

Specific IgE recognition of pollen allergens from subtropic grasses in patients from the subtropics

Emmanuel Nony, PhD^{*}; Victoria Timbrell, BMS[†]; Maud Hrabina, MSc^{*}; Mélanie Boutron, MSc^{*}; Graham Solley, MBBS[‡]; Philippe Moingeon, PhD^{*}; and Janet M. Davies, PhD[†]

^{*} Stallergenes, Antony, France

[†] The University of Queensland, School of Medicine, Brisbane, Australia

[‡] Watkins Medical Centre, Brisbane, Australia

ARTICLE INFO

Article history:

Received for publication September 13, 2014.

Received in revised form November 17, 2014.

Accepted for publication December 8, 2014.

ABSTRACT

Background: Pollens of subtropical grasses, Bahia (*Paspalum notatum*), Johnson (*Sorghum halepense*), and Bermuda (*Cynodon dactylon*), are common causes of respiratory allergies in subtropical regions worldwide.

Objective: To evaluate IgE cross-reactivity of grass pollen (GP) found in subtropical and temperate areas.

Methods: Case and control serum samples from 83 individuals from the subtropical region of Queensland were tested for IgE reactivity with GP extracts by enzyme-linked immunosorbent assay. A randomly sampled subset of 21 serum samples from patients with subtropical GP allergy were examined by ImmunoCAP and cross-inhibition assays.

Results: Fifty-four patients with allergic rhinitis and GP allergy had higher IgE reactivity with *P notatum* and *C dactylon* than with a mixture of 5 temperate GPs. For 90% of 21 GP allergic serum samples, *P notatum*, *S halepense*, or *C dactylon* specific IgE concentrations were higher than temperate GP specific IgE, and GP specific IgE had higher correlations of subtropical GP ($r = 0.771–0.950$) than temperate GP ($r = 0.317–0.677$). In most patients (71%–100%), IgE with *P notatum*, *S halepense*, or *C dactylon* GPs was inhibited better by subtropical GP than temperate GP. When the temperate GP mixture achieved 50% inhibition of IgE with subtropical GP, there was a 39- to 67-fold difference in concentrations giving 50% inhibition and significant differences in maximum inhibition for *S halepense* and *P notatum* GP relative to temperate GP.

Conclusion: Patients living in a subtropical region had species specific IgE recognition of subtropical GP. Most GP allergic patients in Queensland would benefit from allergen specific immunotherapy with a standardized content of subtropical GP allergens.

© 2015 American College of Allergy, Asthma & Immunology. Published by Elsevier Inc. All rights reserved.

Introduction

Population-based studies in the United States reveal a high prevalence of allergic sensitization to grass pollens and an underestimation of the burden of allergic rhinitis.^{1–3} Pollens of subtropical grasses, such as Bahia (*Paspalum notatum*), Bermuda (*Cynodon dactylon*), and Johnson (*Sorghum halepense*) grasses, are a common cause of respiratory allergy and asthma in tropical and subtropical regions, including parts of the southern United States, Australia, Africa, and India.^{4–10} Climate change is likely to increase

the biomass of subtropical grasses¹¹ and extend the current geographic distribution of subtropical grasses.¹² These factors are likely to increase human exposure to subtropical grass pollen (GP) allergens, potentially increasing their contribution to an expanding global burden of allergic rhinitis and asthma.^{13,14}

Our research has revealed qualitative differences in the allergen components of subtropical and temperate GPs.^{4,15} Previous research from ourselves and others has indicated that subtropical GP allergens have subfamily specific IgE reactivity that is not represented in allergen components of temperate GPs.^{16–21} Furthermore, CD4⁺ T-cell recognition of major allergens from subtropical GPs (ie, Pas n 1 and Cyn d 1^{22,23}) includes several non-cross-reactive epitope peptides in addition to a T-cell epitope shared with Lol p 1 and Phl p 1.^{24,25}

Standardized GP allergen immunotherapy based on temperate grasses is available for patients sensitized to pollen allergens from temperate grasses.^{26–28} However, there is now a need to further investigate whether such treatments would suffice to optimally address clinical needs of patients with GP allergy in subtropical

Reprints: Janet M. Davies, PhD, The University of Queensland, School of Medicine, Translational Research Institute, 37 Kent St, Brisbane, QLD 4102 Australia; E-mail: j.davies2@uq.edu.au.

Disclosures: Drs Nony, Hrabina, Boutron, and Moingeon declare they were employees of Stallergenes and UQ has received research funds from Stallergenes. Dr Davies is an inventor on a patent and pending patent applications with potential use for management of pollen allergy.

Funding: This research was funded by Stallergenes Pty via a Collaborative research Agreement with The University of Queensland.

<http://dx.doi.org/10.1016/j.anai.2014.12.005>

1081-1206/© 2015 American College of Allergy, Asthma & Immunology. Published by Elsevier Inc. All rights reserved.

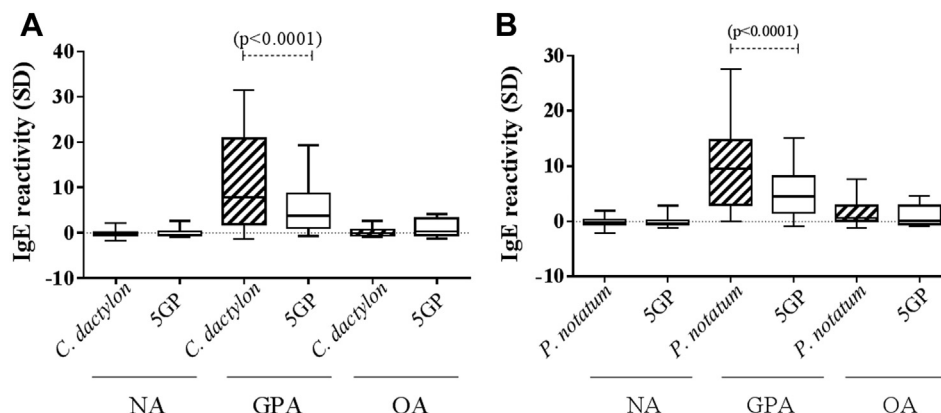


Figure 1. Serum IgE reactivity with subtropical grass pollen (GP) compared with the 5GP mixture. NA indicates nonatopic (n = 19); GPA, grass pollen allergic (n = 54); OA, other allergy (n = 10).

regions of the world who are primarily sensitized to subtropical GPs. To this aim, we studied in detail IgE cross-reactivity among 5 temperate grass (ie, *Poa pratensis* [Kentucky bluegrass], *Dactylis glomerata* [orchard grass], *Lolium perenne* [perennial ryegrass], *Anthoxanthum odoratum* [sweet vernal grass], and *Phleum pratense* [timothy grass]) pollens (5GP) with the aforementioned subtropical grasses within serum samples obtained from patients in subtropical Queensland with allergic rhinitis due to GP.

Methods

Patients who were residents of subtropical parts of Queensland and diagnosed as having allergic rhinitis to GP based on positive skin prick test results were recruited to this study. Patients provided written informed consent, and the study was approved by the Metro South Human Research Ethics Committee. Skin prick testing was performed according to the guidelines of the Australasian Society for Clinical Immunology and Allergy²⁹ using a panel of common aeroallergens, including pollen extracts from *P. notatum* (1/10 wt/vol), *S. halepense* (1/10 wt/vol), *C. dactylon* (10,000 BAU/mL), and the temperate grass *L. perenne* (10,000 BAU/mL; Hollister-Stier, Spokane, Washington). Nonatopic donors reported no clinical history of allergy and had no positive skin prick test results to any of the 10 allergen extracts tested. Serum samples were obtained by venepuncture.

A randomly selected subset of GP allergic patients were included in the cross-inhibition study if sufficient serum was obtained, they had a skin prick test response against one or more of *P. notatum*, *S. halepense*, *C. dactylon*, and *L. perenne*, and serum IgE reactivity with any or all of the subtropical GPs. These serum samples were tested by ImmunoCAP assays for specific IgE reactivity with *P. notatum* (g17), *S. halepense* (g10), *C. dactylon* (g2), and 5GP directly coupled to cyanogen bromide-activated ImmunoCAP by published methods (Thermo Fisher Scientific, Uppsala, Sweden).³⁰

ELISA for Serum IgE Reactivity With *P. notatum* Pollens, *C. dactylon* Pollens, and 5GP

Proteins from nondefatted pollen grains of *P. notatum* and *C. dactylon* (Greer Laboratories, Lenoir, North Carolina) were extracted in ammonium bicarbonate with a complete protease inhibitor cocktail (Roche Diagnostics, Basel, Switzerland) for 3 hours on a rotating wheel at 4°C and clarified as previously described.¹⁶ Then 5GP (*D. glomerata*, *P. pratensis*, *L. perenne*, *A. odoratum*, and *P. pratense*) was extracted in ammonium bicarbonate as described elsewhere.³¹ All extracts were diafiltrated against phosphate-buffered saline before coating on the microtiter plates. The presence of expected allergen components in each extract was confirmed by

immunoblotting (eFig 1). Serum samples diluted 1/10 were tested simultaneously for IgE reactivity with *P. notatum* or *C. dactylon* GP and the temperate grass extract with microtiter plate wells coated with 5 µg/mL of whole GP extract as described previously.¹⁸ Data are expressed as the number of SDs above the mean IgE reactivity of nonatopic controls. In the first assay, the mean (SD) optical densities for 19 nonatopic controls for IgE reactivity with *C. dactylon* GP and the 5GP mixture were 0.078 (0.061) and 0.104 (0.115), respectively. In the second assay, the mean (SD) optical densities for 19 nonatopic controls for IgE reactivity with *P. notatum* GP and the 5GP mixture were 0.115 (0.070) and 0.106 (0.120), respectively.

IgE Cross-inhibition ELISA

Briefly, IgE cross-inhibition enzyme-linked immunosorbent assay (ELISA) experiments were performed after overnight coating of allergenic extracts at 5°C in microtiter plates, with each well containing 0.4 µg of *C. dactylon*, 1.67 µg of *P. notatum*, 1.67 µg of *S. halepense*, or 1.37 µg of *L. perenne* GP protein extract. After blocking, serum samples from GP allergic patients (diluted 1 volume in 4 to 1 volume in 32) were incubated overnight in the presence of serial dilutions of (1) *C. dactylon*, *P. notatum*, *S. halepense*, or *L. perenne* GP extracts; (2) the 5GP mixture; or (3) buffer as a no inhibitor control. After overnight incubation, bound IgE were detected with a peroxidase-labeled anti-IgE antibody. The reactivity was revealed with hydrogen peroxide in the presence of the 3,3',5,5'-tetramethylbenzidine chromogenic substrate (KPL, Gaithersburg, Maryland) using a Sunrise (TECAN, Lyon, France) plate reader.

Statistical Analysis

Data were assessed for normality by the Kolmogorov-Smirnov test. Differences within groups in specific IgE to different allergens were assessed by a Wilcoxon signed rank test for paired data. Skin prick test data were analyzed with the Friedman analysis of variance with the Dunn multiple comparison test. The correlation between specific IgE concentrations to each subtropical GP and the temperate GP extract were assessed by the Spearman test. Inhibitory concentration that results in a 50% reduction in proliferation (IC₅₀) and maximum inhibition, respectively, of IgE reactivity to each GP were determined for both the temperate GP extract and the target extract as inhibitor.

Results

Serum samples were obtained from 54 patients living in the subtropical region of Queensland presenting with allergic rhinitis due to sensitivity to GP, as well as 19 nonatopic controls and 10 controls with other allergies. Because only *C. dactylon* and *P.*

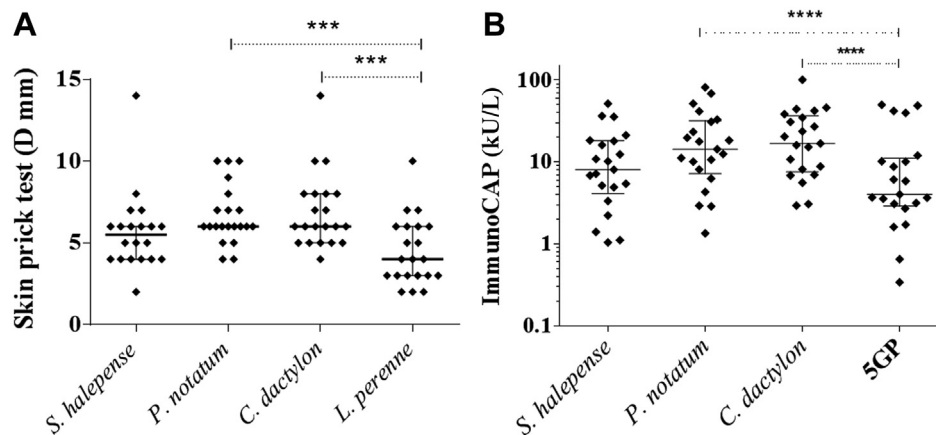


Figure 2. Allergic sensitivity by skin prick test wheal diameters (A) and specific IgE (B) of patients with allergic rhinitis from subtropical regions of Queensland whose serum IgE reactivity with grass pollen (GP) was quantified (n = 21). ImmunoCAP classes are indicated by shading as shown in the key.

notatum and not *S. halepense* produced significantly elevated SPT responses over *L. perenne* pollen in patients from Brisbane,¹⁸ this panel of sera was tested by ELISA for serum IgE reactivity with *C. dactylon* (Fig 1A) or *P. notatum* (Fig 1B) GP compared with simultaneously to the 5GP mixture. In the GP allergic patients, IgE reactivity with pollen extracts from both *C. dactylon* and *P. notatum* grasses was significantly higher than with the 5GP mixture ($P < .0001$).

The allergic sensitivities of a subset of 21 randomly sampled patients with GP allergy for whom sufficient serum was obtained were examined in further detail. All these patients had allergic rhinitis, and 42.8% also had asthma confirmed by a physician. Eighty-five percent were female, and the median age was 39.5 years (interquartile range [IQR], 30.2–47.8). Their median total IgE concentration was 168 kU/L (IQR, 103–261 kU/L). These patients had higher SPT response to subtropical *C. dactylon* and *P. notatum* GP than *L. perenne* pollen extract (Fig 2A). For 19 (90%) of these 21 serum samples, subtropical GP allergen specific IgE concentrations were higher than levels of the 5GP specific IgE (Fig 2B).

The concentrations of specific IgE in serum of these patients was significantly higher with *C. dactylon* ($P < .005$) and *P. notatum* ($P < .005$) GP than with the 5GP mixture (Fig 3). There were very high correlations between concentrations of IgE specific for *P. notatum* and *S. halepense* GP ($r = 0.951$, $P < .0001$) and between these Panicoideae GP and *C. dactylon* GP IgE ($r = 0.774$ and $r = 0.814$, respectively, $P < .0001$ for both). The concentrations of IgE specific for *P. notatum* and *S. halepense* pollen allergens were not as tightly correlated with the 5GP specific IgE concentrations ($r = 0.677$, $P = .0008$, and $r = 0.568$, $P = .007$, respectively) as they were to each other (Fig 3). There was no significant correlation between *C. dactylon* GP specific IgE and 5GP specific IgE.

Qualitative Analysis of Cross-inhibition of Serum IgE Reactivity With Subtropical Grass Pollens

In 19 (90.5%) of 20 serum samples tested, inhibition of IgE with *C. dactylon* GP by *C. dactylon* was higher than with the 5GP mixture (Fig 4A). For 7 (35%) of these serum samples, inhibition by the 5GP mixture reached 0% to 25% of the inhibition obtained with *C. dactylon* GP. For one serum sample, inhibitions by the 5GP and *C. dactylon* GP were similar.

For 15 (71.4%) of 21 serum samples, inhibition of IgE with *P. notatum* GP by *P. notatum* was higher than for the 5GP mixture (Fig 4B). For 1 of these 21 serum samples, inhibition by the 5GP mixture reached 0% to 25% of the inhibition by *P. notatum* GP. For 6 (28.6%) of the serum samples, a similar maximum level of inhibition by *P.*

notatum GP and the 5GP mixture was observed at a high inhibitor dose. However, the slope of the inhibition curve was steeper for the 5GP mixture, suggesting a difference in avidity of IgE interaction between *P. notatum* GP and the 5GP mixture.

For all 21 serum samples tested, inhibition of IgE with *S. halepense* GP by *S. halepense* was higher than by the 5GP mixture (Fig 4C). For 2 of these 21 serum samples, the 5GP mixture had only 0% to 25% of the inhibition by *S. halepense*. None of 21 serum samples exhibited similar inhibition with the 5GP mixture and *S. halepense* GP. In contrast, for all 12 serum samples tested, IgE with *L. perenne* GP was inhibited similarly by the 5GP mixture and *L. perenne* GP (Fig 4D).

Quantitative Analysis of Cross-inhibition of Serum IgE Reactivity With Subtropical Grass Pollens Compared With the 5GP Mixture

The maximum percentage inhibition of IgE reactivity achieved for each GP by the target GP and by the 5GP mixture was calculated for each serum IgE interaction with *P. notatum*, *S. halepense*, *C. dactylon*, and *L. perenne* GP. The maximum inhibition of IgE reactivity with each GP was expressed as a ratio of the value for the 5GP mixture to the target GP (Fig 4E). For *L. perenne*, the ratio of maximum inhibition of IgE reactivity by the 5GP mixture to itself was 1, indicating complete cross-inhibition. The median maximum inhibition by the 5GP mixture compared with the target GP for IgE reactivity with *P. notatum*, *S. halepense*, and *C. dactylon* was 0.55 (IQR, 0.45–0.80), 0.66 (IQR, 0.50–0.78), and 0.32 (IQR, 0.14–0.45), respectively. There was a significant difference between *L. perenne* GP and each of the subtropical GPs in the ratio of maximum inhibitory capacity of the 5GP mixture to the target GP (Fig 4E).

The 5GP mixture did not reach IC_{50} of IgE reactivity with *C. dactylon* GP in 17 of 20 cases, with *P. notatum* GP in 10 of 21 cases and with *S. halepense* GP in 9 of 21 cases (Table 1 and eTable 1). Inhibition of IgE reactivity with *L. perenne* GP revealed median IC_{50} values of 1.8 μ g/mL (IQR, 1.0–4.2 μ g/mL) by *L. perenne* GP and 2.9 μ g/mL (IQR, 1.2–8.7 μ g/mL) by the 5GP mixtures. The IC_{50} values for *L. perenne* and the 5GP mixture for inhibition of IgE reactivity with *L. perenne* were similar with a median fold difference of 0.57 (IQR, 0.48–0.81). In contrast for those individuals in whom the IC_{50} could be determined for the 5GP mixture, there were large fold differences in IC_{50} values for *P. notatum* GP (median, 67.3; IQR, 8.1–182) and *S. halepense* GP (median, 39.6; IQR, 7.3–91.9) compared with the 5GP mixture (Table 1). Comparison of the relative inhibitory capacity between *C. dactylon* and the 5GP mixture was not possible for *C. dactylon* specific IgE because in most cases the IC_{50} was not reached by the 5GP mixture.

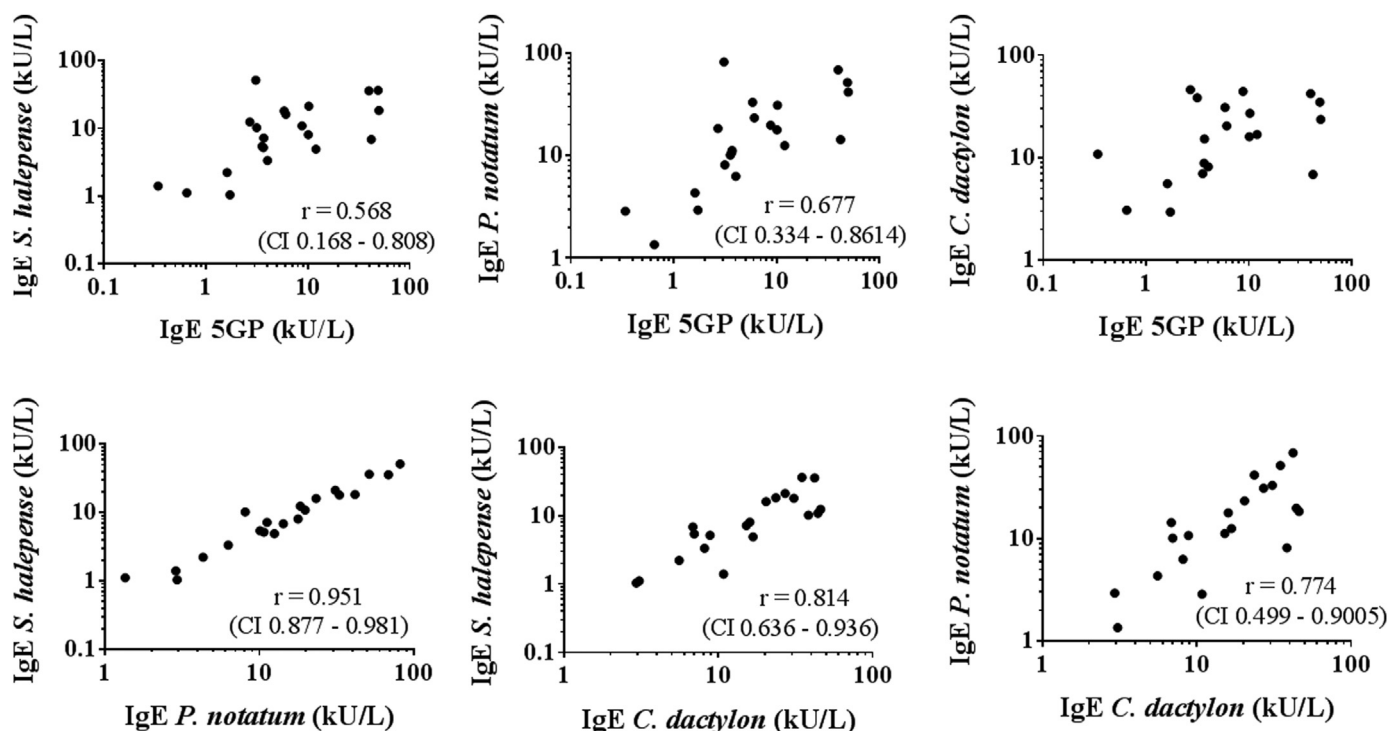


Figure 3. Correlations of specific IgE concentrations among subtropical grass pollens and between subtropical grass pollen (GP) and the 5GP mixture. Spearman r values and the 95% confidence intervals are given.

Discussion

We found that concentrations of serum *P. notatum* GP and *C. dactylon* GP specific IgE were higher than specific IgE to the extract of the 5GP mixture in this cohort of patients with grass pollen allergy from a relevant subtropical region. Moreover, the concentrations of specific IgE to subtropical GP were more highly correlated with IgE to other subtropical GP than with IgE to the 5GP mixture. This study confirms earlier observations that patients living in Queensland have significantly higher rates of allergic sensitivity to subtropical GP than ryegrass pollen assessed by ELISA and skin prick testing.¹⁸ The cross-inhibition studies reveal the existence of some in vitro IgE cross-reactivity between subtropical GP and the 5GP mixture but also provide clear evidence of species-specific immune recognition of subtropical GP that was not effectively blocked by allergenic determinants represented in the 5GP mixture.

Although we previously reported qualitatively subfamily specific IgE recognition of *P. notatum*, *S. halepense*, and *C. dactylon* GP compared with one temperate GP (*L. perenne*) in 5 patients,¹⁸ the IgE inhibition assays performed herein with a larger panel of 21 patients allowed quantitative assessment of cross-inhibition between subtropical GP and the 5GP mixture. Aalberse³² outlined a framework for the interpretation of specificity and affinity from cross-inhibition assays in the context of monoclonal antibody interactions with different antigen preparations. This conceptual framework is to a certain extent applicable to polyclonal serum IgE interactions with different GP extracts. However, because of the complexity of the serum IgE repertoire and the array of molecular components within pollen extracts, the data from the cross-inhibition assays performed in this study indicate the overall avidity of serum IgE interaction with allergens contained within GP extracts arising from the sum of multiple epitope-paratope interactions. This overall avidity of multiple interactions between allergen and IgE is likely to be of clinical importance for activation of basophils on exposure of patients to particular allergen sources.^{33,34}

The maximum percentage of inhibition indicated the relative maximum capacity for IgE cross-reactivity between subtropical and temperate GPs. The ratios of maximum inhibitory capacity of the subtropical GP to the 5GP mixture emphasizes the difference between IgE recognition between subtropical and temperate GPs. The outcomes of this study indicate that allergens of the 5GP mixture represented at most only 55%, 66%, and 32% for *P. notatum*, *S. halepense*, and *C. dactylon* GP, respectively, of the epitopes recognized by serum IgE in this cohort of patients. This analysis also revealed that *C. dactylon* GP had a greater degree of difference from the 5GP mixture than *P. notatum* and *S. halepense* GP. This finding is evident from the cross-inhibition assays and the lack of correlation between specific IgE concentrations between *C. dactylon* IgE and the 5GP IgE.

The IC₅₀ values indicate the relative avidity of binding of a serum to a given GP relative to the 5GP mixture. The marked difference in IC₅₀ values for the 5GP mixture and each subtropical GP indicate significant differences in the relative strength of binding of patient IgE to these allergen sources. For many cases in this cohort of GP allergic patients from a subtropical region, there was insufficient interaction between IgE and allergens in the 5GP mixture to achieve 50% inhibition of IgE reactivity with subtropical GP. This finding reveals significant antigenic differences between subtropical and temperate GPs. That there was a slight difference between the IC₅₀ values for *L. perenne* GP and the 5GP mixture is consistent with earlier reports on heterogeneity of T- and B-cell responses among different temperate GPs.^{35,36}

The observed difference in IgE recognition of subtropical GP and temperate GP may not have been anticipated from studies from Europe and North America reporting high correlations between specific IgE to various GPs.^{37,38} However, it is notable even in those studies that the degree of correlation between subtropical GP and temperate GP was not as high as among temperate GP. Most of the patient serum samples examined in those studies are likely to have been sourced from patients primarily exposed to temperate GP,

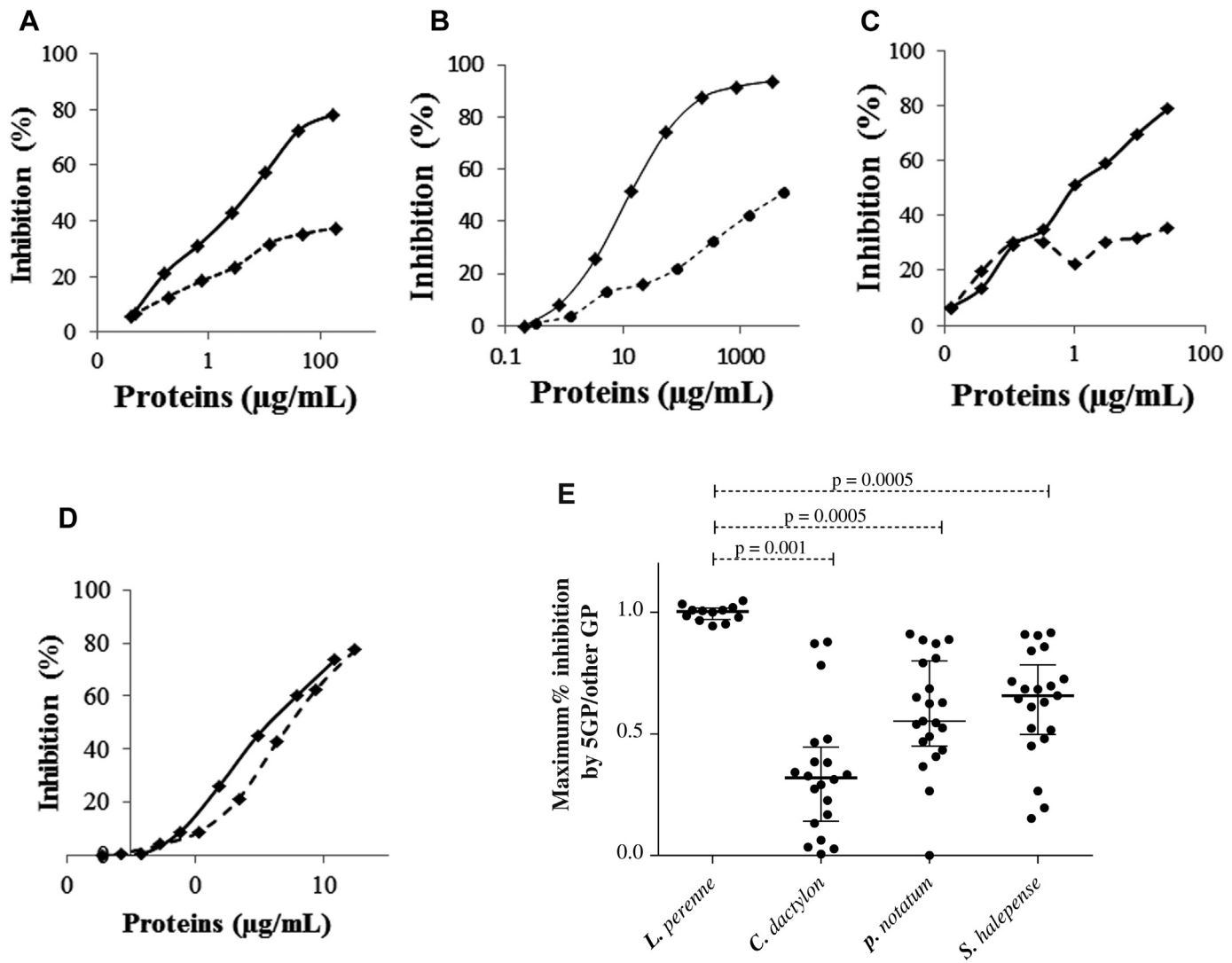


Figure 4. Incomplete IgE cross-reactivity between subtropical grass pollens (GPs) and the 5GP mixture. Exemplary graphs for inhibition of IgE with *Cynodon dactylon* (A), *Paspalum notatum* (B), *Sorghum halepense* (C), or *Lolium perenne* (D) by the target allergen (solid line) compared with the 5GP mixture (dotted line). Summary of the maximum inhibition by the 5GP mixture divided by maximum inhibition of the target allergen (E). Cross-inhibition of subtropical GP by the 5GP mixture tested by Wilcoxon matched-pairs signed rank test. Bar and whiskers show medians and interquartile ranges.

although the origin of patients was not revealed, whereas our study focused on patients from a relevant subtropical region. Hensel and Griffith⁷ observed in 429 patients from Louisiana, a subtropical region in the United States, that *P notatum* GP was the most frequently recognized GP but that patterns of sensitization included various combinations of skin prick test positivity to *P notatum*, *P dilatatum* (Dallis), *S halepense*, *C dactylon*, and *P pratense* GP.⁷ Passive transfer of serum from 5 *P notatum* GP allergic patients to nonallergic recipients resulted in the highest cutaneous

sensitivity responses to *P notatum* GP, as well as cross-reactivity to other species of GP. Even though *P notatum* and *P dilatatum* GP are closely related the *Paspalum* species, there was greater intensity of reactivity with serially diluted extract of *P notatum* GP compared with *P dilatatum* GP, suggesting a high level of specificity in the allergic response even between these closely related subtropical GPs and/or differences in allergen content of the extracts. A study of serum pooled from 5 GP allergic patients recruited from the northern temperate state of Minnesota revealed that *C dactylon* GP

Table 1
IC₅₀ Values for inhibition of IgE with target allergens bound to solid phase by the target allergen and the 5GP mixture in solution phase^a

Target allergen	<i>Lolium perenne</i>			<i>Cynodon dactylon</i>			<i>Paspalum notatum</i>			<i>Sorghum halepense</i>		
	5GP IC ₅₀ , μg/mL	<i>L perenne</i> IC ₅₀ , μg/mL	Fold difference	5GP IC ₅₀ , μg/mL	<i>C dactylon</i> IC ₅₀ , μg/mL	Fold difference	5GP IC ₅₀ , μg/mL	<i>P notatum</i> IC ₅₀ , μg/mL	Fold difference	5GP IC ₅₀ , μg/mL	<i>S halepense</i> IC ₅₀ , μg/mL	Fold difference
Median	2.9	1.8	0.57	ND	7.9	ND	891	20.7	67.3	212	12.5	39.6
IQR	1.3–7.9	1.3–4.2	0.48–0.81	ND	1.6–24.9	ND	168–3610	7.0–34.4	8.1–182	22.9–639	3.0–30.7	7.3–91.9

Abbreviations: 5GP, 5 grass pollen; IC₅₀, inhibitory concentration that results in a 50% reduction in proliferation; IQR, interquartile range; ND, value not determined.
^aData are presented as the median IC₅₀ for each inhibitor and the fold difference of IC₅₀ with 5GP divided by the IC₅₀ for the target GP.

failed to inhibit 50% of IgE with a range of temperate GP.²¹ In the converse experiment, the concentration of *P pratense* GP required to inhibit 50% of IgE to *C dactylon* GP was 3 orders of magnitude greater than *C dactylon* GP,²¹ exemplifying the nonreciprocal pattern of cross-inhibition of IgE binding to temperate vs subtropical GP in those patients. Similarly, IgE cross-inhibition studies with pooled sera of army volunteers revealed that IgE reactivity with subtropical grasses was not completely inhibited by a range of temperate GPs.²⁰ Further evidence of unique allergenicity between *P notatum* GP and *P pratense* GP was a lack of in vivo cross-reactivity in nasal challenge studies of a small number of patients from the southern state of Florida.¹⁹

In all 21 individual serum samples tested from patients living in a subtropical region, the reverse pattern of nonreciprocal cross-reactivity was evident compared with the previous studies from temperate regions as outlined above and elsewhere.^{17,39} When inhibition occurred in this study, the 5GP mixture had significantly lower maximum inhibition of IgE reactivity with subtropical GP and markedly different IC₅₀ concentrations compared with the target subtropical GP. The allergens within the 5GP mixture did not appear to cover the range of epitopes of the subtropical GP allergens that were recognized by serum IgE. Moreover, research indicates that T-cell epitopes of temperate grass pollens do not fully represent dominant T-cell epitopes delineated within major allergen component of *P notatum* and *C dactylon* GP, namely, Pas n 1 and Cyn d 1,^{22,23} that may be required for optimal regulatory T-cell engagement during allergen specific immunotherapy for patients primarily sensitized to subtropical GP.

The outcomes of this study indicate that most patients from the subtropical region of Queensland would be likely to benefit from allergen immunotherapy optimized to contain standardized content of subtropical GP allergens (ie, Panicoideae [*P notatum* and *S halepense*] and Chorioideae [*C dactylon*] grass subfamilies). Similarly, patients exposed and sensitized to temperate grasses should be treated with extracts from temperate (ie, Pooideae) grasses. Although the distribution of subtropical grasses is predominantly within regions close to the equator,⁴⁰ patients from temperate regions can be exposed to pollen of subtropical grasses later in the grass pollen season. Secondary peaks in pollen season correlate with flowering of subtropical species; *C dactylon* GP in Italy⁴¹ and *S halepense* grass pollen in Washington, DC,⁴² have been reported. Moreover, with the advent of climate change and the expansion of the biogeographical range of subtropical grasses, exposure to these kinds of pollen is expected to increase in future.⁴³

To achieve optimal efficacy of immunotherapy targeting subtropical Panicoideae GP, it will be essential to standardize allergen content of extracts of subtropical GP. In the absence of group 5 allergens in subtropical grass pollens,^{4,44–46} it will be necessary to use other dominant allergenic molecular components, such as the major group 1 allergen,^{10,47} for standardization of allergen content of pollen extracts from subtropical grasses.

Acknowledgments

We thank Preethi Guru (formerly University of Queensland, School of Medicine) for technical assistance; respiratory physician John W. Upham of the Princess Alexandra Hospital, Brisbane, Australia, and the research nurses Michelle Towers and Tina Collins of the Lung and Allergy Research Centre for assistance with study recruitment and phlebotomy; and Justin Scott of Queensland Facility for Advanced and Bioinformatics for statistical advice.

Supplementary Data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.anai.2014.12.005>.

References

- [1] Meltzer EO, Blaiss MS, Derebery MJ, et al. Burden of allergic rhinitis: results from the Pediatric Allergies in America survey. *J Allergy Clin Immunol*. 2009;124:S43–S70.
- [2] Arbes SJ Jr, Gergen PJ, Elliott L, Zeldin DC. Prevalences of positive skin test responses to 10 common allergens in the US population: results from the third National Health and Nutrition Examination Survey. *J Allergy Clin Immunol*. 2005;116:377–383.
- [3] Salo PM, Calatroni A, Gergen PJ, et al. Allergy-related outcomes in relation to serum IgE: results from the National Health and Nutrition Examination Survey 2005–2006. *J Allergy Clin Immunol*. 2011;127:1226–1235.e7.
- [4] Davies J. Grass pollen allergens globally: the contribution of subtropical grasses to burden of allergic respiratory diseases. *Clin Exp Allergy*. 2014;44:790–801.
- [5] Sridhara S, Singh BP, Kumar L, et al. Antigenic and allergenic relationships among airborne grass pollens in India. *Ann Allergy Asthma Immunol*. 1995;75:73–79.
- [6] Prescott RA, Potter PC. Allergenicity and cross-reactivity of buffalo grass (*Stenotaphrum secundatum*). *S Afr Med J*. 2001;91:237–243.
- [7] Hensel AE Jr, Griffith RC. Clinical experiences with *Paspalum notatum* (Bahia grass): a new grass antigen. *South Med J*. 1972;65:690–693.
- [8] Calabria CW, Dice J. Aeroallergen sensitization rates in military children with rhinitis symptoms. *Ann Allergy Asthma Immunol*. 2007;99:161–169.
- [9] Ghobrial G, Naser SA, Sweeney M, White R. Identification and characterization of the allergenic proteins of Bahia grass (*Paspalum notatum*) pollen. *Int Arch Allergy Immunol*. 2002;128:304–309.
- [10] White JM, Majidi A, Nasar SA, Sweeney M, White RS. Characterisation of the group I allergen of Bahia grass pollen. *Open Allergy J*. 2009;2:27–29.
- [11] Morgan JA, LeCain DR, Pendall E, et al. C4 grasses prosper as carbon dioxide eliminates desiccation in warmed semi-arid grassland. *Nature*. 2011;476:202–205.
- [12] Gupta A. Geoindicators for tropical urbanization. *Environ Geol*. 2002;42:736–742.
- [13] Beggs PJ. Climate change and plant food allergens. *J Allergy Clin Immunol*. 2009;123:271–272.
- [14] Ziska LH, Beggs PJ. Anthropogenic climate change and allergen exposure: the role of plant biology. *J Allergy Clin Immunol*. 2011;129:27–32.
- [15] Campbell B, Gilding E, Timbrell V, et al. Total transcriptome, proteome and allergome of Johnson grass pollen important for allergic rhinitis in subtropical regions. *J Allergy Clin Immunol*. 2015;135:133–142.
- [16] Davies JM, Bright ML, Rolland JM, O'Hehir RE. Bahia grass pollen specific IgE is common in seasonal rhinitis patients but has limited cross-reactivity with ryegrass. *Allergy*. 2005;60:251–255.
- [17] Davies JM, Dang TD, Voskamp A, et al. Functional immunoglobulin E cross-reactivity between Pas n 1 of Bahia grass pollen and other group 1 grass pollen allergens. *Clin Exp Allergy*. 2011;41:281–291.
- [18] Davies JM, Li H, Green M, Towers M, Upham JW. Subtropical grass pollen allergens are important for allergic respiratory diseases in subtropical regions. *Clin Transl Allergy*. 2012;2:4.
- [19] Phillips JW, Bucholtz GA, Fernandez-Caldas E, Bukantz SC, Lockey RF. Bahia grass pollen, a significant aeroallergen: evidence for the lack of clinical cross-reactivity with timothy grass pollen. *Ann Allergy*. 1989;63:503–507.
- [20] Martin BG, Mansfield LE, Nelson HS. Cross-allergenicity among the grasses. *Ann Allergy*. 1985;54:99–104.
- [21] Leiferman KM, Gleich GJ. The cross-reactivity of IgE antibodies with pollen allergens. I: analyses of various species of grass pollens. *J Allergy Clin Immunol*. 1976;58:129–139.
- [22] Etto T, de Boer C, Prickett S, et al. Unique and cross-reactive T cell epitope peptides of the major Bahia grass pollen allergen, Pas n 1. *Int Arch Allergy Immunol*. 2012;159:355–366.
- [23] Eusebius NP, Papalia L, Suphioglu C, et al. Oligoclonal analysis of the atopic T cell response to the group 1 allergen of *Cynodon dactylon* (bermuda grass) pollen: pre- and post-allergen-specific immunotherapy. *Int Arch Allergy Immunol*. 2002;127:234–244.
- [24] Burton MD, Blaher B, Suphioglu C, et al. T-cell receptor contact and MHC binding residues of a major rye grass pollen allergen T-cell epitope. *J Allergy Clin Immunol*. 1999;103:255–261.
- [25] Schenk S, Breiteneder H, Susani M, et al. T-cell epitopes of Phl p 1, major pollen allergen of timothy grass (*Phleum pratense*): evidence for crossreacting and non-crossreacting T-cell epitopes within grass group I allergens. *J Allergy Clin Immunol*. 1995;96:986–996.
- [26] Didier A, Mallin HJ, Worm M, et al. Post-treatment efficacy of discontinuous treatment with 300IR 5-grass pollen sublingual tablet in adults with grass pollen-induced allergic rhinoconjunctivitis. *Clin Exp Allergy*. 2013;43:568–577.
- [27] Cox LS, Casale TB, Nayak AS, et al. Clinical efficacy of 300IR 5-grass pollen sublingual tablet in a US study: the importance of allergen-specific serum IgE. *J Allergy Clin Immunol*. 2012;130:1327–1334.e1.
- [28] Nelson HS, Nolte H, Creticos P, et al. Efficacy and safety of timothy grass allergy immunotherapy tablet treatment in North American adults. *J Allergy Clin Immunol*. 2011;127:72–80.e1–e2.
- [29] Australian Society for Clinical Immunology and Allergy Working Party. *Skin prick testing for the diagnosis of allergic disease: a manual for practitioners* 2009, <http://www.allergy.org.au>. Accessed June 1, 2010.
- [30] Marknell DeWitt A, Niederberger V, Lehtonen P, et al. Molecular and immunological characterization of a novel timothy grass (*Phleum pratense*) pollen allergen, Phl p 11. *Clin Exp Allergy*. 2002;32:1329–1340.

- [31] Bussieres L, Bordas-Le Floch V, Bulder I, et al. Recombinant fusion proteins assembling Der p 1 and Der p 2 allergens from *Dermatophagoides pteronyssinus*. *Int Arch Allergy Immunol*. 2010;153:141–151.
- [32] Aalberse RC. Assessment of allergen cross-reactivity. *Clin Mol Allergy*. 2007;5:2.
- [33] Christensen LH, Riise E, Bang L, Zhang C, Lund K. Isoallergen variations contribute to the overall complexity of effector cell degranulation: effect mediated through differentiated IgE affinity. *J Immunol*. 2010;184:4966–4972.
- [34] Davies JM, Platts-Mills TA, Aalberse RC. The enigma of IgE+ B cell memory in humans. *J Allergy Clin Immunol*. 2013;131:972–976.
- [35] Chabre H, Gouyon B, Huet A, et al. Molecular variability of group 1 and 5 grass pollen allergens between Pooideae species: implications for immunotherapy. *Clin Exp Allergy*. 2010;40:505–519.
- [36] Fenaille F, Nony E, Chabre H, et al. Mass spectrometric investigation of molecular variability of grass pollen group 1 allergens. *J Proteome Res*. 2009;8:4014–4027.
- [37] Johansen N, Weber RW, Ipsen H, et al. Extensive IgE cross-reactivity towards the Pooideae grasses substantiated for a large number of grass-pollen-sensitized subjects. *Int Arch Allergy Immunol*. 2009;150:325–334.
- [38] Andersson K, Lidholm J. Characteristics and immunobiology of grass pollen allergens. *Int Arch Allergy Immunol*. 2003;130:87–107.
- [39] Gonzalez RM, Cortes C, Conde J, et al. Cross-reactivity among five major pollen allergens. *Ann Allergy*. 1987;59:149–154.
- [40] Esch RE. Grass pollen allergens. *Clin Allergy Immunol*. 2004;18:185–205.
- [41] Frenguelli G, Passalacqua G, Bonini S, et al. Bridging allergologic and botanical knowledge in seasonal allergy: a role for phenology. *Ann Allergy Asthma Immunol*. 2010;105:223–227.
- [42] Kosisky SE, Marks MS, Nelson MR. Pollen aeroallergens in the Washington, DC, metropolitan area: a 10-year volumetric survey (1998–2007). *Ann Allergy Asthma Immunol*. 2010;104:223–235.
- [43] Seidel DJ, Fu Q, Randel WJ, Riechler TJ. Widening of the tropical belt in a changing climate. *Nat Geosci*. 2008;1:21–24.
- [44] Smith PM, Ong EK, Knox RB, Singh MB. Immunological relationships among group I and group V allergens from grass pollen. *Mol Immunol*. 1994;31:491–498.
- [45] Flicker S, Laffer S, Steinberger P, et al. Engineering, purification and applications of His-tagged recombinant antibody fragments with specificity for the major birch pollen allergen, bet v1. *Biol Chem*. 2000;381:39–47.
- [46] Focke-Tejkl M, Campana R, Reininger R, et al. Dissection of the IgE and T-cell recognition of the major group 5 grass pollen allergen Phl p 5. *J Allergy Clin Immunol*. 2014;133:836–845.e11.
- [47] Davies JM, Mittag D, Dang TD, et al. Molecular cloning, expression and immunological characterisation of Pas n 1, the major allergen of Bahia grass *Paspalum notatum* pollen. *Mol Immunol*. 2008;46:286–293.

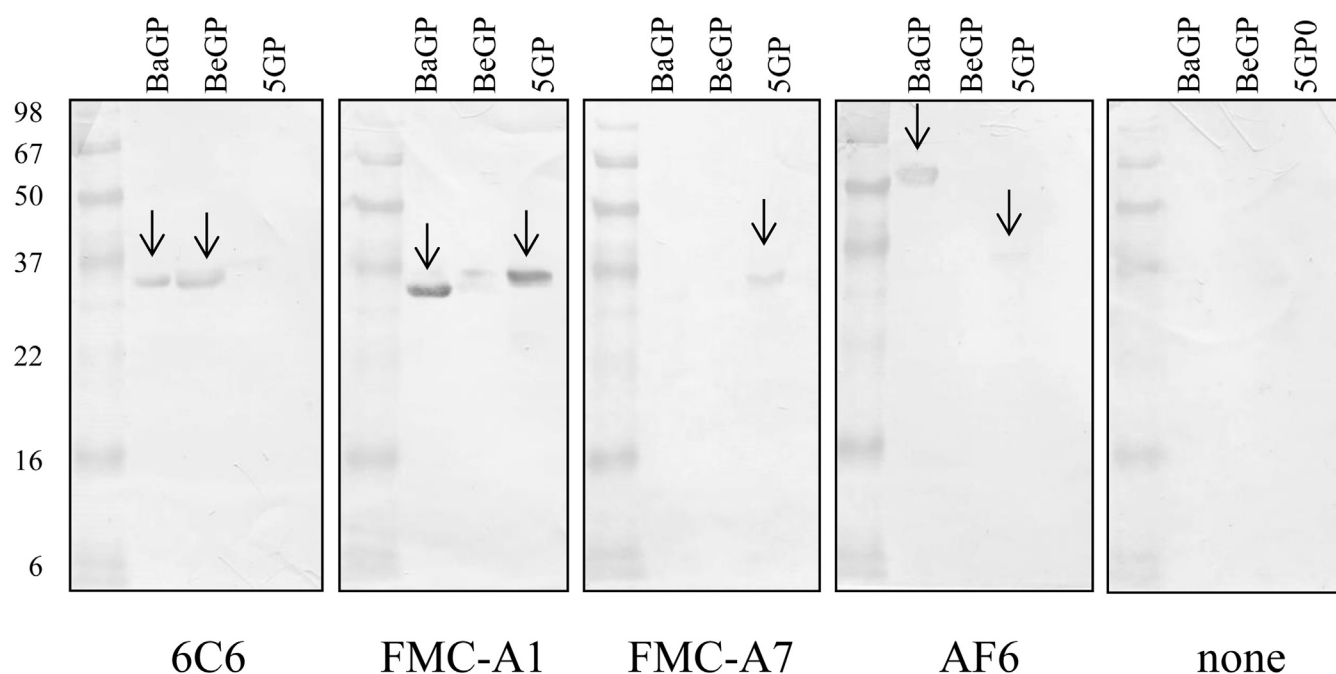


Figure 1. Immunoblotting of *Paspalum notatum* (BaGP), *Cynodon dactylon* (BeGP), and the 5 grass pollen (5GP) extract preparations to confirm the presence of allergen components. Five micrograms of each extract was immunoblotted by protocols described previously¹ to qualitatively assess immunoreactivity with a panel of allergen specific monoclonal antibodies: 6C6,¹ which binds Pas n 1 and Cyn d1 and not temperate grass pollen group 1 allergens; FMC-A1,² which binds group 1 allergens of all temperate grass pollens and Pas n 1; AF6,³ which binds group 13 allergens (group 13 allergen is present in *Paspalum notatum*⁴ and temperate grass pollens; however, as observed previously for Phl p 13³ the group 13 allergen component of the 5GP appears to have degraded); and FMC-A7,² which binds only the group 5 allergen Lol p 5). Molecular weight of marker proteins are given on the left, and arrows mark the location of expected allergen bands.

References

- [1] Davies JM, Dang TD, Voskamp A, et al. Functional immunoglobulin E cross-reactivity between Pas n 1 of Bahia grass pollen and other group 1 grass pollen allergens. *Clin Exp Allergy*. 2011;41:281–291.
- [2] Smart I, Heddle RJ, Zola H, Bradley JE. Development of monoclonal mouse antibodies specific for allergenic components of Ryegrass (*Lolium perenne*) pollen. *Int Arch Allergy Appl Immunol*. 1983;72:243–248.
- [3] Petersen A, Suck R, Hagen S, et al. Group 13 grass allergens: structural variability between different grass species and analysis of proteolytic stability. *J Allergy Clin Immunol*. 2001;107:856–862.
- [4] Davies JM, Voskamp A, Dang TD, et al. The dominant 55kDa allergen of the subtropical Bahia grass (*Paspalum notatum*) pollen is a group 13 pollen allergen, Pas n 13. *Mol Immunol*. 2011;48:931–940.

eTable 1Individual ratios of IC₅₀ for inhibition of IgE with target allergens bound to solid phase by the target allergen and the 5GP mixture in solution phase^a

Target allergen	<i>Lolium perenne</i>		<i>Cynodon dactylon</i>		<i>Paspalum notatum</i>		<i>Sorghum halepense</i>	
Serum	5GP/L <i>perenne</i> , µg/mL	Fold	5GP/C <i>dactylon</i> , µg/mL	Fold	5GP/P <i>notatum</i> , µg/mL	Fold	5GP/S <i>halepense</i> , µg/mL	Fold
1	1.1/0.5	0.47	NT		>/6.6	ND	1640/7.2	230
2	4.6/2.0	0.44	>/4.4	ND	1569/5.6	280	242.3/5.8	41.5
3	3.2/1.7	0.54	>/5.1	ND	7973/18.9	421	87.6/11.2	7.84
4	0.5/1.0	2.10	>/34.7	ND	>/24.0	ND	182.1/3.3	55.3
5	1.4/1.2	0.85	>/1.7	ND	>/1371	ND	>/62.5	ND
6	NT		>/17.9	ND	4628/25.7	180	423/21.8	19.4
7	2.6/1.6	0.63	>/24.3	ND	9.4/17.7	0.53	56.1/15.9	3.53
8	2.5/2.6	1.01	>/9.4	ND	426/6.3	67	1054/12.9	82.0
9	9.2/4.4	0.48	>/18.5	ND	>/26.6	ND	11.8/0.3	37.6
10	NT		>/0.3	ND	168/20.7	8.11	335/46.9	7.13
11	NT		>/32.0	ND	>/4.2	ND	>/27.4	ND
12	1.2/0.9	0.72	2.4/6.6	0.36	891/4.9	182	2.2/0.0	114
13	NT		>/6.0	ND	>/35.8	ND	>/1.2	ND
14	NT		>/1.5	ND	>/23.1	ND	>/30.0	ND
15	NT		>/9.1	ND	>/9.9	ND	711/7.5	95.2
16	NT		>/28.7	ND	1382/138	10.0	>/32.9	ND
17	NT		>/26.4	ND	3610/48.9	73.8	>/0.0	ND
18	7.6/3.7	0.50	5.4/0.0	479	>/33.0	ND	7.5/12.2	0.62
19	15.2/7.7	0.51	18.0/1.2	14.9	>/791	ND	>/31.4	ND
20	15.0/8.7	0.59	>/74.4	ND	135/16.5	8.18	>/2.8	ND
21	NT		>/1.3	ND	341/7.3	46.7	>/124	ND

Abbreviations: >, IC₅₀ greater than maximum inhibitor concentration; 5GP, 5 grass pollen; IC₅₀, inhibitory concentration that results in a 50% reduction in proliferation; ND, value not determined; NT, not tested.

^aData are presented as the IC₅₀ for each inhibitor and the fold difference of IC₅₀ divided by the IC₅₀ for the target GP.