

Cross-reactive and unique grass group I antigenic determinants defined by monoclonal antibodies

Robert E. Esch, Ph.D.,* and David G. Klapper, Ph.D. Chapel Hill, N. C.

As part of a study to probe the immunochemical basis for allergenic cross-reactivity among grass pollens, a series of useful reagents has been prepared. The major grass-pollen allergen, designated group I (GpI), was isolated from five grass pollens (meadow fescue, June grass, sweet vernal grass, redtop grass, and perennial ryegrass). The purified GpI antigens were used to immunize individual groups of BALB/c mice. A total of 123 hybridoma-derived anti-GpI monoclonal antibodies (mAbs) was produced. These mAbs were used to evaluate the antigenic relationship among the GpI antigens by means of two types of ELISA. The experiments revealed a high level of epitope diversity and demonstrated a wide range of antibody specificities. A cross-reactivity ELISA was used to identify and compare antigenic determinants on the GpI molecules, and it was possible to define mAbs with specificities unique for the immunizing allergen and other mAbs that cross-reacted with one or more other members of the test panel of allergens. These murine mAbs reflect the range of specificities present in sera from grass-allergic individuals. (J ALLERGY CLIN IMMUNOL 1987;79:489-95.)

The principal allergenic fraction of many temperate grasses important in hay fever is a group of related glycoproteins designated GpI antigens.¹ Cross-reactivity among the GpI antigens of several grasses of the subfamily Festucoideae has been well established. This has primarily been demonstrated by the ability of antiserum raised against the perennial ryegrass GpI antigen to bind to components present in crude pollen extracts derived from the related grasses.² In addition to the GpI antigens, it appears that grass pollens have a number of cross-reacting allergens.^{3,4} These observations support the idea that at least in some individuals multiple sensitivities to different grass-pollen allergens follow exposure and sensitization by a single grass pollen. If this is correct, then it might be possible to reduce both the number of grass pollens used as therapeutic extracts and the complexity of the extracts without compromising the efficacy of these materials.⁵ These possibilities emphasize the need to identify and characterize the allergenic determinants of GpI antigens and other cross-reactive allergen systems. The

Abbreviations used

GpI:	Group I
mAb:	Monoclonal antibody
MF:	Meadow fescue, <i>Festuca elatior</i>
PR:	Perennial ryegrass, <i>Lolium perenne</i>
J:	June grass, <i>Poa pratensis</i>
RT:	Redtop grass, <i>Agrostis alba</i>
SV:	Sweet vernal grass, <i>Anthoxanthum odoratum</i>
PVC:	Polyvinyl chloride
PBS:	Phosphate-buffered saline
SDS-PAGE:	Sodium dodecyl sulfate-polyacrylamide gel electrophoresis

availability of mAbs with the desired specificities might provide a means to investigate the molecular basis of this cross-reactivity. With this in mind, panels of mAbs were produced against the purified GpI antigens derived from five different species of grass previously demonstrated to possess cross-reactive GpI antigens based on serologic criteria. The ability of these mAbs to identify both unique and cross-reactive determinants on the five GpI molecules with a simple direct-binding ELISA developed for this purpose serves as the basis of this study.

MATERIAL AND METHODS

Antigens

GpI antigens from MF, J, PR, SV, and RT were purified from whole pollen extracts (Greer Laboratories, Lenoir,

From the Department of Microbiology and Immunology, University of North Carolina School of Medicine, Chapel Hill, N. C.

Supported by National Institutes of Health Grant AI14908.

Received for publication Jan. 30, 1986.

Accepted for publication Aug. 23, 1986.

Reprint requests: David G. Klapper, M.D., Dept. of Microbiology and Immunology, U. of North Carolina School of Medicine, 804 Faculty Laboratory Office Bldg. 231-H, Chapel Hill, NC 27514.

*Present address: Greer Laboratories, Inc., P.O. Box 800, Lenoir, NC 28645.

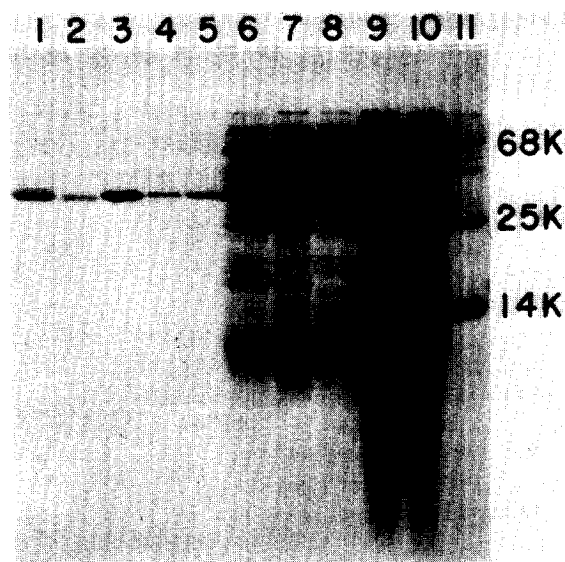


FIG. 1. SDS-PAGE of crude extracts and purified Gpl antigens from the five grasses studied in this article. Lane 11, molecular weight markers; lanes 1 and 6, MF; 2 and 7, PR; 3 and 8, J; 4 and 9, SV; 5 and 10, RT (purified Gpl and crude pollen extracts, respectively).

N. C.) with a modification of the method described by Marsh.¹

Briefly, the lyophilized crude extracts were reconstituted with 0.01 mol/L of sodium acetate buffer, pH 5.5, applied to a CM-biogel column (Bio-Rad Laboratories, Richmond, Calif.), and eluted with a 0 to 0.2 mol/L NaCl gradient. Fractions were used to coat PVC microtiter plates (Dynatech Laboratories, Alexandria, Va.) after dilution in pH 9.6 carbonate-coating buffer (6.67 mmol/L of sodium carbonate, 35 mmol/L of sodium bicarbonate, 0.02% sodium azide). A direct-binding ELISA with a goat antiperennial rye Gpl antiserum (a gift of D. G. Marsh, Johns Hopkins University, Baltimore, Md.) was used to detect Gpl containing fractions. The Gpl antigens were further purified by gel filtration chromatography on Sephadex G-75 (Pharmacia, Piscataway, N. J.) and anion exchange chromatography on a diethylaminoethyl-biogel column (Bio-Rad) with a linear NaCl gradient (0 to 0.2 mol/L). The purity of the isolated antigen was assessed by SDS-PAGE under reducing conditions.⁶ All five purified Gpl preparations yielded a single polypeptide with a molecular weight of approximately 27,000.

Immunization

Groups of female BALB/c mice (Jackson Laboratory, Bar Harbor, Me.) were immunized intraperitoneally with 0.2 ml of an emulsion containing 100 μ g of Gpl antigen and Freund's complete adjuvant. The mice were boosted at least twice at 4- to 5-week intervals with the same material. The mice were bled 3 to 7 days after each booster injection to obtain polyclonal antisera. A final booster injection (Gpl antigen in saline) was administered to the mice 3 to 4 days

before the mice were sacrificed to obtain spleens for hybridization.

Cell fusion and production of mAbs

Spleen cells from immunized BALB/c mice were hybridized with 45.6TG1.7 BALB/c plasmacytoma cells (a gift of D. Scott, University of Rochester, Rochester, N. Y.) or with nonsecreting P3X63-Ag8.653 cells (a gift of Dr. Gillespie, University of North Carolina, Chapel Hill, N. C.) with 33% polyethylene glycol 1500 (Fisher Scientific Co., Pittsburgh, Pa.) as the fusogen. The day after fusion, the cells were plated into 96-well microculture wells. Cells from the 45.6TG1.7 fusions were plated in Dulbecco's modified Eagle medium containing 10 mmol/L of hypoxanthine, 1 mmol/L of aminopterin, 3 mmol/L of thymidine, 1 mmol/L of sodium pyruvate, 2 mmol/L of L-glutamine, penicillin (100 U/ml), streptomycin (100 μ g/ml), fungizone (2.5 mg/ml), 50 μ mol/L of 2-mercaptoethanol, and 15% v/v of fetal calf serum. Cells from the P3X63-Ag8.653 fusions were plated in Dulbecco's modified Eagle medium mixed with Ham's F12 at 1:1 v/v, containing the same supplements as above. The resulting hybridoma cultures were screened for the presence of anti-Gpl antibodies by a direct-binding ELISA.

Positive hybridomas were cloned with limit dilution or soft agar cloning methods. The hybridoma cell lines were expanded, and their culture supernatants were harvested and saved for subsequent screening. Twelve fusions were performed from which 749 hybridoma-containing wells were screened for antibodies against the respective immunogen. Of the 253 positive hybridomas, 123 were successfully cloned and characterized.

ELISAs

ELISAs were performed essentially as described by Voller et al.⁷ Antigens, diluted in the pH 9.6 carbonate-coating buffer (2000 μ l), were used to coat PVC microtiter plate wells by overnight incubation at 4° C. After washing the wells three times with PBS-Tween, dilutions of anti-Gpl antibody preparations (200 μ l) prepared in PBS-Tween containing 1.0 mol/L NaCl were added and incubated for 3 hours at room temperature for direct ELISAs. In the ELISA-inhibition assays, the antibody preparations were premixed with varying concentrations of inhibitor immediately before addition. The plates were again washed with PBS-Tween before the addition of 200 μ l of the second antibody in PBS-Tween. The dilutions of the second antibodies, alkaline phosphatase-labeled goat antimouse IgG (H and L chain specific, Sigma Chemical Co., St. Louis, Mo.), or alkaline phosphatase-labeled goat antihuman IgE (epsilon chain specific, Sigma Chemical Co.) were determined by titration to the highest dilution eliciting maximal absorbance values (1:2000 and 1:500 dilutions, respectively). After 3 hours of incubation at room temperature followed by three washes with PBS-Tween, the enzyme substrate p-nitrophenyl phosphate was added at a concentration of 1.0 mg/ml in diethanolamine buffer at pH 9.8. Absorbance was measured at 405 nm with a Dynatech au-

tomated ELISA reader. The time course of color development under the assay conditions was found to be linear during the first 60 minutes of incubation at room temperature.

Optimal conditions for performing the direct binding and competitive inhibition assays were determined in preliminary experiments. For ELISAs used to investigate cross-reactivity, dilutions of hybridoma culture supernatants yielding absorbance values approximately 50% of the maximum attainable with homologous GpI antigen in 30 minutes were determined. A typical direct-binding experiment consisted of conducting titrations with varying concentrations of solid-phase GpI antigen (0.001 to 10.0 µg/ml) and a fixed dilution of a hybridoma cell-culture supernatant. Saturation of the plastic surface with antigen was obtained with antigen-coating concentrations in the 1.0 to 10 µg/ml range. Measurable adsorption values were observed at antigen concentrations as low as 0.01 µg/ml. In the paratyping experiments, a fixed dilution of hybridoma cell-culture supernatant was incubated with the various GpI antigens under saturating conditions. Since most of the GpI antigens demonstrated extensive cross-reactivity, an irrelevant antigen and mAb, human immunoglobulin light chain and an antishort ragweed antigen E mAb (HaAl) were used as specificity controls. Nonspecific effects were consistently <5%.

Inhibition assays based on the measurement of human IgE antibodies were accomplished by preincubating dilutions of sera from grass-allergic humans with various concentrations of inhibitor before adding the mixture to antigen-coated wells. After overnight incubation at room temperature, IgE antibodies bound to the allergosorbent were detected with enzyme-labeled antihuman IgE. Absorbance measurements were made after adjusting the background with a normal human serum pool. Percent inhibition was calculated as follows:

$$\% = \frac{A_o - A_i}{A_o} \times 100$$

where A_o is the absorbance at 405 nm in the absence of inhibitor and A_i is the absorbance in the presence of inhibitor.

RESULTS

Purification of GpI allergens

Pollen (50 to 100 gm) was extracted overnight at 4° C, and a combination of molecular sieve and ion exchange chromatography after the general protocol of Marsh¹ was followed to purify the GpI activity. Purity and activity were monitored by SDS-PAGE and solid-phase ELISA with an antiperennial ryegrass GpI allergen kindly provided by Dr. David G. Marsh. As presented in Fig. 1, a single major protein was isolated from each sample of grass pollen. This material is highly reactive with the anti-GpI antiserum and is the appropriate molecular weight for GpI allergen. The yield of each GpI allergen was >50% of theoretical, and each preparation of allergen was positive for car-

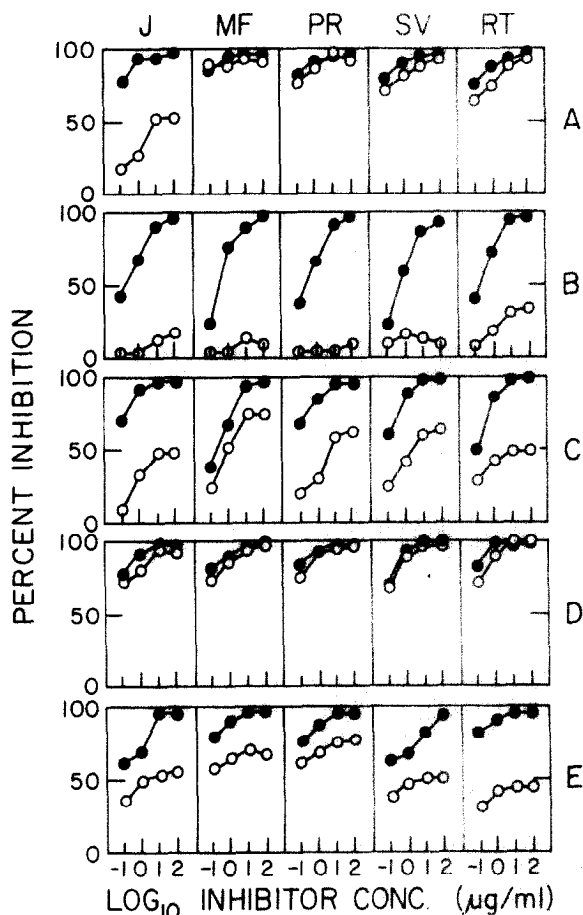


FIG. 2. IgE ELISA inhibitions demonstrating the allergenic activity of purified GpI antigens with respect to the whole grass-pollen extracts prepared from J, MF, PR, SV, and RT grasses that were used to coat PVC microtiter-plate wells. The binding of IgE antibodies in sera from grass-allergic individuals to these allergosorbents was inhibited with either the same whole pollen extract (●) or the purified GpI antigen isolated from the respective grass pollens (○).

bohydrate (3% to 5% w/w) by means of phenol-sulfuric acid analysis.

Allergenic activity of purified GpI antigens

Purified GpI antigens were tested for their ability to compete with whole pollen extracts for human IgE antibodies from grass-allergic individuals. Fig. 2 presents the results of IgE ELISA inhibitions with GpI antigens as inhibitors of IgE binding to solid-phase whole grass-pollen extracts from which the purified antigens were isolated. Sera from five different sources of allergic patients were used for this evaluation. The serum in panel A was a pool obtained from the Research Resources Branch of the National Institutes of Health and collected by the Mayo Clinic. In this assay, whole pollen extract was coated onto a

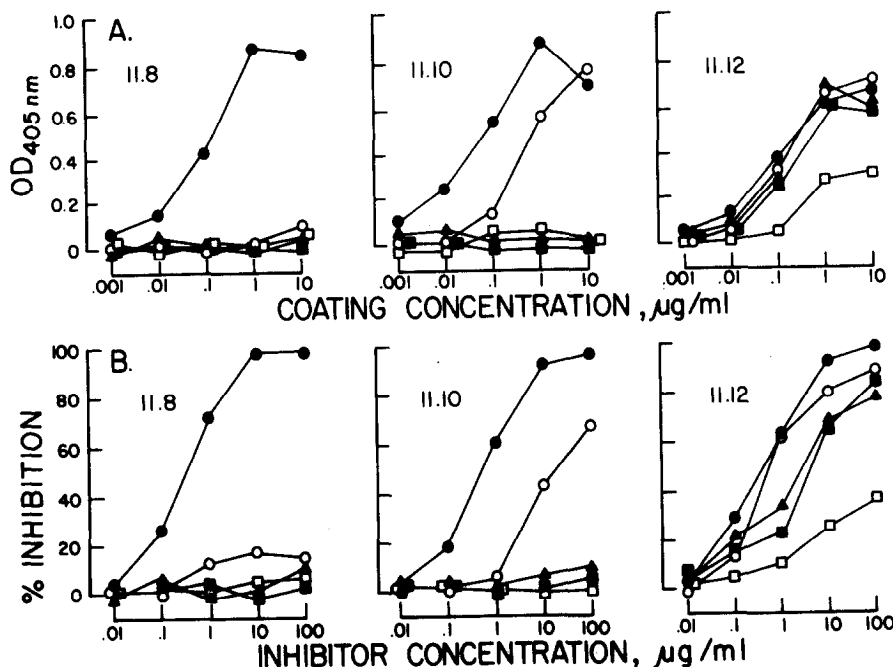


FIG. 3. A, Direct-binding ELISAs. B, ELISA inhibitions. Both ELISAs demonstrating differences in fine specificity of three representative anti-MF GpI mAbs (11.8, 11.10, and 11.12). The direct-binding ELISA compared the ability of each of the three mAbs to bind solid-phase MF (●), PR (○), J (▲), RT (■), or SV (□) GpI antigens. The ELISA inhibitions compared the ability of each of the five GpI antigens to compete for mAb binding to solid-phase MF GpI antigen.

microtiter plate, and the ability of one of the five purified GpI proteins to inhibit IgE binding to the coated material was observed. In this particular serum pool, with the exception of the J extract, it appeared that virtually all of the allergic activity was directed against GpI allergenic determinants. Approximately 50% of the activity against J extract in this serum pool could not be inhibited by purified GpI allergen and was therefore directed against other noncross-reactive components in the crude extract. In panel B, an example of serum of an allergic individual is depicted; it can be observed that most IgE binding could not be inhibited by purified GpI allergen. Panel C is another serum from an individual that demonstrated variable, but significant, reactivity of IgE with GpI determinants. Panel D depicts serum from an individual in which virtually all the IgE activity was apparently directed against GpI antigens. Panel E represents another pool of sera from grass-allergic individuals obtained from the Biomedical Reference Laboratory (Burlington, N. C.). The data obtained with this pool were similar to data obtained with serum from the allergic individual in panel C, i.e., variable but significant inhibition of IgE binding to crude extract with purified GpI allergen. These results suggest that there is great variability in the allergic response to GpI allergen, potentially both in terms of quantitative re-

sponse (percent of specific anti-GpI activity) and qualitative response (activity against epitopes on GpI that are cross-reactive with epitopes on other allergens in the crude extract).⁸ Regardless of these possibilities, the results of these assays demonstrate that purified GpI antigens account for a significant percentage of the total allergenic activity of whole grass-pollen extracts and provide evidence for minimal structural and antigenic deterioration of these GpI molecules during the purification steps.

Cross-reactivity ELISAs

Antigenic relationships among the GpI antigens were initially evaluated in two separate assay systems, a competitive ELISA-inhibition assay and a noncompetitive direct-binding ELISA. In the competitive ELISA-inhibition assay, each of the five purified GpI antigens was used to compete for binding of a particular mAb to its homologous antigen. Each competing antigen was tested for inhibition of binding > a 100,000-fold concentration range in tenfold increments. The data are expressed as the percent inhibition of binding versus concentration of inhibitor. Fig. 3, B, presents representative competitive ELISA-inhibition results obtained with three selected anti-MF GpI mAbs. Cross-reactivity among the GpI antigens with respect to the epitope being recognized by the

mAbs can be detected by comparing the ability of each heterologous antigen to inhibit a mAb binding to its homologous immunogen. In the direct-binding ELISA, depicted in Fig. 3, A, the purified GpI antigens, diluted serially in tenfold increments, were coated onto PVC microtiter plate wells, and a fixed amount of mAb-containing hybridoma culture supernatant was added. MABs were tested for binding to each GpI antigen, and cross-reactivity was detected by comparing the ability of each mAb to bind to either homologous or heterologous antigen.

As presented in Fig. 3, the two assay systems elicited essentially identical results with representative anti-MF GpI mAbs. MAb MF 11.8 demonstrated exquisite fine specificity for the homologous MF GpI antigen in both assays, i.e., only the MF GpI antigen inhibited mAb binding (Fig. 3, B) and the mAb bound only to the MF GpI antigen (Fig. 3, A). Clearly, mAb MF 11.8 recognized an epitope present only on the MF GpI antigen.

The other mAbs appeared to recognize epitopes shared by one or more heterologous GpI antigens. Only the MF GpI and the PR GpI antigens inhibited the binding of mAb MF 11.10 to the solid-phase MF GpI antigen, and this mAb bound only to these GpI antigens in the direct-binding ELISA. The relative position of the dose-response curve suggests that mAb MF 11.10 reacted with the cross-reactive PR GpI epitope with lower affinity. This result implies that the cross-reactive determinant is similar to, but not identical, with the determinant on the MF GpI molecule. MAb MF 11.12 appeared to recognize a cross-reactive epitope shared by all five GpI antigens. When the MF 11.12 mAb was examined in the ELISA-inhibition assay, the SV GpI antigen was significantly less potent as an inhibitor relative to the inhibiting potencies of the other four GpI antigens. The direct-binding ELISA results were consistent with the ELISA inhibition data in that binding of mAb MF 11.12 to the SV GpI antigen was significantly reduced.

The close correlation between the two assay systems and the similar shapes of the dose-response curves allowed for a simplification of cross-reactivity assays based on a comparison of the relative direct binding of a mAb to each GpI antigen with respect to the homologous GpI antigen. This direct assay is technically much more simple than the inhibition assay previously described. With the direct-binding ELISA, a cross-reactivity pattern (paratype) for each of the 123 mAbs raised against the five purified GpI preparations was determined. Results of this assay obtained with the anti-MF GpI mAb panel are presented in Fig. 4. Relative binding was expressed by way of a modification of the convention of Gerhard

mAbs to MF GpI	GpI Antigens				
	MF	PR	J	SV	RT
11.1	■	■	■	■	■
21.2	■	■	■	■	■
21.4	■	■	■	■	■
11.12	■	■	■	■	■
21.1	■	■	■	■	■
11.16	■	■	■	■	■
11.20	■	■	■	■	■
11.7	■	■	■	■	■
11.13	■	■	■	■	■
11.10	■	■	□	□	□
11.2	■	□	□	□	□
11.8	■	□	□	□	□

FIG. 4. Paratypes of mAbs raised against MF GpI. Relative binding of the 12 anti-MF GpI mAbs to five different grass GpI antigens was determined in a direct ELISA (see Material and Methods). The absorbance measured for the homologous MF GpI antigen was normalized to 100%, and the binding to the heterologous GpI antigens was graded as follows: >50% (■), 25% to 50% (▨), twice background to 25% (▩), and less than twice background (□).

et al.⁹ The absorbance values measured for the homologous system were normalized to 100%, and binding to the heterologous GpI antigens was graded as described in the figure. Of the 12 individual anti-MF GpI mAbs, nine cross-reacted with all four heterologous GpI antigens to some degree, one demonstrated restricted reactivity, and two reacted only with the homologous MF GpI antigen. Eight different paratypes were observed.

This same assay was performed with mAbs raised against the remaining four GpI antigens isolated in this study, and similar distributions of paratypes were observed with these anti-GpI mAb panels. Table I is a compilation of those results and illustrates that most mAbs in all five panels (91/123) recognized cross-reactive epitopes present on all GpI antigens, con-

TABLE I. Summary of paratyping results with antigress GpI monoclonal antibodies

	Anti-GpI mAb panels					Total
	MF	PR	J	SV	RT	
mAbs*	12	13	29	21	48	123
Specific	2	1	2	4	6	15
Restricted	1	3	2	1	11	17
Common	9	9	25	16	32	91
No. of different paratypes	8†	10	17	5	27	60

*The antigress GpI mAbs were divided into three categories: specific, binding only to the homologous (immunizing) GpI antigens; restricted, binding to one or more, but not all, heterologous GpI antigens; common, binding to all five GpI antigens to some degree.

†See Fig. 4.

firming the close antigenic relationship among these antigens. Seventeen mAbs demonstrated restricted reactivity, i.e., reacted with some, but not all, GpI antigens, and 15 mAbs reacted only with the homologous GpI antigen. In this set of mAb reagents, at least one mAb that reacted selectively with the homologous GpI antigen was represented in each panel. These mAbs apparently recognize antigenic determinants unique to each of the respective GpI molecules and thus might prove useful in the development of species-specific GpI antigen immunoassays.

A total of 60 distinct paratypes were observed among the 123 mAbs. This finding demonstrated the use of the paratyping assay in that a wide range of antibody specificities can be detected by a relatively simple assay. Despite the complex array of cross-reactivities detected with the mAbs, the presence of both unique and common antigenic determinants was clearly identified with the mAb panels.

DISCUSSION

Complete information about the antigenic and allergenic relationship among the GpI antigens on the molecular level must await sophisticated biochemical and biophysical analysis, such as determination of the amino acid sequence of the GpI antigens and their three-dimensional structure. Valuable information, however, can be derived from detailed immunochemical studies.^{10, 11} Studies comparing related proteins obtained from a variety of sources¹² suggest that the degree of cross-reactivity is directly related to the sequence homology of the tested proteins. Statistical analysis of the reported data predicts that about 80% of all evolutionary amino acid substitutions can be detected with monospecific polyvalent sera. The polyvalent allergic sera examined in this study represented a wide range of specificities for grass GpI allergens, and certain sera might be useful for evaluating GpI cross-reactivity and for pursuing questions relating to the structural correlates of that cross-reactivity.

The use of mAbs in cross-reactivity assays, however, might provide an added dimension to this technique. The homogeneous nature of mAb reagents will allow for further resolution of the structural and antibody-binding domains within a specific molecule. For example, the anti-GpI mAbs described in this article are currently being used as site-specific probes for the localization of human IgE binding sites on GpI antigens. These probes could be used to establish the range of cross-reactive allergenic determinants on GpI molecules. In addition, these anti-GpI mAbs may be useful in defining the relationship between these allergens and other allergens (GpII, GpIII, and GpIV) isolated from the same pollen.

The results obtained with the anti-GpI mAb panels revealed the presence of both cross-reactive and unique antigenic determinants among GpI allergens. A question arising from these results is whether both cross-reactive and unique allergenic determinants can be isolated and characterized. The ability to characterize actual allergenic determinants of GpI molecules would be of practical value for the accurate diagnosis and treatment of grass-pollen allergy.

In connection with standardization of allergenic extracts, mAbs will undoubtedly prove useful.¹³ It has been demonstrated by Krillis et al.¹⁴ with antishort ragweed AgE mAbs, and Kahn and Marsh¹⁵ with antirye grass GpI mAbs, that mAb-based immunoassays capable of detecting nanogram quantities of the specific antigen in whole pollen extracts can be developed. In the latter case, a mAb recognizing a cross-reactive determinant was used to estimate GpI content of extracts derived from several grass species. In view of the complex reactivity patterns displayed by mAbs recognizing cross-reactive determinants on the GpI molecule, further refinements in the assay appear necessary. Anti-GpI mAbs possessing uniform affinities toward the heterologous GpI antigens could be selected in order to achieve more accurate measurements. Furthermore, immunoassays based on mAbs

that possess selective reactivity toward GpI antigens derived from a single species could be used to ensure the identity and purity of allergenic extracts.

The use of mAbs in studies of cross-reactivity and protein structure requires the production of large numbers of hybridoma cell lines recognizing a diverse range of epitopes. The paratyping technique described in this article should facilitate and simplify the screening procedure.

We thank Ms. Joyce Bradshaw for skillful secretarial assistance and Dr. Joseph Olson for helpful discussions.

REFERENCES

1. Marsh DG. Allergens and the genetics of allergy. In: Sela M, ed. *The antigens*, vol. 3. New York: Academic Press, 1975:271.
2. Baer H, Maloney CJ, Norman PS, Marsh DG. The potency and group I antigen content of six commercially prepared grass pollen extracts. *J ALLERGY CLIN IMMUNOL* 1974;54:157.
3. Chakrabarty S, Lowenstein H, Ekramoddoullah AKM, Kisil FT, Sehon AH. Detection of cross-reactive allergens in Kentucky bluegrass pollen and six other grasses by cross radioimmunoelectrophoresis. *Int Arch Allergy Appl Immunol* 1981;66:142.
4. Lowenstein H. Immunological partial identity and in vitro inhibitory effect of two major timothy pollen allergens to whole pollen extract of four grasses. *Int Arch Allergy Appl Immunol* 1978;57:379.
5. Doward AJ, Waclawski E, Kerr JW. A comparison of the clinical and immunological effects of an alum-precipitated five-grass extract with a conjugated two-grass extract in the desensitization of hay fever. *Clin Allergy* 1984;14:561.
6. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 1970;227:680.
7. Voller A, Bidwell D, Bartlett A. Enzyme-linked immunosorbent assay. In: Rose NR, Friedman H, eds. *Manual of clinical immunology*. Washington, D. C.: American Society for Microbiology, 1980:359.
8. Hull WM, Welsh PW, Adolphson CR, Gleich GJ. Reactivity of purified ryegrass-pollen antigens: antigenic and allergenic cross-reactivity [Abstract]. *J ALLERGY CLIN IMMUNOL* 1979;63:193.
9. Gerhard W, Yewdell J, Frankel ME, Webster R. Antigenic structure of influenza virus haemagglutinin defined by hybridoma antibodies. *Nature* 1981;290:713.
10. Berzofsky JA, Schechter AN. The concepts of cross-reactivity and specificity in immunology. *Mol Immunol* 1981;18:751.
11. Diamond AG, Larkins AP, Wright B, Ellis ST, Butcher GW, Howard JC. The alloantigenic organization of RT1A^a, a class I major histocompatibility complex molecule of the rat. *Eur J Immunol* 1984;14:405.
12. White TJ, Ibrahimi IM, Wilson AC. Evolutionary substitutions and the antigenic structure of globular proteins. *Nature* 1978;274:92.
13. Baldo BA. Standardization of allergens. *Allergy* 1983;38:535.
14. Krilis B, Baldo BA, Raison RL, Callard RE, Basten A. Standardization of antigen E in ragweed pollen extracts using a monoclonal antibody-based enzyme immunoassay. *J ALLERGY CLIN IMMUNOL* 1983;71:261.
15. Kahn C, Marsh DG. Analysis of grass group I allergens using monoclonal antibodies [Abstract]. *J ALLERGY CLIN IMMUNOL* 1983;71:95.