

THE EXPRESSION OF ANTI-POLY(LGLU⁶⁰,LPHE⁴⁰) IDIOTYPIC DETERMINANTS DICTATED BY THE GENE PRODUCTS IN THE MAJOR HISTOCOMPATIBILITY COMPLEX (H-2^a)*

BY UMA MAHESH BABU AND PAUL H. MAURER

From the Department of Biochemistry, Thomas Jefferson University, Philadelphia, Pennsylvania 19107

The murine immune response to the linear random terpolymer poly(LGlu⁵⁶,Lys³⁵,LPhe⁹) (GLPhe)¹ is under multi-Ir gene control. It has been postulated that complementation between two Ir gene products is required for the response to GLPhe, with the two genes, Ir- α and Ir- β , mapping to I-E/C and I-A subregions of the major histocompatibility complex (MHC), respectively (1, 2). It has been found that the complementing gene product(s) must be present on both T and B cells for responses to GLPhe to occur (3). We have recently reported (4, 5) that some murine strains previously determined to be "nonresponders" to GLPhe, especially mice of H-2^k haplotype, do respond to GLPhe. This was demonstrated by measure of splenic plaque-forming cells (PFC), specific antigen-binding activity of serum, and a helper-factor-production assay. We have tentatively proposed that, in addition to the two complementing Ir genes, some responders to GLPhe may respond via recognition of poly(LGlu⁶⁰,LPhe⁴⁰) (GPhe) determinants on GLPhe and then generate immune responses apparently against the whole GLPhe molecule (5). We have, therefore, used the random copolymer (GPhe) to study some aspects of the complementation responses to GLPhe. We have reported that the murine responses to GPhe are under genetic control of the genes in the MHC and that these genes map to the I-A subregion (6, 7). The purpose of our present investigation was twofold: (a) to determine whether antibodies produced against GPhe by the responding haplotypes might share common idiotypic determinants and (b) whether antibodies produced against GPhe and GLPhe share common idiotypic determinants. In this study we have used SWR/J (H-2^a) mice as the source of anti-GPhe idotype because this strain has been shown to be the best responder to GPhe (6, 7). Rabbit anti-(SWR anti-GPhe) was used as the source of anti-idiotypic antiserum. The present report deals with the characterization of this anti-idiotypic antiserum using the inhibition of both antigen binding by antibody and plaque-forming cells. It will be shown that this anti-idiotypic antiserum is antigen specific and inhibits the anti-GPhe-GPhe binding reactions, but only when mice of H-2^a haplotype are used as the source of anti-GPhe antibodies. This anti-idiotypic

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¹ Abbreviations used in this paper: CFA, complete Freund's adjuvant; GA, poly(LGlu⁶⁰,LAla⁴⁰); GAT, poly(LGlu⁶⁰,LAla³⁰,LTyr¹⁰); GL, poly(LGlu⁵⁰,Llys⁵⁰); GLA, poly(LGlu³⁶,Llys²⁴,LAla⁴⁰); GLPhe, poly(LGlu⁵⁶,Llys³⁵,LPhe⁹); GPhe, poly(LGlu⁶⁰,LPhe⁴⁰); Ig allotype, immunoglobulin heavy chain allotype; MBSA, methylated bovine serum albumin; MHC, major histocompatibility complex; PBS, phosphate-buffered saline; PFC, plaque-forming cell; SRBC, sheep erythrocytes; (TGAGly)_n, poly(LTyr²⁵,LGlu²⁵,LAla²⁵,Gly²⁵).

antiserum also inhibits binding of GLPhe by some anti-GLPhe antisera. Our data will show that anti-GPhe and anti-GLPhe antibodies produced in mice of the H-2^a haplotype share common idiotypic determinants and that the expression of these idiotypic determinants is dictated by genes in the MHC. This report is the first demonstration of such clear-cut involvement of gene(s) of MHC in governing murine idiotypic determinants. Our findings with anti-idiotypic (anti-GPhe) sera from other responding murine haplotypes and using guinea pig as a source of anti-idiotypic antisera will be presented separately.

Materials and Methods

Animals. Mice of inbred strains BUB, SWR/J, and DBA/1 (all H-2^a) were purchased from The Jackson Laboratory, Bar Harbor, Maine. The congenic mice C3H.Q and B10.Q were raised in our animal facilities. All other mice were purchased from Charles River Breeding Laboratories, Inc., Wilmington, Mass. Mice were of 6–16 wk of age at the beginning of immunization. New Zealand red rabbits were purchased from Three Springs Kennel, Zelienopoe, Pa.

Polymers. The synthesis of the random copolymer GPhe has been reported (6, 7). The random linear terpolymer GLPhe (lot GLP 1-71-199) was purchased from Miles Laboratories, Inc., Ames Div., Elkhart, Ind. GPhe was complexed to methylated bovine serum albumin (MBSA) to prepare the aggregated (GPhe-MBSA) as described previously (8).

Immunizations. Mice were injected initially in the hind footpads with 0.1 ml of solution containing 100 μ g of polymer in complete Freund's adjuvant (CFA). 3 wk later, the animals were boosted with the same amount of polymer in aqueous solution, given intraperitoneally. 10 d later, the animals were bled from the retro-orbital plexus, and the sera were kept frozen at -20°C until analyzed.

Coupling of GPhe to Sepharose 4B. 5 ml of cyanogen bromide-activated Sepharose 4B gel (Pharmacia Fine Chemicals, Div. of Pharmacia Inc., Piscataway, N. J.) were mixed with 1.5 ml of 1,6-hexanediamine in 10 ml water. The mixture was adjusted to pH 10 with 6 N HCl and then incubated overnight at 4°C with constant stirring. The gel was then washed with 0.1 M NaHCO_3 followed by distilled water, until the pH was 5.8. GPhe polymer (also at pH 5.8) and 50 μ l of water-free isobutylchloroformate were added to the gel and the reaction was continued overnight at 4°C on a rotary shaker. The coupled gel was washed successively with phosphate-buffered saline (PBS), 3 M NaSCN, and again with PBS. More than 95% of the input (GPhe) was bound to the gel. The gel was packed into a 10-ml plastic syringe and stored at 4°C in PBS containing 0.01% NaN_3 .

Production and Purification of Anti-GPhe Antibody. To produce ascitic fluid, we used the procedure described by Tung et al. (9), with some modifications. Mice were injected with 100 μ g of polymer in a volume of 0.2 ml of 9:1 CFA to saline. 3 d later, 0.5 ml of pristane was administered intraperitoneally. The animals were then boosted with 100 μ g of polymer on d 14, 21, and 28 in the same CFA/saline emulsion. After 4 wk, ascitic fluids were collected, pooled, centrifuged, and clarified by passing through glass wool. Antibodies were adsorbed to a column of GPhe-Sepharose 4B. After extensive washing with PBS, the bound anti-GPhe antibodies were eluted with 3 M NaSCN, and fractions were collected and dialyzed extensively against PBS. The fractions with antigen-binding activity were pooled and concentrated by Millipore immersible ultrafilters (Millipore Corp., Bedford, Mass.). Titration of the pooled ascitic fluid before passage over the affinity column and the pooled fractions after elution showed <10% loss of antigen-binding activity during purification. The isoelectric focusing pattern on the purified antibodies revealed moderate heterogeneity with 12–15 bands when run on a pH 5–8 gradient. By Ouchterlony double diffusion in agarose it was determined that the SWR anti-GPhe was predominantly of IgG₁ class and of kappa light chain.

Rabbit Anti-Idiotypic Antiserum. New Zealand red rabbits (2 kg) were "tolerized" with a total of 20 mg of purified SWR immunoglobulins injected intravenously; 10 mg were injected on day 0 and on day 2. Each rabbit was then immunized (subcutaneously on day 5 at 8–10 sites) with 2 mg of purified SWR anti-GPhe mixed with an equal volume of CFA. 2 and 4 wk later

the rabbits were boosted intradermally at 8–10 sites with a total of 2 mg of purified SWR anti-GPhe mixed with equal volume of incomplete Freund's adjuvant. Beginning 2 wk after the second boost, the rabbits were bled every 10–15 d. The sera were absorbed 3–6 times on Sepharose 4B coupled to purified normal SWR immunoglobulins using a batch method. The absorbed sera were tested in the antigen-binding inhibition assay (see below) before pooling and storing at -20°C .

Inhibition of Antigen-binding Assay. The antigen-binding ability of mouse antisera was titrated using the serum antibody determination assay described previously (10) but using 3 ng GPhe or GLPhe iodinated by the Bolton-Hunter reagent (11). The percent of the polymer bound was found to be linear between 10 and 60% for all antibody dilutions used in this report. Aliquots of 12.5 μl mouse antisera at final dilutions that bound 30–50% of the labeled antigen were mixed with 12.5 μl of various dilutions of absorbed rabbit anti-(SWR anti-GPhe). This mixture was allowed to stand at room temperature for 15 min. To each sample was added 50 μl of solution containing 3 ng of labeled antigen, and the mixture was incubated at 37°C for 1 h. 300 μl of a previously titrated goat anti-mouse immunoglobulin was added to each sample and incubated for another 2 h at 37°C . The samples were centrifuged and a 200- μl aliquot of the supernatant fluid was assayed for radioactivity. Heat-inactivated normal rabbit serum was used as the control. The percentage of antigen bound was expressed as:

$$\left(1 - \frac{\text{counts per minute in test serum}}{\text{counts per minute in 1\% bovine serum albumin}}\right) \times 100.$$

And the percent inhibition with the anti-idiotypic serum was calculated as follows:

$$\left(1 - \frac{\text{Percent binding in the presence of anti-idiotypic serum}}{\text{Percent binding in the presence of normal rabbit serum}}\right) \times 100.$$

Inhibition of Antigen-specific PFC by Anti-Idiotypic Antiserum. PFC assay was performed as described previously (7). Briefly, GPhe was coupled to sheep erythrocytes (SRBC) by the "aged" CrCl_3 method. Lymph node cells from animals immunized 14 d earlier with 100 μg of GPhe were mixed with GPhe-SRBC and graded amounts of anti-idiotypic antisera in agarose. PFC were counted after developing with rabbit anti-mouse immunoglobulins (N. L. Cappel Laboratories, Inc., Cochranville, Pa). The PFC values given are a total of IgM and IgG plaques per 10^6 cells. The anti-GPhe PFC and anti-poly(LGlu³⁶,LLys²⁴,LAla⁴⁰) (GLA) PFC were enumerated by coupling GLPhe or GLA to SRBC by the tannic acid procedure (12).

Results

Inhibition of GPhe-Anti-GPhe Reactions by Anti-Idiotypic Antiserum. The binding of ^{125}I -GPhe by anti-GPhe antisera produced in a variety of mice of H-2^a haplotype was strongly inhibited by the anti-idiotypic antiserum (Table I). Mice of H-2^a haplotype and having different immunoglobulin (Ig) allotypes were used, yet the inhibition of GPhe-binding by antisera from all strains of mice was virtually identical. In the controls we used, the binding of GLA by SWR anti-GLA was unaffected by the anti-idiotypic antiserum at all the dilutions used, which showed the specificity of the reaction. The specificity of the anti-idiotypic antiserum was further investigated by studying its effect on other, unrelated, polymer-binding reactions such as poly(LGlu⁶⁰,LAla⁴⁰) (GA) and anti-GA, and (LTyr²⁵,LGlu²⁵,LAla²⁵,Gly²⁵) (TGAGly)_n and anti-TGAGly. These reactions also were not affected by the anti-idiotypic antiserum (data not shown).

Effect of Anti-Idiotypic Antiserum on the Binding of GPhe by Anti-GPhe Antisera from Mice of Other Haplotypes. Based upon our report of the murine response pattern to GPhe (6, 7), we tested the effect of the anti-idiotypic antiserum on binding of GPhe by anti-GPhe antisera from other responding mice of H-2^k and H-2^p haplotypes. The above

TABLE I
Inhibition of Binding of ¹²⁵I-GPhe by Immune Sera from H-2^a Mice by Rabbit Anti-Idiotypic Antiserum

Source of anti-GPhe	Ig allo-type	Antigen bound*	Dilution of anti-GPhe studied	Inhibition by anti-idiotypic antiserum‡		
				1:10	1:50	1:100
		%				
SWR	c	42	1:2,000	100	60	20
DBA/1	c	43	1:1,000	92	38	8
C3H.Q	a	36	1:1,000	90	49	12
BUB	a	43	1:500	88	46	20
B10.Q	b	48	1:1,600	95	58	16
SWR§	c	41	1:200	8	2	4

* Percent 3 ng of ¹²⁵I-GPhe bound in presence of normal rabbit serum. All values are the average of duplicates and showed <10% variation.

‡ Final dilutions of rabbit anti-idiotypic antiserum used.

§ SWR anti-GLA was used as the control.

TABLE II
Failure of Rabbit Anti-Idiotypic Antiserum to Inhibit the Binding of ¹²⁵I-GPhe by Anti-GPhe Produced by H-2^k and H-2^p Mice

Source of anti-GPhe	H-2 haplotype	Ig allo-type	Antigen bound*	Dilution of anti-GPhe studied	Inhibition by anti-idiotypic antiserum‡	
					1:3	1:10
			%			
C3H	k	a	38	1:50	4	6
RF	k	c	41	1:40	3	2
AKR	k	d	52	1:50	6	4
B10.P	p	b	49	1:50	6	0
P/J	p	h	43	1:40	2	3
SWR	q	a	50	1:1,600	100	100

* Same as in Table I.

‡ Same as in Table I.

anti-idiotypic antiserum showed no inhibitory effect on these reactions (Table II). We have tested anti-GPhe antisera from three murine strains of H-2^k and two strains of H-2^p having different Ig allotypes. None of these reactions was affected by the anti-idiotypic antiserum. Therefore, those determinants on the anti-GPhe antibodies that are recognized by the rabbit anti-idiotypic antiserum show no association with Ig allotype, but their expression is uniquely associated with the MHC of mice of H-2^a haplotype.

Effect of Anti-Idiotypic Antiserum on GPhe-Anti-GPhe-MBSA-binding Reactions. All mice respond to GPhe when the polymer is complexed to an immunogenic carrier such as MBSA (6, 7). The effect of the rabbit anti-idiotypic antiserum was tested on a variety of anti-GPhe antisera produced with the GPhe-MBSA aggregate. Data obtained with antisera from a few representative strains of mice are given in Table III. Again, only the GPhe-binding reactions with anti-GPhe-MBSA antisera from mice of H-2^a haplotype are inhibited by the anti-idiotypic antiserum.

TABLE III
Effect of Anti-Idiotypic Antiserum on the Binding of ^{125}I -GPhe by Anti-GPhe
Produced by Immunization with GPhe-MBSA

Source of anti-GPhe	H-2 haplotype	Antigen bound	Dilution of anti-GPhe studied	Inhibition by anti-idiotypic antiserum‡		
				1:10	1:50	1:100
		%		%		
SWR	q	44	1:500	100	95	22
DBA/1	q	43	1:400	100	100	18
B10.Q	q	47	1:500	100	90	27
C57BL/6	b	43	1:50	8	0	2
C3H	k	41	1:50	4	2	4
BALB/c	d	37	1:40	6	1	5

* Same as in Table I.

‡ Same as in Table I.

TABLE IV
Effect of Anti-Idiotypic Antiserum on the Binding of ^{125}I -GPhe by Immune Sera from F_1
Mice of Various Haplotypes

Source of anti-GPhe	H-2 haplotype	Antigen bound*	Dilution of anti-GPhe studied	Inhibition by anti-idiotypic antiserum‡		
				1:10	1:50	1:100
		%		%		
(SWR \times BALB/c) F_1	(q \times d)	43	1:500	100	96	26
(DBA/1 \times C57BL/6) F_1	(q \times b)	37	1:300	100	85	25
(C57BL/6 \times A) F_1	(b \times a)	33	1:150	5	8	0
(SJL \times BALB/c) F_1	(s \times d)	41	1:300	4	1	0
(B10.S \times B10.D2) F_1	(s \times d)	47	1:300	6	3	1
(B10.S \times B10.M) F_1	(s \times f)	46	1:250	3	10	2

* Same as in Table I.

‡ Same as in Table I.

Effect of the Anti-Idiotypic Antiserum on the Binding of GPhe by Anti-GPhe Antisera from F_1 Mice. We have reported that F_1 mice of (H-2^a \times H-2^b) and (H-2^a \times H-2^d) respond strongly to GPhe. In addition, F_1 mice of (H-2^a \times H-2^d) and (H-2^a \times H-2^f) exhibit a complementation phenomenon of two nonresponders and respond very well to GPhe (6, 7). The effect of the anti-idiotypic antiserum on the binding of GPhe by anti-GPhe antisera produced in some of these F_1 mice is shown in Table IV. The anti-GPhe antisera produced in (H-2^a \times H-2^b) F_1 and (H-2^a \times H-2^d) F_1 mice were inhibited by the anti-idiotypic antiserum. However, the anti-idiotypic antiserum had no effect on the binding of GPhe by anti-GPhe antisera produced in the F_1 mice of (H-2^a \times H-2^d) and (H-2^a \times H-2^f) haplotypes that exhibited the complementation of immune response gene phenomenon.

Presence of Common Idiotype Determinants on Anti-GLPhe Antibodies. Anti-GLPhe antibodies from many strains of mice are highly cross-reactive with both poly(LGlu⁵⁰, Llys⁵⁰) (GL) and GPhe (5). We therefore tested the effect of anti-idiotypic antiserum on the binding of GLPhe by anti-GLPhe antisera. As shown in Table V,

TABLE V
Effect of Anti-Idiotypic Antiserum on the Binding of ¹²⁵I-GPhe by Various Anti-GLPhe Antisera

Source of anti-GLPhe	H-2 haplotype	Ig allo-type	Antigen bound*	Dilution of anti-GLPhe studied	Inhibition by anti-idiotypic antiserum‡	
					1:3	1:25
			%		%	
SWR	q	c	38	1:200	88	18
DBA/1	q	c	41	1:200	82	16
B10.Q	q	b	43	1:200	76	10
C3H.Q	q	a	43	1:200	86	18
BALB/c	d	a	37	1:400	4	2
C3H	k	a	41	1:50	5	3
SWR§	q	c	42	1:1,500	100	80

* Same as in Table I.

‡ Same as in Table I.

§ Internal positive control of ¹²⁵I-GPhe binding by SWR anti-GPhe.

TABLE VI
Effect of Anti-Idiotypic Antiserum on the Antigen-specific PFC Response Measured in Immune Lymph Nodes

Strain of mice	H-2 haplotype	Immunogen used	Control* PFC/10 ⁶ cells	Inhibition by anti-idiotypic antiserum‡		
				1:10	1:25	1:100
				%		
SWR	q	GPhe	410	92	68	19
B10.Q	q	GPhe	370	95	63	17
C3H	k	GPhe	180	16	12	10
SWR	q	GPhe-MBSA	280	85	53	21
C3H	k	GPhe-MBSA	120	14	8	0
SWR	q	GLPhe	520	56	21	6
B10.Q	q	GLPhe	430	61	18	8
BALB/c	d	GLPhe	650	14	6	3
SWR	q	GLA	380	16	6	12

* Total (IgM + IgG) PFC/10⁶ lymph node cells.

‡ Final dilution of anti-idiotypic antiserum. GLPhe and GLA were coupled to SRBC by tannic acid procedure (12).

the anti-idiotypic antiserum exhibits an inhibitory effect only on the GLPhe-anti-GLPhe-binding reactions when the anti-GLPhe antisera were produced in mice of H-2^a haplotype. However, as expected, the inhibitory effect was weaker here than on the "homologous" GPhe-anti-GPhe reactions because anti-GLPhe antisera from these strains of mice are known to have a considerable amount of anti-GL in addition to anti-GPhe activity. On the other hand, the anti-idiotypic antiserum strongly inhibited the binding of GPhe by the anti-GLPhe antisera from the mice, but only those of H-2^a haplotype (data not shown).

Effect of Anti-Idiotypic Antiserum on Antigen-specific PFC Assay. The effect of the above anti-idiotypic antiserum on the PFC responses of different strains of mice responding to GPhe, GPhe-MBSA, GLPhe, and GLA was studied. The data in Table VI show

that the anti-idiotypic antiserum exhibited inhibitory effects only on PFC responses of mice of H-2^a haplotype responding to GPhe, GPhe-MSBA, and GLPhe. The anti-idiotypic antiserum had no effect on the PFC responses of mice of H-2 haplotypes d, k, and p to GPhe and GLPhe. The anti-GLA PFC responses of H-2^a mice were unaffected by the anti-idiotypic serum, which shows the specificity of the anti-idiotypic serum. We also confirmed the fine specificity of the inhibition reaction involving GPhe by studying the effect of the anti-idiotypic sera on the reactions of other polymers, such as GA and (TGAGly)_n. No inhibitory effects were shown with these systems (data not shown).

Discussion

We report here the production of an anti-idiotypic antiserum (rabbit anti-SWR [H-2^a] anti-GPhe) and some of its unique properties. We have studied the effect of the anti-idiotypic antiserum on the binding of ¹²⁵I-GPhe by anti-GPhe antisera produced in several strains of mice. This type of inhibition by anti-idiotypic antisera of antigen-specific binding has been used to characterize the anti-idiotypic antisera (13). The anti-idiotypic antiserum binds to the determinants found in or closely associated with the antibody-combining site for the antigen GPhe. Our data indicates that the anti-idiotypic antiserum used here exhibits inhibitory effects on binding of GPhe by anti-GPhe antisera. However, the anti-idiotypic antiserum shows the following unique properties: (a) only GPhe-anti-GPhe-binding reactions are inhibited. The binding reactions of unrelated polymers to GPhe such as GA, GLA, and (TGAGly)_n by their specific antisera are unaffected; (b) the binding of ¹²⁵I-GPhe by anti-GPhe antisera produced in H-2^a mice was strongly inhibited by the above anti-idiotypic antiserum. It is of considerable interest that the binding of GPhe by anti-GPhe antisera produced in a number of H-2^a mice having different Ig allotypes was inhibited to the same degree. The anti-idiotypic antiserum showed no inhibitory effect on the antisera produced in the responding strains of mice other than H-2^a haplotype, namely, H-2^k and H-2^p haplotypes and in the guinea pig. Only the anti-GPhe-MBSA antisera produced in mice of H-2^a haplotype showed any inhibition by the anti-idiotypic antiserum. The binding of GPhe by anti-GPhe antisera produced in (H-2^a × H-2^b)F₁ and (H-2^a × H-2^d)F₁ mice was also strongly inhibited by the anti-idiotypic antiserum, showing the dominant presence in these sera of idiotypic determinants whose expression is dictated by the H-2^a MHC. However, the anti-GPhe antibodies produced in the complementing F₁ strains (H-2^s × H-2^d)F₁ and (H-2^s × H-2^f)F₁ were not inhibited by the anti-idiotypic antiserum.

The above specificity of the anti-idiotypic antiserum was also confirmed by the inhibition of the GPhe-specific PFC produced in the lymph nodes and spleens of the responding mice. The anti-idiotypic antiserum exhibited inhibitory effects only on PFC responses of mice of H-2^a haplotype to GPhe or GPhe-MBSA. Again, the anti-idiotypic antiserum showed no significant effect on the PFC responses of mice of H-2 haplotypes k, p, and (d × s)F₁ to GPhe or GPhe-MBSA.

It is clear that the anti-idiotypic antiserum recognizes only those antigenic determinants on the anti-GPhe antibodies that are not associated with any Ig allotypes but whose expression is uniquely associated with the H-2^a haplotype. With other anti-polymer systems such as poly(LGlu⁶⁰,LAla³⁰,LTyr¹⁰) (GAT) and GLPhe, there are

differing reports as to whether the anti-idiotypic antisera exhibit an allotype linkage. A guinea pig anti-(D1·LP anti-GAT) anti-idiotypic antiserum showed no allotype linkage (14). However, the same laboratory reported (15) an allotype linkage using guinea pig anti-(A/J anti-GAT). An allotype linkage in a rabbit anti-(BALB/c anti-GAT) has been noted in our laboratory.² An allotype linkage has been demonstrated in the guinea pig anti-[B10.A(5R) anti-GLPhe] anti-idiotypic antiserum (13). In a recent report the same group has shown that the guinea pig anti-(B10.WB anti-GLPhe) anti-idiotypic antiserum exhibits no allotype linkage (16). However, to our knowledge none of the reports with polymers and proteins has shown that the expression of the idiotypic determinants in the antibody molecule may be dictated by the H-2 locus of the MHC, as we are reporting here. Other studies have shown that neither MHC nor allotype-linked genes can control the expression of V region genes (17, 18). To exclude the possibility that such genes may be common to an H-2^a background, we are now investigating the segregation of H-2 and Ig constant and variable regions. This involves the studies of the response patterns to GPhe in the backcross progenies involving SWR/J and other H-2^a mice.

The data we have reported here are unique as we have also prepared anti-idiotypic antisera against other anti-GPhe antisera, such as [C3H (H-2^k) anti-GPhe] and a complementing responder [(BALB/c × SJL)_{F1} (H-2^d × H-2^b)_{F1} anti-GPhe]. Neither of these anti-idiotypic antisera exhibited any allotype linkage nor the "H-2 specificity" exhibited by the above anti-idiotypic antiserum. A detailed report of these findings will be submitted separately.

We have considered two possible models to explain the H-2 control of the expression of idiotypic determinants produced by mice of H-2^a haplotype. A direct linkage to H-2 is unlikely because chromosomes housing the genes for antibody synthesis and the Ia expression are very far apart for such an association to easily exist. A plausible explanation can involve the macrophages in mice of H-2^a haplotype that present unique determinants of GPhe polymer in the response process of H-2^a mice to GPhe. On the other hand, the immune processes to GPhe by other responder mice, H-2^b, H-2^k, and (H-2^d × H-2^b)_{F1} include these unique determinants as well as others that are not preferentially recognized by mice of H-2^a haplotype. The anti-idiotypic antiserum studied here inhibits such B cell functions as PFC and antigen-binding by the antibodies, and so far we have not been able to elicit any effect on the T cell proliferative responses to GPhe in all the responding strains of mice, including SWR/J (H-2^a) (data not shown).

The anti-idiotypic antiserum also inhibits the binding of GLPhe by anti-GLPhe antisera where the anti-GLPhe antibodies are produced only in H-2^a mice. Inhibition of GPhe-anti-GPhe binding is virtually total, which shows the strength of the anti-idiotypic antiserum; and the inhibition of GLPhe and anti-GLPhe binding is only partial, which shows that anti-GLPhe antibodies have antibodies to determinants other than GPhe, namely, GL in the polymer GLPhe. This is to be expected from the reports that anti-GLPhe antibodies have cross-reactivity against GL as well as GPhe (5, 12).² When the binding reaction was carried out with GPhe instead of GLPhe, the anti-GLPhe antisera from mice of H-2^a haplotype were strongly inhibited by the above anti-idiotypic antiserum. It can be concluded, therefore, that the antibodies

² Sporer, R., H. J. Callahan, and P. H. Maurer. Characterization of idiotypic determinants of anti-GAT¹⁰ antibodies of BALB/c mice. Manuscript submitted for publication.

produced against GPhe and GLPhe share common idiotypic determinants that are recognized by the anti-idiotypic antiserum. It has also been reported that all the responders to GPhe are also responders to GLPhe (4-8). It is evident to us that the response to GLPhe of mice of H-2^a haplotype is via recognition of GPhe in the GLPhe polymer, whereas the recognition of GLPhe by mice of other responding haplotypes such as H-2^d and (H-2^a × H-2^b)F₁ may be via a different recognition pattern.

Summary

Antibodies raised in SWR/J mice (H-2^a, Ig^g) to the random copolymer poly(LGlu⁶⁰,LPhe⁴⁰) (GPhe) were purified by immunoabsorbent chromatography and used to immunize a New Zealand red rabbit. The rabbit anti-idiotypic antiserum thus produced strongly inhibited the binding of ¹²⁵I-GPhe by anti-GPhe antisera produced only in mice of H-2^a haplotype, and had no effect on the binding of GPhe by anti-GPhe antisera produced in mice of other haplotypes, namely, H-2^k and H-2^p. The anti-idiotypic antiserum also inhibited the binding of GPhe by anti-GPhe-methylated bovine serum albumin antisera produced only in mice of H-2^a haplotype. No linkage to Ig allotype was observed. The anti-GPhe antisera produced in F₁ mice of (H-2^a × H-2^d) and (H-2^a × H-2^b) haplotypes have antibodies that are inhibited by the anti-idiotypic antiserum demonstrating the dominant presence in these F₁ mice of idiotypic determinants whose expression is dictated by the H-2^a major histocompatibility complex (MHC). The anti-idiotypic antiserum also inhibited the binding of ¹²⁵I-poly(LGlu⁵⁶,LLys³⁵,LPhe⁹) (GLPhe) and ¹²⁵I-GPhe antisera produced only in mice of H-2^a haplotype. These specificities were also confirmed by the inhibition of the plaque-forming cells. It was concluded that the antibodies produced in mice of H-2^a haplotype against GPhe and GLPhe share common idiotypic determinants that are recognized by the anti-idiotypic antiserum. A possible explanation for the unique findings that the expression of anti-GPhe idiotypic determinants in mice of H-2^a haplotype are dictated by the gene product in the MHC is that the macrophages in mice of H-2^a haplotype present unique determinants of GPhe polymer in the response process to GPhe.

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