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Laboratory and Wild-Derived Mice with Multiple Loci for Production of Xenotropic Murine Leukemia Virus

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Mendelian segregation analysis was used to define genetic loci for the induction of infectious xenotropic murine leukemia virus in several laboratory and wild-derived mice. MA/My mice contain two loci for xenotropic virus inducibility, one of which, *Bxv-1*, is the only induction locus carried by five other inbred strains. The second, novel MA/My locus, designated *Mxv-1*, is unlinked to *Bxv-1* and shows a lower efficiency of virus induction. The NZB mouse carries two induction loci; both are distinct from *Bxv-1* since neither is linked to the *Pep-3* locus on chromosome 1. Finally, one partially inbred strain derived from the wild Japanese mouse, *Mus musculus molossinus*, carries multiple (at least three) unlinked loci for induction of xenotropic virus. Although it is probable that inbred strains inherited xenotropic virus inducibility from Japanese mice, our data suggest that none of the induction loci carried by this particular *M. m. molossinus* strain are allelic with *Bxv-1*.

Genetic determinants of xenotropic murine leukemia viruses (X-MuLVs) are inherited as chromosomal genes in mice. Although all inbred mice carry multiple X-MuLV genomes (>20 copies) (6), production of infectious virus differs qualitatively and quantitatively among mouse strains. Genetic crosses between strains which differ in their patterns of virus expression have been used to identify several loci governing production of infectious X-MuLV. High-virus NZB mice carry two independently assorting induction loci (2, 3). These unmapped loci show distinct patterns of virus expression and produce biochemically distinguishable viruses (3, 5). By comparison, at least five different low-virus mouse strains, including BALB, C57, and AKR, carry the same single locus for inducibility (*Bxv-1*), and this locus has been mapped to chromosome 1 (9). An induction locus at or near *Bxv-1* is also present as the only inducible locus of the high-xenotropic virus mouse F/St (13), although the high level of spontaneous virus expression in F/St is controlled by a gene linked to the *H-2*^{p} complex of this mouse (21). Thus, although these mice all have multiple germ line copies of X-MuLV proviruses, few of these chromosomal loci are expressed as infectious MuLV.

In the present study, three mouse strains inducible for xenotropic virus were examined to determine the induction characteristics and chromosomal location of loci which govern production of infectious X-MuLV.

MATERIALS AND METHODS

Mice. NZB/BlN and the NIH Swiss inbred line NFS/N were obtained from the Small Animal Section, National Institutes of Health, Bethesda, Md. *Mus musculus molossinus* (colony III) was a kind gift of T. Roderick, The Jackson Laboratory, Bar Harbor, Maine. Partial NFS/N congenic mice carrying the high-expression X-MuLV locus of NZB/BlN have been described previously (2). All other mice were from The Jackson Laboratory. Hybrid mice were bred in our laboratory.

Virus induction and assay. Spontaneous virus production was scored in thymus and spleen cells of the parental strains, F_1 hybrids, and mice from the NZB crosses. Single-cell suspensions were treated with mitomycin C (25 μg/ml at 37°C for 30 min) and plated as infectious centers. Virus was scored by focus formation on the mink S+L− line of Peebles (16). Alternatively, mink lung cells (CCL64) inoculated with infectious centers were passed to cover slips, and virus was detected by the fluorescent antigen focus assay with a fluorescein-conjugated anti-MuLV antibody (7).

For induction, tissue cultures were prepared from the tail biopsy tissues of weanling mice (11). When the cultures were in subconfluent growth, 20 μg of 5-iododeoxyuridine per ml was added for 48 h. The culture fluid was then changed, and the cultures were overlaid with mink cells and maintained for 12 days with twice-weekly fluid changes. Cells and culture fluids were then harvested, frozen and thawed, clarified by centrifugation, and tested for focus formation on mink S+L− cells.

Alternatively, 1×10^7 to 2×10^7 spleen cells from individual mice were treated with bacterial lipopolysaccharide (100 μg/ml) plus 5-iododeoxyuridine (20 μg/ml) for 48 h and then added to cultures of mink lung cells as described previously (9). These mixed cultures were maintained for 2 weeks and then tested for xenotropic virus as above.

Genetic markers. Isozyme phenotypes of individual backcross mice were determined by histochemical staining after starch gel electrophoresis of clarified kidney extracts. The following 10 isozyme markers, mapped to eight mouse chromosomes, were used in linkage testing: *Pep-3*, *Gpi-1*, *Mod-1*, *Got-2*, *Gus*, *Gpd-1*, *Mpi-1*, *Pgm-1*, *Es-3*, and *Glo-1*. Methods for the electrophoretic separation and identification of alleles at these loci are described elsewhere (10, 14, 15).

Hybrid mice were also typed for expression of the immunological markers *Ly-17* (*Ly-20*) and *Ly-9* by flow microfluorometry. The antisera used were anti-Ly 17.1 (4, 18), monoclonal anti-Ly-20.2 (provided by N. Tada and U. Hammerling), and fluorescein-conjugated monoclonal anti-Ly-9.1 (anti-Lgp100) (12). It is now agreed that anti-Ly-17.1 and anti-Ly-20.2 detect allelic products of the same locus (8a). The genetic locus defined by these antisera is designated *Ly 17* in this report and has the alleles *Ly 17*^{a} and *Ly 17*^{b} with gene products Ly 17.1 and Ly 17.2.

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TABLE 1. Segregation of virus inducibility and chromosome 1 markers in backcross mice

Cross	No. of mice	Chromosomal markers	No. of X-MuLV-inducible mice/no. with (+) or without (−) MA chromosome 1 (%)	
			+	−
NFS × (MA × SEA)[a]	55	*Pep-3*	19/23 (83)	15/32 (46)
NFS × (NFS × MA)	38	*Ly-9*	22/23 (96)	10/15 (66)
		Ly-17	21/21 (100)	11/17 (64)

[a] MA mice are virus inducible; SEA and NFS mice are not. Parental strains have the following genotypes: MA, *Pep-3^b Ly-17^b Ly-9^b*; SEA/GnJ, *Pep-3^a Ly-17^a Ly-9^a*; and NFS/N, *Pep-3^b Ly-17^a Ly-9^a*.

TABLE 2. Linkage testing of the X-MuLV induction loci of NZB mice

Marker locus	Chromosome no.	No. of recombinants/total (%)	
		Nzv-1[a]	Nzv-2
Pep-3	1	35/63 (56)	6/15 (40)
Mod-1	9	27/57 (47)	6/11 (55)
Gpd-1	4	20/44 (45)	6/14 (43)
c	7	17/33 (52)	
Gpi-1	7	21/46 (46)	
Pgm-1	5	10/19 (53)	5/13 (38)
Gus	8	11/15 (73)	
Got-2	5	14/38 (37)	

[a] Numbers represent combined results from crosses with NZB and NFS.*Nzv-1* partial congenics.

RESULTS

MA/My. Xenotropic virus was not spontaneously produced by spleen or thymus cells of MA/My mice younger than 6 months. However, X-MuLV was readily induced by 5-iododeoxyuridine treatment of tail fibroblasts or lipopolysaccharide treatment of spleen cells. To determine whether this induction phenotype was controlled by the locus commonly found in other strains (*Bxv-1*), MA mice were mated with virus induction-negative SEA/GnJ males or NFS/N females, and the F_1 males of both crosses were mated with NFS females. Of the 93 backcross progeny, 66 (71%) produced X-MuLV in either tail or spleen induction tests, suggesting that virus inducibility in MA mice is controlled by two independently assorting genes.

Progeny of the two crosses were tested for segregation of alleles either at *Pep-3* or *Ly-17* and *Ly-9*, three chromosome 1 markers linked to *Bxv-1* (*Pep-3* - 19 - *Bxv-1* - 3 - *Ly-17* - 1 - *Ly-9*) (Table 1; 8a). Almost all of the segregants inheriting chromosome 1 alleles from the MA grandparent (i.e., mice typed as *Pep-3$^{b/b}$* or *Ly-17^b Ly-9^b/Ly-17^a Ly-9^a*) were inducible for virus (Table 1). The data also suggest a higher incidence of recombination between virus and *Pep-3* than between virus and the *Ly* markers, which is consistent with inheritance of *Bxv-1*. Finally, about half of the segregants not inheriting the MA chromosome 1 were inducible for virus. These results indicate that there are two induction loci in MA mice and that one of these loci is *Bxv-1*.

Subsequent backcross generations were followed to isolate the second gene, designated *Mxv-1*, from *Bxv-1* to characterize its induction phenotype and to determine its chromosomal location. First-backcross mice lacking the *Ly-17^b* allele of the MA mouse are unlikely to carry *Bxv-1* because of the close linkage of these loci. Thus, several inducible backcross segregants were splenectomized, and those typed as *Ly-17$^{a/a}$* were mated with NFS females. Analysis of their progeny indicated that virus inducibility segregated as a single locus which produced infectious virus in both tail and spleen induction tests. However, this locus differs from *Bxv-1* in that the virus titers induced from the *Mxv-1*-positive segregants were consistently lower (by 1 to 2 logs) than the titers from control cells carrying *Bxv-1*.

All second-backcross mice and first-backcross mice that lacked *Bxv-1* (mice which were virus negative or Ly 17.1) were typed for segregation of a variety of isozyme markers on a number of chromosomes. No close linkage was observed between *Mxv-1* and markers on chromosome 7 (*Gpi-1*, r [recombination] = 9/16 = 0.5), chromosome 9 (*Mod-1*, r = 9/23 = 0.39; *Mpi-1*, r = 18/45 = 0.4), chromosome 17 (*Glo-1*, r = 7/16 = 0.44), chromosome 5 (*Pgm-1*, r = 10/26 = 0.38), chromosome 11 (*Es-3*, r = 15/29 = 0.52), and chromosome 4 (*Gpd-1*, r = 10/21 = 0.47).

NZB/BlN. NZB mice carry two independently assorting loci for production of xenotropic virus, with distinct patterns of virus expression (2, 3, 5). Infectious virus production from *Nzv-1*, the high-virus locus, can be detected by plating NZB spleen cells as infectious centers in a direct focus assay on the mink S+L− line. *Nzv-2* is characterized by a much lower level of constitutive expression which can be detected in *Nzv-1*-negative mice by the fluorescent antigen focus assay 2 weeks after inoculation of mink lung cells with spleen cells (2).

NZB/BlN mice were crossed and backcrossed with virus-negative 129/J mice. Half of the progeny (15/30) prduced high titers of X-MuLV characteristic of *Nzv-1*. Of the remaining 15 mice, 9 produced mink infectious virus in the fluorescent antigen test, indicating inheritance of *Nzv-2*. In a second cross, partially congenic mice carrying the *Nzv-1* locus on an NFS/N genetic background (2) were mated with SEA/GnJ mice and backcrossed with NFS females. These NFS.*Nzv-1* congenics showed the high endogenous levels of X-MuLV characteristic of NZB. Half of the backcross progeny (29/58) inherited the *Nzv-1* phenotype by the direct focus assay.

Although the phenotypes associated with *Nzv-1* and *Nzv-2* distinguish these loci from *Bxv-1*, this does not exclude the possibility that one of these genes may represent an allelic variant of the *Bxv-1* locus. Therefore, animals of both crosses were examined for segregation of markers on chromosome 1 (Table 2). The results indicate that neither *Nzv-1* nor *Nzv-2* was inherited with *Pep-3*, indicating that both of these loci are distinct from *Bxv-1* ($P < 0.0005$ for *Nzv-1* and $P < 0.01$ for *Nzv-2*).

In an attempt to localize these genes in the mouse gene map, segregants were also typed for markers on chromosomes 4, 5, 7, and 9 (Table 2). No linkage was detected.

M. m. molossinus. Mice from a partially inbred stock of *M. m. molossinus* were tested for induced and spontaneous expression of X-MuLV. In infectious center assays, 11 of 11 mice 3 to 9 months of age spontaneously produced X-MuLV in both spleen and thymus (10 to 10^3 focus-forming units per 10^5 cells). Spontaneous virus production was detected in cultured tail cells of 5 of 13 mice tested; virus was readily induced by 5-iododeoxyuridine from tail cultures of all 13 mice.

F_1 hybrids between *M. m. molossinus* and various virus-negative inbred strains, including NFS, NZC, SEA, NZW, and the recombinant inbred strain BXD-14, were routinely negative for spontaneously produced X-MuLV in both thy-

TABLE 3. Segregation of X-MuLV inducibility in second-backcross mice from *M. m. molossinus*

Molossinus parent[a]	First-backcross parent	Virus in second-backcross mice
		No. positive/total (%)
♀ 1157	5588	7/8 (88)
	5590	10/11 (91)
	6281	23/28 (82)
	6277	18/24 (75)
♂ 1177	1–8	8/8 (100)
	1–1	16/22 (73)

[a] 1157 and 1177 are siblings.

mus and spleen cells when tested at 2 to 6 months of age. However, all F_1 mice tested were positive in tail and spleen induction assays.

F_1 males from matings between *M. m. molossinus* and either SEA or NFS were crossed with NFS females. Analysis of these first-backcross mice showed that of 93 mice tested, 84 (92%) were inducible for X-MuLV. These data suggest that three or four independently assorting genes control virus expression.

For a further estimate of the number of *M. m. molossinus* induction loci, six virus-inducible first backcross mice were crossed with NFS mice, and their progeny were tested for xenotropic virus by induction (Table 3). The data suggest that all six of the parental first-backcross mice contained at least two induction loci. These results indicate that *M. m. molossinus* carries at least three and probably four X-MuLV induction loci. (For five loci, 73% of the inducible first-backcross mice should have inherited more than one X-MuLV locus; for three loci, 43% of these mice should have inherited more than one.)

The pattern of virus induction seen in first- and second-backcross mice also indicated that most, but not all, of the X-MuLV loci of *M. m. molossinus* are high-induction loci. Virus was generally detectable in mixed cell cultures within 1 week after the removal of the inducer. However, 11 of the inducible first-backcross mice (or 12%) produced detectable xenotropic virus only after prolonged cocultivation (more than 2 weeks) with mink lung cells. In the second backcross, litters from backcross males 1-1, 1-8, and 6277 produced virus-positive offspring with both high and low virus induction phenotypes. Mice with the low-virus phenotype consistently showed the same response to induction. Thus, the induction loci carried by *M. m. molossinus* show patterns of both high and low X-MuLV expression.

To determine whether *M. m. molossinus* carries the *Bxv-1* locus, genetic crosses were carried out to test for linkage of virus inducibility to chromosome 1. Cells from splenectomized first-backcross mice were typed for alleles at *Ly-9*. Two virus-inducible male segregants carrying the *M. m. molossinus* $Ly\text{-}9^b$ allele were mated with virus-negative mice. At each successive backcross generation, progeny of each breeding male were typed for viral and *Ly-9* phenotypes to select breeding males for the next generation. At the fourth-backcross generation, progeny testing indicated that one of two parental males now produced litters with single-gene segregation for X-MuLV inducibility. However, this viral phenotype did not assort in association with alleles at *Ly-9* (r = 7/11). The close linkage between *Bxv-1* and *Ly-9* (4 cM) makes it unlikely that *Bxv-1* was lost through recombination in four backcross generations ($P = 0.15$). The data therefore suggest that the parental *M. m. molossinus* mouse used in these crosses did not contain *Bxv-1*.

TABLE 4. X-MuLV induction loci in *M. musculus*

Mouse strain	Locus	Induction phenotype[a]	Chromosome no.
BALB/c, AKR, C58, C57L, C57BL	*Bxv-1*	Low	1
F	*Bxv-1?*	High	1
MA	*Bxv-1*	Low	1
	Mxv-1	Low	?
NZB	*Nzv-1*	High	?
	Nzv-2	Low	?
	At least 3	High and low	?
M. m. molossinus (III)			

[a] High-virus loci are associated with spontaneous virus production in vivo and produce detectable levels of X-MuLV after short-term cocultivation (1 week) of the induced cells with mink cells. Mice carrying low-virus loci rarely express virus in vivo, and in vitro induction requires cocultivation with mink cells for 2 or more weeks for titers to be detectable.

DISCUSSION

We have used Mendelian segregation analyses to identify and characterize genetic loci for the induction of infectious xenotropic virus in various inbred and wild-derived mice. Table 4 summarizes the X-MuLV induction loci that have now been described in *M. musculus*.

Previous genetic studies identified three loci for X-MuLV expression in mice, the two NZB loci and *Bxv-1* (3, 9), and suggested that *Bxv-1* is the major induction locus of the older inbred strains. We have now demonstrated that another virus-inducible strain, MA/My, also carries *Bxv-1*. MA/My is an albino strain developed by Marsh from animals obtained from the colony of A. Lathrop. This same colony also provided the progenitors of other strains carrying *Bxv-1* (C57L and C58) and may have contributed to the genetic background of the X-MuLV-positive F/St strain (19).

The presence of *Bxv-1* in so many inbred strains suggests that this X-MuLV locus was widely disseminated in the colonies used to establish inbred strains. The *Bxv-1*-positive strains all originated from the collections of private dealers who imported mice from Western Europe (*Mus domesticus*) and Japan (*M. m. molossinus*). The introduction of xenotropic viral loci into these collections can probably be traced to their Japanese ancestry, since the only wild mice inducible for X-MuLVs are *M. m. molossinus* (1, 20; Kozak, unpublished data). Our failure to identify *Bxv-1* in one strain derived from *M. m. molossinus* does not contradict this hypothesis, since our results may simply reflect some polymorphism of X-MuLV loci in wild Japanese mice.

Our data demonstrate that mice can contain inducible X-MuLV loci other than *Bxv-1*. The two loci of NZB mice and the *Mxv-1* locus of MA mice differ from *Bxv-1* in their patterns of induced and spontaneous expression. Data from genetic crosses show that these three loci are nonallelic with *Bxv-1*, although these studies do not indicate whether *Mxv-1* is allelic with *Nzv-1* or *Nzv-2*. However, all three loci have different patterns of expression, suggesting nonidentity. Finally, the wild-derived *M. m. molossinus* (III) was shown to

carry multiple loci for X-MuLV expression, none of which appear to be present at or near *Bxv-1*.

M. m. molossinus is unusual among the virus-inducible mice in the large number of expressed X-MuLV loci carried in its germ line. For endogenous MvLVs with an ecotropic host range, it has been suggested that the acquisition of novel proviruses in the mouse germ line occurs through a mechanism involving virus infection and chromosomal reintegration (17). Although this is unlikely to occur for xenotropic virus in laboratory mice which restrict replication of these viruses, many Asian mice are, in fact, susceptible to exogenous X-MuLV infection (Kozak, unpublished data). *M. m. molossinus* may therefore provide a useful genetic background for studies on the origin, stability, and dissemination of X-MuLV genomes in mouse chromosomes.

Inbred mice contain multiple sequences homologous to the X-MuLV retroviral envelope (X-*env*) (6). Analysis of genomic DNAs from both virus-positive and -negative mice indicates that the bulk of these X-*env*-reactive fragments is large enough to contain full-length proviruses (6). One study on the endogenous X-*env*-reactive sequences cloned from a BALB library indicates that the great majority of these clones shows the organization expected for complete integrated retroviral genomes and that half contain long terminal repeats (8). However, most of these proviral sequences cannot be induced to produce infectious virus, and it has not yet been possible at the DNA level to identify the inducible proviruses among the 20 or more genomic DNA fragments containing X-MuLV sequences. Knowledge of specific induction loci and of their distribution among inbred and feral mice may ultimately aid in the identification of these biologically functional proviruses in germ line DNA.

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