

Oral immunotherapy with short ragweed extract in a novel encapsulated preparation: A double-blind study

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Background: In the past, oral immunotherapy with allergens has had limited clinical effectiveness, presumably because of gastrointestinal destruction of allergens.

Objective: We have developed a new technique for microencapsulating protein antigens that permits them, when given orally, to bypass the stomach and be delivered to the small intestine in a highly immunogenic form. This study's purpose was to confirm the immunologic potency of orally administered short ragweed pollen extracts (SRW) microencapsulated (mSRW) by this new technique and to study the effectiveness of mSRW in controlling the symptoms of ragweed-induced hay fever.

Methods: Twenty-one SRW-sensitive patients were treated with mSRW in a double-blind placebo-controlled study. Serum SRW IgG and IgE antibodies and nasal secretory IgA antibodies were determined. During the ragweed season, symptoms were quantified by symptom-medication scoring. **Results:** The treated patients had high titers of serum SRW IgG antibodies (1.15 µg/ml at baseline, increasing to 21.21 µg/ml), experienced regulation of the seasonal increase in serum SRW IgE antibodies (+9% vs +59% in placebo-treated patients), and produced a small amount of nasal SRW IgA antibodies. Despite an insubstantial pollen count, the symptom-medication scores in the treated group were lower than those in the placebo group (4.28 vs 6.18, $p = 0.059$), but the differences were statistically significant only in the subgroup that tolerated high doses (>20 µg of Amb a 1 in 19 of 21 patients, $p = 0.04$). These effects were accomplished without inducing any systemic reactions with a dose of mSRW (mean, 23.8 µg of Amb a 1) only slightly higher than that used in high-dose subcutaneous immunotherapy.

Conclusion: Oral mSRW seems a safe, easily administered, and immunologically potent treatment for ragweed-induced hay fever, but its ultimate utility requires further study. (*J Allergy Clin Immunol* 1997;100:30-8.)

Abbreviations used

mSRW:	Microencapsulated short ragweed pollen extract
OIT:	Oral immunotherapy
PRU:	Phadebas RAST units
SIT:	Subcutaneous immunotherapy
SRW:	Short ragweed pollen extract

Key words: Oral immunotherapy, microencapsulation, immunotherapy, short ragweed, nasal IgA antibodies

The efficacy of subcutaneously administered allergen immunotherapy (SIT) in the treatment of allergic rhinitis and allergic asthma and in the prevention of anaphylactic reactions to Hymenoptera stings is well established.¹⁻⁴ To accomplish the same goal but improve the ease of administration, reduce cost, and curtail immunotherapy-associated systemic reactions, many investigators have examined oral administration of allergens for treatment of these conditions. Despite numerous attempts, oral immunotherapy (OIT) has not proven to be a viable alternative. The principal reason for this failure is a lack of potency of allergens when given by this route. In the many prior studies, the oral doses of allergen required for either a serologic or clinical effect were extraordinarily large: 100 to 200 times the cumulative dose required by the subcutaneous route.^{5,6} Also, with some allergens, such as grass pollen or cat allergen extracts, there was no effect despite use of high doses of oral allergen.⁷⁻¹⁰

We believe that this requirement for excessive doses of orally administered allergen is due to the destruction of allergens in the stomach by low pH and pepsin, which is followed by further degradation by intestinal proteolytic enzymes. These enzyme-induced alterations not only considerably reduce antigenicity, but may even convert antigens or allergens to tolerogens.¹¹⁻¹³ Indeed, in a number of earlier studies, we demonstrated that pepsin-derived fragments of proteins administered orally or even injected intraperitoneally into mice engender antigen-specific immune tolerance.^{14,15}

To improve the immunogenicity of orally administered protein antigens, we have developed a new technology for encapsulation of proteins. This encapsulation technique permits antigens, on oral administration, to

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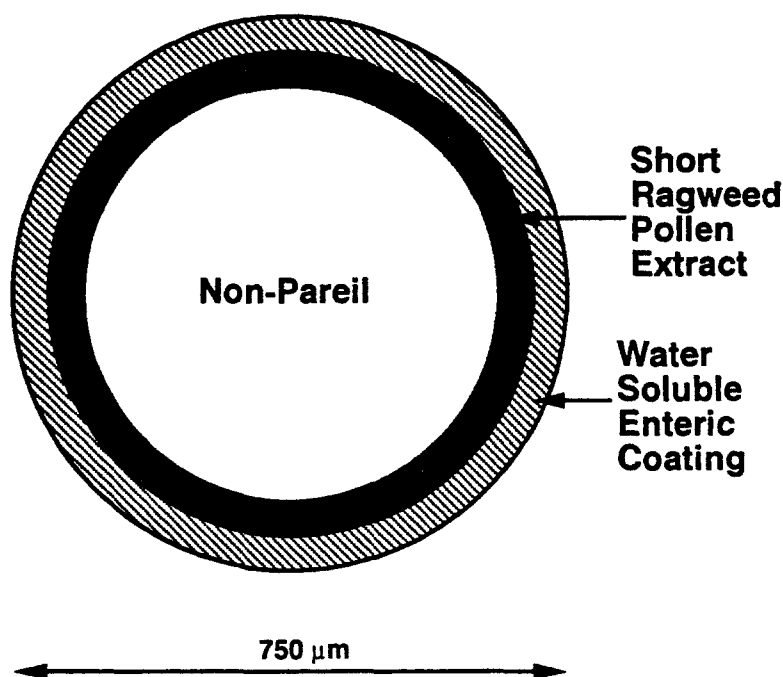


FIG. 1. Schematic diagram of structure of a microcapsule of SRW.

avoid gastric digestion and be delivered unaltered to Peyer's patches in the small intestine for an effective immune response. Murine studies with a variety of orally administered protein antigens encapsulated by this method including ovalbumin, bovine serum albumin, tetanus toxoid, diphtheria toxoid, and a number of inhalant allergen extracts have confirmed that such encapsulation produces antigens with significantly enhanced immunologic potency when given orally.¹⁶⁻¹⁸ An open OIT trial with orally administered encapsulated short ragweed pollen extract (SRW) in the treatment of ragweed-sensitive individuals confirmed the immunologic efficacy of this encapsulation technique in human beings.¹⁹ These patients had high titers of IgG antibodies (similar to the titers induced by SIT) but required only 7 weeks of treatment to achieve this result. Moreover, in contrast to previous attempts by others at OIT, the doses of allergen required for this effect were only slightly higher than those used in high-dose SIT. Despite an aggressive dosing schedule, no systemic reactions were observed. To confirm these previous findings and to determine whether oral immunotherapy with encapsulated allergens is clinically effective, as well as immunologically potent, this study was undertaken wherein encapsulated SRW was administered orally to ragweed-sensitive patients in a double-blind, placebo-controlled trial.

METHODS

Subjects

Each subject was required to be between the ages of 21 and 50 years, to have a history of isolated symptoms of allergic rhinitis limited to August and September for at least 2 years, to

have not received allergen immunotherapy for at least 3 years, and to be free of gastrointestinal and cardiovascular disease. In addition, each subject had to have a positive skin prick test response to SRW with appropriate positive and negative control skin test responses, a RAST titer of class 2 or higher, and an intradermal end-point skin test titer to SRW of 10^{-2} μ g/ml, a level of skin test sensitivity that allows separation of ragweed-sensitive individuals from nonsensitive ones.²⁰ A physical examination, complete blood count, sedimentation rate, urinalysis, and biochemical profile were done initially and again at the end of the study. This study was approved by the Institutional Review Board of the University of Cincinnati. All patients signed an informed consent document.

Allergen preparation

Standardized, lyophilized SRW was prepared by Greer Laboratories (Lenoir, N.C.). It contained 14.5 μ g of Amb a 1 per milligram of dry weight. The SRW was microencapsulated with an aqueous coating system of polymethacrylic acid co-polymers over a core of allergen-coated nonpareil microspheres (≈ 500 μ) (Fig. 1). This was done under good manufacturing practice conditions. Briefly, the lyophilized SRW was solubilized in a solution containing polyvinylpyrrolidone (Plasdone K29; FMC Corp., Wayne, N.J.) as a binding agent. This solution was spray-coated onto a sugar-starch core (nonpareils) by using a Wurster bottom spray process. After application of the allergen coat, the particles were enterocoated with an aqueous dispersion of a methacrylic acid copolymer (Eudragit L30D; Rhom Tech, Boston, Mass.). All processes were conducted at low temperatures to minimize possible denaturation of proteins. Microencapsulated SRW (mSRW) was resistant to acid at pH 1.2 for at least 6 hours and dissociated rapidly at a pH greater than 6.0, releasing the SRW proteins in their native form as confirmed by RAST inhibition, ELISA inhibition, and isoelectric focusing patterns (data not shown). These dissolution characteristics enabled the mSRW, when administered

orally, to evade gastric digestion and to rapidly release the allergen on entering the duodenum. Each microcapsule contained 0.015 μg of Amb a 1. For oral administration, mSRW was dispensed in opaque gelatin capsules. Stability studies at 4° and 21° C have shown that the Amb a 1 content of mSRW was stable for more than 4 years.

Placebo microcapsules were prepared as described above but with lactose instead of SRW.

Skin tests

Prick tests were done with SRW 1:10 and *Alternaria alternata* 1:10 in 50% glycerin-saline solution (Miles Laboratories, Spokane, Wash.). Glycerin-saline solution was used as a negative control and histamine (1 mg/ml) as a positive control. The SRW used for prick testing contained 141.6 $\mu\text{g}/\text{ml}$ Amb a 1. The criterion for a positive prick skin test response was the presence of a wheal of at least 3 mm induration, in cross diameter greater than that measured with the negative control, which was associated with erythema 15 minutes after application of the test solution.

End-point intradermal skin test titrations were performed by intradermal injection of 0.02 ml of SRW extract into the volar surface of the forearm with fivefold dilutions of the SRW preparation used for encapsulation (reconstituted with albumin-saline, Albay; Miles Laboratories). A negative control (albumin-saline) and a positive control (histamine, 0.01 mg/ml) were used. An intradermal skin test result 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 was associated with erythema 15 minutes after application.

Total SRW-specific serum IgG antibodies

Total SRW-specific IgG antibodies were measured by ELISA.^{21,22} Ninety-six-well plates (Dynatech Laboratories, Chantilly, Va.) were coated with our SRW preparation (100 $\mu\text{g}/\text{ml}$) re-solubilized in a bicarbonate coating buffer. Diluted sera were then added to the plate. The initial dilution of each patient's serum was 1:10. Each serum was then diluted serially 1:2 on the ELISA plate. Some individual sera were repeated at higher or lower dilutions as the need arose. The plates were incubated at 37° C for 2 hours and washed three times; and the second antibody, a polyclonal affinity-purified goat anti-human IgG conjugated to alkaline phosphatase (Boehringer Mannheim, Indianapolis, Ind.), was added (1:1000 dilution). Then the plates were incubated at 37° C for another hour. After the addition of the substrate (p-nitrophenyl phosphate), the absorbency at 405 nm was read. Titers of total SRW-specific IgG antibodies were converted to a concentration expressed in micrograms per milliliter in comparison with a standardized serum for which the content of total SRW IgG had been previously determined.

SRW-specific IgG4 antibodies

SRW-specific IgG4 antibodies were measured in sera from mSRW-treated patients by using IgG4 FAST kits and allergen-coated microtitration wells (Biowhittaker, Walkersville, Md.) with proper serum dilutions.²³ The lowest detectable limit of this assay was 2.0 $\mu\text{g}/\text{ml}$.

SRW-specific IgE antibodies

Phadebas RAST kits for measuring SRW IgE antibodies were purchased from Pharmacia (Piscataway, N.J.). The double overnight procedure with proper serum dilutions was used.²⁴

All samples were done in triplicate, and the data are expressed in Phadebas RAST units (PRU).

Total nasal IgA and allergen-specific nasal IgA antibodies

Determinations of both total nasal IgA and allergen-specific nasal IgA antibodies were done by an ELISA. Aliquots of nasal washings from each patient were first centrifuged to remove any aggregated material. Then total nasal IgA was detected by using a sandwich ELISA, utilizing a polyclonal anti-human IgA with an alkaline phosphatase label (Boehringer Mannheim) on plates coated with anti-human IgA antibodies. Briefly, sample wells of the ELISA plates were coated with overnight at 4° C with the goat anti-human IgA polyclonal antibody (0.1 $\mu\text{g}/\text{well}$). Then standard wells were coated with a 1:2 titration of an affinity-purified IgA from human colostrum at concentrations ranging from 1000 to 15.6 ng/well. Plates were washed thoroughly, and nasal samples were titrated 1:2 down the plate in phosphate-buffered saline-Tween-20 with 5% dry milk and 0.5% gelatin (blotto) from a 1:20 dilution to 1:2560 dilution. Plates were incubated overnight (4° C) and washed three times with phosphate-buffered saline-Tween-20, then 100 μl of alkaline phosphatase-labeled goat anti-human IgA (0.4 $\mu\text{g}/\text{ml}$ in blotto) was added to each well. Plates were incubated for 4 hours at room temperature, washed in phosphate-buffered saline-Tween-20, and read at 405 nm after the addition of 100 μl of 4-nitrophenyl phosphate (1 mg/ml in diethanolamine buffer). Sample and standard titrations were run in duplicate, and the IgA content of each sample titration was calculated from the standard curve formed by plotting the amount of IgA in the standard wells to the absorbance. The reported sample IgA concentrations are the mean of at least three determinations at different dilutions. The procedure for determining specific IgA was quite similar, except that sample wells were coated with re-solubilized SRW (100 μl of a 100 $\mu\text{g}/\text{ml}$ solution). Standard wells were coated with human IgA as described above but at a dilution range of 20 to 0.3 ng/well. Samples were titrated 1:2 on the plate, beginning at a 1:2 dilution. The remaining steps were as described above for total IgA.

The standard for both ELISAs was a human IgA, affinity-purified from colostrum (Sigma, St. Louis, Mo.) and measured on wells coated with dilutions of anti-human IgA. The measured concentration of SRW-specific IgA in each sample was normalized to a constant amount of total nasal IgA (5000 ng/ml) to correct for the dilution of nasal fluid by saline solution.²⁵⁻²⁷

Nasal washings

Nasal washings were obtained by instillation of 5 ml of sterile, warmed saline solution into each nostril through vinyl tubing. The tubing was inserted through a cork, which was fitted to each subject's nostrils by wrapping it with Parafilm (American National Can, Greenwich, Conn.). The samples were obtained with the subjects holding their breath for 10 seconds, with their heads slightly inclined forward. The washings were centrifuged, frozen, and stored at -20° C until they were analyzed.

Symptom-medication scores

The effect of each regimen on ragweed-induced hay fever symptoms was assessed by having each patient fill out a symptom-medication scoring card twice daily from the beginning of August to mid-October. This scoring system had been used by us and others successfully in many prior studies.^{21,28} Briefly, each subject evaluated his or her symptoms at noon and

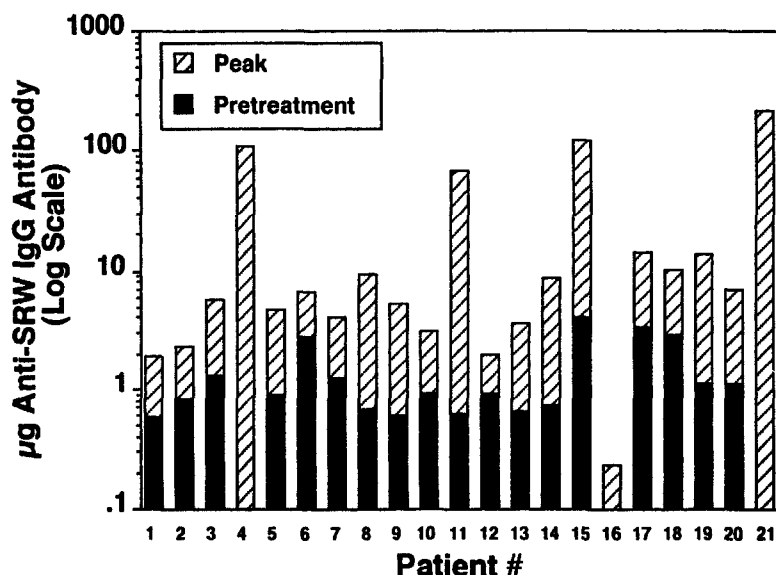


FIG. 2. Individual serum IgG anti-SRW antibody titers in patients treated with mSRW.

at midnight with separate ratings given for: (1) sneezing, (2) stuffy or runny nose, (3) red itchy eyes, (4) coughing, and (5) number of antihistamine-decongestant tablets taken during that 12-hour period (chlorpheniramine, 4 mg; tripelemamine, 50 mg; pseudoephedrine, 60 mg; or carbinoxamine, 4 mg, with pseudoephedrine, 60 mg tablets). Each symptom was scored as: 0, no symptoms; 1, symptoms for less than 30 minutes; 2, symptoms for 30 minutes to 2 hours; or 3, symptoms for more than 2 hours. The number of antihistamine-decongestant tablets was added to the symptom score to obtain the symptom-medication score.

Experimental plan

Forty-five patients fulfilled the criteria for acceptance to this study. After matching the patients by RAST scores and their estimated hours of exposure to unfiltered air during the ragweed season, patients were randomly assigned to receive either mSRW or an identical appearing placebo. Thus 22 patients were to receive active treatment, and 23 were to receive placebo. One patient dropped out of the mSRW group, and three dropped out of the placebo group before receiving any treatment. The reasons for leaving the study were inability to attend the clinic regularly (3 patients) and dislike of nasal lavage (1 patient). The two groups were closely matched in age (mean age: 36.8 years in the mSRW group vs 36.2 years in the placebo group), gender (15 women in each group), race (19 Caucasians in the mSRW group and 18 in the placebo group), RAST titer (geometric means: 23.8 PRU in the mSRW group [range, 2.2 to 519.5 PRU] and 23.8 PRU [range, 3.8 to 696.9 PRU] in the placebo group), and end-point skin test titers (means: $10^{-2.3}$ µg/ml in the mSRW group vs $10^{-2.6}$ µg/ml in the placebo group). None of these differences were statistically significant.

Doses of mSRW in this study are expressed in terms of their Amb a 1 content. Dosing was done daily, first thing in the morning, with the patients refraining from eating for 1 hour. Each dose was taken for 2 days before proceeding to the next higher dose. The dosage was doubled every third day between doses of 0.03 µg and 1.92 µg of Amb a 1; it was increased 50% every third day between doses of 1.92 µg and 6.48 µg; by 2 µg

every third day between doses of 6.48 µg and 21.48 µg; and finally, by 1 µg every third day between doses of 21.48 µg and 24 µg. During the ragweed season, the dosage was reduced to 16 µg of Amb a 1 taken on alternate days. If a gastrointestinal reaction occurred, the dosage was reduced three levels and then raised by the usual increments. If a second reaction occurred, the dosage was reduced by 50% and subsequently increased by 50% of the usual increments. Venous blood was drawn every 2 weeks during the study and assayed for SRW-specific IgG and IgE antibodies. Each day of the study, the patients filled out a reaction report for any gastrointestinal or systemic symptoms that they experienced during the previous 24 hours. Each patient had two Epi-Pens for self administration in the event of a systemic reaction and a supply of antihistamine tablets (chlorpheniramine, 4 mg) and prednisone tablets (to be taken only on advice of a physician). The patients were seen in the clinic weekly. At that time, they took a dose of mSRW in the presence of the study nurse (all patients had been warned that failure to take the previous doses at home could result in this dose causing a severe reaction). During the ragweed pollination season, each subject filled out a daily symptom-medication score card. The antihistamine-decongestants listed above were to be taken only as required for control of symptoms, not for their prevention.

Data analysis

The symptom-medication score data were normalized by using square root transformation and then subjected to a two-way analysis of variance.²⁹ The serologic data had a one-way analysis of variance performed on the log of the ratio of pretreatment and posttreatment sera. Nasal IgA antibodies were analyzed by the one-tailed Wilcoxon test.

RESULTS

Immunotherapy dose and associated adverse events

The mean total preseasonal dose of mSRW was 659 µg of Amb a 1 (range, 261 to 724 µg). The mean daily highest dose was 23.8 µg of Amb a 1 (range, 14.5 to 24.0

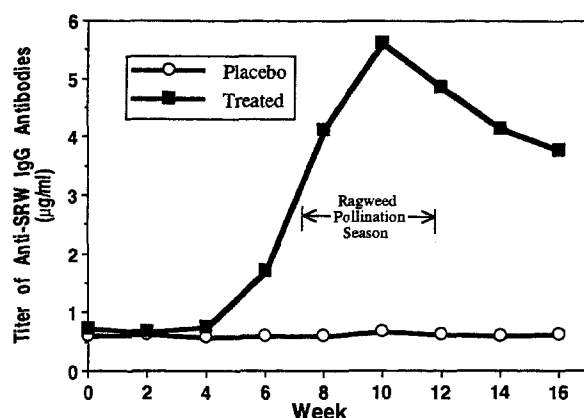


FIG. 3. Biweekly group geometric mean titer of anti-short ragweed IgG antibodies (in micrograms per milliliter) in treated patients and patients receiving placebo.

µg), and only two patients were unable to achieve this dose because of gastrointestinal side effects. Seventeen of 21 patients tolerated all doses of mSRW without any adverse reactions. Although the mSRW-treated patients overall had statistically no more gastrointestinal adverse events than the placebo-treated patients, four patients receiving mSRW had some gastrointestinal symptoms (mild diarrhea and abdominal cramps) that seemed attributable to their ingestion of mSRW. The inciting doses were 12 µg in one patient, 14 µg in two other patients, and 20 µg in a fourth patient. Two of these patients eventually tolerated doses of mSRW in excess of 20 µg, whereas the other two were only able to tolerate doses of 7.65 µg and 13.24 µg, respectively, before experiencing gastrointestinal side effects again. The incidence of other adverse events was not significantly different between the two groups of patients. No systemic or anaphylactic reactions were observed in any patient.

Total SRW IgG antibody levels induced by mSRW treatment

Fig. 2 shows the maximum increase of total SRW IgG antibodies found in each patient compared with the pretreatment level. The average increase was 1665% (geometric mean), but the range was wide (12% to 15,557%). The baseline total SRW IgG level failed to double in only three patients. Ten patients increased their total SRW IgE antibody levels two to five times higher than their baseline; two increased their levels five to 10 times their baseline; and six increased their levels more than 10 times their baseline. The mean time to reach the peak titer after starting administration mSRW was 10.4 weeks (range, 6 to 16 weeks). Fig. 3 shows the course of the weekly mean level of total SRW IgG antibodies observed in each group of patients during the study. The group peak level for the mSRW-treated patients occurred during the ragweed season (tenth week of treatment), and this difference between the two was significant ($p = 0.0001$). During the remainder of

TABLE I. Effect of oral administration of mSRW on SRW-specific IgE titers

Group	IgE titer (PRU)		
	Pretreatment	Mid-August	October
Placebo ($n = 21$)	23.8	20.1 (−16%)*	31.9 (+59%)
Treatment ($n = 20$)	23.8	58.5 (+146%)	63.7 (+9%)

Results are expressed as geometric means.

*Values in parentheses indicate percent change from previous titer.

the ragweed season, the levels fell. No change in total SRW IgG antibodies was detected in placebo-treated patients.

Effect of mSRW treatment on serum IgG4 SRW antibodies

With mSRW treatment, levels of SRW-specific IgG4 antibodies rose in 14 of 21 patients. In these 14 patients, the level of SRW IgG4 antibodies was 2.26 µg/ml (geometric mean) before they received mSRW and rose to 9.37 µg/ml (geometric mean) immediately before the onset of the ragweed season. After the ragweed season, SRW IgG4 antibodies fell in a manner similar to the change observed in levels of total SRW IgG antibodies.

Effect of mSRW treatment on SRW IgE antibodies

Table I shows the effect of mSRW treatment on SRW IgE antibodies. Before the ragweed season, treatment with mSRW induced a 146% (geometric mean) increase in SRW IgE antibodies in treated subjects, whereas a fall in SRW IgE antibodies was observed in placebo-treated patients. During the ragweed pollination season, the placebo-treated patients experienced an increase in SRW IgE antibodies (geometric mean, +59%), which in the actively treated patients was blunted because their SRW IgE antibodies rose only slightly (geometric mean, +5.4%). This difference between the two groups was statistically significant ($p = 0.009$).

Onset of the rise of SRW IgE and total IgG antibodies

Fig. 4 shows the onset the increase in short ragweed IgE and IgG antibodies in the patients treated with mSRW. The curves parallel each other, indicating relatively similar synthetic processes. When the arbitrary criteria of a 50% increase in IgG antibodies and a 25% increase in IgE antibodies are used to represent a significant increase, in 16 of 21 mSRW-treated patients the onset of the increase in the two isotypes was simultaneous; in four patients, the onset of increased synthesis of SRW IgE antibodies preceded that of total SRW IgG antibodies; but in all four of these patients, a rise in total SRW IgG antibodies was detected 2 weeks later. In one patient the onset of increase in isotypes could not be determined because of rapidly changing titers.

TABLE II. Effect of oral mSRW treatment and seasonal pollen exposure on nasal SRW-specific IgA antibodies

Group	Geometric mean titer (ng/5000 ng total IgA)		
	Pretreatment	Mid-August	October
Placebo (<i>n</i> = 21)	1.69	0.91	3.04
Treatment (<i>n</i> = 20)	1.31	1.20	1.89

Nasal secretory SRW-specific IgA antibodies

Nasal washings were obtained before the study, in mid-August before the advent of significant atmospheric ragweed pollen, and in October after ragweed pollen had disappeared. The results of nasal secretory SRW IgA antibody determinations are shown in Table II. By mid-August, the geometric mean titers of nasal SRW IgA remained unchanged in mSRW-treated patients, whereas they fell in the patients receiving placebo. The percentage change between the two groups (median percent change: -13% in mSRW-treated patients vs -27% in placebo-treated patients) was significantly different ($p = 0.04$; one-tailed Wilcoxon test). During the ragweed season, SRW nasal IgA antibodies rose dramatically in both groups but reached higher levels in placebo-treated patients. The percentage change between the two groups (median percent change: +37% in mSRW-treated patients vs +150% in placebo-treated patients) was significantly different ($p = 0.01$, one-tailed Wilcoxon test).

Symptom-medication scores

Fig. 5 shows the weekly mean symptom-medication scores found in the two patient groups and contrasts these to the weekly mean pollen count. The scores for the two groups are distinctly separated. The ragweed season of 1993 was a very mild one (highest count, 170 grains/m³). Despite this mild environmental challenge, a close but nonsignificant statistical difference was detected between the two groups during the peak weeks of this ragweed season (mean scores, 4.28 vs 6.18; $p = 0.059$), the period encompassed by counts of 100 grains/m³ or more (4 days in all) from August 30 through September 15. This difference became significant ($p = 0.03$) when the analysis considered only the 19 of 21 patients who were able to tolerate doses of mSRW in excess of 20 μ g of Amb a 1. These 19 subjects also had significantly decreased symptom-medication scores compared with placebo-treated patients for the entire ragweed season ($p = 0.04$).

Safety surveillance studies

Before the initiation of immunotherapy and at the completion of the study, physical examinations, complete blood counts, urinalyses, sedimentation rates, and biochemical profiles were done; and the results showed either no significant abnormalities or no change during the course of the study.

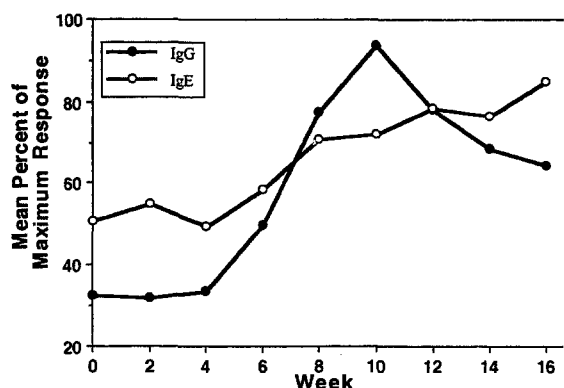


FIG. 4. Kinetics of IgG and IgE short ragweed-specific responses in patients receiving mSRW.

DISCUSSION

The results of this double-blind, placebo-controlled study suggest that allergens encapsulated by this new technique could make OIT a realistic alternative to subcutaneous allergen administration, if future studies extend and verify the results.

The immunologic potency of orally administered mSRW was validated by three sets of observations: the increase in SRW-specific serum IgG antibodies, the regulation of the expected seasonally induced increase in serum SRW IgE antibodies, and the modest increase in nasal secretory SRW IgA antibodies.

In the patients receiving mSRW, SRW-specific IgG antibodies rose more than 16 times the pretreatment levels with at least a doubling of the baseline levels in 18 of 21 treated patients. Changes in SRW IgG4 antibodies paralleled the changes found in total SRW IgG antibodies.

The significance of this increase in allergen-specific IgG antibodies induced by immunotherapy remains an area of contention, excluding the observation that the extent of the rise in allergen-specific IgG antibodies usually parallels the overall clinical effectiveness of an immunotherapy regimen.³⁰ The therapeutic role of IgG antibodies in the clinical effectiveness of immunotherapy is the conundrum. These antibodies have been postulated to but not proven to act as "blocking" antibodies,³¹⁻³³ as inactivators of mast cells by cross-linking of the Fc γ RII and the Fc ϵ RI receptors on the cell surface,³⁴ and as downregulators of the late-phase reaction.³⁵ The role for subclasses of IgG antibodies in the efficacy of immunotherapy, especially the IgG4 subclass antibodies, is also unsettled, although antibodies of this subclass are expanded more than other subclasses in the late IgG response to immunotherapy.^{22, 36}

The magnitude of this rise in allergen-specific IgG antibodies found in the mSRW-treated patients is similar to the rise in IgG antibody levels observed in short-term studies of SIT. For example, Peng et al.²² found a threefold increase in SRW-specific IgG antibodies after 1 year of SIT with SRW and a sixfold increase after 2 years (20 μ g Amb a 1/dose), using an ELISA

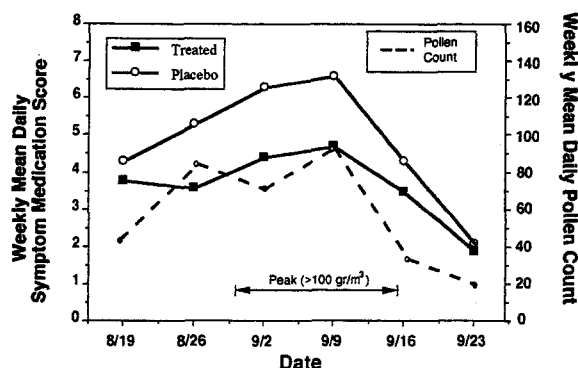


FIG. 5. Mean weekly symptom-medication score in mSRW-treated and placebo-treated patients as compared with mean weekly pollen counts.

similar to that used in this study. However, this rise in IgG elicited by oral mSRW treatment is in contrast to the results of previous OIT studies in which the levels of allergen-specific IgG antibodies induced have usually been far less than those observed in this study.³⁷

An increase in serum-specific IgE antibodies during the initiation of immunotherapy, such as that found in this study, has been reported in many prior SIT studies and is thought to be related to allergen potency.³⁸⁻⁴⁰ More importantly, during the ragweed season, the expected seasonal anamnestic rise in allergen-specific IgE antibodies in mSRW-treated patients was significantly blunted ($p = 0.009$) when compared with placebo-treated patients, again an effect seen with successful SIT studies.^{41, 42}

The level of allergen-specific nasal secretory IgA antibodies was maintained with mSRW treatment, whereas it fell in placebo-treated patients, a significant difference ($p = 0.04$, one-tailed Wilcoxon test). This maintained level with mSRW treatment would be expected because of migration of activated lymphocytes from the gut to the nasal mucosa through the common mucosal immune system.⁴³ The natural exposure to ragweed pollen induced a substantial increase in allergen-specific IgA antibodies in both mSRW-treated and placebo-treated patients, which was far higher in the placebo-treated patients ($p = 0.01$, one-tailed Wilcoxon test). This induction of a major increase in allergen-specific nasal IgA by exposure to environmental ragweed pollen has been previously observed in untreated allergic patients.⁴⁴ However, the blunted increase in secretory SRW IgA antibodies found in mSRW-treated patients has not been described until now. This immunoregulation of the anamnestic increase in nasal secretory IgA antibodies induced by mSRW treatment appears similar to the regulation of seasonally induced increases in serum allergen-specific IgE, an effect regularly observed in patients receiving immunotherapy. This is not surprising because both antibody isotypes are induced and regulated by T_{H2} . This anamnestic increase in allergen-specific secretory IgA antibodies observed in untreated

patients could act adversely because it has been reported that of all immunoglobulin classes, secretory IgA antibodies furnish the strongest stimulus for eosinophil degranulation, possibly a critical factor in the pathogenesis of mucosal diseases such as allergic rhinitis and bronchial asthma.⁴⁵

Granting that this new oral form of immunotherapy is immunologically vigorous, to be useful it must also be clinically effective. The low level of environmental pollen that occurred in Cincinnati in 1993 induced only mild symptoms in placebo-treated patients. Their symptom-medication scores for the entire season were lower than the usual scores that we and others have observed during previous ragweed seasons when using the same scoring system (5.5 vs 7.7 and 9.9).^{19, 21} For a treatment to exert a discernible beneficial effect in the face of mild symptoms in control subjects, it must be a potent one. During the "peak" of the 1993 ragweed season (defined above), the only period when there was significant pollen in the air, the scores between the two groups were separated but did not achieve statistical significance ($p = 0.059$). However, when the comparison is limited only to patients who tolerated a high dose of mSRW (in excess of 20 μ g of Amb a 1), which 19 of 21 patients did, the differences between the two groups becomes statistically significant not only for this "peak" ($p = 0.03$) but also for the entire ragweed season ($p = 0.04$). Therefore only a large dose of mSRW appeared to be successful in significantly reducing hay fever symptoms, which is similar to the experience with SIT.^{46, 47} Another potential advantage of oral mSRW over previous OIT allergen preparations is that the dose of mSRW needed to achieve these immunologic and clinical effects was only slightly higher than that used in high-dose SRW SIT and was achieved in a short time (7 weeks). Thus the requirement for extraordinarily high uneconomical doses of allergen to obtain a beneficial effect when using OIT seems to have been overcome, at least in the instance of SRW. One of the major reasons for investigators to attempt OIT was to circumvent the incidence of associated systemic allergic reactions elicited when using SIT. The only side effects attributable to mSRW treatment were mild gastrointestinal symptoms. There were no systemic allergic reactions. In our previous open study with mSRW in which slightly higher doses of oral mSRW were used, we had a similar experience.¹⁹ Thus in the two studies a total of 30 patients have received oral mSRW without experiencing any systemic reactions. In a review of 20 published studies of conventional SIT, 12.9% of patients had at least one systemic reaction during their course of treatment.⁴⁸ With rush immunotherapy and with very sensitive patients, such as those in this study, the incidence was even higher. Thus if these two studies were conventional SIT studies, it would be expected that at least a few patients would have had a systemic reaction during treatment.

There are several possibilities to explain this freedom from serious reactions with OIT and in particular, with oral mSRW. As indicated in Fig. 4, the onset of the rise

in allergen-specific IgE and IgG antibodies with mSRW treatment in this study was simultaneous in virtually all patients. In comparison, Creticos et al.³⁹ reported that early during the course of SIT with SRW, allergen-specific IgE antibodies began to increase several weeks before an increase in allergen-specific IgG antibodies. This initial period of increase in SRW IgE antibodies in the absence of a similar increase in SRW IgG antibodies coincided with a period of increased systemic reactions in the patients.

Other explanations for the decreased frequency of systemic reactions with OIT would include sequestering of allergen locally in the vast gastrointestinal surface with little spillage into the systemic circulation or differences in the involved mast cell populations between OIT and SIT.

Thus overall, the findings in this study support the assertion that SRW encapsulated by this new technique may make its oral administration a genuine alternative to traditional subcutaneous allergy "shots." This new encapsulation technique preserves the allergenic epitopes of SRW during manufacture, enhances the immunogenicity of SRW, allows administration of SRW to be free from systemic reactions, and could make oral SRW clinically effective when used in high doses. These properties of encapsulated SRW may well apply to other allergens when they are similarly encapsulated, a proposal supported by our successful experiments in mice with orally administered encapsulated grass pollen and oak pollen extracts.⁴⁹

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