
Immunotherapy with mosquito (*Culex quinquefasciatus*) extract: a double-blind, placebo-controlled study

Deepsikha Srivastava, MSc*†; Bhanu P. Singh, PhD*; V. Thangam Sudha, MSc*†; Naveen Arora, PhD*; and Shailendra N. Gaur, MD‡

Background: Mosquito allergy is well established, but mosquito immunotherapy requires validation using clinical and immunologic variables.

Objective: To evaluate the tolerability and efficacy of specific immunotherapy with *Culex quinquefasciatus* (mosquito) extract.

Methods: We performed a randomized, double-blind, placebo-controlled trial of immunotherapy for 1 year in 40 patients with asthma, rhinitis, or both. Patients were evaluated by means of intradermal testing, symptom and drug scores, and histamine provocation testing before and after 1 year of immunotherapy. Mosquito specific IgE and IgG subclass antibody responses were evaluated at the basal level and after 1 year.

Results: Patients receiving allergen immunotherapy for 1 year showed a significant improvement compared with baseline and patients receiving placebo regarding skin reactions, symptom scores (rhinitis and asthma), and forced expiratory volume in 1 second. Provocation concentration of histamine that caused a decrease in forced expiratory volume in 1 second of 20% by inhalation was elevated in the group receiving immunotherapy. In the active group serologic analysis showed a slight reduction in IgE levels ($P = .02$) but a significant elevation in IgG4 levels ($P = .001$), with a significant decrease in the IgE/IgG4 ratio ($P = .001$). All these changes in the placebo group were nonsignificant.

Conclusions: Allergen immunotherapy with mosquito extract was well tolerated, with improvement in symptoms and airway reactivity. Good clinical outcome was associated with increased IgG4 antibody levels.

Ann Allergy Asthma Immunol. 2007;99:273–280.

INTRODUCTION

Allergic reactions to insects is a global problem. Up to 3% of the population is at risk for anaphylaxis to insect stings, with approximately 40 documented deaths annually.¹ Mosquito is one of the most common insects, and its bite results in a variety of diseases. Infection, mild discomfort, and cutaneous reactions are extremely common, and severe inflammatory reactions are not unusual.² In addition, mosquito-derived particles are important inhalant allergens for IgE-mediated respiratory allergic disorders.^{3,4} There are more than 3,000 mosquito species worldwide, of which *Aedes vexans*, *Aedes aegypti*, and *Culex quinquefasciatus* are the most common distributed globally.⁵

Allergic reactions are caused by interaction of IgE with allergen on mast cells or basophils, leading to release of an array of inflammatory mediators, resulting in inflammation of airway mucous membrane, leading to clinical symptoms in the target organ.⁶ Pharmacotherapy controls symptoms, which recur once treatment is stopped,⁷ and has adverse effects because it acts at the last stage of mediator release. In contrast, allergen immunotherapy modifies the underlying pathologic immune response in an antigen-specific manner.⁸ Controlled studies have convincingly demonstrated the beneficial effects of immunotherapy for allergic rhinitis and asthma treatment.⁹ Immunotherapy also restricts seasonal increases in bronchial hyperresponsiveness and prevents the onset of new sensitizations.¹⁰ Immunologic events in immunotherapy include formation of blocking antibodies IgG, reduction in specific IgE antibody levels, and induction of suppressor T cells.¹¹ However, allergen immunotherapy has long been a controversial treatment for asthma.¹² In India, only a few studies^{13–15} were performed to evaluate the effects of immunotherapy with *Cocos nucifera* pollen and mixed allergen preparations (vaccines).

Both IgE- and T-lymphocyte-mediated hypersensitivities are involved in mosquito-induced allergy, and mosquito bite causes specific sensitization.^{16,17} Immunotherapy with whole-body extracts has demonstrated efficacy in children and adults.^{18–20} *Culex quinquefasciatus*, a common mosquito spe-

* Allergy and Immunology Sections, Institute of Genomics and Integrative Biology, Delhi, India.

† Department of Biotechnology, University of Pune, Ganeshkhind, Pune, India.

‡ Department of Respiratory Medicine, Vallabhbhai Patel Chest Institute, University of Delhi, Delhi, India.

Authors have nothing to disclose.

This study was supported by the Department of Science and Technology, New Delhi.

Received for publication January 23, 2007.

Received in revised form April 18, 2007.

Accepted for publication May 6, 2007.

cies in India, plays an important role in the etiology of IgE-mediated respiratory diseases.²¹ However, no systemic study, to our knowledge, is available on immunotherapy with *C quinquefasciatus* extract. The present study is a randomized controlled trial evaluating the effects of immunotherapy with whole-body mosquito (*C quinquefasciatus*) extract in patients with allergic rhinitis and bronchial asthma sensitized to mosquito. Mosquito immunotherapy was evaluated by analyzing changes in clinical and immunologic variables before and after 1 year of treatment in the active and placebo groups.

MATERIALS AND METHODS

Patients and Study Design

One hundred twenty consecutive patients were screened by means of history, skin testing, and specific IgE estimation for the diagnosis of allergy at the outpatient department of Vallabhbhai Patel Chest Institute between July 1, 2004, and April 30, 2005. The diagnosis of asthma or rhinitis was confirmed following the guidelines of the American Thoracic Society²² or Adamopoulos et al.²³ Forty patients with a history of exposure to mosquito, marked positive skin test reactions using 2 dilutions (1:500 and 1:5,000 wt/vol), and raised specific IgE levels to mosquito were included in the study. These patients were randomly divided into 2 groups, active and placebo, in a double-blind manner. Immunotherapy was given with mosquito extract for 1 year starting July 1, 2005, to June 30, 2006, in the active group (n = 20) along with pharmacotherapy, whereas the placebo group (n = 20) received pharmacotherapy and placebo injections. The study protocol was approved by the human ethics committee of Vallabhbhai Patel Chest Institute, and written consent of the participants was obtained for inclusion in the study.

Allergen Extract

Mosquitoes (*C quinquefasciatus*) were freeze dried, crushed to a fine powder, and defatted using diethyl ether. Antigen extraction was performed in 1:50 wt/vol in phosphate-buffered saline (PBS).²⁴ Extracted antigen was dialyzed against distilled water and was filtered through a 0.45- μ m membrane (Millipore, Billerica, Massachusetts). The extract was lyophilized and stored at -20°C . Reconstituted extract was finally filtered through 0.22- μ m membrane and dispensed in 5-mL aliquots in sterile vials. For skin testing and immunotherapy, the extracts were prepared and standardized using the protocols of the antigen laboratory (Institute of Genomics and Integrative Biology), approved by the drug controller of India.

Skin Tests

Intradermal tests were performed with mosquito (*C quinquefasciatus*) and other common insect allergens, including housefly, cockroach, and moth extracts, in 1:500 wt/vol dilution. The tests were repeated with 1:5,000 wt/vol of mosquito extract for confirmation. Skin tests were also performed with pollen and fungal aeroallergens to rule out other asso-

ciated sensitivities in participants. Histamine diphosphate (100 $\mu\text{g/mL}$) was used as positive control and PBS as negative control. Approximately 0.02 mL of allergen extract was injected into the forearm of the patient, raising a bleb of 2 to 3 mm. The reactions were graded after 20 minutes on the basis of the wheal size of the positive control, ie, histamine diphosphate (100 $\mu\text{g/mL}$). A wheal size equal to the positive control was given a score of 2+.²⁵ Blood was collected from patients showing marked positive skin reactions at least the wheal size of histamine. Blood was also collected from 10 nonallergic volunteers (negative intradermal test results) to serve as controls.

Bronchial Provocation Tests

Bronchial provocation testing was performed at baseline and after 1 year of immunotherapy according to American Thoracic Society guidelines using a bronchoconstrictor. In brief, spirometric analysis was performed to obtain forced expiratory volume in 1 second (FEV₁) values. Airway reactivity was evaluated after a 2-minute tidal breathing protocol where aerosolized histamine in PBS was administered in increasing 2-fold concentrations from 0.004 to 1.024 mg/mL. The FEV₁ was measured after 30 and 90 seconds, and the highest reading was taken.²⁶ The challenge was stopped when a 20% decrease in FEV₁ from baseline was reached. Finally, patients were allowed to recover up to the baseline value. Airway reactivity was measured as the provocation concentration of histamine that caused a decrease in FEV₁ of 20% (PC₂₀).

Symptom and Drug Scores

Day-to-day symptoms for the entire study were recorded on a diary card provided to the patient. It was collected and evaluated every 3 months. Symptoms were recorded on a scale from 0 to 4 (0 = no symptoms, 1 = morning/night, 2 = evening, 3 = daytime, and 4 = most of the time) for nasal (sneeze, blockage, itching, and running nose) and bronchial (breathlessness, wheeze, chest tightness, and cough) symptoms. The daily symptom score was calculated as the sum of all individual symptom scores. Scores 3 months preceding immunotherapy were taken as baseline values for symptom and drug scores. Patients were allowed to use antiallergic and antiasthmatic drugs when necessary. Patients were instructed to record the use of drugs for symptom treatment, and a specific score was given in relation to the class of drug. Drugs were scored as follows: 1, inhaled short-acting β -agonists (SABAs), inhaled corticosteroid, inhaled corticosteroid nasal sprays, and oral antihistamines; 2, oral SABAs, ipratropium, oral theophylline, and oral corticosteroids; 3, long-acting β -agonists plus inhaled corticosteroids and high-dose oral SABAs; 4, injectable corticosteroids, injectable theophylline, nebulization with long-acting β -agonists, and antibiotics; and 5, hospitalization. Patients with missing data were considered noncompliant with the study and were dropped.

Serologic Analysis

Specific IgE, IgG1, and IgG4 antibodies were determined against mosquito extract using enzyme-linked immunosor-

bent assay at 0, 6, and 12 months.²⁷ Briefly, microtiter plates (Maxisorp; Nunc Immunomodule, Roskilde, Denmark) were coated with mosquito extract, 2 µg/100 µL per well in carbonate buffer (pH 9.6). Nonspecific sites were blocked with 3% defatted milk, washed, and incubated with individual patients' sera (1:10 vol/vol for IgE and IgG4, 1:100 vol/vol for IgG, and 1:50 vol/vol for IgG1). Sera from nonallergic individuals were used as negative controls. After washing, the plates were incubated for 3 hours with anti-human IgE-horse radish peroxidase (1:1,000 vol/vol) (Sigma-Aldrich Corp, St Louis, Missouri) in PBS. For IgG, IgG1, and IgG4, a plate was incubated for 2 hours with biotinylated anti-human IgG, IgG1, and IgG4 (1:1,000 vol/vol) (BD Pharmingen, Franklin Lakes, New Jersey); washed; and incubated with streptavidin-horseradish peroxidase conjugate (1:1,000 vol/vol) (BD Pharmingen) for 45 minutes. Color was developed using o-phenylene diamine, and absorbance was read at 492 nm.

Immunotherapy Schedule

Three consecutively numbered vials of allergen extract containing 10-fold increasing concentrations of allergen (0.03, 0.3, and 3.0 mg/mL) were given to the patients. Depending on the sensitivity of the patient for the allergen, injection doses varied from 0.003 to 3.0 mg/mL. The dose schedule prescribed was 2 injections per week for 0.003, 0.03, and 0.3 mg/mL starting from 0.1, 0.2, and up to 0.9 mL. Afterward, 3.0 mg/mL was taken weekly from 0.1 to 0.5 mL for 5 weeks, and a further 0.5 mL weekly was continued for 4 weeks. The same concentration (3.0 mg/mL) was used in subsequent

vials, with a slow increase from 0.6 to 1.0 mL. Next vials were prescribed as 1 injection every 2 weeks, then 1 injection every 3 weeks, and finally 1 injection per month. Once the maintenance dose is achieved it is continued once a month. The first injection was given in the clinic under supervision, and the patients were monitored for 1 to 2 hours. In the case of a systemic or local reaction, the dose increment was stopped and the next prescribed dose was the second to the last dose, ie, the dose given before the reaction.

Data Analysis

Parametric analysis of data was performed using the 2-tailed *t* test for skin tests, symptom and drug scores, and immunologic variables. A Wilcoxon test taking log-transformed values was performed for airway reactivity. A paired *t* test was used for intragroup comparisons, and an unpaired *t* test was used for intergroup comparisons. *P* ≤ .01 was considered statistically significant.

RESULTS

Analysis of Participants and Skin Tests

Demographic details of the 35 patients who completed 1 year of immunotherapy are given in Table 1. All 40 patients were sensitized to mosquito, as revealed by skin test and enzyme-linked immunosorbent assay results for specific IgE, and 30 were positive to mosquito and other insects. Ten patients were also sensitized to pollen and mold. Three patients dropped out of the active group (1 because of adverse family events and 2 were noncompliant), and 2 patients discontinued

Table 1. Skin Test Results and Specific IgE Values at Baseline and After 1 Year of Immunotherapy

Patient No.	Sex/age, y		Clinical history		Intradermal skin test				Specific IgE, OD at 492 nm			
	Active	Placebo	Active	Placebo	Active		Placebo		Active		Placebo	
					T0	T1	T0	T1	T0	T1	T0	T1
1	F/18	F/22	BA/AR	BA/AR	+++	++	++	++	0.545	0.410	0.483	0.866
2	F/32	M/18	BA/AR	AR	+++	++	+++	+++	0.540	0.500	0.300	0.456
3	M/25	F/32	BA/AR	BA/AR	+++	++	++	++	0.294	0.299	0.447	0.630
4	F/39	M/15	BA/AR	BA/AR	++++	+++	++	+++	0.400	0.390	0.559	0.629
5	M/32	M/22	AR	BA	+++	+	+++	++	0.216	0.324	0.320	0.450
6	M/43	M/17	BA/AR	BA/AR	+++	++	++++	+++	0.257	0.300	0.579	0.620
7	M/22	F/25	BA/AR	BA/AR	++++	+++	+++	++	0.425	0.450	0.503	0.530
8	F/27	M/42	BA/AR	BA/AR	+++	++	++	++	0.384	0.300	0.738	0.580
9	M/15	F/23	BA	BA/AR	++	-	++	++	0.364	0.240	0.415	0.416
10	F/18	M/24	AR	BA	+++	++	++	+++	0.380	0.287	0.515	0.435
11	F/29	F/23	BA/AR	BA/AR	+++	++	++	++	0.483	0.400	0.250	0.350
12	M/22	M/21	BA	AR	+++	++	+++	++	0.393	0.287	0.631	0.629
13	M/35	M/18	BA/AR	BA/AR	+++	++	++	++	0.326	0.228	0.403	0.530
14	M/21	M/27	BA/AR	BA/AR	+++	++	+++	++++	0.650	0.343	0.415	0.450
15	M/15	F/18	BA/AR	BA/AR	+++	++	+++	++++	0.467	0.470	0.537	0.732
16	M/19	F/30	BA/AR	AR	++++	++	+++	+++	0.308	0.212	0.430	0.330
17	F/27	F/28	BA/AR	BA	+++	++	++	++	0.239	0.267	0.383	0.468
18	NA	M/29	NA	BA/AR	NA	NA	+++	++	NA	NA	0.320	0.433

Abbreviations and symbols: BA, bronchial asthma; AR, allergic rhinitis; NA, not applicable; OD, optical density; T0, baseline (ie, before immunotherapy); T1, after 1 year of immunotherapy; ++, equal to histamine; +++, greater than histamine with 2 pseudopodia; +++++, greater than histamine with multiple pseudopodia; -, no reactivity/equal to negative control phosphate-buffered saline.

from the placebo group (for personal reasons). Intradermal testing with mosquito extract performed after 1 year of treatment in 17 patients in the active group showed a reduction in skin reactivity compared with baseline values. Twelve of the 17 patients also showed a reduction in specific IgE values, and the remaining 5 patients showed a reduction in skin reactivity only. In the placebo group 6 of the 18 patients showed increased sensitization, 3 showed decreased skin reactivity but no change in specific IgE levels, and 9 showed no change in skin reactions or specific IgE levels with the basal level. None of the patients in the active group showed increased sensitization to mosquito extract. Patients in both groups showed some reduction in skin reactivity to other insect allergens as well. Figure 1 shows skin test reactivity in terms of wheal size ($P = .02$ for the active group). Skin test response with allergens other than mosquito was nearly similar to baseline skin reactivity.

Symptom and Drug Scores

The median symptom scores for the active and placebo groups were 196 and 220, respectively. After 1 year of immunotherapy the median values for the active and placebo groups were 71 and 163, respectively. There was an improvement in symptom scores in patients receiving immunotherapy at the 6th (50%) and 12th (85%) months. Of these (85%) patients 25% had no symptoms of asthma or rhinitis after 1 year of immunotherapy. In the placebo group 30% of the patients showed some reduction in symptom score. The group receiving allergen immunotherapy had a significant difference from baseline ($P < .001$), whereas the placebo group did not show a statistically significant reduction. After 12 months

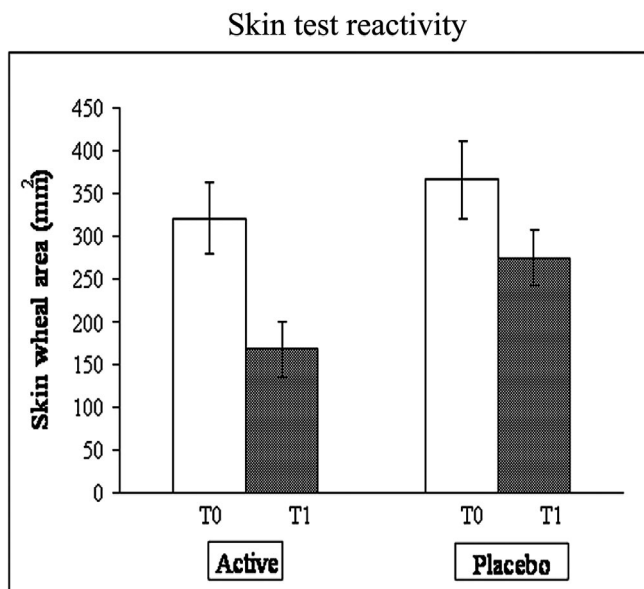


Figure 1. Skin test reactivity (mean wheal size) of the active and placebo groups at baseline, ie, before immunotherapy (T0), and after 1 year of immunotherapy (T1). Error bars represent SEM.

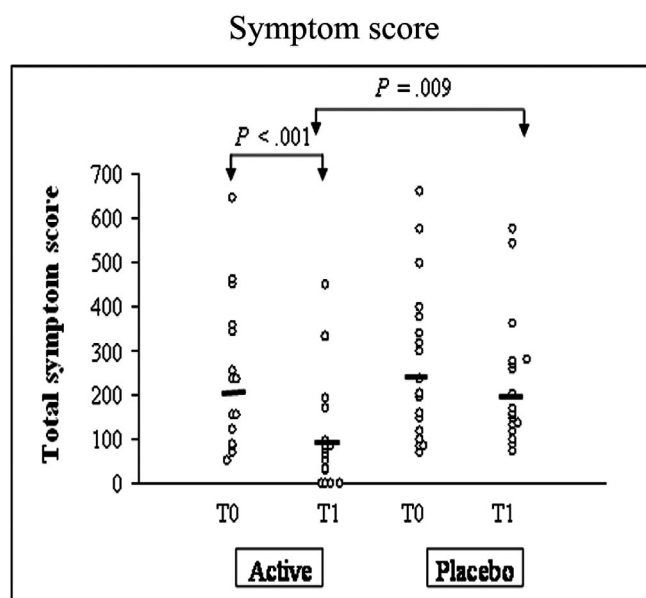


Figure 2. Scattergram of the total symptom scores of the active and placebo groups at baseline, ie, before immunotherapy (T0), and after 1 year of immunotherapy (T1). $P < .01$ for the active group compared with baseline and the placebo group after 1 year of immunotherapy. Horizontal bars indicate medians.

Table 2. Drug Scores for the Active and Placebo Groups at Baseline and After 1 Year of Immunotherapy^a

Disease	Active		Placebo	
	T0	T1	T0	T1
BA	504.0	472.0 ± 28.2	494.6 ± 31.0	494.0 ± 5.2
AR	84.0	81.5 ± 3.5	82.6 ± 2.3	90.0 ± 6.0
BA/AR	459.6 ± 158.3	453.0 ± 124.2	526.4 ± 74.3	573.2 ± 34.4

Abbreviations: AR, allergic rhinitis; BA, bronchial asthma; T0, baseline (ie, before immunotherapy); T1, after 1 year of immunotherapy.

^a Values are given as mean ± SD.

of immunotherapy, the active group showed a significant reduction in symptoms compared with the placebo group ($P = .009$) (Fig 2).

Drug scores were not significantly different between groups at the start of the study (Table 2). After 1 year of immunotherapy, 70% of patients in the active group demonstrated a reduction in drug use, but it was not statistically significant ($P = .07$). In the placebo group drug use reduction was seen in 20% of patients. Here, a change of 10% to 15% constituted a reduction in drug use (Fig 3). A decreased requirement for SABAs and oral antihistamines was also observed in the active group.

Airway Reactivity

In the active group 76% of patients showed a significant increase in PC₂₀ compared with baseline values ($P = .009$), and 41% of patients tolerated the highest dose, ie, 1.024 mg/mL, of histamine after 1 year of immunotherapy (Fig 4).

Change in symptom and drug score

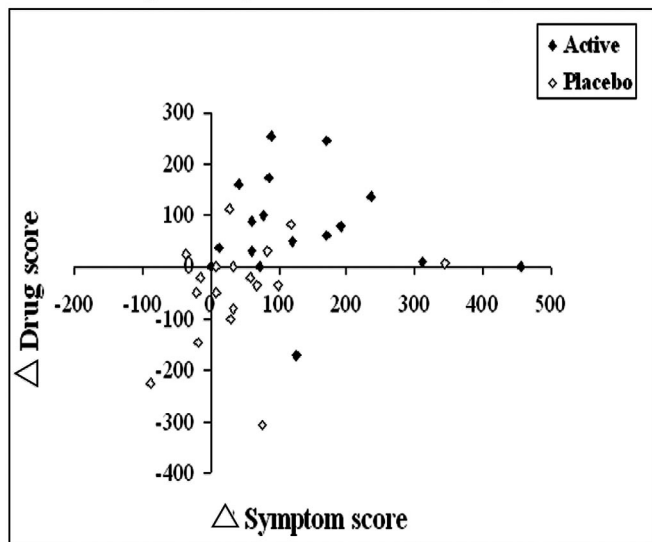


Figure 3. Scattergram of change (Δ) in symptom and drug scores from baseline, ie, before immunotherapy, to after 1 year of immunotherapy for the active and placebo groups.

In the placebo group 80% of patients showed a decrease in PC_{20} . The active group also showed a significant increase in PC_{20} compared with the placebo group ($P = .004$).

Airway reactivity

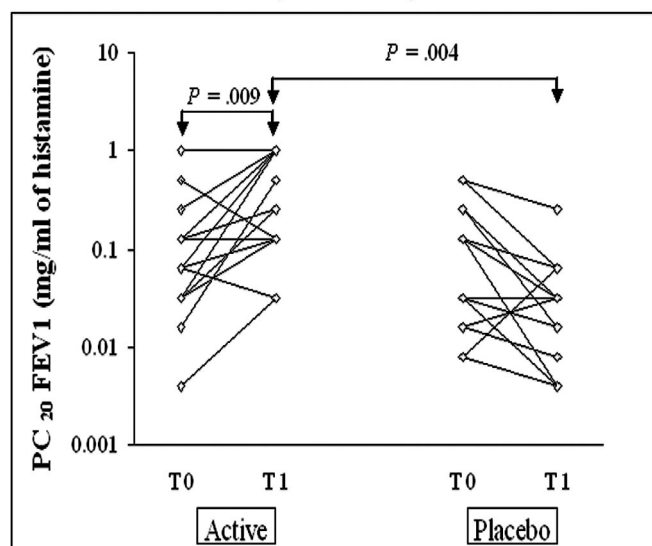


Figure 4. Airway reactivity (provocation concentration of histamine that caused a decrease in forced expiratory volume in 1 second of 20% [$PC_{20}FEV_{11}$]) in the active and placebo groups at baseline, ie, before immunotherapy (T0), and after 1 year of immunotherapy (T1). $P < .01$ for the active group compared with baseline and the placebo group after 1 year of immunotherapy.

IgE and IgG4

Serum IgE concentration did not differ in the active and placebo groups at baseline ($P = 0.03$). After 1 year of immunotherapy there was a reduction in specific IgE levels in the active group compared with the placebo group (Fig 5A). Specific IgG levels increased in the group receiving allergen immunotherapy, but there was not much change in the placebo group (data not shown). Among the subclasses of IgG, IgG4 levels showed a marked increase in the active group compared with baseline and the placebo group after 1 year of immunotherapy ($P < .001$) (Fig 5B). Levels of IgG1 decreased compared with baseline values for both groups ($P = .001$) (data not shown). In the active group the IgE/IgG4 ratio showed a significant decrease after 1 year of immunotherapy ($P < .001$) (Fig 6). The IgG4/IgG1 ratio correlated well with symptom scores in the active group (Fig 7).

DISCUSSION

Allergen immunotherapy continues to be the treatment of choice worldwide for allergic rhinitis and allergic asthma. The consensus reports and meta-analyses of placebo-controlled studies show allergen immunotherapy to be effective in reducing allergic symptoms and drug consumption in cases of respiratory allergy due to pollens, mites, animal danders, and molds.^{8,15} However, there is no correlation between clinical improvement and immunologic changes.²⁸ Until now, 3 studies on mosquito immunotherapy have been conducted based on clinical variables such as skin reactivity, nasal reactivity, and symptom and drug scores.^{18–20}

In the present study patients in the active group receiving immunotherapy with whole-body mosquito (*C quinquefasciatus*) extract for 1 year demonstrated significant improvement in clinical variables (skin reactivity, airway reactivity, and symptom and drug scores) compared with the placebo group. The decrease in skin reactivity to mosquito allergens was more than 50% compared with baseline in the active group, whereas skin reactivity to mosquito in the placebo group remained nearly the same, with just 10% to 20% change on either side. This finding is in agreement with a Cochrane meta-analysis²⁹ of 62 randomized controlled trials of immunotherapy performed between 1954 and 1998 that demonstrated significant improvements in symptoms, reductions in rescue medication use, and improvements in allergen specific and nonspecific bronchial hyperresponsiveness. A recent immunotherapy trial¹⁹ with mosquito allergen also showed a reduction in symptoms, local reactions, and allergen specific nasal reactivity. Similar results were observed with dust mite and ragweed immunotherapy.^{30,31}

In this study, with the progression of immunotherapy 50% of patients showed improvement in symptom scores after 6 months and 85% after 1 year of immunotherapy in the active group. Of these patients nearly 25% demonstrated no symptoms after completion of 1 year of immunotherapy, signifying the success of immunotherapy in treating patients with asthma, rhinitis, or both, where a reduction of 30% or more in symptom scores is considered clinically relevant.⁸ Only 30%

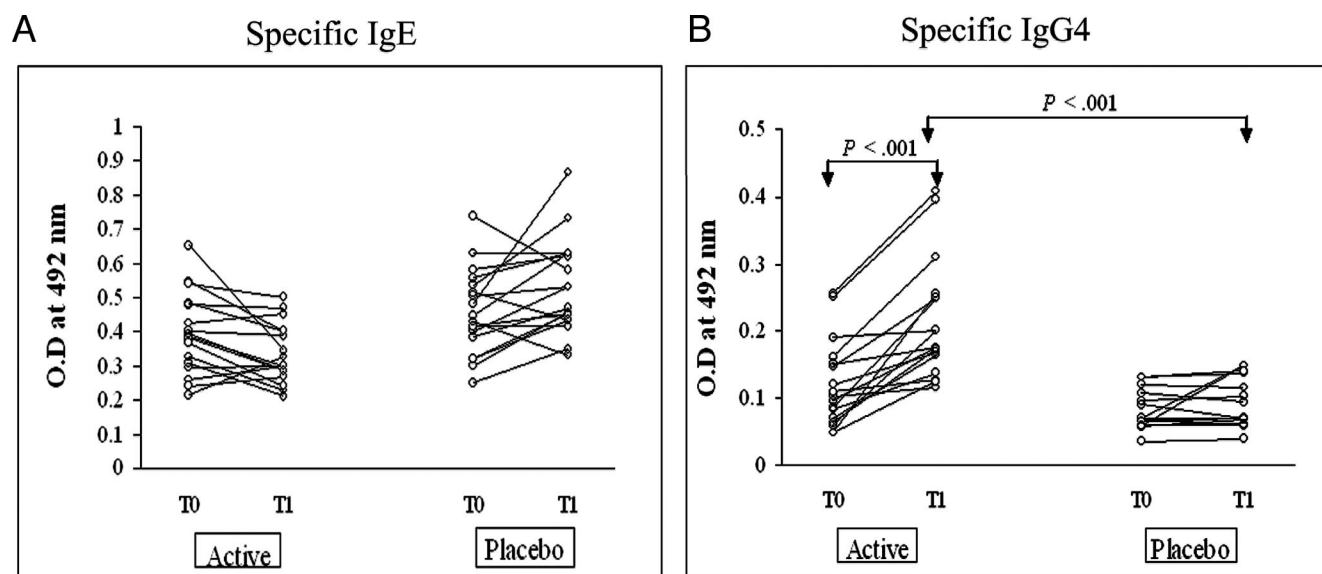


Figure 5. Specific IgE and IgG4 levels in the active and placebo groups at baseline, ie, before immunotherapy (T0), and after 1 year of immunotherapy (T1). A, Specific IgE levels ($P < .01$, active group vs placebo group). B, Specific IgG4 levels ($P < .01$ for the active group compared with baseline and the placebo group after 1 year of immunotherapy). O.D. indicates optical density.

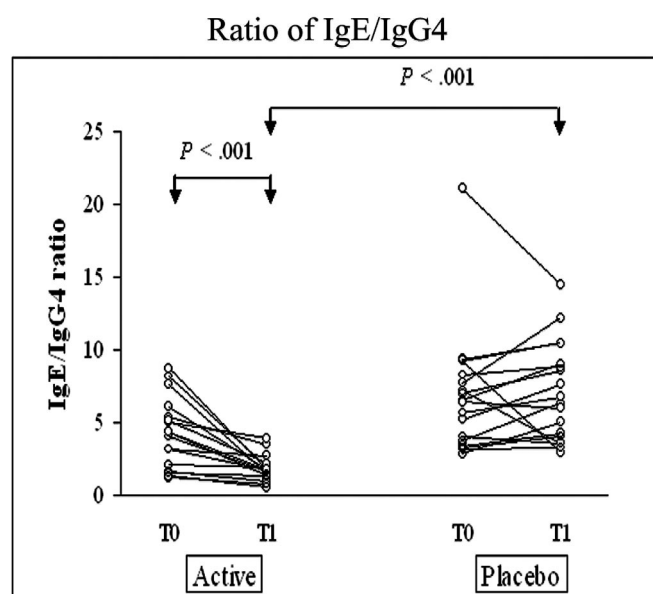


Figure 6. Ratio of specific IgE/IgG4 in the active and placebo groups at baseline, ie, before immunotherapy (T0), and after 1 year of immunotherapy (T1). $P < .01$ for the active group compared with baseline and the placebo group after 1 year of immunotherapy.

of patients in the placebo group showed a reduction in symptom scores that can be attributed to the medication taken by the patients.³² There was a significant reduction in symptom scores in the active group compared with baseline and the placebo group. In the active group, 70% of patients showed a reduction (10%–15%) in drug scores compared with 20% of

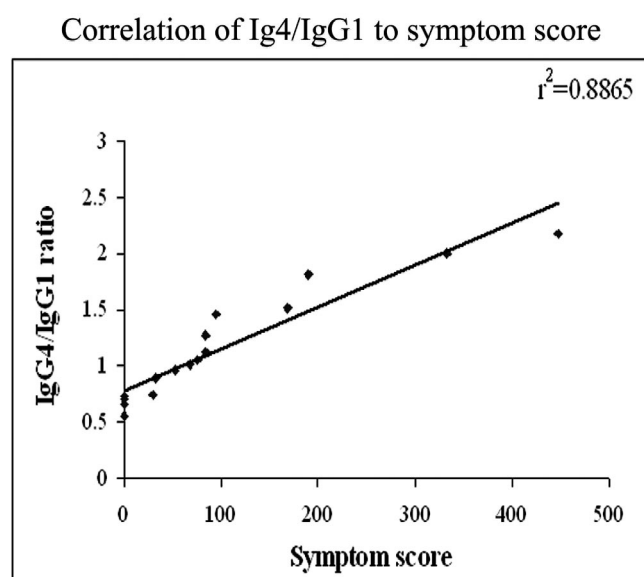


Figure 7. Correlation of the IgG4/IgG1 ratio with the symptom score.

patients in the placebo group. Besides, most patients in the placebo group showed exacerbations in symptom and drug scores. Immunotherapy with *C nucifera* and grass pollen has also demonstrated a reduction in symptom and drug scores.^{13,33}

Bronchial provocation testing performed before and after 1 year of immunotherapy is an important variable for evaluating clinical efficacy.³⁴ In the present study significant improvement in PC₂₀ was observed in the active group com-

pared with baseline and the placebo group. Approximately 76% of patients in the active group showed an increase in PC₂₀. In an open, parallel, comparative trial³⁵ use of inhaled corticosteroids showed faster clinical improvement in the first few months than immunotherapy. Similar to this study we also observed an increase in FEV₁ after 1 year of treatment in both groups. The increase in FEV₁ in the placebo group may be attributed to the use of inhaled corticosteroids.

Several studies have shown a modest reduction in allergen specific IgE levels and induction of allergen specific IgG subclasses.³⁶ IgG has been proposed as blocking antibody, and IgE competes with IgG for allergen binding to mast cells, basophils, and other IgE receptor-expressing cells. Immunotherapy has been shown to modulate allergen specific antibody responses, with a decrease in the IgE/IgG4 ratio leading to a reduction in the recruitment and activation of proinflammatory cells in target mucosa.³⁷ However, some of the studies³⁸ show no change in IgE levels associated with a significant increase in IgG subclasses. Immunotherapy with pollen extract demonstrated changes in clinical and immunologic variables.³⁹ Similar to previous studies, a modest reduction in IgE levels and a significant increase in IgG subclasses was found in the active group. During immunotherapy, IgG1 levels increased for up to 6 months, reached a plateau, and then decreased, whereas IgG4 levels increased after 6 months. Levels of IgG4 showed a significant increase in the active group compared with the placebo group, and clinical improvement correlated with increased IgG4 levels. IgG4 antibody blocks allergen-induced IgE-dependent inflammatory release.⁴⁰ Increased IgG4 levels also correlated with decreased skin reactivity and airway reactivity. The increase in IgG4 concentration may be a parallel epiphenomenon reflective of other immune changes, such as generation of regulatory T cells, that may be more directly responsible for improvement in airway hyperresponsiveness and systemic allergic responses. Similar to the study by Gehlhar et al³⁸ of grass pollen immunotherapy, the present study shows a correlation of clinical improvement with IgG4/IgG1 ratio. In conclusion, patients receiving allergen immunotherapy (the active group) showed improvement in clinical and immunologic variables in mosquito-sensitized patients.

ACKNOWLEDGMENTS

We thank Ashok Kumar Sinha and Dinesh Chand, Vallabh-bhai Patel Chest Institute, for technical support in *in vivo* testing and Harsh Srivastava for help in statistical analysis.

REFERENCES

- Golden DBK. Epidemiology of allergy to insect venoms and stings. *Allergy Proc.* 1989;16:103–107.
- Fan P-C, Chang H-N. Hypersensitivity to mosquito bites: a case report. *Gaoxiong Yi Xue Ke Xue Za Zhi* 1995;11:420–424.
- Gupta S, Jain S, Chaudhry S, Agarwal MK. Role of insects as inhalant allergens in bronchial asthma with special reference to the clinical characteristics of patients. *Clin Exp Allergy.* 1990;20:519–524.
- Agarwal MK, Chaudhry S, Jhamb S, Gaur SN, Chauhan UP, Agarwal HC. Etiologic significance of mosquito (*Anopheles stephensi*) in respiratory allergy in India. *Ann Allergy.* 1991;67:598–602.
- Christophers SR. *Aedes aegypti (L.) the Yellow Fever Mosquito: Its Life History, Bionomics and Structure.* London, England: Cambridge University Press; 1960:739.
- Pawankar R, Yamagishi S, Takizawa R, Yagi T. Mast cell-IgE and mast cell-structural cell interactions in allergic airway disease. *Curr Drug Targets Inflamm Allergy.* 2003;2:303–312.
- Ariano R, Berto P, Tracci D, Incorvaia C, Frati F. Pharmacoeconomics of allergen immunotherapy compared with symptomatic drug treatment in patients with allergic rhinitis and asthma. *Allergy Asthma Proc.* 2006;27:159–163.
- Abramson M, Puy R, Weiner J, Mailing HJ. Immunotherapy as an effective tool in allergy treatment. *Allergy.* 1998;53:461–472.
- Crimi N, Li Gotti F, Mangano G, et al. A randomized, controlled study of specific immunotherapy in monosensitized subjects with seasonal rhinitis: effect on bronchial hyperresponsiveness, sputum inflammatory markers and development of asthma symptoms. *Ann Ital Med Int.* 2004;19:98–108.
- Pajno GB, Barberio G, De Luca F, Morabito L, Parmiani S. Prevention of new sensitizations in asthmatic children monosensitized to house dust mite by specific immunotherapy: a six-year follow-up study. *Clin Exp Allergy.* 2001;31:1392–1397.
- Wachholz PA, Soni NK, Till SJ, Durham SR. Inhibition of allergen-IgE binding to B cells by IgG antibodies after grass pollen immunotherapy. *J Allergy Clin Immunol.* 2003;112:915–922.
- Abramson M, Puy R, Weiner J. Immunotherapy in asthma: an updated systematic review. *Allergy.* 1999;54:1022–1041.
- Karmakar PR, Das A, Chatterjee BP. Placebo-controlled immunotherapy with *Cocos nucifera* pollen extract. *Int Arch Allergy Immunol.* 1994;103:194–201.
- Chowdhury I, Chatterjee B. The immunological and clinical effects of immunotherapy in patients suffering from house dust allergy. *Ann Agric Environ Med.* 1999;6:91–97.
- Gaur SN, Gupta S. Clinical response of immunotherapy in case of nasobronchial allergy. *Indian J Allergy Appl Immunol.* 1996;10:65–68.
- Peng Z, Yang M, Simons FE. Measurement of mosquito *Aedes vexans* salivary gland-specific IgE and IgG antibodies and the distribution of the antibodies in human sera. *Ann Allergy Asthma Immunol.* 1995;74:259–264.
- Peng Z, Yang M, Simons FER. Immunological mechanisms in mosquito allergy: correlations of skin reactions with specific IgE and IgG antibodies and lymphocyte proliferation response to mosquito antigens. *Ann Allergy Asthma Immunol.* 1996;77:238–244.
- McCormack DR, Salata KF, Hershey JN, Carpenter GB, Engler RJ. Mosquito bite anaphylaxis: immunotherapy with whole body extracts. *Ann Allergy Asthma Immunol.* 1995;74:39–44.
- Ariano R, Panzani RC. Efficacy and safety of specific immunotherapy to mosquito bites. *Allerg Immunol (Paris).* 2004;36:131–138.
- Benaim-Pinto C, Fassrainer A. Intradermal immunotherapy in children with severe skin inflammatory reactions to *Aedes aegypti* and *Culex quinquefasciatus* mosquito bites. *Int J Dermatol.* 1990;29:600–601.

21. Kausar MA, Vijayan VK, Bansal SK, Gupta M, Menon BK, Agarwal MK. Mosquito (*Culex quinquefasciatus*) induced IgE response is type I allergic respiratory disorders: a clinico immunologic studies. *Indian J Allergy Asthma Immunol.* 2005;19:102–103.
22. American Thoracic Society. Lung function testing: selection of reference values and interpretative strategies. *Am Rev Respir Dis.* 1991;144:1202–1218.
23. Adamopoulos G, Manolopoulos L, Giotakis I. A comparison of the efficacy and the patient acceptability of budesonide and beclomethasone dipropionate aqueous nasal sprays in patients with perennial rhinitis. *Clin Otolaryngol.* 1995;20:340–344.
24. Thangam Sudha V, Arora N, Sridhara S, Gaur SN, Singh BP. Biopotency and identification of allergenic proteins in *Periplaneta americana* extract for clinical applications. *Biologicals.* 2007;35:131–137.
25. Shankar J, Singh BP, Gaur SN, Arora N. Recombinant glutathione-S-transferase a major allergen from *Alternaria alternata* for clinical use in allergy patients. *Mol Immunol.* 2006;43:1927–1932.
26. Crapo RO, Casaburi R, Coates AL, et al. Guidelines for methacholine and exercise challenge testing—1999. *Am J Respir Crit Care Med.* 2000;161:309–329.
27. Singh AK, Mehta AK, Sridhara S, et al. Allergenicity assessment of transgenic mustard (*Brassica juncea*) expressing bacterial codA gene. *Allergy.* 2006;61:491–497.
28. Soderlund A, Gabriellson S, Paulie S, Hammarstrom ML, Rak S, Troye-Blomberg M. Allergen induced cytokine profiles in type I allergic individuals before and after immunotherapy. *Immunol Lett.* 1997;57:177–181.
29. Abramson MJ, Puy RM, Weiner JM. Allergen immunotherapy for asthma. *Cochrane Database Syst Rev.* 2003;(4):CD001186.
30. Maestrelli P, Zanolla L, Pozzan M, Fabbri LM. Effect of specific immunotherapy added to pharmacologic treatment and allergen avoidance in asthmatic patients allergic to house dust mite. *J Allergy Clin Immunol.* 2004;113:643–649.
31. Iliopoulos O, Proud D, Adkinson NF, et al. Effects of immunotherapy on the early, late and rechallenge nasal reaction to provocation with allergen: changes in inflammatory mediators and cells. *J Allergy Clin Immunol.* 1991;87:855–866.
32. Lipworth BJ, Jackson CM. Second-line controller therapy for persistent asthma uncontrolled on inhaled corticosteroids: the step 3 dilemma. *Drugs.* 2002;62:2315–2332.
33. Walker SM, Pajno GB, Lima MT, Wilson DR, Durham SR. Grass pollen immunotherapy for seasonal rhinitis and asthma: a randomized, controlled trial. *J Allergy Clin Immunol.* 2001;107:87–93.
34. Garcia-Robaina JC, Sanchez I, de la Torre F, Fernandez-Caldas E, Casanovas M. Successful management of mite-allergic asthma with modified extracts of *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae* in a double-blind, placebo-controlled study. *J Allergy Clin Immunol.* 2006;118:1026–1032.
35. Sheikh WA. Immunotherapy vs. inhaled budesonide in bronchial asthma: an open, parallel, comparative trial. *Clin Exp Allergy.* 1997;27:1279–1284.
36. Durham SR, Till SJ. Immunologic changes associated with allergen immunotherapy. *J Allergy Clin Immunol.* 1998;102:157–164.
37. Nagakawa T, Kozeki H, Katagiri J, et al. Changes of house dust mite-specific IgE, IgG and IgG subclass antibodies during immunotherapy in patients with perennial rhinitis. *Int Arch Allergy Appl Immunol.* 1987;82:95–99.
38. Gehlhar K, Schlaak M, Becker W, Bufe A. Monitoring allergen immunotherapy of pollen-allergic patients: the ratio of allergen-specific IgG4 to IgG1 correlates with clinical outcome. *Clin Exp Allergy.* 1999;29:497–506.
39. Keskin O, Tuncer A, Adalioglu G, Sekerel BE, Sackesen C, Kalayci O. The effects of grass pollen allergoid immunotherapy on clinical and immunological parameters in children with allergic rhinitis. *Pediatr Allergy Immunol.* 2006;17:396–407.
40. Garcia BE, Sanz ML, Gato JJ, Fernandez J, Oehling A. IgG4 blocking effect on the release of antigen-specific histamine. *J Investig Allergol Clin Immunol.* 1993;3:26–33.

Requests for reprints should be addressed to:
 Shailendra N. Gaur, MD
 Department of Respiratory Medicine
 Vallabhbai Patel Chest Institute
 University of Delhi
 Delhi-110007, India
 E-mail: sngaur@yahoo.com