

A controlled study of the effectiveness of the Rinkel method of immunotherapy for ragweed pollen hay fever

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In a double-blind study, we compared the effects of the Rinkel method of immunotherapy with ragweed pollen extract and placebo on symptoms of ragweed hay fever and immunologic parameters in 24 ragweed-sensitive patients. Each had a skin-test end point by Rinkel serial dilution titration to ragweed pollen extract at 1:312,500 w/v or greater dilution, a 2+ skin test to ragweed AgE at 0.1 µg/ml or greater dilution, and in vitro leukocyte histamine release by ragweed pollen extract. None had had immunotherapy for at least 7 yr. Patients matched on the basis of leukocyte histamine release by ragweed were assigned to two treatment groups (12 patients in each group). One group received ragweed pollen extract, and the other, placebo, both administered by the Rinkel method between June and October, 1978. Treatment doses were derived from skin-test end points. The median maintenance ("optimal dose") for patients receiving ragweed pollen extract was 0.53 ml of 1:312,500 w/v and the mean cumulative dose of ragweed pollen extract given during the study contained 0.094 µg of ragweed AgE. Symptom-medication scores of all patients rose and fell with ragweed pollen counts. No significant differences were observed in mean daily symptom-medication scores, antiragweed IgG or IgE levels, leukocyte histamine release by ragweed, total IgE levels, or skin-test end-point dilutions with ragweed pollen extract between the group receiving ragweed pollen extract and the group receiving placebo. Despite the absence of specific effect on symptom-medication scores and measured immunologic variates, 10 of the 12 ragweed-treated patients and 10 of the 12 placebo-treated patients were of the opinion that their hay fever symptoms during the ragweed pollen season were less severe in 1978 than in 1977 and that they had been helped by Rinkel method immunotherapy. Under the conditions of the study, Rinkel method immunotherapy with ragweed pollen extract was no more effective than placebo given in an imitation of the Rinkel method.

Through the years, two quite different methods of immunotherapy for ragweed pollen hay fever have evolved. For the purpose of this discussion, they will

be called the current standard method and the Rinkel method.

The current standard method dates back to the earliest studies by Noon.¹ As adapted for ragweed pollen extract, therapy is begun with a low dose which has been shown to be tolerated by both experience and skin testing. Dosage is progressively increased slowly thereafter, according to the tolerance of the patient, at intervals of 1 to 7 days until the highest tolerated dose is reached or at least until the patient is receiving a final maintenance dose containing 2.7 to 22 µg or more of ragweed AgE (usually 0.5 ml or more of 1:50 w/v). This maintenance dose may be continued at regular intervals perennally, or achieved each year

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by a new but shortened course of treatment. Twenty-five or more injections may be required to reach the final maintenance dose, which is usually 1,000 to 10,000 or more times the starting dose and is often close to the highest tolerated dose. It may be necessary to adjust the progression of dosage downward to avoid local and constitutional reactions.

A number of suitable controlled, double-blind studies have shown that the current standard method of immunotherapy will relieve symptoms of ragweed pollen hay fever better than placebo,²⁻¹³ and that this effect is specific^{4, 5} and dose related.^{2, 3, 5, 7-9, 11, 12} Furthermore, it will produce measurable immunologic changes which include the following: (1) increase in specific serum antiragweed IgG antibody in most or all patients^{7-10, 12, 14-24}; (2) decrease in the rise of specific serum antiragweed IgE antibody which would be expected to occur in the ragweed season in most or all patients^{13, 16, 17, 24, 25}; (3) Decrease in leukocyte histamine release from exposure to ragweed pollen extract in some patients^{7-10, 13-17, 20-23}; and (4) increase in IgG and IgA antibodies in nasal secretions.²⁶

The Rinkel method of immunotherapy has its roots in the very low-dose coseasonal immunotherapy which was commonly employed for relief of hay fever symptoms in the previously unimmunized patient in the era before effective pharmacologic therapy became available in the form of antihistamines and corticosteroids.²⁷⁻³¹ Between 1922 and 1931, Vaughan noted that a proper low dose of ragweed pollen extract was promptly followed by satisfactory relief of symptoms in 80% of previously unimmunized patients, even during the 1931 ragweed pollen season when he found pollen counts that were very high in comparison to the preceding 5 yr.³² He reported that the dose must be low—no more than 0.5 ml of 1:1000 w/v—and that higher doses were less effective in neutralizing symptoms. He noted the convenience of the method and suggested its use as the sole form of immunotherapy for patients who responded. Hansel also became interested in the method.^{33, 34}

Rinkel, exposed to the concept of minute, optimal-dose therapy while taking a course with Hansel, began to use end-point skin testing with serial dilutions of extract to determine sensitivity, and found the optimal dose for treatment to be closely related to the end-point dilution.³⁵⁻³⁷ The optimal dose is reported to relieve symptoms of ragweed pollen hay fever within 4 hr and to provide relief for a period of from several days to a week or more. Relief can be maintained in some instances by repeating the injection of the optimal dose when symptoms return.

The optimal dose for ragweed pollen is calculated

by the following method. (1) First, the end-point dilution is determined by intradermal testing employing approximately 0.01 ml of serial fivefold dilutions of ragweed pollen extract. It is the weakest dilution which produces a positive reaction and initiates progressive increase in the diameter of the wheals with each stronger dilution tested. (2) The optimal dose is then determined by injecting at weekly intervals progressively larger subcutaneous doses beginning at 0.1 ml of the end-point dilution. The optimal dose is usually 1 to 5 times the size of the starting dose, is reached after giving 1 to 9 injections, and is the dose which best relieves symptoms. It is very close to the lowest dose producing a positive skin test and, if properly determined, is unlikely to produce untoward reaction. The average optimal dose has been reported to be 0.5 ml of the end-point dilution.^{37, 38}

The Rinkel method of immunotherapy is widely used in the United States at the present time. Proponents claim that it provides very satisfactory relief from symptoms of hay fever. However, it has not been proven effective for this purpose by suitable controlled, double-blind studies. The objectives of our study were (1) to compare by means of a double-blind study the effectiveness of the Rinkel method of immunotherapy with ragweed pollen extract with that of an appropriate placebo in providing relief from symptoms of ragweed pollen hay fever, and (2) to ascertain whether the Rinkel method of immunotherapy with optimal dose of ragweed pollen extract produces specific changes in serum levels of antiragweed IgG or antiragweed IgE, or in leukocyte histamine release by ragweed pollen extract, such as are found in patients treated with ragweed pollen extract by the current standard method.

MATERIALS AND METHODS

Patients

We recruited 24 patients, aged 18 to 50 yr, with a history of seasonal rhinitis in late August and September for at least the 2 prior years. Each had a positive skin test to ragweed pollen extract at a concentration of 1:312,500 w/v or less, and to ragweed AgE at a concentration of 0.01 μ g/ml or less. Their leukocytes released histamine when incubated in vitro with ragweed pollen extract. None had had immunotherapy for at least 7 yr preceding the study. Patients with major concomitant rhinitis symptoms in the mold-dominated seasons in July and late October and November were excluded. Where possible, we recruited patients with negative skin tests to mold extracts (*Alternaria*, *Hormodendrum*, *Helminthosporium*, *Botrytis*, *Aspergillus*, *Penicillium*, *Fusarium*, and *Mucor*) at a concentration of 1,000 protein nitrogen units (PNU)/ml. Thirteen patients with positive skin tests to molds were included in the study.

Few patients fulfilling all of these criteria were present in

TABLE I. Comparison of the diameter of wheals produced 10 min after intradermal tests with serial dilutions of ragweed pollen extract (5^{-4} to 5^{-10}) and placebo (5^{-5} to 5^{-9}) in 12 patients treated with ragweed pollen extract and in 12 treated with placebo

Patients and treatment groups	Diameter (mm) of wheal produced by intradermal test									
	Ragweed extract dilutions							Placebo dilutions		
	5^{-10}	5^{-9}	5^{-8}	5^{-7}	5^{-6}	5^{-5}	5^{-4}	5^{-9}	5^{-8}	5^{-5}
Ragweed										
1		4	4	6*	7	11		5	5	8*
2			4	4	4	8*	11	4	4	7*
3		9/8/78	3	3	3	7*		6/4/79	5	5
4			3	3	8*	10			4	4
5	5	4	4	6*	7	13		5/24/79	5	5
6		4	4	4	4	10*	14		4	4
7		3	3	3	6*	12	20		4	4
8		4	4	6*	7	13		5/30/79	4	4
9			0	0	0	10*	17		4	4
10			4	4	6*	9	12		4	4
11		3	3	3	6*	14	25		4	4
12	5	5	6*	7	8	15			4	4
Placebo										
1	5	5	5	7*	9	19			4	5
2	3	3	3	3	3	9*	14		4	4
3	4	4	4	7*	9	13			4	4
4	4	4	5	6*	7	9		9/25/78	4	4
5		4	4	6*	7	11			5	5
6		4	5	7*	8	12			4	4
7		5	5	9*	11	13			4	4
8		5	5	5	7*	10	20		5	5
9	3	5	4	4	7*	11			4	4
10	4	4	4	4	4	7*	11	8/28/78	5	5
11	4	5	4	8*	11	14			5	5
12	4	4	9*	11	13	15			3	3

All tests done October to December, 1978, unless otherwise stated.

*Marks end-point dilution.

the Johns Hopkins Allergy Clinic or in the authors' allergy practices and most had to be recruited by advertisement.

Reagents

Short ragweed pollen allergenic extract, 1:10 w/v freeze-dried, was provided by Hollister-Stier Laboratories, Spokane, Washington. Short ragweed pollen was defatted by shaking with acetone for 8 hr, rinsed in acetone, then in diethyl ether, dried, and combined with distilled water at 1:10 w/v. Extraction of water-soluble materials was carried out at 4° C for 18 to 24 hr with continuous stirring. The extract was clarified and sterilized by filtration and lyophilized and packaged in vacuum in vials, each of which contained the lyophilized residue of 2.5 ml of 1:10 w/v extract. The material in these vials was reconstituted with 5 ml of appropriate diluent to make 1:20 w/v extract for skin testing, immunotherapy, and in vitro tests.

The 1:20 w/v short ragweed pollen extract was tested by Dr. David Marsh by double-diffusion in gel and was shown

to contain AgE, 187 µg/ml; Ra 3, 47 µg/ml; and Ra 5, 34 µg/ml.

For skin testing and immunotherapy, the lyophilized short ragweed pollen extract was reconstituted with 50% glycerol saline* to provide 1:20 w/v concentrate solution, and serial fivefold dilutions of this concentrate in isotonic saline containing 0.4% phenol* were made according to the method of Rinkel.^{36, 38} A new vial of lyophilized extract was reconstituted and new dilutions were made every 2 wk. Antigens were stored at approximately 4 ± 3° C in a refrigerator or in a special ice box.³⁹ Extracts used for skin testing and immunotherapy were dispensed in coded vials so that patients and participating physicians could not distinguish active extract from placebo.

Histamine caramelized glucose solution placebo was modeled on that designed by Dr. S. Roger Hirsch for the

*Diluents and vials for both ragweed pollen extract and placebo were supplied by Hollister-Stier Labs.

Committee on Alternate Forms of Therapy, Research Council, The American Academy of Allergy. It was made by Hollister-Stier Laboratories in the following manner. D-glucose was caramelized by heating. Sufficient 50% glycerol saline (equal parts glycerine and isotonic saline was added to produce a color darker than that obtained when the freeze-dried short ragweed pollen extract was reconstituted to 1:20 w/v by addition of 50% glycerol saline. Sufficient histamine phosphate 2.75 mg/ml and glycerine were added to make a final coloring solution which contained 0.275 mg histamine phosphate per milliliter and 50% glycerine. A sufficient quantity of 50% glycerol saline containing 0.275 mg histamine phosphate per milliliter was added to lighten the color to approximate that obtained when the freeze-dried short ragweed pollen extract was reconstituted to 1:20 w/v by addition of 50% glycerol saline. The solution was filtered through a 0.2- μ membrane filter to sterilize and obtain final histamine caramelized glucose solution.

For skin testing and immunotherapy, serial fivefold dilutions in isotonic saline containing 0.4% phenol were made every 2 wk. Placebo was stored at $4 \pm 3^\circ \text{C}$ in the same manner as the ragweed pollen extract. It was dispensed in coded vials so that patients and participating physicians could not distinguish placebo from ragweed extract.

Initial screening evaluation

Initial evaluation of prospective subjects included a history, physical examination, and allergy skin testing to seasonal and perennial inhalant allergens as routinely performed in The Johns Hopkins Hospital Allergy Clinic.

Comprehensive initial evaluation

Patients meeting the criteria for entry into the study were further evaluated as follows.

Blood tests. Hematocrit, white blood count, differential count, and total eosinophil count were performed in the Clinical Laboratories of The Johns Hopkins Hospital. Ragweed extract-induced in vitro leukocyte histamine release was performed utilizing the automated technique of Siraganian.⁴⁰ Ragweed extract for histamine release was the same as that used for skin testing and treatment. Serum levels of antiragweed IgE were measured by an agarose-based radioallergosorbent test (RAST),⁴² and total IgE levels by a non-competitive radioimmunosorbent test as described previously.⁴² Determinations of levels of antiragweed IgG by ¹²⁵I *Staphylococcus aureus* protein A solid-phase radioimmunoassay were carried out using the same allergosorbent as for the RAST.⁴¹ The ragweed allergosorbent used for IgE and IgG determinations was prepared from the ragweed extract employed for skin testing and treatment.

End-point dilution skin testing. Determinations of skin-test end points were performed according to the method of Rinkel.^{36, 38} Intracutaneous testing with fivefold serial dilutions of skin-testing extract of ragweed pollen were carried out using disposable 1-ml tuberculin-type syringes with 26-gauge, 0.5-inch needles. Testing was performed from least to most-concentrated extracts. A volume of approximately 0.01 ml was placed superficially into the skin so as

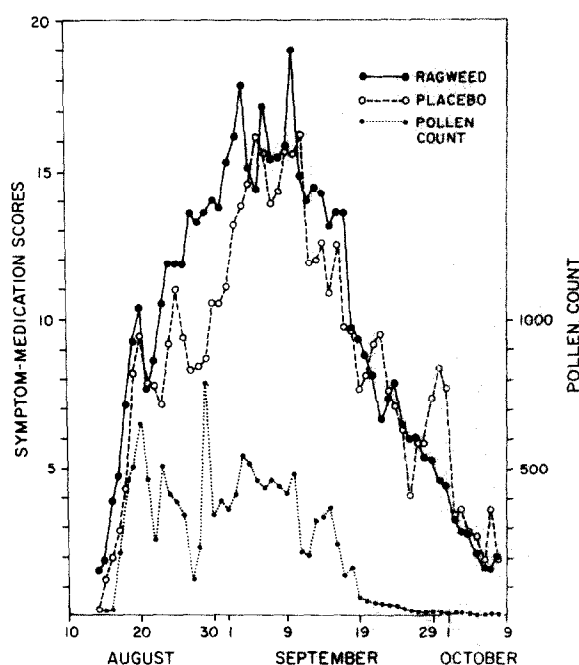


FIG. 1. Arithmetic mean of daily symptom-medication scores for the groups indicated, compared with the pollen count, reported as ragweed pollen grains per square centimeter of roto-slide.

to create a tense wheal about 4 mm in diameter. The test was read at 10 min, with the end-point dilution being that which initiated a progressive increase in the diameter of the wheals with each stronger dilution tested. Typical skin-test end-point titration measurements are shown in Table I. The end point was redetermined periodically as described below.

Skin testing with the caramelized glucose histamine solution placebo was done in the same manner as with the active extract. This procedure produced a skin-test end point which was indistinguishable in appearance from that produced by the active extract (Table I).

Treatment

Patients were divided into two comparable groups, each composed of 12 patients selected on the basis of cell sensitivity to short ragweed pollen extract as determined by leukocyte histamine release. Histamine-release values were divided equally into four ascending categories of values (low, medium low, medium high, and high), and patients in each category were assigned in a random manner to the two treatment groups. Treatment groups were balanced also with regard to patients who had had immunotherapy previously (three in each group had had immunotherapy but discontinued it 7 yr or more before the study) and with regard to patients with positive skin tests to molds (five in the ragweed-treated group, eight in the placebo-treated group). By random selection, one group received ragweed pollen extract by the Rinkel method and the other received placebo. Treatment was given in a double-blind fashion so

TABLE II. Medians of ragweed pollen extract end-point dilutions measured in each patient before immunotherapy, during ragweed pollen season, and after ragweed pollen season in 12 patients treated with ragweed pollen extract and 12 patients treated with placebo

Patients and treatment groups	End-point dilutions		
	Before therapy	During season	After season
Ragweed			
12	5 ⁻⁸	5 ⁻⁸	5 ⁻⁸
11	5 ⁻⁸	5 ⁻⁵	5 ⁻⁵
5	5 ⁻⁸	5 ⁻⁷	5 ⁻⁷
1	5 ⁻⁷	5 ⁻⁷	5 ⁻⁷
3	5 ⁻⁷	5 ⁻⁵	5 ⁻⁵
7	5 ⁻⁷	5 ⁻⁶	5 ⁻⁶
9	5 ⁻⁷	5 ⁻⁵	5 ⁻⁵
10	5 ⁻⁷	5 ^{-5.5}	5 ⁻⁶
4	5 ^{-6.5}	5 ⁻⁶	5 ⁻⁶
2	5 ⁻⁶	5 ⁻⁵	5 ⁻⁵
6	5 ⁻⁶	5 ⁻⁶	5 ⁻⁶
8	5 ⁻⁶	5 ⁻⁶	5 ⁻⁷
Median	5 ⁻⁷	5 ⁻⁶	5 ⁻⁶
Geometric mean	5 ⁻⁷	5 ⁻⁶	5 ^{-6.1}
Placebo			
6	5 ⁻⁹	NM	5 ⁻⁷
2	5 ⁻⁸	NM	5 ⁻⁵
3	5 ⁻⁸	NM	5 ⁻⁷
8	5 ⁻⁸	NM	5 ⁻⁶
11	5 ⁻⁸	NM	5 ⁻⁷
12	5 ⁻⁸	NM	5 ⁻⁸
4	5 ⁻⁷	NM	5 ⁻⁷
5	5 ⁻⁷	NM	5 ⁻⁷
7	5 ⁻⁷	NM	5 ⁻⁷
1	5 ⁻⁷	NM	5 ⁻⁷
10	5 ⁻⁷	NM	5 ⁻⁵
9	5 ⁻⁶	NM	5 ⁻⁶
Median	5 ^{-7.5}		5 ⁻⁷
Geometric mean	5 ^{-7.5}		5 ^{-6.6}

NM: Not measured.

that neither patient nor evaluating physician knew which patients were in the various treatment groups.

Treatment began about 2 mo prior to the onset of ragweed pollination with administration of 0.1 ml of the end-point dilution of extract (or placebo). Further treatment was given at weekly intervals, usually with consecutive doses increased by 0.05 ml. Since the optimal dose has been reported to be, on the average, 0.5 ml of the end-point dilution,^{37, 38} an attempt was made to reach this dose in the treatment program by the onset of the pollen season. The optimal dose was administered at weekly intervals during the ragweed pollination season. All patients received an optimal dose of ragweed pollen extract or placebo on, or as close as possible to, Friday, September 1, just before the

Labor Day weekend, the traditional peak of the ragweed pollen count and hay fever symptom scores. This allowed assessment of the ability of an optimal dose of ragweed pollen extract and placebo to relieve hay fever symptoms due to inhalation of ragweed pollen. Repeat determinations of the end-point dilution were usually done each week just prior to immunotherapy between August 2 and September 25 to pick up changes in end point due to pollen exposure, and the dose was adjusted so that patients received 0.5 ml of the new end-point dilution. During the course of treatment, redeterminations of end-point titration were accomplished with the patient's individual treatment solution (either active short ragweed pollen extract or placebo, as appropriate) so that placebo-treated patients did not receive ragweed extract during the period when the effects of short ragweed pollen extract and placebo were being compared. Treatment was continued until the end of ragweed pollination on October 3. The patients were provided with tablets containing chlorpheniramine 4 mg and tablets containing carbinoxamine maleate 4 mg and pseudoephedrine 60 mg (Rondec) for relief of hay fever symptoms not controlled by the subcutaneous injections, and instructed to take one tablet every 4 hr if necessary for relief of these symptoms.

Observations

Patients recorded symptoms and medication twice daily from mid-June to October 8 on a patient diary form.^{7, 14} Forms were reviewed by physician and patient weekly to ensure completeness.

A physician recorded symptoms and physical findings weekly during the ragweed season.

Ragweed pollen counts were made daily with a roto-slide sampler located on the grounds of the Good Samaritan Hospital, Baltimore, Maryland, and were recorded as pollen grains per square centimeter of roto-slide.⁴⁶

The following determinations were made at the onset of treatment (prior to the first injection), prior to the onset of ragweed pollination (August 7±), and at the end of ragweed pollination (Oct. 10±): serum levels of antiragweed IgE, antiragweed IgG, and total IgE. Skin end-point titrations with ragweed extract were done prior to onset of immunotherapy and in October. Leukocyte histamine release by ragweed pollen extract was measured at the start of the study and at the end of the ragweed season in October.

Analysis of data

Symptom and medication scores from patient diary forms were evaluated as described previously.^{7, 14} Daily symptom and medication scores and total symptom-medication scores were calculated for each patient. Average daily symptom-medication scores were calculated for each group for graphic display (Fig. 1) but nonparametric (rank-order) statistics were used throughout for analysis. The Mann-Whitney U test was employed to test for differences between treatment groups with reference to average daily symptom and medication scores and total symptom-medication scores.⁴⁵ The nonparametric Mann-Whitney U test was used also for test of differences in levels of antiragweed

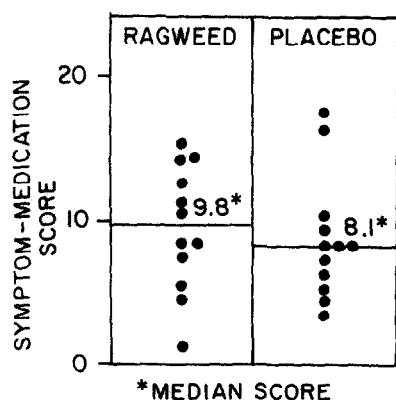


FIG. 2. Arithmetic mean of the daily symptom-medication scores between August 14 and October 8, 1978, for each patient in the groups indicated.

IgG, antiragweed IgE, total IgE, and in histamine release by ragweed in the two groups.⁴⁵

One of the phenomena regularly described by advocates of the Rinkel technique is that patients experience an amelioration of symptoms within a few hours after receiving an optimum dose, and that improvement is maintained for days or weeks after the dose. We therefore looked at each patient for differences between symptom-medication scores on the day before, the day of, and the day following injections during a 2-wk period of peak ragweed pollination between August 25 and September 8.

RESULTS

Matching

The group receiving ragweed pollen extract and that receiving placebo proved to be well matched with regard to leukocyte histamine release by ragweed, antiragweed IgG, antiragweed IgE, and total IgE levels, and skin-test end points with ragweed pollen extract (Table II, Figs. 3 to 6).

Dosage achieved

Ragweed-treatment group. Median ragweed pollen extract skin-test end-point dilutions determined during the ragweed pollen season in the 12 patients treated with ragweed were 5^{-8} for one patient, 5^{-7} for two, 5^{-6} for four, and 5^{-5} to $5^{-5.5}$ for five (Table II). Each week during the ragweed pollen season, each patient received an "optimal dose" which was close to or equal to 0.5 ml of the end-point dilution. The median "optimal dose" for these patients was 0.53 ml of the 5^{-6} dilution. The mean cumulative dose of ragweed pollen extract given during the study was $0.094 \mu\text{g}$ of AgE (range, 0.004 to $0.151 \mu\text{g}$).

Placebo-treatment group. Median placebo skin-test end-point dilutions determined during the ragweed pollen season in the 12 patients treated with placebo were 5^{-3} for every patient. For the purpose of blind-

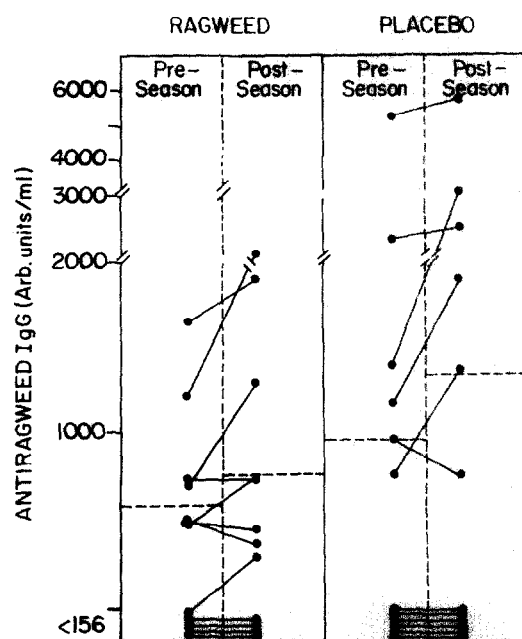


FIG. 3. Serum antiragweed IgG levels before and after ragweed pollen season of 12 patients who received ragweed pollen extract and 12 who received placebo by the Rinkel method. Dotted lines indicate the arithmetic mean levels, with values less than 156 considered equal to 156.

ing, 5^{-3} was labeled 5^{-7} and thus matched the 5^{-7} dilution of ragweed which was the most common end-point dilution before immunotherapy (Tables I and II). Each patient received an "optimal dose" which was close to or equal to 0.5 ml of the end-point dilution weekly during the ragweed season.

Symptoms

Symptom-medication scores rose and fell in both groups concurrently with the rise and fall of the pollen count (Fig. 1). There was no significant difference between the symptom-medication scores of the group receiving ragweed pollen extract and the group receiving placebo. A comparison of the differences in each patient between the symptom-medication scores on the day before injection (D0), the day of injection (D1), and the day after injection (D2) during a 2-wk period of peak ragweed pollination, August 25 to September 8, revealed no significant differences between the ragweed-treated group (symptom-medication scores: D0 - D1 = range -13 to +6, mean -0.1; D0 - D2 = range -8 to +18, mean +3.4; D1 - D2 = range -6 to +17, mean +3.1) and the placebo-treated group (symptom-medication scores: D0 - D1 = range -11 to +13, mean +0.88; D0 - D2 = range -7 to +15, mean +2.29; D1 - D2 = range -8 to +11, mean +1.42). All patients in both groups reported an increase in symptoms during the

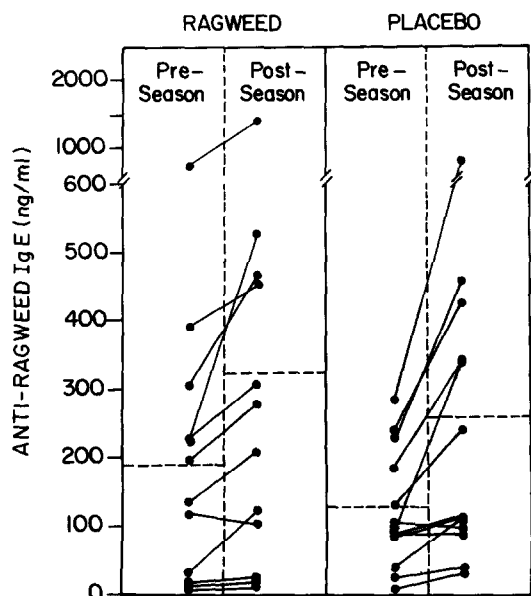


FIG. 4. Serum anti-ragweed IgE levels before and after ragweed pollen season of 12 patients who received ragweed pollen extract and 12 who received placebo by the Rinkel method. Dotted lines indicate the arithmetic mean levels.

ragweed pollination season and thus appeared to be clinically sensitive, but symptom-medication scores were widely variable among the patients (Fig. 2).

Immunologic variates

There were no significant differences in pre- or postseason immunologic variates between the ragweed- and placebo-treated groups. Pre- and post-seasonal anti-ragweed IgG levels were similar in both treatment groups (Fig. 3). The small seasonal rise occasionally seen in anti-ragweed IgG occurred in five patients in both groups. No detectable anti-ragweed IgG was found in 11 of the 24 patients before season and in 10 of the 24 after season. Anti-ragweed IgE levels were comparable in the two treatment groups (Fig. 4). The expected twofold seasonal rise occurred in both groups. Leukocyte histamine release by ragweed was similar in both treatment groups (Fig. 5). The leukocytes of one patient in the ragweed treatment group did not release histamine when tested after season. Total IgE levels were similar in the two treatment groups (Fig. 6). End-point dilutions with ragweed pollen extract were also similar in the two treatment groups (Table I).

Patients' appraisals of results of therapy

Notwithstanding the absence of demonstrated specific effect of ragweed pollen extract given by the Rinkel method on either symptom-medication scores

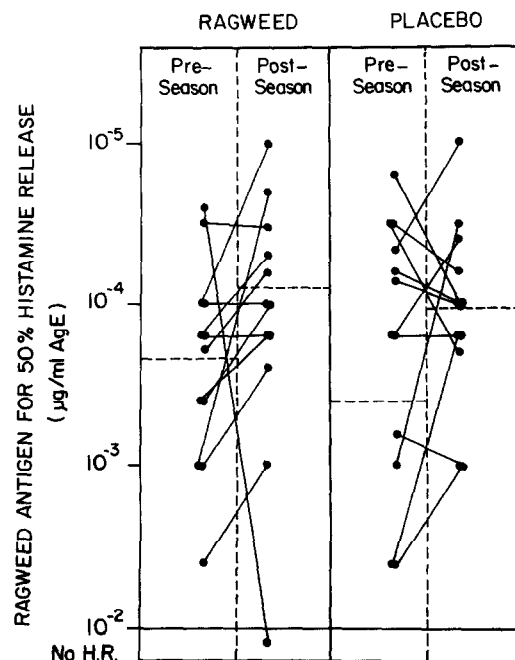


FIG. 5. Ragweed AgE content of ragweed pollen extract required for 50% leukocyte histamine release before and after ragweed pollen season in 12 patients who received ragweed pollen extract and 12 who received placebo by the Rinkel method. Dotted lines indicate the arithmetic mean levels.

or measurements of immunologic response, 10 of the 12 patients in both the ragweed- and placebo-treated groups were of the opinion that their symptoms of hay fever were less severe in 1978 than in 1977 and that they had been helped by the Rinkel method immunotherapy (Table III). Baltimore ragweed pollen counts in 1978 (peak day 781; cumulative August 10 to October 2, 13,002; cumulative August 24 to September 13, 8,271 pollen grains per square centimeter) were lower than those in 1977 (peak day 1,269; cumulative August 10 to October 2, 16,807; cumulative August 24 to September 13, 11,661 pollen grains per square centimeter of roto-slide).

Side effects

No systemic reactions were noted in any patient treated with either ragweed pollen extract or placebo. Local reactions were either absent or small and insignificant in all patients.

DISCUSSION

The purpose of this study was to compare the effects of "optimal doses" in the range of 0.5 ml of the end-point dilution of ragweed pollen extract with those of similar doses of the end-point dilution of an appropriate placebo. This dose was chosen because it

has been reported to be the average maintenance dose in patients treated by the Rinkel method. Rinkel³⁷ (p. 214) reported that "most patients will obtain their best relief with a 50× dose," which in his terminology is 50 times the 0.01-ml dose of extract used for the end-point skin test, or 0.5 ml of the end-point dilution. Willoughby³⁸ (p. 608) also stated that "the average maintenance dose for pollen is 0.5 ml" of "the end point dilution."

Treatment with these doses of ragweed pollen extract did not significantly reduce symptom-medication scores and did not affect measured immunologic variates, which included levels of antiragweed IgG and IgE, leukocyte histamine release by ragweed, total IgE levels, and ragweed pollen extract skin-test end-point dilutions as compared with placebo.

These results with the Rinkel method, in which, we employed a median maintenance dose of 0.006 μ g of AgE, differ from those obtained by this group with the current standard method of immunotherapy, in which we employed a maintenance dose of 2.7 to 22 μ g of AgE. When these higher doses were used, ragweed pollen extract was more effective than placebo for reducing hay fever symptom-medication scores^{2, 7-10, 12, 13} and produced striking changes in measured immunologic variates, which included increase in antiragweed IgG levels,^{7-10, 12, 14-17} decrease in the magnitude of the expected seasonal rise in antiragweed IgE levels,^{13, 16, 17, 25} and, in some patients, a decrease in ragweed-induced leukocyte histamine release.^{7-10, 13-17}

The doses of ragweed pollen extract employed in this study differ from those received by some patients who undergo Rinkel method immunotherapy. Willoughby³⁸ has reported that he employed the following end-point dilutions in 100 "selected" patients who received ragweed pollen extract by this method: 5⁻⁸, 2 patients (2%); 5⁻⁶, 3 (3%); 5⁻⁵, 5 (5%); 5⁻⁴, 16 (16%); 5⁻³, 15 (15%); 5⁻², 16 (16%); and 5⁻¹, 43 (43%). Had Willoughby's patients been given the ragweed pollen extract employed in the present study (which contained 187 μ g of AgE/ml in the concentrated solution), those who received 0.5 ml of the 5⁻¹ dilution would have received a maintenance dose of 18.7 μ g of AgE, and those who received 0.5 ml of the 5⁻² dilution would have received 3.7 μ g of AgE. Such doses are comparable to the 2.7 to 22 μ g maintenance doses which have been shown to reduce ragweed pollen induced symptom-medication scores and to produce immunologic responses when given by the current standard method.

The skin-test end-point dilutions in our patients were in the 5⁻⁹–5⁻⁵ range. We selected patients with high skin sensitivity because we have shown previ-

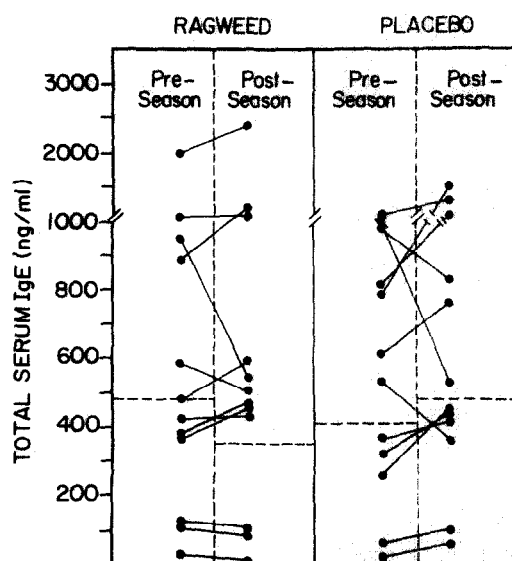


FIG. 6. Total serum IgE levels before and after ragweed pollen season of 12 patients who received ragweed pollen extract and 12 who received placebo by the Rinkel method. Dotted lines indicate geometric mean levels.

TABLE III. Patients' comparison of severity of hay fever symptoms in 1978 and 1977

	Less severe	Same	More severe
Ragweed	10	1	1
Placebo	10	2	0

ously that these patients have a good correlation between the rise and fall in symptom-medication scores and the rise and fall of ragweed pollen counts.⁴³ We purposely did not select patients with low skin sensitivity to ragweed pollen because we have shown previously that many such patients have a poor correlation between symptom-medication scores and ragweed pollen counts.⁴³

It is possible that a group of patients who have symptoms only in the ragweed pollen season and end-point dilutions in the 5⁻¹ to 5⁻² range can be recruited. If such patients are treated by this method, it will be important to prove in an appropriately controlled study that the placebo-treated patients with end-point dilutions in the 5⁻¹ to 5⁻² range have an increase in symptom-medication scores in the ragweed season such as was so clearly demonstrated by the placebo-treated patients in the present study.

The absence of systemic and significant local reactions to 0.1 to 0.5 ml of end-point dilution of ragweed pollen extract in this group of highly sensitive patients suggests that immunotherapy by either the Rinkel or current standard method can be begun safely with 0.1

ml of the end-point dilution and that there is no need to waste time and expense by beginning with a more dilute solution. Therefore, some form of titration may be useful prior to immunotherapy to determine the starting dilution.

In prior studies,^{7, 9, 14} it has been our custom to give an initial dose of 0.1 ml of a solution of ragweed pollen extract which is close to a 1:250,000 to 1:500,000 w/v dilution (dose was 0.1 to 0.2 PNU of a solution which contained either 27⁹ or 38 μg ^{7, 14} of AgE per 10,000 PNU). This has not resulted in systemic or significant local reactions. For this reason we think it likely that highly sensitive patients with a skin-test end-point dilution of 5⁻⁷ (1:1,562,500 w/v) or less (5⁻⁸, 5⁻⁹, and so on) can safely receive a starting dose that is higher than that predicted by the Rinkel method of titration. It should be noted that 19 of 24 patients in the present study had a skin-test end point of 5⁻⁷ or less at the start of the study (Table II).

It is important to emphasize that 10 of 12 patients in both the placebo- and the ragweed-treated groups felt that hay fever symptoms during the 1978 ragweed pollen season were less than those during the 1977 season and that they had been helped by immunotherapy. Baltimore ragweed pollen counts were lower in 1978 than in 1977, which may explain, at least in part, the improvement noted by both groups of patients. The improvement may also be due to the beneficial effects of therapeutic agents such as antihistamines and decongestants and to the well-recognized placebo effects derived from participation in a structured study, injection therapy, and desire to do well and please the physician. This clearly points out the need for proper design and controls in such studies.

The reduction in total IgE levels by Rinkel method immunotherapy with multiple antigens reported by Fadal¹⁴ was not found in this study. Total IgE levels were similar in both ragweed and placebo-treated groups before and after immunotherapy.

We plan to continue this work for another year in order to study larger numbers of patients. In this second year, a positive control will be included in the form of a third group of patients who will be receiving ragweed pollen extract by the current standard method in weekly doses which, in season, will equal or exceed the 2.7 to 22 μg of ragweed AgE shown in the past to produce favorable results.

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