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# NOTES

## Capillary Endothelial Cell Tropism of PVC-211 Murine Leukemia Virus and Its Application for Gene Transduction

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**PVC-211 murine leukemia virus (MuLV) causes neurodegenerative disease following inoculation of neonatal, but not adult, mice and rats. It was previously shown that tropism for brain capillary endothelial cells (CEC) was a determinant of the viral neuropathogenicity. In this study, we demonstrate that host age-dependent replication of PVC-211 MuLV in vivo occurs in CEC in the brain as well as in other organs, such as the liver, kidney, and heart. In contrast, primary explant cultures of CEC derived from brains and livers of adult and neonatal rats could be infected by PVC-211 MuLV, suggesting that the age-dependent susceptibility was abrogated in vitro. Although CEC were generally less susceptible to MuLV-mediated gene transduction than fibroblasts, treatment of CEC with 2-deoxyglucose followed by inoculation of a PVC-211 MuLV-pseudotyped vector in the absence of heparin improved the transduction efficiency. These observations support the possibility that PVC-211 MuLV may be useful for establishing models of CEC gene transduction.**

PVC-211 murine leukemia virus (MuLV) is a neuropathogenic variant of Friend MuLV (F-MuLV) which causes rapidly progressive neurodegenerative disease when injected into neonatal, but not adult, rats and mice (7, 10). Despite the presence of dramatic destructive lesions in the central nervous system (CNS), productive virus infection cannot be demonstrated in either neurons or astroglia. Rather, the primary target for PVC-211 MuLV infection was determined to be brain capillary endothelial cells (CEC) (7). In vitro studies demonstrated that PVC-211 MuLV, but not the nonneuropathogenic parent F-MuLV, infected cultured rat brain CEC efficiently and that the brain CEC tropism of PVC-211 MuLV correlated with its neuropathogenicity (13). Therefore, PVC-211 MuLV-infected CEC appear to play an essential role in induction of neurological disease.

In this study, we provide additional support for the role of virus-infected CEC in neuropathogenicity. Our results indicate that PVC-211 MuLV, unlike F-MuLV, replicates efficiently in brain CEC after neonatal inoculation. This replication in brain CEC is necessary for viral neuropathogenicity and is age dependent. PVC-211 MuLV, but not F-MuLV, also infected CEC of other organs following neonatal, but not adult, inoculation. The age-dependent restriction of PVC-211 MuLV replication in CEC observed in vivo was abrogated when the cells were put into culture and inoculated with the virus in vitro.

To assess the utility of PVC-211 MuLV as a tool for transduction of genes into CEC, we compared the abilities of PVC-211 MuLV and other MuLVs to transduce a retroviral vector into cultured CEC under various conditions. Although CEC were more refractory to transduction of the retroviral vector than fibroblasts, PVC-211 MuLV was more efficient at transducing CEC than other ecotropic MuLVs. Prior treatment of cultured CEC with 2-deoxyglucose (2-DG), followed by inoculation of virus in a heparin-deficient medium, increased the transduction efficiency of PVC-211 MuLV as well as other MuLVs. The efficiency with which PVC-211 MuLV infects CEC supports the possibility that this MuLV system could serve as a useful tool for establishing animal models for CEC-mediated gene therapy.

**PVC-211 MuLV replication in brain CEC in vivo is controlled in a host age-dependent manner and correlates with neurological-disease manifestations.** To analyze the effects of host age on PVC-211 MuLV replication in vivo and neurological-disease manifestations, the virus was inoculated into 2-, 10-, and 21-day-old F344 rats. Three weeks after PVC-211 MuLV inoculation, animals were sacrificed and examined for virus replication in the brain and spleen, as well as for histopathological changes in the CNS. When rats were inoculated with the virus at 2 days of age, virus titers in the brain and spleen homogenates were high (Table 1), and a large proportion of brain CEC were positive for expression of the viral envelope surface (SU) protein (Table 1 and Fig. 1A and B). Reactive gliosis was detected in the brain and spinal cord by immunohistological analysis with the anti-GFAP antibody. Examination of formalin-fixed sections stained with hematoxylin and eosin confirmed the presence of spongiform neurodegeneration in animals demonstrating reactive gliosis (data not shown). Most of the animals inoculated with PVC-211 MuLV at 2 days of age exhibited abnormal neurological signs, such as tremor and hind limb weakness, by 3 weeks postinoculation (7, 10). When 10-day-old rats were inoculated with PVC-211 MuLV, the virus titers in the brain and spleen homogenates

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TABLE 1. Replication and neuropathogenicity of PVC-211 MuLV in F344 rats inoculated at different ages[a]

| Virus | Age of animal (days) at time of inoculation | Gliosis[b] | Virus titer[c] ($10^5$ PFU/ml) in: | | % SU-positive brain CEC[d] |
|---|---|---|---|---|---|
| | | | Brain | Spleen | |
| PVC-211 MuLV | 2 | + | 1.0 ± 0.3 | 15.6 ± 7.2 | 45–50 |
| | 10 | − | 0.012 ± 0.016 | 1.4 ± 1.0 | 0–1 |
| | 21 | − | <0.005 | <0.005 | 0 |
| F-MuLV-57 | 2 | − | <0.005 | 10.7 ± 4.1 | 0–5 |

[a] Two-day-old F344 rats (Harlan Sprague-Dawley, Indianapolis, Ind.) were inoculated intracerebrally with 0.03 ml of culture supernatant from PVC-211 MuLV clone 3d- (15) or F-MuLV-57 (19)-producing NIH 3T3 cells containing approximately $10^3$ PFU of virus. Ten- and 21-day-old rats were inoculated with the same amount of virus retro-orbitally since the cranium was already ossified. Three weeks after virus inoculation, anesthetized rats were killed and perfused with heparinized phosphate-buffered saline and tissue specimens were obtained for further analysis.

[b] The presence (+) or absence (−) of reactive gliosis was determined by the appearance of GFAP-expressing cells in the brain stem as determined by immunofluorescence microscopy, as previously described (7).

[c] Brains and spleens were obtained from the rats 3 weeks after virus inoculation and homogenized in Dulbecco's modified Eagle medium to prepare 10% (wt/vol) homogenates. The virus titers of cleared homogenates were measured by the XC cell fusion assay (24). The numbers are averages ± standard deviations of the values obtained for three animals.

[d] Expression of the viral SU protein was detected by indirect immunofluorescence microscopy with a goat anti-SU envelope protein antibody and a fluorescein isothiocyanate-conjugated donkey anti-goat immunoglobulin G, as previously described (7). CEC were identified by *Ricinus communis* agglutinin 1 (Rca-1) binding and factor VIII expression, as previously described. The numbers indicate the range of percentages of viral SU protein-positive cells among Rca-1 binding cells observed for three animals. For each animal, 300 Rca-1 binding cells were examined for SU protein expression.

were decreased by about 100- and 10-fold, respectively, compared with those obtained after neonatal inoculation. The proportion of brain CEC infected with virus was also markedly decreased, and there was no indication of reactive gliosis or other pathological changes in the CNS. When 21-day-old rats were inoculated with PVC-211 MuLV, no significant virus replication was detected in either the brain or the spleen, and neither SU envelope protein-positive brain CEC nor astrogliosis were detected. None of the rats inoculated with the virus at 10 or 21 days of age showed abnormal neurological signs. The control F-MuLV clone 57 (F-MuLV-57) replicated efficiently in the spleen following neonatal inoculation. However, the titer of F-MuLV-57 in the brain homogenate was at least 200-fold lower than that of the corresponding PVC-211 MuLV-infected sample (Table 1). Only a small proportion of brain CEC expressed SU envelope protein after neonatal inoculation of F-MuLV-57, and no evidence of astrogliosis or other neuropathological changes were observed.

**PVC-211 MuLV infects rat CEC of various organs following neonatal inoculation.** To further analyze PVC-211 MuLV replication in vivo, F344 rats inoculated with PVC-211 MuLV at 2 days of age were sacrificed 3 weeks postinoculation. The brain, spleen, heart, kidneys, and liver were examined for evidence of virus replication. Virus-infected cells were located in frozen sections, taken from infected organs, by indirect immunofluorescence using an anti-SU protein antibody. Endothelial cells were identified by their reactivity with anti-factor VIII antibody. Confocal microscopy demonstrated that CEC expressing factor VIII were the only SU protein-expressing cells observed in the brain (Fig. 1A) and kidney (Fig. 1C), while other cells, such as epithelial cells in the kidney, did not express SU protein. However, in the liver (Fig. 1D), a significant number of SU protein-expressing cells did not demonstrate factor VIII staining and were morphologically identified as hepatocytes. Unlike the case of the PVC-211 MuLV-infected CNS, histopathology was not observed in these other organs in which virus-infected CEC were detected. Neonatal inoculation with F-MuLV-57 resulted in SU protein expression in splenic lymphoid cells but not in CEC from the kidney or liver (data not shown).

**PVC-211 MuLV, but not F-MuLV-57, can infect cultured rat CEC derived from the brain and liver.** To test the susceptibility of CEC to PVC-211 MuLV or F-MuLV-57 infection in vitro, we prepared primary cultures of CEC from the brains and livers of F344 rats. Cultures were inoculated with PVC-211 MuLV or F-MuLV-57 after two to six in vitro passages. Hybridization analysis of integrated proviral DNA showed that PVC-211 MuLV and F-MuLV-57 replicate equally well in Rat-1 fibroblasts (Fig. 2A, lanes 2 and 3). In primary brain CEC from 2-day-old rats, however, PVC-211 MuLV showed a much higher level of proviral integration than F-MuLV-57 (Fig. 2B, lanes 2 and 3). Although PVC-211 MuLV replication was restricted in 21-day-old rats, cultured brain CEC prepared from 21-day-old rats were as susceptible to PVC-211 MuLV infection as the cells from 2-day-old animals (Fig. 2C, lane 2). Brain CEC from 21-day-old rats were highly resistant to F-MuLV-57 infection, and the signal for proviral integration was undetectable (Fig. 2C, lane 3). We also prepared a primary culture of liver CEC from 21-day-old rats and inoculated equivalent portions with PVC-211 MuLV or F-MuLV-57. The results indicated that PVC-211 MuLV infected primary liver CEC efficiently but F-MuLV-57 did not (Fig. 2D, lanes 2 and 3).

**Differential susceptibility of the CEC cell line RTEC-6 to different MuLVs.** To further analyze the susceptibility of cultured CEC to different MuLVs, we prepared neomycin resistance gene-bearing N2 vectors (4) pseudotyped with PVC-211 or F-MuLV-57 and tested their transduction efficiencies on the rat CEC line RTEC-6 as previously described (12) (Table 2). Both of the viruses transduced Rat-1 fibroblasts efficiently, and their levels of transduction were comparable. RTEC-6 cells were less susceptible to vector transduction than fibroblasts, but PVC-211 MuLV was almost 300-fold more efficient at transducing RTEC-6 cells than was F-MuLV-57. Other ecotropic MuLVs, such as the TB strain and the *ts*1 mutant of Moloney MuLV (31), Cas-Br-E MuLV (9), and Fr-Cas$^E$ MuLV (20), also failed to efficiently transduce RTEC-6 cells (data not shown). Amphotropic MuLV 4010A (2) was as effective as PVC-211 MuLV at transducing RTEC-6 cells (Table 2), but it was unable to transduce primary CEC efficiently (see below).

**Susceptibility of cultured CEC to MuLV-mediated gene transduction that was increased by 2-DG treatment of the cells and vector inoculation in the absence of heparin did not elevate expression of the viral receptor gene.** Although PVC-211 MuLV was the most efficient of the MuLVs tested at transducing CEC, its transduction efficiency was 3 logs lower on RTEC-6 cells than it was on rat fibroblasts. In order to determine if it was possible to render CEC more susceptible to MuLV-mediated gene transduction, we inoculated RTEC-6 cells with the vector under various conditions. It has previously been shown that hamster cell lines and *Mus dunni* tail fibroblasts, which are normally resistant to ecotropic MuLV infection, can be rendered susceptible by treatment with the glycosylation inhibitor tunicamycin (5, 18, 30). To investigate whether similar treatment could increase the susceptibility of CEC to ecotropic MuLVs, we treated RTEC-6 cells with various concentrations of tunicamycin and determined their susceptibility to gene transduction by PVC-211 MuLV and F-

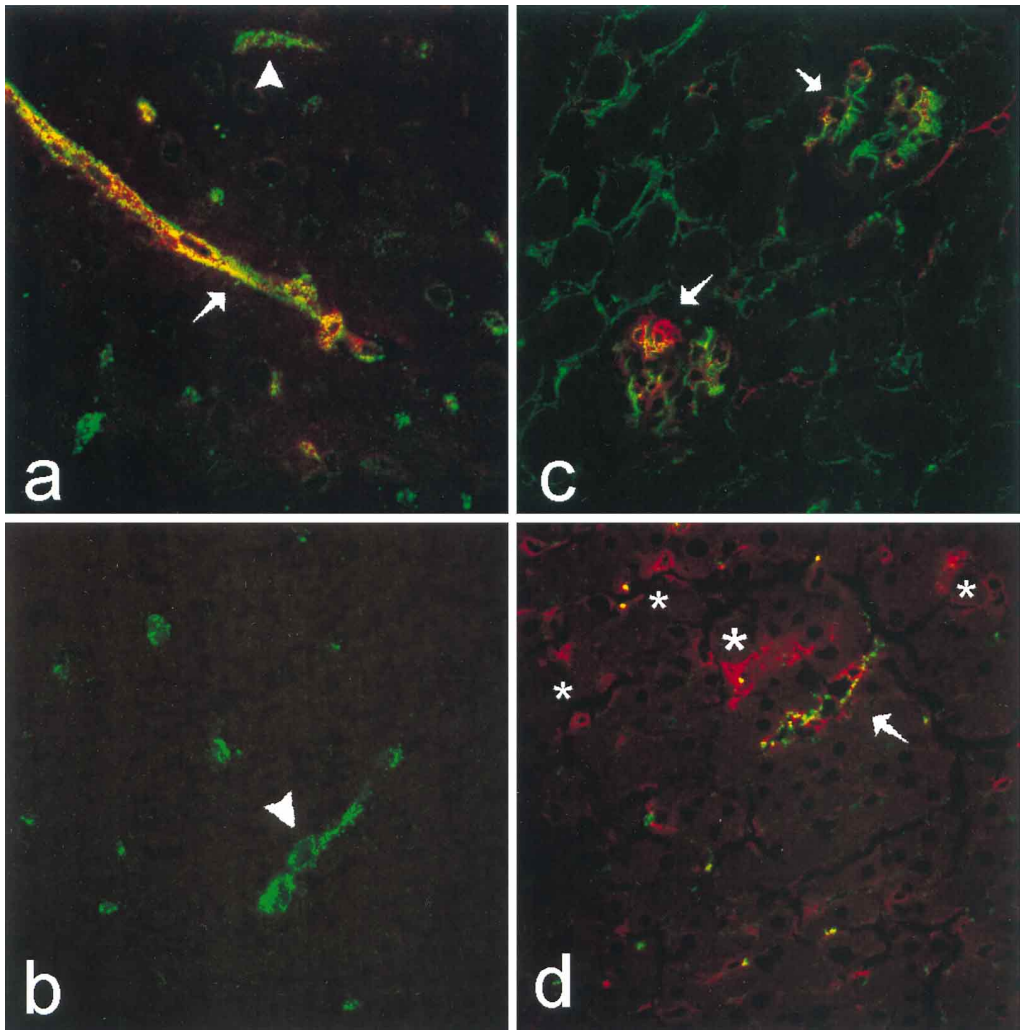


FIG. 1. CEC infection after neonatal inoculation of PVC-211 MuLV. Two-day-old F344 rats were inoculated intracerebrally with 0.03 ml of culture supernatant from PVC-211 MuLV-producing NIH 3T3 cells containing approximately $10^3$ PFU of virus. Three weeks after virus inoculation, anesthetized rats were killed and perfused with heparinized phosphate-buffered saline. Fresh frozen tissue sections (5 μm thick) of the brain (A), kidney (C), and liver (D) of PVC-211 MuLV-infected rats and from the brain of a 21-day-old uninfected F344 rat (B) were acetone fixed for immunofluorescent staining and viewed under a Zeiss 410 laser scanning confocal microscope. Viral SU protein expression (red) was detected by addition of a goat anti-SU protein antibody followed by a rhodamine-conjugated donkey anti-goat immunoglobulin G (IgG) antibody as previously described (7). CEC (green) were identified by addition of a rabbit anti-human factor VIII antibody (Dako Corp) followed by a fluorescein-conjugated anti-rabbit IgG antibody (Jackson ImmunoResearch). Arrows indicate colocalization of factor VIII and SU envelope protein expression (A, C, and D). Arrowheads indicate uninfected CEC (A and B). Asterisks indicate SU protein expression in cells not expressing factor VIII in the liver (D). Shown are representative fields from two animals. Magnification, ×300.

MuLV-57. When the cells were treated with tunicamycin at a concentration of 0.5 or 1 μg/ml, the transduction efficiency of F-MuLV-57 was increased by sixfold while that of PVC-211 MuLV was not significantly affected (Table 2). At concentrations higher than 1 μg/ml, tunicamycin severely affected the viability of RTEC-6 cells. We also tested the effectiveness of adding the metabolic inhibitor 2-DG on the susceptibility of RTEC-6 cells to MuLV-mediated gene transduction. At the optimal concentration of 2.5 to 5 mM, 2-DG was considerably more efficient than tunicamycin at increasing the susceptibility of RTEC-6 cells to PVC-211 and F-MuLV-57, increasing the transduction efficiency of these viruses by 5- and 30-fold, respectively (Table 2).

Since the medium used to grow RTEC-6 cells, unlike that used to grow fibroblast lines, contains heparin to activate heparin-binding growth factors essential for CEC growth (6), we examined the effects of heparin by inoculating the N2 vector pseudotyped with PVC-211 MuLV or F-MuLV-57 onto RTEC-6 cells in medium with or without heparin. As shown in Table 2, inoculation of the vector in medium without heparin greatly increased the transduction efficiencies of both viruses (40-fold for PVC-211 MuLV and 100-fold for F-MuLV-57). Treatment of RTEC-6 cells with 2-DG followed by inoculation of the vector in medium without heparin dramatically increased the transduction efficiencies of F-MuLV-57 (1,000-fold) and PVC-211 MuLV (100-fold) (Table 2). Other ecotropic MuLVs, such as Moloney MuLV, Cas-Br-E MuLV, and Fr-Cas$^E$ MuLV, were also transduced more efficiently (40- to 1,000-fold) by this treatment (data not shown). Although inoculation of the vector in medium without heparin increased the transduction efficiency of amphotropic MuLV on RTEC-6 cells by sevenfold, 2-DG treatment of the cells did not show a significant effect (Table 2). In addition, 2-DG treatment and inoculation of the vector in medium without heparin signifi-

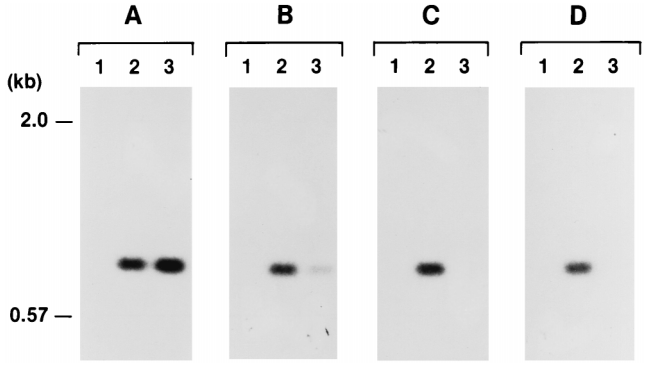


FIG. 2. Susceptibility of cultured rat CEC to PVC-211 MuLV infection. Primary cultures of rat brain CEC were prepared as described previously (7) and grown in minimum essential medium (MEM) with D-valine (Life Technologies, Inc., Gaithersburg, Md.) supplemented with 20% fetal calf serum, 2 mM L-glutamine, 50 U of penicillin per ml, 50 μg of streptomycin per ml, 1/100 volume of MEM nonessential amino acids solution (Life Technologies, Inc.), 1/100 volume of MEM vitamin solution (Life Technologies, Inc.), 16 U of heparin (Life Technologies, Inc.) per ml, and 20 μg of endothelial cell mitogen (Biomedical Technologies, Inc., Stoughton, Mass.) per ml. Rat liver CEC were isolated by the same procedure and grown under the same conditions. Portions of the CEC preparation were examined for factor VIII expression and Rca-1 binding as described previously (7), and more than 95% of the cells were positive for these CEC markers. Cells between the second and sixth passages were used for the experiments. Control Rat-1 fibroblasts (A), primary brain CEC from 2-day-old (B) and 21-day-old (C) F344 rats, and primary liver CEC from 21-day-old F344 rats (D) were mock infected (lane 1) or infected with PVC-211 MuLV (lane 2) or F-MuLV (lane 3), and the 0.83-kb *Bam*HI-*Bam*HI *env* gene-specific fragment of the integrated viral DNA was detected by hybridization analysis as described previously (13). The positions of the molecular size markers are indicated on the left in kilobases.

cantly increased the transduction efficiencies of PVC-211 MuLV and F-MuLV-57, but not of amphotropic MuLV, on primary brain CEC (Fig. 3A).

To examine the effects of the different conditions used for gene transduction on the expression of the ecotropic MuLV receptor (*ecoR*) gene in RTEC-6 cells, we examined the *ecoR* mRNA levels in cells grown under various conditions. As shown in Fig. 3B, lane 1, a signal corresponding to the 7.5-kb *ecoR* mRNA was clearly detected in total RNA from RTEC-6 cells grown under standard conditions. Although treatment of RTEC-6 cells with 2-DG and removal of heparin during vector inoculation increased the susceptibility to ecotropic MuLV, these procedures did not increase, but rather slightly decreased, the level of *ecoR* mRNA (Fig. 3B).

In this study, we provide additional support for the role of CEC in PVC-211-induced neurological disease and extend our studies of the CEC tropism of the virus to demonstrate that it may be a useful tool for both in vivo and ex vivo gene therapy targeted to CEC. Following neonatal inoculation, PVC-211 MuLV, but not F-MuLV-57, infects CEC of multiple organs, including the CNS. However, histopathology was detected only in the brains and spinal cords of PVC-211 MuLV-infected rats. PVC-211 MuLV could therefore cause functional abnormalities specific to brain CEC. An obvious difference in brain CEC and CEC in other tissues is their ability to form tight junctions, an essential feature of the functional blood-brain barrier. However, in our previous studies (7), we were unable to demonstrate either disruption of tight junctions or functional defects in the blood-brain barrier even in severely paralyzed rats infected with PVC-211 MuLV. Alternatively, PVC-211 MuLV infection may activate or inactivate specific genes in brain CEC which, by their proximity to cells such as macrophages and astrocytes, affect their function and initiate the neurodegenerative process. This possibility is currently under investigation. The age-dependent restriction of PVC-211 MuLV replication in CEC observed in vivo was abrogated in vitro, since the virus was as infectious in primary CEC prepared from 21-day-old rats as in CEC from 2-day-old rats. Therefore, restriction of PVC-211 MuLV replication in older animals does not appear to be caused by irreversible changes in CEC per se. Since MuLV can replicate only in proliferating cells (29), it is possible that the proliferation rate of CEC is an important host factor that determines the level of virus replication in these cells. It has been reported that the proliferation rate of CEC in the rat brain is maximal between 5 and 9 days of age and then declines rapidly (23). This correlates well with our finding that the degree of PVC-211 MuLV replication in brain CEC is much higher when the virus is injected into 2-day-old rats rather than when it is inoculated into 10- or 21-day-old rats. Once CEC are explanted, however, the cells may proliferate at comparable rates whether they are derived from newborn or adult rats.

The widespread replication of PVC-211 MuLV in CEC of various organs following neonatal inoculation and the possibility that it may infect proliferating CEC in adult rodents suggest that this virus may be a useful tool for in vivo transduction of genes into CEC in rodent models. We are currently evaluating the usefulness of PVC-211 MuLV, as well as CEC-tropic chimeric MuLVs with attenuated neuropathogenicity (12), for in vivo transduction of genes into rat CEC. Those in vivo protocols may be useful for establishing not only animal models for CEC-mediated gene therapy but also models of human diseases, such as Kaposi's sarcoma, for which a CEC origin of the tumor has been suggested (21, 25, 26, 28).

PVC-211 MuLV may also be useful for ex vivo gene therapy targeted to CEC since we have demonstrated conditions for efficiently transducing cultured CEC with the virus. A combination of 2-DG treatment and inoculation of the vector in

TABLE 2. Effects of 2-DG treatment and heparin on transduction efficiencies of various MuLVs

| Target cells | Treatment | Transduction efficiency ($10^3$ G418-resistant CFU/ml) of[a]: | | |
|---|---|---|---|---|
| | | PVC-211 MuLV | F-MuLV-57 | Amphotropic MuLV |
| RTEC-6 | Heparin | 0.26 ± 0.01 | 0.01 ± 0.001 | 0.22 ± 0.02 |
| | Heparin and tunicamycin | 0.30 ± 0.01 | 0.06 ± 0.003 | ND[b] |
| | Heparin and 2-DG | 1.2 ± 0.1 | 0.31 ± 0.06 | 0.31 ± 0.01 |
| | None | 11 ± 2 | 1.1 ± 0.2 | 1.5 ± 0.5 |
| | 2-DG | 28 ± 6 | 12 ± 1 | 2.3 ± 0.1 |
| Rat-1 | None | 550 ± 50 | 290 ± 10 | 75 ± 5 |

[a] The N2 vector transduction assay was carried out as previously described (12). Cloned NIH 3T3 cells harboring the N2 vector were inoculated with each MuLV, and after two passages, culture fluids containing PVC-211 MuLV/N2 and F-MuLV-57/N2 were collected. RTEC-6 cells, a cell line derived from rat brain CEC (12), were seeded at a density of $10^5$ per 60-mm-diameter culture dish in medium with or without 0.5 μg of tunicamycin (Calbiochem Corp., La Jolla, Calif.) per ml or 2.5 mM 2-DG (Sigma Chemical Co., St. Louis, Mo.) for 16 h and then inoculated with each serially diluted MuLV/N2 stock in the presence or absence of 16 U of heparin per ml. After 24 h, the medium was replaced with fresh medium containing G418 (400 μg/ml), and after 10 to 14 days, G418-resistant colonies were stained and counted. As a control, Rat-1 fibroblasts were also inoculated with the same virus preparations under standard conditions, and the titers were $5.5 \times 10^5$/ml for PVC-211 MuLV/N2 and $2.9 \times 10^5$/ml for F-MuLV-57/N2. The results of duplicate experiments (means ± standard deviations) are shown.

[b] ND, not done.

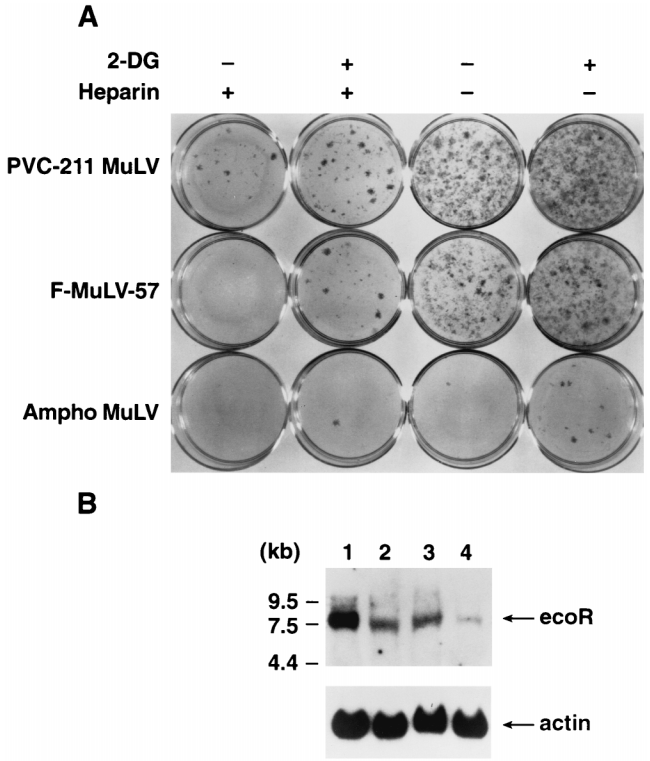


FIG. 3. (A) Effects of 2-DG treatment and heparin on susceptibility of primary rat brain CEC to MuLV-mediated gene transduction. Primary cultures of brain CEC prepared from 21-day-old F344 rats were seeded at a density of $10^5$ cells per 60-mm-diameter culture dish and treated with (+) or without (−) 2.5 mM 2-DG for 16 h. The cells were inoculated with 1 ml of undiluted N2 vector pseudotyped with PVC-211 MuLV, F-MuLV-57, or amphotropic MuLV in the presence (+) or absence (−) of heparin (16 U/ml). After selection with G418 (400 μg/ml), G418-resistant colonies were stained. (B) Expression of the *ecoR* mRNA in RTEC-6 cells. Ten micrograms of total RNA extracted from RTEC-6 cells grown under standard conditions (lane 1), treated with 0.5 μg/ml of tunicamycin (lane 2), treated with 2.5 mM of 2-DG (lane 3), or grown overnight in the absence of heparin (lane 4) was subjected to formalin-agarose gel electrophoresis, transferred to a nitrocellulose filter, and hybridized with a $^{32}$P-labeled mouse ecotropic MuLV receptor (*ecoR*) cDNA probe (1) kindly provided by James Cunningham (Harvard University, Boston, Mass.). The positions of the molecular size markers are indicated to the left in kilobases, and the position of the 7.5-kb *ecoR* mRNA is indicated by the arrow on the right.

heparin-deficient medium dramatically increased the efficiency of PVC-211 MuLV-mediated transduction of genes into CEC and also increased the susceptibility of CEC to other MuLVs. It has previously been shown that genetically manipulated CEC transplanted to a recipient animal can be incorporated into a vascular structure (11, 16, 17). Thus, there appears to be a good possibility that CEC implants which have gone through PVC-211 MuLV-mediated ex vivo gene transduction will function in vivo. This system may provide a useful animal model for CEC-mediated gene therapy.

The reasons that CEC are more resistant to MuLV infection than fibroblasts are still unclear. Comparisons of the closely related viruses PVC-211 MuLV and F-MuLV (22) in our previous studies revealed that subtle changes in the envelope SU protein are important for the rat CEC (12) and the hamster cell (14) tropism of PVC-211 MuLV. Since the viral SU protein is responsible for binding to the cellular receptor, it is possible that the susceptibility of CEC to MuLV infection is determined by the level of expression of the viral receptor. However, RTEC-6 cells expressed a high level of *ecoR* mRNA, in agreement with a previous report describing a high level of *ecoR* mRNA in primary rat brain CEC (27). In addition, procedures which increased the susceptibility to ecotropic MuLV did not increase the amount of *ecoR* mRNA. Thus, the level of *ecoR* gene expression does not appear to explain the relative resistance of CEC to ecotropic MuLV infection. Alternatively, metabolic modification of the *ecoR* protein molecule expressed in CEC may be responsible for its relative resistance to MuLVs since treatment with the metabolic inhibitor 2-DG increased susceptibility of CEC to some extent. The mechanism by which heparin affects the susceptibility of CEC to MuLV infection is unclear. Since the *ecoR* mRNA level was not increased in RTEC-6 cells grown in the absence of heparin, it is unlikely that removal of heparin from the medium increased the susceptibility of CEC to MuLVs by allowing a higher level of *ecoR* gene expression. It has been previously shown that heparin has an inhibitory effect on retroviral reverse transcriptase activity (3) and can also affect the interaction of certain enveloped viruses with their receptors (8). Therefore, the removal of these negative effects may be responsible for the increase of MuLV susceptibility in the absence of heparin. Additional studies to elucidate the cellular factors governing susceptibility of CEC to MuLV infection should be useful for further improving the efficiency of MuLV-mediated transduction of genes into CEC.

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