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IMMUNOCHEMICAL CHARACTERIZATION OF A
HIGH MOLECULAR WEIGHT BASIC ALLERGEN (HMBA)
OF RYE GRASS (*LOLIUM PERENNE*) POLLEN*

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Abstract—A high molecular weight basic allergen (HMBA) was isolated from the mixture of non-dialysable components of the aqueous extract of defatted rye grass pollen by a combination of gel filtration and isoelectrofocussing. HMBA, a glycoprotein of mol. wt 56,800 (17% carbohydrate) contained all naturally occurring amino acids. A hyperimmune rabbit anti-HMBA serum gave only a single precipitin band with the crude extract of the rye grass pollen in crossed immunoelectrophoresis. Thus, it was concluded that HMBA was a unique and highly purified antigen. The allergenicity of HMBA was revealed by its ability to elicit immediate skin reactions in grass allergic patients. Moreover, all patients' sera tested had IgE antibodies to HMBA detectable by direct RAST with HMBA allergosorbent discs. These observations indicated that HMBA was a major allergenic constituent of rye grass pollen. Treatment of HMBA by 6 M guanidine HCl led to a significant reduction in its ability to combine with human IgE antibodies. The treatment also resulted in the complete loss of allergenicity (i.e. inability to elicit PCA reactions with a murine reagent antiserum to HMBA) and antigenicity (inability to form precipitins with rabbit anti-HMBA); hence, it would appear that the allergenic and antigenic determinants of HMBA are 'conformational'.

INTRODUCTION

The common form of treatment for allergies to the various constituents of pollens consists of administering a series of injections of the crude extract of the offending pollen containing a heterogeneous mixture of components. As was shown in a number of studies, this treatment may elicit (or promote) the production of IgE antibodies to some pollen constituents to which the allergic individuals had not been originally sensitive (Richter *et al.*, 1957; Marsh *et al.*, 1972). Obviously, therefore, it would be preferable to establish first the sensitivity profile of each allergic individual to the corresponding allergenic constituents and then to treat the patients with the purified allergen(s)

to which they are actually sensitive. With this aim in mind we began isolating and purifying allergens from Kentucky blue grass (KBG)† pollen (Ekramoddoullah *et al.*, 1980; 1981a; Chakrabarty *et al.*, 1981).

Following the demonstration that cytochrome *c* of KBG pollen was allergenically active (Ekramoddoullah *et al.*, 1981a) we embarked on a program for isolating the cytochromes *c* from related grass pollens [e.g. Timothy (*Phleum pratense*) (Ekramoddoullah *et al.*, 1981b) and rye grass (*Lolium perenne*) (Ekramoddoullah *et al.*, 1982)] and comparing their allergenic specificities. As a by-product of these studies, a highly allergenic and apparently pure allergenic component with an estimated mol. wt of the order of 56,800 and a pI of 9.9 was readily isolated from RG pollen. Since all of the known allergens isolated to date from related pollen, i.e. KBG pollen, have mol. wts in the range of 4,800–16,500, the component isolated from rye grass pollen was designated as high mol. wt basic allergen (HMBA). The present study describes the immunochemical characterization of HMBA and its immunologic relationships with groups I, II and III allergens described by Marsh *et al.* who have extensively studied the rye grass pollen allergens (Johnson & Marsh 1965a, 1965b,

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† Abbreviations used: RG, rye grass; Rye-R, Rye-Retentate; KBG, Kentucky blue grass; SDS PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; RAST, radioallergosorbent test; PCA, passive cutaneous anaphylaxis; i.v., intravenously; i.p., intraperitoneally; i.d., intradermally; GAME, affinity chromatography purified goat antibodies to mouse IgE; C.I.E., crossed immunoelectrophoresis; PBS, phosphate buffered saline; IB, incubation buffer; BSA, bovine serum albumin; HSA, human serum albumin; RIA, radioimmunoassay; PIEF, preparative isoelectrofocussing; HMBA, high molecular weight basic allergen.

1966a, 1966b; Marsh *et al.*, 1966; Lichtenstein *et al.*, 1969; Marsh, 1975).

MATERIALS AND METHODS

Preparation of HMBA from RG pollen:

HMBA was isolated according to the procedures recently developed for the isolation of cytochrome *c* from KBG pollen (Ekramoddoullah *et al.*, 1981a). Briefly, an aqueous extract of RG pollen (Hollister-Stier Laboratory, Mississauga, Ontario, Canada) was prepared by extracting defatted pollen with distilled water at room temp. The aqueous extract was dialyzed through spectrapor membrane tubing (mol. wt cut off = 6-8,000; Spectrum Medical Industries Inc., Los Angeles, CA, U.S.A.) and the non-dialyzable components designated as Rye-Retentate (Rye-R) were fractionated on Sephadex G-50 (Pharmacia AB, Uppsala, Sweden) with 0.05 M Tris-HCl, pH 8.6, as the eluant. Fraction Rye-R-I (Fig. 1) was rechromatographed on Sephadex G-50 and was separated by preparative isoelectrofocusing using ampholyte buffer, pH range 3.5-10 (LKB Products AB, Stockholm-Bromal, Sweden). At the end of the isoelectrophoretic run, the gel was equally divided into 38 fractions. The extreme cathodal fraction was further separated by gel filtration on Ultrogel

AcA 44 (LKB 2204-440, Industrie Biologique Française, Gennevilliers, France) equilibrated with 0.05 M phosphate buffer, pH 7.2, containing 0.15 M Na₂SO₄. Fraction II (Fig. 1) was rechromatographed on AcA 44 to obtain HMBA.

Protein determination

Unless stated otherwise, the amounts and concentration of various fractions are expressed in terms of their protein content which was determined by the method of Lowry *et al.*, 1951).

Amino acid analysis

Samples containing HMBA were hydrolyzed with HCl at 110-120°C for 24, 48 and 72 hr and the hydrolysates were analyzed with the Beckman automatic amino acid analyzer, Model 120-139 (Beckman, Palo Alto, CA). The concentrations of serine, threonine and tyrosine were calculated by extrapolation to zero time hydrolysis and those of valine and isoleucine were calculated from the analysis of the 72 hr hydrolysate. Cysteine was determined as cysteic acid following oxidation of the sample with performic acid (Hirs, 1956) prior to acid hydrolysis and the tryptophan content was measured spectrophotometrically (Edelhoc, 1967). The concentrations of other amino acids were calculated from the analysis of the 48 hr hydrolysate.

Determination of carbohydrate content

The phenol sulfuric acid method (Montgomery, 1961) was employed to determine the carbohydrate content of HMBA. A volume of 0.1 ml of an aqueous solution of phenol (80%) was added to a volume of 2 ml of an aqueous solution containing 10-20 µg of HMBA. Five ml of concentrated H₂SO₄ was added to the mixture with constant agitation. After standing at room temp. for 30 min the absorbance of the solution at 490 nm was measured in a Coleman UV-VIS spectrophotometer. A reference blank solution was similarly prepared by substituting water for the solution of HMBA. A standard curve was established with mannose in the concentrations ranging from 5-40 µg.

Polyacrylamide gel-electrophoresis

Disc electrophoresis in polyacrylamide gel was performed at pH 4.3 using the method described by Reisfeld *et al.* (1962). For the estimation of molecular weight, SDS PAGE was

used as described by Laemmli (1970). The gel was stained according to the method of Fairbanks *et al.* (1971) and the mobility of the bands was measured as described by Weber & Osborn (1969).

Skin tests

The stock solution of HMBA in saline (0.1 mg/ml) was passed through a sterile Millipore membrane, 0.45 µm pore size (Millipore Corporation, Bedford, MA, U.S.A.). A volume of 20 µl of the stock solution, appropriately diluted, was injected i.d. into patients sensitive to grass pollen(s); saline was injected into control sites. After 15 min, the size of the wheal was measured.

Immunization protocols

Murine reagenic antisera containing IgE antibodies to HMBA were prepared in accordance with a procedure previously established for the production of IgE antibodies to KBG pollen allergens (Ekramoddoullah *et al.*, 1977). HMBA (dose range 0.1-50 µg) admixed with freshly prepared Al(OH)₃ (1 mg or 5 mg) was injected i.p. into C3H/HeJ mice. The sera obtained from the blood of 8 mice immunized in an identical manner were pooled; each pool is referred to as a murine reagenic serum.

The levels of IgE antibodies present in the murine reagenic sera were established by PCA in hooded rats obtained from the University of Manitoba vivarium (Gunton, Manitoba, Canada) (Ekramoddoullah *et al.*, 1977).

To produce precipitating antibodies to HMBA, New Zealand white rabbits (Kleefeld Rabbitry, Box 5, Tourond, Manitoba, Canada) were immunized by i.d. injections into multiple sites, each animal receiving on any one occasion 200 µg HMBA emulsified with Freund's complete adjuvant (Difco Laboratories, Detroit, MI, U.S.A.). The rabbit antisera to HMBA used in this study were collected from rabbits which received immunizing regimens (i.e. a total of 600 µg HMBA) at intervals of 4 weeks.

Immunodiffusion

Ouchterlony (Ouchterlony, 1949) double diffusion analysis was performed in 1% agar (Difco) in PBS (0.9% NaCl, 0.05 M phosphate buffer, pH 7.4, 0.05% sodium azide) in plastic Petri dishes. To avoid the formation of the immune precipitates adjacent to the antibody wells, the antiserum was allowed to diffuse into

the agar for a period of 3 hr prior to the addition of antigens into their respective wells.

Crossed immunoelectrophoresis (CIE)

CIE was performed essentially as described by Axelsson *et al.* (1973) and as modified by Lowenstein (1978). The electrophoresis (Electrophoretic Apparatus, Safety Model V, Holm-Nielsen Aps, 17, Generatorvej, Post Base 41, DK 2730, Herlev, Denmark) was carried out in 1% agarose (Indubiose A-37, Industrie Biologique Française, Gennevilliers, France) in buffer (0.073 M Tris, 0.025 M barbital, 0.006 M calcium lactate and 0.003 M sodium azide, pH 8.6). Electrophoresis in the first dimension was performed at 10 V/cm for 1/2 hr and in the second dimension at 1-2 V/cm for 18 hr in gels containing rabbit anti-HMBA antiserum.

Treatment of HMBA with guanidine-HCl

A solution of HMBA (4 mg/ml) was prepared in 6 M guanidine HCl (Schwartz/Mann, Division of Becton, Dickinson and Company, Orangeburg, NY, U.S.A.). The solution was kept at room temp. for 1 hr and then extensively dialyzed in spectrapor membrane tubing (mol. wt cut off = 6-8 × 10³) against water to remove guanidine hydrochloride and the protein concentration of the treated preparation was then determined.

Labelling of antibodies

Antibodies were labelled with ¹²⁵I by the procedure of Hunter & Greenwood (1962). The sp. act. of the labelled protein was of the order of 6 × 10⁶ cpm/µg protein.

Allergenic activity of HMBA as compared to that of the Rye-R by direct RAST

The RAST was performed in disposable flexible polyvinyl chloride microtiter plates (12.7 cm × 9.5 cm, 96 round "U" wells; Dynatech Laboratories, Inc. 900 Slaters Lane, Alexandria, VI, U.S.A.). The wells were coated with a solution of allergen consisting of 10 µg in 100 µl of coating buffer (1.59 g of Na₂CO₃, 2.93 g of NaHCO₃, and 0.2 g of NaN₃ made up to 1 liter with distilled water, pH 9.6) by maintaining the plates at 4°C in a moist chamber for 18 hr. The solutions of allergen were then withdrawn and the plates washed with PBS (200 µl/well). The capacity of the wells to further adsorb proteins was blocked by coating with an unrelated protein such as HSA using the incubation buffer (IB) (PBS containing

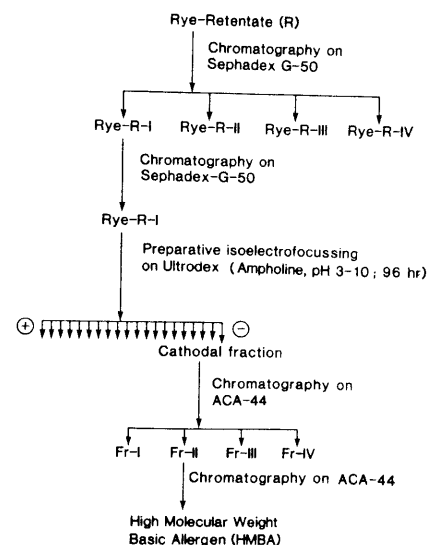


Fig. 1. Flow sheet for the isolation of HMBA.

0.3% HSA) according to the conditions described for the coating procedure. Finally, the plates were washed 3 times with PBS (200 μ l/well). Volumes of 100 μ l of an allergic serum (which had been diluted two-fold with IB) were added to the allergen-coated wells and kept overnight at room temp.; the serum samples were then withdrawn and the wells washed 3 times with IB containing 1% Tween 20 (Fisher Scientific Company, Fair Lawn, NJ, U.S.A.). ^{125} I-labelled rabbit anti-human IgE antibodies (Pharmacia, AB, Uppsala, Sweden, 5 μ Ci/0.8 μ g) were added to the wells ($\sim$ 100,000 cpm/well) and incubated overnight at room temp. The wells were washed 3 times with PBS containing 1% Tween 20, and cut out from the plates. The levels of radioactivity were measured in a Beckman gamma counter.

Allergenic and antigenic relationship of HMBA with rye grass allergens gr-I, gr-II, gr-III

(i) *Inhibition of RAST.* To establish the degree of allergenic cross-reactivity between HMBA and rye grass allergens gr-I, gr-II and gr-III generously supplied by the National Institutes of Health, (Bethesda, MD, U.S.A.), the procedure of inhibition of RAST (Gleich *et al.*, 1974) was employed with the aid of pool of six sera from patients allergic to grass pollen. Allergosorbent discs were prepared by coupling HMBA to cyanogen bromide activated paper discs (Whatman 3 mm filter paper, 6 mm dia., 10 μ g of HMBA/disc). Volumes of 20 μ l each of the serum pool were mixed with varying quantities of a given allergenic fraction expected to cross-react with HMBA (i.e. expected to inhibit the RAST, and after standing for 3 hr at room temp. each mixture was added to an allergosorbent disc. The discs were incubated overnight at 4 C and then washed with PBS. The extent to which the IgE antibodies bound to the allergosorbent was evaluated by the addition of ^{125} I-labelled rabbit anti-human IgE antibodies. The reduction in the uptake of the ^{125} I-anti-IgE antibodies by the allergosorbent disc, in relation to the amount of radioactivity bound to the disc in absence of the test fraction, was expressed as the percent of inhibition of RAST by the formula: % inhibition = (A-B)/A $\times$ 100, where A = cpm in the absence of inhibitor, B = cpm in the presence of inhibitor.

(ii) *RAST with murine anti-HMBA reagent sera.* The allergenic cross-reactivity between HMBA and gr-I, gr-II, and gr-III allergens was

evaluated in terms of the ability of these allergens to combine with murine anti-HMBA reagent sera. For this purpose, microtiter plates were coated with each allergen and the RAST was performed as outlined for the direct RAST with the following modifications: for the blocking stage, PBS containing 1% bovine serum albumin was used; murine reagent sera were serially diluted with PBS containing 2% normal rat serum (before adding to the allergen-coated plate); ^{125} I-labelled GAME was used for determination of the extent to which murine IgE antibodies had become attached to the antigen-coated wells. GAME was prepared according to the procedure described elsewhere (Kelly *et al.*, 1980).

(iii) *RIA with rabbit anti-HMBA.* The antigenic cross-reactivity between HMBA and gr-I, gr-II and gr-III allergens was evaluated in terms of the ability of these allergens to combine with the rabbit antiserum to HMBA. For this purpose, the RIA was performed as outlined above with the following modifications: the rabbit anti-HMBA serum was serially diluted with PBS containing 0.1% BSA and 0.05% Tween 20; the amount of the IgG antibody bound was evaluated with ^{125} I-labelled sheep antibodies to rabbit IgG.

RESULTS

Physicochemical properties of HMBA

As stated earlier, HMBA was isolated from RG with the aid of the protocol (shown in Fig. 1) which had been described for the isolation of KBG cytochrome c. As is evident from Fig. 2, HMBA migrated primarily as a single component on PAGE and its mol. wt. determined by SDS-PAGE, was of the order of 56,800. Disc electrophoresis also revealed the presence of a trace amount of a component that was not observed on SDS-PAGE. Based on measurements made on the preparative isoelectrofocussing run, the pI of HMBA was estimated as 9.9. HMBA contained approximately 17% carbohydrate and its protein moiety contained all the naturally occurring amino acids (Table 1). Although HMBA is a highly basic protein (pI value = 9.9), the acidic amino acids, i.e. aspartic acid and glutamic acid, were found to be present in amounts higher than the basic amino acids, i.e. arginine, histidine and lysine. Since asparagine and glutamine in a protein are converted into glutamic

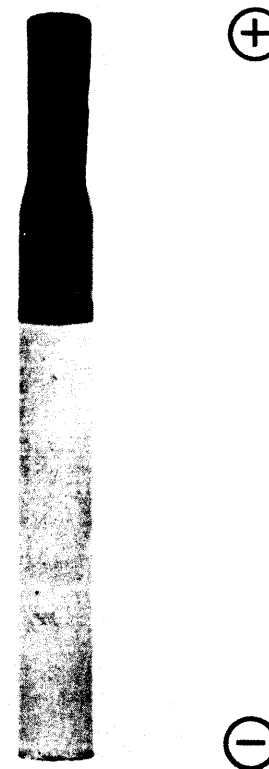


Fig. 2. Polyacrylamide gel electrophoresis pH 4.3 of HMBA (25 μ g sample).

and aspartic acids, respectively, during acid hydrolysis, the amounts of aspartic and glutamic acid determined by the acid hydrolysis of HMBA may indeed be higher than their actual values. To obtain the true values, it will be necessary to identify the individual amino acids in the course of the complete sequence analysis of HMBA.

Allergenic activity of HMBA

By means of the direct RAST, the allergenic activity of HMBA was compared with that of Rye-R which contains most of the allergenic constituents of rye grass pollen. Twenty-five sera from individuals allergic to grass pollens were analyzed by this method and all the sera had significant levels of anti-HMBA antibodies (Fig. 3). These results clearly indicate that sera of all the grass allergic individuals reacted with HMBA. Furthermore, the majority ($\sim$ 75%) of

Table 1. Amino acid composition of HMBA

Amino Acid	Residue/Molecule
Alanine	21
Arginine	24
Aspartic acid	48
Cysteine	7
Glutamic acid	40
Glycine	47
Histidine	7
Isoleucine	28
Leucine	37
Lysine	31
Methionine	11
Phenylalanine	21
Proline	34
Serine	27
Threonine	23
Tryptophan	38
Tyrosine	24
Valine	40

the sera had RAST values higher with the HMBA allergosorbent than with the Rye-R allergosorbent. In direct skin tests on two untreated allergic patients the lowest concentration of HMBA able to elicit an immediate skin reaction of 2⁺ (i.e. erythema, 21-30 mm; wheal, 5-10 mm) was 0.01 μ g/ml. These results further support the conclusion that HMBA is an important allergenic component of rye grass pollen.

Immunogenic properties of HMBA

From the profiles of the IgE antibody responses following a single immunization of C3H/HeJ mice with HMBA at different doses (Fig. 4), it is obvious that HMBA was capable of inducing an IgE antibody response in C3H/HeJ mice and that the highest response was elicited at a dose of 5 μ g of HMBA with 1 mg of Al(OH)₃; hence, in subsequent experi-

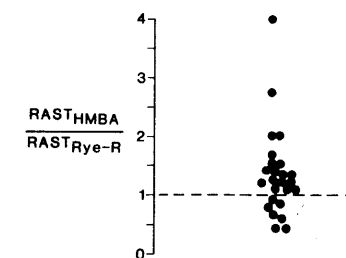


Fig. 3. Allergenic activity of HMBA relative to that of Rye-R. Each serum was tested by RAST using allergosorbent discs prepared with HMBA or Rye-R.

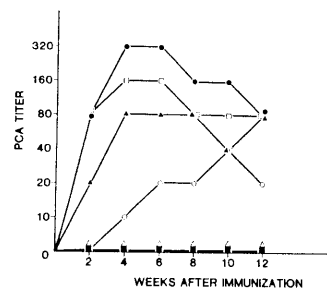


Fig. 4. IgE antibody responses following single injections of HMBA at different doses in C3H/HeJ mice. PCA titers were determined in hooded rats; ●, 5 µg HMBA in 1 mg Al(OH)₃; ▲, 1 µg HMBA in 1 mg Al(OH)₃; ■, 0.1 µg HMBA in 1 mg Al(OH)₃; ○, 5 µg HMBA in 5 mg Al(OH)₃; △, 5 µg HMBA in 5 mg Al(OH)₃; □, 1 µg HMBA in 5 mg Al(OH)₃.

ments this dose was used to produce murine anti-HMBA reaginic sera.

HMBA was also capable of inducing the formation of precipitating antibodies in rabbits. The CIE pattern of Rye-R obtained with rabbit anti-HMBA (Fig. 5a) indicated that the antiserum was monospecific for HMBA. Moreover, both HMBA and Rye R (the latter consisting of a heterogeneous mixture of components) elicited a single precipitin peak following CIE analysis with the rabbit antiserum to HMBA (Fig. 5). Hence it may be concluded that the HMBA, which had been used as the immunizing antigen, was pure and was capable

of eliciting the formation of antibodies which recognized only one component (i.e. HMBA). In a separate analysis, utilizing CIE and a hyperimmune rabbit antiserum to the crude extract of RG pollen, Lowenstein (1982) in a personal communication, confirmed that HMBA had a high degree of purity. He also noted that HMBA apparently shared immunochemical identity with the major antigenic component present in Ag30 of Timothy grass pollen (Lowenstein, 1978), and Allergen C of KBG pollen (Chakrabarty *et al.*, 1981).

Effect of guanidine-HCl on the allergenic and antigenic activities of HMBA

The guanidine-HCl treated HMBA, at a challenging dose of 200 µg, did not elicit any PCA reactions in rats passively sensitized with undiluted murine reaginic antiserum to HMBA. By contrast, the untreated HMBA, at the same challenging dose, elicited strong PCA reactions with reaginic serum diluted 320 fold. Moreover, a challenging dose of 2 µg of the untreated HMBA gave PCA reactions with the reaginic antiserum diluted 20-fold. This result suggests that the allergenic determinants of HMBA, recognized by the murine IgE antibodies, are destroyed by guanidine-HCl. However, no attempt was made to estimate the extent of denaturation, if any, following treatment with guanidine-HCl. It was also noted that the ability of the guanidine HCl treated

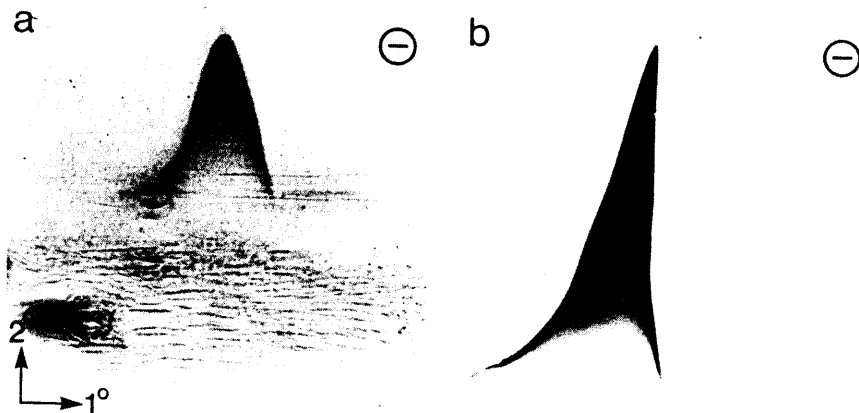


Fig. 5. CIE analysis of (a) Rye-R and (b) HMBA. Sample applied: 70 µg of Rye-R and 14 µg of HMBA. The electrophoresis in the first direction was performed at 10 V/cm for a period of 30 min and in the second dimension at 1.2 V/cm for 18 hr in gels containing rabbit anti-HMBA antisera.

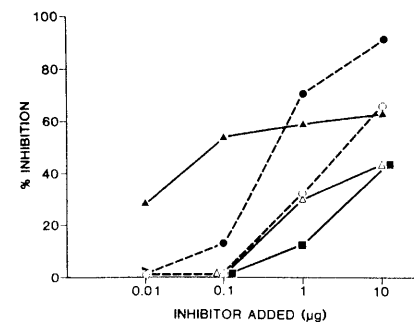


Fig. 6. Demonstration of allergenic cross-reactivity of HMBA and rye grass pollen allergens gr-I, gr-II and gr-III by inhibition of RAST using HMBA allergosorbent discs and a pool of human allergic sera. Inhibitors: ●, HMBA; ○, guanine hydrochloride treated HMBA; ▲, gr-I; △, gr-II; ■, gr-III (■).

HMBA to combine with human IgE antibodies was significantly reduced (Fig. 6).

The treatment also affected the antigenicity of HMBA. Thus, whereas a native HMBA at a concentration of 1 mg/ml gave a strong precipitin line with rabbit anti-HMBA antisera, the treated HMBA (at the same concentration) elicited only a weak precipitin line having a partial identity with the native HMBA. In view of the known denaturing effect of guanidine-HCl on proteins, our findings may suggest that the allergenic and antigenic activities of HMBA are dependent on the conformational integrity of the molecule.

RELATIONSHIPS BETWEEN HMBA AND GR-I, GR-II AND GR-III ALLERGENS

Allergenic cross-reactivity

The extent of the allergenic cross-reactivity among the four different purified rye grass allergens was evaluated by the inhibition of RAST using allergosorbent discs prepared with HMBA. It is evident from Fig. 6 that the addition of the three allergens (i.e. gr-I, gr-II or gr-III) in increasing amounts over the range of 0.01–10 µg to the serum from grass sensitive patients resulted in increasing inhibition of the binding of human IgE antibodies to the HMBA allergosorbent and that the gr-I allergen was the most effective inhibitor among the three. Hence, it may be concluded that HMBA cross-reacts allergenically with gr-I, gr-II and gr-III allergens.

The allergenic relationship was also exam-

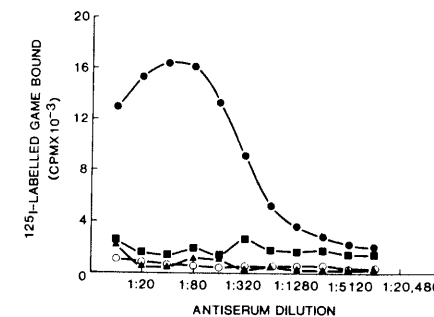


Fig. 7. Evaluation of allergenic activity of HMBA and Rye Grass pollen allergens gr-I, gr-II and gr-III by direct RAST utilizing murine anti-HMBA reaginic sera and allergosorbents prepared with: ●, HMBA; ▲, gr-I; ■, gr-II; ○, gr-III.

ined by PCA utilizing murine anti-HMBA reaginic sera. Remarkably, although this anti-HMBA serum had a PCA titer of 320 in relation to HMBA, none of the other three RG allergens elicited any PCA reaction even at challenging doses of 200 µg per rat. Consistent with these findings, the RAST utilizing ¹²⁵I-GAME (Fig. 7) also indicated that allergens gr-I, gr-II and gr-III failed to combine with the murine anti-HMBA reaginic sera. It was concluded that immunization of C3H/HeJ mice with HMBA did not elicit the production of IgE antibodies capable of recognizing allergenic determinants present on the gr-I, gr-II and gr-III allergens.

Antigenic cross-reactivity

An examination of the antigenic relationships of HMBA with gr-I, gr-II and gr-III by immunodiffusion analysis on agar gel did not prove useful since gr-I, gr-II and gr-III did not form precipitin lines with the rabbit anti-HMBA antisera. However, when the antigenic relationships were analyzed by radioimmunoassay, gr-I, gr-II and gr-III allergens were found to combine with the rabbit anti-HMBA antisera (Fig. 8). Moreover, the binding profiles for the three allergens were very similar to each other which suggested that the antibodies to HMBA were recognizing an antigenic specificity that was common to all these allergens.

DISCUSSION

A high molecular weight basic allergenic constituent, HMBA, was isolated from RG pol-

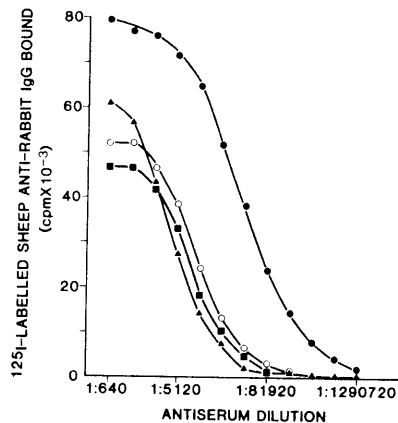


Fig. 8. Demonstration of antigenic cross-reactivity of HMBA and rye grass pollen allergens gr-I, gr-II and gr-III by RIA employing rabbit antiserum to HMBA and microtiter plates coated with: ●, HMBA; ▲, gr-I; ■, gr-II; ○, gr-III.

len according to a protocol recently developed in this department for the isolation of cytochromes *c* from KBG and RG pollens (Ekramoddoullah *et al.*, 1981a, 1982). HMBA was found to be antigenically related to various allergenic components of KBG and Timothy grass pollens (Lowenstein, 1982). This observation is consistent with an earlier report (Chakrabarty *et al.*, 1981) which demonstrated extensive antigenic and allergenic cross-reactivity of components of related grass pollens.

It was noted that the molecular size and charge of the purified HMBA were very similar to those of a partially purified fraction of RG pollen previously designated as gr-IV (Marsh, 1975). However, whereas Marsh had stated that only a small percentage of grass allergic individuals were sensitive to gr-IV, this observation, in association with a consideration that high molecular weight components are not readily permeable across mucosal membranes, led Marsh to postulate that gr. IV was not an important allergen (Marsh, 1975). In marked contrast, the high molecular weight basic allergen described in this study was clearly shown to be recognized by all grass allergic patients studied and therefore should be regarded as a major allergen of RG.

In numerous studies on the allergens of rye grass pollen (Johnson *et al.*, 1965a, 1965b, 1966a, 1966b; Marsh *et al.*, 1966; Lichtenstein *et al.*, 1969; Marsh, 1975) the purified allergens, I, II and III and partially purified allergens of Group IV, were shown by immunodiffusion

(utilizing rabbit antisera) to be antigenically distinct from one another. In the present study we found that the Groups I, II and III allergens did not form a precipitin band with rabbit anti-HMBA antiserum and it would therefore appear that HMBA possessed an antigenic determinant(s) that was antigenically distinct from those present on the Group I, II and III allergens. On the other hand, utilizing a radio-immunoassay, the presence of a common antigenic determinant(s) detectable with the rabbit antiserum to HMBA among these allergens was demonstrated. These observations may be interpreted as indicating that each of gr. I, II and III allergens possessed only a single determinant on each molecule that could be recognized by the rabbit antibody to HMBA. As a consequence, immunoprecipitation would not be readily achieved. On the other hand, the specific binding to the insolubilized allergen (i.e. allergosorbent) by the rabbit antibody, which recognized a determinant common to this group of allergens, was readily detected. An allergenic cross-reactivity of HMBA with the Groups I, II, and III allergens was also demonstrated by RAST using human sera from grass allergic individuals. Although a similar pattern of cross-reactivity of these allergens was established with either the rabbit or human sera, our studies have not resolved the question as to whether or not the different antisera recognized the same or similar determinant(s). An alternate possibility, that the two antisera recognized different determinant(s), cannot be ruled out at the present time.

The fact that HMBA possessed at least one allergenic determinant that was not detectable on Groups I, II and III allergens was clearly demonstrated with the murine reagenic antiserum to HMBA. This apparent absence of cross-reactivity may be attributable to the failure of the inbred strain of mice to produce IgE antibodies to a common determinant(s) which was detected by the human IgE antibodies. Our studies on the cross-reactive nature of the allergens serve to emphasize the need for exercising caution in comparing the results of experiments where antisera produced in different animals were employed. In spite of this shortcoming, antisera which recognize a specific allergenic determinant(s) are valuable in the fact that they can be used to selectively identify and quantitate an allergen which may be present in a mixture of different allergenic components.

A pollen allergen, ragweed antigen E, that incurred a loss of allergenic activity on treatment with a denaturing agent such as urea, has been shown to be capable of suppressing the IgE antibody response to the native antigen in mice (Ishizaka *et al.*, 1975). Thus, the finding that the allergenic activity of HMBA could be destroyed by treatment with guanidine-HCl suggests that further studies be made to evaluate the capacity of the treated HMBA to suppress the murine IgE antibody response to the native allergen.

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