

Relevance of Allergenic Sensitization to *Cynodon dactylon* and *Phragmites communis*: Cross-reactivity With Pooideae Grasses

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J Investig Allergol Clin Immunol 2016; Vol. 26(5): 295-303

doi: 10.18176/jiaci.0049

■ Abstract

Background and Objectives: The homologous group of sweet grasses belongs to the Pooideae subfamily, but grass pollen species from other subfamilies can also cause allergy, such as *Cynodon dactylon* (Chloridoideae) and *Phragmites communis* (Arundinoideae). *C. dactylon* and *P. communis* have not been included in the sweet grasses homologous group because of their low cross-reactivity with other grasses. The aims of this study were to investigate the profile of sensitization to *C. dactylon* and *P. communis* in patients sensitized to grasses and to analyze cross-reactivity between these 2 species and temperate grasses.

Methods: Patients were skin prick tested with a grass mixture (GM). Specific IgE to GM, *C. dactylon*, *P. communis*, Cyn d 1, and Phl p 1 was measured by ImmunoCAP. A pool of sera was used for the immunoblot assays. Cross-reactivity was studied by ELISA and immunoblot inhibition.

Results: Thirty patients had sIgE to GM. Twenty-four (80%) had positive results for *C. dactylon*, 27 (90%) for *P. communis*, 22 (73.3%) for nCyn d 1, and 92.9% for rPhl p 1. Bands were detected in the 3 extracts by immunoblot. Inhibition of GM was not observed with *C. dactylon* or *P. communis* by immunoblot or ELISA inhibition. When *C. dactylon* or *P. communis* were used in the solid phase, GM produced almost complete inhibition.

Conclusions: Eighty percent of patients sensitized to grasses were also sensitized to *C. dactylon* and 90% were sensitized to *P. communis*. Sensitization to these species seems to be induced by allergens different to those in sweet grasses.

Key words: Grass allergy. Cross-reactivity. *Cynodon dactylon*. *Phragmites communis*. Phl p 1. Cyn d 1.

■ Resumen

Antecedentes y Objetivos: Desde un punto de vista taxonómico, el grupo homólogo de las gramíneas pertenece a la sub-familia Pooideae. Sin embargo, existen también otras especies de gramíneas alergénicas que pertenecen a sub-familias diferentes como son *Cynodon dactylon* (Chloridoideae) o *Phragmites communis* (Arundinoideae). *C. dactylon* y *P. communis* no están incluidas en este grupo homólogo debido a que la reactividad cruzada con otras gramíneas es limitada. Los objetivos del estudio fueron investigar el perfil de sensibilización a *C. dactylon* y *P. communis* en pacientes sensibilizados a gramíneas y analizar la reactividad cruzada entre estas dos especies y las gramíneas más comunes.

Métodos: A los pacientes se les realizó una prueba cutánea con una mezcla de gramíneas (MG). Mediante ImmunoCAP se midió la IgE específica para MG, *C. dactylon*, *P. communis*, Cyn d 1 y Phl p 1. Un pool de sueros se utilizó para ensayos de inmunoblot. La reactividad cruzada se estudió mediante ELISA e inmunoblot inhibición.

Resultados: Treinta pacientes tuvieron IgE específica para MG. Veinticuatro (80%) fueron positivos a *C. dactylon*, 27 (90%) a *P. communis*, 22 (73,3%) a nCyn d 1 y 92,9% fueron positivos a rPhl p 1. Se detectaron bandas en los tres extractos mediante inmunoblot. No se observó inhibición de MG con las otras dos especies mediante inmunoblot o ELISA inhibición. Cuando *C. dactylon* o *P. communis* se usaron en fase sólida, MG produjo una inhibición casi completa.

Conclusiones: El 80% de los pacientes sensibilizados a gramíneas estaban también sensibilizados a *C. dactylon* y el 90% a *P. communis*. La sensibilización a estas especies parece estar inducida por diferentes alérgenos que en el caso de gramíneas.

Palabras clave: Alergia a gramíneas. Reactividad cruzada. *Cynodon dactylon*. *Phragmites communis*. Phl p 1. Cyn d 1.

Introduction

Grasses, and particularly species from the Poaceae family, are the main cause of pollen allergy worldwide. The Poaceae family has different subfamilies, of which the temperate Pooideae subfamily contains the most allergenic species. However, there are other subfamilies with species capable of inducing allergic symptoms, such as Chloridoideae (*Cynodon dactylon*) and Arundinoideae (*Phragmites communis*). Both *C dactylon* and *P communis* have been identified in warm temperate and subtropical areas in Africa, Asia, Australia, and America [1], and also in Europe up to a latitude of approximately 53°N [2]. Although *C dactylon* is now cosmopolitan, it is generally recognized that its present distribution is largely due to human activities [3] as it is used as livestock herbage and turf. In recent years, climate change has contributed to modifications in pollen release and distribution patterns, and different species are colonizing new areas, modifying the allergenic composition of the environment. In countries with temperate climates such as Spain [4,5] and Italy [6], Pooideae and subtropical grasses coexist in the same areas. In Spain, *C dactylon* is found throughout the country, while *P communis* is more frequent in humid areas such as coastal regions and close to rivers [7].

Pollen grains from different grass families have a similar morphology and it is very difficult to distinguish them by visual inspection. Studies of flowering may help to identify specific grasses responsible for pollen allergy by identifying the pollination season of different species [8]. *C dactylon* has been found to flower later than temperate grasses [6], possibly explaining the onset of symptoms in grass-sensitized patients in late June or July. Patterns of sensitization depend on primary grass sensitization and differ from one geographic region to the next [1].

To date, species of the Pooideae family have been grouped in the same homologous group: the sweet grasses. Membership of this group is based on the presence of 3 allergen families: group 1, 2, and 5 [9]. Cross-reactivity studies with different species have shown that the majority of Pooideae species are highly cross-reactive [10-12]. *C dactylon* is not included in this homologous group because it shows limited cross-reactivity with other grasses [9]. There have, however, been recent calls for the inclusion of *C dactylon* in this group, but considering only the allergen Cyn d 1 [13]. Seven allergens have been described to date in *C dactylon*: Cyn d 1, Cyn d 7, Cyn d 12, Cyn d 15, Cyn d 22w, Cyn d 23, and Cyn d 24 (IUIS Allergen Nomenclature Subcommittee) [14], although an additional

8 allergens (Cyn d 2, Cyn d 3, Cyn d 5, Cyn d 6, Cyn d 11, Cyn d 13, Cyn d CP, and Cyn d EXI) have been reported in Allergome.org [15]. Several publications have described the lack of group 2 and 5 allergens in *C dactylon* [16], although they are mentioned in Allergome. Only Cyn d 1 and Cyn d 7 have been found to exhibit some cross-reactivity with other grasses, but the results are not consistent [9]. Moreover, group 1 allergens in *C dactylon* have different epitopes to group 1 allergens in Pooideae grasses [1,17]. These differences are probably responsible for the low cross-reactivity between *C dactylon* and members of the Pooideae subfamily [1,18].

Five allergens have been described in *P communis*: Phr a 1, Phr a 4, Phr a 5, Phr a 12, and Phr a 13, (Allergome database) [15]. There are reports of a lack of group 2 and 6 allergens in *P communis* [19] and of low cross-reactivity with grasses from the Pooideae family [20]. *P communis* has been insufficiently studied to be considered for inclusion in the homologous group of sweet grasses.

The aims of the study were to investigate profiles of sensitization to *C dactylon* and *P communis* in patients sensitized to grasses in Catalonia (northeast Spain) and to analyze cross-reactivity between these 2 species and a mixture of temperate grasses.

Materials and Methods

Patient Population

Patients were recruited from 6 hospitals in the northeast of Spain: Hospital Universitari Joan XXIII (Tarragona), Allergo Center (Barcelona), Hospital de Terrassa (Terrassa, Barcelona), Hospital Clinic (Barcelona), Hospital Vall d'Hebron (Barcelona), and Hospital Arnau de Vilanova (Lleida). The selection criteria were the presence of respiratory symptoms (rhinitis and/or asthma) during the grass pollen season and a positive skin prick test (wheal diameter >3 mm) with a standardized grass mixture (GM) containing equal amounts of *Dactylis glomerata*, *Festuca elatior*, *Lolium perenne*, *Phleum pratense*, and *Poa pratensis* (Laboratorios LETI S.L.U., Tres Cantos, Madrid, Spain). A serum sample was obtained after oral consent from each of the patients enrolled in the study.

All patients were also skin prick tested with a battery of biologically standardized aeroallergens including pollens (*Olea europaea*, *Artemisia vulgaris*, *Parietaria judaica*, *Cupressus arizonica*, *Salsola kali*, *Platanus acerifolia*, and *Plantago lanceolata*) as well as mites, molds, and epithelia.

Extract Preparation

Pollen extracts were prepared following internal manufacturing procedures (Laboratorios LETI). Briefly, grass pollen (*D. glomerata*, *F. elatior*, *L. perenne*, *P. pratense*, *P. pratensis*, *C. dactylon*, and *P. communis*) was extracted consecutively for 4 and 8 hours in phosphate-buffered saline (PBS) 0.01M, pH 7.4. After each extraction the sample was centrifuged and the supernatant recovered. Supernatants from both extractions were pooled, filtered, and freeze dried. The protein content was measured using the Bradford method (Thermo Fisher Scientific).

SDS-PAGE and 2-D Electrophoresis

SDS-PAGE analysis was used to determine the protein profile of the grass extracts. Fifty micrograms of protein from every extract were loaded. Bands were analyzed via densitometry with the ImageQuant TL 8.1 software (GE Healthcare).

For 2-D electrophoresis, the extracts were purified and concentrated with a solution of ammonium sulfate in 2 separate steps until the saturation percentages of 40% and 80% were reached; they were then stored at 4°C overnight. Thereafter, the samples were centrifuged and the pellets were collected and reconstituted in ultrapure water. Concentrated extracts were washed using the ReadyPrep 2-D Cleanup Kit (BioRad) following the manufacturer's instructions. Proteins were separated according to their isoelectric point on ReadyStrip IPG strips (BioRad) with a pH range of 3 to 10, using Protean IEF Cell (BioRad). After the first dimension, the strips were equilibrated with ReadyPrep 2-D Kit buffers (BioRad) and proteins were separated in the second dimension according to their molecular weight. Gels were stained with Oriole fluorescent solution (BioRad) following the manufacturer's instructions.

Specific IgE

Specific IgE to GM, *C. dactylon*, *P. communis*, and the allergens Phl p 1 and Cyn d 1 was determined for all serum samples by ImmunoCAP (Thermo Fisher Scientific) following the manufacturer's instructions. A specific IgE (sIgE) of over 0.35 kUA/L was considered positive.

ELISA Assays

Direct ELISA was performed with a pool of sera prepared by mixing equal quantities of sera with an sIgE to the GM of over 1 kUA/L (27 sera). Briefly, a microplate (Immulon 4HBX, Thermo Fisher Scientific) was coated with 20 µg of lyophilized extract per milliliter, and the pool of sera (1:1 diluted in 0.01M PBS) was incubated for 2 hours at room temperature. After 3 washes, peroxidase-conjugated monoclonal anti-human IgE (Ingenasa, Madrid, Spain) was added. Two hours later, the reaction was developed, stopped, and read at 450 nm. Results were expressed in optical density units. ELISA inhibition was performed with the pool of sera as well as with individual sera. For the inhibition assay, sera were preincubated with the inhibitory extract for 2 hours before the addition to the microplate.

Immunoblot Experiments

Fifty micrograms of protein of each extract were electrophoresed by SDS-PAGE and electrotransferred to a Trans Blot Turbo Transfer Pack (BioRad). Membranes were incubated overnight with the pool of sera (dilution 1/5 in 0.01 M PBS Tween 0.1%). After washing, membranes were incubated with anti-human IgE-PO (Ingenasa), developed with luminol solutions (Immuno-Star HRP Chemiluminescent Kit, BioRad) and detected by chemiluminescence (ChemiDoc XRS, Bio-Rad). The same procedure was used for the immunoblot inhibition experiments; in this case the pool of sera was incubated for 2 hours at room temperature with 500 µg of the inhibitory extract before its addition to the blot membrane.

Quantification of Group 5 Allergens

Group 5 allergens were quantified in the 3 extracts. Briefly, microplates (MaxiSorp; Thermo Scientific) were coated with MA-1D11 anti-Phl p 5 monoclonal antibody (Indoor Biotechnologies) at a dilution 1/1000. After blocking with 1% BSA, PBS-Tween 0.05%, the samples were added to the plate in serial dilutions from 1 µg/mL to 31.25 ng/mL. The European Pharmacopoeia Reference Standard (EDQM) was used as a standard. After an hour of incubation, the biotinylated anti-Phl p 5 mAb Bo1 (Indoor Biotechnologies) was added and incubated for 1 hour. Finally, streptavidin-PO was added and the reaction was developed and measured at 450 nm.

Statistical Analysis

Descriptive statistical analyses were used for the calculation of variables and the Mann-Whitney rank sum Test was used to compare sIgE values. The relationship between sIgE values was compared by linear regression. SigmaStat 3.5 software (Point Richmond, California, USA) was used for the statistical analysis.

Results

Patient Population

Thirty-one patients were recruited (Table 1). They all had rhinitis; 20 had conjunctivitis (64.5%), 6 had asthma (19.4%), and 6 had cutaneous symptoms (19.4%). Only 3 individuals (9.7%) were monosensitized to grass pollen, 24 (77.4%) were sensitized to other pollen extracts, mainly olive tree pollen (19 patients, 61.3%), 16 were sensitized to mites (51.6%), and 12 were sensitized to animal dander (38.7%).

SDS-PAGE and 2-D electrophoresis

In SDS-PAGE, the 3 extracts (GM, *C. dactylon*, and *P. communis*) showed protein bands with a molecular weight in the range of 10 to 100 kDa. The densitometry assay showed a very similar profile for the 3 extracts (Figure 1A). The most intense bands were at 10 and 13 kDa for the GM extract and at 34 kDa for the *C. dactylon* and *P. communis* extracts (Figure 1). In 2-D electrophoresis, we observed that most proteins in the 3 extracts were located in the acidic region (left middle part of the gel) and had different isoforms (Figure 1B). Some spots

Table 1. Characteristics of the Study Population

No.	Age, y	Sex	IgE (kUA/L)					Symptoms	Other Sensitizations
			GM	Phl p 1	Cyn	Cyn d 1	Phr		
1	32	M	8.14	8.82	1.52	2.44	0.84	R, C	
2	44	F	3.53	0.94	Neg	0.51	0.4	R, C	
3	34	F	6.86	4.91	1.3	2.26	2.9	R, C, S	P, Mi, D
4	40	F	1.94	Neg	3.51	Neg	4.05	R, C, S	P, Mi, D
5	50	M	4.22	3.43	1.1	2.15	1.83	R, C, A	P
6	23	F	>100	NA	21.4	65.8	>100	R, A	Mi, D
7	19	M	>100	NA	6.82	33.4	55.4	R	P, Mi, D
8	25	M	24.9	Neg	Neg	Neg	2.53	R	P
9	52	M	21.8	2.3	5.43	2.51	6.34	R	P
10	9	M	>100	>100	6.77	62.2	48.7	R	Mi
11	37	M	29.6	10.3	1.11	0.4	3.32	R, C	P
12	29	M	1.55	14.2	Neg	Neg	0.58	R, C, S	P, Mi, Fu, D
13	20	F	84.5	92.9	28.3	17.6	32.1	R, C, S	P, Mi, D
14	39	F	14.1	17	1.46	Neg	1.77	R, C	Mi
15	36	M	1.23	2.04	0.45	Neg	0.61	R, C, A	P, Mi, Fu, D
16	42	F	3.16	4	1.14	0.93	1.14	R	P
17	8	M	27.7	42	15.8	11.2	13.9	R	P, D
18	62	F	2.82	0.99	0.62	1.66	1.56	R, C	P
19	27	M	0.82	0.79	Neg	Neg	Neg	R, C	Mi
20	60	F	0.59	1.36	Neg	Neg	Neg	R	
21	39	F	66.6	23.2	10.4	18.2	16.7	R, C	P, Mi, D
22	27	F	61.2	12.8	1.94	5.11	6.54	R, C	P, Mi, D
23	42	F	2.38	1.32	0.43	0.7	0.49	R, S	P, Mi, D
24	36	M	Neg	Neg	Neg	Neg	Neg	R, C	P
25	39	F	13.8	11.9	6.29	6.91	10.2	R	P, Mi
26	27	F	0.43	0.61	Neg	Neg	Neg	R, C	P, Mi
27	41	M	27.8	2,10	31.7	33.6	0.79	R, C, S	P, Mi, Fu, D
28	35	F	0.46	3.35	0.63	0.66	4.56	R, C, A	P
29	34	M	0.87	1.97	1.32	0.93	2.76	R	P
30	40	M	1.94	7.58	2.27	3.6	7.73	R, C, A	P
31	28	F	1.73	30.4	2.51	5.08	36.6	R, C, A	P

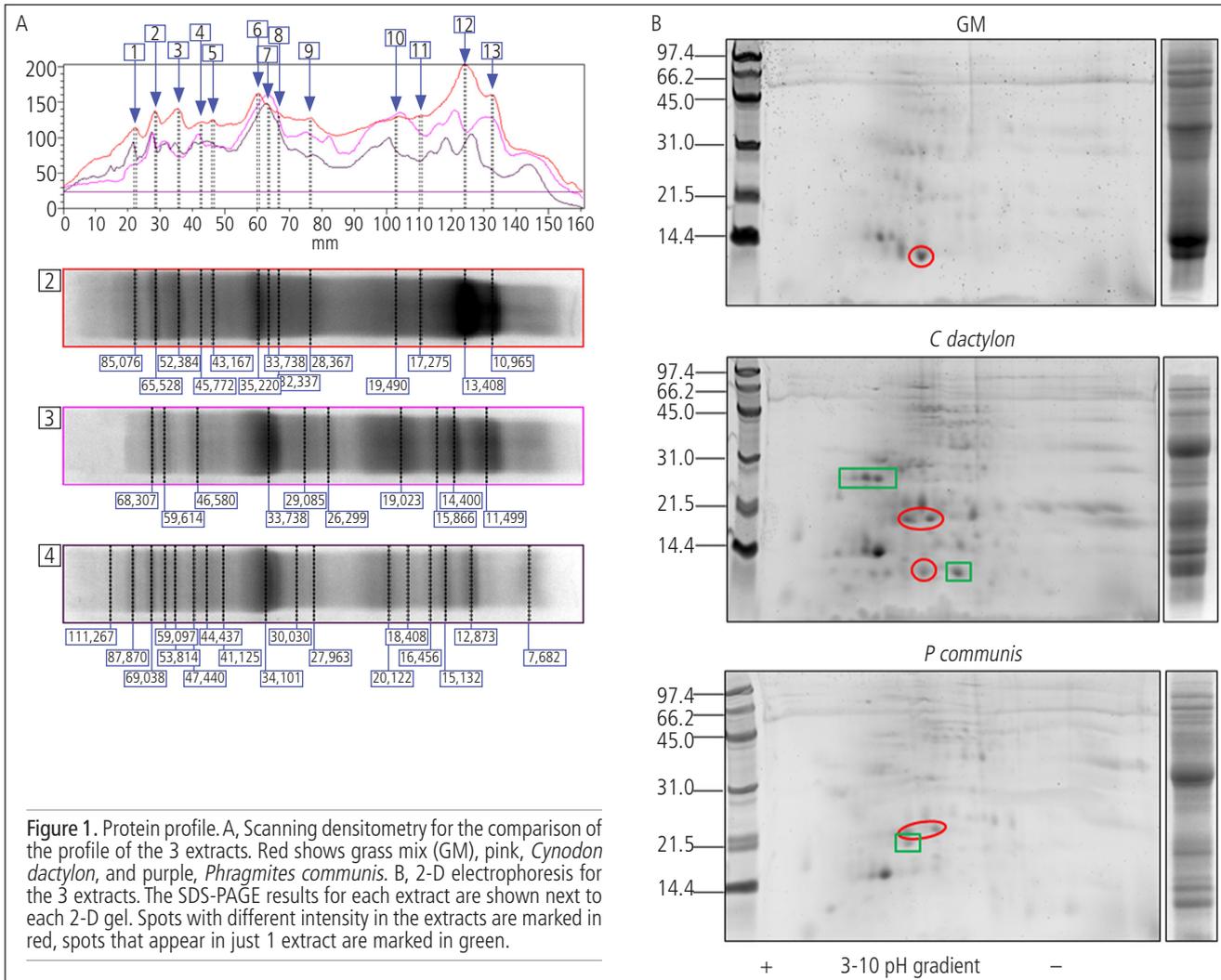
Abbreviations: A, asthma; C, conjunctivitis; Cyn, *Cynodon dactylon*; D, animal dander; F, female; Fu, fungi; GM, mixture of grasses; M, male; Mi, mites; NA, specific IgE not analyzed; Neg, negative (<0.35 kUA/L); P, other pollen; Phr, *Phragmites communis*; R, rhinitis; S, skin symptoms.

had a different intensity depending on the extract. There were, for instance, 2 intense spots around 21 kDa in *C dactylon* and *P communis* that were unremarkable in the GM extract. Conversely, there was an intense spot at 10 kDa in the GM extract that was less intense in the *C dactylon* extract and not detected in the *P communis* extract. These spots are circled in Figure 1B. We also detected some characteristic spots for the *C dactylon* and *P communis* extracts (Surrounded by a

square in Figure 1B). Other visible differences may be due to the specific method variations for the 3 gels.

Specific IgE

Positive specific IgE to GM was detected in 30 patients. One patient (#24, see Table 1) had negative results to all the extracts and was not included in further assays. Twenty-four



individuals (80%) were positive to *C dactylon* and 27 (90%) to *P communis*. The highest mean (SD) IgE values were obtained with GM (23.8 [33.4] kUA/L) and the lowest with *C dactylon* (6.4 [8.9] kUA/L). There were no statistical significant differences between the values obtained for the different extracts (Figure 2). For the group 1 allergens, 26 individuals (92.9%, 26/28) were positive to Phl p 1 (this test was not performed in 2 patients due to a lack of serum sample) and 22 (73.3%) were positive to Cyn d 1 (Figure 2). In the regression analysis of sIgE values, we found the highest correlation between *P communis* and Phl p 1 ($R^2=0.8$). For *C dactylon*, the correlation with all the other extracts was low (0.2-0.4). Correlation between Cyn d 1 and Phl p 1 was 0.5 (Figure 3).

ELISA and ELISA Inhibition

The pool of sera recognized the 3 extracts by direct ELISA. Values obtained with GM in the solid phase were 2 to 4.4 times higher than those obtained with *C dactylon* and 1.3 to 2.4 times higher than with *P communis* (Figure 4).

In the ELISA inhibition assay, 0.05 ng of GM extract was necessary to obtain the 50% inhibition point, compared with 5.7 and 4.9 μ g for *C dactylon* and *P communis*, respectively. These assays were performed with GM in the solid phase. Valid inhibition lines were obtained only with 3 individual serum samples (serum 3, 6 and 17). To obtain the 50% inhibition point with *C dactylon* compared with GM, the quantity needed was 650 times higher for serum 3, 353 times higher for serum 6, and 337 times higher for serum 17. The respective increases in quantity for *P communis* were 3360 for serum 3, 436 for serum 6, and 300 for serum 17. All the assays were performed with GM in the solid phase.

Immunoblot Experiments

The pool of sera recognized 2 main bands with a molecular weight of around 30 kDa in the GM extract, 1 band in the *C dactylon* extract, and 2 bands in the *P communis* extract (Figure 5).

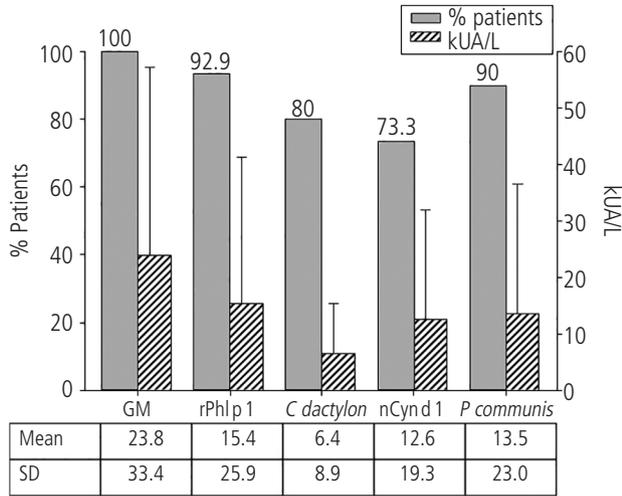
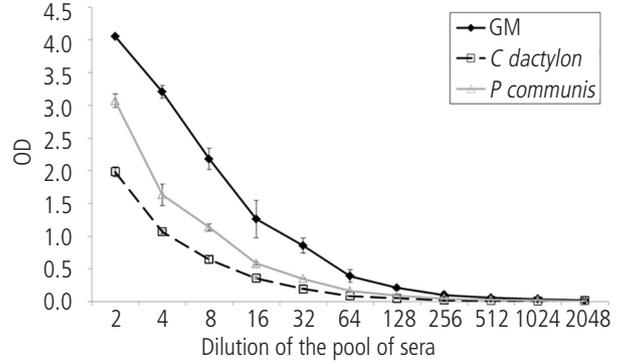


Figure 2. Percentage of patients sensitized to each extract and to the individual allergens and the mean value of sIgE (kUA/L). Error bars correspond to SD. Mean values and SD are shown in the table below.



	4.05	3.21	2.18	1.26	0.86	0.39	0.21	0.10	0.06	0.04	0.02
GM	4.05	3.21	2.18	1.26	0.86	0.39	0.21	0.10	0.06	0.04	0.02
<i>C dactylon</i>	1.98	1.07	0.64	0.36	0.19	0.09	0.05	0.02	0.01	0.01	0.01
<i>P communis</i>	3.07	1.63	1.13	0.59	0.35	0.16	0.09	0.05	0.03	0.02	0.01

Figure 4. Direct ELISA with grass mix (GM), *Cynodon dactylon*, and *Phragmites communis* extracts in the solid phase and incubation with serial dilutions of the pool of sera. The specific optical density (OD) values are shown in the table.

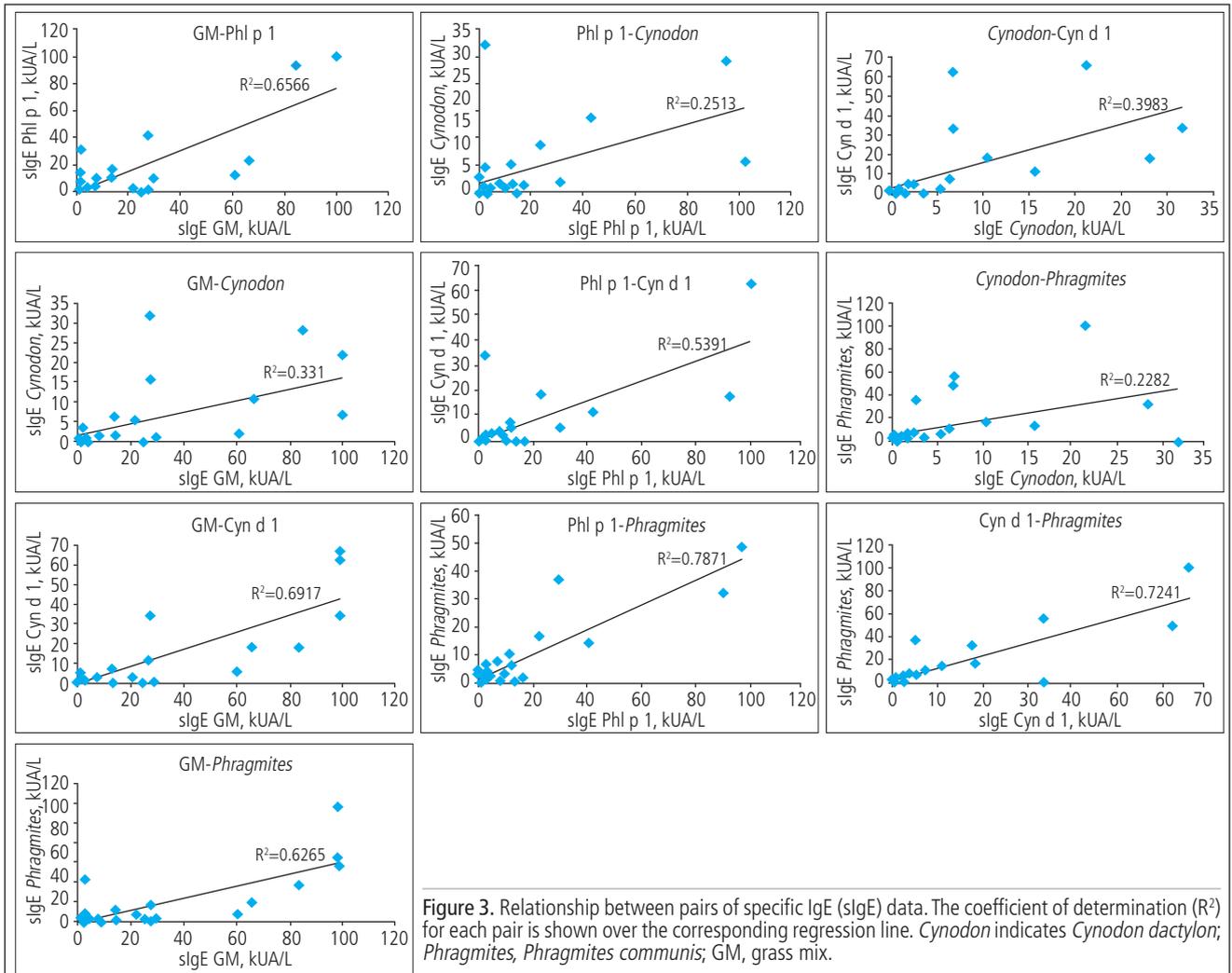


Figure 3. Relationship between pairs of specific IgE (sIgE) data. The coefficient of determination (R^2) for each pair is shown over the corresponding regression line. *Cynodon* indicates *Cynodon dactylon*; *Phragmites*, *Phragmites communis*; GM, grass mix.

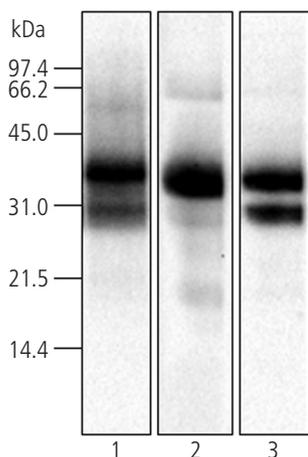


Figure 5. Immunoblot with the pool of sera (dilution 1/5). Fifty micrograms of each extract were run in the solid phase. Lane 1, grass mix; lane 2, *Cynodon dactylon*, and lane 3, *Phragmites communis*.

Inhibition Experiments

When GM extract was used in the solid phase, no inhibition was observed with the *C dactylon* or *P communis* extracts. Conversely, when *C dactylon* or *P communis* were used in the solid phase, GM inhibited the binding of the sera similarly to when the inhibition was performed with the same extract. In both cases, the other extract produced an intermediate inhibition (Figure 6).

Quantification of Group 5 Allergens

The GM extract contained 12.3 µg of group 5 allergens/mg of lyophilisate. However, no group 5 allergens were detected in the *C dactylon* or *P communis* extracts.

Discussion

We have assessed cross-reactivity between a mixture of 5 grasses (GM) from the homologous sweet grasses group (*D glomerata*, *F elatior*, *L perenne*, *P pratense*, and *P pratensis*) and 2 species from different subfamilies: *C dactylon* (Chloridoideae) and *P communis* (Arundinoideae). *C dactylon* and *P communis* are 2 very abundant subtropical grasses in the study area (Catalonia, northeast Spain). The patients analyzed had positive SPTs to the GM extract. *C dactylon* and *P communis* extracts were unable to inhibit IgE binding to the GM extract, but this extract inhibited IgE binding to *C dactylon* and *P communis*. Our findings are consistent with the fact that GM is the primary sensitizer in the study population. Asymmetric cross-reactivity between temperate and subtropical grasses has been reported [21] and varies according to the geographic origin of patients.

In areas where different Poaceae subfamilies grow together in the same habitat, it is difficult to determine which species are responsible for triggering symptoms in sensitized patients. The species have similar pollen grain characteristics and are morphologically indistinguishable. To overcome this problem, Frenguelli et al [6] performed a phenology study in Italy and

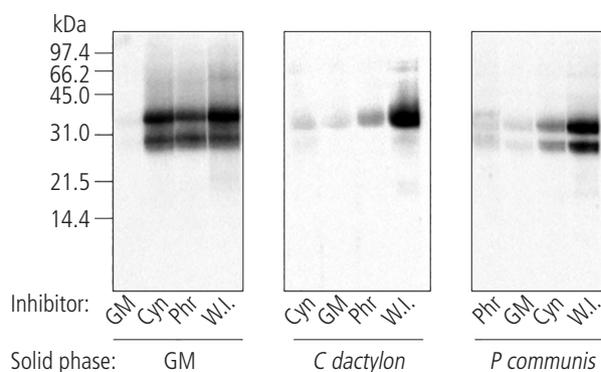


Figure 6. Immunoblot inhibition with the pool of sera (dilution 1/5). Fifty micrograms of each extract were run in the solid phase, the corresponding extracts are indicated at the bottom of figure. The inhibitory extract is indicated in each lane. GM indicates grass mixture, Cyn, *Cynodon dactylon*, Phr, *Phragmites communis*, and W.I., without inhibition.

demonstrated that *C dactylon* flowered later than the other grasses. Knowing that patients with symptoms in summer months (June-July) may be sensitized to *C dactylon* can aid a correct diagnosis. Comparisons of phenology data with symptoms and the use of component-resolved diagnosis will help to determine individual sensitization profiles and potentially aid in the selection of the most adequate specific immunotherapy.

Eighty percent of the individuals in this study had positive sIgE to *C dactylon* and 90% had positive sIgE to *P communis*. In the case of group 1 allergens, 92.9% of the patients had positive results for Phl p 1 versus 73.3% for Cyn d 1. Two patients (#12 and #14 in Table 1) had high sIgE to Phl p 1 but negative results for Cyn d 1. This observation confirms previous reports of immunologic differences between the 2 allergens [17] in terms of recognition of T-cell epitopes [21,22] and in amino acid sequence and 3D structure [13]. In addition, the correlation coefficient (R^2) between Phl p 1 and Cyn d 1 sIgE values was 0.5, corroborating the presence of different IgE binding epitopes. *P communis* sIgE values showed the highest correlation with Phl p 1 sIgE ($R^2=0.8$), although the correlation was also high with Cyn d 1 ($R^2=0.7$). Duffort et al [23] developed a monoclonal antibody to quantify Cyn d 1 that also recognized *P communis*. Both results suggest that Phr a 1 shares epitopes with Cyn d 1 and with Phl p 1. Moreover, previously published data show that Phl p 1 has specific epitopes that are not present in group 1 allergens of *C dactylon* or *P communis* [18]. Our findings confirm differences in IgE binding in Phl p 1 and Cyn d 1, probably due to differences in their epitopes. Although the Phr a 1 sequence is still unknown, our results suggest that it shares more epitopes with Phl p 1 than with Cyn d 1. This hypothesis should be confirmed with purified allergens.

The group 5 allergen in the Poaceae subfamily is a major allergen [20,24], and to date is the only allergen that has been quantified in immunotherapy extracts. However, no authors have described group 5 allergens in *C dactylon* to date. We confirmed that the group 5 allergen was undetectable in *C dactylon* with the monoclonal antibody used for the

quantification of group 5 allergens in sweet grasses (EDQM standard). Although this standard consists of the Phl p 5a isoform, it can be used for the identification and quantification of other Pooideae members. Using the same method, we were also unable to detect group 5 allergens in *P communis*, suggesting that Phr a 5 has different antigenic determinants to group 5 allergens from sweet grasses. Therefore, patients sensitized to *C dactylon* and/or *P communis* do not recognize group 5 allergens. Accordingly, it is important to identify the primary sensitizer in each case, as immunotherapy with an extract enriched in group 5 allergens (treatment with a mixture of sweet grasses) may not be effective in patients sensitized to *C dactylon* or *P communis*, and moreover it could induce new group 5 sensitizations [25-27].

One of the limitations of this study is the small number of patients included. It would be interesting to study a large group of patients as well as patients selected specifically for sensitization to *C dactylon* and/or *P communis*. The second limitation is the absence of clinical results, which are the only way to confirm our in vitro immunological results. It is important to identify primary sensitizers in specific areas to guide choice of immunotherapy, as proposed by Nony et al [28] for Australian patients. In our population, immunotherapy with sweet grasses (Poaceae) would be adequate as all the allergens recognized in *C dactylon* and *P communis* were inhibited by the GM extract. A population with primary sensitization to *C dactylon* and/or *P communis* could show different cross-reactivity patterns and would probably need treatment with the specific species involved.

In summary, 80% of patients sensitized to grasses were also sensitized to *C dactylon* and 90% were sensitized to *P communis*, but with lower sIgE levels. The GM and *C dactylon* and *P communis* extracts have different IgE-binding epitopes, precluding their inclusion in the homologous sweet grasses group. Sensitization to *C dactylon* and *P communis* seems to be induced by allergens other than those in sweet grasses. Further clinical studies should be considered to confirm the immunological results.

Acknowledgments

We thank Raquel Reyes, from the R&D Department, for her technical work and María Dolores García Arnedo, Jordi García, Jordi Muñoz, Daniel Resina, Aristides Rius and Raul Ramirez for their support.

Funding

The authors declare that no funding was received for the present study.

Conflicts of Interest

López-Matas MA, Moya R, and Carnés J are employees of Laboratorios LETI S.L.U. The rest of the authors declare that they have no conflicts of interest.

Previous Presentation

Part of the results were presented as a poster at the European Academy of Allergy and Clinical Immunology Congress held in June 2015 in Barcelona (Spain).

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- *Manuscript received October 27, 2015; accepted for publication, December 29, 2015.*
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