

Allergic rhinitis to ragweed pollen

I. Reassessment of the effects of immunotherapy on cellular and humoral responses

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This work presents a double-blind, placebo-controlled study of 27 patients with allergic rhinitis to ragweed who received preseasonal desensitization immunotherapy [IT] with alum-precipitated aqueous ragweed extracts. We reassessed the following parameters in relation to clinical responses: clinical scores, nasal reactivity to a provocative dose of ragweed causing a 75% fall in airflow rate (PD_{75}), ragweed IgE and IgG, and ragweed-induced basophil histamine release (BHR). First, the nasal PD_{75} correlated with the severity of nasal symptoms ($p < 0.05$). Second, we confirmed a significant symptomatic improvement in the IT-treated group either by clinical scores ($p < 0.05$) or the prevention of the seasonal fall of the PD_{75} ($p < 0.005$). Also, IT reduced the seasonal rise of IgE ($p < 0.02$) and induced an increase in IgG ($p < 0.01$) and a decrease in BHR ($p < 0.03$). There was a significant correlation between IgE and BHR ($r = 0.80$; $p < 0.01$). After selecting out the effects of IgE, the BHR was still higher in the placebo-treated group than in the IT-treated group ($p < 0.02$), suggesting the involvement of other modulating factors. Symptomatic improvement after IT correlated only with the summation of both IgE and BHR (PD_{75} ; $r = 0.64$; $p < 0.005$). This observation suggests that the severity of clinical symptoms is determined by several interacting factors and not by the antibody response alone. (J ALLERGY CLIN IMMUNOL 1992;89:76-86.)

Key words: Immunotherapy, rhinitis, basophil, releasability, histamine, ragweed, nasal reactivity, clinical symptoms, IgE, IgG

The efficacy of IT in the treatment of AR to pollen has been clearly established in a number of studies.^{1, 2} Quantitative measurements of IR have then permitted a more comprehensive definition of the pathophysiologic mechanisms involved in allergic diseases and their modulation by IT. Indeed, it was demonstrated that pollen desensitization prevents the seasonal rise of specific IgE antibodies,³⁻⁵ induces a specific IgG antibody response speculated to have a blocking effect,⁶⁻¹⁰ corrects a defective down regulation by suppressor T cells on IgE antibody production,^{11, 12} changes the nasal reactivity after specific or

Abbreviations used

AR:	Allergic rhinitis
IT:	Immunotherapy
NAR:	Nasal airflow rate
PNU:	Protein nitrogen unit
PBS:	Phosphate-buffered saline
PD_{25} , PD_{50} , PD_{75} :	Provocative dose of ragweed extract causing a 25%, 50%, and 75% drop in airflow rate, respectively
HR:	Histamine release
BHR:	Basophil histamine release
HSA:	Human serum albumin
IR:	Immune response

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nonspecific challenges,¹³ and reduces mast cell-mediator release in the nasal secretions collected after pollen challenge.¹⁴⁻¹⁷ Finally, diminished basophil responsiveness has been observed in some, but not all, subjects responding to IT¹⁸⁻²¹ and was best correlated with clinical status when patients were analyzed as a

group. However, correlations between changes of IRs resulting from desensitization and the degree of clinical improvement remain a matter of controversy when each set of parameters is analyzed individually. This finding may result, in part, from the standard evaluation of the severity of AR that uses a less reliable measurement of daily symptoms/medication scores. In addition, since pollen IT results in complex humoral and cellular immunologic changes, it is possible that a combination of factors, rather than only one, could determine the magnitude of clinical amelioration. Therefore, the object of the present study was, first, to ascertain whether, in ragweed pollinosis, the intensity of nasal symptoms can be quantified by the objective measurement of nasal reactivity with nasal provocation with pollen antigen, followed by calculation of nasal airflow responses. Second, since previous studies have focused interest on mediator release as an important factor of clinical improvement after desensitization, our work was designed to examine this point in greater detail and to find contributing determinants of the clinical amelioration.

More specifically, our study included 27 ragweed-allergic subjects suffering from rhinoconjunctivitis without asthma and who were randomly assigned to receive preseasonal IT or a placebo. Before the initiation of therapy and during the ragweed-pollen season, the following parameters were performed for all volunteers: ragweed IgE and IgG antibody levels, symptom/medication scores, physician evaluation, assessment of nasal reactivity to ragweed by determining the dose of ragweed needed to change significant the NAR, and specific ragweed-induced BHR. The relationships between the laboratory findings and the clinical response to IT were analyzed in the perspective that some combination of factors could determine the intensity of therapeutical success.

MATERIAL AND METHODS

Study groups

Twenty-seven ragweed-sensitive subjects, aged 19 to 56 years, were included in this study after an informed consent was obtained. They were selected at the Allergy and Immunology Clinic of Le Centre Hospitalier de l'Université Laval according to the following characteristics: a clear-cut history of ragweed AR without asthma, a positive skin prick test to ragweed extracts (1:20 wt/vol of glycerinated extract; Omega Laboratories, Montreal, Canada), and significant titers (less than class 2) of antiragweed IgE antibodies, as determined by RAST (Pharmacia, Piscataway, N.J.). None had any significant nonrelated diseases, and none were receiving previous specific IT. Thirteen nonatopic volunteers with no history of AR and with negative skin tests and negative RAST to common inhalants were also included in this study.

IT regimens

All atopic patients were first stratified into two groups by their skin sensitivity to ragweed, as determined by end point titration. This procedure was done to obtain groups with even distribution of moderately and highly sensitive subjects. Then, patients underwent prick testing with serial threefold dilutions of ragweed extracts (10,000 PNU/ml in sodium chloride solution containing HSA [0.3 mg/ml] and phenol [5 mg/ml]; Omega Laboratories) starting at a concentration of 10,000 PNU/ml. Fifteen minutes after injection, wheal reactions were measured, and the dilution (end point) eliciting a wheal reaction equal to that of the reaction of the positive control (histamine, 1 mg/ml in buffer solution with 50% glycerine)² was determined. Afterward, patients were randomly assigned to receive between May and July subcutaneous injections of either alum-precipitated extracts of ragweed (50, 100, 200, 500, 1000, 2000, 3000, 3000, and 3000 PNU) in 9 consecutive weeks, or placebo (alum-precipitated HSA containing histamine phosphate [0.5 mg/ml] and caramelized glucose, which made it indistinguishable in color and appearance from treatment). All coded solutions were kept at 4° C during the treatment period, and neither the physician administering the injections nor the patient were aware of the individual's therapeutic regimen. Reactions to IT were minimal. All subjects receiving placebo or ragweed injections had only a local wheal reaction of <2 cm of induration. In one case only, a patient receiving ragweed extracts developed a late (6 hours) local wheal reaction of 8 cm accompanied with dyspnea; at that time, an additional dose of 50 PNU was repeated before continuing the schedule.

Assessment of clinical status

During the pollen season (mid-August to mid-September), all patients were asked to complete daily symptom diary cards. Then, each patient was requested to evaluate his symptoms for the previous 12 hours at noon and bedtime. Separate ratings were listed for sneezing, itchy nose, rhinorrhea, nasal obstruction, lacrimation, and/or itchy eyes, according to the following scale²²: 0: none, no symptoms evident; 1: mild, trivial, definitely present, but not bothersome; 2: moderate, bothersome, but not disabling or intolerable; and 3: severe, disabling and/or interfering with daily activities and/or sleep.

Also the patients were asked to report the type and the amount of permitted medication (chlorpheniramine, 4 mg tablets; pseudoephedrine, 60 mg tablets; and naphazoline hydrochloride, pheniramine maleate, eyedrops). The numerical scores were totaled to derive a daily symptom score for each patient's day. During the ragweed season, the patients were clinically evaluated twice at 14-day intervals, according to the same criteria, by an allergist not aware of the treatment received by the patients.

Nasal challenge technique

All nasal provocations were performed before the beginning of desensitization in the month of May and during the ragweed season in September by a technician not aware of

the treatment received. The basal NARs of each nostril were obtained by anterior rhinomanometry with a Connell rhinomanometer, as previously reported by us.¹³ Specific nasal reactivity to ragweed was done by insufflating, in each nostril, increasing doses of daily, fresh PBS solutions containing 1 to 3000 PNU in fivefold increments of aqueous ragweed extracts prepared from a solution of 40,000 PNU/ml in phenol, 5 mg/ml (Omega Laboratories). NAR was measured at 3 minutes before challenge in each nostril. Serial challenges were performed until the mean NAR of the two nostrils decreased by 75% from baseline. Results were expressed as PD of ragweed extracts in PNUs inducing PD₂₅, PD₅₀, and PD₇₅, respectively.

Ragweed-induced BHR

Heparinized blood was drawn from each subject in May and September, and coded specimens were sent to the laboratory for BHR assay, as previously reported by our group.^{23,24} Briefly, leukocytes were enriched by the passage of subject's whole blood on a dextran gradient (Dextran T-500, Pharmacia, Piscataway) for 30 minutes. The buffy coat was centrifuged, and the leukocyte-enriched pellet containing basophils was washed in Tris buffer (25 mmol/L, pH 7.4) containing albumin (0.03%) and ethylenediaminetetraacetic acid (8 mmol/L), and resuspended at a final concentration of 3×10^6 cells per milliliter in RPMI 1640 alone or with various concentrations (0.001 to 1.0 µg/ml) of aqueous ragweed extracts. Then, ragweed extracts (40,000 PNU in phenol, 5 mg/ml; Omega Laboratories) were previously dialyzed against H₂O, lyophilized, resuspended in RPMI 1640 at a concentration of 10 mg/ml, and stored at -70° C until use. After a 30-minute incubation at 37° C, the reaction was stopped by cooling, and the supernatant was recovered for histamine determination with a single radioenzymatic assay.²⁵ The results were expressed as a percentage of HR (% HR) and calculated as follows:

$$\% \text{ HR} = (A - B)/T \times 100$$

where A = allergen-induced HR from basophils, B = buffer-induced HR from basophils, and T = total histamine content of basophils.

Ragweed-specific IgE antibody levels

Serum antiragweed IgE antibody levels were determined on each coded samples by RIA, as previously described.²⁶ Briefly, disks were first coated (500 ng per disk) with concentrated form of crude ragweed extract described as above, saturated with ethanolamine, 0.9%, and then incubated with HSA for 3 hours at 37° C. Unbound immunoglobulins were removed by a wash with Tween (0.01%)-PBS, 0.1 mol/L, pH 7.2, and ¹²⁵I-labeled anti-IgE was added to disks and incubated for 6 hours at 37° C. After extensive washes with Tween-PBS, the radioactivity fixed on disks was counted in a gamma counter (LKB, Bromma, Sweden). Results were means of duplicate analyses and expressed in counts per minute.

Ragweed-specific IgG antibody levels

Serum-specific IgG antibodies to ragweed were measured by ELISA with a modification of the technique previously described.²⁷ Briefly, 96-well microtiter plates were coated with a concentrated form of crude ragweed allergen described as above (100 ng per well), saturated with 10% bovine serum albumin, and then incubated with HSA for 2 hours at room temperature. After washes, alkaline-phosphatase-labeled antihuman IgG antiserum (Zymed Laboratories, San Francisco, Calif.; 1:1000) was added for 1 hour before addition of 4-nitrophenylphosphate (1 mg/ml). The reaction was stopped 1 hour later by the addition of sodium hydroxide (3 mol/L), and the absorbance was read at 405 nm with an automated spectrophotometer (Dynatech Laboratories, Chantilly, Va.).

Statistical analysis

Results were expressed as means $\pm$ SE. The values for symptoms scores were compared by Wilcoxon's rank-sum test, and values for specific IgE and IgG antibody levels, by Wilcoxon's signed-rank and rank-sum tests. The results of specific nasal challenge were analyzed via point-by-point comparisons with the least-square means procedure (SAS Institute, GLM procedure),²⁸ supplemented by a test for heterogeneity of slopes and common intercept. Finally, correlations between various parameters were computed by Spearman's rank-correlation coefficient. Nonparametric tests were used because of lack of homogeneity of variances.

RESULTS

Characteristics of the study populations

This study included 27 patients allergic to ragweed, randomly divided into two treatment groups: 14 receiving placebo and 13 receiving ragweed-specific IT. A group of 13 normal volunteers was also included in this study. These three study groups were comparable as to the number of subjects, the age, and the basal NAR measured before the initiation of IT (Table I).

Effects of IT on IgE and IgG antibody

Analysis of immunologic parameters revealed that the significant rise of IgE antibody in the placebo-treated group observed during the ragweed-pollen season, as compared to preseasonal level (21551 ± 3779 versus 26159 ± 4222 counts per minute; $p < 0.02$), was prevented by specific IT (23196 ± 3985 versus 24938 ± 3952 counts per minute; p was not significant) (Fig. 1). However, the IgG antibody level was significantly higher in the group of treated patients as compared to levels in untreated patients (Fig. 2), and levels could be detected in serum from nonatopic volunteers as previously reported.²⁷

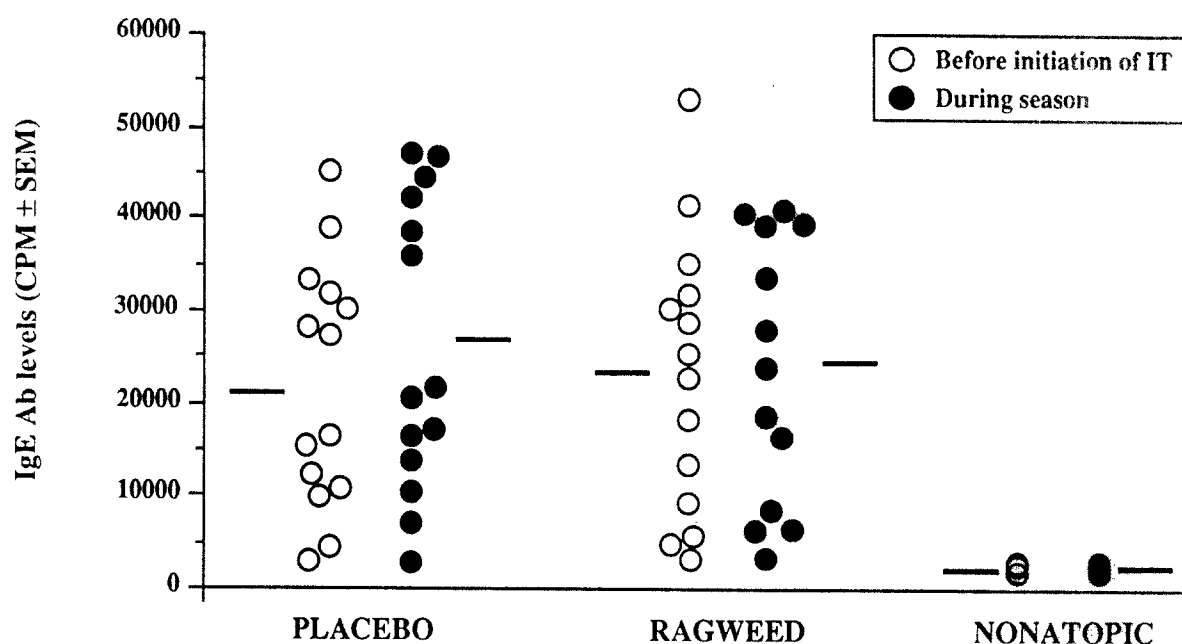


Fig. 1. Specific IgE antibody levels in placebo- and ragweed-treated patients before initiation of IT and during pollen season (* $p < 0.02$). Each circle represents individual patient; plain lines indicate group average.

Effects of IT on clinical symptoms

Symptom scores. The clinical response of atopic patients, based on the total score of their symptom/medication diary card, is illustrated in Fig. 3. During the ragweed-pollen season, daily symptom scores were significantly lower in the actively treated patients than in patients receiving placebo (4.7 ± 0.7 versus 7.5 ± 1.2 ; $p < 0.05$). Patients' scores were in agreement with scores given by the allergist ($r = 0.7$; $p < 0.05$; not presented). The types and amounts of symptomatic medication taken by patients of either groups were not statistically different (0.9 ± 0.2 versus 0.7 ± 0.2 ; $p = 0.6$; not presented), and most importantly, patients with lower symptom scores (IT-treated group) actually took less medications.

Nasal reactivity to ragweed. First, the severity of nasal reactivity was evaluated by measuring the dose of ragweed extracts necessary to induce a PD_{25} , PD_{50} , or PD_{75} in all subjects before initiation of IT. The doses of ragweed needed to induce such drops of NAR were significantly lower in ragweed-sensitive subjects (182 ± 116 , 501 ± 116 , and 818 ± 116 PNU, respectively) than in nonatopic subjects (PD_{75} , >6000 PNU) (not presented). Second, the effect of natural exposure to ragweed pollen on the specific nasal reactivity in the placebo-treated group was assessed. As

TABLE I. Characteristics of the study groups

	Placebo treated	Ragweed treated	Nonatopic
No. of subjects	14	13	13
Age (range)	21-52	19-45	18-42
Sex (M/F)	8/6	10/3	8/5
Clinical history (yr)	7.1 ± 2.1	7.6 ± 2.5	0
Basal NAR (ml/sec)	297 ± 19	283 ± 28	347 ± 26

illustrated in Figs. 4 and 5, the doses of ragweed required to induce significant drops of NAR were significantly lower during the pollen season than before the season, suggesting an increased nasal sensitivity to pollen. This conclusion is reached whether each PD points of the curves were compared, particularly to PD_{50} (146 ± 190 versus 665 ± 182 PNU; $p = 0.05$) and PD_{75} values (227 ± 190 versus 1000 ± 182 PNU; $p = 0.005$), or when the dose-response curves were analyzed (heterogeneity of slopes, $p < 0.02$; common intercept, $p = 0.99$).

As illustrated in Figs. 6 and 7, the ragweed-specific nasal reactivity measured during the pollen season was significantly higher in the placebo-treated group than

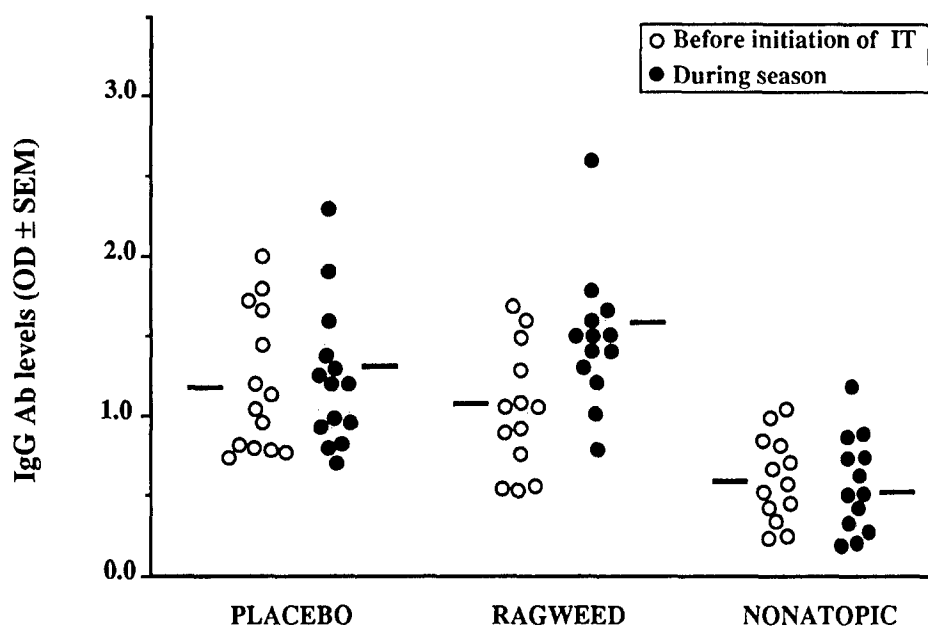


Fig. 2. Specific IgG antibody levels in ragweed-sensitive patients (placebo- and ragweed-treated) and in nonatopic subjects before initiation of IT and during pollen season ($*p < 0.01$). Each circle represents individual patient; plain lines indicate group average.

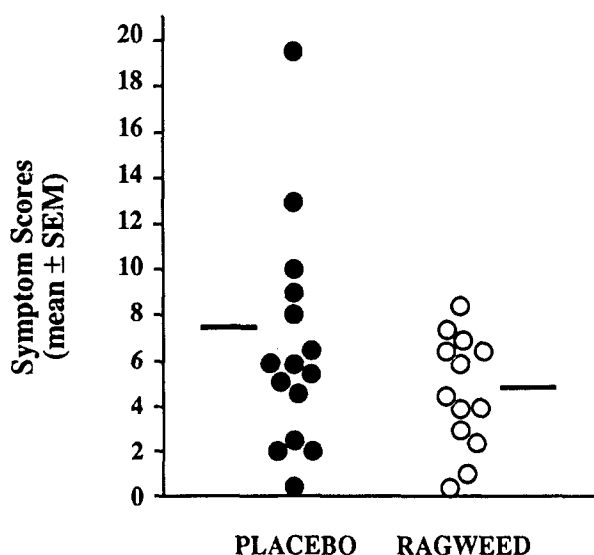


Fig. 3. Daily symptom diary scores in placebo- and ragweed-treated patients during the ragweed-pollen season ($*p < 0.05$). Each circle represents individual patient; plain lines indicate group average.

in the actively treated group (heterogeneity of slopes, $p = 0.02$; common intercept, $p = 0.9$). Indeed, more ragweed extracts are needed to induce a drop of NAR in ragweed-treated patients than in placebo-treated subjects, especially at PD_{50} (614 ± 114 versus 118 ± 98 PNU; $p < 0.002$) and PD_{75} (901 ± 113

versus 163 ± 114 PNU; $p < 0.0001$). Furthermore, natural exposure to pollen did not affect, significantly, the nasal reactivity to ragweed in ragweed-treated patients ($p = 0.44$). Indeed, the dose-response curves obtained in season and out of the pollen season were comparable (heterogeneity of slopes, $p = 0.5$; common intercept, $p = 0.5$; not presented), suggesting that specific IT prevented the increase of nasal reactivity to ragweed observed in untreated patients. This observation is not explained by differences of basal NAR, which were comparable in the two groups before the initiation of IT (test for heterogeneity of slopes, $p = 0.4$; common intercept, $p = 0.5$; not presented).

Effects of IT on ragweed-induced BHR

The effects of our desensitization protocol on BHR is demonstrated in Figs. 8 and 9. First, it could be observed that placebo-treated patients release more histamine during the pollen season compared to preseasonal values (heterogeneity of slopes, $p < 0.03$), particularly at 0.1 ($61.2\% \pm 9.2\%$ versus $45\% \pm 8.5\%$) and 1 $\mu\text{g}/\text{ml}$ ($61.3\% \pm 9.1\%$ versus $42.8\% \pm 7.0\%$) of ragweed extracts (Fig. 6; $p < 0.05$). In contrast, for patients receiving IT, the dose-response curves before and during the ragweed season are not significantly different (heterogeneity of slopes, $p = 0.99$; not presented), suggesting that specific desensitization prevents the seasonal increase of

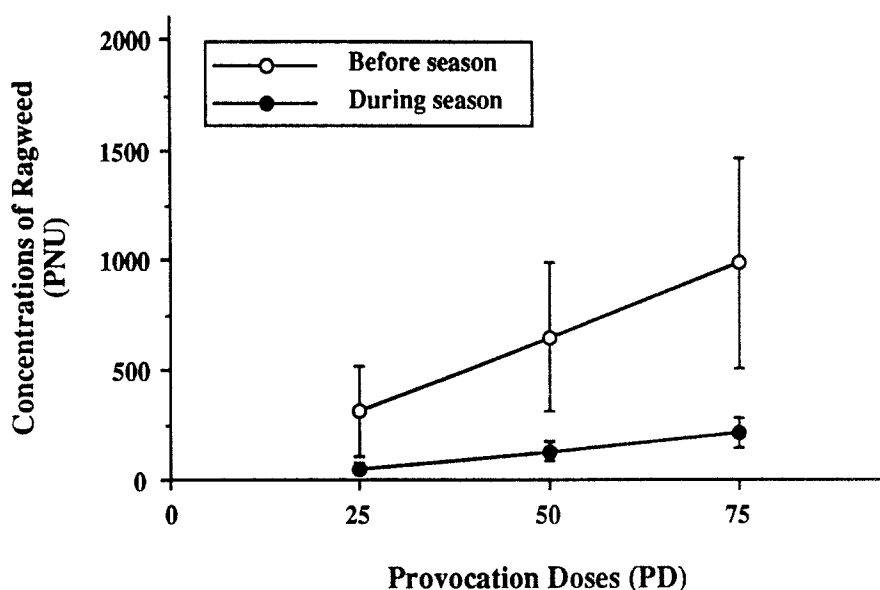


Fig. 4. Determination of specific nasal reactivity (PD_{25} , PD_{50} , and PD_{75}) in placebo-treated patients before and during the pollen season (* $p < 0.02$).

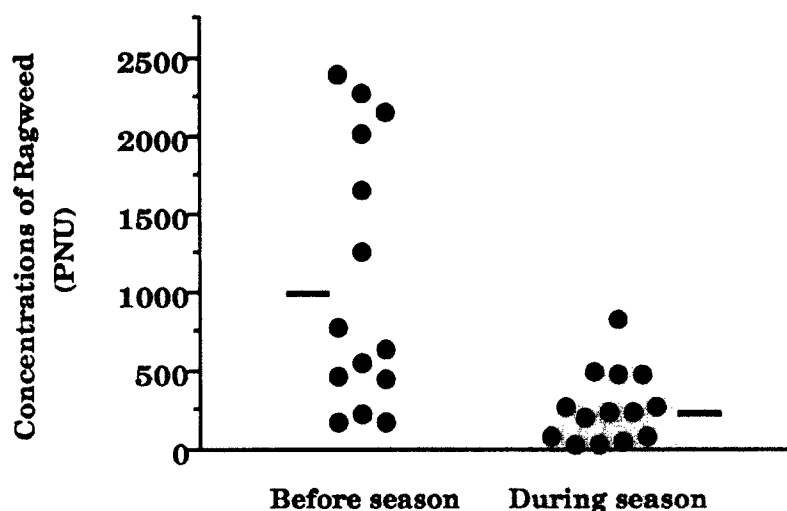


Fig. 5. PD_{75} levels in placebo-treated patients before and during pollen season. Each circle represents individual patient; plain lines indicate group average.

the antigen-induced HR. Furthermore, after use of a covariance analysis procedure that selected out the possible discrepancy between the two groups under study before the pollen season, we compared the amounts of HR by cells from each study group during the pollen season (Fig. 9). We demonstrated that basophils from placebo-treated patients released more histamine on challenge at each concentration of ragweed extracts tested (heterogeneity of slopes, $p < 0.02$). Finally, as expected in the nonatopic in-

dividuals, the ragweed-induced HR was insignificant compared to HR of the atopic group when basophils were stimulated with 0.001 ($1.4\% \pm 0.6\%$ versus $29.6\% \pm 9.4\%$), 0.01 ($4.4\% \pm 1.3\%$ versus $38.9\% \pm 9.7\%$), 0.1 ($3.1\% \pm 0.8\%$ versus $43.3\% \pm 8.0\%$) and $1 \mu\text{g/ml}$ ($4.4\% \pm 1.3\%$ versus $42\% \pm 6.5\%$) of ragweed extracts ($p < 0.001$) and was not influenced by natural exposure to pollen (not presented).

To confirm that specific IgE antibody has a role in

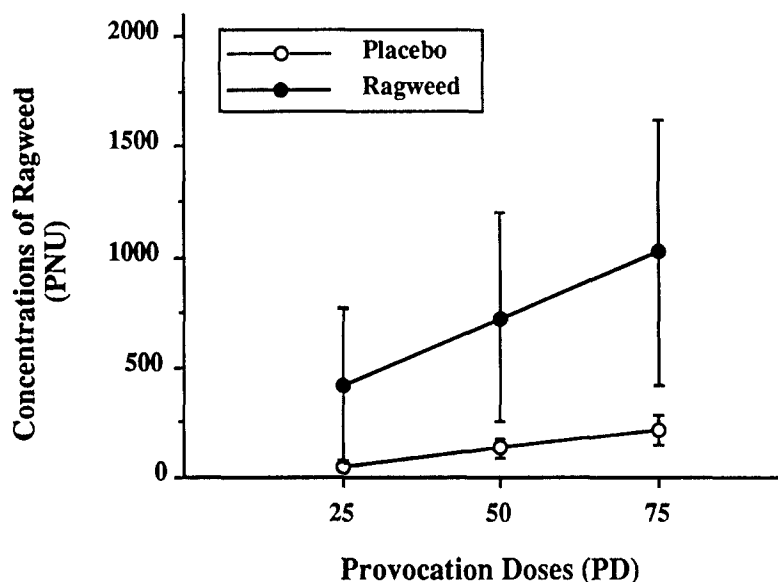


Fig. 6. Determination of specific nasal reactivity (PD_{25} , PD_{50} , PD_{75}) in placebo- and ragweed-treated patients during pollen season (* $p < 0.02$).

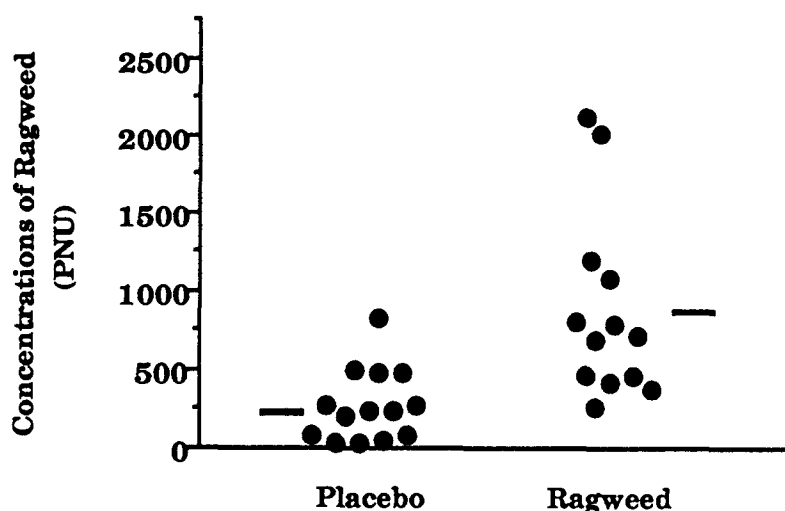


Fig. 7. PD_{75} levels in placebo- and ragweed-treated patients during the pollen season. Each circle represents individual patient; plain lines indicate group average.

the ragweed-induced HR, we correlated, in the placebo-treated group, the percentage of HR with the ragweed-specific IgE antibody levels. One patient withdrew from this analysis. The significant correlation ($r = 0.80$; $p < 0.01$) found between the specific IgE antibody levels and the basophil responses to ragweed in atopic subjects before and during the pollen season (Fig. 10) supports the role of IgE in this phenomenon. However, when we selected out the effect of IgE in a multiway statistical analysis procedure, the profiles of antigen-induced histamine secretion by

basophils were still significantly higher in the placebo-treated group than in the other group ($p < 0.02$) and were, in fact, comparable to profiles reported above for all doses of ragweed tested, suggesting that other factors than IgE may be involved as well.

Correlations between clinical and immunologic parameters

There is a significant correlation between the PD_{75} values and the daily symptom scores reported by each patients ($r = 0.51$; $p < 0.02$; not presented). The

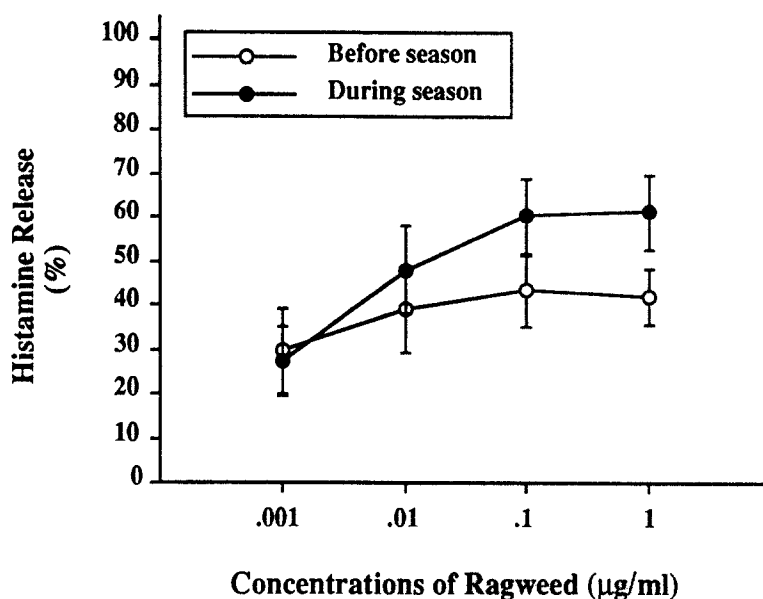


Fig. 8. Leukocyte sensitivity to ragweed in placebo-treated subjects before and during pollen season (* $p < 0.03$).

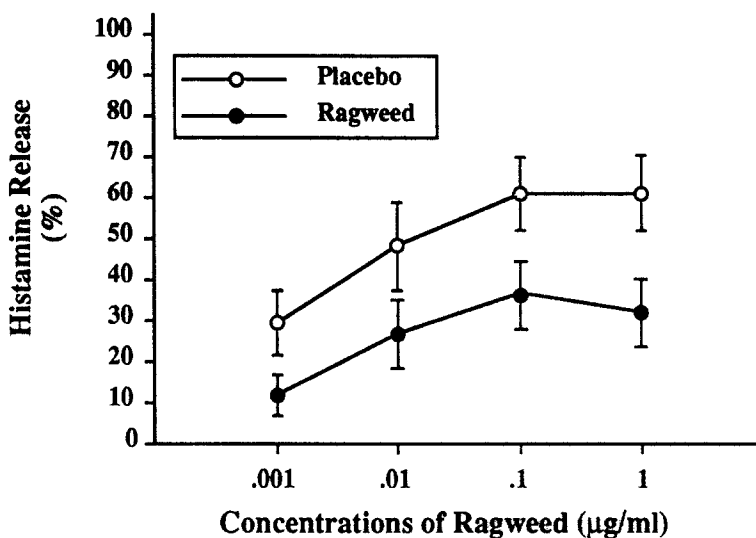


Fig. 9. Leukocyte sensitivity to ragweed in placebo- and ragweed-treated patients during the pollen season (* $p < 0.02$).

clinical response to pollen expressed in symptom scores or PD_{75} did not correlate significantly with either IgE antibody level ($r = 0.36$; $p < 0.6$; and $r = 0.54$; $p < 0.12$) or basophil responses to the allergen ($r = 0.42$; $p < 0.2$; and $r = 0.41$; $p < 0.4$), or IgG antibody level (not presented). However, a significant correlation was found between clinical parameters and the summation of IgE antibody level and

basophil responsiveness (PD_{75} : $r = 0.64$; $p < 0.05$; symptom scores: $r = 0.51$; $p < 0.07$; not presented).

DISCUSSION

The clinical efficacy of IT for pollen AR has been established,^{1,2} but its mode of action remains a matter of controversy. The aim of this double-blinded, placebo-controlled study, including 27 ragweed-sensitive

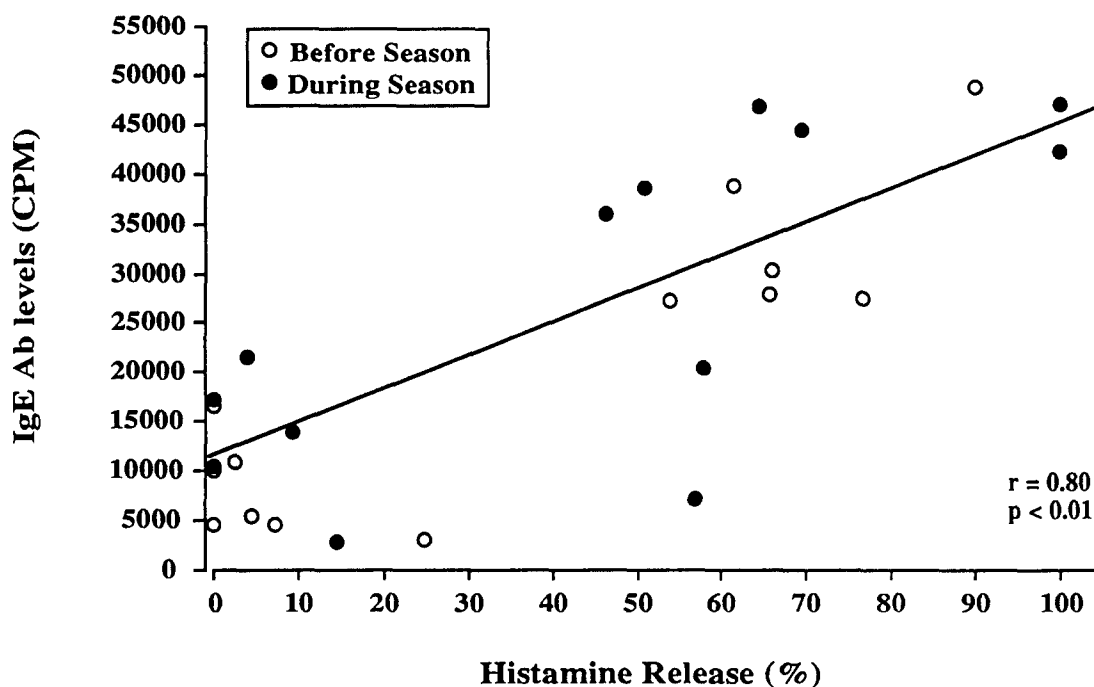


Fig. 10. Correlation between specific IgE antibody levels and leukocyte sensitivity (percent of histamine release at 1 μ g/ml of ragweed) in ragweed-allergic subjects receiving placebo. Values were obtained before and during pollen season.

patients, was to reassess the correlations between immunologic and clinical parameters, based on the effects of treatment. The careful selection of patients was based on the presence of at least moderate sensitivity to ragweed and the absence of nasal structure deformity. The nasal mucosa reactivity to ragweed was evaluated by anterior rhinomanometry rather than by the posterior technique for the reasons underlined in a previous study.¹³ The preseasonal desensitization with alum-precipitated ragweed extracts has demonstrated good efficacy by us,² and its short duration facilitated both clinical and in vitro assessment by avoiding interacting factors that can occur over a longer period of time. Most studies on pollen desensitization, including our studies,^{2, 13, 22} have used the symptom/medication diary-card method to assess the severity of symptoms. Since this method is subjective, poorly quantified, and difficult to correlate with quantitative measurements of IRs, we also took advantage of the nasal-challenge technique to correlate the nasal sensitivity to ragweed, expressed in PDs of ragweed extracts needed to induce PD₂₅, PD₅₀, and PD₇₅ of NAR, with the symptom scores obtained during the pollen season. By this method, we have confirmed herein both the greater specific nasal reactivity of atopic patients over nonallergic subjects and their increase in reactivity on natural exposure to pollen. This finding held true at each point tested, but the differ-

ences were more significant at PD₇₅. These data are in keeping with the increase in reactivity of the nasal membrane after repeated exposures to pollens in laboratory and referred to as "priming effect," and confirms the clinical relevance of the observation by Connell.²⁹ Furthermore, the PD₇₅ values recorded during the pollen season were closely correlated with the severity of symptoms. In spite of the fact that the obstructive responses to nasal challenge do not take into account either the amount of the nasal secretions or the other nasal symptoms and the extranasal symptoms, this study suggests that the PD₇₅ value is a reliable and a sensitive determination to assess the clinical responses to pollen exposure and its modulation by IT. In fact, this approach confirms the efficacy of specific IT that not only improves the symptom scores but also prevents the seasonal deterioration of specific nasal reactivity. These results are parallel to results found in bronchial asthma in which the PC₂₀ represents an objective end point, even though the severity of wheezing, cough, and dyspnea are not taken into account. The PD₇₅ is easy to generate, readily accessible to most investigators or clinicians, and may improve some of the inherent limitations possible in correlations between laboratory results, clinical status, and the effects of treatment. Therefore, we believe that PD₇₅ has the potential to become an objective tool to assess the severity of rhinitis.

One of the objectives of our study was to correlate the degree of clinical symptoms to immunologic parameters regarding efficacy of ragweed IT. As previously reported in many other clinical trials,³⁻⁵ we demonstrate herein that IT prevents the seasonal rise of IgE antibody levels and the increased allergen-induced HR on natural exposure to pollen and induces an IgG antibody response. It should be stressed that, in this study, a preseasonal IT with alum-precipitated extracts was administered as opposed to other studies in which dose and duration of treatment were greater. These differences could explain the less vigorous ragweed-IgG response than that reported by other investigators.⁵ It could also be postulated that a more prolonged course of IT should produce a clearer difference in the clinical responses of the two groups. Data from earlier studies could not determine if this reduced basophil HR was attributable to a change in cell-bound IgE antibodies or to a modification of cellular sensitivity.^{5, 19, 20} Our data confirm a role for IgE in the HR process. Indeed, a significant correlation was observed between the increased basophil responsiveness to allergen and specific IgE antibody level, which should reflect the amount of cell-fixed IgE. However, the dose-response curves of ragweed-driven HR are not modified in selecting out the effects of IgE antibody, which suggest that other modulatory factors and/or an intrinsic defect of the basophil itself could also be involved. In that respect, it is interesting to speculate that the recently described histamine-releasing factor could have a role to play.

The absence of correlation between neither IgE antibody level nor basophil responsiveness and the severity of clinical symptoms expressed in symptom scores or PD₇₅ suggest that levels of IgE antibodies are not sufficient to predict the severity of clinical symptoms and that basophil releasability is not the only phenomenon involved in the pathophysiology of allergic diseases. However, a significant correlation was observed between the summation of IgE antibody level and basophil responsiveness and the symptom score or PD₇₅. These data suggest that pollen IT is effective in blunting separately the seasonal rise of both IgE antibody level and basophil responsiveness. Allergic diseases, characterized by both high levels of serum IgE antibody and the release of a number of mediators by IgE-coated basophils/mast cells on exposure to allergens, involve several pathophysiologic mechanisms acting in synergy to determine the clinical symptomatology.

In summary, we demonstrated that the clinical response to pollen could be assessed by nasal provocation, which appears to be as accurate but much simpler and less time consuming than the clinical rat-

ing by symptoms and medications. Also, this study confirms the efficacy of specific IT in preventing the seasonal-induced priming effects at both the nasal mast cell and basophil levels. Finally, it could be observed that the severity of nasal symptoms is determined by several interacting factors, namely, the IgE antibody level and the basophil responsiveness, underlying the multifactorial aspects of the pathogenicity of allergic diseases. Furthermore, it could be speculated that histamine-releasing factor is then involved in this IgE-independent change in basophil sensitivity.

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REFERENCES

1. Creticos PS, Norman PS. Immunotherapy with allergens. *JAMA* 1987;258:2874-80.
2. Hébert J, Small P. Comparison of alum-precipitated aqueous extracts and modified ragweed tyrosine adsorbate vaccine in the treatment of ragweed hay fever. *Ann Allergy* 1988;60:226-30.
3. Gleich GJ, Zimmermann EM, Henderson LL, Yunginger JW. Effect of immunotherapy on immunoglobulin E and immunoglobulin G antibodies to ragweed antigens: a six-year prospective study. *J ALLERGY CLIN IMMUNOL* 1982;70:261-71.
4. Levy DA, Osler AG. Studies on the mechanism of hypersensitivity phenomena. XVI. In vitro assays of reaginic activity in human sera: effect of therapeutic immunization on seasonal titer changes. *J Immunol* 1967;99:1068-77.
5. Levy DA, Lichtenstein LM, Goldstein ED, Ishizaka K. Immunologic and cellular changes accompanying the therapy of pollen allergy. *J Clin Invest* 1971;50:360-9.
6. Cooke RA, Barnard JH, Hebal S, Stull A. Serologic evidence of immunity with coexisting sensitization in a type of human allergy (hay fever). *J Exp Med* 1935;62:733-50.
7. Lichtenstein LM, Holtzman NA, Burnett LS. A quantitation in vitro study of the chromatographic distribution and immunoglobulin characteristics of human blocking antibody. *J Immunol* 1968;101:317-24.
8. Loveless MH. Immunological studies of pollinosis. IV. The relationship between thermostable antibody in the circulation and clinical immunity. *J Immunol* 1943;47:165-80.
9. Sherman WB. Reaginic and blocking antibodies. *J ALLERGY* 1957;28:62-75.
10. Sobotka KA, Valentine MD, Ishizaka K, Lichtenstein LM. Measurement of IgG-blocking antibodies: development and application of radioimmunoassay. *J Immunol* 1976;117:84-90.
11. Hébert J, Beaudoin R, Tremblay C. The regulatory effect of histamine on the immune response. III. Defect on in vitro IgE production in atopics. *Ann Allergy* 1984;53:138-42.
12. Rocklin RE, Sheffer AL, Greineder DF, Melmon DK. Generation of antigen-specific suppressor cells during allergy desensitization. *N Engl J Med* 1980;302:1213-9.
13. Bédard PM, Jobin M, Clément L, Mourad W, Hébert J. Evaluation of nonspecific nasal reactivity to histamine during and after natural ragweed pollen exposure. *Am J Rhinology* 1989;3:211-5.
14. Naclerio RM, Meier HL, Kagey-Sobotka A, Adkinson NF, Meyers DA, Norman PS. Mediator release after nasal airway

- challenge with allergen. *Am Rev Respir Dis* 1983;128:597-602.
15. Proud D, Togias A, Naclerio RM, Crush SA, Norman PS, Lichtenstein LM. Kinins are generated in vivo following nasal airway challenge of allergic individuals with allergens. *J ALLERGY CLIN IMMUNOL* 1983;72:1678-85.
 16. Creticos PS, Marsh DG, Proud D, et al. Responses to ragweed-pollen nasal challenge before and after immunotherapy. *J ALLERGY CLIN IMMUNOL* 1989;84:197-205.
 17. Baumgarten CR, Togias AG, Naclerio RM, Lichtenstein LM, Norman PS, Proud D. Influx of kininogens into nasal secretions after antigen challenge of allergic individuals. *J Clin Invest* 1985;76:191-7.
 18. Irons JS, Pruzansky JJ, Patterson R, Zeiss R. Studies of perennial ragweed immunotherapy: associated changes in cellular responsiveness, total serum antigen-binding capacity, and IgE antibody concentrations. *J ALLERGY CLIN IMMUNOL* 1977;59:190-9.
 19. Lichtenstein L, Osler A. Studies on the mechanisms of the hypersensitivity phenomena. IX. Histamine release from human leukocytes by ragweed pollen antigen. *J Exp Med* 1964;10:50-8.
 20. Lichtenstein LM, Norman PS, Winderwerder WL, Osler AG. In vitro studies of human ragweed allergy: changes in cellular and humoral activity associated with specific desensitization. *J Clin Invest* 1966;45:1126-36.
 21. Van Arsdel PP, Middleton D. The effect of hyposensitization on the in vitro histamine release by specific antigen. *J ALLERGY* 1961;32:348-56.
 22. Hébert J, Bédard P, Carpio JD, et al. Loratadine and pseudoephedrine sulfate: a double-blind, placebo-controlled comparison of the combination table (SCH434) and its individual components in seasonal allergic rhinitis. *Am J Rhinol* 1988;2:71-5.
 23. Mecheri S, Mourad W, Lapeyre J, Jobin M, David B, Hébert J. Demonstration of idiotypes expressed on basophil-bound IgE antibodies by using anti-idiotypic-induced histamine release in grass pollen-allergic patients. *Immunology* 1988;64:11-5.
 24. Mourad W, Bernier D, Jobin M, Hébert J. Mapping of *Lol p I* allergenic epitopes by using murine monoclonal antibodies. *Mol Immunol* 1989;26:1051-7.
 25. Bédard PM, Brunet C, Pelletier G, Hébert J. Increased compound 48/80 induced local histamine release from nonlesional skin of patients with chronic urticaria. *J ALLERGY CLIN IMMUNOL* 1986;78:1121-5.
 26. Mourad W, Pelletier G, Hébert J. Suppression by relevant allergen of the spontaneous in vitro production of rye group I specific IgE antibodies by human peripheral blood lymphocytes. *Clin Exp Immunol* 1987;67:391-7.
 27. Beaulieu L, Valet JP, Beaudoin J, et al. Development of a reverse enzymeallergosorbent test (REAST) to detect timothy-specific IgE antibodies: comparison with RAST. *J Immunol Methods* 1986;86:131-8.
 28. SAS user's guide. Statistics. 5th ed. Cary, N.C.: SAS Institute, Inc., 1985:956.
 29. Connell JT. Quantitative intranasal pollen challenges. III. The priming effect in allergic rhinitis. *J ALLERGY* 1969;43:33-44.

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