
Efficacy of immunotherapy to ragweed antigen tested by controlled antigen exposure

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Background: Immunotherapy is a recognized component in the management of allergic rhinitis. Its efficacy has been evaluated in a number of clinical field trials. These methods of evaluation are limited by control of antigen exposure.

Objective: A study was designed to evaluate the efficacy of immunotherapy in ragweed-induced rhinoconjunctivitis using an environmental exposure unit.

Methods: Forty-three subjects were grouped into (1) immunotherapy group: ragweed-allergic subjects on maintenance ragweed immunotherapy for at least 2 years ($N = 16$), (2) positive control group: ragweed-allergic subjects who had never received immunotherapy ($n = 16$), and (3) negative control group: ragweed-nonallergic subjects ($N = 11$). Ragweed specific skin tests and ragweed IgE levels were obtained prior to exposure. The study was done in a room where levels of 2,500 to 3,000 grains m^3 of ragweed were maintained over three hours. Symptoms were recorded every 15 minutes.

Results: Nasal symptoms in the immunotherapy group were significantly less than in the positive control group after 45 minutes ($P = .025$). Significant differences were not observed for ocular symptoms. Combined nasal and ocular scores were 50% less in the immunotherapy group than in the positive control group by 75 minutes ($P = .039$). Ragweed-specific skin tests and IgE were significantly less in the immunotherapy group than in the positive control group. Rhinoconjunctivitis symptoms in the negative control group were absent throughout.

Conclusions: Controlled ragweed pollen exposure in this setting demonstrated that ragweed immunotherapy significantly reduced symptoms of ragweed-allergic rhinitis but had no significant effect on ocular symptoms. This system presents opportunities for additional studies on immunotherapy for allergic respiratory conditions.

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INTRODUCTION

Immunotherapy is an established component in the management of pollen-induced allergic rhinitis/conjunctivitis.¹ Decisions to employ this mode of management are usually influenced by a number of factors including the compliance with treatment,² the history of adverse reactions,³ and the increasing availability of effective pharmacologic options.⁴ Despite the general accep-

tance of immunotherapy, there is a lack of controlled clinical studies that document the efficacy of immunotherapy as determined by subject response to pollen in an exposure situation that reproduces the clinical setting.

Recent advances in the development of topical corticosteroids, nonsedating antihistamines, and sodium cromoglycate have established their position in the treatment of ragweed induced rhinitis/conjunctivitis.^{4–6} Nevertheless, immunotherapy has continued to have a place in therapy either alone or in conjunction with other treatments for allergic respiratory disease. In spite of its acceptance as a treatment option, its efficacy and safety should continue to

be examined as appropriate means for doing so become available.

Nasal challenges using aqueous allergen extract have demonstrated the efficacy of ragweed immunotherapy and these findings have been supported by evaluation of allergic mediators.^{7,8} Other studies have used nasal provocation technique in a clinical setting to confirm the diagnosis of nasal allergy and determine the duration of immunotherapy required to achieve efficacious levels.^{9–11} Such studies however, are limited by apparent differences to natural pollen exposure. Connell,¹² using a facial apparatus to allow direct pollen inhalation, demonstrated an increase in nasal airway resistance in allergic subjects. This approximated but still did not duplicate usual exposure conditions. The development of the Environmental Exposure Unit has allowed for a controlled pollen challenge in an environmental setting where a limited number of individuals can be monitored for allergic reactivity. Up to 120 persons may be tested simultaneously providing optimal conditions for evaluation of anti-allergic treatments including efficacy and onset of action of medications.^{13–18}

In this study, the Environmental Exposure Unit was used to assess the response to environmental levels of ragweed in three groups of subjects: ragweed-allergic persons with upper respiratory symptoms on long-term high dose maintenance immunotherapy, ragweed-allergic individuals not receiving immunotherapy, and non-ragweed-allergic subjects as controls.

METHODS

Inclusion Criteria

Forty-three individuals age 18 to 65 were recruited for the study. The study

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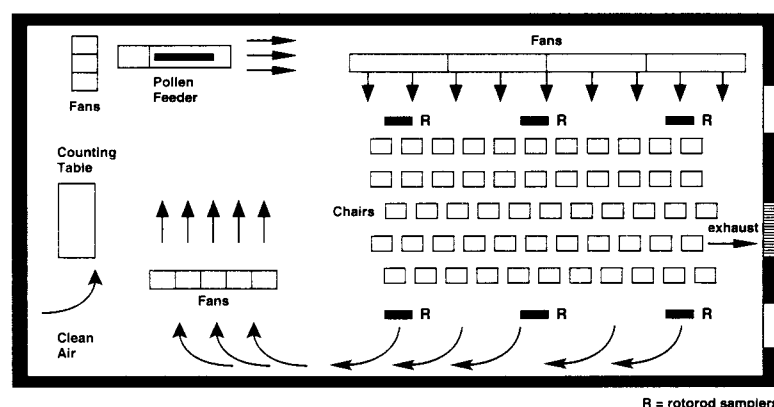


Figure 1. Design of environmental exposure unit (EEU).

subjects were selected based on their suitability for admission into one of three treatment groups: (1) immunotherapy group: ragweed-allergic individuals having received immunotherapy at a maintenance level for at least the previous 2 years ($N = 16$). (2) Positive control group: ragweed-allergic individuals who have never received immunotherapy for ragweed rhinitis ($N = 16$). (3) Negative control group: non-ragweed-allergic individuals ($N = 11$). Five subjects in the immunotherapy group were mildly asthmatic individuals who used anti-asthmatic medication intermittently only during the ragweed season.

All individuals were screened by skin prick testing with mixed aqueous ragweed allergen at a strength of 116.8 PNU/mL (1:20 wt/vol) (Hollister-Stier, Miles Lab, Inc) in order to confirm reactivity to ragweed allergen in the immunotherapy group and positive control group, and a lack of reactivity in the nonallergic group. Only patients with both clear clinical histories of ragweed allergy and 3+ (wheal and flare greater than or equal to positive control or at least 3 mm greater than the wheal and flare diameter for the negative control) or greater reactions to prick testing were entered into the immunotherapy group and positive control group. Patients in the negative control group were chosen based on negative histories of clinical hay fever and negative skin prick test.

Subjects in the immunotherapy group were required to be at mainte-

nance doses (500 pnu every 4 weeks–2.07 μg antigen E/mL) for at least the previous 2 years. The immunotherapy for those in the immunotherapy group was co-ordinated through the Division of Allergy at Kingston General Hospital with ragweed antigen obtained from Hollister-Stier (Division of Miles laboratories Inc) and prepared as an aqueous solution by mixing with albumin buffered saline.

Blood samples were obtained prior to pollen exposure. Ragweed-specific IgE titers were determined using RAST methodology (Pharmacia Diagnostics, Uppsala, Sweden) and were compared retrospectively with reaction to ragweed exposure.

Written informed consent was obtained from all patients before the conduct of any procedure. The protocol and consent form for the study were reviewed and approved by the Queen's University Health Sciences Human Research Ethics Board.

Exclusion Criteria

Subjects were excluded from the study for symptoms of rhinitis at the time of pre-exposure screening; active or quiescent tuberculosis; untreated fungal, viral, or bacterial respiratory infections; diseases or conditions that could interfere with the evaluation of response to ragweed exposure such as perennial rhinitis (allergic or nonallergic), acute or chronic infectious sinusitis, or asthma of moderate to severe classification requiring daily use of inhaled or oral corticosteroids; oral cor-

ticosteroid treatment within 60 days; topical corticosteroid treatment within 30 days; astemizole within 30 days; or the use of other short-acting medications (antihistamines, intranasal sodium cromoglycate, ketotifen, oral decongestants, and vasoconstrictors) within three days prior to enrollment.

Controlled Pollen Exposure

Subject-allergic symptoms were assessed during exposure to controlled pollen levels for up to three hours in the Environmental Exposure Unit. The Environmental Exposure Unit is a modified classroom with the ability to contain up to 120 individuals. Clean air was admitted into the room at the rate of $85\text{m}^3 \cdot \text{min}^{-1}$; both the intake and exhaust systems were equipped with filters to prevent room contamination. A feeder system introduced commercially obtained short ragweed (*Ambrosia elatior*) non-defatted pollen (Greer Laboratories Inc, Lenoir NC) into the Environmental Exposure Unit while a modified laser counter measured grains emitted and recorded the concentrations on a microcomputer. A system of fans was arranged to continuously circulate the air in the room and six impact type rotorod samplers (Sampling Technologies, St Paul MN) were arranged to determine pollen levels throughout the subject seating area. Feedback from the rotorod counters at 20-minute intervals was used to modify the pollen emission rate such that pollen concentration was maintained within a narrow range over the 3-hour period. To avoid bias from possible pollen level fluctuations in the Environmental Exposure Unit all patients were rotated to new positions every 30 minutes. Subjects whose symptoms did not permit them to remain in the Environmental Exposure Unit for the full three hours were moved to a pollen-free room and monitored for up to one hour (Fig 1).

Symptom Assessment

Patients were instructed to score their nasal symptoms, (rhinorrhea, congestion, itching, and sneezing) ocular symptoms (tearing and itching), and

Table 1. Characteristics of the Patient Population

Characteristic	Immunotherapy	Positive Control	Negative Control
Number of patients	16	16	11
Age, (yr)			
mean	39	34	35
range	19–60	20–55	20–63
Sex, n(%)			
male	6 (38%)	8 (50%)	5 (45%)
female	10 (62%)	8 (50%)	6 (55%)
Years on IT*			
mean	7		
range	2–18		

* IT = immunotherapy.

overall symptom assessment prior to pollen exposure and at 15-minute intervals during the 3-hour exposure. Four assessment groups containing equivalent randomized members from each of the three treatment groups were assigned to a physician and nurse. The assigned physician and nurse team were responsible for monitoring patient compliance, scoring patient eye redness, and the physician's overall symptom assessment for each patient. Symptom scores were recorded using the following scale: 0 (no symptoms), 1 (mild-occasional symptoms), 2 (moderate-recurrent symptoms), and 3 (severe-continuous symptoms). Asthmatic patients were monitored for respiratory problems by comparing baseline peak flow rates with periodic peak flow readings throughout the 3-hour study. Eligibility for the study was assessed by reviewing a patient history questionnaire given to the patients, which provided

information regarding their nasal and ocular symptom status for 2 weeks prior to the study.

Data Analysis

The primary variables for analysis were the combined nasal symptoms (discharge, congestion, itching, and sneezing), combined ocular symptoms (tearing, itching, and redness), and combined nasal/ocular symptoms. The combined nasal and ocular measures were calculated by summing the individual nasal and ocular symptoms respectively and the combined nasal/ocular measure was calculated by summing all the individual symptoms. The secondary variables for analysis were skin test results in mm, and IgE results in pru and class. Data analysis was divided into two main objectives.

The first objective was to determine whether there were any differences in response to ragweed challenge among immunotherapy, positive control, and

negative control groups. In the first step of this analysis the null hypothesis that ragweed challenge was not associated with a systemic variation in symptoms was tested by performing a Friedman nonparametric two-way analysis of variance on the primary variables for each group. All tests for the first objective were performed with the software package SYSTAT version 5.03 (©1990, SYSTAT Inc, Evanston IL) run on IBM PC with a DOS operating system. The null hypothesis was rejected when the probability of the variation in symptoms over time being due to chance was less than 0.05. In the second step the null hypothesis that groups had an identical response to ragweed challenge was tested by performing a Kruskal-Wallis one-way analysis of variance on the primary variables of the groups. The null hypothesis was rejected at sampling times where the probability of the difference between groups being due to chance was less than 0.05.

The second objective was to determine whether there were any differences between the skin test and IgE results for the immunotherapy and positive control groups. The null hypothesis that there was no difference in skin test and IgE results between groups was tested by performing a Mann-Whitney U Test on the secondary variables. All tests for the second objective were performed with the software package SAS version 5 (©1984, 1986, SAS Institute Inc, Cary, NC) run on an IBM ES9000 mainframe computer. The null hypothesis was rejected for

Table 2. Descriptive Statistics of Treatment Groups for Each Primary Variable

	Nasal Symptoms			Ocular Symptoms			Nasal and Ocular Symptoms		
	Immuno-therapy*	Positive Control*	Negative Control*	Immuno-therapy	Positive Control	Negative Control	Immuno-therapy	Positive Control	Negative Control
Number of time points	14	14	14	14	14	14	14	14	14
Mean symptom score	2.047	4.536	.1755	1.757	2.281	.2924	3.804	6.817	.4676
Std. error	.515	1.352	.0754	.475	.786	.1521	.934	2.122	.1504
Minimum	1.000	2.125	.0910	.813	.938	.000	1.813	3.125	.2730
Maximum	2.688	6.063	.2730	2.375	3.313	.5450	4.813	9.063	.7270

* Treatment group.

Table 3. Test for Systematic Variation in Symptoms During Pollen Exposure for Each Primary Variable of the 3 Treatment Groups

Group	Measure	P Value*
Immunotherapy	Combined nasal	.083
	Combined ocular	.005
Positive control	Combined nasal/ocular	<.001
	Combined nasal	<.001
Negative control	Combined ocular	<.001
	Combined nasal/ocular	<.001
	Combined nasal	1.000
	Combined ocular	.899
	Combined nasal/ocular	.979

* Friedman two-way ANOVA.

any secondary variable where the probability of the difference between groups being due to chance was less than 0.05. The relationship between the secondary variables was also examined. The null hypothesis that there was no correlation between an individual's skin test result and IgE result (pru) was tested by computing the Spearman rank correlation coefficients for skin test result and IgE in pru. The null hypothesis was rejected when the probability of the correlation being due to chance was less than 0.05.

RESULTS

Patient Population

Forty-three subjects were recruited and enrolled in the study, all of whom completed the three-hour pollen expo-

sure. For subjects receiving immunotherapy the mean duration of therapy was 7 years with a range of 2 to 18 years. The demographic characteristics of the three treatment groups are displayed in Table 1, and there were no significant differences in these characteristics between groups.

Response to Treatment

Data for the primary variables (sum of nasal scores, sum of ocular scores, and sum of combined nasal and ocular scores) were analyzed and compared among the three treatment groups. In general the three major findings of this study were (1) the positive control group scores were significantly greater than the immunotherapy group for nasal and combined nasal/ocular, but not

different for ocular symptoms alone, (2) positive control group and immunotherapy group scored significantly greater than the negative control group for all symptoms, and (3) there was no difference between the negative control group and zero for any of the three variables (Table 2).

The data were analyzed in two ways. The first method employed a Friedman nonparametric two-way ANOVA to look for changes in the primary variables within the three treatment groups over the course of the study. The ANOVA revealed a significant variation in all three variables during pollen exposure for the positive control group ($P = .001$). As one would expect, no significant variation was found in these variables for the negative control group. For the immunotherapy group, significant variation existed in ocular scores ($P = .005$) and in combined nasal/ocular scores ($P = .001$), but the variation in nasal symptom score during pollen exposure was not significant ($P = .083$). These results are summarized in Table 3 and Figures 2 to 4.

The second method of data analysis relied on a Kruskal-Wallis one-way ANOVA to look for changes in the primary variables among the three treatment groups at each 15-minute interval. A comparison of immunotherapy group and positive control group revealed a greater overall treatment response for nasal symptoms than for ocular symptoms. Differences in nasal symptoms between these groups were apparent by 45 minutes ($P = .025$) and remained throughout the exposure (Fig 2). A comparison of ocular symptoms revealed a lack of significant difference at any point throughout the exposure (Fig 3). When combined nasal and ocular symptoms were compared, a difference between the groups became evident at 75 minutes ($P = .039$) and remained throughout the pollen exposure (Fig 4).

Baseline ocular symptoms were similar between the negative control group and positive control group, ($P = .059$) and were significantly different at all subsequent times (Fig 3). In com-

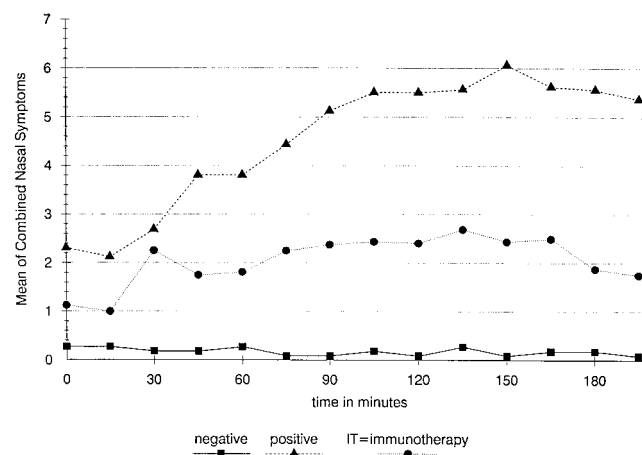


Figure 2. The combined nasal symptoms (discharge, congestion, itching, and sneezing) were calculated by summing the individual nasal symptoms. This graph demonstrates the changes in mean of the combined nasal symptoms for the three different test conditions at 14 separate time points.

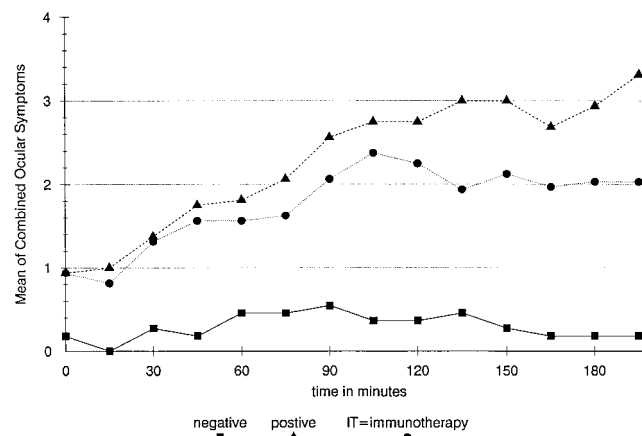


Figure 3. The combined ocular symptoms (tearing, itching, and redness) were calculated by summing the individual ocular symptoms. This graph demonstrates the changes in mean of the combined ocular symptoms for the three different test conditions at 14 separate time points.

parison, nasal symptoms were significantly different at baseline between the negative control group and positive control group ($P = .002$). They remained significantly different, while the absolute difference increased over the course of the study (Fig 2). In comparing the negative control group and immunotherapy group, differences were found by 30 minutes for nasal symptoms and combined nasal/ocular symptoms, but by 15 minutes for ocular symptoms.

The secondary variables (skin test scores and IgE levels) were analyzed and compared between the positive control group and immunotherapy

group. Using the Mann-Whitney U Test, the positive control group had significantly greater skin test scores (in mm) than the immunotherapy group ($P = .002$). IgE values were also found to be greater in the positive control group when compared with the immunotherapy group for both pru values and IgE class ($P = .034$ and $P = .032$, respectively). Using the Spearman Rank correlation coefficient, a significant positive correlation was found to be evident with skin test scores and IgE values (pru). This indicates for an increasing skin test score a corresponding IgE value was evident (Spearman's $Rho = 0.672$; $P = .0002$).

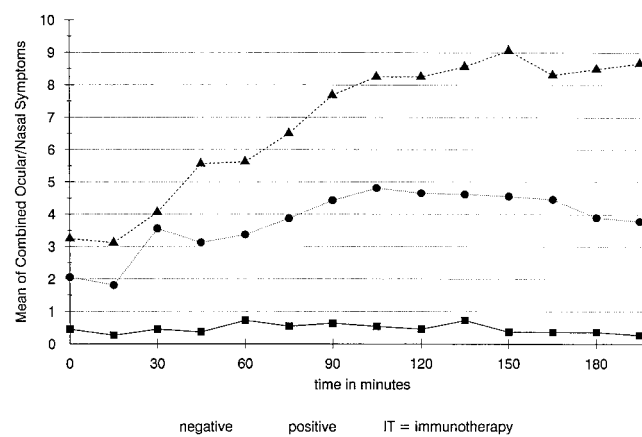


Figure 4. The combined nasal/ocular symptoms measure was calculated by summing all the individual symptoms. This graph demonstrates the changes in the mean sum of all the individual symptoms for the three different test conditions at 14 different separate time points.

Peak flow values decreased in four of the five asthmatic individuals during pollen exposure. Two of the subjects had values which dropped by 20% of their baseline scores (23% and 27%), while the other two patients had peak flow scores drop by 13% and 17% of their baseline values. The remaining unaffected individual had identical peak flow scores prior to and at the end of the 3-hour exposure.

DISCUSSION

In this study immunotherapy was found to have a significant moderating effect on ragweed-allergic rhinitis but a less apparent effect on ocular symptoms of ragweed allergy. The symptom response during pollen exposure in the positive control group indicated that the Environmental Exposure Unit produced upper respiratory and conjunctival symptoms in allergic individuals when these were absent in negative controls. This demonstrated that the Environmental Exposure Unit, while inducing specific responses, did not produce nonspecific reactions.

Symptom assessment postchallenge for the immunotherapy group reveal that nasal symptoms respond more than ocular symptoms to immunotherapy, perhaps indicating that immunotherapy produces greater benefit to allergic rhinitis and lesser to allergic conjunctivitis. These findings are also supported from the results comparing the positive control group and the immunotherapy group, where differences were found for nasal symptoms at 45 minutes and continued throughout the exposure, but ocular scores were comparable in both groups. Although these findings are suggestive of a lesser efficacy of immunotherapy for ocular symptoms, a steady trend towards achieving significant differences was observed during the 3-hour exposure period.

An attempt to explain ocular results involved analyzing the symptom scoring system used by each study personnel. Each physician was responsible for scoring for eye redness, while patients scored for itching and tearing. Statistical analysis revealed no differ-

ence between the physician and patients assessment of eye symptoms ruling-out the possibility of biased scoring by either party.

Various studies evaluating the efficacy of immunotherapy have been primarily concerned with rhinitis, but have indicated that immunotherapy is efficacious at controlling both nasal and ocular symptoms.¹ A similar observation was made by Juniper et al⁶ who compared the efficacy of aqueous steroid nasal spray with short-term immunotherapy in ragweed-allergic individuals. This study also reported a difference in control of nasal symptoms between the two treatment groups but no difference for ocular relief. Controlled studies using different means of ocular provocation have been undertaken¹⁹⁻²¹; however, the Environmental Exposure Unit has permitted assessment of ocular symptoms under controlled but also natural conditions and has proven to be valuable at determining efficacy of ophthalmic medication for the treatment of allergic conjunctivitis.¹⁵ A possible explanation for the findings in this study is that allergic individuals have a continued high level of non-specific reactivity of conjunctival mucous membrane surfaces and are more responsive to airborne pollen particulates. It is also possible that factors affecting efficacy of immunotherapy are represented differently on the nasal and ocular mucous membranes. As to its nonspecific nature of reactivity, it should be noted that the normal control group had no eye symptoms.

Skin test results and IgE levels were greater in the positive control group than the immunotherapy group. These findings coincide with clinical symptoms observed with these groups, but the use of skin test scores as an indicator of severity of allergy symptoms has been controversial.²² The controversy centers on the precise reliability of skin testing. Certainly individual variation in response to allergens is well known, but our findings and other studies suggest that skin testing may provide an indication of response to immunotherapy.^{23,24} Our results showed a strong correlation in the immuno-

therapy group between ragweed-specific skin test results and ragweed-specific IgE levels. These findings for antigen-specific IgE support work by Fei et al²⁵ who found IgE interference can lead to a reduction in clinical symptoms. In their study, antigen-specific IgE values were an indicator of patient symptomology and suggested these to be useful to assess efficacy of immunotherapy. Bousquet et al²⁶ found a correlation between skin test score and symptom score but no significant correlation between antigen-specific IgE levels and symptom score. These observations emphasize that patient symptom score may be the best indicator of the efficacy of immunotherapy.

Monitoring the five asthmatic individuals in our study indicated the potential of the Environmental Exposure Unit for assessing anti-asthmatic treatments. A small sample size and lack of controls ruled out an accurate interpretation of the efficacy of immunotherapy for asthma in this study. Four of the five individuals showed a reduction in peak flow scores after a 3-hour pollen exposure suggesting benefit of immunotherapy on asthma. Various studies indicate that immunotherapy has a place in treating allergy-induced asthma, but problems remain in evaluating its efficacy in pollen-induced asthma.^{1,27} The Environmental Exposure Unit presents an opportunity to further determine information about this condition.

The Environmental Exposure Unit has provided a reproducible exposure environment for testing the efficacy of various therapies for ragweed-allergic rhinitis. The absence of symptoms in the nonreactive group provided important evidence that nonspecific factors associated with the Environmental Exposure Unit environment were not responsible for nasal or ocular symptoms. The level of pollen approximated that which was found in some environments during peak ragweed season,^{28,29} an important control over a variable encountered in usual studies of pollen induced disease. Furthermore, this can be done independent of weather. The advantage of this

method over direct nasal and ocular application techniques includes the ability to provide reproducible uniform exposure to a large number of persons simultaneously in a situation quite comparable to natural exposure.

This study demonstrates that ragweed immunotherapy for ragweed allergy significantly modifies nasal symptoms under the test conditions of an Environmental Exposure Unit. This is another application of a system that has been used to test efficacy of other treatments of allergic rhinitis and conjunctivitis and may be applied to comparable studies of allergic asthma.

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