

Local intranasal immunotherapy for grass-allergic rhinitis

John W. Georgitis, M.D.,* Robert E. Reisman, M.D., William F. Clayton, M.D., Ulrich R. Mueller, M.D., John I. Wypych, Ph.D., and Carl E. Arbesman, M.D.
Buffalo, N. Y.

In a double blind controlled study, local intranasal immunotherapy was evaluated for the treatment of grass pollenosis. On the basis of serum grass-specific IgE levels, 50 grass-allergic patients were randomly divided into three groups and treated with either an aqueous solution of mixed-grass extract, a formaldehyde-modified mixed-grass extract (allergoid), or a histamine solution (placebo). Intranasal solutions were administered in gradually increasing doses over a preseasonal 10 wk period. Adverse local reactions from the aqueous grass extract were frequent during treatment. Few adverse reactions occurred from the allergoid or histamine solutions. During the pollen season, patients receiving both grass extracts had much lower symptom/medication scores than patients receiving placebo. The severity of eye symptoms was the same in all groups. After treatment, serum grass-specific IgE rose in patients receiving aqueous and allergoid extract; no change was noted in patients receiving placebo therapy. Grass nasal secretory-specific IgA titers in each group did not change during the study. The results of this study suggest that local intranasal immunotherapy with either aqueous or allergoid grass extracts is clinically effective for the treatment of grass pollenosis. Adverse reactions associated with the aqueous extract may limit its usefulness. No correlation was present between the secretory immune response and clinical benefit. (J ALLERGY CLIN IMMUNOL 71:71, 1983.)

Previous studies have suggested that LNIT with ragweed extracts is effective in the treatment of ragweed-allergic rhinitis.^{1, 2} Because the grass pollen season is considerably longer, this approach was evaluated in patients with grass-allergic rhinitis. In addition, the use of polymerized ragweed extracts caused fewer reactions and stimulated a greater local immune response when compared with aqueous extracts.³ For these reasons, modified grass extracts also were used for optimal evaluation of LNIT.

A double-blind controlled study was designed to evaluate the clinical and immunologic response to aqueous and formaldehyde-treated grass extracts. The

results of these studies suggest that LNIT is effective for the treatment of grass pollenosis and that the modified extracts, in particular, because of the lower incidence of adverse reactions, are a promising therapeutic regimen.

MATERIALS AND METHODS

Antigens

The aqueous grass extracts and chemically modified grass allergoid were kindly supplied by EM Laboratories, Inc. The mixed-grass extract consisted of equal parts of timothy, orchard, and june grasses in a total concentration of 20,000 PNU/ml. The allergoid grass extract was prepared from equal parts of the same grasses by means of the method of Haddad et al.⁴ The allergoid-grass extract was supplied in a concentration of 100,000 PNU/ml. Serial 10-

From the Allergy Research Laboratory, Buffalo General Hospital, Department of Medicine, Division of Allergy, State University of New York at Buffalo, Buffalo, N. Y.

Funded in part by NIH/NIAID grant 2R01 AI01303-24.

Received for publication June 1, 1982.

Accepted for publication Aug. 9, 1982.

Reprint requests to: Robert E. Reisman, M.D., 50 High St., Buffalo, NY 14203.

*Recipient of a Dr. Henry C. and Bertha Buswell Research Fellowship at the State University of New York at Buffalo.

Abbreviations used

LNIT:	Local intranasal immunotherapy
PNU:	Protein nitrogen unit
NPT:	Nasal provocation testing
SMS:	Symptom medication scores

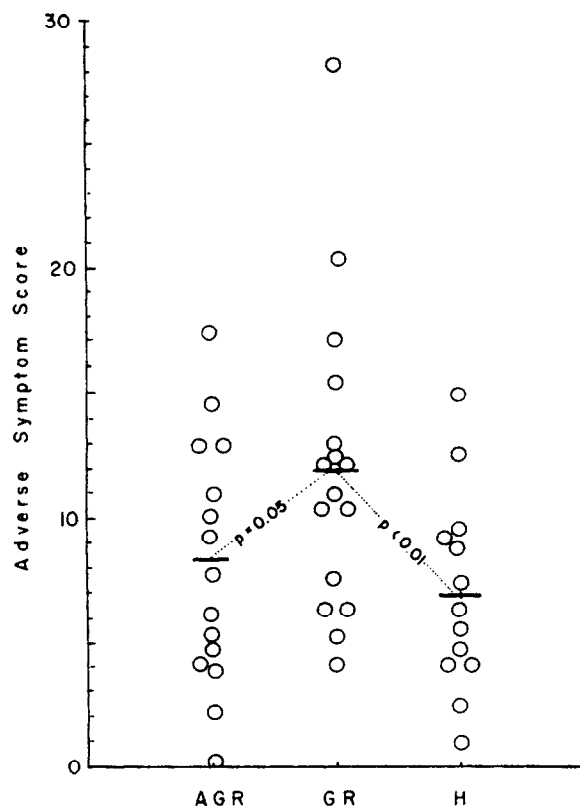


FIG. 1. Adverse reaction score by treatment group. Each circle represents an individual subject. Horizontal bars denote group means. Aqueous grass treatment (GR) vs allergoid grass treatment (AGR), $p = 0.05$; aqueous vs histamine (H), $p < 0.01$ (Mann-Whitney U test).

fold dilutions were prepared of both extracts. Histamine phosphate was used as a placebo nasal aerosol.

Patient selection

Patients were recruited through a local advertising program. Qualified patients had a history of seasonal grass-allergic rhinitis for at least 3 yr, a positive prick test to 1:20 w/v mixed-grass extract, and had received no parenteral immunotherapy for at least 3 yr. Criteria for exclusion were perennial rhinitis, asthma requiring daily medication, tree- or mold-induced allergic symptoms, pregnancy, breast feeding, or a serious systemic disorder. At the initial visit, a complete history was recorded and a physical examination was performed. Prick skin tests were done with selected trees, *Alternaria*, ragweed, *Hormodendrum*, timothy, orchard and June grasses, and mixed-grass extracts. Histamine and saline controls served as positive and negative controls. Blood samples and nasal secretions were taken for immunologic analysis and nasal challenge was carried out to assess nasal reactivity.

Study design

In a double-blind study, patients were randomly matched into three groups based on serum grass-specific IgE levels. Treatment with nasal spray aerosol was started in March

TABLE I. Treatment protocol

Week	Active treatment		Placebo (histamine, mg/ml)
	Aqueous grass (PNU/ml)	Allergoid grass (PNU/ml)	
0	10	100	0.1
2	100	1,000	1.0
4	1,000	5,000	2.0
6	10,000	25,000	5.0
8	20,000	100,000	5.0

1981 and carried out for 10 wk. Coded bottles of the nasal spray were used as a blind. The spray was given as a single dose on alternate days to avoid the priming phenomenon described by Connell et al.⁵ Bottles of stronger extract solutions were supplied to the patients every 2 wk (Table I). Subjects could use oral antihistamines or decongestants if local nasal symptoms developed from the nasal spray treatment. Physicians evaluated subjects on alternate weeks, at which time treatment compliance was assessed by weighing bottles before and after use. Nasal spray therapy was completed prior to the appearance of large quantities of grass pollen in mid-May. This study was approved by the Investigative Review Board of the Buffalo General Hospital.

Serum and nasal secretions were collected prior to therapy (March 1981), at the end of the treatment period (May 1981), and at the end of the pollen season (August 1981).

NPT

NPT was performed by a modification of the Okuda technique.⁶ The mixed-grass extract served as the challenging antigen. In brief, 3 mm paper discs were impregnated with serial 10-fold concentrations of the grass extract. The challenge was begun with a control disc that was visually placed on the inferior turbinate. If no reaction occurred with the control disc, a high dilution of antigen (10^{-6} dilution of grass extract) was applied to the turbinate. At 5 min intervals, the concentrations were increased until a positive challenge occurred. A positive response was defined as the appearance of at least three nasal signs or symptoms, e.g., rhinorrhea, congestion, itching, sneezing, or mucosal palor and edema. Nasal reactivity was assigned a rating based on the negative log of the allergen concentration that elicited a positive challenge. Nasal secretions were collected for immunologic analysis at the end of the nasal challenge.

Evaluation

Clinical. Patients maintained a daily diary of nasal and eye symptoms and medication use during the grass pollen season from May 1 through July 15. This scoring system has been used in previous LNIT studies.¹ Patients were seen every 2 wk during the pollen season. Daily pollen counts were taken with an intermittent rotorod sampler (Ted Brown Associates, Los Altos, Calif.). Pollen counts were reported as grains per cubic meter, and the peak pollen period was defined as that in which daily counts were consistently >20 grains/m³.

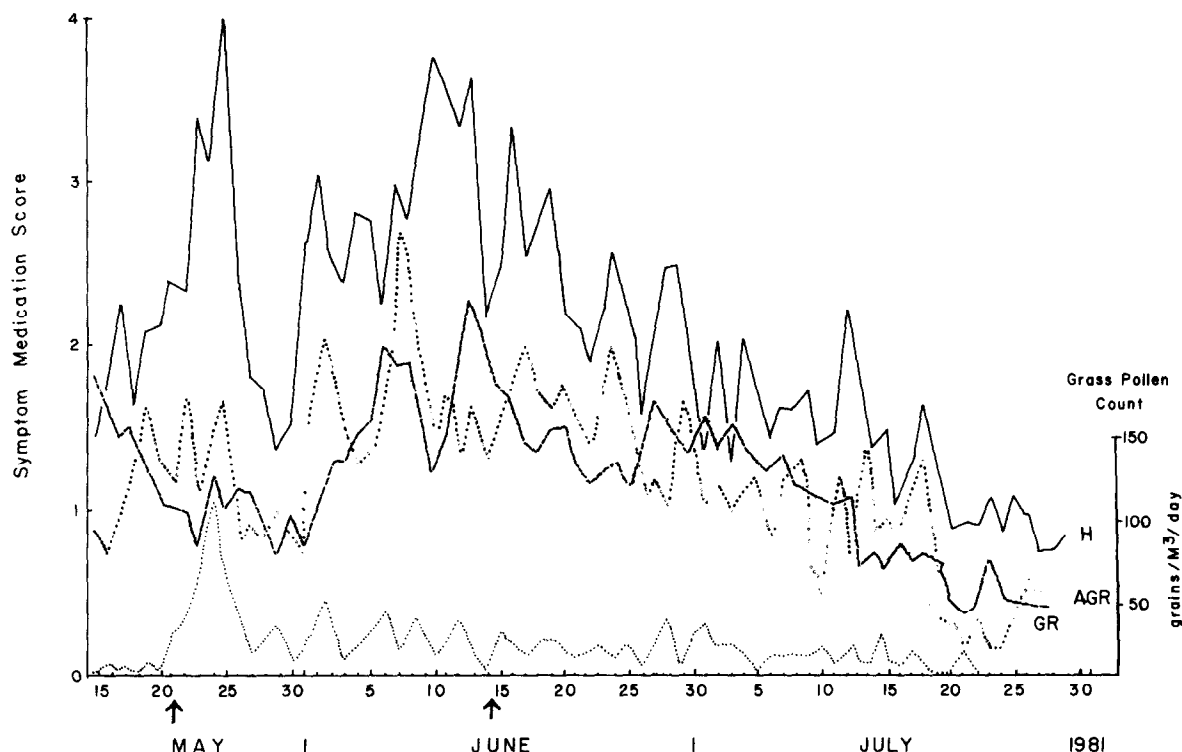


FIG. 2. Group mean daily SMS. Scores for histamine placebo group (*solid line, H*), aqueous grass treatment group (*hatched line, GR*), and allergoid grass treatment group (*dotted line, AGR*) are shown. Daily pollen counts are plotted at the bottom (grains/m³). The peak pollen period is identified by the arrows on the date axis.

TABLE II. Demographic data

	Aqueous grass	Allergoid grass	Histamine placebo
Mean age (yr)	35.2	33.3	37.2
(range)	(17-60)	(16-57)	(20-67)
Sex (M/F)	8/8	12/3	7/6
Previous immunotherapy	5	6	7
Grass RAST	25.2 ± 31.5*	26.2 ± 23.7	18.2 ± 17.9
(range)	(1-110)	(1-74)	(1-57)
NPT	2.9 ± 1.5*	2.5 ± 1.1	2.7 ± 1.6

RAST = radioallergosorbent test.

*Expressed as mean ± SD.

Immunologic. As described previously,³ radioimmunoassays were used to measure serum grass-specific IgE and IgG, and grass-specific IgG, IgA, and IgE in the nasal secretions. The mixed-grass extract was used as the coupling antigen. IgE titers were reported as IU per milliliter; IgA and IgG antibodies were reported as percentage of counts bound. The albumin concentration in nasal secretions was determined by the Lowry technique.

Statistics

SMS were calculated from patient symptom diaries. An average SMS over the peak pollen period was calculated for each patient. For intergroup statistical analysis, the

Mann-Whitney rank-sum test was used for daily SMS and the average SMS over the peak pollen period. The paired t test was used for intragroup variation in NPT score and antibody titers in the serum and nasal secretions. The unpaired t tests were used for intergroup analysis for NPT score and antibody titers. Significance was a p value of ≤0.05.

RESULTS

Course of treatment

Forty-four of 50 patients who had initially enrolled in the study completed the treatment course. Two pa-

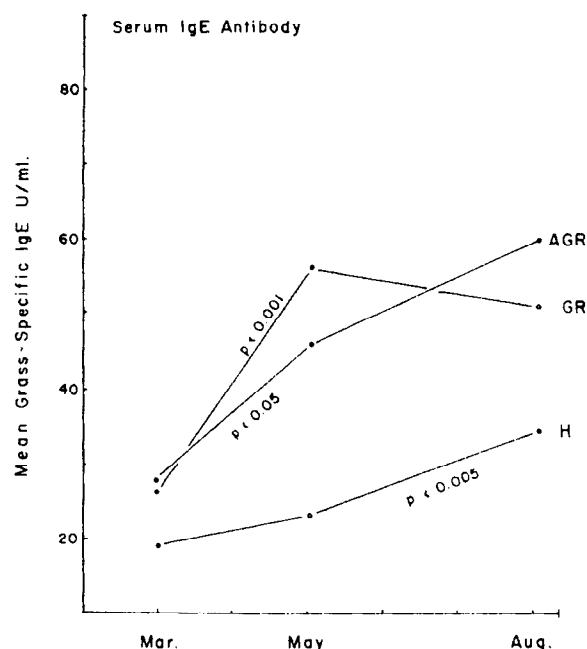


FIG. 3. Group mean serum grass-specific IgE titers at each evaluation period. H, Histamine; GR, aqueous grass treatment; AGR, allergoid grass treatment.

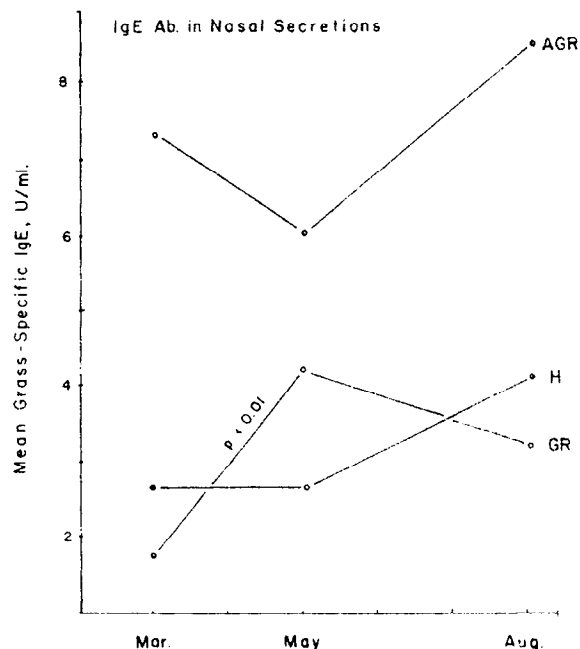


FIG. 4. Group mean nasal secretory grass-specific IgE titers at each evaluation period. H, Histamine; GR, aqueous grass; AGR, allergoid grass.

TABLE III. Group mean SMS during the peak pollen period

	SMS treatment			Significance		
	Allergoid (AGR)	Aqueous (GR)	Histamine (H)	Gr vs AGR	AGR vs H	GR vs H
Sneezing	1.06	1.12	1.84	NS	<0.05	<0.05
Rhinorrhea	1.29	0.80	2.09	NS	<0.05	<0.01
Congestion	1.61	1.38	2.42	NS	NS	<0.05
Nasal symptoms	1.32	1.10	2.11	NS	<0.05	<0.005
Eye symptoms	0.98	1.10	1.45	NS	NS	NS
Overall severity	1.46	1.40	2.45	NS	<0.05	<0.05
Medication	0.18	0.17	0.67	NS	<0.001	<0.001
Average total score	1.46	1.33	2.79	NS	<0.01	<0.005

NS = not significant ($p < 0.05$).

tients withdrew, one because of transportation difficulties and the other because of severe local adverse reactions to treatment. Four patients were disqualified for failure to comply with the protocol. The 44 patients completing the study are characterized in Table II. The groups were well matched for age, previous immunotherapy, pretreatment mean serum total IgE levels, pretreatment mean serum grass-specific IgE levels, and nasal provocation sensitivity.

The total amount of aqueous grass extract administered ranged from 600 to 44,000 PNU, with a mean of 17,000 PNU; the amount of allergoid administered

ranged from 12,000 to 192,000 PNU, with a mean of 71,000 PNU.

Most subjects experienced some local adverse reactions to the nasal immunotherapy treatment, which were limited to the upper airway. Symptoms consisted of sneezing, nasal itching, rhinorrhea, a local burning sensation of the nose and throat, itching of the throat, and postnasal drip. These reactions were brief and rarely lasted more than a few hours. Patients receiving aqueous grass treatment had a significantly higher adverse reaction rate than those patients receiving allergoid or placebo (Fig. 1). The reaction rate did

not differ between the allergoid- and placebo-treatment groups.

Clinical response

The clinical effectiveness of LNIT was assessed by evaluating daily SMS. The SMS for each group are shown in Fig. 2, along with the daily pollen counts. The aqueous- and allergoid-treated patients had significantly lower mean SMS than the placebo group during the peak pollen period from May 21 through June 14 ($p < 0.002$ and $p < 0.01$, respectively). Throughout the entire grass pollen season there was no significant difference in SMS between the two active-treatment groups ($p > 0.10$).

In evaluating individual symptoms, both active-treatment groups had significant lower nasal symptom scores than did the placebo group (Table III). Sneezing, rhinorrhea, nasal congestion, total nasal symptoms, and overall severity of symptoms were significantly lower in the aqueous-treated group. Eye symptoms were diminished in both active-treatment groups, but this improvement did not reach statistical significance. Both active-treatment groups used medication to a lesser degree than did the placebo group.

Immunologic response

Mean titers of serum grass-specific IgE are shown in Fig. 3. After treatment, there was a rise in serum grass-specific IgE in both aqueous- and allergoid-treated groups ($p < 0.001$ and $p < 0.05$, respectively). The titers did not change in the placebo-treated group. During seasonal pollen exposure, serum grass-specific IgE levels rose in both placebo- and allergoid-treated patients. Only in the placebo group did this reach a level of statistical significance ($p < 0.005$). The customary seasonal rise was suppressed in the aqueous-treated patients.

Serum grass-specific IgG titers were below the level of sensitivity of the assay in most patients. In the three groups, patients with demonstrable titers exhibited no significant change after treatment or pollen exposure.

Grass-specific IgE in nasal secretions is shown in Fig. 4. During the treatment period there was a rise in grass nasal secretory-specific IgE in patients receiving aqueous grass extracts ($p < 0.01$). No change was noted in the other two groups. During the pollen season, an increase in antibody titers was noted in the allergoid and placebo groups. Throughout the study, patients in the allergoid group had higher mean titers of secretory-specific IgE.

Titers of grass-specific secretory IgA and IgG did not differ between the treatment groups during any

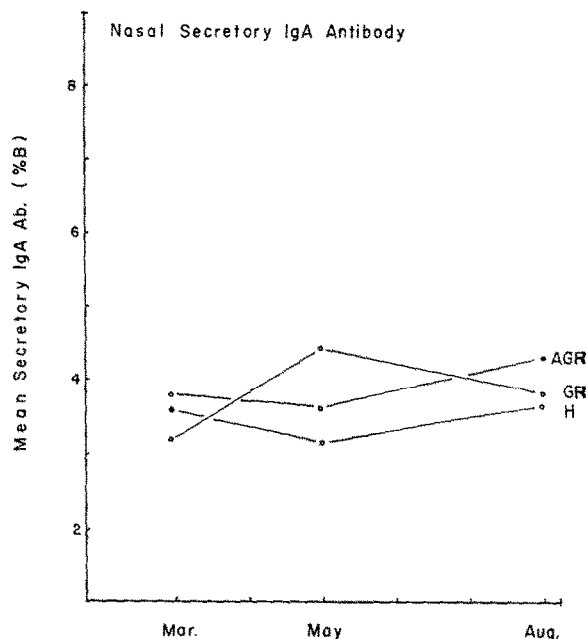


FIG. 5. Group mean nasal secretion grass-specific IgA titers at each evaluation period. H, Histamine; GR, aqueous grass; AGR, allergoid grass.

period in the study. There was no effect of treatment or seasonal pollen exposure on these titers (Fig. 5).

The range of nasal sensitivity before treatment was similar in placebo- and both grass-treated patients. After treatment, mean reactivity scores did not differ significantly between groups at any time or within each group over time.

DISCUSSION

This study demonstrates the clinical effectiveness of LNIT in the treatment of grass-induced allergic rhinitis. The aqueous grass and the allergoid grass extracts are clearly superior to a histamine placebo in reducing SMS during the grass pollen season.

The clinical findings in this study agree with other previously reported nasal immunotherapy studies using aqueous grass extracts. Taylor and Shivalkar⁷ found a significant reduction in symptom scores during the pollen season and a prolonged reduction in nasal sensitivity lasting for months after immunotherapy with an aqueous grass extract. Mehta and Smith⁸ and Cook⁹ also reported that preseasonal nasal immunotherapy was effective in reducing nasal symptoms during the pollen season.

Chemically modified allergens have been evaluated in studies of LNIT.¹⁻¹⁰ Previous work in our own laboratory has shown that a glutaraldehyde-modified ragweed extract is clinically effective and associated

with minimal adverse reactions. Mathews et al.¹⁰ could not confirm this clinical benefit. Johansson et al.¹¹ noted definite improvement with a glutaraldehyde-treated tyrosine-adsorbed grass extract. Possible reasons for discrepancies in results of these various studies include differences in pollen exposure, duration of treatment, total administered dosage, and extract formulation.

In this present study, neither active-therapy group demonstrated a significant decrease in eye symptoms. Prior studies with intranasal administration of ragweed extracts have shown a reduction in allergic eye symptoms.¹ It is assumed that relief of eye symptoms is related to the systemic effect of therapy rather than local response. The serum antibody response suggested that both grass extracts were systemically absorbed. Thus we have no explanation for failure of eye symptoms to improve in this study.

The administration of both extracts to the nasal membranes did stimulate a rise in serum grass-specific IgE, suggesting systemic absorption. In the nasal secretions there was a rise in grass-specific IgE levels after aqueous treatment, whereas after allergoid treatment the IgE antibody titers dropped. For this reason we believed that the rise in serum IgE antibody titers reflected systemic allergen absorption rather than local antibody production in the nasal tissue. In our prior studies with the glutaraldehyde-modified extract, there was no rise in serum antibodies, implying that this extract was not systematically absorbed.³ The allergoid has a lower range in molecular weight than the glutaraldehyde-modified extract (50,000 to 200,000 vs 200,000 to 1,000,000). The lower molecular weight of the allergoid may allow for systemic absorption and subsequent stimulation of a systemic immune response. The absence of this systemic immune response by the glutaraldehyde-modified extract is probably due to its much higher molecular weight. The IgE antibody in nasal secretions may result from either local production or transudation from serum.

In this study, no significant change in secretory IgA and IgG antibodies was observed after aqueous or allergoid treatment. This contrasts with our findings in the ragweed LNIT studies, where the polymerized ragweed extract did stimulate a local immune response. The reason for this discrepancy is unclear. However, these findings suggest that there is no correlation between local antibody response and clinical benefit.

Nasal reactivity did not change dramatically in either treatment group, although there was a definite decrease in reactivity scores during the time of sea-

sonal exposure. Possibly NPT may not accurately reflect actual inhalation of pollen grains.

Adverse reactions during LNIT with aqueous extracts continued to be a significant deterrent for its use. Local reactions frequently occurred, particularly with higher concentrations. Similar results were found in previous studies with aqueous ragweed extracts.^{1, 2} The allergoid elicited far fewer adverse reactions, agreeing with our studies with glutaraldehyde-polymerized extracts.

LNIT is an effective treatment for grass-induced allergic rhinitis. It is safe and inexpensive and can be administered at home. Its major drawback appears to be the local adverse reactions. The immunologic data suggest that the aqueous antigen acts like parenteral immunotherapy with systemic absorption. Similarly, the allergoid grass extract also appeared to be systemically absorbed. At this point, we believe that this therapeutic modality merits further investigation, particularly with the use of modified extracts in higher concentrations.

REFERENCES

1. 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.
2. 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.
3. 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.
4. Haddad ZH, Marsh DG, Campbell DH: Studies on "allergoids" prepared from naturally occurring allergen. II. Evaluation of allergenicity and assay of antigenicity of formalinized mixed grass pollen extracts. *J ALLERGY CLIN IMMUNOL* 49:197, 1972.
5. Connell JT: Quantitative intranasal pollen challenges. III. The priming effect in allergic rhinitis. *J ALLERGY* 43:33, 1969.
6. Okuda M: Basic study of nasal provocative test, first report: side, site of the nose, size of site and allergen amount. *Arch Otorhinolaryngol* 214:24, 1977.
7. Taylor G, Shivalkar PR: Local nasal desensitization in allergic rhinitis. *Clin Allergy* 2:125, 1972.
8. Mehta SB, Smith FM: Nasal hyposensitization and hayfever. *Clin Allergy* 5:279, 1975.
9. Cook N: Preseasonal local nasal desensitization in hayfever. *J Laryngol* 88:1169, 1974.
10. Mathews KP, Bayne NK, Banas TM: A controlled study of intranasal immunotherapy with polymerized ragweed antigen. *J ALLERGY CLIN IMMUNOL* 65:191, 1980.
11. Johansson SGO, Deuschl H, Zetterstrom O: The use of glutaraldehyde modified timothy grass pollen extract in nasal hyposensitization treatment of hayfever. *Int Arch Allergy Appl Immunol* 60:467, 1979.