

IDENTIFICATION AND LOCALIZATION OF ALLERGENIC DETERMINANTS ON GRASS GROUP I ANTIGENS USING MONOCLONAL ANTIBODIES¹

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Epitopes recognized by five mAb which block the binding of human IgE antibodies to grass group I (GpI) Ag were characterized and partially mapped. Site specificity studies defined four apparently non-overlapping blocking antibody binding sites on the meadow fescue GpI molecule, *Fes e I*. One of these sites (site A) was localized to a 14,000 m.w. fragment designated P3 generated by CNBr cleavage of purified *Fes e I*. The P3 peptide possessed human IgE binding sites as well as other epitopes (non-site A) defined by 19 other anti-GpI mAb. All of the P3 reactive antibodies recognized cross-reactive determinants found on GpI Ag isolated from five different grasses suggesting that P3 is a conserved portion of grass GpI molecules. The P3 fragment from *Fes e I* was used to immunize mice and induced antibodies which reacted with intact GpI Ag from all 5 different grasses currently being studied in this laboratory.

There is considerable evidence indicating that pollen allergens of botanically related grasses are cross-reactive. This cross-reactivity has been demonstrated using a variety of in vitro and in vivo techniques using crude pollen extracts and polyclonal antisera (1-9) or mAb (10-12). Polyclonal antibody preparations have allowed for the identification of cross-reacting Ag in complex mixtures, but have lacked the resolving power required to obtain more precise information about the immune structure of allergens. To overcome these limitations, we produced and characterized 123 murine mAb toward a major cross-reacting grass pollen allergen, GpI, from five different grass species (10). The finding was that most of the mAb cross-reacted with some or all of the other GpI proteins, although some were found to have unique specificity toward immunizing GpI Ag. The broad range of specificities represented by these mAb panels suggested that a large portion of the GpI molecule could be probed. Although complete information about the antigenic and allergenic structures and the molecular basis of cross-reactivity among the grass GpI Ag must await the deter-

mination of their covalent and tertiary structure, valuable information can be derived from detailed immunochemical studies using mAb.

One of the goals of this laboratory is to define and localize epitopes on the GpI Ag that represent targets of the human allergic response. Specific mAb can be used to block the binding of human IgE antibodies in a competitive binding immunoassay to identify allergenic epitopes. This approach was previously used in this laboratory to identify two major allergenic sites on a short ragweed pollen allergen, *Amb a I* (13). In this report, we extended this approach to identify the allergenic sites on the grass GpI molecules.

The highly conformation-dependent nature of the antibody binding sites of some protein allergens, most notably *Amb a I*, have restricted our attempts to localize epitopes on antigenic fragments (13). In contrast, the grass GpI Ag retain much of their antigenic reactivity after denaturation and fragmentation by cyanogen bromide cleavage. This finding has allowed us to produce immunoreactive GpI Ag fragments which possess epitopes recognized by the blocking mAb and human IgE antibodies. One of the isolated peptide fragments when used directly as an immunogen in mice, induced IgG antibodies which cross-reacted with GpI Ag from five different grass species. The work presented in this manuscript is a step toward delineating the epitope structure of an important protein allergen and may provide information for the design of synthetic peptides potentially useful in the treatment of allergy.

MATERIALS AND METHODS

Ag. GpI Ag from meadow fescue (*Festuca elatior*), June grass (*Poa pratensis*), perennial ryegrass (*Lolium perenne*), sweet vernal grass (*Anthoxanthum odoratum*), and redtop grass (*Agrostis alba*) were purified from whole pollen extracts using a combination of ion-exchange and gel filtration chromatography as previously described (10).

Production of mAb. Hybrid cell lines were prepared from fusions between spleen cells from BALB/c mice (The Jackson Laboratory, Bar Harbor, ME) immunized with purified GpI Ag and 45.6TG1.7 BALB/c plasmacytoma cells (gift of D. Scott, Rochester University, Rochester, NY) or non-secreting X63-Ag8.653 cells (gift of Y. Gillespie, University of North Carolina, Chapel Hill, NC) using 33% polyethylene glycol 1500 as the fusogen. An initial characterization of the anti-GpI mAb produced by the hybridoma cell lines has been reported (1). Affinity purification of mAb from hybridoma culture supernatants or Ig containing ascites fractions was accomplished by passage of mAb preparations over a *Fes e I*-coupled Sepharose 4B-CL column and elution with 0.5 M acetic acid. The eluates were immediately neutralized with saturated TRIS and extensively dialyzed against PBS pH 7.4.

Direct binding ELISA. Direct binding ELISA used to determine the cross-reactivity profile of anti-GpI mAb were performed essentially as described previously (10). Briefly, a fixed concentration of mAb in 200 μ l PBS-Tween was allowed to react with each of the five

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purified Gpl Ag adsorbed to PVC⁴ microtiter plate wells. After 4 h of incubation, the wells were washed three times with PBS-Tween and the antibodies bound were detected with a 1/2000 dilution of an alkaline phosphatase-labeled goat anti-mouse IgG (Sigma Chemical Co., St. Louis, MO). After 3 h of incubation at room temperature with the second antibody, the wells were washed three times with PBS-Tween and the enzyme substrate, *p*-nitrophenyl phosphate was added in diethanolamine buffer. The absorbance was read at 405 nm using a Biotek automated ELISA reader (Biotek, Burlington, VT). Relative binding to each of the heterologous Gpl Ag was calculated as follows:

$$\% \text{ relative binding} = (B/A) \times 100$$

where A is the absorbance at 450 nm when reacted with the homologous (immunizing) Gpl Ag and B is the absorbance when reacted with the heterologous Gpl Ag.

Direct binding ELISA were also used to detect immunoreactive Gpl Ag fragments. Isolated peptide fragments were diluted in coating buffer and allowed to adsorb to PVC microtiter plate wells for 6 to 16 h. The wells were washed three times with PBS-Tween and a dilution of an anti-Gpl antibody preparation was added to the wells. When murine polyclonal or mAb were used, an alkaline phosphatase-labeled goat anti-mouse IgG was used as the second antibody. An alkaline phosphatase-labeled goat anti-human IgE (Sigma) was used as the second antibody when human grass-allergic serum was added in the previous step. Absorbance was read at 405 nm after the addition of enzyme substrate as described above.

Blocking assay. The ability of the anti-Gpl mAb to block the binding of human anti-Gpl IgE antibodies was determined in an IgE-ELISA competition assay using a pool of sera collected from grass-allergic patients (pool S2 from the Office of Biologics, FDA, Bethesda, MD). Preliminary experiments with this serum pool showed that it contained IgE antibodies which bound to Gpl Ag purified from each of the five grass pollens. The serum dilution required to obtain 50% maximal binding of IgE antibodies to each solid-phase Gpl Ag was used throughout the blocking studies. A total of 100 μ l of the appropriate dilution of the serum pool was mixed with 100 μ l of a dilution of an anti-Gpl mAb and the mixture was added to Ag-coated PVC microtiter plate wells. mAb concentrations sufficient to saturate the available binding sites on the Gpl allergosorbents were used. After overnight incubation at room temperature and washing with PBS-Tween, the amount of IgE antibodies bound to the Gpl allergosorbent was measured with an alkaline phosphatase-labeled goat anti-human IgE. After 6 h incubation at room temperature, the enzyme substrate was added and the absorbance at 405 nm was read. The blocking activity of the anti-Gpl mAb was expressed in terms of the percent inhibition of IgE binding in the absence of competitor using the formula:

$$\% \text{ inhibition} = ((A - B)/A) \times 100$$

where A is the absorbance at 405 nm in the absence of competitor and B is the absorbance in the presence of competitor.

Double-bind ELISA. A double-bind ELISA was used to define non-overlapping sites recognized by anti-Gpl mAb. In this assay, PVC microtiter plate wells were coated with 200 μ l of a 1/500 dilution of an affinity purified mAb preparation. A 200- μ l volume of Gpl Ag at 2 μ g/ml was incubated with the solid phase mAb for 4 h at room temperature. This concentration of Ag was shown in preliminary experiments to be more than 10 times the amount sufficient to saturate the available antibody combining sites. After three washes with PBS-Tween to remove unbound Ag, the alkaline phosphatase labeled second mAb (25) was added in 200 μ l at a 1/500 dilution. After 4 h incubation at room temperature, the plate wells were washed with PBS-Tween and the binding of the second mAb was detected after addition of the substrate. A positive result was interpreted to suggest that the two mAb recognized distinct and non-overlapping sites on the Gpl Ag molecule.

Cyanogen bromide cleavage. *Fes e I* was digested with CNBr as described by Gross and Witkop (14). To 10 mg of reduced and alkylated *Fes e I* (in 0.5 ml 0.1 N HCl), 15 mg CNBr (in 0.5 ml 0.1 N HCl) was added and the mixture was incubated for 24 h at room temperature. The digested *Fes e I* was diluted five-fold with distilled water and lyophilized to remove volatile side products, excess CNBr, and HCl. SDS-PAGE analysis of the digest revealed three major components, consistent with the methionine content (two residues) calculated from an amino acid analysis (D. G. Klapper, unpublished data). Two fragments corresponded to m.w. of about 12,000 and 14,000 and one fragment migrated close to the tracking dye front, indicating a m.w. of less than 5,000 D.

RESULTS

Blocking antibody activity of anti-Gpl mAb. A total of 53 mAb was screened for their ability to inhibit the binding of human IgE antibodies from a grass allergic serum pool to solid phase Gpl Ag. Three anti-*Fes e I* mAb, MF11.8, MF11.10, MF21.4, one anti-*Loi p I* mAb, PR16.9, and one anti-*Ant o I* mAb, SV24.64 significantly inhibited (>20%) the binding of IgE antibodies to the homologous Gpl Ag. The five blocking mAb were further tested for their ability to inhibit IgE binding to each of the four heterologous Gpl Ag (Fig. 1A). The blocking profiles correlated well with the cross-reactivity profiles of the mAb (Fig. 1B). The specificity of the blocking assays was confirmed by the finding that inhibition was not observed when a mAb did not bind to the respective Ag and that control anti-Gpl mAb did not inhibit IgE binding.

Site specificity studies. The five blocking mAb were tested with the double-bind ELISA to determine whether a pair of mAb recognized overlapping antigenic sites. The test was performed with *Fes e I* because this Gpl Ag possessed epitopes recognized by all five mAb (Fig. 1B). The assay is based on the assumption that when two mAb can simultaneously bind to the Gpl Ag, they are binding to distinct and non-overlapping determinants on the Gpl Ag. The 25 cell matrix of all possible binding pairs of mAb is shown in Table I.

Each of the mAb apparently bound to non-repeated sites because in no case were identical mAb able to bind to the Ag simultaneously. mAb PR16.9 and MF21.4 were the only non-identical antibody pair that were not able to bind to *Fes e I* simultaneously and this result was interpreted to mean that the antigenic determinant(s) recognized by PR16.9 and MF21.4 are sterically overlapping. Thus, the site recognized by PR16.9 and MF21.44 was designated as site A. The results of this experiment suggest that mAb MF11.8, MF11.10, and SV24.64 recognize independent antigenic sites, designated sites B, C, and D, respectively. Inasmuch as mAb are exquisitely sensitive in defining fine specificities, it was decided to compare the specificities of this panel of mAb with the specificity of human IgE for Gpl Ag. If each mAb inhibits human IgE binding by competing for some site on the surface of the Gpl Ag, then it might be expected that combinations of blocking mAb would give rise to additive inhibition of IgE binding. This possibility was tested by examining whether the blocking activities of the mAb were additive when two mAb were added together in the blocking assay. If each of the four sites represented truly distinct IgE binding sites, then additivity should be observed. The results of this experiment (Table II) show that combinations of anti-site A, B, and C did not yield additive inhibitions. Increased inhibition was observed only when anti-site A, B, or C was added simultaneously with anti-site D. These results suggest that sites A, B, and C do not define distinct IgE binding sites but may in fact overlap a common IgE binding site (site I). It is evident, however, that the anti-site D mAb (SV24.64) inhibits the binding of a set of IgE antibodies different from that blocked by the anti-site A, B, and C mAbs (site II).

Localization of blocking antibody and human IgE binding sites on MF Gpl Ag fragments. Preliminary experiments revealed that reduced and alkylated Gpl Ag retained the ability to bind human IgE antibodies and

⁴ Abbreviation used in this paper: PVC, polyvinylchloride.

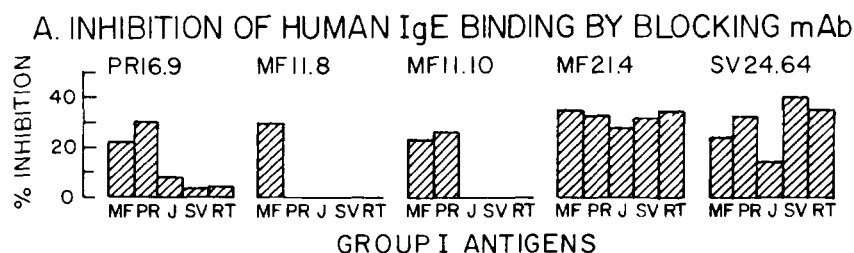


Figure 1. Specificity of (A) blocking and (B) binding activities of five anti-Gpl mAb. In the blocking assay, the ability of the mAbs to inhibit the binding of anti-Gpl IgE antibodies from a grass allergic pool to each of the five Gpl allergosorbents (see below) was measured using a standard IgE ELISA competition as described in *Materials and Methods*. A direct binding ELISA was used to establish the binding specificity of each mAb. The binding to each of the five Gpl allergosorbents was measured relative to that obtained with the homologous Gpl allergosorbent as described in *Materials and Methods*. The Gpl allergosorbents used are as follows: MF, *F. elatior*; PR, *L. perenne*; J, *P. pratensis*; SV, *A. odoratum*; and RT, *A. alb.a*

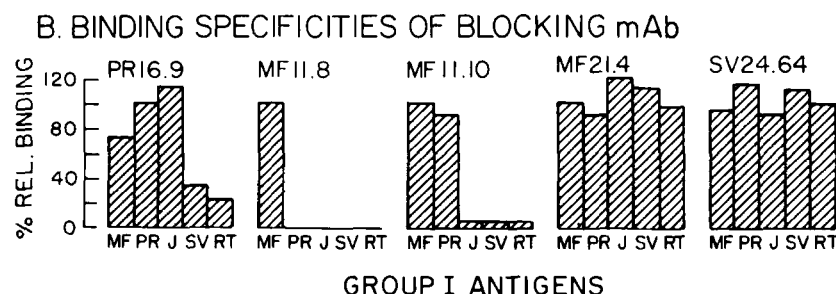


TABLE I

Double bind ELISA defining four non-overlapping blocking mAb sites^a

Solid Phase First Antibody	Labeled Second Antibody				
	PR16.9	MF21.4	MF11.8	MF11.10	SV24.64
PR16.9	—	—	+	+	+
MF21.4	—	—	+	+	+
MF11.8	+	+	—	+	+
MF11.10	+	+	+	—	+
SV24.64	+	+	+	+	—

^a The double bind ELISA used to identify non-overlapping sites is described in *Materials and Methods*. +, detectable binding by the labeled second mAb; —, no detectable binding.

TABLE II

Additivity of blocking activity by pairs of blocking mAb^a

	% Inhibition ^b	
	Theoretical	Observed
Pairs showing additivity		
Anti-A + anti-D	54.3	55.4 (0.5)
Anti-B + anti-D	45.3	49.2 (2.4)
Anti-C + anti-D	44.9	43.1 (2.5)
Pairs not showing additivity		
Anti-A + anti-B	52.6	31.6 (2.8)
Anti-B + anti-C	43.2	22.4 (0.8)
Anti-A + anti-C	52.0	24.5 (1.2)
Negative controls		
Anti-A + anti-A	30.7	28.9 (4.2)
Anti-B + anti-B	21.9	22.4 (2.0)
Anti-C + anti-C	21.3	21.1 (2.0)
Anti-D + anti-D	23.6	24.5 (1.5)

^a The blocking assay described in *Materials and Methods* was modified to accommodate a mixture of blocking mAb: human allergic serum (pool S2) was coincubated with pairs of blocking mAb added in amounts that corresponded to twice the concentration required for maximal inhibition when the mAb were added alone.

^b The theoretical inhibition values were estimates based on the additive values of the maximal blocking activities of individual mAb in the pair: anti-A (mAb MF21.4) = 30.7%; anti-B (mAb MF11.8) = 21.9%; anti-C (mAb MF11.10) = 21.3%; and anti-D (mAb SV24.64) = 23.6%. The observed values were recorded as the mean and SD (in parentheses) obtained from three separate experiments. Negative controls showed that saturating concentrations of each mAb were obtained.

each of the five blocking mAb. These observations suggest that at least some of the human IgE binding sites as well as sites A, B, C, and D constitute "continuous" antigenic sites. Peptide fragments generated by CNBr cleavage of *Fes e I* were prepared with the hope that the Ag

would be cleaved into fragments which retained IgE and/or blocking mAb binding sites. The CNBr peptides were separated by HPLC on a reverse phase C-18 (Fig. 2) column and tested for antigenicity using a direct bind ELISA (Table III).

Antigenic peptide fractions P1, P2, and P3 were identified using a murine polyclonal anti-*Fes e I* antibody preparation. The human grass allergic serum pool contained IgE antibodies which bound to the same three fractions. The inability of mAb MF11.10 (anti-site C) to bind any peptide fraction indicated that site C was destroyed as a result of the CNBr treatment. The possibility that a fragment possessing site C was present in the digest but was unable to coat PVC plastic or that site C was rendered inaccessible to antibody binding after coating was excluded by testing the CNBr peptides in ELISA inhibition assays where fluid phase fragments were used to inhibit the binding of antibodies to intact *Fes e I*. Neither the unfractionated CNBr digest nor any of the peptide fractions inhibited the binding of mAb MF11.10 to intact *Fes e I*. mAb MF11.8 (anti-site B) and SV24.64 (anti-site D) bound to both fractions P1 and P2. These fractions have not been sufficiently well characterized to exclude such explanations as incomplete cleavage and contamination with partial cleavage products. Fraction P3 was further characterized in this study because the two anti-site A mAb (MF21.4 and PR16.9) and all allergic human sera tested bound this peptide.

Characterization of peptide fraction P3. Using SDS-PAGE analysis, it was found that the P3 fraction contained a single peptide with a m.w. of 14,000. The antigenic activity of the P3 fragment with respect to the whole molecule was examined using an ELISA inhibition. A competition curve using P3 peptide as the inhibitor of anti-*Fes e I* antibody binding to solid phase *Fes e I* reached a plateau of approximately 40% inhibition (data not shown).

Inasmuch as a peptide fragment accounting for roughly half the molecule size of the parent protein and approximately 40% of the total immunochemical activity is expected to possess numerous antigenic sites, the collection of anti-Gpl mAb were tested and 22 were found to react

Figure 2. C-18 HPLC fractionation of the MF GpI CNBr digest. Reduced and alkylated MF GpI was reacted with CNBr as described in *Materials and Methods*. The digest was chromatographed on a Phenomenex C-18 reverse phase column equilibrated with 0.01% trifluoroacetic acid pH 3.4 and eluted at 1 ml/min with a 20 to 80% acetonitrile gradient (solvent B). Fractions were pooled as indicated by the bars (P1, P2, and P3).

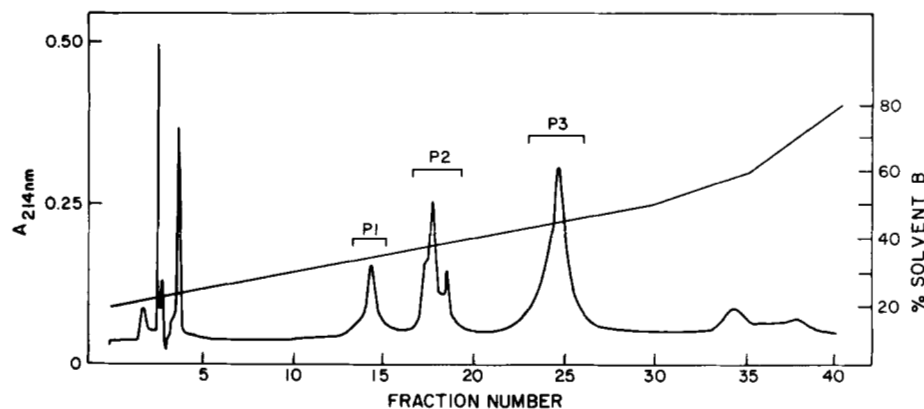


TABLE III
Immunoreactivity profiles of CNBr peptides^a

Antibody Preparation	HPLC Fraction			Unfractionated CNBr Digest
	P1	P2	P3	
Mouse anti-Fes e I serum pool	+	+	+	+
Human allergic sera				
Pool S2	+	+	+	+
DL	-	-	+	+
DR	-	-	+	+
04	+	+	+	+
09	-	-	+	+
56	-	-	+	+
Blocking mAb				
MF 11.8	+	+	-	+
MF 11.10	-	-	-	-
MF 21.4	-	-	+	-
PR 16.9	-	-	+	+
SV 24.64	+	+	-	+

^a CNBr peptides separated by HPLC on a C-18 column (Fig. 2) were adsorbed to PVC microtiter plate wells and reacted with various human and mouse antibody preparations in a direct binding ELISA (see *Materials and Methods*). +, detectable binding to the peptide fractions; -, no detectable binding.

with the P3 peptide. The epitopes recognized by these mAb were not restricted to site A as only three were found to inhibit the binding of the anti-site A mAb (MF21.4 and PR16.9) to solid phase MF GpI in competitive binding assays. The paratypes of these 22 P3-reactive mAb were examined and it was found that all recognized cross-reactive determinants found on the five different GpI Ag, as do the anti-site A blocking mAb and polyclonal human IgE antibodies. The fine specificity defined by these additional anti-P3 mAb has not yet been mapped.

Immunogenicity of the P3 fragment was tested in BALB/c mice. Pooled sera from four mice immunized with the unconjugated P3 peptide in CFA contained IgG antibodies that recognized the native Ag as well as the peptide (Fig. 3). The antibodies were specific for determinants on the P3 fragment because there was no cross-reactivity with either P1 or P2. Cross-reactivity assays showed that the anti-P3 antiserum reacted with all 5 GpI Ag.

DISCUSSION

The antigenic structure of grass GpI Ag is being investigated to identify allergenically important sites on the molecules. Two general approaches have been adopted: one involves topologic mapping using both polyclonal and panels of anti-GpI mAb. The other, more recent approach uses peptide fragments to directly identify antigenic and/or allergenic sites.

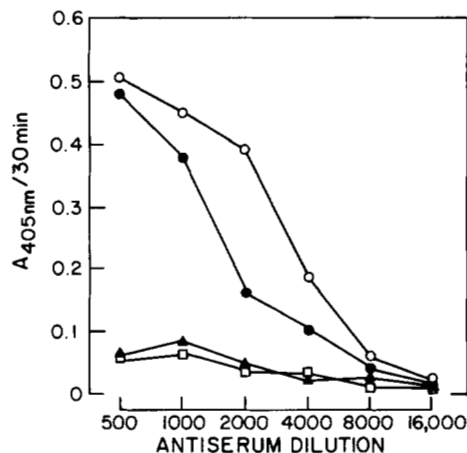


Figure 3. Immunogenicity of the P3 fragment in BALB/c mice. The sera from four female BALB/c mice injected four times at weekly intervals with approximately 50 µg P3 in CFA were pooled and tested using a direct binding ELISA for binding against solid phase native Fes e I (●) and the Fes e I CNBr peptide fractions P1 (□), P2 (▲), and P3 (○).

Panels of anti-GpI mAb raised against five different grass species have been screened and five mAb blocking the binding of human IgE antibodies to the homologous GpI Ag have been identified. The vast majority of mAb examined in this study (48 of 53) did not significantly block human IgE binding. This finding suggests that mAb from mice hyperimmunized with GpI Ag recognize a more diverse array of epitopes than those that stimulate an allergic response.

Cross-reactivity assays revealed that three of the five blocking mAb recognized determinants shared by GpI Ag isolated from the five different grass species. One mAb (MF 11.8) recognized a determinant specific to the Fes e I, and one (MF 11.10) recognized a determinant shared by Fes e I and Lol p I. Thus, with these mAb selected for their ability to inhibit human IgE binding to GpI Ag, it appears that there are both cross-reactive and unique blocking antibody binding sites on the GpI molecules.

Fes e I was selected for structural characterization studies because all five blocking mAb recognized determinants on this GpI Ag. Site specificity studies suggested that the five blocking mAb recognized four distinct, non-overlapping sites on the Fes e I molecule, which are designated sites A, B, C and D. Two mAb, PR 16.9 and MF 21.4, were found to recognize overlapping determinants in site A. These two anti-site A mAb probably do not recognize identical determinants since differences in their cross-reactivity profiles as well as differences in their blocking profiles can be detected (Fig. 1).

Additivity tests suggested that the anti-site A, B, and C blocking mAb inhibit binding of the same set of anti-GpI IgE antibodies because the combined inhibitory effect of any two blocking mAb was not additive even under saturating conditions. If the anti-site A, B, and C mAb block IgE antibodies that recognize determinants within a single site, then these results can be explained by the presence of an IgE binding site in close proximity to the three blocking antibody sites. Spatially, each of the three distinct sites could be envisioned as overlapping a common IgE binding site. When the anti-site D blocking mAb was combined with an anti-site A, B, or C blocking mAb, additive inhibition was observed. The additive result suggests that the anti-site D mAb blocks IgE antibodies that bind to a site (or sites) different from that blocked by the anti-site A, B, or C mAb. These mapping experiments have led to the proposal that the five blocking mAb identify two major allergenic sites and that these two sites account for approximately 50% of the total anti-GpI IgE antibodies present in the grass allergic serum pool. The proposed map of these sites is illustrated in a Venn diagram (Fig. 4).

The role of conformation in determining antigenic activity of protein antigens is widely recognized (15–17). Evidence obtained with a variety of proteins, including the major allergen of short ragweed pollen, AgE, indicated that profound changes in antigenic properties occur after protein denaturation or unfolding. The denaturation of Amb a I by reduction and alkylation or heating is accompanied by the total loss of antigenic and allergenic activity when tested with antibodies raised against native Amb a I. Thus, attempts to isolate immunoreactive fragments of Amb a I have been difficult (13). In contrast, reduced and alkylated GpI Ag retain their reactivity with antibodies raised against native Ag. Additionally, denatured GpI Ag retain their ability to bind human IgE antibodies from grass allergic individuals (Esch, unpublished results). This important property of GpI Ag will enable the pursuit of studies to localize blocking and IgE antibody binding sites on peptide fragments.

CNBr digestion of Fes e I yielded three antigenically active fractions, P1, P2, and P3. The direct ELISA results presented in Table III also indicate that all three fractions contain IgE binding sites. The P3 fragment possessed an IgE binding site (or sites) recognized by the allergic serum pool as well as all individual grass allergic sera tested. In

contrast, fractions P1 and P2 possessed sites that were recognized by IgE antibodies in only the serum pool and one of five individual allergic sera. Additional experiments with a larger number of individual allergic sera will be required to obtain more precise information about the distribution and range of IgE binding sites on the GpI Ag fragments.

Of the four antigenic sites defined by the five mAb used in this report, site C was destroyed by CNBr treatment, sites B and D were associated with fractions P1 and P2. Site A was localized on the P3 peptide, the major IgE binding fraction. There are 34 other mAb in the collection maintained in this laboratory which react with Fes e I and 22 of these recognize epitopes on the P3 fragment. The paratypes of these 22 anti-P3 mAb were examined and it was found that all recognized cross-reactive epitopes on the five GpI Ag. Thus, it appears that the P3 peptide represents a highly conserved domain of grass GpI molecules. Further site specificity studies with the 22 anti-P3 mAb are in progress to characterize the epitope structure of the P3 peptide. Evidence that three of these mAb recognize epitopes close to site A was obtained in a competitive binding assay where the mAbs were used to inhibit the binding of enzyme labeled anti-site A mAb.

The use of mAb as probes for Ag and allergen structure has become well established (13, 18–24). In a previous report from this laboratory (13), two allergenically important sites on the major allergen of short ragweed pollen, Amb a I were defined by two murine blocking mAb. These two mAb could block the binding of up to 80% of the IgE antibodies to Amb a I. In the present study with anti-GpI mAb, 40 to 55% inhibition of IgE binding was achieved with two blocking mAb. These results suggest that there are a limited number of allergenic determinants on Amb a I and grass GpI Ag. Inasmuch as the entire protein surface is potentially antigenic, the apparent restriction of IgE antibody specificity may be controlled by a number of possible regulatory factors (17). Ultimately, elucidation of the immune structure of allergenic proteins will be critical for an understanding of the IgE immune response and for development of immunotherapeutic strategies. This goal with grass pollen allergy may be simplified by the likely presence on grass allergens of structurally similar, cross-reactive determinants.

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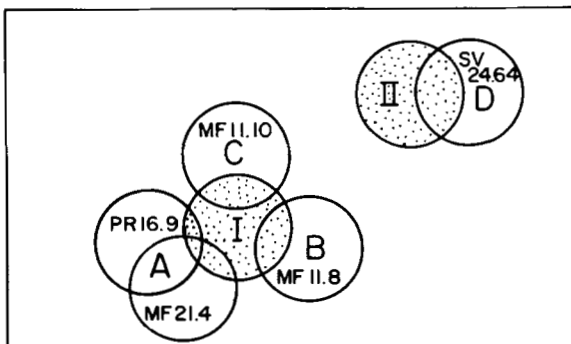


Figure 4. Venn diagram of five murine mAb reacting with two major allergenic epitopes of GpI Ag. The data suggest that one mAb (representing site D of the murine response) reacts uniquely with an epitope called site II (in the human response). The other mAb, representing three different sites of the murine response, all overlap a single different epitope called site I in the human response.

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