

House dust mite sublingual immunotherapy: Results of a US trial

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Background: Few trials of sublingual immunotherapy (SLIT) in the United States have been reported.

Objective: This randomized, placebo-controlled feasibility SLIT study compared the safety and physiologic effects of high-versus low-dose *Dermatophagoides farinae* vaccine.

Methods: Thirty-one *D farinae*-sensitive adults with allergic rhinitis with or without mild intermittent asthma were eligible for randomization to high-dose maintenance vaccine (n = 10, 4200 allergen units [approximately 70 µg of Der f 1/d]), low-dose maintenance vaccine (n = 10; 60 allergen units [approximately 1 µg of Der f 1/d]), or placebo (n = 11) over 12 to 18 months. Medication-symptom scores and adverse events were monitored, serum *D farinae*-specific IgE and IgG4 levels were measured, and bronchial reactivity to *D farinae* was determined at baseline and 6-month intervals.

Results: Of the 31 randomized subjects, 6 withdrew because of non-treatment-ascribed events. Four withdrew because of treatment-ascribed effects: high-dose group, 1 of 10 (gastrointestinal symptoms); low-dose group, 1 of 10 (gastrointestinal symptoms); and placebo group, 2 of 11 (headache and increased nasal symptoms). Thus 21 subjects completed the study: high-dose group, 9; low-dose group, 7; and placebo group, 5. Eleven of the 21 subjects experienced mild-to-moderate gastrointestinal symptoms, throat irritation, or both (high-dose group, 5/9; low-dose group, 4/7; and placebo group, 2/5). No severe systemic reactions were noted. No differences in symptom-medication scores were found. High-

dose SLIT increased the bronchial threshold to allergen challenge and increased serum *D farinae*-specific IgG4 levels, whereas low-dose SLIT and placebo had no significant effect. **Conclusions:** High-dose *D farinae* SLIT was generally tolerable, increased serum *D farinae*-specific IgG4 levels, and improved the bronchial threshold to allergen challenge. Larger US trials are warranted. (J Allergy Clin Immunol 2011;■■■:■■■-■■■.)

Key words: Sublingual immunotherapy, immunotherapy, house dust mites, *Dermatophagoides farinae*, house dust mite allergy

House dust mites are ubiquitous in humid areas worldwide. Sensitization to house dust mite allergens is common and is strongly linked to allergic rhinitis and asthma.¹ Pharmacotherapy can effectively control the symptoms of allergic rhinitis and improve lung function in patients with asthma, but this approach does not modify the course of these diseases.²

Allergen immunotherapy is effective in the treatment of allergic respiratory diseases^{3,4} and is the only currently available therapy that modulates the underlying immune-based mechanisms of these conditions.⁴ Allergen injection subcutaneous immunotherapy is widely used in the United States for the treatment of allergic respiratory disease. However, because of safety concerns and inconvenience, alternative forms of administration, including sublingual immunotherapy (SLIT) have been studied.⁵

Since the first report of a successful trial of SLIT in the treatment of allergic rhinitis caused by house dust mite sensitivity in 1986,⁶ several meta-analyses based on studies largely conducted in Europe have examined the role of SLIT in the management of allergic respiratory disease.⁷⁻¹² These meta-analyses have generally supported the use of high-dose SLIT regimens as being relatively safe and effective, although recently, concerns about the robustness of the meta-analyses' data and other issues have arisen.¹³ Nonetheless, SLIT has gained increasing acceptance, and its use has been thoroughly reviewed in a recent World Allergy Organization position paper.¹⁴

A few practitioners in the United States have used low-dose SLIT therapy over the years, but few controlled trials have been reported.¹⁵ Because of differences in allergen immunotherapy products and use of multiple allergens in the United States, studies on the use of SLIT in the United States are beginning to emerge.¹⁶⁻¹⁸

Because sensitivity to house dust mite allergens is an important factor in the development of allergic rhinitis and asthma, we conducted a double-blind, placebo-controlled trial of high-dose and low-dose SLIT in adults with allergic rhinitis with or without mild intermittent asthma sensitized to the house dust mite *Dermatophagoides farinae* to evaluate the safety and potential benefits of SLIT.

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Abbreviations used

AE: Adverse event
 AgPD₂₀: Allergy provocative dose producing a 20% decrease in FEV₁
 AU: Allergen units
 SLIT: Sublingual immunotherapy

METHODS**Subjects**

Male and female adults aged 18 to 50 years were recruited from the Madison, Wisconsin, area. All subjects had a 2-year or greater history of allergic rhinitis, a positive skin prick test response to *D farinae*, and an *in vitro* *D farinae*-specific serum IgE level greater than 2 times that of serum from a nonallergic control subject.

Screening procedures

Additional skin prick tests to 19 aeroallergens (grass, elm, ash, maple-box elder mix, oak, hickory, birch, ragweed, late fall pollen mix, plantain-sorrel mix, *Aspergillus fumigatus*, *Penicillium* species mix, *Alternaria alternata*, *Cladosporium herbarum/cladosporoides* mix, cat, dog, murine epithelium, cockroach, and *Dermatophagoides pteronyssinus*; Greer Laboratories, Lenoir, NC), a positive control (histamine), and a negative control (diluent) were performed. All subjects had positive skin test responses to 1 or more of the allergens tested.

Subjects with mild intermittent asthma as defined by the National Heart, Lung, and Blood Institute criteria¹⁹ were included as long as the FEV₁ at screening was 80% or greater of predicted value and only intermittent short-acting β_2 -agonists were required to control asthma symptoms.

Subjects with positive skin test responses to cats, dogs, or both were excluded if a pet was kept in the home. Subjects who had previously received immunotherapy with house dust mite vaccines were excluded. Female subjects who were pregnant at the time of screening or were planning to become pregnant during the study were excluded. Additional exclusion criteria included subjects receiving β -adrenergic antagonists and those requiring topical nasal or inhaled corticosteroids or other asthma controller medications. Subjects were permitted to use oral loratadine, 10 mg/d, as needed and topical azelastine nasal spray twice daily, as needed, if rhinitis symptoms were not controlled with oral loratadine. Inhaled albuterol was permitted for as-needed use to control asthma symptoms (maximum of 2 inhalations 4 times per day).

The study was conducted under an Investigational New Drug Application (IND 12762 CBER) issued by the US Food and Drug Administration. The University of Wisconsin-Madison Health Sciences Institutional Review Board approved the study, and all subjects signed the appropriate informed consent form before participating.

Study design

This was a single-center, randomized, double-blind, placebo-controlled study. Subjects were enrolled over a period of 18 months (January 2006–July 2007). After an initial screening examination, eligible subjects recorded daily nasal symptoms and medication use on diary cards for a 4-week period before randomization. Subjects received daily treatment with sublingually administered US Food and Drug Administration–standardized *D farinae* vaccine in a 50% glycerinated saline diluent or placebo that contained histamine in a caramelized sugar, glycerinated saline diluent (Greer Laboratories). A single lot of *D farinae* vaccine was used throughout the study. The 10,000 AU/mL extract contained 169 μ g/mL of the major *D farinae* allergen Der f 1. Dose-escalating regimens (see Tables E1–E3 in this article's Online Repository at www.jacionline.org) were conducted under medical supervision until the highest daily maintenance dose was achieved (high dose, 4200 AU/d [approximately 70 μ g of Der f 1]; low dose, 60 AU/d [approximately 1 μ g of Der f 1]; or placebo, 0.038 mg of histamine per day). Doses were delivered by using vials equipped with a metered-dose pump in glass

bottles (Greer Laboratories), and subjects were instructed to hold the solution under the tongue for 2 minutes and not to eat or drink for 15 minutes after the dose.

Once the maintenance dose of SLIT was achieved, subjects self-administered the treatment at home. They returned each month to review adverse events (AEs) and to receive new supplies of the vaccine. If subjects experienced treatment-related AEs (eg, mouth itching or gastrointestinal effects) during the escalation phase, the dosage was continued at the same level until the AE resolved (usually within a few days). Then the dose escalation was resumed.

Clinical assessments

A schedule of evaluations and list of procedures performed at each study visit can be found in Fig E1 (available in this article's Online Repository at www.jacionline.org). Subjects completed diary cards that recorded their symptoms and medication use for a 4-week period before dosing (baseline) and the visits at 6, 12, and 18 months after randomization to treatment. Subjects received a physical examination and were assessed for AEs. Subjects also underwent routine spirometry and phlebotomy for serum IgE and IgG4 antibody titers to *D farinae*. In addition, allergen bronchoprovocation with *D farinae* was performed.

AEs

AEs were monitored at each escalating dose visit and by subject report at any time. Subjects also recorded AEs in the symptom-medication diary cards before each visit. AEs were ascribed as possibly or not treatment related by the blinded principal investigator. Subjects rated AEs as mild, moderate, or severe. An independent safety-monitoring board (blind to the treatment regimen) was in place to monitor each subject's risk. Subjects were permitted to withdraw voluntarily or on the recommendation of the investigator or the safety-monitoring board.

Symptom and medication scoring

Subjects completed a diary card for 4 weeks before randomization (baseline) and 4 weeks before the study visits at 6, 12, and/or 18 months. Symptoms were scored twice a day (morning and evening) on a 4-point scale (0, none; 1, mild; 2, moderate; and 3, severe) for each nasal symptom of congestion, rhinorrhea, pruritis, and sneezing. Similarly, asthma symptoms of cough, chest tightness, shortness of breath, and wheeze were scored as above. The maximum morning and evening scores would be 24, respectively. Medication use was scored as 1 for each 10-mg tablet of loratadine used and for each puff of albuterol used. Each spray of azelastine was scored as 2.

***D farinae*-specific immunoglobulin measurements**

Serum was obtained at baseline and at 6, 12, and/or 18 months after randomization to treatment. Undiluted serum samples were analyzed for *D farinae*-specific IgE levels by using a fluoroenzyme immunoassay (Phadia ImmunoCap System, Uppsala, Sweden) with an automated instrument (Uni-cap 100; Phadia, Portage, Mich). The detection limit of the assay is 0.35 kUA/L. A similar FEIA method was used to determine *D farinae*-specific IgG4 serum levels. All samples were diluted 1:100. The detection limit of the IgG4 assay is 0.001 to 300 mg/L.

Allergen bronchoprovocation

Allergen bronchoprovocation was conducted at baseline and at the end of treatment (12–18 months). The dosage of *D farinae* allergen required to cause a 20% decrease in FEV₁ from the baseline value was determined by using the method of Tan and Spector.²⁰ After obtaining the baseline FEV₁ by means of spirometry (KoKo System; Pulmonary Data-Systems, Louisville, Colo), doubling doses of *D farinae* extract were administered through a nebulizer (DeVilbiss Model 645; Sunrise Medical, Somerset, Pa) connected to a dosimeter (KoKo DigiDoser, Pulmonary Data Systems) until the FEV₁ had decreased

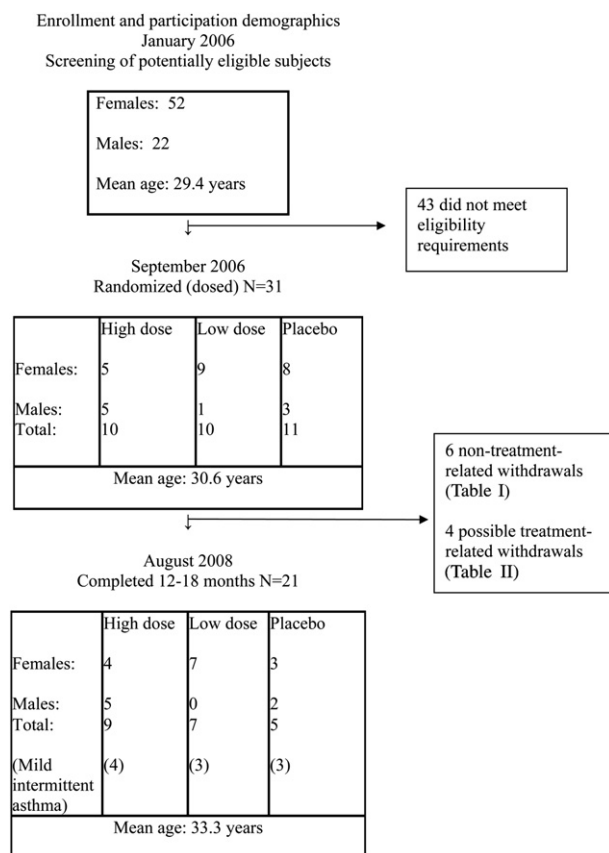


FIG 1. Enrollment and participation demographics, January 2006: screening of potentially eligible subjects.

by 20% from the baseline value. The results were expressed as cumulative breath units.

Environmental house dust mite allergen levels

Settled dust samples were collected within a 2-week period before the baseline, 6-month, and final (12-18 month) visits. After appropriate training, subjects collected settled dust samples from their bedroom floors within a 2-week period before the baseline, 6-month, and final (12-18 months) visits. Subjects vacuumed a 0.25-m² template area for 2 minutes. Samples were then assayed for the concentration of Der f 1, expressed as micrograms per gram of dust, by using a commercially available ELISA kit (Indoor Biotechnologies, Charlottesville, Va) with a detection limit in the range of 0.02-0.06 ng/mL.

Statistical analysis

Data analyses were based on subjects completing 12 to 18 months of treatment. The primary analyses were based on a comparison between the 2 active treatment arms versus the placebo arm. These analyses were performed with analysis of covariance models of the 6- or 12- to 18-month observations, with treatment group and baseline observation as covariates. The relationship between the changes in IgG4 and allergy provocative dose producing a 20% decrease in FEV₁ (AgPD₂₀) levels was analyzed by using the Pearson correlation coefficient. Der f 1 levels in dust samples were compared by treatment group by using repeated-measures ANOVA. AgPD₂₀ values, serum *D farinae*-specific IgE and IgG4 levels, and settled dust sample Der f 1 levels were log transformed for analysis. Results were expressed as means ± SEMs. A *P* value of less than .05 was considered significant.

TABLE I. Non-treatment-related withdrawals

Dose group	No.	Cause	Days on treatment	Outcome
High dose (n = 10)	0	—	—	—
Total	0/0			
Low dose (n = 10)	1	Relocation	251	—
	1	Too busy	392	—
Total	2/10			
Placebo (n = 11)	1	Optic neuritis	68	Stable
	1	Pregnancy	21	Normal delivery, healthy infant
	1	Relocation	271	—
	1	Poor adherence	?	—
Total	4/11			

Overall total = 6 (19.4%) of 31.

RESULTS

AEs

Seventy-four subjects were screened for enrollment (Fig 1). Thirty-one met the eligibility requirements and were randomized to treatment with high-dose SLIT (n = 10), low-dose SLIT (n = 10), or placebo (n = 11). Twenty-one subjects completed 12 to 18 months of the assigned treatment (high-dose SLIT group, n = 9; low-dose SLIT group, n = 7; and placebo group, n = 5). Four subjects in the high-dose SLIT group and 3 each in the low-dose SLIT and the placebo groups carried a diagnosis of mild intermittent asthma. Six subjects withdrew or were withdrawn for non-treatment-related reasons (Table I): none in the high-dose SLIT group, 2 in the low-dose SLIT group, and 4 in the placebo group. Four subjects withdrew for possible treatment-related reasons (Table II): 1 in the high-dose SLIT group for abdominal cramps and diarrhea during escalation, 1 in the low-dose SLIT group for nausea and diarrhea during the dose escalation, and 2 in the placebo group for increased headache intensity and reduced hearing during the dose escalation (n = 1) and unrelieved rhinitis symptoms during maintenance treatment (n = 1). All recovered after discontinuing treatment (13-44 days).

All completed subjects eventually tolerated the highest targeted daily dose of treatment; however, possible treatment-related AEs were common (52% overall) in all treatment groups that completed the trial (5/9 [55.6%] in the high-dose SLIT group, 4/7 [57.1%] in the low-dose SLIT group, and 3/5 [60%] in the placebo group; Table III). Mouth and throat irritation were the most commonly reported AEs (4 in the high-dose SLIT group, 3 in the low-dose SLIT group, and 2 in the placebo group). Most of these events occurred during the maintenance phase. Only 2 events occurred during the escalation phase (high-dose group, n = 1; low-dose group, n = 1). One subject in the high-dose group experienced increased asthma symptoms of moderate severity that did not require treatment other than occasional use of an albuterol metered-dose inhaler. In most instances the AEs resolved within 1 to 39 days, although in the 1 subject with asthma symptoms, the AE persisted for 92 days. Dosing was interrupted in 3 subjects (high-dose group, n = 1; low-dose group, n = 2) but resumed once the AE had resolved.

TABLE II. Possible treatment-related withdrawals

Dose group	No.	AE	Severity	Days on treatment	Duration (d)
High dose (n = 10)	1	Abdominal cramps	Mild	13	13
		Diarrhea	Mild		
Total	1/10 (10%)				
Low dose (n = 10)	1	Nausea	Moderate	6*	9
		Diarrhea	Moderate	8*	43
Total	1/10 (10%)				
Placebo (n = 11)	1	↑Headache	Moderate	10*	44
		↓Hearing	Moderate	10*	44
	1	Nasal congestion	Moderate	207	19
Total	2/11 (18.2%)				

Overall total = 4 (12.9%) of 31.

*Escalation phase.

TABLE III. Possible treatment-related adverse events: completed subjects

Dose group	No.	AE	Severity	Days on treatment	Duration (d)
High dose (n = 9)	1	Oral lesion	Mild	51	15
	1	Oral lesion	Mild	148	39
	1	Oral lesion	Moderate	1*†	<1
	1	Throat irritation	Mild	128	1
	1	Asthma symptoms	Intermittent moderate	263	92
Total	5/9 (56.6%)				
Low dose (n = 7)	1	Oral lesion	Mild	253†	3
	1	Oral lesion	Moderate	51	7
	1	Sore throat	Intermittent mild	183	1
	1	Diarrhea	Intermittent moderate	4*†	30
Total	4/7 (57%)				
Placebo (n = 5)	1	Oral lesion	Intermittent moderate	362	5
	1	Oral lesion (2 episodes)	Severe	221	2
				225	2
Total	2/5 (40%)				

Overall total = 11 (52%) of 21.

*Escalation phase.

†Dose interrupted.

Symptoms and medication use

Symptom and medication scores were analyzed only at the 12-month visit to reduce seasonal variations in environmental allergen exposure as a confounder. No significant differences were observed in total symptom scores (data not shown) or in medication use (see Figs E2 and E3 in this article's Online Repository at www.jacionline.org) at the end of 12 months of treatment in either the low-dose or high-dose treatment groups versus the placebo group. When analyzed for outcomes based on the presence or absence of mild intermittent asthma, no significant differences were found. No subject was withdrawn from the study because of concomitant use of permitted symptomatic medications (antihistamines, albuterol, or both).

Immunoglobulin measurements

D farinae-specific IgE serum levels showed a slight but statistically nonsignificant increase from baseline values in the high-dose SLIT group at 6 months and at the end of the study (12-18 months, Fig 2).

D farinae-specific IgG4 serum levels in the high-dose SLIT group were significantly increased at 6 months ($P = .03$) and at the end of the study (12-18 months, $P = .02$) compared with those

in the placebo group. There was no significant change in the *D farinae*-specific IgG4 serum levels in the placebo or low-dose SLIT groups (Fig 3).

Allergen bronchoprovocation

In the high-dose SLIT group we observed a significant increase in the bronchial threshold to allergen challenge (AgPD₂₀) with *D farinae*. The AgPD₂₀ value increased from 70 ± 18 cumulative breath units at baseline to 101 ± 13 cumulative breath units ($P = .04$ vs placebo) after treatment (12-18 months). No significant change in AgPD₂₀ value was found in the placebo or low-dose SLIT groups (Fig 4). A highly significant correlation was noted between the increase in *D farinae*-specific IgG4 serum levels and the increase in AgPD₂₀ values from baseline ($r = +0.89$, $P < .0001$; Fig 5).

Environmental house dust mite allergen levels

Relatively low levels of the major *D farinae* allergen Der f 1 were found in the settled dust samples. Levels ranged from non-detectable to $8 \mu\text{g/g}$ of dust. Mean Der f 1 levels were similar among the 3 treatment groups (see Fig E4 in this article's Online Repository at www.jacionline.org).

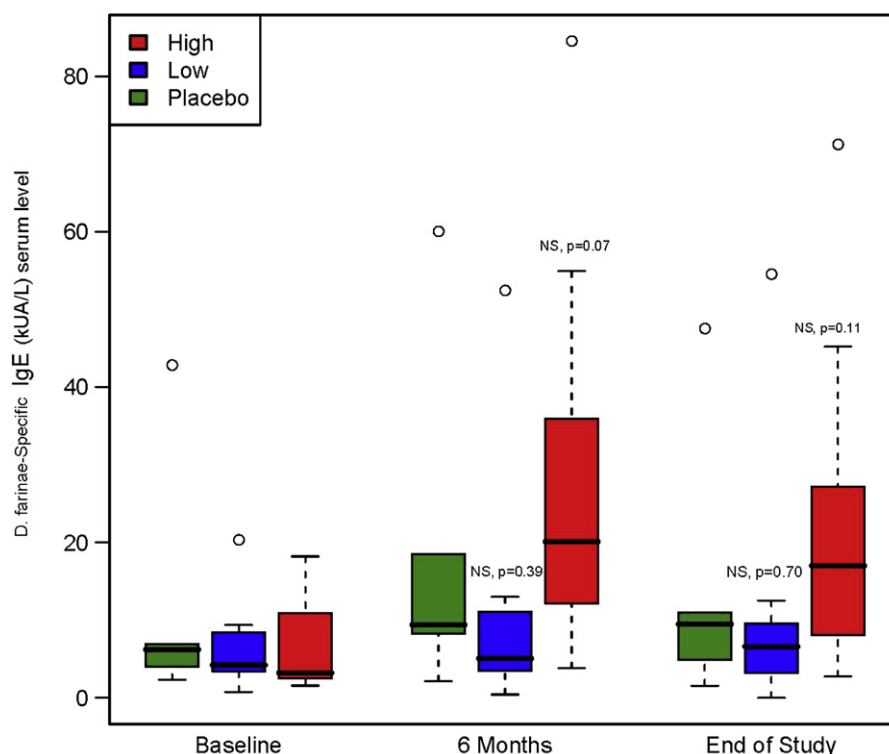


FIG 2. *D. farinae*-specific IgE serum levels showed a slight but statistically nonsignificant increase from baseline values in the high-dose SLIT group. NS, Not significant.

DISCUSSION

Sublingual therapy with high-dose *D. farinae* vaccine (4200 AU/d maintenance [approximately 70 μ g of Der f 1/d]) for 12 to 18 months appears to be generally safe and tolerable. All completed subjects eventually tolerated the highest targeted dose for their assigned group. No severe systematic reactions (anaphylaxis) or urticaria were observed. One subject had a moderate increase in asthma symptoms that persisted for 3 months, but the symptoms resolved with continued therapy and did not require treatment beyond metered-dose inhaler-administered albuterol on an as-needed basis. We could not attribute the increased symptoms to concomitant sensitizations or to respiratory tract viral infection.

Treatment-ascribed withdrawals in the high-dose ($n = 1$ because of abdominal cramps and diarrhea), low-dose ($n = 1$ because of nausea and diarrhea), and placebo ($n = 2$ because of headache/decreased hearing and increased nasal congestion) groups tended to occur during the escalating dose phase of the study (3/4 subjects, Table II). AEs were similarly distributed among the subjects who completed the treatment (high-dose group, 5/9 [55.6%] subjects; low-dose group, 4/7 [57.1%] subjects; and placebo group, 2/5 [40%]; Table III). Most of these occurred during maintenance treatment (9/11). AEs were of mild-to-moderate severity (10/11) and resolved with continual treatment, although dosing was temporarily interrupted in 3 instances (high-dose group, $n = 1$ during escalation; low-dose group, $n = 2$, 1 during escalation and 1 during maintenance; Table III). One subject with an oral aphthous ulcer experienced localized swelling and pruritis at the site lasting for 4 to 6 hours after receiving the initial dose of the high-dose vaccine. One subject with underlying irritable bowel syndrome noted increased diarrheal symptoms of moderate severity that continued for 30 days while

receiving low-dose SLIT. Although most studies^{16,17,21-24} have demonstrated that high-dose SLIT is generally safe, scattered reports of anaphylaxis^{14,25} have appeared, and approximately 3% of subjects might experience systemic AEs (urticaria, increased asthma or rhinitis symptoms, and worsening atopic dermatitis). Our results suggest that subjects with underlying mucosal lesions (eg, aphthous ulcers) and gastrointestinal conditions (eg, gastroesophageal reflux disease and irritable syndrome) might be vulnerable to adverse risks from SLIT.

In one of the first SLIT trials conducted in the United States, we found that 12 to 18 months of high-dose *D. farinae* SLIT (4200 AU daily maintenance [approximately 70 μ g of Der f 1/d]) improved the bronchial threshold to allergen challenge (Fig 4). This finding is consistent with the results of other SLIT trials.^{5,26} Although we did not conduct nasal challenges, several studies have also demonstrated improvements in response to nasal allergen challenge.^{6,17}

We also found a significant increase in *D. farinae*-specific IgG4 serum levels in the high-dose SLIT group (Fig 3) that highly correlated with the increase in AgPD₂₀ values (Fig 5). Although the mechanisms of the beneficial effects of SLIT are not fully elucidated, it is believed that specific IgG4 levels might reflect the production of IL-10 by regulatory T cells. Our finding is in keeping with other SLIT studies,²⁷⁻²⁹ but some investigators have not observed changes in specific IgG4 levels.³⁰⁻³²

The improvement in airway response to allergen challenge that we observed suggests a possible local effect of SLIT. Because the *D. farinae*-specific IgG4 serum levels were highly correlated with the improvement in the airway response to allergen challenge in our study (Fig 5), potentially beneficial humoral immunologic responses might also be involved.

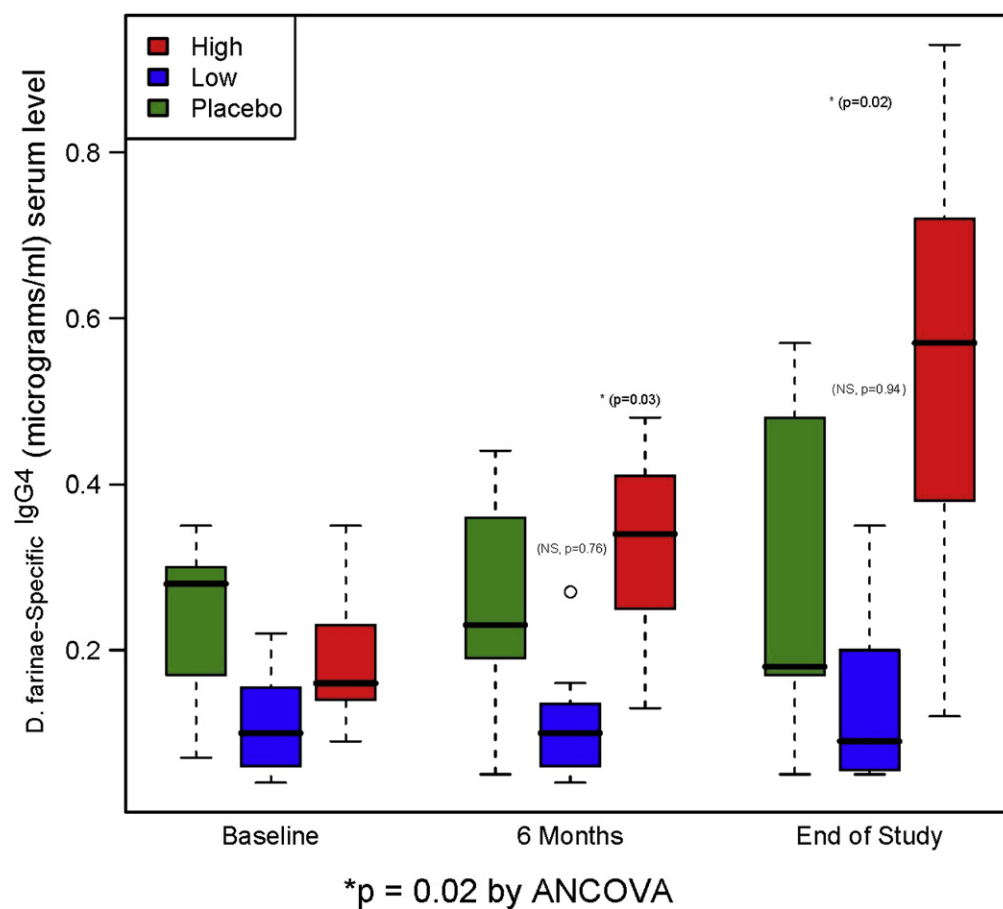


FIG 3. *D. farinae*-specific IgG4 serum levels at baseline, 6 months, and the end of the study showed a significant increase from baseline values in the high-dose SLIT treatment group. ANCOVA, Analysis of covariance; NS, not significant.

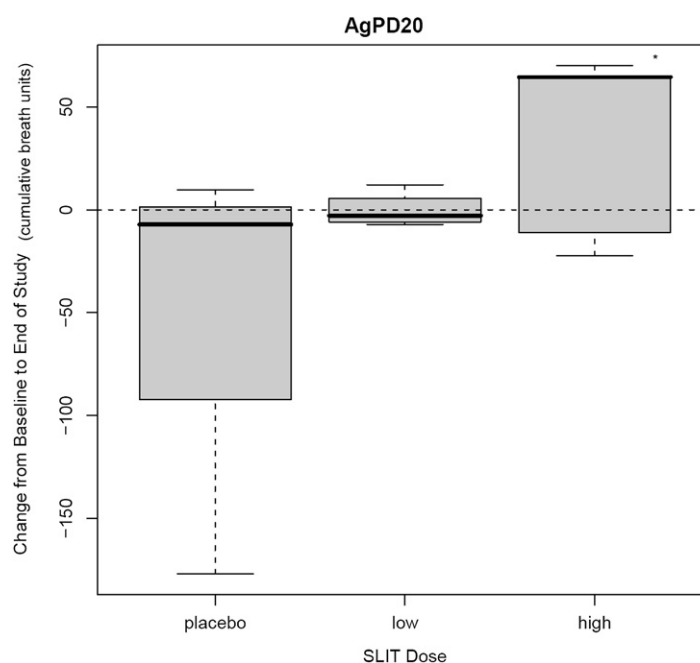


FIG 4. AgPD₂₀ values at baseline and the end of the study were significantly increased in the high-dose SLIT treatment group.

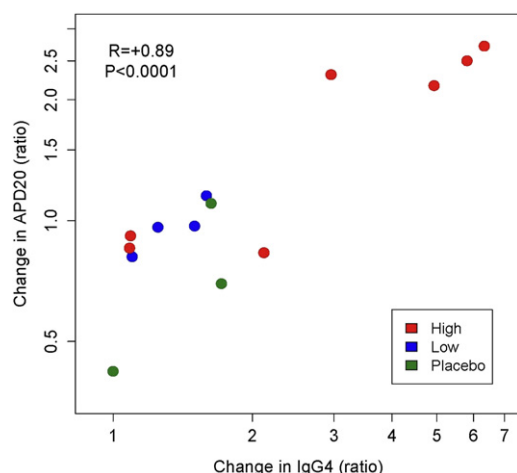


FIG 5. Relationship of change in AgPD₂₀ and serum specific IgG4 ratio (end of study vs baseline) showed a significantly positive correlation.

In this study we found *D farinae*-specific IgE serum levels were slightly but not statistically increased in the high-dose SLIT group (Fig 2). Others have observed similar changes in specific IgE levels in their trials.^{29,32} Because specific IgE is a fundamental part of the mechanism of allergic disease and others have demonstrated a reduction in specific IgE levels in long-term SLIT trials,³³ further studies will be needed to determine the long-term effects of SLIT on allergen-specific serum IgE levels.

Although we did not detect any significant differences among the treatment groups (high-dose SLIT, low-dose SLIT, or placebo) in symptom scores or medication use (see Figs E2 and E3), there are several possible explanations. Indoor settled dust samples from the subjects' homes showed relatively low concentrations of the major *D farinae* allergen Der f 1 (see Fig E4) that ranged from undetectable to 8 µg/g of dust. Because the settled dust samples were self-collected, this could have affected the results as well. Furthermore, all of the subjects were sensitized and exposed to other aero-allergens, except for pets in the home. Although many studies of SLIT, both in adults and children, have demonstrated improvement in allergic rhinitis and asthma symptoms and reduction in medication use,^{7,8,21} other trials have not.^{22,32} A recent SLIT study¹⁵ with a multiallergen mix containing grass pollen was less effective than SLIT grass pollen monotherapy. Further studies of SLIT will be needed to determine its role in the treatment of allergic subjects who are typically sensitized to multiple allergens.

In conclusion, we found that high-dose SLIT with *D farinae* vaccine (4200 AU [approximately 70 µg of Der f 1 daily maintenance) induced a potentially protective immune response that was associated with an increase in the bronchial threshold to allergen challenge with a *D farinae* extract. Although mild-to-moderate AEs, largely mouth lesions/pruritis and gastrointestinal symptoms (nausea and diarrhea), were common (57% in the high-dose group), high-dose SLIT was generally safe (no severe systemic reactions).

Several questions remain to be answered before the role of SLIT in the treatment of allergic diseases can be fully defined.^{14,25} Among these questions are the effective dosage, the duration of treatment, and the use of multiple allergens. Our study supports previous observations that high-dose SLIT might become an effective tool in the treatment of allergic respiratory disease. Larger-scale trials of SLIT in the United States are warranted.

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Pulmonary function support: Ryan Burton

Study design: Mary Morris, MD, and Christine Sorkness, PharmD

Manuscript preparation: Rebecca Forbes

Clinical implications: SLIT for allergic respiratory disease is widely used in many countries. Larger US trials might lead to US Food and Drug Administration approval.

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Schedule of Evaluations

Procedure	Baseline	Randomization	6 months	12 months	18 months
Symptom-medication diary two weeks before visit		+	+	+	+
Skin testing (HDM)	+		+	+	+
Serum-specific IgE and IgG ₄	+		+	+	+
Bronchial challenge to HDM allergen	+		+	+	+
Home dust collection	+		+	+	+

FIG E1. Schedule of evaluations. *HDM*, House dust mite.

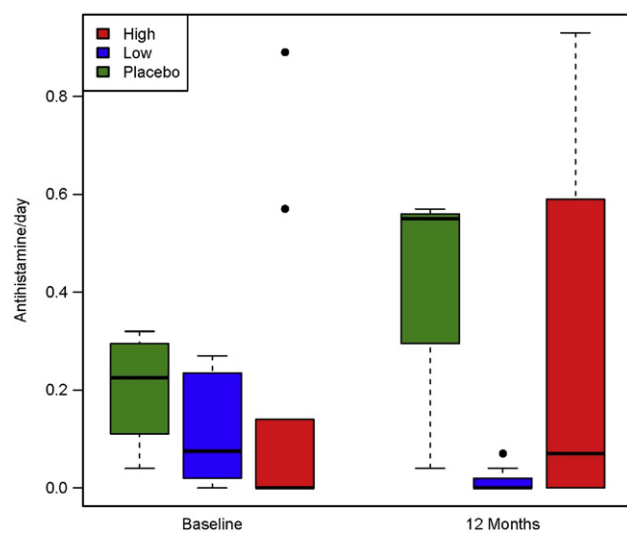


FIG E2. Daily antihistamine medication scores. No significant differences were found between treatment groups. Maximum score = 5.

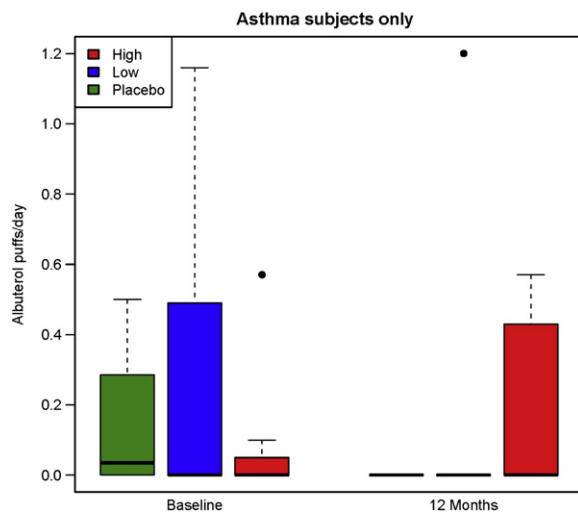


FIG E3. Albuterol use by asthmatic subjects (high-dose SLIT, $n = 4$; low-dose SLIT, $n = 3$; and placebo, $n = 3$). No significant differences were found between treatment groups. Maximum score = 8.

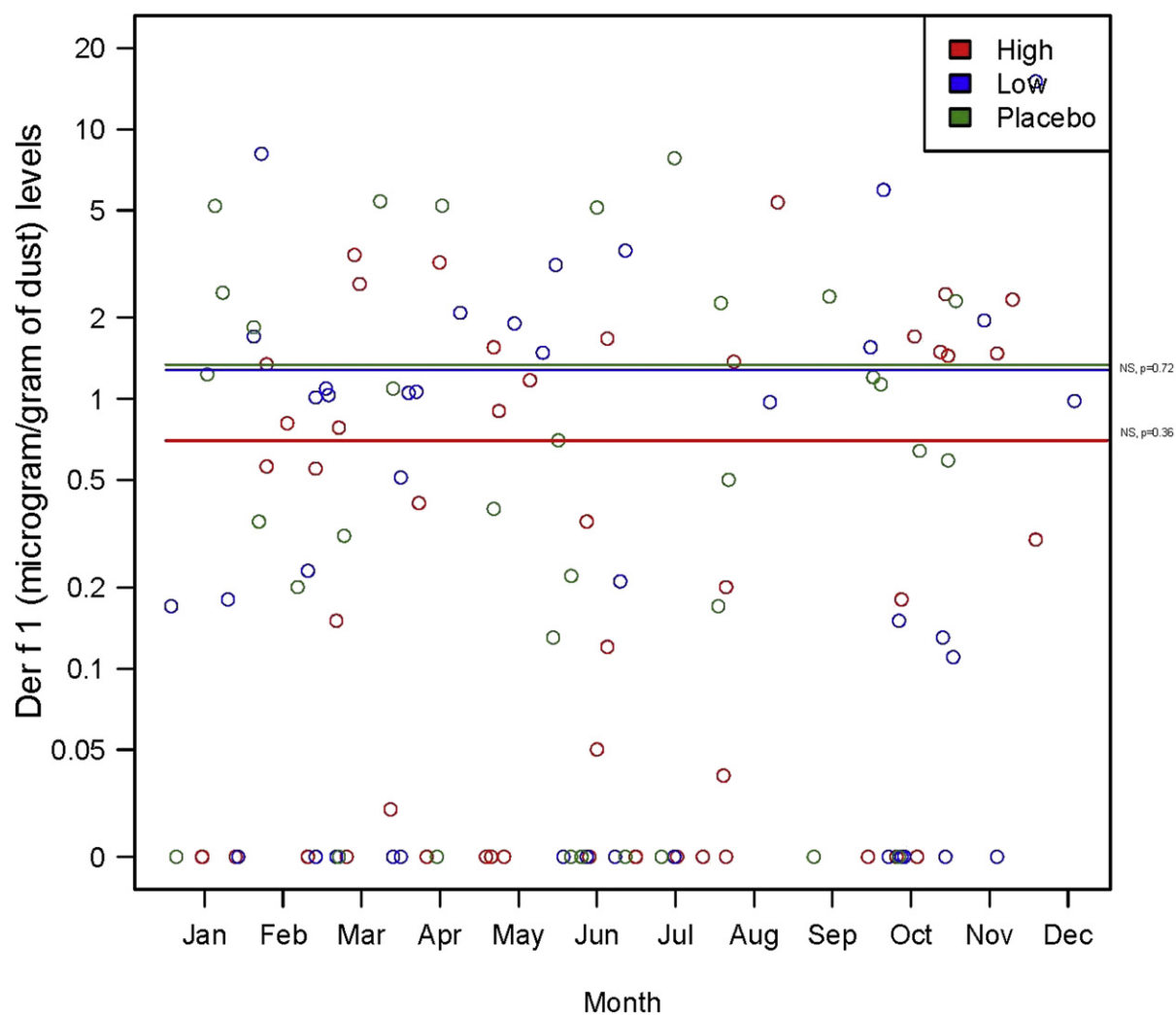


FIG E4. *D. farinae* allergen (Der f 1) content of settled dust samples showed low levels that were similar across treatment groups.

TABLE E1. Escalating dose regimen: high-dose *D farinae*

	Concentration (AU/mL)	Volume (μL)	Dose (AU)	Cumulative (AU)
Day 1*	0	140	0	0
	1,000	50	50	50
	1,000	140	140	190
	10,000	50	500	690
	10,000	140	1,400	2,090
Day 2	10,000	140	1,400	3,490
Day 15	10,000	140 × 2	2,800	6,290
Day 29	10,000	140 × 3	4,200	10,490
Then daily thereafter	10,000	140 × 3	4,200	—

*Administered at 15-minute intervals with a 30-minute observation after the last dose.

TABLE E2. Escalating dose regimen: low-dose *D farinae*

	Concentration (AU/mL)	Volume (μ L)	Dose (AU)	Cumulative (AU)
Day 1*	0	40	0	0
	0	50	0	0
	0	140	0	0
	0	50	0	0
Day 2	143	140	20	20
	143	140	20	40
Day 15	143	140×2	40	80
Day 29	143	140×3	60	140
Then daily thereafter	143	140×3	60	—

*Administered at 15-minute intervals with a 30-minute observation after the last dose.

TABLE E3. Escalating dose regimen: placebo (histamine)

	Concentration (mg/mL)	Volume (μ L)	Dose (mg)	Cumulative (mg)
Day 1*	0	140	0	0
	0.001	50	0.0005	0.0005
	0.001	140	0.0014	0.0019
	0.09	50	0.0045	0.0064
	0.09	140	0.0130	0.0194
Day 2	0.09	140	0.0130	0.0324
Day 15	0.09	140×2	0.025	0.0574
Day 29	0.09	140×3	0.038	0.0954
Then daily thereafter	0.09	140×3	0.038	—

*Administered at 15-minute intervals with a 30-minute observation after the last dose.