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Endogenous Ecotropic Mouse Type C Viruses Deficient in Replication and Production of XC Plaques

ULF R. RAPP AND ROBERT C. NOWINSKI*
McArdle Laboratory for Cancer Research, University of Wisconsin, Madison, Wisconsin 53706

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Endogenous ecotropic type C viruses were induced by iododeoxyuridine from nontransformed and chemically or spontaneously transformed clones of the C3H/10T1/2 cell line. Viruses produced by cells of certain transformed clones were N-tropic and formed large XC plaques. In contrast, viruses produced by nontransformed C3H/10T1/2 cells were not detectable in the XC plaque test. These XC$^-$ viruses infected mouse cells with high efficiency, as shown by the induction of murine leukemia virus group-specific antigens in infected cells, but virus production, as determined by DNA polymerase-containing particles, was extremely low. Upon growth in certain mouse cells these replication-deficient, XC$^-$ viruses converted to type C viruses that were similar in XC assays to N-tropic AKR virus (XC$^+$).

Endogenous N-tropic type C viruses have been isolated in vivo and in vitro from mice of high and low leukemic strains (3, 6, 7). These viruses appeared to have similar antigenic properties (2), although they showed differences upon comparison of their abilities to establish productive infections. N-tropic viruses that were induced from C58 mouse embryo fibroblasts (MEF) were transmitted to cells with high efficiency and formed large XC plaques, whereas N-tropic virus derived from BALB/c or C57BL/6 MEF required co-cultivation of induced cells with NIH/3T3 cells for detection in the XC assay, and the plaques formed were small and diffuse (3, 14).

We reported previously (10) that endogenous type C viruses induced by iododeoxyuridine (IUdR) from some transformed C3H cell lines were highly infectious for both mouse and rat cells in the XC and focus induction assays. These viruses were N-tropic and formed large XC plaques, similar to those caused by N-tropic virus derived from AKR mice. In contrast, viruses induced from nontransformed cells of the low leukemic mouse strains C3H and BALB/c appeared to be noninfectious for mouse as well as for rat cells in XC or focus induction assays.

The present report describes further biological properties of these apparently noninfectious particles. We have found that these viruses do infect mouse cells with an efficiency similar to that of N-tropic viruses from AKR mice; however, the infected cells produce minimal amounts of virus and do not form syncytia upon contact with XC cells. Further, we have found that growth of these viruses in certain mouse cells leads to conversion of these viruses to N-tropic type C virus that is indistinguishable in the XC test from the N-tropic virus of AKR mice.

MATERIALS AND METHODS

Cells. MEF and cell lines were maintained in vitro in Eagle minimum essential medium containing 10% heat-inactivated fetal calf serum with penicillin (200 U/ml) and streptomycin (200 μg/ml). The orgins of the C3H/10T1/2. C3H/ST3, C3H/MCA5, and AKR cell lines have been described in detail (10, 11). Briefly, C3H/10T1/2 is a clonal nontumorigenic cell line (established from MEF) that is sensitive to postconfluence inhibition of growth. C3H/MCA5 and C3H/ST3 are clonal tumorigenic cell lines that have been established by chemical and spontaneous transformation of C3H/10T1/2 cells, respectively. Other cell lines included the previously described BALB/3T3, SC-1 (7), and the rabbit cornea SIRC (4).

Virus assays. The XC test was a slight modification of that developed by Rowe et al. (13). XC and SC-1 embryo cells were maintained in minimum essential medium with 10% fetal calf serum. Petri dishes (60 mm) were seeded with 10^5 SC-1 cells. Twenty-four hours later the SC-1 cells were pretreated for 1 h with polybrene (20 μg/ml) and then infected with 0.2 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. Four days after infection the SC-1 cells were lethally irradiated with a UV germicidal lamp and overlaid with 10^6 viable XC cells. Another medium change was performed 2 days later and, depending upon the confluence of the XC monolayer, the test was terminated after 1 or 2 days. The cells were fixed in methanol and stained with Giemsa to score for plaques.

Immunofluorescence tests (6) were performed on fixed target cells for the determination of murine leukemia virus (MuLV) group-specific (gs) antigens. Tissue culture cells were attached to individual wells of "Toxoplasmosis Slides" (Bellco, Inc.) 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. Immunofluorescence tests were performed in three steps. (i) Cells were incubated in a drop of antiserum dilution for 1 h at 37 C in a humidified incubator. The slides were washed twice in phosphate-buffered saline (PBS), pH 7.2, and twice in distilled water (5-min intervals for each wash). (ii) The slides were dried, and 1 drop of fluorescenated antiglobulin was added to each well. The cells were incubated for 1 h at 37 C, and the slides were washed twice in PBS and distilled water. (iii) The cells were counter stained by incubation in Evans blue dye (0.06%) for 10 min, washed twice in distilled water, and then dried. A small drop of glycerine-PBS (1:1) was placed on each spot, and the slide was covered with a long (20 by 50 mm) cover slip. MuLV antiserum was prepared in (W/Fu × BN)F_1 hybrid rats bearing a syngeneic tumor W/Fu C58(NT)D, which was originally induced by murine Gross leukemia virus. Goat anti-rat 7*S* gamma globulin (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.

For DNA polymerase assays (7), culture fluids were cleared of cells and cellular debris by centrifugation at 10,000 × *g* for 10 min. DNA polymerase activity (released into the culture fluid during a 24-h interval) was then concentrated 100- to 500-fold by centrifugation at 82,500 × *g* for 60 min. Viral pellets were disrupted with 0.1% Nonidet P-40 for 10 min at 4 C. Thirty microliters 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 A_{260} units of poly(rA)·poly(dT), annealed at a ratio of 4:1, and 20 μM [^{3}H]TTP, 11,000 dpm/pmol (New England Nuclear Corp.). Ten-microliter samples 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 3MM 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. Filters were washed in 10% trichloroacetic acid and counted by liquid scintillation. Culture fluids for control cells (e.g., SC-1) had a background incorporation of 3 × 10^2 to 7 × 10^2 dpm/30 min; culture fluids from virus-infected cells (virus produced at a titer of 2 × 10^5 PFU/ml in XC plaque assay) had a DNA polymerase activity of 1 × 10^6 to 2 × 10^6 dpm/min per 60-mm dish.

Infectious-center assays (9) were used to determine the percentage of cells in a population that were producing oncornaviruses. This technique (based on AKR MuLV-producing cells) was sufficiently sensitive to detect single virus-producing cells. For the assay of cells producing nontransforming MuLV, test cells were seeded in 60-mm plastic petri dishes at various concentrations (10^1 to 10^6), incubated for several hours to permit attachment to the substrate, and then lethally irradiated with UV light and overlaid with 10^6 XC cells. Plaques were scored 2 to 3 days later.

RESULTS

Infectivity of type C viruses stably produced by C3H cells after IUdR induction. Treatment of C3H/10T1/2 cells in vitro with IUdR resulted in the induction of DNA polymerase-containing particles that were stably produced in large amounts after all cells in the IUdR-treated cultures had become positive for the production of MuLV gs antigens (10).

Virus produced by nontransformed C3H/10T1/2 cells late after IUdR treatment differed markedly from viruses released by certain IUdR-treated transformed C3H/10T1/2 cells. Thus, only the virus from the transformed cells could be shown to be infectious for mouse cells with the XC assay (10). As shown in Table 1, similar amounts of DNA polymerase-containing parti-

TABLE 1. *Expression of endogenous type C viruses by nontransformed and transformed C3H/10T1/2 cells after IUdR induction*[a]

IUdR-treated cells[b]	DNA polymerase in culture fluid[c]	Titer of infectious virus	
		Ecotropic type C virus XC test (PFU/ml on SC-1)	Xenotropic type C virus polymerase test (PIU/ml on SIRC)[d]
C3H/10T1/2	11,600	–[e]	10^0
C3H/ST3	19,800	–	10^0
C3H/MCA5	21,100	$10^{6.1}$	10^0

[a] Fluids of cultures in late log phase of growth were harvested 24 h after medium change. Culture fluids were freed of cells and cell debris by centrifugation, and the viruses were concentrated 100-fold by centrifugation (9) for determination of DNA polymerase-containing particles. At the same time aliquots of nonconcentrated culture fluids were obtained for the infectivity assays, and cells were trypsinized and counted.

[b] C3H/ST3 and C3H/MCA5 are transformed cells; C3H/10T1/2 is the nontransformed parental cell.

[c] Picomoles of [^{3}H]TTP incorporated/30 min per 10^7 cells.

[d] Titer represents DNA polymerase-inducing units (PIU) per milliliter of virus stock. One PIU equals the dilution of stock that yields DNA polymerase-containing particles in infected cells with activity of 10 pmol of [^{3}H]TTP incorporated/30 min per ml of medium.

[e] –, < 1 PFU/ml of culture fluid.

cles were produced by both cell types, although infectious ecotropic type C virus was detected only in the culture fluid of the chemically transformed C3H/MCA5 cells. Furthermore, direct co-cultivation of the nontransformed virus-producing C3H/10T1/2 cells with XC cells also did not result in syncytial cell or plaque formation (10). This XC^- phenotype of the IUdR-treated C3H/10T1/2 cells was stable over at least 20 passages in culture.

Xenotropic MuLV was not detected in the culture fluids of either nontransformed or transformed C3H/10T1/2 virus-producing cells.

Host range analysis of type C viruses constitutively produced by C3H cells after IUdR induction. The XC assay is an indirect test for the replication of type C viruses in mouse cells, as has been shown previously (10). Thus, the production of large amounts of type C virus by infected cells may not be sufficient for syncytial cell formation. Therefore, in our infectivity assays we chose criteria for infection of target cells that also included the induction of DNA polymerase-containing particles and the induction of MuLV gs antigens.

Table 2 shows the results obtained from host range analyses with target cells that were susceptible to infection by eco- and/or xenotropic type C viruses. Virus produced by C3H/MCA5 cells showed plating efficiencies characteristic of N-ecotropic MuLV and formed large XC plaques. Virus that was produced by the C3H/10T1/2 and C3H/ST3 cells did not produce plaques or syncytia in the XC test. However, virus from C3H/10T1/2 cells did infect mouse cells with an efficiency similar to that of

TABLE 2. *Host range analysis of MuLV in vitro*

Cell lines used as source of MuLV	DNA polymerase in virus stock[a]	Target cell	Infectivity assay		
			XC test (PFU/ml)	DNA polymerase (PIU/ml)[b]	gs Antigen (gsIU/ml)[c]
C3H/MCA5 IUdR treated	1,700	NIH Swiss	$10^{4.5}$	$10^{3.8}$	$10^{6.6}$
		C3H/10T1/2	$10^{4.4}$	$10^{3.3}$	$10^{6.5}$
		BALB/3T3	$10^{2.6}$	$10^{1.4}$	$10^{4.3}$
		SC-1	$10^{5.7}$	$10^{5.6}$	$10^{6.8}$
		SIRC	NT[d]	10^{0}	$<10^{2.0}$
C3H/10T1/2 IUdR treated	300	NIH Swiss	Negative	10^{0}	$10^{5.7}$
		C3H/10T1/2	Negative	10^{0}	$10^{5.6}$
		BALB/3T3	Negative	10^{0}	$10^{3.3}$
		SC-1	Negative	$10^{1.1}$	$10^{5.9}$
		SIRC	NT	10^{0}	$<10^{2.0}$
C3H/ST3 IUdR treated	710	NIH Swiss	Negative	NT	NT
		C3H/10T1/2	Negative	$10^{0.4}$	NT
		BALB/3T3	Negative	$10^{0.3}$	NT
		SC-1	Negative	$10^{1.3}$	NT
		SIRC	NT	10^{0}	NT
AKR	1,900	NIH Swiss	$10^{4.8}$	$10^{3.6}$	NT
		C3H/10T1/2	$10^{4.2}$	$10^{3.4}$	$10^{7.1}$
		BALB/3T3	$10^{2.6}$	$10^{1.3}$	$10^{4.8}$
		SC-1	$10^{5.9}$	$10^{5.4}$	$10^{7.3}$
		SIRC	NT	10^{0}	$<10^{2.0}$
BALB V. 2 (from SIRC-infected cells)	NT	C3H/10T1/2	NA[e]	10^{0}	NT
		BALB/3T3	NA	10^{0}	NT
		SC-1	NA	10^{0}	NT
		SIRC	NA	$10^{2.2}$	NT

[a] Picomoles of [^{3}H]TTP incorporated/30 min per milliliter of culture fluid.

[b] Titer represents DNA polymerase-inducing units (PIU) per milliliter of virus stock. One PIU equals the dilution of stock that yields DNA polymerase-containing particles in infected cells with activity of 10 pmol of [^{3}H]TTP incorporated/30 min per ml of medium.

[c] Titer calculated from the percentage of gs-positive cells 1 day after infection, assuming that virus spread had not occurred with these conditions. The number of cells per 60-mm dish at the time of infection was $2 \times 10^5 \pm 2.5 \times 10^4$.

[d] NT, Not tested.

[e] NA, Not applicable.

the virus produced by C3H/MCA5. On the basis of the induction of gs antigens in infected cells, this virus showed N-tropism.

The replication of XC^- type C virus produced by the nontransformed C3H/10T1/2 cells was very inefficient in all target cells examined; only small amounts of DNA polymerase-containing particles were released from infected cells into the culture medium (Table 3). XC^- type C virus produced by the spontaneously transformed C3H/ST3 cells was produced in significantly larger amounts (data not shown).

We have provisionally designated these XC-defective type C viruses as ecotropic XC^-. Ecotropic type C of the high leukemic strains AKR and C58 have been designated as ecotropic XC^+.

Conversion of XC^- virus to XC^+ virus by passage of infected cells in culture. In attempts to investigate the possibility that ecotropic XC^+ virus also was present as a minority component in culture fluids of nontransformed C3H/10T1/2 virus-producing cells, we performed co-cultivation experiments of these cells with SC-1 mouse cells. Upon continued passage of these cultures, XC^+ virus was invariably detected.

The findings led us to the question of whether these XC^+ viruses had preexisted as contaminants of the XC^- virus in low titer, or whether the XC^+ viruses were formed as a consequence of the co-cultivation procedure. To examine this further, SC-1 and C3H/10T1/2 cells (non-IUdR treated) were inoculated with serial dilutions of culture fluids from C3H/10T1/2 cells that were producing XC^- virus and then subcultured for 10 passages. At each passage cells were examined for the production of DNA polymerase-containing particles and for XC^+ virus (by overlay with XC cells); in addition, cells from each passage were seeded on microscope slides and examined by immunofluorescence for the production of MuLV gs antigen. The results of this study are shown in Table 3.

Infection of C3H/10T1/2 cells with XC^- virus was virtually abortive, and only with undiluted culture fluids was there a low productive infection that yielded XC^+ virus. Infection of SC-1 cells also was relatively inefficient, although with continued subculture there was emergence of XC^+ virus in cells that were infected with a dilution of culture fluid as low as 10^{-4}. The occurrence of XC^+ virus in cells infected with highly diluted XC^- virus stocks suggests that the appearance of XC^+ virus in these cultures was not the consequence of selection of a minority component that preexisted in the initial virus stock, but rather was due to the emergence of a virus with new growth characteristics from the subcultured cells. The XC^+ viruses that were derived by subculturing of SC-1 cells infected with XC^- viruses were genetically stable and could be serially propogated in SC-1 cells; thus, these viruses did not appear to represent phenotypic variants.

XC plaques formed by the SC-1 converted (XC^+) virus were mostly acellular in the center; in contrast, the majority of plaques formed by the C3H/10T1/2 converted (XC^+) virus still contained cells in the center.

The appearance of ecotropic XC^+ virus was observed concomitant with both an increase in the percentage of cells that were positive for MuLV gs antigens and an increase in the production of DNA polymerase-containing particles. However, the frequency of this virus conversion (from XC^- to XC^+) was not a linear function of the multiplicity of infection (MOI) (Table 3), and it remains to be determined whether infection of a given cell with more than one XC^- virus is required or increases the probability with which it gives rise to an ecotropic XC^+ virus.

The result of an infective-center test of cultures infected wth the C3H/10T1/2 XC^- virus (from the experiment in Table 3) is shown in Table 4. The fraction of SC-1 cells producing XC^+ virus increased over a twofold range when the fraction of virus-free cells (available for infection) was increased by a factor of 10^1 to 10^3. The frequency of appearance of XC^+ virus-producing cells upon infection with undiluted fluids was 30-fold higher on SC-1 than on C3H/10T1/2 cells; differences in the converting ability of these cells were more dramatically seen with infections at higher dilutions of the virus stock (e.g., infection with fluids diluted 10^{-3} resulted in 61% XC^+-producing SC-1 cells; at the same dilution the C3H/10T1/2 cells were incapable of converting the XC^- virus). It should be pointed out that the frequency of conversion of XC^- to XC^+ virus on both SC-1 and C3H/10T1/2 cells was variable from one experiment to another and that in only one of four experiments was the infective-center assay of C3H/10T1/2 cells as high as 1%.

A host range analysis of the converted XC^+ virus produced by SC-1 cells showed this virus to be similar in tissue culture to the N-tropic MuLV that is endogenous in mice of high leukemic strains. Thus, the converted XC^+ virus was produced at high levels by infected cells, it plated with 100-fold greater efficiency on NIH than on BALB/c cells, and mouse cells infected with the virus formed large syncytia upon contact with XC cells.

TABLE 3. *Conversion of XC⁻ type C virus to XC⁺ type C virus in C3H/10T1/2 and SC-1 cells*[a]

Target cell	Tissue culture passage	Infective-center assay (% cells yielding plaques)					DNA polymerase in culture fluid (pmol incorporated/30 min per 60-mm dish)					MuLV gs antigens (% cells gs positive)				
		10^0 [b]	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^0	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^0	10^{-1}	10^{-2}	10^{-3}	10^{-4}
C3H/10T1/2	24 h p.i.[c]	NT[d]	NT	NT	NT	NT	NT	NT	NT	NT	NT	41.9	7.10	0.71	0.05	–[g]
	0	–[e]	–	–	–	–	26	–[f]	–	–	–	47.1	7.85	0.71	0.05	–
	1	–	–	–	–	–	25	–	–	–	–	50.9	7.42	0.70	0.06	–
	2	–	–	–	–	–	24	–	–	–	–	48.0	7.48	0.72	0.06	–
	3	–	–	–	–	–	32	2	–	–	–	47.9	7.23	0.80	0.07	–
	4	0.023	–	–	–	–	320	4	–	–	–	87.8	7.50	0.89	0.05	–
	5	0.051	–	–	–	–	330	3	–	–	–	>99	7.42	0.88	0.08	–
	6	0.181	–	–	–	–	1,800	3	–	–	–	>99	10.4	1.23	0.11	–
	10	>1	–	–	–	–	1,200	11	–	–	–	>99	13.7	2.36	0.12	–
SC-1	24 h p.i.	NT	NT	NT	NT	NT	NT	NT	NT	–	–	60.2	9.2	1.50	0.17	–
	0	–	–	–	–	–	59	28	3	–	–	57.4	9.1	1.93	0.11	–
	1	0.030	–	–	–	–	124	30	2	–	–	70.6	10.8	1.20	0.20	–
	2	0.347	–	–	–	–	1,980	38	3	–	–	93.5	12.0	1.17	0.18	–
	3	>1	–	–	–	–	4,200	292	4	–	–	>99	11.7	1.20	0.19	–
	4	>1	0.035	0.004	0.001	–	6,800	1,600	328	19	–	>99	65.6	2.70	NT	–
	5	>1	0.456	0.282	0.008	–	7,100	7,400	2,000	82	–	>99	>99	74.1	25.7	0.04
	6	>1	>1	>1	0.316	–	4,900	7,800	3,300	1,800	–	>99	>99	>99	87.3	0.18
	10	>1	>1	>1	>1	–	6,400	7,600	6,200	9,200	1,300	>99	>99	>99	>99	12.4

[a] C3H/10T1/2 and SC-1 cells were infected as described previously (9). The MOI was determined 24 h after infection by immunofluorescence of infected cells. Cultures were maintained in minimum essential medium with 10% fetal calf serum and 2 μg of polybrene per ml; at confluency, the media was changed to minimum essential medium with 10% fetal calf serum. Twenty-four hours later the culture fluids were harvested for determination of DNA polymerase-containing particles. Cells were then trypsinized and used for infective-center assays (9) and for immunofluorescence assays (9). The range of cells seeded for infective centers was 2×10^4 to 2×10^5 per 60-mm petri dish. In the case of negative reactions, approximately 14,000 cells were screened by immunofluorescence for each time point.

[b] Dilution at which cultures were infected with C3H XC⁻ MuLV.

[c] p.i., Postinfection.

[d] NT, Not tested.

[e] –, <1 in 10^4 cells gs positive.

[f] –, <2 in 2×10^5 cells.

[g] 1 pmol of [^{3}H]TTP incorporated/30 min per 60-mm dish.

TABLE 4. *Infective-center assay of SC-1 and C3H/10T1/2 cells infected with C3H/10T1/2 XC⁻ MuLV*[a]

Infected cells	% Virus-producing cells				
	10^{0}[b]	10^{-1}	10^{-2}	10^{-3}	10^{-4}
SC-1	35.1	43.6	47.8	61.2	17.9
C3H/10T1/2	1.2	−[c]	−	−	−

[a] Infective-center assays (9) were performed with cells that were subcultured 12 passages after infection.

[b] Dilution at which cultures were infected with C3H XC⁻ MuLV.

[c] −, <1 out of 2 × 10^5 cells.

DISCUSSION

We have identified by infectivity studies three major characteristics of endogenous ecotropic type C viruses of the low leukemic mouse strain C3H. (i) Infected cells do not form syncytia in contact with XC cells, even when they produce large amounts of DNA polymerase-containing particles. (ii) Infection of Fv-1^n or Fv-1$^-$ mouse cells with XC$^-$ viruses occurs efficiently and as a linear function of virus dose (at MOI > 1). Infected cells release only small or undetectable amounts of DNA polymerase-containing particles, although they do express MuLV gs antigens in their cytoplasm. (iii) Continued subculture of infected SC-1 mouse cells leads to a high frequency conversion of ecotropic XC$^-$ viruses to XC$^+$ viruses that are similar in XC tests to N-tropic MuLV of AKR mice. This conversion of XC$^-$ virus to XC$^+$ virus also has been observed on the cells of origin, C3H/10T1/2, but at much lower frequency and only in cultures infected with the highest MOI that was used in these experiments (0.5 to 1.2 as judged from the fraction of cells expressing MuLV gs antigens 24 h after infection).

It is unlikely that the large differences in conversion frequencies that were observed between SC-1 and C3H/10T1/2 cells were due to differences in the number of cell divisions that ensued upon continued subculture of infected cells, since both cell lines showed post-confluence inhibition of division and their saturation densities were similar. It is also apparent from the conversion experiments that the production of DNA polymerase-containing particles and the appearance of converted virus were not processes that simply required a fixed number of divisions by infected cells; if this were the case, all the cultures would have started producing converted virus at about the same time after infection, independent of the initial MOI. Instead, the frequency and time of onset of conversion was a nonlinear function of the fraction of initially infected cells.

It is possible that the differences in conversion frequencies that were observed between SC-1 and C3H/10T1/2 cells were a result of differences in the endogenous viruses that were present in these cells. Although both cells contain DNA sequences of MuLV proviruses, MuLV can be induced from C3H/10T1/2 cells by IUdR but not from SC-1 cells. In this regard, we have recently found (unpublished data) that infection of C3H/MCA5 (but not C3H/10T1/2) cells with the C3H/10T1/2 XC$^-$ MuLV results in a rapid conversion of the XC$^-$ virus to XC$^+$ comparable to that observed with SC-1 cells.

The infective-center data from cultures producing converted virus indicate that spread of XC$^-$ virus occurred before the appearance of XC$^+$ virus. Thus a significant fraction of cells in the converted culture was stably infected with the XC$^-$ virus (independent of MOI), indicating that this agent initially established the early rounds of infection in these cells. Our interpretation of this finding is that conversion of XC$^-$ virus to XC$^+$ virus required more than one round of infection in converting cells.

IUdR or bromodeoxyuridine induction experiments with normal cell lines or secondary MEF cultures derived from the low leukemic strains BALB/c and C57BL/6 (14) and from BALB/c, C57BL, and B10.D2 (12) have been described previously. Little or no infectious ecotropic virus was detected in the XC test unless the induced cells were co-cultivated with NIH/3T3 cells (1, 14). Viruses obtained by co-cultivation of cells were detectable in the XC test, although the plaques were small and difficult to score (3). In more recent studies, assays have been used that would not be expected to distinguish XC$^-$ viruses from XC$^+$ viruses (1). The findings reported here demonstrate the need to use infectivity assays in addition to the XC test in studies with MuLV and murine sarcoma virus.

Our findings also indicate that the ability of endogenous type C virus to replicate efficiently on the cells of origin may be an important determinant of their potential in their natural host. Further studies on the pathogenic properties of ecotropic XC$^-$ and converted XC$^+$ virus in vivo and in vitro are in progress.

Recently, we learned that an XC$^-$ type C virus of BALB/c mice has been independently described by Hopkins and Jolicoeur (8). This virus shows similar properties to those described in this manuscript; taken together with our previous observation of XC$^-$ viruses in BALB/c mice (9) and with the occurrence of an XC$^-$ virus in L cells (13) it appears that these XC$^-$ agents may constitute a widely distributed virus group in mice.

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