Grass pollen immunotherapy: A single year double-blind, placebo-controlled study in patients with grass pollen-induced asthma and rhinitis

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Fifteen grass pollen–sensitive asthmatic patients were selected from 200 patients with grass pollenosis on the basis of positive SPTs and RASTs that were restricted to grass pollens (except Bermuda grass), no previous IT, and residence and occupation in an area monitored by serial pollen counts. They underwent a double-blind trial of specific IT with a mixture of three grass pollen–aqueous extracts (velvet, sweet vernal, and timothy) or placebo. After 10 mo, the mean maintenance dose of pollen extract (assayed by RAST inhibition) in eight actively treated patients was 6000 RAST units (range 3000 to 8000) and the mean total dose was 18,700 RAST units (range 10,200 to 30,000). Results were assessment done by the following clinical and immunological data: (1) during the pollen season, daily symptom scores; (2) PD 20% FEV_1, IgE antibody to timothy by RAST in serum and in nasal secretions, serum IgG antibody to purified timothy allergen D by solid-phase radioimmunoassay, and the four IgG subclass antibodies by enzyme immunoassay were all measured before treatment and before and after the pollen season. Symptom scores of both treated patients and controls correlated with pollen counts ($R = 0.88$, $p < 0.05$ and $R = 0.71$, $p < 0.05$, respectively). There was a significant difference between the mean symptom score values of treated patients versus controls (Kruskal-Wallis test, $p < 0.001$). No significant differences or changes either in the PD 20% FEV_1 or IgE antibody to timothy in serum and nasal secretions were found in the two groups before or after IT. Total IgG and all four IgG subclass antibodies to timothy antigen D increased significantly in actively treated patients ($p < 0.005$ for each). The increase in IgG4 subclass antibodies was greater than in that of the other three IgG subclasses ($p < 0.05$). We conclude that a single year of aqueous grass pollen IT is clinically effective in properly selected patients without demonstrable decreases in serum or secretory IgE antibody and is associated with significant rises in all IgG subclass antibodies, especially IgG4. (J ALLERGY CLIN IMMUNOL 73:283-290, 1984.)

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Since the introduction of specific IT for respiratory allergy in 1911,[1] several double-blind trials (a majority with aqueous ragweed extracts) have been conducted. The first controlled clinical trial of specific IT was reported in 1954 when Frankland and Augustin[2] reported the results of a single year of treatment on 99 subjects with active grass pollen extract and 99 subjects with placebo. The clinical result was evaluated on the basis of daily symptom scores that were supplied by the patients. A greater efficacy ($p < 0.001$) of the active extract versus placebo was demonstrated. Similar findings have been reported by

Abbreviations used

RAST:	Radioallergosorbent test
PD 20% FEV_1:	Allergen-specific bronchial provocation dose required to lower forced expiratory volume in 1 sec by 20%
SPT:	Skin prick test
IT:	Immunotherapy
BPT:	Bronchial provocation test

many others, and the results of 16 controlled trials, 14 of which used ragweed allergens, were analyzed in 1978 by Patterson et al.[3] The overall conclusion was that aqueous inhalant allergen extracts are efficacious in about two thirds of cases versus placebo efficacy in less than a third, which was a highly significant difference.

Although the clinical efficacy of inhalant allergen IT has thus been amply verified (mainly for ragweed pollenosis), the mechanism or mechanisms of action remain controversial. It has not been possible to demonstrate unequivocally that improvement of symptomatology is due to a decrease of specific IgE, an increase of specific IgG either in serum or in local secretions, a critical IgE:IgG ratio, or other factors.[4–9] The purpose of this study was to evaluate the clinical efficacy of IT for isolated grass pollenosis in patients with both asthma and rhinitis. The main focus was to evaluate the improvement of asthma. The experimental design was a double-blind, placebo-controlled trial while monitoring potentially useful clinical and seroimmunologic parameters.

MATERIAL AND METHODS

Patients. Fifteen patients (six male patients, nine female patients) who were allergic only to grass pollen completed the study (Table I). The age ranged from 15 to 45 yr. The patients were selected from 200 pollenosic patients in accordance with the following criteria: (1) positive SPT and RAST for grass pollens and clinical correspondence of symptoms with the seasonal pollenation pattern; (2) negative SPT and RAST for Bermuda grass pollen and for all other inhalant allergens (pollens, mites, moulds, etc.); (3) presence of bronchial asthma and rhinitis; (4) no previous specific IT; (5) no other chronic disease; (6) no pregnancy in progress; (7) residence and work in the Milan metropolitan area where the pollen count was determined.

Allergens. For IT an aqueous lyophilized extract (Hollister-Stier, Spokane, Washington) that was purified by dialysis of all the components of molecular weight under 5000 daltons was used and consisted of ⅓ velvet, ⅓ sweet vernal, and ⅓ timothy grass pollen. Extracts were standardized by RAST inhibition. One RAST unit was defined as the amount of extract that induced 50% inhibition of RAST binding by standard serum pool of grass-sensitive individuals.[10] Ten thousand RAST units corresponded to 7000 PNU and to 0.18 mg of total nitrogen. The allergen extract composition was based on SPT and RAST frequency of positivity for these three pollens with the intention of including a maximum of potential minor noncross-reacting allergens in the treatment program. Extracts were prepared for IT by dissolving in coca solution, and extracts for specific BPT were prepared by lyophilization of extracts followed by restoration before use in coca solution. A riboflavin-colored coca solution was used for placebo.

Specific IT. IT started in January 1981 and was carried out in accordance with a perennial schedule with an increase to maintenance (maximal tolerated dose) by the end of April 1981. The average single maximum dosage was 6000 RAST units (range 3000 to 8000) and the mean cumulative dose was 18,700 RAST units (range 10,000 to 30,000).

Specific BPT. The lyophilized allergenic extract was resolubilized before use and administered at increasing doses by means by a De Vilbiss 646 nebulizer. Atomization was regulated by a dosimeter that was provided with a relay/thermistor–timer circuit in order to limit the atomizing to the inspiratory phase that was in accordance with Rosenthal.[11] For each patient a provocative dose was determined to produce PD 20% FEV_1. The dose of the allergen that was used for provocation testing varied from 1 to 500 RAST units.

Nasal secretions. Secretions were elicited according to Merrett et al.[12] with a spray of 2 ml of 20% sodium chloride solution. The hypertonic saline was first cleared in a plastic tube after spraying. Polysorbate 20 (Tween 20; Pharmacia, Uppsala, Sweden) was added to each sample that was then homogenized in a stirrer for 30 min. Samples were stored at −20° C.

Pollen counts. Pollen counts were done with a volumetric spore trap (Burkard Manufacturing Company Ltd, Rickmansworth, England) with an intake of 10 L/min placed at a height of 25 m on an exposed terrace that was open to the four winds. Pollen was collected for a week on a greased cellophane tape that was taken out afterwards and cut up into small portions that corresponded to the day involved. The daily cellophane portions were placed on a glass slide, stained with 2% glycerinated eosin, and then examined microscopically. Pollen counts are expressed as grains per cubic meter of air.

Grass pollen–specific IgE antibody. Timothy–specific IgE antibody in serum and nasal secretion was assessed by RAST with commercial Phadebas reactants (Pharmacia).

Solid-phase radioimmunoassay for IgG-class antibody to timothy antigen D. IgG-class antibody to timothy was quantitated by a modification of microtiter solid phase radioimmunoassay as described in detail elsewhere.[13] Briefly, alternate rows of wells of a polyvinyl chloride microtiter plate were coated with 50 μl/well of purified timothy antigen D (Hollister-Stier) at a concentration of 500 μg/ml in normal saline for 1 hr at 37° C. After aspiration all wells were filled with 0.1% human-serum albumin for 1 hr at 37° C. After aspiration wells were washed three times with phosphate-buffered saline-0.5% Tween 20 (Pharmacia) and air dried. Fifty μl/well of test serum diluted 1:1000 in 10%

TABLE I. Patient characteristics

	Treatment	
	Active	**Placebo**
Male patients	4	2
Female patients	4	5
Mean age	25 (15 to 45)	22 (15 to 38)
Mean of the maximal treatment	6,000	—
Dose (RAST U)	(3,000 to 8,000)	
Cumulative treatment	18,700	—
Dose (RAST U)	(10,200 to 30,000)	

normal goat serum was then added and incubated overnight at 4° C. Wells were then aspirated, washed three times, and air dried. Goat anti-human IgG (Tago, Berkeley, Calif.) was radiolabelled with ^{125}I by the chloramine T method.[14] Fifty μl/well of indicator antibody was added and incubated overnight at room temperature. After aspiration, washing, and drying, the wells were cut from the plate and counted in a gamma counter. Results were expressed as average duplicate cpm in antigen-coated wells minus average of duplicate filler-coated wells.

Enzyme-linked immunoassay for IgG subclasses 1 to 4 antibodies to timothy antigen D. IgG subclasses 1 to 4 antibodies to timothy antigen D were quantitated by an amplified microtiter solid phase, enzyme-linked immunoassay as previously described.[15] Purified antigen was used to minimize nonspecific binding. Briefly, antigen (500 mcg/ml in phosphate-buffered saline) was used to coat alternate rows of Immulon I microtiter plates (Dynatech, Alexandria, Va.) by 1 hr incubation at 37° C. After aspiration, washing with phosphate-buffered saline-0.1% gelatin −0.05% Tween 20 (Pharmacia), and drying, the wells were filled with 0.1% gelatin for 1 hr at 37° C. After aspiration, washing, and drying, test serum diluted 1:100 in wash buffer was added to the wells and incubated for 2 hr at 37° C. After aspiration, washing, and drying, swine anti-human IgG1, IgG2, IgG3, or IgG4 antiserum (Nordic Immunological Labs, Tilburg, Holland) diluted 1:1000 in wash buffer was added to all wells and incubated overnight at room temperature. After aspiration, washing, and drying, rabbit anti-swine IgG conjugated to peroxidase (Nordic) diluted 1:10,000 in wash buffer was added to all wells for 1 hr at 37° C. After aspiration and washing, O-phenylene-diamine 0.5 mg/ml in 0.15M citrate phosphate buffer, pH 6 was added to the wells for 1 hr at room temperature. The reaction was stopped by addition of 5M sulfuric acid, and results were read in a microtiter spectrophotometer (Bio-Tek Instruments, Burlington, Vt.) at 492 nm. Results were expressed as net optical density of average duplicate antigen-coated wells minus duplicate filler-coated control wells.

Assessment of results

Daily symptom-score diaries were kept by each patient during May and June 1981. Symptoms were scored from 0 to 3 in each of the following categories: eye itch, throat itch, ear itch, sneezing, runny nose, nasal congestion, eye discharge, shortness of breath, wheezing, cough, headache, and tiredness. Pollen counts in the months of May and June were recorded as described earlier. The patients were subjected to BPTs, blood drawing, and collection of secretion for IgE antibody to timothy in serum and secretions and for IgG and IgG subclass antibodies 1 to 4 to timothy D in serum before the onset of IT in December 1980, before the pollen season in April 1981, and after 10 mo of IT in October 1981. During May and June 1981 the symptom scores of each patient were correlated with the aerometric data.

Statistical analysis for clinical differences between treatment groups was performed by the nonparametric analysis of Kruskal-Wallis. The relation between symptoms and pollen concentration was analyzed with the use of Pearson's correlation coefficient. Data that concerned the immunoglobulin levels were analyzed by the paired Student's t test.

RESULTS

At the end of the trial, the eight actively treated subjects had received a cumulative dose of 18,700 RAST units (range 10,200 to 30,000), with a mean maximal dose of 6000 RAST units (range 3000 to 8000). The total cumulative dose for each patient in RAST units was: B. W., 30,000; D. R., 10,500; D. S., 30,000; D. H., 10,200; I. L., 17,700; N. R., 23,100; D. A., 13,000; and P. M., 15,000.

Pollen concentrations showed two seasonal peaks, one in the second week and the other one in the fourth week of May 1981 (Fig. 1). The diphasic behavior was more evident when the mean weekly values were considered (Fig. 2).

Symptom scores of subjects who were treated with placebo had a similar diphasic behavior, which showed two peaks that corresponded to pollen concentration (Fig. 2). Again this diphasic peak was more evident when the mean weekly values were considered. Symptom scores in patients treated with placebo correlated with pollen counts ($R = 0.71$, $p < 0.05$).

Patients who were treated with active extract showed symptom score patterns that were similar to that of patients treated with placebo (Fig. 1). Symptom scores of the actively treated patients were also

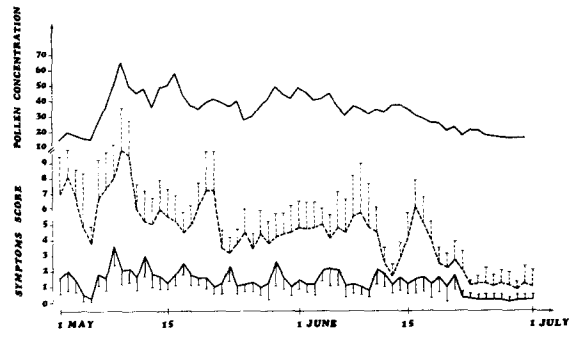

FIG. 1. Pollen concentration, *above,* in grains per cubic meter and symptom scores, *below* (mean ± SE), in actively treated subjects and subjects treated with placebo (*solid* and *dashed lines,* respectively) for the May to June grass pollen season during the study.

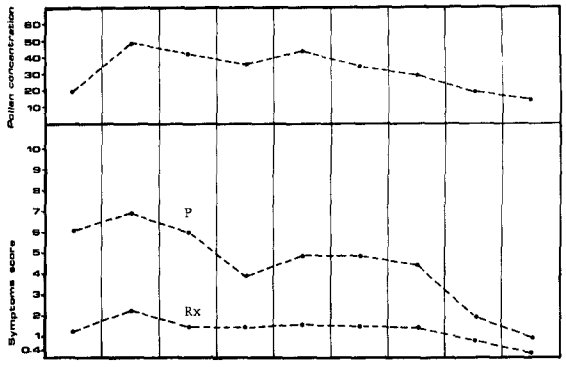

FIG. 2. Comparison of weekly average pollen count (ordinate, *upper section*) versus weekly average symptom scores *(lower section)* in placebo *(P)* and actively treated *(Rx)* subjects during the May to June grass pollen season (weeks marked on abscissa). For both actively treated group and group treated with placebo, there was a correlation between pollen exposure and symptoms ($r = 0.71$, $p < 0.05$ and $r = 0.88$, $p < 0.05$, respectively).

correlated to the pollen count ($R = 0.88$, $p < 0.05$). Actively treated patients had significantly lower mean score values than controls had during similar natural allergen exposure (Kruskal-Wallis test $p < 0.001$). Also when only the score values of the three symptoms most related to lower respiratory allergy (shortness of breath, wheezing, and cough) were considered, the treated patients again had lower mean score values than the controls had who were treated with placebo. (Kruskal-Wallis test $p < 0.01$).

IgE antibody to timothy did not show significant variations either in the serum or in the secretions of treated subjects with respect to the controls. No important seasonal variations were noted (Table II).

In contrast, specific IgG antibody showed significant increases in treated subjects on comparison with controls after 4 mo of IT ($p < 0.005$) that started from very low levels before institution of IT. Significant increases were observed in each of the four IgG subclasses with a statistically greater increase of IgG4 ($p < 0.05$) relative to baseline when compared with IgG antibodies 1 to 3 (Table III). After achieving maintenance dose around the fifth month of therapy, a decrease in IgG antibodies of all subclasses was observed. This decrease was significant for total IgG class antibodies in Oct. as compared to April 1981 ($p < 0.05$). Nevertheless, differences in IgG and IgG antibodies 1 to 4 between treated subjects and controls remained highly significant at the end of the trial.

The threshold of specific bronchial reactivity to provocation test PD 20% FEV_1 did not show any variations either in patients who were treated with placebo or in those patients who received active antigen. Fig. 3 reveals that the median PD 20% FEV_1 after inhalation of allergen was unchanged in the second and third provocation tests in both groups on comparison with the initial challenge.

A total of seven reactions occurred in the treated patients (three large local reactions, two with generalized urticaria, one with asthma, and one with anaphylaxis). All reactions were easily treated.

DISCUSSION

This study has demonstrated that IT carried out with aqueous grass pollen extract in grass pollenosis is more effective than placebo after a few months of treatment with the attainment of a maximally tolerated cumulative allergen dose ranging between 8000 and 10,000 RAST units. The degree of symptomatic difference in our small subject group between active and placebo treatment is of the same order as that found by Frankland and Augustin[2] in a much larger population (198 subjects). The high correlation between symptom scores in both treated and nontreated subjects with pollen counts confirms the reliability of the scoring system used, which, since it is based upon subjective daily evaluation by each study subject, might have been misleading. The wide range of scores at the subjects' disposal and the limitation of the study to subjects who were living and working in Milan near the pollen collector probably account for the correlations observed.

Comparison of data for atmospheric pollen concentration and symptom scores in both actively treated subjects and subjects treated with placebo strongly suggests that during natural exposure symptomatology of patients with grass pollenosis is highly related to the concentration of allergen in the air. We found that actively treated subjects have a significant diminution of symptoms, which confirms earlier controlled studies.[3] Also we found that during higher pol-

TABLE II. RAST to timothy

	Treatment	Mean specific-bound reactivity (%)		
		Dec. 80	April 81	Oct. 81
Serum	Active	39.1 ± 10.6	39.6 ± 10.9	38.2 ± 9.7
	Placebo	42.8 ± 15.3	45.7 ± 19.4	43.6 ± 15.3
Nasal secretions	Active	22.4 ± 16.1	16.1 ± 11.1	19.6 ± 11.5
	Placebo	27.4 ± 15.8	24.1 ± 18.3	23.3 ± 14.8

TABLE III. Serum antibody values of IgG (total and subclasses) to purified timothy allergen D

Antibody class or subclass	Treatment	Results in net units (±SD)		
		Dec. 1980	April 1981	Oct. 1981
Total IgG	Active	4350 ± 2255*	21917 ± 5817	15356 ± 3667
	Placebo	4303 ± 3059	6551 ± 4109 ‡	3729 ± 3580‡
IgG1	Active	0.488 ± 0.253†	1.713 ± 0.394	1.595 ± 0.406
	Placebo	0.412 ± 0.280	0.428 ± 0.294‡	0.407 ± 0.218‡
IgG2	Active	0.525 ± 0.176	1.742 ± 0.335	1.539 ± 0.211
	Placebo	0.500 ± 0.262	0.519 ± 0.246‡	0.479 ± 0.297‡
IgG3	Active	0.362 ± 0.148	1.635 ± 0.423	1.282 ± 0.335
	Placebo	0.283 ± 0.207	0.307 ± 0.203‡	0.245 ± 0.153‡
IgG4	Active	0.388 ± 0.217	2.169 ± 0.435	1.869 ± 0.607
	Placebo	0.344 ± 0.178	0.465 ± 0.271‡	0.416 ± 0.294‡

*Unit = net cpm in solid phase radioimmunoassay.
†Unit = net OD_{492} in enzyme-linked immunosorbent assay.
‡$p < 0.005$.

len concentrations, symptomatology in treated patients is worsened although diminished with respect to nontreated patients. These results lead us to believe that the threshold of bronchial reactivity in spontaneous exposure to aeroallergen is increased by IT in actively treated patients.

In contrast, the threshold of bronchial reactivity to allergen that is measured by specific bronchial provocation testing did not show any notable change during or after active IT. Both actively treated and group treated with placebo had comparable and unchanged bronchial sensitivity to grass pollen. The discrepancy between bronchial provocation testing and naturally induced symptoms suggests that provocation testing may create artificial conditions that do not mimic physiologic exposure and therefore might be unreliable in the clinical evaluation of allergic asthma.

Several differences between natural and provocative exposures can be discerned. In the natural exposure the subject inhales (for the most part) airborne pollen grains, whereas in provocation testing an aqueous and micronized extract is prepared. This physical difference may be the basis of different clinical response. Natural grass pollen grains consist of particles ranging from 20 to 50 μm in diameter that deposit at the level of the upper airways from the pharynx to the first bronchial branches. Atomization for bronchial provocation testing creates particles of much smaller diameter, which in the case of the apparatus used in this study, averages 1.3 μm in mass medium diameter.[11] Studies with radioactively labelled albumin have demonstrated that particles of such smaller diameter will deposit at the site of the sixth bronchial branch point down to the most peripheral structures including nonciliated epithelium and distal to the terminal bronchioles.[16] Busse et al.[17] demonstrated the presence of ragweed antigen in airborne particles smaller than 5 μm which suggests that these particles may also penetrate the distal bronchi and directly produce asthma. Although similar evidence has not been obtained for grass pollen, it must also be considered plausible. In any case the allergenic stimulus during bronchial provocation testing reaches the terminal respiratory airways more rapidly and deeply than airborne particles. The nonphysiologic nature of bronchial provocation testing is strongly suggested by bronchospastic reactions in nonasthmatic patients with allergic rhinitis.[18] Thus the failure to demonstrate an increased threshold for bronchial reactivity in treated patients by no means rules out this possibility in the setting of natural exposure. Several other studies have in fact been able to

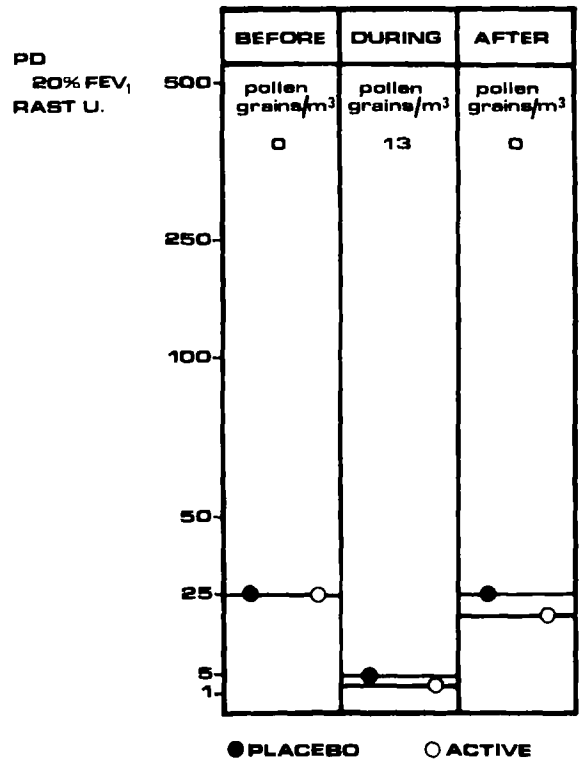

FIG. 3. Specific bronchial threshold to allergen PD 20% FEV_1 that is measured in RAST units before (Dec), during (Apr), and after (Oct) treatment of subjects with placebo *(open circles)* and actively treated *(closed circles)* subjects. The grass pollen aeroallergen concentration is indicated.

demonstrate increases in the threshold of specific bronchial reactivity after allergen IT.[19-21] Provocation testing more closely adhering to natural exposure conditions as, for example, those proposed by Connell[22] may help to answer this question in the future.

One theory of efficacy in IT emphasizes decreases in specific IgE antibodies. However, we found no variation in the level of either serum or secretory IgE antibody to timothy grass pollen in actively treated patients after 10 mo of IT with respect to controls treated with placebo. Our data and other reports on single year IT suggest that the efficacy of IT in the first year does not appear to depend upon changes in secretory or circulating specific IgE.[23-25]

Another and more widely accepted theory of mechanism of action of IT is the potential blocking role of specific-IgG antibodies, although the site and mechanism of action for such antibodies are unknown. We found that serum-IgG antibodies to timothy grass pollen were at low levels in nontreated patients with pollenosis, and no particular variations in relation to natural exposure were seen in the placebo group. In sharp contrast, at the end of the most intense period of IT, all actively treated patients demonstrated a notable increase in these antibodies with a mean IgG-antibody increase of approximately fivefold over baseline. This increase appeared to be distributed within all four IgG subclasses. A relatively selective induction of IgG_4 subclass antibodies was suggested in that a greater increase in the IgG_4 subclass relative to baseline on comparison with the IgG antibodies 1 to 3 was found. This result differs from that of Van der Giessen et al.[26] who found an increase almost exclusively of IgG_4 antibodies after grass IT. As our assay is semiquantitative, conclusions with regard to the magnitude of IgG subclass responses cannot be made. In the latter 6 mo of maintenance IT, a modest fall in IgG antibody was found in our treated patients, and this was reflected in all four IgG subclasses. This reduction in blocking antibody titer after only 6 mo of maintenance IT may indicate both a spontaneous reduction of IgG response as Norman and Lichtenstein[27] suggest and a tachyphylaxis if not overcome by an adequate and continuous stimulus. One might speculate that atopic subjects with pollenosis have a stronger tendency to synthesize IgE antibodies toward aeroallergens that does not dramatically change after specific IT and conversely have a reduced ability to synthesize IgG antibodies toward aeroallergens. This hypothesis is supported by low levels of IgG antibodies after spontaneous exposure and the relatively rapid fall after reaching maintenance IT at maximal tolerated doses. Whether these IgG antibodies provide the ameliorative effect is not clear from our study. All the treated patients improved, and all also showed increases in specific-IgG antibodies of all four subclasses, whereas no change was observed in any subject treated with placebo. IgG antibodies, and in particular IgG4, appear to be associated with the protective effect of specific IT. Much more work in investigating the biologic activity of IgG subclass antibodies in such in vitro systems as leukocyte histamine release needs to be done to explore any possible direct or indirect (e.g., immunoregulatory) protective role of IgG subclass antibodies. In the case of ragweed pollenosis, it has been observed that a relationship between IgG-class antibody response and clinical results exists, and that tolerance to the extract dose is related to the rise in IgG antibodies.[5] But it was also observed that after the first year of treatment, IgG-antibody levels remained unchanged despite further increases in the cumulative ragweed extract dose.[26] Similarly in the situation of insect venom IT, serum levels of IgG-class antibodies increase during IT,[28-30] and the passive administration of IgG antibodies has

been demonstrated to block the anaphylactic response.[31] On the other hand, some patients have had systemic reactions after sting challenge despite significant increases in specific IgG to insect venom, and conversely other patients, despite low values of specific IgG, are clinically protected to sting challenge.[32] On the basis of these and similar data, it has been suggested that immunity to insect venom may depend on critical ratios between IgG and IgE antibodies rather than absolute values of specific antibodies in either class considered independently.[30, 32-34] These considerations should also be applied to grass pollen IT and relations between IgG (in particular IgG4) antibodies to IgE should be investigated.

Finally we must consider the high incidence of local and systemic reactions in actively treated patients that receive aqueous pollen extracts. This point, which has been well emphasized in the literature, advises the physician to consider risks for every patient in deciding to embark on a course of IT on the one hand, and on the other should further stimulate preparation of allergenic extracts with reduced allergenicity and retained or enhanced immunogenicity.[34]

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REFERENCES

1. Noon L: Prophylactic inoculation for hay fever. Lancet 1:1572, 1911
2. Frankland AW, Augustin R: Prophylaxis of summer hay fever and asthma: a controlled trial comparing crude grass pollen extracts with the isolated main protein component. Lancet 1:1055, 1954
3. Patterson R, Lieberman P, Irons SS, et al: Immunotherapy. *In* Middleton E Jr, Reed CE, Ellis EF, editors: Allergy, principles and practice. St. Louis, 1978, The CV Mosby Co, p 877
4. Lichtenstein LM, Norman PS, Winkenwerder WL: In vitro studies on the role of immunotherapy in ragweed hay fever. Am J Med 44:514, 1968
5. Lichtenstein LM, Norman PS, Winkenwerder WL: A single year of immunotherapy for ragweed hay fever: immunologic and clinical studies. Ann Intern Med 75:663, 1971
6. Platts-Mills TAE, Von Maur RK, Ishizaka K, Norman PS, Lichtenstein LM: IgA and IgG anti-ragweed antibodies in nasal secretions. J Clin Invest 57:1041, 1976
7. Foucard T, Johansson SGO: Allergen specific IgE and IgG antibodies in pollen-allergic children given immunotherapy for 2-6 years. Clin Allergy 8:349, 1978
8. Tse KS, Wicher K, Arbesman CE: Effect of immunotherapy on appearance of antibodies to ragweed in external secretions. J ALLERGY 51:208, 1973
9. Nickelsen J, Goldstein S, Mueller U, Wypych J, Reisman R, Arbesman CE: Local nasal immunotherapy with aqueous and polymerized ragweed extract. J ALLERGY CLIN IMMUNOL 65:192, 1980
10. Gleich GJ, Larson JB, Jones RT, Baer H: Measurement of the potency of allergy extracts by their inhibitory capacities in radioallergosorbent tests. J ALLERGY CLIN IMMUNOL 53:158, 1974
11. Rosenthal R: Inhalation challenge: procedure, indication and techniques. The emerging role of bronchoprovocation. J ALLERGY CLIN IMMUNOL 64:569, 1979
12. Merrett TG, Houri M, Mayer ALR, Merett J: Measurement of specific IgE antibodies in nasal secretion-evidence for local production. Clin Allergy 6:69, 1976
13. Moss RB, Hsu YP, Lewiston NJ: ^{125}I-C1q binding and specific antibodies as indicators of pulmonary disease activity in cystic fibrosis. J Pediatr 99:215, 1981
14. Greenwood FC, Hunter WM, Glower JS: The preparation of ^{131}I-labelled human growth hormone of high specific radioactivity. Biochem J 89:114, 1963
15. Hsu YP, Moss RB: Microtiter immunoenzymatic assay (microELISA) for antigen-specific IgG_4 subclass antibody. Clin Rev Allergy 1:231, 1983
16. Sanchis JR, Dolovich R, Chalmers R, et al: Quantitation of regional aerosol clearance in the normal human lung. J Appl Physiol 33:757, 1972
17. Busse WW, Reed CE, Hoehne JH: Where is the allergic reaction in ragweed asthma? II. Demonstration of ragweed antigen in airborne particles smaller than pollen. J ALLERGY CLIN IMMUNOL 50:289, 1972
18. Ortolani C, Miadonna A, Adami R, Restuccia M, Zanussi C: Correlation of the specific IgE in serum and nasal secretions with clinical symptoms in atopics. Clin Allergy 11:241, 1981
19. Aas K: Hyposensitization in house dust allergy asthma. A double-blind controlled study with evaluation of the effect on bronchial sensitivity to house dust. Acta Paediatr Scand 60:264, 1971
20. Tuchinda M, Chai H: Effect of immunotherapy in chronic asthmatic children. J ALLERGY CLIN IMMUNOL 51:131, 1973
21. Taylor WW, Ohman JL, Lowell FC: Immunotherapy in cat-induced asthma. Double-blind trial with evaluation of bronchial responses to cat allergen and histamine. J ALLERGY CLIN IMMUNOL 61:283-287, 1978
22. Connell JT: Quantitative intranasal pollen challenges. I. Apparatus, design and technique. J ALLERGY 39:358, 1967
23. Osterhage F, Wipper M, Kalden JR, Deicher H: Determination of specific IgE and IgG serum antibodies during immunotherapy in hay fever patients. Z Immunitaetsforsch 153:189, 1977
24. Wide L: A RAST neutralization test for detection of blocking antibodies in serum after hyposensitization. Int Arch Allergy Appl Immunol 52:219, 1976
25. Weyer A, Doinel C, Debbia M, L'Heritier C, Rivat L, Le Mao J, Hirth C, David B: Grass pollen hyposensitization versus placebo therapy. Allergy 36:319, 1981
26. Van der Giessen M, Homan WL, van Kernebeek G, Aalberse RC, Dieges PH: Subclass typing of IgG antibodies formed by grass pollen allergic patients during immunotherapy. Int Arch Allergy Appl Immunol 50:625, 1976
27. Norman PS, Lichtenstein LM: Comparisons of alum-precipitated and unprecipitated aqueous ragweed pollen extracts in the treatment of hay fever. J ALLERGY CLIN IMMUNOL 63:180, 1979
28. Reisman R, Wypych J, Arbesman CE: Stinging insect allergy: detection and clinical significance of venom IgE antibody. J ALLERGY CLIN IMMUNOL 56:443, 1975
29. Antrach SPJ, Peterson CC, Yunginger JW: Comparison of diagnostic tests of Hymenoptera sting allergy. Ann Allergy 45:130, 1980
30. Hoffman DR, Gillman S, Cummings LH, Korak PP, Oswald A: Correlation of IgG and IgE antibody levels to honey bee

allergy with protection to sting challenge. Ann Allergy 46:17, 1981

31. Lessof MH, Sobotka HY, Lichenstein LM: Effects of passive antibody in bee venom anaphylaxis. Johns Hopkins Med J 142:1, 1978
32. Golden DBK, Meyers DA, Kagey-Sobotka A, Valentine MD, Lichtenstein LM: Clinical relevance of the venom-specific immunoglobulin G antibody level during immunotherapy. J ALLERGY CLIN IMMUNOL 69:489, 1982
33. Reisman R, Arbesman CE, Carrell M: Observation of the etiology and natural history of stinging insect sensitivity: application of measurement of venom-specific IgE. Clin Allergy 9:303, 1979
34. Norman PS: An overview of immunotherapy: implications for the future. J ALLERGY CLIN IMMUNOL 65:87, 1980

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