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Mechanism of Restriction of Ecotropic and Xenotropic Murine Leukemia Viruses and Formation of Pseudotypes Between the Two Viruses

PETER BESMER AND DAVID BALTIMORE*

Department of Biology and Center for Cancer Research, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

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Ecotropic and xenotropic murine leukemia viruses (MuLV's) constitute separate interference groups; within each group there is cross-interference, but between the groups there is no detectable interference. Interference is manifest against pseudotypes in which the vesicular stomatitis virus genome is contained within the coat of one of the murine leukemia viruses. The pseudotypes display the cell specificity of the leukemia viruses: pseudotypes with an ecotropic MuLV coat infect mouse cells but not rabbit or mink cells; pseudotypes with a xenotropic MuLV coat infect rabbit or mink cells well but mouse cells very poorly. Efficient pseudotype formation also occurs between the two MuLV classes, and both the interference patterns and the cell specificity of these pseudotypes are entirely determined by their envelope. Using these pseudotypes, ecotropic MuLV infection could be established in xenogeneic cells, and the resulting progeny could be scored by using a conventional XC cell assay. Also, xenotropic MuLV infection could be established in a mouse cell, showing that no absolute intracellular barrier against xenotropic virus growth exists in murine cells. The major barriers against both xenotropic and ecotropic MuLV therefore are cell surface barriers. Xenogeneic cells probably lack receptors for ecotropic MuLV, but murine cells may either lack receptors for xenotropic MuLV or have receptors that are blocked by endogenous expression of the glycoprotein of endogenous xenotropic MuLV.

The host range of RNA tumor viruses is determined by a composite of surface receptor specificities and intracellular restriction phenomena. For avian and feline viruses, the major characterized determinant of cell susceptibility is the specificity of cell surface receptors; the best-characterized determinant of the host range of murine leukemia viruses (MuLV) is the Fv-1 gene product, which causes an intracellular restriction (18, 21, 22).

There is a class of endogenous mouse viruses that are unable to infect mouse cells; they have been called xenotropic viruses because they can infect the cells of other animal species (2, 5, 23, 26). From work on interspecies hybrid cells, it has been suggested that the barrier to infection of mouse cells by these viruses is an intracellular restriction (14, 33). Using several methods, we have investigated whether there might be a barrier at the cell surface. First, we have determined cross-interference patterns of the xenotropic and ecotropic MuLV's and have shown that mouse-tropic (ecotropic) murine viruses interfere with each other because they all use the same receptors. However, the xenotropic viruses were found not to interfere with ecotropic viruses, suggesting that the xenotropic viruses cannot absorb to the receptor for ecotropic virus. Second, we have made pseudotypes of vesicular stomatitis virus (VSV) having the coat of xenotropic MuLV. These pseudotypes did not penetrate mouse cells but did penetrate rabbit cells, showing that no functional receptor for xenotropic murine leukemia virus exists on fibroblastic murine cells. In a similar fashion, it was shown that ecotropic MuLV's do not penetrate rabbit and mink cells. Finally, we have produced pseudotypes in which an ecotropic genome is found in a xenotropic coat, and vice versa. In both cases the tropism is determined by the coat, allowing establishment of an infection of a xenotropic virus in mouse cells and an ecotropic virus in mink cells.

To simplify description of these experiments, we have adopted the nomenclature of $MuLV_E$ and $MuLV_X$ to designate ecotropic and xenotropic MuLV's, respectively.

MATERIALS AND METHODS

Cells and leukemia viruses. The designation and

origin of each of the viruses used in this study are given in Table 1. The description of the cell lines used here is given in Table 2.

NIH/3T3 cells were a gift of S. A. Aaronson (National Institutes of Health [NIH]). JLS-V9 cells were obtained from Ken Manly (Roswell Park Memorial Institute). The rabbit cell line (SIRC) was obtained from G. Todaro (National Cancer Institute). XC cells were provided by J. Hartley (NIH). NIH cells producing Moloney MuLV (M-MuLV) clone 1 virus were derived in this laboratory (11). The 15176 cell line producing murine sarcoma virus (MSV) and $MuLV_X$ was kindly provided by J. Levy. SIRC cells producing the $MuLV_X$ from BALB/c cells are the end point of a titration of bromodeoxyuridine (BUdR)-induced virus from JLS-V9 cells (6). NIH cells producing the endogenous N-tropic virus from JLS-V9 cells were obtained by infecting NIH/3T3 cells with virus from BUdR-induced JLS-V9 cells (6). The 8c subclone 81, a sarcoma-positive, leukemia-negative (S^+L^-) cat cell line transformed by M-MSV, was kindly provided by P. Fischinger. The mink cell line, CCL64, was obtained from the American Type Culture Collection. NRK cells were obtained from D. Livingston, and SIM.R cells were provided by A. Axelrad.

TABLE 1. *Murine leukemia virus nomenclature*

Designation	Characteristics
$MuLV_E$	Any MuLV able to grow on some mouse cells.
$MuLV_X$	Any MuLV unable to grow on mouse cells but able to grow on cells of other species.
IN-MuLV	N-tropic MuLV recovered after induction of JLS-V9 cells with IUdR, cloned on NIH/3T3 cells. Makes small plaques (11).
N-C1-35-MuLV	N-tropic MuLV derived from BALB/c animals and cloned (20).
B-C1-11-MuLV	B-tropic MuLV derived from BALB/c animals and cloned (20).
M-MuLV	Clone 1 Moloney MuLV. NB-tropic (11).
$MuLV_{Xa}$	Subclass of xenotropic MuLV that previously was designated Xa by Callahan et al. (7) and as class II MuLV by Aaronson and Stephenson (3).
$MuLV_{Xb}$	Subclass of xenotropic MuLV that previously was designated Xb by Callahan et al. (7) and as class III MuLV by Aaronson and Stephenson (3). $MuLV_{Xb}$ differs from $MuLV_{Xa}$ by nucleic acid sequences and the immunological type specificity in the structural protein p12.

TABLE 2. *Origin of cell lines*

Cells	Species of origin	Reference
JLS-V9	Mouse (BALB/c)	45
NIH/3T3	Mouse (NIH Swiss; $Fv\text{-}1^{n/n}$)	19
NRK	Rat	9
SIRC	Rabbit	5
Cat S^+L^- (subclone 81)	Cat (transformed by M-MSV)	12
CCL64	Mink	16
XC	Rat (transformed by Rous sarcoma virus)	40
SIM.R	Mouse (Swiss inbred; $Fv\text{-}1^{b/b}$)	42
15176	Human [infected with MSV($MuLV_{Xb}$)]	J. Levy (personal communication)

Cells were grown in Dulbecco modified Eagle medium with 10% calf serum (NIH/3T3, SIRC, XC) or with 10% heat-inactivated fetal calf serum (JLS-V9, 15176, CCL64, and SIM.R). The cat S^+L^- cells were grown in McCoy 5A medium plus 14% heat-inactivated fetal calf serum.

Induction with halogenated pyrimidines. On the day after plating of 5×10^5 cells/plate, the medium was changed to medium containing 20 μg of BUdR or iododeoxyuridine (IUdR) and 25 μg of deoxycytidine per ml. (27). After 20 to 24 h, the BUdR- or IUdR-containing medium was removed, and the plates were washed once with regular medium. Peak virus production occurs 1.5 to 3 days after removal of the BUdR- or IUdR-containing medium (6).

MuLV infections. Approximately 1×10^5 to 2×10^5 cells were seeded onto a 60-mm dish. Twenty-four hours later the cells were inoculated with 0.5 ml of the virus sample in medium containing 8 μg of polybrene per ml. After 2 h of adsorption, 5 ml of regular medium was added. The XC plaque assay with NIH/3T3 as indicator cells was used for quantitation of $MuLV_E$ (31). For quantitation of $MuLV_X$, a focus assay described by Fischinger et al. (12) was used.

VSV. VSV wild type (Indiana serotype) was kindly provided by A. S. Huang (35). A cloned stock of the VSV mutant *tl*17 was provided by R. Weiss and J. Zavada. This mutant has a thermolabile envelope glycoprotein and is easily inactivated at 45°C (47).

VSV titration was carried out as described earlier (34). To produce VSV pseudotypes with a MuLV coat [VSV(MuLV)], subconfluent cells were infected with a 1-ml (per 10-cm plate) virus inoculum in Dulbecco modified Eagle medium (multiplicity of infection of 2 to 5). After 1 h of adsorption at 32 or 37°C for VSV *tl*17 or VSV wild type, respectively, the unadsorbed virus was washed off two times and regular medium was added back. After 12 to 15 h of incubation at 32 or 37°C, respectively, the supernatant was collected and cell debris was removed by low-speed centrifugation. Heat inactivation was carried out at 45°C (±0.2°C) for 1 h or as indicated.

Neutralizations. Anti-M-MuLV serum from tumored Fischer rats was provided by the Virus Cancer Program. Rabbit neutralizing antiserum against VSV was provided by A. S. Huang, and rabbit antiserum made against NZB-$MuLV_X$ was provided by Jay Levy.

VSV antiserum was used at a final concentration of 1:20 if not otherwise indicated. A virus sample of 0.5 ml was mixed with 0.5 ml of VSV antiserum in phosphate-buffered saline. The neutralizations with the $MuLV_X$ antiserum were done at a final antibody

concentration of 1:20. Controls contained equal volumes of virus and phosphate-buffered saline. The samples were incubated for 1 h at 37°C. Neutralizations with antiserum made against M-MuLV were done at a final antibody concentration of 1:40. At the concentration used in these experiments, this antiserum neutralizes M-MuLV to less than 10^{-5}.

RESULTS

Interference among ecotropic viruses. Existing data suggest that all $MuLV_E$ cross-interfere (32). We have tested this generality for two very different $MuLV_E$ strains. One is an N-tropic virus, IN-MuLV, that makes turbid small XC cell plaques. The other is M-MuLV, a virus that makes large XC cell plaques and is NB-tropic. The plaque morphology marker allowed the two viruses to be distinguished. When uninfected NIH/3T3 cells and NIH/3T3 cells preinfected with IN-MuLV were exposed to M-MuLV, the preinfected cells produced a yield after 5 days that was 10^{-3} of the yield from uninfected cells (Table 3). An XC cell titration of M-MuLV on either NIH/3T3 or NIH/3T3 preinfected with IN-MuLV showed a titer of 10^6 PFU/ml on NIH/3T3 cells and no M-MuLV plaques on the preinfected cells. These two different assays indicate a strong interference between the two viruses.

To investigate whether the interference among $MuLV_E$ strains was a surface interference, VSV(M-MuLV) was produced by infecting M-MuLV-producing cells with the heat-labile VSV *tl*17. The VSV(VSV) in the stock was then killed by heating at 45°C (47; Fig. 1). Neutralization of the heat-inactivated samples with anti-M-MuLV antibody further decreased the remaining fraction 1,000-fold. The fraction that can be neutralized with M-MuLV antisera after heat inactivation is composed of the VSV(M-MuLV) pseudotypes (Fig. 1). The VSV(M-MuLV) produced in this way was used to infect control mouse cells and mouse cells preinfected with various $MuLV_E$ strains (Table 3). Different uninfected cell lines show as much as 10-fold variation in intrinsic susceptibility to VSV. To control this variability, the VSV(M-MuLV) pseudotypes were assayed before and after neutralization with an anti-M-MuLV antiserum. Positive pseudotype susceptibility was indicated by at least a 100-fold drop in titer after antiserum treatment.

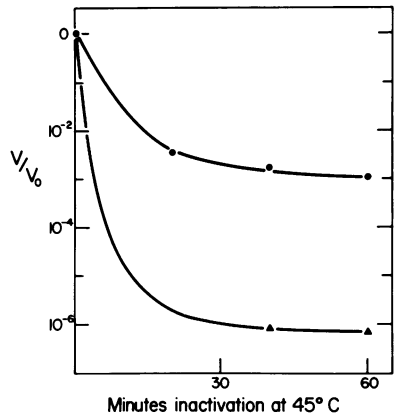

FIG. 1. *Preparation and characterization of VSV tl17(M-MuLV$_E$). M-MuLV$_E$-producing clone 1 cells were infected with VSV tl17 at 32°C at a multiplicity of infection of 1 to 2. The virus was adsorbed in a volume of 0.5 ml per 5-cm dish. After 45 min of adsorption, 4 ml of medium was added to the culture. After 14 h of incubation at 32°C, the virus yield was harvested and aliquots were heat inactivated for different lengths of time at 45°C. The residual fraction of VSV was then determined in a VSV plaque assay on NIH/3T3 cells at 32°C* (●). *Some samples that had been inactivated at 45°C were also neutralized with antiserum made against M-MuLV as indicated in Materials and Methods, and the remaining fraction of VSV was determined as described above* (▲).

TABLE 3. *Interference of MuLV$_E$-producing cells with M-MuLV*

(A) Superinfection with M-MuLV and determination of M-MuLV yield

Cells	PFU/ml after 5 days
NIH	4×10^6
NIH(IN-MuLV)	4×10^3

(B) Superinfection with VSV*tl*17(M-MuLV)

Cells	PFU/ml after given antibody treatment		Ratio none/anti-M-MuLV
	None	Anti-M-MuLV	
NIH	1.8×10^5	1.4×10^2	1.3×10^3
NIH(N-C1-35-MuLV)	8×10^1	9×10^1	0.9
NIH(IN-MuLV)	1.1×10^3	6×10^2	1.8
JLS-V9	1.4×10^5	3×10^1	4.7×10^3
JLS-V9(M-MuLV)	5×10^2	1.2×10^2	4.2
JLS-V9(B-C1-11-MuLV)	4×10^2	3×10^2	1.3

VSV(M-MuLV$_E$) could infect normal mouse cells as previously reported (18, 30, 46) but could not infect the cells previously infected with $MuLV_E$ (Table 3). Therefore, the $MuLV_E$ strains tested all cross-interfere at the cell surface, suggesting that they use the same receptor-penetration mechanism.

Interference between a xenotropic virus and an ecotropic virus. Although $MuLV_X$ does not infect mouse cells, it might be able to block the surface receptor used by $MuLV_E$. To test such a possibility, a mouse cell line producing a

high titer of $MuLV_X$ was infected with a $MuLV_E$. The cells used were JLS-V9 cells induced with IUdR. Although untreated JLS-V9 cells produced no detectable $MuLV_X$, the induced cells produced 10^4 to 3×10^5 infectious particles per ml of $MuLV_X$ as determined by end-point dilutions on rabbit (SIRC) and rat (NRK) cells or by focus assay on cat S^+L^- cells (Table 4). By supernatant DNA polymerase assay, IUdR-induced cells produced almost as much virus as the best $MuLV_E$-producing cell lines (6). The induced cells also produced a low amount of N-tropic $MuLV_E$ detected by end-point dilution on NIH/3T3 cells (Table 4), but no detectable B-tropic virus (6). Induced and uninduced JLS-V9 cells were infected with M-$MuLV_E$ at a multiplicity of infection of 0.5, and the M-$MuLV_E$ yield was determined by XC assay 40 h after infection. The induced cells produced 2.4-fold less M-$MuLV_E$ than did the uninduced cells (Table 5). This should be compared with the 1,000-fold difference produced by preinfection of cells with $MuLV_E$ (Table 3A) and suggests that $MuLV_X$ interferes, at most, slightly with $MuLV_E$. The lack of interference between $MuLV_X$ and $MuLV_E$ was confirmed by titering VSV(M-$MuLV_E$) on uninduced and BUdR-induced JLS-V9 cells; again the induced cells gave only a slightly lower titer of VSV (Table 5). The twofold difference between induced and uninduced cells seen in these assays could have been a result of the small amount of IN-$MuLV_E$ produced by the induced cells or could have been a result of toxicity. Whatever the explanation, $MuLV_X$ does not appear to bind effectively to the receptor that allows $MuLV_E$ to infect murine cells.

TABLE 4. *Host range of the induced viruses from JLS-V9 cells*[a]

Cells	Infectious units/ml	FFUs/ml
NIH/3T3	2×10^1	–
NRK	1×10^4	–
SIRC	1×10^5	–
Cat S^+L^-	–	3×10^5

[a] Infectious units were determined by end-point dilutions on the indicated lines and DNA polymerase assays of the supernatant fluids from the infected cultures (6). FFU were determined as by Fischinger et al. (12).

TABLE 5. *Lack of interference of $MuLV_X$-producing cells with M-$MuLV_E$*

Cells	Yield or titer (PFU/ml) after superinfection with:[a]	
	M-MuLV	VSV(M-MuLV) pseudotypes
JLS-V9	8.6×10^6	3.5×10^5
IUdR-treated JLS-V9	3.5×10^6	1.4×10^5

[a] For M-MuLV, yield was determined 40 h after infection of the indicated cells; for VSV(M-MuLV), the indicated cells were used to titer a pseudotype preparation.

Preparation and characterization of VSV pseudotypes with a $MuLV_X$ coat. To more directly analyze the ability of $MuLV_X$ to penetrate different cells, VSV($MuLV_X$) was produced. Two classes of $MuLV_X$ have been found and classified (3, 7, 38). They differ with respect to their nucleic acid sequences and the immunological type specificity in their structural protein p12. The $MuLV_X$ from NZB mice described by Levy (23) and Levy and Pincus (26) is referred to here as $MuLV_{Xb}$, and the one isolated from BALB/c mice is referred to as $MuLV_{Xa}$. We have often used them interchangeably because their interference patterns appear to be identical (16).

To investigate whether VSV($MuLV_{Xb}$) was actually produced upon superinfection of 15176 cells with VSV, the yield of VSV from these cells was neutralized with various dilutions of anti-VSV antiserum and then assayed on rabbit cells permissive for $MuLV_X$ (5). A residual titer of about 10^{-4} could not be neutralized by excess anti-VSV serum (Fig. 2). Addition of an antiserum made against $MuLV_X$, however, reduced the residual VSV titer from 10^{-4} to less than 10^{-6}, indicating that more than 100 PFU of VSV($MuLV_{Xb}$) were present. Another way to demonstrate the VSV($MuLV_X$) was to titrate this VSV($MuLV_X$) on either SIRC cells or SIRC cells preinfected with $MuLV_X$ after treatment with either anti-VSV serum or anti-VSV serum plus anti-$MuLV_X$ serum. When the virus was assayed on SIRC cells, the anti-$MuLV_X$ antiserum inactivated at least 59-fold more virus than the VSV antiserum alone (Table 6). On SIRC cells producing $MuLV_X$, no reduction could be detected. VSV(VSV) gave a fivefold higher titer on the $MuLV_X$-infected cells. VSV($MuLV_X$) is therefore detectable in two ways: as virus that can be interfered with by $MuLV_X$, and as virus that can be neutralized by anti-$MuLV_X$ antiserum.

To determine whether $MuLV_X$ can penetrate mouse cells, VSV($MuLV_{Xb}$) was titrated on JLS-V9 mouse cells. On the mouse cells, the anti-$MuLV_X$ antiserum inactivated only twofold more virus than the VSV antiserum alone (Table 6). It appears, therefore, that $MuLV_{Xb}$ is restricted at the cell surface in mouse cells.

VSV($MuLV_{Xa}$) was made by superinfecting SIRC cells producing $MuLV_{Xa}$ with VSV. With this preparation, a set of experiments similar to those described above for VSV($MuLV_{Xb}$) was

performed using mink cells as permissive cells and NIH/3T3 mouse cells (Table 7). The titer of VSV($MuLV_{Xa}$) was 350-fold above the background of the doubly neutralized sample on the permissive mink cells. Mouse cells showed a fivefold lower titer after double neutralization than after anti-VSV treatment alone. The receptor concentration for $MuLV_{Xa}$ on mink cells and for $MuLV_{Xb}$ on SIRC cells was much higher than on mouse cells, but some receptors on mouse cells appeared to exist (Table 7). We do not know whether these apparent receptors on mouse cells were due to a contaminating pseudotype, low levels of $MuLV_X$ receptors on mouse cells, or an experimental artifact.

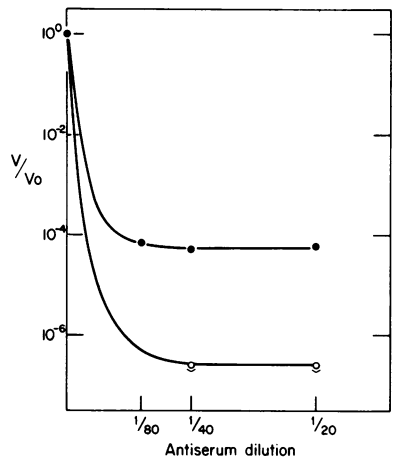

FIG. 2. *Demonstration of VSV($MuLV_X$) on rabbit cells. Human 15176 cells producing $MuLV_{Xb}$ were superinfected with VSV at a multiplicity of infection of 2 to 4. The virus was adsorbed for 45 min at 37°C (1 ml/10-cm dish), and then 9 ml of medium was added. The virus yield was harvested after 14 h of incubation at 37°C. Aliquots were neutralized with different concentrations of anti-VSV antiserum as described in Materials and Methods. The remaining fraction of VSV was determined in a VSV plaque assay on SIRC cells as indicator cells at 37°C (●). Some samples were neutralized with anti-VSV antiserum as indicated and with anti-$MuLV_X$ antiserum at a concentration of 1/40, and the remaining fraction of VSV was determined as above* (○).

Restriction of VSV($MuLV_E$) on xenogeneic cells. To determine whether xenogeneic cells restrict growth of $MuLV_E$ at the level of penetration or as an intracellular event, rat, mink, rabbit, and mouse cells were infected with VSV(M-$MuLV_E$) (Table 8). The VSV(M-$MuLV_E$) pseudotype was made by using VSV *tl*17 and heat inactivation of VSV(VSV) (Fig. 1). The titer of VSV(M-$MuLV_E$) neutralized with antiserum made against M-$MuLV_E$ was reduced by 10^4 for NIH/3T3 cells, 10^2 for NRK cells, 2.2 for mink cells, and 1.1 for rabbit cells. It thus appears that the pseudotypes were severely restricted at the level of penetration in mink and rabbit cells. The reduced susceptibility of NRK cells to VSV(M-$MuLV_E$) appeared to be concordant with the observed reduced susceptibility to M-MuLV (28; E. Rothenberg, unpublished observations).

Phenotypic mixing between $MuLV_E$ and $MuLV_X$. To further characterize the penetration block of mouse cells and xenogeneic cells toward $MuLV_X$ and $MuLV_E$, respectively, we made pseudotypes between $MuLV_E$ and $MuLV_X$. Phenotypic mixing has been observed to occur very readily between different subgroups of the avian leukosis viruses (41). Similarly, phenotypic mixing might be expected if cells were producing $MuLV_E$ and $MuLV_X$ simultaneously. JLS-V9 cells, upon induction with halogenated pyrimidines, produced 3 ×

TABLE 6. *Host restriction of VSV($MuLV_{Xb}$) in homogeneic and xenogeneic cells*

Indicator cells	PFU/ml after given antibody treatment			Ratio anti-VSV/(anti-VSV and anti-$MuLV_X$)
	None	Anti-VSV	Anti-VSV and anti-$MuLV_X$	
SIRC producing $MuLV_{Xa}$	20×10^7	40	40	1
SIRC	4×10^7	590	≤10	59
JLS-V9	20×10^7	130	80	1.6

TABLE 7. *Host restriction of VSV($MuLV_{Xa}$) in mouse cells*

Indicator cells	PFU/ml after given antibody treatment			Ratio anti-VSV/(anti-VSV and anti-$MuLV_X$)
	None	Anti-VSV	Anti-VSV and anti-$MuLV_X$	
Mink (CCL64)	2.3×10^8	7×10^3	20	350
Mouse (NIH)	7.2×10^7	110	20	5.5

TABLE 8. *Host restriction of VSVtl17(M-MuLV$_E$) in xenogeneic and homogeneic cells*

Indicator cells	PFU/ml after given antibody treatment		Ratio none/anti-M-MuLV$_E$
	None	anti-M-MuLV$_E$	
Mouse (NIH)	1.5×10^5	10	1.5×10^4
Rat (NRK)	1.1×10^3	10	1.1×10^2
Mink (CCL64)	5.3×10^2	2.4×10^2	2.2
Rabbit (SIRC)	3.4×10^2	2.6×10^2	1.3

10^5 PFU/ml of MuLV$_X$ (Table 4). JLS-V11 cells are JLS-V9 cells chronically infected with M-MuLV$_E$ (45). They are producing 10^6 PFU/ml of MuLV$_E$. Upon induction with BUdR, these JLS-V11 cells produced 10^6 PFU/ml of MuLV$_E$ and 3×10^5 PFU/ml of MuLV$_X$. BUdR-induced JLS-V11 cells therefore produce similar titers of MuLV$_E$ and MuLV$_X$, a situation in which phenotypic mixing is expected. It appears that induction of MuLV$_X$ by BUdR in JLS-V11 cells does not affect their production of M-MuLV$_E$.

MuLV$_E$(MuLV$_X$) pseudotypes were sought in the following way. SIRC cells and SIRC cells already producing MuLV$_X$ were infected with the yield from BUdR-treated JLS-V11 cells containing the presumptive MuLV$_E$(MuLV$_X$) pseudotypes. Both types of cells were also infected with pure MuLV$_E$ as a control. Five days after the infections the number of infective centers producing MuLV$_E$ was determined, and 12 days after infection the yield of MuLV$_E$ produced by these infected SIRC cell cultures was determined (Table 9). The SIRC cells infected with the presumptive MuLV$_E$(MuLV$_X$) produced a titer of 10^4 PFU/ml of MuLV$_E$ as assayed on mouse cells, and more than 50% of the cells were scored as infective centers for MuLV$_E$. SIRC cells producing MuLV$_X$ could not be infected by the MuLV$_E$(MuLV$_X$), nor did pure MuLV$_E$ infect the rabbit cells. Experiments carried out with mink cells gave essentially the same result.

Thus the MuLV$_E$(MuLV$_X$) appears to exist and to allow the MuLV$_E$ genome to be established in xenogeneic cells. Cells infected by the pseudotype produce virus that grows on mouse cells and therefore has the phenotype of an MuLV$_E$. Xenogeneic cells that are already producing MuLV$_X$ interfere with the superinfection of MuLV$_E$(MuLV$_X$). MuLV$_E$ provided with an MuLV$_X$ envelope can thus pass the penetration block otherwise exerted towards MuLV$_E$-(MuLV$_E$). An interesting observation was made in the course of these experiments. It was found that MuLV$_E$ growing in xenogeneic cells causes syncytia formation with XC cells. MuLV$_X$ can therefore easily be distinguished from MuLV$_E$ because it is negative in an XC cell assay. We thus could measure MuLV$_E$(MuLV$_X$) directly in a conventional XC assay on rabbit and mink cells, since the MuLV$_E$ genome can only get into xenogeneic cells if it is provided with an MuLV$_X$ envelope as described above. On rabbit cells as well as on mink cells, a titer of approximately 10^4 XC PFU was observed. These results again showed that phenotypic mixing is a very efficient process (Table 10).

MuLV$_X$(MuLV$_E$) pseudotypes were then sought in the following way. NIH/3T3 cells, SIM.R cells, and NIH/3T3 cells producing M-MuLV$_E$ were infected with the yield from

TABLE 9. *Infection of rabbit cells with MuLV$_E$(MuLV$_X$) pseudotypes*

Cells	Virus used for infection	XC assay on NIH/3T3 cells	
		Infective centers (%)	PFU/ml
SIRC	MuLV$_E$(MuLV$_X$)[a]	>50	2×10^4
SIRC producing MuLV$_X$	MuLV$_E$(MuLV$_X$)	–	<10
SIRC	MuLV$_E$ (pure)	–	<10

[a] Presumptive phenotypic mixture containing MuLV$_E$(MuLV$_X$), MuLV$_X$(MuLV$_E$), MuLV$_E$, and MuLV$_X$.

TABLE 10. *XC assay of MuLV$_E$(MuLV$_X$) on rabbit and mink cells*[a]

Assay cells (result scored)	Virus detected	Prepn used to infect	
		Pure M-MuLV	Virus from BUdR-induced JLS-V11 cells
Cat S$^+$L$^-$ (FFU/ml)	MuLV$_X$	0	3×10^5
Rabbit (XC plaques/ml)	MuLV$_E$(MuLV$_X$)	0	1.5×10^4
Mink (XC plaques/ml)	MuLV$_E$(MuLV$_X$)	0	2×10^4
NIH/3T3 (XC plaques/ml)	MuLV$_E$	4×10^6	3×10^6

[a] For XC assays on SIRC indicator cells, 10^5 cells/5-cm dish were plated. The following day the cultures were infected with virus in 8 μg of polybrene per ml. On day 2 after infection the medium was changed, and on day 4 the cultures were UV irradiated and overlaid with 2×10^6 cells. For XC assays on mink cells, 5×10^4 cells/5-cm dish were plated. The following day the cultures were infected with virus in medium containing 8 μg of polybrene per ml. On day 2 after infection the medium was changed, and on day 3 or 4 the cultures were UV irradiated and overlaid with 2×10^6 cells/5-cm dish. For the SIRC as well as the mink XC assay, the cultures were fixed and stained on day 7 after infection.

BUdR-induced JLS-V11 cells and with $MuLV_E(MuLV_E)$ as control. After 5 days, the titers of $MuLV_X$ and $MuLV_E$ were determined (Table 11). All cultures produced similar amounts of M-MuLV as determined by XC assay on NIH/3T3 cells. NIH/3T3 cells infected with the presumptive $MuLV_X(MuLV_E)$ pseudotypes produced 3×10^3 FFU/ml of virus having an $MuLV_X$ phenotype as determined by the cat S^+L^- focus assay. NIH/3T3 cells infected with M-$MuLV_E$ did not produce any $MuLV_X$. NIH/3T3 cells already producing M-$MuLV_E$ did not produce detectable $MuLV_X$ after superinfection with the putative $MuLV_X(MuLV_E)$. $MuLV_X$ provided with an $MuLV_E$ envelope can thus pass the penetration block otherwise exerted towards $MuLV_X$ in mouse cells. Subsequently, these cells will produce virus that has the phenotype of $MuLV_X$.

The amounts of $MuLV_X$ produced by NIH/3T3 cells and SIM.R cells infected with $MuLV_X$(M-$MuLV_E$) (M-$MuLV_E$ being an NB-tropic M-MuLV) were not significantly different. NIH/3T3 cells and SIM.R cells differ with respect to their Fv-1 locus (29, 30): NIH/3T3 cells have the $Fv\text{-}1^n$ allele and SIM.R cells have the $Fv\text{-}1^b$ allele (42). It therefore appears that the Fv-1 locus does not affect the establishment of an infection by $MuLV_X$ in mouse cells if these cells were infected by a phenotypic mixture of an NB-tropic $MuLV_E$ and $MuLV_X$.

DISCUSSION

These results as well as extensive previous work from a number of laboratories delineate two distinct classes of retroviruses of inbred mice. One class is the ecotropic viruses, those that can infect mouse cells. The other class is the xenotropic viruses, those that can infect cells of other species (2, 5, 23–26). Both classes of viruses are considered to be murine viruses because they can be recovered from uninfected mice and their DNA appears to be inherited as part of the genome of murine cells. They all have murine group-specific antigenic determinants (39).

The major difference between the ecotropic and xenotropic murine viruses from our results, as well as those of Levy (23) and Fischinger et al. (13), appears to be the type of cell surface receptor used by the virus in the initial steps of infection. The mouse fibroblast cells we have tested have receptors available for the ecotropic viruses but few if any for xenotropic viruses; cells of rabbit, mink, and other species have receptors for xenotropic virus but few if any receptors for ecotropic viruses. Our evidence for receptors on a given cell comes mainly from studies using VSV pseudotypes with an appropriate MuLV-derived coat. $VSV(MuLV_E)$ infects mouse cells but not cells of other species (18, 46). $VSV(MuLV_X)$ infects cells of rabbit and mink but infects poorly, if at all, cells from mice.

Murine ecotropic viruses interfere with each other at the cell surface, presumably by competition for a limited number of surface receptor sites (32, 36, 37). Viruses that bind to the receptors, even if they cannot infect mouse cells, would be expected to interfere with $MuLV_E$. Therefore, the ability of $MuLV_X$-producing mouse cells, such as the IUdR-induced JLS-V9 cells, to be infected by an $MuLV_E$ is evidence that the $MuLV_E$ receptor cannot even bind $MuLV_X$ and that $MuLV_X$ receptors must be distinctly different from $MuLV_E$ receptors. Even if some of the cells in the $MuLV_X$-producing cultures were not producing virus, the large amount of infectious $MuLV_X$ in the culture medium should have blocked all the available receptors if the $MuLV_E$ receptor could bind $MuLV_X$.

The phenotypic mixing experiments described here show that $MuLV_E$ provided with an $MuLV_X$ envelope will penetrate and establish an infection in xenogeneic cells and that $MuLV_X$ can infect mouse cells if provided with a $MuLV_E$ envelope. These pseudotypes penetrate the cells, using the receptors corresponding to the envelope of the virus particle, as demonstrated by interference experiments. The $MuLV_X$ genome does not appear to be sensitive to the intracellular restriction exerted by the products of either the $Fv\text{-}1^n$ or $Fv\text{-}1^b$ alleles. Results in agreement with ours about phenotypic mixing between $MuLV_E$ and $MuLV_X$ were obtained independently by A. Ishimoto and W. P. Rowe (personal communication).

The penetration block exerted by mouse cells towards $MuLV_X$ could either be due to the lack of $MuLV_X$-specific receptors on mouse cells or possibly to the production of $MuLV_X$ glycoprotein by mouse cells that then could block

TABLE 11. *Infection of mouse cells with $MuLV_X(MuLV_E)$ pseudotypes*

Cells	Virus used for infection	$MuLV_X$ titer (FFU/ml)	$MuLV_E$ titer (PFU/ml)
NIH/3T3	$MuLV_X(MuLV_E)$[a]	3×10^3	3×10^5
SIM.R	$MuLV_X(MuLV_E)$[a]	4×10^3	9×10^4
NIH/3T3 producing M-$MuLV_E$	$MuLV_X(MuLV_E)$[a]	3	7×10^5
NIH/3T3	M-$MuLV_E$ (pure)	0	3×10^5
SIM.R	M-$MuLV_E$ (pure)	0	10^5

[a] Presumptive phenotypic mixture containing $MuLV_X$-($MuLV_E$), $MuLV_E(MuLV_X)$, $MuLV_E$, and $MuLV_X$.

$MuLV_X$-specific receptors in a fashion reminiscent of the restriction of the subgroup E avian leukosis viruses on chf^+ cells (43). Small amounts of $MuLV_X$ glycoprotein have been found on the mouse fibroblasts from the strains used in these experiments (17). It is not clear whether the amount of glycoprotein formed by these cells could be sufficient to cause significant interference. The experiments with VSV pseudotypes suggest that mouse cells may have a small number of $MuLV_X$ receptors, and one mouse cell line, derived from a wild mouse, has receptors for $MuLV_X$ (15). The possibility that most or all mouse cells do have $MuLV_X$ receptors but that they are usually blocked by synthesis of endogenous $MuLV_X$ glycoprotein must therefore be seriously considered.

The observation by Scolnick and Parks (33) and by Gazdar et al. (14) that $MuLV_X$ is restricted in a dominant fashion in mouse fibroblasts would agree well with such an explanation.

A different approach to determine $MuLV_E$ receptors on cell surfaces was reported recently by De Larco and Todaro (8), who measured binding of radiolabeled glycoprotein of ecotropic viruses to various cells. Their results and ours using VSV pseudotypes are in agreement.

There have been reports that mouse viruses can be grown on human cells (1, 4, 44). In view of the results presented here, those reports are best explained by phenotypic mixing of $MuLV_X$ with $MuLV_E$ in the virus stocks that were used. The $MuLV_X$ envelope thus can serve as a vehicle for either the MSV or the $MuLV_E$ genome in interspecies viral transfer.

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ADDENDUM IN PROOF

In a recent publication Hartley and Rowe also describe the lack of interference between $MuLV_E$ and $MuLV_X$ (J. W. Hartley and W. P. Rowe, J. Virol. 19:19-25, 1976).

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