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Attenuation of allergen sensitivity early in the course of ragweed immunotherapy

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*During the course of an open immunotherapy (IT) study of ragweed (RW)-allergic patients, nasal mediator release was studied by provocation testing. All subjects had a history of seasonal RW rhinitis, positive skin puncture test to RW, and RW-specific IgE by RAST. Nasal challenge was performed with serial dilutions of RW extract, before and after 12 weekly injections, providing a cumulative dose of 0.22 µg of Amb a 1. Serum IgE and IgG and basophil histamine release with RW were also measured. By 12 weeks of IT, when only 1% of the usual maintenance level dose had been administered, mean histamine release and TAME-esterase activity in nasal washes decreased significantly ($p < 0.05$ and $p < 0.01$). Prostaglandin D_2 release did not change. Skin sensitivity decreased ($p < 0.05$), whereas RW-specific IgE increased ($p < 0.05$). No significant change in basophil histamine release was observed for RW or a control antigen. Only six of 40 subjects had an RW-specific IgG rise >0.05 µg/ml. Changes in nasal sensitivity did not correlate with the increases in IgE or IgG or with the change in skin test sensitivity. These present data indicate that there is a significant decline in nasal sensitivity to inhaled RW very early in the course of IT. There is, however, no indication of a relationship between the decreased nasal sensitivity and the production of RW-specific IgG antibodies (*J ALLERGY CLIN IMMUNOL* 1989;84:390-9.)*

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Most studies of the effect of IT in patients with rhinitis have focused on the clinical and immunologic response to the highest tolerated dose or a certain defined maintenance dose. Several studies have demonstrated that the clinical efficacy of the treatment increases progressively with increasing dose.¹⁻³ There has, however, been little evaluation of the early course of IT, the biologic effects of which are poorly understood. The aim of this open study was to investigate early biologic effects of RW IT in subjects with RW-induced rhinitis. We have previously established a nasal-challenge model of airway reactivity⁴ to evaluate

Abbreviations used

IT:	Immunotherapy
RW:	Ragweed
Amb a I:	<i>Ambrosia artemisiifolia</i> (eliator) I (formerly ragweed antigen E)
BHR:	Basophil histamine release
ED ₅₀ :	Allergen concentration required to induce a 30% histamine release
TAME:	<i>N</i> - α -P-tosyl-L-arginine methyl ester
PGD ₂ :	Prostaglandin D ₂
RW IgE:	Ragweed-specific IgE
RW IgG:	Ragweed-specific IgG
SPT:	Skin puncture test

the clinical and immunologic consequences of aeroallergen IT.⁵ During nasal challenge with RW, inflammatory mediators in nasal lavages were lower in patients who had received RW IT for 3 to 5 years compared to untreated subjects matched for RW sensitivity.⁵ In particular, TAME-esterase activity, which is largely due to mast cell tryptase and a plasma kallikrein/ α -macroglobulin complex,⁶ was lower in the nasal wash of treated subjects. We have now applied this nasal-challenge technique to evaluate effects on nasal allergen challenge early in the course of RW IT. After 12 weekly injections, when the cumulative RW dose administered was only 0.22 μ g of Amb a I, which is about 1% of the expected maintenance dose, we, nevertheless, found significant decrease in histamine release and TAME-esterase activity in nasal secretion during nasal provocation. This desensitization effect could not be accounted for by IgE and IgG antibody fluxes.

MATERIAL AND METHODS

Patients

Twenty-one adults (aged 22 to 40 years) and 22 children (aged 7 to 14 years) were recruited by advertisement from the general population of Baltimore. Seventeen females and 23 male subjects completed the study. Three patients withdrew during the first 2 months because of lack of compliance. All patients had a history of rhinitis during the RW season for at least the 2 previous years. None had previously received IT. The study was approved by The Johns Hopkins Joint Committee on Clinical Investigation, and all subjects (including parents of minors) gave written informed consent. Most patients also had perennial rhinitis symptoms or symptoms during the spring-pollen season. Two adults and 13 children had symptoms of allergic asthma. The diagnosis of RW rhinitis was confirmed by SPT and RAST. All patients had a wheal reaction to RW extract similar to (3+) or larger than (4+) the wheal produced by histamine chloride (10 mg/ml).

Study protocol

Before therapy was started, all patients were evaluated by screening SPTs with a panel of common inhalant allergens and skin puncture titrations with ragweed, and when this was possible, a control allergen (cat, dog, or cockroach) not included in IT. Nasal challenges with incremental increases in RW-antigen concentration were performed, and the serial dosing was stopped only if the patient had more than five sneezes. Blood was drawn for RW IgE and RW IgG, BHR to RW, and a control allergen. An identical nasal challenge was performed, and skin tests and serum antibody measurements were repeated after 12 weekly injections when each patient had received a cumulative RW dose equivalent to 0.22 μ g of Amb a I.

Allergen extracts

The short RW extract provided by ALK (Copenhagen, Denmark) was a partly purified, well-standardized extract containing 80 μ g/ml of Amb a I. The same lot of extract was used for all skin tests, nasal challenges, specific IgE and IgG assays, and BHR. Other partly purified and standardized extracts from ALK were used for skin testing and IT. The panel included a three-grass mixture of *Lolium perenne* (ryegrass), *Phleum pratense* (timothy), and *Dactylis glomerata* (orchard grass), as well as extracts of *Plantago lanceolata* (English plantain), *Cynodon dactylon* (Bermuda grass), *Quercus alba* (white oak), a 50/50 mixture of *Dermatophagoides pteronyssinus* and *D. farinae*, *Alternaria*, *Cladosporium*, *Aspergillus*, and cat. A dog extract from ALK, and a crude cockroach extract from Hollister-Stier, Spokane, Wash., and were used for skin testing and BHR, but not for IT. The crude tree-pollen extracts, white ash, silver and red maple, birch, and American elm were obtained from Hollister-Stier.

Skin tests

An SPT with each of the 17 extracts mentioned above in a 50% glycerinated diluent was used on the first visit to confirm RW sensitivity and to evaluate sensitivity to other common allergens. In addition, skin test sensitivity to RW was quantitated by serial puncture testing with five half log dilutions of the RW extract (80, 24, 8, 2.4, and 0.8 μ g/ml of Amb a I) in duplicate. A skin titration was, when this was applicable, also performed with an allergen of minor clinical importance to which the patient had a positive reaction (>2 mm) on the screening test but which was not to be included for therapy. The highest concentration used for the control skin test was arbitrarily named 10 U/ml for all extracts. All skin tests were performed on the volar side of the forearm as SPTs with a Wyeth needle (Wyeth Laboratories, Inc., Philadelphia, Pa.), as previously described.⁷ Histamine chloride (10 mg/ml) was used as a positive control, and the glycerinated diluent (0.3% human serum albumin in phenol saline) as a negative control. The wheal reaction was outlined on the skin after 15 minutes, and the outline was transferred to a squared millimeter paper with plastic tape. The average diameter of the wheal was calculated by measuring the largest diameter, determining its orthogonal perpendicular, and dividing the sum by 2. The

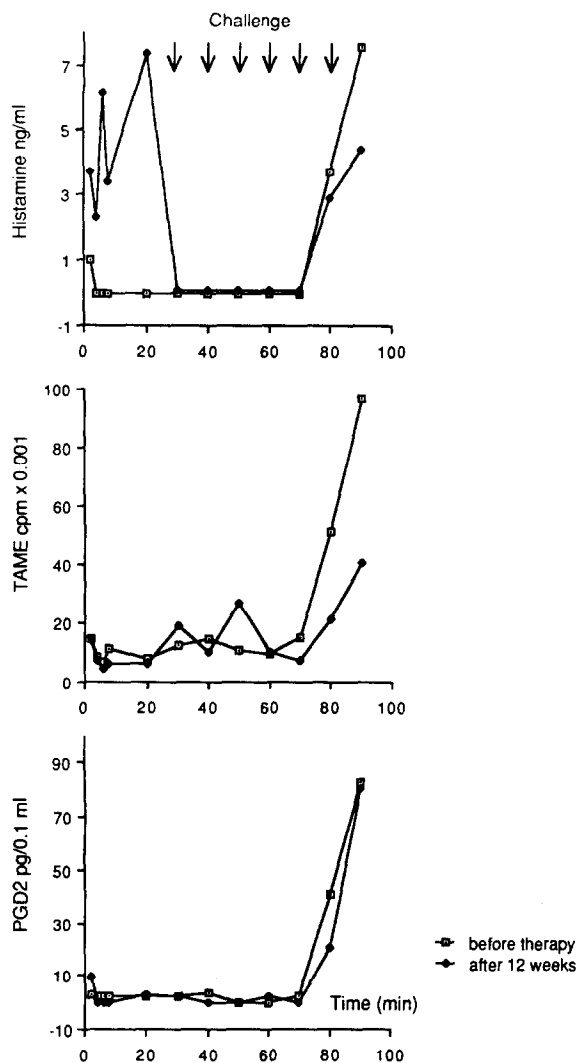


FIG. 1. An example of mediator release in nasal secretions in one subject during nasal challenges with six tenfold-increasing concentrations of RW antigen (0.00008 to 8 $\mu\text{g/ml}$ of *Amb a 1*) before and after 12 weekly RW injections. The first six points represent prechallenge nasal washouts and the control wash (diluent); before therapy (— $\square$ —); after 12 weeks (— $\blacklozenge$ —).

results are presented as the concentration of *Amb a 1* ($\mu\text{g/ml}$) or control allergen (units per milliliter) that induced a wheal reaction of the same size as the wheal produced by histamine.

Nasal challenge

Identical challenges were performed once during February or March and once in June or July, both before the RW season. The subjects were free of symptoms at the time of the challenge. The protocol followed has been extensively described before.⁴ Briefly, the challenge was started with four prewashes with instillation of 2.5 ml of normal saline into each nostril while the patient tilted his head 30 degrees from the horizontal in a sitting position. The saline-secretion mixture was recovered and stored on ice. The patient was

then administered oxymetazoline hydrochloride nasal spray (Afrin; Schering Corp, Kenilworth, N.J.) to avoid extensive congestion during the allergen challenge. Diluent was thereafter administered, then tenfold increasing doses of the RW extract (8×10^{-5} $\mu\text{g/ml}$ to 8 $\mu\text{g/ml}$ of *Amb a 1*). The diluent and 0.1 ml of the different concentrations of RW extract were instilled into the right nostril from a tuberculin syringe. Ten minutes after the administration of the oxymetazoline hydrochloride nasal spray (Afrin), the diluent, and each challenge dose, a nasal lavage was performed, as described above. The wash after the diluent was considered the control wash. The first challenge was stopped when the subject reacted with five or more sneezes or was otherwise continued until the highest challenge dose (8 $\mu\text{g/ml}$ of *Amb a 1*) was administered. Identical challenges, regardless of the symptomatic response, were performed again after 12 weekly injections. The samples were centrifuged at 800 g for 15 minutes to separate the gel from the sol phase. For histamine determinations, 0.8 ml of the sol phase was mixed with 0.2 of 8% HC104 and stored at -20°C until analysis. For TAME-esterase activity and PGD_2 sol phase, samples were stored at -20°C until analysis.

Mediator assays

Histamine. The concentration of histamine was determined by an automated fluorometric technique, which is sensitive to approximately 1 ng/ml with a coefficient of variation of 20% to 30% at the 1 to 2 ng level.⁸

TAME-esterase. The TAME-esterase activity was determined according to the method of Imanari et al.⁹ which is based on the liberation of tritiated methanol from the synthetic substrate ^3H -TAME. The sensitivity of the method was 1000 cpm.

PGD_2 . PGD_2 was measured by a competitive radioimmunoassay sensitive to 2 pg/0.1 ml, as previously described.¹⁰ The cross-reactivity of the antiserum was $<1\%$ with PGE_1 , PGE_2 , thromboxane B_2 , 6-keto- $\text{PGF}_{1\alpha}$, and $\text{PGF}_{2\alpha}$.

RW-specific IgE. The RW extract was coupled to agarose with a cyanogen bromide method, as described previously.¹¹ The allergosorbent was standardized by reciprocal plot analysis to determine optimal concentrations for use in the antibody assay by RAST. The concentration of IgE in patient's serum was determined by interpolation from an RW reference—serum curve previously standardized in nanograms per milliliter by RAST-elution technique.¹² IgE determinations were undertaken in three dilutions to detect and correct for IgG interference.¹³ Samples from before therapy and after 3 months were analyzed within the same assay.

RW IgG. Samples were analyzed with the *Staphylococcus aureus* protein A solid-phase radioimmunoassay, which has previously been described,¹² and the results were expressed in micrograms per milliliter with a calibrated reference serum. All samples were analyzed within the same assay in duplicates. Assay sensitivity was 0.1 $\mu\text{g/ml}$ corrected to undiluted serum.

BHR.^{14, 15} Whole blood was allowed to sediment in 20% dextran, and the leukocyte-rich plasma was removed and centrifuged at 800 g. The resulting pellet was washed in *N, N'*-bis[2-ethanesulfonic acid]-buffered saline without

TABLE IA. Mediator release and number of sneezes after the highest challenge dose of RW used before therapy and at the equivalent challenge dose after 12 weekly injections in 40 subjects

Mediator	Before therapy	After 12 weeks	% Change	<i>p</i> *
Histamine ng/ml	3.5 ± 1.3 (0.9)	1.2 ± 0.4 (0)	-66	<i>p</i> < 0.05
TAME esterase cpm × 10 ⁻³	37.4 ± 5.6 (32.4)	22.3 ± 5.4 (8.2)	-40	<i>p</i> < 0.01
PGD ₂ pg/0.1 ml	13.4 ± 3.0 (7.4)	13.3 ± 3.6 (2.7)	-1	NS
Sneezes No.	5.1 ± 0.8 (4.5)	3.6 ± 0.8 (2.0)	-29	NS

NS, not significant.

Mean ± SEM; medians within parentheses.

*Statistics by Wilcoxon paired signed-rank test.

Ca⁺⁺. For testing, cells were resuspended in *N*, *N'*-bis[2-ethanesulfonic acid] buffer with Ca⁺⁺. Dose-response curves more than a 6 log range for RW antigen and the control allergen (an allergen not included in the therapy) were performed with the washed leukocytes from 20 cc of blood. Dose-response curves were constructed by plotting the percentage of histamine release versus the concentrations of *Amb a* I and the control allergen. The results were expressed as ED₅₀.

IT

IT was administered as 12 weekly injections, starting with a single dose of 0.00013 µg of *Amb a* I, progressing to a dose of 0.11 µg. IT with RW was administered simultaneously with other allergens, chosen according to standard clinical criteria, including history, skin test, and RAST. A maximum of five different allergens were administered to each subject. Six of the subjects had five allergens included in their therapy, seven had four allergens, eleven had three and two allergens, respectively, and five subjects were only administered RW IT. The most common allergens added to the RW therapy were the grass mix (53%) and dust mite (38%).

Statistics

Wilcoxon signed-rank test was used to compare changes related to therapy. The Spearman rank-correlation coefficient was calculated to evaluate the relationship between variables. Fisher's exact test was used to evaluate the relationship between dichotomized variables.

RESULTS

Nasal challenge

Before therapy, 28 subjects reacted with sneezes at the respective highest challenge dose, whereas 12 subjects reported symptoms like stuffy and/or runny nose. The latter subjects had reached the highest challenge dose (8 µg/ml of *Amb a* I), as had 13 of the "sneezes." After 12 weeks of IT, 23 subjects still

sneezed after an identical challenge, 13 reported other symptoms, and four subjects remained symptom free during the whole challenge.

An example of the response to a nasal challenge before therapy and after the 12 weekly injections in one patient is illustrated in Fig. 1. Both during the challenge, before, and after the 12 weekly injections, this patient became symptomatic only at the highest dose of allergen (8 µg/ml of *Amb a* I). At this dose, histamine rose from <1 ng/ml to 8 ng/ml before IT, but only to 4.5 ng/ml in the second challenge 12 weeks later. TAME-esterase activity also rose less after IT, whereas the PGD₂ response was similar both times.

At a challenge dose equivalent to the highest pre-treatment challenge dose, 19 subjects had a decrease in histamine concentration ranging from 0.4 to 33 ng/ml, whereas 10 subjects did not release histamine before or after the treatment period, and 10 subjects had a rise in their histamine concentrations (*p* < 0.05 for the whole group) (Table IA). A decline of TAME-esterase activity >50% was observed in 24 of 40 patients after 12 weeks of therapy. The mean percent decrease for the whole group was 40%, which was significant (*p* < 0.001). Fourteen patients had a fall of the PGD₂ concentration by >2 pg/0.1 ml (2 to 60 pg/0.1 ml) and 14 had an increase of their PGD₂ release. The change for the whole group was not significant. The mean change in mediator release and the change in individual patients of the respective mediators before and after the 12 weekly injections is presented in Table IA and IB.

The cumulative percent of patients who had significant nasal mediator release at each respective challenge dose before and after the 12 weekly injections is illustrated by Fig. 2. A significant response was defined as an increase of the histamine level to

TABLE IB. Mediator release in individual subjects before (B) and after 12 weekly injections (A)

Subject No.	TAME (cpm $\times 10^{-3}$)		Histamine (ng/ml)		PGD ₂ (pg/0.1 ml)	
	B	A	B	A	B	A
1	0	66.5	0	4.8	0	62.4
2	31.3	18.6	0	1.0	0	2.3
3	33.0	4.4	2.0	0	24.7	0
4	36.4	0.3	32.0	0	31.5	0
5	93.9	1.2	0.9	0	10.7	0
6	40.6	9.2	0.4	0.4	13.2	0
7	60.4	50.8	1.0	0	17.4	33.6
8	63.8	6.6	2.2	0	0	0
9	62.5	16.4	0.9	0	60.4	15.2
10	55.8	3.1	4.7	0	23.4	0
11	11.7	2.8	0	0	0	0
12	29.7	0.7	2.4	0	10.9	0
13	12.0	3.9	0.4	0	0	0
14	102.0	99.0	0	0	13.7	26.4
15	9.4	0	0	0	0	0
16	24.0	2.1	0	0	0	0
17	42.5	10.8	0	0	0	0
18	56.5	62.0	1.8	2.3	25.6	29.4
19	0	0	0	0	0	4.4
20	11.0	6.7	0	0	0	4.2
21	12.5	8.5	1.0	0	0	0
22	43.6	0	0	0	42.3	8.4
23	2.0	8.1	0	0	0	0
24	38.0	0	1.3	0	8.3	6.3
25	18.5	53.0	0	2.3	6.5	36.2
26	51.3	13.5	0	0.8	0	0
27	31.7	14.8	33.0	0	0	2.9
28	38.7	68.8	1.0	7.6	17.6	13.8
29	51.9	1.8	29.8	0	2.6	4.5
30	3.6	0.8	0	0	6.5	0
31	22.0	19.8	1.7	2.6	63.8	2.4
32	82.1	30.9	7.6	4.4	79.8	80.3
33	0	4.5	0	0.35	1.8	5.7
34	192.0	98.0	8.3	1.2	0	12.6
35	10.2	5.8	1.1	0	19.8	8.0
36	6.8	157.0	0	14.8	8.7	85.9
37	24.6	8.3	1.4	0	0	0
38	37.1	0	2.0	1.5	24.4	0
39	36.6	17.2	0	1.7	12.8	26.3
40	14.5	15.9	3.9	1.9	9.6	58.9

>1 ng/ml above the control wash, a threefold increase in TAME-activity from the control wash, and a PGD₂ increase of >3 pg/0.1 ml above the control wash. Ninety percent (36 of 40 patients) had a significant TAME-esterase activity before treatment and 61% after 12 weeks. Sixty-three percent (25 of 40) had a significant histamine release before therapy and 47% after. By chi-square analysis, the distribution of the TAME and histamine responses to escalating chal-

lenge doses was significantly different after 12 weeks of IT ($p < 0.05$ for both). The PGD₂ response was not affected by the therapy, 23 (55%) of the patients had a PGD₂ response before, and 20 patients after. There was a significant correlation (Spearman rank) between the decrease in TAME activity and the decrease of the histamine and PGD₂ release ($r = 0.63$; $p < 0.001$ and $r = 0.44$; $p < 0.01$, respectively). There was a weak correlation between the change of

the histamine and the PGD_2 release ($r = 0.28$, $p < 0.05$).

Skin test

Skin sensitivity to ragweed decreased in 28 of the 40 subjects (Fig. 3) compared with a decrease in 12 patients for the control (nontreatment) allergen (Fig. 3). The concentration of *Amb a* I required to produce a 3+ skin reaction increased from a mean of 26.3 $\mu\text{g}/\text{ml}$ to 36.7 $\mu\text{g}/\text{ml}$ ($p < 0.05$), whereas the concentration of the control allergen required to elicit a 3+ skin test reaction did not change (Table II). The size of the wheal produced by histamine did not change during IT (mean 3.0 mm before and 3.2 mm after).

RW IgE

The mean pretreatment level of serum IgE *Amb a* I antibody was 200 ng/ml (median, 29.0 ng/ml). During the course of IT, RW IgE increased an average of 14% to 227 ng/ml (median, 42.5 ng/ml; $p < 0.05$) (Table II).

RW IgG

Low levels of RW IgG were detectable (>0.1 $\mu\text{g}/\text{ml}$) in 11 of the 40 patients at baseline (mean, 0.23 $\mu\text{g}/\text{ml}$; median, 0). After 12 weeks of therapy, only 10 patients had an increase of >0.5 $\mu\text{g}/\text{ml}$. Mean level rose to 0.48 $\mu\text{g}/\text{ml}$ (median, 0.27 $\mu\text{g}/\text{ml}$; $p < 0.05$) (Table II; Fig. 4).

BHR

Before therapy was started, the median concentration of RW required for a 30% BHR (ED_{30}) was 0.8 $\mu\text{g}/\text{ml}$ of *Amb a* I, and after therapy, 0.12 ng/ml (Table II). Six subjects (four children and two adults) had $<20\%$ BHR to RW antigen. After 3 months of therapy, four of these subjects still did not have 20% BHR at the highest allergen dose. Altogether basophil sensitivity to RW decreased in 15 patients but increased in 20 patients, and did not change in five patients. There was thus no significant change in BHR as a result of the therapy, nor was there any change of BHR to the control allergen.

Correlations

Correlations between changes in nasal sensitivity, as measured by TAME-esterase activity in nasal-challenge wash fluids, and the changes in skin sensitivity, RW IgE, RW IgG, or BHR are presented in Table III. There was no significant correlation by Fisher's exact test between any of the immunologic parameters and the decline in TAME-esterase activity during the nasal provocation. Furthermore, the Spear-

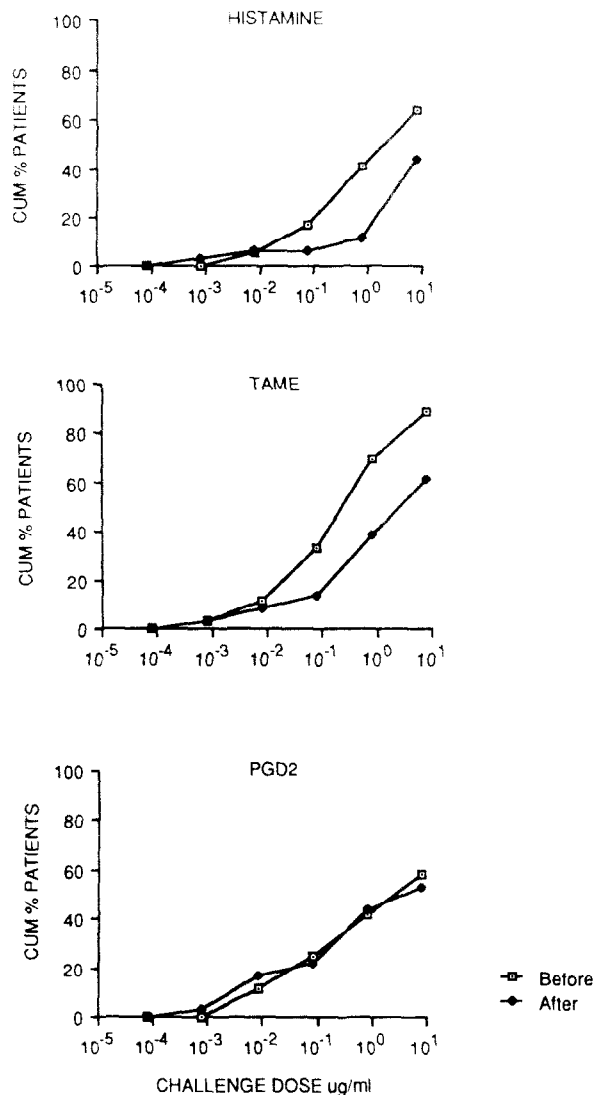


FIG. 2. The cumulative percent of subjects demonstrating mediator release at each challenge dose (for definition of response, see text) before and after 12 weekly injections of RW antigen. Challenge-dose concentrations ranged from 0.00008 to 8 $\mu\text{g}/\text{ml}$ of *Amb a* I. By chi-square analysis, the percentage of patients responding at each challenge dose with TAME-esterase activity and histamine release was greater before than after the 12 injections, $p < 0.05$ (for histamine, $n = 0/0/2/4/10/9$ before and $n = 0/1/1/0/2/13$ after therapy at each respective dose; for TAME, $n = 0/1/4/9/14/8$ before and $n = 0/1/2/2/10/9$ after therapy; for PGD_2 , $n = 0/0/4/5/7/7$ before and $0/1/4/2/9/4$ after 12 weekly injections); before therapy (—○—); after 12 weeks (—●—).

man rank-correlation test also revealed no significant correlations between the change in mediator release of any of the mediators and changes in immunologic measurements. Likewise there was no significant correlation between the changes in RW IgE, RW IgG, BHR, or skin sensitivity.

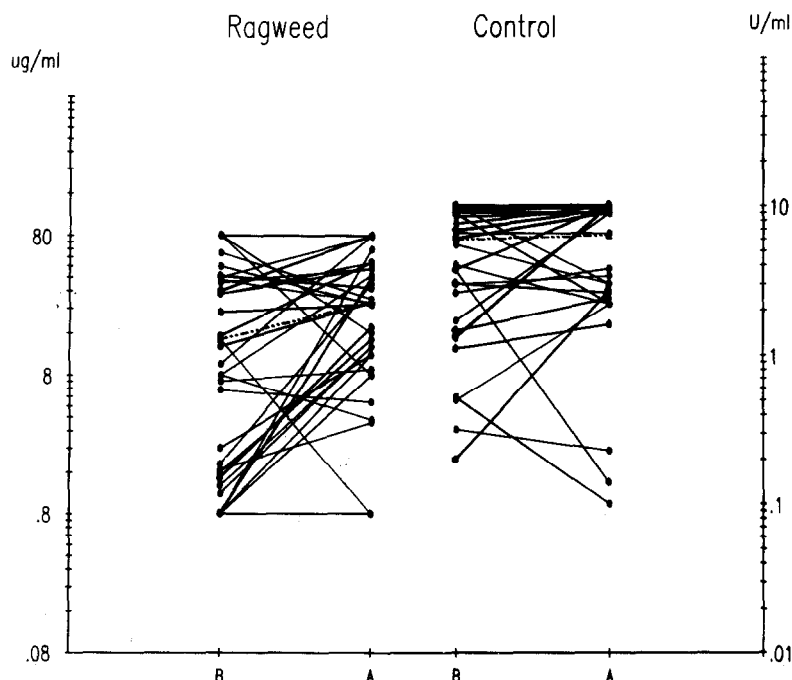


FIG. 3. Skin test sensitivity to RW in 38 RW-treated patients and to a control (nontreatment) allergen in 29 of these subjects before (B) and after 12 weekly injections (A). Sensitivity was expressed as the concentration of *Amb a 1* (micrograms per milliliter) or control allergen (units per milliliter) required to induce a wheal of the same size as that produced by histamine. Cat, dog, and cockroach extracts served as control allergens. Medians are indicated.

TABLE II. Immunologic effects of a cumulative dose of 0.22 μ g of *Amb a 1*

	No. subjects	Before therapy	After 12 weeks	<i>p</i> *
SPT-RW (log μ g/ml)	38	0.26	0.52	<i>p</i> < 0.05
SPT-co (log U/ml)	29	0.80	0.99	NS
ED ₃₀ RW (ng/ml)	40	0.8	0.12	NS
RW IgE (ng/ml)	38	29.0	42.5	<i>p</i> < 0.05
RW IgG (μ g/ml)	38	0	0.27	<i>p</i> < 0.05

NS, Not significant.

Skin test sensitivity (SPT-RW and SPT-co [control allergen]) is expressed as the log extract concentration of *Amb a 1* and control allergen required to produce a wheal of the same size as the wheal produced by histamine. BHR is presented as ED₃₀ RW (the *Amb a 1* concentration needed to induce a 30% histamine release). Medians are given.

*Statistics by Wilcoxon paired signed-rank test.

DISCUSSION

We have used a nasal-challenge model of allergic rhinitis to evaluate the clinical effect of IT in an objective way.⁴ Several controlled studies have reported decreased nasal sensitivity in patients receiving IT for allergic rhinitis.¹⁶⁻¹⁸ However, most of the posttreatment nasal provocation testing has been performed only after the subjects have reached the maintenance dose.

One attempt to measure early in vivo effects was made by Osterballe¹⁶ in a grass-pollen IT study. Nasal

challenges were performed after 8 weeks of therapy when the treatment dose administered was about 25% of the maintenance dose. Nasal sensitivity was assessed by number of sneezes, fall in nasal peak flow, and amount of nasal secretion after nasal provocation with tenfold increasing doses of allergen. No significant decrease of nasal sensitivity was found after 8 weeks of therapy. Dantzer et al.¹⁸ studied RW-treated patients in whom nasal challenges were performed after 1, 3, and 6 months of therapy. No significant change in nasal sensitivity measured by nasal airway

resistance or sneezing was found after 1 or 3 months (halfway, dose wise, to maintenance level treatment).

In contrast to these studies, we were able to demonstrate a significant decline in mediator release in nasal secretion achieved after 12 weeks of IT, building up to a single dose of 0.11 μg of *Amb a* I (about 1% of the target single dose at maintenance). Our positive results may reflect the increased sensitivity of our nasal-challenge model that used nasal-lavage mediators as criteria, rather than measurements of nasal airway resistance, symptom scores, or number of sneezes.

The present study did not include a nontreated or placebo-treated control group, and it is therefore possible that the observed changes in nasal sensitivity were due to experimental variability. However, we have previously examined the reproducibility of our nasal challenge model.¹⁹ We found that the mean TAME-esterase activity and the mean release of histamine in identical challenges in eight subjects about 2 weeks apart were not different. In another study involving 26 subjects, there was no significant change of the release of TAME-esterase activity and histamine when challenges were repeated 2 to 5 months apart.⁵ A third ongoing study involving two identical challenges of 10 grass-pollen allergic, symptom-free subjects has also failed to demonstrate any significant change of mediator release in untreated subjects. It is therefore unlikely that the decline in mediator release that we observed can be attributed to experimental variation.

A tentative explanation of the decrease of nasal sensitivity to RW could be that the second challenge was performed later after the RW season than the first challenge. However, almost half the subjects (16) were also allergic to grass pollen. Their second challenge was done during the grass-pollen season, and it would thus be expected that they would be primed to an increased nasal sensitivity. However, 14 of the subjects had lower TAME activity and 12 had lower or unchanged histamine levels. The perennial allergies, for instance, toward dust mite, could also affect the mast cell sensitivity. There are, however, two sides to that phenomenon. The subjects could either have become less sensitive by the dust mite part of the therapy or they could be considered as primed by a continuous exposure to the allergen. In either case, nine of 13 dust mite-allergic subjects improved, whereas four subjects were more sensitive on the second challenge. IT with multiple allergens was administered to 36 of the 40 subjects.

The question could therefore be raised whether multiple allergen treatment might accelerate nasal mast cell desensitization nonspecifically. In a study by Lowell and Franklin,²⁰ multiple allergen treatment was

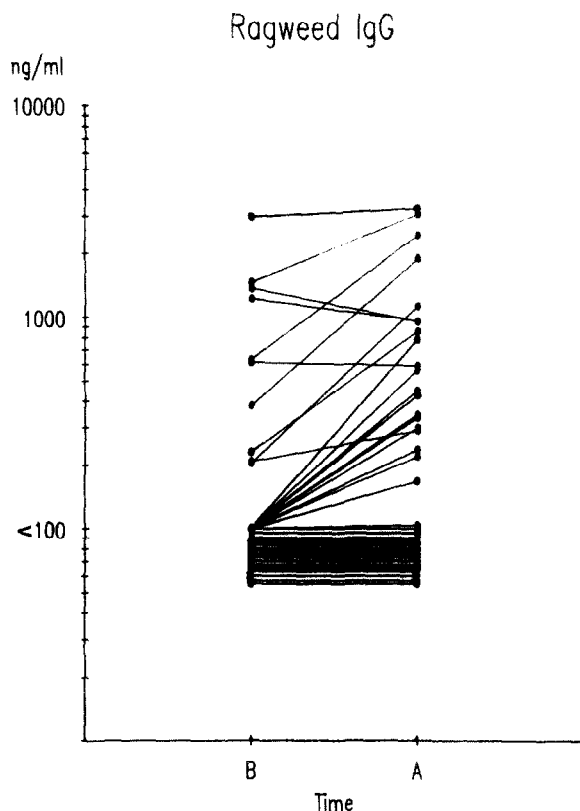


FIG. 4. Serum RW IgG in 38 subjects before therapy (B) and after 12 ragweed injections (A). Note that 17 patients did not have detectable levels of RW IgG before or after 12 weeks of therapy.

administered with or without RW. The patients whose RW treatment was withheld reported significantly more severe symptoms during the RW season than patients who were administered the RW extract, suggesting that treatment with other allergens did little for RW-induced symptoms. The question of the clinical and immunologic specificity of RW IT has also been addressed by Norman and Lichtenstein.²¹ They reported that treatment with RW-pollen extracts had little or no effect on grass-pollen symptoms. Admittedly, effects measured by symptom scores during a pollen season are probably less sensitive than measurements of mediator release during nasal challenge. Therefore, clinical evaluations may not reflect more subtle effects of therapy on mast cell function. As of now, there is little indication that treatment with one allergen influences mast cell responsiveness to another allergen. The question, however, needs further investigation.

Another observation that deserves comment is the discrepancy between the findings with histamine and PGD₂ release. We believe this to be a result of the PGD₂ assay being less sensitive than the histamine assay. In two thus far unpublished studies done by us,

TABLE III. Correlation between nasal sensitivity measured by TAME-esterase activity in nasal secretion and immunologic indices

	RW skin test sensitivity		BHR ED ₃₀ RW		RW IgE		RW IgG	
	Dec	Inc	Dec	NC or Inc	Dec	NC or Inc	Inc	NC
Nasal sensitivity								
Dec	24	6	11	20	9	21	6	22
Inc	4	5	4	5	3	5	4	6
Fisher's exact test								
<i>p</i>	>0.1		>0.5		>0.5		>0.5	

Dec, Decrease; *Inc*, increase; *NC*, no change.

Decrease of nasal sensitivity was defined as before TAME-after TAME >0; of skin sensitivity, as requirement of a higher concentration of *Amb a I* to produce a 3+ reaction; of RW IgE, as before IgE-after IgE >0. Increase of RW IgG was defined as a rise >0.5 µg/ml.

we have found the PGD₂ levels to decrease after 6 to 12 months of RW IT in comparable groups of patients.

We also observed an early significant decline in skin test sensitivity that was specific for the treatment allergen (RW), whereas the sensitivity did not decrease to the control allergens not included in IT. A decline of skin sensitivity was also found at 3 months in a study of IT in cat- and dog-allergic subjects; however, most subjects had reached maintenance at the time of the repeat skin test.²² Osterballe¹⁶ found decreased skin sensitivity among patients receiving grass-pollen IT, but the skin tests were repeated only after subjects reached their maintenance dose. Dantzler et al.¹⁸ found an attenuation of skin responsiveness when subjects were halfway to their target maintenance dose.

It is not clear why doses of RW as low as 0.1 µg of *Amb a I* should result in desensitization of nasal and skin mast cells. Lichtenstein and Osler¹⁴ and also Mendoza and Minigawa²³ have reported that human basophils can be desensitized in vitro by exposure to subthreshold doses of antigen. This in vitro phenomenon can be observed as analogous to the decreased skin and nasal sensitivity that we report.

We have previously reported in detail IgE and IgG antibody fluxes throughout a course of RW IT.²⁴ We found that the mean threshold dose of *Amb a I* required for an antibody response was 0.13 µg for IgE and 0.56 µg for IgG. The present findings are in good agreement with our previous results, since after the single dose of 0.11 µg of *Amb a I*, there was an RW IgE response in 27 of 38 patients, whereas only 10 patients had a significant RW IgG rise.

The relationship between antibody fluxes and the clinical response has been addressed in numerous

studies. In Hymenoptera-venom IT, the protective role of IgG antibody is clear,²⁵ but in aeroallergen disease, the role of IgG in clinical relief is questionable. Patients receiving pollen^{1, 17, 26} and animal dander²² IT and who are high responders for IgG antibody appear to fare better than patients who are low antibody responders. Conversely, low-dose IT regimens that do not produce a significant IgG-antibody response have frequently been found to be clinically ineffective.²⁷ It is thus remarkable that in the present study in vivo effects indicating partial nasal desensitization were found before most patients produced an IgG response. The significance of this observation was further emphasized by the fact that most subjects had a rise in their serum RW IgE levels. Therefore, an increase rather than a decrease in nasal sensitivity at this stage of IT might be expected. The discrepancy observed in this study between depressed mast cell responsiveness in both the nose and skin and an increase of RW IgE suggests that cellular desensitization may occur early in IT independent of fluxes in allergen-specific IgE and IgG antibodies. Also noteworthy in this regard is the lack of correlation between the IgG (and IgE) responses and the attenuation of nasal sensitivity. This finding could be due to the IgG assay not being sensitive enough to detect low levels of IgG that, nevertheless, may exert a "protective" effect. This is unlikely, however, since low, but detectable, IgG levels occur frequently in nontreated patients who may often have severe clinical symptoms.

We thus conclude that there is a significant decline in nasal sensitivity very early during the course of RW IT that can be demonstrated by a decrease of histamine release and TAME-esterase activity in nasal secretion during repeated nasal challenges. There is no evidence

of a relationship between this biologic sensitivity change and the production of RW IgG.

Further studies of low-dose IT that include placebo-treated control groups are needed to confirm our observations and extend them to include assessments of clinical efficacy.

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