

Immunotherapy with standardized extract of *Dermatophagoides pteronyssinus* in bronchial asthma: a dose-titration study

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According to the maximum tolerated dose (MTD) achieved, we assessed the changes in clinical and laboratory parameters, induced by specific immunotherapy (SIT), in a group of 43 asthmatic patients sensitized to *Dermatophagoides pteronyssinus*, over a period of 18 months. A standardized extract (100 Bu/ml; 40 µg/ml of Der p 1; 20 µg/ml of Der p 2) was used. The patients were divided into two groups: the high-dose immunotherapy (HDI) group (MTD $\geq$ 4 µg Der p 1) and the conventional immunotherapy (CI) group (MTD $<$ 4 µg Der p 1). Changes in clinical severity index, medication, and symptom scores; in cutaneous and conjunctival reactivity; and in the levels of specific IgE, IgG, IgG1, and IgG4 to *D. pteronyssinus* (Der p 1 and Der p 2) were measured (ELISA monoclonal antibodies). Safety was monitored according to the EAACI guidelines. The range of the MTD was 0.8–16 µg of Der p 1. Ninety percent of the patients tolerated a dose of 3.2 µg, but only 18% of the patients reached a maintenance dose of 16 µg. The medians of the accumulated dose were 197 µg of Der p 1 for the HDI group, and 50 µg for the CI group. Conjunctival and cutaneous reactivity was significantly lowered ($P < 0.001$) after SIT, as were the clinical severity score and medication score in both groups, without significant differences between the groups, except for cutaneous reactivity. Levels of specific IgE decreased significantly ($P < 0.01$) in both groups, again without significant differences between the groups. The range of the increase in medians of specific IgG, IgG1, and IgG4 was 4.4–120-fold for the HDI group and 3–24-fold for the CI group ($P < 0.01$). The increase in the levels of Der p 1 and Der p 2 IgG4 were correlated to the changes in cutaneous and conjunctival reactivity ($P < 0.01$). These results show that a maintenance dose of 3.2 µg Der p 1 (8 BU) can induce pronounced clinical and immunologic changes with an excellent safety profile.

Key words: bronchial asthma; dose; house-dust mite; immunotherapy.

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Specific immunotherapy (SIT) with high-quality extracts of allergens is considered an effective treatment of allergic bronchial asthma (1, 2). In spite of this, there are many controversial aspects such as the mechanisms of action of immunotherapy, the selection of the patients, the most effective administration schedules, and the predictable parameters of its tolerance (3). A relationship exists between the dose administered and both its clinical efficacy and the appearance of adverse effects (4–7). There are other factors that have been related

to the appearance of adverse reactions to SIT apart from the dose, such as the type of allergens, the levels of allergenic exposure, and the clinical severity (8).

The study of the optimum doses of SIT runs parallel to that of the standardization of allergenic extracts (9, 10). The technology of monoclonal antibodies now allows us to quantify exactly the levels of the main major allergens in complex mixtures such as allergenic extracts for diagnosis or SIT. This standardization level permits the design of SIT dosification studies and the easy comparison of the

results obtained (7, 11). Often, the optimal doses of SIT have been identified with the maximal tolerated doses, although this identification does not have a clear scientific basis (11, 12). It seems to be more logical to think of optimal doses as those capable of modifying the clinical picture of the patients while giving a clear margin of good tolerance (13).

This study aimed to assess the changes observed in clinical and immunologic parameters after treatment with SIT, according to the dose administered, in asthmatic patients sensitized to *Dermatophagoides pteronyssinus* (Dp). This was done by comparing two groups of patients according to the maximum tolerated dose (MTD) they achieved:

- 1) the high-dose immunotherapy (HDI) group: patients tolerating doses of 4–16 µg Der p 1
- 2) the conventional immunotherapy group (CI): patients tolerating doses lower than 4 µg Der p 1.

For this SIT, we used a biologically standardized extract, in which the quantities (µg) of major mite allergens of groups 1 and 2 were also known.

Material and methods

The study was carried out in the Section of Allergology of the Hospital Virgen del Camino in Pamplona (Spain). The protocol was approved by the hospital's Committee of Investigation and Ethics, and all subjects gave informed consent.

Study design

The study was designed as an open-dose titration trial. The patients could not be randomized, because we specified that all patients had individually to reach the MTD in the SIT protocol. The study consisted of the following two phases:

- 1) An observation phase (run-in): (November 1990–January 1991) of 8 weeks' duration. In this phase, 43 patients were evaluated, and given a daily score for their symptoms and medication; quantification of allergenic exposure levels was also done. At the end of this phase, the two clinical investigators who participated in the study carried out a grading of the severity of the disease which will be described later.
- 2) A treatment phase: this consisted of three basic periods:
 - T₀ (February 1991): the start of the SIT program
 - T₁ (September 1991): after the administration of the doses of the initiation period and three maintenance doses
 - T₂ (May–June 1992): after the administration of the following eight maintenance doses.

In T₁, the patients were divided into two groups according to their MTD:

- 1) the HDI group: patients tolerating doses of 4–16 µg Der p 1 (vial IV)
- 2) the CI group: patients tolerating doses lower than 4 µg Der p 1 (vial III).

The patients were not informed of the SIT doses they were receiving, and the two investigators who carried out the clinical assessment were unaware of which group each patient belonged to until the end of the study. At T₀, T₁, and T₂, we determined the cutaneous and conjunctival reactivity, as well as serum IgE and IgG levels; specific IgG1 and IgG4 levels against the complete extract of Dp and against Der p 1 and Der p 2 allergens were also determined. As data of the clinical follow-up, the grading of the severity of the disease was again determined at T₂ by the same clinical investigator. Between T₁ and T₂ (December 1995/January 1996), daily scoring of symptoms and the consumption of drugs over 8 weeks was again carried out.

Study population

Forty-three patients from our outpatient clinic, diagnosed with bronchial asthma (14) and sensitized to Dp, were selected according to the following criteria:

- 1) positive skin prick test with Dp extract (Abelló, Madrid, Spain)
- 2) level of serum-specific IgE to Dp ≥ 3.5 kU/l (CAP System, Pharmacia, Sweden)
- 3) typical history of bronchial asthma of at least 2 years
- 4) absence of sensitization against other perennial allergens.

Patients not disposed to regular attendance at our SIT unit, those with contraindications for SIT (3), and those who had received previous SIT were excluded from the study.

The demographic and clinical data of the patients are presented in Table 1. All had mild to moderate asthma, diagnosed according to published guidelines (14), and none required treatment with oral steroids. Both groups were comparable, although 11 patients (50%) in the HDI group were on inhaled budesonide (eight receiving 400 µg/day) but only five (33%) patients in the CI group.

Allergen extracts

All preparations were supplied by Abelló (Madrid, Spain). The extracts used were partially purified, standardized extracts of Dp whole-mite culture from which molecular components smaller than

Table 1. Demographic and clinical data

	Group 1 (HDI)	Group 2 (CI)
<i>n</i>	15	22
Sex (M/F)	11/4	13/9
Age (years)	12 (6–50)	15 (6–32)
Duration of asthma (years)	5 (2–30)	5 (2–18)
Medication		
Inhaled B ₂ -agonist	5	7
Inhaled cromoglycate	5	4
Inhaled budesonide	5	11

Median values given for age and duration of asthma; range in parentheses.

5 kDa were removed. Total allergenic activity was measured by RAST inhibition, and the biologic activity was determined by skin tests, as described by Brighton et al. (15) and Lombardero et al. (16). The content of the major allergens Der p 1 and Der p 2 was quantified by monoclonal antibody enzyme immunoassays (17). At a concentration of 100 BU/ml, the extract contained 40 µg/ml of Der p 1 and 20 µg/ml of Der p 2. A depot extract adsorbed in aluminum hydroxide (Pangramin® depot, Abelló, Madrid) was used for SIT. The same batch of the extract in different diluents was used throughout the study for skin prick test (SPT), conjunctival provocation test (CPT), and SIT.

Specific immunotherapy

The dose-increment schedule is presented in Table 2. The maintenance dose was tailored to each patient in order to administer the MTD. This was defined as the highest dose not inducing a systemic reaction, or the one just before that inducing three consecutive large local reactions. In patients who presented a mild systemic reaction before reaching the dose of 2.4 µg of Der p 1 (6 BU), the last dose tolerated was again administered, and later another attempt to raise it was made. In any case, maintenance doses higher than 16 µg of Der p 1 (40 BU) were not administered. After the MTD were reached, the next two maintenance injections were given quarterly and the other eight injections every 4 weeks. The safety of SIT was monitored according to the EAACI guidelines (3). All doses of SIT were administered in our SIT Unit. At every visit, patients were asked about the status of their disease and the appearance of reactions to the previous injections, and peak expiratory flow (PEF) rate was recorded. After allergen injections, the patients stayed at the clinic for 30 min, and any sign or symptom of systemic or large local reactions, and another PEF record were carefully registered. Indications for dose reduction were as follows: a systemic reaction (immediate or delayed); an early local reaction defined as local swelling at the

Table 2. Schedule for dose increment

No.	Week	Vial	Volume (ml)	Dose UB	Dose µg Der p 1
1	1	I (0.1 BU/ml)	0.1	0.001	0.004
2	2	0.04 µg/ml Der p 1	0.2	0.02	0.008
3	3		0.4	0.04	0.016
4	4	0.02 µg/ml Der p 2	0.8	0.08	0.032
5	5	II (1 BU/ml)	0.1	0.1	0.04
6	6	0.4 µg/ml Der p 1	0.2	0.2	0.08
7	7		0.4	0.4	0.16
8	8	0.2 µg/ml Der p 2	0.8	0.8	0.32
9	9	III (10 BU/ml)	0.1	1	0.4
10	10		0.2	2	0.8
11	11	4 µg/ml Der p 1	0.4	4	1.6
12	12		0.6	6	2.4
13	13	20 µg/ml Der p 2	0.8	8	3.2
14	14	IV (100 BU/ml)	0.1	10	4
15	15		0.2	20	8
16	16	0.4 µg/ml Der p 1	0.4	40	16
18	20	20 µg/ml Der p 2	0.4	40	16

injection site 5 cm or greater in diameter, occurring 30 min after the injection; or a late local reaction 10 cm or greater in diameter occurring from 30 min to 48 h after an injection.

Clinical assessment

Diary cards. During two 8-week periods, the patients noted down their symptoms and daily consumption of bronchodilatory medication. The first period was completed before the start of the SIT (run-in period) and the second period, 1 year later. The severity of symptoms was assessed on a scale of 0–3 for cough, wheezing, chest tightness, and night symptoms (range 0–12). Every two puffs of salbutamol (200 µg) scored 1 point. Patients were highly committed to use their maintenance drugs (cromoglycate or inhaled steroids), if prescribed, daily in these periods. The consumption of these drugs was analyzed as an ordinal variable, and patients were divided into two groups: no such treatment (salbutamol prn) and anti-inflammatory treatment (cromoglycate or budesonide). No patient needed doses of inhaled steroids higher than 800 µg/day.

Clinical severity score. A severity score for the disease was registered by the two clinical investigators who participated in the study at the beginning and at the end of the SIT, according to the clinical status of the previous 3 months. This score was a combined one, based on the sum of a symptom score (Aas's symptom score [0–4 points]) (18), the maintenance medication needed for the control of the

disease (none: 0 points; salbutamol prn: 1 point; cromoglycate: 2 points; budesonide: 3 points), and the value of forced expiratory volume (FEV_1) on a basal day ($FEV_1 > 80\%$: 0; $FEV_1 < 80\%$: 1). The range of the score was 0–8.

Skin prick test (SPT)

SPT was performed in duplicate on the volar side of the forearm with three concentrations: 6, 30, and 150 BU/ml. Glycerinated normal saline and histamine HCl 10 mg/ml were used as controls. Prick-Lancett BN (Dome/Hollister-Stier) lancets were used. After 15 min, the wheal reactions were marked with a soft, fine-tip pen, and transparent adhesive tape was placed over the wheals. The tape was transferred to a record sheet and the area of the wheals was measured. The change in skin sensitivity was estimated by parallel-line bioassay (19, 20). Results were omitted in two cases (one in each group) of nonparallel regression lines. The precision of the technician who performed the SPT had been determined previously, as recommended (21). Her variation coefficient (histamine 10 mg/ml in quadruplicate, measuring wheal areas) was 13%.

Conjunctival provocation test (CPT)

The method described by Möller et al. (22) was used. The concentrations used were 0.24, 1.2, 6, 30, and 150 BU/ml. The criteria for a positive test were congestion of the conjunctival mucosa and itching.

Determination of specific IgE, IgG, IgG1, and IgG4 antibodies

Serum IgE, IgG, IgG1, and IgG4 specific for *D. pteronyssinus* extract, as well as for the main allergens Der p 1 and Der p 2, were determined by ELISA (23, 24). Briefly, the whole extract and purified Der p 1 and Der p 2 were adsorbed to plastic wells. After incubation, the serum to be tested was added to the wells and incubated, and, finally, mouse monoclonal antibodies specific for the respective immunoglobulins were used as tracers. The sensitivity and specificity of the technique has been reported elsewhere (25–27). Results were expressed in arbitrary units per ml (AU/ml).

Allergen exposure levels

Dust samples from the bedrooms were collected by the patients with their own vacuum cleaners in a standardized fashion (28). Aqueous extracts of the samples were prepared (15% w/v), and levels of Der p 1, Der f 1, and Der p 2 were determined by a commercial ELISA (DEA-Test, Abelló,

Spain), which contained monoclonal antibodies (17, 29).

Statistical analysis

The data were analyzed with the statistical packages BMDP and PLA. The data of the *in vitro* tests were log transformed to increase symmetry. The results were compared with the nonparametric test, the Wilcoxon test, or the Mann-Whitney U test for continuous variables, or the chi-square test for categorical variables. Correlation between variables was determined by the Spearman correlation test. All *P* values were two-tailed. A difference was considered statistically significant if the *P* value was below 0.05.

Results

Of the 43 patients initially selected, 37 reached the T_1 period and 34 completed the SIT (T_2). The nine patients who did not complete the study were lost for the following reasons: stopping treatment after systemic reaction (one patient), bad clinical control of asthma (one patient), appearance of general diseases that contraindicated SIT (three patients), voluntarily stopping the study protocol (two patients), and change of residence (two patients). For the study of the safety of and the adverse reactions to SIT, all the data obtained from the 43 patients were used. In the analysis of the changes induced by SIT in the effect of the doses, only the data of the 34 patients that completed the study were used.

Safety monitoring and tolerance of SIT

A total of 1100 doses (64% buildup/36% maintenance periods, respectively) were administered, registering a total of 54 adverse reactions; 40 of these were systemic, appearing in 30 patients. These data represent a frequency of systemic reactions per dose administered of 4.10%. Only in three of the 37 patients who reached the maintenance period was the MTD determined by the appearance of repeated local reactions; therefore, most of the systemic reactions were not preceded by significant local reactions. The most frequent local reactions were those of late appearance ($n=9$ [64%]). Systemic reactions appeared only after administering of doses from vials III (0.4–3.2 µg/Der p 1) and IV (4–16 µg Der p 1). In the buildup period, the frequency of appearance of systemic reactions after administering of doses of vial IV was 26.4%, which was significantly higher than the frequency registered after administering vial III doses (4.8%) (odds ratio 7.04; 95% confidence

interval 2.91–17.45). Eighty-two percent of all the immediate systemic reactions registered were grade 1 or 2 on the EAACI scale (3). The remaining 18% were grade 3; no case of serious (i.e., grade 4) anaphylaxis was registered. The most common symptom presented by these patients was bronchospasm. Response to treatment was rapid and complete with the disappearance of the symptoms and normalization of the peak expiratory flow-rate values. The 13 delayed systemic reactions comprised of four cases of urticaria and nine of bronchospasm.

The MTD medians and ranges (in μg of Der p 1) of the accumulated doses for both HDI and CI groups of patients were 69 (29–93) and 25 (6–41) at T_1 and 197 (61–221) and 50 (12–66) at T_2 , respectively. The distribution of the MTD frequencies and the tolerance percentage is shown in Fig. 1. The range of the MTD oscillated between 2 BU and 40 BU (0.8–16 μg of Der p 1; 0.4–8 μg for Der p 2). The mode value of the MTD was 8 BU (3.2 μg of Der p 1); this dose was reached by more than 90% of our patients.

The only characteristic that differed between the two groups at the baseline period was skin sensitivity. As assessed by PLA, skin sensitivity to Dp was 3.9 times lower for the HDI group ($P < 0.001$). All other clinical and laboratory parameters, including the allergen exposure level, were quite similar (Tables 1 and 3). In general, the level of exposure of the patients to mite allergens was mild or moderate. The geometric means and range for all the samples were 0.70 (0.01–44) for Der p 1, 0.04 (0.01–6) for Der f 1, and 0.72 (0.01–22) $\mu\text{g/g}$ for Der p 2.

Clinical efficacy

Diary cards. During the run-in period, a diary card was completed by 80% of patients in group HDI and by 95% in group CI. After 1 year, the corresponding figures were 75% of patients in group HDI and 80% in group CI. The daily symptom scores were very low at the beginning of the study. The median score at T_0 was 4.5 for the HDI group and 6.5 for the CI group. There was a small but

Table 3. *In vitro* parameters at baseline period (T_0) (medians)

Parameter	HDI group	CI group
IgE (AU/ml)		
<i>D. pteronyssinus</i>	2.2	4.7
Der p 1	13.1	14.7
Der p 2	21.8	26.4
IgG (AU/ml)		
<i>D. pteronyssinus</i>	2569	3676
Der p 1	213	216
Der p 2	432	536
IgG1 (AU/ml)		
<i>D. pteronyssinus</i>	2106	2502
Der p 1	136	111
Der p 2	414	219
IgG4 (AU/ml)		
<i>D. pteronyssinus</i>	48	71
Der p 1	60	84
Der p 2	52	72
Allergen exposure		
Der p 1 ($\mu\text{g/g}$ dust)	2.1	0.64

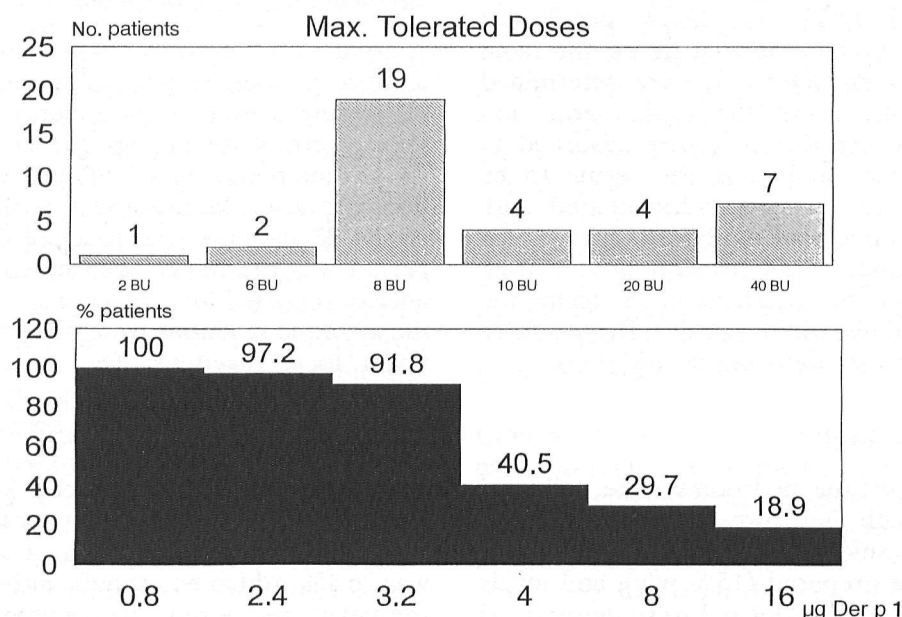


Fig. 1. Maximum dose administered and percentage of tolerance.

Table 4. Changes in medication use

Study phase	Bronchodilators Median score		Anti-inflammatory No. patients (%)	
	HDI group	CI group	HDI group	CI group
Observation phase	22	37	10 (66%)	15 (68%)
Treatment phase	2	8	2 (9%)	3 (20%)

nonsignificant reduction at T_2 in both groups (median HDI score: 4; median CI score: 4.9). On the other hand, there was a significant reduction in the use of bronchodilators (Wilcoxon's test, $P < 0.01$) and anti-inflammatory treatment (Fisher's test, $P < 0.01$) for both groups. At the beginning of the study, 70% of patients in group HDI and 75% in group CI used anti-inflammatory treatment regularly (CGS or budesonide), but only two patients (7%) in group HDI and three (15%) in group CI continued on this treatment. Results are shown in detail in Table 4.

Clinical severity score. The clinical severity score, as shown in Fig. 2, was significantly reduced from the baseline period (T_0) in both groups at T_2 (Wilcoxon's test, $P < 0.01$), again without significant differences between them.

Skin and conjunctival sensitivity

The changes in skin sensitivity to Dp are shown in Fig. 3. In both groups HDI and CI, the skin sensitivity decreased at T_1 : 6.5- and 4.9-fold, respectively. An additional improvement was observed at T_2 . The cutaneous tolerance index was 22.7-fold the pretreatment value in the HDI group (95% confidence interval: 12.6–40.9). This was significantly higher than the 6.2-fold increase in the CI group (95% confidence level: 3.7–10.4) ($P < 0.001$).

Changes in conjunctival sensitivity at T_1 and T_2 from the baseline period (T_0) were significant for both groups (Wilcoxon's test, $P < 0.01$), but there were no differences between the groups.

Dp-specific antibodies

Dp-specific antibody concentrations relative to pretreatment levels (medians) for both groups at T_1 and T_2 are shown in Table 5.

Dp-specific IgE antibodies. In both groups HDI and CI, Dp-specific IgE increased significantly ($P < 0.05$) at T_1 : 1.36 and 3.97, respectively (Mann-Whitney test, $P < 0.05$). On the other hand, these levels decreased equally at T_2 in both groups (0.61 and 0.64; $P < 0.01$ for both).

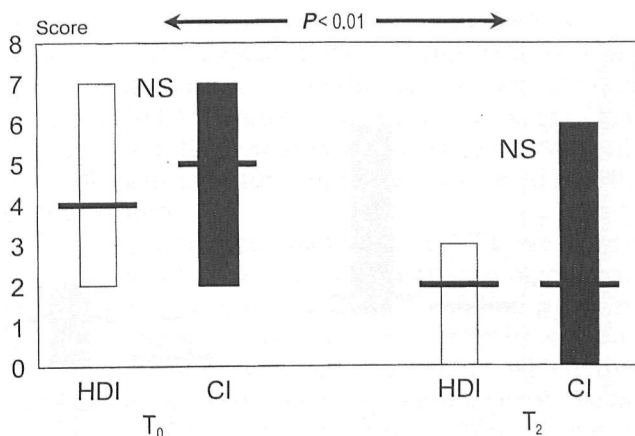


Fig. 2. Clinical severity score for both groups. Box: interquartile range with median as horizontal line.

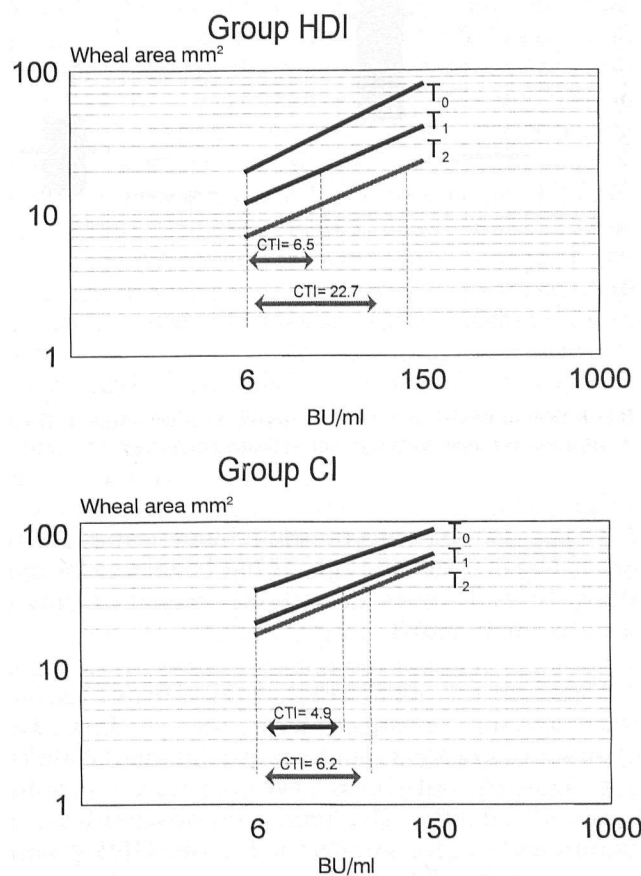


Fig. 3. Results of PLA at different times of study.

Changes in the levels of Der p 1- and Der p 2-specific IgE relative to pretreatment are presented in Fig. 4. There was a progressive fall that reached significant values in T_1 for the patients of the HDI group, while in T_2 the descent was significant for both groups. Significant differences between both groups were registered for specific IgE levels against Der p 2 in T_1 (Mann-Whitney test;

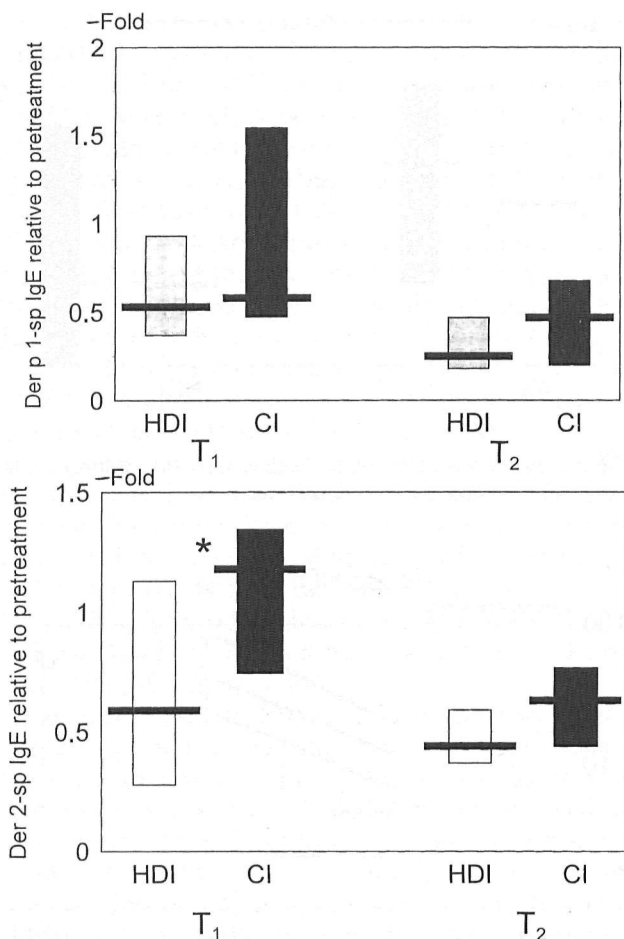


Fig. 4. Box plots of specific IgE relative to pretreatment. Box: interquartile range with median as horizontal line. * $P < 0.05$.

$P < 0.05$). Two patients presented undetectable levels of Der p 1-specific IgE at the beginning of the study. In both cases, these levels remained undetectable in T_1 and T_2 .

Dp-specific IgG antibodies. A significant increment of the levels of both IgG against the complete Dp extract and against the major allergens Der p 1 and Der p 2 was registered at both times ($P < 0.001$). The increases observed were significantly more important for the HDI group in T_1 and T_2 . IgG1 levels registered the most important increases in T_1 , with a later decrease of the same in T_2 , although its levels were still found to be significantly much above those observed in the basal situation. The modifications observed in Der p 1- and Der p 2-specific IgG4 were significantly greater for the patients of the HDI group. The maximum increase was that registered in the specific IgG4 levels against Der p 2 (36 and 17 times in T_1 ; 119 and 23 times in T_2 for the HDI and CI groups, respectively). The changes registered against Der p 1 were also

Table 5. *Dermatophagoides pteronyssinus*-specific antibody concentration relative to pretreatment levels (medians)

	T_1			T_2		
	HDI group	CI group		HDI group	CI group	
IgE						
<i>D. pteronyssinus</i>	1.36	3.97	■	0.61	0.64	NS
Der p 1	0.51	0.69	NS	0.24	0.46	NS
Der p 2	0.58	1.21	■	0.44	0.62	NS
IgG						
<i>D. pteronyssinus</i>	4.88	3.03	■ ■ ■	4.36	3.04	■
Der p 1	15.76	8.01	■	17.31	8.18	■
Der p 2	10.5	3.85	■ ■ ■	12.26	4.07	■ ■
IgG1						
<i>D. pteronyssinus</i>	15.74	7.42	NS	10.91	5.78	NS
Der p 1	19.46	10.48	■ ■	9.41	6.06	■
Der p 2	23.95	8.90	■ ■	9.21	9.23	NS
IgG4						
<i>D. pteronyssinus</i>	31.98	17.37	■	64.00	24.27	■ ■
Der p 1	36.78	17.49	■	119.5	23.31	■ ■
Der p 2	39.75	13.43	■	53.00	16.68	■

Significant differences between groups: ■ $P < 0.05$; ■ ■ $P < 0.01$; ■ ■ ■ $P < 0.001$

highly pronounced (39 and 13 times in T_1 ; 53 and 16 times in T_2).

Changes in specific IgG or the subclasses IgG4 or IgG1 did not correlate to the decrease observed in the levels of specific IgE.

Correlation between clinical and immunologic changes

Changes in the levels of specific IgE were not correlated to changes in cutaneous or conjunctival sensitivity except for Der p 1-specific IgE, which was modestly related to the decrease in cutaneous sensitivity (Spearman's test, $r = 0.45$, $P = 0.007$). Changes in the levels of specific IgG4 to Dp and to Der p 1 and Der p 2 were correlated to changes in cutaneous sensitivity ($r = 0.51$, $P = 0.002$; $r = 0.47$, $P = 0.005$; $r = 0.54$, $P = 0.001$, respectively). The increase observed in IgG4 levels against Der p 1 and Der p 2 were also discretely correlated with the changes in the conjunctival sensitivity ($r = 0.42$, $P = 0.013$; $r = 0.41$, $P < 0.05$).

No significant correlation was seen between clinical parameter improvement (clinical severity score, medication score) and changes in IgG, IgG1, IgG4, IgE, or skin and conjunctival sensitivity.

Discussion

In this study, we carried out a close follow-up of two groups of asthmatic patients sensitized to Dp,

who reached different maintenance doses. This close monitoring allowed us to verify the changes in the various immunologic *in vivo* and *in vitro* parameters, the clinical changes, and the appearance of adverse reactions, and to study their interrelationships according to the maintenance dose achieved.

The frequency of the systemic reactions we registered was much higher than that referred to in the daily clinical use of SIT with standardized extracts (30), although it was similar to those of other studies designed with the intention of reaching MTD (31). In spite of the potency and the high doses of the extract used, all the systemic reactions were mild or moderate, with an excellent response to the treatment. The bronchospasm attack was the most frequent symptom observed even in the late reactions; this finding agrees with the observations of other SIT studies with mites in bronchial asthma (30–33).

The range of the MTD oscillated between 0.8 (this reflects the extreme sensitivity to Dp presented by some of our patients) and 16 µg of Der p 1. These results reflected the recommendations of Norman (7) in SIT agreed with pollens, and agreed with the results of Wahn et al. (34) and Mosbech et al. (35) in SIT with mites. In these trials, the ranges of MTD were 0.6–12 µg of Der p 1 and 0.6–48 µg of Der p 1, respectively. From our previous experience, we chose the cutoff point of 8 BU (Brighton) (3.2 µg/Der p 1; 1.6 µg Der p 2) to separate the groups of the study, as a dose usually well tolerated (30). In effect, our results show that 90% of our patients tolerated this dose; however, the number of patients whose MTD was equal to or higher than 4 µg of Der p 1 (10 BU) declined tremendously, and only 18% of the patients reached the programmed maximum dose of 16 µg of Der p 1. These data show that, just as suggested by other authors (12, 36), the therapeutic range of SIT may be very narrow, since small increments in the doses notably increased the frequency of adverse reactions. In the study of possible basal parameter predictions of the tolerance of SIT, only basal cutaneous reactivity was related to it; patients with lower basal cutaneous sensitivity reached much higher maintenance SIT doses. Our results corroborated the observations of Turkeltaub in SIT with pollens (12) and those of Mosbech et al. in SIT with Dp (31). Therefore, the inclusion of patients with high cutaneous sensitivity to the allergen is essential in studies of the safety and efficacy of SIT in order to ensure its applicability in clinical practice. Delayed local reactions are well-known features of SIT; these were mild and subsided with local measures. In general, no predictable effect of local reaction has been found in

studies on SIT with pollens (37), molds (38), or mites (31). The exposure level to mite allergens in our patients was low or moderate (39), and did not influence SIT tolerance. Only one other study (31) has assessed the relationship between levels of mite-allergen exposure and tolerance of SIT with similar results.

In general, all the changes that we have registered in the *in vivo* or *in vitro* immunologic parameters agree with the findings described in most of the SIT studies in the literature (3, 6, 10, 40, 41). However, unlike most SIT studies, in which the changes in the IgE levels described have been modest (32, 34, 36), we registered a strong decrease of the serum-specific IgE levels on finishing the study (in spite of an initial increase in the levels at T₁ for the CI group). This decrease was important in both groups of patients for specific IgE both against the complete extract of Dp and, more strikingly, against the major allergens Der p 1 and Der p 2. In a comparative study of conventional SIT with extracts modified with polyethylene glycol, Mosbech et al. (42) also described a decrease in the specific IgE levels against Der p 1 in various patients, in the maintenance phase. Although we found a modest correlation with the changes in cutaneous sensitivity, it is difficult to determine the role played in the mechanisms of action in SIT of these quantitative changes of specific IgE, especially if we bear in mind that these changes do not correlate with the modifications in the conjunctival sensitivity against Dp, or with any other clinical parameter.

Our study clearly showed a close relationship between the doses of allergen administered and the modifications in the specific IgG levels against Dp and the main allergens Der p 1 and Der p 2, as well as of the IgG1 and IgG4 subclasses. We observed a great difference between both groups of patients, according to the maintenance doses reached. The change in most of these *in vitro* parameters was 15–100-fold for patients of the HDI group, in contrast to other studies of SIT with mites, in which the change observed was 3–10-fold (42–44). The general kinetics of both IgG1 and IgG4 subclasses follows a classical pattern, which has been already described (44–46). We have not observed a correlation of these increases in IgG with changes in IgE levels, and the correlations we registered with the changes in the cutaneous and conjunctival reactivity were modest and of similar magnitude to those described by Mosbech et al. (42) and Einarsson et al. (43). Therefore, this relationship could be considered a mere epiphenomenon; in our opinion, IgG cannot be used to monitor the outcome of immunotherapy, even if an isolated, major allergen-specific IgG is assessed.

With regard to the clinical parameters, we observed a marked decrease of the cutaneous sensitivity to the allergen, an observation well documented in the literature (36, 42, 47). We note that this decrease not only appeared in the buildup phase, but also persisted markedly during the maintenance phase, for both groups of patients. On the other hand, conjunctival reactivity was lower at the end of the study, but the magnitude of its change was similar for both patient groups (as was the case for specific IgE). Furthermore, we verified a clear clinical improvement of all the patients, with a decrease in the score of the clinical severity of the disease and a decrease in the consumption of bronchodilators and of anti-inflammatory medication. This clinical improvement was of equal magnitude for both patient groups, independently of the MTD reached. The drastic decrease in the consumption of anti-inflammatory treatment that we observed in both groups was remarkable. Therefore, the administration of maintenance doses equal to or higher than 4 µg of Der p 1, although it led to a positive increase in the response of various significant immunologic parameters (such as cutaneous reactivity or specific IgG), was not accompanied by a clear increase in the clinical therapeutic benefit to the patients. It did cause, as has been emphasized, a notable increase (sevenfold higher) in the risk of adverse reactions. However, it can be argued, as we admit, that the sample size of the study was small and the possibility of a type II error in the data must be considered. Due to the trial design, we did not know what would have happened if HDI patients had received a lower dose. However, the clinical and demographic characteristics of the two groups of patients were so similar (except for cutaneous sensitivity to *D. pteronyssinus*) that it can be assumed that changes of similar magnitude would have occurred if they had received the same doses of SIT. As far as we know, cutaneous reactivity to the allergen is not related to the clinical expression (activity, severity, or prognosis) of allergic respiratory diseases.

After administering cumulative doses of 80–100 µg of Der p 1, which were close to the cumulative dose of our group CI, Wahn et al. (34) induced pronounced changes in various clinical and immunologic parameters. Recently, in a dose titration study of SIT with Dp in asthmatic patients, Haugaard et al. (48) have proposed that a maintenance dose of 7 µg of Der p 1 has the best risk/benefit ratio.

The present therapeutic method for bronchial asthma, which proposes the introduction of anti-inflammatory drugs in the early phase in such a way that the symptomatology of the patient is minimal (49), is clearly responsible for our diffi-

culties in evaluating the symptom score. The evaluation of the changes in the symptomatology of the patients in many SIT studies has been problematic for various reasons (50), and what we have pointed out should be added to this list.

In conclusion, the tolerance range of SIT in the treatment of bronchial asthma due to Dp with standardized extracts, in which the quantity of major allergens is well known, is ample and is correlated with the cutaneous sensitivity of the patient against the allergen. A maintenance dose of 3.2 µg of Der p 1 and 1.6 µg of Der p 2 (8 BU) induced intense clinical and immunologic changes, with a very low risk of adverse reactions. A very few patients tolerated doses equal to or higher than 4 µg of Der p 1 or 2 µg of Der p 2 (10 BU). In spite of receiving a higher maintenance dose, this group of patients achieved the same level of clinical benefit as those who tolerated small doses, provided that these small doses were their respective maximum tolerated doses.

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