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Analysis of the Unique Hamster Cell Tropism of Ecotropic Murine Leukemia Virus PVC-211

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PVC-211 murine leukemia virus (MuLV) is a neuropathogenic variant of Friend MuLV (F-MuLV). Previous studies from our laboratory demonstrated that unlike the parental F-MuLV, PVC-211 MuLV can infect rat brain capillary endothelial cells efficiently and that it has acquired genetic changes responsible for its expanded cellular tropism. To determine if PVC-211 MuLV also has expanded its host range, we tested its infectivity on Chinese hamster ovary-derived CHO-K1 cells, which are generally resistant to ecotropic MuLV. The results indicated that PVC-211 MuLV, but not F-MuLV, was highly infectious for CHO-K1 cells. Studies using glycosylation inhibitors and glycosylation mutants of CHO-K1 cells, as well as interference studies, suggested that PVC-211 MuLV has acquired the ability to interact with the ecotropic MuLV receptor on CHO-K1 cells that has undergone glycosylation-dependent modification. Using chimeric viruses between PVC-211 MuLV and F-MuLV, we were able to localize the viral genetic element crucial for CHO-K1 cell tropism within the *env* gene of PVC-211 MuLV and show that glycine at position 116 and lysine at position 129 of the envelope glycoprotein SU were important. These viral determinants also appear to confer tropism for other hamster cells resistant to ordinary ecotropic MuLVs. Further studies on the interaction between PVC-211 MuLV and the receptor on hamster cells may provide novel insights into the molecular mechanisms for receptor recognition and binding by viral envelope glycoproteins.

We have been characterizing a neuropathogenic variant of Friend murine leukemia virus (F-MuLV), PVC-211, which causes rapidly progressive neurodegenerative disease in susceptible rats and mice (17, 19, 25–27, 41). Interference properties of PVC-211 MuLV on mouse fibroblasts (25), as well as its structural relationship with F-MuLV (41), classifies this virus as an ecotropic MuLV. Our previous studies have shown that PVC-211 MuLV can infect rat brain capillary endothelial cells (BCEC) that are resistant to F-MuLV (26) and other ecotropic MuLVs (14). We further showed that the BCEC tropism of PVC-211 MuLV was attributable to two amino acid changes: glutamic acid (Glu) to glycine (Gly) at position 116 and Glu to lysine (Lys) at position 129 in the putative receptor-binding domain of the envelope surface glycoprotein SU (25). Thus, the subtle changes in the SU protein of PVC-211 MuLV expand the cellular tropism of the virus.

In this study, to examine if PVC-211 MuLV has an expanded host range in addition to its expanded cellular tropism, we tested its infectivity on cultured hamster cells. Hamster cells are generally resistant to MuLV of various classes, including ecotropic MuLV (35, 45). For example, previous studies have shown that Chinese hamster ovary-derived CHO-K1 cells are highly resistant to transduction of a retroviral vector pseudotyped by ecotropic Moloney MuLV (M-MuLV) under normal conditions (30, 48). It appears that the SU protein of ecotropic MuLV is unable to bind to the ecotropic virus receptor (ecoR) on the CHO-K1 cell surface due to N-linked glycosylation-dependent modification of the receptor (30, 48). Our results demonstrate that PVC-211 MuLV, unlike other ecotropic MuLVs, is highly infectious for CHO-K1 cells. Further studies suggest that PVC-211 MuLV has acquired genetic changes which enable the virus to overcome the modification of the ecoR on CHO-K1 cells. By using chimeric viruses constructed between PVC-211 MuLV and F-MuLV, we have identified the viral genetic determinants within the *env* gene important for CHO-K1 cell tropism of PVC-211 MuLV. These determinants also appear to confer upon the virus the ability to infect several other hamster cell lines normally resistant to other ecotropic MuLVs.

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MATERIALS AND METHODS

Cells. NIH 3T3 cells and Rat-1 cells were grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum (FCS). CHO-K1 cells, and their glycosylation mutants Lec2 and Lec8, Chinese hamster lung-derived Don and E36 cells, Syrian hamster kidney-derived BHK-21 cells, and Armenian hamster lung fibroblasts (AHL cells) were provided by M. Eiden (National Institute of Mental Health, Bethesda, Md.). Wild-type CHO-K1 cells and Lec2 and Lec8 mutants were propagated in Ham's F12 medium supplemented with 10% FCS. Other hamster cell lines were maintained in Dulbecco's modified Eagle's medium supplemented with 10% FCS.

Viruses. Molecular clones of PVC-211 MuLV and F-MuLV clone 57 (F-MuLV-57) were described previously (27, 37). Construction of chimeric viruses between these two viruses, such as PVF-L, PVF-N, PVF-e1 through -e6, PVF-7-1, and PVF-1-15, has also been described in our previous studies (25, 26). Each virus was prepared by transfecting NIH 3T3 cells with its DNA clone as described previously (27). 10A1 MuLV-infected NIH 3T3 cells (38) were provided by Alan Rein (NCI-FCRDC, Frederick, Md.).

Virus infection. Target cells were seeded at 10^5 cells per 60-mm-diameter dish on day 1. On day 2, the medium was removed, and fresh medium, virus, and Polybrene (5 μg/ml) were added. On day 3, the medium was replaced by fresh medium. For G418 selection, medium containing G418 (1 mg/ml for hamster cells or 400 μg/ml for Rat-1 cells) was used on day 3. For treatment of target cells with glycosylation inhibitors, 2-deoxyglucose (2-DG; Sigma Chemical Co., St. Louis, Mo.) or tunicamycin (Calbiochem Corp., La Jolla, Calif.) was added to the culture medium at a final concentration of 2.5 mM or 1.0 μg/ml, respectively, 4 h

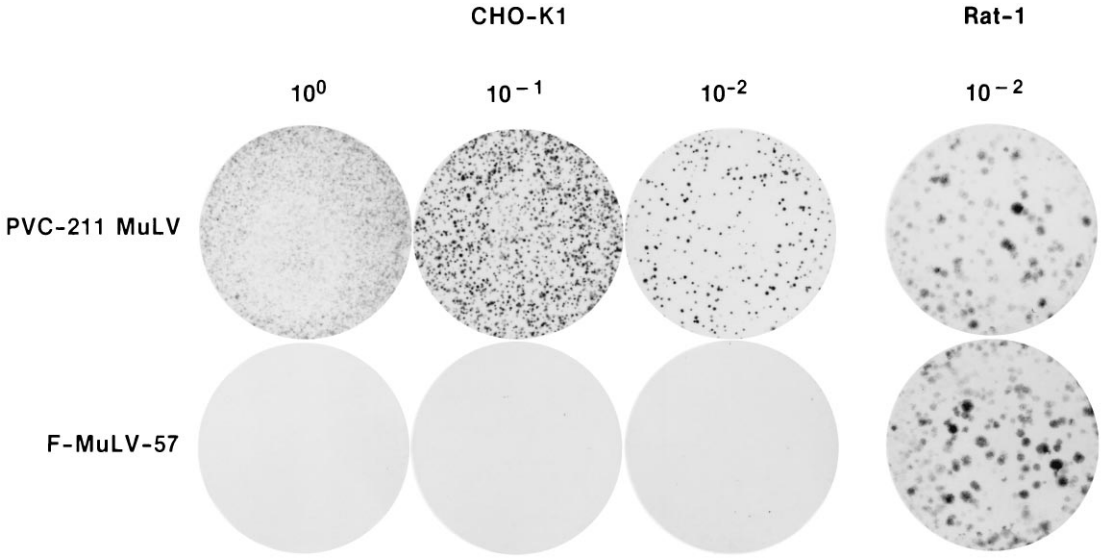

FIG. 1. Efficient N2 vector transduction into CHO-K1 cells by PVC-211 MuLV. CHO-K1 cells seeded at a density of 10^5 cells per 60-mm-diameter culture dish were inoculated with serially diluted N2 vector pseudotyped by PVC-211 MuLV or F-MuLV-57 as described in Materials and Methods. After growth of the cells in the presence of G418, surviving colonies were stained. As a control, Rat-1 cells were similarly inoculated with N2 vector pseudotyped by each virus and selected in the presence of G418. Experiments were carried out in duplicate, and representative culture dishes are shown.

after the cells were seeded. After 16 h, the medium was removed and virus was inoculated as described above.

Preparation of N2 virus pseudotyped with MuLV. NIH 3T3-derived cell line 3T3/N2-1, which harbors the neomycin resistance gene-bearing N2 retroviral vector (8), was established as previously described (25). To prepare the N2 vector pseudotyped with PVC-211 MuLV, F-MuLV-57, chimeric viruses, and 10A1 MuLV, 3T3/N2-1 cells were inoculated with each of these viruses at a multiplicity of infection of 1.0, and after two passages, 24-h culture fluids were collected from the confluent cells and stored at −80°C.

RESULTS

PVC-211 MuLV, but not F-MuLV, can infect CHO-K1 cells efficiently. To investigate whether PVC-211 MuLV has a distinct host range compared with other ecotropic MuLVs, we tested its infectivity on Chinese hamster ovary-derived CHO-K1 cells, which are normally resistant to ecotropic MuLV. Infectivity was evaluated by examining the ability of the neomycin resistance gene-containing N2 retroviral vector pseudotyped with PVC-211 MuLV to transduce G418 resistance into CHO-K1 cells. As a control, the transduction efficiency of the N2 vector pseudotyped by F-MuLV-57 was also examined. As shown in Fig. 1, PVC-211 MuLV was highly efficient in transducing the N2 vector into CHO-K1 cells, whereas F-MuLV-57 failed to transduce CHO-K1 cells at a detectable level. Transduction efficiencies of PVC-211 MuLV and F-MuLV-57 on Rat-1 cells were comparable (Fig. 1).

Inhibition of protein glycosylation does not affect susceptibility of CHO-K1 cells to PVC-211 MuLV. It has been previously shown that treatment of CHO-K1 cells with the glycosylation inhibitor tunicamycin renders the cells susceptible to M-MuLV (30, 48). To examine the effects of protein glycosylation on susceptibility of CHO-K1 cells to PVC-211 MuLV and the parental F-MuLV, we treated the cells with the glycosylation inhibitors tunicamycin and 2-DG and challenged them with the N2 vector pseudotyped by each of these viruses (Table 1). Although CHO-K1 cells were highly resistant to F-MuLV-57, treatment of the cells with tunicamycin or 2-DG increased the susceptibility dramatically. In contrast, treatment of CHO-K1 cells with glycosylation inhibitors had no effect on the high susceptibility of these cells to PVC-211 MuLV.

We also tested the transduction efficiency of PVC-211 MuLV and F-MuLV-57 on two glycosylation mutants of CHO-K1, Lec2 and Lec8 (Table 1). F-MuLV-57 was able to infect Lec8 cells, which are deficient in protein galactosylation and sialylation (6), but not Lec2 cells, which are deficient in only protein sialylation (7). In contrast, PVC-211 MuLV was able to infect both mutant CHO-K1 cell lines as well as wild-type CHO-K1 cells.

PVC-211 MuLV does not appear to depend on availability of the amphotropic or GaLV receptor to enter CHO-K1 cells. CHO-K1 cells have been shown to be susceptible to a few retroviruses, such as gibbon ape leukemia virus (GaLV) and 10A1 MuLV (30, 38, 48). Therefore, in addition to expressing a modified ecoR, CHO-K1 cells also express functional receptors for these viruses. Recently, it has been shown that the GaLV receptor is a phosphate symporter, PiT1 (formerly designated GLVR-1) (20, 34, 36). It has also been demonstrated that 10A1 MuLV can utilize not only PiT1 but also another phosphate symporter, PiT2 (formerly designated Ram-1 and

TABLE 1. Transduction efficiencies of PVC-211 MuLV and F-MuLV-57 on glycosylation-altered CHO-K1 cells

Cells	Treatment	Transduction efficiency ($G418^r$ CFU/ml)[a]	
		PVC-211 MuLV	F-MuLV-57
CHO-K1		1.6×10^5	<1
CHO-K1	Tunicamycin	2.5×10^5	1.2×10^4
CHO-K1	2-DG	2.0×10^5	5.3×10^4
Lec2		1.4×10^5	<1
Lec8		3.4×10^5	1.2×10^4
Rat-1		8.5×10^4	1.3×10^5

[a] CHO-K1 cells seeded at a density of 10^5 per 60-mm-diameter culture dish were treated with or without tunicamycin or 2-DG as described in Materials and Methods and inoculated with serially diluted N2 vector pseudotyped by PVC-211 MuLV or F-MuLV-57. Untreated Lec2, Lec8, and Rat-1 cells were similarly inoculated with the N2 vector. After growth of the cells in the presence of G418, surviving colonies were counted. Experiments were carried out in duplicate, and the average $G418^r$ colony-forming titers are indicated.

TABLE 2. Transduction efficiencies of PVC-211 MuLV on 10A1 MuLV-infected CHO-K1 cells

Cells	Transduction efficiency (G418^r CFU/ml)[a]	
	PVC-211 MuLV	10A1 MuLV
CHO-K1	2×10^5	1.5×10^3
CHO-K1/PVC-211 MuLV	2	10^2
CHO-K1/10A1 MuLV	1.5×10^4	3

[a] Uninfected CHO-K1 cells or CHO-K1 cells chronically infected with PVC-211 MuLV or 10A1 MuLV were seeded at a density of 10^5 per 60-mm-diameter culture dish and inoculated with serially diluted N2 vector pseudotyped by PVC-211 MuLV or 10A1 MuLV. After growth of the cells in the presence of G418, surviving colonies were counted. Experiments were carried out in duplicate, and the average G418^r colony-forming titers are indicated.

GLVR-2), which serves as the amphotropic MuLV receptor (29, 31, 46, 49, 50). To determine if PVC-211 MuLV depends on either PiT1 or PiT2 to enter CHO-K1 cells, we carried out interference assays using CHO-K1 cells chronically infected with PVC-211 MuLV or 10A1 MuLV (Table 2). PVC-211 MuLV-infected CHO-K1 cells were highly resistant to transduction by a PVC-211 MuLV-pseudotyped vector, indicating efficient interference. In contrast, 10A1 MuLV-infected CHO-K1 cells were highly susceptible to PVC-211 MuLV, although the cells showed a 10-fold reduction in susceptibility compared with uninfected CHO-K1 cells. Transduction of a 10A1 MuLV-pseudotyped vector was blocked on 10A1 MuLV-infected CHO-K1 cells, whereas its transduction efficiency was reduced by only 10-fold on PVC-211 MuLV-infected CHO-K1 cells. The partial reduction in transfection efficiency of PVC-211 MuLV on 10A1-infected CHO-K1 cells and of 10A1 MuLV on PVC-211 MuLV-infected CHO-K1 cells does not appear to be due to any toxicity of the MuLVs on CHO-K1 cells leading to lower colony counts. Transfection of N2 vector DNA into uninfected and virus-infected CHO-K1 cells generated comparable numbers of G418^r colonies (data not shown).

The *Afl*II-*Age*I region within the SU protein coding region of the *env* gene of PVC-211 MuLV determines the CHO-K1 cell tropism of the virus. In an attempt to localize the genetic elements in the PVC-211 MuLV genome responsible for its CHO-K1 cell tropism, we tested chimeric viruses between PVC-211 MuLV and F-MuLV-57 (25, 26) for the ability to transduce CHO-K1 cells (Fig. 2). The chimeras contain the 5′ *Kpn*I-*Eco*RI region of PVC-211 MuLV and various amounts of the 5′ *env* gene from PVC-211 MuLV on an F-MuLV background. Chimera PVF-L, which contains the *Xba*I-*Bam*HI region of the PVC-211 MuLV *env* gene, was as highly CHO-K1 cell tropic as PVC-211 MuLV. In contrast, chimera PVF-N, which has the same structure as PVF-L except that the *Xba*I-*Bam*HI region is derived from F-MuLV-57, failed to efficiently infect CHO-K1 cells. Additional chimeras bearing different structures in the *Xba*I-*Bam*HI region were also tested for their CHO-K1 cell tropism, and those containing the *Afl*II-*Age*I region of the PVC-211 MuLV *env* (i.e., PVF-e2, -e4, and -e5) were significantly infectious for CHO-K1 cells. On the other hand, chimeras containing the *Afl*II-*Age*I region from F-MuLV-57 (i.e., PVF-e1, -e3, and -e6) failed to infect CHO-K1 cells at a comparable efficiency. Replacement of the *Afl*II-*Age*I region between F-MuLV-57 and PVC-211 MuLV results in substitution of two amino acids in the SU protein (Glu-116 and Glu-129 in F-MuLV-57 and Gly-116 and Lys-129 in PVC-211 MuLV when the N-terminal alanine of the mature SU protein is numbered as 1). To examine the effects of each amino acid residue, point mutant viruses PVF-7-1 and PVF-1-15, carrying only a Glu-116-to-Gly and a Glu-129-to-Lys substitution, respectively, were also tested. Neither of these point mutant viruses transduced CHO-K1 cells efficiently. All of the chimeric and mutant viruses, as well as the parental PVC-211 MuLV and F-MuLV-57, showed comparable levels of transduction efficiency on Rat-1 cells (data not shown and reference 25). A chimera containing the *Bgl*I-*Age*I region of PVC-211 MuLV in the background of the F-MuLV *env* gene was also constructed, but no infectious virus was produced upon transfection of this construct into NIH 3T3 cells (28).

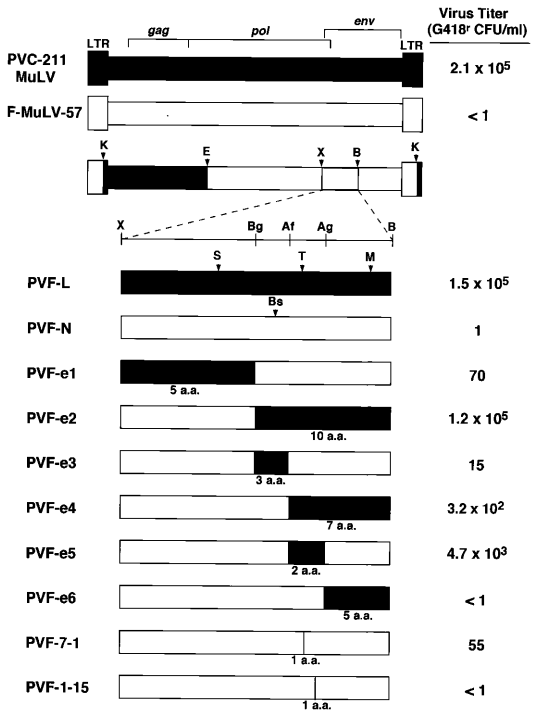

FIG. 2. Transduction efficiencies of PVC-211 MuLV, F-MuLV-57, and chimeric viruses on CHO-K1 cells. CHO-K1 cells seeded at a density of 10^5 cells per 60-mm-diameter culture dish were inoculated with serially diluted N2 vectors pseudotyped by each of the viruses and grown in the presence of G418. Experiments were carried out in duplicate, and the average G418^r colony-forming titer of each virus is shown on the right. Structures of the viruses used are shown on the left. Genomic organizations of PVC-211 MuLV and F-MuLV-57, including the positions of the *gag*, *pol*, and *env* genes and the long terminal repeat (LTR), are shown at the top. A backbone structure of chimeric viruses is shown in the middle, with solid and open regions derived from PVC-211 MuLV and F-MuLV-57, respectively. The *Xba*I-*Bam*HI region, whose structure differs from virus to virus, is shaded. Chimeric viruses are represented by the structures of their *Xba*I-*Bam*HI regions, with solid and open boxes derived from PVC-211 MuLV and F-MuLV-57, respectively. Positions of point mutations in PVF-7-1 and PVF-1-15 are indicated by solid lines. For PVF-e1 through -e6, PVF-7-1, and PVF-1-15, the number of amino acids (a.a.) substituted by the replacement of each fragment is indicated. Restriction enzyme sites used for chimeric virus construction are shown: Af, *Afl*II; Ag, *Age*I; B, *Bam*HI; Bg, *Bgl*I; E, *Eco*RI; K, *Kpn*I; X, *Xba*I. Recognition sites of the diagnostic restriction enzymes used for confirming the structure of each chimera are also shown: Bs, *Bsg*I; M, *Mun*I; S, *Ssp*I; T, *Taq*I.

PVC-211 MuLV and chimera PVF-e5 show tropism for various hamster cell lines in addition to CHO-K1 cells. To examine the tropism of PVC-211 MuLV and F-MuLV-57 for hamster cell lines other than CHO-K1, we inoculated the N2 vector pseudotyped with each of these viruses on Chinese hamster-derived Don and E36 cells, Syrian hamster-derived BHK-21 cells, and Armenian hamster-derived AHL cells and compared their transduction efficiencies (Table 3). PVC-211 MuLV was able to infect all of these hamster cell lines efficiently except

TABLE 3. Transduction efficiencies of PVC-211 MuLV, F-MuLV-57, and chimeric viruses on various hamster cell lines

Cells	Transduction efficiency ($G418^r$ CFU/ml)[a]			
	PVC-211 MuLV	F-MuLV-57	PVF-e5	PVF-N
Don	3.9×10^5	<1	2×10^3	7
E36	2.3×10^6	55	1.2×10^5	80
BHK-21	1.3×10^4	<1	1.2×10^4	9.5
AHL	54	<1	<1	<1
Rat-1	4.9×10^6	5.3×10^5	1.7×10^6	8.5×10^5

[a] Chinese hamster-derived Don and E26 cells, Syrian hamster-derived BHK-21 cells, and Armenian hamster-derived AHL cells were seeded at a density of 10^5 cells per 60-mm-diameter culture dish and inoculated with serially diluted N2 vector pseudotyped by each virus. After the cells were grown in the presence of G418, surviving colonies were counted. As a control, Rat-1 cells were similarly inoculated with N2 vector pseudotyped by each virus. Experiments were carried out in duplicate, and the average $G418^r$ colony-forming titers are indicated.

AHL, while F-MuLV-57 failed to infect any of these hamster cell lines at a significant level. To examine the effects of the *Afl*II-*Age*I region of the viral genome on tropism for these additional hamster cell lines, we also tested chimeric viruses PVF-e5, which contains the *Afl*II-*Age*I region from PVC-211 MuLV, and PVF-N, which has the same structure as PVF-e5 except for the F-MuLV-derived *Afl*II-*Age*I region (Table 3). Chimera PVF-e5 was able to efficiently transduce all of the hamster cell lines tested except AHL, although its efficiency appeared to be somewhat lower than that of PVC-211 MuLV on some hamster cell lines. In contrast, PVF-N, which differs from PVF-e5 by only two amino acids in the SU protein, did not transduce any of the hamster cell lines at a high level.

DISCUSSION

Chinese hamster ovary-derived CHO-K1 cells have been shown to be resistant to various retroviruses, including ecotropic MuLV. Previous studies showed that M-MuLV and F-MuLV were unable to infect CHO-K1 cells under normal conditions (30, 48). In this study, we have demonstrated that a neuropathogenic variant of F-MuLV, PVC-211 MuLV, has an unexpectedly high level of infectivity for CHO-K1 cells and other hamster cell lines due to changes in the receptor-binding domain of its envelope glycoprotein.

The exact mechanism by which PVC-211 MuLV enters CHO-K1 cells is not known. CHO-K1 cells express at least three classes of retroviral receptors: a modified ecoR, a GaLV receptor (PiT1), and an amphotropic MuLV receptor (PiT2) (30, 31, 38, 48). Our previous studies have shown that PVC-211 MuLV uses the ecoR on mouse and rat cells (25). In addition, our present interference studies indicated that PiT1 and PiT2 were unnecessary for PVC-211 MuLV to enter CHO-K1 cells. Therefore, it is likely that PVC-211 MuLV is able to use the glycosylation-modified ecoR on CHO-K1 cells. However, since we observed partial interference (a 10-fold reduction in transfection efficiency) between 10A1 MuLV and PVC-211 MuLV, the possibility cannot be excluded that PVC-211 MuLV also binds to PiT1 or PiT2 in addition to the ecoR. It is also possible that PVC-211 MuLV uses another unknown receptor on CHO-K1 cells. However, if this were the case, one would have expected the titer of PVC-211 MuLV to increase, as did the titer of F-MuLV, once the ecoR in CHO-K1 cells was cleared of modification by glycosylation inhibitors. In an attempt to directly examine whether PVC-211 MuLV depends on the ecoR for infecting CHO-K1 cells, efforts are being made to establish CHO-K1 cells chronically infected with F-MuLV by

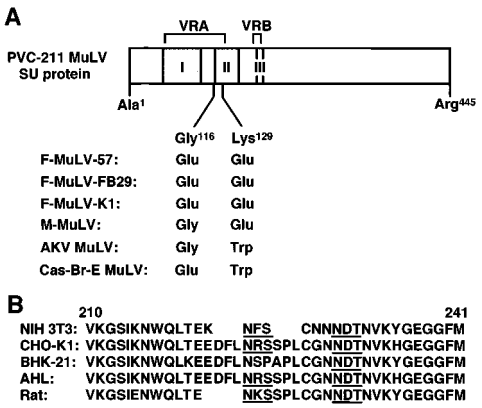

FIG. 3. (A) Schematic representation of the structure of the mature SU protein of PVC-211 MuLV. Positions of the N-terminal Ala-1, Gly-116, and Lys-129 and the C-terminal Arg-445 are indicated. Structural elements I, II, and III defined in previous studies (23) are shown by shaded boxes. Positions of VRA and VRB (15) are also shown. Amino acid sequences of the SU protein of F-MuLV-57 (GenBank accession number X02794), F-MuLV-FB29 (39), F-MuLV-K1 (33), M-MuLV (42), AKV MuLV (16), and Cas-Br-E MuLV (40) were aligned with that of PVC-211 MuLV by using the GAP program in the Genetics Computer Group software package (12), and the amino acid residues at positions corresponding to Gly-116 and Lys-129 are indicated. (B) Comparison of the amino acid residues contained in the TED of the ecoR expressed in various species. The region of the NIH 3T3 ecoR encompassed by amino acids 210 and 241 (2) and the corresponding regions of the ecoR expressed in CHO-K1 (10), BHK-21 (10), AHL (9), and rat (44, 51) cells are aligned. Potential N-linked glycosylation sites are underlined.

treating the cells with the glycosylation inhibitor prior to virus inoculation and to test their susceptibility to PVC-211 MuLV.

The major factor that determines the receptor usage by MuLVs is thought to be the structure of its envelope surface glycoprotein, SU. Therefore, it is likely that PVC-211 MuLV has acquired CHO-K1 cell tropism through genetic changes leading to alteration of this protein. Using chimeric viruses between PVC-211 MuLV and F-MuLV-57, we have shown that the structure of the SU protein is in fact important for the CHO-K1 cell tropism of the virus. All of the viruses carrying both Gly-116 and Lys-129 in their SU protein, such as PVC-211 MuLV, PVF-L, PVF-e2, PVF-e4, and PVF-e5, showed a significant level of infectivity on CHO-K1 cells. On the other hand, those viruses, such as F-MuLV, PVF-N, PVF-e1, PVF-e3, and PVF-e6, carrying Glu-116 and Glu-129 derived from F-MuLV-57 failed to infect CHO-K1 cells at a comparable level. Since point mutant viruses having either Gly-116 or Lys-129 alone failed to infect CHO-K1 cells efficiently, a combination of these two amino acid residues appears to be important for conferring CHO-K1 cell tropism. Gly-116 and Lys-129 are localized within the region of the ecotropic SU protein that binds to the receptor (3, 15, 32) or, more specifically, at the carboxyl terminus of variable region A (VRA) (15) (Fig. 3A). These residues are also in the vicinity of or within structural element II of the SU protein (Fig. 3A), which is thought to play a role in receptor binding (23). Alignment of the SU protein sequences of PVC-211 MuLV and other ecotropic MuLVs revealed that PVC-211 MuLV is the only virus that carries both Gly-116 and Lys-129 among the viruses compared (Fig. 3A).

Comparison between PVF-e5 and PVF-N clearly illustrates significant effects of Gly-116 and Lys-129. However, the transduction efficiency of PVF-e5 was considerably lower than that of the parental PVC-211 MuLV or chimeras PVF-L and -e2. The transduction efficiency of PVF-e4 was even lower, although it has a larger *env* gene region and three additional

amino acid residues derived from PVC-211 MuLV compared with PVF-e5. An attempt to increase the transduction efficiency of PVF-e5 by adding back the *Bgl*I-*Afl*II region of the PVC-211 MuLV *env* gene to the chimera failed to result in a viable virus (28). Therefore, the structural context in which Gly-116 and Lys-129 are localized appears to have supplementary effects on the SU protein function.

All of the chimeric viruses used in this study have the *Kpn*I-*Eco*RI region of PVC-211 MuLV. Our previous studies showed that this region had supplementary effects on viral tropism for rat BCEC (26). Although the levels of CHO-K1 cell tropism of the chimeras studied are determined by their *env* gene structure, it is possible that the *Kpn*I-*Eco*RI region of PVC-211 MuLV has additional effects on CHO-K1 cell tropism of the virus. By using a different set of constructs containing chimeric structures of the *Kpn*I-*Eco*RI region, studies are in progress to examine this possibility.

In addition to showing that PVC-211 MuLV is highly infectious for CHO-K1 cells, we also showed that it could efficiently infect other cell lines derived from Chinese and Syrian hamsters. Chimera PVF-e5, but not PVF-N, was also highly infectious on most of these hamster cells, indicating that Gly-116 and Lys-129 of the SU protein play a crucial role in virus-receptor interaction on these hamster cells as well. It has been shown that the ecoR is a cationic amino acid transporter (2, 22, 47) and that its third extracellular domain (TED) is important for interaction with the viral SU protein (1, 52). Alignment of the TED structures of the ecoRs expressed in various cells (Fig. 3B) revealed that compared with the mouse ecoR, CHO-K1 and BHK-21 ecoRs have additional amino acid residues at two positions: aspartic acid-phenylalanine-leucine between Lys-222 and Asn-223 and serine-alanine-proline-leucine between Ser-225 and Cys-226. Among the two N-linked glycosylation sites found in the mouse ecoR TED, Asn-Asp-Thr, which was shown to affect susceptibility to M-MuLV infection in a context-dependent manner (10), is conserved in these hamster ecoRs, whereas the other glycosylation site is missing from the BHK-21 ecoR. Thus, it is possible that glycosylation at the Asn-Asp-Leu site in the context of two sets of additional amino acids inactivates the ecoR function. The rat ecoR, like the CHO-K1 ecoR, carries a Ser-Pro-Leu insertion (Fig. 3B). Since PVC-211 MuLV was generated by passaging Friend virus complex in F344 rats (19), it is possible that this virus is more adapted to rat cells than the parental F-MuLV due to an increased ability to interact with the rat ecoR. In fact, our previous interference studies suggested that PVC-211 MuLV had a higher affinity for rat ecoR than F-MuLV (25). Thus, PVC-211 MuLV, which acquired Gly-116 and Lys-129 through adaptation to growth in rat cells, may have acquired the ability to interact with an ecoR bearing the Ser-Pro-Leu insertion, such as the hamster ecoR. A previous study (18) showed that F-MuLV could be adapted to grow at high titers in Syrian hamster cells. It would be interesting to determine if the hamster-adapted F-MuLV has undergone the same genetic changes in its *env* gene as PVC-211 MuLV. Neither PVC-211 MuLV nor PVF-e5 was able to infect Armenian hamster-derived AHL cells. Since the structure of the TED of the AHL ecoR is identical or highly homologous to that of the CHO-K1 and BHK-21 ecoRs (Fig. 3B), the molecular basis for the resistance of AHL cells to PVC-211 MuLV infection is unclear. Since expression of mouse ecoR cDNA in AHL cells confers susceptibility to M-MuLV on these cells, it is unlikely that PVC-211 MuLV replication in AHL cells is blocked at the postentry step. Previous studies have suggested that the resistance of AHL cells to ecotropic MuLVs, in contrast to CHO-K1 cells, is due to a glycosylation-independent mechanism (48). Although ecoR transcripts can be detected in AHL cells (48), it is not known whether the receptor protein is expressed on the cell surface. If it is, the envelope glycoprotein of PVC-211 MuLV is apparently unable to interact with it.

The structure-function relationship of the ecotropic MuLV SU protein has been the scope of extensive studies (3–5, 11, 13, 15, 21, 24, 25, 32, 43, 53). However, most of the studies were carried out by examining the interaction between the ecotropic SU protein and the ecoR on mouse cells. It was proposed in a recent study that the region encompassed by amino acids 81 to 88 of the M-MuLV SU protein is directly involved in its binding to the mouse ecoR (24). In contrast, our present study demonstrates that amino acids at positions 116 and 129 of the SU protein are the major determinants of the hamster cell tropism of PVC-211 MuLV, consistent with our previous studies indicating that the same two amino acids are the major determinants for the tropism of PVC-211 MuLV for rat BCEC (25). Therefore, it is possible that the mode of virus-receptor interaction depends on host species and that Gly-116 and Lys-129 are the primary determinants of receptor choice or binding on rat and hamster cells. Further studies of the interaction between the ecotropic SU protein and nonmouse ecoRs may provide novel and useful insights into the molecular mechanism for virus entry.

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