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Fv-1 Determinants in Xenotropic Murine Leukemia Viruses Studied with Biological Assay Systems: Isolation of Xenotropic Virus with N-Tropic *Fv-1* Activity in the Cryptic Form

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By a biological assay system using phenotypically mixed ecotropic and xenotropic murine leukemia viruses, we investigated whether in the virions of a xenotropic virus there is N- or B-tropic *Fv-1* determinant in active form. The existence of N-tropic *Fv-1* determinant was demonstrated in SL-XT-1 xenotropic virus isolated from the spleen of a 3-month-old SL mouse, and the N-tropic *Fv-1* tropism was confirmed by analysis of the phenotypically mixed viruses harvested from clonal SC-1 cells doubly infected with the SL-XT-1 and B-tropic ecotropic viruses. However, neither N- nor B-tropic *Fv-1* determinant was demonstrated in any xenotropic viruses isolated from embryo cells of BALB/c, NZB, or DBA/2 mice, or Cas E #1-IU, a xenotropic-like virus isolated from a wild mouse.

The in vivo generation of B-tropic viruses upon recombination of N-tropic ecotropic and xenotropic viruses has been suggested (2, 4). Hopkins et al. (10), who demonstrated the altered electrophoretic mobility of p30 of six NB-tropic viruses derived from a B-tropic virus, raised the possibility that p30 may be involved in determining the N-, B-, or NB-tropism of murine leukemia viruses (MuLVs). Gautsch et al. (3) observed that one of the seven tyrosine-containing tryptic peptides of p30 is associated with B-tropic and some xenotropic viruses, and recently recombinants generated in vitro between ecotropic and xenotropic viruses with *Fv-1* host ranges were isolated according to the origin of their p30 proteins (1, 4). These findings raised the question of whether the N- or B-tropic target of the *Fv-1* gene product, which may be called a determinant of *Fv-1* tropism or the *Fv-1* determinant of the virus, is present in active, albeit cryptic, form in such a xenotropic virion.

We previously reported (12, 14) that no *Fv-1* determinant was detected in BALB-IU-1 and NZB-IU-1 xenotropic viruses; in phenotypically mixed virus pools, the xenotropic genomes of viruses with ecotropic host range showed the *Fv-1* restriction pattern of the ecotropic virus. In those studies, the *Fv-1* sensitivity of the ecotropic virus was not affected by coinfection with xenotropic virus. Therefore, we hypothesized that the xenotropic genomes of the viruses may be considered to be analogous to NB-tropic genomes. In the present investigation, we again assessed the presence of *Fv-1* determinant in BALB-IU-1, NZB-IU-1, and other xenotropic viruses, using a biological assay system and an improved method in which phenotypically mixed virus pools of higher titers were prepared. We now report precise analyses of the *Fv-1* tropism of this phenotypically mixed virus and demonstrate the presence of N-tropic *Fv-1* determinant in a xenotropic virus isolated from an SL mouse. Our biological assay system, which employs phenotypic mixing of viruses to detect cryptic *Fv-1* tropism of xenotropic MuLV, is based on the hypothesis that the sensitivity of a virion to *Fv-1* restriction is not determined by the genome carried in that virion but by some virion component, possibly p30. This hypothesis is supported by findings that (i) N- and B-tropic, but not NB-tropic, viruses can contribute *Fv-1* tropism determinants, i.e., viral determinants of tropism make viruses sensitive to the *Fv-1* restriction gene; (ii) NB-tropic genomes can acquire the N- or B-tropism phenotype in mixed infection harvests; (iii) the N- and B-tropism determinants are "codominant," in that virions produced by cells dually infected with N- and B-tropic viruses show host range restrictions of both viruses, i.e., they are dually restricted, namely, virions with both N-tropic and B-tropic determinants are subject to restriction in both $Fv\text{-}1^b$ and $Fv\text{-}1^n$ cells (15, 18); (iv) the N- and B-tropism determinants can interact similarly with xenotropic and amphotropic genomes (12, 14).

Based on these considerations, we attempted

to detect the cryptic *Fv-1* determinants in the xenotropic virus by studying the *Fv-1* tropism of virus phenotypically mixed between the xenotropic and ecotropic viruses.

To reexamine the possible existence of cryptic *Fv-1* tropism of various xenotropic MuLVs, clonal lines of mink cells producing N-tropic (AKRL-1), B-tropic (WN1802B), and NB-tropic (Friend) ecotropic virus, designated AKR-mink, B-mink, and Fr-mink cell lines (13), respectively, were superinfected with various xenotropic viruses isolated from BALB/c, NZB, SL, DBA/2, and wild mice, in addition to BALB-IU-1 and NZB-IU-1 xenotropic viruses. Phenotypically mixed virus pools were harvested daily, and their *Fv-1* tropism was assayed. The change of *Fv-1* tropism in the acute and subacute phase of infection was carefully examined, since it tended to change between acute and chronic infection in mixed infection between N-tropic amphotropic and B-tropic ecotropic MuLV (14). In the present investigation, we first tried to detect the *Fv-1* determinant in Cas E #1-IU xenotropic-like MuLV, in which the existence of B-tropic determinant was expected, since Gautsch et al. (4) isolated a B-tropic ecotropic virus in vitro from the progeny of mouse cells doubly infected with N-tropic ecotropic virus and this Cas E #1 xenotropic virus (they designated the xenotropic Cas E #1-IU MuLV WM-X). Based on p30 fingerprint analysis and in vitro recombination studies, Cas E #1-IU xenotropic-like MuLV isolated from wild mouse was expected to have the genetic information enabling it to induce B-tropic *Fv-1* determinant. However, using our biological assay system, we could not detect either B- or N-tropic *Fv-1* determinant in the Cas E #1-IU virus, as well as the other five kinds of xenotropic viruses listed in Table 1, except SL-XT-1 xenotropic virus.

During these experiments, we found a very interesting xenotropic virus among the seven xenotropic viruses listed in Table 1. After superinfection of B-mink cells with a xenotropic virus isolated from the spleen of a 3-month-old SL mouse, the *Fv-1* restriction of the X(e) particle, xenotropic virus genome with ecotropic host range, was converted from B- to N-tropic; this strongly suggests that the SL-XT-1 xenotropic virus can contribute N-tropic *Fv-1* determinant of its own. Figure 1 shows the results of experiments in which B-mink cells were superinfected with xenotropic SL-XT-1 virus. Fluids were harvested daily, and xenotropic (Fig. 1A) and ecotropic genomes (Fig. 1B and C) were assayed. E(e) particles, ecotropic virus genome with ecotropic host range, in the mixed virus pool were assayed by infecting *Fv-1*^{nn} (NFS-ME), *Fv-1*^{bb} (BALB/c-ME), *Fv-1*^{nb} [(NFS × BALB/c)F_1], and *Fv-1*$^{--}$ (SC-1) cells (6) and by UV-XC procedures (19). The X(e) particles were assayed by infecting the same mouse embryo cells and *Fv-1*$^{--}$ (3T3FL) cells (5) and by overlaying them with S^+L^- mink cells after UV irradiation (12). In this experiment, the harvested phenotypically mixed xenotropic genomes with ecotropic host range showed prominent conversion of *Fv-1* restriction from B- to N-tropic in the course of time after mixed infection, i.e., on the second postinfection day, the xenotropic genomes were B-tropic; on day 8, they were N-tropic. On intermediate days 4 and 5, the genomes showed dual rather than N- or B-tropic restriction. By several independent experiments, we carefully confirmed the *Fv-1* conversion of the phenotypically mixed virus

TABLE 1. Contribution of *Fv-1* determinants to the virion obtained by phenotypic mixing of various xenotropic viruses

Xenotropic virus examined	Origin	Cryptic *Fv-1* tropism detected by the contribution of *Fv-1* determinant
BALB-IU-1 (8)	BALB/c mouse embryo cells, IUdR-induced	NB
NZB-IU-1 (8)	NZB mouse embryo cells, IUdR-induced	NB
NZB-XT-1[a]	NZB mouse embryo cells, IUdR-induced	NB
BALB-XT-1[a]	BALB/c mouse embryo cells, IUdR-induced	NB
DBA/2-XT-1[a]	DBA/2 mouse embryo cells, IUdR-induced	NB
SL-XT-1[b] (11)	SL mouse spleen cells	N
Cas E #1-IU[c]	Wild mouse	NB

[a] Isolated from embryo cells of NZB, BALB/c, and DBA/2 mice, respectively. The primary cultures were treated for 24 h with 20 μg of iododeoxyuridine (IUdR) overlaid with mink lung cells (9), and xenotropic viruses were usually harvested 1 week later.

[b] Isolated from spleen cells of a 3-month-old SL mouse (11). The spleen cells were cultured in the presence of mink cells, and virus was harvested from the mixed culture.

[c] Isolated by J. W. Hartley from a wild mouse. This virus is neither a clear-cut xenotropic nor a mink cell focus-inducing (MCF) virus. It induces MCF-like cytopathic effect in mink cells (personal communication, J. W. Hartley).

harvested from B-mink cells superinfected with SL-XT-1. In addition to embryo cells from NFS and BALB/c mice, we used embryo cells from DDD and C57BL/6 mice as *Fv-1*^{nn} and *Fv-1*^{bb} cells and got almost the same viral titers as were obtained in NFS and BALB/c mouse embryo cells (data not shown). As illustrated in Fig. 1B and C, ecotropic genomes from the same culture retained their B-tropism; the xenotropic genomes did not contribute N-tropism to a coinfecting B-tropic ecotropic virus. When AKR- and Fr-mink cells were superinfected with SL-XT-1 xenotropic virus, no clear contribution of *Fv-1* determinant from SL-XT-1 to the phenotypically mixed viruses was noted; the X(e) virions in the mixed viral pool always showed the *Fv-1* restriction pattern of the N- and NB-tropic ecotropic virus.

To study the properties of SL-XT-1 xenotropic MuLV in more detail, SL-XT-1 xenotropic MuLV-infected SC-1 cells, superinfected with B-tropic ecotropic WN1802B virus, were cloned, and several doubly infected lines were established from single cells. The properties of progeny virus from each clonal line were compared with those of the mass cultures. We have previously documented (14) that ecotropic and amphotropic genomes (7) tend to maintain their homologous tropism when phenotypically mixed virus pools are harvested from mass cultures of SC-1 cells chronically infected with N-tropic amphotropic MuLV and B-tropic ecotropic MuLV. We also showed that there are marked differences in virus production and tropism between the clonal lines upon cloning of the superinfected SC-1 cells. In Table 2, the virus populations harvested from doubly infected clonal SC-1 cells are characterized. Some interesting points are apparent. First, clones varied with respect to the production of ecotropic and xenotropic virus titers. Second, both viruses tended to show dual restriction. Especially clones 19 and 52 produced ecotropic and xenotropic MuLV with distinct dual restriction, suggesting that the SL-XT-1 xenotropic virus has N-tropic *Fv-1* determinant. As shown by the growth curves of ecotropic and xenotropic viruses (Fig. 1A and B), the *Fv-1* tropism of xenotropic genomes with ecotropic host range, X(e), was affected by the coinfection with the xenotropic virus; that of the ecotropic genome was not. However, the *Fv-1* tropism of ecotropic and xenotropic genomes from some clonal lines was affected by the coinfection with the xenotropic virus. Conversion of *Fv-1* tropism of ecotropic virus from B-tropic to dual restrictive was clearly demonstrated in clones 19 and 52. However, the isolation of clonal lines which showed distinct dual restriction, such as clone 19, was very difficult; only 2 of 18 clones established showed dual restriction. Ecotropic and xenotropic virus harvested from uncloned mass culture chronically infected with both viruses was always B-tropic (Table 2).

If our hypothesis to detect *Fv-1* determinant in xenotropic viruses is correct, then all data observed in the growth curves of phenotypically mixed X(e) virion and in the *Fv-1* restriction patterns of virions produced by clonal lines of doubly infected SC-1 cells firmly support the suggestion that SL-XT-1 xenotropic virus has an N-tropic *Fv-1* determinant in active form, although these data do not elucidate whether p30 is an *Fv-1* determinant.

An important weak point of our biological detection system is the technical difficulty of preparing a phenotypically mixed virus pool adequate for detecting the cryptic *Fv-1* tropism of xenotropic virus. When preparing viruses mixed between ecotropic and xenotropic virus, our detection system may be adversely affected by the weaker activity, or the lesser amount of *Fv-1* determinant produced by the xenotropic MuLV gene than by the ecotropic MuLV gene in the phenotypically mixed virus or both. In general, the *Fv-1* tropism of the phenotypically mixed E(e) and X(e) virion between ecotropic and xenotropic viruses showed the *Fv-1* restriction pattern of the ecotropic virus. When NRK cells or SC-1 cells were doubly infected, even with SL-XT-1 xenotropic virus and N-, B-, or NB-tropic ecotropic virus, the harvested E(e) and X(e) virions always showed the *Fv-1* tropism of the ecotropic virus (data not shown). In obtaining adequate phenotypically mixed virus between ecotropic viruses with different *Fv-1* tropism or between ecotropic and amphotropic viruses, selection of the cells to be infected, the time of harvest, and the multiplicity of infection were very important factors. Conversion of NB-tropic ecotropic virus to N- or B-tropic by mixed infection with N- or B-tropic virus was particularly difficult. When superinfecting NB-tropic ecotropic virus-producing Fr-mink cells with xenotropic virus with cryptic N-tropic activity, the contribution of the N-tropic *Fv-1* determinant from the xenotropic virus to X(e) and E(e) virions could be expected. However, we could not obtain phenotypically mixed N-tropic X(e) or E(e) virions from Fr-mink cells superinfected with SL-XT-1 virus. Therefore, the failure to demonstrate *Fv-1* determinant in Cas E #1-IU MuLV may not be indicative of a lack of *Fv-1* determinant but, rather, may demonstrate the difficulties in preparing an adequate phenotypically mixed virus pool in which cryptic *Fv-1* tropism is detectable. Furthermore, isolation of clonal cell lines producing adequate phenotypically mixed virus was also difficult.

Another question is whether SL-XT-1 is a real

TABLE 2. Host range of viruses produced by clones of SC-1 cells isolated after chronic infection with xenotropic (SL-XT-1) and B-tropic ecotropic (WN1802B) MuLVs

Virus	Cells infected (*Fv-1* type)																	
	Titer (log) of xenotropic genomes[a], X(e) and X(x) virion									Titer (log) of ectropic genomes[a], E(e) and E(x) virion								
	NFS-ME (*Fv-1*^{n})	DDD-ME (*Fv-1*^{n})	BALB/c-ME (*Fv-1*^{b})	C57BL/6-ME (*Fv-1*^{b})	(N × B) F_1[b] (*Fv-1*^{nb})	FL (*Fv-1*$^-$)	S^+L^- mink	N/B ratio[c] (log) (N-B)	Tropism[d]	NFS-ME (*Fv-1*^{n})	DDD-ME (*Fv-1*^{n})	BALB/c-ME (*Fv-1*^{b})	C57BL/6-ME (*Fv-1*^{b})	(N × B) F_1[b] (*Fv-1*^{nb})	SC-1 (*Fv-1*$^-$)	Mink lung cell	N/B ratio[c] (log) (N-B)	Tropism[d]
Phenotypically mixed virus																		
From uncloned SC-1	0.5	0.3	2.3	2.0	0.8	2.5	4.0	−1.8	B	0.6	0.5	3.0	2.8	0.5	5.0	3.5	−2.4	B
From cloned SC-1, Cl-16	0.5	0.1	2.2	2.0	1.0	2.9	4.2	−1.7	B	0.1	Neg.	3.2	3.4	0.5	5.3	3.4	−3.1	B
From cloned SC-1, Cl-19	0.4	0.5	0.5	0.3	0.5	2.6	4.4	−0.1	D-R	0.5	0.6	0.8	1.0	Neg.	3.6	2.2	−0.3	D-R
From cloned SC-1, Cl-51	0.1	0.1	1.6	1.8	0.3	2.6	3.8	−1.5	B	Neg.	Neg.	2.8	3.0	Neg.	4.6	3.5	−2.8	B
From cloned SC-1, Cl-52	Neg.	0.0	0.0	Neg.	Neg.	1.5	2.8	0	D-R	0.1	0.2	0.7	0.7	Neg.	3.2	3.0	−0.6	D-R
Standard virus																		
4070A (N-tropic amphotropic)	4.0	4.2	1.7	1.5	2.3	4.6	5.6	+2.3	N	Neg.		Neg.		Neg.	Neg.	Neg.		
WN1802B (B-tropic ecotropic)	Neg.		Neg.		Neg.	Neg.	Neg.			0.1		3.7		1.8	4.7	Neg.	−3.6	B
Rauscher (NB-tropic ecotropic)	Neg.		Neg.		Neg.	Neg.	Neg.			5.0		4.6		5.0	5.7	Neg.	0.4	NB
SL-XT-1 (xenotropic)	Neg.		Neg.		Neg.	Neg.	5.2			Neg.		Neg.		Neg.	Neg.			

[a] To detect X(e) virions, 3 days postinfection the mouse cells were UV-irradiated and overlaid with S^+L^- mink cells (17). S^+L^- cells were also infected to titrate xenotropic virus with xenotropic host range, X(x). E(e) virions were detected by infecting mouse cells and by the UV-XC test (19). Mink cells were infected to titrate ecotropic genomes with xenotropic host range; E(x) virions were detected by UV-irradiating the mink cells 3 days postinfection and overlaying them with SC-1 cells and by the UV-XC test (12, 13). Neg., Negative.

[b] Embryo cells from a (NFS × BALB/c) F_1 mouse.

[c] Titers in NFS-ME minus titer in BALB/c-ME.

[d] D-R, Dual restrictive.

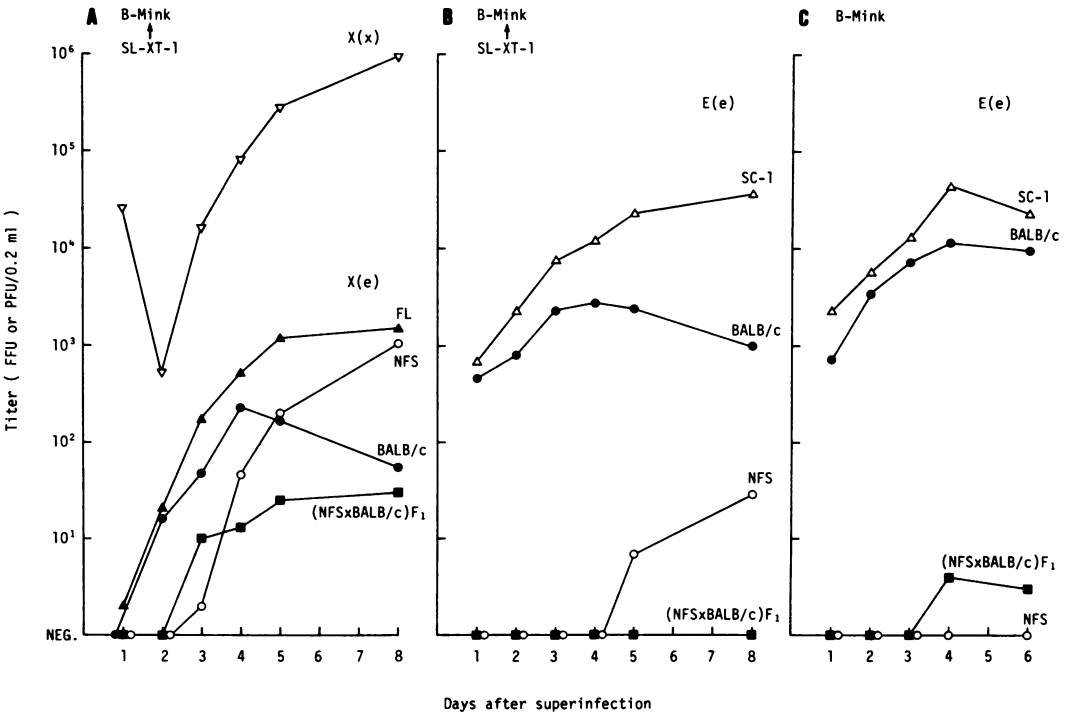

FIG. 1. Growth curves of phenotypically mixed viruses in B-tropic WN1802B virus-producing B-mink cells after superinfection with SL-XT-1 xenotropic virus isolated from a 3-month-old SL mouse. On day −2, 2×10^6 clonal cells per 75 cm^2 were plated in plastic flasks; the next day they were exposed to 20 μg of iododeoxyuridine per ml, and on day 0 they were superinfected with SL-XT-1 virus at an input multiplicity of approximately 20 focus-forming units per cell. The culture medium was replaced every 24 h. (A) Xenotropic virus with xenotropic host range X(x) was detected by infecting S^+L^- cells, and xenotropic virus with ecotropic host range X(e) was detected by infecting NFS-ME (*Fv-1*^{nn}-type cell), BALB/c-ME (*Fv-1*^{bb}-type cell), (NFS × BALB/c)F_1 (*Fv-1*^{nb}-type cell), or FL cells (*Fv-1*$^{--}$-type cell) and overlaying them with S^+L^- mink cells after UV irradiation. (B) Ecotropic virus with ecotropic host range E(e) was detected by infecting NFS-ME, BALB/c-ME, (NFS × BALB/c)F_1-ME, or SC-1 cells (*Fv-1*$^{--}$-type cells) and by using UV-XC procedures. (C) Ecotropic virus in B-mink cells in the absence of superinfection.

endogenous xenotropic virus or a recombinant virus between an N-tropic ecotropic and xenotropic virus. Generally, it is difficult to isolate an endogenous xenotropic virus from SL mice; our attempts to isolate xenotropic virus from leukemic or preleukemic SL mice usually resulted in the isolation of xenotropic viruses with properties of mink cell focus-inducing virus rather than endogenous xenotropic virus without these properties. Our fingerprint analysis of the viral RNA suggested these mink cell focus-inducing xenotropic viruses to be recombinant viruses (unpublished data). However, whether SL-XT-1 is a real endogenous xenotropic virus remains an open question since we could not isolate a virus which seemed to be a real endogenous xenotropic virus from SL mouse embryo cells. If SL-XT-1 is a recombinant virus between xenotropic and N-tropic virus, then the possibility arises that *Fv-1* tropism contributed by SL-XT-1 virus is not coded for by a gene originally derived from xenotropic virus but, rather, by a gene from an endogenous ecotropic virus of the SL mouse. In other words, the endogenous xenotropic virus may not have a gene coded for the *Fv-1* determinant. Gautsch et al. (3) have identified two different xenotropic viruses from BALB/c mice on the basis of the p30 structure. However, a single Mendelian gene has been identified for induction of the endogenous xenotropic virus in BALB/c mice (16), suggesting that one of the xenotropic viruses from BALB/c mice classified by Gautsch et al. (3) is also a recombinant virus between endogenous xenotropic virus and an unknown gene from the mouse. The unknown gene coding for B-tropism may exist within the cellular gene of BALB/c mice, out of the viral gene. This means that a B-tropic ecotropic virus need not always be a recombinant virus between xenotropic and ecotropic virus, but could be a direct recombinant virus between ecotropic virus and an unknown gene in the mouse.

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