

Responses to ragweed pollen in a pollen challenge chamber versus seasonal exposure identifies allergic rhinoconjunctivitis endotypes

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Background: The level of concordance between allergic symptoms induced on exposure to pollen in a pollen challenge chamber (PCC) versus the natural season is unknown.

Objective: We sought to test the hypothesis that the symptom levels of allergic rhinoconjunctivitis elicited after out-of-season exposure to short ragweed in a PCC and during the natural season for giant ragweed pollen are highly correlated.

Methods: Thirty-one ragweed-sensitive participants recorded symptoms for 15 days during the natural giant ragweed season in San Antonio, Texas. Twenty-six of these participants were challenged to short ragweed pollen in a PCC for 3 hours per day for up to 4 days.

Results: In the PCC participants were dichotomized into those in whom low versus high levels of symptoms developed slowly or rapidly (ie, slow/low vs rapid/high). Each successive exposure visit associated with a progressive increase in symptom levels that approximated those experienced during the natural season. Hierarchic clustering identified 3 endotypes: endotypes I and II reflected concordantly low ($n = 7$) versus high ($n = 14$) total symptom scores (TSSs) in both the natural season and the PCC, respectively. Accordingly, the correlation between the TSSs recorded in the natural season and in the PCC for these 21 participants was very high. Although participants with endotype III ($n = 5$) had greater TSSs in the natural season than in the PCC, the degree of correlation between the TSSs remained high.

Conclusions: Our findings affirm our hypothesis, underscore the high cross-reactivity between distinct pollens, and highlight the utility of the PCC to identify novel allergy endotypes that might have contrasting mechanistic underpinnings and potentially therapeutic responses. (J Allergy Clin Immunol 2012;■■■■:■■■■-■■■■.)

Key words: Allergic diseases, environmental chamber, ragweed, endotypes

Although seasonal allergy to pollen is a very common diathesis, more effective and durable therapies are needed. Confounding environmental factors (eg, dust and mold spores) during the natural allergy season can be hard to control and thus might negatively affect the assessment of novel therapies. Environmental exposure chamber studies can offer the advantage of inducing allergic rhinoconjunctivitis after exposure to pollen in the context of a highly controlled environment.¹⁻⁶ This strategy might potentially mitigate uncontrolled confounding factors that are inherent in phase III therapy trials conducted in sensitive subjects during the natural allergy season (eg, variable exposure to pollen or a short in-season period).⁶

However, there are 2 key knowledge gaps that need to be bridged before this goal can be achieved. Foremost, the relative contributions of pollen itself versus additional environmental cofactors in triggering allergic rhinoconjunctivitis during the natural allergy season are unknown.^{7,8} Additionally, it is unknown whether participants in one geographic area exposed during the natural season, primarily to different species of given plants, will respond adequately to a single nondominant species being used in environmental chamber studies in multiple geographic sites. For example, in South Texas the dominant ragweed species are giant ragweed (*Ambrosia trifida*) and false ragweed (*Ambrosia acanthicarpa*).⁹ By contrast, short ragweed (*Ambrosia artemisiifolia*), the most widespread plant of the genus *Ambrosia* in North America, is present, as is western ragweed (*Ambrosia psilostachya*), although both are present at lesser levels of exposure. For these reasons, here we determined whether the extent to which allergic symptoms induced by an out-of-season challenge of short ragweed in a pollen challenge chamber (PCC) correlated with symptoms induced by giant ragweed pollen in the natural season. The choice of comparing short versus giant ragweed pollen was based on the study design. This was an important objective because a high degree of cross-reactivity would allow for testing the efficacy of novel therapies against an allergen in a PCC in which the pollen-induced allergy is geographically less prevalent (eg, short ragweed in Texas).

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Abbreviations used

PCC: Pollen challenge chamber
 TNSS: Total nasal symptom score
 TOSS: Total ocular symptom score
 TSS: Total symptom score

METHODS**Study participants**

The protocol, amendments, and informed consent documents were approved by the IntegReview Institutional Review Board (Austin, Tex). All participants provided written informed consent, and the study was conducted according to Good Clinical Practice standards. Thirty-one participants met the inclusion/exclusion criteria outlined in Fig E1 in this article's Online Repository at www.jacionline.org.

Study design

An overview of the study design is shown in Fig 1. The natural season phase of the study was performed during the height of the natural ragweed season (September 27, 2010, to October 11, 2010), and the PCC phase was 6 weeks later, when no pollen was detected in San Antonio, Texas (Fig 1).

Natural seasonal exposure

Visit 1. This visit day was within the peak of the natural ragweed allergy season (Fig 1). Medical/medication histories and results of physical examinations (and urine pregnancy test for female participants) were obtained. Participants were dispensed a medical events calendar to record any changes in their health status and instructed on the use of participant daily diaries and symptoms evaluation diaries. Symptoms were self-scored on a Likert scale, with values of 0 (absent), 1 (mild), 2 (moderate) and 3 (severe). Total nasal symptom scores (TNSSs) were based on nasal congestion, sneezing, rhinorrhea, and itching. Total ocular symptom scores (TOSSs) were based on redness, tearing, and itching. The total symptom score (TSS) resulted from adding the scores of the 7 symptoms, yielding a minimum score of 0 and a maximum score of 21.

Visit 2. Participants were evaluated at the end of the peak of the natural season to determine eligibility to enter the PCC phase (Fig 1).

Visit 3 (pre-PCC visit). The following study-related items were performed: medical events calendars were reviewed, and changes in concomitant medications that might have occurred since the natural seasonal exposure were recorded. Participants who continued to maintain inclusion/exclusion criteria were provided symptoms evaluation diaries. Symptoms were recorded twice (morning and evening) for the 4 days preceding the PCC visit and once (morning) on the day of the first exposure visit (Fig 1). The average of these prechallenge symptom scores was considered the baseline value.

PCC visits

Four exposure (PCC) visits were planned. PCC visit 1 occurred on November 29, 2010. Those who continued to meet the study criteria ($n = 26$, Fig 1) were dispensed PCC diaries. Each participant was instructed to record TNSSs and TOSSs at 30-minute intervals during the 3-hour exposure visits (Fig 1). All participants underwent at least 2 exposure visits (Fig 1) and were free to withdraw from the study on any account. During each exposure visit, 3 PCC physicians (R.L.J., C.P.A., and D.A.R.) were present. The study protocol had an *a priori* requirement that if a participant reached a maximum score of 21 or experienced side effects that were of concern, they would be released from the study. Defatted short ragweed pollen (Greer Laboratories, Lenoir, NC) was delivered (3500 ± 500 grains/m³) by a computer-controlled feeder, as described previously.¹⁰ Pollen levels were counted at 30-minute time intervals and at the end of the exposure visit, as previously described.¹⁰

Statistical analysis

The Pearson correlation coefficient was used to determine correlations among the individual components that comprise the TNSSs and TOSSs, as well as between TOSSs and TNSSs. The effects of (1) increasing duration of exposure time in the PCC and (2) each additional exposure visit on TSSs were assessed by using linear generalized estimating equation models. The mean TSS and the SD of the TSS for each subject within each environment (PCC or natural season) were calculated to determine the maximum TSS in the natural season and exposure visits. The maximum value that was within 2 SDs from the mean TSS was considered the maximum PCC or natural season TSS and abbreviated as PCC TSS^{max} and seasonal TSS^{max}, respectively.

To determine the correlation of allergic symptoms during the natural season and those elicited after exposure to pollen in the PCC, it was assumed that the ideal scenario would be complete correlation in symptoms, where the line of best fit was as follows:

$$\text{Seasonal TSS}^{\text{max}} = 0 + 1.00 \times \text{PCC TSS}^{\text{max}} \quad (X = Y).$$

The residual from this ideal line was calculated, and the SD of these residuals was then computed. A maximum TSS that was within 2 SDs from this line was considered concordant, and the remainder were considered discordant. Linear regression was used to examine the relationship between PCC TSS^{max} and seasonal TSS^{max}. Unsupervised hierarchic clustering with Euclidean distances and average linkage was performed to further examine potential groupings of subjects based on the maximum TSS. Where applicable, χ^2 , Fisher, Mann-Whitney *U*, and paired *t* tests were used.

RESULTS**Natural season phase**

Thirty-one participants completed the natural season phase of the study (Fig 1). Seventeen and 13 were non-Hispanic and Hispanic white subjects, respectively, and 1 was African American. The average age was 41 years (range, 18-65 years). During the height of the natural season, the average pollen count was 774 grains/m³ (range, 290-1520 grains/m³). Twenty-six of the 31 participants recorded TOSSs of 5 or greater (84%) and 26 participants recorded TNSSs of 7 or greater (84%) on 4 consecutive days.

The mean symptom scores of the 31 participants were 4.4, 8.4, and 11.8 for the TOSS, TNSS, and TSS, respectively. The scores were consistent across the 15 days of recording (see Fig E2 in this article's Online Repository at www.jacionline.org). Although TSSs did not correlate significantly with the same-day pollen count (see Fig E2 and Table E1 in this article's Online Repository at www.jacionline.org), they correlated with the ambient pollen count recorded 1 or 2 days before the day symptoms were recorded (see Table E1).

PCC phase

Of the 31 participants completing the natural season phase, 26 met the eligibility criteria for PCC studies (Fig 1). Of these 26 participants, 10 were male, and 15, 10, and 1 were non-Hispanic white, Hispanic white, and African American subjects, respectively. The average age was 42 years (range, 22-62 years).

Pollen induced symptoms within 30 minutes of exposure (Fig 2, A-C). Fourteen participants completed all 4 exposure visits (Fig 2, B), whereas 12 participants withdrew from the study before completing 4 visits, mostly because of rapid induction of heightened symptoms (Fig 2, C). On the basis of their responses, those completing all exposure visits versus those exiting the PCC early were designated as slow/low responders (Fig 2, B) versus rapid/high (Fig 2, C) responders, respectively. Rapid/high responders had a trend toward higher TSSs at baseline (ie, before

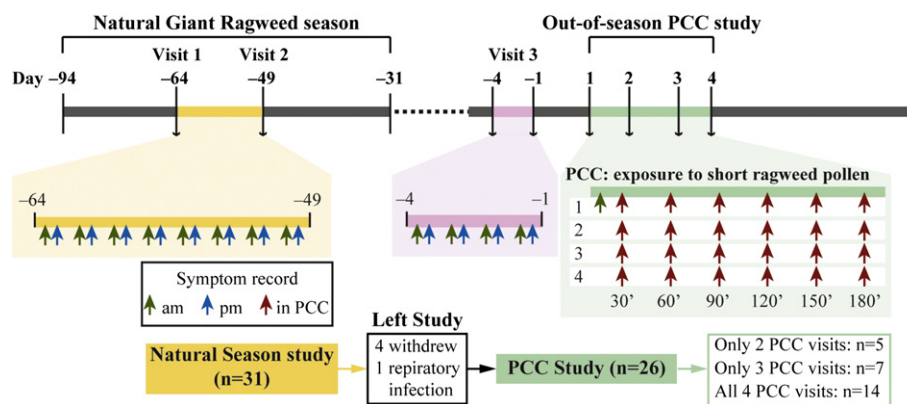


FIG 1. Study design and participants. Study visits and timings of symptoms were recorded by participants in the natural season and in the PCC. The number of participants in each phase of the study is shown.

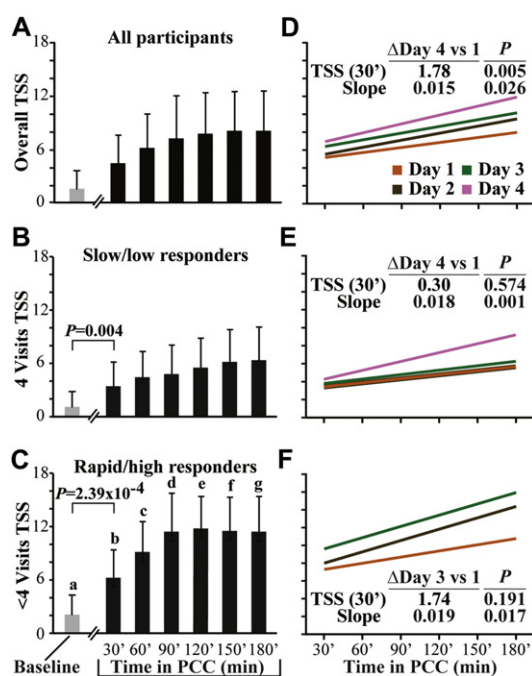


FIG 2. Average TSSs and trend lines in the PCC. Means $\pm$ SDs of the TSSs for all exposure visits recorded in all participants, regardless of the number of PCC visits (**A**); those who completed all visits (slow/low responders; **B**), and those not completing all visits (rapid/high responders; **C**). Baseline is the average TSS from 4 days before and the morning of the first PCC visit. *P* values for data in Fig 2, C, versus Fig 2, B are as follows: a, .197; b-g, <.001. **D-F**, Changes in TSSs over time during the PCC visits were estimated by using linear generalized estimating equation modeling. Δ , Difference.

challenge) and had significantly greater symptom levels at each of the 6 measurements made during an exposure visit compared with slow/low responders (compare Fig 2, C, vs Fig 2, B). Slow/low responders manifested a progressive increase in TSSs during the course of the exposure visit (Fig 2, B), whereas rapid/high responders exhibited a brisk increase in TSSs, reaching a plateau by 90 minutes of exposure (Fig 2, C).

We next investigated, using generalized estimating equation modeling, the nature of the response to pollen challenge with each successive visit (Fig 2, D-F). In this analysis the slope of each line reflects how fast participants responded, and the intercept of each

line reflects the average TSS at 30 minutes after pollen exposure. In the overall study group the slope increased by 0.015 TSS/min between days 1 and 4 ($P = .026$), suggesting that each additional day of exposure increases the rate at which symptoms were induced (Fig 2, D). Study participants reach, on average, a TSS of 5.16 after 30 minutes of allergen challenge during the first-day visit, and by day 4, the intercept increases by 1.78 ($P = .005$; Fig 2, D). Among slow/low responders, the TSSs 30 minutes after pollen exposure were comparable in each successive exposure visit, and the rate of increase in TSS during each exposure visit was fairly stereotypical on PCC days 1 to 3, whereas on day 4, there was a rapid increase in TSSs (Fig 2, E). By contrast, among rapid/high responders there was a trend for the TSS 30 minutes after pollen exposure to be higher on day 3 versus those on day 1, and the rate of increase in TSSs was progressively faster during each successive exposure visit (Fig 2, F).

Comparison of PCC versus natural season phases

Similar to what was observed in the natural season, TSSs and TNSSs in the exposure visits contributed to one third and two thirds, respectively, of the overall TSS (see Fig E3, A, in this article's Online Repository at www.jacionline.org). We categorized participants who achieved a TNSS of 7 or greater or a TOSS of 5 or greater during the natural season or the exposure visits as significant responders. The proportion of participants who were classified as significant responders increased progressively with each successive exposure visit (see Fig E3, B). With each successive visit, those who were categorized as rapid/high responders had symptom scores in the PCC that were comparable with those experienced during the peak of the natural season, whereas those who were slow/low responders had symptom scores in the PCC that were slightly lower than those experienced in the peak of the natural season (see Fig E4 in this article's Online Repository at www.jacionline.org). A significant correlation between the diameter of the skin test to ragweed and the TSS in the PCC or natural season was not observed (see Fig E5 in this article's Online Repository at www.jacionline.org).

There was a high degree of correlation among the individual ocular and nasal symptoms recorded in the natural season and in the PCC, as well as between the TNSS and the TOSS (Table I). However, as a general rule, the correlations among individual symptoms were lower after exposure to pollen in the PCC

TABLE I. Symptom correlation in the natural season versus the PCC

Symptom comparison	Natural season (n = 31)		PCC (n = 26)	
	R	P value	R	P value
TOSS components				
Eye itch vs tears	0.83	<.0001	0.68	<.0001
Eye itch vs redness	0.81	<.0001	0.62	.0005
Tears vs redness	0.76	<.0001	0.55	.003
TNSS components				
Congestion vs nasal itch	0.79	<.0001	0.71	<.0001
Congestion vs sneezing	0.52	.0057	0.28	.168
Congestion vs runny nose	0.88	<.0001	0.85	<.0001
Nasal itch vs sneezing	0.53	.0047	0.17	.41
Nasal itch vs runny nose	0.79	<.0001	0.61	.0007
Sneezing vs runny nose	0.54	.0038	0.39	.048
TSS components				
TOSS vs TNSS	0.86	<.0001	0.52	.0057

Correlations were derived based on the maximum score recorded in each subject.

compared with in the natural season, especially for the comparisons between congestion versus sneezing and nasal itch versus sneezing (Table I). Comparison of the scores in the natural season versus those in the PCC revealed that with the exception of sneezing and runny nose, the correlations for the scores of the other symptoms recorded during the natural season versus the PCC were high (Table II). Concordantly, the correlations for the TOSS, TNSS, and TSS between the natural season versus exposure visits were 0.57, 0.56, and 0.65, respectively (Table II).

The abovementioned data point to the possibility that symptom levels experienced during the natural season might be higher than in the PCC. However, it was also conceivable that these results might be skewed by a subset of participants who were more responsive to pollen during the natural season than in the PCC. To test this possibility, we compared the relationship between the maximum TSS ($TSS^{\max}$) recorded for each participant during the PCC visits versus those recorded during the natural season. The overall correlation between PCC $TSS^{\max}$ and seasonal $TSS^{\max}$ was high ($R = 0.65$, $P < .001$). The plot of PCC $TSS^{\max}$ versus seasonal $TSS^{\max}$ revealed that those participants (letter and color coded) clustered into 3 main groups (Fig 3): clusters I and II had concordant responses because they were comprised of participants who had concordantly low (Fig 3, blue) versus high (Fig 3, green) TSSs, respectively, in both the PCC visits and the natural season, and cluster III, with the exception of 1 participant, had discordant responses because it comprised subjects with low TSSs in the PCC but high TSSs in the natural season (Fig 3, purple).

The slopes for the correlations between maximum TSSs in the PCC and those in the natural season derived for participants with concordant versus discordant responses did not significantly differ from those of the ideal (perfect) match line ($X = Y$; Fig 3, A). In general, the 3 lines (ideal and those for the concordant and discordant responders) were nearly parallel to each other, except for the line for group III being shifted to the upper left (Fig 3, A). This observation, together with the similar R^2 values for the concordant and discordant groups, indicates a high correlation between symptom scores recorded in the natural season and the PCC visits, although the discordant group experienced higher symptomatology in the natural season than during the pollen challenge in the PCC (Fig 3, A). Notably, except for 1 study participant

TABLE II. Correlation between natural season versus PCC visits

Variables	R	P value
TOSS components		
Eye itch	0.49	.01
Tears	0.56	.003
Redness	0.54	.004
TNSS components		
Congestion	0.58	.001
Sneezing	0.21	.298
Runny nose	0.17	.397
Nasal itch	0.63	.0004
TSS components		
TOSS	0.57	.002
TNSS	0.56	.002
TSS	0.65	.0002

Correlations were derived based on the maximum score recorded in each subject (n = 26).

(participant O), all the other participants who were categorized as rapid/high responders (ie, who did not complete 4 exposure visits) clustered to group II; most rapid/high responders also had high symptom scores during the natural season (Fig 3). Thus comparison of the symptoms elicited in the PCC versus those elicited in the natural season identified 3 allergy endotypes (Fig 3, B). Comparison of the baseline characteristics of participants who were categorized in these 3 endotypes indicated that 85.7%, 92.9%, and 80.0% of those of endotypes I, II, and III, respectively, had a history of other seasonal allergic rhinitis (>0.1 ; see Table E2 in this article's Online Repository at www.jacionline.org). A higher proportion of participants with endotype II worked indoors and were non-Hispanic white subjects compared with the other participants (see Table E2).

To evaluate the capacity of TSSs in the PCC to predict TSSs in the natural season, we conducted logistic regression analyses (see Table E3 in this article's Online Repository at www.jacionline.org). Although sex, race, age, and skin test results for ragweed were not significant predictors, the maximum PCC TSS was a strong predictor of the maximum TSS recorded in the natural season (see Table E3). As noted, working indoors associated with endotype II and hence also predicted a higher TSS (data not shown).

DISCUSSION

In this study we demonstrated that ragweed-sensitive subjects in South Texas, when exposed to short ragweed pollen in a controlled environment (ie, a PCC), had allergic rhinoconjunctivitis symptoms that mimicked those elicited by giant ragweed during the natural season. These data suggest a high degree of cross-sensitivity between these 2 allergens. There was also significant correlation among individual symptom scores between the natural season and exposure to pollen in the PCC, and on the basis of the symptom levels elicited in the PCC versus the natural season, we identified 3 clinical allergy endotypes. Most of our studied participants were either Hispanic white subjects or non-Hispanic white subjects, and we found that race, age, sex, or skin test results to ragweed did not associate with symptom scores, whereas the symptom scores elicited in the PCC were strong determinants of the level of symptoms experienced during the natural season. Consistent with previous studies,¹¹⁻¹⁴ our study

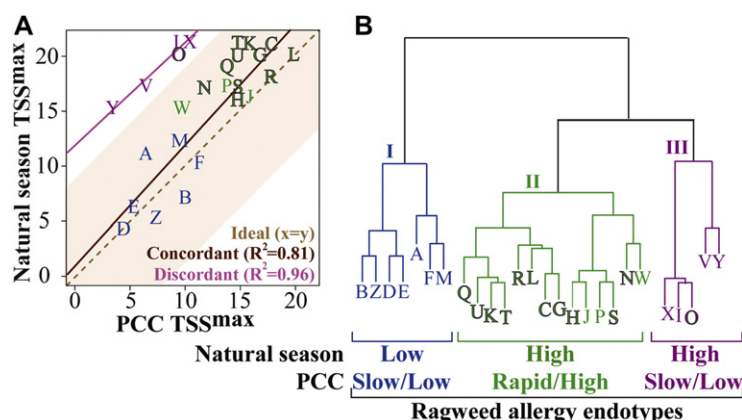


FIG 3. Correlation of seasonal TSS^{max} and PCC TSS^{max} and cluster analysis. **A**, Correlation of natural season TSS^{max} and PCC TSS^{max}. Brown and purple regression lines represent subjects with concordant versus discordant (ie, PCC TSS^{max} < natural season TSS^{max}) symptoms between the natural season and the PCC, respectively. The shaded band represents $\pm$ 2 SDs from the ideal line ($Y = X$). **B**, Cluster analysis based on both seasonal TSS^{max} and PCC TSS^{max}. Participants were letter and color coded. Black letters indicate participants with less than 4 PCC visits. Endotypes are noted by Roman numerals I, II, and III.

confirmed that the rate of symptom development or maximal symptom level achieved by participants exposed to ragweed pollen in the PCC or natural season was unrelated to the size of their ragweed skin test response. Together, these results substantiate the value of PCC studies for the identification of allergy endotypes and evaluation of novel therapeutics.

Interestingly, in the PCC the correlations in symptom scores between sneezing versus congestion, nasal itch, or rhinorrhea were lower than the same correlations during the natural season. We also observed that, in general, symptom scores were higher in the natural season compared with those experienced in the PCC. These differences in symptom scores could relate to the presence of additional particulate material or allergens during the natural season compared with the PCC. Thus, although speculative, having higher particulate/allergen concentrations in the air could cause more irritation of the nose and more sneezing in a similar fashion as when dust is blown into a person's face.

In the South Texas area there are 4 species of ragweed that pollinate at levels sufficient to cause symptoms in sensitized subjects.⁹ Giant ragweed and false ragweed are more abundant than common (or short) ragweed, with western ragweed in the mix in certain areas of the state. There is controversy concerning the level of cross-reactivity between these various species. Bernstein et al¹⁵ and Leiferman et al¹⁶ reported a very strong cross-reactivity using a RAST inhibition technique. However, Asero et al¹⁷ suggested that cross-reactivity between short and giant ragweed is only approximately 50% when considering their responses to subcutaneous immunotherapy. Other environmental chambers in North America have also used short ragweed pollen. Berkowitz et al¹ reported 48% of participants did not have sufficient symptoms on 2 priming visits of 3 hours in duration. Studies by Day et al¹⁸ and Patel et al⁴ reported nonpriming rates of 34% and 26%, respectively. However, in agreement with Ellis et al,¹¹ we found that all our participants had symptoms, with 65% achieving priming levels, suggesting that cross-reactivity is very strong between the ragweed species.

On the basis of the rate at which subjects had symptoms and the degree of their symptoms in the PCC, ragweed-sensitive participants were categorized broadly into 2 categories: rapid/high versus slow/low responders. Successive exposure visits

associated with progressively greater symptom scores that approximated the scores recorded in the natural season, particularly in rapid/high responders. These data suggested that multiple priming visits might be needed to “awaken” fully allergic responsiveness, particularly in the slow/low sensitive participants. However, because rapid/high responders might have adequate symptoms with only 1 or 2 priming visits, this could obviate the need for additional exposures.

Prior data show that 2 to 3 priming visits might be required to obtain high levels of symptoms.^{4,11} However, these studies did not correlate the findings obtained in the natural season versus those obtained in the PCC. In our study comparative analyses of symptomatology during the natural season versus the PCC identified 3 ragweed allergy endotypes: endotype I reflects slow/low responders in the PCC who also exhibited concordantly low symptom scores in the natural season, endotype II reflects rapid/high responders in the PCC who also manifested concordantly high symptom scores in the natural season and PCC, and endotype III reflects slow/low responders in the PCC who had higher symptom scores in the natural season than in the PCC. These endotypes highlight the heterogeneity of the allergenic response to pollen challenge in sensitive subjects.

Thus endotypes I and II represent the concordant phenotype and were present in 81% of our study participants. The consistency of low versus high symptom levels in both the PCC and the natural season proffers the pathogenic possibility that ragweed-sensitive subjects maintain stable high versus low “preprimed steady states” in the off season. On priming in the natural season or the PCC, sensitive subjects with endotypes I and II transition from these preprimed steady states to a “primed or symptomatic steady state,” having stably low versus high symptomatology, respectively. Conceivably, novel and contrasting immunologic pathways might underlie endotypes I and II. One possibility, although speculative, is that rapid/high responders (endotype II) maintain higher preprimed steady states because of genetically or epigenetically mediated heightened inflammatory status that, on priming with allergen (pollen), promotes exaggerated immunologic responses. Ongoing studies are addressing these mechanistic possibilities because specific targeting of these endotypes might point to novel personalized therapies.

The third endotype we identified represents a discordant phenotype. Possible contributors to the higher symptom scores in the natural season than PCC could be that participants with higher symptoms in the natural season might manifest reactivity to pollens other than ragweed. Additionally, weather fronts, changing temperatures, mold spores, pets, dust mites, and environmental debris might also contribute to endotype III. Conversely, the absence of these factors in the PCC and the shorter durations of exposure to allergen in the PCC relative to the natural season might together explain why none of our study participants had features consistent with the fourth endotype (ie, higher symptomatology in the PCC compared with that in the natural season). Identifying these cofactors that promote symptomatology in the natural season compared with those observed in the PCC might lead to the development of novel strategies for mitigating symptoms.

The main strengths of the present study are its prospective design because we determined allergic symptoms after exposure to ragweed pollen in a controlled environmental setting and compared them with symptoms elicited during the natural ragweed season in the same set of sensitive participants, as well as the focus on understanding the degree of cross-sensitivity to contrasting pollen challenges in the natural season versus the PCC. The lack of biological correlates of the described endotypes represents the main limitation of the present study. Additionally, psychological factors, for which we could not control, might alter a participant's perceptions of the intensity of symptoms.¹⁴

Recent studies in asthmatic patients have identified distinct endotypes that have distinct pathogenic mechanisms and therapeutic implications (eg, upregulated IL-13 pathway in those with steroid-resistant asthma).^{19,20} As a corollary, the distinct endotypes we identified herein might have implications for understanding allergy pathogenesis and use of the PCC for evaluation of therapeutics. For example, dose-response characteristics of therapies directed against pollen/allergen-induced symptomatology might differ among subjects with these distinct endophenotypes. The strong cross-reactivity between giant and short ragweed together with the high correlation of symptom levels in the PCC and the natural season points to the applicability of using a single common allergen (eg, short ragweed) to conduct phase III clinical trials in multiple PCCs that are geographically dispersed.

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Clinical implications: Symptoms of allergic rhinoconjunctivitis elicited after exposure to ragweed in an environmental chamber and during the ragweed season are largely concordant. Environmental chambers have utility for evaluating therapies for and mechanisms of rhinoconjunctivitis endotypes.

REFERENCES

- Berkowitz RB, Woodworth GG, Lutz C, Weiler K, Weiler J, Moss M, et al. Onset of action, efficacy, and safety of fexofenadine 60 mg/pseudoephedrine 120 mg versus placebo in the Atlanta allergen exposure unit. *Ann Allergy Asthma Immunol* 2002;89:38-45.
- Day JH, Briscoe MP. Environmental exposure unit: a system to test anti-allergic treatment. *Ann Allergy Asthma Immunol* 1999;83:83-93.
- Hashiguchi K, Tang H, Fujita T, Suematsu K, Tsubaki S, Nagakura H, et al. Validation study of the OHIO EEC in patients with Japanese cedar pollinosis. *Int Arch Allergy Immunol* 2009;149:141-9.
- Patel D, Garadi R, Brubaker M, Conroy JP, Kaji Y, Crenshaw K, et al. Onset and duration of action of nasal sprays in seasonal allergic rhinitis patients: olopatadine hydrochloride versus mometasone furoate monohydrate. *Allergy Asthma Proc* 2007;28:592-9.
- Ronborg SM, Mosbech H, Johnsen CR, Poulsen LK. Exposure EEC for allergen challenge. The development and validation of a new concept. *Allergy* 1996;51:82-8.
- Akerlund A, Andersson M, Leflein J, Lildholdt T, Mygind N. Clinical trial design, nasal allergen challenge models, and considerations of relevance to pediatrics, nasal polyposis, and different classes of medication. *J Allergy Clin Immunol* 2005;115(suppl):S460-82.
- Badorrek P, Dick M, Hecker H, Schaumann F, Sousa AR, Murdoch R, et al. Anti-allergic drug testing in an environmental challenge EEC is suitable both in and out of the relevant pollen season. *Ann Allergy Asthma Immunol* 2011;106:336-41.
- Hohlfeld JM, Holland-Letz T, Larbig M, Lavae-Mokhtari M, Wierenga E, Kapsenberg M, et al. Diagnostic value of outcome measures following allergen exposure in an environmental challenge EEC compared with natural conditions. *Clin Exp Allergy* 2010;40:998-1006.
- Everitt JH, Lonard RL, Little CR. Weeds in South Texas and Northern Mexico. Lubbock (TX): Texas Tech University Press; 2007.
- Jacobs RL, Ramirez DA, Andrews CP. Validation of the Biogenics Research Chamber for *Juniperus ashei* (mountain cedar) pollen. *Ann Allergy Asthma Immunol* 2011;107:133-8.
- Ellis AK, Ratz JD, Day AG, Day JH. Factors that affect the allergic rhinitis response to ragweed allergen exposure. *Ann Allergy Asthma Immunol* 2010;104:293-8.
- Andersson M, von Kogler B, Andersson P, Pipkorn U. Allergen-induced nasal hyperreactivity appears unrelated to the size of the nasal and dermal immediate allergic reaction. *Allergy* 1987;42:631-7.
- Bacon JR, McLean JA, Mathews KP, Banas JM. Priming of the nasal mucosa by ragweed extract or by an irritant (ammonia). *J Allergy Clin Immunol* 1981;67:111-6.
- Graif Y, Goldberg A, Tamir R, Vigiser D, Melamed S. Skin test results and self-reported symptom severity in allergic rhinitis: the role of psychological factors. *Clin Exp Allergy* 2006;36:1532-7.
- Bernstein IL, Perera M, Gallagher J, Michael JG, Johansson SG. In vitro cross-allergenicity of major aeroallergenic pollens by the radioallergosorbent technique. *J Allergy Clin Immunol* 1976;57:141-52.
- Leiferman KM, Gleich GJ, Jones RT. The cross-reactivity of IgE antibodies with pollen allergens. II. Analyses of various species of ragweed and other fall weed pollens. *J Allergy Clin Immunol* 1976;58:140-8.
- Asero R, Weber B, Mistrello G, Amato S, Madonini E, Cromwell O. Giant ragweed specific immunotherapy is not effective in a proportion of patients sensitized to short ragweed: analysis of the allergenic differences between short and giant ragweed. *J Allergy Clin Immunol* 2005;116:1036-41.
- Day JH, Briscoe MP, Ratz JD, Ellis AK, Yao R, Danzig M. Onset of action of loratadine/montelukast in seasonal allergic rhinitis subjects exposed to ragweed pollen in the environmental exposure unit. *Allergy Asthma Proc* 2009;30:270-6.
- Corren J, Lemanske RF, Hanania NA, Korenblat PE, Parsey MV, Arron JR, et al. Lebrikizumab treatment in adults with asthma. *N Engl J Med* 2011;365:1088-98.
- Kraft M. Asthma phenotypes and interleukin-13—moving closer to personalized medicine. *N Engl J Med* 2011;365:1141-4.

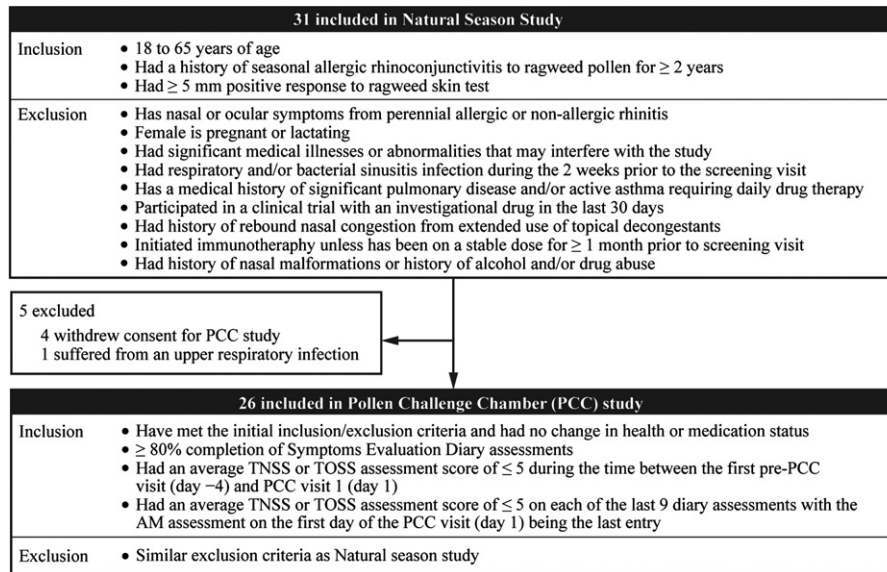


FIG E1. Inclusion/exclusion criteria for the natural season and the PCC.

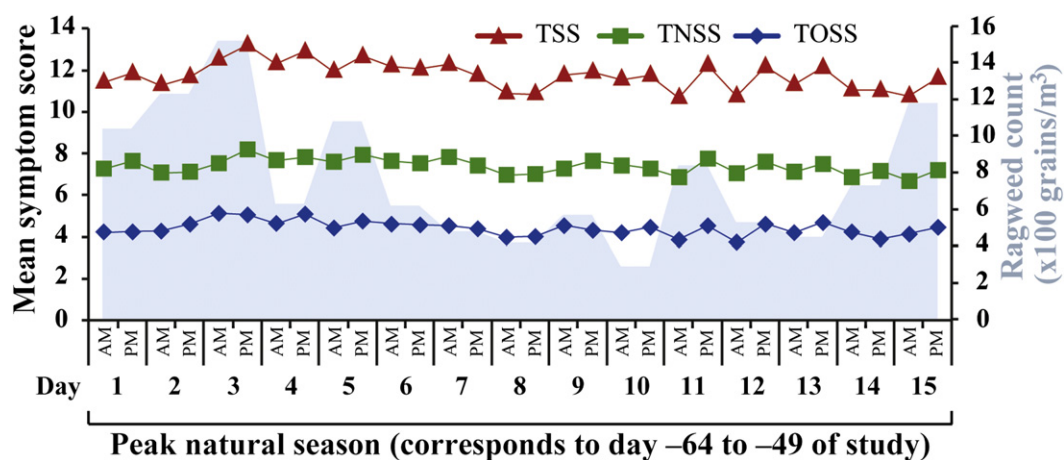


FIG E2. Average symptom scores and pollen counts during the natural season. Shown is the mean TSS (triangles), TNSS (squares), and TOSS (diamonds), as well as pollen counts (blue shading), in the 31 participants who recorded symptoms for 15 days during the peak of the natural season. Symptoms were recorded twice a day (morning [AM] and evening [PM]).

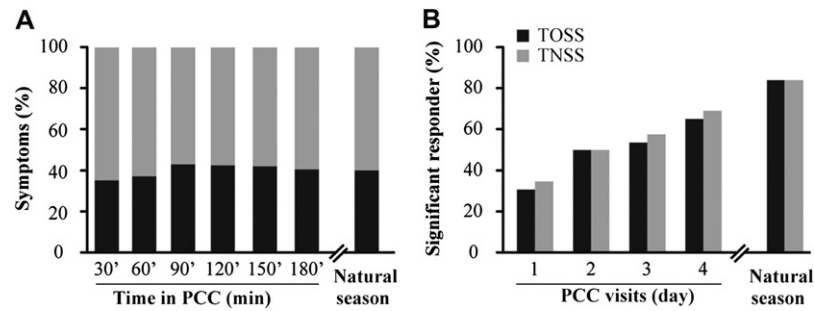


FIG E3. Correlation among symptom scores between natural season versus PCC visits. **A**, Relative contributions of TOSSs and TNSSs to the TSS. **B**, Proportion of significant responders by TOSS (score ≥ 5) or TNSS (score ≥ 7).

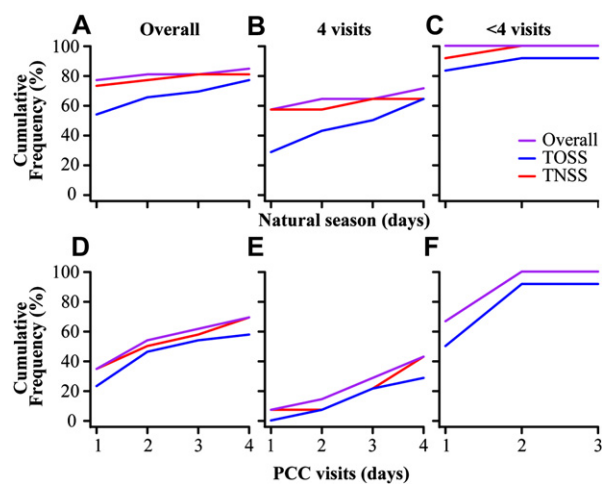


FIG E4. Cumulative frequency of symptom scores for the first 4 days of the peak of the natural season (**A-C**) and during chamber runs (**D-F**). Symptom scores are color coded. We chose the TSSs from the first 4 days in the peak natural season for comparisons. In Fig E4, *F*, the *blue* and *red lines* are superimposed.

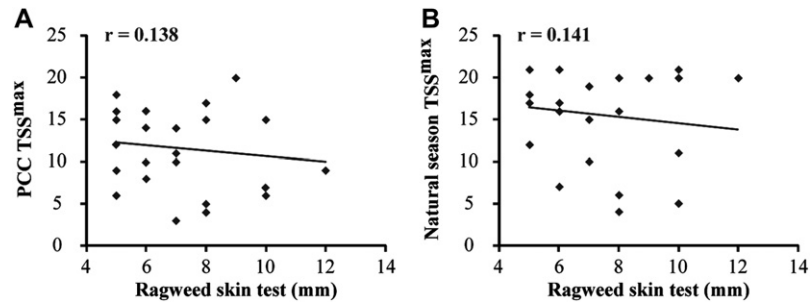


FIG E5. Correlation between the maximum TSS in the chamber (**A**) and natural season (**B**) with the ragweed skin test. Pearson correlation r values and trend lines were shown.

TABLE E1. Association of average daily pollen count and TSS in the natural season*

Time of pollen count	Estimated TSS/ pollen count	P value
Same day	0.0006	.131
1 Day prior	0.0011	.003
2 Days prior	0.0010	.003

*Depicted are the associations of pollen counts on same day of, 1 day before, and 2 days before the average daily TSS recorded for a given day. Values were calculated by using a generalized least-squares model with a compound symmetric correlation structure.

TABLE E2. Baseline characteristics of the participants with the 3 endotypes

	Endotype			P value		
	I	II	III	I vs II	I vs III	II vs III
No.	7	14	5			
Male sex,* no. (%)	3 (42.9)	6 (42.9)	1 (20.0)	1.000	.579	.603
Non-Hispanic white,*,§ no. (%)	1 (14.3)	12 (85.7)	2 (40.0)	.003	.523	.084
Age,† mean $\pm$ SD	41.4 $\pm$ 12.6	43.5 $\pm$ 7.2	39.1 $\pm$ 10.8	.694	.743	.437
Ragweed skin test diameter (mm),‡ median (IQR)	8 (7-9)	7 (5-8)	6 (5-7)	.322	.410	.886
Asthma history,* no. (%)	0 (0.0)	1 (7.1)	0 (0.0)	1.000	1.000	1.000
Perennial allergic rhinitis,*, no. (%)	7 (100.0)	14 (100.0)	5 (100.0)	1.000	1.000	1.000
Other seasonal allergic rhinitis,*,¶ no. (%)	6 (85.7)	13 (92.9)	4 (80.0)	1.000	1.000	.468
Indoor work environment,*,# no. (%)	1 (20.0)	11 (78.6)	3 (75.0)	.034	.206	1.000
Other concurrent diseases,*,** no. (%)	4 (57.1)	11 (78.6)	2 (40.0)	.354	1.000	.262

*The Fisher exact test was used to compare proportions between endotypes.

†The Student *t* test was used to compare mean values between endotypes.‡The Mann-Whitney *U* test was used to compare medians between endotypes.

§Participants categorized into non-Hispanic white versus the rest.

||Participants categorized into those who do have perennial allergic rhinitis versus those who do not.

¶Participants categorized into those who have other seasonal allergic rhinitis versus those who do not.

#Participants categorized into those whose work environment is primarily indoors versus those whose work environment includes the outdoors. Endotype I had 2 participants with an unknown work environment, and endotype III had 1 participant with an unknown work environment. Those 3 participants were excluded from the analyses.

**Participants categorized into those who do have other diseases versus those who do not. Those diseases, including hypertension, migraine, obesity, and hyperlipidemia, are not allergy related.

TABLE E3. TSSs in the PCC predict TSSs in the natural season*

	OR	95% CI	<i>P</i> value
Sex (male vs female)	3.30	0.59-18.36	.185
Race (Hispanic vs white)	0.67	0.12-3.59	.641
Age	0.95	0.85-1.07	.416
Skin test	1.16	0.77-1.74	.489
TSS ^{high} vs TSS ^{low} unadjusted	12.38	1.69-90.58	.021
TSS ^{high} vs TSS ^{low} adjusted for sex	12.45	1.65-93.80	.022

*High and low TSSs were stratified by the median of the maximum TSS in the chamber and the natural season. Logistic regression was conducted by using the chamber TSS as a predictor and the natural season TSS as the outcome.