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Simple, Quantitative Assay for Both Xenotropic Murine Leukemia and Ecotropic Feline Leukemia Viruses

PETER J. FISCHINGER, CHARLOTTE S. BLEVINS, AND SHIGEKO NOMURA

Viral Leukemia and Lymphoma Branch, National Cancer Institute, Bethesda, Maryland 20014

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A cat cell line carrying the genome of a murine sarcoma virus developed discrete foci in linear response to infection with feline leukemia virus or xenotropic murine C-type virus.

Mammalian leukemia type oncornaviruses can be distinguished on the basis of their host range into those which can grow (ecotropic) and those which are restricted for growth (xenotropic) in cells from which they originate. Up to now no direct assay was available for xenotropic mouse leukemia viruses (MuLV-X), and indirect methods such as murine sarcoma virus (MSV) pseudotype assay or group-specific (gs) antigen induction had to be used (1, 8, 9). Sarcoma-positive, leukemia-negative mouse cells transformed only by Moloney (M-) MSV react to replicating ecotropic MuLV by the induction of lytic-type foci in the monolayer (3). In this study, an analogous M-MSV-transformed cat cell line readily detected both replicating MuLV-X and feline leukemia virus (FeLV).

Several CCC cat cell sublines were transformed after single-hit M-MSV infection. The cells were positive for the gs-1 of MuLV and initially yielded no virus. After several weeks the endogenous, xenotropic RD-114-like oncornavirus, (CCC) virus and MSV(CCC) pseudotype were relased (5). Relatively flat sublines and subclones 81, 89 of the cloned 8C line reacted with a transforming effect after the addition of FeLV. Large amounts of FeLV or MuLV-X caused the cell layer to round up completely, but at higher dilutions discrete focal lesions could be seen as piled up, loosely attached cells (Fig. 1). Foci produced by either FeLV or MuLV-X were indistinguishable. The foci released pseudotype MSV coated with the infecting virus as determined by host range and neutralization with specific antisera. Because of attending MSV(CCC) and CCC (5), a new pseudotype virus was purified by two passages through cat embryo cells (FEF) which were virus negative and completely restricted CCC virus growth.

A variety of both FeLV and MuLV-X stocks could be detected with high sensitivity (Table 1). In comparative assays for FeLV, the quantity of FeLV focus-inducing units (FIU) in 8C cells was about twofold less than replicating FeLV as determined by the induction of interference in FEF cells (4). The parental CCC cells are also fourfold less sensitive to MSV (FeLV) or FeLV than are FEF cells (7). We induced an MuLV-X from BALB/c cells into FEF cells and compared this virus to some other MuLV-X isolates (P. J. Fischinger, manuscript in preparation). This MuLV-X grown in several host cells produced identical foci. MuLV-X emerging from mouse cells could be assayed directly. Thus, standard MuLV or MSV stocks could be monitored for contamination by MuLV-X. Pure Moloney or Gross MuLV could be obtained by passage through outbred Swiss cells; these viruses ($>10^6$ FIU in sarcoma-positive, leukemia-negative mouse cells) did not give foci in 8C assay cells. In contrast, infection by pure ecotropic MuLV of Kirsten MSV-transformed BALB 3T3 cells (1) yielded more than 10^3 FIU per ml of MuLV-X. CCC virus or the RD-114 virus isolates did not induce foci in 8C cells.

A titration pattern of either FeLV or MuLV-X was performed in 8C cells (Fig. 2). The observed numbers of foci were directly proportional to virus dilution, indicating a one-hit response for both MuLV-X and FeLV. Other MuLV-X and FeLV strains grown in different species tested were also linear.

This simple assay has a number of advantages: (i) it is direct, sensitive, and relatively rapid; (ii) mouse-cell-derived MuLV-X can be

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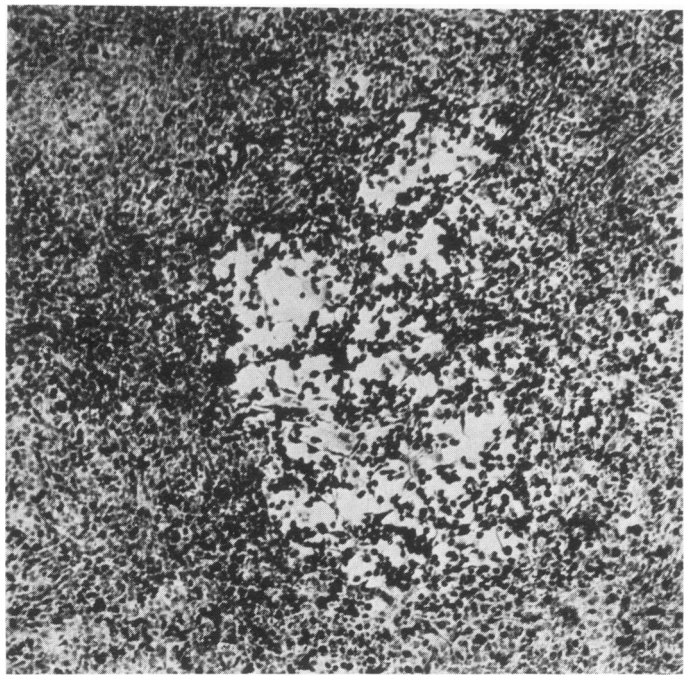

FIG. 1. *A focus induced by FeLV in the cloned 8C subline. 8C cells were grown in McCoy 5A medium supplemented with antibiotics and 15% fetal calf serum as described (4). Cells (2×10^5) were seeded in 6-cm plastic petri dishes. On the next day the medium was withdrawn, and the cells were treated with 1 ml of DEAE dextran (25 μg/ml) for 30 min at 37 C. Cells were then infected for 1 h with the virus. The inoculum was not withdrawn, and 5 ml of medium with 15% fetal calf serum was added for further maintenance. On days 3 and 10, 3 ml of medium was added. On day 7, medium was changed completely. Discrete foci appeared on days 8 and 10 after infection and were usually counted on day 12 without staining. An unstained focus contained many dark, rounded, loosely attached cells in its center which were removed by washing. The A substrain of FeLV-422 additionally had within the foci prominent polykaryocytes with nuclei arranged in rings. As in the assay for MuLV with sarcoma-positive, leukemia-negative mouse cells (2), focus number did not increase with time, and the distribution of foci in plates infected with terminal virus dilutions followed Poissonian statistics. May-Greenwald-Giemsa stain, ×42 magnification.*

TABLE 1. *Quantitation of several mammalian oncornaviruses in the focus-inducing assay*

Virus[a]	Derived from cell (species) of:	Titers	
		Focus-inducing units/ml in line 8C	Leukemia virus replicating units
FeLV-161	FEF (cat)	5.7×10^5	6.3×10^5
FeLV-422	Thymus (cat)	1.1×10^4	NT
MLV-IC	3T3FL (mouse)	0	1.4×10^6
GLV	NRK (rat)	0	7.5×10^4
RD-114	RD-114 (human)	0	NT
CCC	CCC3aV (cat)	0	2.1×10^3
MuLV-X	FEF (cat)	1.7×10^4	NT
MuLV-X	BALB/c (mouse)	10^0–10^3	NT
BALB-2	NRK (rat)	1.1×10^2	NT
AT-124	RD (human)	4.3×10^3	NT

[a] FeLV 161 and 422 are Rickard strains of FeLV passed in either FEF- or 422-transformed thymus cells in culture. MLV and GLV are Moloney and Gross MuLV isolates grown in 3T3FL cells; GLV was then adapted to NRK cells. RD-114 and CCC isolates of the xenotropic cat oncornavirus were described (4). The MuLV-X was an atypical MuLV-X derived from BALB/c mouse cells or Kirsten sarcoma virus-transformed BALB/c cells which could be specifically neutralized by sera from old NZB mice (P. J. Fischinger, manuscript in preparation). BALB-2 and AT-124 are two of the MuLV-X strains described previously (2, 8). Several cloned sublines of 8C, 81, and 89 gave titers similar to that of the 8C line. Leukemia virus replicating units were calculated from dilutions of virus which gave a 50% infectious dose inducing interference with homologous sarcoma virus pseudotype in the most sensitive cell system available. This was done in FEF cells for FeLV as described (3) and in 3T3FL for ecotropic MuLV strains. NT, Not tested.

detected; (iii) it measures replicating virus, and specific MSV pseudotypes can be purified; (iv) it can detect MuLV-X in MuLV or MSV stocks.

This assay may be able to detect xenotropic viruses of other species.

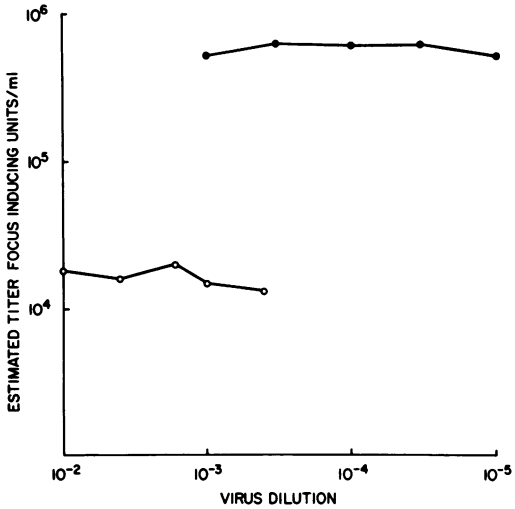

FIG. 2. *Titration patterns of FeLV and MuLV-X in 8C cells. Logarithm of estimated virus titer (foci times dilution) plotted against logarithm of virus dilution gave a straight line for one-hit kinetics (5). The FeLV stock was the Rickard F-161 passed for 6 years in virus-free FEF cells in our laboratory. The MuLV-X was chemically induced from Kirsten sarcoma virus-transformed BALB/c cells and was passed directly into FEF cells. Because MuLV-X titer exceeded that of the accompanying sarcoma pseudotype, MuLV-X was isolated by the end-point dilution technique and passed in FEF cells. Symbols:* O, *MuLV-X,* ●, *FeLV.*

LITERATURE CITED

1. Aaronson, S. A., and J. R. Stephenson. 1973. Independent segregation of loci for activation of biologically distinguishable RNA C-type viruses in mouse cells. Proc. Nat. Acad. Sci. U.S.A. **70**:2055–2058.
2. Aaronson, S. A., and C. A. Weaver. 1971. Characterization of murine sarcoma virus (Kirsten) transformation of mouse and human cells. J. Gen. Virol. **13**:245–252.
3. Bassin, R. H., N. Tuttle, and P. J. Fischinger. 1971. Rapid cell culture assay technique for murine leukaemia viruses. Nature (London) **229**:564–566.
4. Fischinger, P. J., and D. K. Haapala. 1971. Quantitative interactions of feline leukaemia virus and its pseudotypes of murine sarcoma virus in cat cells: requirement for DNA synthesis. J. Gen. Virol. **13**:203–214.
5. Fischinger, P. J., P. T. Peebles, S. Nomura, and D. K. Haapala. 1973. Isolation of an RD-114-like oncornavirus from a cat cell line. J. Virol. **11**:978–985.
6. Hartley, J. W., and W. P. Rowe. 1966. Production of altered foci in tissue culture by defective Moloney sarcoma virus particles. Proc. Nat. Acad. Sci. U.S.A. **55**: 780–786.
7. Lee, K. M., S. Nomura, R. H. Bassin, and P. J. Fischinger. 1972. Use of an established cat cell line for investigation and quantitation of feline oncornaviruses. J. Nat. Cancer Inst. **49**:55–60.
8. Levy, J. A. 1973. Xenotropic viruses: murine leukemia viruses associated with NIH Swiss, NZB, and other mouse strains. Science **182**:1151–1153.
9. Todaro, G. J., P. Arnstein, W. P. Parks, E. H. Lennette, and R. J. Huebner. 1973. A type-C virus in human rhabdomyosarcoma cells after inoculation into NIH Swiss mice treated with antithymocyte serum. Proc. Nat. Acad. Sci. U.S.A. **70**:859–862.