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Oncogenicity of AKR Endogenous Leukemia Viruses

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Four biologically distinct groups of endogenous murine leukemia virus (MuLV) have been isolated from AKR mice. These viruses include (i) ecotropic XC^+ MuLV that occur in high titer in normal tissues and serum of AKR mice throughout their life span, (ii) ecotropic XC^- MuLV that are produced in high titers by leukemia cells, (iii) xenotropic MuLV that are readily demonstrable only in aged mice, and (iv) polytropic MuLV that arise in the thymuses of aged mice as a consequence of genetic recombination between ecotropic and xenotropic MuLV. Viruses of each of these biological classes were assayed in AKR mice for their ability to accelerate the occurrence of spontaneous leukemia. Certain isolates of ecotropic XC^- MuLV and polytropic MuLV were found to have high oncogenic activity. These viruses induced 100% leukemias within 90 days of inoculation. In contrast, ecotropic XC^+ MuLV that were obtained from AKR embryo fibroblasts and xenotropic MuLV that were obtained from the lymphoid tissues of aged AKR mice did not demonstrate oncogenic activity. These findings demonstrate fundamental differences between XC^- and XC^+ ecotropic MuLV that are found in leukemic and normal tissues, respectively. Furthermore, these findings point to the role of ecotropic XC^- and polytropic MuLV in the spontaneous leukemogenesis of AKR mice.

Mice of diverse genetic backgrounds contain multiple integrated copies of endogenous murine leukemia viruses (MuLV) (1, 8). In inbred populations, mice of certain strains (e.g., AKR) express high titers of MuLV throughout their life spans and develop a high incidence of leukemia. In mice of other strains (e.g., C57BL/6), the expression of endogenous MuLV is repressed and the incidence of leukemia is considerably lower. Although persistent virus production occurs in leukemia-prone mice, the onset of leukemia does not occur in these animals until relatively late (8 to 12 months) in life. This suggests that age-related changes occur in the host before the development of leukemia. Analysis of the endogenous viruses produced by aged AKR mice supports this conjecture.

The endogenous viruses of AKR mice are a polymorphic group of agents. These viruses have been separated into two broad groups by host range analysis and antigenicity: ecotropic MuLV (2) that efficiently infect mouse cells, but not xenogeneic cells; and xenotropic MuLV (7) that efficiently infect xenogeneic cells, but not mouse cells. The expression of these two classes of endogenous viruses is independently controlled by mechanisms that show age-related effects (3, 6, 10, 12). Ecotropic MuLV are produced in high titers in AKR mice over 2 weeks old and can be readily isolated from the serum and tissues of normal and leukemic mice (12). Xenotropic MuLV, on the other hand, are produced in low titers in young AKR mice and can only be readily isolated from aged animals (6, 10). In addition, the lymphoid tissues of preleukemic and leukemic AKR mice produce defective (XC^-) ecotropic MuLV (9) and a heterogeneous group of recombinant viruses that have acquired an expanded (polytropic) host range (3). As a result of this complexity of viruses in AKR mice, it has been difficult to determine the actual oncogenic agent responsible for the induction of disease.

We describe here the characterization of a variety of endogenous MuLV of AKR mice. These viruses have been analyzed in tissue culture for their infectious properties, host range restrictions of growth, and oncogenicity upon inoculation into newborn AKR mice. We have found that certain isolates of ecotropic XC^- MuLV and polytropic MuLV rapidly accelerate the occurrence of leukemia in susceptible animals.

MATERIALS AND METHODS

Viruses and cells. Fibroblast cell lines were maintained in Eagle minimum essential medium containing 10% fetal calf serum. Leukemia cell lines were maintained in RPMI 1640 medium containing 15% fetal calf

serum (9). The AKR pII cell line was established from a culture of AKR mouse embryo fibroblasts; these cells produced high titers of XC^+ ecotropic MuLV. Leukemia cell lines were established from the thymuses of mice with spontaneous leukemias (SL). Culture fluids from leukemia cell lines were used to infect SC-1 fibroblast cells. These viruses initially established abortive infections, although upon continued growth in culture, the SC-1 cells produced highly infectious XC^+ ecotropic MuLV (9, 11). The converted viruses ($XC^- \rightarrow XC^+$) produced by SC-1 cells were designated by the postscript "-SC1" (e.g., SL2-SC1 and SL3-SC1). AKR xenotropic MuLV [designated AKR X1(N)] was isolated from a spontaneous leukemia by cocultivation with mink CCL64 cells. AKR polytropic MuLV [designated AKR MCF1(N)] was isolated from another spontaneous leukemia by cocultivation with mink CCL64 cells. Both AKR X1(N) and AKR MCF1(N) were cloned by end-point dilution on mink cells; AKR MCF1(N) was additionally cloned by end-point dilution on murine NIH 3T3 cells. The xenotropic virus AKR X6 and polytropic viruses AKR MCF 13 and AKR MCF 247 were kindly provided by W. Rowe (National Institutes of Health, Bethesda, Md.). Polytropic and xenotropic viruses were routinely carried in mink CCL64 cells; viruses propagated on mink cells were designated by the postscript "-m" (e.g., AKR MCF 247-m). Cultures of thymic fibroblasts and epithelial cells were established from the pooled thymuses of 2- to 3-month-old AKR mice; these cells produced high titers of XC^+ ecotropic MuLV. Cell extracts were prepared from the pooled lymphoid tissues of 2- to 3-month-old AKR mice by gentle homogenization in phosphate buffer, low-speed centrifugation, and filtration of supernatants through 0.45-μm membrane filters (Millipore Corp., Bedford, Mass.)

Virus assays. Petri dishes (60 mm) were seeded either with NIH 3T3 cells (5×10^4) or with mink CCL64 cells (10^5). Twenty-four hours later, the cells were treated for 1 h with polybrene (20 μg/ml) and then infected with 0.25 ml of virus suspension. Two hours after infection, 5 ml of medium was added to each dish and the cells were returned to the incubator. Two days later the cells were trypsinized and either transferred to fresh petri dishes or used in immunofluorescence (IF) assays for the determination of virus infection by the production of MuLV group-specific (gs) antigen.

For IF assays (5), tissue culture cells were attached to individual wells of "Toxoplasmosis Slides" (Bellco Glass, Inc., Vineland, N.J.) by growth for several hours in tissue culture medium; after the cells had spread sufficiently to enable visualization of cytoplasmic detail, they were fixed by immersion in acetone for 10 min. IF tests were performed in three steps. In step 1, cells were incubated in a drop of diluted antiserum for 1 h at 37°C in a humidified incubator. The slides were washed twice in phosphate-buffered saline (pH 7.2) and twice in distilled water (5-min intervals for each wash). In step 2, slides were dried under a stream of warm air and 1 drop of fluoresceinated antiglobulin was added to each well. The cells were incubated for 1 h at 37°C, and the slides were washed twice in phosphate-buffered saline and distilled water. In step 3, cells were counterstained by incubation in Evans blue dye (0.06%) for 10 min, washed twice in distilled water, and then dried. A small drop of glycerine–phosphate-buffered saline (1:1) was placed on each spot, and the slide was covered with a cover slip (20 by 50 mm). The presence of MuLV gs antigen in infected cells was determined with goat anti-p30 antiserum. Rabbit anti-goat immunoglobulin G (fluorescein-conjugated) was purchased from Hyland Laboratories, Inc., Los Angeles, Calif. Both antisera were initially absorbed in vivo in C57BL/6 mice to remove heterophile antibodies against mouse antigens. Titers of infecting viruses (referred to as gs antigen-inducing units per ml [gsIU/ml]) were determined by the following calculation: gsIU/ml = (% cells gs^+) $\times$ (number of cells in petri dish at time of infection) $\times$ (volume of fluid in milliliters used for infection).

For DNA polymerase assays (13), culture fluids were cleared of cells and cellular debris by centrifugation at 10,000 $\times$ *g* for 10 min. DNA polymerase activity (released in culture fluid during a 24-h interval) was then concentrated 100- to 500-fold by centrifugation at 82,500 $\times$ *g* for 60 min. Viral pellets were disrupted with 0.1% Nonidet P-40 for 10 min at 4°C. A 30-μl portion of the disrupted virus suspension was then mixed with 70 μl of reaction mixture at 37°C. The reaction mixture contained 50 mM Tris-hydrochloride (pH 8.3), 45 mM KCl, 0.2 mM $MnCl_2$, 30 mM dithiothreitol, 0.05 absorbancy unit at 260 nm of polyriboadenylic acid·polydeoxythymidylic acid annealed at a ratio of 4:1, and 20 μM [^{3}H]TTP, 11,000 dpm/pmol (New England Nuclear Corp., Boston, Mass.). Samples (10 μl) were removed from the polymerase reaction at 0-, 15-, and 30-min intervals to determine the incorporation of [^{3}H]TTP into trichloroacetic acid-precipitable material. Samples from the polymerase reaction were spotted on Whatman 3 MM filter disks that had been prewashed in a solution of 0.1 M NaP_i, 0.1 M $NaPP_i$, 10 mM rATP, and 20 mM EDTA, pH 7.4. Polymerase activity was expressed as the number of picomoles of [^{3}H]TTP incorporated per minute per milliliter of culture fluid.

Oncogenicity assays. AKR mice (from the UCLA animal colony) were inoculated within 72 h of birth with filtrates from tissue culture supernatants. Acceleration of the onset of leukemia (i.e., disease prior to 150 days postinjection) in these mice was considered evidence for the presence of oncogenic virus in the inoculum. The incidence of spontaneous leukemias in 100 noninjected AKR mice was 94% at 556 days, with a median of 280 days.

RESULTS

Host range analysis of AKR MuLV. Culture fluids from AKR cells were characterized for their content of MuLV by infection of mouse (NIH 3T3) and mink (CCL64) cells. Infection of these target cells was determined 10 days later by IF assays for MuLV gs antigen.

Culture fluids from six of the eight AKR SL cell lines contained infectious ecotropic MuLV (titers ranging from 10^3 to 10^5 infectious units per ml; Table 1). These viruses, although infectious for NIH cells, failed to induce plaques in

TABLE 1. *Host range properties of AKR viruses*

Cell line as source of virus	Fluid infection[a]			Cocultivation infection[b] (% gs$^+$ cells)			
	Polymerase activity of infecting fluids[c]	% gs$^+$ cells (10 days p.i.)[d]		(10 days p.i.)		(22 days p.i.)	
		NIH	Mink	NIH	Mink	NIH	Mink
AKR SL1	0.10	<0.02	<0.05	1.05	<0.03	1.05	1.65
AKR SL2	1.11	3.45	<0.05	2.45	<0.03	100.00	<0.03
AKR SL3	0.62	9.15	<0.05	ND[e]	<0.03	97.50	<0.03
AKR SL4	2.21	28.75	20.00	2.95	2.15	100.00	98.18
AKR SL5	0.59	0.55	<0.05	1.65	2.15	100.00	75.57
AKR SL6	2.60	23.61	<0.05	ND	1.25	100.00	56.25
AKR SL7	0.13	<0.05	<0.05	0.10	0.90	23.39	1.45
AKR SL8	0.05	0.22	<0.05	5.75	0.05	100.00	0.05
AKR pII	1.16	100.00	<0.05	100.00	0.05	ND	<0.03

[a] Infections were performed with undiluted tissue culture fluids.
[b] Infections were performed with 5×10^6 mitomycin-treated cells.
[c] Picomoles of [^{3}H]TTP incorporated per minute per milliliter of culture fluid.
[d] p.i., Postinfection.
[e] ND, Not determined.

the XC assay; furthermore, their initial rounds of infection in NIH cells were abortive and yielded extremely low levels of progeny virus. As a result, these viruses were classified as defective XC$^-$ MuLV. In most instances, continued passage of the NIH cells infected with XC$^-$ MuLV resulted in the release of highly infectious (converted) XC$^+$ MuLV.

In addition to containing ecotropic XC$^-$ virus, the culture fluids of AKR SL4 cells contained virus that infected both mink and mouse cells (Table 1). The mink-infectious virus from SL4 was passaged by end-point dilution on mink cells and then reexamined for host range properties. Data presented in Table 2 demonstrate that after end-point dilution this virus was still polytropic. The polytropic property of AKR SL4 MuLV was also stable to end-point dilution on mouse cells.

The culture fluids from AKR pII embryo fibroblast cells contained high titers of XC$^+$ ecotropic MuLV (Table 1). This XC$^+$ MuLV rapidly established an efficient infection in NIH cells. As a general property, the virus preparations from AKR pII cells contained 100- to 1,000-fold-greater specific infectivity (infectivity per unit of DNA polymerase activity) for NIH cells than did viruses from the AKR SL cell lines.

To increase the sensitivity of the host range analysis, NIH and mink cells were cocultivated with mitomycin-treated (50 μg/ml) AKR cells. Infection of the NIH and mink target cells was then determined 10 and 22 days later by IF assays for MuLV gs antigens.

Seven of the eight AKR SL cell lines and the AKR pII cell line produced high titers of ecotropic MuLV (Table 1). Although AKR SL1 did not demonstrate the production of infectious virus, other tests of this leukemia cell line (unpublished data) demonstrated the production of low titers of ecotropic XC$^-$ MuLV.

TABLE 2. *Host range properties of mink-infectious viruses obtained from AKR SL cell lines*

Cell line as source of virus[a]	Viral infectivity (% gs$^+$ cells)[b]	
	NIH	Mink
AKR SL4-m	37.00	35.00
AKR SL5-m	<0.05	38.00
AKR SL6-m	77.00	89.00

[a] AKR SL4-m was obtained from the infection of mink cells with culture fluids of SL4; AKR SL5-m was obtained from the infection of mink cells by cocultivation with SL5 cells; AKR SL6-m was obtained from the infection of mink cells by cocultivation with SL6 cells. For details see Table 1.
[b] Ten days postinfection.

The increased sensitivity afforded by cocultivation enabled the detection of two new viruses that were produced by the AKR SL5 and SL6 cell lines. Both of these viruses were infectious for mink cells. In subsequent experiments, the SL5 and SL6 MuLV were passed by end-point dilution on mink cells and reexamined for host range properties. Data presented in Table 2 demonstrate that the mink-infectious viruses from AKR SL5 was xenotropic, while the mink-infectious virus from AKR SL6 was polytropic. The polytropic properties of AKR SL6 MuLV was also stable to end-point dilution on mouse cells.

Cocultivation of mitomycin-treated SL1 and SL7 with mink cells resulted in a low-level infection (ca. 1%) of the mink fibroblasts. Since this infection did not spread throughout the culture, it was assumed that these gs$^+$ cells were infected with pseudotyped ecotropic virions (consisting of a xenotropic envelope and an ecotropic ge-

nome) or with defective xenotropic viruses.

A summary of the host range properties of viruses from different cell lines is presented in Table 4.

Oncogenicity of AKR MuLV. Newborn AKR mice were inoculated intraperitoneally with filtrates of tissue culture fluids from a variety of cell lines. For this purpose, each tissue culture fluid was frozen in four portions in liquid nitrogen; within 24 h one of the portions was thawed and tested for DNA polymerase activity and for infectivity on NIH and mink cells. The remaining portions were then used for the inoculation of mice. The oncogenic activity of several AKR MuLV are shown in Table 3 and Fig. 1.

High oncogenic activity was detected in the filtrates of culture fluids from the AKR SL2, SL3, and SL4 cell lines. Inoculation of $10^{3.4}$ infectious units of SL2 and SL3 MuLV into newborn AKR mice induced 100% leukemias with a median latency of 88 and 83 days, respectively. Culture fluids from SC-1 cells infected with SL2 and SL3 viruses also were oncogenic, demonstrating that the leukemogenic agent from these cell lines was capable of replication in fibroblast cells. Within the sensitivity of the host range analysis, the viruses produced by SL2, SL3, SL2-SC1, and SL3-SC1 cell lines were exclusively ecotropic MuLV.

Filtrates of culture fluids from the SL4 cell line contained both ecotropic and polytropic MuLV and were highly oncogenic upon inoculation into AKR mice. Inoculation of $10^{3.6}$ infectious units of SL4 MuLV induced 100% leukemias with a median latency of 76 days.

Filtrates of culture fluids from other AKR SL cell lines (SL1, SL5, SL6, SL7, and SL8) contained only low or nondetectable levels of oncogenic activity. The limited oncogenicity of SL1 and SL7 MuLV may reflect the poor infectivity of these viruses (Tables 1 and 3). In other assays with different preparations of virus (Table 5), we have found oncogenic activity with MuLV from SL1 and SL7.

High oncogenic activity also was detected in filtrates of culture fluids of the AKR MCF 13 and AKR MCF 247 cell lines (Table 3). Inoculation of $10^{4.0}$ infectious units of MCF 13 induced 100% leukemias with a median latency of 98 days, whereas inoculation of $10^{5.2}$ infectious units of MCF 247 induced 100% leukemias with a latency of 122 days. In contrast, culture fluids from AKR MCF1(N) cells did not demonstrate oncogenic activity upon inoculation into AKR mice.

TABLE 3. *Host range properties and oncogenicity of AKR viruses*

Cell line as source of virus	Viral infectivity in culture fluids[a]			Oncogenicity of culture fluid[b] (no. of leukemias/no. inoculated)
	Polymerase activity of infecting fluid[c]	% gs^+ cells (48 h p.i.)[d]		
		NIH	Mink	
AKR SL1	0.08	0.20	<0.05	2/13
AKR SL2	0.15	8.00	<0.05	10/10
AKR SL2-SC1	ND	37.00	<0.05	6/6
AKR SL3	0.25	8.00	<0.05	9/9
AKR SL3-SC1	ND	19.00	<0.05	9/9
AKR SL4	0.46	11.00	0.10	10/10
AKR SL5	0.27	0.60	<0.05	0/8
AKR SL6	2.60	32.00	<0.05	2/4
AKR SL7	0.10	0.20	<0.05	0/15
AKR SL8	0.04	0.05	<0.05	0/8
AKR pII	0.49	93.00[e]	<0.05	0/11
AKR X6-m	0.48	0.30	60.00	0/12
AKR X1 (N)-m	0.42	0.30	52.00	0/9
AKR MCF 13-m	2.08	27.00	74.00	6/6
AKR MCF 247-m	0.90	>95.00[f]	81.00	8/8
AKR MCF 1(N)-m	0.53	51.00	58.00	0/11

[a] Infections were performed with undiluted tissue culture fluids.

[b] Each mouse was inoculated intraperitoneally with 0.1 ml of undiluted culture fluid and then observed for the development of leukemia for a period of 150 days.

[c] Picomoles of [^{3}H]TTP incorporated per minute per milliliter of culture fluid. ND, Not determined.

[d] p.i., Postinfection.

[e] Infection with 10^{-1} diluted culture fluid scored 46% gs^+ NIH cells, <0.05% gs^+ mink cells.

[f] Infection with 10^{-1} diluted culture fluid scored 52% gs^+ NIH cells, 50% gs^+ mink cells.

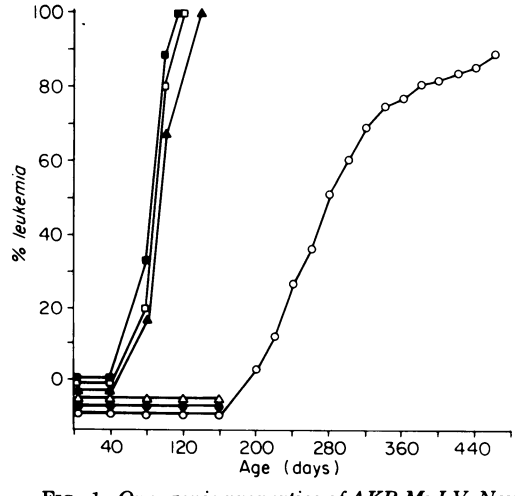

FIG. 1. *Oncogenic properties of AKR MuLV. Newborn AKR mice were inoculated intraperitoneally with 0.1 ml of culture fluid from AKR SL2 (□), AKR SL3 (■), AKR MCF 13 (▲), AKR X6 (△), and AKR pII embryo fibroblasts (●). Also shown is the cumulative incidence curve of spontaneous leukemia for 100 nontreated controls (○).*

Filtrates of culture fluids from the AKR pII embryo fibroblast cell line were not oncogenic. Inoculation of $10^{5.4}$ infectious units into AKR mice failed to accelerate the appearance of leukemia. In both host range properties and growth characteristics the AKR pII virus was indistinguishable from the XC^+ ecotropic MuLV that occurred in high titers in the serum and tissues of AKR mice. In fact, oncogenicity assays with other isolates of AKR XC^+ ecotropic MuLV (derived from embryo fibroblasts or from the tissues of young normal mice) have been consistently negative in our hands (Table 5).

Filtrates of culture fluids from the AKR X6 and AKR X1(N) cell lines also were negative in the oncogenicity assays. Inoculation of $10^{5.6}$ infectious units of these viruses into newborn AKR mice failed to induce leukemias within 150 days. In host range analysis these viruses were exclusively xenotropic.

TABLE 4. *Summary of host range characteristics and oncogenicity of AKR viruses*

Cell line[a]	Host range characteristics of viruses in culture fluids	Oncogenic properties[b]
AKR SL1	XC^- N-ecotropic	+
AKR SL2	XC^- N-ecotropic	++
AKR SL3	XC^- N-ecotropic	++
AKR SL4	XC^- N-ecotropic and polytropic	++
AKR SL5	XC^- N-ecotropic and xenotropic	−
AKR SL6	XC^- N,B-ecotropic and polytropic	+/−
AKR SL7	XC^- N-ecotropic	+
AKR SL8	XC^- N-ecotropic	−
AKR SL2-SC1	XC^+ N-ecotropic	++
AKR SL3-SC1	XC^+ N-ecotropic	++
AKR embryo fibroblast	XC^+ N-ecotropic	−
AKR thymus epithelium	XC^+ N-ecotropic	−
AKR thymus fibroblast	XC^+ N-ecotropic	−
AKR X6-m	Xenotropic	−
AKR X1(N)-m	Xenotropic	−
AKR MCF 247-m	Polytropic	++
AKR MCF 13-m	Polytropic	++
AKR MCF 1(N)-m	Polytropic	−
AKR SL4-m	Polytropic	NT
AKR SL5-m	Xenotropic	NT
AKR SL6-m	Polytropic	NT

[a] "-SC1" designates viruses propagated on the SC-1 fibroblast cell line; "-m" designates viruses propagated on mink cells.

[b] ++, Strong oncogenicity (100% leukemias); +, moderate oncogenicity (30 to 70% leukemias); +/−, weak oncogenicity (<20% leukemias); −, non-oncogenic; NT, not tested.

Additional oncogenicity assays of AKR viruses are shown in Table 5. Culture fluids used in these studies were initially screened for activity in the XC plaque assay. High oncogenic activity was observed with culture fluids from the AKR leukemias SL1, SL2, SL3, SL4, and SL7; however, viruses from each of these cells failed to induce XC plaques when plated on NIH cells. In contrast, viruses produced by AKR embryo cells, AKR thymic fibroblasts, and AKR thymic epithelial cells induced XC plaques, but were non-oncogenic upon inoculation into newborn AKR mice. In addition, filtrates of tissue extracts prepared from pooled lymphoid tissues of young AKR mice contained high titers of XC^+ MuLV, but failed to induce accelerated leukemias.

DISCUSSION

On the basis of infectious properties in tissue culture, four distinct types of MuLV have been demonstrated (3, 6, 9, 10) in AKR mice: (i) highly infectious XC^+ ecotropic MuLV, (ii) poorly infectious XC^- ecotropic MuLV, (iii) xenotropic MuLV, and (iv) polytropic XC^- MuLV. All four types of AKR virus can be isolated from the thymuses of leukemic mice. However, leukemia cell lines in cultures tend to produce a predominance of XC^- ecotropic MuLV (4, 9), suggesting that the other classes of MuLV may be produced by nonleukemic cells in the thymus. In line with this hypothesis, we have found that the cells of the thymic epithelium and stroma produce high titers of XC^+ ecotropic MuLV in culture.

TABLE 5. *Oncogenicity of AKR viruses*

Source of inoculum	Activity in XC plaque assay	No. of experiments	Total no. of mice inoculated[a]	% Mice with leukemia
AKR SL1	−	5	37	78
AKR SL2	−	2	19	95
AKR SL3	−	1	6	100
AKR SL4	−	2	10	60
AKR SL5	−	1	7	0
AKR SL7	−	1	7	100
AKR SL8	−	1	9	0
AKR embryo fibroblast	+	3	28	0
AKR thymic fibroblast	+	1	7	0
AKR thymic epithelium	+	2	17	0
AKR normal lymphoid tissue	+	6	36	0

[a] Each mouse was inoculated intraperitoneally with 0.1 ml of undiluted culture fluid and then observed for 150 days for the development of leukemia.

Although leukemic cells produce predominantly XC^- ecotropic MuLV, low levels of polytropic and xenotropic MuLV also can be detected in these cells. The production of polytropic and xenotropic MuLV by most leukemia cell lines tends to be a sporadic event and is only occasionally detected in host range assays. In four different tests (involving either infection of NIH and mink cells with culture fluids or by cocultivation with mitomycin-treated SL cells), the SL2 and SL3 leukemias were found to produce exclusively ecotropic MuLV. Polytropic MuLV has been isolated repeatedly from the culture fluids of SL4, but not from the culture fluids of other SL cell lines. In one cocultivation assay we isolated polytropic MuLV from SL6 and xenotropic MuLV from SL5; however, in three other assays of culture fluids the SL5 and SL6 cells were found to produce only ecotropic MuLV.

The oncogenic activity of AKR viruses has been examined by inoculation of tissue culture filtrates into AKR mice. Ecotropic XC^- MuLV from SL2 and SL3 cells, as well as polytropic MCF 13 and MCF 247 MuLV, were found to be highly oncogenic in AKR mice. These viruses could be contrasted to the XC^+ ecotropic MuLV from AKR embryo fibroblasts, thymic fibroblasts, and thymic epithelial cells which did not have demonstrable oncogenicity. As shown in Fig. 1, $10^{5.4}$ infectious units of XC^+ ecotropic MuLV was totally non-oncogenic, whereas $10^{3.4}$ infectious units of XC^- ecotropic MuLV induced 100% leukemias within 90 days. Of further interest was the finding that not all XC^- ecotropic MuLV and polytropic MuLV were oncogenic. These differences in the oncogenic potential of the XC^- ecotropic MuLV and the polytropic MuLV suggest that the host range properties of viruses may not reflect their oncogenicity in vivo.

Finally, it should be stressed that the virus-accelerated oncogenicity assay involves the inoculation of tissue culture-derived viruses into mice that are productively infected with an XC^+ endogenous virus and who will naturally develop an oncogenic state upon aging. Furthermore, the virus preparations that we have utilized were not cloned and may represent, to some degree, mixtures. It is conceivable, therefore, that the accelerated induction of leukemia in these mice may be a consequence of the interaction in vivo between several different viruses. In fact, it is our present hypothesis that XC^- MuLV, due to inefficient replication properties, preferentially recombine with xenotropic MuLV to yield an efficiently replicating polytropic MuLV that is highly oncogenic.

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