	0.10 BU
II 0.5 BU/ml	0.4 ml	0.20 BU
II 0.5 BU/ml	0.8 ml	0.40 BU
III 5 BU/ml	0.1 ml	0.50 BU
III 5 BU/ml	0.2 ml	1 BU
III 5 BU/ml	0.4 ml	2 BU
III 5 BU/ml	0.6 ml	3 BU
III 5 BU/ml	0.8 ml	4 BU
IV 50 BU/ml	0.1 ml	5 BU
IV 50 BU/ml	0.2 ml	10 BU
IV 50 BU/ml	0.3 ml	15 BU
IV 50 BU/ml	0.4 ml	20 BU
IV 50 BU/ml	0.5 ml	25 BU
IV 50 BU/ml	0.6 ml	30 BU
IV 50 BU/ml	0.7 ml	35 BU
IV 50 BU/ml	0.8 ml	40 BU

Patient's self-evaluation and symptom-medication scores

All patients recorded their symptoms and the medication required according to the previously described SMS. ²⁹ Initially, the daily treatment was reduced to a minimum and the different medications were introduced, one at a time and in the same order, in each patient. On the first day of treatment, patients were taught how to indicate their symptoms on a standardized chart and which medication they had to take to counteract the symptoms. These charts were provided for the patients and subsequently brought to our clinic. After 1 year of treatment, we calculated each patient's average daily score, and the patients were asked to grade their symptoms during direct contact with cats in relation to

those symptoms occurring before the immunotherapy: 100% corresponded to total success, and 0% to complete failure.

Skin tests

The skin tests were done in duplicate in accordance with the prick-test technique and a previously described method.³⁰ All patients were tested by the same investigator between 9:00 and 11:00 AM. Medication that might alter the skin response was suspended sufficiently in advance.³¹ The following substances were used: (1) three fivefold concentrations (200, 40, and 8 BU/ml) of the extract used for immunotherapy, (2) half dilutions of the cat extract for ET, (3) purified extract of *Fel d I* (33 µg/ml), (4) histamine, histamine chlorhydrate 10 mg/ml (positive control), and (5) normal saline solution (negative control). Reading was done after 15 minutes, and a planimeter was used to measure the area of the papule.

A program especially designed for these purposes (AIASA Clinical Research Software - PLA; Alergia e Immunologia Abello, S. A., Madrid, Spain)³² was used to record and estimate the change in skin sensitivity by a parallel line assay (PLA).

The ET skin test was defined as the highest dilution (twofold) of the extract that produced a wheal of 3 mm in diameter.³³

CPT

All patients were tested by the same investigator with a method based on that described by Möller et al.³⁴ The material used was lyophilized and reconstructed on the same day as the test. The dilutions to be tested were obtained at a rate of 1 to 5 (0.16 to 100 BU/ml). Once it was verified that the patient had not received any medication that might alter the test results,³¹ the test was begun with a saline solution as a negative control.

ABT and MBT

Nonspecific bronchoprovocation tests were carried out with inhaled methacholine, with the use of the procedure and concentrations proposed by Cockcroft³⁵ and in compliance with the requirements of the European Committee on the Standardization of Bronchial Provocation Tests.³⁶ A Devilbiss 646 nebulizer (Devilbiss Health Care, Inc., Somerset, Pa.) of known input, output, and particle diameter was used. The parameter chosen in this study was FEV₁, recorded with a Vitalograph spirometer (Vitalgraph, Inc., Lenexa, Kan.).

ABT with cat extract was carried out by using the technical system described previously. The dilutions (twofold) used were made up each day from lyophilized extract. After nebulization, spirometry was carried out at 5, 10, 20, and 30 minutes. The results were expressed as PC₂₀. Spirometry was performed every 2 hours, except at night for 24 hours or until the patient experienced symptoms.

Statistical analysis of data

Data from ABT, MBT, and SPT and from measurements of specific IgE were log-transformed to normalize their distributions and homogenize their variances and the geometric means shown in all cases. Arithmetic means were used to represent age, PSE, and SMS; whereas median values were chosen for CPT data. Nonparametric statistics

were applied for group comparison (Mann-Whitney U test) and for intragroup evolution (Wilcoxon's signed-rank test). Correlations were established with Spearman's rank-correlation test.

RESULTS

Homogeneity of patient groups

The actively treated group was compared with the placebo group on the basis of age, sex, ET, SPT with purified *Fel d I* extract, MBT, ABT, CPT, and specific IgE (Table I). In terms of the Mann-Whitney U test, there were no statistically significant differences between the two groups for the parameters considered before the immunotherapy began.

Tolerance

With the exception of two patients in the active treatment group, all reached the preset dose of 13.20 µg of *Fel d I* (40 BU). The average maintenance dose was 11.3 ± 4.7 µg of *Fel d I* (34.4 ± 14.3 BU). The dose accumulated until maintenance levels (T1) of 100 ± 51 µg of *Fel d I* were reached, corresponding to 303 ± 154 BU. Table III shows the average dose administered after 12 months of treatment.

TABLE III. Average dose administered

	ID	MD	CD1	TCD
<i>Fel d I</i> (µg)	0.001	13.2	100	170
Albumin (µg)	0.026	260	1969	3347
<i>Fel d Bd</i> 4.1/30K (µg)	0.003	39.6	299	510
BU	0.004	40	303	515

ID, Initial dose; MD, maintenance dose; CD1, cumulative dose in T1; TCD, total cumulative dose (1 year).

In general, the extract was well tolerated. Ten patients showed a total of 14 reactions (1.90 reactions for every 100 injections administered). Of these 10, seven had local reactions, representing 1.5% of all injections administered (0.14% immediate and 1.36% delayed reactions). The other three had systemic reactions, representing 0.41% of all injections administered. There was no anaphylactic shock. Notably, one reaction appeared with the maintenance dose but was subsequently tolerated when the dose was reduced and then augmented progressively. All general reactions took place during the first hour of administration of the extract, and there was a good response to treatment with epinephrine.

The lowest dose producing a local or systemic reaction varied greatly from patient to patient. We did not find any alterations in the elementary analytic parameters (hemogram, biochemistry, urinalysis), nor was there any reference to alteration in the general state.

PSE and SMS

After 1 year of treatment, the patients in the group receiving immunotherapy noted a significantly greater improvement than those in the placebo group ($81.3\% \pm 15.5\%$ in the treated group, $20.7\% \pm 33.2\%$ in the placebo group; $p < 0.001$ Mann-Whitney U test) (■Fig. 1). The SMS (■Fig. 2) confirmed this improvement, ($0.14\% \pm 0.36\%$ treated group, $1.42\% \pm 0.51\%$ placebo group; $p < 0.001$ Mann-Whitney U test). There was no correlation in the improvement noted between the subjective and objective consultative parameters (SPT, CPT, MBT, and ABT).

FIG. 1. Patients' self-evaluation of efficacy in the immunotherapy and placebo groups.

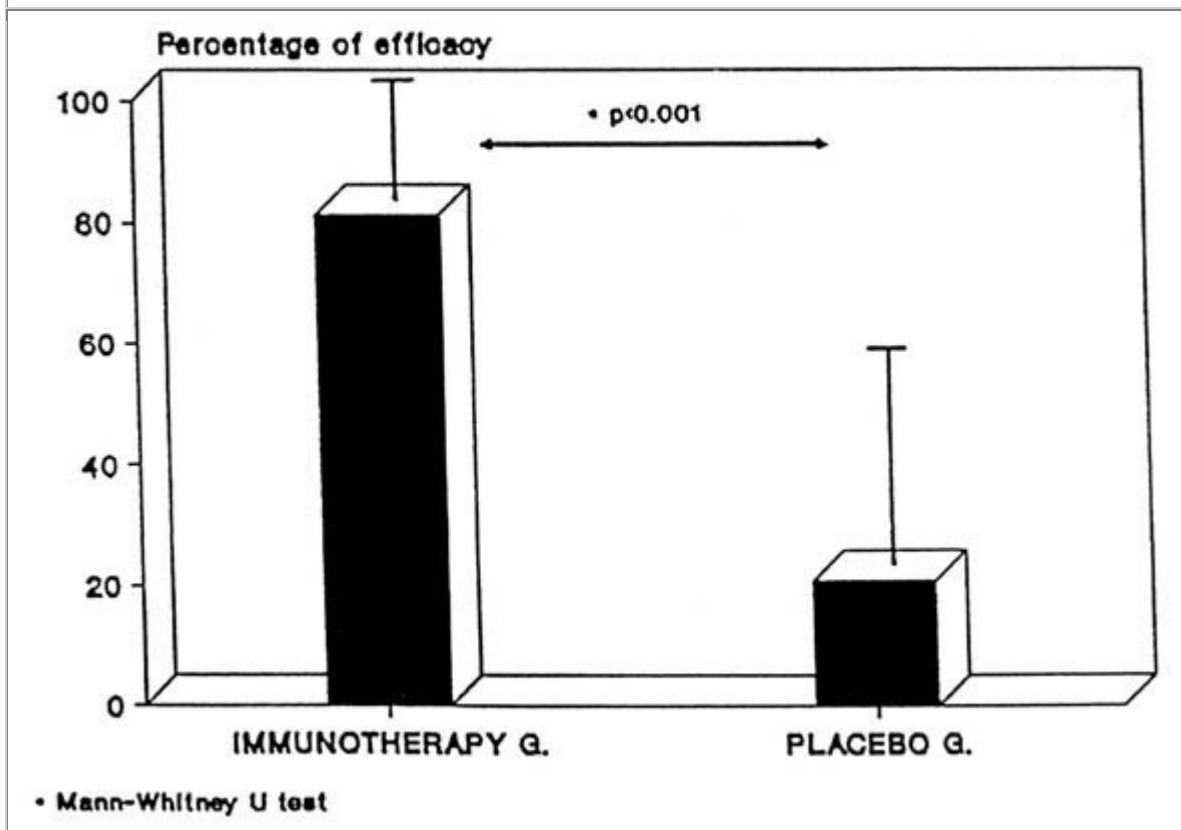
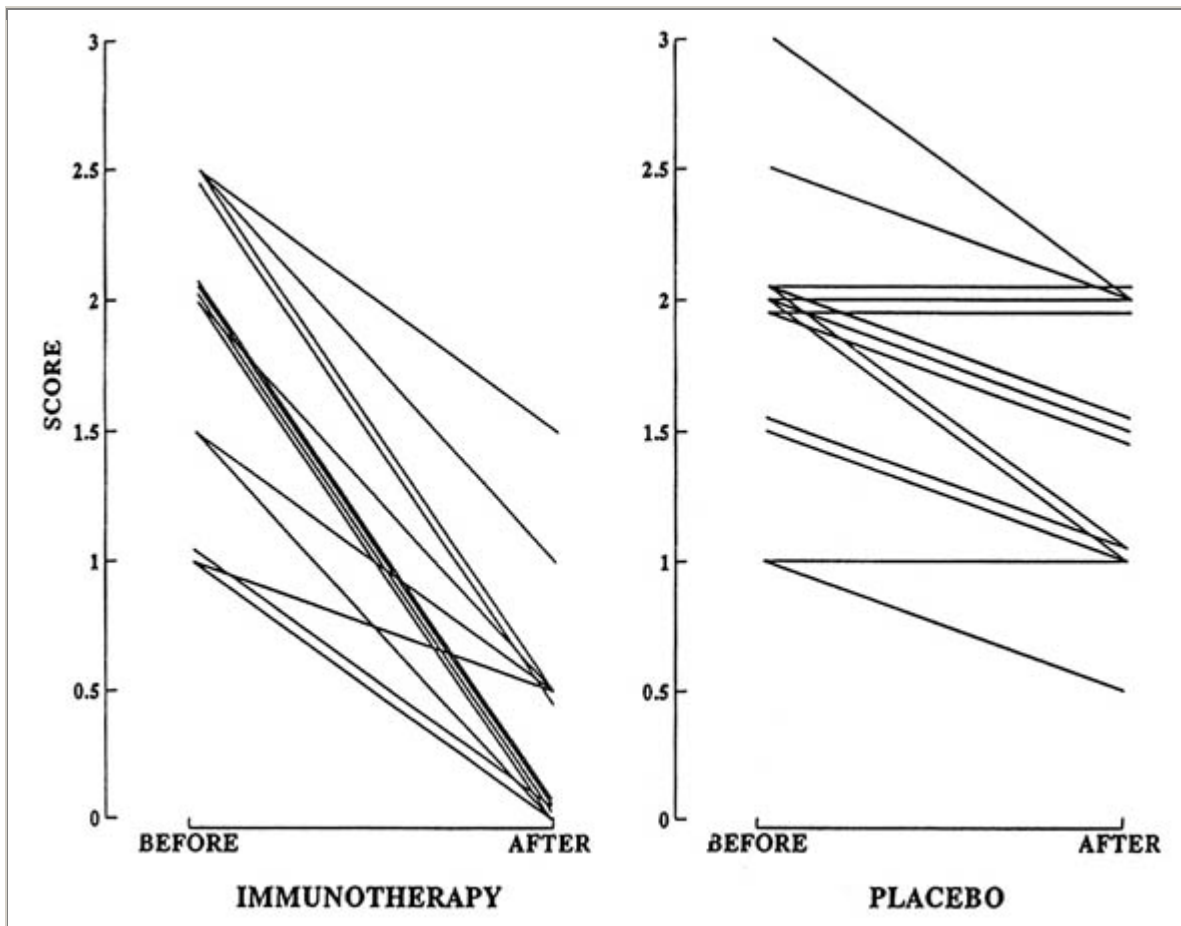


FIG. 2. Average SMS before treatment and after 1 year of treatment.

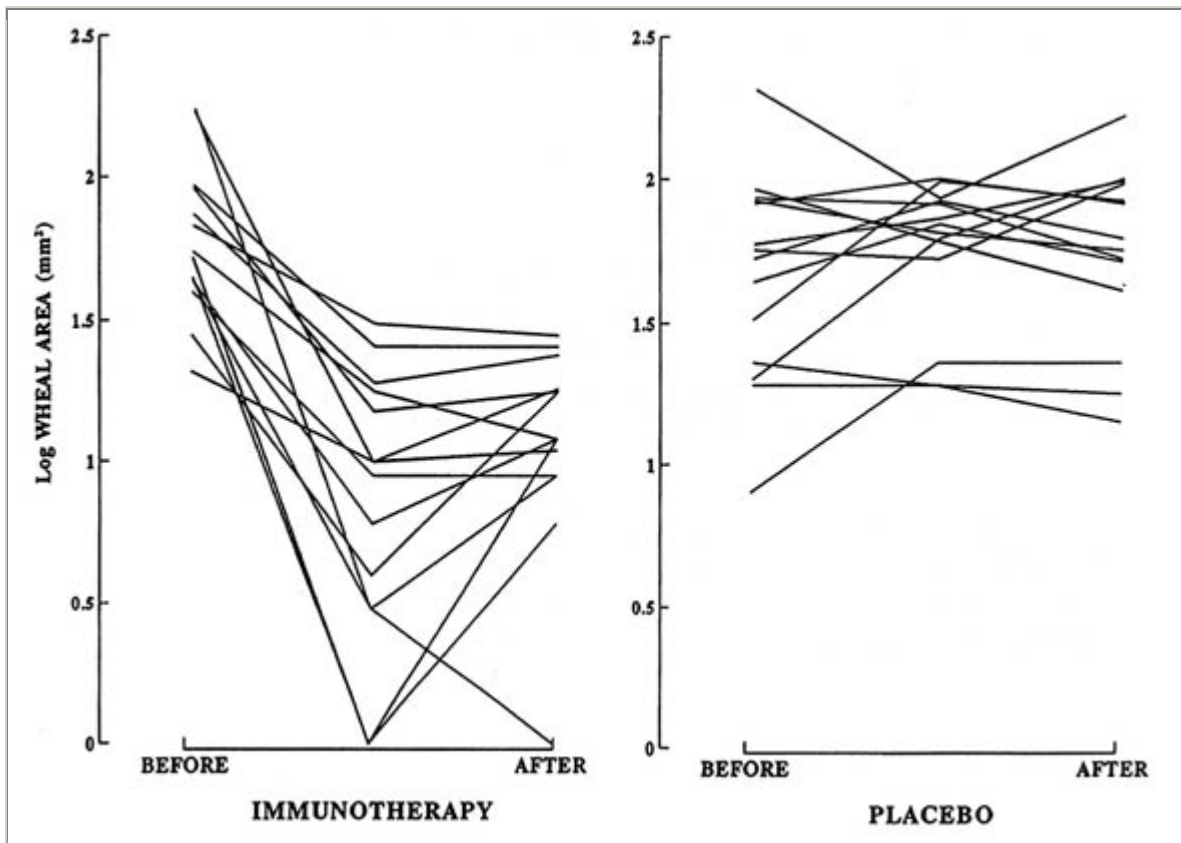


SPT

Purified Fel d I extract

In all 14 patients (100%) in the active treatment group, skin sensitivity decreased both at T1 and after 1 year of treatment (T2) from $60 \pm 0.27 \text{ mm}^2$ before immunotherapy to $6.6 \pm 0.53 \text{ mm}^2$ at T1 and $11.7 \pm 0.36 \text{ mm}^2$ at T2. This was a statistically significant decrease ($p < 0.001$, Wilcoxon W test). There were no statistically significant modifications in the placebo group (■Fig. 3).

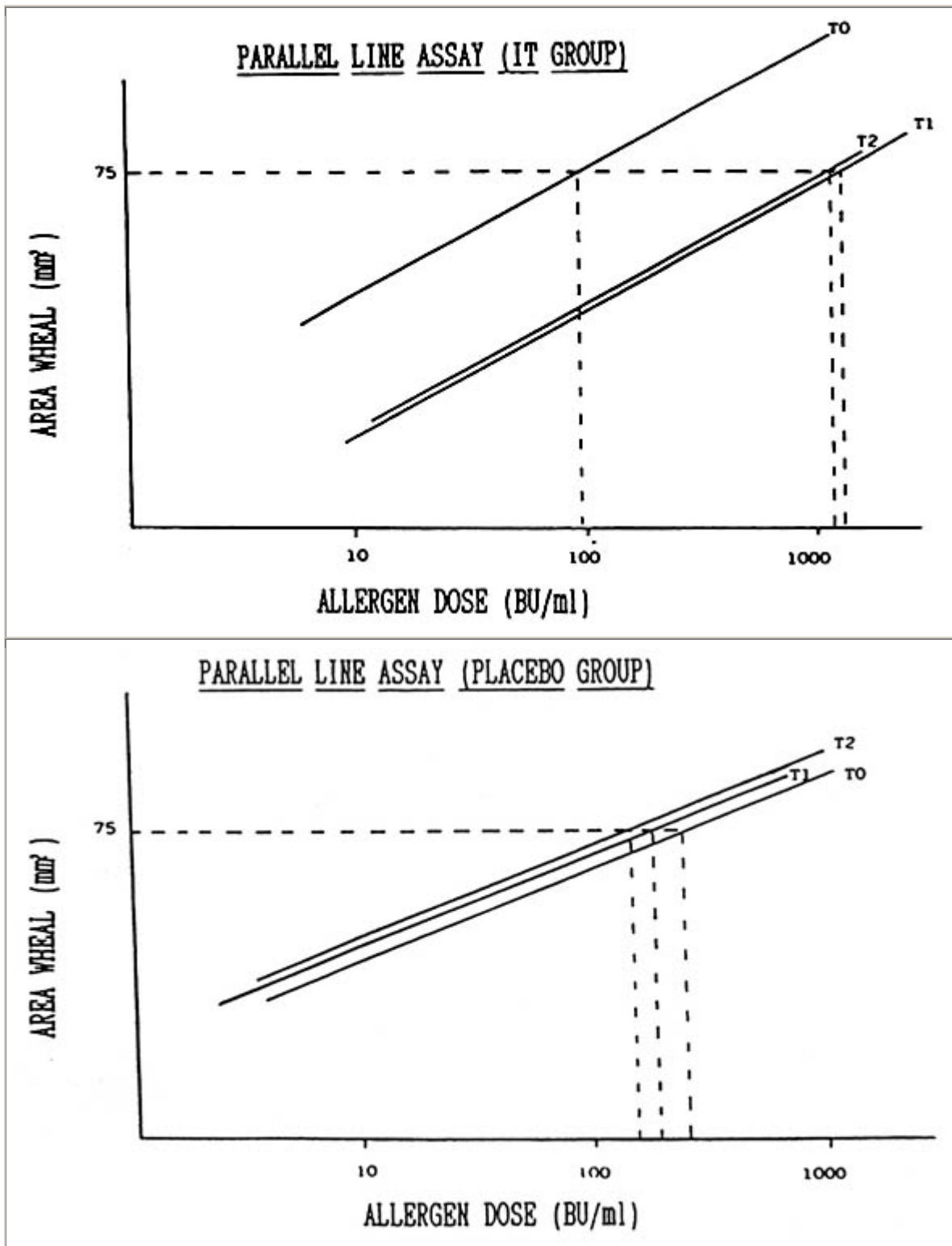
FIG. 3. Change in cutaneous reactivity with purified *Fel d I* extract.



PLA

The cat extract-treated group required a 10-fold stronger antigen concentration to produce the same skin reaction as that produced before immunotherapy. There were no significant differences in the placebo group (■Fig. 4).

FIG. 4. Change in cutaneous reactivity by PLA in immunotherapy (IT) and placebo groups.



ET

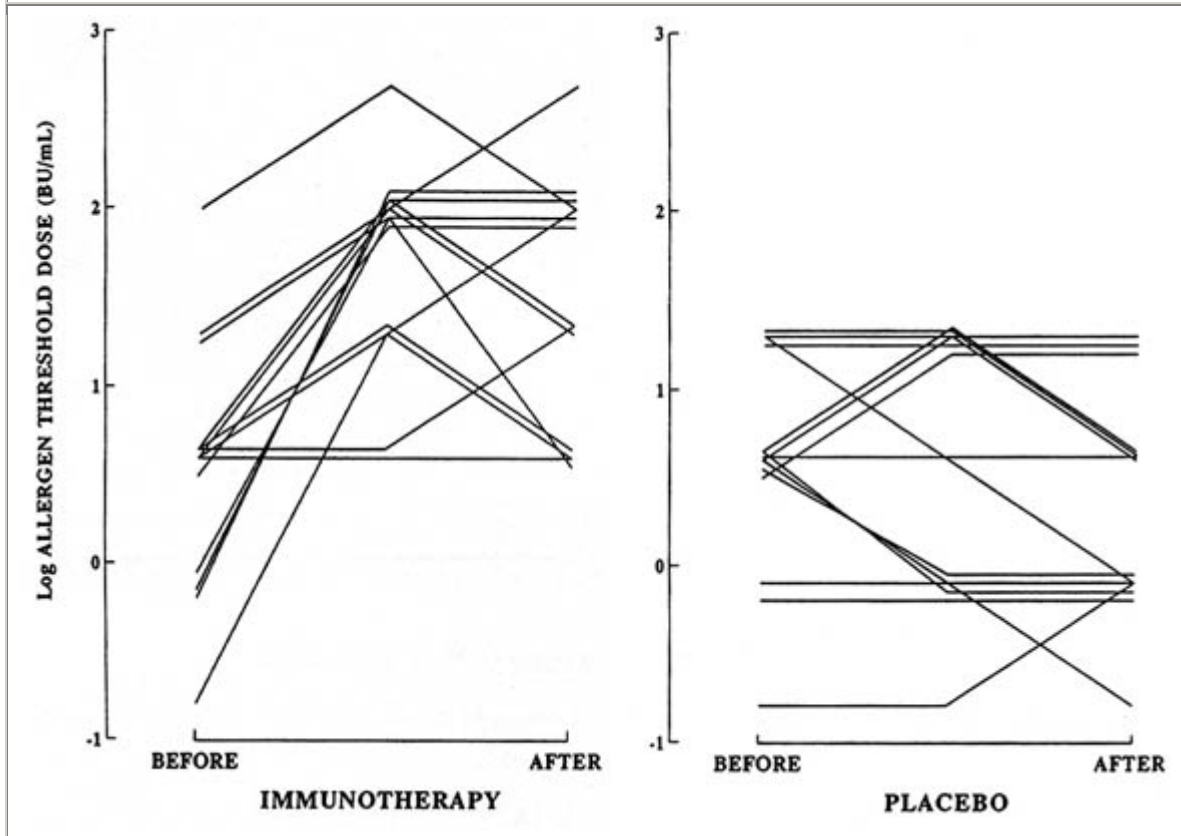
In the active treatment group skin sensitivity to the antigen decreased in 13 of 14 patients (92%) and was maintained in one (7%). It was significant in T1 and T2 in comparison with T0 ($p < 0.01$). There were no significant differences in the placebo group.

CPT

No patient had complications during the challenge. Twelve of 14 patients (86%) in the

active treatment group had improved conjunctival sensitivity on reaching T1; this was the case for 11 of 14 (78%) at T2. There were no changes in sensitivity in the remaining cases, that is 2 of 14 patients (14%) in T1 and 3 of 14 (21%) in T2 (Fig. 5). There were no cases of increased sensitivity. In the placebo group conjunctival sensitivity decreased in three of the 14 patients (21%), remained unchanged in five (35%), and increased in six (42%).

FIG. 5. Evolution of the conjunctival provocation test.



In T1 and T2, there were significant differences between patients in the active treatment and placebo groups when the threshold dose that produced a positive pruritic response was compared with conjunctival redness. A comparison of the values of these measurements in T1 with the value of measurements made before treatment in patients in the active treatment and placebo groups revealed a median value of 100 in the active group and a median value of 4 in the placebo group ($p < 0.001$). After 1 year of immunotherapy, these differences continued to be significant.

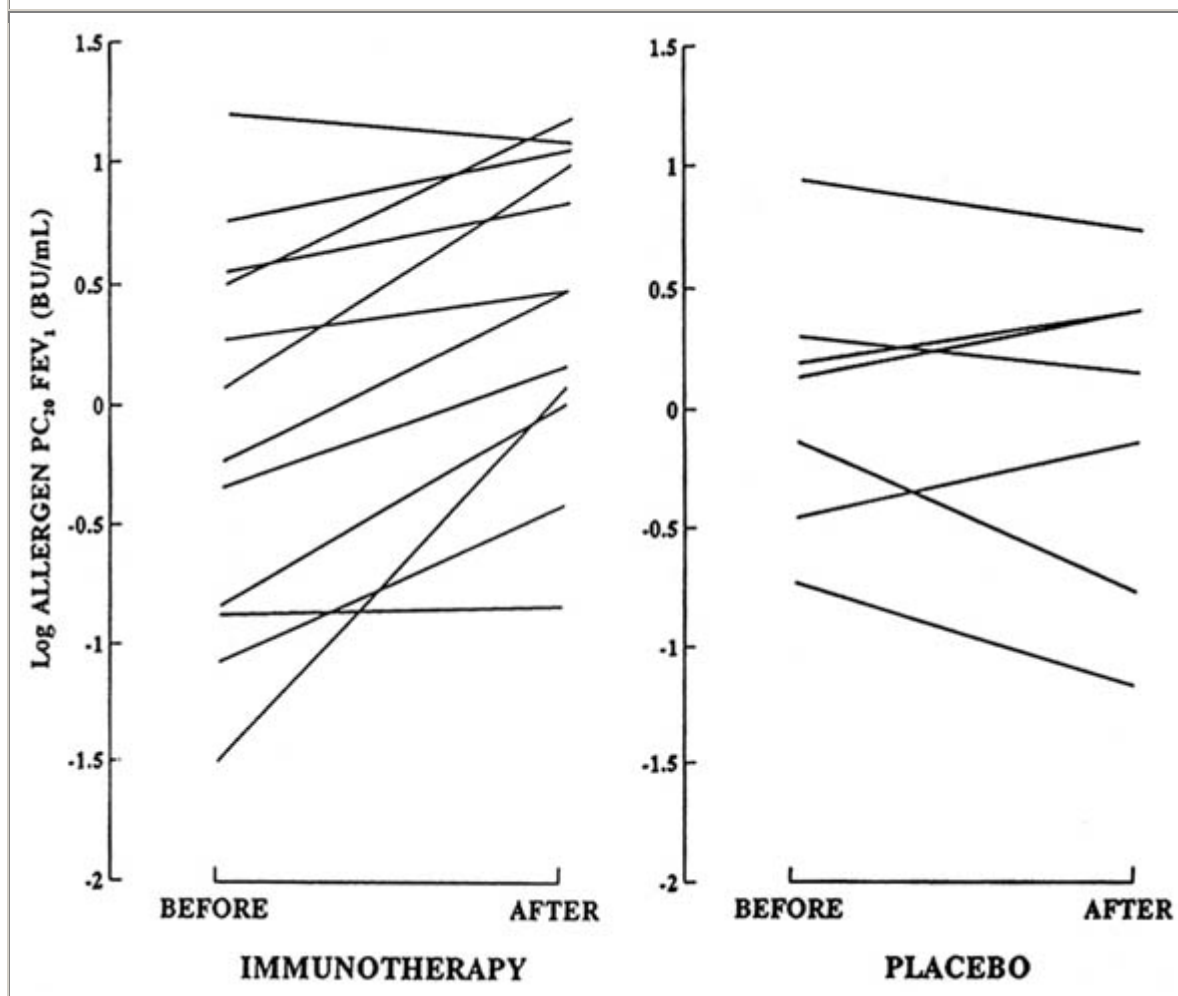
MBT

Throughout the study, methacholine PC_{20} could be established and followed up in 13 of the 14 patients in the treatment group and in 7 of the 14 in the placebo group. In the former group there was a statistically insignificant improvement in methacholine PC_{20} (before immunotherapy, 0.56 ± 0.47 mg/ml; 12 months after immunotherapy, 0.57 ± 0.42 mg/ml). In the placebo group, methacholine PC_{20} worsened (before immunotherapy, 0.81 ± 0.54 mg/ml; after immunotherapy, 0.58 ± 0.42 mg/ml [NS]).

ABT

Throughout the study, antigen PC₂₀ could be established in 12 of 14 patients in the active treatment group and 7 of 14 in the placebo group. In 11 of 12 patients (92%), antigen PC₂₀ improved, and in 1 of the 12 (8%) it worsened (■Fig. 6): this was the patient receiving the lowest maintenance dose (0.16 µg of *Fel d I*). The antigen PC₂₀ improved in three of seven patients (42%) and worsened in four of seven (57%) in the placebo group. After a year of treatment, the antigen PC₂₀ improved 3.42 ± 2.55 times the initial value ($p < 0.05$) in the active treatment group, but no significant change was observed for the placebo group (1.08 ± 0.77 , NS). At the end of the treatment, comparison of the two groups showed a statistically significant difference ($p < 0.05$).

FIG. 6. Evolution of change in allergen PC₂₀.

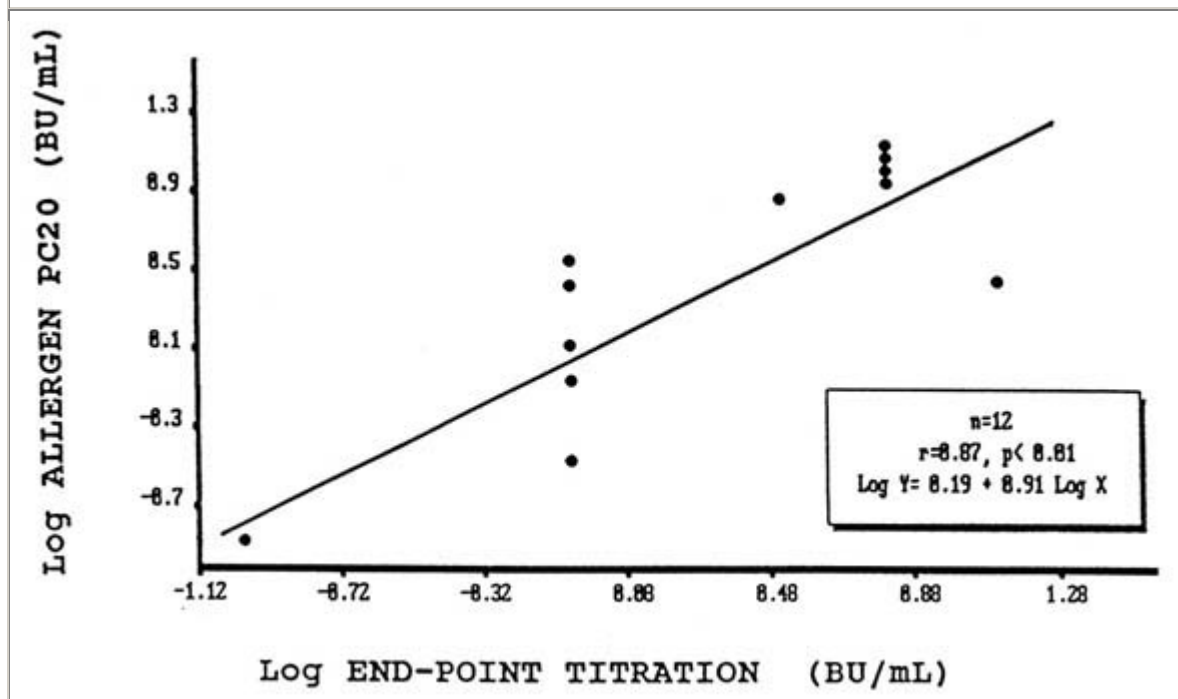


Correlations between parameters

A correlation between the SPT results with the cat extract and with *Fel d I* ($r = 0.66$; $p < 0.01$), but not between the SPT and ET results, was found. There was also a correlation between the SPT results measured by the ET and the antigen PC₂₀ both at the commencement of the study ($r = 0.84$, $p < 0.01$) and after 1 year of immunotherapy ($r = 0.87$, $p < 0.01$) (■Fig. 7) and between the values for methacholine PC₂₀ and antigen PC₂₀, both at the commencement of the study ($r = 0.76$, $p < 0.01$) and after 1 year of immunotherapy ($r = 0.84$, $p < 0.01$). A multiple correlation existed between the values for ET and methacholine PC₂₀ with antigen PC₂₀ ($r = 0.89$, $p < 0.01$), and correlations

between increases in ET and antigen PC₂₀ after 1 year of treatment ($r = 0.64$, $p < 0.01$) and between the increase in methacholine PC₂₀ and antigen PC₂₀ ($r = 0.84$, $p < 0.01$) were observed. A multiple correlation between initial ET values and their increase with antigen PC₂₀ ($r = 0.87$, $p < 0.01$) was seen. However, there was no correlation between the parameters studied in vivo and the dose of antigen administered or of the in vivo parameters with the results of the CPT, although the association is notable between the improvement in the ABT and CPT results because the only patient for whom antigen PC₂₀ did not improve also failed to show improvement in CPT results.

FIG. 7. Relationship between allergen PC₂₀ and cutaneous sensitivity to allergen (ET) after 1 year of immunotherapy.



DISCUSSION

Monoclonal antibodies have represented a major advance in the field of immunology. As early as 1981, Krilis et al. [37](#) suggested standardizing allergen extracts with monoclonal antibodies, but this only became a real possibility several years later with the development of a significant number of monoclonal antibodies specific to major antigens. Our study is the first article on immunotherapy in which an extract that is biologically standardized and quantified with monoclonal antibodies was used. In our opinion, and according to other authors, [38-40](#) use of these extracts will mark one of the most important advances in immunotherapy in coming years.

Benefits were measured by subjective clinical parameters confirmed through objective clinical parameters and immunologic monitoring. The subjective clinical parameters used were the SMS and the PSE. Because there is no internationally accepted standard SMS, we have used the one designed by Dreborg et al. [29](#) as the basis for ours. As in previous studies, skin tests showed a 10-fold reduced skin sensitivity according to the PLA. The

CPT showed that there was a significant improvement in cat-induced atopic conjunctivitis. Our results do not show that it is possible to monitor immunotherapy in bronchial asthma with the CPT in the absence of a correlation between it and the changes in ABT and MBT results. Our patients demonstrated an improvement in methacholine PC₂₀ that was not statistically significant. Antigen PC₂₀ improved in the group receiving treatment with the cat extract, confirming the findings of earlier studies that immunotherapy with cat extract improves specific bronchial hyperreactivity. [11-14, 41](#) As shown by Van Metre et al., [42](#) the antigen PC₂₀ improvement with the ABT is correlated with that obtained after natural exposure to the animal.

The present study supports the hypothesis of Tiffenau [43](#) and Zuidema [44](#) and the results of Cockcroft et al. [33](#) that the immediate air pathway response to allergens can be predicted if we are aware of the nonallergic reactivity of the airway to certain chemical mediators and of the skin response to the antigen. We also found that, after immunotherapy, there continues to be a close relationship between the antigen PC₂₀ with methacholine PC₂₀ and the ET. If there is an improvement in cutaneous sensitivity, there will be an improvement in the IgE-mediated illness. In our daily medical practice, we consider that ET before immunotherapy and its variations during treatment may be a marker of the evolution of asthma (antigen PC₂₀) during immunotherapy monitoring because of the correlation between ET and antigen PC₂₀, a theory that needs to be confirmed by other authors.

Previous studies of immunotherapy with cat extract have been associated with a high incidence of systemic reactions, as much as 40% (10 reactions in 4 of the 10 patients in the treatment group) in the study by Ohman et al. [12](#) and 60% (3 of the 5 patients in the treatment group) in the study by Taylor et al. [11](#) These studies used rapid immunotherapy patterns, leading to the suspicion that the high rate of severe reactions is a consequence of the type of pattern and the type of antigen used, cat extract, in this particular case. In the study of immunotherapy with cat extract used in a conventional pattern, Van Metre et al. [13](#) reported two systemic reactions in two (18%) of the 11 patients receiving immunotherapy, 0.6% of the total injections administered. Twenty-one percent of our patients had systemic reactions, 0.41% of all injections administered. These results are similar to those presented by Van Metre et al. [13](#) in support of the hypothesis that the high rate of systemic reactions is not the result of the extract type but rather of the type of pattern used for dosage increase. In all cases, severe reactions occurred within the first hour of administration of the extract, and subcutaneous administration of epinephrine was useful, effective, safe, and swift. The fact that systemic reactions did not occur after local reaction in the previously administered dose shows the need for strict control of the dose, despite the good tolerance of the previous one. A priori, we found no in vivo indicator that predicted improvement as a result of immunotherapy or the appearance of systemic reactions.

The average accumulated dose after 1 year was 170 µg of *Fel d* I, 3347 µg of albumin, and 510 µg of *Fel d* Bd 4.1/30K. In recent years some immunotherapy studies have been published in which the amount of predominant allergen present in the extract was determined by radioimmunodiffusion or crossed radioimmunoelectrophoresis. [14, 45-47](#) We believe the reason that the quantity of antigen (*Fel d* I) administered in immunotherapy studies with cat extract varies from 3.4 to 3200 µg, according to different authors, [14, 45](#) may be that the anti-serum against the allergen to be evaluated has an enormous variety and that these techniques have lower sensitivity and specificity than the immunoassays that use monoclonal antibodies. [38](#)

In conclusion, we believe that: (1) in evaluating the risk-benefit quotient of the possible optimal dose studied (13.2 µg of *Fel d* I, 39.6 µg of *Fel d* Bd 4.1/30K, and 260 µg of albumin) in cat-induced asthma, we have shown its efficacy with subjective parameters (SMS and PSE), confirmed by objective parameters (SPT, CPT, and ABT) with a perfectly admissible tolerance and (2) this article may provide a future reference in immunotherapy for defining the ideal proportion of antigens that a cat extract should contain for immunotherapy and the optimum dose that should be administered. This would be possible by means of standardization with monoclonal antibodies, precisely defining the antigen content, which could be compared in different studies.

REFERENCES

1. American Veterinary Medical Association. The veterinary services market. vols. I and II. Overland Park, Kansas: Charles, Charles & Associates, 1983:140.
2. Kjellman B, Pettersson R. The problem of furred pets in childhood atopic disease. *Allergy* 1983;38:65-73.
3. Rudolph R. Frequency of animal sensitization. In: Kerr JW, Gannderton MA, eds. *Proceedings of the XI International Congress of Allergology and Clinical Immunology*. London: The Macmillan Press Ltd., 1983:437-44.
4. Sarsfield JK, Boyle AG, Rowell EK, Moriarty SC. Pet sensitivities in asthmatic children. *Arch Dis Child* 1976;51:186-9.
5. Haahtela T, Bjorksten F, Heiskala M, Suoniemi I. Skin prick test reactivity to common allergens in Finnish adolescents. *Allergy* 1980;35:425-31.
6. Murray AB, Fergusson AC, Morrisson BJ. The frequency and severity of cat versus dog allergy in atopic children. *J ALLERGY CLIN IMMUNOL* 1983;72:145-9.
7. Wood RA, Eggleston PA, Lind P, et al. Antigenic analysis of household dust samples. *Am Rev Respir Dis* 1988;137:358-63.
8. Barber D, Chamorro MJ, Carpizo JA, et al. Valoración de la presión alérgica ambiental. Interés de esta determinación en la prevención, diagnóstico, y tratamiento de las enfermedades alérgicas. *Rev Esp Alergol Inmunol Clin* 1990;5:125-32.
9. Dreborg S, Munir AKM, Einarsson R. The level of *Fel d* I in school dust is sufficiently high to induce symptoms in asthmatics [Abstract]. *J ALLERGY CLIN IMMUNOL* 1991;87:169.
10. De Blay F, Chapman MD, Platts-Mills TAE. Airborne cat allergen (*Fel d* I). Environmental control with the cat in situ. *Am Rev Respir Dis* 1991;143:1334-9.
11. Taylor WW, Ohman JL Jr, Lowell FC. Immunotherapy in cat-induced asthma. Double-blind trial with evaluation of bronchial responses to cat allergen and histamine. *J ALLERGY CLIN IMMUNOL* 1978;61:283-7.
12. Ohman JL Jr, Findlay SR, Leitermann KM. Immunotherapy in cat-induced

asthma. Double-blind trial with evaluation of in vivo and in vitro responses. J ALLERGY CLIN IMMUNOL 1984;74:230-9.

13. Van Metre TE, Marsh DG, Adkinson NF, et al. Immunotherapy for cat asthma. J ALLERGY CLIN IMMUNOL 1988;82:1055-68.

14. Sundin B, Lilja G, Graff-Lonnevig V, et al. Immunotherapy with partially purified and standardized animal dander extracts. I. Clinical results from a double-blind study on patients with animal dander asthma. J ALLERGY CLIN IMMUNOL 1986;77:478-87.

15. Bousquet J. Specific immunotherapy in asthma. Postgraduate Education Course Syllabus. 46th Annual Meeting. Baltimore, Maryland, March 23-28, 1990. Milwaukee: American Academy of Allergy and Immunology, 1990: 183-220.

16. Duffort O, Carreira J, Lombardero M. Characterization of the main IgE-binding components of cat dander. Int Arch Allergy Appl Immunol 1987;84:339-44.

17. Duffort O, Carreira J, Nitti G, Polo F, Lombardero M. Studies on the biochemical structure of the major cat allergen *Felis domesticus* I. Mol Immunol 1991;28:301-9.

18. Duffort O, Carreira J, Lombardero M. Monoclonal antibodies against *Fel d* I and other clinically relevant cat allergens. Immunol Lett 1988;17:71-7.

19. Lombardero M, Carreira J, Duffort O. Monoclonal antibody based radioimmunoassay for the quantification of the main cat allergen (*Fel d* I or Cat 1). J Immunol Methods 1988;108:71-6.

20. Van Metre TE Jr, Adkinson NF Jr, Lichtenstein LM, et al. A controlled study of the effectiveness of the Rinkel method of immunotherapy for ragweed pollen hay fever. J ALLERGY CLIN IMMUNOL 1980;65:288-97.

21. Malling HJ, Dreborg S, Weeke B. Diagnosis and immunotherapy of mould allergy. V. Clinical efficacy and side effects of immunotherapy with *Cladosporium herbarum*. Allergy 1986;41:507-19.

22. Bousquet J, Guerin B, Michel FB. Clinical trials with standardized extract. In: Brede HD, Stevens E, eds. Proceedings of the Third Paul Ehrlich Seminar on Regulatory Control and Standardization of Allergenic Extracts. Stuttgart: Gustav Fischer Verlag, 1985:291-8.

23. Fernández-Caldas E, Fox RF. Environmental control of indoor air pollution. Med Clin North Am 1992;76:935-52.

24. Platts-Mills TAE, Chapman MD. Dust mites: immunology, allergic disease, and environmental control. J ALLERGY CLIN IMMUNOL 1987;80:755-75.

25. Colloff MJ, Ayres J, Carswell F, et al. The control of allergens of dust mites and domestic pets: a position paper. Clin Exp Allergy 1992;22:1-28.

26. Immunotherapy Subcommittee EAACI. Immunotherapy position paper. Allergy

1988;43(suppl 6):9-13.

27. Brighton WD, Topping MD, Henocq E. Activity units for allergen extracts. Clin Allergy 1979;9:591-6.

28. Lombardero M, Gonzalez R, Duffort O, et al. Evaluación de la actividad biológica total y composición alergénica de extractos alergénicos. Allergol Immunopathol 1986;14:189-98.

29. Dreborg S, Agrell B, Foucard T, Kjellman N-IM, Koivikko A, Nilsson S. A double-blind, multicenter immunotherapy trial in children using a purified and standardized *Cladosporium herbarum* preparation. I. Clinical results. Allergy 1986;41:131-40.

30. Skin Test Subcommittee EAACI. Skin tests used in type I allergy testing. Allergy 1989;44(suppl 10):22-30.

31. Pipkorn U. Pharmacological influence of antiallergic medication on in vivo allergen testing. Allergy 1988;43:81-6.

32. Martin S, Cuesta P, Rico P, Cortés C. A program to analyse cutaneous responses by means of parallel line assay [Abstract]. Allergy 1992;47:129.

33. Cockcroft DW, Murdock KY, Kirby J, Hargreave F. Prediction of airway responsiveness to allergen from skin sensitivity to allergen and airway responsiveness to histamine. Am Rev Respir Dis 1987;135:264-7.

34. Möller CH, Björkstén B, Nilsson G, Dreborg S. The precision of the conjunctival provocation test. Allergy 1984;39:37-41.

35. Cockcroft DW. Bronchial inhalation tests. I. Measurement of nonallergic bronchial responsiveness. Ann Allergy 1985;55:527-34.

36. SEPCR Working Group "Bronchial Hyperreactivity": guidelines for standardization of bronchial challenges with (nonspecific) bronchoconstricting agents. Bull Eur Physiopathol Respir 1983;19:495-514.

37. Krilis S, Baldo BA, Basten A. Standardization of allergens: lectins and monoclonal antibodies as primary standards for house dust mite allergens. Int Arch Allergy Appl Immunol 1981;66:252-8.

38. Chapman MD, Aalberse RC, Brown MJ, Platts-Mills TAE. Monoclonal antibodies to the major feline allergen *Fel d I*. II. Single step affinity purification of *Fel d I*, N-terminal sequence analysis, and development of a sensitive two-site immunoassay to assess *Fel d I* exposure. J Immunol 1988;140:812-8.

39. Chapman MD, Platts-Mills TAE. Standardization of allergens using monoclonal antibodies. Annual Meeting of EAACI: Allergen Standardization Symposium. Mallorca, Spain: Artes Graficas Gala, 1987:65-71.

40. Baldo BA. Standardization of allergens. Examination of existing procedures and

the likely impact of new techniques on the quality control of extracts. *Allergy* 1983;38:535-46.

41. Bertelsen A, Andersen JB, Christensen J, Ingemann L, Kristensen T, Ostergaard PA. Immunotherapy with dog and cat extracts in children. *Allergy* 1989;44:330-5.

42. Van Metre TE, Marsh DG, Adkinson NF, et al. Dose of cat (*Felis domesticus*) allergen I (*Fel d I*) that induces asthma. *J ALLERGY CLIN IMMUNOL* 1986;78:62-75.

43. Tiffeneau R. Hypersensibilite cholinergo-histaminique pulmonaire de l'asthmatique. *Acta Allergol* 1958;12 (suppl 5):187-221.

44. Zuidema P. Value of inhalation tests in bronchial asthma. *Respiration* 1969;26(suppl):141-50.

45. Ohman JL Jr, Marsh DG, Goldman M. Antibody responses following immunotherapy with cat pelt extract. *J ALLERGY CLIN IMMUNOL* 1982;69:320-6.

46. Wahn U, Schweter C, Lind P, Lowenstein H. Prospective study on immunologic changes induced by two different *Dermatophagoides pteronyssinus* extracts prepared from whole mite culture and mite bodies. *J ALLERGY CLIN IMMUNOL* 1988;82:360-70.

47. Creticos PS, Marsh DG, Proud D, et al. Responses to ragweed pollen nasal challenge before and after immunotherapy. *J ALLERGY CLIN IMMUNOL* 1989;84:197-205.

Publishing and Reprint Information

- From ^aHospital Ramon y Cajal, Madrid; and ^bHospital Universitario, Valladolid.
- Received for publication Oct. 14, 1992.
- Revised Aug. 2, 1993;
- Accepted for publication Aug. 3, 1993.
- Reprint requests: Emilio Alveraz-Cuesta, MD, PhD, C/ Claudio Coello 43, 4 Izq, 28001 Madrid, Spain.
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- 1/1/50445

Articles with References to this Article

This article is referenced by these articles:

Allergic Rhinitis and Its Impact on Asthma

Journal of Allergy and Clinical Immunology

November 2001, part 2 • Volume 108 • Number 5

Jean Bousquet, MD, PhD, Paul van Cauwenberge, MD, PhD, Nikolai Khaltaev, MD In collaboration with the World Health Organization

The role and remediation of animal allergens in allergic diseases

Journal of Allergy and Clinical Immunology

March 2001, part 2 • Volume 107 • Number 3
Martin D. Chapman, PhD^a, Robert A. Wood, MD^b

Allergic and immunologic disorders of the eye. Part II: Ocular allergy

Journal of Allergy and Clinical Immunology
December 2000 • Volume 106 • Number 6
Leonard Bielory, MD

The use of standardized extracts in allergen immunotherapy

Journal of Allergy and Clinical Immunology
July 2000, part 1 • Volume 106 • Number 1
Harold S. Nelson, MD

Allergen immunotherapy: Therapeutic vaccines for allergic diseases

A WHO position paper

Journal of Allergy and Clinical Immunology
October 1998, part 1 • Volume 102 • Number 4
Jean Bousquet, MD, Richard Lockey, MD, Hans-Jorgen Malling, MDthe WHO panel members*

A method for quantitation of food biologic activity: Results with peach allergen extracts

Journal of Allergy and Clinical Immunology
August 1998 • Volume 102 • Number 2
Javier Cuesta-Herranz, MD, PhD^a, Milagros Lázaro, MD, PhD^b, Alberto Martínez, PhD^c, Emilio Alvarez-Cuesta, MD, PhD^b, Elena Figueredo, MD^a, Jorge Martínez, PhD^c, Carlos Cuesta, MD^a, Manuel de-Las-Heras, MD^b
Madrid and Bilbao, Spain

Laboratory animal allergy

Journal of Allergy and Clinical Immunology
July 1998 • Volume 102 • Number 1
Robert K. Bush, MD^{a,b}, Robert A. Wood, MD^c, Peyton A. Eggleston, MD^c
Madison, Wis., and Baltimore, Md.

The use of standardized allergen extracts

Journal of Allergy and Clinical Immunology
May 1997 • Volume 99 • Number 5
American Academy of Allergy, Asthma and Immunology (AAAAI)

Asthma caused by Dermestidae (black carpet beetle): A new allergen in house dust

Journal of Allergy and Clinical Immunology
January 1997 • Volume 99 • Number 1
Javier Cuesta-Herranz, MD, PhD^a, Manolo de las Heras, MD^a, Joaquin Sastre, MD, PhD^a, Magdalena Lluch, MD^a, Manolo Fernández, MD^a, Carlos Lahoz, MD, PhD^a, Emilio Alvarez-Cuesta, MD, PhD^b
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