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Allergenic and Antigenic Cross-Reactivities of Group IX Grass Pollen Allergens

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Abstract. The allergenic and antigenic cross-reactivities between a major recombinant *Poa pratensis* (*Poa p*) IX allergen, rKBG8.3, and its corresponding proteins of different grass pollens were examined. Immunoblotting of the proteins of thirteen different grass pollens using anti-rKBG8.3 antibodies indicated that *Poa p* IX-like proteins are present in ten other grass pollens, albeit in variable amounts and polymorphic forms. These proteins ranged in size from 20 to 88 kDa in different grass pollens. The percent relative binding determined for each grass pollen extract using allergic human sera showed a significant correlation ($r = 0.891$) with that of anti-rKBG8.3 antiserum. Moreover, there was a strong association ($r = 0.901$) between the Kentucky bluegrass extract and rKBG8.3 with respect to their inhibition of the binding of human IgE antibodies to allergens in grass pollen extracts. Taken together, these results suggest that the allergenic and antigenic epitopes of the *Poa p* IX-related proteins in some but not all grass pollens are similar in structure and specificities. It is concluded that the group IX allergens constitute a major family of homologous proteins in several grass pollens.

Introduction

The major drawbacks that undermine the efficacy of immunotherapy in relation to allergies to plant pollens are: (1) the large number of undefined allergenic components to which an individual may be allergic, (2) lack of knowledge of a given pollen allergen and of the precise allergenic and antigenic relationships among components of various pollens, and (3) most importantly, the lack of sufficient amounts of pure and standardized preparations of allergenic proteins which could be used for a diagnostic identification of the allergenic components responsible for the sensitivity of a patient. In attempts to demonstrate allergenic cross-reactivity among pollens of different grasses in vitro techniques, such as the radioallergo-sorbent test and immunodiffusion analysis [1-5], and the in vivo skin prick test [6-8] have been employed. Recently, murine monoclonal antibodies (Mabs) to purified allergenic components have been used to investigate cross-reactivities among grass pollens [9-12],

whereby each Mab is directed against a unique epitope. However, this approach has two potential pitfalls: (1) the single epitope recognized by Mab may not be representative of relative allergenic potency of the protein(s), and (2) it may be difficult to identify Mabs to minor component(s) which may, nevertheless, constitute important allergens in patients.

We reported recently the molecular cloning and expression in *Escherichia coli* of a new group of isoallergens of Kentucky bluegrass (KBG) pollen, which were designated as *Poa pratensis* (*Poa p*) IX allergens [13-17]. Furthermore, a polyclonal antiserum was raised in mice to the fusion protein which consisted of a truncated β -galactosidase fused to a polypeptide encoded by a cDNA clone, KBG8.3. The KBG8.3 cDNA consisted of a conserved sequence of all three *Poa p* IX isoallergens and included the sequence encoded by previously reported KBG7.2 [13]. This antiserum was shown to react with three components of KBG pollen [15]. In this study, this antiserum was used to establish that *Poa p* IX-related proteins are present in

eleven different grass pollens. Furthermore, immunochemical studies utilizing the above antiserum, the pooled sera of allergic individuals and the purified rKBG8.3 have demonstrated that *Poa p* IX-corresponding allergens in some but not all grass pollens are similar with respect to their antigenic and allergenic epitopes.

Materials and Methods

Preparation of the Recombinant Allergen rKBG8.3

The cDNA insert of KBG8.3 was isolated from the purified recombinant λ gt11 phage DNA and was ligated to the pWR590.1 vector [18] and transformed into *E. coli* strain JM105. The transformed cells were plated onto LB-agar plate containing ampicillin, and the transformants were screened with a pool of 11 sera of patients allergic to grass pollens as described earlier [13, 17]. One of the positive colonies was grown overnight at 37°C. The cells were then harvested and lysed by adding 2× sodium dodecyl sulfate (SDS) sample buffer (0.125 M Tris-HCl, pH 6.8, 4% SDS, 10% glycerol, 2% β -mercaptoethanol) and boiling for 5 min. The solubilized proteins were electrophoresed on a SDS-polyacrylamide gel electrophoresis (SDS-PAGE) slab gel and a part of the gel was stained with Coomassie brilliant blue (Bio-Rad, Mississauga, Ont.) to localize the fusion protein; the corresponding area of the unstained gel was cut out and the protein was eluted in 50 mM Tris-HCl buffer pH 9.5 [19]. The protein was precipitated by adjusting the pH to 7.0 by addition of acetic acid and stored at -20°C.

Murine Antibodies to rKBG8.3 Allergenic Polypeptide

BDF1 mice, female 6–8 weeks old (Jackson Laboratories, Bar Harbor, Mich.), were immunized with rKBG8.3 protein in dextran sulfate (Sigma) as adjuvant. Each mouse was primed with 5 μ g recombinant protein and 2.5 mg dextran sulfate. The mice were boosted 23 days later with the same dose of the protein and 1 mg of dextran sulfate; 7 days later they were bled and their sera were stored at -20°C. Murine antibodies to the crude extract of KBG pollen and β -galactosidase were also produced by the same method.

Preparation of Grass Pollen Proteins

The proteins of 13 different grass pollens (Kentucky blue, Bermuda, Canary, Orchard, Redtop, Smooth brome, Tall oat, perennial Rye, Timothy, Colonial bent, Quack, Johnson, and Reed canary, Hollister-Stier Laboratory, Mississauga, Ont.) were extracted with distilled water at 4°C overnight [20]. The protein concentration of each extract was determined by Lowry's method [21].

SDS-PAGE and Immunodetection

SDS-PAGE was performed on a vertical slab gel apparatus using a 15% polyacrylamide gel according to the method of Laemmli [22]. The molecular weights of the components were estimated with reference to the mobilities of prestained protein standards (Bio-Rad Laboratories, Mississauga, Ont.). The separated proteins were either stained with Coomassie blue or electroblotted onto nitrocellulose membrane. The membrane was then probed with a 4,000-fold diluted murine antiserum to the rKBG8.3 poly-

peptide, followed by incubation with a conjugate of goat antibodies to murine immunoglobulins and alkaline phosphatase (Zymed, South San Francisco, Calif.) [23].

Enzyme-Linked Immunoassay

The enzyme-linked immunoassay (ELISA) used for the determination of the specificities of antiserum to rKBG8.3 and to KBG pollen extract is described below. One microgram of KBG extract in 100 μ l of carbonate/bicarbonate buffer, pH 9.6, was coated onto each well of a Nunc-immuno plate overnight at room temperature. After washing 3 times with PBS-tween buffer, the plate was blocked with 1% gelatin in PBS for 2 h at 37°C followed by addition of serially diluted murine antiserum to rKBG8.3, to β -galactosidase, to KBG extract, or normal mouse serum (a pool of 3 mice). The bound antibodies were detected by adding the alkaline phosphatase-conjugated goat antimouse IgG (Zymed) at 1/1,000 dilution and the enzyme substrate *p*-nitrophenyl phosphate in diethanolamine buffer. The ability of antisera to rKBG8.3 of 13 different grass pollen extracts was examined essentially as described above except that the plates were coated in turn with each different pollen extract, and the antiserum to rKBG8.3 was used at a dilution of 1:2,000.

The allergenic cross-reactivities between the different grass pollens was evaluated by ELISA utilizing a pool of sera of humans allergic to grass pollens [17]. Briefly, 1 μ g protein in 100 μ l of carbonate/bicarbonate buffer of each of the 13 grass pollen extracts was coated onto Nunc-immuno plates by overnight incubation at room temperature. The plates were then washed 3 times with PBS-tween buffer, blocked with 1% gelatin in PBS, and then incubated with the pool of allergic sera overnight at room temperature. Bound IgE antibodies were detected with alkaline phosphatase-conjugated goat antihuman IgE (Tago, Calif.) and its substrate *p*-nitrophenyl phosphate. The absorbance was read at 405 nm using a microplate reader (Dynatech). The reactivity of the KBG allergen-specific antibodies with other grass pollen extracts was calculated as percent relative binding = binding to heterologous pollen extract/binding to KBG extract $\times$ 100 according to a previously described method [9].

Immunoinhibition

Antigenic cross-reactivity of rKBG8.3 with different grass pollen allergens was analyzed by competitive binding to plate-bound pollen proteins of individual grasses. A Nunc-immuno plate was coated with pollen proteins of each grass pollen, each well containing 1 μ g of protein in 100 μ l of carbonate/bicarbonate buffer, at room temperature overnight. After washing and blocking with 1% gelatin in PBS, 3 of the 6 wells were incubated with the pooled allergic human serum in absence of inhibitor, the other 3 with the same pooled serum but in presence of KBG pollen protein or the serum absorbed with rKBG8.3.

KBG pollen protein (1 mg/ml) was used in solution phase for the inhibition assay, whereas for the inhibition assay involving rKBG8.3 the serum was first absorbed with rKBG8.3 (0.5 mg/ml), centrifuged at 10,000 rpm for 10 min to remove the insoluble rKBG8.3 antibody complexes and diluted 80-fold prior to its use in ELISA. These assays were performed in triplicates. After overnight incubation at room temperature, the plate was washed 3 times. The IgE bound was detected with alkaline phosphatase-conjugated goat anti-human IgE (Tago) and its substrate *p*-nitrophenyl phosphate. The absorbance was read at 405 nm. The re-

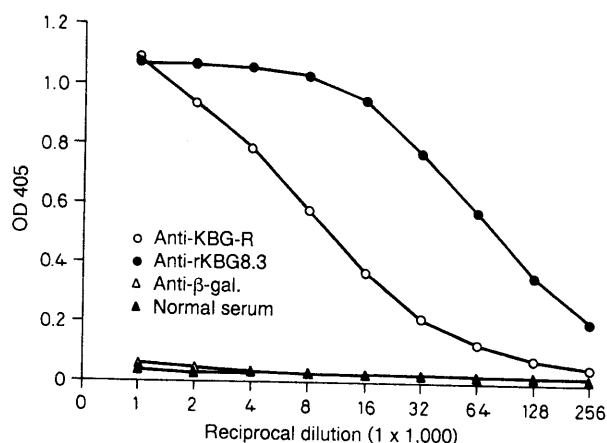


Fig. 1. Nonoverlapping specificity between antibodies to KBG extract (KBG-R) and β -galactosidase (β -gal.) demonstrated by ELISA. 1 μ g of KBG extract was used for coating each well. Four murine antisera as indicated with serial dilutions were tested for their reactivity with KBG extract. Antibodies to both KBG extract and rKBG8.3 strongly reacted with the coating antigen, but antibodies to β -galactosidase and the normal murine serum did not exhibit any reactivity with the coating antigen.

sults were expressed as a percentage of inhibition, calculated as follows: $[1 - (\text{OD in presence of inhibitor} / \text{OD in absence of inhibitor})] \times 100$.

Results

The specificity of the murine anti-rKBG8.3 antibodies was analyzed by both SDS-PAGE immunoblotting assay and ELISA using proteins of KBG, short ragweed pollens, and β -galactosidase as antigens. The antibodies recognized KBG proteins and β -galactosidase but not short ragweed proteins [data not shown]. Since the rKBG8.3 allergen expressed by *E. coli* was associated with a truncated β -galactosidase protein, the antibodies raised against this fusion protein were directed not only to the recombinant KBG polypeptide but also to β -galactosidase protein. The possibility of cross-reactivity between KBG proteins and β -galactosidase was examined by an ELISA assay (fig. 1). The antibodies to both KBG proteins and rKBG8.3 reacted with the solid-phase KBG proteins, whereas there was no detectable reaction of β -galactosidase antibodies as well as the normal mouse serum pool.

An electrophoretogram of the SDS-PAGE gel which was stained with Coomassie Blue is shown in figure 2. One 28-kDa component and a very faint 32-

kDa component were detected in protein extract of KBG pollen as well as protein extracts of 12 other grass pollens. Antiserum to rKBG8.3 was employed as a probe to examine its ability to recognize the cross-reactive components in the 13 grass pollen protein extracts (fig. 2). Three components with molecular mass of 28, 32 and 34 kDa were detected in KBG pollen extract. Except for the Bermuda and Johnson grass pollen extracts, all other 11 grass pollen extracts possessed components recognized by the anti-rKBG8.3 antiserum. Moreover, 7 out of 11 grass pollens examined possessed multiple components which reacted with the above antiserum. The components ranged in molecular weight from 20 to 38 kDa. In addition, a high molecular weight component of about 88 kDa was detected in the Smooth brome and Canary extracts (table 1). These results indicate that components corresponding to rKBG8.3 exist in at least 10 other grass pollens.

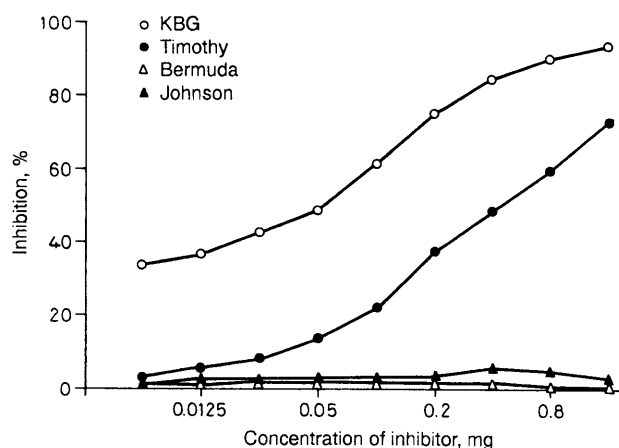
To rule out the possibility that some antigens which may bind poorly to nitrocellulose membrane and therefore escape detection in the western blotting assay, we performed a cross-inhibition experiment. Four grass pollen extracts were chosen to inhibit the binding of rKBG8.3-antibodies to solid-phase KBG proteins (fig. 3). Pollen proteins of Bermuda and Johnson grasses which lacked reactivity with the anti-rKBG8.3 antiserum in western blots had no inhibiting effect. Of the two grass pollen extracts which had exhibited the highest reactivity with the antiserum to rKBG8.3, Timothy grass pollen extract inhibited to a large extent the binding ability of the solid phase of KBG extract. However, the inhibition was lower than that seen using KBG protein extract itself. It is inferred from these studies that the proteins recognized by anti-rKBG8.3 are present in variable quantities in 10 other grass pollens.

The cross-reactivities among the grass pollen components were expressed as relative bindings using KBG pollen proteins as standard and the results are shown in figure 4a and b. The pool of sera of patients allergic to KBG, which were selected on the basis of their reactivity with rKBG8.3, contained a variety of specificities of IgE antibodies with the other 12 grass pollens (fig. 4a). On the other hand, anti-rKBG8.3 antiserum showed relatively restricted specificities in its reaction with different grass pollen extracts (fig. 4b). Thus, the pollen extracts of Orchard, Timothy, Canary, Smooth brome and Tall oat grasses showed higher reactivity than Quack, Rye, Colonial bent and Red-

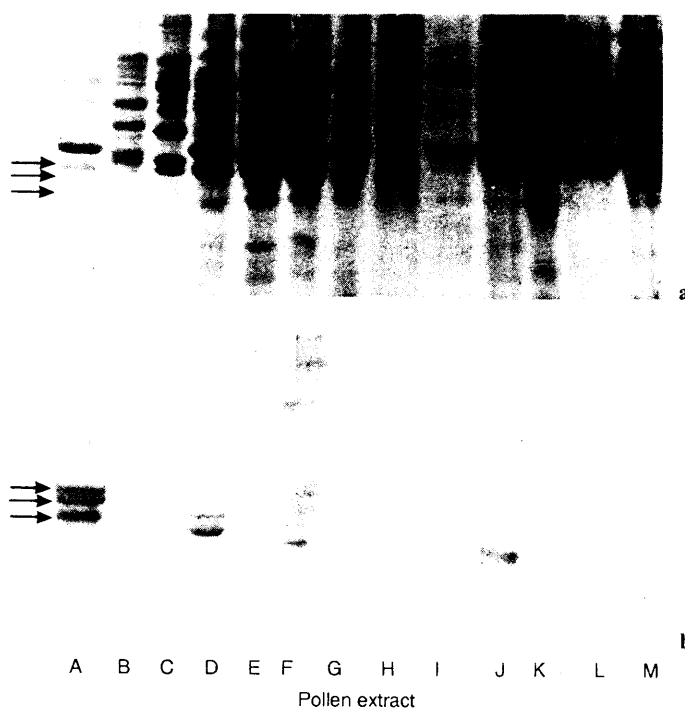
Table 1. Summary of components recognized by anti-rKBG8.3 antiserum in different grass pollens

Code	Grass pollen	Molecular sizes (kDa)
A	KBG	28.0 ^a , 29.0, 32.0, 34.0
B	Bermuda grass	ND
C	Canary grass	29.4 ^a , 35.1 ^a , 88 ^a
D	Orchard grass	24.2, 28.6, 32.9 ^a
E	Redtop grass	22.0 ^a
F	Smooth grass	several bands above 22.0
G	Tall oat grass	27–32 (several bands)
H	Ryegrass	28.0 ^a , 36 ^a
I	Timothy grass	31–38 (several bands)
J	Colonial grass	19.8, 28
K	Quack grass	28.6
L	Johnson grass	ND
M	Reed Canary grass	27.7 ^a

ND = Not detectable.

^a Bands of faint color reaction.**Fig. 3.** Cross-inhibition of anti-rKBG8.3 antibodies to KBG extract. Serial concentration of 4 grass pollen extracts in solution phase was employed to determine their ability to inhibit binding of rKBG8.3-antibodies to KBG extract in ELISA assay. KBG extract was coated onto ELISA plate with 1 µg well. Each point represents the mean of three experimental values.

top grasses, whereas the extracts of Bermuda and Johnson grass pollens exhibited no reactivity whatsoever. Furthermore, the percent relative binding determined for each grass using allergic human sera showed a significant correlation ($r = 0.891$) with that using anti-rKBG8.3 antibodies. These results indicate that *Poa p* IX cross-reactive components in different grass pollen extracts vary in their specificities.

**Fig. 2.** SDS-PAGE analysis and immunodetection of Group IX proteins in 13 grass pollen extracts. (a) Protein extracts were electrophoresed on 15% polyacrylamide gel under reduced conditions. The bands were visualized by staining with Coomassie Brilliant Blue. The arrows indicate the positions of the Group IX allergens in KBG. The lanes containing protein extracts from different grass pollens are marked with letters as: Kentucky Blue (A), Bermuda (B), Canary (C), Orchard (D), Redtop (E), Smooth brome (F), Tall oat (G), Rye (H), Timothy (I), Colonial (J), Quack (K), Johnson (L). Canary Reed (M). (b) The proteins were separated on SDS-PAGE gel and electroblotted onto nitrocellulose membrane. The membrane was probed with anti-rKBG8.3 antibodies, followed by incubation with enzyme-conjugated second antibody and its substrate.

The inhibition assay was employed to assess the relative capacities of the KBG extract and the rKBG8.3 to inhibit the binding of human IgE to different grass pollens. The results in figure 5a showed that KBG extract blocked most IgE binding to Canary, Orchard, Smooth brome, Tall oat, Rye and Timothy; relatively low inhibition of binding to Quack, Colonial and Redtop; and little or no inhibition to Johnson and Bermuda grass extracts. The inhibition profile of rKBG.3 had a similar pattern as the KBG extract (fig. 5b). Moreover, there was a significant correlation ($r = 0.901$) between the KBG extract and rKBG8.3 with respect to their inhibition of the bind-

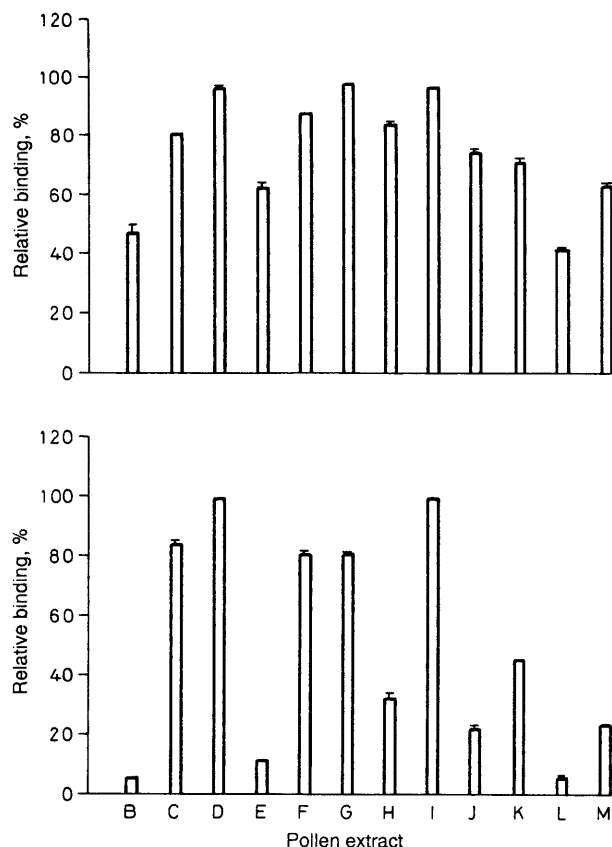


Fig. 4. Binding of a KBG-allergic human serum pool and anti-rKBG8.3 antibodies to different grass pollen extracts. Similarities of allergenicity and antigenicity between KBG pollen and the other 12 grass pollens were measured as relative bindings, in which OD values of each grass pollen were compared with that of KBG extract in ELISA assay. The grass pollens in both panels are marked as in figure 1.

ing of human IgE antibodies to allergens in grass pollen extracts. These studies confirm the results of relative binding studies and suggest that purified allergens can be readily employed in studies of allergenic and antigenic cross-reactivities.

Discussion

Cloning and expression of allergens with recombinant DNA techniques have greatly facilitated their identification, characterization and analysis of the epitopes. In the present study, we have shown the application of recombinant allergens to the study of cross-reactivities among grass pollen components by employing (1) the purified recombinant allergen

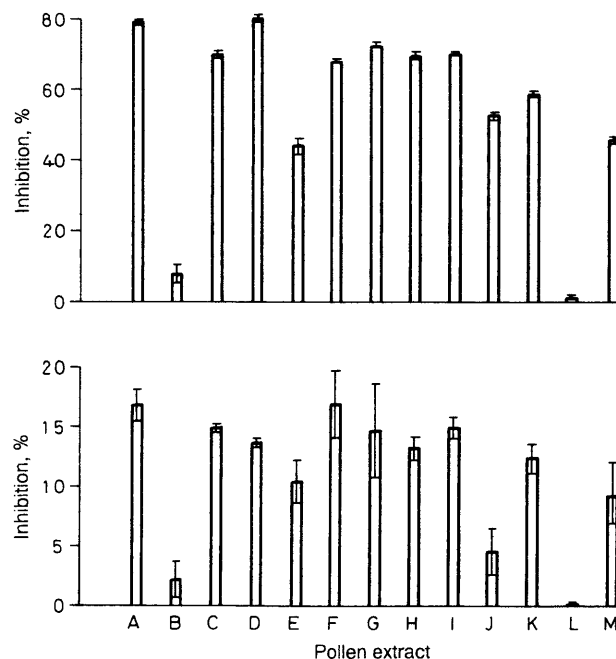


Fig. 5. Immunoinhibition of human IgE binding. The bindings of human IgE to 13 grass pollens marked as in figure 1 were inhibited with both KBG extract and purified rKBG8.3 polypeptide. The former was used in the solution phase to compete the IgE bound to the fixed antigens, and the latter was first used to directly absorb the IgE antibodies from the serum. Both profiles were obtained by ELISA assay.

rKBG8.3 and allergic human sera for the analysis of cross-reactivities among allergenic components, and (2) a murine monospecific polyclonal antiserum to the above recombinant allergen for the detection of cross-reactive antigenic components.

Previously, several investigators have employed monoclonal antibodies and demonstrated that cross-reactive allergens, which are similar in molecular weight, exist among different grass pollens [11, 12, 24, 25]. By contrast, immunodetection of allergens in 11 different grass pollens with antibodies to rKBG8.3 revealed that the cross-reacting allergens ranged in molecular weight from 20 to 88 kDa, although in most of the grasses, the components were in the range of 30–38 kDa. On the other hand, Bermuda and Johnson grass pollens clearly lacked the *Poa p* IX-like compo-

nents. These results are in agreement with previous findings [1, 3] which have indicated that these latter two grasses appear to possess allergenic molecules distinct from other grasses.

The observation that the antibodies to rKBG8.3 recognized several components in some of the grass pollen extracts is consistent with our previous report that *Poa p* IX was comprised of a group of at least three isoallergenic proteins [15]. The different degree to which the individual components were recognized suggest that the density of epitopes in the individual components or the amount of each of the components may vary from one grass pollen to another. Furthermore, it appeared that diversity of epitopes in the molecules played a major role in the degree of staining on the nitrocellulose membrane since the 34-kDa component in KBG pollen showed strong reaction even if the amount of the protein in the preparation was extremely low. This was also supported by the results of cross inhibition since the highly reactive Timothy extract could not totally abolish the reaction with KBG extract.

The relationship of 12 different grass pollens to KBG in terms of allergenicity and antigenicity was analyzed with relative binding to an allergic human serum pool and anti-rKBG8.3 antibodies. Clearly, the serum pool contained IgE antibodies to all grass pollens examined. This observation is consistent with our previous finding of a strong positive correlation in levels of IgE antibodies corresponding to *Poa p* IX allergenic peptide and a mixture of 10 grass pollen extracts [17]. However, the reason for this reactivity, i.e. whether the binding results from IgE antibodies produced as a consequence of contact of human to all grass pollens or due to cross-reactivity, is unknown. Moreover, the basis for the observed differences in reactivity of 12 grass pollens to rKBG8.3 antibodies is unclear. This may be attributed to the geographical distribution of these grasses, or to the similarities in the structures of their allergenic proteins. It is to be noted that the antigenic relationships of these proteins in different grasses did not necessarily agree with their relationships established on the basis of taxonomic classification. For example, Timothy and Redtop grasses both belong to tribe Agrostideae, but Timothy exhibited a higher reactivity with rKBG8.3 than Redtop. Similarly, Reed canary, Ryegrass and KBG although they belong to the same tribe Festuceae, Reed canary and Ryegrass showed weak reactivity with the anti-rKBG8.3 antibodies. This suggests

that for each newly cloned allergen the distribution of its epitopes has to be confirmed among other grass components by similar studies as reported here.

In conclusion, this study has clearly pointed out that allergenic and antigenic cross-reactivities widely exist in grass pollens with *Poa p* IX proteins. These results in conjunction with our previous studies of the allergenic properties of recombinant *Poa p* IX allergens [17] indicate that the group IX proteins constitute a major group of allergenic components of several grass pollens. Furthermore, the correlations of reactivities of grass pollen extracts with human allergic serum versus anti-rKBG8.3 antiserum suggest that, in general, the epitopes may bind to both IgE and IgG antibodies. However, it is to be pointed out that whether an individual epitope would preferentially bind or recognize the IgE and/or IgG antibodies remains unclear. Work in progress is aimed at determining the epitopes of the rKBG8.3 [25] and other cloned polypeptides of *Poa p* IX allergens. The knowledge of epitopes is expected to facilitate the understanding of the molecular and structural basis of allergenic and antigenic cross-reactivities among different grass pollen components.

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References

- 1 Martin BG, Mansfield LE, Nelson HS: Cross allergenicity among the grasses. *Ann Allergy* 1985;54:99.
- 2 Bernstein IL, Perera MM, Gallagher J, Johansson SGO: In vitro cross-allergenicity of major aeroallergenic pollens by the radioallergosorbent technique. *J Allergy Clin Immunol* 1976; 57:141.
- 3 Leiferman KM, Gleich GH: The cross-reactivity of IgE antibodies with pollen allergens. I. Analysis of various species of grass pollen. *J Allergy Clin Immunol* 1976;58:129.
- 4 Marsh DG, Haddad ZH, Campbell DH: A new method for determining the distribution of allergenic fractions in biological materials: Its application to grass pollen extracts. *J Allergy* 1970;46:107.
- 5 Feinberg JG: Immunological inadequacy of randomly selected grass pollen extracts in specific hay fever therapy. *Int Arch Allergy* 1960;16:1.
- 6 Augustin R: Grass pollen allergens. II. Antigen-antibody preci-

- pitiation patterns in gel: Their interpretation as a serological problem in relation to skin reactivity. *Immunology* 1959;2:148.
- 7 Coca AF, Grove E: Studies in hypersensitiveness. XIV. A study of atopic reagents. *J Immunol* 1925;10:445.
 - 8 Rackemann FM, Wagner HC: The desensitization of skin sites passively sensitized with serum of patients with hay fever. Crossed reactions of different pollens. The variation in the recipient. *J Allergy* 1936;7:319.
 - 9 Esch RE, Klapper DG: Identification and localization of allergenic determinants on grass group I antigens using monoclonal antibodies. *J Immunol* 1989;142:179.
 - 10 Mourad W, Mecheri S, Pelter G, David B, Hebert J: Study of the epitope structure of purified *Dac G I* and *Lol p I*, the major allergens of *Dactylis glomerata* and *Lolium perenne* pollens, using monoclonal antibodies. *J Immunol* 1988;141:3486.
 - 11 Kahn CR, Marsh DG: Monoclonal antibodies to the major *Lolium perenne* (Rye grass) pollen allergen *Lol p I* (Rye I). *Mol Immunol* 1986;23:1281.
 - 12 Singh MB, Knox RB: Grass pollen allergens: Antigenic relationship detected using monoclonal antibodies and dot blotting immunoassay. *Int Arch Allergy Appl Immunol* 1985;78:300.
 - 13 Mohapatra SS, Hill R, Astwood J, Ekramoddoullah AKM, Olsen E, Hatton T, Kisil FT, Sehon AH: Isolation and characterization of a cDNA clone encoding an IgE binding protein from Kentucky bluegrass (*Poa pratensis*) pollen. *Int Arch Allergy Appl Immunol* 1990;91:362.
 - 14 Mohapatra SS, Hill RD, Sehon AH: Molecular cloning of grass pollen allergens: Progress and perspectives. *Aerobiologia* 1990;6:205.
 - 15 Silvanovich A, Astwood J, Zhang L, Olsen E, Kisil F, Sehon A, Mohapatra S, Hill R: Nucleotide sequence analysis of three cDNAs for *Poa p IX* isoallergens of Kentucky bluegrass pollen. *J Biol Chem* 1991;266:1204.
 - 16 Olsen E, Zhang L, Hill RD, Kisil FT, Sehon AH, Mohapatra SS: Identification and characterization of the *Poa p IX* group of basic allergens of Kentucky bluegrass pollen. *J Immunol* 1991;147:205.
 - 17 Yang M, Olsen E, Dolovich J, Sehon A, Mohapatra S: Immunologic characterization of a recombinant Kentucky bluegrass (*Poa pratensis*) allergenic peptide. *J Allergy Clin Immunol* 1991;87:1096.
 - 18 Guo L, Stepien PP, Tso JY, Brousseau R, Narang S, Thomas DY, Wu R: Synthesis of human insulin gene. VIII. Construction of expression vectors for fused proinsulin production in *Escherichia coli*. *Gene* 1984;29:251.
 - 19 Carroll SB, Laughon A: Production and purification of polyclonal antibodies to the foreign segment of β -galactosidase fusion protein; in Glover D (ed): *DNA Cloning*. Oxford IRL, 1987, vol 3, p 89.
 - 20 Ekramoddoullah AKM, Chakrabarty S, Kisil FT, Sehon AH: Isolation of a purified allergen from Kentucky bluegrass pollen. *Int Arch Allergy Appl Immunol* 1980;63:220.
 - 21 Lowry OH, Rosebrough NH, Farr AL, Randall RJ: Protein measurement with Folin phenol reagent. *J Biol Chem* 1951;193:265.
 - 22 Laemmli UK: Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 1970;227:680.
 - 23 Towbin H, Staehelin T, Gordon J: Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: Procedure and application. *Proc Natl Acad Sci USA* 1979;76:4350.
 - 24 Standring R, Spacman V, Porter SJ: Distribution of a major allergen of rye grass (*Lolium perenne*) pollen between other grass species. *Int Arch Allergy Appl Immunol* 1987;83:96.
 - 25 Zhang L, Kisil FT, Sehon H, Mohapatra SS: Cross-reactivity and IgE binding of recombinant *Poa p IX* peptides (abstract). *J Allergy Clin Immunol* 1991;87:326.

Note Added in Proof

The serum pool used for the assay of allergenic cross-reactivities comprised a pool of 11 individuals who were allergic to grass pollens as determined by (i) skin-prick test, and (ii) the presence of specific IgE antibodies by ELISA. Differences between the cross-reactivity pattern of individual serum was not examined in this study.

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