

# Evaluation of Molecular Basis of Cross Reactivity between Rye and Bermuda Grass Pollen Allergens

Ruby Tiwari<sup>1,2</sup>, Prem L Bhalla<sup>1</sup> and Mohan B Singh<sup>1</sup>

## ABSTRACT

**Background:** Allergenic cross reactivity between the members of the Pooids (*Lolium perenne*, *Phleum pratense*, and *Poa pratensis*) and Chloridoids (*Cynodon dactylon* and *Paspalum notatum*) is well established. Studies using crude extracts in the past have demonstrated limited cross reactivity between the Pooids and the Chloridoids suggesting separate diagnosis and therapy. However, little is known regarding the molecular basis for the limited cross reactivity observed between the 2 groups of grasses. The present study was undertaken to gain insights into the molecular basis of cross allergenicity between the major allergens from rye and Bermuda grass pollens.

**Methods:** Immunoblot inhibition tests were carried out to determine the specificity of the proteins involved in cross reactivity. Crude pollen extract and bacterially expressed and purified recombinant Lol p 1 and Lol p 5 from rye grass were subjected to cross inhibition experiments with crude and purified recombinant Cyn d 1 from Bermuda grass using sera from patients allergic to rye grass pollen.

**Results:** The immunoblot inhibition studies revealed a high degree of cross inhibition between the group 1 allergens. In contrast, a complete lack of inhibition was observed between Bermuda grass group 1 allergen rCyn d 1, and rye grass group 5 allergen rLol p 5. Crude rye grass extract strongly inhibited IgE reactivity to Bermuda grass, whereas crude Bermuda grass pollen extract showed a weaker inhibition.

**Conclusions:** Our data suggests that a possible explanation for the limited cross reactivity between the Pooids and Chloridoids may, in part, be due to the absence of group 5 allergen from Chloridoid grasses. This approach of using purified proteins may be applied to better characterize the cross allergenicity patterns between different grass pollen allergens.

## KEY WORDS

Bermuda grass, cross reactivity, immunoblot inhibition, rye grass, SDS-PAGE

## INTRODUCTION

Grasses constitute a very large and diverse group of plants counting over 10000 individual species. Bermuda grass (*Cynodon dactylon*) and Bahia grass (*Paspalum notatum*) are found mainly in the warmer climate zones, whereas rye grass (*Lolium perenne*), Timothy grass (*Phleum pratense*) and Kentucky blue grass (*Poa pratensis*) are found worldwide, but prefer-

entially in the temperate zones.<sup>1</sup> Geographical diversity as well as climate conditions affect the diversity of grasses found in a certain area, as a result of differences found in sensitization patterns and conditions in these areas. A majority of clinically important grass pollens belong to the Pooids and allergens identified in any one species often have homologues in other species, displaying similar physicochemical and immunological properties.<sup>2</sup> Based on the prevalence of

<sup>1</sup>ARC Centre of Excellence for Integrative Legume Research, Faculty of Land and Food Resources, University of Melbourne, Parkville, Australia and <sup>2</sup>Present address: Institute of Food Nutrition and Human Health, Massey University, Palmerston North, New Zealand.

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revision of the manuscript for important intellectual content and general supervision. All authors have read and approved the final manuscript.

Correspondence: Ruby Tiwari, PhD, Institute of Food Nutrition and Human Health, Riddet Road Massey University, Private Bag 11222, Palmerston North 4442, New Zealand.

Email: rtiwari14@yahoo.com

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IgE antibody recognition among grass pollen sensitised individuals, several allergens may qualify as major, but members of 2 groups, namely groups 1 and 5, have been shown to dominate the immune response to grass pollen extracts. Group 1 grass pollen allergens are recognized as the most prominent and potent allergenic determinants in grass pollen extracts with approximately 90% of grass pollen allergic individuals producing IgE antibodies specific to this component. Group 1 allergens share significant sequence homology with each other.<sup>3,4</sup> Prevalence of sensitization to group 5 allergens range between 65 and 85% among grass pollen allergic individuals, and together with group 1 allergens, dominate the allergenicity of grass pollen species.<sup>5</sup> It is notable that while the Pooid grasses have both group 1 and 5 allergens, only the group 1 allergen has been identified in Chloridoids and to date, no presence of group 5 allergen in any of the Chloridoid grasses have been reported.

Clinical evidence of cross allergenicity among grass pollens has suggested that diagnosis and immunotherapy can be achieved with a limited number of grasses, the selection of species being based upon their regional prevalence and taxonomic relationship. Grass pollen cross reactivity when investigated by inhibition techniques revealed extensive cross reactivity among taxonomically related grasses, with the exception of Bermuda grass.<sup>6,7</sup> Several inhibition studies performed with sera from North American and European subjects confirmed that there is little allergen cross reactivity between the Pooids and the Chloridoids.<sup>8</sup> The diversity in phylogenetic relationship of Bermuda grass with Pooid grasses or presence of unique allergenic components in Bermuda grass were proposed as possible reasons for the limited cross reactivity.<sup>6,8</sup> Although several studies have demonstrated a low degree of cross reactivity between rye grass and Bermuda grass, little is known regarding the molecular basis for this observation. Since most studies in the past have frequently used crude pollen extracts, the degree of IgE cross reactivity between Bermuda grass and rye grass at a molecular level needs further investigation.

Approaches used to identify cross reactivity of allergenic proteins have strongly implied that cross reactivity is based on IgE recognition of structural similarities between the sensitizing antigens. Since structural studies rely heavily on the use of purified proteins, we investigated the cross reactivity pattern by paying particular emphasis on using both crude pollen extracts as well as purified recombinant proteins Lol p 1, Lol p 5 and Cyn d 1 to further characterize their cross reactivity pattern. Such information would have clinical relevance in current diagnostic and treatment protocols.

## METHODS

### SERUM

Patients with a case history and positive skin test to rye grass pollen were selected. A blood sample collection was carried out according to the University of Melbourne ethics guidelines.

### GRASS POLLEN EXTRACTS

Grass pollens were purchased from Greer Laboratories (NC, USA) as dry, non-defatted pollen and stored at -20°C until use. Aqueous pollen extracts were prepared by gently agitating 500 mg of pollen in 1 mL of extraction buffer (15 mM phosphate buffered saline, 1 mM phenylmethyl-sulfonyl fluoride) at 4°C for approximately 30 minutes. The mixture was centrifuged at 12,000 g for 5 minutes and the supernatant collected. The concentration of proteins in the supernatant was determined using BIORAD reagent (Biorad, CA, USA). Supernatants were frozen and stored as 1 ml aliquots at -20°C until use. The quality of the protein extracts was analysed by SDS PAGE and Coomassie blue staining.

### ISOLATION OF MESSENGER RNA FROM BERMUDA GRASS POLLEN

Total RNA was isolated using the guanidine hydrochloride method. Five hundred milligrams of grass pollen was finely ground with a mortar and pestle in liquid nitrogen, and resuspended in 5 mL of extraction buffer (7.5 M guanidine HCl in 0.1 M  $\beta$ -mercaptoethanol, 0.5% w/v lauryl sarcosinate and 25 mM sodium citrate) dissolved in DEPC (diethyl pyrocarbonate) treated water. This mixture was centrifuged at 12000 g for 20 minutes. The supernatant was collected and extracted twice with an equal volume of phenol: chloroform: isoamylalcohol (25 : 24 : 1 v/v) and the suspension was mixed gently for 20 minutes followed by centrifugation at 12000 g for 20 minutes at 4°C. Final extraction was carried out with equal volume of chloroform: isoamylalcohol. The RNA was precipitated with 1/10 volume of 3 M sodium acetate and equal volume of cold isopropanol. The mixture was stored at -20°C and RNA pellet was collected by centrifugation at 4°C for 20 minutes at 12000 g. The pellet was washed with 70% ethanol prepared in DEPC water; vacuum dried and dissolved in DEPC treated water. DNase treatment was given to degrade any traces of DNA contamination. The quality of RNA was checked by agarose gel electrophoresis and the concentration was measured at OD<sub>260</sub> before aliquoting and storing at -70°C. Poly (A) messenger RNA was affinity purified using oligo-dT cellulose (Microfast trak, Invitrogen, CA, USA), following the manufacturer's instructions.

### CLONING AND SEQUENCING OF Cyn d 1 ALLERGEN FROM BERMUDA GRASS POLLEN

A cDNA expression library from Bermuda grass pollen was constructed in a lambda gt11 vector. Five micrograms of mRNA was used for generating double-stranded cDNA, according to manufacturer's instructions (Pharmacia LKB, Uppsala, Sweden). The cDNA was ligated into Eco R1/Not 1 adaptors and cloned into Lambda gt 11 vector (Amersham, Uppsala, Sweden). The clones were packaged into bacteriophages (Gigapak Gold III Stratagene, CA, USA) to construct a cDNA expression library. *E. coli* Y1090 were transduced with in vitro packaged phages, which resulted in a plaque forming unit per millilitre of  $2.2 \times 10^6$  in the primary library containing 98% recombinant clones. The cDNA library was screened with anti-Cyn d 1 mouse monoclonal antibody mAb 3A2. Briefly, the pool of phages containing the cDNA inserts was used to infect *E. coli* strain Y1090 cell which were then placed onto LB plates containing 100 µg/mL ampicillin. After 3 hours growth at 42°C, the plates were overlaid with nitrocellulose filters (Hybond-C extra, Amersham) soaked with 1 mM IPTG (isopropyl-β-D-thiogalactopyranoside). The incubation temperature was reduced to 37°C in order to induce the production of fusion protein. The filters were inverted 4 hours later and left for an additional 4 hours. Two lifts were taken from each plate. The unoccupied binding sites were blocked by incubating the filters in 10% skim milk powder in PBS. After blocking, the protein plaque lifts were washed twice in PBST (PBS containing 0.1% Tween 20) followed by 2 washes with PBS. The filters were incubated in Anti-Cyn d 1 mAb 3A2 (1 : 1000 in 1% BSA/PBS) for 5-6 hours. After washing, colorimetric detection of filters was carried out with 5% NBT (nitro blue tetrazolium chloride) dissolved in 70% dimethylformamide, and BCIP (bromochloroindolyl phosphate) (Astral Scientific, New South Wales, Australia) in 100% dimethylformamide. Positive clones were excised from the master plate and different dilutions of clones were re-screened to isolate pure individual plaques. The insert sizes of various clones were determined using PCR with lambda gt11 Forward and reverse primers. The PCR products were gel purified and sequenced on automated DNA sequencer (ABI; Applied Biosystems, CA, USA).

### HOMOLOGY SEARCH AND FULL LENGTH Cyn d 1 CLONE ISOLATION

The open reading frame (ORF) of the Cyn d 1 sequence was identified by homology search in the NCBI genebank at both nucleotide and amino acid levels. A clone encoding full length Cyn d 1 gene was selected for preparing the expression construct.

### PREPARATION OF Cyn d 1 EXPRESSION CONSTRUCT

The sequence covering the full length Cyn d 1 gene was sub-cloned from the lambda gt11 vector into the pQE30Xa expression vector (Qiagen, CA, USA). Gene specific primers were designed to introduce restriction enzyme sites at both ends. The insert was cloned into the expression vector downstream from the six residue histidine tag in the correct reading frame. University of Melbourne research guidelines were followed for preparing these constructs.

### EXPRESSION AND PURIFICATION OF RECOMBINANT ALLERGEN COMPONENTS

Expression constructs for Lol p 1 and Lol p 5 were kindly provided by Dr. Nicole de Weerd (University of Melbourne). Recombinant rye grass allergens (rLol p 1 and rLol p 5) and Bermuda grass pollen allergen (rCyn d 1) were expressed with an N-terminal, six residue, histidine tag in the pQE30xa expression vector (Qiagen) in *E. coli* M15 cells (Qiagen). In brief, a 250 mL volume of LB broth containing 50 mg/ml kanamycin (Sigma Aldrich, St. Louis, USA) and 100 mg/mL ampicillin (Sigma Aldrich) was seeded with 5 ml overnight culture of *E. coli* M15 cells (Qiagen) harbouring the appropriate plasmid and grown at 37°C for 4 hours in a conical flask. Isopropyl-βD-thiogalactoside (IPTG) (Progen, Heidelberg, Germany) was then added to a final concentration of 1 mM to induce expression of the fusion protein. Following incubation at 37°C for an additional 3 hours, bacterial cells were harvested by centrifugation at 10000 g for 20 minutes. The supernatant was discarded and cells were stored at -20°C overnight. The cells were given multiple freeze thaw cycles in liquid nitrogen to encourage cell lysis. The partially lysed cells were then resuspended in 4 ml of lysis buffer (50 mM NaH<sub>2</sub>PO<sub>4</sub>, 300 mM NaCl, 10 mM imidazole pH 8.0). Lysozyme (Sigma, Aldrich) at a concentration of 1 mg/ml was added to the resuspension and incubated on ice for 30 minutes for further cell lysis. RNase A (Sigma, Aldrich) (10 µg/ml) and DNase (Sigma, Aldrich) (5 µg/ml) was added to the lysate and incubated for 15 minutes to remove bacterial RNA and DNA in order to reduce viscosity of the cell lysate. The lysate was centrifuged at 10000 g for 20 minutes at 4°C. The supernatant was harvested and added to 1 mL TALON Ni-NTA resin (Clontech, CA, USA). The mixture was rocked gently on ice for 1 hour. The resin and supernatant mixture was pipetted gently into a disposable 1 mL column (Qiagen). The resin column was allowed to form by gravity before washing with further 2 column volumes of wash buffer 50 mM NaH<sub>2</sub>PO<sub>4</sub>, 300 mM NaCl, 10 mM imidazole pH 8.0). Fusion proteins were eluted from the column 4 times with 0.5 mL of elution buffer (50 mM NaH<sub>2</sub>PO<sub>4</sub>, 300 mM NaCl, 250 mM imidazole pH 8.0). Purified fusion proteins were visualized by Coomas-

sie staining of 12.5% SDS-PAGE. The concentration of proteins present in eluted aliquots was determined at OD<sub>280</sub>.

### IMMUNOBLOT INHIBITION ASSAY

Protein samples 10 µg per well for mini gels were denatured by reducing the conditions by boiling for 5 minutes in the presence of 25 mM Tris-HCl, 2% dithiothreitol and 10% glycerol with Bromophenol blue dye at pH 6.8. Proteins were resolved on a 12.5% SDS-PAGE. Protein standards (Invitrogen) were run in conjunction with the extracts to determine its molecular weight. Gels were electrophoresed at 200 V for 1 hour, until the dye front reached the bottom of the gel. Gels were stained with Coomassie blue to visualise proteins or left unstained for electrophoretic transfer. Proteins separated by SDS-PAGE were transferred to PVDF membranes (Amersham) in 48 mM Tris/HCl, pH 9.0, containing 39 mM glycine, 0.0375% SDS, and 20% (v/v) methanol, for 1 hour. The individual sera ( $n = 7$ ) or the pool ( $n = 10$ ) of sera from allergic patients (diluted 1/5) was pre-incubated overnight at 4°C, with crude pollen extracts (100 µg of total protein) from rye and Bermuda grass pollens before the binding assay on the immobilized antigen. For recombinant purified proteins Lol p 1, Lol p 5 and Cyn d 1, sera were pre-adsorbed with 10 µg of purified protein prior to performing the binding assay. Proteins separated by SDS-PAGE were transferred to PVDF membranes (Amersham) in 48 mM Tris/HCl, pH 9.0, containing 39 mM glycine, 0.0375% SDS, and 20% (v/v) methanol, for 1 hour. Membranes were blocked in 10% nonfat milk for 1 hour then probed with individual sera or pooled sera diluted 1/5 in 1% BSA in PBS overnight at room temperature. Strips were washed with 0.1% Tween-20 in PBS and incubated overnight with I<sup>125</sup> labelled anti-human IgE (Bioclone, Sydney, Australia). After washing the blots, autoradiography was performed at -70°C using X-ray films (MS Kodak, Sigma Aldrich) and intensifying screen.

## RESULTS

### CROSS REACTIVITY BETWEEN CRUDE EXTRACTS FROM RYE AND BERMUDA GRASS POLLEN

All grass pollen allergic sera showed a positive IgE reaction to crude extracts from both Bermuda and rye grass pollen. The IgE antibodies recognized expected band sizes at 28-30 kDa corresponding to Lol p 5 and Lol p 1 from rye grass and Cyn d 1 from Bermuda grass. The IgE binding to rye grass was stronger compared to Bermuda grass. The stronger signals observed in the rye grass extract may have been due to the overlap of group 1 and group 5 allergens because of their similar molecular weights. Complete inhibition to Bermuda grass was observed by immunoblot inhibition when crude extract from

rye grass was used as the inhibitor. However, Bermuda grass produced minimal inhibition of IgE binding to rye grass for all sera tested. Partial inhibition was observed in the case of pooled sera (P1) and patient 2 (P2). The comparison of remaining signals of pollen proteins, with and without an inhibitor is shown in Figure 1.

### CROSS REACTIVITY BETWEEN RECOMBINANT Lol p 1 AND Cyn d 1

To analyse the ability of the specific allergen groups to cross react with each other, IgE inhibition immunoblots were performed with recombinant purified proteins rLol p 1 and rCyn d 1 representing one of the major allergenic components. For both pooled, as well as individual patient sera, nearly complete inhibition was observed when group 1 allergens, rLol p 1 and rCyn d 1 were used as inhibitors against each other, indicating the presence of shared allergenic epitopes. Results are shown in Figure 2.

### CROSS REACTIVITY BETWEEN RECOMBINANT Lol p 5 AND Cyn d 1

When cross reactivity was compared between purified rLol p 5 and rCyn d 1, all sera pre-incubated with rLol p 5 displayed IgE reactivity with purified rCyn d 1 immobilised on the PVDF membrane. In the reciprocal assay, pre-adsorption of sera with rCyn d 1, showed no detectable inhibition of IgE reactivity to rLol p 5 as shown in Figure 3.

## DISCUSSION

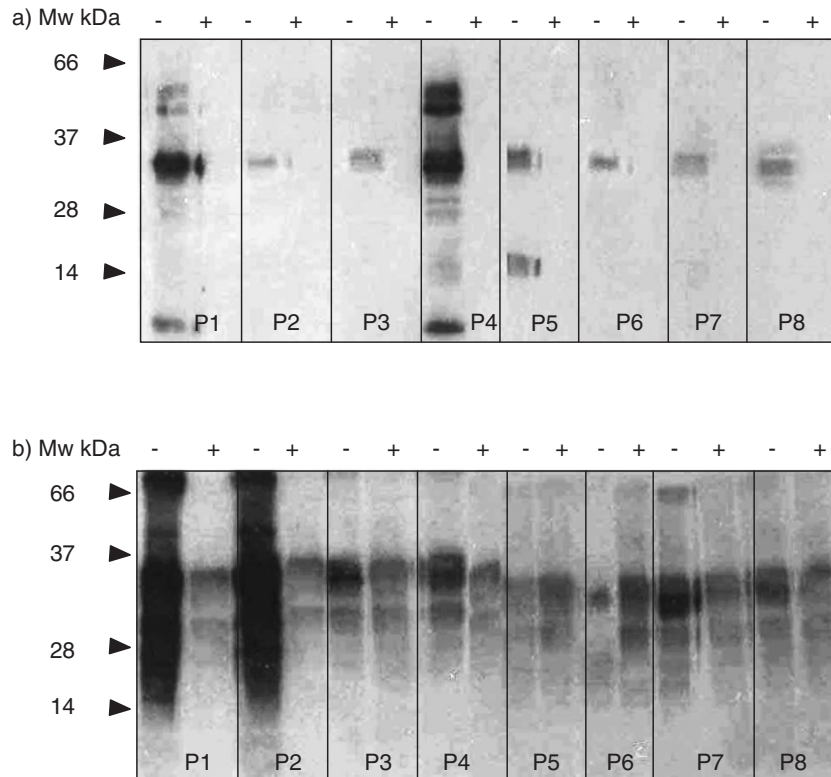
The aim of this study was to investigate the molecular basis for limited cross reactivity between rye and Bermuda grass pollen allergens.

Rye grass pollen is a potent seasonal aeroallergen, considered as a major source of pollen in early summer in temperate zones. The widespread use of Bermuda grass in turfs and lawns has made it a source for triggering allergic rhinitis throughout the autumn and summer. Clinical tests such as radioallergosorbent test (RAST) and skin tests use total grass pollen protein extracts to establish allergenicity. Recombinant allergens offer a number of advantages compared with their natural counterparts. The technology permits the production of defined allergens with high purity, permitting the development of new diagnostic assays that are of far greater specificity than was possible, thereby allowing the determination of specific patient sensitization profiles.<sup>9</sup>

In order to precisely identify the cross reactivity profile, we analysed the IgE profile by immunoblot inhibition using crude extracts and purified recombinant proteins that constitute the major allergenic components of both grasses.

Our data showed patient IgE reactivity predominantly to crude rye grass extract in comparison to Bermuda grass. There could be two possible explana-

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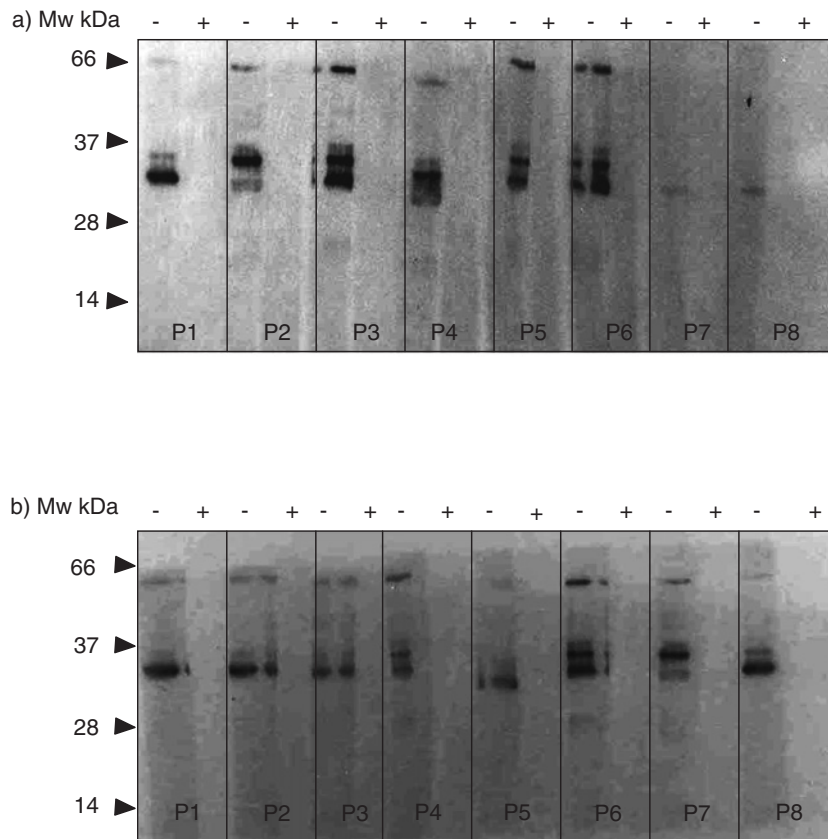


**Fig. 1** a) SDS-PAGE of crude protein extract of Bermuda and Rye grass pollen. SDS-PAGE of crude protein extract of Bermuda immunostained with pooled (P1) and individual (P2-P8) patient sera are displayed in lanes marked (-). Inhibition of binding was analysed after preincubation of sera with inhibitor crude Rye pollen extract marked (+). b) SDS-PAGE of crude protein extract of Rye grass pollen immunostained with pooled (P1) and individual (P2-P8) patient sera are displayed in lanes marked (-). Inhibition of binding was analysed after preincubation of sera with crude Bermuda pollen extract marked (+).

tions for this observation. First, the presence of 2 major allergens (Group 1 and 5) in rye grass may have led to increased IgE binding with sera containing IgE antibodies directed against both allergen groups. Secondly, rye grass may have been the primary sensitizing allergen source in the panel of sera with majority of the IgE antibodies directed against rye grass. In contrast, the IgE binding to Bermuda grass was weak, which may be attributed to the presence of only a single major group 1 allergen in Bermuda grass. Moreover, sera pre-adsorbed with rye grass extract showed complete inhibition of IgE reactivity with Bermuda grass, whereas Bermuda grass did not inhibit IgE reactivity with rye grass. These results suggest that rye grass is the more potent or primary sensitizing agent of the 2 allergen sources, thereby making it a stronger inhibitor.

When inhibition assays were performed using bacterially expressed and purified recombinant proteins, a complete cross inhibition was achieved with purified rLol p 1 and rCyn d 1 in reciprocal inhibition as-

says (Fig. 2). Generally, the IgE inhibition of protein bands on an allergenic extract only occurs when structurally homologous counterparts are present in the inhibitory extract. The primary structures of known allergens has allowed for grouping of allergens into families (e.g. Pfams) based on structural similarities that may be reflected in their amino acid sequence similarity using a bioinformatics approach.<sup>10,11</sup> With grass group 1 allergens exhibiting 85-90% sequence identity at the amino acid level, the high sequence and probable structural similarity of these proteins may account for the shared IgE epitopes. Evidence for the location of IgE epitope for Lol p 1 has been reported in a previous study using recombinantly expressed Lol p 1 fragments. In this study, 2 fragments of Lol p 1, corresponding to amino acids 1-168 and 169-240, could inhibit human IgE antibody binding to rLol p 1 by 17 and 51%, respectively suggesting the presence of a major IgE epitope at the C-terminal end.<sup>12</sup> The IgE epitope mapping study on Cyn d 1 has also identified 2 immunodominant epi-



**Fig. 2** a) SDS-PAGE of purified recombinant Bermuda grass rCyn d 1 immunostained with pooled (P1) and individual (P2-P8) patient sera are displayed in lanes marked (-). Inhibition of binding was analysed after preincubation of sera with Rye grass rLol p 1 marked (+). b) SDS-PAGE of purified recombinant protein of Rye grass rLol p 1 immunostained with pooled (P1) and individual (P2-P8) patient sera are displayed in lanes with (-). Inhibition of binding was analysed after preincubation of sera with rCyn d 1 marked (+).

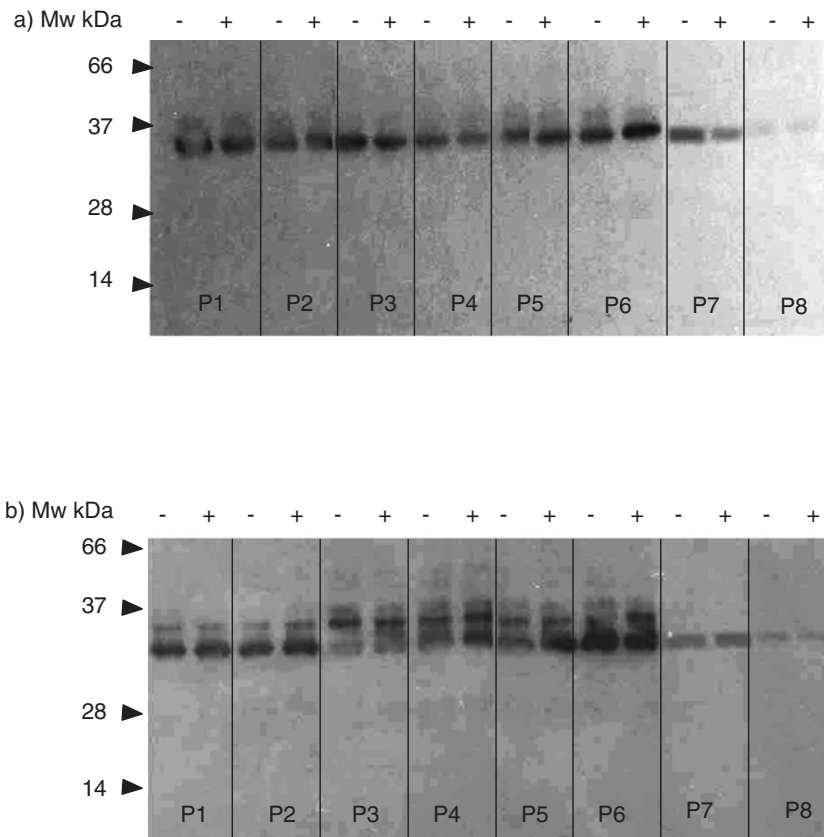
topes at the C-terminus of the molecule.<sup>13</sup> One could speculate that the common C-terminal between Lol p 1 and Cyn d 1 may play a role in total cross inhibition.

On the other hand, there was no notable inhibition between rLol p 5 and rCyn d 1 in the reciprocal inhibition assays. The apparent lack of inhibition of IgE reactivity to rCyn d 1 by rLol p 5 and vice versa may be due to significantly low sequence and structural homology. Group 1 and 5 allergens are primarily composed of  $\beta$ -sheet and  $\alpha$ -helix secondary structures, respectively, greatly reducing the possibility of common structural features or shared IgE epitopes.<sup>2</sup>

Bahia grass is a member of the Chloridoideae family and phylogenetically close to Bermuda grass. In a previous study performed by Davies *et al.*, a low degree of cross reactivity was reported between Bahia grasses and rye grass when tested with sera from patients allergic to rye grass pollen. Although, 78% of the sera reacted with Bahia grass, their reactivity was weak with respect to rye grass. Furthermore, rye

grass inhibited IgE reactivity with Bahia grass, whereas Bahia grass did not inhibit IgE reactivity with rye grass.<sup>14</sup> Our results are in agreement with those reported by Davies *et al.*, where rye grass was found to be the predominant allergen and a stronger inhibitor compared to Bermuda grass, when tested with rye grass pollen allergic sera. In a recent study by Rossi *et al.*, 411 patients with known sensitivity to Bermuda grass pollen were evaluated for their IgE reactivity with both crude and purified Timothy grass pollen allergens. All patients had higher IgE against Timothy grass allergens compared to Bermuda grass allergens with the exception of those subjects who were sensitised to calcium binding protein Phl p 7 and/or profilin Phl p 12. Interestingly, more than 68% of patients reacted to purified Phl p 1 as well as Phl p 5. They concluded that since the IgE levels were higher against Timothy grass allergens, the Bermuda grass positive patients were in reality sensitised to Timothy grass and that the patients may have been

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**Fig. 3** a) SDS-PAGE of purified recombinant Bermuda grass rCyn d 1 immunostained with pooled (P1) and individual (P2-P8) patient sera are displayed in lanes marked (-). Inhibition of binding was investigated after preincubation of sera with Rye grass rLol p 5 (+). b) SDS-PAGE of purified recombinant Rye grass rLol p 5 immunostained with pooled (P1) and individual (P2-P8) patient sera are displayed in lanes marked (-). Inhibition of binding was analysed after preincubation of sera with Bermuda grass rCyn d 1 marked (+).

co-sensitized. Since their study did not extend into IgE inhibition analysis using the various purified grass allergens, the role of each of the allergen groups could not be clearly defined.<sup>15</sup> Our findings can shed some light on the observations from the study by Rossi *et al.* The higher IgE reactivity with Timothy grass allergens may be due to the absence of group 5 allergen from Bermuda grass pollen.

Cross inhibition analyses, such as those used in our study, involving both crude extracts and purified recombinant proteins from different pollen sources, provide a better tool for detecting and distinguishing specific allergens for a given pollen extract. Our results suggest that patients sensitized to rye grass are likely to experience allergy symptoms when exposed to Bermuda pollen, and other allergen sources which have group 1 allergen-like proteins. Furthermore, the results from this study suggest that the limited cross reactivity between rye and Bermuda grass as demonstrated by earlier RAST tests may be as a result of the

absence of the group 5 allergen from Bermuda grass. However, individual variation in immune response and cross reactive profiles of allergic patients' may also play a role, depending on environmental conditions and geographic and climatic differences which may influence the diversity and dominance of the allergen source. Furthermore, the frequency of sensitivity to different pollens and their specific allergens and climatic conditions in an area need to be given careful consideration when screening for patients that may require separate therapy.

In conclusion, we investigated the molecular basis of cross reactivity between 2 groups of clinically relevant grasses. The similarity in the molecular weight, sequence homology and structural features of the group 1 grass allergens and the finding that they cross inhibit each other suggests that both components are related. The lack of cross-inhibition between rCyn d 1 and rLol p 5 provides further evidence in support of the view that the 2 groups are



structurally different. Taken together, the findings from this study suggest that the low degree of cross reactivity between rye and Bermuda grasses may, in part, be as a result of the absence of group 5 allergens in Bermuda grass. Further studies using purified proteins promises opportunities to better characterize the cross allergenicity patterns between different grass pollen allergens. Such information should allow integration of this information into diagnosis and immunotherapy of grass pollen allergy.

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