

Allergen-controlled study of intranasal immunotherapy for ragweed hay fever

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Previous studies of intranasal immunotherapy have not included control groups treated with an irrelevant allergen. In the present double-blind study, we tested the effectiveness of intranasal immunotherapy in 20 patients sensitive to both short ragweed (SRW) and orchard grass (OG). Patients sprayed increasing concentrations of either SRW (n = 11) or OG (n = 9) extract intranasally six times per day for 8 wk before the SRW pollination season. The effects of this treatment were determined by analysis of symptom score diaries and clinical examinations during the SRW pollination season. SRW-treated patients received cumulative AgE doses from 3 to 59 μ g (mean 21); this mean dose was approximately sevenfold less than that used in a previous study from our laboratory. All patients reported immediate hay fever symptoms after use of the nasal spray. Five patients (four SRW- and one OG-treated) reported episodes of mild epistaxis during treatment; no other unexpected side effects were noted. During the treatment period, more SRW-treated patients showed signs of nasal obstruction and edematous nasal mucosa than OG-treated control patients ($p < 0.03$). During the SRW pollination season, the SRW-treated patients reported lower mean weekly symptom scores than the OG-treated control patients, but the difference was not statistically significant. Supplemental antihistamine use was significantly higher ($p < 0.016$) in the OG-treated control patients during the SRW pollination period. Subjective assessment of treatment efficacy by patients was similar in both treatment groups. We conclude that intranasal immunotherapy was of only marginal benefit in this study. (J ALLERGY CLIN IMMUNOL 71:454, 1983.)

Previous studies by our group¹ and by other investigators²⁻⁷ have shown that preseasonal intranasal administration of pollen allergen extracts significantly reduced hay fever symptoms in sensitive patients during the subsequent pollination season. In our previous study, we reported that preseasonal intranasal immunotherapy with SRW extract produced significant relief of seasonal ragweed hay fever symptoms when compared with histamine as a placebo.¹ However, we were concerned that the use of histamine placebo might not have been appropriate in that it did not completely mimic the effects of an allergen. None of

these previous intranasal immunotherapy studies included a control group treated with an unrelated antigen. Therefore we studied patients who were sensitive to two allergens, SRW and OG, and administered OG extract as the placebo in a double-blind study of nasal immunotherapy during the summer and fall of 1980.

MATERIALS AND METHODS

Allergen extract

The lyophilized SRW extract used in the 1978 study and described previously¹ was again used as the test allergen; this extract contains 3.86% AgE. A freeze-dried extract of OG (Lot L20-31) was obtained from Greer Laboratories, Inc., Lenoir, N. C. The SRW and OG extracts were reconstituted with Sorensen's buffer (0.0017M NaH_2PO_4 , 0.005M Na_2HPO_4 , 0.15M NaCl, pH 7.4) containing 0.5% human serum albumin and were used for both intranasal immunotherapy and diagnostic tests.

Patients

Twenty-three patients with clinical histories suggestive of sensitivity to both SRW and OG and one patient with seasonal allergic rhinitis to SRW only were selected for the

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Abbreviations used

SRW:	Short ragweed
OG:	Orchard grass
RAST:	Radioallergosorbent test
PNU:	Protein nitrogen unit

15 wk study, which extended from June 9 through September 22, 1980. Because of some difficulty with patient recruitment, the present study began approximately 4 wk later than our previous study.¹ Each patient was in good general health and was not receiving allergen immunotherapy. Sera from all patients showed an elevated preseasonal IgE antibody level to SRW (greater than 20 times that of control sera from nonsensitive patients), and 23 of 24 sera showed elevated IgE antibody levels to OG (greater than twice the binding given by sera of nonsensitive patients) by means of RAST. Endpoint skin-test titrations and nasal provocation tests were performed as described previously.¹ All patients had positive intradermal skin tests (0.04 to 25.6 ng/ml, total solids) and nasal provocation tests (640 to 400,000 ng/ml, total solids) to SRW. The 23 OG-sensitive patients had positive intradermal skin tests (0.4 to 20 PNU/ml) to OG; however, four of these patients failed to show a positive nasal challenge to OG at a maximum concentration of 500 PNU/ml.

IgE antibody measurement

Baseline serum samples (May 19 to 30) were obtained from each patient for quantitative measurement of IgE antibody levels to both SRW and OG by means of a calibrated mini-RAST procedure as described previously.^{8, 9} The SRW assay included a standard curve from a serum pool of sera from four untreated SRW-sensitive patients, which contained 2370 ng of IgE antibody to SRW/ml, while the OG assay included a standard curve from a serum pool of sera from five untreated grass-sensitive patients, which contained an arbitrary 10,000 units of IgE antibody to grass allergens/ml. The correlation coefficients for the regression lines of both the SRW and OG standard curves were +0.99. Subsequent serum samples, obtained on June 30, August 11, and October 20, were tested for IgE antibody in a similar manner.

IgG antibody measurement

Sera were tested for the presence of IgG antibody to SRW by the *Staphylococcus aureus* protein A assay.¹⁰ Sera were diluted 1:10 in RAST diluent containing 0.2% bovine serum albumin and were added to solid-phase microcrystalline cellulose coupled to SRW extract suspended in RAST diluent. ¹²⁵I-*Staphylococcus* protein A (50 µg) was added as the second-stage reagent. The results were expressed as percent counts bound of IgG antibody.

Pollen count

Ragweed pollen counts were monitored throughout the study period with a roto-slide sampler.¹¹ The sampler was

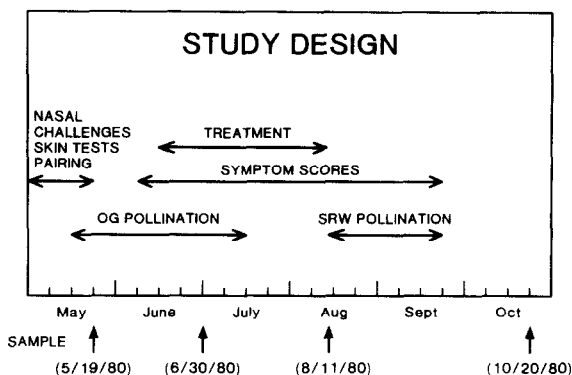


FIG. 1. Design of the study. Dates when sera were obtained for measurement of IgE antibody levels are shown by the vertical arrows.

located in a more rural area than previously¹ and was placed 6 ft above ground level.

Statistical analyses

Differences between the study groups were tested either by the Mann-Whitney rank sum test, Wilcoxon signed-rank test, Spearman rank correlation test, or chi-square analysis¹² with a programmable Hewlett-Packard 9810 calculator or a Hewlett-Packard 9845A computer. One-tailed tests of significance were used.

Conduct of the study

Fig. 1 shows the study design. Clinical histories, skin tests, nasal provocation tests, and preseasonal serum IgE antibody measurements to both SRW and OG were obtained from each patient before the study. The data were reviewed by P. W. W. and J. W. Y., who paired patients according to IgE antibody levels to SRW and randomly assigned patients to the SRW or OG group. Patients were seen thereafter by J. H. B., who had had no prior contact with the patients, thus maintaining the double-blind protocol. The clinical characteristics of the study groups are shown in Table I; no significant differences between the groups were seen.

Test solutions consisted of either crude SRW extract (0.2 to 125 µg/ml by weight) diluted in 0.15M NaCl containing 0.5% human serum albumin with benzalkonium chloride (0.2 mg/ml) as a preservative, or OG extract (4 to 2500 PNU/ml) diluted in the same manner. Test solutions were dispensed in low-density polyethylene squeeze bottles and stored at 4° C until used. Treatment consisted of one spray in each nostril six times per day. Compliance was assessed by subtracting the mass of the bottles returned from the original mass of the bottles as dispensed. Five random test solutions were cultured for bacteria before being dispensed and were sterile; in addition, ten random test solutions returned by the patients were cultured for bacteria and were sterile.

All patients kept daily symptom diaries beginning June 9. Symptoms of runny nose, stuffy nose, and eye irritation were expressed on a semiquantitative scale from 0 to 4 (0 = none, 1 = mild, 2 = moderate, 3 = severe, 4 =

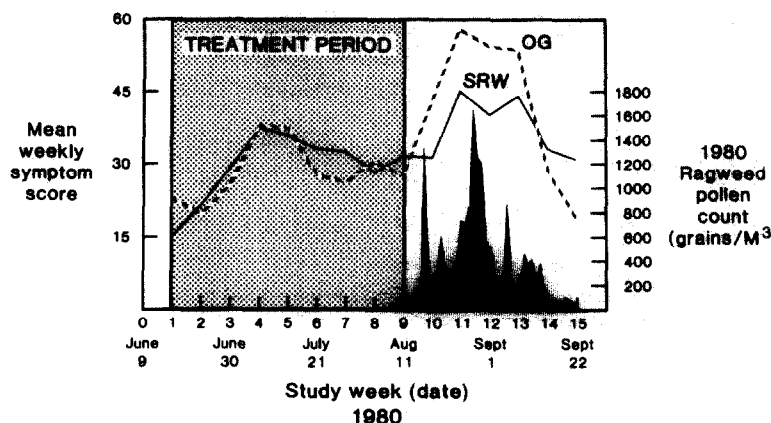


FIG. 2. Mean weekly symptom scores in the SRW- and OG-treated groups; the daily ragweed pollen counts are shown by the dark area. The treatment period is shown by the shaded area (June 16 to August 10). Mean weekly symptom scores were calculated by summing the individual symptom scores from each patient for each week (week 1 = week 0 to 1, etc.) and dividing by the number of patients within that group.

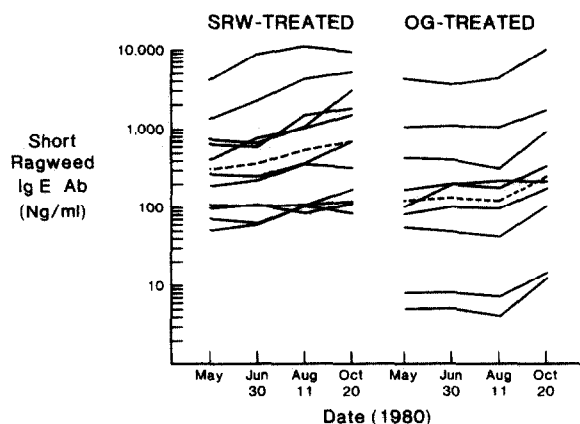


FIG. 3. Changes in serum IgE antibody to SRW from May to October 1980 in SRW- and OG-treated patients. *Dashed line*, Mean IgE antibody levels. The OG pollination extended from May 15 to July 15 and the SRW pollination from August 10 to September 20.

very severe). The actual number of sneezing episodes was recorded. The presence of throat irritation, breathlessness, wheezing, cough, and common cold symptoms were scored as + or 0. Supplemental antihistamine tablets (chlorpheniramine maleate, 4 mg) were provided, and the use of these and any other supplemental medications was recorded; all scores were recorded once a day. Prior to receiving test solutions, patients kept symptom scores for 1 wk to determine baseline symptom levels. At the beginning of week 2 (June 16), SRW-treated patients and OG-treated control patients received bottles containing SRW or OG solutions in individual graded doses, with the starting concentration approximately 10-fold less than that needed to provoke symptoms during the pretrial nasal challenge testing. The four patients without a positive nasal challenge to OG received the SRW test solutions. After week 2, SRW and OG

doses were increased to maximally tolerated levels based on symptoms experienced. Treatment continued six times daily for 8 wk until the beginning of the SRW pollination season (August 11). Patients returned once every 2 wk during the study to exchange diary cards and test solutions (where applicable) and for follow-up examination to assess the severity of induced symptoms. At the end of the study period (September 22) patients were questioned regarding their subjective evaluation of the treatment and any side effects experienced.

RESULTS

Compliance of patients

One patient assigned to the OG group voluntarily withdrew from the study for personal reasons after baseline symptoms were recorded. Two patients receiving the OG preparation were dismissed from the study after 6 wk because of failure to keep scheduled appointments. Data from these three patients were omitted from further analyses. Data from one SRW-treated patient were also omitted from the analyses because of the patient's use of intranasal beclomethasone throughout the SRW pollination season.

Compliance was similar in both groups. SRW- and OG-treated patients used an average of 38 and 35 ml of solution during the treatment period, respectively; this difference was not significant. Patients treated with SRW received from 3 to 59 μ g (mean 21) of AgE during therapy; the OG-treated control patients received from 5810 to 20,024 PNU (mean 12,334) during treatment.

Patient evaluation

Fig. 2 shows the mean weekly total symptom scores for both groups and the 1980 pollen counts.

TABLE I. Study groups: clinical characteristics

	Treatment	
	SRW	OG
Patients (No.)	12	12
Age (yr)		
Range	13-47	15-43
Arithmetic mean	22	25
Sex (M/F)	6/6	6/6
Duration hay fever (yr)		
Range	5-45	5-30
Arithmetic mean	14	17
Symptom severity*		
Range	11-29	8-25
Geometric mean	19	17
Preseasonal IgE antibody to SRW (ng/ml)		
Range	36-1891	3-1177
Geometric mean	161	82

*These numbers were generated by summing the semiquantitative values (0 = none, 1 = mild, 2 = moderate, 3 = severe, 4 = very severe) for the nine symptoms (itchy nose, runny nose, stuffy nose, itchy eyes, itchy throat, sneezing, nose blowing, wheezing, and coughing) according to the severity of symptoms recollected by the patients from previous SRW pollination seasons.

Both groups reported symptom scores that were similar during the treatment period. During the SRW pollination season the SRW-treated group had lower mean weekly symptom scores than the OG-treated group; however, these differences were not statistically significant ($p = 0.1$). Analysis of symptom scores by day rather than by week did not affect statistical results, nor did the symptom scores in the two groups differ significantly when only the peak pollen season (August 25 to September 1) was considered. When hay fever symptoms were analyzed individually, the SRW-treated group reported milder symptoms of runny nose, stuffy nose, eye irritation, and fewer sneezing episodes during the SRW pollination season and during the peak pollination period; again, these differences were not significant. The SRW-treated group used significantly fewer supplemental medication tablets (294 vs 421, $p < 0.016$) than the OG-treated group. The presence of throat irritation, breathlessness, wheezing, cough, and common cold symptoms were similar in each group, both during and after treatment.

Two OG-treated control patients had lower pre-seasonal levels of IgE antibody to SRW (< 10 ng/ml) than the remainder of the patients. If these patients are eliminated from consideration, the remainder of the patients in the SRW-treated group still did not differ from the OG placebo group except at week 12, when

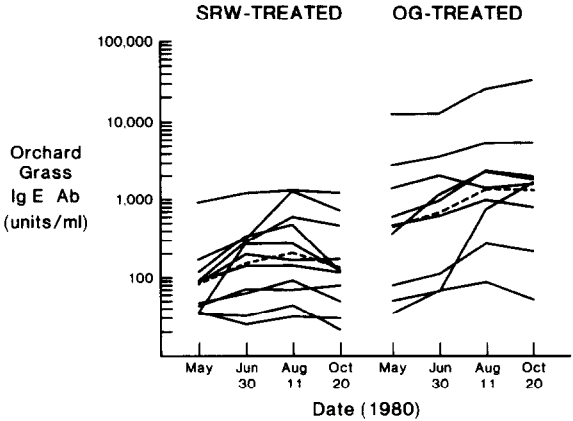


FIG. 4. Changes in serum IgE antibody to OG from May to October 1980 in SRW- and OG-treated patients. Dashed line, Mean IgE antibody levels.

the symptoms of the treated group differed only at the $p < 0.05$ level.

Biweekly examination findings are shown in Table II. During the treatment period, SRW-treated patients showed more signs of nasal obstruction and edematous nasal mucosa upon examination than seen in the OG-treated control patients ($p < 0.03$). However, during the SRW pollination season, both groups showed similar examination results. Five patients (four SRW-treated, one OG-treated) reported episodes of intermittent mild epistaxis only during the treatment period.

Table III shows the patients' assessments of treatment. During the treatment period, all patients experienced hay fever symptoms from their test sprays, lasting from 5 min to all day, with the mean duration similar in each group (geometric mean, 1.0 hr for the SRW-treated patients and 0.5 hr for the OG-treated control patients). Eight SRW-treated patients and six OG-treated control patients reported a reduction in hay fever symptoms and an overall benefit of therapy during this study; this difference was not significant by chi-square analysis. Ten of 11 SRW-treated patients and six of nine OG-treated control patients thought they had used the SRW test solutions. Fourteen patients (nine SRW and five OG) stated they would use this form of therapy again; those who would not claimed the symptoms suffered pre-seasonally were not tolerable.

Changes in IgE and IgG antibodies

Fig. 3 shows changes in SRW IgE antibody levels. During the treatment period (June 16 to August 11), nine of 11 SRW-treated patients showed an increase in their IgE antibody levels to SRW, while only three of nine OG-treated patients had a slight increase in

TABLE II. Biweekly examination results in both study groups

Physical findings	Frequency*			
	Treatment period (June 16-August 11)		Pollination period (August 11-September 22)	
	SRW-treated	OG-treated	SRW-treated	OG-treated
Nasal obstruction (p/c)†	73	55	73	77
Edematous nasal mucosa	82	33	100	77
Excess nasal secretions	27	11	64	66
Excoriated external nares	18	11	9	22
Inflamed conjunctiva	64	66	64	88
Injection/pharyngeal mucosa	9	0	0	0
Uvular/tonsillar edema	0	0	36	44
Epistaxis	27	11	0	0

*Data expressed as the percent of patients showing this abnormality at least once during the study.

†Partial/complete blockage by inferior turbinate upon nasal examination.

TABLE III. Assessment of treatment by each study group

	Treatment	
	SRW	OG
Severity of symptoms*		
Much less severe	3	2
Slightly less severe	5	4
About the same	2	0
Slightly more severe	1	3
Much more severe	0	0
Benefit of therapy		
Very good	3	2
Good	5	3
Slight	1	1
None	2	3

*Recollection of previous pollen seasons.

IgE antibody to SRW; this difference was significant ($p < 0.0025$). Three OG-treated control patients showed a slight rise in IgE antibody to ragweed; the rises were slight and most likely reflected variance in the radioimmunoassay for IgE antibodies. The OG-treated control patients showed the expected increase in IgE antibody levels to SRW during and after the SRW pollination season (August 11 to October 20), while the increases in the SRW-treated group were less marked ($p < 0.004$).

Fig. 4 shows changes in OG IgE antibody levels. All patients sensitive to OG showed an increase in their IgE antibody levels to OG during or after the OG pollination season (May 15 to July 15); the OG-treated control patients tended to show a greater rise in IgE antibody to OG during this period ($p = 0.07$).

Six of 11 SRW-treated patients and three of nine OG-treated control patients showed minimal rises in

IgG antibodies to SRW during the treatment period (< 5 times baseline levels); this difference was not statistically significant.

DISCUSSION

The findings in the present study differ in several respects from those of our previous study, although a similar study design was used. First, we were unable to show a significant reduction in hay fever symptoms in the present study. Perhaps the fewer subjects enrolled in the present study or the lower amount of AgE used by SRW-treated patients during the treatment period were key elements. In the earlier study, SRW-treated patients used from 6 to 694 μg of AgE (mean 151) during 12 wk of treatment; SRW-treated patients in this study used only from 3 to 59 μg of AgE (mean 21) during an 8 wk treatment period, a sevenfold decrease in the mean dose received. Because dosages were pushed to the maximum tolerated in both studies, the additional 4 wk of treatment in our first study accounted for the difference in the mean AgE dose received. We were unable to correlate the quantity of AgE received to the degree of clinical symptoms experienced in either of our studies.

Connell¹³ has shown in one patient that daily intranasal challenge with an unrelated allergen may "prime" the nasal mucosa nonspecifically. If this were the case in the present study, one would expect increased symptoms due to SRW pollen inhalation in both the OG and SRW-treated groups. On the contrary, the majority of patients in both treatment groups felt that they did not experience more symptoms during this SRW pollination season as compared with the previous one. Another possible explanation for the lack of significant benefit would be deterioration of the potency of the extract during the 2 wk period

when the spray was carried at ambient temperature. This possibility is unlikely because all patients continued to have reactions from the spray during the entire treatment period.

Second, we noted some differences in the immunologic results between the studies; the lower doses of AgE delivered in this study may have influenced the immunologic findings. In this study, the SRW-treated group had a significant increase in mean IgE antibody levels to SRW during the treatment period ($p < 0.0025$), and the rises during the SRW pollination season in this group were dampened. Similar immunologic results were seen by Nickelsen et al.¹⁴ in their crude-SRW-treated group. In our earlier study, we did not see this preseasonal immunologic response. The reasons for these differences are unclear. As expected, patients treated with OG showed no mean change in their IgE antibody levels to SRW during the treatment period and they exhibited the expected rise in SRW IgE antibody during the SRW pollination season. There was a tendency for OG-treated control patients to show greater rises in IgE antibody levels to OG during the treatment period, but this difference was not significant. No significant production of serum IgG antibody to SRW was seen in either group.

Side effects in this study were minimal. Clinical examinations were similar in both groups during the SRW pollination season; however, more signs of nasal obstruction and edematous nasal mucosa were seen in the SRW-treated patients during the treatment period. Immediate hay fever symptoms were reported by all patients after use of either treatment solution. In fact, six of nine OG-treated control patients thought that they were using SRW solutions, suggesting that the allergen placebo was effective.

We believe that OG extract in this study is a better placebo than the histamine solution used in the previous study. Symptoms provoked by OG were similar to those provoked by SRW in this study, whereas the symptoms provoked by histamine in the previous study were more immediate and transitory than those provoked by the SRW. Furthermore, it should be obvious that an appropriate control for an allergen treatment is an allergen placebo, a point appreciated by Lowell and Franklin¹⁵ in their study of parenteral immunotherapy. Complex changes that could occur in the nose as a consequence of administration of an allergen, particularly the recruitment of cells into the nasal tissues, and possible changes in sensitivity not only to histamine but also to other mediators ultimately demand that the placebo be of the same class of materials as the test substance. Although the OG spray was administered in part during the OG pollination season, the symptoms reported by the OG-treated

control group during the treatment period were primarily due to the OG test spray and not to airborne grass pollen (Fig. 1); the peak OG pollination period is from June 10 to 20, and in Rochester, Minn., 71% of grass pollen is released before July,¹⁶ whereas the peak symptom scores in the OG subjects were reported from June 30 to July 21. Furthermore, the effect of the OG placebo paralleled chronologically the ragweed treatment. Finally, one could argue that the occurrence of grass pollen symptoms due to the presence of grass pollen in the air alters the nasal cavity in such a manner as to negate a positive treatment effect. Clearly, one cannot rule out such a possibility. However, we believe our results have value even in the face of this possibility because many patients are allergic to both grass and ragweed pollens. If a coexisting pollen sensitivity negates a therapeutic effect of ragweed intranasal immunotherapy, then all patients who are contacting other pollens to which they are allergic would not benefit from this form of treatment. This would include not only patients with ragweed and grass sensitivity but also those with allergy to *Alternaria* or animal danders, who for one reason or another continue to be exposed to these allergens.

We believe that the value of intranasal immunotherapy with crude SRW may provide some marginal relief of hay fever symptoms during the SRW pollination season. However, the usefulness of the treatment is questionable, keeping in mind that hay fever symptoms are provoked during the treatment period. The use of a chemically modified allergen to reduce allergenicity during the treatment period may make nasal immunotherapy more tolerable; this approach has been tested by other investigators with varying results.^{7, 17-19}

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REFERENCES

1. Welsh PW, Zimmermann EM, Yunginger JW, Kern EB, Gleich GJ: Preseasonal intranasal immunotherapy with nebulized short ragweed extract. *J ALLERGY CLIN IMMUNOL* 67: 237, 1981.
2. Swineford O Jr, Cumbia JW, Berger KW, Lucas D: Treatment of pollen hay fever and asthma with aerosols of pollen extracts. *J ALLERGY* 26:305, 1955.
3. Taylor G, Shivalkar PR: Local nasal desensitization in allergic rhinitis. *Clin Allergy* 2:125, 1972.
4. Cook N: Pre-seasonal local nasal desensitization in hay fever. *J Laryngol Otol* 88:1169, 1974.
5. Mehta SA, Smith JM: Nasal hyposensitization and hay fever. *Clin Allergy* 5:279, 1975.
6. Guercio JP, Birch S, Fernandez RJ, Sackner MA: Deposition of ragweed pollen and extract on nasal mucosa of patients with

- allergic rhinitis: effect on nasal airflow resistance and nasal mucus velocity. *J ALLERGY CLIN IMMUNOL* **66**:61, 1980.
7. Nickelsen JA, Goldstein S, Mueller U, Wypych J, Reisman RE, Arbesman CE: Local intranasal immunotherapy for ragweed allergic rhinitis. I. Clinical response. *J ALLERGY CLIN IMMUNOL* **68**:33, 1981.
 8. Gleich GJ, Jacob GL, Yunginger JW, Henderson LL: Measurement of the absolute levels of IgE antibodies in patients with ragweed hay fever. *J ALLERGY CLIN IMMUNOL* **60**:188, 1977.
 9. Gleich GJ, Adolphson CR, Yunginger JW: The mini-RAST: comparison with other varieties of the radioallergosorbent test for the measurement of immunoglobulin E antibodies. *J ALLERGY CLIN IMMUNOL* **65**:20, 1980.
 10. Hamilton RG, Sobotka AK, Adkinson NF Jr: Solid-phase radioimmunoassay for quantitation of antigen-specific IgG in human sera with ¹²⁵I protein A from *Staphylococcus aureus*. *J Immunol* **122**:1073, 1979.
 11. Ogden EC, Raynor GC: A new sampler for airborne pollen: the roto-slide. *J ALLERGY* **40**:1, 1967.
 12. Dixon WS, Massey FJ Jr: Introduction to statistical analysis, ed. 3. New York, 1969, McGraw-Hill Book Co, Inc., pp. 341-345.
 13. Connell JT: Quantitative intranasal pollen challenges. III. The priming effect in allergic rhinitis. *J ALLERGY* **43**:33, 1969.
 14. Nickelsen JA, Goldstein S, Mueller U, Wypych J, Reisman RE, Arbesman CE: Local intranasal immunotherapy for ragweed allergic rhinitis. II. Immunologic response. *J ALLERGY CLIN IMMUNOL* **68**:41, 1981.
 15. Lowell FC, Franklin W: A double-blind study of the effectiveness and specificity of injection therapy in ragweed hay fever. *N Engl J Med* **273**:675, 1965.
 16. Karau M: Statistical Report of the Pollen and Mold Committee of the American Academy of Allergy. 1970 (booklet).
 17. Deusch H, Johansson SGO: Hyposensitization of patients with allergic rhinitis by intranasal administration of chemically modified grass pollen allergen. *Acta Allergol* **32**:248, 1977.
 18. Mathews KP, Bayne NK, Banas JM, McLean JA, Bacon J: Controlled studies of intranasal immunotherapy for ragweed pollenosis. *Int Archs Allergy Appl Immunol* **66**:218, 1981.
 19. Schumacher MJ, Pain MCF, Mitchell GF: Controlled study of intranasal immunotherapy with modified allergen. *J ALLERGY CLIN IMMUNOL* **67**(Suppl.):8, 1981. (Abst.)