

The nasal response to histamine challenge: Effect of the pollen season and immunotherapy

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To evaluate changes in the nasal response to histamine, we challenged 19 subjects with allergic rhinitis caused by ragweed (RW) before, during, and after the RW season with increasing doses of histamine diphosphate. We compared their response, as measured by symptoms and the levels of TAME-esterase activity and albumin recovered in the nasal lavage fluid, with response of two groups with allergic rhinitis undergoing immunotherapy with moderate-dose (N = 16) and high-dose (N = 11) RW (2 and 24 µg of antigen E [Amb a I] as maintenance dose, respectively). Four challenges with histamine were performed in each group: before, at the peak of, near the end of, and 2 weeks after the RW season. The three groups of subjects had similar skin sensitivity to antigen and levels of TAME-esterase activity and albumin recovered from nasal lavages after histamine challenge performed before seasonal exposure. Symptom diaries obtained throughout the season revealed a significant reduction only in the high-dose immunotherapy-treated group. At the peak of the season, the untreated group had more symptoms in response to the challenge compared with the challenges before and after the season (p = 0.04 for both groups). The saline challenge occurring before challenging with histamine also demonstrated a significant increase at the peak of the season compared with increases before the season (p = 0.02). This observation was also true for the levels of albumin and TAME-esterase activity. If the response after saline challenge was subtracted from each response after histamine challenge, no difference was found in the results between any of the visits. No changes occurred for the net albumin and TAME-esterase activity scores through the season. The saline challenges for the two immunotherapy-treated groups did not reveal a significant change, nor did the overall challenge vary during the season. We speculate that the increased responsiveness of the nasal mucosa during the allergen season may represent an increase in the baseline reactivity of the mucosa and not an increased responsiveness to histamine. Immunotherapy prevents this change in baseline reactivity. (J ALLERGY CLIN IMMUNOL 1992;90:85-91.)

Key words: Ragweed, histamine, allergy, immunotherapy, rhinitis, nasal mucosa, hyperresponsiveness

Hyperresponsiveness to irritants is a hallmark of continuing allergic states. During the pollen season, allergic subjects with asthma demonstrate increased responsiveness to both allergic and nonallergic stim-

Abbreviations used

HDI: High-dose immunotherapy
MDI: Moderate-dose immunotherapy
RW: Ragweed
TAME: N- α -tosyl-L-arginine methyl ester
MW: Molecular weight

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uli, such as histamine.^{1, 2} Increased responsiveness after seasonal exposure has also been demonstrated in the upper airways.³⁻⁵ In the laboratory, increased sensitivity to histamine has been noted in the upper airways after antigen exposure associated with an inflammatory cell infiltration. In contrast, histamine provocation does not induce increased reactivity to itself or to antigen.⁶ Immunotherapy alters the inflam-

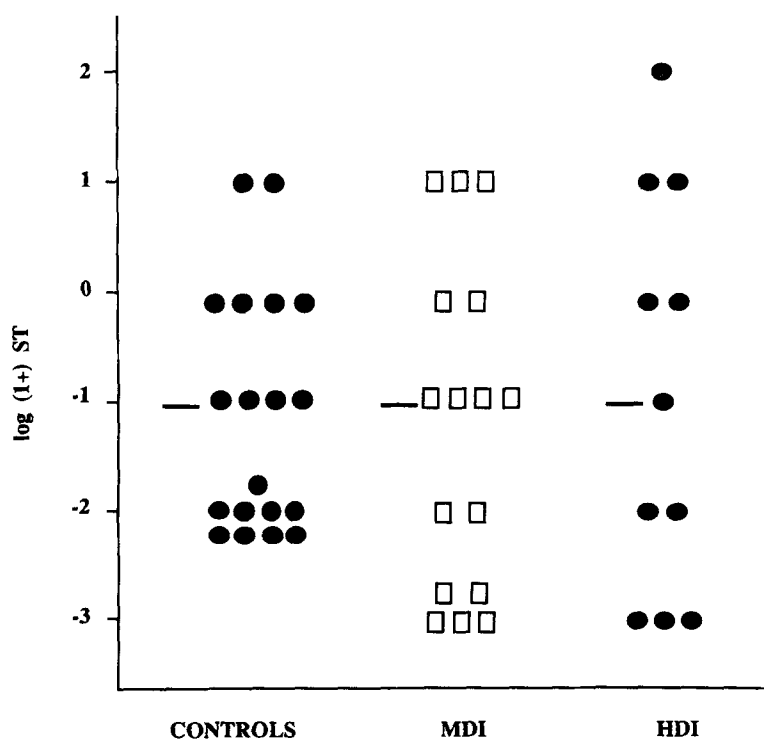


FIG. 1. Immediate response to skin tests with RW-antigen extract. Values in *ordinate* represent log value of concentration of antigen that elicited (1+) response on skin testing; *horizontal lines*, median values for each group. No difference regarding skin sensitivity to antigen was found amount the three groups ($p = 0.9$).

matory response to nasal antigen challenge, as demonstrated by (1) the requirement of larger doses of pollen to induce sneezing or mediator release after treatment, (2) a decrease in eosinophil infiltration and release of inflammatory mediators, and (3) the suppression of the late-phase response.⁷⁻¹⁰ In this study we sought to confirm the increased sensitivity of the nose to histamine during the pollen season and to determine if this increased response was reduced by immunotherapy.

MATERIAL AND METHODS

Subject selection

We recruited volunteers between the ages of 23 and 57 years (34 ± 8 , mean $\pm$ SD) with a history of RW hay fever. Nineteen subjects with a history of seasonal allergic rhinitis caused by RW and a positive intradermal skin test served as control subjects, whereas two immunotherapy-treated groups with similar histories and skin tests received maintenance injections of whole RW extract equivalent to 2 μ g for 6 months (MDI-treated group, N = 16) or 24 μ g for 18 months (HDI-treated group, N = 11) of *Amb a* I. Immunotherapy injections were administered weekly until maintenance dose was reached. Subsequently, injections were administered biweekly. Each volunteer gave informed consent after the nature of the study had been explained. The protocol was approved by the Joint Committee on Clin-

ical Investigation of The Johns Hopkins Medical Institutions.

Methods

Diaries of symptoms and medication use were kept daily during the RW season. Subjects scored sneezing, stuffy, itchy, or runny nose twice daily (midnight to noon and noon to midnight).¹¹ Each symptom was scored based on duration: 0, none; 1, less than 30 minutes; 2, 30 minutes to 2 hours; 3, more than 2 hours. Medication scores were based on dose and ranged from 1 to 3. The total daily symptom-medication score represented the sum of all symptom and medication scores for a 24-hour day. If any of the scores were not reported by a subject, the day was not counted for that subject.

Subjects underwent four graded nasal challenges with histamine before (July 7 to August 14, 1987), at the peak of (August 31 to September 11, 1987), near the end of (September 28 to October 17, 1987), and 2 weeks after (November 2 to November 20, 1987) the RW-pollen season. Before each histamine challenge, six lavages with lactated Ringer's solution were performed to reduce the levels of cell-free mediators initially present in the nasal cavities; only the first lavage fluid was saved for mediator assay. Immediately after these lavages, normal saline, the diluent for the histamine, was sprayed in the nose, and after 10 minutes, a lavage was performed and the resultant fluid was saved. Progressively increasing doses of histamine diphosphate

(0.01, 0.03, 0.1, and 0.3 mg) (Sigma Chemical Co., St. Louis, Mo.) were sprayed in one nostril, a lavage with 5 ml of lactated Ringer's solution was performed 10 minutes after each dose, and the resultant fluids were prepared for assay. Before and during each nasal challenge, sneezes were counted and symptom scores were recorded with a 4-point scale (0, none; 1, mild; 2, moderate; 3, severe). No subjects were using cromolyn or intranasal steroids at any time during the study. Any subject requiring medication (antihistamines and/or decongestants) stopped their medication 48 hours before the challenge. Astemizole was not taken by any subject before or during this study.

Intradermal skin testing was performed with tenfold dilutions of an RW extract (Greer Laboratories, Lenoir, N.C.). The first dose that clearly demonstrated a response was considered a (1+) reaction. The pollen extract used for immunotherapy (Greer Laboratories) was the same as that used for skin testing.

Pollen counts

Daily pollen counts were performed with the aid of a Rotorod sampler (Sampling Technologies, Inc., Los Altos Hills, Calif.). The pollen counts are expressed as the mean daily number of pollen grains per cubic meter of sampled air.

Assays

All recovered lavages were placed in polypropylene tubes, shaken vigorously, and then maintained on ice until the challenge was completed. The tubes were then centrifuged for 14 minutes at 5000 rpm (3090g) at 4° C to separate the cells and the thick gel phase from the remainder of the lavage fluid. Untreated aliquots from the latter were taken for measurement of TAME-esterase activity and albumin and frozen at -20° C until the assays were performed.

TAME-esterase activity was measured by a radioenzymatic assay that measures the release of [³H]methanol from the synthetic substrate, TAME.¹² This assay measures enzymes that have arginine-esterase activity, designated TAME-esterases. During the response to histamine challenge, TAME-esterase activity is due primarily to a plasma kallikrein/ α_2 -macroglobulin complex, with an additional contribution from tissue kallikrein.¹³ Its presence in nasal lavage after nasal challenge with histamine represents a large (900,000 MW) protein leaking from the plasma. Human serum albumin was measured by a specific RIA sensitive to 1 ng/ml.¹⁴ All the samples from one participant were analyzed in the same assay.

Statistics

Nonparametric statistics were used. The Wilcoxon's matched-pairs signed-rank test was used to determine differences between visits within each of the three groups of subjects. The Mann-Whitney U test was used to evaluate the differences between groups.

First, we analyzed the sum of the net changes from baseline (the diluent response before histamine challenges). For example, if the concentration after diluent is 1, the first challenge 5, the second challenge 6, and the third challenge

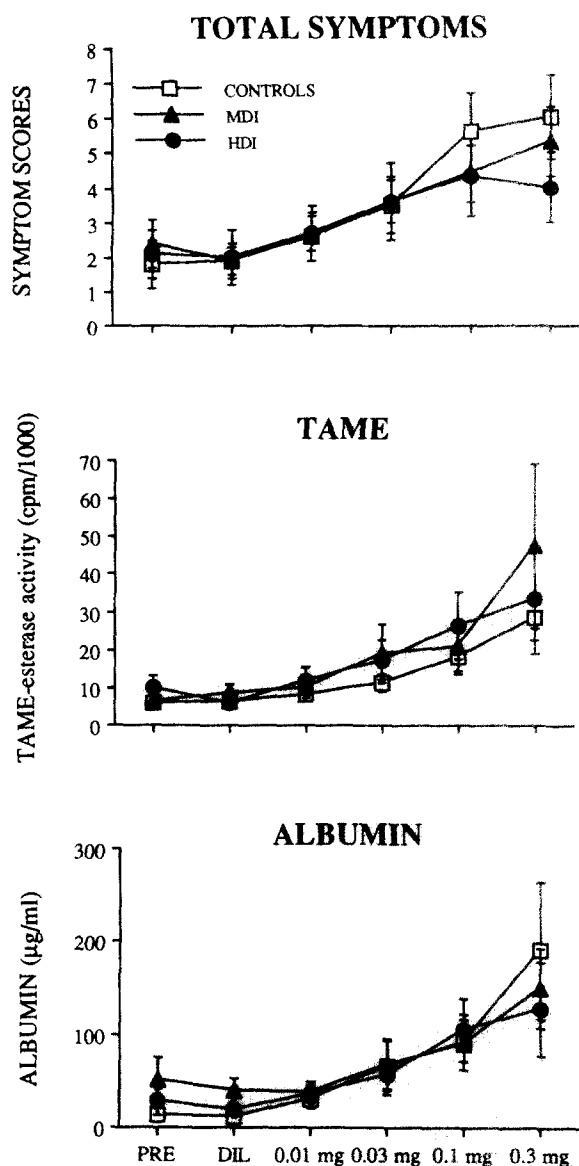


FIG. 2. Histamine challenges before start of RW season for three groups in study. Protocol of challenge displayed on *abscissa*; Values, mean $\pm$ SEM. No significant differences were found in any parameters evaluated; control group, N = 19; MDI treated, N = 16; HDI treated, N = 11.

7, the net change is $(5 - 1) + (6 - 1) + (7 - 1)$ or 15. Second, we analyzed the differences between responses to diluent challenges. Last, we analyzed the gross responses (without subtracting the diluent response before histamine challenges). In the above example, the gross change would be $5 + 6 + 7$ or 18. Two-tailed *p* values are reported.

RESULTS

Comparison of groups before the season

Sensitivity to RW antigen by skin testing was similar among the three groups. The medians of the log of the concentration of antigen that induced a (1+)

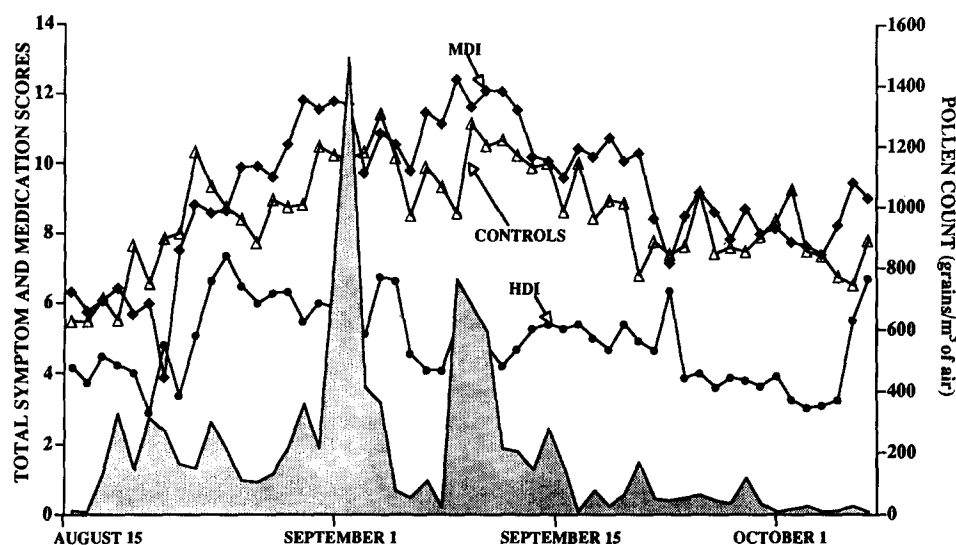


FIG. 3. Median of total symptoms plus medication scores for three groups of study during RW season; shaded area, pollen count during 1987 RW season in Baltimore. HDI-treated group demonstrated significantly fewer symptoms and medication use compared with that of control subjects and MDI-treated group ($p < 0.05$).

response to skin testing were -1 for all groups ($p = 0.9$) (Fig. 1). The histamine challenge before the season induced a response in all three groups, and this response was not different between groups. Each group demonstrated similar patterns and levels of TAME-esterase activity and albumin recovery (Fig. 2) after challenge.

Symptoms and medication diaries

In 1987, the pollen counts in Baltimore started rising in mid-August, had peaks at the beginning of September, and again 1 week later. The counts started to decline by the end of September. The symptom and medication scores of the control group increased in response to pollen. The MDI-treated group reported symptoms that were not different from the symptoms of the control group. The HDI-treated group had significantly fewer symptoms and medication scores compared with symptoms and scores of the control group ($p < 0.05$) (Fig. 3).

Histamine challenges

Control group. At the peak of the season, the control group demonstrated a greater response to histamine with respect to total symptoms compared with that of other time points (Fig. 4). When all the histamine challenges were compared, the challenges at the peak of the season demonstrated a significantly increased response to histamine compared with responses to challenges before ($p = 0.04$) and after the season ($p = 0.04$). When the symptom scores for

the saline challenge were compared, this group demonstrated a significant ($p = 0.02$) increase in symptoms during the peak of the season compared with symptom increase before the season. However, if the diluent symptom scores were subtracted, no difference was found ($p = 0.38$) when these visits were compared.

The TAME-esterase activity levels during the various visits did not demonstrate any significant differences. However, for the diluent challenges, the levels of TAME-esterase activity measured during visit 2 were significantly higher than levels at visit 1 ($p = 0.003$).

The levels of albumin measured in the control group after histamine challenge at the peak of the season were higher than levels detected near the end of the season ($p = 0.01$). For the diluent challenges, levels of albumin were higher at the peak compared with levels before the season ($p = 0.0007$). If albumin levels after saline challenge are subtracted from levels after histamine challenge, the peak of the season would still be significantly increased compared with increases near the end of the season ($p = 0.007$).

MDI-treated group. In the MDI-treated group, symptom scores and levels of TAME-esterase activity and albumin after histamine challenge were not different throughout the study (Fig. 5). The diluent challenges also did not demonstrate a significant difference.

HDI-treated group. In the HDI-treated group, the symptom scores before the season were significantly

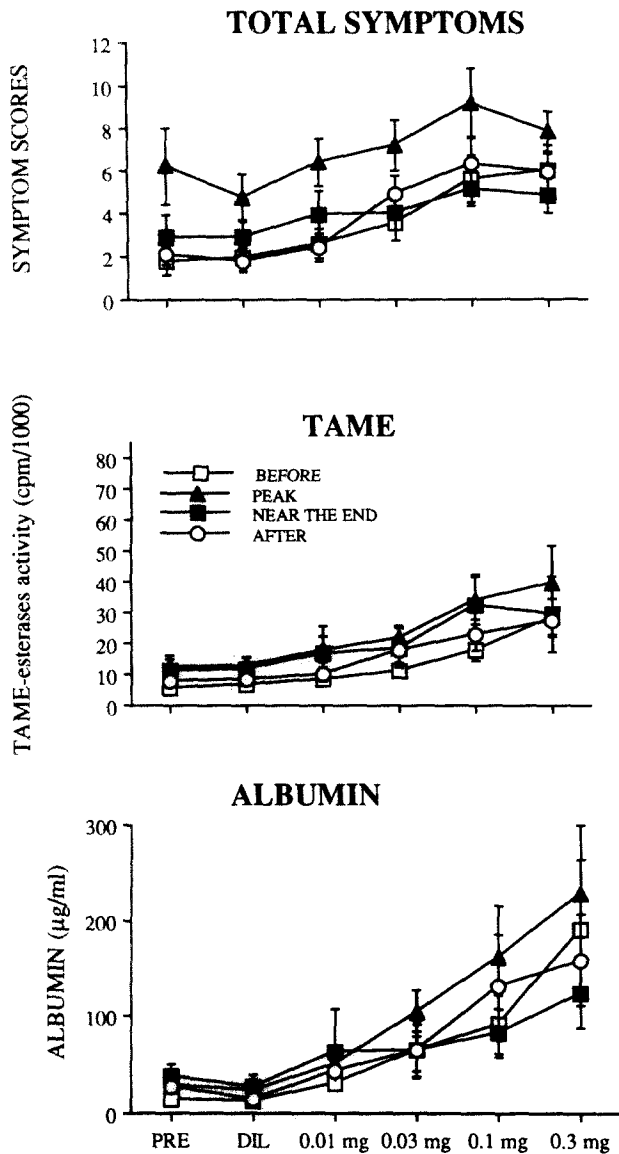


FIG. 4. Control group (N = 19). Symptom scores, levels of TAME-esterase activity, and albumin recovered from nasal lavages after histamine challenge. Total symptom scores were significantly higher during peak of season compared with scores before and after season ($p = 0.04$ and $p = 0.04$, respectively). TAME-esterase activity levels were not significantly different among visits. Only albumin levels differed between peak of season and near end of season ($p = 0.01$); values, mean $\pm$ SEM.

higher ($p = 0.05$) compared with scores after the season (Fig. 6). The saline challenges did not demonstrate any significant differences throughout the study.

The levels of TAME-esterase activity were significantly higher ($p = 0.04$) at the peak of the season compared with levels after the season but not com-

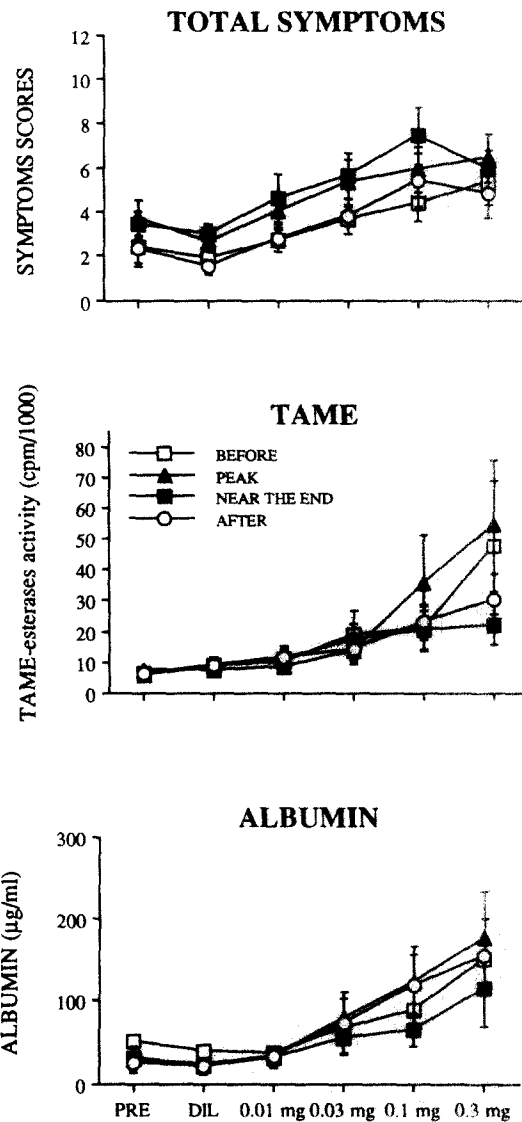


FIG. 5. MDI-treated group (N = 16). Symptom scores and levels of TAME-esterase activity and albumin recovered from nasal lavages after histamine challenge. No parameters demonstrated significant differences among visits; values, mean $\pm$ SEM.

pared with levels before the season. The results of saline challenges were not different in this group.

When albumin was analyzed, levels were higher during the peak of the season compared with levels before ($p = 0.03$) and after the season ($p = 0.04$). The saline challenges did not demonstrate any statistically significant difference among the visits.

DISCUSSION

Nasal histamine challenge in allergic subjects induces sneezing, rhinorrhea, and nasal congestion.

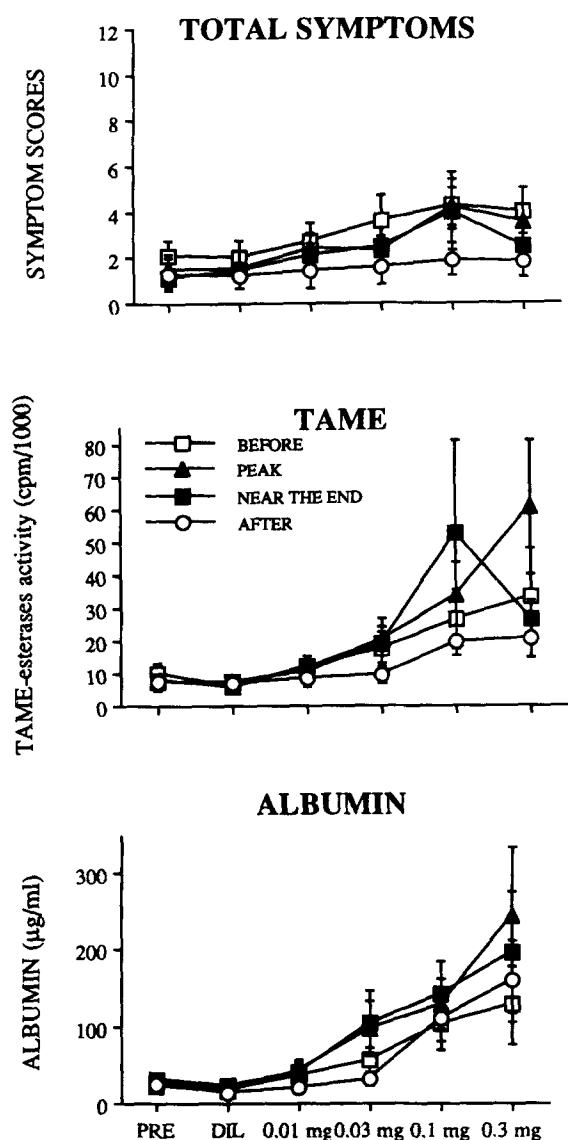


FIG. 6. HDI-treated group (N = 11). Symptom scores and levels of TAME-esterase activity and albumin recovered from nasal lavages after histamine challenge; values, mean $\pm$ SEM. No consistent differences were found among visits. See text for specific details.

These symptoms are accompanied by the recovery in nasal lavage fluid of increased levels of markers of vascular permeability, such as TAME-esterase activity (900,000 MW) and albumin (68,000 MW). This study demonstrated an increased symptom response to histamine nasal challenge during the peak of the pollen season in subjects with allergic rhinitis, as previously reported,¹⁵ whereas subjects undergoing immunotherapy did not demonstrate the same effect. However, if the saline response is considered baseline, then no change in symptoms from baseline occurred. The results for the levels of albumin and TAME-esterase

activity demonstrated similar patterns. Both parameters measured after saline challenge increased at the peak of the season only in the control group. When net changes in these levels were calculated, there was essentially no difference during the season.

Previous studies¹⁶ have demonstrated that the increased responsiveness to histamine after antigen is associated with an influx of inflammatory cells, neutrophils, eosinophils, and basophils, suggesting a causal relationship between cell influx and changes in reactivity. Since the changes observed occurred during the peak of the pollen season, when the inflammatory status of the nasal mucosa is expected to be at its maximum,¹⁷ we suspect that the inflammatory influx caused the changes we observed. Without a direct measure of inflammation in this study, the strength of the speculation is weakened. Since the effect dissipated as the pollen exposure decreased, the effect is reversible.

We previously demonstrated¹⁸ that the optimal maintenance dose for RW immunotherapy is between 0.6 and 12.4 μ g of Amb a I. Further increase in dosage does not decrease the symptom medication scores or the levels of histamine and TAME-esterase activity recovered after nasal challenge with RW. This same study demonstrated that the subjects receiving immunotherapy release less histamine and have less vascular permeability, as demonstrated by a decrease in recovered TAME-esterase activity after challenge. In this study, the MDI (2 μ g) did not affect the symptom-medication diaries but did affect the response to diluent during the histamine challenge.

In the HDI-treated group, no change in the diluent challenges was found throughout the study. Why there was a significant increase in the response to histamine with respect only to albumin from the baseline at the peak of the season compared with responses before and 2 weeks after the RW season is not readily explainable.

Naturally occurring antigen exposure to RW during the season induces a significant increase in symptoms and medication scores in control subjects after histamine challenge. However, this increase is not significant when the response to saline is subtracted. This finding may mean that the apparent hyperresponsiveness of the nasal mucosa to histamine during the antigen season can be explained on the basis of an increase in the baseline reactivity of the mucosa and not on an increased response to histamine. Our observation would not have been possible if our histamine challenges had not used a saline challenge before provoking with increasing doses of histamine. A single histamine challenge (0.1 mg) without a baseline measure would have reached the conclusion reported in the literature.¹⁹

This study raises questions about the definition of hyperresponsiveness. In our model of histamine challenge, in which the time interval between challenge and lavage is fixed, we examined the rate of recovered substances (in this case, albumin and TAME-esterase activity) in a fixed interval. The difference found after saline challenge could reflect either a true nonspecific hyperreactivity to the delivery system or, more likely, a change in the baseline level of vascular permeability. Although we present no data in this study to support the notion, we believe the inflammatory mucosal response to antigen caused the change. Subtracting the baseline response to saline eliminates any conclusions about seasonal increases in response to histamine, as previously reported. The fact that the changes do not persist beyond the peak of the pollen season we believe favors the argument that baseline status was altered rather than a true change in reactivity. This differs from laboratory models. The discrepancy may reflect the time of challenge and the uniformity of antigen exposure. A more vigorous pollen season may have produced a different outcome.

We believe the baseline shift is not observed in the initial lavage because multiple factors affect the initial lavage. The fixing of the time interval and exposure with the laboratory yield a more controlled situation in which observations can be made.

In sum, our study supports the benefits of immunotherapy but raises issues regarding the definition of hyperresponsiveness.

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