

A comparison of immunotherapy schedules for injection treatment of ragweed pollen hay fever

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In 44 patients highly sensitive to ragweed, we compared weekly injections of single doses of ragweed extract (RW-Wk, 15 patients) with clustered doses of ragweed extract at 3-wk intervals (RW-Cl, 18 patients) and with placebo (11 patients) for effects on ragweed hay fever symptom-medication scores and immunologic variates. Patients were matched and randomly assigned to treatment groups. Ragweed doses were advanced to the highest tolerated dose. Doses and number of visits were lower in the RW-Cl group than in the RW-Wk group. Despite lower doses, systemic reactions were not reduced and antiragweed IgE levels increased significantly more in the RW-Cl group than those in the RW-Wk group. Both the RW-Cl and RW-Wk groups had significant increases in antiragweed IgG levels, decreases in seasonal rise in antiragweed IgE levels, and lower symptom-medication scores ($p < 0.01$) in comparison with the placebo group. We conclude that the RW-Cl regimen offered no important advantage over RW-Wk. Seventeen patients had previously received Rinkel-method immunotherapy with 0.5 ml of end-point dilution of ragweed extract for 1 to 2 yr without significant clinical improvement or immunologic changes. After adequate treatment with either RW-Wk or RW-Cl, these patients had significantly lower symptom-medication scores than those of the placebo group and immunologic changes similar to those of the entire active-treatment group. Therefore, treatment failures on Rinkel immunotherapy respond well to adequate dose immunotherapy by either schedule. (J ALLERGY CLIN IMMUNOL 69:181, 1982.)

A number of controlled studies have demonstrated that immunotherapy with a sufficient dose of potent ragweed pollen extract will relieve symptoms of ragweed pollen hay fever better than placebo.¹⁻¹⁵ The relief is specific for ragweed pollen and other allergies are not improved,^{3, 4, 13} and it is dose related.^{1, 2, 4,}

6-8, 10, 11, 13-15

Such immunotherapy will also produce measurable immunologic changes, including increase in specific serum antiragweed IgG antibody in most or all patients,^{6-9, 14-26} decrease in the rise of specific serum antiragweed IgE antibody that would be expected to occur in the ragweed season in most or all patients,^{12, 14, 15, 18, 19, 26, 27} decrease in in vitro leukocyte histamine release by ragweed pollen extract in some patients,^{6-9, 12, 14-19, 22-25} and increase in IgG and IgA antiragweed antibodies in nasal secretions.²⁸

In the studies discussed above, ragweed pollen extract was usually given at weekly intervals during the period in which the dose was being increased. Therapy was begun with a low dose that had been shown to be tolerated by the patient by both experience and skin testing. Dosage was progressively increased slowly thereafter according to the tolerance of the patient until a final maintenance dose containing 2.7 to 46.8 μ g of ragweed AgE was reached.

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Abbreviations used

RW-Wk:	Weekly ragweed immunotherapy schedule
RW-Cl:	Clustered ragweed immunotherapy schedule
HSA:	Human serum albumin

Results of a recent study of immunotherapy with Hymenoptera venoms by some members of this group indicate that for venoms, an alternative clustered schedule could be used safely and effectively to reach the final maintenance dose.²⁹ On the initial visit, three injections were given at half-hour intervals, followed by visits every 2 wk in which one injection was given with a rapid escalation of dose. In comparison with a weekly schedule, this clustered schedule provided equivalent protection against allergic reaction to a sting and somewhat greater antibody responses but required only about one third the number of visits and injections; it was also associated with fewer untoward reactions. The objective of the present study was to determine whether a clustered regimen could achieve similar results with ragweed immunotherapy.

This study served an additional purpose. We have previously demonstrated in patients highly sensitive to ragweed pollen that the Rinkel method of immunotherapy with 0.5 ml of end-point dilution of ragweed pollen extract was no more effective than placebo for providing relief of ragweed hay fever symptoms^{14, 15} and was much less effective than immunotherapy that employed a maintenance dose containing 3.7 to 46.8 μ g (median 11 μ g) of ragweed AgE.¹⁵ In the present study, patients who had formerly received Rinkel-method immunotherapy without significant favorable effect¹⁵ now received a sufficient maintenance dose (18.6 μ g of AgE or nearest tolerated dose) by either weekly or clustered schedules and had significant relief of symptoms and immunologic response.

MATERIALS AND METHODS

Patients

Patients were recruited from three sources: (1) 17 patients who received Rinkel-method immunotherapy with ragweed pollen extract in 1979,¹⁵ (2) 11 patients who received placebos in 1979,¹⁵ and (3) 16 new subjects recruited for study. Each patient had a history of seasonal rhinitis in late August and September for at least the two preceding years and a positive skin test to ragweed pollen extract at a concentration of 1:312,500 w/v or greater dilution and to ragweed AgE at a concentration of 0.01 μ g/ml or less. Eleven patients had had immunotherapy prior to the study, but none of these had had immunotherapy since 1974 (Table I). Patients with major concomitant rhinitis symptoms in the mold-dominated seasons in July, late October, and Novem-

ber were excluded. When possible, we recruited patients with negative skin tests to mold extracts (*Alternaria*, *Hor-moendrum*, *Helminthosporium*, *Botrytis*, *Aspergillus*, *Penicillium*, *Fusarium*, and *Mucor*) at a concentration of 1000 PNU/ml. Nineteen patients with positive skin tests to molds were included in the study (Table I). Few patients fulfilling all of these criteria were present in the Johns Hopkins Allergy Clinic or in the authors' allergy practices and most had to be recruited by news media releases. Initial evaluation of new prospective patients was done as described previously.¹⁴

Reagents

The short ragweed pollen allergenic extract and the histamine caramelized glucose placebo solution were the same as those used in the 1978 and 1979 studies.^{14, 15} For treatment, serial 10-fold dilutions of the ragweed pollen extract or of the placebo were employed as shown in Table II. For diagnosis, serial fivefold dilutions were employed as described previously.¹⁴ Dilutions were made with HSA saline (normal saline with 0.03% HSA, preserved with 0.4% phenol) to stabilize the ragweed pollen extract.^{30, 34}

Comprehensive initial evaluation

Patients meeting the criteria for entry into the study were evaluated by the methods previously described for hematocrit, white blood cell total and differential counts, total eosinophil counts, ragweed extract-induced in vitro leukocyte histamine release, serum levels of antiragweed IgE, total IgE, and antiragweed IgG, and skin-test end-point dilutions with ragweed pollen extract.¹⁴

Treatment

Subjects who received immunotherapy in 1979 by the Rinkel method with either very low doses of ragweed extract or with placebo were paired on the basis of cell sensitivity to ragweed extract as determined by in vitro leukocyte histamine release. One member of each pair was randomly assigned to the weekly treatment schedule with ragweed extract and the other to the clustered treatment schedule with ragweed extract. Newly recruited patients were ranked according to cell sensitivity to ragweed extract, and matched patients were randomly assigned to the treatment group as follows: one third to weekly treatment with placebo, one third to placebo therapy on a clustered dose schedule, one sixth to weekly treatment with ragweed extract (RW-Wk), and one sixth to a clustered dose schedule with ragweed extract (RW-Cl).

Treatment was given between February 25 and October 6, 1980. The schedules are shown in Table II. Group I (RW-Wk) consisted of 15 patients (seven from source 1, six from source 2, and two from source 3) who received weekly* single injections of ragweed pollen extract. Group II consisted of five patients from source 3 who received weekly* single injections of placebo. Group III (RW-Cl)

*After week 21, group I (RW-Wk) and group II (placebo) were treated every 1 to 3 weeks (Table 2).

TABLE I. Patient's characteristics at time of admission to the study

Characteristic	No. of patients	Treatment groups			
		Weekly groups		Clustered groups	
		Ragweed (I)	Placebo (II)	Ragweed (III)	Placebo (IV)
Total no. patients	44	15	5	18	6
Sex					
Male	30	11	4	13	2
Female	14	4	1	5	4
Age (yr)					
18-35	27	8	2	13	4
36-50	17	7	3	5	2
Mold skin test positive	19	7	2	8	2
Immunotherapy with ragweed before study	11	3(1969) (1949-55) (1958-59)	2(1969-71) (1969-73)	5(1963) (1967) (1966-74) (1967) (1971)	1(1941-46)
Immunotherapy with ragweed by Rinkel method (1978-1979 or 1979)	17	7(4,1978-9; 3,1979)		10(4,1978-9; 6,1979)	

consisted of 18 patients (10 from source 1, five from source 2, and three from source 3) who received clustered injections of ragweed pollen extract every 3 wk. Group IV consisted of six patients from source 3 who received clustered injections of placebo every 3 wk.

Patients were provided with tablets containing chlorpheniramine maleate (4 mg) or with tablets containing carbinoxamine maleate (4 mg) and pseudoephedrine (60 mg) (Rondec) for relief of hay fever symptoms not controlled by the subcutaneous injections and were instructed to take one tablet every 4 hr if necessary for relief of these symptoms.

Blinding

Personnel who conducted the study were divided into an evaluating team and an injecting team. The evaluating team interviewed and examined the patients, checked diaries, and monitored symptom-medication scores. They checked end-point titrations at suitable intervals. The evaluating team remained completely blinded to the treatment given to the newly recruited patients.

The injecting team injected the appropriate dose of ragweed pollen extract or placebo into each patient. They evaluated and treated any reactions to the injections. They were not blinded with reference to the treatment given to the patients and had no role in evaluating the results of therapy.

The newly recruited patients did not know what treatment they were receiving. The other patients knew that they were receiving ragweed pollen extract.

Observations

We obtained daily patient symptom-medication diaries from June 16 to October 6, physician record of symptoms

and physical findings at the time of each visit for injection (Table II) from February 25 to October 6, daily ragweed pollen counts from August 1 to October 12, serum levels of antiragweed IgG, antiragweed IgE, and total IgE every 3 wk from February 1 to October 8, and measurements of in vitro leukocyte histamine release by ragweed pollen extract before (October 1979 to February 1980) and after (October 1980) treatment with use of methods described previously.¹⁴

Analysis of data

Data were analyzed by use of nonparametric statistical methods for group comparisons as described previously,¹⁵ by means of the CLINFO computer system.

RESULTS

Placebo groups

For all variates under study, the two placebo groups (group II and group IV) did not differ. Therefore, for the purpose of contrast to the active treatment groups, these two placebo groups were combined and analyzed together. In Figs. 1 and 4 to 8, placebo group patients a to e were from group II and patients f to k were from group IV.

Matching of treatment groups

All three treatment groups (RW-Wk, RW-Cl, and placebo) proved to be well matched with reference to incidence of positive skin tests to molds (Table I), pretreatment in vitro histamine release by ragweed pollen extract (Fig. 1), pretreatment serum levels of antiragweed IgG (Fig. 5), antiragweed IgE (Fig. 6)

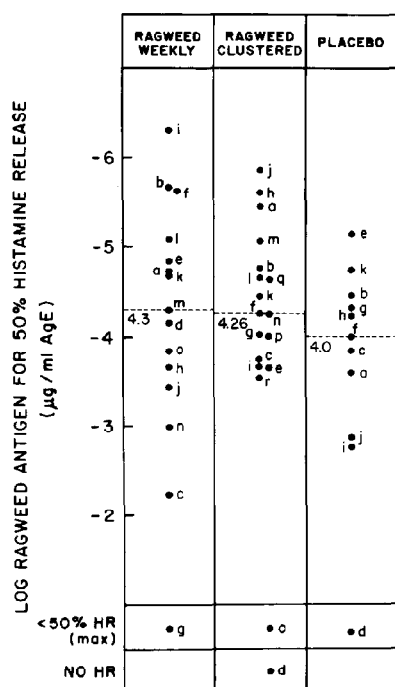


FIG. 1. Ragweed AgE content of ragweed pollen extract required for 50% leukocyte histamine release at entry into study. Dashed lines, Median measurements. Comparison of the values found in each of the three groups by Wilcoxon's non-paired rank sums test revealed no significant differences (RW-Wk vs RW-Cl, $p = 0.77$; RW-Wk vs placebo, $p = 0.38$; RW-Cl vs placebo, $p = 0.36$).

and total IgE (Fig. 7), and skin-test end-points with ragweed pollen extract (Fig. 8). When RW-Wk, RW-Cl, and placebo groups were compared by the Wilcoxon non-paired rank sum test, there were no statistically significant pretreatment differences among the groups with regard to any of these variables.

Dosage achieved, number of injections, and number of visits for injection (Fig. 2)

Only three patients in RW-Wk group and four in RW-Cl group reached the planned top dose of 0.1 ml of concentrated solution (18.7 µg of AgE), but all patients in RW-Wk group and all but three in the RW-Cl group reached a maximum dose that exceeded 1 µg of AgE. Maximum single doses were not significantly different between the two groups (RW-Wk group range 1.7 to 18.8 µg of AgE, median 9.4; RW-Cl group range 0.4 to 28.1 µg of AgE, median 4.7; $p > 0.75$). Cumulative doses were significantly

TABLE II. Schedules employed for ragweed immunotherapy

Week*	Weekly		Clustered	
	I. RW-Wk (n = 15)	II. Placebo (n = 5)	III. RW-Cl (n = 18)	IV. Placebo (n = 6)
Dilution	Dose (ml)	Dilution†	Dose (ml)	
1	1:10,000	0.1	1:10,000	0.1, 0.5, 0.9
2		0.2		
3		0.4		
4		0.8	1:1,000	0.2, 0.5
5	1:1,000	0.15		
6		0.3		
7		0.6	1:100	0.15, 0.4
8	1:100	0.1		
9		0.15		
10		0.25	1:10	0.1, 0.2
11		0.35		
12		0.5		
13		0.7		0.3, 0.3
14		Holiday		
15	1:10	0.1		
16		0.15		0.9
17		0.25		
18		0.35		
19		0.5	Conc.	0.1
20		0.7		
21	Conc.	0.1		

Conc. = concentrate.

*Doses were advanced as close as possible to the schedule shown or until the highest tolerated dose was achieved. After week 21, groups I and II were treated every 1 to 3 wk and groups III and IV approximately every 3 wk.

†Dilutions were made with HSA saline. Conc. ragweed extract was a 1:20 w/v extract containing 187 µg AgE/ml. Conc. placebo contained 0.275 mg histamine phosphate/ml.

higher in the RW-Wk group (RW-Wk group range 16.4 to 252 µg of AgE, median 70; RW-Cl group range 2.2 to 147 µg of AgE, median 17.5; $p < 0.01$). The median number of visits for injection per patient was 10 in the RW-Cl group (range eight to 11) and 27 in the RW-Wk group (range 18 to 30). The median number of injections per patient was 16 in the RW-Cl group (range 13 to 17) and 27 in the RW-Wk group (range 18 to 30).

Placebo group. Placebo-treated subjects reached a dose of 0.5 ml of 1:10 dilution of the concentrated placebo solution (0.014 mg of histamine phosphate). Higher doses were not given because they produced localized stinging at the site of injection.

Untoward reactions

The number of large local reactions (local reactions of 5 cm or greater diameter without systemic reaction)

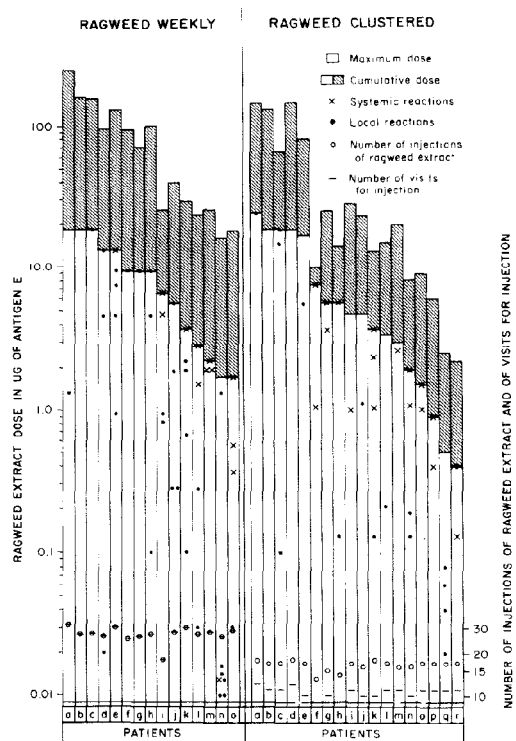
TABLE III. Total number of large local reactions without systemic reactions, systemic reactions, injections, and visits for injection for patients in RW-Wk and RW-CI groups

	RW-Wk	RW-CI
Large local reactions		
Subjects	15	18
Reactors	13	9
Reactions	33	15
Systemic reactions		
Subjects	15	18
Reactors	7	10
Reactions	13	19
Injections	405	298
Visits	405	181

per patient was about twice greater for the RW-Wk than for the RW-CI group ($p < 0.01$), but the large local reaction rates per visit and per injection were similar ($p > 0.3$) (Fig. 2 and Table III).

Systemic reactions occurred in both treatment groups (Fig. 2 and Table III). Some reactions (RW-Wk, nine in six patients; RW-CI, 15 in 10 patients) were of moderate severity and were treated with epinephrine. Others were milder (RW-Wk, three in two patients; RW-CI, two in two patients) and were treated with antihistamines. Still others were very mild (RW-Wk, one in one patient; RW-CI, two in two patients) and were not treated. The number of systemic reactions per patient was not reduced by the clustered regimen and was in fact slightly but not significantly greater in the clustered program than in the weekly program (RW-Wk, 13 reactions in 15 patients; RW-CI, 19 reactions in 18 patients; $p > 0.5$). Furthermore, the total number of reactors per group were also not significantly different (RW-Wk, seven of 15; RW-CI, 10 of 18; $p > 0.8$). The Mann-Whitney two-tailed U test revealed no significant ($p > 0.1$) difference between the doses of ragweed pollen extract ($\mu\text{g}/\text{ml}$ of AgE) at which systemic reactions occurred in the RW-Wk group and in the RW-CI group (Fig. 2). However, because the number of visits and injections in the RW-CI group was about one third to one half of those in the RW-Wk group, the rates of reactions per visit and injection were two or three times greater (RW-CI, 19 reactions in 181 visits = 0.099; RW-Wk, 13 reactions in 405 visits = 0.032; $p < 0.001$; RW-CI, 19 reactions in 298 injections = 0.064; RW-Wk, 13 reactions in 405 injections = 0.032, $p < 0.05$).

None of the placebo-treated patients had a large local or systemic reaction.

**FIG. 2.** Semi-logarithmic plot of the maximum single dose and cumulative dose of ragweed pollen extract given to each patient in the RW-Wk and the RW-CI groups between February 25 and October 6, 1980. Also plotted for each patient is the dose at which each large local reaction (local reaction of 5 cm or greater diameter without systemic reaction) and each systemic reaction occurred, the number of injections given, and the number of visits for injection.

Symptoms

Mean daily symptom-medication scores for each group (Fig. 3) were similar and low at the beginning and end of the ragweed pollination season. During the season, symptom-medication scores rose and fell in a pattern that for each group was correlated significantly ($p < 0.001$) with the ragweed pollen count. The mean daily symptom-medication scores of the patients in the RW-Wk and RW-CI groups were significantly lower ($p < 0.01$) than those of the placebo group; those of RW-Wk and RW-CI groups were not significantly different. The arithmetic means of the daily symptom-medication scores between August 11 and October 12, 1980, for each patient in each treatment group were widely variable (Fig. 4). The scores

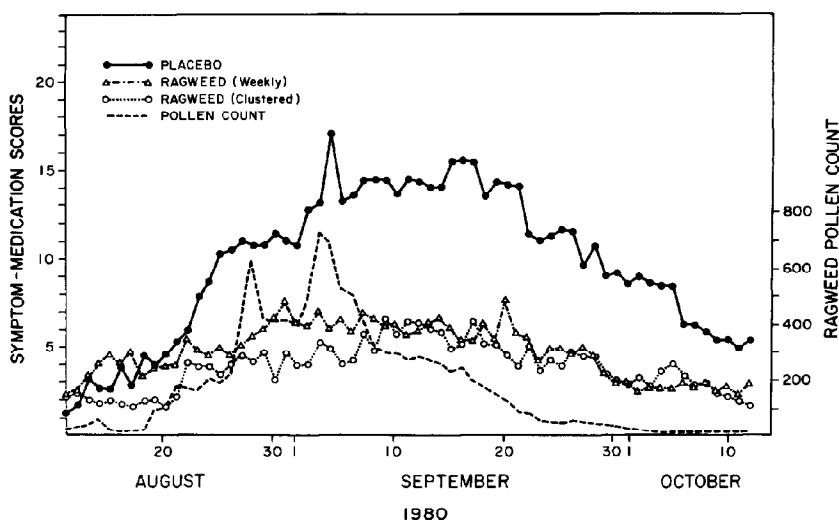


FIG. 3. Arithmetic mean of daily symptom-medication scores for the groups indicated compared with the pollen count reported as ragweed pollen grains per square centimeter of roto-slide. Rise and fall of symptom-medication scores were significantly correlated with rise and fall of ragweed pollen counts for the RW-Wk group (Spearman correlation coefficient, $r_s = 0.850$, $p < 0.001$), the RW-Cl group ($r_s = 0.664$, $p < 0.001$), and the placebo group ($r_s = 0.664$, $p < 0.001$).

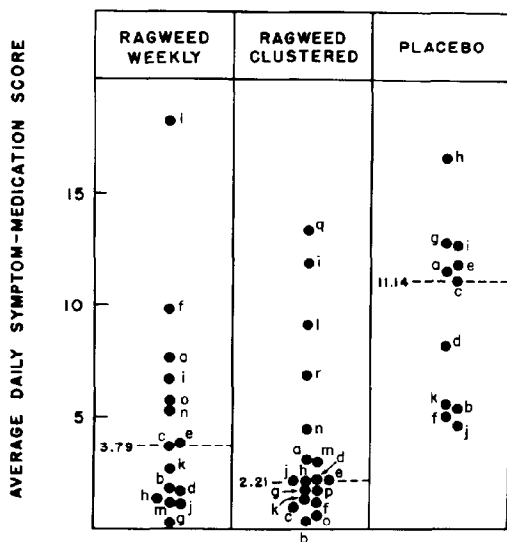


FIG. 4. Arithmetic means of the daily symptom-medication scores between August 11 and October 12, 1980, for each patient in the RW-Wk, RW-Cl, and placebo groups. Dashed lines, Median scores. Comparisons of scores by Mann-Whitney U test reveal that the scores of the RW-Wk and RW-Cl groups were significantly lower ($p = 0.01$) than the scores of the placebo group and that the scores of the RW-Wk and RW-Cl groups were not significantly different from each other ($p > 0.10$).

of the RW-Wk and RW-Cl groups were significantly lower than those in the placebo group ($p = 0.01$) but were not significantly different from each other ($p > 0.10$). There was no difference in the results when symptom scores instead of symptom-medication scores were used for the analysis.

Immunologic variates

Antiragweed IgG levels in serum are shown in Fig. 5. The levels of the RW-Wk group rose significantly from a median of <156 arbitrary units at entry into study to a median of 4601 before season to a median of 11,327 units after season. The levels of the RW-Cl group rose significantly from a median of <156 arbitrary units at entry to a median of 5307 units before season to a median of 8506 units after season. The change in levels of antiragweed IgG did not differ significantly between entry into study and before season in RW-Wk and RW-Cl groups, but the change from before season to after season was significantly greater ($p = 0.007$) in the RW-Wk than that in the RW-Cl group. Four patients in the RW-Cl group (patients o, p, q, and i) had levels of antiragweed IgG of <156 before season. They received relatively low single and cumulative doses of ragweed extract (Fig. 2) in comparison with the other actively treated patients. With injections continued through the season, each of these four patients had a significant rise in

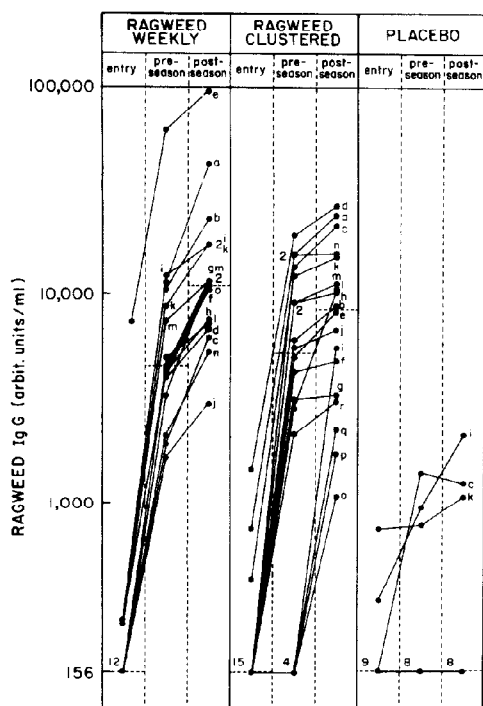


FIG. 5. Semi-logarithmic plot of serum antiragweed IgG levels in arbitrary units at entry into study (February 21 to March 12), before season (July 21 to August 6), and after season (October 10 to 27) for patients in the RW-Wk, RW-Cl, and placebo groups. Horizontal dashed lines, Median levels. Values less than 156 arbitrary units are considered equal to 156. RW-Wk group levels rose from a median of 156 arbitrary units at entry to 4601 before season and to 11,327 after season. RW-Cl levels rose from a median of 156 arbitrary units at entry to 5307 before season and to 8506 after season. Placebo group levels remained at a median of 156 arbitrary units for the duration of the study. Significance of differences in levels within and between groups at indicated times by Wilcoxon's non-paired rank sums test: RW-Wk entry vs RW-Wk before, $p < 0.001$; RW-Wk before vs RW-Wk after, $p < 0.001$; RW-Cl entry vs RW-Cl before, $p = 0.001$; RW-Cl before vs RW-Cl after, $p < 0.001$; placebo entry vs placebo before, $p > 0.3$ (N.S., not significant); placebo before vs placebo after, $p > 0.3$ (NS); difference between RW-Wk before and RW-Wk entry vs difference between RW-Cl before and RW-Cl entry, $p = 0.914$ (NS); difference between RW-Wk after and RW-Wk before vs difference between RW-Cl after and RW-Cl before, $p = 0.007$.

level by after season. The preseason and postseason antiragweed IgG levels of both RW-Wk and RW-Cl groups were significantly higher than the corresponding levels of the placebo group, which remained at a median level of <156 arbitrary units for the duration of the study. The small rise occasionally seen between

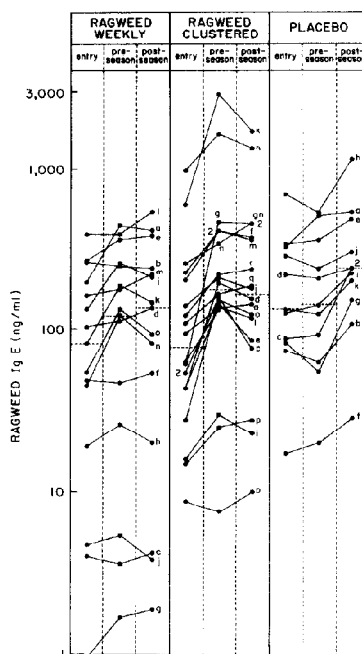


FIG. 6. Semi-logarithmic plot of serum antiragweed IgE levels (ng/ml) at entry into study (February 21 to March 12), before season (July 21 to August 6), and after season (October 10 to 27) for each patient in the RW-Wk, RW-Cl, and placebo groups. Horizontal dashed lines, Median levels. Levels of RW-Wk group rose from a median of 81 ng/ml at entry to 123 ng/ml before season and to 137 ng/ml after season. Significance of differences in levels at indicated times by Wilcoxon's non-paired rank sums test: entry vs before, $p = 0.008$; before vs after, $p > 0.3$ (NS). Levels of RW-Cl group rose from a median of 132 ng/ml at entry to 141 ng/ml before season and to 243 ng/ml after season. Significance of differences at indicated times: entry vs before, $p > 0.3$ (NS); before vs after, $p = 0.007$. The difference between the RW-Wk and RW-Cl groups with reference to the rise in serum levels between entry and before season is significant ($p = 0.015$).

preseason and postseason levels noted previously in some placebo-treated patients occurred in two patients in the placebo group. No detectable antiragweed IgG antibody (<156 arbitrary units) was found in nine patients before treatment and in eight after season in the placebo group.

Serum antiragweed IgE levels are shown in Fig. 6. The levels of the RW-Wk group rose significantly from a median of 81 ng/ml at entry to 123 ng/ml before season, and rose slightly but insignificantly to

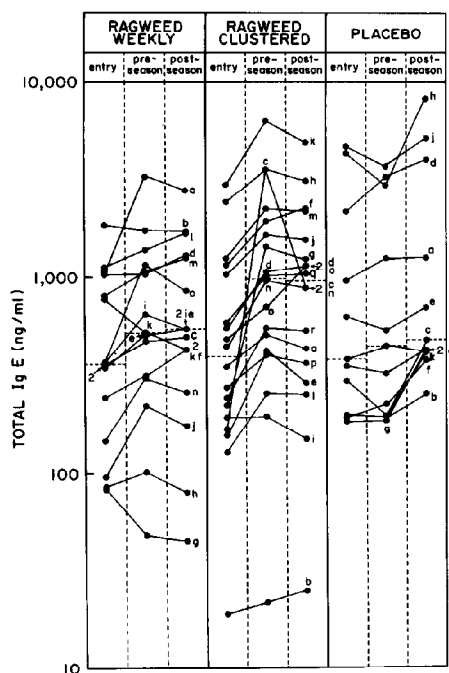


FIG. 7. Semi-logarithmic plot of total serum IgE levels (ng/ml) at entry into study (February 21 to March 12), before season (July 21 to August 6), and after season (October 10 to 27) for each patient in the RW-Wk, RW-Cl, and placebo groups. Horizontal dashed lines, Median levels. Levels of RW-Wk group rose from a median of 361 ng/ml at entry to 523 ng/ml before season and to 542 ng/ml after season. Significances of differences in levels at indicated times by Wilcoxon's non-paired rank sums test: entry vs before, $p = 0.035$; before vs after, $p > 0.3$ (NS). Levels of RW-Cl group rose from a median of 398 ng/ml at entry to 985 ng/ml before season and fell to 962 ng/ml after season. Significance of differences in levels at indicated times: entry vs before, $p < 0.001$; before vs after, $p = 0.16$ (NS). Median levels of placebo group rose from 387 ng/ml at entry to 450 ng/ml before season and to 487 ng/ml after season. Significance of differences at indicated times: entry vs before, $p > 0.3$ (NS); before vs after, $p = 0.017$. The difference between RW-Wk and RW-Cl with reference to the rise in serum levels between entry and before season is significant ($p = 0.018$).

137 ng/ml after season. The levels of the RW-Cl group rose significantly from a median of 80 ng/ml at entry to 176 ng/ml before season, and fell slightly and probably significantly ($p = 0.034$) to 166 ng/ml after season. The levels of the placebo group rose insignificantly from a median of 132 ng/ml at entry to 141 ng/ml before season, and rose significantly to 243 ng/ml after the season. The rise in ragweed IgE levels from entry to before season was significantly greater

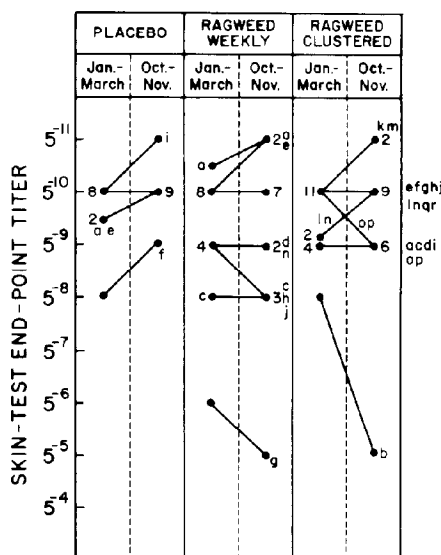


FIG. 8. Median skin-test end-point titer (end-point dilution) of ragweed pollen extract, January to March 1980 and October to November 1980, for each patient in RW-Wk, RW-Cl, and placebo groups. Measurements were made with ragweed pollen extract that had been diluted from freshly reconstituted lyophilized extract with HSA saline 1 to 30 days before test.

($p = 0.015$) in the RW-Cl group than that in the RW-Wk group.

Total IgE levels in serum for the patient groups are shown in Fig. 7. The levels of the RW-Wk group rose moderately and possibly significantly from a median of 361 ng/ml at entry to 523 ng/ml before season, and rose slightly but insignificantly to 542 ng/ml after season. The levels of the RW-Cl group rose significantly from a median of 398 ng/ml at entry to 985 ng/ml before season, and fell slightly but insignificantly to a median of 962 ng/ml after season. The levels of the placebo group behaved differently. They rose slightly but insignificantly from a median of 387 ng/ml at entry to 450 ng/ml before season and rose significantly to 487 ng/ml after season ($p = 0.017$). The rise in total IgE levels from entry to before season was significantly greater ($p = 0.018$) in the RW-Cl group than that in the RW-Wk group.

Median skin-test end-point titer (end-point dilutions) of ragweed pollen extract from January to March and October to November are shown in Fig. 8. These remained remarkably constant during this time. As might be expected, fivefold dilutions did not reflect the modest rise in antiragweed IgE (usually

less than twofold) observed during this period (Fig. 5). There were no significant differences between the end-point titers of the three groups from January to March and October to November and no significant change in titer in any group. One patient (patient b) in the RW-CI group had a decline in titer from 5^{-8} to 5^{-5} .

Effect of HSA diluent on skin-test end-points

In 1980, measurements of skin-test end-point titers were made with ragweed pollen extract that had been diluted with HSA saline from freshly reconstituted lyophilized extract 1 to 30 days before the test. The median end-point was the 5^{-10} dilution of ragweed extract in all groups at all times (Fig. 8). Dilutions of ragweed pollen extract were prepared with HSA saline and plain saline and stored at 4°C for 7 days (plain saline dilutions) or 7 to 28 days (HSA dilutions) before simultaneous measurements of skin-test end-point titer (Fig. 9). It is evident that the end-point with HSA diluent (median 5^{-10} , range 5^{-8} to 5^{-11}) was usually three logs to the base five (125-fold) more dilute than the end-point with saline diluent (median 5^{-7} , range 5^{-5} to 5^{-8}). Simultaneous measurements of end-points with serial dilutions of ragweed extract in HSA made 1 to 21 (median 7) days before the test and with serial dilutions of ragweed extract in HSA saline made 31 to 105 (median 93) days before the test produced the same results in 17 of 18 patients.

Patient appraisal of therapy

Most patients (RW-Wk, 14 of 15; RW-CI, 16 of 18; placebo, eight of 11) were of the opinion that their symptoms of hay fever were less severe in 1980 than they had been in the year before immunotherapy was started (Table IV). Ragweed pollen counts in Baltimore were slightly higher in 1980 than those in 1979 and somewhat less than those in 1978 and 1977 (Table V).

Response of patients in the RW-Wk and RW-CI groups who had received Rinkel-method immunotherapy in 1979

The subgroup of 17 patients in the RW-Wk group and RW-CI group who had received 0.5 ml of skin-test end-point dilution of ragweed pollen extract by the Rinkel method in 1978-79 or 1979 were RW-Wk patients a, b, e, g, h, i, and n, and RW-CI patients a, b, d, e, f, h, j, o, q, and r. This subgroup behaved like the RW-Wk and RW-CI groups and was indistinguishable from the remainder of the groups with regard to maximum single dose, cumulative dose, and local and systemic reactions (Fig. 2); symptom-

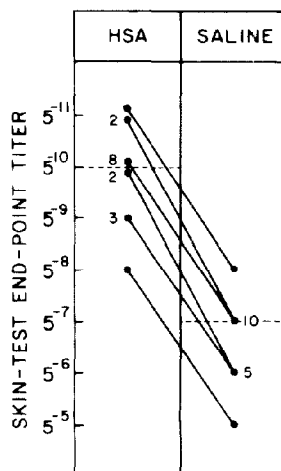


FIG. 9. Comparison at same sitting of skin-test end-point titers of ragweed pollen extract diluted with HSA saline and of ragweed pollen extract diluted with normal saline in 17 patients highly sensitive to ragweed. HSA saline dilutions were prepared from lyophilized concentrate 7 to 28 days before the test; normal saline dilutions were prepared 7 days before the test. Dashed lines, Median levels.

TABLE IV. Patient comparison of severity of ragweed hay fever symptoms in 1980 and in year before start of immunotherapy

	Less severe	Same	More severe
RW-Wk	14	1	0
RW-CI	16	1	1
Placebo weekly	4	1	0
Placebo clustered	4	1	1

medication scores (Figs. 4 and 10); antiragweed IgG levels (Fig. 5); antiragweed IgE levels (Fig. 6); and total IgE levels (Fig. 7). This subgroup also had significantly lower symptom-medication scores ($p < 0.001$) than those of the placebo group (Figs. 4 and 10); the median score of the ragweed subgroup was 2.3 and that for the placebo group was 11.14.

DISCUSSION

Several issues were addressed in this study in addition to the central problem of dosage schedule. As in our previous studies,^{14, 15} we have found that despite significantly more severe ragweed hay fever symptoms in the placebo group than in the treatment groups receiving an adequate dose of ragweed extract, most

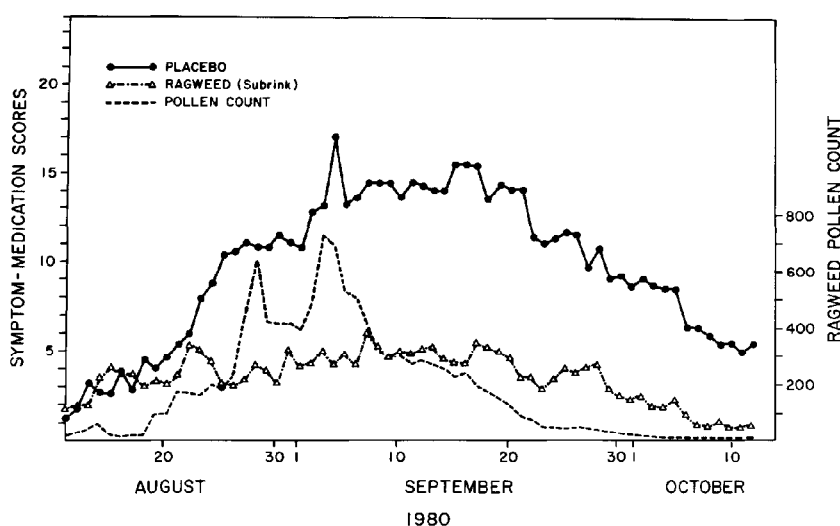


FIG. 10. Arithmetic mean of daily symptom-medication scores for the groups indicated, compared with the pollen count reported as ragweed pollen grains per square centimeter of roto-slide. Rise and fall of symptom-medication scores of patients in the ragweed subrink group (patients in either RW-Wk group or RW-Cl group in 1980 who had received ragweed immunotherapy by Rinkel method in 1978-79 or 1979) and in the placebo group were significantly correlated with rise and fall of ragweed pollen counts (Spearman correlation coefficient ragweed subrink group, $r_s = 0.740$, $p < 0.001$; placebo group, $r_s = 0.664$, $p < 0.001$). Symptom-medication scores of ragweed subrink group were significantly lower ($p < 0.01$) by Mann-Whitney U test than those of the placebo group.

TABLE V. Comparison of Baltimore ragweed pollen counts (pollen grains/cm² of roto-slide) in 1977, 1978, 1979, and 1980

Year	Maximum count	Date of maximum count	Cumulative count	
			Aug. 10-Oct. 2	Aug. 24-Sept. 13
1977	1,269	Sept. 7	16,807	11,661
1978	781	Aug. 29	13,002	8,271
1979	761	Sept. 4	9,340	7,299
1980	721	Sept. 3	10,413	8,273

patients in all treatment groups (RW-Wk, 14 of 15; RW-Cl, 16 of 18; placebo eight of 11) were of the opinion that hay fever symptoms in the 1980 ragweed season were less severe than those during the season before immunotherapy and that they had been helped by the immunotherapy (Table IV). Baltimore ragweed pollen counts in 1979 and 1980 were very similar and possibly slightly higher in 1980 than in 1979 (Table V) and do not explain the improvement described by all groups of patients. This improvement may have been due to the beneficial effects of therapeutic agents such as antihistamines and decongestants and to the well-recognized placebo effects derived from participating in a structured study, under-

going injection therapy, and desiring to do well and please the physician. This state of affairs clearly points out the need for proper design and controls in such studies.

We and others have previously demonstrated in matched groups of patients highly sensitive to ragweed that the Rinkel method of immunotherapy with 0.5 ml of end-point dilution of ragweed pollen extract was no more effective than placebo for providing relief of symptoms of ragweed hay fever and for producing increase in serum levels of antiragweed IgG and decrease in seasonal rise in levels of antiragweed IgE.^{14, 15, 32} The Rinkel method has been found to be much less effective for these purposes than immuno-

therapy that employed a maximum dose of ragweed extract containing 3.7 to 46.8 μg of AgE.¹⁵ In the present study, we have shown that 17 highly sensitive patients who did no better than matched placebo-treated patients when treated in 1978-79 or 1979 with 0.5 ml of end-point dilution¹⁵ did much better than matched placebo-treated patients with regards to both symptoms and specific IgG and IgE responses when given an adequate dose of ragweed pollen extract by either a clustered or weekly schedule. Another trial has produced essentially the same results.³³ These results show unequivocally that patients who do not respond to 1 or 2 yr of low-dose Rinkel-method immunotherapy have a good therapeutic and immunologic response when treated with an adequate dose.

The highly dilute ragweed pollen extract employed in this study for measurement of skin-test end-point titer was 125-fold more active when diluted with HSA saline than when diluted with saline. Furthermore, ragweed extract diluted with HSA saline retained essentially the same potency for this measurement for at least 90 days. These findings are similar to those previously reported by our group, which were derived by another method of skin-test end-point titration.³⁴ The stabilizing effect of the HSA on highly dilute ragweed extract has been attributed to reduction of absorption of AgE to glass and reduction of denaturation of protein at the liquid-to-air interface.³⁴

With regard to the main issue under study, our results agree in part with a recent study by some members of this group with Hymenoptera venoms²⁹ in which a clustered schedule provided equivalent relief of symptoms but required one third to one half the number of visits and injections. The two studies differ in that the clustered schedule was associated with fewer untoward reactions with venom than was the weekly schedule, whereas with ragweed extract, the clustered schedule was associated with slightly more systemic reactions per patient and twice the number per injection. The clustered schedules, however, were not identical for venom and ragweed; venom was given every 2 wk and ragweed every 3 wk. Three weeks was chosen for ragweed because studies of the kinetics of ragweed antibody response have indicated that most of the immunologic response to a single dose has occurred by this time (Norman PS, Marsh DG: unpublished data). It is possible that a shorter interval, perhaps 1 or 2 wk between clustered injections, might be superior.

In our ragweed extract-treated patients, systemic reactions limited dose in four RW-CI patients so that they entered the season with no detectable rise in IgG antibody. The 3-wk interval in the clustered regimen made it difficult to reach an immunologically ade-

quate dose in time for the season in patients who had large local or systemic reactions. There was not time to reduce doses and then raise them again before the onset of ragweed pollination. Nevertheless, continued injections during the season did raise IgG antibody levels in these patients once the dose was sufficient and the RW-CI group actually reported slightly less severe symptoms during the season than those in the RW-Wk group. The higher preseason and postseason specific IgG levels in the RW-Wk group are probably related to the significantly higher cumulative doses given to them. In contrast, in the RW-CI group, anti-ragweed IgE levels rose between entry into study and before season significantly more, a finding that could relate to the higher rate of systemic reactions.

Because half or more of the patients in both ragweed extract-treated groups had systemic reactions and many of these had more than one, we consider the rate of untoward reactions too high for either regimen to be used in daily practice with patients as sensitive as these. Yet, our results are consistent year after year and indicate that single maximum and cumulative doses on the order of those used here are necessary for optimum relief of hay fever symptoms. Achieving these doses without reactions in highly sensitive patients with unaltered ragweed extracts in the short time allowed by the schedules employed here appears to be impossible. Although it is possible that regimens of immunotherapy involving many more injections over a longer period will save patients from having systemic reactions before reaching an adequate dose of extract, this point has not been addressed by past controlled studies of immunotherapy. We and others, however, have provided evidence that a practical solution to this impasse may be provided by employment of depot products such as alum-precipitated extracts⁹ or altered extracts such as allergoids³⁵ or polymerized materials,³⁶ where in each case the ability of the extract to induce immediate allergic reactions is reduced and the ability to reduce symptom-medication scores and to induce IgG antibody responses is largely maintained.

If it were possible to identify those patients at risk of systemic reactions, a trial with a less aggressive regimen early in their course would be in order. Until now, we have not found pretreatment determinations of sensitivity by histamine release, skin test, or specific IgE antibody helpful in identifying patients likely to have reactions. The same was true many years ago with titrations of skin-sensitizing antibody. In another report, we plan to analyze data from several studies for the relationships between the various immunologic variates and the occurrence of systemic and local reactions.

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