Jpn. J. Cancer Res. 81, 768–772, August 1990

Fv-1 Restriction of Endogenous Feline C-Type RD114 Virus Genome Phenotypically Mixed with Ecotropic Murine Leukemia Viruses

Kazuhiro Kakimi, Yoshiko Kishida, Ikuko Higuchi, Takahiro Kiyomasu, Hiroyuki Sakai, Riri Shibata, Shin-ichiro Yanagawa, Akio Adachi and Akinori Ishimoto[1]

Laboratory of Gene Analysis, Department of Viral Oncology, Institute for Virus Research, Kyoto University, Shogoin-Kawaracho, Sakyo-ku, Kyoto 606

Endogenous feline leukemia RD114 virus genome rendered capable of infecting mouse cells by phenotypic mixing with an ecotropic murine leukemia virus (MuLV) exhibited the Fv-1 restriction pattern of the ecotropic murine virus. However, RD114 genomes phenotypically mixed with ecotropic MuLV showed one-hit dose-response kinetics, even when titrated with murine cells with the restricted Fv-1 phenotype.

Key words: Murine leukemia virus — Feline C-type RD114 virus — Fv-1 gene

Restriction of murine leukemia virus (MuLV) infection by the murine Fv-1 gene is a major element in the pathogenesis of MuLV infection. The mechanism of the restriction is not clear, but blocking of viral replication appears to occur prior to integration of the provirus.[1–3] Reports that linear DNA is synthesized normally while circular forms and integrated DNA are greatly reduced in the restricted cells have suggested that inhibition of circular DNA formation is a part of the Fv-1 restriction mechanism.[4–7] However, recent studies on the mechanism of retroviral DNA integration have shown that linear DNA is an intermediate in the integration process rather than circular DNA.[8,9]

Phenotypic mixing of ecotropic MuLVs with different degrees of tropism has provided several important insights into Fv-1 restriction.[10,11] In particular, it has been shown that the Fv-1 restriction phenotype is not determined directly by the genome carried by the virion, but by the p30 *gag*-gene product carried by the virion.[12–15] The infectivity of defective murine leukemia virus genomes and retrovirus vector genomes constructed with MuLV was also reported to be restricted by the Fv-1 gene.[16] However, the phenomenon of multihit kinetics was not observed in the case of infection by virions with defective replication genomes carried by a retrovirus vector system. Although much is known about biological phenomena which are related to the Fv-1 gene, little is known about the mechanisms underlying these phenomena. We investigated which kinds of viruses in addition to the murine retroviruses were restricted by the Fv-1 gene when phenotypically mixed with ecotropic MuLV, in the hope that analysis of the properties of different viruses restricted by the Fv-1 gene might give us some new knowledge about the mechanisms of the Fv-1 gene system.

In this paper, we report that RD114 viral genomes, which were rendered capable of infecting mouse cells by phenotypic mixing with an ecotropic MuLV, exhibited the Fv-1 restriction pattern of the ecotropic MuLV.

MATERIALS AND METHODS

Tissue culture Secondary mouse embryo cultures (ME) were prepared by standard techniques,[17] and were grown and maintained in 5% heated fetal calf serum (FCS) in Eagle's minimal essential medium (EMEM). Mink lung cells,[18] mink S+L− cells,[19] and ecotropic MuLV-producing AKR-mink, B-mink and Fr-mink cells[20] were grown and maintained in 5% heated FCS in the Dulbecco-Vogt modification of EMEM.

Viruses Endogenous feline leukemia RD114 virus[21] was obtained from Dr. P. S. Sarma, NIH, USA. The various virions in the phenotypically mixed harvests are referred to as the genotype followed by the host range phenotype in parenthesis; i.e., RD114(MuLV) virions contain RD114 genomes in an envelope capable of penetrating mouse cells.

Assay Ecotropic MuLV genome with an MuLV host range, MuLV(MuLV), was detected by infecting mouse cells (embryonic cells from NFS mice) and 5 days later carrying out the UV-XC test.[22] RD114 virus genome with an ecotropic MuLV host range, RD114(MuLV), was detected by infecting the mouse cells. Three days later UV irradiation and overlaying with mink S+L− cells[19] were performed, and 7 days later the cultures were assessed for foci of transformation. Ecotropic MuLV genome with an RD114 virus host range, MuLV(RD-114), was detected by infecting mink cells. Three days later UV irradiation and overlaying with SC-1 cells[23] were performed, and after 3 more days the UV-XC test was performed. RD114 virus genome with an RD114 virus host range, RD114(RD114), was detected by in-

[1] To whom correspondence should be addressed.

fecting S+L− cells and 7 days later reading the culture for foci of transformation.

RESULTS

RD114 virus is an endogenous feline C-type retrovirus which infects mink cells and does not infect mouse cells.[21] However, a phenotypically mixed RD114 viral genome with a murine leukemia host range can infect mouse cells and produce RD114 viral progeny. Phenotypic admixture between the RD114 virus and ecotropic MuLVs was achieved by superinfecting ecotropic MuLV-producing mink cells with RD114 virus. Clonal lines of mink cells producing N-tropic, B-tropic, and NB-tropic ecotropic MuLVs, which are referred to as AKR-mink, B-mink, and Fr-mink, respectively, were isolated by infecting mink cells with phenotypically mixed ecotropic MuLVs with a xenotropic MuLV host range.[20] The infectivities of RD114 virus and MuLV singly and when phenotypically mixed are shown in Table I. RD114 genomes were rendered capable of infecting mouse cells and MuLV genomes were rendered capable of infecting mink cells by the phenotypic mixing process. Mixed harvests were titrated in duplicate in a variety of mouse cell cultures. One set of cultures was assayed for foci of ecotropic MuLV production by the XC test, i.e., the MuLV genome with an MuLV host range shown as MuLV(MuLV) in Table II. The other set of cultures was assayed for cells producing RD114 virus, i.e., cells infected by the RD114 genome with an MuLV

Table I. Infectivity of the RD114 Virus and MuLVs Singly and when Phenotypically Mixed

Virus	Harvested from	Titer (log/0.2 ml, PFU or FFU) in assays for			
		MuLV(MuLV)[a]	RD114(MuLV)[b]	MuLV(RD114)[c]	RD114(RD114)[d]
RD114	mink cell	neg.	neg.	neg.	4.3
MuLV					
AKRL-1	AKR-mink[e]	3.9	neg.	neg.	neg.
WN1802B	B-mink[f]	0.8(2.8)[h]	neg.	neg.	neg.
Friend	Fr-mink[g]	4.0	neg.	neg.	neg.
Mixed infection harvest					
AKRL-1 & RD114	AKR-mink	3.5	1.3	2.0	3.3
WN1802B & RD114	B-mink	0.6(3.6)[h]	1.2(2.7)[h]	1.9	3.9
Friend & RD114	Fr-mink	3.6	2.6	2.5	4.0

a) Ecotropic murine leukemia virus (MuLV) genome with an MuLV host range.
b) RD114 virus genome with an ecotropic MuLV host range.
c) Ecotropic MuLV genome with an RD114 virus host range.
d) RD114 virus genome with an RD114 virus host range.
e) Clonal mink cell line producing N-tropic ecotropic AKRL-1 MuLV.
f) Clonal mink cell line producing B-tropic ecotropic WN1802B MuLV.
g) Clonal mink cell line producing NB-tropic ecotropic Friend MuLV.
h) Titers infecting permissive mouse cells (embryonic cells from BALB/c mice).
neg.=negative

Table II. Fv-1 Restriction of RD114 Virus with an Ecotropic MuLV Host Range

Cell titrated on (Fv-1 type)	Titer (log.PFU or FFU/0.2 ml) of RD114 virus phenotypically mixed with		
	AKR-L1 MuLV (N-tropic)	WN1802B MuLV (B-tropic)	Friend MuLV (NB-tropic)
NFS-ME[a] (Fv-1^{nn})	2.3[b] (3.5)[c]	0.6 (1.0)	2.0 (3.6)
BALB/c-ME (Fv-1^{bb})	0.8 (1.0)	2.7 (3.6)	2.7 (3.4)
SC-1 (Fv-1^{--})	2.9 (3.6)	3.0 (2.7)	2.8 (3.9)
BALB/c×NFS-F_1-ME (Fv-1^{nb})	1.0 (0.6)	0.9 (1.1)	1.8 (3.4)

a) Secondary culture of mouse embryonic cells.
b) Titer of RD114 virus with an ecotropic MuLV host range (see Table I).
c) Titer of MuLV detected by the XC test.

host range shown as RD114(MuLV). The different classes of virions in Table I are shown as the genotype followed by the host range phenotype in parenthesis.[17] Data in Table II show that the infectivity of RD114 virus with a murine leukemia virus host range was regulated by the murine Fv-1 gene system, since RD114(MuLV) particles produced by mixture with ecotropic N-, B-, or NB-tropic viruses showed the corresponding Fv-1 tropism. For example, RD114 virus with an MuLV host range produced by AKR-mink cells achieved the infection of N-type mouse embryo cells prepared from NFS mouse embryos (Fv-1^{nn}) more efficiently than that of B-type cells prepared from BALB/c mouse embryos (Fv-1^{bb}) or heterozygous cells prepared from (NFS×BALB/c)F_1 embryos. The magnitude of restriction for MuLV(MuLV) in restricted cells was more than 100-fold greater than in permissive cells, but the magnitude of restriction for the RD114(MuLV) virion in restricted cells was less than 100-fold greater than in permissive cells.

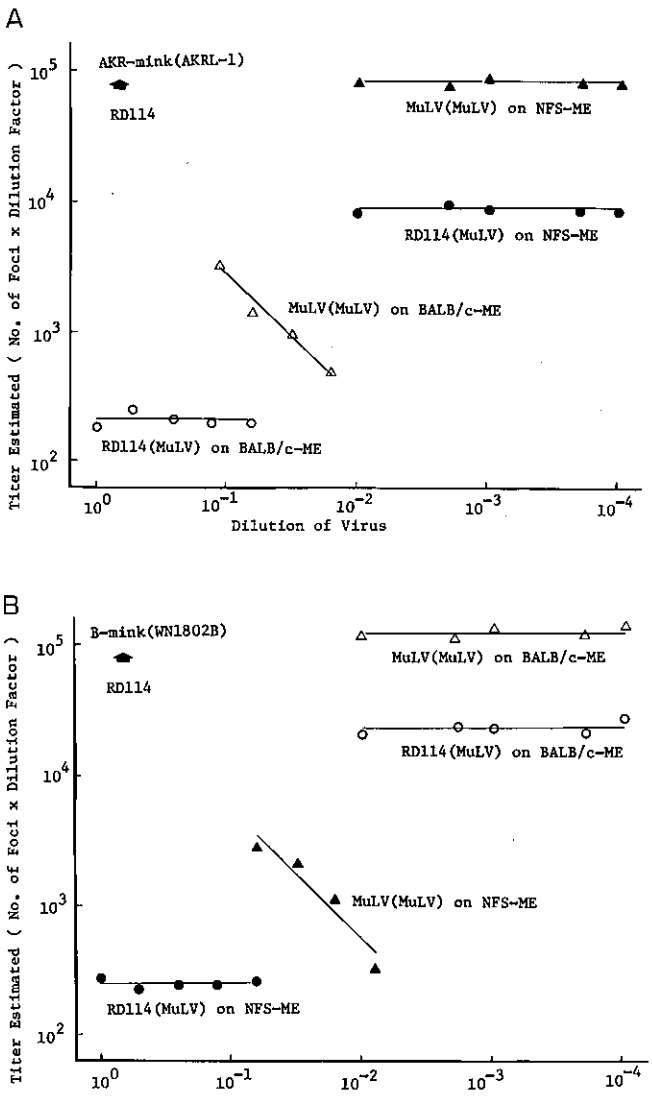

Fig. 1. Titration of MuLV(MuLV) and RD114(MuLV) virions in a mixed harvest of N-tropic ecotropic MuLV (A) and B-tropic ecotropic MuLV (B) with RD114 virus in permissive or restricted cells. The phenotypic mixtures between ecotropic MuLV and RD114 virus and the methods for assay were as described in the text.

Investigation of phenotypic mixing among MuLVs has shown that N-tropic and B-tropic viruses, but not NB-tropic viruses, can contribute Fv-1 tropism determinants, while NB-tropic viruses can acquire the B-tropism phenotype in mixed infection harvests.[13, 15, 24] Our findings indicated that the RD114 virus does not contribute Fv-1 determinants of its own to the ecotropic MuLVs, as has been shown in the case of xenotropic MuLV.[25] Thus, the RD114 virus can be considered as being analogous to NB-tropic MuLV genomes in this respect.

Fv-1 restriction may be due in part to a multihit requirement for initiation of infection, i.e., in permissive cells an ecotropic MuLV would show single-hit dose-response kinetics.[26] Experiments were conducted to determine whether or not RD114(MuLV) showed a similar phenomenon. The infection procedure used to obtain the dose-response curves was slightly different from the one used to obtain the data in Tables I and II, since the titers of ecotropic MuLVs and RD114 virus in the mixed harvests from mink cells were low to give dose-response curves in restricted cells. To obtain the data shown in Tables I and II, embryo cells grown in 5-cm dishes with 4 ml of tissue culture medium were inoculated with 0.2 ml of diluted virus. The virus inoculated was diluted again 21-fold by the medium in the dish. However, to obtain the dose-response curves shown in Fig. 1, dishes without tissue culture medium were inoculated with 4 ml of diluted virus.

The MuLV(MuLV) particles from the mixed harvest infected permissive cells with one-hit kinetics and showed clear two-hit dose-response kinetics in the restricted cells (Fig. 1). However, the RD114(MuLV) particles showed one-hit kinetics for the both permissive and restricted cells.

DISCUSSION

The phenomenon of multihit titration kinetics in restricted cells has been one of the controversial problems with respect to the Fv-1 gene system. The lack of a two-hit kinetic response by restricted cells infected with RD114(MuLV) particles may have been due to the effects of coinfection by MuLV(MuLV) particles present in the mixed virus pool,[27, 28] as has been shown for

ecotropic and xenotropic MuLVs. Coinfection with MuLV(MuLV) might have converted the two-hit titration pattern of RD114(MuLV) virions in restricted cells into a one-hit pattern. However, the titer of RD114-(MuLV) virions in restricted cells, even with the MuLV-(MuLV) helper, was more than 10 times lower than the titer in permissive cells, and coinfection with excess MuLV(MuLV) virions (>m.o.i. 10) did not raise the titer detected in permissive cells.

Another possibility for the lack of two-hit kinetics with the RD114(MuLV) virus is that RD114(MuLV) particles do not produce a two-hit kinetic pattern in restricted cells like the retrovirus vector particles irrespective of coinfection with the MuLV(MuLV) helper.[16]

Results obtained with RD114 virus and a retrovirus vector suggest that some of the viral RNA sequences carried by the MuLV virus, but not by the RD114 virus may have a role in the production of multihit titration kinetics in Fv-1-restricted cells.

Although the evidence that there is a recognition factor of the viral or cellular genome for the Fv-1 determinant p30[29-31] does not necessarily mean that this determinant directly interacts with the viral or cellular genome DNA, it is possible that there is some specificity of p30 binding to viral RNA through p12 during formation of the inner core of the virion. Alternatively, p30 *gag* gene product may be secondarily aasociated with the homologous viral genomic RNA by specific binding of the uncleaved *gag* gene precursor to the genomic RNA,[32] since it is known that p12 *gag* gene product binds to the viral RNA.[33]

The RD114 virus is reported to be distantly related to MuLV, and to be closely related to BaEV,[34, 35] which suggests that the infectivity of BaEV and the feline leukemia virus may also be restricted by the Fv-1 gene. Our findings suggest that Fv-1 gene products and MuLV p30 regulate a ubiquitous process which is necessary for retrovirus replication, i.e., interaction between MuLV p30 and Fv-1 gene products may occur not only in MuLV but also in retrovirus replication in general.

(Received January 5, 1990/Accepted May 11, 1990)

REFERENCES

1) Jolicoeur, P. and Baltimore, D. Fv-1 gene product on proviral DNA formation and integration in cells infected with murine leukemia viruses. *Proc. Natl. Acad. Sci. USA,* **73,** 2236–2240 (1976).
2) Sveda, M. M. and Soeiro, R. Host restriction of Friend leukemia virus: synthesis and integration of the provirus. *Proc. Natl. Acad. Sci. USA,* **73,** 2356–2360 (1976).
3) Hsu, I. C., Yang, W. K., Tennant, R. W. and Brown, A. Transfection of Fv-1 permissive and restrictive mouse cells with integrated DNA of murine leukemia viruses. *Proc. Natl. Acad. Sci. USA,* **75,** 1451–1455 (1978).
4) Jolicoeur, P., and Rassart, E. Effect of Fv-1 gene product on synthesis of linear and supercoiled viral DNA in cells infected with murine leukemia virus. *J. Virol.,* **33,** 183–195 (1980).
5) Yang, W. K., Kiggans, J. O., Yang, D. M., Ou, C. Y., Tennant, R. W., Brown, A. and Bassin, R. H. Synthesis and circularization of N- and B-tropic retroviral DNA in Fv-1 permissive and restrictive mouse cells. *Proc. Natl. Acad. Sci. USA,* **77,** 2994–2998 (1980).
6) Chinsky, J. and Soriro, R. Fv-1 host restriction of Friend leukemia virus: analysis of unintegrated proviral DNA. *J. Virol.,* **40,** 45–55 (1981).
7) Iwamoto, A., Masuda, M. and Yoshikura, H. Synthesis of proviral DNA in inbred mouse-derived clones of cells expressing different Fv-1 phenotypes. *J. Gen. Virol.,* **66,** 2265–2269 (1985).
8) Brown, P. O., Bowerman, B., Varmus, H. E. and Bishop, J. M. Correct integration of retroviral DNA *in vitro. Cell,* **49,** 347–356 (1987).
9) Fujiwara, T. and Mizuuchi, K. Retroviral DNA integration: structure of an integration intermediate. *Cell,* **54,** 497–504 (1988).
10) Huang, A. S., Besmer, P., Chu, L. and Baltimore, D. Growth of pseudotypes of vesicular stomatitis virus with N-tropic murine leukemia virus coats in cells resistant to N-tropic viruses. *J. Virol.,* **12,** 659–662 (1973).
11) Krontiris, T. G., Soeiro, R. and Fields, B. N. Host restriction of Friend leukemia virus. Role of outer viral coat. *Proc. Natl. Acad. Sci. USA,* **70,** 2549–2553 (1973).
12) Bassin, R. N., Gerwin, B. I., Duran-Troise, G., Gisselbrecht, S. and Rein, A. Murine sarcoma virus pseudotypes acquire a determinant specifying N- or B-tropism from leukemia virus during rescue. *Nature,* **256,** 223–225 (1975).
13) Rein, A., Kashmiri, S. V. S., Bassin, R. H., Gerwin, B. I. and Duran-Troise, G. Phenotypic mixing between N-tropic and B-tropic murine leukemia viruses: infectious particles with dual sensitivity to Fv-1 restriction. *Cell,* **7,** 373–379 (1976).
14) Duran-Troise, G., Bassin, R. H., Rein, A. and Gerwin, B. I. Loss of Fv-1 restriction in BALB/3T3 cells following infection with a single N-tropic murine leukemia virus particle. *Cell,* **10,** 479–488 (1977).
15) Kashmiri, S. V. S., Rein A., Bassin, R. H., Gerwin, B. I. and Gisselbrecht, S. Donation of N-tropic or B-tropic phenotype to NB-tropic murine leukemia virus during mixed infections. *J. Virol.,* **22,** 626–633 (1977).
16) Boone, L. R., Innes, C. L., Glover, P. L. and Linney, E. Development and characterization of an Fv-1 sensitive

retrovirus-packaging system: single-hit titration kinetics observed in restrictive cells. *J. Virol.*, **63**, 2592–2597 (1989).
17) Ishimoto, A., Hartley, J. W. and Rowe, W. P. Detection and quantitation of phenotypically mixed viruses: mixing of ecotropic and xenotropic murine leukemia viruses. *Virology*, **81**, 263–269 (1977).
18) Henderson, I. C., Lieber, M. M. and Todaro, G. J. Mink cell line MulLu(CCL64). Focus formation and generation of "non-produced" transformed cell lines with murine and feline sarcoma viruses. *Virology*, **60**, 282–287 (1974).
19) Peebles, P. T. An *in vitro* focus-induction assay for xenotropic murine leukemia virus, feline leukemia virus C, and the feline-primate viruses RD114/ccc/M-7. *Virology*, **67**, 288–291 (1975).
20) Ishimoto, A., Hartley, J. W. and Rowe, W. P. Phenotypic mixing between murine leukemia virus: characteristics of ecotropic virus infection of heterologous cells. *Virology*, **91**, 464–471 (1978).
21) McAllister, R. M., Nicolson, M., Gardner, M. B., Rongey, R. W., Rasheed, S., Sarma, R. S., Huebner, R. J., Hatanaka, M., Oroszlan, S., Gieden, R. V., Kabigting, A. and Vernon, L. C-type virus released from cultured human rhabdomyosarcoma cells. *Nature New. Biol.*, **235**, 3–6 (1972).
22) Rowe, W. P., Pugh, W. E. and Hartley, J. W. Plaque assay techniques for murine leukemia viruses. *Virology*, **42**, 1136–1139 (1970).
23) Hartley, J. W. and Rowe, W. P. Clonal cell lines from a feral mouse embryo which lack host range restriction for murine leukemia viruses. *Virology*, **65**, 128–134 (1975).
24) Ishimoto, A., Hartley, J. W. and Rowe, W. P. Fv-restriction of xenotropic and amphotropic murine leukemia virus genomes phenotypically mixed with ecotropic virus. *Virology*, **93**, 215–225 (1979).
25) Sakai, K., Narita, H., Adachi, A., Tsuruta, S., Yorifuji, T. and Ishimoto, A. 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. *J. Virol.*, **42**, 331–336 (1982).
26) Pincus, T., Hartley, J. W. and Rowe, W. P. A major genetic locus affecting resistance to infection with murine leukemia viruses. IV. Dose-response relationships in Fv-1 sensitive and resistant cell culture. *Virology*, **65**, 333–342 (1975).
27) Yoshikura, H. Ultraviolet inactivation of murine leukemia virus and its assay in permissive and non-permissive cells. *Int. J. Cancer*, **11**, 739–746 (1973).
28) Niwa, O., Decleve, A. and Kaplan, H. S. Conversion of restrictive mouse cells to permissiveness during sequential and mixed double infection by murine leukemia viruses. *Virology*, **74**, 140–153 (1976).
29) Hopkins, N., Schindler, J. and Hynes, R. Six NB-tropic murine leukemia viruses derived from a B-tropic virus of BALB/c have altered p30. *J. Virol.*, **21**, 309–318 (1977).
30) Schindler, J., Hynes, R. and Hopkins, N. Evidence for recombination between N- and B-tropic murine leukemia viruses: analysis of three virion proteins by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. *J. Virol.*, **23**, 700–707 (1977).
31) Gautsch, J. W., Elder, J. H., Schindler, J., Jensen, F. C. and Lerner, R. A. Structural markers on core protein p30 of murine leukemia virus: functional correlations with Fv-1 tropism. *Proc. Natl. Acad. Sci. USA*, **75**, 4170–4174 (1978).
32) Bolognesi, D. P., Montelaro, R. C., Frank, H. and Schafer, W. Assembly of type C oncornaviruses: a model. *Science*, **199**, 183–186 (1978).
33) Sen, A., Sherr, C. J. and Todaro, G. J. Specific binding of the type C viral core protein p12 with purified viral RNA. *Cell*, **7**, 21–32 (1976).
34) Todaro, G. J., Benveniste, R. E., Lieber, M. M. and Sheur, C. J. Characterization of a type C virus released from the porcine cell line PK(15). *Virology*, **58**, 65–74 (1974).
35) Barbacid, M., Stephenson, J. R. and Aaronson, S. A. Evolutionary relationships between *gag* gene-coded proteins of murine and primate endogenous type C RNA viruses. *Cell*, **10**, 641–648 (1977).