

IDENTIFICATION AND CHARACTERIZATION OF THE *Poa p IX* GROUP OF BASIC ALLERGENS OF KENTUCKY BLUEGRASS POLLEN¹

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We reported previously the primary structure of three full-length cDNA clones that encode a new group of IgE-binding proteins of Kentucky bluegrass (KBG) pollen, designated as *Poa p IX*. In the present study we have further characterized the cloned *Poa p IX* proteins, identified the corresponding proteins in KBG pollen extract, and determined their antigenic relationships with other known grass pollen allergens. A recombinant IgE-binding polypeptide rKBG7.2 that represents the C-terminal fragment, conserved in *Poa p IX* proteins, appeared to contain epitopes unique to these proteins and served as an immunosorbent for the isolation of the corresponding human IgE antibodies. On two-dimensional PAGE blots these IgE antibodies bound selectively to five distinct KBG pollen proteins with molecular mass 28 to 34 kDa and isoelectric point > 9.5. These proteins differ in size and charge from known allergens, but are very similar to those of the recombinant *Poa p IX* proteins. The rKBG3.1, which represents the N-terminal region of the *Poa p IX* clone KBG31, as well as the corresponding natural allergens were shown to possess epitopes that crossreact with the acidic group V allergens of Timothy. Comparison of amino acid sequences of recombinant *Poa p IX* proteins with those of *Lol p I* isoallergens revealed no significant sequence similarities. In contrast, partial homology was demonstrated between the N-terminal sequences of these proteins and the *Phl p V* proteins. Our results confirm that the *Poa p IX* clones represent a distinct and major group of allergens of KBG pollen, and demonstrate structural similarities and antigenic cross-reactivities among different groups of allergenic proteins in grass pollens.

Knowledge of structural similarities and immunologic cross-reactivities among protein components of different grass pollens is considered to be of clinical importance.

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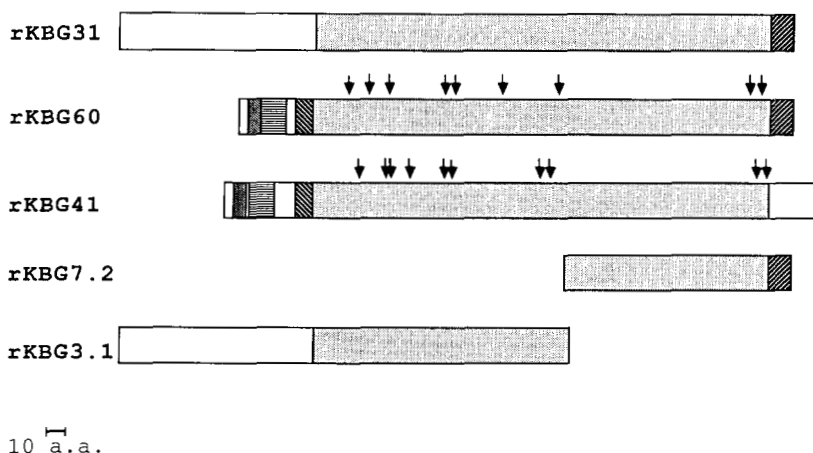
because this may lead to identification of the components that would be useful in development of products for the treatment of allergies caused by these aero-allergens (1). Furthermore, understanding of the interactions between the allergens and the immune system requires characterization of epitopes that interact with 1) antibodies and B cells, 2) the TCR of T cells, and 3) HLA-D on APC. There is a demonstrated correlation between MHC phenotype and the IgE antibody response in humans (2–4) and mice (5) against certain allergens. In both mouse and human systems, IL-4 and INF- γ have been shown to be involved, respectively, in the induction and suppression of IgE antibodies (6, 7). However, the mechanism as to how the allergenic molecules are involved, either directly or indirectly, in the regulation of the specific IgE synthesis remains to be elucidated. Such studies have been limited because most allergens or their fragments have not been readily available in pure form and their primary sequences have not been established.

Grass pollen extracts are complex mixtures of allergenic and nonallergenic proteins, and may consist of up to 14 IgE-binding components (8). Extensive physicochemical studies comprising the determination of the m.w., pI³ and partial or complete amino acid sequences of these proteins have led to their categorization into nine groups of grass pollen allergens (9–22) in accordance with the IUIS Allergen Nomenclature system (23). The complete amino acid sequences of two of the above allergens, *Lol p II* (12) and *Lol p III* (13), were determined by sequencing the purified proteins. More recently, the primary structures of *Lol p I* isoallergens were determined by the application of cDNA cloning and sequencing procedures (24).

We have used gene cloning procedures for the isolation and characterization of KBG pollen allergens. An IgE-binding cDNA clone, KBG7.2, was isolated and the corresponding recombinant polypeptide (rKBG7.2) was synthesized in *Escherichia coli* (25–27). Furthermore, three homologous full length cDNA, KBG31, KBG41, and KBG60, were isolated and sequenced (26, 28, 29). The KBG7.2 encompasses the most conserved domain of these clones (Fig. 1). On the basis of 1) the minor sequence homologies of the deduced proteins, which have molecular mass in the range of 28.3 to 34.9 kDa and pI of 9.7 to 10.0, with other grass pollen allergens, and 2) the immunologic characterization of the rKBG7.2 (30), the corresponding KBG pollen proteins were designated

³ Abbreviations used in this paper: pI, isoelectric point; KBG, Kentucky bluegrass; TBS, Tris-buffered saline; TBST, TBS added 0.05% Tween 20; 2D, two-dimensional; AP, alkaline phosphatase.

Figure 1. Comparison (schematic) of the deduced primary amino acid sequences of the full length cDNA clones KBG31, KBG60, and KBG41 and of the partial clones KBG7.2 and KBG3.1. Each differently shaded domain represents a sequence present in two or more of the molecules. Blank domains represent non-homologous sequences. The arrows represent single amino acid substitutions.



as *Poa p IX*. In the present study, the recombinant and natural *Poa p IX* proteins have been further characterized in terms of their allergenic and antigenic properties, from which it is concluded that *Poa p IX* proteins represent a major group of allergens of KBG pollen.

MATERIALS AND METHODS

Human sera. A total of 55 individual sera from patients in Norway was kindly provided by Nycomed AllergenLab, Oslo, Norway. These patients had a history of allergy to grass pollen, and serum with IgE RAST g6 (Timothy) score 4, and high IgE EIA titre against KBG and Rye grass.

mAb. The mAb PpV1 and PpV4 [31], raised against group V allergens of Timothy, *Phl p V*, were kindly provided by Drs. F. Matthiesen and H. Lowenstein of ALK Research, Horsholm, Denmark. The mAb 61 [32] and mAb 90 [33] which are specific for group I and group IV allergens, respectively, were described previously.

Preparation of KBG pollen extracts. A 1% (w/v) suspension of pollen (*Poa pratensis*, Hollister-Stier, Ontario, Canada) in TBS containing 0.01% NaN_3 and 1 mM PMSF was incubated with shaking overnight at 4°C. Pollen grains were removed by filtration (Whatman 3MM, Clifton, NJ), and the pollen extract was further concentrated 10× by ultrafiltration (YM5, Amicon, MA) and stored in aliquots at -20°C. The protein concentration was determined by the Bio-Rad (Richmond, CA) protein assay.

Synthesis and purification of recombinant polypeptides. rDNA techniques were performed as described previously [28]. The two cDNA clones, KBG3.1 and KBG7.2, were synthesized by using expression vector pWR 590-1 [34] in *E. coli* JM 105. The polypeptides were expressed as fusion proteins and isolated from inclusion bodies of the bacterial lysate and further purified by SDS-PAGE. The band containing fusion protein was excised and the protein was eluted with 0.01 M Tris-HCl pH 7.5 containing 0.1% SDS and 3 M urea or with 6 M guanidine-HCl, pH 7.5/1 mM PMSF. Protein concentration was estimated by dot-blotting diluted samples onto nitrocellulose membrane (Schleicher & Schuell, Keene, NH, 0.45 μm), followed by staining with 0.1% amido black in isopropanol/acetic acid (25%/10%). BSA diluted in the same solvent served as the protein standard.

Absorption of human serum with recombinant polypeptide and recovery of polypeptide-specific antibodies. A total of 10 μl of purified fusion proteins (10 mg/ml) in 6 M guanidine-HCl, pH 7.5, with 1 mM PMSF was dot-blotted onto nitrocellulose membrane discs of 14 mm in diameter. After drying for 20 min at room temperature, the discs were blocked with 1% gelatin in TBS for 6 h at room temperature, washed 6×, 5 min each time in TBST, and were incubated with 1 ml of human serum diluted 1/15 in TBST with 0.1% gelatin overnight at room temperature. After removal of the serum, the discs were washed 6× (5 min each time) in TBST and the bound antibodies were eluted by adding 200 μl 0.1 M glycine-HCl buffer, pH 2.5, containing 0.5% BSA. This step was repeated 5× and the eluates were immediately dialysed against PBS/ NaN_3 . Both the absorbed serum and the dialysed eluates were stored at 4°C.

SDS- and 2D-PAGE. SDS-PAGE was performed in a discontinuous system according to Laemmli [35] in a Fisher mini slab gel apparatus. Samples were prepared in loading buffer; 0.06 M Tris-HCl buffer, pH 6.8, containing 2% SDS and 5% 2-ME. 2D-PAGE was performed in Protean Minicell (Bio-Rad, Richmond, CA) according to the manufacturer's instructions. Nine μg of pollen protein were applied per

gel. The gels were either stained in Coomassie brilliant blue or directly electroblotted onto nitrocellulose. The pH gradient of the first dimension gel was measured by eluting the ampholines from a parallel gel. Western blotting was performed by PolyBlot (American Bionetics, Inc., Hayward, CA) using a discontinuous buffer system as described by the manufacturer. The membranes were subsequently blocked by incubation in TBS/1% gelatin for at least 6 h, and then incubated overnight with mAbs (1 $\mu\text{g}/\text{ml}$) or human IgE-containing serum diluted 1/100 in TBST/0.1% gelatin. Antibody binding was detected by incubation for 2 h with AP-conjugated goat anti-mouse Ig (Zymed, South San Francisco, CA) diluted 1000-fold or AP-conjugated goat anti-human IgE (Tago Inc., Burlingame, CA), 2000-fold diluted in TBST with 0.1% gelatin. The membranes were washed five times for 4 min in TBST between each incubation step, and nitroblue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate were used as the enzyme substrates.

Enzyme immunoassay in microtiter plates. The purified fusion protein, rKBG7.2 (10 $\mu\text{g}/\text{ml}$ solubilized in either 7 M guanidine-HCl or 0.01 M Tris-HCl, pH 7.5, containing 0.1% SDS and 3 M urea) was applied overnight onto microtiter plates (Nunc, Copenhagen, Denmark). Coating with all other proteins was performed in TBS with 10 μg protein/ml overnight. After washing five times with TBS, the wells were blocked by TBS/1% gelatin for 3 h. Subsequent to washing with TBST the wells were incubated with human serum diluted with TBST/0.1% gelatin overnight. After washing with TBST, AP-conjugated secondary antibodies (goat anti-human IgE (Tago), diluted 3000-fold) were added to the wells and were incubated for 3 h. When mAb were used as primary antibodies they were incubated with the solid phase for 3 h, and the secondary antibody, AP-conjugated goat anti-mouse Ig (Zymed) diluted 1000-fold, for 2 h. After washing with TBST the color reaction was developed by adding para-nitro phenyl phosphate, 1 mg/ml Tris-HCl buffer containing NaCl and MgCl_2 , and measured at 405 nm in a Titertek Multiscan spectrophotometer (Flow Laboratories, McLean, VA). All washing steps were performed with the Nunc "Elisa hand washer" and all incubation steps were performed at room temperature.

RESULTS

Allergenic and antigenic properties of *Poa p IX* clone KBG31. To investigate further the allergenic and antigenic properties of the previously cloned *Poa p IX* allergens [30], the two partial clones, KBG3.1 and KBG7.2, which, respectively, represent the N- and C-terminal part of rKBG31 (see Fig. 1), were expressed in *E. coli*. The purified fusion proteins, rKBG3.1 and rKBG7.2, were analyzed by enzyme immunoassay. All sera from 55 grass pollen allergic individuals contained IgE antibodies to rKBG7.2 and more than 90% of them contained also IgE antibodies specific for rKBG3.1 (results not shown). None of the fusion proteins was bound by the murine mAb 61 or mAb 90, which are specific for groups I [32] and IV [33] of grass pollen allergens, respectively. The rKBG3.1 was recognized by both mAbs PpV1 and PpV4 that were raised against group V allergens of Timothy grass pollen [31]. These results indicated that KBG31 encodes a pre-

dominant allergen that possess several IgE-binding epitopes, of which at least one was located in the N-terminal part and another at the C-terminal part of the conserved region of *Poa p* IX. Furthermore, antigenic epitopes cross-reacting with group V allergens were found in the N-terminal part of the molecule. These similarities to group V prompted further investigations to identify and characterize *Poa p* IX proteins in KBG pollen.

Characterization of allergens of KBG pollen and identification of *Poa p* IX proteins. Several IgE-binding components with molecular mass of 55, 36, 34, 32, 29/28, and 12 kDa, were detected by Western immunoblots of KBG pollen extract, using a pool of 13 sera of patients allergic to grass pollen. Whereas seven individual sera selected for their high level of IgE binding to rKBG7.2 and to components in the KBG pollen extract contained IgE antibodies to the 36, 34, 32, and 29/28-kDa components (Fig. 2A), five of these sera recognized the 55-kDa component and only four sera recognized the 12-kDa component. These results indicate that the proteins corresponding to the 36-, 34-, 32-, and 29/28-kDa components are most frequently recognized by human IgE antibodies.

In view of the requirement for well defined human IgE antibodies specific for rKBG7.2, which would be essential for identification of *Poa p* IX allergens in extracts of pollens and the indication that rKBG7.2 possessed unique allergenic and antigenic epitopes, human antibodies were isolated by affinity absorption on immobilized rKBG7.2 from three individual human sera, represented by 60, 89, and 97. After complete depletion of IgE antibodies specific for rKBG7.2 from each of these sera their EIA titers against the KBG pollen extract were reduced by 20 to 30% (results not shown). The three eluates of IgE antibodies from the rKBG7.2 adsorbents, referred to as IgE-rKBG7.2, bound to the 28/29-, 32-, and 34-kDa components but not to the 36- and 55-kDa components in the KBG pollen extract (Fig. 2B). These results indicate that the KBG extract contains several *Poa p* IX-like components with molecular mass in the range of 28 to 34 kDa. Moreover, the lack of reactivity of the eluted IgE antibodies with the major 36- and 55-kDa components, are considered as evidence for the specificity of both the

affinity absorption method yielding IgE antibodies reacting with rKBG7.2, and the assay system.

To further confirm the identity of the allergenic components, the blots of KBG pollen extract were reacted with murine mAb previously shown to react with protein of molecular mass 28 to 36 kDa (Fig. 3). On the basis of reactivity with mAb 90 and mAb 61, the 55- and 36-kDa components were thus shown to belong to groups IV and I allergens, respectively. Furthermore, the 32-kDa component was bound by mAbs 61, PpV1 and PpV4, which indicated the presence of epitopes associated with both group I and group V-like allergens. In addition, mAb PpV1 bound to the 34- and 29-kDa components and PpV4 to the 28- and 29-kDa bands. Taken together, these results clearly show that the proteins with molecular mass in the range of 28 to 36 kDa include both the basic *Poa p* IX and the acidic group I and group V allergens.

2D-electrophoretic examination of allergens in KBG pollen extract. To discriminate between *Poa p* IX allergens from those of groups I and V allergens, the KBG pollen extract was subjected to 2D-PAGE analysis. IEF in the range of pH 4 to 9 and pH 9 to 11 was used to examine both acidic (Fig. 4) and basic (Fig. 5) proteins. The 36-kDa component was further resolved into five isoallergenic components with pI values in the range of 6.0 to 6.6, all of which bound to IgE antibodies in sera of grass sensitive patients and to mAb 61. The 34-kDa component consisted of a single basic protein, 10, with a pI > 9.5, which bound to both IgE-rKBG7.2 and mAb PpV1, but not to mAb PpV4. The 32-kDa component was resolved into three proteins, 11 to 13, all with pI > 9.5, which bound to IgE antibodies of human sera, but only 11 and 12 were recognized by IgE-rKBG7.2 (Fig. 4) and by mAb PpV1 (Fig. 5). Moreover, 11 was also bound to mAb 61 in addition to mAb PpV4 (Fig. 5). The resolution on 2D-PAGE revealed that the 28/29-kDa components consisted of at least three acidic IgE-binding proteins, 6 to 8, of molecular mass 28 kDa, which were bound by mAb PpV1 and PpV4 but not by IgE-rKBG7.2, and four basic IgE-binding proteins, 14 to 17, of which 15 and 17 were bound by IgE-rKBG7.2 (Fig. 4). Protein 15 was also bound by mAbs PpV1 and PpV4 (Fig. 5).

The physicochemical and immunochemical character-

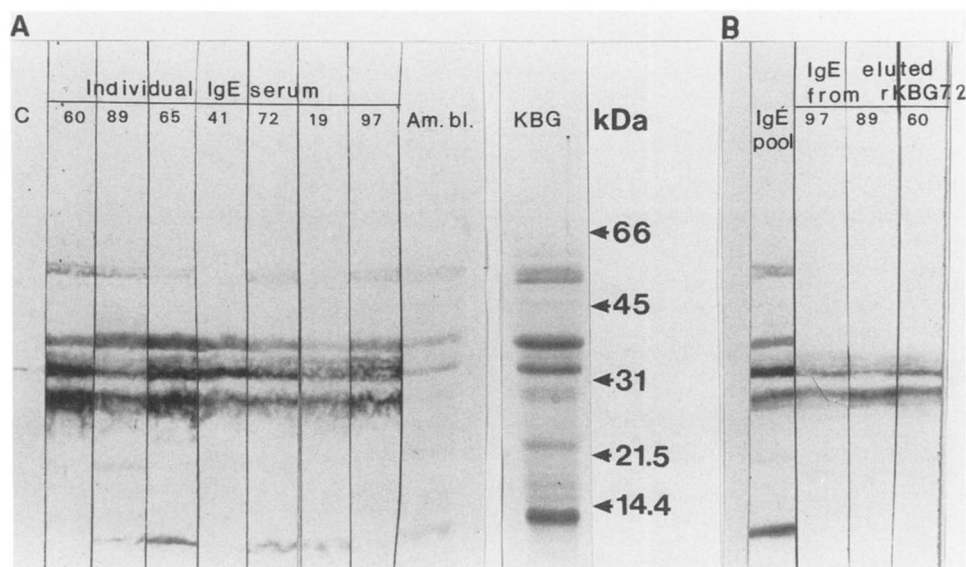


Figure 2. A. Binding of IgE in individual serum from grass pollen allergic patients to proteins in KBG extract, analyzed by Western blotting after separation by SDS-PAGE (15%). A. Except for one strip stained with amido black (*Am. bl.*), the blot was blocked by gelatin and individual strips were subsequently incubated with individual patient serum and developed for IgE antibody binding as described in *Materials and Methods*. B. Strips, as in A, were incubated with IgE specific for rKBG7.2, from individual serum. Binding patterns of IgE-rKBG7.2 were compared to binding of IgE from serum pool and related to protein band pattern of KBG as in A.

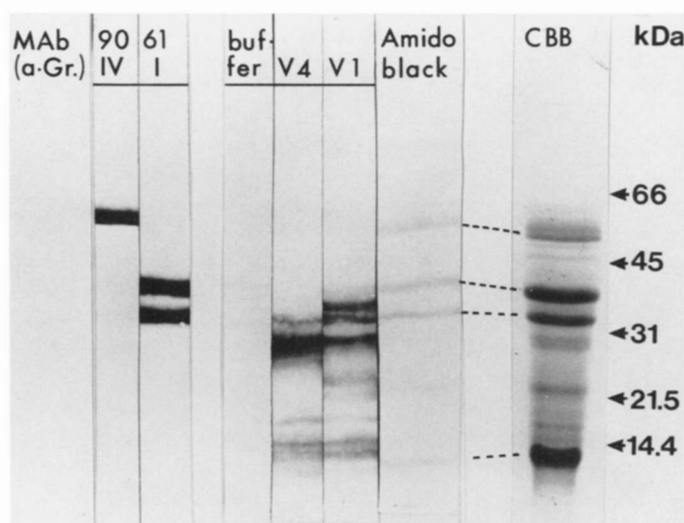


Figure 3. Characterization of KBG extract by Western immunoblot analysis with allergen group specific mAbs followed by AP-conjugated anti-mouse Ig, as described in *Materials and Methods*. The mAb 90 and 61 are specific for *Lol p* IV and *Poa p* I, respectively, whereas mAb V4 and V1 were produced against *Phl p* V. Part of the membrane was stained directly with amido black and compared to Coomassie brilliant blue (CBB) stained gel. Identical bands in the membrane blot and the gel are indicated by broken lines.

istics of the IgE-binding proteins in the KBG pollen extract derived from 2D-immunoblots are summarized in Table I. It is to be noted that both mAbs PpV1 and PpV4 reacted with allergenic components of the KBG pollen extract that had the physicochemical properties of the group V allergens, against which these mAb had been produced (31). Whereas mAb PpV1 recognized both basic and acidic allergens in the KBG pollen extract, the mAb PpV4 preferentially bound to acidic proteins of which some were IgE binding. All these results support the view that *Poa p* IX group allergens consist of five basic components of KBG pollen, and that four of these reacted with mAb(s) to group V allergens and that one of these reacted with a mAb to group I allergen.

Comparison of amino acid sequences of *Poa p* IX with sequences of *Lol p* I and *Phl p* V. The deduced amino acid sequences of the three *Poa p* IX cDNA clones were compared to the recently published complete sequences of isoallergens of *Lol p* I (24). There was no significant homology between any of the *Poa p* IX sequences and the *Lol p* I sequences. Furthermore, comparison of the *Poa p* IX sequences with the revised N-terminal sequence of *Phl p* V (36) revealed significant similarity among these se-

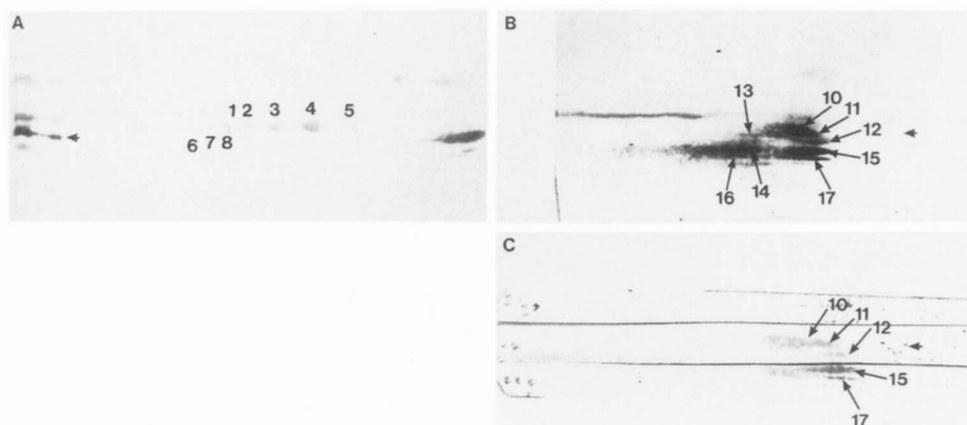
quences when aligned for optimal homology (Fig. 6). In particular a stretch of five amino acids of *Phl p* V sequence was identical to the N-terminal end of the conserved regions (Fig. 1) of *Poa p* IX sequences and the overall homology of the *Phl p* V sequence was of the same order as that seen among the corresponding *Poa p* IX fragments.

This alignment indicated that rKBG60 and *Phl p* V possess the same number of amino acids in front of their homologous pentameric peptide. Inasmuch as the molecular mass calculated for rKBG60, 28.2 kDa, is within the range of group V allergens (31), the possibility existed that the group V proteins of KBG pollen might also share homologies with conserved domain of *Poa p* IX allergens. However, the theoretical pI value calculated for the simulated chimeric protein consisting of the N-terminal peptide of *Phl p* V and the C-terminal homologous region of the *Poa p* IX, was calculated to be 9.7. It is inferred from these sequence analyses that groups IX and V allergens contain sequence homologies in their primary structures. However, they appear to be significantly different from each other with respect to their overall amino acid composition giving rise to their basic and acidic characteristics, respectively.

DISCUSSION

The main purpose of this study was to further characterize the previously cloned *Poa p* IX proteins (29) with respect to their allergenic and antigenic properties and to identify the proteins in the KBG pollen that represent the natural counterparts of these recombinant proteins. Allergenic proteins were previously identified and classified into different groups with the aid of murine mAbs (32, 35–38). By contrast, in this study the *Poa p* IX allergens were identified by cDNA cloning of pollen proteins that reacted with IgE antibodies of grass pollen-sensitive patients (25–30). In this report we present evidence that human IgE antibodies specific to *Poa p* IX proteins could be affinity purified using one of the recombinant allergens as an immunosorbent. The recombinant KBG7.2 was selected for this purpose because 1) it constituted the most conserved domain of *Poa p* IX allergens, and 2) it appeared to contain epitopes unique to this group of proteins. We demonstrated that the natural allergens closely resemble the recombinant *Poa p* IX allergens with respect to their physicochemical properties, i.e., their molecular masses and pI values, and to their antigenic cross-reactivities, particularly with the group V allergens.

Figure 4. Binding pattern of human IgE to KBG pollen proteins analyzed by 2D-PAGE and Western blotting. First dimension IEF with pH gradient 4–9 (A) or 9–11 (B and C) (gradients left to right). Electrobots were blocked by gelatin and were incubated with serum pool, 1:100, from grass pollen allergic patients (A and B) or human antibodies eluted from rKBG7.2 (C). AP-conjugated anti-human IgE was used for the secondary antibodies as described in *Materials and Methods*. The antibody-binding components were numbered arbitrarily. The arrows indicate the position of the 32-kDa band as shown in the pattern of KBG applied in the reference lane.



Molecular Mass ^a (kDa)	Protein No. ^b	pI	IgE-rKBG7.2	Antibodies Used		
				mAb 61	mAb PpV1	mAb PpV4
36	1-5	6.0-6.6	-	+	-	-
34	10	>9.5	+	-	+	-
32	11-13	>9.5	+ ^c	+ ^d	+ ^e	+ ^f
29/28	14-17	>9.5	+	-	+	+ ^f
29/28	6-8	5.2-5.7	-	-	+	+

max 1 p v 4 binds only protein nos. 11 and 18.

61
rKBG31 V K A P P A S K F P A K P A P K V A A Y T P A A F
7
rKBG41 Y G A P A T L A T P A T P A A P A A G Y T P A A F
1
rKBG60 Y A A D L S Y G A P A T P A A P A A G Y T P A A F
1
Phl p V A D A G Y A P A T P A A P G A G Y T P A T P A A F

specific for group I allergens generally identifies protein(s) of similar m.w. in a number of grasses (39–41). In view of the observations that a large number of components including group I (1, 8, 32) and group V (20, 36) allergens with molecular mass in the range of 25 to 36 kDa were detected by SDS-PAGE immunoblotting procedures using sera from allergic individuals, in this study 2D-PAGE immunoblotting with affinity purified human IgE antibodies was used for the identification of *Poa p* IX proteins in KBG pollen extract. The results have clearly revealed that this procedure has greater capacity to resolve the mixture of proteins of a similar size than that of SDS-PAGE alone. In addition to identifying isoallergenic proteins of each of the major groups, this procedure

enabled a detail study of their cross-reactivities using mAb and human IgE antibodies specific for the allergenic polypeptides. Therefore, we are of the opinion that identification of new allergenic proteins based on data obtained by SDS-PAGE and immunoblotting using murine mAb may not be adequate for classification of allergens. Instead we propose that the precise classification of allergens be based on their characteristics involving the determination of their 1) complete amino acid sequence(s), and 2) immunochemical properties revealed by 2D-PAGE blotting not only with murine mAb but also with affinity purified IgE antibodies that state their allergenic properties.

It is to be noted that in addition to the existence of interspecies cross-reactivities of individual allergens among different grass pollens there exist also intraspecies cross-reactivities among allergenic components. The latter point is documented by the results of this study that demonstrate remarkably that both recombinant and natural allergens representing basic *Poa p* IX allergens share some of the antigenic determinants of acidic group V and group I allergens. Thus, all five *Poa p* IX allergens were bound by mAb PpV1, which was shown to react with an epitope located in the N-terminal part of these proteins. However, because one of these allergens (no. 15) was bound by mAb PpV4 only and another (no. 11) by both mAb PpV4 and 61, the epitopes recognized by these two mAb are expected to be located elsewhere in the N-terminal site of these proteins.

Comparison of the deduced amino acid sequences of the recently described *Lol p* I (24) with those of the *Poa p* IX proteins (29) did not show any significant homology, but noncontinuous conformational homologies could not be ruled out. Furthermore, mAb61 recognized one of the *Poa p* IX allergens that seemed comparable to a protein previously referred to as *Poa p* Ib (32). However, since the N-terminal sequence of *Poa p* 1b (unpublished observations) does not match with the N-terminal sequence of the three cloned *Poa p* IX proteins, it is likely that the mAb61 binding basic allergen is encoded by another, yet to be isolated *Poa p* IX cDNA clone. It is noteworthy that the cross-reactivity between group I allergens and proteins of different M_r has been observed previously (41).

Interestingly, there appears to be a striking similarity among some antigenic determinants (as revealed by two mAb) between the basic *Poa p* IX and acidic group V allergens. Consistent with the reactivity of the N-terminal recombinant polypeptide rKBG3.1 with *Phl p* V mAb, a recently revised N-terminal amino acid sequence of *Phl p* V showed similarity to the sequences of *Poa p* IX proteins. We have no proof that this homologous sequence constitutes the recognition site for the mAb PpV1, but this explanation appears plausible. Further information of the amino acid sequence of the group V allergens is expected to reveal the molecular basis for the observed cross-reactivity and the overall similarity between these two groups of allergens. It is, however, to be noted that such cross-reactivities and considerable homologies between basic and acidic proteins is not unprecedented. For instance, acidic *Lol p* II (12) and basic *Lol p* III (13), which have 57 of a total of 97 of their residues identically positioned, possess both common and unique antigenic epitopes.

On the basis of 1) sequence similarities among different

groups of allergenic proteins established in our laboratory (29) and other laboratories (24), and 2) the cross-reactivity studies presented here using human IgE antibodies and mAb, we postulate that the antigenic cross-reactivities may constitute a more common phenomenon within each group of aero-allergens than previously assumed. The molecular basis for the observed cross-reactivities is presently unknown. However, we suggest that this is a reflection of the presence of a limited number of exons initially present in different grasses that encoded immunogenically active protein domains those might have been responsible for binding with antibodies. It has been recently reported that processes resulting in protein diversification, i.e., exon reassortment followed by gene duplication and divergence, sharply restrict the sequence diversity (42). This hypothesis is testable and requires extensive sequencing of genes corresponding to several major allergens.

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