

Efficacy and safety of oral immunotherapy with short ragweed extract

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Background: Oral immunotherapy, if proven safe and effective, could be an alternative to subcutaneous immunotherapy.

Objective: This pilot study investigated the clinic and immunologic effects of ragweed immunotherapy using a new microencapsulated, pH-sensitive, oral delivery system.

Methods: A double-blind, placebo-controlled trial was conducted in 23 patients with allergic rhinitis to short ragweed. Following a baseline nasal challenge with ragweed allergen, oral immunotherapy with encapsulated short ragweed extract or placebo was administered once daily, 6 days/week. Doses began at 3 μ g Amb a 1 per day and were increased by 3 μ g every three days as tolerated, to a maximum daily maintenance dose of 24 μ g. A nasal challenge was repeated 6 weeks later, followed by the continuation of maintenance therapy through the natural ragweed season. Daily allergy symptoms and relief medication usage was recorded. A final nasal challenge was performed at the end of the natural season. Short ragweed-specific serum IgE, IgG, and IgG₄ antibody levels were measured every 2 weeks during the study.

Results: Maximum tolerated doses ranged from 6 to 24 μ g Amb a 1 per day (74% reached 24 μ g). Adverse events were not serious or different between the active and placebo groups. The active treatment group showed increases in short ragweed-specific serum IgG and IgG₄ antibody levels. Symptom scores during the natural season were numerically but not statistically lower in the active treatment group. This group also experienced a greater reduction from baseline in nasal reactivity as assessed by nasal challenge.

Conclusions: These pilot data suggest that the encapsulated, pH-sensitive oral immunotherapy delivery system was safe, induced a brisk serologic response, and attenuated the symptomatic response to both experimental and environmental ragweed exposure.

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INTRODUCTION

Although subcutaneous immunotherapy is an important mode of therapy for patients with allergic rhinitis,^{1,2} several problems are associated with its administration. These include the high cost, inconvenience, pain, and frequency of systemic reactions, including death (~1/2.5 million injections).

For these reasons, alternative methods of delivering immunotherapy have been developed. Examples of these delivery methods include sublingual,^{5,6} nasal,⁷ and oral immunotherapy.^{8–13}

Previously, gastric protein degradation has precluded the use of orally administered peptide immunotherapy. Recently, an oral delivery system that uses enteric-protected, antigen-coated polysaccharide microspheres has been developed.⁸ By virtue of pH-targeting, duodenal, rather than gastric, protein release and absorption is encouraged. By stimulating the mucosal immune system, oral immunotherapy may offer

additional benefits over conventional subcutaneous immunotherapy.⁹

The purpose of this double-blind, placebo-controlled pilot study was to assess the efficacy and safety of oral ragweed immunotherapy when administered using the microencapsulated, pH-targeted system. Efficacy outcome parameters included systemic immunologic responses and nasal responses to nasal ragweed provocation in the laboratory and natural ragweed exposure in the environment.

METHODS

Subjects

Healthy, adult volunteers with ragweed allergic rhinitis were identified ($n = 23$, age range 19 to 49, mean 30 ± 10 years). Ragweed-allergic rhinitis was defined by: (1) the presence of a positive history for seasonal rhinitis during the months of August, September, and October for at least the past 2 consecutive years, (2) a positive skin puncture (≥ 3 mm wheal and flare, $n = 20$) or a positive intradermal skin test (≥ 9 mm wheal and flare, $n = 3$) to short ragweed extract, and (3) elevated short ragweed-specific serum IgE antibody levels (≥ 0.50 IU/ml, class 0/1 or higher). All subjects were asymptomatic at the time of enrollment in June. None of the subjects had received immunotherapy within the previous 3 years. None of the subjects had asthma or any other chronic diseases and none of the subjects had any recent medication use except oral contraceptives. The protocol was approved by the Children's Hospital of Pittsburgh Human Rights Committee and informed consent was obtained.

Experimental Protocol

A schematic diagram describing the plan of the study is provided in Figure

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1. Subjects underwent a baseline nasal challenge with incremental doses of short ragweed allergen. Subjects were then randomized to active treatment ($n = 12$) or placebo ($n = 11$). To ensure that mildly sensitive and moderately sensitive subjects were equally distributed among the two groups, stratification was based on the short ragweed-specific serum IgE antibody levels obtained at baseline together with the sum of the sneezing counts and secretion weights collected during the baseline nasal challenge. Subjects in the active treatment group received gelcaps containing antigen-coated polysaccharide microspheres. These antigen-coated microspheres were protected with pH-sensitive outer coats composed of polymethacrylic acid copolymers.⁸ Subjects in the placebo group received identical capsules containing polysaccharide microspheres protected with pH-sensitive outer coats; however, these polysaccharide microspheres were not coated with short ragweed antigen.

Beginning three to four days after the baseline nasal challenge, subjects received oral capsules in the clinic on a daily basis (Monday through Saturday for weeks 1 to 4 and Monday through Friday for weeks 5 to 6) followed by a one hour observation period. The short ragweed antigen dosage level (Amb a 1) was $3 \mu\text{g}$ Amb a 1 initially, and was advanced by up to $3 \mu\text{g}$ every third day

as tolerated to a maximum dosage level of $24 \mu\text{g}$ or the highest tolerated dose. Following the occurrence of a mild adverse reaction (gastrointestinal or systemic), the dosage level was repeated. If the adverse reaction persisted, the dosage level for that subject was reduced by 50%. If the adverse reaction did not persist, the subject resumed the normal dosing schedule. After 6 weeks of treatment, subjects underwent a second nasal challenge. Doses reached at the end of week 6 were considered maintenance and continued at home (Mon-Fri) through the natural ragweed season. Subjects underwent a final nasal challenge at week 14, after the conclusion of the natural ragweed season.

A rescue kit was provided for "as-needed" use in the event of a systemic reaction to the study medication and for relief of allergic symptoms that occurred during the natural ragweed season. Rescue medications included chlorpheniramine, oral prednisone, and subcutaneous epinephrine (1:1000; Epi-pen, Center Laboratories, Port Washington, NY).

Nasal Challenge

Short ragweed doses of 0, 10, 100, and 1000 PNU were administered by nebulizer (Pulmo-Aide, DeVilbiss, Somerset, PA) every 15 minutes with half the total dose in each nostril. Nasal patency was assessed by active posterior

rhinomanometry. Sneezes were counted and symptoms of nasal congestion, runny nose, and itchy throat were assessed using a visual analog scale. The weight of expelled nasal secretions was determined using preweighed tissues.¹⁴ Vital signs and pulmonary function testing were also performed following each challenge dose.

Symptom/Medication Recording

Adverse reactions were scored on a 0 to 3 scale corresponding to absent, mild, moderate or severe degrees for gastrointestinal (nausea, vomiting, abdominal cramps, diarrhea, and anal itching) and systemic symptoms (hives/rash, difficulty breathing, lightheadedness, swelling/flushing, and nasal congestion). In addition, symptoms of nasal congestion, runny nose, sneezing, itchy eyes, and itchy throat were scored during the natural ragweed season extension using the same 0 to 3 scale. Any medication use or adverse event was recorded.

Immunologic

Venous blood samples were collected every 2 weeks during the study to determine the effects of the treatment on systemic immune parameters. Sera were analyzed for short ragweed-specific IgG, IgE, and IgG4 levels. Total short ragweed-specific serum IgG antibodies were measured by ELISA (ICN Biomedicals, Aurora, OH), using plates coated with freeze-dried short ragweed (Greer Laboratories, Lenoir, NC). A polyclonal affinity purified goat anti-human IgG, conjugated to alkaline phosphatase (Jackson ImmunoResearch, West Grove, PA) was applied. Serum IgG levels were determined as that dilution which produced an absorbency equivalent to that produced by a 1:160 dilution of a standard anti-short ragweed serum prepared by pooling several high titer antibody sera. The lowest detectable limit of this assay was $\leq 16 \text{ U/mL}$. To reduce interassay variability, all samples from a given individual were assayed for specific IgG serum antibody levels at the same time. Short ragweed-specific serum IgE antibodies were

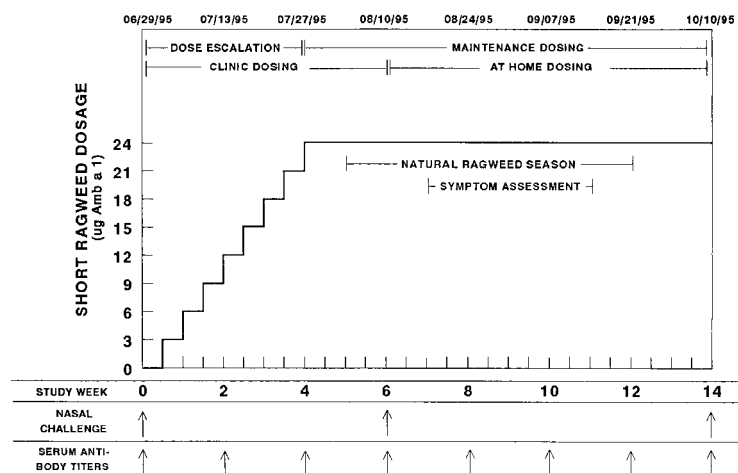


Figure 1. Study protocol/time table.

measured using IgE FAST-Plus kits and allergen-specific coated microtitration wells (Biowhittaker, Walkersville, MD). The lowest detectable limit of this assay was ≤ 0.25 IU/mL.

Short ragweed-specific serum IgG4 antibodies were measured using IgG4 FAST kits and allergen-specific coated microtitration wells (Biowhittaker, Walkersville, MD) with proper serum dilutions. The lowest detectable limit of this assay was ≤ 2.0 μ g/mL. Both the IgE and IgG4 FAST assays are highly reproducible (with-in run and run-to-run coefficients of variation of approximately 15%).

Pollen Counts

Pollen counts were obtained twice weekly using The Rotorod Sampler (Sampling Technologies, Inc, Los Altos, CA).

Statistical Analysis

Immunologic parameters including IgG, IgE, and IgG4 antibody levels were averaged at each assessment period. In addition, two criteria for determining an IgG response were defined as: (1) IgG titer more than two standard deviations above the average IgG titer for the placebo treatment group and (2) a 4-fold or greater increase with respect to the baseline level. For the data collected during the natural ragweed season extension, a 4-week period was selected for analysis based on completeness of symptomatic data collection. Each subject's average weekly and total scores for the 4-week period were calculated. The total score for each symptom was compared between the active and placebo groups using a one-tailed Student's *t* test evaluated at $P \leq .05$. For the nasal challenge sessions, response scores for symptoms and sneeze count were calculated for each subject using a baseline-adjusted sum over the challenge doses. Nasal secretions expelled during the sessions were weighed. Nasal patency was measured as the maximum change in conductance during the sessions. The difference recorded at challenge sessions #1 and #2 for each

subject was compared, along with the averages and standard deviations for the active and placebo groups using a one-tailed Student's *t* test. The total scores and their standard deviations for

each symptom in the active and placebo groups and their respective *t* values for between-group comparisons were calculated. The natural season symptom scores were also analyzed

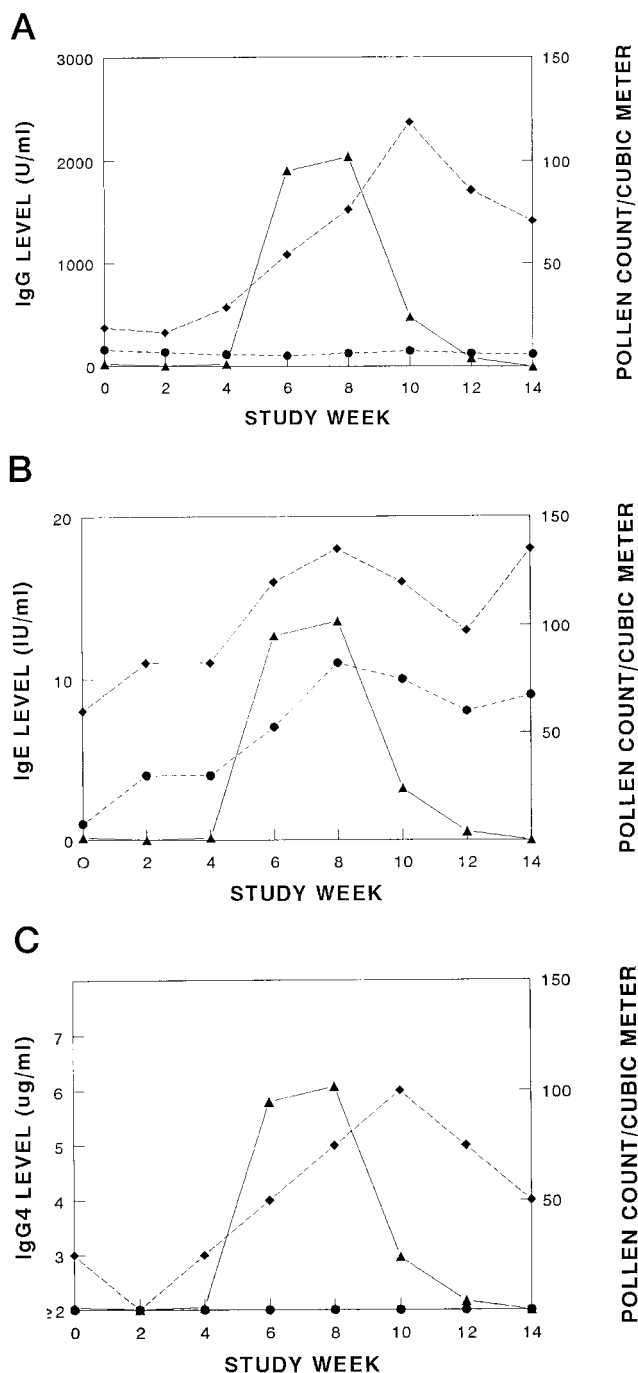


Figure 2. Mean serum short ragweed-specific antibody levels of IgG (A), IgE (B), and IgG4 (C), and mean pollen counts by study week. —▲— ragweed, --●-- placebo, and --◆-- active.

Table 1. Number of Subjects Meeting IgG Response Criteria by Study Week (A = active and P = placebo)

		Study Week							
		0	2	4	6	8	10	12	14
Criterion #1*	A	1	2	4	5	4	5	6	5
	P	0	0	0	0	0	0	0	0
Criterion #2†	A	0	0	2	5	6	5	4	5
	P	0	0	0	0	0	0	0	0

* IgG titer more than 2 standard deviations above the average IgG titer for the placebo group.

† Four-fold or greater increase in IgG titer with respect to baseline.

using a non-parametric method (Kruskal-Wallis).

RESULTS

Protocol Compliance

Twelve subjects (7 of 12 active, 5 of 11 placebo) reached the maximum dosage level (24 μ g) in 4 weeks. The remaining subjects experienced one or more mildly adverse reactions that prevented them from following the proposed dosage schedule. Three subjects in the active group reached the maximum dosage level at week 5 and two subjects in the placebo group reached the maximum dosage level at week 6. Each of the six remaining subjects was given the option to continue with increasing doses for 1 additional week in an effort to attain a higher maintenance dosage level. Only one subject elected to continue with increasing doses. The maximum dosage level attained by these six subjects (two actives and four placebos) ranged from the 6- μ g to the 21- μ g dosage level.

All 23 subjects completed the first and second nasal challenges. Two subjects did not participate in the natural ragweed season extension and two subjects were unable to complete the natural ragweed season extension for personal reasons. Nineteen subjects (ten actives and nine placebos) completed the natural ragweed season extension and the third nasal challenge. Due to the presence of an upper respiratory infection, two subjects remained on maintenance dosing for an additional 2 to 4 weeks before undergoing

the final nasal challenge. For the 19 subjects completing the natural season extension, the mean cumulative dose of short ragweed extract during the entire study was 1264 μ g Amb a 1 (doses ranged from 432 to 1614 μ g Amb a 1).

Immunologic

The short ragweed-specific serum IgG, IgE, and IgG4 antibody levels for the 19 subjects who completed the study are shown in Figures 2a, b, and c, respectively. In addition, the pollen counts collected during the study are shown in these figures. Specific IgG and IgG4 antibody levels showed steady increases up until week 10 only in the active treatment group. Specific IgE antibody levels increased in both groups with no apparent blunting of the seasonal increase in the active treatment group. The number of subjects meeting response criterion #1 and #2 are shown in Table 1. At baseline, one subject in the active treatment group had an IgG titer more than two standard deviations above the average IgG titer for the placebo group. This subject met criteria #1 throughout the entire study. The most notable increase in the number of subjects meeting these criteria was at weeks 4 to 6. Overall, 90% (nine of ten) of the subjects in the active group met one or both of the response criteria at some point during the study. None of the subjects in the placebo group met either response criterion at any time during the study.

Symptom Scores/Medication Usage During the Ragweed Season

The total daily symptom scores during the natural ragweed season are shown in Figure 3. Eighteen subjects (nine actives and nine placebos) had complete data for this period. Although the score for each symptom and for the total symptoms was higher in the placebo than in the active treatment group, these differences were not statistically significant. Also, there was no difference between the groups in either the number of subjects requiring relief medications (five actives and four placebos) or in the mean number of doses administered (10.1 ± 13.3 doses for the active group, 10.0 ± 17.3 doses for the placebo group) during this period.

The results for the natural season symptom scores were unchanged when analyzed by the Kruskal-Wallis test, except that a statistically significant difference between treatment groups was demonstrated at week 3 for the symptom of nasal congestion.

Nasal Challenge Data

Symptom scores and objective outcome parameters from the first and second nasal challenges are shown in Figures 4a and b, respectively. Although numerically larger posttreatment decreases in all symptom responses were observed in the active versus placebo treatment group, the small sample size did not provide sufficient power to permit detection of a statistically significant effect. One exception was the degree of congestion reported after intranasal ragweed challenge when only the highest challenge dose (1000 PNU) was considered. The effect of oral immunotherapy on this parameter was statistically significant ($P \leq .05$).

Safety

No systemic reactions requiring prednisone or subcutaneous epinephrine were observed or reported. The most commonly reported adverse events during the study are listed in Table 2. Gastrointestinal complaints (abdominal cramping, nausea, vomiting, anal

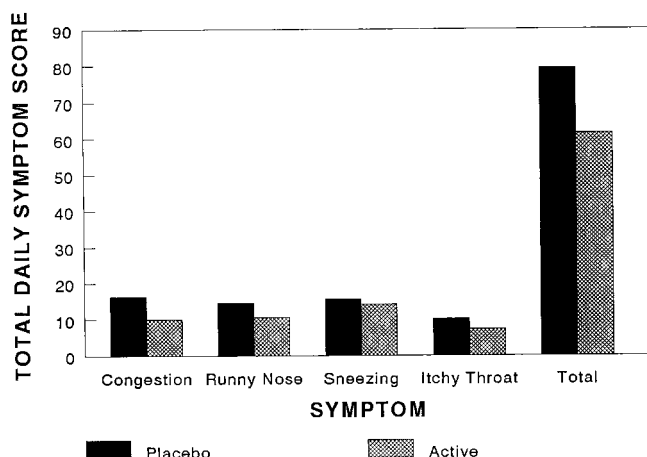


Figure 3. Mean symptom scores over a 4-week period of the natural ragweed season extension.

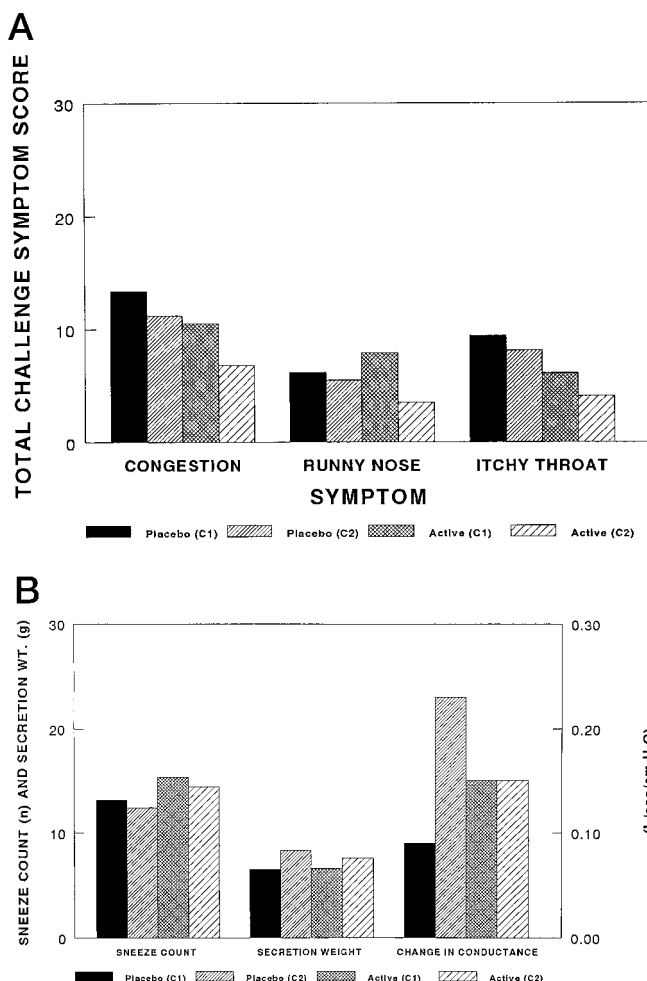


Figure 4. Subjective (upper panel) and objective (lower panel) outcome parameters of nasal challenge 1 and 2.

itching, and diarrhea) were most commonly reported. Although the number of diarrhea events was higher in the active treatment group, the number of persons reporting that event was higher in the placebo group. There was no clear difference between the active and placebo treatment groups. There were no serious adverse events during the study and no subject dropped out due to an adverse event.

DISCUSSION

The results of this pilot study provide evidence for the safety and clinical efficacy of oral immunotherapy with short ragweed extract. Treatment with oral immunotherapy was safe, as evidenced by the lack of serious adverse events and the reported frequency of non-serious adverse events. Oral immunotherapy invoked a brisk serologic response as evidenced by the increase in the short ragweed-specific serum IgG and IgG4 levels of subjects in the active group. Finally, oral immunotherapy modestly attenuated the symptomatic response to ragweed exposure. Not only were symptom scores uniformly less in the active group during the natural ragweed season extension, but there was also a greater reduction in response scores during the nasal challenge sessions following active treatment when compared with placebo.

The incidence of gastrointestinal effects observed in this study is consistent with that observed in previous studies noting an increased frequency of gastrointestinal reactions.^{11,12,15,16} In comparison to the study conducted by Litwin et al using the same pH-sensitive, encapsulated delivery system, the incidence of gastrointestinal effects in the present study is slightly higher. This can likely be attributed to the differences in the dosing regimens of the two studies.⁸ In the current study, subjects began at a much higher short ragweed dosage (3 μ g Amb a 1 per day versus 0.03 μ g Amb a 1 per day) and increased the dosage at a more aggressive rate. In the patients studied to date ($n > 40$), there have been no indications of delayed reactions occurring.

Table 2. Frequently Reported Adverse Events

Event	Active	Placebo
Gastrointestinal Complaints	16 (n = 4)*	11 (n = 6)
Diarrhea	16 (n = 5)	7 (n = 6)
Headache	8 (n = 6)	15 (n = 4)
Lightheadedness	4 (n = 4)	8 (n = 6)
Hives/generalized itchiness	1 (n = 1)	9 (n = 4)
All other events	18 (n = 5)	27 (n = 8)
Total events	65 (n = 10)	76 (n = 10)

* Number of events (n = number of subjects reporting).

Increases in immunologic parameters, specifically short ragweed-specific IgG and IgG4 levels, were seen following active treatment with oral immunotherapy. Such increases have been associated with clinical efficacy in trials of injectable immunotherapy.^{17–21} The present study, however, did not attempt to determine the correlation between serum IgG, IgE, and IgG4 levels and symptomatology or sensitivity due to the small sample size. These results are generally consistent with those of the study conducted by Litwin et al using the same pH-sensitive, microencapsulated delivery system.⁸ Short ragweed-specific IgE levels increased in both the actively treated and placebo groups during the course of the study, an effect commonly observed during the natural ragweed season; thus, an amnestic response in serum IgE value during the season was observed. It was of interest that, in the context of clear effects on serum short ragweed-specific IgG and IgG4 antibody levels, there was no blunting of the seasonal rise in serum short ragweed-specific IgE antibody levels in the active treatment group. This may relate to the small sample size or to a dissociation of the effects of active treatment on different parameters of the immune system.

Previous studies of oral immunotherapy have yielded conflicting results. While several double-blind studies have indicated reductions in allergy symptoms during natural and experimental antigen exposure,^{10–13} other trials utilizing similar methods and doses have failed to confirm those results.^{21–23} Recently, Giovane et al demonstrated the efficacy of oral immuno-

therapy with aqueous *Dermatophagoides pteronyssinus* extract.²⁴ In another trial, Creticos et al documented the efficacy of aqueous oral ragweed immunotherapy in the treatment of seasonal allergic rhinitis.⁹ Evidence supporting the efficacy of oral immunotherapy in these trials included symptom score reductions, decreases in relief medication usage, and improved responses to various provocative challenges as well as changes in immunologic parameters.^{9,24} In contrast, a recent study conducted by Oppenheimer et al indicated that oral immunotherapy with aqueous high-dose standardized cat extract was ineffective in reducing nasal blockage and symptoms following exposure to an apartment contaminated with cat dander.¹⁵

In addition to evaluating the efficacy of oral immunotherapy, previous studies have shown that administration of allergen immunotherapy via the oral route is relatively safe. Oral immunotherapy has been associated with an increased frequency of gastrointestinal complaints in some studies^{11,12,15,16}; however, other studies have shown no overall difference in adverse events between the actively treated and placebo groups.^{13,21,22} Rarely, instances of urticaria have been reported,^{9,10,12,15,23} occasionally with wheezing and rhinitis.¹² Interestingly, no side effects were reported in a 3-year study of oral immunotherapy with aqueous extract of *Dermatophagoides pteronyssinus*.²⁴ Systemic reactions secondary to oral immunotherapy have been uncommonly reported, although an anecdote concerning an anaphylactic reaction that occurred 24 hours after adminis-

tration of oral extract has been reported.²⁵ Oppenheimer et al also reported that 2 of 23 subjects treated with oral cat allergen may have had systemic reactions.¹⁵ One subject developed pulmonary edema seven days after beginning therapy and one subject experienced urticaria nine days after the initiation of therapy.¹⁵ While the causal relationship was unclear, these reports illustrate the need for caution when investigating any form of immunotherapy.

Advantages of this microencapsulated delivery system include (1) due to the enteric-protected outer coat, antigen survives gastric pH and targets the antibody-secreting cells associated with the mucosal lymphoid tissue of the small intestine and (2) a “burst delivery” occurs when the antigen-coated polysaccharide microspheres reach the small intestine, resulting in rapid absorption. In turn, amounts of antigen comparable to those employed using injectable immunotherapy may be utilized. This is favorable, as previous studies of oral immunotherapy suggest that doses 50 to 200 times greater than those of injectable immunotherapy must be used to obtain efficacy.^{10,11} Conversely, gastrointestinal reactions, including diarrhea, nausea, abdominal cramping and vomiting, may result from irritation of the gastrointestinal mucosa, as suggested by Taudorf et al,¹⁶ or may represent a specific allergic reaction. Previous studies suggest that the frequency at which these gastrointestinal reactions occur is related to the aggressiveness of the dosing schedule.^{8,11,16}

Despite the pilot nature of this investigational trial with a small sample size, the results support a positive trend of clinical efficacy for the orally administered immunotherapy. Evidence includes the virtually unidirectional beneficial effects on outcome parameters during allergen exposure in both the laboratory and natural environment. The size of those effects approximated that observed during similar controlled clinical trials conducted with antihistamines and injectable immunotherapy.^{26,27} The small sample

size of this study, however, did not provide sufficient power to permit detection of a statistically significant effect. Indeed, conservative sample size estimates require a minimum of 80 subjects per group to determine statistically significant effects.

One possible variable influencing the outcome of this study was the degree of allergic sensitivity of the subjects. Specifically, the inclusion criteria utilized in this study may not have been rigorous enough to provide a population of individuals with sufficient ragweed sensitivity to evaluate efficacy adequately. Nelson et al demonstrated that a positive intradermal test to timothy grass in the presence of a negative skin prick test was not indicative of clinically significant sensitivity.²⁸ In the current study, higher sensitivity could have been attained by restricting eligible subjects to those with (1) skin test reactivity by the puncture method only, (2) high serum-specific IgE antibody level (class II or higher), and (3) a pre-defined high degree of responsiveness to the baseline intranasal ragweed challenge. Such inclusion criteria would also have resulted in a lower degree of variance in the sample and provide more confidence for detection of a statistically significant effect.

A second variable that may have influenced the outcome of this study was the early onset of the natural ragweed season. The second nasal challenge was scheduled to precede the onset of the natural ragweed season; however, low ragweed pollen counts (1 to 3 particles/m³) were unexpectedly detected at the time of that challenge. Due to the short period of exposure to relatively low counts, it is unlikely that this variable had a significant effect on the results.

Two study design modifications will be necessary in future trials of this microencapsulated delivery system. The pivotal trial must be sufficiently powered to detect a statistically significant effect. Additionally, strict inclusion criteria designed to yield a highly allergic population must be employed. With an improved study design, closer

observations can be made in assessing adequate dosing in the build-up and maintenance phases of therapy, thus, assisting in determining the cost-effectiveness of this product.

CONCLUSIONS

Oral immunotherapy was safe; caused a brisk serological response; and attenuated the symptomatic response to ragweed exposure, indicating a positive trend toward efficacy. The information gleaned from this clinical trial will aid greatly in designing future pivotal trials of this novel allergy therapy.

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