GENETIC CONTROL OF A SHARED IDIOTYPE AMONG ANTIBODIES DIRECTED TO DISTINCT SPECIFICITIES*

By SHYR-TE JU, BARUJ BENACERRAF, and MARTIN E. DORF

From the Department of Pathology, Harvard Medical School, Boston, Massachusetts 02115

Since the discovery of idiotypic determinants on myeloma proteins by Slater et al. 20 yr ago (1), most of the idiotypic systems described in the literature followed the general rule that an idiotype was associated only with antibody molecules of a given specificity but not with antibodies possessing different binding specificities. Oudin and Cazenave reported in 1971 (2) some unexpected sharing of idiotypic specificities among subpopulations of anti-ovalbumin antibodies and also between anti-ovalbumin antibodies and antibodies not bearing specificity for the ovalbumin immunogen. Two individual rabbits were used, and this idiotypic cross-reactivity only occurred among antibodies in the same rabbit. The second exception to this concept was reported by Karol et al. (3), who studied the idiotypes of sheep antibodies specific to the human sickle cell hemoglobin, i.e., antibodies recognizing the N-terminal position 6 residue, valine-associated determinants, termed anti-VAL antibodies. These investigators demonstrated shared idiotype(s) between anti-VAL antibodies and antibodies not possessing detectable anti-VAL activity from a goat and a sheep. Furthermore, Eichmann et al. (4) reported that some lipopolysaccharide (LPS)[1]-induced B cell clones secreted immunoglobulin which expressed A5A idiotypic determinants but failed to bind streptococcal A carbohydrate. Recently, Bona et al. (5) demonstrated that sera from 2- to 4-wk-old BALB/c mice express a defined anti-inulin cross-reactive idiotype, but these sera lack detectable inulin-binding activity. These results were interpreted as evidence that somatic mutational events occurred in the structural V genes encoding antibody for a given specificity such that portions of the gene encoding for idiotypic determinants remained essentially unchanged, whereas other portions vital for antigen-binding activity were modified to code for a new specificity. In the absence of a demonstrable antigenic stimulus, it is difficult to rationalize how such somatically derived clones are selectively triggered and expanded to produce detectable quantities of the unusual shared idiotypic antibodies.

This study describes a set of shared idiotypic determinants in antibodies of distinct

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[1] *Abbreviations used in this paper:* Ar-BGG, *p*-azophenylarsonate conjugate of bovine gamma globulin; CGAT, common idiotypes of murine anti-GAT antibodies; DNP, dinitrophenyl; FGG, fowl gamma globulin; GA, copolymer of L-glutamic acid^{60}-L-alanine^{40}; GAT, copolymer of L-glutamic acid^{60}-L-alanine^{30}-L-tyrosine^{10}; GAT^{33}, copolymer of L-glutamic acid^{33}-L-alanine^{30}L-tyrosine^{33}; GL, copolymer of L-glutamic acid^{60}-L-lysine^{40}; GLA, copolymer of L-glutamic acid^{55}-L-lysine^{35}-L-alanine^{10}; GLϕ, copolymer of L-glutamic acid^{54}-L-lysine^{35}-L-phenylalanine^{11}; GLpro, copolymer of L-glutamic acid^{57}-L-lysine^{38}-L-proline^{5}; GLT, copolymer of L-glutamic acid^{57}-L-lysine^{38}-L-tyrosine^{5}; Gϕ, copolymer of L-glutamic acid^{60}-L-phenylalanine^{40}; GT, copolymer of L-glutamic acid^{50}-L-tyrosine^{50}; GTGL, common idiotype of anti-GT and anti-GL antibodies; LPS, lipopolysaccharide; OVA, ovalbumin.

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specificity and uses various inbred strains of mice to demonstrate a genetic basis for this phenomenon. The idiotypic assay system is designed to eliminate any possibility of contamination of assay reagents. This strategy involved independent preparations of the main components in the radioimmunoassay system. First, the ^{125}I-labeled ligand was a monoclonal anti-copolymer of L-glutamic acid^{60}-L-alanine^{30}-L-tyrosine^{10} (GAT) antibody obtained from a hybridoma cell line of DBA/2 origin. Second, the immunogen used to prepare a xenogeneic guinea pig anti-idiotypic antiserum was specifically purified anticopolymer of L-glutamic acid^{54}-L-lysine^{35}-L-phenylalanine^{11} (GLϕ) antibody from an individual B10.WB mouse immunized only with GLϕ. Third, all of the immune antisera used as inhibitors were obtained independently from various sources neither involved in the production of ligand nor of the anti-idiotypic antiserum. This assay system permits us to demonstrate unequivovally a new set of idiotypic determinants, termed common idiotype of anticopolymer of L-glutamic acid^{50}-L-tyrosine^{50}(GT) and anticopolymer of L-glutamic acid^{60}-L-lysine^{40}(GL) (GTGL) idiotype, demonstrated to be shared by antibodies possessing distinct binding specificities. In contrast with previous studies, the GTGL idiotype is readily induced and frequently expressed in antisera obtained from 25 different mouse strains immunized with various (Glu, Tyr)- or (Glu, Lys)-containing polypeptide polymers. The results indicate that GT- and GL-related antigenic stimulation is required for the induction and expansion of the GTGL idiotype. Furthermore, we demonstrated that Igh-linked genes control the expression of the GTGL idiotype.

Materials and Methods

Animals. All mice were obtained from either The Jackson Laboratory, (Bar Harbor, Maine) or from the animal colonies at Harvard Medical School (Boston, Mass.) Guinea pigs and rabbits were obtained from the animal colonies at Harvard Medical School.

Polymers and Antigens. The synthetic polymers of L-amino acids of L-Glu^{60}-L-Ala^{30}-L-Tyr^{10} (GAT^{10}), lot 6 average mol wt 39,000; L-Glu^{60}-L-Ala^{40} (GA), lot 1, average mol wt 36,000; GT, lot 9, average mol wt 133,000; L-Glu^{33}-L-Ala^{33}-L-Tyr^{33} (GAT^{33}), lot GTA 1, average mol wt 8,100; GL, lot 1641, average mol wt 73,000, and GLϕ, lot 2, average mol wt 45,100, were obtained from Miles Yeda, Ltd., Rehovot, Israel. Polymers of L-Glu^{57}-L-Lys^{38}-L-Pro^{5} (GLpro) and L-Glu^{55}-L-Lys^{35}-L-Ala^{10} (GLA) were provided by Dr. Elkan Blout of Harvard Medical School. Polymers of L-Glu^{57}-L-Lys^{38}-L-Tyr^{5} (GLT) and antisera to polymer of L-Glu^{60}-L-Phe^{40} (Gϕ) were kindly provided by Doctors Paul M. Maurer and Uma M. Babu of the Department of Biochemistry, Jefferson Medical College, Philadelphia, Pa. Fowl gamma globulin was purchased from Miles Laboratories Inc., Miles Research Products, Elkhardt, Ind., and was conjugated to GL as described by Cheung et al. (6). GAT conjugates of fowl gamma globulin (FGG) was prepared as described (7). Amino acid analysis of GAT and GLϕ by the company showed that L-Lys and L-Tyr were absent in GAT and GLϕ preparations, respectively.

Immunization. All mice were immunized twice intraperitoneally with a 3-wk interval, by injection of an emulsion containing equal volumes of antigen solution (0.25 mg–1 mg/ml) and complete Freund's adjuvant having 0.5 mg/ml *Mycobacterium butyricum* (Difco Laboratories, Detroit, Mich.). Sera were collected 7 d after the second immunization. Individual or pooled sera were stored at −20°C until use.

Idiotypic Immunogens. Pooled and individual D1.LP anti-GAT antibodies, B10.WB and C3H.Q anti-GLϕ antibodies were specifically purified from Sepharose 4B beads conjugated with GAT and GLϕ, respectively (8, 9).

Anti-Idiotypic Antisera. Guinea pig anti-idiotypic antisera against D1.LP anti-GAT antibodies and B10.WB anti-GLϕ antibodies were prepared and characterized as previously described (8, 9). These immune sera were absorbed at equivalence with (B6 × D2)F1 gamma globulin followed by adsorption with (B6 × D2)F1 gamma globulin conjugated to Sepharose 4B gel. These reagents did not form precipitin lines with mouse immunoglobulin and did not exhibit

detectable binding activity with ^{125}I-labeled TEPC-183 (μ, κ) and MOPC 21 (γ1, κ) myeloma proteins.

Hybridoma Anti-GAT Antibodies. The production of hybridoma cell lines of DBA/2 origin and purification and characterization of the secreted IgM anti-GAT antibodies has been reported (10, 11).

Hemagglutination. Hemagglutination of GAT-coupled sheep cells was carried out as described (10).

Radioiodination and Radioimmunoassay. Proteins and polymers were radiolabeled with carrier-free Na[^{125}I] (New England Nuclear, Boston, Mass.) by using the chloramine-T method. Activity of antisera was measured by a modified Farr assay using 8 ng of either ^{125}I-GAT or ^{125}I-GLT as ligand (8). Idiotype binding and inhibition of idiotype-binding assays were established according to previously described methods (8). The combinations of anti-idiotypic antisera and ^{125}I-labeled idiotype ligands used were: (*a*) guinea pig anti-idiotypic antiserum against pooled D1.LP anti-GAT antibodies and ^{125}I-labeled D1.LP (6.1) anti-GAT antibodies; this reaction defines common idiotypes of murine anti-GAT antibodies (CGAT) (12), and (*b*) guinea pig anti-idiotypic antiserum against B10.WB anti-GLϕ antibodies and ^{125}I-labeled hybridoma D anti-GAT antibodies; this binding defined GTGL idiotype. In the second idiotypic interaction, 15 μl of MOPC 21 and 15 μl of TEPC 183 ascitic fluids were added to block any possible antibodies to IgM or to MOPC 21 κ-chain.

Immunoadsorption. 100 μl of pooled DBA/2 anti-GAT or pooled B10.WB anti-GLϕ antisera was mixed in a 10- × 75-mm test tube with 300 μl packed Sepharose 4B beads conjugated with either GAT or GLϕ or an unrelated antigen. After 30 min at room temperature, the unbound fractions were collected by centrifugation. The beads were then washed four times with 3 ml saline, and the bound fractions were eluted twice with 0.2 M glycine-HCl, pH 2.33, and immediately buffered with 2 M Tris-HCl solution, pH 7.9. Both unbound and eluted fractions were stored at −20°C and used within 2 d.

Results

Detection of the GTGL Idiotypic Determinants. Fig. 1 depicts the source of components which constitute the idiotypic analysis system used in the present study. There are three important compartments: (*a*) the ligands are *DBA/2* monoclonal IgM hybridoma anti-GAT antibodies bearing defined CGAT idiotypes; (*b*) the guinea pig anti-idiotypic antisera are prepared against individual *B10.WB* anti-GLϕ antibodies; these anti-idiotypic antisera were used to characterize the GL-1 idiotypes (9); and (*c*) the inhibitors are derived from sera of various mouse strains immunized with one of the following polymers: GAT, GAT^{33}, GAT-FGG, GT, GA, GLϕ, GLA, GLpro, Gϕ, and GL-FGG. The unique features of the idiotypic system included (*a*) the labeled ligands and the idiotypic antibodies used to prepare anti-idiotypic antisera are obtained separately and independently from mouse strains bearing different Igh-1 allotypes; (*b*) the GAT and GLϕ polymers appear unrelated with respect to net charge and amino acid composition; and (*c*) nearly all mouse strains immunized with a diverse series of polymers expressed this shared idiotypic determinants (see below).

Idiotype Binding. The specific idiotypic interactions between six ^{125}I-labeled CGAT positive DBA/2 hybridoma IgM anti-GAT antibodies and a guinea pig anti-idiotypic antiserum prepared against an individual B10.WB anti-GLϕ antibodies are shown in Table I. This reagent bound 72% of the ^{125}I-labeled homologous B10.WB anti-GLϕ antibodies. Under the conditions employed, maximal binding of hybridoma anti-GAT antibodies ranged between 50 and 72%. Quantitative binding studies indicated that the binding did not plateau with 3 μl of anti-idiotype (data not shown). The specificity of binding was shown by the fact that this anti-idiotypic antiserum failed to bind ^{125}I-labeled TEPC-183 and MOPC-21 myeloma proteins. The IgM hybridoma

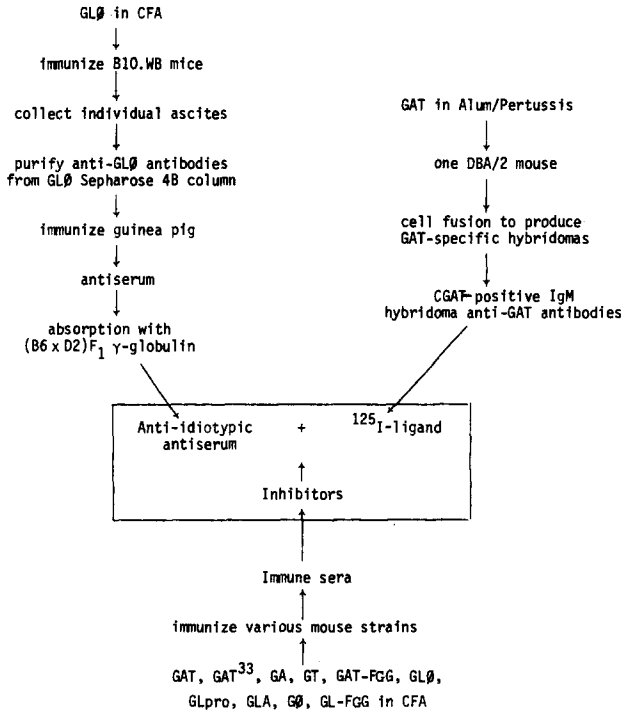

FIG. 1. Inhibition of GTGL idiotype-binding system. Schematic presentation of GTGL idiotype assay system. The anti-idiotypic antiserum has been used to characterize the interstrain GL-1 idiotypes associated with GL-specific antibodies (9). The fine idiotypic specificities and interrelationships of the IgM hybridoma anti-GAT antibodies have been characterized (10, 11).

F9-102.2 anti-GAT antibodies which were obtained from the same fusion as the six CGAT-bearing IgM anti-GAT antibodies but lacked CGAT idiotypic determinants, were not bound. Furthermore, the heavy-chain amino terminal sequence of F9-102.2 is identical with that of the other CGAT-positive hydriboma antibodies up to position 27 (M. Pierres, C. Tonnelle, and M. Fougereau. Personal communication.). Interestingly, weak binding was found with ^{125}I-labeled D1.LP anti-GAT antibodies (6.1), 65% of which could be bound with guinea pig anti-idiotypic antiserum prepared against pooled D1.LP anti-GAT antibodies. This latter reagent reacted strongly with the hybridoma anti-GAT antibodies (11) and exhibited weak binding of ^{125}I-B10.WB anti-GLϕ antibodies.

Strain Distribution of GTGL Idiotype

STRAINS IMMUNIZED WITH GAT AND ITS RELATED POLYMERS. The specific idiotypic interaction between ^{125}I-labeled hybridoma D (F9-195.6) anti-GAT antibody and guinea pig anti-idiotypic antiserum-designated GTGL idiotype was chosen to further analyze this unusual idiotypic cross-reaction. Table II demonstrates and compares the distribution patterns of GTGL and CGAT idiotypes in various mouse strains immunized with GAT, GAT^{33}, GAT-FGG, GT, and GA polymers. ^{125}I-GAT- and ^{125}I-GLT-binding activities of 5 μl antisera were also determined. 10 μl of preimmune or normal mouse sera was included as specificity controls in all experiments. Other specificity controls included the lack of inhibition of GTGL idiotype binding with 25

TABLE I

Guinea Pig Anti-Idiotypic Antiserum to B10.WB Anti-GLϕ Antibodies Bound DBA/2 IgM Hybridoma Anti-GAT Antibodies

^{125}I-labeled ligand‡	Percent labeled ligand bound*			
	Normal guinea pig serum	Anti-idiotype to		Anti-Ig antiserum
		B10.WB anti-GLϕ antibody	D1.LP anti-GAT antibody	
F9-102.2 (A)	3	5	78	80
F9-238.9 (B)	19	60	69	77
F9-195.6 (D)	3	63	91	91
F9-38.1 (E)	28	79	92	93
F9-231.3 (F)	2	65	89	89
F9-32.2 (G)	3	62	88	91
F9-94.6 (H)	5	65	93	94
MOPC 21	1	1	2	95
TEPC 183	3	5	2	89
B10.WB anti-GLϕ Antibody	3	72	7	90
D1.LP anti-GAT Antibody	2	9	65	86

* 4 μl of normal guinea pig serum and anti-idiotypic antiserum and 3 μl of rabbit anti-mouse Ig were used in the binding experiments.

‡ Letter in parentheses is the code used for our laboratory designations of the hybridoma anti-GAT antibodies.

μg of TEPC-183 or MOPC-21 myeloma proteins (<10%). Preimmune sera and normal mouse sera caused an average of 15 ± 4% inhibition of GTGL idiotype binding. Furthermore, sera from GAT[33] nonresponder mice (C3H.Q) also failed to inhibit GTGL idiotype binding. In contrast, anti-GAT, anti-GAT-FGG, and anti-GAT[33] antisera obtained from all responder strains except C.AL-20, A/J, and its H-2 congenic strains, strongly inhibited GTGL idiotype binding. In most cases, more than 85% inhibition was observed. Experiments carried out simultaneously demonstrated that all these sera including GTGL idiotype-negative strains, expressed a high level of CGAT idiotype. The expression of GTGL idiotypic specificities appeared to be a dominant trait as CAF_1 anti-GAT antisera exhibited high levels of GTGL idiotypic antibodies. Interestingly, the Igh-congenic C.AL-20 mice, a strain considered similar to A/J with respect to V-gene repertoire, also failed to produce anti-GAT antibodies bearing GTGL idiotype. The data obtained with anti-GAT-FGG antisera from GAT responder and nonresponder strains indicated that GAT nonresponder mice (C3H.Q and DBA/1) immunized with GAT-FGG produced GTGL idiotype.

The antigenic moieties on GAT molecules responsible for the induction of GTGL idiotype are GT-related determinants. This is demonstrated by the ability of all the GT-containing polymers including the GT polymer, to induce the GTGL idiotype. Conversely, anti-GA antisera from four different mouse strains, although capable of binding GAT polymer, did not exhibit detectable levels of GTGL idiotype.

STRAINS IMMUNIZED WITH GLϕ AND ITS RELATED POLYMERS. Table III shows the distribution pattern of GTGL idiotype in various mouse strains immunized with GLϕ, GLpro, GLA, Gϕ, and GL-FGG. The ability of the immune sera to inhibit GTGL and CGAT idiotype binding was assessed. In addition, we tested the ability of these

TABLE II

*Strain Distribution of GTGL Idiotype in Antisera to GAT and Related Polymers**

Strains‡	Antigen	Percent antigen binding		Percent inhibition of idiotype binding§	
		GAT	GLT	CGAT	GTGL
BALB/c	GAT	88	42	100	95
C.AL-20	GAT	57	20	95	22
B10.BR	GAT	59	43	62	95
SM/J	GAT	89	54	89	96
DBA/2	GAT	100	70	100	90
A/J	GAT	75	33	91	20
A.BY	GAT	90	55	90	23
A.CA	GAT	93	59	95	12
SEA/GN	GAT	82	47	98	100
CAF_1	GAT	87	55	99	100
BALB/c	GAT^{33}	93	49	100	96
B10.D2	GAT^{33}	94	61	100	98
C3H.SW	GAT^{33}	73	25	80	54
C3H.Q	GAT^{33}	−5	−1	−3	12
GD	GAT-FGG	88	51	100	96
DBA/1	GAT-FGG	60	37	100	99
DBA/2	GAT-FGG	89	53	100	100
C3H.Q	GAT-FGG	84	62	74	100
C3H.SW	GAT-FGG	87	65	89	85
GT	GT	31	5	87	87
BALB/c	GA	34	1	−5	5
A/J	GA	62	6	7	13
SEA/Gn	GA	51	4	−3	23
CE/J	GA	43	5	0	6

* Most samples tested were pooled immune sera. A number of individual sera tested gave similar results. The average percent inhibition of idiotype binding with 10 B10 and 10 DBA/2 normal mouse sera was 15 ± 4%.

‡ Additional sera tested not shown are: anti-GAT^{33} antisera from 15R, B10.SM, C3H, and PL; anti-GAT-FGG antisera from C3H.NB, B10.BR, and DBA/2. All sera tested were positive for CGAT and GTGL idiotypes. C3H.Q mice are genetic nonresponders to GAT^{33}; this serum is included as a negative control.

§ The quantities of unlabeled autologous ligands needed for 50% inhibition of idiotype binding were: 250 and 600 ng for CGAT and GTGL idiotype, respectively.

antisera to bind ^{125}I-GAT and ^{125}I-GLT. The majority of these sera did not express detectable levels of CGAT idiotype. This is particularly evident for anti-GLpro, anti-GLA, anti-Gϕ, and anti-GL-FGG antisera of all strains tested. However, under identical conditions, anti-GLϕ antisera from B10.WB, RIII, BALB/c, C3H.Q and LG/J, and CAF_1 strains caused 20–48% inhibition of CGAT idiotype binding. This level of inhibition is considered significant because experiments carried out at the same time with a large number of individual or pooled normal mouse sera consistently caused <10% inhibition. Furthermore, when 50 μl of 20 individual normal mouse sera and 20 individual anti-GLϕ antisera from B10.WB, C3H.Q, and BALB/c strains were tested simultaneously for CGAT idiotype, higher levels (40–50%) of inhibition were observed with anti-GLϕ antisera, whereas all of the control sera caused less the than 15% inhibition of CGAT idiotype binding (data not shown). Interestingly, some of these anti-GLϕ antisera also exhibited weak cross-reactive binding to GAT.

TABLE III

Strain Distribution of GTGL Idiotype in Antisera to GLϕ-related Polymers *

Strains‡	Antigen	Percent antigen binding		Percent inhibition of idiotype binding§	
		GAT	GLT	CGAT	GTGL
BALB/c	GLϕ	13	41	28	84
B10.WB	GLϕ	17	70	33	97
SWR	GLϕ	29	100	21	95
RIII	GLϕ	68	100	38	100
C3H.Q	GLϕ	15	50	19	97
LG/J	GLϕ	53	87	40	99
2R	GLϕ	5	1	−3	7
CAF_1	GLϕ	56	91	48	90
SJL	GLpro	8	49	−4	65
9R	GLpro	4	31	−1	85
A.TH	GLpro	0	38	−1	0
BALB/c	GLA	0	64	−4	80
B10.M	GLA	10	74	0	82
DBA/2	GLA	−15	64	4	57
A/J	GLA	0	81	6	12
A.TFR-3	GLA	15	87	7	7
B10.F	GLA	−6	7	−3	1
P/J	GL-FGG	4	70	12	65
B10.BR	GL-FGG	2	54	7	87
DBA/1	GL-FGG	9	81	21	78
A/J	GL-FGG	19	92	7	29
CE/J	GL-FGG	9	94	−4	55
2R	Gϕ	6	2	10	18
SWR	Gϕ	36	2	12	18

* Most samples are pooled sera. Over 20 individual B10.WB, BALB/c, and C3H.Q anti-GLϕ antisera tested gave similar results. For normal mouse serum controls see legend to Table II.

‡ Additional sera tested not shown are: DBA/2, B10.D2, and B10.A(5R) anti-GLϕ antisera; B10.BR, SJL, and A.BY anti-GLA antisera. Only A.BY anti-GLA antisera was negative for GTGL idiotype. As controls, sera from genetic nonresponder strains 2R (GLϕ nonresponder) and B10.F (GLA nonresponder) were included.

§ For sensitivity of the idiotypic systems, see footnotes to Table II.

When the same antisera were tested for the expression of GTGL idiotype, some interesting observations emerged. 2R GLϕ nonresponder mice and B10.F GLA nonresponder mice did not produce detectable GLT binding activity and consequently their antisera did not inhibit GTGL idiotype binding (Table III). In contrast, anti-GLϕ, anti-GLA, anti-GLpro, and anti-GL-FGG antisera from all responder mouse strains except C.AL-20, A/J, and its H-2 congenic strains expressed GTGL idiotype as shown by the ability of these sera to inhibit idiotype binding. Antisera from A/J mice immunized with GLA and GL-FGG possessed high levels of GLT-binding activity, but expressed little or nondetectable levels of GTGL idiotype. Thus, the strain distribution pattern of GTGL idiotype obtained with antisera from various responder mouse strains immunized with GLϕ and some of its related compounds is identical with that obtained with antisera specific to GAT and some of its related compounds (Tables II and III).

It is important to note that these anti-GLpro, anti-GLA, and anti-GL-FGG antisera possessed strong GLT-binding activity but exhibited either extremely weak or non-detectable levels of cross-reactive binding to GAT. These results argue that GTGL idiotypic determinants are present on a population of anti-GL antibodies which lack detectable specificity for the GAT polymer. However, some anti-GLϕ antisera weakly cross-reacted with GAT polymer. To determine whether these GAT cross-reactive antibodies can account for the total GTGL idiotype in anti-GLϕ antisera, we specifically purified anti-GAT and anti-GLϕ antibodies and quantitatively compared the levels of GTGL idiotype per unit weight of anti-GAT antibody activity. The results shown in Table IV demonstrate that B10.WB and C3H.Q anti-GLϕ antibodies contained less than 13% of the GAT-binding activity noted with anti-GAT antibodies but expressed more than 50% of the GTGL idiotype on a unit weight basis with D1.LP and DBA/2 anti-GAT antibodies. Similarly, these anti-GLϕ antibodies showed less than 1% of the GAT-agglutinating activity but exhibited 15–18% of the GTGL idiotype as compared with purified hybridoma anti-GAT antibodies. These results strongly suggest that, in addition to GAT cross-reactive antibodies, additional GL- or GLϕ-specific antibodies also possess the GTGL idiotypic determinants (see below).

The demonstration of GTGL idiotype in anti-GLA, anti-GLpro, and anti-GL-FGG antisera argues against the assumption that Gϕ-related determinants on the GLϕ polymer were responsible for the induction of the GTGL idiotype. To demonstrate further this point, we tested antisera obtained from B10.A (2R) and SWR mice immunized with Gϕ, for the presence of CGAT and GTGL idiotypes. Merryman et al. (13) have shown that SWR and 2R mice produce anti-Gϕ antibody activity. In the present experiment, we demonstrated that these antisera also displayed binding activity to GLϕ by solid-phase radioimmunoassay, and the activity of SWR anti-Gϕ antisera is comparable with that obtained with B10.WB anti-GLϕ antibodies (data not shown). Furthermore, SWR anti-Gϕ antisera possessed moderate GAT-binding activity as judged by modified Farr binding assays. In spite of this cross-reactive

TABLE IV

Quantitative Measurement of GTGL Idiotype

Specifically purified antibodies*	Nanograms required for		
	33% GAT binding	GAT agglutination	50% inhibition of GTGL idiotype binding
Anti-GAT hybridoma F9-195.6	>5,000‡	7	600
D1.LP anti-GAT, antibodies	120	103	2,200
DBA/2 anti-GAT antibodies	150	100	2,300
B10.WB anti-GLϕ antibodies	>2,100	1,050	3,800
C3H.Q anti-GLϕ antibodies	3,000	875	4,000
C57BL/6 anti-Ar antibodies	>27,000	>2,500	>27,000

* The purified DBA/2 anti-GAT antibodies and the control C57BL/6 anti-Ar antibodies were obtained from pooled sera, the other antibodies were purified from ascites derived from individual mice.

‡ The hybridoma IgM anti-GAT antibodies had extremely weak GAT-binding activity using either guinea pig anti-IgM antiserum or 50% saturated $(NH_4)_2SO_4$ as a precipitating agent.

binding activity, both B10.A (2R) and SWR anti-Gϕ antisera exhibited nonsignificant levels of GTGL idiotype. In contrast, GLϕ responder SWR, B10.A (5R), B10.WB, and B10.D2 (B10.A [2R] is a nonresponder) produced GTGL idiotype upon GLϕ immunization. These results strengthen the conclusion that the antigenic moieties on GLϕ and related compounds responsible for the induction of GTGL idiotype are the GL-related determinants. Furthermore, the results also strongly suggest that Gϕ-related determinants on GLϕ do not induce high levels of GTGL idiotype.

Fractionation of GTGL Idiotypic Antibodies. To establish whether the GTGL idiotypic determinants found in anti-GAT and anti-GLϕ antisera are localized in a fraction of antibody molecules bearing cross-reactive specificity for GAT and GLϕ, or that anti-GAT and anti-GLϕ antibodies bearing GTGL idiotype actually possess distinct antigen binding specificity. We carried out reciprocal immunoadsorption experiments using GAT- and GLϕ-Sepharose 4B beads with pooled DBA/2 anti-GAT and B10.WB anti-GLϕ antisera and tested both the unbound and eluted fractions for GTGL idiotype and for GAT- and GLT-binding activity. The results were shown in Table V. Control experiments established that (*a*) GAT beads and GLϕ beads do not nonspecifically retain irrelevant antibody molecules by simple charge interactions and (*b*) anti-GAT and anti-GLϕ antisera do not nonspecifically adsorb onto dinitrophenyl (DNP)-ovalbumin (OVA) (DNP-OVA)- and *p*-azophenylarsonate conjugate of bovine gamma globulin (Ar-BGG)-conjugated beads. Both GAT beads and GLϕ beads were able to remove completely GTGL idiotype-bearing anti-GAT and anti-GLϕ antibodies, respectively, as evidenced by the inability of the unbound fraction to inhibit GTGL idiotype binding. Concomitant with the removal of GTGL idiotype, the loss of antigen-binding activities for GAT and GLT were observed. These GTGL-bearing anti-GAT and anti-GLϕ antibodies could be partly recovered by acid elution. Under

TABLE V

Cross-Immunoadsorption of GTGL-bearing Idiotype in Anti-GAT and Anti-GLϕ Antisera

Inhibitor	Treatment*		Percent antigen binding		Percent inhibition of GTGL idiotype binding
	Column	Fraction	GAT	GLT	
Pooled DBA/2 anti-GAT antisera	Ar-BGG beads	U	97	51	94
		E	3	8	3
	GAT beads	U	13	7	2
		E	70	21	76
	GLϕ beads	U	85	13	51
		E	60	36	38
Pooled B10.WB anti-GLϕ antisera	DNP-OVA beads	U	14	52	91
		E	1	3	8
	GLϕ beads	U	0	4	9
		E	12	41	38
	GAT beads	U	2	24	38
		E	9	28	52

* Experimental procedure was described in Materials and Methods. U and E represent the unbound and eluted fractions, respectively. For antigen binding, 5 and 20 μl of the U and E were tested. For GTGL idiotype assay, 10 and 30 μl of U and E were used. Control experiments with B10 and DBA/2 normal mouse serum and B6 anti-NP antisera demonstrated that no inhibiting materials for GTGL idiotype binding could be obtained from GAT and GLϕ beads and that these beads did not nonspecifically retain unrelated NP^b idiotype.

identical conditions, cross-immunoadsorption of anti-GAT and anti-GLϕ antisera with GLϕ and GAT beads, respectively, resulted in partial loss of the GTGL idiotype-bearing antibodies as indicated by the lower inhibition values of GTGL idiotype binding (compared with DNP-OVA- and Ar-BGG beads). Both acid eluted fractions possessed GTGL idiotype-bearing antibodies. Furthermore, such cross-immunoadsorption appeared to remove the cross-reactive antibodies, leaving antibodies bearing specificity for autologous antigen untouched. Collectively, these results clearly demonstrated that distinct anti-GAT and anti-GLϕ antibodies, as well as GAT- and GLϕ-cross-reactive antibodies, can share common GTGL idiotypic determinants. Thus, two populations of GTGL idiotype-bearing antibodies are present in both anti-GAT and anti-GLϕ antisera; one uniquely reacts with autologous antigen and the other exhibited dual binding specificities to both GLϕ and GAT polymers.

Discussion

The data we presented establish four major points. First, shared GTGL idiotypic determinants demonstrated on antibodies bearing distinct binding specificities, are present in various mouse antisera directed against a diverse series of polypeptides containing either GT- or GL-related determinants. The antigenic moieties responsible for the induction of GTGL idiotypic antibodies were identified to be GT- and GL-related determinants. Second, the occurrence of GTGL idiotype in inbred strains of mice is extremely frequent; both individual and pooled antisera obtained from 25 of the 31 mouse strains immunized with 8 GT-related or GL-related polymers exhibited high levels of GTGL idiotype. Third, the ability to produce GTGL idiotype is controlled by Igh-linked gene(s) as evidenced by the inability of C.AL-20 anti-GAT and anti-GLϕ antisera to express GTGL idiotype. BALB/c mice, which differ from C.AL-20 mice only at the Igh-linked genes, express GTGL idiotype upon immunization with either GT- or GL-related polymers. Furthermore, antisera from A/J mice (Igh-1^e) and all of its H-2 congenic strains failed to express GTGL idiotype despite having high levels of CGAT idiotype and strong GAT-binding activity. Lastly, antibodies bearing distinct antigen-binding specificities to either GAT- or GLϕ-related determinants, and antibodies exhibiting dual GAT- and GLϕ-binding activity expressed GTGL idiotype.

It is important to emphasize the uniqueness associated with the GTGL idiotypic system. The ^{125}I-labeled ligand was obtained from monoclonal IgM anti-GAT antibody of DBA/2 origin, and the idiotypic immunogens that were used to prepare anti-idiotypic antiserum were purified from individuals of a different strain (B10.WB) immunized with a different polymer (GLϕ). In addition, all of the immune antisera tested as inhibitors in the radioimmunoassays were obtained from various sources neither involved with the production of ligand nor the anti-idiotypic antiserum. Such completely independent processes excluded any possibility of contamination of experimental reagents and provided unequivocal evidence for the validity of the shared GTGL idiotypic determinants reported in this study.

Our method of detecting shared idiotypic determinants is different from those of Oudin and Cazenave (2), Karol et al. (3), and of Eichmann et al. (4). The implications are also different. The above investigators used homologous idiotypic interactions to detect shared idiotypes (in which the anti-idiotypic antiserum was made against the same idiotypic antibodies used as ligand). Their demonstration of shared idiotypic

specificities, therefore, reflected the presence of virtually all idiotypic determinants in the fraction not bearing known antibody specificity. In contrast, presence of shared GTGL idiotype among antibodies against GLϕ, GAT, and related polymers are most convincingly demonstrated by using a heterologous idiotypic interaction (DBA/2 monoclonal IgM anti-GAT antibody as ligand and anti-idiotypic antiserum made against B10.WB anti-GLϕ antibodies). In our experience homologous idiotypic interactions (^{125}I-labeled D1.LP anti-GAT antibodies and guinea pig anti-idiotypic antiserum made against D1.LP anti-GAT antibodies) fail to detect shared idiotypic determinants in antisera against GL-related polymers (Table IV). These results imply that homologous idiotypic interactions detect a more complex set of idiotypic determinants whereas heterologous idiotypic interactions selectively detect a limited number of idiotypic determinants and thereby permit the demonstration of GTGL idiotypic specificities.

Using reciprocal immunoadsorptions with GAT and GLϕ beads, we demonstrated some GTGL-bearing idiotypic antibodies that were specific for GAT, whereas others were specific for GLϕ. In addition, we identified some GTGL idiotypic antibodies possessing dual specificity for GAT and GLϕ. Whether the latter antibodies induced by GAT and GLϕ in a given strain are the products of the same B cell clones remains to be determined. Furthermore, all of the anti-GLpro, anti-GLA, and anti-GL-FGG antisera that exhibited cross-reactive GTGL idiotypic antibodies failed to express detectable levels of GAT-binding activity as judged by highly sensitive immunoassay (Tables III and IV).

The above results have important implications with respect to the genetic mechanisms accounting for the inheritance and generation of antibody specificities. The presence of GTGL-idiotypic determinants on antibodies bearing distinct specificities are compatible with the hypothesis that these antibodies are coded for by two separate germ line V genes. It is possible that the two germ line V-region genes are derived from one ancestral gene by duplication and then underwent independent evolutionary pressure such that one became specific for GT- and the other for GL-related determinants; similar arguments have been made in another idiotypic system (5). Alternatively, one germ line V gene could be derived by a process of somatic mutation. Our results suggest that such a somatic diversification process must have occurred in a predictable manner to generate GT- and GL-binding antibodies, without affecting the GTGL idiotypic determinants. This restriction on the somatic mutation model is similar to the programmed somatic generation of antibody specificity proposed by Klinman et al. (14). Another possibility is that DNA segments encoding the GTGL idiotypic determinants could be incorporated into, and involved in the formation of, two separate V genes for anti-GT and anti-GL antibodies. This hypothesis is compatible with the insertion (minigene) theory of antibody specificities (15). It is also possible that the GTGL-idiotypic determinants in anti-GAT and anti-GLϕ antibodies may be governed by genes controlling the J segments responsible for the joining of V gene to C gene; then the recombination of GAT- and GLϕ-specific V genes to J segments may occur in a nonrandom fashion. In this regard, it should be noted that Schilling et al. (16) reported that hybridoma anti-α-(1,3) dextran antibodies and some myeloma proteins bearing different specificities possess identical J segments as determined by amino acid sequence analysis.

Summary

We developed an idiotypic radioimmunoassay system that detects shared idiotypic determinants, termed GTGL idiotype, on antibodies bearing distinct antigen-binding specificities in various mouse strains. Either poly-(Glu, Tyr) (GT)- or poly-(Glu, Lys) (GL)-related determinants are able to induce anti-GT and anti-GL (GTGL)-idiotypic antibodies. Strain distribution studies indicate that GTGL-idiotypic antibodies are readily induced and frequently expressed in antisera obtained from 25 different mouse strains immunized either with GT-related or GL-related polymers. The ability to express GTGL-idiotypic antibodies is a dominant trait and is controlled by Igh-linked gene(s). In addition, we demonstrated that in anticopolymer of L-glutamine acid^{60}-L-alanine^{30}-L-tyrosine (GAT) and anticopolymer of L-glutamic acid^{54}-L-lysine^{35}-L-phenylalanine^{11} (GLϕ) antisera, both antibodies uniquely specific to GAT or GLϕ, respectively, and antibodies bearing dual specificities for GAT and GLϕ, expressed GTGL idiotype. The genetic implications of these findings are discussed.

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