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A *Mus dunni* Cell Line That Lacks Sequences Closely Related to Endogenous Murine Leukemia Viruses and Can Be Infected by Ecotropic, Amphotropic, Xenotropic, and Mink Cell Focus-Forming Viruses

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A *Mus dunni* cell line has been developed that is permissive for all four classes of murine leukemia viruses (MuLV): ecotropic, amphotropic, xenotropic, and mink cell focus-forming viruses. The *M. dunni* cells contain fewer MuLV-related sequences than do feral or domestic mouse, rat, or mink cells. Infection of the line by ecotropic MuLV induces a distinct cytopathic effect, and the cells can be readily transfected by MuLV DNA. The *M. dunni* line has been used to isolate an endogenous MuLV from the SC-1 feral mouse cell line.

A variety of cell lines are routinely employed to propagate the four major classes of murine leukemia viruses (MuLV), depending on the tropism of the viruses under study. Cells from feral (SC-1) (7) or laboratory (NIH or BALB/c) (1, 12) mice are used for analysis of ecotropic (9) or amphotropic MuLV (8), whereas mink (CCL 64) (11) cells are used to study xenotropic (14), mink cell focus-forming (MCF) (10), and amphotropic MuLV. Despite their proven utility for the isolation and characterization of MuLV, all of these cells share at least two potential disadvantages: none is permissive for all classes of MuLV, and each possesses endogenous DNA sequences that are closely related to MuLV. Although it is usually preferable to compare viruses propagated in the same cell, the lack of a single cell line that can be efficiently infected by all four MuLV classes has in some instances precluded such a direct comparison. The presence of closely related endogenous MuLV sequences in all of these cells raises the possibility of aberrent results arising as a consequence of recombination between input virus and these endogenous viral sequences.

In the course of studies on lines established from cultured tail cells of several different *Mus* species, we have found that a line developed from *Mus dunni* can circumvent both of these problems. In contrast to mouse, rat, and mink cells, there is very little cross-reactivity between MuLV and *M. dunni* DNA sequences. Furthermore, the *M. dunni* line is permissive for growth of virtually all viruses of the four MuLV classes, with the notable exception of ecotropic Moloney MuLV. The line can also be transfected with DNA, although with less efficiency than NIH 3T3 cells.

A tail from an adult female *M. dunni* animal (kindly provided by T. C. Hsu, M. D. Anderson Hospital and Research Center, Houston, Texas) was used to initiate a culture that was developed into a cell line as described (13). Briefly, a portion of the tail was minced and digested for 1 h in a 1:19 mixture of pronase (2.5 mg/ml)-collagenase (160 U/ml) and grown in modified McCoy 5a medium (GIBCO Laboratories) supplemented with heated (56°C for 30 min) 10% fetal bovine serum. SC-1, mink and mink S^+L^- cell lines were also grown in this medium, whereas NIH 3T3 cells were maintained in Dulbecco minimal essential medium (high-glucose formulation, Meloy Laboratories) supplemented with 10% heated fetal bovine serum. Cells used for virus assays were treated with either DEAE-dextran (25 μg/ml) or polybrene (16 to 32 μg/ml) and maintained in either Dulbecco or Eagle minimal essential medium (Microbiological Associates) with 10% fetal bovine serum. Virus titers were determined by fluorescent-antibody staining, the XC plaque assay (16), or a UV–SC-1 overlay technique (Table 1). Certain infectivity studies were carried out with a reverse transcriptase assay.

The following viruses have been grown in *M. dunni* cells. Ecotropic viruses were AKR L1, BALB/c (WN1802N and WN1802B), 1504M, Rauscher, and Friend. Xenotropic viruses were AKR-6, AKR-40, Kyushu, BALB-IU-1, BALB-IU-2, C58, NFS-1, NFS-2, NZB-6, NZB-IU-6, and *Mus musculus castaneus* (4, 6). Amphotropic viruses were 1504A, 292A, 4070A, 1740A, and 1313A (6). MCF viruses were AKR 247, AKR 13, AKR 1375-2, AKR 6A spleen, AKR 6A thymus, *Akv*-1-36, *Akv*-1-M111A, *Akv*-2-34, *Akv*-2-M66, C3H/Fg L3, and *C58v*-1-C77 (4, 3).

We have used several MuLV DNA probes in Southern blot analysis to compare the endogenous MuLV sequences in the *M. dunni* cell line with those found in mouse (*Mus musculus*), rat, and mink cells (Fig. 1). As shown in Fig. 1A, DNA from the feral mouse cell line SC-1 and from the laboratory mouse strains NIH, BALB/c, and NZB contain multiple copies of DNA that hybridize to a 5′ probe that includes the MuLV sequences which are most highly conserved (5). Rat and mink DNA contain two or more fragments that hybridize strongly to the MuLV probe, but *M. dunni* does not contain sequences that cross-react strongly with this probe. When an MCF *env* probe is used (3), multiple fragments hybridize with all mouse DNAs, but not with mink, rat, or *M. dunni* DNA. These results indicate that MuLV-related sequences in *M. dunni* are much less closely related than those present in rat or mink.

The genus *Mus* is subdivided into three subgenera, i.e., *Mus*, *Pyromys*, and *Coelomys*. *M. dunni* belongs to the subgenus *Mus* along with *M. caroli*, *M. cervicolor*, *M. cookii*, and *M. musculus*. The minimal hybridization of MuLV DNA sequences with cellular DNA from *M. dunni*, *M. caroli*, *M. cervicolor* and *M. cookii* (5) indicates that this group of MuLV genomes entered into *M. musculus* after

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TABLE 1. Quantitative virus titer assay on *M. dunni* cells as compared with other commonly used cell lines

MuLV[a]	Virus titer (log_{10} per ml) on the following cells[b]:					
	NFS	BALB/c	SC-1	*M. dunni*	Mink	Mink S^+L^-
Ecotropic						
AKRL1	7.1	5.1	6.9	5.8	—	—
WN1802N	5.7	3.3	6.2	5.4	—	—
WN1802B	2.5	6.0	6.6	4.7	—	—
1504-E	4.5	—	5.0	5.0	—	—
Xenotropic						
BALB/c-Iu-1	—	—	—	4.9	5.2	4.1
Amphotropic						
1504-A	—	—	—	5.4	5.4	4.7
MCF						
Akv-2-34	—	—	—	4.2	5.0	—

[a] Titers of ecotropic viruses were determined as follows. Cells (3 days postinfection) were UV irradiated, and 2×10^5 SC-1 cells were added; 3 days after addition of SC-1 cells, plates were UV irradiated and XC cells were added. Three days later, dishes were stained and plaques were counted. Titers on SC-1 cells were determined by direct UV-XC tests. Xenotropic and amphotropic virus titers determined by a fluorescent antibody technique with fluorescein-conjugated anti-Moloney antibody, except for Mink S^+L^-, on which foci were counted. MCF virus titers were determined by counting direct cytopathic foci.

[b] —, Not done.

speciation occurred. However, these four species do harbor other unrelated classes of endogenous retroviral genomes (2; our unpublished data).

Acute and chronic infection with various MuLV classes has been done in the *M. dunni* cells. In acute infectivity studies, the *M. dunni* line was found to be less efficient for quantitation of MuLV when compared with mink or SC-1 cells (Table 1). The line could be readily infected by several different ecotropic MuLV, but it was almost totally resistant to infection by Moloney ecotropic MuLV. Cytopathic effects (CPE) were noted in cells infected with ecotropic AKR (toxic CPE, Fig. 2B) and Rauscher (syncytia, Fig. 2D) MuLVs. These morphological changes were not seen when the same viruses were used to infect lines from other species (data not shown). M. W. Cloyd (Rocky Mountain Laboratory, National Institute of Allergy and Infectious Diseases) has also noted syncytia formation induced by ecotropic Friend and Casitas brain MuLV (8) in the *M. dunni* line (personal communication). MCF viruses induced CPE in the *M. dunni* line similar to those seen in mink cells (Fig. 2F). These morphological changes of *M. dunni* cells can be used for virus quantitation (Table 1 for MCF virus and personal communication from John L. Portis of Rocky Mountain Laboratory, who used the *M. dunni* cells to quantitate various strains of Friend virus and an ecotropic virus from Casitas brain [8]). In his hands, these viruses gave titers equal to those obtained with mouse SC-1 cells by the XC assay.

Amphotropic and xenotropic viruses infected the *M. dunni* line with an efficiency similar to that seen in mink cells, whereas SC-1 cells were more efficiently infected by amphotropic MuLV and less efficiently infected by xenotropic viruses. Viruses possessing these two host ranges did not usually induce CPE during acute infection, although mink and *M. dunni* cells chronically infected with *M. musculus castaneus* xenotropic virus (6) displayed some cytopathic effects (data not shown).

Virus yields from chronically infected *M. dunni* cells equaled or exceeded those obtained from mink or SC-1 cells (data not shown). For this reason and because of the range of viruses for which it is permissive together with its low endogenous MuLV background, the line has been extremely useful for restriction endonuclease analysis and molecular cloning of many exogenous proviral DNAs (4, 6).

M. dunni cells are transfectable with DNA although with less efficiency than NIH 3T3 cells. Transfection experiments with various molecularly cloned Harvey murine sarcoma virus DNAs yielded 5- to 10-fold-less foci as compared with NIH 3T3 cells (data not shown).

Because the *M. dunni* cells are susceptible to such a wide variety of MuLVs, we reasoned that this line might be useful for the isolation of MuLVs that had not been propagated in other cells. We have indeed isolated a virus from SC-1 cells (a line in which infectious endogenous MuLV has not previously been found) by cocultivation of these two lines. In data to be reported elsewhere, biochemical studies demonstrate that the virus represents an endogenous SC-1 MuLV.

In summary, the *M. dunni* line is susceptible to infection by viruses from all four classes of MuLV, and its genomic DNA does not contain sequences that hybridize to MuLV probes under stringent conditions. The low, endogenous, MuLV background has already proved useful in several MuLV studies, and the wide spectrum of viruses for which the line is permissive has permitted direct comparison of different MuLVs in the same host cell. Since this line contains receptors for all four classes of MuLV (15), it should also be useful as a standard line for characterizing the interference pattern of a given MuLV to determine the *env* genotype of the virus.

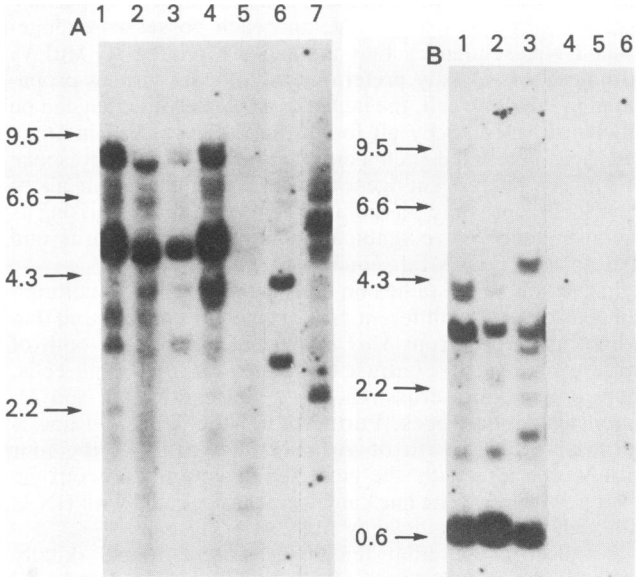

FIG. 1. Hybridization of restriction endonuclease-digested DNAs. DNAs were digested with *Pst*I (A) and *Bam*HI plus *Eco*RI (B), electrophoresed in 0.5% agarose gels (35 V, 20 h), transferred to nitrocellulose filters, and hybridized with ^{32}P-labeled, nick-translated viral DNAs in the presence of 0.6 M Na^+ at 65°C as described previously (6) to a (A) ^{32}P-labeled 5′ viral probe (4.6-kilobase-pair *Sma*I fragment from molecularly cloned AKR 623 virus [5]) or to a (B) ^{32}P-labeled *Bam*HI plus *Eco*RI fragment from spleen focus-forming virus (3). The numbers in the vertical lines represent the length (in kilobase-pairs) of the marker DNAs. (A) lane 1, BALB/c; lane 2, NFS; lane 3, SC-1; lane 4, NZB-Q; lane 5, *M dunni*; lane 6, mink; lane 7, rat cells. (B) Lane 1, BALB/c; lane 2, 129; lane 3, SC-1; lane 4, *M. dunni*; lane 5, mink; lane 6, rat cells.

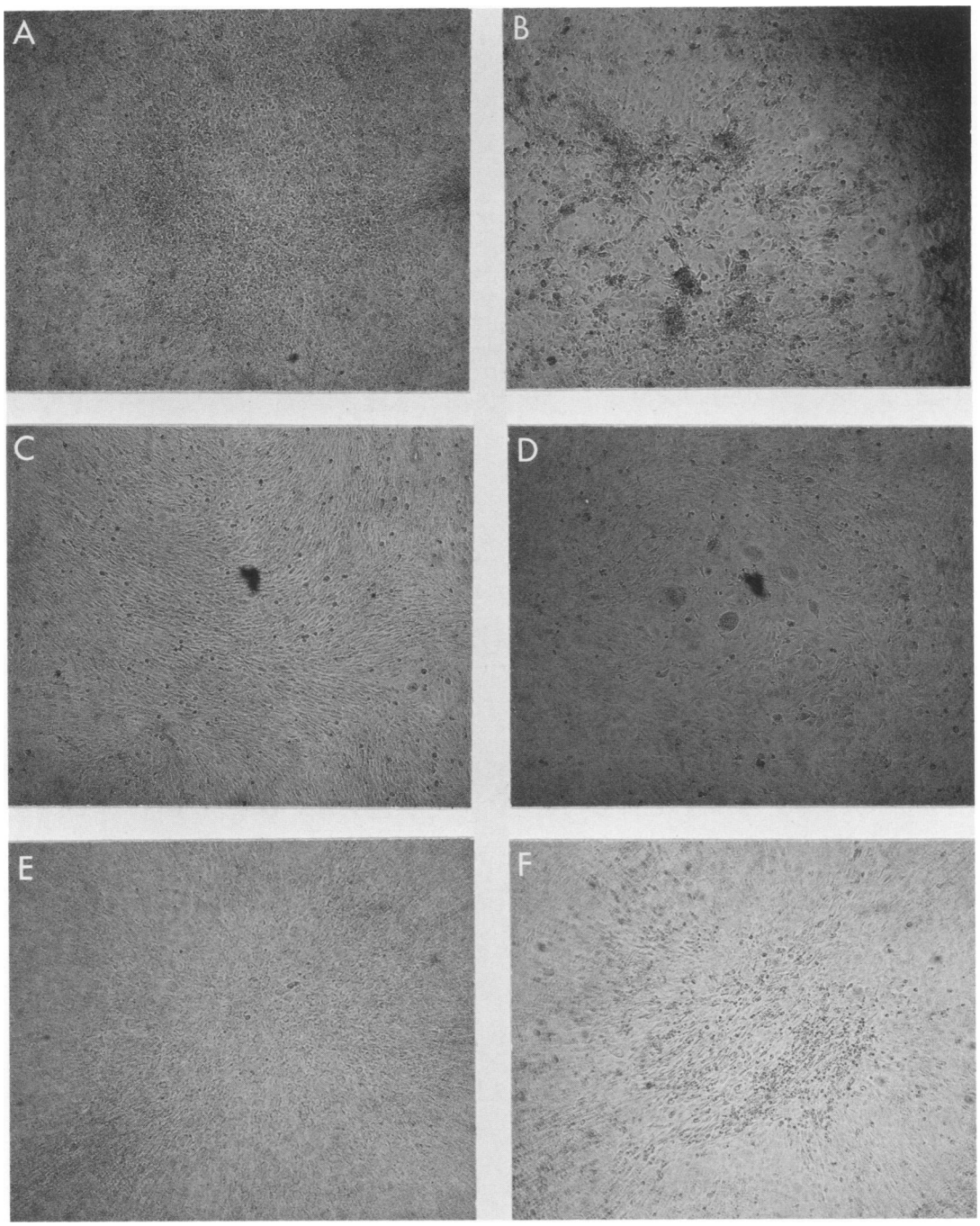

FIG. 2. CPE from infection with MuLVs on *M. dunni* cells. (A), (C), and (E) are control *M. dunni* cells. (B) *M. dunni* cells infected with AKRL1. (D) *M. dunni* cells infected with Rauscher MuLV. (F) *M. dunni* cells infected with *Akv*-2-34 MCF virus.

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