Selective Host Range Restriction of Goat Cells for Recombinant Murine Leukemia Virus and Feline Leukemia Virus Type A

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We isolated a strain of normal goat fibroblasts which was uniquely selective in that it allowed the replication of xenotropic murine leukemia virus but not polytropic recombinant murine leukemia virus. In addition, feline leukemia virus type A replication was severely diminished in these goat cells, whereas feline leukemia virus type B and feline endogenous RD114-CCC viruses replicated efficiently. No other known cells exhibit this pattern of virus growth restriction. These goat cells allow the study of xenotropic murine leukemia virus in mixtures which also contain recombinant murine leukemia virus and may be helpful in eliminating feline leukemia virus type which often coexists in feline sarcoma or leukemia virus mixtures with other feline leukemia virus types.

Several groups of mammalian oncornaviruses consist of distinct types that contain different envelope (env) glycoproteins which confer specific host range, interference, and neutralization properties (19, 21). Based on host range, the murine leukemia virus (MuLV) system has the following four distinct, identifiable env gene-specific subgroups: the ecotropic MuLV's, which can grow only in mouse and rat cells; the xenotropic MuLV's (designated MuX), which cannot grow in mouse cells but can grow in cells of many other mammals; the recombinant MuLV's (RM-MuLV's), whose env gene is composed of both ecotropic and xenotropic sequences and whose host range is polytropic, so that these viruses can grow in both mouse and nonmouse cells; and, finally, the amphotropic MuLV's, which are derived from wild mice and can grow in mouse cells as well as heterologous cells, but are distinct from RM-MuLV's on the basis of interference and neutralization (11, 15, 19, 21). The viruses isolated from animals with murine leukemia appear to be all ecotropic, but careful genetic analyses have revealed that RM-MuLV and MuX genomes are also present, either as phenotypically mixed or genomically masked virions (5, 18). Circumstantial evidence has strongly implicated RM-MuLV, which is itself potentially oncogenic in pure form, as the agent causative of murine leukemia (8, 16). RM-MuLV's can be isolated easily in pure form from mixed MuLV stocks by manipulating the polytropic host range properties of the virus. Although the ecotropic MuLV parents of RM-MuLV's can be traced easily, the precise xenotropic parent has not been identified because until now no cell system selective for MuX and against RM-MuLV's has been described (24).

In the feline oncornavirus system, the agent that causes the disease is feline leukemia virus (FeLV). Three types (types A, B, and C) exist, all of which can grow not only in cat cells but also in cells of many species (3, 4). Although FeLV type A (FeLV-A) has been isolated from nature in pure form, FeLV-B and FeLV-C have always appeared admixed with FeLV-A. A second group of viruses has been isolated from cat cells; these are the RD-CCC group, which are endogenous and inducible and do not share group-specific antigenic determinants with FeLV (20). These viruses can also appear in FeLV stocks. The RD-CCC group is generally restricted from de novo growth in cat cells, but otherwise these viruses have a broad host range (3, 4, 13, 20). Several species of mammalian cells are known to restrict FeLV-A as opposed to FeLV-B, FeLV-C, or the RD-CCC group (4). However, the Rickard 161 FeLV-A isolate grows well in human cells, which otherwise restrict other FeLV-A isolates (12, 22, 25). Here we describe a strain of goat fibroblast cells which differs from all other host cell types in that it allows the growth of MuX isolates but completely restricts both ecotropic MuLV's and RM-
MuLV's. These cells also selectively essentially eliminate the growth of FeLV-A Rickard 161 but allow the growth of FeLV-B and the RD-CCC groups.

A fibroblastic cell strain was established from a skin biopsy of a young Nubian goat by standard cell culture techniques. The cells were grown initially in F-10 medium, then in Dulbecco modified Eagle medium, and finally in McCoy 5A medium, all containing 10% fetal calf serum. These goat cells could be transformed with simian sarcoma virus, and nonproducer cells were isolated (Thiel et al., Virology, in press). Direct infections of these goat cells with murine sarcoma virus (MSV) or feline sarcoma virus (FeSV) pseudotypes were performed by adding various helper viruses to the system. Propagation of various host cell types, replication of transforming and helper viruses, and assays of transforming and helper viruses have been described previously (1, 6, 7, 11, 12, 14). In initial assays, some sarcoma virus pseudotypes transformed goat cells, but individual limiting dilution foci were difficult to identify. Of specific interest was the fact that whereas MuX, RD-CCC, FeLV-B, and simian sarcoma-associated virus pseudotypes of MSV or FeSV transformed goat cells well, two virus pseudotypes which otherwise had broad-host ranges (FeLV-A and RM-MuLV) appeared to have no effect on goat cells. Murine ecotropic virus pseudotypes also had no effect on goat cells. MSV coated either with ecotropic MuLV's or with a number of specific RM-MuLV isolates, including RM-M1ix, RM-M11, RM-M12, RM-Gr (Graffi), and RM-AKR-F11, did not transform these goat cells (9).

To determine the selective permissiveness of goat cells for these viruses, we compared growth curves by using MSV pseudotypes coated with the MuX isolate B-MuX and with RM-M1ix, a prototype RM-MuLV isolate. Both of these preparations were genetically pure based on biological and biochemical criteria (11, 17). MSV focus-forming (FFU) to helper virus-focus-inducing (FIU) ratios were 1:1 in both virus stocks. FFU assays were performed on diploid cat embryo (FEP) cells, and FIU assays were done on cat sarcoma-positive leukemia-negative (S°L°) clone 81 cells. Growth tests with both virus pseudotypes were performed at a multiplicity of infection (MOI) of 1 (Fig. 1A and B). Unabsorbed virus was measured in both cases after initial exposure. All viruses, including MSV(B-MuX), B-MuX, MSV(RM-M1ix), and RM-M1ix, were absorbed at between 40 and 60% of the initial input during the 2-h incubation period when they were assayed in appropriate cells.

MSV(B-MuX) grew very rapidly in goat cells, attained maximal titers in about 2 days, and persisted at that level for more than 18 days. The B-MuX component of the complex grew to very high titers (≥10⁷ FIU/ml) (Fig. 1A and B).

**Fig. 1.** Growth curves of murine and feline helper viruses and their MSV pseudotypes in goat cells. A total of 2.0 × 10⁶ goat cells were grown for 24 h in 6-cm plastic dishes. Cells were pretreated with DEAE-Dextran for 30 min at 37°C. Except where indicated below, virus input was adjusted to 1.0 pseudotype and 1.0 helper virus per cell. After a 2-h incubation, the residual inoculum and wash fluids were combined to assay for unabsorbed virus. Virus was harvested at the indicated times with a preceding 24-h medium change. Each point represents the pooled harvest from two individual dishes. (A) Assay for FFU or MSV(B-MuX) (O) and MSV(RM-M1ix) (□) in mink cells. Both input viruses were grown in cat FEF cells. FeLV-A was added as a helper virus at the concentration optimal for better focus visualization in mink cells (4 × 10⁶ FIU/2 × 10⁶ cells). (B) Assays for B-MuX (O) and RM-M1ix (□) from the same samples as in (A) in cat S°L° clone 81 cells. Both B-MuX and RM-M1ix were detected with optimal efficiency. (C) Growth of MSV(FeLV-A) (△) derived from FeLV cells and MSV(CCC) (●) derived from dog cells. Both preparations were assayed in normal mink cells with FeLV at the optimal concentration as helper virus. (D) Growth of FeLV-A (△) and CCC virus (●), using the same samples as in (C). FeLV was assayed in cat S°L° clone 81 cells. CCC was assayed at an MOI of 0.2 virus per goat cell by the immunofluorescent focus assay. The CCC growth curve was also assessed as RD-114gp70-positive immunofluorescent foci induced after 7 days on mink cell cover slips exposed to different dilutions of virus harvests. Mink S°L° cells were not used because compared with immunofluorescent assays, assays with these cells underscored the actual replicating virus by 10- to 100-fold (6).
Other MuX variants, such as AT124, also grew in goat cells. In contrast, when MSV(RM-M$_{\text{HIX}}$)-infected goat cells were harvested for virus, no progeny was detected in either mink (Fig. 1A), or mouse, or cat cells. No free RM-M$_{\text{HIX}}$ was found when this virus was assayed in either mouse or cat S$^L$- cells (Fig. 1B). Analogueous growth tests were attempted with MSV(FeLV-A), whose FeLV-A Rickard 161 isolate was passaged only in our diploid FEF cells for 10 years (12, 22, 25). These FEF cells were not inducible for the RD-CCC endogenous viruses and restricted the growth of exogenously applied RD-CCC viruses (6, 13). The MSV(CCC) pseudotype was propagated in dog cells as described previously; the ratio of MSV to CCC in this pseudotype was about 8:1, as determined from FFU analyses and from the limiting dilution replicating units of the helper virus in mink cells. As Fig. 1C shows, an MOI of 1 resulted in relatively rapid growth of MSV(CCC); the titers increased with time, and production of virus continued for 18 days. MSV(FeLV) production dropped to very low levels but remained barely detectable throughout the growth period. Virus titers did not appear to increase even after 3 weeks of incubation. When the CCC virus was assessed for the helper virus component (assayed as RD114 gp70-positive immunofluorescent goat cell foci), the CCC virus grew at about the same rate as its MSV(CCC) component. The FeLV helper virus of the MSV(FeLV) input was also generally detectable at extremely low levels as FIU in S$^L$- clone 81 cells throughout the 21-day test period. When a second FeLV-A isolate (the virus derived from line 422) was used, a similar growth curve revealed no virus progeny.

The FeLV-B pseudotype of Snyder-Theilen FeSV derived from nonproducer mink cells superinfected with FeLV-B was also assessed for FFU and FIU production (14). FeSV FFU were assayed in normal mink cells, and the helper virus was assayed in cat S$^L$- clone 81 cells. In contrast to FeLV-A and its MSV pseudotype, FeSV(FeLV-B) grew well in goat cells, producing $\geq 10^5$ FFU in mink cells and $\geq 10^6$ FIU of helper virus in S$^L$- clone 81 cells. Accordingly, FeLV-B and its FeSV pseudotype appeared as well as the endogenous RD-CCC virus in goat cells.

Although all of the viruses tested adsorbed adequately to goat cells, we did not know where the restriction occurred. One ready measure of the level of restriction was whether the entering MSV pseudotype could integrate and give rise to its MuLV-derived gag region-encoded precursor polypeptide (10, 23). Table 1 shows the results of an immunofluorescence examination of infected goat cells. At an MOI of MSV(CCC) of 2, most of the cells were positive for the MuLV p30 group-specific determinant within 7 days, indicating translation of MSV-encoded Pr60$^{\text{agg}}$. Most of the cells were also positive for RD114 gp70. In contrast, no goat cells were positive for either MuLV p30 or gp70 after an analogous infection with MSV(HIX). Only about 1% or fewer of the MSV(FeLV)-infected cells (MOI 2) stained positive for pr60. None of the cells were positive for RD114 gp70, indicating that MSV(FeLV-A) passed through FEF cells contained no detectable RD-CCC virus.

Another measure of the entry of the MSV pseudotype was its rescuability after infection with a helper virus that was capable of growth

<table>
<thead>
<tr>
<th>MSV input*</th>
<th>% Of viral antigen-positive cells in the immunofluorescence test</th>
<th>Rescue of MSV (FFU/ml)*</th>
<th>No additional helper B-MuX added</th>
</tr>
</thead>
<tbody>
<tr>
<td>mlMSV(FeLV-A)$^d$</td>
<td>$&lt;0.5$</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>mlMSV(RM-M$_{\text{HIX}}$)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>mlMSV(CCC)</td>
<td>$&gt;90$</td>
<td>85</td>
<td>85</td>
</tr>
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* MOI, 2.

$^b$ Goat cells were grown on cover slips, treated with DEAE-dextran for 30 min at 37°C, and infected with MSV pseudotypes. After 7 days of infection, the cells were washed, fixed in acetone, and exposed to anti-Friend MuLV p30 and gp70 sera, which have been well characterized previously (9, 23). A potent, highly specific anti-RD114 gp70 goat serum was used. Fluorescein isothiocyanate-conjugated pig anti-goat immunoglobulin G sera and anti-RD114 gp70 serum were obtained from Huntington Research Laboratories.

$^c$ DEAE-dextran-treated cells in 6-cm plastic dishes were infected with MSV at an MOI of 1. Preparations in other dishes were coinfect ed with B-MuX at an MOI of 2. After 7 days, virus was harvested and assayed on normal mink cells by itself and in the presence of added helper FeLV ($4 \times 10^4$ FIU/dish). B-MuX grew well in all sets of goat cells, as determined by its harvest titers in cat S$^L$- clone 81 cells ($>10^6$ viruses per ml).

$^d$ The prefix ml indicates the ml isolate of MSV which codes for Pr60$^{\text{agg}}$ (23).
in goat cells. Goat cells were infected at an MOI of 1 with MSV(RM-MHIX) or MSV(FeLV), and B-MuX was added at an MOI of 2 to each preparation as a helper virus. After 7 days, tests for MSV(RM-MHIX) and RM-MHIX were performed in mouse T3FL cells and mouse S"L"-FG10 cells, both of which were capable of detecting RM-MHIX and its pseudotype but were insensitive to B-MuX. No mouse cell-tropic RM-MHIX or its MSV pseudotype was detected after 7 days. However, infection with MSV(FeLV) and B-MuX yielded more than 10^6 MSV after 7 days because of sequential cycles of replication of rescued MSV(B-MuX). The emerging pseudotype was coated with B-MuX, as determined by its susceptibility to normal mouse serum derived from oncornavirus inhibitory factor (19). In both cases, ≥10^6 FIU of B-MuX was detected in virus harvests from S"L"-clone 81 cells.

To eliminate the possibility that contaminating RD-CCC virus was responsible for growth of FeSV and marginal detection of MSV(FeLV-A) in goat cells, we examined viruses from goat cells by neutralization with FeLV or RD114 antiserum. Table 2 shows that progeny of both FeLV-A and FeLV-B stocks produced FIU in cat S"L"-clone 81 cells, whereas CCC was negative. The FeLV-A parental virus was neutralized substantially by the anti-FeLV serum, but the CCC virus was not affected. The amount of virus emerging from goat cells was so low that significant neutralization was not feasible. Conversely, the anti-RD114 serum did not neutralize the presumptive FeLV-B progeny from goat cells significantly but it did inactivate the CCC virus. These findings confirmed that it was FeLV-B and not CCC that grew in goat cells.

These goat cells provide a unique system for further examinations of events in the generation of RM-MuLV's. It is now possible to identify and quantitate the MuX component in complex MuLV stocks containing all three varieties of MuLV. In the feline system, purification of FeLV-B and FeLV-C stocks was accomplished previously by eliminating the otherwise ever-present FeLV-A by neutralization and dilution techniques. These goat cells, which restrict Rickard 161 FeLV-A but allow the growth of FeLV-B or endogenous CCC virus, should facilitate further investigations of the role of FeLV-A in naturally polytropic FeLV or FeSV stocks.

Whether recombinant FeLV's exist or are at all relevant in feline leukemia could be examined with such a selective cell system. Based on our experience with MuLV's, the FeLV's from leukemic cats are being examined specifically for the presence of recombinant variants.

### LITERATURE CITED


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