

Immunologic characterization of a recombinant Kentucky bluegrass (*Poa pratensis*) allergenic peptide

Ming Yang, MD,* Egil Olsen, PhD,* Jerry Dolovich, MD,**

Alec H. Sehon, PhD,** and Shyam S. Mohapatra, PhD*

Winnipeg, Manitoba, and Hamilton, Ontario, Canada

A recombinant peptide of Kentucky bluegrass (KBG) pollen was synthesized as a fusion protein (FP) in Escherichia coli by recombinant DNA procedures and was compared with its natural counterparts with respect to its allergenic properties. The FP was demonstrated to bind to the IgE antibodies (Abs) of $\geq 95\%$ of 55 individual sera examined. A positive correlation ($r = 0.90$) was observed between the levels of IgE Abs corresponding to the FP and the grass-pollen extract(s). With sera of five allergic patients, the IgE binding of three different protein preparations were compared, namely, KBG pollen proteins, 27 to 35 kd gel-purified pollen proteins, and the FP. Results indicated that about 50% of the total IgE binding of KBG pollen proteins was due to the IgE Abs specific to FP. Comparison of the above protein preparations with respect to their abilities to specifically stimulate murine popliteal lymph node cells in vitro indicated that the total pollen proteins stimulated the highest proliferation of lymph node cells. Interestingly, the FP supported higher proliferation of lymph node cells than the gel-purified proteins. Collectively, these results suggest that the recombinant peptide constitutes a major allergenic constituent of grass pollens and may be of diagnostic and therapeutic value. (J ALLERGY CLIN IMMUNOL 1991;87:1096-1104.)

Molecular cloning of allergens is expected to be useful for two major areas of allergy research. First, it would facilitate the production of defined allergens in virtually unlimited quantities that is essential for the development of (1) highly discriminating diagnostic assays for allergies, (2) international standards of major allergens for use in immunotherapy,^{1,2} and (3) tolerogenic derivatives of allergens that may serve as effective therapeutic modalities.³ Second, cloning of allergens would aid in the establishment of the struc-

From the *Department of Immunology and **Medical Research Council Group for Allergy Research, University of Manitoba, Winnipeg, and the ***Department of Pediatrics, McMaster University, Hamilton, Ontario, Canada.

Supported by the Manitoba Health Research Council and the Paul H. T. Thorlakson Foundation of Winnipeg, and the Medical Research Council of Canada.

Received for publication April 24, 1990.

Revised Nov. 28, 1990.

Accepted for publication Feb. 20, 1991.

Reprint requests: S. S. Mohapatra, PhD, Department of Immunology, The University of Manitoba, Winnipeg, Canada, R3E 0W3.

Dr. Shyam S. Mohapatra holds a scholarship from the Manitoba Health Research Council.

Dr. Egil Olsen is partly supported by the Norwegian Council for the Sciences and Humanities.

1/1/29009

Abbreviations used

Poa p:	<i>Poa pratensis</i>
KBG:	Kentucky bluegrass
FP:	Fusion protein
Ab:	Antibody
PLN:	Popliteal lymph node
SPT:	Skin prick test
SDS-PAGE:	Sodium dodecyl sulfate-polyacrylamide gel electrophoresis
PBS:	Phosphate-buffered saline
PBST:	PBS with Tween 20
TBS:	Tris-buffered saline
TBST:	TBS with Tween 20
MW:	Molecular Weight
GPP:	Gel-purified <i>Poa p</i> proteins
KPP:	KBG pollen proteins
EDTA:	Ethylenediaminetetraacetic acid
Con-A:	Concanavalin A
BSA:	Bovine serum albumin
OD:	Optical density
Ag:	Antigen

tures (or epitopes) of allergens, which interact with the components of the immune system, such as IgE and IgG Abs, T cell receptor V gene products, and the major histocompatibility complex molecules.

Knowledge of the epitopes of allergens would lead not only to the designing of immunotherapeutic vaccines but also would provide the tools for investigations of the cellular and humoral mechanisms involved in the induction, progression, and suppression of hypersensitivity diseases. Recently, isolation of cDNA clones and analysis of their corresponding primary amino acid sequences were reported for the major mite,^{4,6} tree pollen,⁷ and hornet⁸ allergens. However, the progress of similar studies of the grass-pollen allergens, which constitute an important group of aeroallergens, the grass-pollen allergens, has been slow.⁹

Previously, several cDNA clones encoding IgE-binding polypeptides of KBG pollen were isolated and analyzed for their nucleotide and the corresponding amino acid sequences.^{10, 11} Furthermore, one of these cDNAs was inserted into an expression vector to achieve high-level expression of the allergenic polypeptide in *E. coli* as an FP in association with a truncated β -galactosidase.¹² In the present study, by immunoblotting experiments, it was demonstrated that the recombinant peptide constituted a fragment common to three polypeptides that appeared to be minor components of KBG pollen. Moreover, the recombinant polypeptide was characterized and compared with its natural counterpart(s) with respect to its ability to bind to human IgE and to stimulate proliferation of murine PLN cells from immunized animals. The results revealed that this peptide is a major allergenic constituent of grass pollens.

MATERIAL AND METHODS

Human sera and skin tests

Sera were obtained from 55 individuals attending Chedoke-McMaster Hospital, Hamilton, Ontario, who exhibited positive skin tests for grass-pollen allergens. The skin test grass mix (Bencard, Mississauga, Ontario) contained extracts of KBG, redtop, June grass, orchard, and timothy grass pollens. The epicutaneous SPTs were performed on the forearm with a 26-gauge hypodermic needle with the consent of the subject. Grading was as follows: no wheal, negative; 1 to 2 mm wheal, 1+; 3 to 3 mm wheal, 2+; 6 to 9 mm wheal, 3+; and >9 mm wheal, 4+. Histamine and salines were used as controls. The skin tests were read after 15 minutes.

Grass pollens

Pollens of the following 10 different species of grasses were obtained from Hollister-Stier Laboratory, Mississauga, Ontario: KBG (*Poa pratensis*), ryegrass (*Lolium perenne*), timothy (*Phleum pratense*), Johnson (*Sorghum halepense*), quack (*Agropyron repens*), tall oat (*Arrhenatherum elatius*), smooth brome (*Bromus inermis*), redtop (*Agrostis maritima*), Canada blue (*Poa compressa*), and orchard (*Dactylis glomerata*).

Protein isolation from grass pollens

A 1% (wt/vol) suspension of pollens in TBS with 1 mmol/L of phenylmethylsulfonyl fluoride was incubated with shaking overnight at 4° C. The debris was removed by filtration and centrifugation, and the supernatant containing pollen proteins was stored in aliquots at -20° C. One preparation of KPP was used in all comparative experiments. In addition, a gel-purified fraction of KPP consisting of proteins in the range of 27 to 35 kd, designated as GPP, which included the proteins corresponding to the FP and group I proteins,¹³ was also used in these experiments. The GPP was isolated by separation of the KBG pollen extract in a 10% SDS-polyacrylamide gel according to the method of Laemmli¹⁴ and excision of the gel at the MW 27 to 35 kd. The proteins were eluted from the gel with an electroelution apparatus (Bio-Rad, Rockville Centre, N.Y.). The protein concentration was determined by Bio-Rad protein assay. The GPP preparation was sterilized by filtration through 0.45 μ m membrane (Millipore Corp., Bedford, Mass.) and was stored in aliquots (at a concentration of 1 mg/ml) at -20° C. With the above procedure, 1 gm of pollen was found to yield ~10 mg of total protein and ~1 mg of GPP protein.

Expression of KBG 7.2 in pWR590 plasmid

The pWR590 series of expression plasmids were kindly provided by Dr. D. Thomas,¹⁵ and recombinant DNA techniques were performed as described previously.¹¹ Based on the sequence data of KBG 7.2, pWR590.1 was chosen for the expression of the allergenic peptide encoded by KBG 7.2.¹⁰ The plasmid pWR590.1 was digested with *Eco* RI, precipitated with ethanol, and then treated with bacterial alkaline phosphatase. The KBG 7.2 insert was prepared by *Eco* RI digestion and isolated with "gene clean" procedures (Bio Can Scientific, Mississauga, Ontario). The insert was ligated to the pWR590.1 and transformed in *E. coli* strain JM105. Ampicillin-resistant transformant colonies were screened for expression of the FP with a procedure previously described.¹¹

Purification of the FP

A liter of culture of one of the IgE binding transformants, carrying the recombinant plasmid, was grown overnight in Luria broth containing 100 μ g/ml of ampicillin at 37° C without any inducer. Cells were harvested and resuspended in a 3 ml of lysis buffer (50 mmol/L of Tris HCl, pH 8.0, 1 mmol/L of EDTA, 100 mmol/L of NaCl, 0.8 mg lysozyme, and 4 mg Na-deoxycholate) per gram of wet weight of cells at 4° C. DNA was fragmented either by sonication or by treatment with DNase, 20 μ g/gm of cells, and the cell lysate was centrifuged at 12,000 g for 15 minutes at 4° C. The pellet was resuspended in 9 vol (wt/vol) of lysis buffer containing 0.5% (vol/vol) of Triton X100 and 10 mmol/L of EDTA for 5 minutes at 30° C. The inclusion bodies were obtained by centrifugation (25,000 g for 15 minutes at 4° C) of the above suspension in a 30% (wt/vol) sucrose cushion prepared in the lysis buffer. Proteins were isolated from inclusion bodies in lysis buffer containing 3

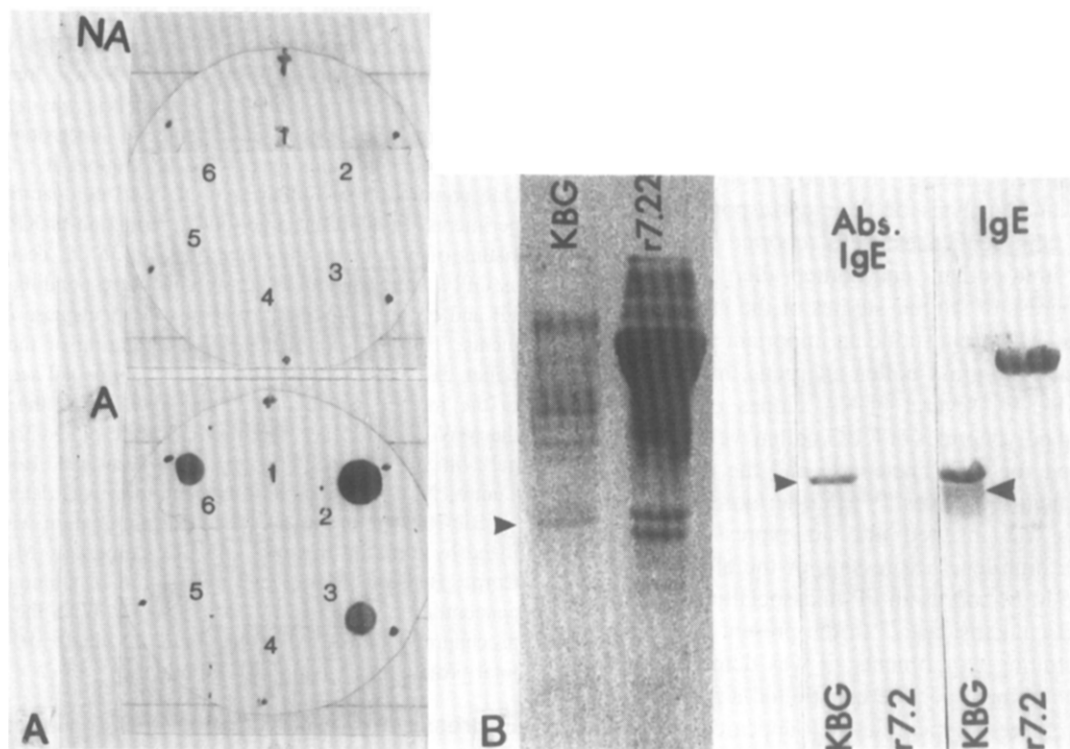


FIG. 1. **A**, Expression of a cDNA encoding an IgE-binding peptide in *E. coli*. Proteins isolated from three separate transformants, one nonrecombinant (1) and two recombinants (2 and 3), JM105 host bacteria (4), buffer alone (5), and KBG pollen (6) were dotted onto two nitrocellulose membranes. One of these membranes was reacted with a pooled sera from grass pollen-allergic subjects and the other membrane with pooled sera from two nonatopic subjects. The IgE reactions were detected as described in MATERIAL AND METHODS. **B**, Identification of pollen polypeptides encoded by the recombinant cDNA fragment by immunoblotting. Proteins were isolated from KBG pollen and *E. coli* (KBG r7.2), separated by SDS-PAGE, and blotted onto nitrocellulose. The membranes were treated (1) with a pool of sera of patients allergic to grass pollen (panel IgE) and (2) with the sera used in (1), which was preabsorbed with the FP (panels Abs and IgE). The large arrowhead indicates the position of the polypeptides that react to IgE Abs specific to FP. The small arrowheads indicate the position of described¹⁷ 35 kd *Poa p* polypeptide.

mol/L of guanidine HCl, and subjected to SDS-PAGE. The FP band was excised from the gel, and the protein was eluted in a buffer containing 50 mmol/L of Tris HCl, pH 8.0, 1 mmol/L of CaCl₂, and 0.1% (wt/vol) of SDS that solubilized the FP.¹⁶ This preparation was filter sterilized before use. With this procedure from 1 L of culture, it was possible to obtain ~10 mg of FP.

Immunoblotting

For immunoblotting, the proteins were either dot blotted or electroblotted onto a nitrocellulose membrane subsequent to SDS-PAGE (10%).¹³ The IgE-reactive proteins on blot were detected by procedures described previously.¹¹ Briefly, the membrane was blocked with a solution of 1% gelatin in TBS for 6 hours at room temperature. The membrane was then treated separately with a pool of sera of six allergic patients at a dilution of 1:10 overnight and with pooled sera that was preabsorbed with insoluble FP. For the purpose of absorption, 300 μ l of pooled IgE sera was added to 250 mg

of FP in 20 ml of TBST with 0.1% gelatin. The mixture was incubated at room temperature for 4 hours, and then the FP-Ab complexes were separated by centrifugation. The supernatant served as the source of pooled sera preabsorbed with FP. The IgE binding proteins were detected with alkaline phosphatase-conjugated goat antihuman IgE (1:1000 dilution) (Tago Inc., Burlingame, Calif.) in TBST and 0.1% gelatin for 3 hours. The subsequent color reaction was based on nitroblue tetrazolium/5-bromo-4-chloro-3-indolyl phosphate system and stopped by the addition of TBS/EDTA.

ELISA

The proteins were coated onto microtiter plates (Costar, Mississauga, Ontario) in a buffer, containing 0.01 mol/L of Na₂CO₃ and 0.01 mol/L of NaHCO₃ (pH 9.6), blocked by 1% BSA in PBS, and then incubated with human sera at a dilution of 1:10 for a minimum of 6 hours. Bound IgE was detected by treatment with a murine monoclonal anti-

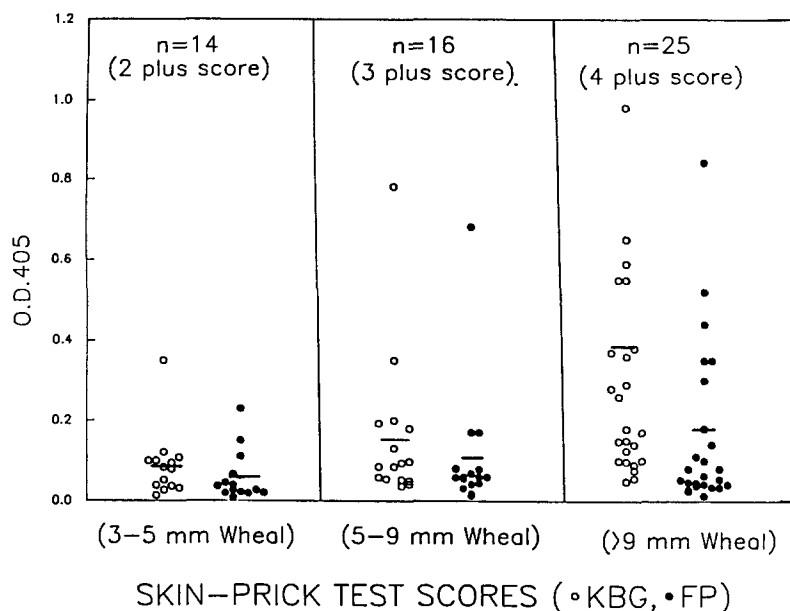


FIG. 2. IgE reactivity of sera of patients with distinct clinical symptoms (as measured by SPT score) with FP or KBG proteins by ELISA. The individual OD represents the value after subtraction of the background. The background was measured by use of a pool of five sera (as control) of individuals who were not allergic to grass pollens, as measured by SPTs. No IgE reactivity was detectable by immunodot assay. The ELISA was considered positive with values above background.

human IgE Ab (1 μ g/ml, 4 hours), washed, and incubated with alkaline phosphatase-conjugated goat antimouse IgE (1/1000, 1 hour) (Zymed Corp., South San Francisco, Calif.). For the detection of the Ab reactions in ELISA, p-nitrophenyl phosphate was used as substrate, and the OD was measured by an ELISA reader. All reagents were diluted in PBST, 0.1% of BSA, and each incubation step was followed by washing with PBST.

The ELISA background was measured by use of a pool of sera of five nonatopic individuals and was found to have OD values of 0.02 units. At OD values higher than 0.02 units, the ELISA was considered positive. The specificity of ELISA was established by including unrelated Ag and no Ag as controls in these experiments. Each ELISA experiment was repeated twice.

Lymphocyte proliferation assay

The PLNs were isolated from C₃H/HeJ mice that had been primed with either KBG pollen extract or FP (60 μ g per mouse in Freund's complete adjuvant). Proliferation of cells in the presence of Ag was carried out after the procedure as described.¹⁷ The extent of lymphocyte proliferation, as indicated by the degree of incorporation of [³H]thymidine (37 MBq, Amersham International, Toronto, Canada), added at 1 μ Ci per well during the last 12 hours of culture, was expressed as counts per minute. The proliferation experiments were repeated at least twice. A single cell suspension of PLNs was prepared in RPMI 1640 medium. The viability of the cells was checked by their ability to exclude trypan blue. The cells were finally suspended at a concen-

tration of 5×10^6 /ml in freshly made culture media (10% fetal calf serum, 1 ml of 2-mercaptoethanol [5×10^{-5} mol/L], 1 ml of L-glutamine [2×10^{-5} mol/L], 10,000 units of penicillin, and 5 mg of streptomycin and made up to 100 ml with supplemented RPMI 1640). One hundred microliters of this cell suspension (5×10^5 cells) was then pipetted into each of the 96 flat-bottom wells of tissue-culture plates (Flow Laboratories, Mississauga, Ontario). The stock solution of various Ags, KBG-pollen extract, FP, and BSA, β -galactosidase (Sigma Chemical Co., St. Louis, Mo.), and Con A (Pharmacia, Uppsala, Sweden) were diluted to appropriate concentration with the culture media.

RESULTS

In Fig. 1, A, an immunoblot of two representative transformants that produced the FP that reacted with the pooled sera of six allergic subjects, but not with another pool of sera of two nonallergic subjects, is illustrated. No Ab binding was observed for the proteins isolated from host *E. coli* strain with or without the nonrecombinant plasmid or the buffer in which the FP was solubilized. Furthermore, the reactivity of human IgE Abs with the proteins isolated from KBG pollen and with FP was examined by Western blotting. In the left panel in Fig. 1, B, is illustrated an electrophoretogram of extracts of KBG pollen and of FP-producing *E. coli* separated by SDS-PAGE. The FP appeared as a major 81 kd component of *E. coli* ex-

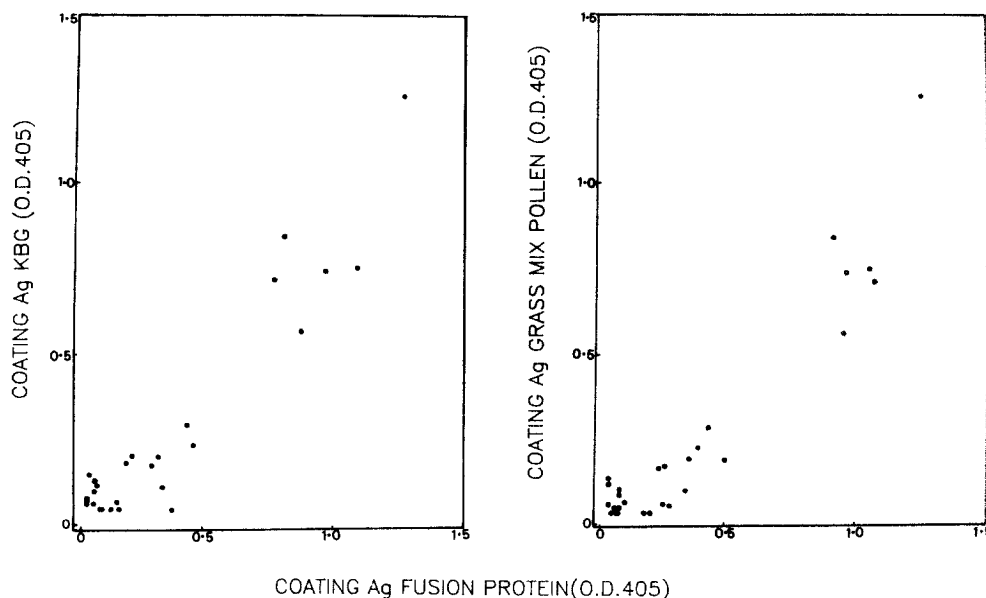


FIG. 3. Correlation analysis of IgE reactivity with FP versus KBG extract as coating Ags (**left panel**) and of reactivity with FP versus a protein mixture of 10 grass pollens as coating Ags (**right panel**).

tract. Densitometric analysis revealed that FP comprised about 40% of total cell protein (data not presented).

A nitrocellulose blot of the above gel was treated with sera of patients allergic to grass pollens for detection of IgE-binding proteins (Fig. 1, *B, right panel*). The IgE Abs in this pool of sera reacted with four polypeptides in the range of 28 to 35 kd from the KPP and with the 81 kd FP. To identify the KBG polypeptide that corresponds to the FP, an identical membrane blot was treated with an aliquot of the above IgE pool that had been preincubated with the FP. The immunoblotting results with the absorbed sera is illustrated in Fig. 1, *B, center panel*. Lack of reactivity with the FP band indicated that the IgE corresponding to the FP had been completely removed from these sera. Interestingly, the IgE reactivity of three other KPP components with MW 28 to 34 kd were reduced in intensity, whereas the reactivity with the major 35 kd component was marginally affected. Moreover, the components with MW 28 to 34 kd were barely detectable in the stained gel. It is therefore inferred that these proteins that correspond to FP constitute minor components of KBG-pollen extracts in terms of their relative abundance.

To assess the allergenicity of the recombinant peptide, the capacity of FP to bind the IgE Abs in sera of allergic humans was examined. A comparison of the FP- and KPP-specific IgE Ab levels of individual sera at 1:10 dilution is illustrated in Fig. 2. A total of 55 patients were classified into three groups based on

their SPT results to grass pollen mix; that is, the sera of patients with a score of 2+, 3+, or 4+ were examined separately. Although sera of approximately 50% of the patients revealed low reactivity (equivalent of 0.1 OD unit in ELISA) with IgE Abs, clearly high scores in SPT were associated with high IgE Ab levels, irrespective of the coating Ag, that is, KPP or FP. These results indicate that within a given group of patients with the same SPT scores, the relative IgE binding to the FP was comparable to that of KPP. Furthermore, a higher SPT score was associated with higher specific IgE binding.

Since the FP reacted with $\geq 95\%$ of patients' sera examined, which suggested that the cloned peptide is a clinically relevant allergenic component of grass pollen, we were prompted to compare the IgE Ab reactivity of 31 individual sera of FP with that of KPP, and also with a mixture of proteins from 10 different grass pollens. A positive correlation ($r = 0.90$) was found between the FP- and KPP-specific IgE levels in individual sera (Fig. 3). Similarly, a positive association was observed in levels of IgE Abs corresponding to FP and mixture of 10 grass-pollen extracts.

The result from the microtiter ELISA for IgE Ab levels, specific to the FP, GPP, and KPP, are illustrated in Fig. 4. The individual patient's sera demonstrated different IgE Ab levels, and the Ab levels varied with different coating Ags. The highest OD values were observed with KPP, followed by GPP, and then by FP. For each individual serum, the OD value with FP

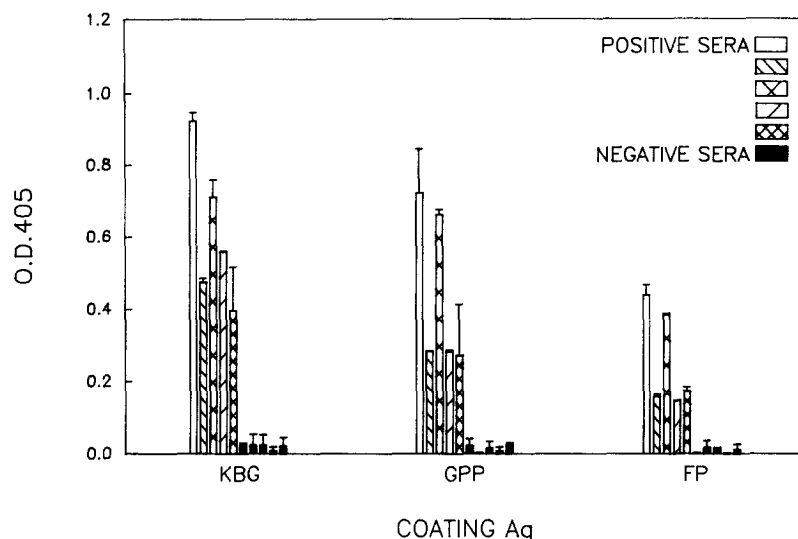


FIG. 4. Comparison of specific IgE reactivity in five patients (indicated by symbols) and five nonatopic individuals as control by ELISA. Either KBG protein extracts GPP, or FP at 5 $\mu\text{g/ml}$ was used as coating Ag.

as coating Ag comprised about 50% and 80% of that with KPP and GPP, respectively. However, sera from five control, nonatopic subjects did not demonstrate any detectable reactivity. Similarly, unrelated Ags, such as β -galactosidase and BSA, did not elicit any reactivity (data not presented) with these five sera from allergic patients. It is inferred that recombinant peptide contains a dominant allergenic epitopes of grass pollens.

Previously, it was found that FP was capable of eliciting an immune response in mice. Thus, both IgG¹¹ and IgE¹⁸ Abs specific to the recombinant peptide were produced in mice by immunization with the FP. To examine the presence of T cell epitopes on the rKBG 7.2, proliferative responses of PLN cells were examined from mice immunized with 60 μg each of the FP (Fig. 5, A) or KPP (Fig. 5, B). At >10 $\mu\text{g/ml}$, FP demonstrated higher proliferation than the background. This increase was not due to its association with β -galactosidase, which by itself induced significantly lower stimulation than FP. A similar proliferative response was observed with the PLN cells isolated from mice that were immunized by KPP (Fig. 5, B). Con A, in contrast, demonstrated a different proliferative response of PLN cells. In this case, the highest proliferation was observed at 10 $\mu\text{g/ml}$, and moreover, higher incorporation was observed with cells isolated from FP-immunized mice than from KPP-immunized mice. Furthermore, the PLN cells from mice immunized with an unrelated protein, such as ovalbumin, when cells were exposed to FP in vitro, demonstrated little stimulation compared to

that of ovalbumin (data not presented). These results suggest that the recombinant peptide contains some epitopes that may be responsible for T cell proliferation.

Finally, the FP and the natural KBG allergens (KPP or GPP preparation) were compared for their abilities to specifically stimulate the murine PLN cells isolated from mice immunized with KPP. The results are summarized in Fig. 5, C. Clearly, the KPP at all concentrations exhibited the highest proliferation of PLN cells, as indicated by [³H]thymidine incorporation. Interestingly, the FP supported higher proliferation of PLN cells than GPP at all concentrations, suggesting that the recombinant peptide may possess a dominant T cell epitope.

DISCUSSION

A unique advantage of the recombinant DNA technology is the production of pure proteins and their peptides in required amounts from corresponding DNA molecule cloned in *E. coli*. Although the number of available recombinant proteins continues to increase at a rapid pace, it is only recently that this technology has been applied to the production of allergens. In this article is reported the high-level expression in *E. coli* of a cDNA coding for a protein of KBG pollen. The allergenic properties of the FP are compared with properties of its counterpart(s) present in the pollen extract with respect to (1) its binding ability to human IgE Abs and (2) its capacity to stimulate proliferation of PLN cells.

Interestingly, absorption of the serum pool with

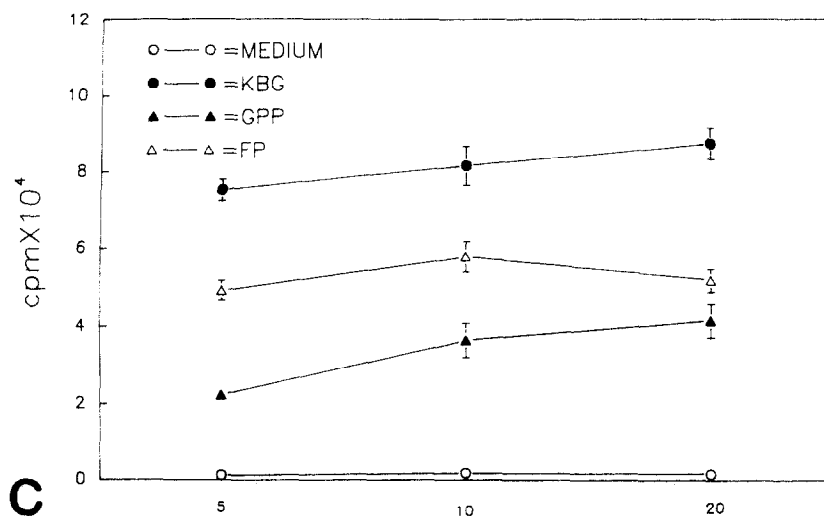
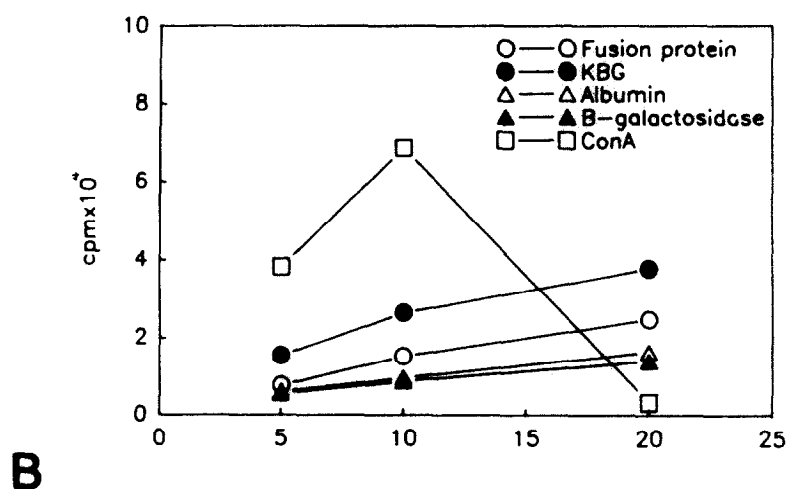
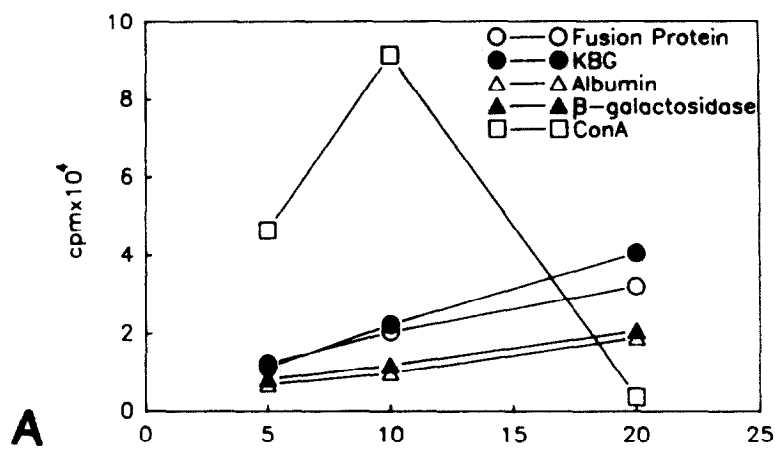


FIG. 5. In vitro proliferation of PLN cells stimulated at different concentrations of Ag, that is 5, 10, and 20 µg/ml. Con A, β-galactosidase, and BSA were used as control. The [³H]thymidine incorporation was measured as counts per minute (after subtracting the background). The values are means of three triplicates ± SE (>5%). **A**, Proliferation of KBG-pollen extract-primed cells, immunized by FP. **B**, Proliferation of FP-primed PLN cells, immunized by KBG. **C**, Comparison of [³H]thymidine incorporation of KBG-pollen extract-primed PLN cells stimulated with KBG proteins, FP, and GPP proteins. No stimulation was observed with medium alone.

insoluble FP led to selective removal of IgE Abs reactive with 28 to 34 kd proteins and the supernatant after absorption reacted only with the major 35 kd band. This effect could be due to either dilution or nonspecific removal of part of the total IgE. However, this is unlikely because of the following: (1) In a dot-blot assay, it was revealed that IgE reactivity to KBG after absorption was comparable to 1:1 dilution of serum pool before absorption, that is, ~50% IgE Abs bound is specific to FP. (2) It was possible to elute the Abs from the FP-Ab complexes, and these Abs reacted to the polypeptides in the range of 28 to 34 kd and not to the 35 kd band (unpublished results). (3) Moreover, murine Abs to another peptide, that is, rKBG 8.3, which is, in part, identical in amino acid composition to the recombinant peptide, rKBG 7.2, recognized three polypeptides in the same MW range on an SDS-PAGE immunoblot.¹¹

It is noteworthy that the recombinant allergenic fragment has enabled us to identify the proteins encoded by the KGB 7.2-homologous cDNAs. Although these proteins appeared to be present in barely detectable amounts in this batch of pollen, as is evident from the SDS-PAGE electrophoretogram, the production of this recombinant peptide at high level made possible the identification of the natural allergenic proteins in KBG pollen. The fact that about three polypeptides were recognized by the IgE Abs corresponding to the FP indicated the existence of a group of isoallergenic proteins in KBG pollen. More recently, three full-length cDNA clones were isolated and sequenced.¹¹ Indeed, KBG 7.2 was found to constitute a common fragment of the above cDNAs. To the best of our knowledge, the allergenic proteins encoded by these cDNAs have not been described before, and therefore, these corresponding KBG-pollen proteins were classified as a new group, designated as *Poa p IX*.

This study exclusively used the ELISA procedure for the determination of the allergenic proteins without any reference to absolute levels of IgE Abs. For this purpose, initially, the sensitivity and reproducibility of the system were established. Thus, with five individual sera, it was clearly demonstrated that 50% of total IgE binding to KPP was due to the IgE Abs specific to FP. Furthermore, the specificity of the system was examined by ELISA-inhibition assay (data not presented). Serum of the above five individuals, in 1:10 dilution, preincubated with FP (30 µg/ml), inhibited in a range of 5% to 60% of specific IgE binding to KPP and 70% to 85% of that binding to FP, whereas IgE binding to KPP and FP were inhibited 85% and 50% to 70%, respectively, by serum that was preincubated with KPP (30 µg/ml). The results obtained by ELISA procedure were therefore consid-

ered valid and conclusive. The observed positive correlation for IgE reactivity with the cloned peptide and total crude extract suggest that the cloned allergenic peptide is a major component of grass pollen. It is apparent from the correlation studies of IgE reactivity with FP versus KPP and FP versus grass mix that the highly purified recombinant allergens not only may facilitate an accurate diagnosis of allergy by in vitro tests (RIA or ELISA), as is presented in this article, but also may aid discriminatory diagnosis of allergy to different grass-pollen components by SPTs.

The studies involving proliferation of murine PLN cells in response to FP suggest the existence of T cell recognition structures on the recombinant peptide. It is yet to be established that murine IgE has a similar role in mice to its role in hypersensitivity in humans. However, it was found that both human and mouse IgG Abs recognized similar epitopes on allergenic molecule¹⁹ and that some of these epitopes also recognized murine T cells. Evidence presented in a previous article¹⁸ from our laboratory indicated that mice also produce IgE Abs in response to the recombinant peptide, rKBG 7.2. Thus, these studies have led to the establishment of a murine model that may be useful for examining the efficacy of therapy involving recombinant allergens and their peptides with or without chemical modification.

In essence, our previous data,¹¹ along with studies of the allergenic properties of the recombinant peptide reported here, indicate that *Poa p IX* group of proteins constitute major allergenic proteins of grass pollens. Experiments are currently underway to synthesize peptides corresponding to the other *Poa p IX* cDNAs and their fragments in *E. coli* and to determine complex profiles of T and B cell epitopes of this important group of allergens, which are responsible for the IgE response in atopic patients. It is anticipated that the precise determination of epitope structures would permit designing appropriate immunotherapeutic modalities for the treatment of allergies.

We thank Ms. Kathy Risk for excellent secretarial assistance, and Mrs. Magon O'Conner for her skillful assistance in skin testing of the subjects.

REFERENCES

1. Norman PS. Immunotherapy for nasal allergy [Symposium]. *J ALLERGY CLIN IMMUNOL* 1988;81:992-6.
2. Creticos BS, Norman PS. Allergoids and immunotherapy. In: Lessof MH, Lee TH, Kemeny DM, eds. *Allergy: an international textbook*. New York: John Wiley, 1987:617-30.
3. Schon AH. Suppression of IgE antibody responses with tolerogenic conjugates of allergens and haptens. In: Ishizaka K, ed. *Progress in allergy*. Basel: S Karger AG, 1982:161-202.
4. Chua KY, Stewart GA, Thomas WR, et al. Sequence analysis of cDNA encoding for a major house dust mite allergen, *Der p I*, homology with cysteine proteases. *J Exp Med* 1988; 167:175-82.

5. Thomas WR, Stewart GA, Simpson RJ, et al. Cloning and expression of DNA coding for the major house dust mite allergen, *Der p 1*, in *Escherichia coli*. *Int Arch Allergy Appl Immunol* 1988;85:127-9.
6. Tovey ER, Johnson MC, Roche AL, Gobon GS, Baldo BA. Cloning and sequencing of cDNA expressing a recombinant house dust mite protein that binds human IgE and corresponds to an important low molecular weight allergen. *J Exp Med* 1989;170:1457-62.
7. Breiteneder H, Pettenburger K, Bito A, et al. The gene coding for the major birch pollen allergen, *Bet v 1*, is highly homologous to a pea disease resistance response gene. *EMBO J* 1989;8:1935-8.
8. Fang KSY, Vitale M, Fehlner P, King TP. cDNA cloning and primary structure of a white-face hornet venom allergen, antigen 5. *Proc Natl Acad Sci USA* 1988;85:895-9.
9. 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.
10. 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.
11. Silvanovich A, Astwood J, Zhang L, et al. Nucleotide sequence analysis of three *Poa p* IX isoallergens of Kentucky bluegrass pollen. *J Biol Chem* 1991;266:1204-10.
12. Mohapatra SS, Sehon AH, Hill RD. Complementary DNA cloning, expression in *Escherichia coli*, and analysis of IgE epitopes of *Poa p* allergen. In: Sehon AH, Kraft D, Kunkel G, eds. *Epitopes of atopic allergens*. Brussels: UCB Institute of Allergy Research, 1990:61-4.
13. Ford SA, Baldo BA. A reexamination of ryegrass (*Lolium perenne*) pollen allergens. *Int Arch Allergy Appl Immunol* 1986;81:193-203.
14. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 1970;227:680-5.
15. Guo L, Stepien PP, Tso JY, et al. Synthesis of human insulin gene. VIII. Construction of expression vectors for fused proinsulin production in *Escherichia coli*. *Gene* 1984;29:251-4.
16. Marston FAO. The purification of eukaryotic polypeptides expressed in *Escherichia coli*. In: Glover D, ed. *DNA cloning*, vol 3. Oxford: IRL, 1987:59-88.
17. Ekramoddoullah AKM, Kisil FT, Cook RT, Sehon AH. Proliferative immune responses by murine popliteal lymph node cells to high molecular weight basic allergen of rye grass pollen and its cyanogen bromide fragments. *Int Arch Allergy Appl Immunol* 1986;79:397-403.
18. Mohapatra SS, Hill RD, Sehon AH. Molecular cloning of allergens: progress and perspectives. In: Vijay H, Comotoia P, Crompton C, eds. *Proceedings of First Pan-American meeting of aerobiology and health, aerobiologia* [in press].
19. Atassi MZ, Atassi H. Antibody and T-lymphocyte recognition regions on ragweed allergen, *Amb a* III (Ra3). In: Sehon AH, Kraft D, Kunkel G, eds. *Epitopes of atopic allergens*. Brussels: UCB Institute of Allergy Research, 1990:33-40.

AVAILABLE NOW! The PROCEEDINGS OF THE INTERNATIONAL CONGRESS OF ALLERGOLOGY AND CLINICAL IMMUNOLOGY can be purchased from the Publisher. This collection of "state-of-the-art" presentations from the XII Congress held October 20-25, 1985, in Washington, D.C., brings together the current advances in basic and applied aspects of allergy and allergic diseases. It includes 528 pages covering such topics as IgE, roles of the different cell types and their products, clinical problems, asthma, rhinitis, and reactions to foods and drugs and occupational agents, collected and reviewed by Editor Charles E. Reed, MD (USA) and Associate Editors Joseph Bellanti, MD (USA), Robert J. Davies, MD (UK), Sidney Friedlaender, MD (USA), Albert Oehling, MD (Spain), and Raymond G. Slavin, MD (USA).

To purchase, call or write: Mosby-Year Book, Inc., 11830 Westline Industrial Dr., St. Louis, MO 63146-3318, or telephone FREE 1-800-325-4177, Journal Fulfillment, ext. 4351 (in Missouri call collect at 314-872-8370, Journal Fulfillment, ext. 4351). Prepayment required. Make checks payable to Mosby-Year Book, Inc. (All payments must be in US funds drawn on a US bank.) Price: \$36.50 in the US, \$40.50 in Canada, and \$41.50 international (surface shipping charges included).