γ-GLUTAMYL CYCLOTRANSFERASE: A NEW GENETIC POLYMORPHISM IN THE MOUSE (*MUS MUSCULUS*) LINKED TO *LYT-2*.

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ABSTRACT

Electrophoretically variant forms of γ-glutamyl cyclotransferase have been identified in red cells of inbred mouse strains. Each inbred strain exhibited a major band of activity and a minor band that migrated more anodally. The polymorphism affects the migration of both the major and minor bands in a similar way. F_1 hybrids between strains with fast forms (A/J) and strains with the slow forms (C57BL/6J) exhibited a four-banded pattern consistent with co-dominant inheritance. The patterns observed in backcross and F_2 mice were consistent with the segregation of a pair of autosomal co-dominant alleles. Recombinant inbred strains and a congenic strain were used to show that the locus controlling γ-glutamyl cyclotransferase (*Ggc*) is linked to *Lyt-2*, a lymphocyte alloantigen locus on chromosome *6*, with an estimated map distance of 5.0 ± 2.5 centimorgans.

THE enzyme γ-glutamyl cyclotransferase catalyzes the following reaction: L-γ-glutamyl-L-amino acid————5-oxo-L-proline + L-amino acid (CONNELL and HANES 1956; ORLOWSKI and MEISTER 1973). The enzyme is of considerable interest because it is part of the γ-glutamyl cycle, which may be involved in the transport of amino acids and the degradation and synthesis of glutathione (ORLOWSKI and MEISTER 1973; ORLOWSKI and WILK 1975).

The enzyme has been found in homogenates of rat kidney, liver, testes, spleen, brain, lung, heart, thymus, thyroid, skeletal muscle and adrenal glands (ORLOWSKI and MEISTER 1973), as well as intestine (CORNELL and MEISTER 1976). γ-Glutamyl cyclotransferase activity has been detected in human brain, kidney, liver, spleen, pancreas, lung, heart muscles, sketal muscle, serum and red cells (CONNELL and SZEWCZUK 1967) and lymphoid cells (NOVOGRODSKY, TATE and MEISTER 1976). The enzyme has been purified from human brain and hog liver (ORLOWSKI, RICHMAN and MEISTER 1969).

Electrophoretically variant forms of γ-glutamyl cyclotransferase can be distinguished in lysates of red cells of both mouse and man (TULCHIN 1977). The isozyme patterns of mice are controlled by a pair of autosomal co-dominant

alleles, designated *Ggc*^{a} and *Ggc*^{b}. Recombinant inbred and congenic strains are used to show that the *Ggc* locus is linked to the *Lyt–2* lymphocyte alloantigen locus on chromosome *6*.

MATERIALS AND METHODS

Mice of all the inbred, RI and congenic strains were raised in the Animal Resources and Research colonies of the Jackson Laboratory, unless otherwise noted. Two mice of each strain were bled from the retroorbital sinus with heparinized capillary tubes. Red cells were separated from plasma by centrifugation and frozen at —80° and subsequently shipped to New York on dry ice for analysis. The C57BL/6JBoy, C57BL6/*Lyt-2*^{a}/Boy, C57BL/6-*Lyt-2*^{a} *Lyt-3*^{a}/Boy, C3H/An, C3H/Bi and C3H/Fg strains were generously provided by E. Boyse, Sloan-Kettering Research Institute. Backcrosses of B6AF$_1$ (A/J × C57BL/6J) to both parental strains, A/J and C57BL/6J, and the respective F$_2$ generation were raised in the facilities of A. Wishnia at Stony Brook, New York.

Extracts from kidney and liver of the parental and hybrid strains were prepared by homogenizing the fresh tissue at 0° for two min with three volumes of Tris-HCl buffer (0.05 M; pH 8.0) in a Potter-Elvehjem glass homogenizer equipped with a motor-driven Teflon pestle. The homogenates were centrifuged for 30 min at 34,000 × g, and the supernatant fluids were tested for their isozymes.

Human red cell lysates were prepared for peripheral blood by routine methods. Extracts from human spleen (supplied by M. Fotino, New York Blood Center) were prepared as described above for mouse tissue. Human lymphocyte extracts were prepared by centrifugation of lymphoblastoid lines, washing in phosphate buffered saline and freeze-thawing.

Gel electrophoresis was performed after the method of Orlowski and Meister (1973), except that horizontal agarose electrophoresis was substituted for vertical starch electrophoresis. The gels were buffered at pH 8.6 with a solution consisting of 0.045 M Tris, 0.25 M boric acid and 0.001 M EDTA; 1.5% agarose (Bio-Rad) was used in the gel. The cathodal buffer was 2.5 times and the anodal buffer 4 times more concentrated than the gel buffer. Samples of 10–30 μl of red cell lysate, white cell lysate or tissue homogenate were applied to slots in the gel and electrophoresis was carried out in a cold room at 20V/cm for 3 hr. The reaction mixture employed for localizing the enzyme was the same as that of Orlowski and Meister (1973) with the addition of 0.1 M $MnCl_2$ (Lewis and Harris 1967). γ-Glutamyl-L-methionine was purchased from the Bachem Corp. (Torrance, CA). Specificity of the reaction for γ-glutamyl cyclotransferase was shown by lack of deposition of oxidized dianisidine when γ-glutamyl-L-methionine was omitted.

RESULTS

The results of a typical electrophoresis run are shown in Figure 1. The γ-glutamyl cyclotransferase isozymes migrate towards the anode at pH 8.6 (some insoluable material remains at the origin in liver extracts) (Figure 1, photograph Channels 2,4).

The phenotypic pattern AB, found in the hybrid B6AF$_1$ tissues, consists of four bands: two dense A and B, and two lighter bands A$_1$ and B$_1$. The parental strain A/J has the phenotypic pattern A, which consists of one dense band A and one lighter band A$_1$; the other parent (C57BL/6J) has the phenotypic pattern B, which consists of one dense band B and one lighter band B$_1$. The red cell lysates from the mouse strains have patterns entirely similar to the tissue extracts; hemoglobin migrates 2.5 cm from the origin when the B band appears at 5 cm.

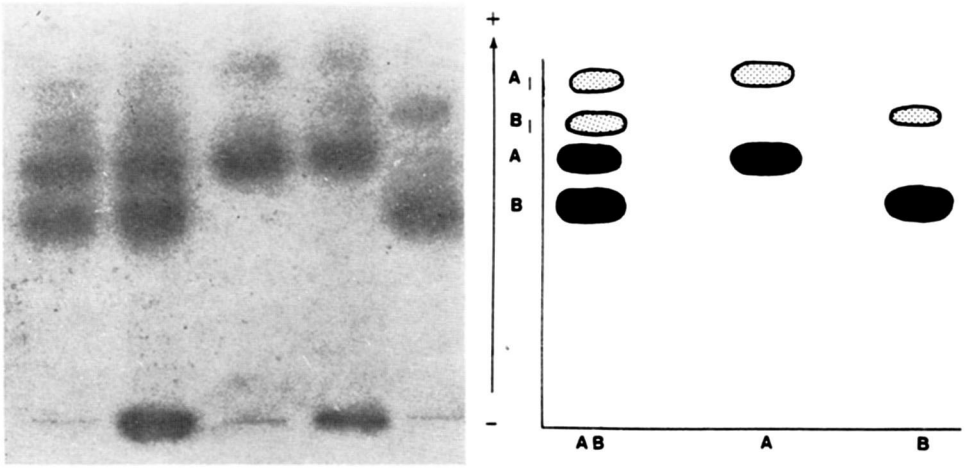

FIGURE 1.—Zymogram of mouse γ-glutamyl cyclotransferase isozymes. The hybrid mouse ($B6AF_1$) kidney and liver extracts consist of two equivalent dense bands (A,B) and two lighter bands (A_1, B_1) (photograph, Channels 1, 2; and drawing 1). The parental strain (A/J) has isozyme pattern A, which consists of one dense band A, and a lighter band A_1 (photograph, Channels 3, 4; and drawing 2). The other parental strain (C57BL/6J) has pattern B, which has a dense band B and a lighter band B_1 (photograph, Channel 5; and drawing 3). The liver extracts have some insoluble material at the origin that strains for γ-glutamyl cyclotransferase (photograph, Channels 2, 4).

The human red cell γ-glutamyl cyclotransferase isozymes migrate faster than the mouse isozymes and appear 5 cm anodal to the hemoglobin band(s) and 7.5 cm anodal to the origin. The common human red cell pattern found in 598 individuals of 600 tested consists of one dense anodal band, and one lighter cathodal band. In the two rare individuals, the pattern was reversed, the cathodal band was the darker one and the anodal band was lighter. Extracts of three human spleens and lymphocyte extracts were identical to the variant pattern, indicating that there was tissue-specific isozyme variation.

Genetic crosses were made to investigate the mode of inheritance of γ-glutamyl cyclotransferase electrophoretic differences. The $B6AF_2$ generation yielded representatives of both parental phenotypes, as well as the hybrid phenotype (A, B, and AB). Backcrosses to A/J were either A or AB in phenotype, while backcrosses to C57BL/6J were either B or AB in phenotype. Although the number of animals in these crosses was insufficient for statistical analysis, the results were consistent with autosomal co-dominant inheritance. The allele carried by strain A/J is designated Ggc^a, while the allele carried by strain C57BL/6J is designated Ggc^b.

Distribution of Ggc *alleles among inbred, RI, and congenic strains*

Inbred strains: A large number of inbred strains maintained at the Jackson Laboratory were classified with respect to the γ-glutamyl cyclotransferase phenotype (Table 1). The phenotype of most of these strains resembled A/J and are presumed to carry the Ggc^a allele. Relatively few strains resembled

TABLE 1

Distribution of Ggc *alleles among inbred strains*

Ggc^a		Ggc^b
A/J	I/LnJ	B10.D2(58N)/Sn
A/HeJ	IS/CamEi	C57BL/KsJ
ABP/J	LG/J	C57BL/6J
AKR/J	LP/J	C57BL/6ByJ
AU/SsJ	LT/Sv	C57BL/6JBoy
BALB/cByJ	MA/MyJ	C58/J
BALB/cJ	MK/Re	FS/Ei
BDP/J	*M.m.castaneus* (F11)	HP/EiTy
BUB/BnJ	*M.m.molossinus*	JGBF/Ty
CBA/J	NZB/B1NJ	MWT/Le
CBA/CaJ	P/J	PRO/Re
CBA/HT6J	Peru/AtteckEi (F19)	RAP/GnLe
CE/J	PH/Re	SK/CamRk (F12)
C3H/An	PL/J	
C3H/Bi	RF/J	
C3H/Fg	RSV/Le	
C3H/HeJ	RIIIS/J	
C3HeB/FeJ	SEA/GnJ	
C57BR/cdJ	SEC/1ReJ	
C57L/J	SF/CamRk (F18)	
DBA/2J	SJL/J	
DW/J	SM/J	
FL/1Re	ST/bJ	
FL/2Re	SWR/J	
HRS/J	129/J	

C57BL/6J and are presumed to carry the Ggc^b allele. All of the latter are known to be related to the C57BL or C58 strains, with the exception of SK/CamRk, which has been inbred from feral mice. Thus the Ggc^b allele appears to be rare among inbred mice, but is represented in at least one feral mouse population.

RI strains: In an effort to assign *Ggc* to a specific chromosome, several sets of RI strains were typed whose progenitors differed at *Ggc* (TAYLOR, BEDIGIAN and MEIER 1977; BRADLEY *et al.* 1979; BAILEY 1971) (See Tables 2 and 3). A high degree of concordance was observed between the segregation of *Ggc* and the *Lyt–2* locus in both the BXD RI strains (five discordant strains among 26 strains) and the BXH RI strains (one discordant strain among 13 strains). These results differ significantly from the expectation based on the assumption of independence ($P < 0.01$). Also noteworthy is the absence of the $Lyt\text{–}2^a$ allele of C3H/HeJ in any of the BXH RI strains. Similar distorted segregation has been observed for another polymorphic locus, *Lvp–1*, in this region (WILCOX, personal communication). One must assume that there was strong selection directed against some gene in the vicinity of *Lyt–2*. We have used the method of TAYLOR *et al.* (1975) to calculate the recombination frequency between *Ggc* and *Lyt–2*. Of 39 independently derived RI strains, only six exhibited recom-

TABLE 2

Inheritance of Ggc *and* Lyt-2 *alleles in the BXD and BXH sets of recombinant inbred strains**

	BXD																								
Locus	1	2	5	6	8	9	11	12	13	14	15	16	18	19	20	21	22	23	24	25	27	28	29	30	31	32
Ggc	*B*	B	*B*	D	D	B	D	B	D	B	B	B	B	D	B	*D*	D	B	*B*	D	D	*D*	B	*D*	B	B
Lyt-2	D	B	D	D	D	B	D	B	D	B	B	B	B	D	B	D	D	B	D	D	D	B	B	B	B	B

	BXH											
Locus	2	3	4	5	6	7	8	9	10	11	12	14	19
Ggc	*H*	B	B	B	B	B	B	B	B	B	B	B	B
Lyt-2	B	B	B	B	B	B	B	B	B	B	B	B	B

* B, D and H are used here as generic symbols for alleles inherited from the progenitor strains C57BL/6J, DBA/2J and C3H/HeJ, respectively. The *Lyt-2* typing was kindly provided by MARIANNA CHERRY (personal communication).

TABLE 3

Inheritance of Ggc *alleles in the CXB, LXB, 58NXL and BRX58N sets of recombinant inbred strains**

Ggc^a	Ggc^b
CXBG	CXBD
CXBI	CXBE
CXBJ	CXBH
CXBK	LXB-2
LXB-3	58NXL-1
LXB-4	58NXL-2
58NXL-4	58NXL-3
BRX58N-1	58NXL-8
BRX58N-3	BRX58N-7
BRX58N-4	BRX58N-8
BRX58N-11	BRX58N-9
BRX58N-12	
BRX58N-13	

* The progenitors of these four sets of RI strains are given parenthetically as follows: CXB (BALB/cBy × C57BL/6By); LXB (C57L/J × C57BL/6J); 58NXL (B10.D2(58N)/Sn × C57L/J); and BRX58N (C57BR/cdJ × B10.D2(58N)/Sn.

binant phenotypes giving an estimate of the recombination frequency of 5.0 ± 2.5 centimorgans.

Congenic strains: Linkage between *Ggc* and the *Lyt-2,3* complex was identified by typing three congenic strains with respect to *Ggc* (Table 4). The B6.PL-*Lyt-2*^{a} *Lyt-3*^{a}/Cy congenic strain had been constructed by transferring the *Lyt-2*^{a} and *Lyt-3*^{a} alleles (closely linked loci) of PL/J onto the C57BL/6J background by 10 generations of backcrossing, followed by brother-sister inbreeding (GIBSON, TAYLOR and CHERRY 1978; CLAFLIN *et al.* 1978). The C57BL/6-*Lyt-2*^{a} *Lyt-3*^{a}/Boy congenic strain was similarly constructed by transferring the *Lyt-2*^{a} and *Lyt-3*^{a} alleles of RF onto the C57BL/6JBoy background by 16 generations of backcrossing, followed by brother-sister inbreeding. The C57BL/6-*Ly-2*^{a}/Boy congenic strain had been constructed by transferring the *Lyt-2*^{a} allele from C3H/An onto the C57BL/6JBoy background by 16 generations of backcrossing, followed by brother-sister inbreeding (KLEIN 1973; BOYSE, per-

TABLE 4

Distribution of Ggc *alleles among* Lyt-2,3 *congenic strains*

Congenic strain		Background strain		Donor strain	
Designation	*Ggc* allele	Designation	*Ggc* allele	Designation	*Ggc* allele
B6.PL-*Lyt-2*^{a} *Lyt-3*^{a}/Cy	*a*	C57BL/6J	*b*	PL/J	*a*
C57BL/6-*Lyt-2*^{a} *Lyt-3*^{a}/Boy	*a*	C57BL/6JBoy	*b*	RF	*a**
C57BL/6-*Lyt-2*^{a}/Boy	*b*	C57BL/6JBoy	*b*	C3H/An	*a*

* Although the donor RF subline was not typed directly, its genotype was inferred to be Ggc^a like the congenic strain derived from it.

sonal communication). Since PL/J, RF, and C3H/An each carry the Ggc^a allele, we could test the derivative congenic strains to determine whether Ggc^a had been co-transferred. The results showed that in two of the three congenic strains (B6.PL-*Lyt-2*^{a} *Lyt-3*^{a}/Cy and C57BL/6-*Lyt-2*^{a}*Lyt-3*^{a}/Boy) this had in fact happened. The third congenic strain, C57BL/6-*Lyt-2*^{a}/Boy, was found to have the Ggc^b allele of the background strain, indicating that *Ggc* had recombined with *Lyt-2* in the construction of this stock. These results confirm the linkage to *Ggc* to the *Lyt-2,3* complex. Furthermore, they support the estimate of recombination frequency obtained in the RI strains. In the absence of data involving three-point crosses with suitable chromosome *6* markers, it is not possible to say on which side of *Lyt-2* the *Ggc* locus lies.

DISCUSSION

We previously demonstrated the presence of electrophoretically variant forms of γ-glutamyl cyclotransferase in red cells of mouse and man (TULCHIN 1977). Data from genetic crosses, recombinant inbred strains and three congenic strains showed that γ-glutamyl cyclotransferase isozymes are under the control of a single gene (*Ggc*) linked to *Lyt-2* on chromosome *6* of the mouse. We estimated the map distance to be 5.0 ± 2.5 centimorgans.

Since the *Lyt-2* locus is closely linked to mouse kappa chain immunoglobulin genes (CLAFLIN *et al.* 1978; GIBSON, TAYLOR and CHERRY 1978), *Ggc* is also linked to the *Igk* complex. Human kappa chain genes have been mapped to chromosome 7 (KEATS, MORTON and RAO 1977). It will be of interest to see if the human homologue of *Ggc* also maps to chromosome 7.

The difference in electrophoretic mobilities between mouse and human isozymes would permit the chromosome localization in man using somatic cell genetics of human-mouse hybrid cells. The scarcity of allelic variation of human red cell γ-glutamyl cyclotransferase has also recently been pointed out by BOARD (1980), who found one electrophoretic form of the enzyme in human red cells of 200 donors. There have, as yet, been no descriptions of deficiencies of the enzyme. Deficiencies of two other enzymes of the γ-glutamyl cycle in human red cells, glutathione synthetase, and γ-glutamyl cysteine synthetase, are inherited and associated with hemolytic anemia (KONRAD *et al.* 1972).

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