

Recombinant allergens and diagnosis of grass pollen allergy

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To evaluate the use of recombinant allergens for the diagnosis of grass pollen allergies, we examined the levels of IgE antibodies specific for grass pollen as by immunoassays. SDS-PAGE analysis of two batches of Kentucky bluegrass pollen extracts demonstrated that there was considerable variability in allergen content of extracts, which in turn affected quantitation of specific IgE antibodies by different immunoassay procedures. Furthermore, the levels of IgE antibodies in human sera specific for a recombinant grass pollen allergen, rKBG8.3, were examined by enzyme immunoassay. The results demonstrated that quantitation of IgE antibodies specific for even one single allergen may be used to discriminate sera of allergic individuals with respect to IgE specific for grass pollen in general. A positive correlation, $r = .82$, was found for IgE binding of the recombinant allergen and the crude extracts of grass pollens. It is concluded from these results that a single recombinant allergen or a combination of a few major recombinant allergens can substitute the crude extract for in vitro as well as in vivo diagnostic purposes.

INTRODUCTION

Diagnosis of atopic allergy is based on clinical history and the detection of specific IgE antibodies by combination of in vivo (skin prick test) and in vitro (RAST) techniques.¹ The measurement of specific IgE antibody binding is influenced by the inherent heterogeneity of most allergen extracts and significant variations in the preparation of these extracts used for the analysis.² Pollen extracts of individual grass species contain about 30 different protein components, which may be present in variable concentrations, and more than ten of these are capable of inducing specific IgE antibodies.^{3,4} The current procedures for diagnosis of IgE-mediated allergies employing the extracts, which may be considered adequate only for categorizing the class of aeroallergens, lack the resolution to pin-

point the allergenic component the individual is allergic.

It is expected that replacement of crude extract with a mixture of a few major allergens, against which almost all patients have IgE antibodies, would standardize the analysis and minimize the existing uncertainties. Molecular cloning techniques provide the potential means to produce necessary amounts of pure allergens; some of which may be difficult, if not impossible to isolate from natural sources. This technology has recently been employed to obtain structural information of many allergens, and several recombinant allergens have been synthesized in a pure and water soluble native form.⁵⁻⁷

We have recently cloned and expressed a group of allergens, *Poa p* IX, from Kentucky bluegrass (KBG) pollen,⁸⁻¹¹ which are shown to be present in most other grasses.¹² Our previous results demonstrated that the *Poa p* IX allergens constitute a major allergens of grass pollens.¹¹ In this study, we have investigated the use of a single allergen for quantitation of grass pollen specific IgE antibodies in humans, by using the rKBG8.3 as a model for total IgE, which represents a water soluble, recombinant polypeptide

representing the conserved region of the *Poa p* IX allergens.¹³ Our results demonstrate that a single recombinant allergen or possibly the combination of recombinant allergens could replace the crude extracts for diagnostic purposes.

MATERIAL AND METHODS

Preparation of Grass Pollen Extracts

A 1% (wt/vol) suspension of pollen, *Poa pratensis* or *Phleum pratensis* (Hollister-Stier, Ontario, Canada), in TBS (0.01 M Tris-HCl, pH 7.4, 0.15 M NaCl) containing 0.01% NaN₃ and 1 mM PMSF was incubated with shaking overnight at 4 °C. Pollen grains were removed by filtration (Whatman 3MM), and the pollen extract was further concentrated 10× by ultrafiltration (YM5, Amicon, MA, USA) and stored in aliquots at -20 °C. The protein concentration was determined by the Bio-Rad protein assay.

Human Sera

Individual sera from allergic patients in Norway were kindly provided by Nycomed AllergenLab, Oslo, Norway. The sera were originally analyzed by RAST for different allergens and by PRIST* as described by the manufacturer. These analyses were performed based on

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requests by clinicians. Of 94 selected sera, 20 gave RAST score class <1 (class 0), 20 sera gave >1 and <2 (class 1), 20 sera gave >2 and <3 (class 2), 20 sera gave >3 and <4 (class 3), and 14 sera gave >4 (class 4). These sera were originally selected based on their reactivity in a Phleum (timothy g6) RAST. The use of these sera for extended examinations was approved by health authorities in Norway.

Expression and Purification of Recombinant Allergenic Proteins

The recombinant allergenic proteins were isolated as described recently.¹³ Briefly, overnight cultures were diluted 1 in 10 into 1 liter of L-broth containing 50 µg/mL of ampicillin and incubated at 37 °C for one to four hours. Then, isopropyl thiogalactopyranoside was added at 0.5 mM to induce high-level expression of the fusion protein. After four hours of incubation, cells were pelleted, resuspended in 20 mL of buffer containing 20 mM phosphate, pH 7.3, and 1% Triton X-100 and frozen at -70 °C. The cells were quick-thawed at 37 °C, sonicated and the lysate was cleared by centrifugation at 18,000 g for 20 minutes at 4 °C. Supernatant was mixed with swollen and washed glutathione agarose beads (Sulphur Linkage, Sigma Chemical Co. St. Louis, MO) in the ratio 10:1 (vol:vol) to allow specific binding of the GST moiety of the fusion proteins to the glutathione on the beads. After incubation on a roller for 30 minutes at 4 °C, the beads were subsequently washed by 5 × 15 mL of phosphate buffer and 1 × 15 mL of 50 mM Tris-HCl, pH 8.0. The recombinant allergenic polypeptide was cleaved from its fusion partner, glutathione-S-transferase, while the latter was still bound to the gel, by thrombin. The washed beads were suspended in 50 mM Tris-HCl, pH 8.0 containing 150 mM NaCl and 125 mM CaCl₂, and subsequently added with thrombin (1:500; wt/wt) at room temperature. The cleaved protein was obtained in the super-

natant after centrifugation of the beads.

Protein concentration of the cleaved product was measured by the Lowry method by dotblotting the diluted samples onto nitrocellulose membrane (Schleicher & Schull, 0.45 µm), followed by staining with 0.1% amido black in isopropanol/acetic acid (25%/10%). Bovine serum albumin, diluted in the same solvent, served as the protein standard. Eluted materials were aliquoted and stored at -20 °C.

SDS-PAGE and Immunoblotting

SDS-PAGE was performed in a discontinuous system according to Laemmli¹⁴ using 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. Western blotting was performed using PolyBlot (American Bionetics, Inc, CA) with a discontinuous buffer system as described by the manufacturer. The membranes were subsequently blocked by incubation in TBS/1% gelatin for at least six hours, and then incubated overnight with human IgE serum diluted 1:100 in TBST/0.1% gelatin. IgE antibody binding was detected by incubation of these filters overnight with alkaline phosphatase-conjugated goat anti-human IgE (Tago, CA, USA), 2000-fold diluted in TBST with 0.1% gelatin. The membranes were washed 5 ×, four minutes each in TBST between each incubation step, and nitroblue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate were used as substrates. The enzymatic reaction was stopped by incubating the membranes in 50 mM Tris-HCl, pH 8.0, 100 mM EDTA.

Enzyme Immunoassay in Microtitre Plates

Proteins, 10 µg/mL of buffer, were coated on wells of microtiter plates (Nunc, Immunoplate, Denmark) overnight. The wells were further blocked by phosphate buffer containing 1% gelatin for eight hours. Subsequent to washing 5 × with

phosphate buffer containing 0.05% Tween 20, the wells were incubated with human serum diluted with the washing buffer containing 0.1% gelatin overnight. After washing, alkaline phosphatase-conjugated secondary antibodies (goat anti-human IgE (TAGO), diluted 3000-fold) were added and the wells were incubated overnight. Following washing with phosphate buffer containing Tween 20, the color reaction was developed by adding the substrate 1 mg/mL *p*-nitrophenyl phosphate in 0.1 M Tris-HCl buffer containing NaCl and MgCl₂ and the optical density was 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

The variations in allergen content of grass pollen extracts. The variations in allergen content of grass pollen extract and its impact on analysis for allergen-specific IgE antibodies was examined. The SDS-PAGE electrophoretogram of two batches of Kentucky bluegrass pollen extracts are illustrated in Figure 1. These two extracts used in our previous studies^{9,11} were prepared by the same procedure but based on two different batches of Kentucky bluegrass pollen. These extracts contained significantly different profiles of proteins, including almost total absence of the major *Poa* *p* IX allergens of Mr 28,000 and 32,000 in extract I.¹¹ Extract II has been shown to contain all previously identified groups of grass pollen allergens¹¹ and was selected for use in this study.

Since the 93 human sera used in this study were selected by screening for grass pollen-specific IgE antibodies using RAST g6 (timothy), and our ELISA immunoassay system differed from the RAST system with respect to the solid phase, the secondary antibodies and the label; a

timothy extract was prepared for the comparison of the initial RAST data with that of the Kentucky bluegrass-specific IgE antibodies. Figure 2A shows the correlation plots of RAST/g6 (timothy) and IgE-EIA/timothy of these sera. The results indicated a low correlation between the two methods ($r = .28$). The analysis, however, of IgE antibodies specific for timothy and Kentucky bluegrass in the EIA-system gave very similar results (Fig 2B; $r = .92$). The correlation plot of binding of IgE to timothy and the total IgE in the same sera (Fig 2C) indicated no significant non-specific binding of IgE in the EIA. These results demonstrated that the variation in allergen content of grass pollen extracts

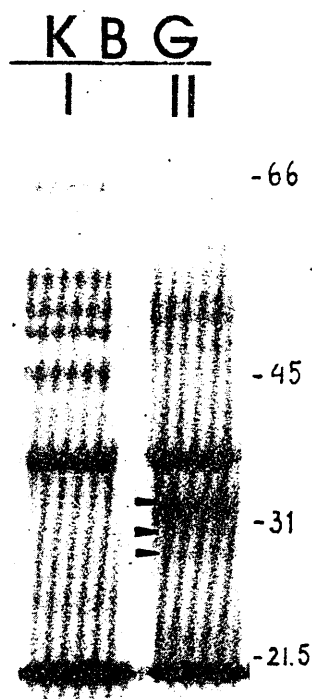


Figure 1. SDS-PAGE, 10% disc. gel, of two extracts, I and II, of two different batches of Kentucky bluegrass pollen. Migration of molecular weight standards as well as IgE-binding proteins of Mrt 28, 29 and 32000 are indicated.

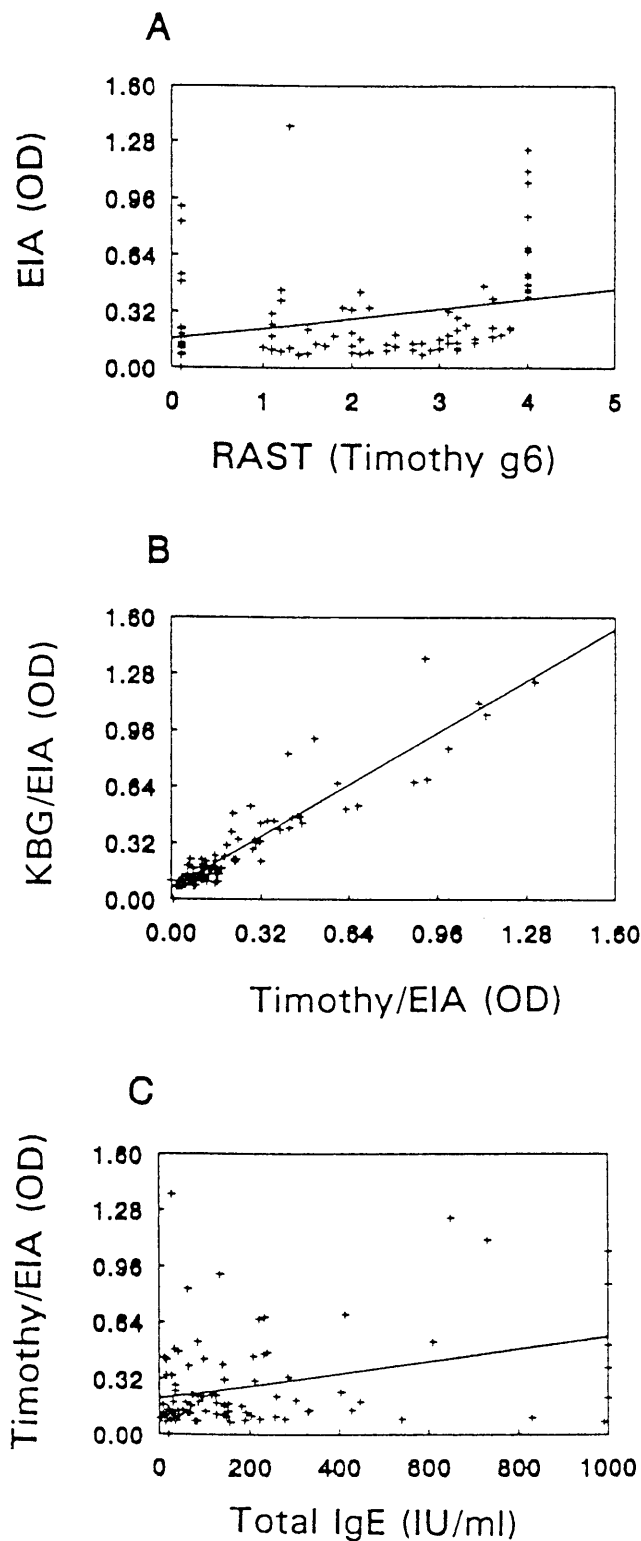


Figure 2. Correlation of IgE-binding to different grass pollen extracts as determined by ELISA immunoassay and RAST using 96 human sera (diluted 1/30). A: correlation between IgE binding of timothy extract determined by ELISA immunoassay (EIA/OD) and RAST/g6. B: correlation of IgE binding of timothy and Kentucky bluegrass extract determined by ELISA immunoassay. C: correlation of IgE binding of timothy extract by ELISA immunoassay and total IgE by PRIST.

and that the type of solid phase used in the assay influenced the quantitation of specific IgE antibodies.

The recombinant Poa p IX allergens and their IgE-binding epitopes. The recombinant *Poa p IX* allergen, rKBG31, encoded by cDNA KBG31, was used to investigate its IgE binding ability and its application in diagnosis of atopic allergy. To be useful in in vitro as well as in vivo analysis, the exposure of more than one IgE-binding epitope on a molecule is mandatory. Several defined fragments of the recombinant allergen prepared by expression of subclones of the cDNA KBG31 (Fig 3A) were used to localize epitopes. The recombinant allergen produced by *E. coli* containing the expression-vector, the pGEX2T-1,¹³ is water-soluble and hence, the native conformation of the allergen is ensured.

The IgE-binding to the recombinant protein was inhibited by preincubation of pooled human sera with different fragments and control proteins (Fig 3B). The non-inhibitory effect of recombinant GST revealed that no IgE was binding to the GST part of FP-rKBG3.1. The partial inhibition by rKBG8.3 demonstrated that the part of rKBG3.1 overlapping with rKBG8.3, expose IgE-binding epitope(s), and that the N-terminal part of rKBG3.1 expose additional IgE-binding epitope(s). The rKBG7.2 which represent the C-terminal part of rKBG8.3 was previously shown to expose IgE-binding epitopes.¹¹ These results demonstrated that the rKBG31 exposes at least three different sets of IgE-binding epitopes and that two of these are located within the rKBG8.3, which previously has

been produced in non-fused form.¹³

Quantitation of Poa p IX Molecules in KBG Pollen Extract

Because rKBG8.3 represent the conserved region of the *Poa p IX* allergens, the soluble rKBG8.3 was used to quantitate the concentration of the *Poa p IX* allergens in Kentucky bluegrass pollen extract, by comparing the ability of Kentucky bluegrass extract and rKBG8.3, respectively, to inhibit binding of human IgE antibodies to rKBG8.3 (Fig 4). Incubation of allergic human sera (a pool of sera of 11 patients allergic to grass pollens, at 1:100 dilution) with 63 $\mu\text{g/mL}$ of rKBG8.3 and 330 $\mu\text{g/mL}$ of Kentucky bluegrass extract inhibited to the same extent of the IgE binding to *Poa p IX* allergens. Incubation of a about five individual serum with the same allergens indicated that the recombinant allergen was able to block from 18% to 33% (mean 20%) the IgE binding of Kentucky bluegrass pollen extract (data not shown). Further, the slopes of the inhibition curves produced by rKBG8.3 and crude Kentucky bluegrass extract were similar. It is inferred from all these studies that the *Poa p IX* constituted approximately 20% of the total proteins in the KBG extract.

Comparison of Serum IgE Binding to Recombinant rKBG8.3 and to Crude Pollen Extracts of KBG

Titration curves of IgE binding to rKBG8.3 and Kentucky bluegrass pollen extract in serum of four high IgE-responders and one non-responder are shown in Figure 5 A and B. The results indicated that high IgE-responders against Kentucky bluegrass are also high responders against *Poa p IX*. The SDS-PAGE immunoblot analysis using the same sera with Kentucky bluegrass extract (data not shown) indicated that the four high-binding sera appear to have similar reactivities.

The 93 sera from potential grass pollen-allergic individuals, already screened by RAST and by ELISA immunoassay using Kentucky blue-

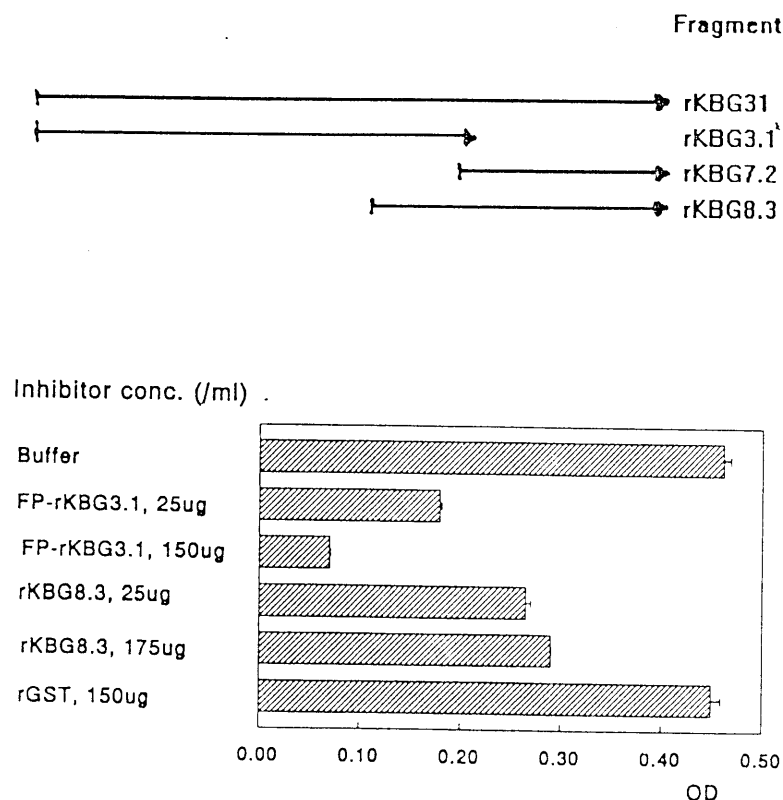


Figure 3. (A) Schematic representation of the recombinant *Poa p IX* allergen, rKBG31, and defined fragments prepared by pGEX2T-1 expression vector system. (B) Inhibition of binding of human IgE antibodies to FP-rKBG3.1 as determined by ELISA immunoassay. A pool of sera, diluted 1/30, was preincubated with different concentrations of FP-rKBG3.1, rKBG8.3 and recombinant glutathione-S-transferase, and subsequently added to wells coated with FP-rKBG3.1. The extent of inhibition was determined by EIA as described.

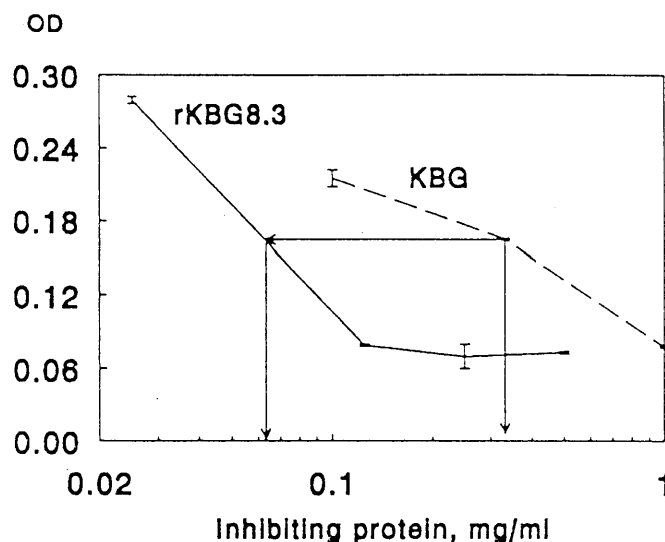


Figure 4. Quantitation of *Poa p* IX-like proteins in Kentucky bluegrass pollen extract. The capacities of Kentucky bluegrass pollen extract and rKBG8.3, respectively, to inhibit binding of human IgE antibodies to rKBG8.3 were compared. The allergic human sera (a pool of sera from 11 patients allergic to grass pollen) at a dilution of 1:100, was preincubated with different protein concentrations of the two inhibitors and subsequently added to rKBG8.3 coated wells for ELISA assay.

grass extract, were further analyzed for IgE binding by immunoassay with rKBG8.3 (Fig 6). The correlation between IgE-binding to rKBG8.3 and to the extract of Kentucky bluegrass, with a correlation coefficient 0.82, demonstrated that the spectra and titer of individual specificities of IgE antibodies varied considerably among the sera (Fig 6A). To discriminate between positive and negative sera, a Kentucky bluegrass extract-specific IgE cut-off value correlating to RAST score class 1 was chosen and indicated that 86 sera (91.5%) were either double positive or negative for rKBG8.3 and KBG, while eight (8.5%) did not agree (data not shown). There was no significant correlation between total IgE level and IgE for rKBG8.3 (Fig 6B), which indicated non-specific IgE-binding in the ELISA immunoassay system.

Taken together, these results lead to the inference that the IgE binding of Kentucky bluegrass extract and the recombinant allergens are correlated in the high IgE responders.

DISCUSSION

Current diagnostic methods utilize crude pollen extracts for quantitation of specific IgE antibodies in human sera, thus the complex mixture of allergenic and nonallergenic proteins are immobilized onto a solid phase for the determination of allergen-specific IgE antibodies. As is demonstrated in this study, the extract based diagnosis has two major drawbacks: first, the SDS-PAGE pattern of the two extracts of Kentucky bluegrass pollen, prepared according to the same procedure, demonstrated significant qualitative and quantitative differences that may exist between extracts from the same species of grass pollen. It is not known whether these differences are related to the original protein content of the pollen or are secondary effects of storage of the pollen caused by enzymatic degradation. Second, the various types of solid phases and coupling techniques employed lead to spurious results relating to the amount of the individual allergens that are present on the

solid phases. The comparison of the ELISA immunoassay and RAST method for quantitation of IgE antibodies specific for timothy pollen demonstrated that sera of certain individuals gave a low reading with ELISA immunoassay and a high reading with RAST and vice versa. In essence, the diagnosis by these methods using the crude allergen extracts do not provide reliable results.

Because of (1) the magnitude of variation in the existing diagnostic procedures due to the extensive differences (up to 100-fold) in allergenic potency of extracts, and (2) the availability of the recombinant allergens, we were prompted to examine the alternative approaches for the diagnosis of grass allergies with *Poa p* IX allergens used here as a model. The Kentucky bluegrass extract employed in this study was previously shown to contain all major grass pollen allergens.¹¹ The similarity of IgE-binding of Kentucky bluegrass extract examined by two different methods, ie, immunoblotting and ELISA immunoassay, and the high degree of correlation between IgE-binding to the Kentucky bluegrass and timothy by the ELISA immunoassay, are consistent with our existing knowledge of cross-reactivity between allergens from different grasses,^{12,15,16} and led to the inference that the Kentucky bluegrass pollen extract employed in this study was a proper reference for quantitation of grass pollen-specific IgE antibodies by the ELISA immunoassay.

A member of the *Poa p* IX allergens, previously demonstrated to be a major group of allergens¹¹ was shown to expose at least three sets of IgE-binding epitopes, two of which were located within the C-terminal fragment rKBG8.3. The rKBG8.3 constituting the conserved part of the *Poa p* IX was used to estimate that the *Poa p* IX allergens make up 20% of the protein content of Kentucky bluegrass pollen extract. The rKBG8.3 was considered

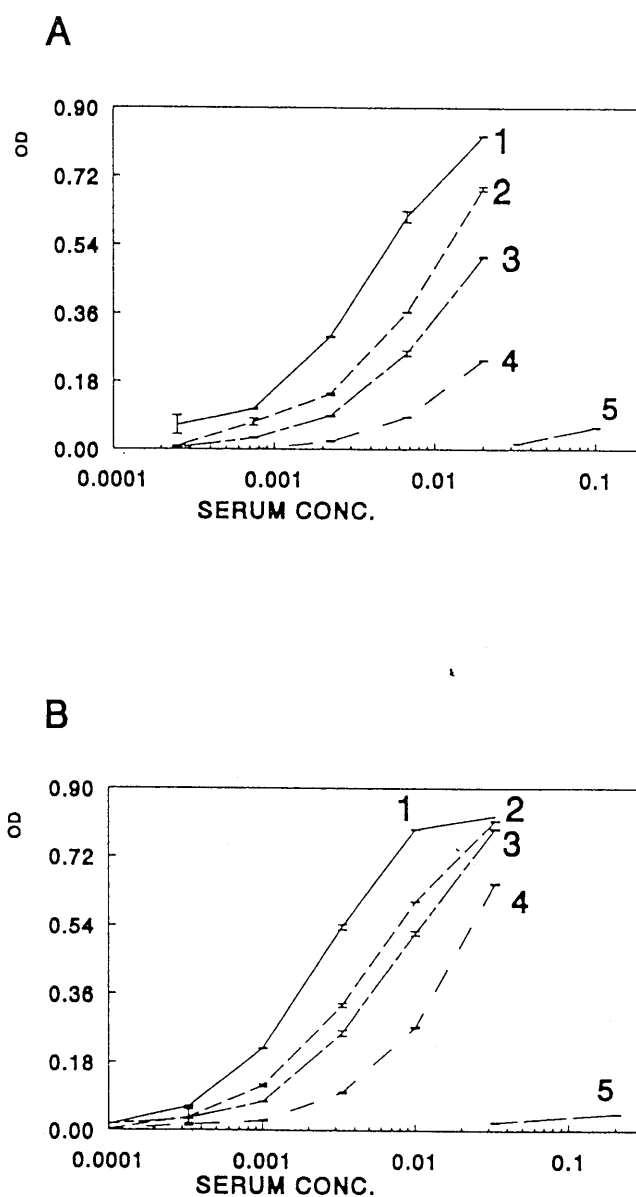


Figure 5. Binding of human IgE by four high responder sera and one nonresponder serum to recombinant and native Kentucky bluegrass pollen allergens. A: Titration of binding to rKBG8.3 and B: Titration of binding to Kentucky bluegrass pollen extract, by ELISA immunoassay.

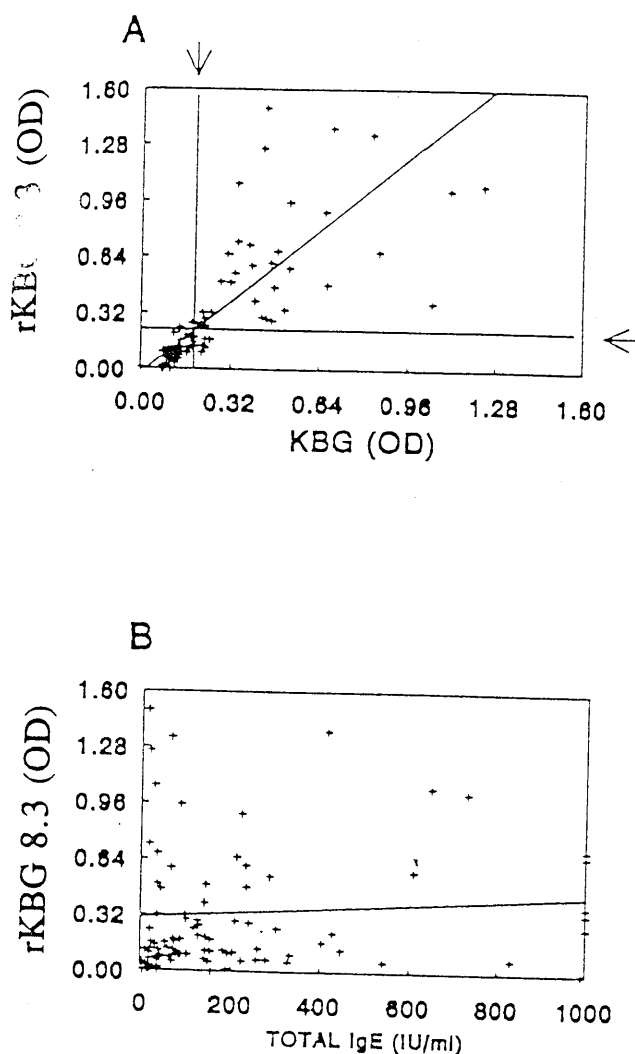


Figure 6. Correlation analysis of IgE-binding to rKBG8.3 and to extracts of Kentucky bluegrass (A). 94 human sera (diluted 1/30) as determined by ELISA immunoassay. Line of correlation indicated, and cut-off OD values for positive specific IgE are marked with arrows. B: Correlation of IgE binding to rKBG8.3 and total IgE in the same sera.

as a possible replacement for the crude pollen extract by the ELISA immunoassay. The results of the comparison, however, indicated that a single major grass pollen allergen can not replace the crude extract for quantitation of specific IgE antibodies towards grass pollen.

While using the IgE antibodies of the high-responder sera, which contain IgE antibodies toward most allergens in an extract,¹⁷ a better correlation was found between the IgE binding of the extract versus the recombinant allergen, as also was indicated by the initial experiments

(Fig 3). A lower correlation value with sera of intermediate and low IgE-binding is probably due to the existence of more pronounced IgE responses of these individuals toward selected allergens. Alternatively, this may be because of the broad spectra of specificities but low titer of each specificity of the IgE antibodies in the sera. Quantitation of antibodies specific to all the allergens in an extract is a formidable task, since both the concentration of antibodies specific for the individual allergen varies from sera to sera and different allergens are pres-

ent in varying amounts on the solid phase. Any method would therefore seldom give a true quantitation of the concentration of all the relevant antibodies in the samples. Our results demonstrated that the recombinant allergen alone could qualitatively discriminate between positive and negative sera equally well as the crude extracts.

In summary, the evidence presented herein demonstrate that indeed for diagnostic purposes it may be feasible to replace the crude extract with the major recombinant allergen(s). Since several of the major allergens of grass pollen have been cloned,^{6,7,18,19} it is expected on the basis of these results that a combination of a few of these allergens would result in a reproducible assay for quantitation of grass pollen-specific IgE in serum. In addition to its use in in vitro analysis, our demonstration that rKBG8.3 exposes at least two IgE-binding epitopes, makes it an interesting molecule for in vivo analysis of grass pollen allergy.

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REFERENCES

1. Bernstein L. Proceedings of the task force on guidelines for standardizing old and new technologies used for the diagnosis and treatment of allergic diseases. *J Allergy Clin Immunol* 1988;82:487-526.
2. Platts-Mills TA, Chapman MD. Allergen standardization. *J Allergy Clin Immunol* 1991;87:621-5.
3. Ford SA, Baldo BA. A re-examination of ryegrass (*Lolium perenne*) pollen allergens. *Int Arch Allergy Appl Immunol* 1986;81:193.
4. Chakrabarty S, Lowenstein H, Ekramoddoullah AKM, et al. Detection of cross-reactive allergens in Kentucky bluegrass pollen and six other grasses by crossed radioimmunoelectrophoresis. *Int Arch Allergy Appl Immunol* 1981;66:142.
5. Bond JF, Garman RD, Keating KM, et al. Multiple *Amb a* I allergens demonstrate specific reactivity with IgE and T cells from ragweed-

- allergic patients. *J Immunol* 1991; 146:3380-5.
6. Walsh DJ, Matthews JA, Denmeade R, Walker MR. Cloning of cDNA coding for an allergen of cocksfoot grass (*Dactylis glomerata*) pollen. *Int Arch Allergy Appl Immunol* 1989;90:78-83.
 7. Singh MB, Hough T, Theerakulpisut P, et al. Isolation of cDNA encoding a newly identified major allergenic protein of rye-grass pollen: intracellular targeting to the amyloplast. *P. N. A. S.* 1991; 88:1384-8.
 8. Mohapatra SS, Hill R, Astwood J, et al. Isolation and characterization of a cDNA clone encoding an IgE binding protein from Kentucky bluegrass (*Poa pratensis*) pollen. *Int Arch Allergy Appl Immunol* 1990;91:362-8.
 9. Yang M, Olsen E, Dolovitch J, et al. Immunological characterization of a recombinant Kentucky bluegrass (*Poa pratensis*) allergenic peptide. *J Allergy Clin Immunol* 1990;87:1096-1104.
 10. Silvanovich A, Astwood J, Olsen E, et al. Nucleotide sequence analysis of three cDNA's coding for major isoallergens of Kentucky bluegrass pollen. *J Biol Chem* 1991;266: 1204-10.
 11. Olsen E, Zhang L, Hill RD, et al. Identification and characterization of the *Poa p IX* group of basic allergens of Kentucky bluegrass pollen. *J Immunol* 1991;147:205-11.
 12. Zhang L, Kisil FT, Sehon AH, Mohapatra SS. Allergenic cross-reactivity of grass pollens with a recombinant *Poa p IX* polypeptide. *Int Arch Allergy Appl Immunol* 1991;96:28-34.
 13. Olsen E, Mohapatra SS. Expression and Thrombin cleavage of *Poa p IX* recombinant allergens fused to glutathione S-transferase. *Int Arch Allergy Appl Immunol* 1992; 98:343-8.
 14. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 1970;227:680-5.
 15. Leiferman KM, Gleich GJ. The cross-reactivity of IgE antibodies with pollen allergens. 1. Analysis of various species of grass pollen. *J Allergy Clin Immunol* 1976;58: 129-39.
 16. Standring R, Spackman V, Porter SJ. Distribution of a major allergen of Rye grass (*Lolium perenne*) pollen between other grass species. *Int Arch Allergy Appl Immunol* 1987; 83:95-103.
 17. Bousquet J, Hejjaoui A, Becker WM, et al. Clinical and immunological reactivity of patients allergic to grass pollens and to multiple pollen species. *J Allergy Clin Immunol* 1991;87:737-46.
 18. Perez M, Ishioka GY, Walker LE, Chesnut RW. cDNA cloning and immunological characterization of the rye grass allergen *Lol p I*. *J Biol Chem* 1990;265:16210-5.
 19. Griffith IJ, Smith PM, Pollock J, et al. Cloning and sequencing of *Lol p I*, the major allergenic protein of ryegrass pollen. *FEBS* 1991;279: 210-5.

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ORAL BECLOMETHASONE SUPPRESSES CALCIUM ABSORPTION

To study possible effects of swallowed beclomethasone on absorption of calcium the authors evaluated calcium absorption by a strontium absorption test in a double-blind, randomized, placebo-controlled trial in 12 normal adult volunteers. Each swallowed beclomethasone dipropionate capsules, 500 µg twice each day, or placebo for 1 week and the alternate capsules for a second week. Ingestion of beclomethasone was associated with a 12% reduction in strontium absorption ($P < .01$) and a 23% reduction in 24 hour urinary cortisol excretion. Mean reduction in strontium absorption was 20.3% in the six subjects who received beclomethasone the first week and placebo the second week ($P < .005$).

Decreased calcium absorption from the swallowed corticosteroid in patients treated with inhaled beclomethasone dipropionate may contribute to long term reduction in bone density.

—RMS

Smith BJ, Phillips PJ, Pannall PR, et al. Effect of orally administered beclomethasone dipropionate on calcium absorption from the gut in normal subjects. *Thorax* 1993;48:890-3.