

Local intranasal immunotherapy for ragweed allergic rhinitis

II. Immunologic response

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Local nasal immunotherapy (LNIT) was administered in a double-blind study to 67 subjects. Twenty-three received an unmodified ragweed extract (RW), 24 received a glutaraldehyde polymer of ragweed extract (PRW), and 21 received placebo. Serum ragweed-specific IgE (S-IgE), ragweed-specific nasal secretory (NS-) IgE, secretory IgA (SIgA) and IgG, and NS-albumin were measured. RW therapy caused a significant increase in ragweed-specific S-IgE ($p < 0.005$) and NS-SIgA ($p < 0.05$). PRW therapy caused a significant rise in ragweed-specific NS-SIgA ($p < 0.001$), NS-IgE ($p < 0.05$), and NS-IgG ($p < 0.01$). Ragweed-specific S-IgG was not affected by any of the treatments. There was no consistent correlation between NS-antibody levels and symptom/medication scores. (J ALLERGY CLIN IMMUNOL 68:41, 1981.)

In an accompanying article¹ we demonstrated the clinical efficacy of local nasal immunotherapy in a placebo-controlled double-blind study of 67 patients. This article deals with the systemic and local immune response in these patients treated by LNIT with either aqueous ragweed (RW), polymerized ragweed (PRW), or placebo. The correlation between these immunologic data and clinical parameters will also be discussed.

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MATERIALS AND METHODS

Patient selection and study design have been described in detail in the accompanying paper.¹

Test samples

Blood and nasal secretions were collected on four occasions: before treatment (April or early May 1979), after treatment (preseason, first week of August 1979), mid-season (first week of September 1979), and postseason (first or second week of October 1979). Serum was stored at -20°C until analyzed. Nasal secretions were obtained after NPT by having the patient blow his nose on Parafilm (American Can Co., Neenah, Wisc.), according to the method of Okuda.² The secretions were transferred to a plastic tube and stored at -50°C until analyzed.

Measurement of albumin concentration in nasal secretions

Albumin concentration was determined by single radial immunodiffusion.³

Measurement of antibody concentration in serum and nasal secretions

Antigens. A paper disc radioimmunoassay was used for measurement of all antibody concentrations in serum and nasal secretions. The antigen, crude short ragweed extract (antigen E content $80\text{ }\mu\text{g/ml}$), was the same as that used for

Abbreviations used

LNIT:	Local nasal immunotherapy
RW:	Ragweed extract
PRW:	Glutaraldehyde polymer of ragweed extract
S-IgE:	Serum ragweed-specific IgE
NS:	Nasal secretory
S-IgA:	Secretory IgA
NPT:	Nasal provocative test

immunotherapy in the ragweed group and for NPT in all patients. To prepare 2000 cellulose discs, 644 mg of ragweed protein were used as previously reported.⁴

Antisera. Anti-IgE and anti-IgG immunosorbent-purified antibodies were prepared and radiolabeled as previously reported.⁵

Goat antiserum to human secretory piece was purified from antiserum obtained after immunization with the SIgA-containing fraction of defatted human colostrum eluted from diethylaminoethyl (DEAE)-Sephadex. The antiserum cross-reacted strongly with commercial antiserum to secretory piece. The antiserum was absorbed with Sephadex-coupled IgA from normal serum, myeloma IgA, and normal IgM until it failed to react with normal human serum. When purified by elution from an immunosorbent prepared with colostrum IgA, it still showed identity reaction with the commercial antiserum. After radiolabeling, the purified immunoglobulin reacted minimally with discs coupled with myeloma IgA but reacted strongly with colostrum IgA discs. Addition of whole normal human serum to the radiolabeled anticolostrum SIgA reduced this unwanted binding to acceptable background levels of < 1% of the total activity added.

Antibody assay. The basic procedure for each serum assay was as follows: 200 μ l of borate-buffered saline, pH 8.0, containing 0.3% human serum albumin, 1% polysorbate (Tween 20), and 0.1% sodium azide was added to the bottom of a polystyrene tube. This was followed by the addition of 10 μ l of either unknown serum, reference serum solution, or normal human serum as control. One ragweed disc was added to each tube. After shaking, the tubes were incubated overnight and washed three times with saline. Fifty microliters of the radiolabeled antiserum were then added, followed by another overnight incubation. The discs were then washed, and the residual radioactivity was counted. The tests for antibodies in nasal secretions were modified to the extent that 10 μ l of saline were added to each tube containing reference or control serum, whereas 10 μ l of normal human serum were added to each tube containing nasal secretions to compensate for the greater protein concentration in sera. Although IgE and IgG antibodies were determined in both serum and nasal secretions, SIgA antibody was measured only in the nasal secretions. Counts for the serially diluted reference serum vs. the antibody concentration were plotted on semilog paper. IgE and IgG antibody levels of the unknowns were read from the corre-

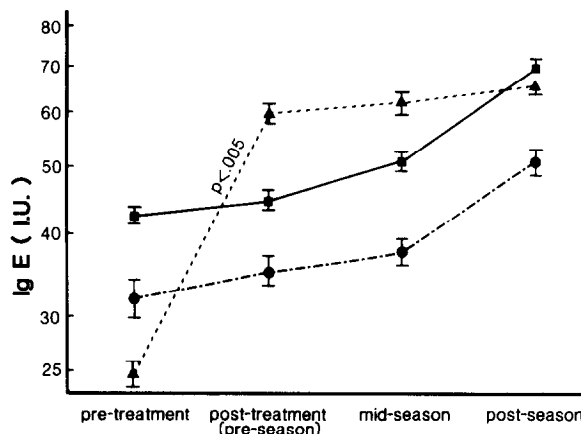


FIG. 1. Group mean serum ragweed-specific IgE levels at each evaluation period. Vertical bars indicate SEM. ■, Placebo; ▲, RW; ●, PRW.

sponding reference curve. Because the absolute amount of ragweed-specific IgE of the IgE reference was known, IgE antibody values could be expressed in international units (IU). The amount of ragweed-specific IgG contained in 1 μ l of reference serum was arbitrarily designated as 100 units. Because no standard was available for SIgA, these results are given as percent of the total activity added:

$$\frac{\text{cpm unknown} - \text{cpm background}}{\text{total cpm background}} \times 100.$$

All assays were done in duplicate.

Statistical analysis. Statistical analysis of the antibody levels was done on log-transformed data. Student's unpaired or paired t tests were used to compare differences between groups and within groups, respectively. Correlation of symptoms with immunoglobulin levels was done with Pearson correlation coefficients. The relationship between patient assessment or NPTs with antibody levels was done with χ^2 analysis.

RESULTS

Systemic immune response

The ragweed-specific IgE levels in serum are shown in Fig. 1. During the treatment period there was a marked increase in serum IgE antibody levels in the RW group only ($p < 0.005$). The placebo and PRW groups had only slight increases during the treatment period, but both showed the expected coseasonal increases in serum IgE antibody ($p < 0.005$ for both groups). The coseasonal increase was suppressed in the RW group. Ragweed-specific IgG antibody levels in the serum were at or below the sensitivity limit of our assay in most of the patients. No substantial changes of ragweed-specific IgG during treatment or season were observed in any of the three groups.

Local immune response

The ragweed-specific antibodies in nasal secretions are shown in Fig. 2. Therapy with PRW was associated with a significant increase in the antibody level of all three immunoglobulin classes during the treatment period. RW therapy was also associated with an increase in all three antibodies but only in SIgA was this change statistically significant. Placebo treatment did not induce any significant changes. In all three groups there was a coseasonal increase in IgE antibodies in nasal secretions, which was not significant at the $p < 0.05$ level. No significant intergroup differences in pretreatment antibody levels were observed. Post-treatment values differed significantly only for the SIgA antibody levels: the PRW group had significantly higher levels at preseason, midseason, and postseason than those in either the ragweed ($p < 0.01$) or the placebo group ($p < 0.001$). The RW group had significantly higher levels of SIgA antibody than those in the placebo group at the posttreatment evaluation only ($p < 0.05$).

Albumin concentration in nasal secretions ranged from 20 to 5200 $\mu\text{g/ml}$ and was highest in the mid-season samples. The increase from preseason to mid-season was significant ($p < 0.05$) in the PRW and placebo groups but not in the RW patients.

Immune response and clinical parameters

Correlations between the antibody levels and symptom/medication scores of each treatment group are summarized in Table I. In the placebo group there was no correlation between antibody levels and symptom/medication scores at any of the evaluation times. In the RW group there was a negative correlation between the ragweed-specific NS-IgG and the symptom/medication scores at midseason. In the PRW group there was a positive correlation between both ragweed-specific S-IgE and NS-IgE and symptom/medication scores. There were no significant correlations for ragweed-specific NS-IgA or NS-albumin and symptom/medication scores in any of the treatment groups. Considering the patients as a group, there was a positive correlation only between symptom/medication scores and NS-IgE antibody levels. It should be noted that of the preseason antibody concentrations, there was significant correlation only between the NS-IgE antibody levels and the clinical outcome. Use of antibody ratios of NS-IgE:NS-IgG or NS-SIgA did not improve the correlation coefficients between antibody levels and symptom/medication scores in any of the treatment groups.

There were no significant correlations between nasal sensitivity (NPT score)¹ and any of the antibody

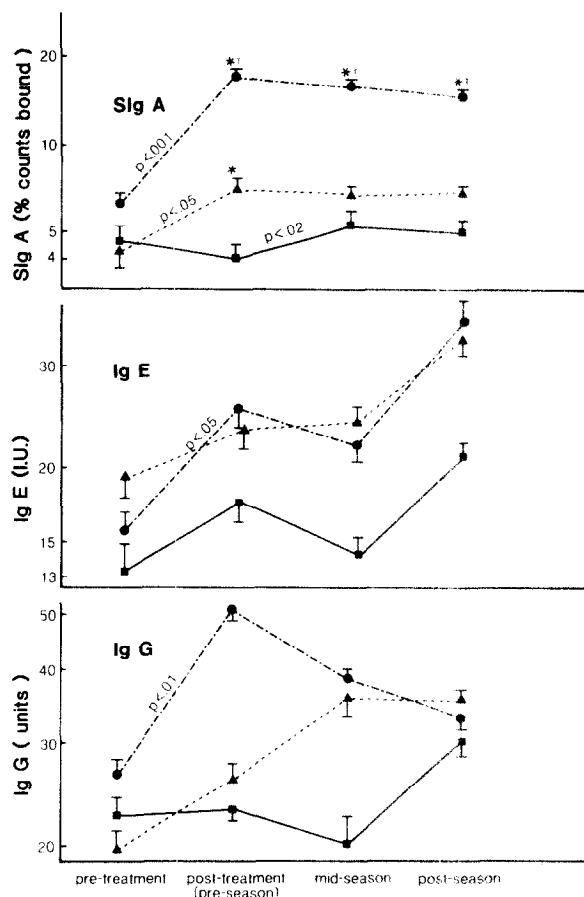


FIG. 2. Group mean nasal secretion ragweed-specific antibody levels at each evaluation period. Vertical bars indicate SEM. ■, Placebo; ▲, RW; ●, PRW. Asterisk, Significantly greater than placebo; superscript T, significantly greater than RW.

levels measured or between change in NPT score and change in antibody levels.

There were, however, significant statistical correlations between the antibody levels of all the immunoglobulin classes measured (Table II). Albumin concentration in the nasal secretions correlated positively with NS-IgE and negatively with NS-IgG; it did not correlate with NS-SIgA.

DISCUSSION

In the accompanying article,¹ LNIT with RW and PRW was shown to be clinically effective when compared with placebo. RW was superior to PRW treatment under the conditions used but was also associated with more side effects. In this paper the systemic and local immune response to LNIT is analyzed.

An immune response was induced by the two active treatments but differed both qualitatively and quantitatively. Only the RW treatment induced a measur-

TABLE I. Correlations between antibody levels and symptom/medication scores

	Pretreatment	Preseason	Midseason	Postseason
RW				
NS-IgG	NS	NS	R = -0.54 p < 0.02	NS
PRW				
S-IgE	NS	R = 0.40 p < 0.05	R = 0.38 p < 0.05	R = 0.43 p < 0.02
NS-IgE	R = 0.49 p < 0.02	R = 0.48 p < 0.01	R = 0.57 p < 0.01	R = 0.56 p < 0.01
All patients				
NS-IgE	R = 0.34 p < 0.01	NS	R = 0.33 p < 0.01	R = 0.30 p < 0.02

NS = not significant (p > 0.05).

TABLE II. Correlation between ragweed-specific antibodies in various immunoglobulin classes and NS-Albumin*

	S-IgE	NS-SIgA	NS-IgE	NS-IgG
S-IgE	—	N.S.	R = 0.42 p < 0.001	R = 0.18 p < 0.05
NS-SIgA	N.S.	—	R = 0.31 p < 0.001	R = 0.16 p < 0.06
NS-IgE	R = 0.42 p < 0.001	R = 0.31 p < 0.001	—	N.S.
NS-Albumin	N.S.	N.S.	R = 0.25 p < 0.001	R = -0.36 p < 0.001

N.S. = not significant (p > 0.05).

*Data includes all samples.

able systemic immune response (Fig. 1). There was a marked increase in S-IgE during the treatment period, which was not followed by the usual coseasonal increase. In both the placebo and PRW groups there was no change in S-IgE during the treatment period, but the expected coseasonal increase did occur. Analysis of the antibody levels in nasal secretions (Fig. 2) shows clearly that PRW is more potent than RW in inducing a local immune response. The NS-SIgA response was pronounced as one would expect for the mucosal immune system. Furthermore there was also a measurable antibody response in the other immunoglobulin classes analyzed.

Previous studies differ in their findings regarding the systemic immune response to LNIT. Welsh et al.⁶ noted no change in either the serum IgE or serum IgG antibody levels. On the other hand, the results of Johansson et al.⁷ are in agreement with ours, they found a significant increase in the NS-IgA, NS-IgG, and NS-IgE antibody concentrations in the actively treated group. The only significant response in the placebo group was a coseasonal increase in NS-IgE.

Thus local immunotherapy has been shown to be

clinically effective and to induce an increase in ragweed-specific antibodies in nasal secretions; these clinical and immunologic findings, however, do not correlate. Clinically the RW treatment was more effective; the PRW treatment induced a greater local immune response. Furthermore there was only inconsistent correlation between each subject's clinical response, as indicated by symptom/medication scores, and nasal antibody levels. Platts-Mills⁸ was also unable to correlate grass-specific NS-IgE or IgA with seasonal symptoms. Because LNIT stimulated antibody production in all three classes of immunoglobulins measured, it is conceivable that the clinical outcome depends on which antibody response prevails—that of the sensitizing (NS-IgE) or of the blocking (NS-IgG or NS-SIgA). Ratios of sensitizing and blocking antibodies in nasal secretions did not, however, improve the correlation between the immune response and symptom/medication scores. We also found no correlation of the secretory antibody levels with the NPT scores. There was, however, a positive correlation between the antibody levels in the various immunoglobulin classes (Table II). It appears that

LNIT did not enhance the development of either blocking or sensitizing antibodies but rather the development of antibodies in all classes.

Concentrations of albumin in nasal secretions varied from 20 to 5200 $\mu\text{g/ml}$ and tended to peak at midseason, especially in the placebo and PRW groups. This suggests that increases in albumin concentration in nasal secretions reflect increased vascular permeability caused by local inflammation. There is no correlation, however, between albumin concentrations and symptom/medication scores in any of the treatment groups, but NS-albumin did correlate with some of the NS antibody levels (Table II). Of interest is the fact that correlation of NS-albumin with NS-IgG was negative. This indicates either a local production of IgG anti-ragweed antibodies or preferential secretion of IgG over albumin. Platts-Mills⁸ also noted this evidence of local IgG antibody production. Several authors have used NS-albumin as a parameter for expressing concentrations of other constituents in nasal secretions. This may be necessary where the secretions are obtained by use of a nasal washing technique, which introduces an unknown factor of exogenous dilution. Because we collected our secretions without diluting them, we do not express our immunoglobulin concentrations as ratios to albumin.

In summary our data confirm that LNIT is a clinically effective treatment for ragweed allergic rhinitis. Furthermore, they demonstrate that this form of immunotherapy does induce a local immune response.

The correlation between this immune response and the clinical parameters of efficacy is poor. The more immunogenic PRW treatment was not as effective clinically as the RW treatment. On the whole, these data give only little support to the concept that locally induced blocking antibodies of the IgG or IgA classes afford protection in allergic rhinitis.

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