

Successful management of mite-allergic asthma with modified extracts of *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae* in a double-blind, placebo-controlled study

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Background: Mite sensitivity is highly prevalent in tropical and subtropical regions, such as the Canary Islands.

Objectives: To evaluate the clinical efficacy and safety of a depigmented polymerized allergen vaccine containing a 50% mixture of *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*.

Methods: Sixty-four patients participated in the study. It was prospective, double-blind, and placebo-controlled, with random allocation of patients to receive active treatment or placebo. The active group received a mixture of modified allergen extracts containing 50% *D pteronyssinus* and 50% *D farinae*; the control group received placebo. All individuals were diagnosed with mild/moderate asthma and rhinoconjunctivitis caused by sensitization to *D pteronyssinus* and *D farinae*. Bronchial provocation test (BPT) was considered the main outcome to document clinical efficacy.

Results: Fifty-four patients completed the study. The active group experienced a significant improvement in BPT ($P < .001$), whereas the placebo group did not ($P = .648$). At the end of the study, 20 patients in the active versus 9 in the placebo group ($P = .013$; odds ratio, 5.71 [1.76, 18.51]) needed more than twice the amount of allergen to obtain a positive BPT. The median improvement in the active group over placebo was 53.76% in total symptom and 58.09% in medication scores.

Conclusion: Immunotherapy with a mixture of modified allergen extracts of *D pteronyssinus* and *D farinae* is safe and efficacious to treat mite-allergic asthma.

Clinical implications: This immunotherapy modifies the natural course of the illness because it improves all clinical outcomes measured and prevents the worsening of specific bronchial hyperreactivity. (J Allergy Clin Immunol 2006;117:1016-1023.)

Key words: Modified allergen vaccines, immunotherapy, depigmentation, allergoids, asthma, mites, *Dermatophagoides* spp, Canary Islands

Dermatophagoides pteronyssinus and *Dermatophagoides farinae* are one of the most common sources of indoor allergens worldwide.¹ In subtropical latitudes, most individuals with allergy are sensitized to house dust mites,²⁻⁴ and mite allergen levels regularly exceed 10 µg of Der p 1 per gram of dust.⁵ The overall treatment of these patients focuses primarily on education, the reduction of allergen exposure, pharmacotherapy, and immunotherapy. However, in the light of recent publications that have shown negative results using environmental control measures,⁶⁻⁸ immunotherapy with mite extracts has emerged as a solid treatment of mite-induced allergic respiratory diseases. Specific immunotherapy with mite extracts has demonstrated clinical benefits in double-blind, placebo-controlled trials using aqueous, physically, or chemically modified extracts.⁹⁻¹³ The threshold dose eliciting an immediate bronchial constriction or nasal obstruction after immunotherapy is significantly increased in most studies using provocation tests as an objective measure of efficacy.

A new type of modified allergen extract has been successfully used to treat patients with allergy. These extracts include a depigmentation step and a subsequent polymerization with glutaraldehyde and adsorption onto aluminum hydroxide. Extracts produced by using this procedure have demonstrated clinical efficacy and safety in open¹⁴⁻¹⁸ and double-blind, placebo-controlled studies.¹⁹⁻²¹

The objective of this study was to evaluate the clinical efficacy and safety of a depigmented, polymerized vaccine containing a mixture of *D pteronyssinus* and *D farinae* using objective (improvement in specific bronchial provocations tests and dose-response skin test) and subjective (visual analog scale [VAS], asthma-related quality of life, and medication and symptom scores) measures.

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Received for publication February 8, 2006; revised July 5, 2006; accepted for publication July 13, 2006.

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doi:10.1016/j.jaci.2006.07.043

Abbreviations used

AQLQ: Asthma Quality of Life Assessment
 BPT: Bronchial provocation test
 IQR: Interquartile range
 VAS: Visual analog scale

METHODS**Study design and patient assignment**

The study was prospective, double-blind, placebo-controlled, and randomized. The active group received a mixture (50%:50%) of modified allergen extracts of *D pteronyssinus* and *D farinae*, whereas the control group received placebo. The placebo contained aluminum hydroxide, phenol, and saline solution with the exception of the modified allergen extracts. The duration of the study was 54 weeks. This study was approved by the Ethics Committee of the University Hospital "Virgen de la Candelaria," Santa Cruz de Tenerife, and the Spanish Health Authorities. The inclusion criteria were clinical history of asthma and rhinoconjunctivitis suggestive of house dust mite allergy, positive skin tests to house dust mites using standardized extracts, negative to other mite species and common aeroallergens, positive specific bronchial provocation test to a standardized *D pteronyssinus*, and detectable specific IgE (CAP ≥ 2) to this mite. All patients who met these criteria had mild or moderate asthma following Global Initiative for Asthma criteria²² and rhinoconjunctivitis caused by sensitization to *D pteronyssinus* and *D farinae*. The severity of rhinitis was persistent in frequency and moderate to severe in symptoms, according to Allergic Rhinitis and its Impact on Asthma.²³ The exclusion criteria were those outlined in the World Health Organization position paper on allergen immunotherapy,²⁴ as well as pregnancy, lactation, and aspirin intolerance. After the initial diagnostic tests, the patients were blindly randomized and treated with active vaccine or placebo. Both groups received pharmacologic treatment. All patients were evaluated weekly (at the build-up phase) and monthly (when maintenance dose was reached). At this time, a comprehensive evaluation of the patient took place, and the medication was adjusted as needed. The allowed medication consisted of loratadine, salbutamol, and topical (nasal and bronchial) glucocorticosteroids (budesonide).

Sample size and patient population

The sample size was calculated on the basis of the number of patients who at the end of the study would need more than twice the amount of native allergen extract needed at baseline to achieve a positive bronchial provocation test (BPT). Assuming that 25% of the patients receiving placebo and 70% in the active group would achieve this improvement, for an $\alpha = 0.05$ (2-tailed) and $\beta = 0.2$ (1-tailed), the required sample size was 52 patients.²⁵ Assuming a 20% of drop-outs, the sample size was adjusted to 64 patients. Demographic data of patients are shown in Table I.

Modified allergen extracts of *D pteronyssinus* and *D farinae*

The vaccines for study were supplied by the allergen manufacturer (Laboratorios LETI, SL, Madrid, Spain). These vaccines were prepared and standardized as previously described.¹⁸ The 50% specific IgE binding inhibition points were determined by ELISA²⁶; 295 ng was needed for the native and 18.52 mcg for the modified extracts of *D pteronyssinus* and 77 ng and 2.38 mcg for *D farinae*, respectively. The IgG binding activity was measured by ELISA;

TABLE I. Demographic data of the patients included in the study

	Active	Placebo
Patients included	32	32
Men	15	12
Women	17	20
Mean age ($\pm$ SD)	23.5 $\pm$ 9.3	23.8 $\pm$ 7.7
Mild asthma	3	3
Moderate asthma	29	29
Inhaled corticosteroids at baseline	29	29

13 ng of the native and 15 ng of the modified extract of *D pteronyssinus* and 17 ng of the native and 15 ng of the modified extract of *D farinae* were needed to produce 50% inhibition. The native extract of *D pteronyssinus* contained 20.35 μ g Der p 1 and 12.30 μ g Der p 2 per mg freeze-dried extract. The *D farinae* extract contained 19 μ g Der f 1. As a result of the polymerization process, Der p 1, Der p 2, and Der f 1 could not be detected in the modified extracts. The same batch of native extract of *D pteronyssinus* was used for all *in vivo* tests (BPT and dose-response skin prick tests).

Treatment schedule

Two consecutively numbered vials containing the polymer were prepared for each patient. The concentration in vial 1 was 3.5 μ g/mL freeze-dried extract of *D pteronyssinus* plus 2.8 μ g of *D farinae*; vial 2 contained 10 times more. The trial medication was administered subcutaneously at regular intervals using the following schedule: 0.1, 0.3, and 0.5 mL vial 1 (1 injection/wk) followed by 3 administrations of 0.1, 0.3, and 0.5 mL vial 2 (1 injection/wk). Finally, 12 administrations of 0.5 mL vial 2 were administered in monthly intervals.

Safety

Side reactions were recorded and classified as local or systemic, or immediate or delayed. Local reactions were expressed as the length of the diameter of the erythema. Local immediate reactions with a diameter of less than 5 cm and delayed of less than 10 cm were considered clinically irrelevant. Systemic reactions were graded in accordance with the European Academy of Allergy and Clinical Immunology guidelines.²⁷

BPT

The BPT was considered the main outcome to document the clinical efficacy.²⁸ It was performed at baseline and after 1 year, according to the method of Cockcroft et al,²⁹ with slight modifications.³⁰ The same batch of standardized native unmodified allergen extract of *D pteronyssinus* at 0.002, 0.02, 0.2, and 2 mg/mL was used throughout the trial. The vial of maximum concentration had a potency of 100 histamine equivalent in prick-testing units (HEP)/mL and contained 40.70 μ g Der p 1/mL and 17.22 μ g Der p 2 (Laboratorios LETI, S.L.). It was supplied freeze-dried and vacuum-closed to be reconstituted just before use. All patients were tested between 8 AM and noon, none was pretreated or challenged under circumstances that could affect the outcome of the study, and all discontinued inhaled steroids 30 days before the BPT. Only immediate responses were recorded and evaluated. The allergen extract was delivered by means of a DeVilbiss 646 nebulizer (DeVilbiss Co., Somerset, Pa) following the method described by Rosenthal et al.³¹ The nebulizer was connected to a dose-metering device that electronically controlled the flow of compressed air at 20 psi through the

nebulizer for a fixed interval. The test was initiated with 5 inhalations of the lowest concentration of the allergen extract (0.002 mg/mL). If the decrease in FEV₁ was less than 20% of the predicted value, the patient performed 5 additional inhalations of the next concentration (0.02 mg/mL), and the FEV₁ was measured again. If the result was negative, the process followed with the remaining concentrations until a decrease greater than 20% of the predicted value was achieved. The results of the BPT were expressed as the PD₂₀FEV₁ value that was calculated according to Chai et al.³² The results for each group were expressed as the median of the PD₂₀FEV₁ and its increase from baseline to the end. Because the reproducibility of the PD₂₀FEV₁ is between the limits of a 2-fold concentration of the antigen,³³⁻³⁵ the results were also expressed as the number of patients who needed at the end of the study more than double or less than the half the initial amount of allergen extract to experience a positive BPT.

Evaluation of the worst-case scenario

Following the recommendations of the medicine based on evidence, the worst-case scenario related to the main outcome was studied.³⁶ This analysis consisted in the consideration that patients who dropped out of the study would have remained during the whole length of the study. This test considers that patients who did not finish the study and were receiving the active treatment had a bad evolution, whereas those on placebo improved. We considered that the patients who abandoned the active group had the same median worsening in PD₂₀FEV₁ as those who worsened in the placebo group, whereas those who left the placebo group had the same median improvement as those who improved in the active group.

Skin prick tests

Patients were skin tested at baseline and at the end. These tests were conducted in duplicate on the volar surface of the forearm using the same batch and at the same concentrations used for BPT. Glycerinated saline solution and histamine HCl 10 mg/mL were used as negative and positive controls, respectively. All the reactions were recorded after 15 minutes of application.²⁸ The area of each wheal was measured by planimetry using a Wacom palette (Wacom Technology Co, Vancouver, Wash) and the computer program MacDraft (Microspot, Inc, Boca Raton, Fla). The results were expressed²⁸ for each patient as the dose of native allergen extract needed to produce the same wheal size as the positive control (10 HEP).

Symptom and medication scores and VAS

All patients recorded daily symptom scores (nose, eyes, and lungs) in a diary card, and the weekly score was calculated. The evaluated symptoms were nose (sneeze, blockage, and running), eye (itching, redness, and swelling), and chest (breathlessness, wheeze, chest tightness). The rating system used in this clinical trial was 0 (absent symptoms), 1 (mild), 2 (moderate), and 3 (severe).³⁷ The total symptom score was calculated as the sum of all individual symptom scores. Medication was recorded and quantified according to Dreborg et al³⁸ with slight modifications: 1 point was given for the intake of 1 tablet (antihistamine), the inhalation of 1 puff of a short-acting β -adrenergic agent, or 1 puff of 200 μ g budesonide. VAS consisted of a 100-mm horizontal line with no intermediate marks or signs. The left end read "very poor" and the right end "very good." It was filled after each injection and was used to evaluate the clinical perception of the patients regarding asthma severity. This scale has a good relation with the perception of bronchial obstruction.³⁹ The results of symptom and medication scores and VAS were expressed as the mean,⁴⁰ as well as the percentage of improvement over placebo. Furthermore, the number of weeks with symptom and medication scores ≤ 1 and with values >10 were counted and compared between groups. Because of the length of the trial, some patients failed to fill their diary

card daily. The number of weeks in which the diary card was not filled and remained blank was also counted to establish potential differences between both groups.

Asthma Quality of Life Assessment

The questionnaire developed by Marks et al⁴¹ was used. The total scale score was measured together with subscale scores for the domains of breathlessness, mood disturbance, social disruption, and health concerns. It was formulated after each injection of the vaccine during the trial. The results were expressed as the mean of all measurements.

Statistical methods

The analysis of the study was on the basis of intention to treat. The Excel spreadsheet (Microsoft, Inc, Seattle, Wash), and the statistical software SPSS v.11.0 (SPSS, Inc, Chicago, Ill) and StatView (SAS Institute, Cary, NC) were used. The results were analyzed for normality (Shapiro-Wilk), showing that practically all the outcomes did not follow a normal distribution. Descriptive statistics for BPT and skin tests were expressed as the median with the first and third interquartile range (IQR), whereas for symptom and medication scores, VAS and Asthma Quality of Life Assessment (AQLQ) were expressed as the mean with 95% CIs. Wilcoxon, Mann-Whitney, and Fisher exact *P* value tests were used for comparative statistics. The Hodges-Lehmann estimator (with the 95% lower and upper confidence limits) was used to measure the effect size of the differences between the 2 groups. The odds ratio with 95% CI was calculated in the main variable of evaluation (BPT).

RESULTS

Patients

Fifty-four patients (84.4%) completed the trial; 5 abandoned in each group. Withdrawals were not caused by lack of efficacy, worsening of the disease, or adverse reactions. One patient withdrew because of pregnancy. Each patient who completed the study received 18 injections (5 in the build-up phase and 13 in maintenance).

Safety

There were 4 local reactions (2 in each group). All these reactions were with the maintenance dose (0.5 mL of vial number 2), and all were immediate with a diameter smaller than 5 cm. No systemic reactions were reported.

BPT

At baseline, the median value of the PD₂₀FEV₁ was 10.05 (IQR, 5.48, 81.00) for the active and 43.50 (IQR, 6.10, 511.00) for the placebo group (*P* = .416, Mann-Whitney test). At the end, these values were 111.06 (IQR, 41.05, 686.00) and 41.00 (IQR, 3.35, 311.00), respectively (*P* = .029). The active group showed a significant difference in PD₂₀FEV₁ from baseline (*P* < .001, Wilcoxon test), whereas the placebo group did not (*P* = .648). Fig 1 shows the evolution of the PD₂₀FEV₁ in the active and placebo groups, respectively.

At the end of the study, 20 patients in the active group needed more than twice the amount of the native allergen to obtain a positive BPT (the median of the ratio for these patients was 5.30; IQR, 3.34, 12.20) versus 9 in the placebo group (the median of the ratio for these patients

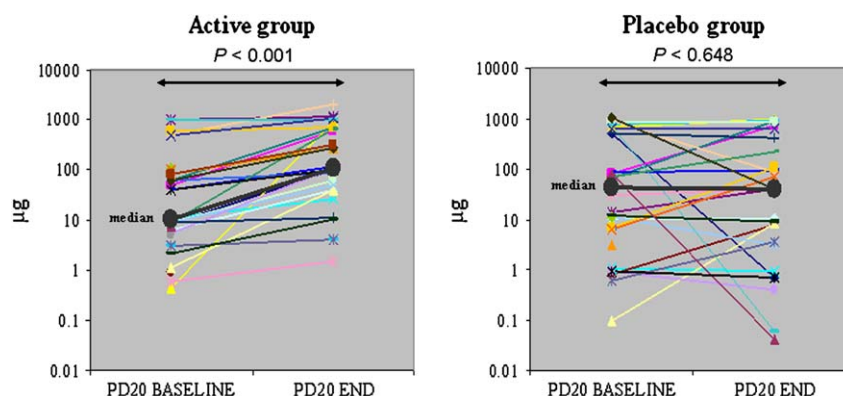


FIG 1. Individual changes in a log scale of the PD₂₀FEV₁ values in the active and placebo groups.

TABLE II. Statistical analysis of the bronchial provocation tests, including all the patients who completed both bronchoprovocations and the worst-case scenario*

	Active	Placebo	Hodges-Lehmann	P†
Study	79.80 (29.50; 225.00)	−0.04 (30.45; 645.90)	97.06 (32.00; 230.54)	<.001
Worst-case scenario	46.90 (1.83; 205.00)	6.06 (−4.08; 73.93)	52.02 (1.41; 147.00)	.0291

*The median values (and interquartile ranges) of the difference of PD₂₀FEV₁ from baseline to the end for each group and the comparative statistics are shown. The line of the worst-case scenario reflects the corrected data once the values from the patients who abandoned the study were incorporated.

†Mann-Whitney test.

was 10.03; IQR, 6.00, 13.08; $P = .013$; odds ratio, 5.71 [1.76; 18.51]). The number of patients who needed less than half the initial amount of allergen extract to experience a positive BPT at the end of the study was 0 in the active and 7 (26%) in the placebo group (among these 7 patients, the median of the ratio was 0.04; IQR, 0.001, 0.019; $P = .024$).

The median amount of allergen needed to produce the PD₂₀FEV₁ at baseline in the 5 patients who withdrew the study in the active group was 8.02 mcg freeze-dried native allergen extract of *D pteronyssinus* (IQR, 5.10, 101) and in the 5 patients in the placebo group, 11.00 (IQR, 6.10, 56.00; $P > .917$). In the worst-case scenario, we supposed that all patients who abandoned were also challenged at the end of the study. After correction of the data, including now the theoretical worsening in the active group (PD₂₀FEV₁ from baseline multiplied by 0.04), or improvement in the placebo group (PD₂₀FEV₁ from baseline multiplied by 5.3), the active group still showed a significant difference from baseline (Wilcoxon $P < .001$), whereas the placebo group did not ($P = .270$). Table II shows the median increment of the amount of native allergen extract needed to achieve the PD₂₀FEV₁ at the end of the study.

Skin prick tests

The active group showed a significant difference from baseline ($P = .029$, Wilcoxon), whereas the placebo group did not ($P = .900$). The active group needed at the end of the study a median of 21.9 μ g (IQR, 8.99, 73.80) more of allergen extract to achieve the value of 10 HEP, whereas in the placebo group the figure was −0.31 μ g (IQR, −21.28, 18.10). The difference between both groups was

significant ($P = .015$, Mann-Whitney). The result of the Hodges-Lehmann test was 24.34 (5.22, 63.70).

Symptom and medication scores and visual scale

The complete treatment time for each patient was 54 weeks. Because of the patient abandons and oversights to fill the diary cards, the median of the weeks completed was 46 (85.2%; IQR, 37, 50) for the active group and 47 (87%; IQR, 39, 49) for the placebo ($P = .54$). The descriptive (median and IQR) and comparative statistics are summarized in Table III. Fig 2 shows the evolution of the sum of symptom and medication scores, respectively. The median improvement in total symptom and medication scores in the active versus placebo group was 53.86% and 58.09%, respectively. For all patients in the active group, there were 567 weeks free of inhaled corticosteroids (median per patient, 13; IQR, 3.5, 30.5) versus 431 (median, 6; IQR, 1.0, 18.5) in the placebo group (Fisher exact P value < .001). The number of weeks with a score higher than 10 for all patients in the active group was 414 (median of 5 weeks/patient; IQR, 0.0, 21.2) and 717 (median of 14 weeks/patient; IQR, 5.7, 41.0) for the placebo group ($P < .001$).

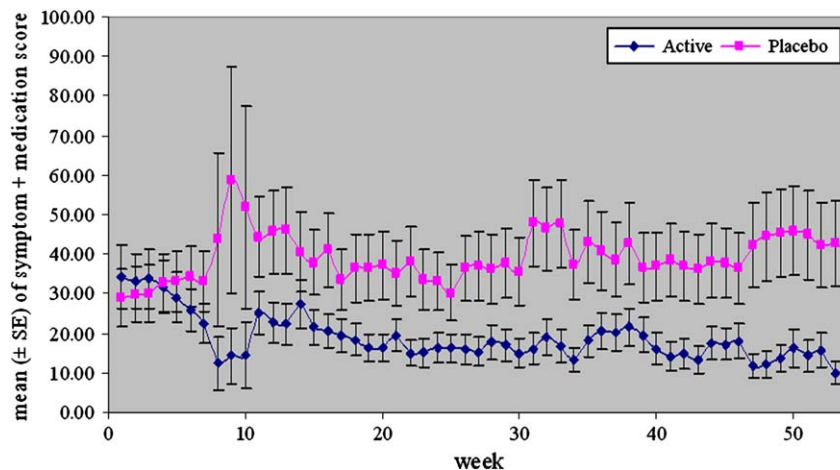
AQLO

The median values for both groups were practically identical at baseline (22 and 23 for active and placebo groups, respectively). There was a sharp improvement from baseline to the third week of treatment in both groups ($P < .05$ for both groups). In the third week, the values were 9 and 11, respectively. After this week, the values of the placebo group were in the range from 14 to 7, whereas in the active group the trend was to improve, with the range

TABLE III. Descriptive statistics (mean and 95% CI) of bronchial, nasal, ocular, and overall symptom scores, medication scores, visual scale, and AQLQ of both groups*

	Active	Placebo	Hodges-Lehmann	P (Mann-Whitney)	Percentage of improvement
Bronchial symptom score	3.16 (2.83, 3.50)	6.06 (5.71, 6.40)	-2.88 (-3.36, -2.43)	<.001	47.75
Nasal symptom score	6.62 (6.03, 7.20)	14.48 (13.69, 15.27)	-7.71 (-8.49, -6.76)	<.001	54.29
Ocular symptom score	1.85 (1.69, 2.01)	4.58 (4.25, 4.92)	-2.71 (-3.09, -2.39)	<.001	59.67
Overall symptom score	11.63 (10.66, 12.61)	25.12 (23.84, 26.40)	-13.02 (-14.67, -11.53)	<.001	53.86
Medication score	10.47 (9.69, 11.25)	22.09 (21.43, 22.75)	-12.67 (-13.22, -10.93)	<.001	58.09
Consumption of inhaled glucocorticosteroids	7.09 (6.40, 7.77)	14.20 (13.71, 14.69)	-7.56 (-8.40, -6.73)	<.001	50.10
Visual scale	7.60 (7.36, 7.83)	6.73 (6.50, 6.96)	0.90 (0.59, 1.17)	<.001	12.88
AQLQ					
Overall	7.44 (5.78, 9.11)	11.44 (9.67, 13.22)	-4.36 (-8.69, -0.10)	.043	34.95
Breathlessness	1.36 (1.07, 1.66)	2.92 (2.51, 3.32)	-1.23 (-2.09, -0.39)	.005	53.33
Mood	2.00 (1.43, 2.57)	2.69 (2.22, 3.17)	-0.50 (-1.87, 0.51)	.254	25.77
Social	0.72 (0.35, 1.10)	3.17 (2.41, 3.93)	-1.44 (-0.01, -3.59)	.043	77.19
Concerns	2.22 (1.66, 2.79)	4.00 (3.34, 4.66)	-1.50 (-3.44, -0.22)	.025	44.44

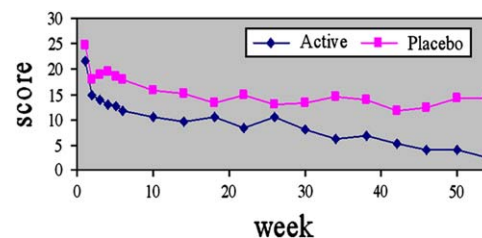
*Comparative statistics (Hodges-Lehmann and Mann-Whitney tests) were used to compare all these variables between both groups. The last column reflects the percentages of improvement in symptom and medication scores, VAS, and AQLQ in the active versus placebo group.

**FIG 2.** Sum of symptom and medication scores for the patients in the active and placebo groups.

from 10 to 3. The active group had a significantly better perception of quality of life in the overall questionnaire and all the domains, with the exception of the mood disturbance, than the patients of the placebo group. The descriptive and comparative statistics are summarized in Table III. Fig 3 shows the evolution of the overall AQLQ.

DISCUSSION

In the current manuscript, we describe the results of a double-blind, placebo-controlled study using a modified therapeutic vaccine containing a mixture of *D peronyssinus* and *D farinae* extracts. The results of the study demonstrate that the treatment was efficacious and well tolerated. The BPT was used as an objective method to document the clinical effect and to calculate the sample size. BPT has good reproducibility when using FEV₁ as an objective marker of obstruction^{42,43} not

**FIG 3.** Evolution of the overall AQLQ.

affected by suggestion.⁴⁴ PD₂₀FEV₁ is the most common outcome used to evaluate BPT. It is reproducible in a range of 1 doubling dose (2-fold variation). Twenty patients in the active group needed more than twice the amount of the native allergen to obtain a positive BPT, versus 9 in the placebo group. The data suggest that an increase in the threshold of the allergen needed to produce a reaction in the shock organ takes place primarily in the group that

received immunotherapy, although some patients in the placebo group also improved. However, the difference was statistically significant. An important clinical finding was that 7 (26%) patients of the placebo group needed less than half the amount of the native unmodified extract to achieve a positive specific BPT at the end of the study ($P < .05$). These patients seem to have experienced a progressive deterioration in $PD_{20}FEV_1$, in spite of a significantly greater consumption of inhaled glucocorticosteroids in the placebo group. This finding is consistent with a chronic inflammatory and remodeling process of the lungs, as has been suggested for several animal models.⁴⁵⁻⁴⁸ This result is also in agreement with the findings of Guerra et al¹⁴ and Ameal et al,²⁰ who reported similar results in patients with allergy and asthma sensitized to the pollen of *Olea europaea* and to *D pteronyssinus*, respectively, and who received treatment for their asthma following current guidelines, including inhaled corticosteroids, but did not receive immunotherapy. A Cochrane review⁴⁹ that examined 54 randomized controlled trials of specific immunotherapy in asthma confirmed the efficacy of immunotherapy in asthma as evidence A. In particular, it emphasized the clinically useful outcomes of decreased symptom scores and medication requirements, as well as improved allergen-specific and nonspecific airway hyperresponsiveness. The results of the Cochrane review were consistent, and the number of patients studied was greater than 1000, making interpretation of the meta-analysis valid.²² Our study confirms the clinical efficacy of immunotherapy when administered to well selected patients and the sparing effect of corticosteroids and other symptomatic medication. Furthermore, the study proves that immunotherapy is safe and prevents the worsening of the specific bronchial hyperreactivity as could be observed in the conventionally treated (placebo) group. Furthermore, the median improvement in the active group over placebo was 53.76% in total symptom and 58.09% in medication scores. Therefore, the early use of immunotherapy in correctly selected patients should be considered.

Patients in the study were advised to use conventional environmental control measures. After the study was completed and the code opened, the patients were contacted to establish who had used encasements and/or acaricides. None of the participants had used these special measures. This study demonstrates that the vaccines used induced a significant clinical improvement in this patient population, in spite of the effectiveness, or not, of the regularly recommended environmental control measures. This finding is not surprising based on recent environmental control studies that, using pragmatic approaches, have shown no clinical benefit of the regularly prescribed environmental control measures.

An important tool in the analysis of clinical trials is the incorporation of the worst-case scenario analysis. To the best of our knowledge, this is the first time that this analysis has been applied to a double-blind, placebo-controlled study of immunotherapy. We applied this concept to the results of BPT and confirmed that in spite of

25-fold reduction (original ratio multiplied by 0.04) in the dose needed for the 5 patients who withdrew from the active group and a 5.3-fold increase in the 5 patients in the placebo group, the results still retained their significant and relevant difference. Studies including more patients and longer observational periods are clearly needed, although ethical problems may arise with the use of placebo, because the specific hyperresponsiveness of these patients may worsen.

The median improvement in total symptom and medication scores in the active versus placebo group was 53.76% and 58.09%, respectively. This significant improvement can be considered highly relevant because it was calculated throughout the study and not only comparing baseline with the end of the study, as was the case in a previous study.²⁰ The difference in the number of weeks free of symptoms and medication was also highly significant between both groups, confirming the clinical efficacy of the treatment.

Quality of life is an outcome related to a fundamental aim of health care.⁵⁰ The analysis of the AQLQ shows that there was no significant difference at baseline ($P > .05$) between both groups. After inclusion in the study, both groups improved. This improvement could be explained as the Hawthorne effect,⁵¹ which is described as an initial improvement in a process of production caused by the obtrusive observation of that process. However, in our study, statistical analysis of the results between both groups demonstrated a significant difference in the overall questionnaire and in all domains, except in mood disturbance.

In conclusion, after 1 year of treatment with a modified vaccine of *D pteronyssinus* and *D farinae*, patients in the active group experienced a significant improvement in BPT and AQLQ and a significant reduction in symptoms, medication scores, and size of skin test reactions to *D pteronyssinus*.

REFERENCES

1. Platts-Mills TAE, de Weck AL. Dust mite allergens and asthma: a worldwide problem. *J Allergy Clin Immunol* 1989;83:416-27.
2. Fernández-Caldas E, Fox RW, Bucholtz GA, Trudeau WL, Ledford DK, Lockey RF. House dust mite allergy in Florida: mite survey in households of mite-sensitive individuals in Tampa, Florida. *Allergy Proc* 1990;11:263-7.
3. Nelson RP Jr, DiNicolo R, Fernández-Caldas E, Seleznick MJ, Lockey RF, Good RA. Allergen-specific IgE levels and mite allergen exposure in children with acute asthma first seen in an emergency department and in nonasthmatic control subjects. *J Allergy Clin Immunol* 1996;98:258-63.
4. Kemp SF, Lockey RF, Fernández-Caldas E, Arlian LG. Skin test and crossreactivity studies with *Euroglyphus maynei* and *Dermatophagoides pteronyssinus*. *Clin Exp Allergy* 1997;27:893-7.
5. García Robaina JC, Torre Morín F, Bonnet Moreno CG, Antolín Arias J, Pérez Santos C, Sánchez Covisa A. House dust mites and Der p 1 in Tenerife (Canary Islands, Spain): the relative importance of other non *Dermatophagoides* spp mites. *Allergol Immunopathol (Madr)* 1996;24:135-8.
6. Terreehorst I, Duivenvoorden HJ, Tempels-Pavlica Z, Oosting AJ, de Monchy JG, Bruijnzeel-Koomen CA, et al. The effect of encasings on quality of life in adult house dust mite allergic patients with rhinitis, asthma and/or atopic dermatitis. *Allergy* 2005;60:888-93.
7. Terreehorst I, Hak E, Oosting AJ, Tempels-Pavlica Z, de Monchy JG, Bruijnzeel-Koomen CA, et al. Evaluation of impermeable covers for bedding in patients with allergic rhinitis. *N Engl J Med* 2003;349:237-46.

8. Woodcock A, Forster L, Matthews E, Martin J, Letley L, Vickers M, et al. Medical Research Council General Practice Research Framework. Control of exposure to mite allergen and allergen-impermeable bed covers for adults with asthma. *N Engl J Med* 2003;349:225-36.
9. Machiels JJ, Lebrun PM, Jacquemin MG, Saint-Remy JM. Significant reduction of nonspecific bronchial reactivity in patients with *Dermatophagoides pteronyssinus*-sensitive allergic asthma under with allergen-antibody complexes. *Am Rev Respir Dis* 1993;147:1407-12.
10. Tabar AI, Echechipia S, García BE, Olaguibel JM, Lizaso MT, Gómez B, et al. Double-blind comparative study of cluster and conventional immunotherapy schedules with *Dermatophagoides pteronyssinus*. *J Allergy Clin Immunol* 2005;116:109-18.
11. Echechipia S, Tabar AI, Lobera T, Muñoz D, Rodríguez A, Blasco A, et al. Immunotherapy with a standardized *Dermatophagoides pteronyssinus* glutaraldehyde-modified extract against an unmodified extract: a comparative study of efficacy, tolerance and in vivo and in vitro modification of parameters. *J Investig Allergol Clin Immunol* 1995;5:325-32.
12. Malet A, Lluh M, Valero AL, Casanovas M. Clinical and immunological effects of immunotherapy with glutaraldehyde modified house dust mite extract. *Allergol Immunopathol (Madr)* 1994;22:226-32.
13. Dokic D, Schnitker J, Narkus A, Cromwell O, Frank E. Spezifische Immunotherapie mit einem neu entwickelten Hausstaubmilbenallergoid (Acaroid®). Eine doppelblinde, placebokontrollierte Studie über 2 Jahre mit einem Fortsetzungsjahr. *Allergo J* 2005;14:337-43.
14. Guerra F, Daza JC, Almeda E. Immunotherapy with a depigmented, polymerized vaccine of *Olea europaea* pollen allergens significantly reduces specific bronchial and skin test reactivity in sensitized patients after one year of treatment. *J Investig Allergol Clin Immunol* 2003;13:108-17.
15. García-Sellés J, Pascual A, Funes E, Pagán JA, López JD, Negro JM, et al. Clinical efficacy and safety of a depigmented and glutaraldehyde polymerized therapeutic vaccine of *Parietaria judaica*. *Allergol Immunopathol (Madr)* 2003;31:63-9.
16. Ferrer A, García-Sellés J. Significant improvement in symptoms, skin test, and specific bronchial reactivity after 6 months of treatment with a depigmented, polymerized extract of *Dermatophagoides pteronyssinus* and *D. farinae*. *J Investig Allergol Clin Immunol* 2003;13:244-51.
17. Íbero M, Castillo MJ. Significant improvement of specific bronchial hyperreactivity in children after 4 months of treatment with a modified extract of *Dermatophagoides pteronyssinus*. *J Investig Allergol Clin Immunol* 2006;16:194-202.
18. Branco Ferreira M, Spinola Santos A, Pereira Santos MC, Palma Carlos ML, Pereira Barbosa MA, Palma Carlos AG. Efficacy and safety of specific immunotherapy with a modified mite extract. *Allergol Immunopathol (Madr)* 2005;33:80-5.
19. Álvarez-Cuesta E, Aragonese-Gilsanz E, Martín-García C, Berges-Gimeno P, González-Mancebo E, Cuesta-Herranz J. Immunotherapy with depigmented glutaraldehyde-polymerized extracts: changes in quality of life. *Clin Exp Allergy* 2005;35:572-8.
20. Ameal A, Vega-Chicote JM, Fernández S, Miranda A, Carmona MJ, Rondón MC, et al. Double-blind and placebo-controlled study to assess efficacy and safety of a modified allergen extract of *Dermatophagoides pteronyssinus* in allergic asthma. *Allergy* 2005;60:1178-83.
21. Colás C, Monzón S, Venturini M, Lezaun A. Double blind, placebo controlled study with a modified therapeutic vaccine of *Salsola kali* (Russian thistle) administered using a cluster schedule. *J Allergy Clin Immunol* 2006;117:810-6.
22. Global Initiative for Asthma (GINA). Global strategy for asthma management and prevention: NHLBI/WHO workshop report, March 1993. National Institutes of Health, Publication No. 953659. 1995. Available at: <http://www.ginasthma.org>. Accessed May 2004.
23. Bousquet J, van Cauwenberge P, Khaltaev N. Allergic Rhinitis and Its Impact on Asthma. *J Allergy Clin Immunol* 2001;108:147s-334s.
24. Bousquet J, Lockey R, Malling HJ. Allergen immunotherapy: therapeutic vaccines for allergic diseases. Geneva: January 27-29 1997. *Allergy* 1998;53(suppl 44):1-42.
25. Shuster JJ. Handbook of sample size guidelines for clinical trials. Boca Raton: CRC Press, Inc; 1990.
26. Casanovas M, Marañón F, Bel I. Comparison of skin-prick test assay and reverse enzyme immunoassay competition (REINA-C) for biological activity of allergens. *Clin Exp Allergy* 1994;24:134-9.
27. EAACI. Position paper: immunotherapy. (EAACI) The European Academy of Allergology and Clinical Immunology. *Allergy* 1993;48:7-35.
28. Nordic Council on Medicines. Registration of allergen preparations. Nordic guidelines. Prepared by the Nordic Council on Medicines in cooperation with the Drug Regulatory Authorities in Denmark, Finland, Iceland, Norway and Sweden. NLN Publication #23. January ed. Uppsala: Nordiska Läkemedelsnämnden; 1989. p. 1-36.
29. Cockcroft DW, Killian DN, Mellon JJ, Hargreave FE. Bronchial reactivity to inhaled histamine: a method and clinical survey. *Clin Allergy* 1977;7:235-43.
30. Frölund L. Bronchial allergen challenge methodological studies. *Allergy* 1996;51:1-41.
31. Rosenthal RR, Norman PS, Summer WR. Bronchoprovocation: effect on priming and desensitization phenomenon in the lung. *J Allergy Clin Immunol* 1975;56:338-46.
32. Chai H, Farr RS, Froehlich LA, Mathison DA, McLean JA, Rosenthal RR, et al. Standardization of bronchial inhalation challenge procedures. *J Allergy Clin Immunol* 1975;56:323-7.
33. Fish JE, Ankin MG, Adkinson NF, Peterman VI. Indomethacin modification of immediate-type immunologic airway responses in allergic asthmatic and non-asthmatic subjects: evidence for altered arachidonic acid metabolism in asthma. *Am Rev Respir Dis* 1981;123:609-14.
34. Ruffin RE, Cockcroft DW, Hargreave FE. A comparison of the protective effect of fenoterol and Sch 1000 on allergen-induced asthma. *J Allergy Clin Immunol* 1978;61:42-7.
35. Kopferschmitt-Kubler MC, Bigot H, Pauli G. Allergen bronchial challenge tests: variability and reproducibility of the early response. *J Allergy Clin Immunol* 1987;80:730-40.
36. Sackett D, Richardson S, Rosenberg W, Haynes R. Evidence-based medicine: how to practice and teach EBM, 1st ed. London: Churchill Livingstone; 1997.
37. CDER. Guidance for industry: allergic rhinitis: clinical development programs for drug products. Draft guidance. 2000. Available at: <http://www.fda.gov/cder/guidance/index.htm>. Accessed June 2005.
38. Dreborg S, Agrell B, Foucard T, Kjellman NI, 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.
39. Roisman GL, Peiffer C, Lacroque JG, Le Cae A, Dusser DJ. Perception of bronchial obstruction in asthmatic patients: relationship with bronchial eosinophilic inflammation and epithelial damage and effect of corticosteroid treatment. *J Clin Invest* 1995;96:12-21.
40. Altman DG. Some common problems in medical research. In: Practical statistics for medical research. London: Chapman & Hall; 1991. p. 396-439.
41. Marks GB, Dunn SM, Woolcock AJ. A scale for the measurement of quality of life in adults with asthma. *J Clin Epidemiol* 1992;45:461-72.
42. Cropp GJ. Relative sensitivity of different pulmonary function tests in the evaluation of exercise-induced asthma. *Pediatrics* 1975;56(suppl 2):860-7.
43. Fish JE, Kelly JF. Measurements of responsiveness in bronchoprovocation testing. *J Allergy Clin Immunol* 1979;64:592-6.
44. Spector S, Luparello TJ, Kopetzky MT, Souhrada J, Kinsman RA. Response of asthmatics to methacholine and suggestion. *Am Rev Respir Dis* 1976;113:43-50.
45. Tran MU, Weir AJ, Fanucchi MV, Rodriguez AE, Pantle LM, Smiley-Jewell SM, et al. Smooth muscle hypertrophy in distal airways of sensitized infant rhesus monkeys exposed to house dust mite allergen. *Clin Exp Allergy* 2004;34:1627-33.
46. Snibson KJ, Bischof RJ, Slocombe RF, Meeusen EN. Airway remodeling and inflammation in sheep lungs after chronic airway challenge with house dust mite. *Clin Exp Allergy* 2005;35:146-52.
47. Kumar RK, Herbert C, Kasper M. Reversibility of airway inflammation and remodelling following cessation of antigenic challenge in a model of chronic asthma. *Clin Exp Allergy* 2004;34:1796-802.
48. McMillan SJ, Lloyd CM. Prolonged allergen challenge in mice leads to persistent airway remodelling. *Clin Exp Allergy* 2004;34:497-507.
49. Abramson M, Puy R, Weiner J. Immunotherapy in asthma: an updated systematic review. *Allergy* 1999;54:1022-41.
50. Spitzer WO. State of life and functional status as target variables for research. *J Chronic Dis* 1987;40:465-71.
51. Draper SW. The Hawthorne effect and other expectancy effects: a note. 2004. Available at: <http://www.psy.gla.ac.uk/~steve/hawth.html>. Accessed June 2005.