
Immunologic effects of encapsulated short ragweed extract: a potent new agent for oral immunotherapy

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Background: Oral allergen immunotherapy with conventional allergens has not been a useful mode of treatment because of the lack of potency of allergens when administered by this route.

Objective: To study the immunologic potency of short ragweed pollen extracts microencapsulated by a new technique administered orally to short ragweed pollen-sensitive humans and to establish the dose of oral microencapsulated short ragweed pollen extract required for these effects.

Methods: Nine short ragweed pollen-sensitive patients were treated with a new oral agent for immunotherapy, microencapsulated short ragweed pollen extract, in an open study. The effectiveness of this treatment was determined by comparison to a group of nine matched short ragweed pollen-sensitive patients who received no treatment. Treated patients developed high titers of short ragweed-specific IgG and IgE antibodies and their expected seasonal increase in IgE antibodies was regulated. The dose of microencapsulated short ragweed pollen extract required to achieve these effects was only slightly higher than the dose of short ragweed pollen extract used in high dose subcutaneous immunotherapy. Furthermore, this dose was achieved in 7 weeks. There were no side effects other than mild gastrointestinal ones. The nine treated patients fared clinically better during the ragweed season than the untreated patients in this open study.

Conclusion: This study suggests that allergens microencapsulated by this new technique may make oral immunotherapy a practical mode of treatment.

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INTRODUCTION

Inhalant atopic allergy affects a significant portion of the population of the industrial world. Since 1900, many attempts have been made to modify allergic symptoms with a variety of im-

munization techniques. In many well-controlled trials of subcutaneous immunotherapy, efficacy with this procedure has been convincingly demonstrated in patients with allergic rhinitis, allergen-induced asthma, and anaphylactic reactions to insect stings.¹⁻⁴ Because of the inconvenience of closely spaced repeated injections, the cost, and the incidence of systemic reactions associated with subcutaneous immunotherapy, a number of investigators have attempted oral administration of allergens, with the hope not only for positive clinical effects but enhanced safety and convenience.

Despite many trials, oral immunotherapy has proven impractical. One reason for failure is that the dose of allergen required for clinical benefit is extremely large: 100 to 200 times the

subcutaneous immunotherapy cumulative dose.^{5,6} Also, no clinical improvement was observed using certain allergens such as grass pollen extracts and cat allergen, even using high doses of the oral allergen.⁷⁻¹⁰

In the present study, we have addressed this lack of potency of orally administered allergens through encapsulation.

Using a newly developed encapsulation technique with short ragweed pollen extract, we found that microencapsulated short ragweed was capable of initiating vigorous primary and secondary immune responses when given orally to mice, whereas nonmicroencapsulated short ragweed extract in the same doses produced no response.¹¹ The present study was our first attempt to administer the same material to humans in a phase I (open label) clinical trial. As the toxicity and safety of this product were unknown in humans, we elected to administer it in a phase I, open-label, unblinded clinical trial.

METHODS

Subjects

Each subject was between 21 and 50 years of age, had a history of isolated symptoms of allergic rhinitis limited to August and September for at least 2 years, had not received allergen immunotherapy for at least 3 years and was free of gastrointestinal disease. In addition, each subject had to have a positive skin prick test to short ragweed pollen extract, a class 2 RAST titer (standard overnight RAST, classes 0 to 4) and an intradermal endpoint skin test titer to short ragweed extract of 10^{-1} μ g/mL. This skin test criterion for selecting sensitive patients is based

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on the study of Norman, et al, who showed that such a cutoff was useful in separating ragweed-sensitive patients from nonsensitive ones.¹² This study was approved by the Institutional Review Board of the University of Cincinnati and all patients signed informed consent.

Of the 18 selected patients, five had received immunotherapy in the remote past. Three were assigned to the active treatment group and two to the control group.

Routine laboratory studies, including complete blood counts, urinalysis, sedimentation rate, and biochemical profiles (liver profile, blood urea nitrogen, creatinine, total protein and albumin:globulin ratio, serum electrolytes, serum calcium and phosphorus, and serum cholesterol) were performed prior to the initiation of the study, in mid-August and at the termination of the study in October 1992. Patients receiving active immunotherapy had three stools tested for occult blood by the guaiac method at mid-study.

Allergen Preparation

Standardized, lyophilized short ragweed pollen extract was prepared by Greer Labs, Lenoir, NC. It contained 14.5 μg Amb a 1/mg dry weight. The short ragweed pollen extract was microencapsulated with an aqueous coating system of a polymethacrylic acid co-polymer over a core of allergen-coated non-pareils ($\sim 500 \text{ m}\mu$) (Fig 1),

prepared under GMP conditions (Good Manufacturing Practices as defined by the FDA). Briefly, the solubilized short ragweed extract was solubilized in a solution containing polyvinylpyrrolidone (Plasdone K29, FMC Corp, Wayne, NJ) as a binding agent. This solution was spray coated onto a sugar/starch core (non-pareils) utilizing a Wurster bottom spray process. Following application of the allergen coat, the particles were enterocoated with an aqueous dispersion of methacrylic acid copolymer (Eudragit L30D, Rhom Tech, Boston, MA). All processes were conducted at low temperatures in order to minimize possible denaturation of proteins. Microencapsulated short ragweed pollen extract was resistant to dissolution in acid at pH 1.2 for at least six hours and dissociated rapidly at pH > 6.0 , releasing the short ragweed proteins. Thus, microencapsulated short ragweed pollen extract is protected during gastric transit with rapid dissolution occurring upon entering the duodenum. The microcapsules, upon dissolution, release the short ragweed allergens in their native form, as shown by RAST inhibition, ELISA inhibition, and isoelectric focusing patterns (data not shown). Each microsphere of microencapsulated short ragweed pollen extract contained 0.015 μg Amb a 1. The microcapsules were dispensed in opaque gelatin capsules.

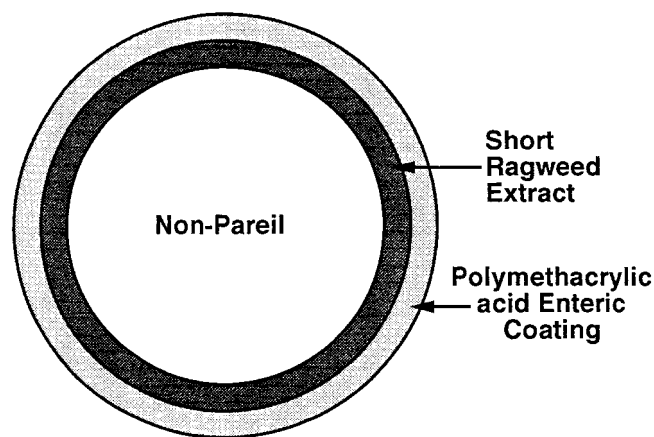


Figure 1. Diagrammatic representation of a microcapsule of microencapsulated short ragweed extract.

Skin Tests

Prick tests were done with both short ragweed pollen extract 1:10 and *Alternaria alternata* 1:10 in 50% glycerin-saline, using a new Lancetter (Miles Labs, Spokane, WA) for each individual test. Glycerin-saline solution was used as a negative control and histamine solution (1 mg/mL) as a positive control. The short ragweed pollen extract used for prick testing contained 141.6 μg Amb a 1/mL. The criterion for a positive skin test was the presence of at least 3-mm induration in cross diameter greater than that measured with the negative control associated with erythema, 15 minutes after application of the test.¹⁴

Endpoint skin test titrations were performed by intradermal injection of 0.02 mL of short ragweed pollen extract into the volar surface of the forearm using 10-fold dilutions of the short ragweed pollen extract preparation used for encapsulation (reconstituted with albumin-saline, Albay, Miles Labs, Spokane, WA). A negative control skin test (albumin-saline) and a positive control skin test (histamine 0.1 mg/mL) were used.

An intradermal skin test was considered positive when the wheal exhibited a cross diameter induration of at least 6 mm greater than that observed with the saline control, and which was associated with erythema measured 15 minutes after application.¹⁵ The results of the intradermal skin test are expressed in micrograms of total short ragweed extract rather than Amb a 1.

SEROLOGIC ASSAYS

Short Ragweed-Specific Serum IgG Antibodies

Total short ragweed-specific IgG antibodies were measured by ELISA.^{16,17} Ninety-six-well plates (Dynatech Laboratories, Chantilly, VA) were coated with 100 μg /mL of our short ragweed pollen extract preparation. Dilutions of the patients' sera were added to these plates. The plates were incubated at 37 °C for two hours, washed three times, and the second antibody, a polyclonal affinity-purified goat anti-hu-

man IgG conjugated to alkaline phosphatase (Boehringer Mannheim, IN), was added (1:1000 dilution). Then the plates were incubated at 37 °C for one hour. Following the addition of the substrate (*p*-nitrophenylphosphate), the absorbency at 405 nm was read. The micrograms of anti-short ragweed extract-specific IgG antibodies were determined by comparison to a standardized serum whose content of short ragweed IgG in μg had been determined previously.

Short Ragweed-Specific IgE Antibodies

Phadebas RAST kits for measuring short ragweed-specific IgE antibodies were purchased from Pharmacia, Piscataway, NJ. The double overnight procedure with proper serum dilutions was used. All samples were done in triplicate and the data expressed in PRU.¹⁸

Specific Nasal IgA and Total Nasal IgA Antibodies

Both determinations were done by an ELISA assay.^{11,17} Thawed aliquots of nasal fluid from each patient were centrifuged to pellet any aggregated material. IgA antibodies were detected, utilizing a polyclonal antihuman IgA with an alkaline phosphatase label (Boehringer Mannheim, Indianapolis, IN). The standard for the ELISA was an affinity purified human IgA (Sigma, St. Louis, MO). The measured concentration of short ragweed-specific IgA in each sample was adjusted to a constant amount of total nasal IgA (5000 ng/mL) to correct for the dilution effects of saline on nasal fluid.¹⁹

Nasal Washings

Nasal washings were obtained by instillation of 5 mL of sterile warmed saline into each nostril through vinyl tubing. The tubing was inserted through a cork that was fitted to each individual subject's nostrils by wrapping it with Parafilm. The samples were obtained with the subjects holding their breath for ten seconds, with their heads inclined forward. The washings were centrifuged, frozen, and stored at -20 °C until analyzed.

Symptom-Medication Scores

The effect of treatment on ragweed hayfever symptoms was assessed by having each patient fill out a symptom-medication scoring card twice daily from the beginning of August to mid-October. The details of the scoring are described in a previous publication.¹⁶

Experimental Plan

Nine patients were treated with microencapsulated short ragweed pollen extract in an open label phase 1 study. Nine other patients, matched for skin test sensitivity by intradermal endpoint titration, served as serologic controls; and, during the ragweed season, as untreated subjects for the symptom-medication scoring. The matching was close, as the treated patients had a mean endpoint skin test titration of $10^{-2.5}$ $\mu\text{g/mL}$ short ragweed extract and a mean RAST titer of 35.4 PRU; whereas the values in controls were $10^{-2.4}$ $\mu\text{g/mL}$ short ragweed extract and 30.2 PRU, respectively.

Doses of microencapsulated short ragweed pollen extract in this study are expressed in terms of their Amb a 1 content (approximately 1% of the dry weight of the lyophilized short ragweed extract). The initial dose contained 0.03 μg Amb a 1. Dosing was done daily with the patients fasting. The same dose was taken for two consecutive days. On the third day, it was doubled until a dose containing 15 μg Amb a 1 was achieved. After reaching 15 μg Amb a 1, doses were increased 2 μg Amb a 1 every third day. If a gastrointestinal reaction occurred, the dose was decreased to the previously tolerated dose and then increased by 2 μg every third day. With repeated gastrointestinal symptoms, the dose was decreased 50% and then increased at a rate of 50% of the previous increases.

Venous blood was obtained every 2 weeks and tested for short ragweed specific IgG and IgE antibodies. Each day of the study, the treated patients filled out a reaction report for any gastrointestinal or systemic symptoms that they experienced during the previous twenty-four hours.

Each patient had two syringes filled with epinephrine for self administration (Epi-Pen, Center Labs, Port Washington, NY) in the event of a systemic reaction, as well as a supply of antihistamine tablets (chlorpheniramine, 4 mg) and prednisone tablets (to be taken on physician advice only). The patients were seen in the clinic weekly. At that time, they took their next dose of microencapsulated short ragweed extract in the presence of the study nurse. All patients had been warned that failure to take the prescribed doses at home could result in this dose causing a severe systemic reaction. During the ragweed pollination season, each subject, including the untreated control subjects, filled out a daily symptom-medication score card. The antihistamines/decongestants furnished to the patients (chlorpheniramine maleate, 4 mg; triphenamine, 50 mg; carbinoxamine maleate, 4 mg, with pseudoephedrine, 60 mg; and pseudoephedrine, 60 mg) were to be taken only as required for control of severe symptoms, not their prevention.

Data Analysis

The significance of the differences between treated and control groups for serologic data was determined by a one way analysis of variance performed on the log of the ratio of pre-treatment and post-treatment sera. The symptom-medication score data were normalized using square root transformation and then subjected to a 3-way analysis of variance.²⁰

RESULTS

Immunotherapy Dose and Associated Adverse Events

As the dose began to exceed 15 μg Amb a 1, adverse gastrointestinal reactions were observed in some patients. These increased in frequency as the dose increased to between 20 to 30 μg Amb a 1; therefore, dosing was curtailed at 30 μg Amb a 1 or less/day in all individuals. Of the nine treated patients, four patients had definite gastrointestinal symptoms while receiving microencapsulated short ragweed ex-

tract and one had a borderline reaction: heartburn. The symptoms included nausea, abdominal cramping, diarrhea, and, rarely, vomiting. These reactions occurred between 1½ to 15 hours after ingestion of the doses. In three patients, these reactions were mild, and, with the previously outlined dosage reduction, they ceased. Eventually, these three patients all tolerated doses in excess of 20 µg Amb a 1 per day. A fourth individual had three episodes of adverse gastrointestinal events and eventually only tolerated a dose of 8 µg Amb a 1.

The mean highest dose achieved for all the patients was 27.8 µg Amb a 1 (range 20 to 36 µg). The mean cumulative dose taken prior to the ragweed pollination season (7 weeks) was 506 µg Amb a 1 (range 291 to 657 µg). During the ragweed pollination season, the doses were decreased to 15 to 20 µg per individual and the dosing frequency changed to alternate days. No individual had any adverse reactions while receiving the maintenance regimen.

No systemic or anaphylactic reactions were observed in any patient during the course of the study.

Short Ragweed IgG Antibody Responses

Threshold Dose

The mean threshold dose was 11.1 µg Amb a 1 (range 0.2 to 20 µg). The mean time to reach the threshold was 5.3 weeks (range 2 to 10 weeks). The threshold dose is defined as the mean dose of microencapsulated short ragweed received during the week that individuals' short ragweed IgG antibodies rose to a level 50% higher than baseline.

IgG Antibody Levels Induced by Microencapsulated Short Ragweed Pollen Treatment

Figure 2 depicts the maximum increase of short ragweed IgG antibodies compared with the pretreatment level found in each patient. The average increase from baseline to peak titer was 440% (range 50% to 1090%). The time the peak occurred varied with the mean being 9.6 weeks with a range of 5 to 12 weeks. Figure 3 depicts the weekly mean level of short ragweed IgG antibodies observed in each group of treated and untreated patients during the course of the study. The group peak level for the treated patients oc-

curred during the ragweed season (12th week of treatment) and was 310% higher than the pretreatment level ($P = .009$). Following this peak, the levels fell 24% during the remainder of the ragweed season. This decline of IgG antibodies is possibly related to the lower dose of microencapsulated short ragweed extract employed during this time. Six months after discontinuation of microencapsulated short ragweed extract, short ragweed IgG antibodies had decreased to approximately one-half the peak levels (data not shown). No increase in ragweed specific IgG antibodies was detected in untreated patients.

Effect of Microencapsulated Short Ragweed Pollen Extract Treatment on Short Ragweed IgE Antibodies

Prior to the ragweed season, treatment with microencapsulated short ragweed pollen extract induced a 210% mean increase in short ragweed specific IgE antibodies in treated subjects (range +987% to -35%), which reflects the potency of the microencapsulated antigen.^{21,22} During the ragweed pollination season, however, the treated subjects exhibited a blunted anamnestic response to the seasonal exposure, having a group mean increase of +6.8% in short ragweed IgE antibodies (range +29 to -35%) compared with control subjects whose short ragweed-specific IgE antibodies increased 40.4% (range +92 to -37%). Because of the large intragroup variations, none of these seasonally induced changes was statistically significant, although there was a positive trend ($P = .08$) for blunting of the IgE anamnestic response in treated patients.

Onset of Rise of Short Ragweed IgE and IgG Antibodies

Figure 4 depicts the onset of rise of short ragweed IgE and IgG antibodies in the microencapsulated short ragweed pollen extract-treated patients. The curves parallel each other indicating relatively identical synthetic processes. Using the arbitrary criteria of a 50% increase in IgG antibodies and a

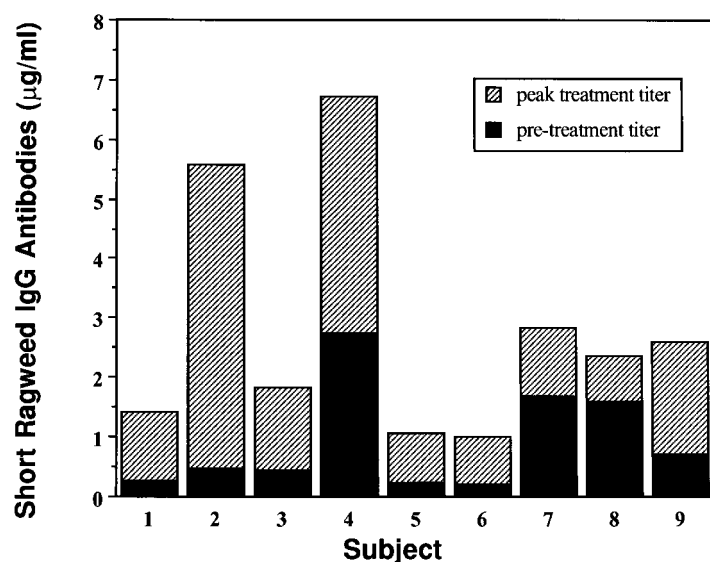


Figure 2. Comparison of pretreatment and peak anti-short ragweed IgG titers in subjects receiving oral microencapsulated short ragweed extract.

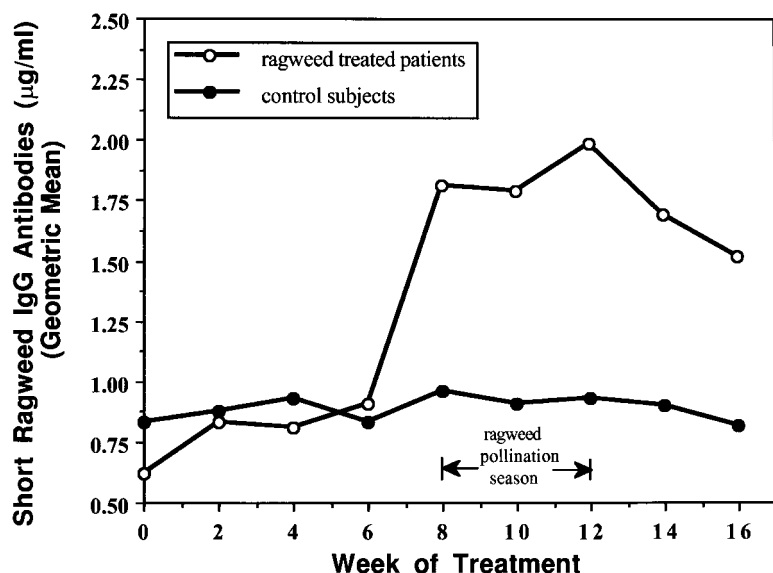


Figure 3. Weekly mean anti-short ragweed IgG titers in each group of patients.

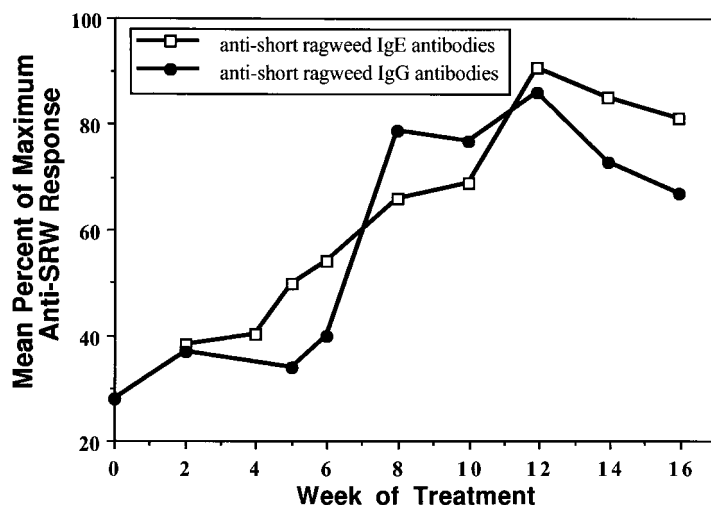


Figure 4. Kinetics of IgG and IgE responses in patients receiving oral micro-encapsulated short ragweed extract.

25% increase in IgE antibodies as representing significant increases, 4/9 treated patients had an increase in short ragweed IgG antibodies preceding the onset of an increase in short ragweed IgE antibodies; in 3/9 individuals the onset of the rise of the two isotypes was simultaneous; and, in only 2/9 subjects did the onset of increased synthesis of short ragweed IgE antibodies precede that of IgG antibodies.

Nasal Secretory Short Ragweed-Specific IgA Antibodies

In mid-August, just prior to the advent of significant environmental ragweed pollen, nasal washings were obtained from treated and control subjects. At this time, treated subjects had a mean level of short ragweed specific IgA of 6.7 ng/mL; compared with the controls who had a level of 4.2 ng/mL. These

differences were not statistically significant. During the ragweed pollination season, short ragweed-specific IgA antibodies rose in both groups: the mean percent increase with microencapsulated short ragweed pollen extract treatment was +625% versus +3426% in untreated controls. These differences were not statistically significant ($P > .10$).

Symptom-Medication Scores

Since this was an open study, the symptom-medication scores are not a rigorous measure of clinical effectiveness. As depicted in Figure 5, the group mean for the season was 3.9 in the treated subjects and 7.7 in control subjects ($P = .056$). During the height of the ragweed season (August 15 through September 9), the difference was significant ($P = .02$). Also, considering only subjects who could tolerate sustained doses of microencapsulated short ragweed pollen extract of 20 μg or more of Amb a 1 (eight of nine treated subjects), the differences were significant for the entire season ($P = .03$). It should be noted that the ragweed pollination season in Cincinnati in 1992 was mild, with the highest counts of only 400 grains/mm³ (usual peak is 1000 to 1500 grains/mm³).

Safety Surveillance Studies

Prior to the initiation of immunotherapy, in mid-August, and at the completion of the study, physical examinations, complete blood counts, urinalyses, sedimentation rates, and biochemical profiles were done and showed no significant abnormalities or any change during the course of the study. Three stools tested for occult blood in mid study in the patients receiving immunotherapy were negative.

DISCUSSION AND CONCLUSIONS

The positive immunologic results of the present study suggest that allergens microencapsulated by our new technique may make oral immunotherapy practical. The improved potency of short ragweed pollen extract microencapsulated by our technique was demonstrated in this study by the increase

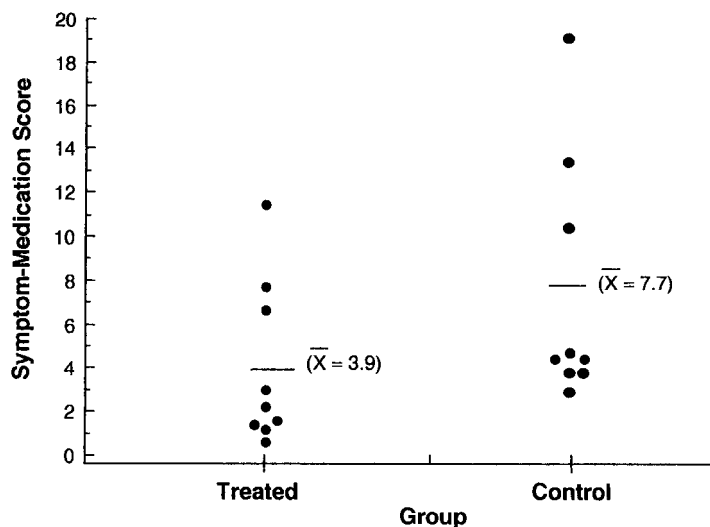


Figure 5. Mean symptom-medication scores in each patient.

in specific serum IgG antibodies (4.4 times pre-treatment levels) and the observed regulation of serum IgE antibodies in the treated patients. The IgG antibody response was higher than that found by us in a previous short-term study using an injectable short ragweed extract allergen during which specific IgG antibodies only doubled.¹⁶ Furthermore, these immunologic effects were achieved with relatively low doses of oral allergen, only slightly higher than those used in high dose subcutaneous immunotherapy.

At present, most investigators are unwilling to ascribe the beneficial effects of immunotherapy entirely to IgG antibodies, as the titer of specific IgG antibodies does not always correlate with the clinical results in individual patients.²³ Nevertheless, the extent of the rise in IgG antibodies found in groups of patients treated with immunotherapy usually parallels the clinical effectiveness of the immunotherapy regimen.²⁴ Many techniques, differing in sensitivity and specificity, have been employed to determine specific IgG antibodies, making interstudy comparisons difficult. Our results can be compared to those obtained by Peng et al, who used an ELISA technique similar to the one employed in this study.¹⁷ In their study, after 2 years of weekly short ragweed pollen extract

subcutaneous immunotherapy (highest dose 20 μ g Amb a 1), short ragweed IgG antibodies increased 6-fold; whereas in the present study, they increased more than four times after only 12 weeks of treatment.

In this open study, clinical effectiveness could not be adequately evaluated. Although the symptom-medication scores of the treated patients during the ragweed pollination season were significantly lower than those of the control subjects, double-blinded placebo-controlled trials will be required before the issue of clinical effectiveness for this new method of oral allergen encapsulation can be decided.

The highest dose of allergen given in this study was achieved in a very short time (7 weeks), and was accomplished without inducing any systemic reactions. Similar dosing with high dose subcutaneous immunotherapy would have required a far longer time using conventional schedules, and is frequently accompanied by a definite incidence of systemic allergic reactions.²⁵ In a review of 20 published immunotherapy studies using conventional subcutaneous dosing, 12.9% of the patients experienced a systemic reaction.²⁵ With rush subcutaneous immunotherapy, it is possible to administer high doses more quickly, but usually with an even higher incidence

of systemic reactions. Other than mild gastrointestinal events, no significant toxicity to microencapsulated short ragweed extract treatment was detected. The rarity of systemic reactions with oral immunotherapy has not been explained. Creticos et al reported that early during the course of subcutaneous immunotherapy, the onset of increased synthesis of IgE antibodies preceded that of IgG antibodies by several weeks.²⁶ During this time, patients had a higher risk of incurring an anaphylactic reaction to their injections. In the present study, as demonstrated in Figure 3, the onset of rise of short ragweed specific IgE and IgG antibodies was simultaneous, thus, eliminating this cause for systemic reactions. Other factors, however, such as the binding of the allergen locally in vast surface area of the gastrointestinal tract, could also contribute to the increased safety of oral immunotherapy.

In addition to short ragweed allergens, murine studies with encapsulated rye grass pollen extracts have shown that these extracts given orally to mice are capable of stimulating a significant IgG antibody response.¹¹

In summary, allergen extracts encapsulated by this new technique may make oral allergen immunotherapy a realistic alternative to the traditional subcutaneous allergy injections. This conclusion seems reasonable because our new technique of encapsulation preserves allergenic epitopes, enhances their immunologic potency, and when such encapsulated allergens are given orally, renders them free from serious reactions. Furthermore, because of these factors, the time required for a therapeutic dose to be achieved is greatly shortened.

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