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Responsiveness to timothy grass pollen in individuals without known natural exposure in an allergen challenge chamber

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ABSTRACT

Background: The responsiveness to a nonendemic grass species is unknown and cannot be research without an allergen challenge chamber.

Objective: To determine the clinical responsiveness to timothy grass pollen (TGP) in participants without known natural exposure in an allergen challenge chamber (ACC).

Methods: Of the 26 screened participants, 22 met screening criteria and completed the 2 chamber exposures. The study consisted of an initial screening visit that included a blood draw for serum specific IgE (ssIgE) to Bermuda grass pollen and TGP followed by a 4½-day run-in phase and two 3-hour ACC exposure visits. This study was performed early in the first week of December 2013, when no seasonal pollens were detected in San Antonio, Texas. Symptom scores were recorded at baseline and every 30 minutes.

Results: Of the 26 screened participants, 22 met the screening criteria and completed the 2 chamber exposures. Thirteen participants had always lived in South Texas without natural exposure, and 9 had previously lived in areas with TGP exposure. All participants tested positive to TGP and Bermuda grass pollen. Twelve and 13 of 22 had positive ssIgE test results to Timothy and Bermuda allergens, respectively, with 11 having positive results for both allergens. There were strong correlations among skin prick test size, a positive ssIgE test result, and high symptoms from TGP exposure. There was little difference in symptoms between those who had lived their entire lives in South Texas and those who had lived elsewhere.

Conclusion: In Texas, where exposure to TGP is minimal, strongly positive SPT and ssIgE test results were predictors of high symptoms to TGP exposure. Never exposed participants in South Texas reacted to TGP similar to those who had previous natural exposure, suggesting that in vivo cross-reactivity may be higher than predicted by prior in vitro data and may allow the use in clinical trials of allergens not endemic to the locale of an ACC.

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Introduction

Allergen challenge chambers (ACCs) have been previously used to determine the efficacy and onset of action of new therapies for allergic rhinitis study and asthma.^{1,7,9–11} These facilities offer many advantages over traditional research methods in that studies can be performed outside the natural pollen seasons, allergen levels can be carefully controlled and monitored in the chamber with no effect from outside weather conditions, and study participants are carefully monitored while being exposed to allergen.

Grass pollen sensitivity is a major cause of allergic rhinoconjunctivitis throughout the world, affecting most atopic individuals.² Asthma, atopic dermatitis, and, rarely, contact dermatitis may also occur as a result of exposure to these pollens. Management of these symptoms may be only partially effective, with treatments ranging from symptomatic (attempting to control symptoms) to specific (attempting to alter the individuals level of sensitivity).³

Exposure to the pollinating grass species is not evenly distributed and varies according to the climate of the geographic areas.² Temperate grasses dominate the northern parts of North America and Europe, with subtropical grasses dominating the warmer climates of parts of Africa, India, Asia, Australia, and Central and South America and the Southern United States.¹ Even though in vitro studies have found significant cross-reactivity among various grass species, no in vivo clinical trials using nonendemic grasses have been performed.

In this investigation, we sought to determine the extent to which allergic symptoms induced by a challenge to timothy grass

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Table 1

Subject inclusion and exclusion criteria

Inclusion Criteria	
Male or female 18 to 70 years of age	
History of AR to grass pollen exposure and a SPT result to Bermuda grass of ≥ 5 mm greater than the diluents control	
Exclusion criteria	
Female who is pregnant or lactating	
Have any significant medical illness that may interfere with the study	
Have any abnormalities on physical examination that may interfere with the study	
Had a respiratory infection during the past 14 days before the screening visit	
Current medical history of pulmonary disease requiring daily drug therapy or asthma requiring treatment >2 times per week	
Has participated in a trial with an investigational drug in the last 30 days	
History of rebound nasal congestion from extended use of topical decongestants	
History of nasal polyps, septal perforation, or significant nasal tract malformations noted on physical examination	
Current alcohol or drug abuse or history of the same in the past 3 years	
Use of disallowed medications	
Immunotherapy within 90 days of screening visit	

Abbreviations: AR, allergic rhinoconjunctivitis; SPT, skin prick test.

pollen (TGP), a temperate grass, in an ACC located in South Texas would mimic the symptoms that would be naturally induced by pollens of subtropical grasses (Bermuda, Bahia, or Johnson grass pollens). The choice of TGP was based on its wide prevalence, the intense symptoms elicited in its endemic regions, and the increasing use of its pollen in new approaches to therapy.

Currently, there is great interest in novel allergy therapies, including safer modes of immunotherapy, such as sublingual immunotherapy. Two new grass sublingual immunotherapy products, Oralair (a tablet containing extracts from sweet vernal, orchard, perennial rye, timothy, and Kentucky blue grass)⁴ and Grastek (a tablet containing timothy grass extract)⁵ have recently been approved for use in the United States by the Food and Drug Administration for the treatment of grass allergy to one of the component grasses in the product. The grass species included in these grass tablets belong to the subfamily Pooideae, which are the dominant grasses in the temperate zones of the world but account for less than 15% to 25% of the grasses in the subtropical Southern United States.⁶ By contrast, the most prevalent grasses in central Texas belong to the subfamilies Chorioideae (Bermuda grass) and Panicoideae (Johnson grass and Bahia grass).⁶

As in our previous investigation with ragweed,⁷ the successful use of a nonendemic allergen was an important objective because sufficient cross-reactivity could allow for testing the efficacy of novel therapies against an allergen in an ACC in which the species of pollen being tested is different from the species that would be used for treatment.

Methods

Study Participants

The protocol, amendments, and informed consent documents were approved by the IntegReview Institutional Review Board (Austin, Texas). All participants provided written informed consent,

Table 2

Likert scale symptom scores

Score	Symptoms	Description
0	Absent	No sign or symptoms evident
1	Mild	Signs or symptoms clearly present but minimal awareness; easily tolerated
2	Moderate	Awareness of signs or symptoms; bothersome but tolerable
3	Severe	Definite awareness of sign or symptoms; hard to tolerate but does not interfere with the activities of daily living
4	Very severe	Difficult to tolerate and interferes with the activities of daily living

and the study was conducted according to Good Clinical Practice standards. Twenty-six participants were screened, and 22 participants met all the study inclusion and exclusion criteria (Table 1) and completed the study. Of the 26 screened participants, 22 met screening criteria and completed the 2 chamber exposures.

Study Design

The study design consisted of an initial screening visit followed by a 4½-day run-in phase and two 3-hour ACC exposure visits on 2 consecutive evenings starting at 6 PM. This study was performed early in the first week of December 2013, when no seasonal pollens were detected in San Antonio, Texas. At the screening visit, participants gave informed consent and underwent assessments and procedures that consisted of a medical history review, physical examination, skin prick testing (SPT) to a screening allergen panel, and a blood draw for serum specific IgE (ssIgE) to Bermuda grass pollen and TGP (Phadia ImmunoCAP: negative <0.35 kUA/L) to determine eligibility to participate in the study. A participant run-in diary was distributed to all participants for assessing and recording nasal symptoms, including congestion, itching, sneezing, and runny nose, and ocular symptoms, including itching, tearing, and redness, every morning and every evening using a 5-point Likert scale (scores, 0–4) (Table 2) up to the first chamber exposure. This scale differs from the current 4-point (scores, 0–3) scoring system,⁷ which may be too restricted at the 3 (severe) category. Therefore, we elected to use a variation of a 5-point (scores, 0–4) scoring system as suggested by Calderon et al,⁸ who also noted that a 0–3-point scale may be inadequate for allergy studies.

Chamber Exposures

Twenty-two participants qualified for inclusion in the study. On confirmation of eligibility and before entry into the ACC, urine pregnancy test results were obtained for all female participants of child-bearing potential. Each participant was assigned a chair and table number for the duration of the consecutive 2-day chamber

Table 3

Participant demographics

Demographic	No. (%) of participants ^a
Age, mean (range), y	42.9 (21–69)
Non-Hispanic white	11
Non-Hispanic black	1
Asian	1
Hispanic white	9
Male sex	12 (54.5)
Lived outside Texas for >5 years	9 (40.9)

^aData are presented as number (percentage) of patients unless otherwise indicated.

Table 4
SPT and sslgE Test Responses

Test	No. (%) of participants
Allergen SPT	
Timothy grass	22/22 (100)
Bermuda grass	22/22 (100)
Johnson grass	21/22 (95.5)
Bahia grass	20/22 (90.9)
Mountain cedar	22/22 (100)
Virginia live oak	14/22 (63.6)
Fall (cedar) elm	12/22 (54.5)
Pecan pollen	16/22 (72.7)
Arizona ash	13/22 (59.1)
Ragweed, short	17/22 (77.3)
Ragweed, mixed	15/22 (68.2)
Spiny pigweed	15/22 (68.2)
Mold mix I	17/22 (77.3)
Mold mix II	16/22 (72.7)
Dog epithelium	17/22 (77.3)
Cat hair	15/22 (68.2)
<i>Dermatophagoides pteronyssinus</i>	21/22 (95.5)
<i>Dermatophagoides farinae</i>	17/22 (77.3)
German cockroach	19/22 (86.4)
Allergen sslgE Phadia ImmunoCAP	
Bermuda sslgE class ≥ 1	13/22 (59.1)
Timothy sslgE class ≥ 1	12/21 (57.1)

Abbreviations: SPT, skin prick test; sslgE, serum specific IgE.

visits. Each participant was issued a chamber visit scoring diary to assess and record his/her nasal and ocular symptoms at baseline and every 30 minutes for the 3-hour chamber exposure visits using the Likert scale. For the run-in and chamber diaries, a total nasal symptom score (TNSS) was calculated by adding the individual scores of nasal congestion, nasal itching, congestion, and rhinorrhea, yielding a range of scores from 0 to 16. The total ocular symptom score was determined by adding the ocular symptoms of pruritus, lacrimation, and ocular erythema with a range of 0 to 12. A total symptom score (TSS) was calculated by adding the TNSS and total ocular symptom score, yielding minimum and maximum scores of 0 and 28, respectively.

Defatted TGP (Greer Laboratories, Lenoir, North Carolina) was delivered into the chamber by a computer-controlled feeder, as described previously.⁹ In the chamber, TGP was collected on

silicone-coated slides with Allergenco-D Posi-track disposable cassettes (Environmental Monitoring Systems Inc, Charleston, South Carolina) and microscopically counted at 30-minute intervals and at the end of the exposure visit to ascertain relative equal distribution in 5 stations of the chamber.⁹ The mean pollen count for both 3-hour exposure sessions in the ACC was 3,916 grains/m³, with a mean range of 3,312 to 4,251 grains/m³.

Statistical Analysis

Simple descriptive statistics were used to summarize data. For each participant, the mean (SD) of the TSS was calculated, and the maximum TSS was defined as the maximum TSS that was within 2 SDs of the mean TSS. The Spearman correlation coefficient (ρ) was used to compare maximum TSS and SPT sizes. The 2-sample *t* test analysis assuming unequal variances was used to compare participants who had always lived in South Texas without natural exposure to TGP and those who had lived elsewhere with TGP exposure and for analysis of the results of the sslgE tests to Bermuda grass pollen and TGP. Linear regression analyses were conducted where appropriate.

Results

Demographics

Most of our studied participants were either Hispanic white or non-Hispanic white. A demographic summary of the study participants is listed in Table 3.

Skin Tests

Participants were chosen for this study based on having a greater than 2-year history of allergic rhinoconjunctivitis symptoms with cut grass exposure and having a positive SPT result (≥ 5 -mm wheal greater than the size of a diluent control) to Bermuda and/or Johnson grass pollen extracts. All study participants reacted to Bermuda grass pollen, TGP, and mountain cedar pollen. For the other 2 grass species, Bahia and Johnson, the reaction rates were 90.9% and 95.5%, respectively. More than 70% of participants had positive SPT results to 14 or more of the 19 allergens tested (Table 4).

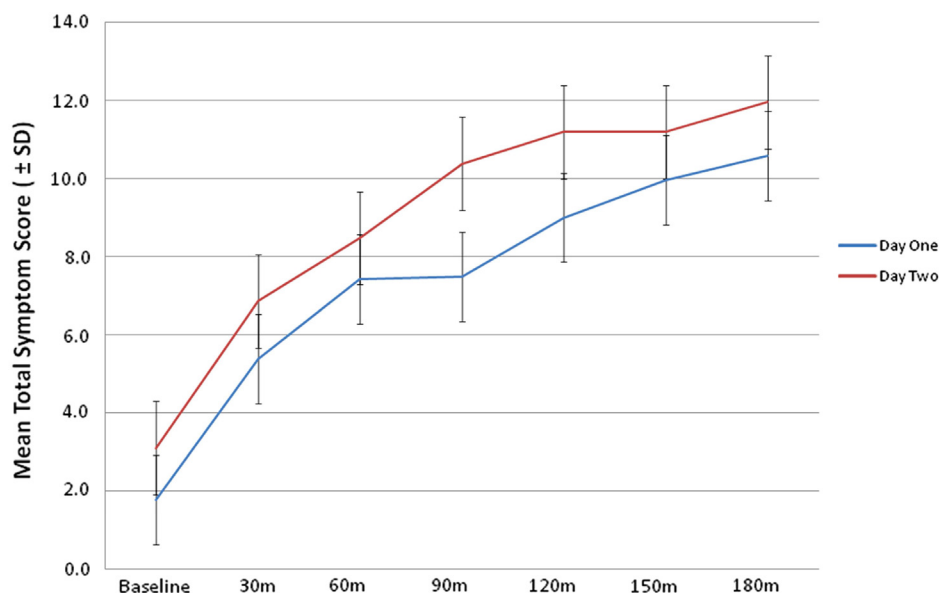


Figure 1. Mean iTSS Results All Subjects for two the Chamber Exposure days. Mean iTSS $\pm$ SD at all study time points in days one and two in the chamber.

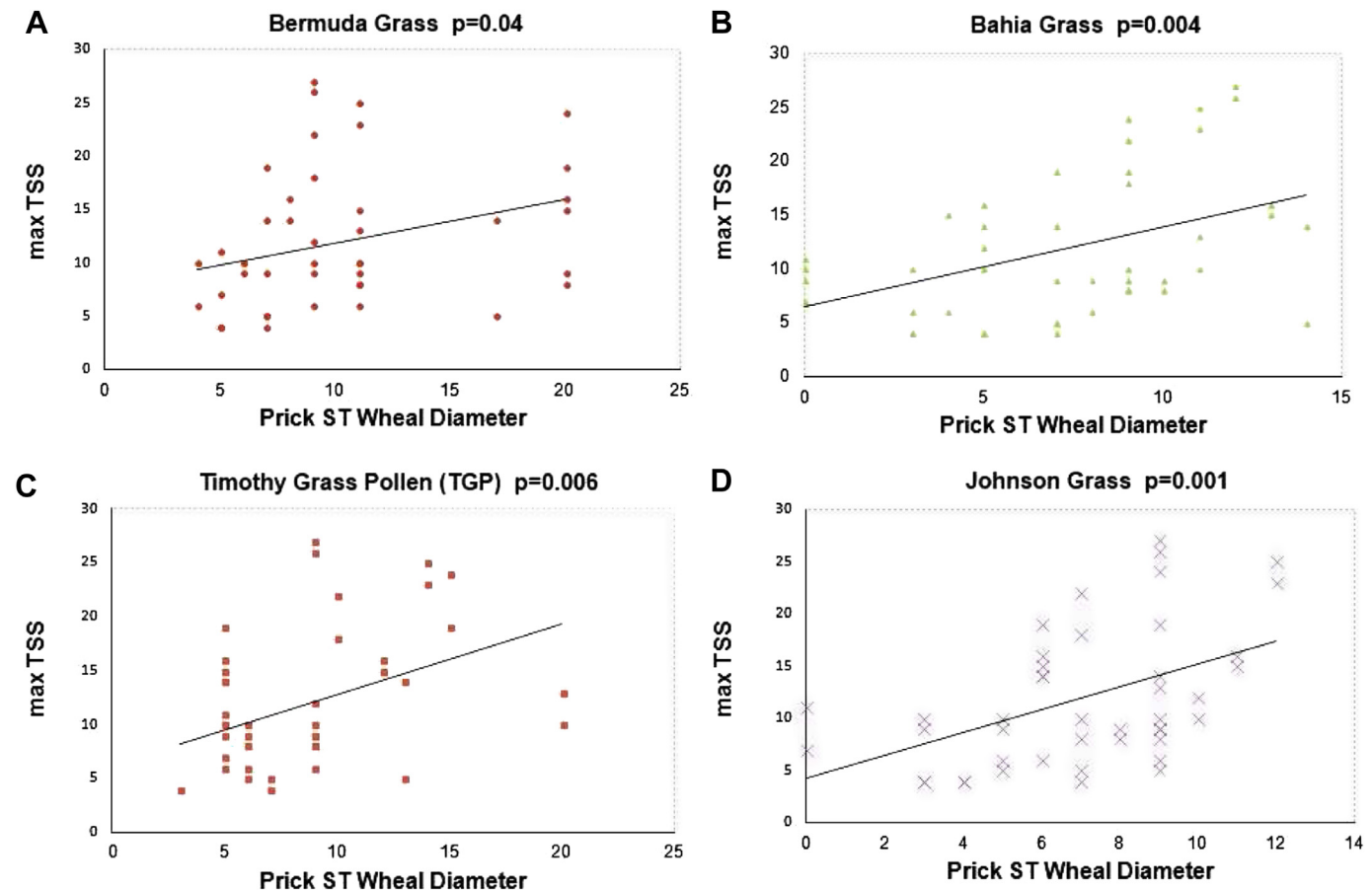


Figure 2. maxTSS in the Chamber vs. Grass Pollen SPT Diameter and Trend Lines and calculated Spearman rho(ρ) values. (A) Bermuda grass wheal diameters vs. maxTSS. ($\rho = 0.305$) (B) Bahia grass wheal diameters vs. maxTSS. ($\rho = 0.429$) (C) Timothy grass wheal diameters vs. maxTSS. ($\rho = 0.404$) (D) Johnson grass wheal diameters vs. maxTSS ($\rho = 0.475$).

Symptom Scores in the ACC

We found that race, age, and sex were not associated with symptom score changes. During the run-in period (4 days and morning of first chamber exposure), the mean reflective TSS was 2.51. At baseline, at the time of entry into the chamber for visit 1 and before pollen exposure, the mean instantaneous total symptom score (iTSS) was 1.8 and increased to 10.6 after the 3-hour exposure. On the second day, the mean baseline iTSS was 3.1, increasing to 12.0 at the end of that exposure (Fig 1). A maximum TNSS (maxTSS) of 8 or greater was recorded in 45% of participants, and a maxTSS of 10 or greater was recorded by 72% of participants. Eight participants reached a maxTSS of 15 or greater. The highest values were reached in the second chamber day. For both chamber exposure days, the mean change from baseline TSS (Δ TSS) was 8.6.

Skin-Test Correlation With Symptoms

The study SPT panel included pollen of Bermuda, Bahia, Johnson, and Timothy grasses. There was a statistical correlation between the sizes of the skin test wheals from the pollen of all grass species with increasing symptom scores from TGP exposure in the ACC. The strongest correlations were seen with Johnson grass pollen ($P = .001$), Bahia grass pollen ($P = .004$), and TGP ($P = .006$). The weakest correlation was with the pollen of Bermuda grass ($P = .04$). The correlation scatter diagrams and trend lines are plotted in Figures 2A–D. Conversely, we found no correlation between symptom scores from TGP exposure and the skin test wheal sizes of dust mite, mountain cedar, dog, cat, short ragweed, and cockroach.

ssIgE Results

The ssIgE results for study participants are summarized in Table 4. The percentages of individuals with class 1 or greater ssIgE (Phadia ImmunoCAP >0.35 kUA/L) were almost equal for Bermuda grass pollen and TGP (59.1% and 57.1%, respectively). Eleven of the participants were positive to both grasses. A positive ssIgE result to TGP and Bermuda grass pollen was associated with larger values for Δ TSS in the ACC at all exposure time points (Fig 3). The mean Δ TSS after 90 through 180 minutes of exposure in the chamber was 10.64 for ssIgE-positive participants vs 5.13 for ssIgE-negative participants. The difference in these values are significant (2-tailed [$T \leq t$] $P < .008$).

Residence Outside South Texas

Nine of the study participants had lived outside South Texas for more than 5 years and presumably would have been exposed and sensitized to TGP by natural exposure in the past (Table 3). All the other study participants had spent their entire lives in South Texas and were exposed only to subtropical grasses. We found, however, that there was little difference in the severity of symptoms elicited by TGP in the ACC between those who had lived their entire lives in South Texas and those who had lived elsewhere (mean TSS, 10.9 vs 10.8, respectively; 2-tailed [$T \leq t$] $P = .96$). The Δ TSS time course for these groups is illustrated in Figure 4.

Adverse Responses

There were no significant adverse responses during the chamber exposures.

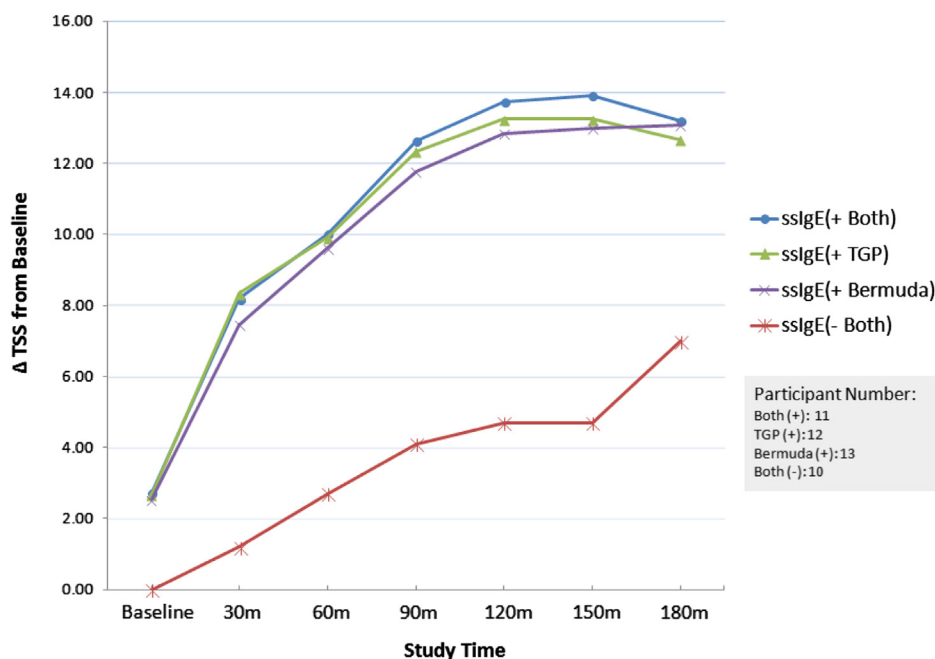


Figure 3. Comparison of the time course of Δ TSS in participants by sslgE status: positive to both grass species (sslgE(+Both)), positive to TGP (sslgE(+TGP)), positive to Bermuda grass pollen (sslgE(+Bermuda)) as well as negative to both grass species (sslgE(-Both)).

Discussion

Study Population

We found that race, age, and sex were not associated with symptom score changes, whereas the symptom scores elicited in the ACC to TGP were statistically correlated with the level of grass pollen skin test reactivity and also with sslgE status. The association between skin test reactivity and symptom levels differs from the findings reported in most previous chamber studies using ragweed,^{7,10} rye grass,¹¹ mountain cedar,⁹ Japanese red cedar,^{12,13} and oak¹⁴. Possible explanations for the discordant results with these other studies may include (1) most of the studied participants (>90%) reacted strongly to all of the grass species tested and therefore there may be a cumulative sensitizing effect; (2) a large number of the study participants were also strongly polysensitized to nongrass allergens, with more than 70% of the participants having large positive SPT results to 14 or more of the 19 allergens tested; (3) although a 5-mm or greater wheal to Bermuda or Johnson grass was required for inclusion in the study, many of the study participants had wheal diameters that were larger than 10 mm and may have been more clinically reactive and respond with higher symptom levels when exposed to TGP.

Intensity of Symptom Scores

Symptoms scores during these TGP chamber exposures are consistent in character, quality, and severity with the scores recorded during our previous pollen chamber studies.^{7,9} In the 2 days of chamber exposures, there was an upward shift in baseline symptoms from the first to the second day, suggesting a priming effect (Fi 1). Despite the upward shift, the high level of response as measured by Δ iTSS from baseline remained almost identical in the 2 study days (Fig 1).

Effects of Prior TGP Exposures

In this study, we found that grass-sensitive participants in South Texas, when challenged to TGP in a controlled environment (ie, an

ACC), developed significant allergic rhinoconjunctivitis symptoms. We found similar symptomatic responses in participants with a history of residence in areas with prior exposure to TGP and in those who had only experienced natural exposure to subtropical grasses.

Most of the grass species responsible for allergic sensitization belong to 3 grass subfamilies: Pooideae (smooth brome, orchard, rye, Kentucky blue, timothy, and sweet vernal grasses), Chloridoideae (Bermuda and grama grass), and Panicoideae (Bahia and Johnson grass).^{2,6,15} Grass allergic individuals are known to almost always be reactive to multiple grasses often belonging to different grass subfamilies. This is thought to be a reflection of extensive allergenic cross-reactivity among the grass species, especially within those in the same grass subfamily.^{16,17} The International Union of Immunological Society's allergen nomenclature subcommittee lists 13 principal grass pollen allergen groups.⁶ The major grass allergens are thought to be in groups 1, 2/3, and 5, although reactivity to group 4 allergens has been reported in up to 75% of patients with grass allergy,^{6,15} and 50% can also have sslgE antibodies to group 12 allergens. Group 1 allergens (β -expansins) are present in all grass species,⁶ and greater than 90% of grass pollen allergic individuals have sslgE reactivity to allergens in this group. There is extensive immunologic cross-reactivity among the group 1 allergens of taxonomically related species. However, there is considerably less cross-reactivity outside the subfamilies (ie, Bermuda group 1 vs the pooid group 1 allergens). Group 2 and 5 allergens seem to have clinical importance and are mostly restricted to the Pooideae subfamily.¹⁵ Group 12 allergens (profilins) have significant intergrass family homology. It has been reported that Bermuda grass pollen possesses unique allergens and has limited overall in vitro cross-reactivity with the pooid grasses, including timothy.^{15,18} Furthermore, TGP seems to have unique allergens not shared with the other grass species in the Pooideae subfamily.¹⁹ Using radioallergen sorbent inhibition techniques, 2 of the grasses common in South Texas (Johnson grass and Bahia grass) can partially overlap with members of the other grass subfamilies.^{17,20} Although most studies report weak in vitro cross-reactivity between subtropical grasses

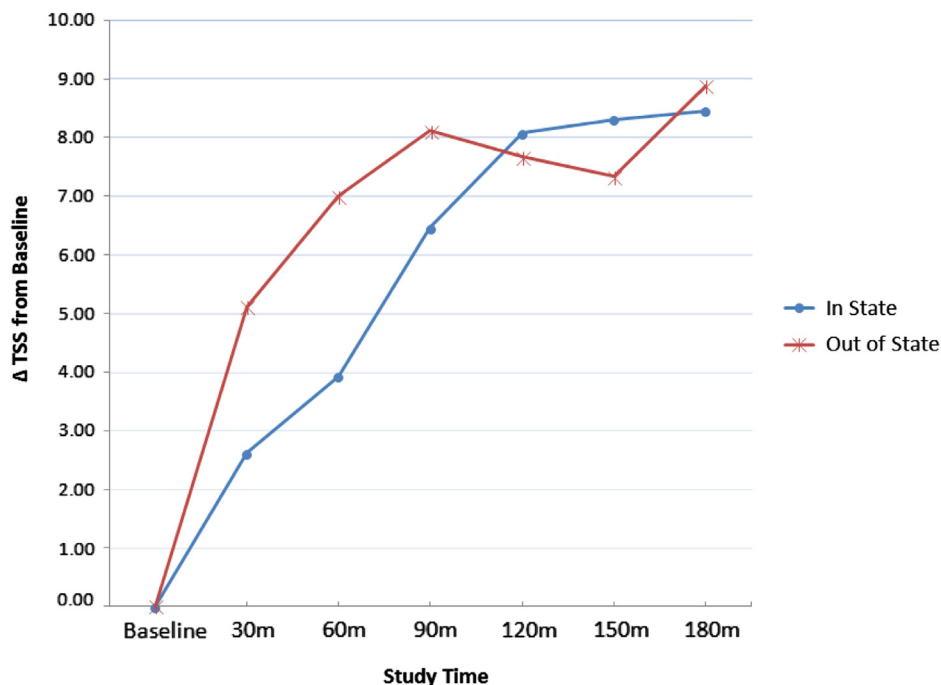


Figure 4. Comparison of the time course of Δ TSS in participants that had lived Out of the state of Texas vs. residing only In Texas.

and temperate grasses,^{2,15–17} this study has documented significant clinical responses to TGP in nonexposed study participants.

Study Summary

The current study found that strongly positive SPT and ssIgE test results to both Bermuda grass pollen and TGP were predictors of high symptoms to TGP exposure in an ACC. Nonexposed participants in South Texas reacted to TGP similar to those who had previous natural exposure, suggesting that a clinical in vivo cross-reactivity may be higher than predicted by prior in vitro data.

Limitations of This Study

Although we have found significant responses to TGP in study participants, we would have liked to study individuals sensitized to one of the subtropical grasses but nonreactive to TGP. Unfortunately, we could not identify any individuals who met this criterion. We also would have liked to compare the responses to TGP in the ACC to the responses using Bermuda grass and/or Johnson grass pollens at the same pollen density in the same study population. This remains a goal for future research in our ACC.

As discussed in the Methods section, the Likert scale used for symptom scoring was extended to a range of 0 to 4 to better define the more severe responses in the ACC. Further studies need to be performed to better define more effective scoring systems.

Implications for the Future

There is a predicted probability that due to climate change, geographic expansion of subtropical grasses into northern latitudes² will contribute to sensitization and, therefore, the allergenic importance of these grass species worldwide. Knowledge of the differences and similarities in immune recognition of grass allergens is likely to affect the approach to allergen immunotherapy in patients who will be exposed and sensitized to subtropical grass pollens not included in interventions directed toward temperate grasses.

The current study and a previous ragweed study⁷ found that significant allergen responses can be elicited with the use of allergens not endemic to the locale of an ACC. Of practical clinical importance is that immunotherapy with a cross-reacting allergen (eg, using *Juniperus ashei* to treat cypress rhinoconjunctivitis) is both safe and effective.²¹ This therapeutic benefit may not extend to other plant species (eg, giant ragweed to treat short ragweed sensitized patients).²² These results and our findings in this study suggest another dimension to the value of ACCs in therapeutic trials. Studies could be uniquely performed in an ACC on participants sensitized to different but cross-reacting allergens, leading to a possible extension of treatment indications for certain novel therapies.

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