

Responses to ragweed-pollen nasal challenge before and after immunotherapy

**Peter S. Creticos, MD, David G. Marsh, PhD, David Proud, PhD,
Anne Kagey-Sobotka, PhD, N. Franklin Adkinson, Jr., MD,
Linda Friedhoff, MA, Robert M. Naclerio, MD,*
Lawrence M. Lichtenstein, MD, PhD, and Philip S. Norman, MD**
Baltimore, Md.

To evaluate whether immunotherapy reduces mediator release after nasal challenge, we followed previously untreated patients with ragweed hay fever through 2 years (three seasons) of treatment. Eleven adult patients started immunotherapy after a season of symptom diaries and graded pretreatment challenges with 0.03, 0.3, 3.3, and 16.5 mg of ragweed pollen. Repeat challenges were performed when the treatment dosage reached 0.6, 12.4, and 24.8 μ g of Amb a 1 (antigen E) equivalents per injection. After the 0.6 μ g dose, there was little change, but after the larger doses, there was a significant reduction in histamine and tosylarginine methyl ester-esterase (TAME-esterase) release in two respects. More pollen was required before any mediator appeared, and the amount of mediator released at each stage of the challenge was reduced. There was no significant difference between the responses at the 12.4 and 24.8 μ g treatment doses. Sneezing after challenge was not apparently changed after immunotherapy; however, patients' seasonal symptom-medication scores were reduced after treatment. These data set the optimal individual treatment dose of ragweed extract for immunotherapy at >0.6 μ g, but probably not more than 12.4 μ g in terms of Amb a 1 equivalents. (J ALLERGY CLIN IMMUNOL 1989;84:197-205.)

Immunotherapy (desensitization) for allergy to airborne pollens, molds, and inhalants is extensively used in therapy, and its efficacy has been proved in numerous clinical studies.¹ The mechanism of action has been difficult to study because clinical responses assessed by the standard clinical method of symptom diaries not only are poorly quantified but also are subject to interference by several environmental factors. Diary scores are therefore difficult to correlate with specific quantitative measurements of immuno-

Abbreviations used

PNU: Protein nitrogen unit
TAME: Tosylarginine methyl ester
ANOVA: Analysis of variance
Ag E: Antigen E

From the Clinical Immunology Division, Department of Medicine, The Johns Hopkins University and the Good Samaritan Hospital, Baltimore, Md.

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Reprint requests: Peter S. Creticos, MD, Clinical Immunology Division, The Johns Hopkins University at The Good Samaritan Hospital, 5601 Loch Raven Blvd., Baltimore, MD 21239.

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logic response. Furthermore, the immunologic response is complex, involving lymphocyte responses to antigen,²⁻⁶ serologic responses in three classes of antibodies, IgE, IgG, and IgA,⁷⁻⁹ and sometimes IgA and IgG antibodies in nasal secretions.^{10, 11}

Clinical improvement, like immunologic responses, depends on the dose of allergen administered.¹² Because local and systemic reactions dictate variations in dose within a group of patients under study, once-a-year seasonal reporting of symptoms has never allowed more than a rough estimate of the dosage necessary.

We have previously reported on a nasal-challenge technique that allows us to characterize the clinical and biochemical mediator response of individual patients to allergen applied directly to the nasal mucosa.¹³⁻¹⁶ A retrospective study of highly allergic pa-

tients with ragweed hay fever demonstrated that a group of immunized patients ($N = 12$), when they were compared to a matched group of nontreated patients ($N = 26$), had a significantly diminished response in three respects: (1) Treated patients required larger pollen doses to excite sneezing or mediator release. (2) Significantly fewer treated subjects released mediator at any dose. (3) At any dose of challenge, the amount of mediator released was less. Three mediators were measured: histamine, TAME-esterase, and prostaglandin D_2 . The treated patients in this study had received maintenance doses of ragweed extract that varied from 1.2 to 18.6 μg (median 6 μg) of *Amb a I* (Ag E) equivalents, leaving the minimum dose required for efficacy undetermined.⁷

Having observed that immunotherapy alters mediator responses to nasal-pollen challenge, we organized a prospective study to explore the effective immunizing dose of ragweed extract. We randomized in a double-blinded fashion the 27 nontreated ragweed-allergic subjects that served as untreated control subjects in the previous study into two immunotherapy regimens. This article concerns the 13 subjects placed on standard ragweed immunotherapy. We examined immunologic change and biochemical mediator response to nasal-pollen challenge when each of the three predetermined doses had been reached during an 18-month period. The results in 11 patients who completed the study indicate that a dose $>0.6 \mu\text{g}$, but probably no more than 12.4 μg in terms of the major allergen, is sufficient to attenuate the immediate allergic reaction to pollen exposure.

METHODS

Patients

Written informed consent was obtained from a group of 27 patients, aged 18 to 55 years, who displayed seasonal ragweed pollenosis of at least a 2-year duration and who had not received immunotherapy in the previous 15 years. Of these 27 patients, 13 were chosen at random to receive immunotherapy with a ragweed extract made in the standard method of 24-hour extraction of ragweed pollen, whereas the other patients received a novel extract, the results of which will be discussed in another article.

Pollen and pollen extracts

Ragweed pollen was purchased from Greer Laboratories (Lenoir, N.C.) within a month after collection and was stored at -20°C in a sealed container until use. The same batch of pollen, used for all experiments, was found to contain $6.7 \pm 0.2 \mu\text{g}$ of ragweed *Amb a I* per milligram of pollen by radial immunodiffusion analysis. An extract of pollen for treatment was prepared as follows: The pollen was defatted with petroleum ether and was extracted (at 1/10 wt/vol) by stirring gently for 24 hours at 4°C with physiologic saline at pH 7.15 to 7.50. During the initial

mixing, and periodically throughout the extraction, the pH was maintained within this range by addition of 0.1 mol/L of Na_2CO_3 , and the final pH was adjusted to 7.50. The extract was centrifuged and sterile filtered. Sterile glycerine (United States Pharmacopeia) was then added in an equal volume to elicit a 1/20 wt/vol of glycerinated extract. The antigen levels in this extract were as follows: *Amb a I* (Ag E), $336 \pm 12 \mu\text{g/ml}$; *Amb a III* (Ra3), $38.0 \pm 1.4 \mu\text{g/ml}$; *Amb a V* (Ra5), $22.1 \pm 1.6 \mu\text{g/ml}$; *Amb a VI* (Ra6), $59.4 \pm 1.5 \mu\text{g/ml}$, as determined by radial immunodiffusion analysis. The PNU content was 59,600 PNU/ml. Thus, 1.0 μg of *Amb a I* is contained in 177 PNU of this ragweed extract. This extract also elicited a crossed immunoelectrophoresis pattern compatible to national (Food and Drug Administration) and international (World Health Organization) standards. The extract was used both for diagnostic skin tests and for treatment.

Skin tests

Intradermal end point titrations were performed according to the method of Norman.⁸

Nasal challenge technique

These techniques have been described in detail.^{13, 15} All challenges were performed outside the ragweed season (either before or more than 6 weeks after) at a time when patients were asymptomatic. Briefly, varying amounts of ragweed pollen were combined with lactose to obtain a total weight of 25 mg in a gelatin capsule. The individual capsules were then insufflated in one nostril via a Spinhaler (Fisons Corp., Bedford, Mass.) apparatus connected to a Rosenthal-French dosimeter (The Johns Hopkins University, Baltimore, Md.). With activation (four activations for 2 seconds with a driving pressure of 10 psi), approximately 22 mg of pollen and lactose (range, $\pm 15\%$) were expelled into the nose. The capsule was examined to ensure that it had been emptied.

Nasal secretions were collected by nasal lavage. The subjects were instructed to tilt the head backward at a 30 degree angle in a sitting position, and 5 ml of normal saline (Cutter Laboratories, Emeryville, Calif.) was instilled into each nostril while the patients held their breath and did not swallow. After 10 seconds, the patients expelled the mixture of mucus and saline into a collection vessel. These samples were maintained on ice until the challenge was complete. Subsequently, all samples were centrifuged at 15000 g at 4°C for 15 minutes, and the sol phase was separated from the gel (mucus) phase. Aliquots of samples were processed as previously reported^{13, 15} for mediator assays.

Four prechallenge saline washes were performed to reduce levels of cell-free mediators typically present in early nasal secretions. Then, oxymetazoline hydrochloride (Schering Corp., Kenilworth, N.J.) was sprayed into the nose (two sprays per nostril) to maintain nasal patency during the allergic reaction so that nasal secretions could be collected. Previous study has demonstrated that this drug dose does not effect mediator release.¹³ Subsequently, two challenges with a lactose diluent were used to

establish a baseline. Thereafter, serial challenges with 0.03/0.33/3.3/16.5 mg (representing about 1000, 10,000, 100,000, and 500,000 grains) of ragweed pollen at 10-minute intervals were undertaken, and a saline wash (8.0 ± 1.5 ml recovered) was collected 10 minutes after each pollen-challenge dose to allow measurement of the various inflammatory mediators produced in nasal secretions. A previous study demonstrated that lactose as a placebo challenge caused essentially no mediator release,¹³ and another study demonstrated that pollen challenges about 3 months apart elicited reproducible TAME-esterase and histamine curves in a group of 26 untreated patients.¹⁷

Mediator assays

Aliquots of the sol phase of nasal secretions were assayed for mediator content.

Histamine. For histamine determinations, 0.8 ml of sample was mixed with 0.2 ml of 8% HClO_4 and centrifuged for 10 minutes at 1000 g. Histamine in the supernatants was assayed by an automated spectrofluorometric assay¹⁹ with a sensitivity of 1 ng/ml and a precision of $\pm 5\%$.

TAME-esterase. Enzyme(s) having arginine esterase activity was assayed according to the method of Imanari et al.²⁰ that is based on the liberation of tritiated methanol from the synthetic substrate [^3H]-TAME. A 40 μl aliquot of nasal sample and 10 μl of 0.2 mol/L of Tris buffer, pH 8.0, were added to a 1.5 ml Eppendorf microtube (Hamburg, West Germany). A 10 μl volume ($1.5 \text{ by } 10^5$ cpm) of [^3H]-TAME (Amersham Corp., Chicago, Ill.) was then added with mixing, and tube was placed in a counting vial containing 10 ml of Econofluor (New England Nuclear, Boston, Mass.) and 50 μl of "stop" solution (1 vol of glacial acetic acid, and 9 vol of 0.02 mol/L of TAME). The counting vial was then tightly capped, and after 1 hour, the reaction was terminated. Tritiated methanol was partitioned into the Econofluor by shaking. The vial was then counted for 4 minutes in a liquid scintillation spectrometer, and the levels of TAME-esterase were computed by reference to standards. The sensitivity of the assay is defined as 1000 cpm above background.

Definitions. For the purpose of defining the threshold of response, a positive clinical response was defined as one or more sneezes in response to pollen challenge. A positive mediator response was defined as an increase above the baseline two times the minimum sensitivity of the assay. Hence, for histamine, with a sensitivity of 1 ng/ml, an increase of 2 ng/ml was required to be considered a positive response. With TAME-esterase (sensitivity of 1000 cpm), an increase of 2000 cpm was required.

Statistics. ANOVA for repeated measures was used to analyze differences in mediator release after each of the four dosage levels, and Friedman's two-way ANOVA was used for threshold of response data.²¹

RESULTS

Skin test sensitivity

Skin test sensitivity in the patients ($n = 13$) before treatment ranged from 3.0×10^{-7} to 1.0×10^{-2} μg

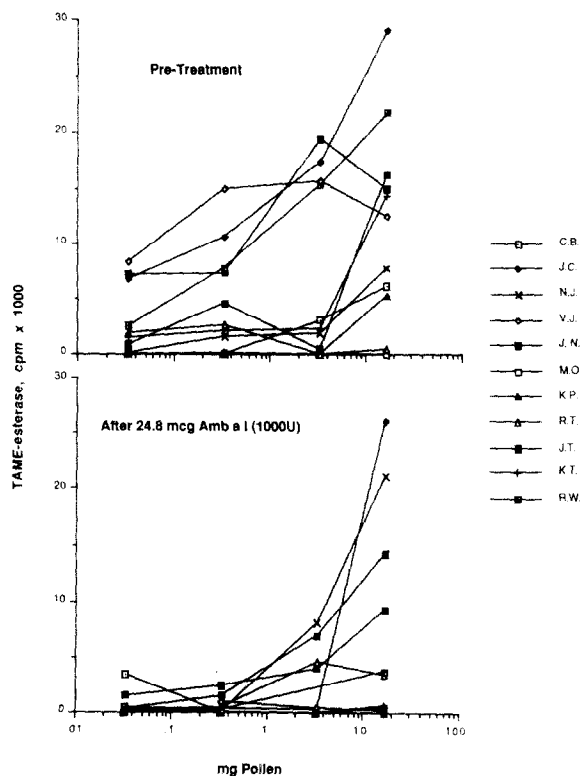


FIG. 1. Dose-response curves of TAME-esterase from 11 patients before and after immunotherapy with a final dose of 24.8 μg of *Amb a I* equivalent of whole ragweed extract.

of *Amb a I* per milliliter, median 3.0×10^{-5} μg of *Amb a I* per milliliter.

Immunotherapy regimen

After the pretreatment studies and after the 1982 ragweed season, patients were started on subcutaneous injections once a week at a dose of 0.01 units (0.0024 μg of *Amb a I* equivalent) with an increase at each subsequent injection unless allergic reactions dictated reducing the dose or holding it at the same level. One patient withdrew before reaching the 0.6 μg level of dosage. Another patient had allergic reactions at 0.14 and 0.19 μg . After 1 year the patient was only receiving a dose of 0.5 μg and then chose to withdraw from the study. In the remaining patients, when the individual dose reached 25 units (0.6 μg of *Amb a I*), the patient was scheduled for a repeat nasal challenge and was maintained at that dose until the challenge was completed. Again at 500 units (12.4 μg of *Amb a I*), another nasal challenge was performed, and finally, at 1000 units (24.8 μg of *Amb a I*), the final nasal challenge was done. One patient (N. J.) had reactions after the 700 units (17.4 μg of *Amb a I*) dose, and after several tries at larger doses, which resulted in reactions, the final dose was

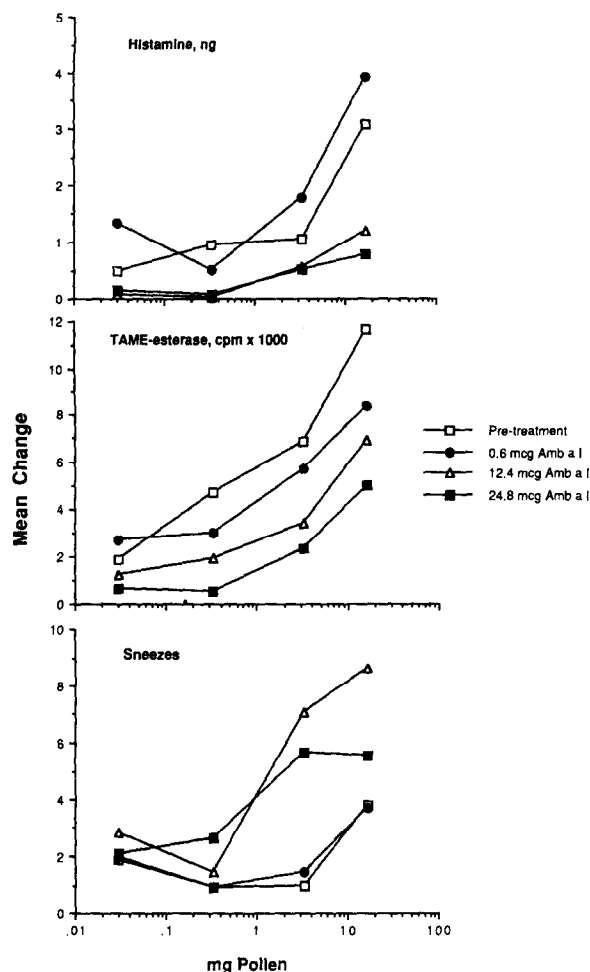


FIG. 2. Dose-response curves demonstrating the mean of the increases from prechallenge levels for histamine and TAME-esterase and the number of postchallenge sneezes are depicted. Curves before treatment and at three challenge levels of ragweed extract are illustrated for 11 patients.

200 units (4.9 μ g of *Amb a I*). Data on her challenges are included because she did accomplish the first two levels of dose and continued injections at a lowered dose until the end of the study. The time to achieve the 0.6 μ g dose was 4 to 5 months; to achieve the 12.4 μ g dose was 6 to 10 months; to achieve the 24.8 μ g dose was 12 to 18 months after initiation of immunotherapy.

Effect of immunotherapy on nasal-pollen challenge

Eleven patients had a complete set of challenges, that is, a pretreatment challenge and a subsequent challenge at each of three dosage levels. In Fig. 1, **top panel**, the pretreatment dose-response curves from nasal-pollen challenges of these 11 patients before treatment for TAME-esterase are illustrated, and

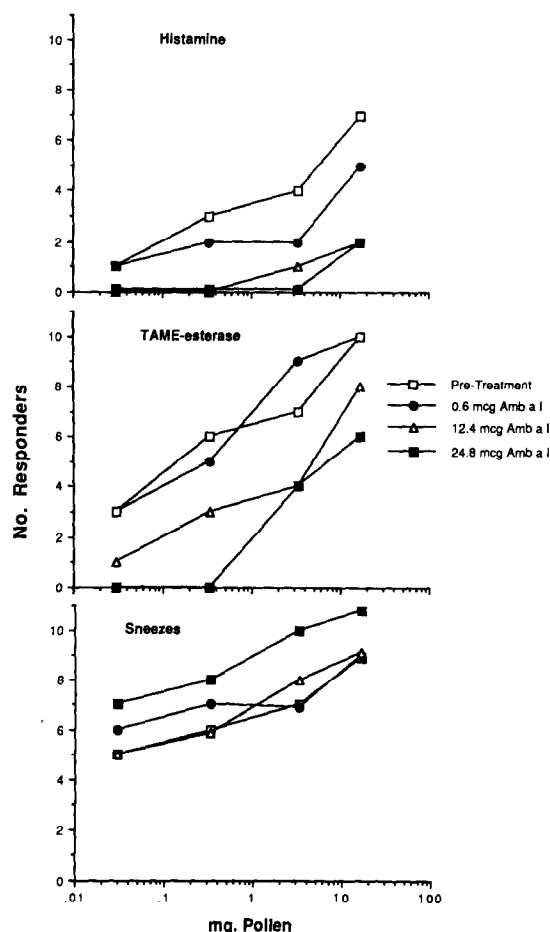


FIG. 3. Number of patients responding with significant (see Methods for definition) rises in histamine, TAME-esterase, and sneezes at each level of pollen challenges before treatment and at three dosage levels of ragweed extract.

these curves 18 months later after reaching the 24.8 μ g of *Amb a I* level are illustrated in Fig. 1, **bottom panel**. Patient N. J., who had reactions and could not be maintained even at the 12.4 μ g level, is depicted by the symbol X and is the only one who released more mediator at the end of the study. The other posttreatment curves differ in several ways. (1) The threshold for the first rise in mediator is increased; that is, the curve is shifted to the right. Five patients do not respond with any mediator release at any level of challenge after treatment. (2) If some mediator is released, the amount is less; that is, the slope of the curve is flatter.

These two somewhat different parameters make it necessary to examine the data in two ways. The means for TAME-esterase, histamine, and sneezes in the 11 patients at each dosage level (pretreatment, 0.6, 12.4, and 24.8 μ g of *Amb a I*) are illustrated in successive panels of Fig. 2. The differences between the family

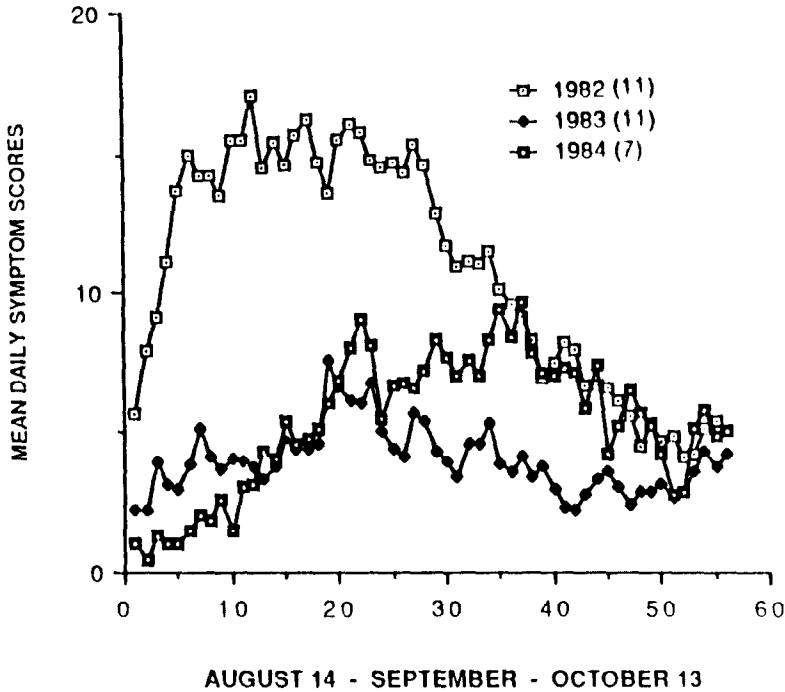


FIG. 4. Daily symptom diary scores during the three ragweed seasons of observation.

of curves were examined by ANOVA for repeated measures. The p values obtained for the several comparisons possible are presented in Table I. For the mediators, the differences between the curves before treatment and after reaching the 12.4 and 24.8 μg dosage level are significant, but the 0.6 μg dose did not elicit a significant change. The difference between responses at 24.8 and 12.4 μg is not statistically significant. The greater clinical response of sneezing after immunotherapy is not significant.

The cumulative number of the patients responding to the challenges with increasing doses of ragweed at the several dosages of immunotherapy is illustrated in Fig. 3. There is essentially no change at the 0.6 μg dose; however, at the 12.4 and 24.8 μg doses, fewer patients respond with histamine and TAME-esterase release, whereas there is no apparent change with sneezing. By Friedman's two-way ANOVA, the trend for less TAME-esterase release is highly significant ($p = 0.006$), and the trend for less histamine release is significant ($p = 0.017$).

Clinical response to immunotherapy

For completeness, the clinical response of these subjects, based on their total symptom-medication diary scores for the 3 years of this study, is illustrated in Fig. 4. Scores in 1982 were taken before injections were begun. Symptoms reported were reduced in every patient during the 1983 and 1984 seasons, but

TABLE I. Significance of differences at different treatment doses: Challenge data

Comparison	TAME-esterase	Histamine	Sneezes
Over all challenges	0.01*	0.08	0.06
Pre vs 0.6 μg	NS	NS	NS
Pre vs 12.4 μg	0.06	0.04	NS
Pre vs 24.8 μg	0.04	0.06	NS
0.6 vs 24.8 μg	0.02	NS	NS
12.4 vs 24.8 μg	NS	NS	NS

NS, Not significant.

* p values by ANOVA for multiple measures.

the patients were aware of the initiation of an active treatment, even though they had been told that they would receive one of two treatment materials and that one of these might not be effective. These data cannot be related to the treatment dosages because seasons arrived when the patients were at different dosages. Daily pollen counts and the total count for the season in Baltimore were remarkably similar in the 3 years under study.²²

Immunologic response to treatment

The IgG antibody (nanograms per milliliter) responses to ragweed for the immunized patients at each therapeutic dose level are illustrated in Fig. 5. A step-wise increase in IgG antibody titer can be demon-

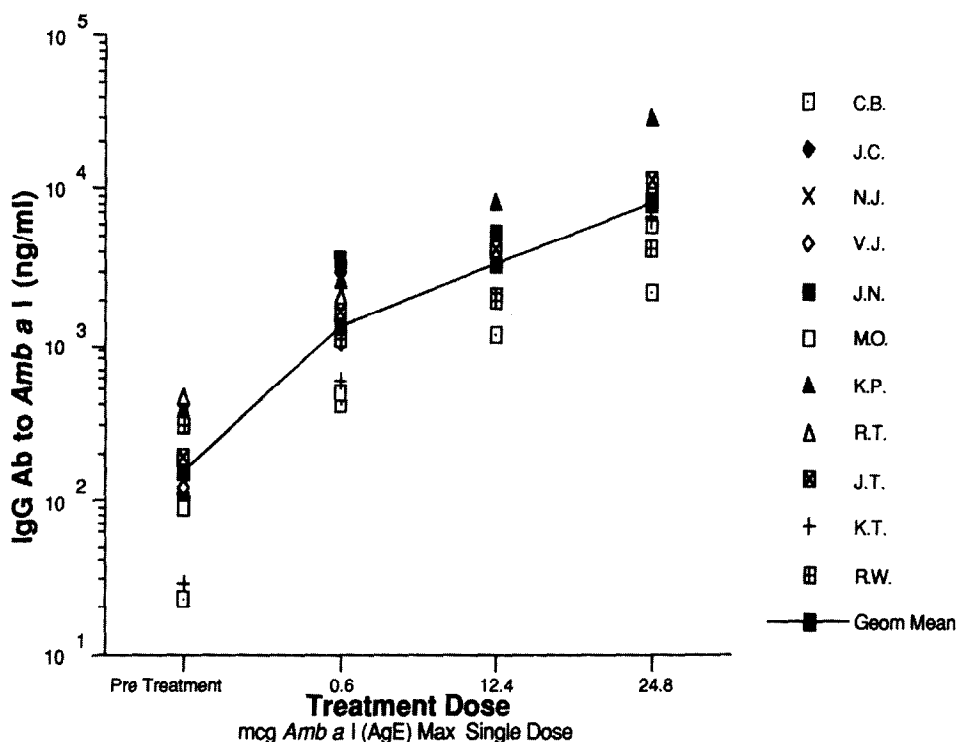


FIG. 5. IgG antibodies to whole ragweed extract in sera drawn before treatment and after each of three dosage levels were achieved. Before treatment: 0.6 and 12.4 μ g, 11 patients; 24.8 μ g, nine patients.

strated at each level, as compared to pretreatment. There was no significant correlation between the change in any of the three parameters tested and the increase in IgG antibody. Changes in IgE antiragweed were not followed.

DISCUSSION

The criteria used for a positive mediator response are to some extent arbitrary. We base it on the sensitivity of the assay (see Methods). In a previous study we used an increase of three times baseline for mediators as the criterion for a positive response instead of the two times increase used in this study. In the current data, with histamine, changing to the two times criterion results in one patient being considered positive at one dose less and another two doses less in the pretreatment challenge. One patient is positive at one dose less at the 0.6 μ g treatment level, and one patient becomes a reactor rather than a nonreactor at the largest pollen dose at the 24.8 μ g treatment level. These minor changes cut both ways and do not materially change the interpretation of the data. With TAME-esterase, all changes were 3000 cpm or more; therefore, the numbers of responders are not changed. We prefer the two times criterion as not introducing any unnecessary restrictions.

We likewise arbitrarily considered a single sneeze as a positive clinical response. Increasing the number to two or three would reduce the number of responders but not change the fact that sneezing was not significantly less on challenge after immunotherapy. We have no ready explanation for this finding since our earlier study indicated significantly less sneezing in treated patients than in control subjects.¹⁷ Sneezing represents an end organ response through neural circuits that involve much more than the amount of mediator released. We note that the patients reported less severe symptoms during natural exposure; hence, the usual clinical effects of immunotherapy were reproduced in this study.

A placebo cohort was not recruited for this study because we feel that the individual patients serve as their own controls because of the reproducibility of the pollen challenge technique. Such data represent objective measurements not subject to observer or patient bias. Since challenge studies can be performed as soon as a specific therapeutic dose is reached without waiting for the next season of natural exposure, it is possible to test the effects of several increasing doses in the same group of patients.

At treatment doses of 0.6 μ g of Amb a I equivalents, repeat nasal-pollen challenge demonstrated so little

change as to be not significant with this number of patients. A significant reduction in mediator response was observed, however, at 12.4 µg of *Amb a 1*, a dose level previously demonstrated to be effective by purely clinical evaluations.¹ The 24.8 µg of *Amb a 1* dose was not clearly more effective. Not enough patients were available to explore still larger doses, but the inability of one patient to remain at this dose is some indication that we were close to a practical limit of dosage. In our previous studies, some patients were not able to reach this dose because of reactions.

The changes in dose-response curves observed *in vivo*, that is, shift to the right and a flatter curve with some patients not responding to any dose, parallel the changes in basophil histamine-release curves observed *in vitro* in earlier studies.¹⁻⁷ We did not observe *in vitro* histamine release in these patients and are unable to say whether the *in vivo* changes observed were accompanied by similar changes in basophil behavior. We believe, however, that the mediators observed *in vivo* originate from mast cells or are stimulated by mast cell mediators. TAME-esterase activity in nasal secretions measures mainly a plasma kallikrein- α_2 -macroglobulin complex, together with a mast cell tryptase.²³ That this measurement is more sensitive than histamine to the effects of challenge probably reflects assay sensitivity. This increased sensitivity is due to the ability of a single enzyme molecule to hydrolyze many molecules of the substrate.

Clinical studies have consistently revealed that immunotherapy can result in significant clinical improvement but not a "total cure" in most patients.¹ Likewise in this study, a reduction in the number of patients releasing any mediators, as well as a significant diminution in the amount of mediators, were observed at several pollen-challenge doses. However, as demonstrated by the dose-response curves (Figs. 1 and 2), the threshold for release is not completely abrogated but rather dramatically shifted to the right, thereby requiring larger pollen challenge doses to elicit a mediator response.

With brief (not continuous) pollen challenges, doses larger than likely to be encountered naturally in a short time are needed to elicit mediator release in some patients with undoubted clinical sensitivity. From experiments of catching pollen issuing from the device on a greased slide, we estimate that only about 40% of the pollen put into the challenge capsule is actually delivered to the mucosa, the remainder staying in the challenge apparatus or lost in the surrounding air. Thus, about 4000, 40,000, 400,000, and 2,000,000 grains are delivered at each stage of the challenge. Calculations from outdoor sampling suggest that, during the peak of the ragweed season, an

individual outdoors at rest, breathing at a rate of 6 to 7 L/min, would breathe in approximately 300 to 400 ragweed-pollen grains per hour. Subjects, however, are uncommonly at rest when they are outdoors. Thus, moderate outdoor activities with a breathing rate of 30 to 40 L/min could easily increase exposure to 1500 to 2600 pollen grains per hour or 6000 to 26,000 grains during a 4- to 10-hour outdoor exposure. Hence, levels of challenge comparable with natural exposure demonstrates almost no response in most of our patients after immunotherapy, and there is partial protection even at levels of pollen exposure unlikely to occur naturally.

Furthermore, Connell^{24, 25} demonstrated that daily laboratory exposures of allergic subjects to pollen, out of season, lowers the threshold for symptomatic response within a few days. After such "priming," the threshold corresponds to the amount of pollen inhaled naturally during the season at rest. Naclerio et al.²⁶ in our laboratory have recently demonstrated that a single laboratory exposure out of season sufficient to cause symptoms results in an increased mediator response to a second challenge only 11 hours later. We are currently exploring the effect of immunotherapy on priming.

We have previously noted that between 6 to 24 µg of ragweed *Amb a 1* equivalent per injection is needed for an effective clinical response to be demonstrable.^{12, 17, 27, 28} Our retrospective study with nasal challenge demonstrated that a median dose of 6 µg of ragweed *Amb a 1* equivalent per injection was clinically effective.¹⁷ When this is put in terms of micrograms of the major allergen, effective final or maintenance doses of grass pollen extract,^{29, 30} cat extracts,³¹⁻³³ and insect venoms^{34, 35} have been similar in controlled studies. It appears more appropriate to state the dose of the final or maintenance injection than to state cumulative seasonal dose, as we have in past studies, since the immunologic response is more likely to be related to the final dose. Both cumulative and maintenance doses are related, of course, since the maintenance dose represents the larger part of the cumulative dose.

Some individuals have reported that a lower dose (<1 µg Ag E per injection) may be sufficient to induce clinical relief.³⁵⁻³⁹ Studies by Van Metre et al.⁴⁰ in our clinic were unable to confirm these findings. Our challenges appear to indicate that the mediator response to exposure is but little altered when nasal pollen challenge is done after 0.6 µg of $\times$, a "low-dose" immunotherapy level. A recent study by Hedlin et al.⁴¹ in our clinic did find a significant reduction in mediator release after ragweed-extract challenge in 40 patients (one half children and one half adults) early

in a course of immunotherapy when the dose had reached only 0.11 μg of *Amb a* I. The extract challenges they used are not readily comparable with pollen challenges, and they accepted a smaller increase of mediator over baseline as a positive response. It does appear that the difference they observed in terms of numbers of responders at the 0.11 μg level is not dissimilar to the change we observed at 0.6 μg . With 40 patients under study, a similar difference may be significant when it is not significant with 11 patients. Their patients also received five to seven other allergens, which may provide a greater stimulus for non-specific effects.

Doses of 6 to 12.4 μg of *Amb a* I can probably be achieved regularly in practice. Ragweed extracts sold in the United States come with information on the Ag E (*Amb a* I) content. Although there is not fixed relationship between *Amb a* I and PNU, a conversion factor can be calculated for each batch of extract from the information furnished by the manufacturer. For instance, with the extract used in this study, 1.0 μg of *Amb a* I equals 177 PNU; hence, the 12.4 μg maintenance dose is equivalent to 12.4×177 , about 2200 PNU. This ratio is lower than found in most commercial extracts, and the PNU dose would be somewhat higher with the average extract. This 12.4 μg dose would correspond to approximately 4000 allergy units as a maintenance dose if a standardized 100,000 allergy unit ragweed product was used (≈ 300 to 350 μg of Ag E).

We hope to explore further intermediate doses with repeat challenges at several dosage levels. Once some minimally effective dose is reached, the therapeutic response to immunotherapy may well not be a threshold phenomenon but a gradual diminution of response. In addition, the "optimum" dose might vary from patient to patient.

Our data indicate suppression of the immediate response; however, the late-phase IgE-mediated component of an allergic reaction may also be an essential feature of allergic disease.^{42, 43} For instance, a nasal-challenge study by Naclerio et al.²⁶ demonstrates a second wave of symptoms and mediator release 3 to 11 hours after the initial challenge. Immunotherapy more readily modifies late-phase skin test responses to allergen than immediate responses.⁴⁴ In children with asthma immunotherapy with *Dermatophagoides pteronyssinus* altered the late-phase response to bronchial challenge more readily than the immediate response.⁴⁵ We are currently engaged in a study of ragweed hay fever to assess the ability of ragweed immunotherapy to suppress the late-phase response to challenge.

We were not able to correlate patient responses with

increases in serum IgG antibody. Even though the challenge response, particularly when appearance of several mediators at the site is measured, would appear to offer a more quantitative evaluation of patient responses than symptom diaries during natural exposure, no relationship between antibody and the suppression of the allergic response appeared. For instance, the 0.6 μg of *Amb a* I dose produced a considerable antibody response, even though it had little effect on the challenge response.

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