Glyoxalase I Polymorphism in the Mouse: A New Genetic Marker Linked to H-2

Abstract. *Two electrophoretically distinct variants of glyoxalase I (Glo-1) are present in mouse* (Mus musculus). *The two forms are controlled by two codominant alleles* Glo-1^a *(common) and* Glo-1^b *(rare) at an autosomal locus. A linkage study showed that* Glo-1 *maps at approximately 3 centimorgans from the* Ss *locus of the H-2 histocompatibility region. A similar linkage relationship exists in man between GLO and HLA, the human homolog of the H-2 gene complex. Thus, the chromosomal segment evolutionarily preserved in the two species is longer than previously suspected, and it includes genes with no obvious functional relation to the other components of the major histocompatibility complex. Several features of the* Glo-1 *polymorphism in the mouse recommend it as a marker of choice for the H-2 region.*

In both mice and humans, a homologous group of genes related to immune defense have been found to be clustered in a small chromosomal segment called the major histocompatibility complex (MHC) (*1–3*). It is now becoming apparent that the relatedness among the products of the mouse H-2 and human HLA histocompatibility genes rests upon a considerable sequence homology of their primary structure (*4*). Available data from classes as diverse as Aves and Amphibia are also compatible with the concept that the linkage relationship among these functionally homologous genes has been evolutionarily conserved (*5*). Whether this association represents coordinate selection of functionally related genes or chance maintenance of an ancestral linkage remains unanswered.

We now report a new genetic marker in the mouse which extends considerably the homology in the genetic organization of the mouse and the human MCH's, although it lacks any manifest relationship to the other constituents of these linkage groups.

Recently it has been shown that the genetic polymorphism of the enzyme glyoxalase I [*S*-lactoylglutathione methylglyoxal-lyase (isomerizing); E.C. 4.4.1.5] in the human is controlled by a locus designated GLO (*6*) which is linked to HLA (*7*). We have investigated the possibility of the existence of a similar linkage relationship in the mouse. Our data provide evidence that erythrocyte glyoxalase I also exhibits genetic polymorphism in the mouse, and that the locus controlling this variation is linked to the H-2 complex.

Glyoxalase I typing was done on blood samples collected from the tail or the retro-orbital plexus and incubated for 2 to 3 hours at room temperature or overnight at 4°C. Serum was removed after centrifugation, and the remaining clot was washed in isotonic saline and homogenized in an equal volume of distilled water. The resulting erythrocyte lysates were clarified by centrifugation and subjected to high-voltage agarose electrophoresis. The gels were stained for 1 hour at 37°C, as described by Weitkamp (*8*). Specificity of the standing reaction was confirmed by deletion of methyl glyoxal from the incubation mixture. Segregation at the *Ss* locus, which has been mapped between the K and D ends of the H-2 complex (*9*), was determined by single radial immunodiffusion (*10*). Rabbit antiserum against the *Ss* serum protein [a later bleeding of the preparation used in (*11*)] was incorporated into agarose plates at a ratio of 30 μl/cm^2.

Metaphase chromosome preparations from lymph node cells that were cultured for 2 days in RPMI 1640 medium plus penicillin (100 unit/ml), streptomycin (100 μg/ml), 2-mercaptoethanol ($5 \times 10^{-5}M$), glutamine (2 mM), fetal calf serum (5 percent), and concanavalin A (2 μg/ml) were processed directly on slides as described by Edwards *et al.* (*12*). Colcemid (5 μg/ml) was added to the cultures during the last 2 hours before the cells were harvested.

Red cell lysates from the inbred mouse strains A/J, AKR/J, AKR/ABOM, BALB/cJ, C3H/HeFib, C3H/HeJ, C3H/Tif, C57BL/6J, C57BL/10J, CBA/HLacSto, CBA/J, CBA/JCrBOM, DBA/2J, DW/J, LP/J, GR/AFibBOM, SJL/J, St/aBOM, St/bBOM, and 129/J demonstrated a single band of glyoxalase I activity, which migrated anodally at a rate indistinguishable from that of the slow (GLO-1) human variant (*6*). Similar results were obtained with outbred Swiss albino mice and with a partially inbred stock homozygous for the genes *cw* and *d*. In contrast, the glyoxalase I activity of strain MA/MyJ migrated as a single band of lower electrophoretic mobility. The C57BL/6J × MA/MyJ F1 hybrids expressed two bands of activity corresponding to the parental types and, in addition, a third band of intermediate mobility (Fig. 1). These patterns are homologous to those reported for humans (*6*), and suggest that the enzyme structure is composed of dimers or a

Table 1. Linkage analysis of *Glo-1* with *Ss* and the centromeric marker of the metacentric chromosomes Rb8Bnr and Rb3Bnr.

Segregating markers			AKR × (Rb8 × AKR)						CBA × (CBA × Rb3) F1♂1		Progeny phenotypes for *Glo-1* and *Ss*	
			F1♂1		F1♂2		F1♂3					
Metacentric chromosome*	*Glo-1*	*Ss*	♀	♂	♀	♂	♀	♂	♀	♂	Parental	Recombinant†
+	b	h	0‡	3	7	5	0	0	6	2		
−	b	h	1	0	1	1	5	1	4	8	46	
N.D.	b	h	0	0	0	0	0	0	0	2		
+	a	1	1	2	1	5	2	1	1	2		
−	a	1	4	4	7	3	4	1	3	4	46	
N.D.	a	1	0	0	0	0	0	0	0	1		
+	a	h	0	0	0	0	0	0	0	0		
−	a	h	0	0	0	0	0	1	0	0		1
+	b	1	0	0	0	0	0	0	0	0		
−	b	1	1	1	0	0	0	0	0	0		2

*Plus and minus indicate presence or absence of the metacentric chromosomes segregating in the crosses; N.D., not karyotyped. †Recombination frequency, 3.2 percent; $\chi^2 = 83.4$. ‡Figures refer to the number of offspring recovered per each combination of the three segregating markers.

multiple thereof (*13*). The segregation data indicate that these variants are determined by allelic genes at a single locus for which we propose the name *Glo-1*. We suggest that the common allele be called *Glo-1*^{a} and the rare allele, which is expressed by strain MA/MyJ, be called *Glo-1*^{b}.

Mice of the two stocks carrying the chromosomal markers Rb (10.11) 8Bnr and Rb (5.15) 3Bnr, respectively, were also found to be either homozygous or heterozygous for *Glo-1*^{b}. We have not been able to determine with certainty whether this gene was contributed by the feral mice from which the marker chromosomes were derived or by the laboratory stocks with which these were crossed (*14*). A further and rather unexpected instance of the *Glo-1*^{b} allele was a single female of the inbred strain AKR/ABOM, which was found to display a heterozygous phenotype which we confirmed by segregation analysis. However, by progeny testing, this female proved to be homozygous for the *Ss*^{1} allele which is characteristic of the AKR/ABOM strain.

Linkage information was obtained from backcross progeny of matings between the aforementioned stocks, homozygous for the Robertsonian (Rb) chromosomal markers, and common laboratory inbred strains. In the first cross, females homozygous for the translocation Rb8 and *Ss*^{h}, which were either *Glo-1*^{a}/*Glo-1*^{b} or *Glo-1*^{b}/*Glo-1*^{b}, were mated to AKR/ABOM males (*Ss*^{1}/*Ss*^{1}, *Glo-1*^{a}/*Glo-1*^{a}). Three of the male progeny that were determined to be triple heterozygotes were backcrossed to AKR/ABOM females. The segregation data for these three males are presented individually in Table 1.

No significant deviation from the expected segregation ratios was found for the albino locus (*c*), sex, Rb8, *Ss*, or *Glo-1*. The low frequencies of the classes involving recombination between *Ss* and *Glo-1* clearly indicate linkage between these loci ($P < .001$). An apparent association of borderline statistical significance ($.05 > P > .02$) was noted between the metacentric marker Rb8 and each of the markers: *Glo-1*, sex, and *Ss*. The underlying cause for this association is not obvious in view of the very close linkage observed between *Glo-1* and the locus *Ss* of the H-2 complex which has been mapped on chromosome 17. Apart from being fortuitous, the phenomenon could also reflect a nonrandom meiotic segregation due to centromeric or chromosomal affinity (*15*).

In the last cross, one male homozygous for the translocation Rb3 with a genotype *Ss*^{h}/*Ss*^{h}, *Glo-1*^{a}/*Glo-1*^{b} was mated to a CBA/J/Cr/BOM female (*Ss*^{1}/*Ss*^{1}, *Glo-1*^{a}/*Glo-1*^{a}). One triply homozygous male offspring was backcrossed to CBA/J/Cr/BOM females. Although the segregation ratios of *Ss*, *Glo-1*, and Rb3 deviate from the expected (Table 1), these differences are not statistically significant ($P > .05$). On the other hand, the absence of any *Ss*–*Glo-1* recombinant progeny confirms the indication of linkage between these loci ($P < .001$). Among the segregating markers, only *Glo-1* and *Ss* showed significant association.

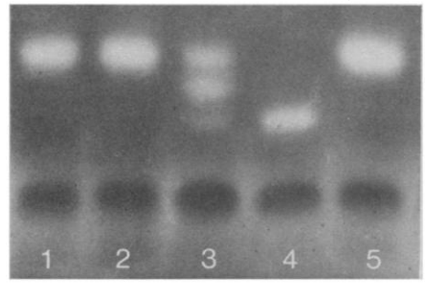

Fig. 1. Mouse erythrocyte glyoxalase I phenotypes. Lysates were from (1) DW/J, (2) C57BL/6J, (3) C57BL/6J × MA/MyJ F1, (4) MA/MyJ, and (5) SJL/J. Note the three bands of activity in the heterozygote. The dark band at the bottom is hemoglobin (cathode). Agarose-gel electrophoresis was carried out at 3°C for 1.5 hours at 15 volt/cm. The bridge buffer consisted of 10.6 m*M* barbital, 61 m*M* sodium barbital, and 1.8 m*M* calcium lactate, *p*H 8.6; the conductivity was 4.8 millisiemens. The gel buffer consisted of a dilution of the bridge buffer with distilled water to a conductivity of 3.75 millisiemens.

A χ^2 analysis was made on the data obtained from the four crosses. The total χ^2 was partitioned into a highly significant linkage component ($\chi^2 = 83.4$) and the two nonsignificant components for the segregation of *Glo-1* ($\chi^2 = 0.01$) and *Ss* ($\chi^2 = 0.01$). Neither the heterogeneity χ^2 for the frequency of recombination nor the deviations from the expected segregation ratios were significant. The cumulative estimate of the recombination fraction is 3.2 percent. Within the fiducial interval of $P = .95$ the extremes of this value are 0.6 and 8.9 percent (*16*).

The *H-2K*–*Ss* and *H-2D*–*Ss* recombination frequencies have been estimated to be 0.14 and 0.19 percent, respectively (*2*). Our data would therefore suggest that *Glo-1* is located outside the H-2 complex, as also appears to be the case with GLO relative to HLA (*7*, *8*). The inclusion of data from female meioses (not available from these crosses) would be expected to strengthen rather than weaken this interpretation. The generally higher rate of recombination in the heterogametic sex has been found for the *H-2* and HLA regions, although sex differences in the recombination frequency between GLO and HLA were observed by Kompf and Bissbort (*7*) but not by Weitkamp (*8*). The orientation of *Glo-1* relative to H-2 could be rapidly resolved by typing of already available congenic and intra–H-2 recombinants. To date, we have typed the strains: A.SW, A.CA, B10.AQR, B10.BR, B10.M, B10.P, B10.T(6R), B10.W7R, and C3H.SW for *Glo-1* but found them uniformative in this respect because they are all homozygous for the common allele *Glo-1*^{a}. A multiple-point analysis of appropriate backcrosses is currently in progress.

The conserved linkage relationship of *Glo-1* with the MHC of mouse and human is remarkable in view of the scarcity of homologous linkage or syntenic groups in the genomes of these two species (*17*). In addition, the recombination frequency between these two markers appears larger than those estimated among any other MHC markers considered homologous in mouse and human. Also, glyoxalase I has no obvious functional relation with the constituents of the MHC (although neither the product of the *Glo-1* gene nor the biological function of this enzyme are known with certainty).

It may be anticipated that similar linkage relationships will also be found in other species. Such confirmation would indicate that the evolutionarily conserved chromosomal region encompassing the MHC is larger than was previously suspected, and might caution the "selectionist" view that the interspecies homologous genes of this region necessarily have related functions.

Several features of the *Glo-1* polymorphism in the mouse recommend it as a marker of choice for the H-2 region. The variants are qualitatively distinct, codominantly expressed, and segregate normally. The enzyme has a broad tissue distribution and can be used in studies of somatic cell genetics (*18*) or for proving induced hemopoietic chimerism. Typing is rapid and does not require the preparation and testing of specific immunologic reagents.

TOMMASO MEO, TOMMY DOUGLAS
ANN-MARIE RIJNBEEK
Basel Institute for Immunology, Postfach 4005 Basel 5, Switzerland

References and Notes

1. P. Ivanyi, *Curr. Top. Microbiol. Immunol.* **53**, 1 (1970).
2. J. Klein, *Biology of the Mouse Histocompatibility-2 Complex* (Springer-Verlag, New York, 1975).
3. G. D. Snell, J. Dausset, S. Nathenson, *Histocompatibility* (Academic Press, New York, 1976).
4. J. Silver and L. Hood, *Proc. Natl. Acad. Sci. U.S.A.* **73**, 599 (19 → C. Terhorst, P. Parham, D. Mann, J. Strominger, *ibid.*, p. 910.

5. L. Du Pasquier, X. Chardonnens, V. C. Miggiano, *Immunogenetics* **1**, 482 (1975).
6. J. Kompf, S. Bissbort, S. Gussmann, H. Ritter, *Humangenetik* **27**, 141 (1975).
7. J. Kompf and S. Bissbort, *Hum. Genet.* **32**, 197 (1976); K. Bender and K.-H. Grzeschik, *Cytogenet. Cell Genet.* **16**, 93 (1976); E. R. Giblett and M. Lewis, *ibid.*, p. 313; M. Lewis *et al.*, *ibid.*, p. 317; P. Meera Khan, W. S. Volkers, B. A. Doppert, A. B. Bijnen, J. Schreuder, J. J. van Rood, *ibid.*, p. 328; L. R. Weitkamp and S. A. Guttormsen, *ibid.*, p. 364.
8. L. R. Weitkamp, *Tissue Antigens* **7**, 273 (1976).
9. D. C. Shreffler, *Transplant. Reviews* **32**, 140 (1976).
10. T. H. Hansen, T. N. Krasteff, D. C. Shreffler, *Biochem. Genet.* **12**, 281 (1974).
11. T. Meo, T. Krasteff, D. C. Shreffler, *Proc. Natl. Acad. Sci. U.S.A.* **72**, 4536 (1975).
12. G. E. Edwards, R. G. Miller, R. A. Phillips, *J. Immunol.* **105**, 719 (1970).
13. L. Uotila and M. Koivusalo, *Eur. J. Biochem.* **52**, 493 (1975).
14. A. Gropp, J. Olert, R. Maurizio, *Experientia* **27**, 1226 (1971); B. M. Cattanach and H. Moseley, *Cytogenet. Cell Genet.* **12**, 264 (1973).
15. D. Michie, *Proc. R. Soc. London Ser. B* **114**, 241 (1955).
16. R. A. Fisher and F. Yates, *Statistical Tables* (Oliver and Boyd, Edinburgh, 1963).
17. F. H. Ruddle and R. P. Creagen, *Annu. Rev. Genet.* **9**, 407 (1975).
18. K. Bender and K.-H. Grzeschik, *Hum. Genet.* **31**, 341 (1976).
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Elastase Release from Human Alveolar Macrophages: Comparison Between Smokers and Nonsmokers

Abstract. *Alveolar macrophages from smokers, in contrast to those of nonsmokers, release elastase into serum-free culture medium. Since enzymes that digest elastin produce pulmonary emphysema in experimental animals, release of elastase by alveolar macrophages from smokers suggests that these cells are important in the pathogenesis of emphysema of smokers.*

Preparations that digest elastin, including papain (*1*), polymorphonuclear leukocyte homogenates (*2*), and pancreatic elastase (*3*), alter the connective tissue framework of the lung in experimental animals, yielding lesions similar to those of emphysema. It has been speculated that elastase activity is important in the pathogenesis of human emphysema. Alveolar macrophages (AM) and polymorphonuclear leukocytes (PMN) are the most likely sources of elastase which may affect human lung tissues.

The presence of elastase (E.C. 3.4.21.–) in PMN's is well established (*4*), but studies to demonstrate elastase in alveolar macrophages have been inconclusive (*5–7*). Most reports of alveolar macrophage elastase have relied on measurements of whole cell homogenates or cell components. Recently, however, Werb and Gordon (*8*) demonstrated that peritoneal macrophages in culture release an enzyme with elastolytic activity. Their finding of elastase as a secretory product is consistent with other data indicating that macrophages are capable of secreting a variety of substances (*9*). In the present investigation we have observed elastase release from human alveolar macrophages in culture. Notably, elastase activity was detected in cell cultures from smokers, but was not detected in cultures from nonsmokers.

Alveolar macrophages were obtained by lung lavage from 12 healthy volunteers (seven males and five females) whose ages ranged from 24 to 33 years. Five of the subjects smoked more than 30 cigarettes daily; the others did not smoke. Lung lavages were performed through a fiber-optic bronchoscope placed in a right lower lobe subsegmental bronchus. Five or six equal volumes of warm (37°C) sterile saline (0.9 percent NaCl, weight to volume) were instilled through the bronchoscope from a sterile 50-ml syringe. The fluid was removed by gentle suction.

The yield of alveolar macrophages ranged from 1 to 2.2×10^7 in nonsmokers and 15 to 28×10^7 in smokers. As noted by others, the material from smokers was dark brown in contrast to the ivory-colored material obtained from nonsmokers. The fluid recovered (approximately 60 percent of the instilled volume) was placed in sterile, plastic, conical centrifuge tubes, chilled to 4°C, and centrifuged at 500*g* for 10 minutes. The supernatant was decanted. The cells were washed twice in an equal volume of Neuman and Tytell medium (serum free); a portion of this cell suspension was counted in a hemocytometer, and the remaining cells were suspended in the same medium to a final concentration of 3×10^6 cells per milliliter. Portions (1 ml) of the cell suspension were placed in 35-mm culture dishes (Falcon). After 2 hours of incubation at 37°C in a humidified incubator under 5 percent carbon dioxide and 95 percent air, the culture medium was removed. The cultures were washed three times with fresh medium to remove nonadherent cells and then 1 ml of fresh medium was added to each dish. The cells were reincubated for 48 hours. Cell viability was assessed by examining representative cells for exclusion of supravital dye and by measuring the distribution of lactic acid (LDH; E.C. 1.1.1.27) dehydrogenase and β-glucuronidase (E.C. 3.2.1.31) activities between the media and the cells. In some experiments the culture period was extended for as long as 14 days. In the prolonged experiments, the medium was removed and replaced at various intervals.

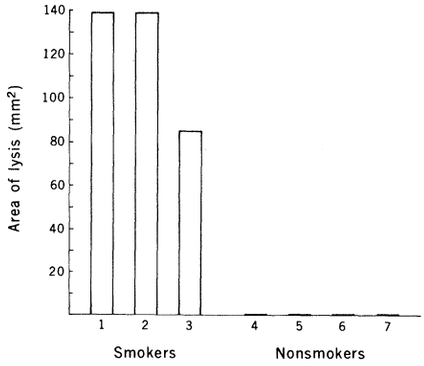

Fig. 1. The effects of reconstituted culture media upon elastin agarose. The activity was determined by calculating the area of lysis.

Medium removed from the cultures was centrifuged at 500*g* for 10 minutes at 4°C and dialyzed for 24 to 48 hours against cold 10 m*M* tris-HCl, *p*H 7.6, containing 1 m*M* $CaCl_2$. This solution was lyophilized and the product stored at −70°C. Prior to enzymatic analysis, dialyzed lyophilized medium was reconstituted with cold distilled water to 0.5 percent of its original volume.

Cell lysates were prepared by exposing freshly washed cultures to 1 percent Triton X-100 for 20 minutes. These cells were scraped from the plates and subjected to sonication at 2500 amp for 15 seconds. Complete cell disruption was confirmed by light microscopy.

Elastolytic activity was measured by solubilization of elastin particles (< 400 mesh) suspended in agarose (*8*), and by the hydrolysis of succinyl-L-alanyl-L-alanyl-L-alanine-*p*-nitroanilide (SLAPN), a synthetic substrate with a high degree of specificity for elastases (*10*).

The medium from three of the smokers' cultures showed easily detectable activity against elastin suspended in agarose, whereas culture medium from four of the nonsmokers' AM failed to show activity even after prolonged incubation (Fig. 1). At 48 hours, more than 80 percent of the cells excluded dye. Enzyme partition studies demonstrated that 15 percent of the total β-glucuronidase and 50 percent of the total LDH activity was extracellular. Elastolytic activity was demonstrable in the media of