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# Genetic Basis for Resistance to Polytropic Murine Leukemia Viruses in the Wild Mouse Species *Mus castaneus*

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**Cultured cells derived from the wild mouse species *Mus castaneus* were found to be uniquely resistant to exogenous infection by polytropic mink cell focus-forming (MCF) murine leukemia viruses (MuLVs). This MCF MuLV resistance is inherited as a genetically recessive trait in the progeny of $F_1$ crosses between *M. castaneus* and MCF MuLV-susceptible laboratory mice. Examination of the progeny of backcrosses demonstrated that susceptibility is inherited as a single gene which maps to chromosome 1. The map location of this gene places it at or near the locus *Rmc1*, the gene encoding the receptor for MCF/xenotropic MuLVs, suggesting that resistance is mediated by the *M. castaneus* allele of this receptor.**

Cells from inbred strains of laboratory mice and wild mouse species differ in their ability to support productive infection by the various subgroups of murine leukemia viruses (MuLVs). Standard genetic and molecular genetic approaches have identified the genetic loci and, in some cases, the specific sequences responsible for these differences. Analyses of genetic crosses between mice of different susceptibilities have defined four loci with allelic variants that alter in vitro virus susceptibility patterns: *Fv1*, which controls susceptibility to the N- or B-tropic subgroups of mouse tropic viruses (20); *Fv4*, which controls resistance to ecotropic MuLVs (8, 22); *Rmcf*, which restricts replication of polytropic MuLVs (10); and *Rmc1*$^{Sxv}$, which renders cells of most wild mouse species susceptible to xenotropic MuLVs (14). Inheritance of resistance alleles at three of these loci (*Fv1*, *Fv4*, and *Rmcf*) is known to provide mice with a measure of protection against virus infection in vivo and against virus-associated diseases. Genetic map locations for all four of these genes have been determined, but the molecular genetic sequences responsible for resistance have been identified for only one *Fv4*. This locus in resistant mice contains viral *env* sequences, which are absent in susceptible mice (12).

The molecular genetic basis for an additional virus resistance phenotype found in wild mice has been determined by comparative sequence analysis of the host cell gene encoding the cell surface receptor. This approach demonstrated that the resistance of *Mus dunni* cells to ecotropic Moloney MuLV but not other ecotropic MuLVs can be attributed to specific sequence differences in the ecotropic viral receptor (5, 6).

In the present study, we describe a novel pattern of in vitro resistance to mink cell focus-forming (MCF) MuLVs in the wild mouse species *M. castaneus*. We use a classical genetic approach to define a single gene responsible for this resistance, and we map this gene in the mouse genome to the chromosomal region which contains the MCF viral receptor *Rmc1*.

## MATERIALS AND METHODS

**Viruses, cells, and virus assays.** The viruses used for inoculation were obtained from J. Hartley, National Institute of Allergy and Infectious Diseases, Bethesda, Md. Three MCF isolates, *Akv1* MCF, AKR13 MCF, and Moloney MCF-HIX (3, 7), and one amphotropic virus, 4070A (9), were used. All viruses were grown in mink lung cells (Mv-1-Lu; ATCC-CCL 64).

Primary cultures of tail biopsy tissue were established from 10- to 20-day-old mice as previously described (18), maintained until confluent, and then passaged. Subconfluent cultures were infected with 0.2 ml of each virus dilution in the presence of Polybrene (4 μg/ml; Aldrich, Milwaukee, Wis.). Polybrene-containing medium was removed the next day, and cultures were maintained for 4 to 5 days and then lethally irradiated. MCF MuLV-infected cultures were overlaid with $2 \times 10^5$ mink lung cells or $6 \times 10^5$ MK $S^+L^-$ cells (19), and amphotropic MuLV-infected cultures were overlaid with mink $S^+L^-$ cells. Cultures were examined for foci after 5 to 8 days. For comparison, virus titers were determined in mink lung cells or in tail cultures of NFS/N-*Bxv1* mice.

**Mice.** DBA/2J mice were obtained from The Jackson Laboratory, Bar Harbor, Maine. NFS/N-*Bxv1* congenic mice carry the AKR/N-*Bxv1* locus on the NFS/N genetic background and were generated in our laboratory; AKR/N and NIH Swiss inbred NFS/N mice were originally obtained from the Small Animal Section, National Institutes of Health, Bethesda, Md. Mice of various wild mouse species were obtained from random-bred colonies maintained at PerImmune, Rockville, Md. (NCI contract NO1-CB-21075) and kindly provided by M. Potter, National Cancer Institute, Bethesda, Md.

$F_1$ hybrids and backcross mice were bred in our laboratory. Specifically, *M. castaneus* males and females were bred with DBA/2J mice to produce $F_1$ hybrids. The $F_1$ males and females were bred to *M. castaneus* to produce first-backcross mice. In a second cross, DBA/2J mice were mated to NFS/N-*Bxv1* mice and the progeny were backcrossed to NFS/N-*Bxv1*.

**Genetic markers.** High-molecular-weight DNA was extracted from parental mouse livers by standard procedures. DNA was extracted from peripheral blood or from cultured tail cells by using the QIAamp blood kit (Qiagen, Chatsworth, Calif.). DNAs were typed for variation in the PCR product sizes of the simple sequence length polymorphic (SSLP) markers *D1Mit33*, *D4Mit60*, *D5Mit1*, and *D5Mit3* (chromosome 1, 4, and 5 DNA markers; Massachusetts Institute of Technology). Primer pairs used for PCR amplification for each marker have been described (Whitehead Institute/MIT Center for Genome Research; *Genetic Map of the Mouse*, database release 10, 28 April 1995). PCRs were done on an MJ Research thermocycler under assay conditions described previously (4).

## RESULTS

**Resistance of *M. castaneus* cells to MCF MuLVs.** Cultured cells derived from tail biopsy tissue of various wild mouse species and laboratory strains were examined for susceptibility to the nonecotropic MCF and amphotropic MuLVs. *M. castaneus* cells were uniquely observed to be resistant to MCF MuLVs. As shown in Table 1, productive viral infection as measured by focus formation on mink lung cells was not detected in these cells. In contrast, $\log_{10}$ virus titers of 3.5 to 4.0 were observed in cells of the susceptible laboratory strain NFS/N-*Bxv1* and the other four wild mouse species tested. The reduction in virus titer seen in *M. castaneus* was more dramatic than that seen in DBA/2J mice, in which the presence of the

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TABLE 1. Virus susceptibility of various wild mouse species, inbred laboratory strains, and $F_1$ hybrids

| Mice | Geographic origin | $Log_{10}$ virus titer[a] | |
|---|---|---|---|
| | | MCF MuLV | A-MuLV[b] |
| *M. castaneus* | Thailand | ND[c] | 4.1 |
| *M. cookii* | Thailand | 3.9 | NT[d] |
| *M. caroli* | Thailand | 4.0 | NT |
| *M. musculus* NYD | Egypt | 3.6 | NT |
| *M. hortulanus* | Yugoslavia | 3.0 | NT |
| NFS/N | | 3.8 | 3.8 |
| DBA/2J | | 2.2 | 4.0 |
| (DBA/2J × *M. castaneus*)$F_1$ | | 2.0 | 3.9 |
| (NFS/N × *M. castaneus*)$F_1$ | | 3.9 | 3.9 |

[a] Virus titers were routinely 1 to 2 log units lower on tail cells than when determined directly on mink lung cells or mink $S^+L^-$ cells.
[b] A-MuLV, amphotropic MuLV.
[c] ND, not detectable.
[d] NT, not tested.

resistance gene *Rmcf*$^r$ reduced titers by approximately 1.5 log units.

In an attempt to amplify low levels of replicating virus in inoculated *M. castaneus* cultures, infected cultures were overlaid with mink cells 4 to 5 days after infection and cocultivated for 10 to 12 days. Cells and culture fluids were harvested, frozen and thawed to disrupt cells, filtered, and used to infect mink and mink $S^+L^-$ cells. No virus was detected.

*M. castaneus* cells were resistant to all three MCF virus isolates used (*Akv1*, AKR13, and Moloney HIX). In contrast, titers of the amphotropic MuLV, 4070A, were essentially identical in all mouse cells examined. These results indicate that *M. castaneus* cells are broadly resistant to MuLVs in the polytropic host range subgroup and that this resistance does not extend to the nonecotropic amphotropic MuLVs.

**Resistance is recessive and controlled by a single gene.** To further characterize this resistance, *M. castaneus* mice, which are interfertile with laboratory mice, were mated with two inbred strains, NFS/N-*Bxv1* and DBA/2J. Virus infectivity in parental mice and $F_1$ hybrids was compared. For both $F_1$ hybrids, cells resembled the inbred mouse parent in their susceptibility patterns (Table 1). NFS/N-*Bxv1* $F_1$ mice were fully susceptible to MCF MuLVs, whereas the reduced titer in DBA/2J $F_1$ mice was consistent with inheritance of the *Rmcf*$^r$ resistance allele from these mice. Thus, the *M. castaneus* MCF resistance is genetically recessive.

To define the *M. castaneus* genetic factor(s) responsible for the resistance phenotype, (DBA/2J × *M. castaneus*)$F_1$ hybrids were backcrossed to *M. castaneus* and typed for resistance. These DBA/2J backcrosses were initially designed to examine *Rmcf*-mediated resistance on the *M. castaneus* genetic background, which is largely free of endogenous MCF *env* genes (16). In this cross, then, half the progeny would be expected to inherit *Rmcf*$^r$, a dominant resistance gene; any deviation from this ratio could be attributed to the *M. castaneus* factor(s).

Tail cultures established from 202 backcross mice were individually typed for susceptibility to MCF viruses. Mice were typed by using Moloney HIX and/or AKR13 MCF, and virus replication was scored by focus formation on both mink lung cells and mink $S^+L^-$ cells. All assays were done in duplicate. Titers varied somewhat in assays done at different times, but mice were scored as resistant if titers were reduced by at least 1.5 log units below those in fully susceptible cells in each assay. Table 2 shows a sample assay of one litter. Of the 202 mice typed, 142 (70%) were scored as resistant and 60 were scored as susceptible. This ratio is consistent with inheritance of two unlinked genes, the presence of either one of which would produce resistant mice ($\chi^2 = 2.14$; $P < 0.20$). Since one of these genes must be *Rmcf*, these results indicate that the *M. castaneus* recessive resistance can be attributed to inheritance of a single gene from these mice.

To identify the progeny whose resistance can be attributed to *Rmcf* versus the *M. castaneus* factor, we typed the progeny of this cross for inheritance of other markers in the chromosomal region containing *Rmcf*. Previous genetic studies had demonstrated that *Rmcf* maps to the proximal region of chromosome 5 (10). The position of *Rmcf* was first independently established relative to several molecular genetic SSLP loci known to map in the same region of chromosome 5 by using the cross NFS/N-*Bxv1* × (NFS/N-*Bxv1* × DBA/2J)$F_1$, in which *Rmcf* resistance was inherited in the absence of other resistance factors. One of the SSLP markers tested, *D5Mit1*, was polymorphic in this cross and showed closest linkage to *Rmcf*. Of the 45 mice typed for the marker *D5Mit1*, 2 were recombinant for *Rmcf* resistance. This indicates that *Rmcf* is located 6.7 ± 3.7 centimorgans (cM) from *D5Mit1*.

The progeny of the *M. castaneus* cross were then typed for *D5Mit1* as well as for *D5Mit3*, markers predicted to flank *Rmcf* (15), in order to identify the resistant mice likely to carry *Rmcf*$^r$. As shown in Table 2, all the mice in this single litter heterozygous for *D5Mit1* and *D5Mit3* were resistant to MCF virus. Among the 202 backcross progeny, 98 were determined to be heterozygous for *D5Mit1*, of which 8 recombinants were typed as virus susceptible, and 101 were heterozygous for *D5Mit3*, of which 14 were susceptible. These data suggest a recombinational distance between *Rmcf* and *D5Mit3* of 13.9 ± 3.4 cM and a distance between *Rmcf* and *D5Mit1* of 8.2 ± 2.8 cM, consistent with the distance obtained in the NFS/N-*Bxv1* cross described above. Of the 104 *D5Mit1*$^{C/C}$ mice which are likely to lack *Rmcf*$^r$, 52 (50%) were also virus resistant. This confirms that resistance is governed by a single gene in *M. castaneus* and defines the distribution of the *M. castaneus* resistance gene in *Rmcf*$^s$ segregants. These 104 mice which did not inherit the chromosome 5 region containing *Rmcf*$^r$ were used to map the resistance gene.

**Resistance maps to chromosome 1.** Since the genetic map locations are known for several previously defined genes which

TABLE 2. Susceptibility to MCF MuLV of individual mice in a single litter of the cross (DBA/2J × *M. castaneus*) × *M. castaneus*

| Mouse | $Log_{10}$ titer[a] on: | | Resistance phenotype | Inheritance of genetic markers[b] | |
|---|---|---|---|---|---|
| | Mink $S^+L^-$ | Mink lung | | *D5Mit1*, *D5Mit3* (*Rmcf*) | *D1Mit33* (*Rmc1*) |
| 635-3-1 | ND[c] | ND | r | CD, CD | CC |
| 635-3-2 | ND | ND | r | CD, CD | CC |
| 635-3-3 | 2.30 | 2.90 | s | CC, CC | CD |
| 635-3-4 | ND | ND | r | CD, CD | CD |
| 635-3-5 | 2.06 | 2.55 | s | CC, CC | CD |
| 635-3-6 | ND | ND | r | CC, CC | CC |
| 635-3-7 | 2.14 | 2.85 | s | CC, CC | CD |
| NFS/N | 2.60 | 3.50 | | | |

[a] The Moloney MCF-HIX MuLV titer was 4.58 on mink lung cells and 4.04 on mink $S^+L^-$ cells.
[b] C, *M. castaneus* allele; D, DBA/2J allele.
[c] ND, not detectable.

TABLE 3. Linkage between MCF MuLV resistance and *Fv1*- or *Rmc1*-linked SSLP markers in backcross mice lacking the *Rmcf*$^r$-linked *D5Mit1* marker (*D5Mit1*$^{C/C}$)

| Locus[a] | Linked resistance gene | No. resistant/total no. (%) | |
|---|---|---|---|
| | | C/C | C/D |
| *D1Mit33* | *Rmc1* | 51/51 (100) | 1/52 (1.9) |
| *D4Mit60* | *Fv1* | 12/20 (60) | 14/23 (61) |

[a] PCR product sizes for DBA/2 and *M. castaneus* were as previously described (Whitehead Institute/MIT Center for Genome Research). For NFS/N-*Bxv1*, sizes were as follows: 104 bp (*D1Mit33*), 186 bp (*D4Mit60*), and 135 bp (*D5Mit1.*

affect virus susceptibility in mouse cells, we typed the backcross progeny for inheritance of additional SSLP markers known to map near the resistance genes. Initial efforts concentrated on markers near two mouse genes known to be involved in virus replication: *Fv1* (chromosome 4) and the MCF/xenotropic viral receptor gene, *Rmc1* (chromosome 1). As shown in Table 3, no correlation was observed between resistance and the *Fv1*-linked marker *D4Mit60*. Of the mice heterozygous for this marker, 60% were resistant, as were 60% of the homozygous mice ($\chi^2 = 2.12$; $P < 0.40$). In contrast, a close correlation was observed between resistance and a marker near, *Rmc1*, *D1Mit33*. Of the 51 mice homozygous for *D1Mit33*, 100% were virus resistant (Table 2, mouse 635-3-6; Table 3); of the 52 heterozygous mice, 98% were susceptible. It is likely that the single resistant mouse identified as *D1Mit33*$^{C/D}$ was not an *Rmcf*$^r$ recombinant, since both *D5Mit1* and *D5Mit3* were homozygous. This indicates that the *M. castaneus* factor responsible for resistance is closely linked to *D1Mit33* ($\chi^2 = 96.12$; $P < 0.0005$). Since this region of chromosome 1 is known to contain the *Rmc1* MCF/xenotropic receptor gene (11, 13), these results strongly suggest that the *M. castaneus* resistance is mediated by its MCF virus receptor.

## DISCUSSION

*M. castaneus* cells show a uniquely powerful resistance to productive infection by MCF virus, and genetic data strongly suggest that this resistance may be due to species variation in the *Rmc1* MCF/xenotropic receptor gene. Involvement of the receptor is also consistent with the observation that all three isolates of the MCF host range subgroup were restricted but that amphotropic virus, which uses a different cell surface receptor, was not restricted. The inheritance patterns observed here are consistent with the possibility that the *M. castaneus* receptor is defective for receptor activity. $F_1$ mice are susceptible, like their inbred strain parents, presumably as a result of inheritance of a normal receptor allele from the inbred mice, and resistance in backcross mice is observed only in mice inheriting both copies of the *Rmc1* chromosome 1 region of the *M. castaneus* parent. In the (DBA/2J × *M. castaneus*)$F_1$ mice, the presence of *Rmcf*$^r$ reduces the MCF virus titer, presumably by interaction with the inbred mouse receptor in these hybrid mice.

Our previous studies on wild mice indicate that wild mouse populations contain an allelic variation of *Rmc1* termed *Sxv*, initially described as responsible for infectibility of cells from wild mouse species such as *M. spretus* with xenotropic MuLVs (14). This gene is now assumed to be an allelic variation of for *Rmc1* several reasons. First, genetic evidence shows that the *Sxv* gene maps to the same chromosomal region as *Rmc1* (14). Second, interference studies show that cells of wild mouse origin with the Sxv phenotype (SC-1 cells and *M. dunni* cells) are not infectible by MCF virus if they are preinfected with xenotropic virus, indicating that xenotropic MuLVs and MCF MuLVs use the same receptor in these cells (2). Our previous studies also indicated that *Rmcf* interferes with xenotropic MuLV infection in *Sxv* mice (14), consistent with the proposal that *Rmcf* encodes *env* glycoproteins which interfere with exogenous virus infection by competing for the same receptors (*Rmc1* or *Rmc1*$^{Sxv}$) (1, 21). The *Rmc1* MCF/xenotropic viral receptor has not yet been cloned, but the data presented here and in our previous studies suggest that the wild mice *M. spretus* and *M. castaneus* carry variants of this receptor which are functionally distinct from the inbred mouse receptor. Molecular cloning and characterization of the *Rmc1* genes of these wild mice should thus contribute to the functional analysis of the receptor gene. The *M. castaneus* resistance described here is analogous to the resistance of *M. dunni* cells to Moloney ecotropic MuLVs (17). The receptor sequence in these mice is now known to differ from the fully functional receptor of inbred mice by four amino acids, and mutagenesis studies have determined that glycosylation plays a role in this resistance (6).

*M. castaneus* is a particularly interesting mouse in its role as a host for naturally occurring MuLVs. This mouse is one of only three populations of wild mice, which also include the Japanese mouse *M. musculus molossinus* and the Lake Casitas mice of southern California, which are known to contain endogenous ecotropic MuLV proviruses capable of producing infectious virus. All three of these mice, which have a common ancestry (16), are also uniquely protected from the deleterious effects of these ecotropic viruses in that they contain *Fv4*$^r$, which protects cells against infection by ecotropic virus. In addition to ecotropic viruses, infectious xenotropic MuLVs related to those of laboratory mice have been specifically isolated only from the wild mouse species *M. castaneus* and *M. m. molossinus*. The present study demonstrates that the resistance of *M. castaneus* to MCF viruses is likely to be mediated by its MCF/xenotropic receptor. It is thus tempting to speculate that this mouse has evolved independent survival mechanisms protecting it from exposure to the deleterious effects of both types of infectious viruses it carries.

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