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Genetic Studies of the *Fv-1* Locus of Mice: Linkage with *Gpd-1* in Recombinant Inbred Lines

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Multiple recombinant inbred lines, derived from crosses between strains permissive to N-tropic murine leukemia viruses (*Fv-1*^{n}) and strains permissive to B-tropic murine leukemia viruses (*Fv-1*^{b}), have been characterized as to *Fv-1* genotype and other chromosome 4 markers, including the closely linked hexose-6-phosphate dehydrogenase isozyme locus (*Gpd-1*). Only one recombinant between *Fv-1* and *Gpd-1* was found among 45 lines tested. On this basis, the distance between *Fv-1* and *Gpd-1* is estimated to be 0.6 centimorgans. None of the lines was either resistant or susceptible to both N- and B-tropic viruses. Nineteen other inbred strains, previously untested, were characterized as either *Fv-1*^{n} or *Fv-1*^{b}.

The Friend virus 1 locus (*Fv-1*) of mice is the major genetic determinant of susceptibility to infection by naturally occurring mouse-tropic murine type C viruses in vivo and in vitro (13, 14, 16). A reciprocal relationship exists between two types of mouse strains (types N and B) and two host range variants of virus stocks (N-tropic and B-tropic), such that type N mice (or cell cultures) are permissive to infection by N-tropic viruses and nonpermissive to infection by B-tropic viruses, whereas type B mice (or cell cultures) are permissive to infection by B-tropic viruses and nonpermissive toward N-tropic viruses. Resistance is dominant: *Fv-1*^{n}/*Fv-1*^{b} heterozygotes are nonpermissive to infection by either N- or B-tropic viruses. The precise nature of the *Fv-1* restriction regarding multiplicity of infection is the subject of contention (2, 10, 15, 21). The mechanism of the *Fv-1* restriction is also under study (7, 11, 17, 22).

The *Fv-1* locus was found to be loosely linked to the brown coat color locus (*b*) on chromosome 4 (19) and subsequently was shown to be closely linked to the hexose-6-phosphate dehydrogenase electrophoretic variant (*Gpd-1*) (20). We have determined the *Fv-1* and *Gpd-1* types of multiple recombinant inbred (RI) lines that were derived by brother-sister mating beginning with the F_2 generations obtained by crossing C57BL/6J (*Fv-1*^{b} *Gpd-1*^{a}) with either DBA/2J (25 lines), C3H/HeJ (14 lines), SJL/J (2 lines), or AKR/J (1 line) (each of the latter strains carries the *Fv-1*^{n} and *Gpd-1*^{b} alleles). These data confirm and further quantify the linkage relationship between *Fv-1* and *Gpd-1*. They also afford an opportunity to detect recombinational events that could produce new genotypes either resistant or susceptible to both N- and B-tropic viruses. We have also typed 19 additional inbred strains with respect to *Fv-1* to further characterize the polymorphism.

MATERIALS AND METHODS

Mice. The BXD, BXH, and BXJ RI lines were derived by B.A.T. from crosses of C57BL/6J with DBA/2J, C3H/HeJ, and SJL/J, respectively. The lines had attained an expected degree of genetic fixation of 0.79 or greater when *Fv-1* testing was initiated. In instances in which a particular line was evidently still segregating for *Fv-1*, the line was retested in later generations. The miscellaneous inbred strains tested for *Fv-1* were obtained from either Production or Research colonies of the Jackson Laboratory.

Cell cultures and viruses. Mouse embryo cultures from the various RI lines were prepared from 14- to 17-day-old embryos as described previously (6). Two embryos from each pregnant female were pooled to establish cultures for *Fv-1* testing. Cultures were maintained in Eagle minimum essential medium supplemented with 2 mM L-glutamine, 10% unheated fetal calf serum, penicillin (100 U/ml), and streptomycin (100 μg/ml).

The murine leukemia viruses (MuLV) used were from two sources. The N (AKR-MuLV no. 781)- and B (BALB/c-MuLV no. 18831)-tropic viruses were from R. Peters, Microbiological Associates, Inc., Walkersville, Md. In addition, the N (WN1802N) and B (WN1802B) viral strains were kindly supplied by Janet Hartley, National Institutes of Health, Bethesda, Md. The viruses were passaged in vitro in the permissive cell lines, and the plaque-forming titer (PFU per milliliter) was determined by the XC assay. Samples of virus ranging in titer from 1×10^5 to 5×10^5 PFU/ml were stored at −70°C.

***Fv-1* typing.** *Fv-1* typing was done using the standard UV-XC procedure (12, 14). Secondary cell cultures, seeded at a density of 2×10^5 cells in 60-mm Falcon dishes, were treated with DEAE-dextran (25 μg/ml) for 1 h before virus infection. Duplicate cultures were inoculated with N- and B-tropic MuLV's at multiplicities of 10^{-1} through 10^{-4} PFU/ml. Plates were UV irradiated at 4 to 5 days postinfection and overlaid with 10^6 XC cells. Four days later the plates were stained, and the plaques were counted microscopically.

Other markers. Vertical starch gel electrophoresis of kidney homogenates was used to type the RI lines for the hexose-6-phosphate dehydrogenase (*Gpd-1*) polymorphism, according to the method of Hutton and Coleman (9). Vertical polyacrylamide gel electrophoresis (5% gel) of urine samples was used to type the RI lines with respect to the major urinary protein (*Mup-1*) polymorphism (4), using the procedures described for serum prealbumin (*Pre*) typing (24), except that electrophoresis time was reduced to 1 h. The brown coat color locus (*b*) provides an additional chromosome 4 locus in the BXD RI lines.

RESULTS AND DISCUSSION

The patterns of inheritance of chromosome 4 markers in the BXD and BXH RI lines are presented in Tables 1 and 2, respectively. All of the RI lines are genetically fixed for all of the loci. With respect to *Fv-1*, each line could be clearly classified as either *Fv-1*^{n}/*Fv-1*^{n} or *Fv-1*^{b}/*Fv-1*^{b}. The restricted virus (N- or B-tropic) was subject to a 30- to 1,000-fold reduction in plaque-forming efficiency in individual tests of RI lines. No major shifts in restriction patterns were observed that would suggest any genetic alteration, such as intragenic or unequal crossing-over involving *Fv-1*, or mutation. The *Fv-1* and *Gpd-1* loci were inherited concordantly in all but a single case, confirming the close linkage between these two loci. Line BXD-27 inherited the *Gpd-1*^{a} allele of C57BL/6J and the *Fv-1*^{n} allele of DBA/2J. Eleven of 25 BXD RI lines exhibit recombinant genotypes with respect to *b* and *Gpd-1*, loci separated by approximately 30 centimorgans. Five of the 25 BXD RI lines exhibit recombination between *b* and *Mup-1*, loci that are separated by only 7 centimorgans (3, 8).

Several miscellaneous RI lines are informative with respect to the *Gpd-1-Fv-1* linkage (Table 3). They are: BXJ-1, BXJ-2, LT/Re, HP/Ei, and TSK/Le. None of these involves recombination between *Fv-1* and *Gpd-1*.

Forty-five lines derived by brother-sister in-

TABLE 1. *Inheritance of chromosome 4 markers Mup-1, b, Gpd-1, and Fv-1 in 25 BXD RI lines*

RI line (or progenitor strain)	Genotype[a]				Crossovers	
	Mup-1	*b*	*Gpd-1*	*Fv-1*	Region	Number of lines
(C57BL/6J); BXD-2, -4, -6, -11, -19, -20, -23, -29	B	B	B	B	None	8
(DBA/2J); BXD-1, -13, -21	D	D	D	D	None	3
BXD-28, -30	B	D	D	D	*Mup-1-b*	2
BXD-22	D	B	B	B	*Mup-1-b*	1
BXD-5, -8, -12, -14, -16, -18	B	B	D	D	*b-(Gpd-1, Fv-1)*	6
BXD-15, -24, -25	D	D	B	B	*b-(Gpd-1, Fv-1)*	3
BXD-9	B	D	B	B	*Mup-1-b, b-(Gpd-1, Fv-1)*	1
BXD-27	B	D	B	D	*Mup-1-b, b-Gpd-1, Gpd-1-Fv-1*	1

[a] Of the 16 genotypes possible only 8 were recovered among the 25 BXD RI lines. B and D are used as generic symbols for alleles inherited from C57BL/6J and DBA/2J, respectively. BXD-4 is extinct.

TABLE 2. *Inheritance of chromosome 4 markers Mup-1, Gpd-1, and Fv-1 in 14 BXH RI lines*

RI line (or progenitor strain)	Genotype[a]			Crossovers	
	Mup-1	*Gpd-1*	*Fv-1*	Region	Numbers of lines
(C57BL/6J); BXH-5, -11, -14, -18	B	B	B	None	4
(C3H/HeJ); BXH-6	H	H	H	None	1
BXH-10, -19	B	H	H	*Mup-1-(Gpd-1, Fv-1)*	2
BXH-2, -3, -4, -7, -8, -9, -12	H	B	B	*Mup-1-(Gpd-1, Fv-1)*	7

[a] Of the eight genotypes possible only four were recovered among the 14 BXH RI lines. B and H are used as generic symbols for alleles inherited from C57BL/6J and C3H/HeJ, respectively. BXH-18 is genetically extinct.

breeding have been tested for potential recombination between *Fv-1* and *Gpd-1*. Multiple opportunities for recombination between linked loci occur during the development of an RI strain before the chance genetic fixation of one or the other progenitor types that precludes further recombinational opportunities. The probability of fixation of a recombinant genotype (R) with respect to two loci that recombine with a frequency r is $4r/1 + 6r$ (5). Equating 1/45 for R, the estimate of r is 0.0057 ± 0.0062 (24). This is consistent with Rowe and Sato's (20) finding of a single recombinant among 107 backcross progeny. Table 4 shows the *Fv-1* typing on 19 other inbred strains. These were tested to further define the polymorphism and to provide a basis for selection of one of these strains for genetic studies of RNA tumor viruses. The distribution of *Fv-1* alleles reflects known strain relationships (23). Among the rarer, *Fv-1*^{b}-bearing, strains probably only BDP/J and RIII/2J can be considered additional independent occurrences of that allele, since the other strains are known to be descended from, or closely related to, other known *Fv-1*^{b} strains. The *Fv-1*^{b} allele has not been reported in wild mouse populations; the IS/CamEi, Peru-Atteck, and SK/CamEi stocks (18), which were derived from such populations, are N-type.

Spontaneous XC plaques were seen in some uninoculated control cultures. These cases presumably reflect the spontaneous expression of endogenous MuLV genomes that are present in many inbred strains. We plan to systematically study the effect of the *Fv-1* locus on the inducibility of ecotropic MuLV in secondary embryo cultures of the BXD RI lines.

As additional polymorphic markers are mapped into the *b–Gpd-1* region of chromosome 4, using the BXD RI lines, it may be possible to assign the correct gene order for *Fv-1*, *Gpd-1*, and other markers. Other markers located on both sides of *Fv-1* would be useful for identifying rare recombinants that could be used to discriminate between the effects of *Fv-1* and other linked loci.

The structural locus for 6-phosphogluconate dehydrogenase (*Pdg*) is closely linked to, but recombines with, *Gpd-1* (1). Since these two enzymes are closely related metabolically, it has been suggested that the genes specifying these enzymes arose from a common ancestral gene by tandem duplication. Other loci in this vicinity, such as *Fv-1*, may have been duplicated as well. Thus, one should be alert to the possibility of other loci affecting viral parameters being located near *Fv-1*.

TABLE 3. *Fv-1 and Gpd-1 types of some miscellaneous RI lines*

RI line[a]	Genotype	
	Gpd-1	*Fv-1*
BXJ-1	*b*	*n*
BXJ-2	*b*	*n*
HP/Ei	*a*	*b*
LT/Re	*b*	*b*
SEA/Gn	*a*	*n*
TSK/Le	*a*	*b*

[a] The BXJ-1 and BXJ-2 RI lines were derived from crossing C57BL/6J (*Gpd-1*^{a} *Fv-1*^{b}) with SJL/J (*Gpd-1*^{b} *Fv-1*^{n}). HP/Ei is an RI strain derived from C57BL/6J and AKR/J (*Gpd-1*^{b} *Fv-1*^{n}). LT/Re and SEA/GnJ are RI strains derived from crossing BALB/c (*Fv-1*^{b} *Gpd-1*^{b}) with C58 (*Fv-1*^{n} *Gpd-1*^{a}) and P/J (*Fv-1*^{n} *Gpd-1*^{a}), respectively. TSK/Le is an RI strain derived from crossing B10.D2 (58N)/Sn (*Fv-1*^{b} *Gpd-1*^{a}) with C3H/Di (*Fv-1*^{n} *Gpd-1*^{b}).

TABLE 4. *Fv-1 and Gpd-1 types of some previously untested inbred strains of mice*[a]

Fv-1^n *Gpd-1*^{a}	*Fv-1*^{n} *Gpd-1*^{b}	*Fv-1*^{b} *Gpd-1*^{a}	*Fv-1*^{b} *Gpd-1*^{b}
P/J	BUB/BnJ	BDP/J	LG/J
Peru-Atteck[b,c]	CBA/CaJ	C57BL/KsJ	RIII/2J
PL/J	C3HeB/FeJ	PRO/Re	SEC/1ReJ
SK/CamEi[c]	DBA/1J		
	IS/CamEi[c]		
	LP/J		
	MA/J		
	NZB/BlNJ		
	SM/J		

[a] The *Gpd-1* types are taken from either the literature (18) or unpublished data (T. H. Roderick, personal communication).

[b] Incompletely inbred.

[c] Derived from wild mice.

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LITERATURE CITED

1. **Chapman, V. M.** 1975. 6-Phosphogluconate dehydrogenase (PGD) genetics in the mouse: linkage with metabolically related enzyme loci. Biochem. Genet. 13:849-856.
2. **Decleve, A., O. Niwa, E. Gelmann, and H. S. Kaplan.** 1975. Replication kinetics of N- and B-tropic murine leukemia viruses on permissive and non-permissive cells in vitro. Virology 65:320-332.
3. **Finlayson, J. S., D. M. Hudson, and B. L. Armstrong.** 1969. Location of the *Mup-a* locus of mouse linkage group VIII. Genet. Res. 14:329-331.
4. **Finlayson, J. S., M. Potter, and C. C. Runner.** 1963. Electrophoretic variation and sex dimorphism of the major urinary protein complex in inbred mice: a new genetic marker. J. Nat. Cancer Inst. 31:91-107.
5. **Haldane, J. B. S., and C. H. Waddington.** 1931. Inbreeding and linkage. Genetics 16:357-374.
6. **Hartley, J. W., W. P. Rowe, W. I. Capps, and R. J. Huebner.** 1969. Isolation of naturally occurring viruses of the murine leukemia virus group in tissue culture. J. Virol. 3:126-132.
7. **Huang, A. S., P. Besmer, L. Chu, and D. Baltimore.** 1973. Growth of pseudotypes of vesicular stomatitis virus with N-tropic murine leukemia virus coats in cells resistant to N-tropic viruses. J. Virol. 12:659-662.
8. **Hudson, D. M., J. S. Finlayson, and M. Potter.** 1967. Linkage of one component of the major urinary protein complex of mice to the brown coat color locus. Genet. Res. 10:195-198.
9. **Hutton, J. J., and D. L. Coleman.** 1969. Linkage analysis using biochemical variants in mice. II. Levulinate dehydratase and autosomal glucose 6-phosphate dehydrogenase. Biochem. Genet. 3:517-523.
10. **Jolicoeur, P., and D. Baltimore.** 1975. Effect of the Fv-1 locus on the titration of murine leukemia viruses. J. Virol. 16:1593-1598.
11. **Jolicoeur, P., and D. Baltimore.** 1976. Effect of *Fv-1* gene product on proviral DNA formation and integration in cells infected with murine leukemia viruses. Proc. Natl. Acad. Sci. U.S.A. 73:2236-2240.
12. **Klement, V., W. P. Rowe, J. W. Hartley, and W. E. Pugh.** 1969. Mixed culture cytopathogenicity: a new test for growth of murine leukemia viruses in tissue culture. Proc. Natl. Acad. Sci. U.S.A. 63:753-758.
13. **Lilly, F.** 1967. Susceptibility of two strains of Friend leukemia in mice. Science 155:461-462.
14. **Pincus, T., J. W. Hartley, and W. P. Rowe.** 1971. A major genetic locus affecting resistance to infection with murine leukemia viruses. I. Tissue culture studies of naturally occurring viruses. J. Exp. Med. 133:1219-1233.
15. **Pincus, T., J. W. Hartley, and W. P. Rowe.** 1975. A major genetic locus affecting resistance to infection with murine leuekmia viruses. IV. Dose-response relationship in *Fv-1* sensitive and resistant cell cultures. Virology 65:333-342.
16. **Pincus, T., W. P. Rowe, and F. Lilly.** 1971. A major genetic locus affecting resistance to infection with murine leukemia viruses. II. Apparant identity to a major locus described for resistance to Friend murine leukemia virus. J. Exp. Med. 133:1234-1241.
17. **Reim, A., and S. V. S. Kashmiri.** 1976. Phenotypic mixing between N- and B-tropic murine leukemia viruses: infectious particles with dual sensitivity to *Fv-1* restriction. Cell 7:373-379.
18. **Roderick, T. H., F. H. Ruddle, V. M. Chapman, and T. B. Shows.** 1971. Biochemical polymorphisms in feral and inbred mice (*Mus musculus*). Biochem. Genet. 5:457-466.
19. **Rowe, W. P., J. B. Humphrey, and F. Lilly.** 1973. A major genetic locus affecting resistance to infection with murine leukemia viruses. III. Assignment of the *Fv-1* locus to linkage group VIII of the mouse. J. Exp. Med. 137:850-853.
20. **Rowe, W. P., and H. Sato.** 1973. Genetic mapping of the *Fv-1* locus of the mouse. Science 180:640-641.
21. **Schuh, V., M. E. Blackstein, and A. A. Axelrad.** 1976. Inherited resistance to N- and B-tropic murine leukemia viruses in vitro: titration patterns in strains SIM and SIM.R congenic at the Fv-1 locus. J. Virol. 18:473-480.
22. **Sveda, M. M., and R. Soeiro.** 1976. Host restriction of Friend leukemia virus: synthesis and integration of the provirus. Proc. Natl. Acad. Sci. U.S.A. 73:2356-2360.
23. **Taylor, B. A.** 1972. Genetic relationships between inbred strains of mice. J. Hered. 63:83-86.
24. **Taylor, B. A., D. W. Bailey, M. Cherry, R. Riblet, and M. Weigert.** 1975. Genes for immunoglobulin heavy chain and serum prealbumin protein are linked in mouse. Nature (London) 256:644-646.