Replication of the Moloney Murine Sarcoma-Leukemia Virus in XC Cells

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The XC rat cell line was found to support the replication of a strain of the Moloney murine sarcoma-leukemia virus. In growth curve experiments cytopathology was paralleled by the production of murine sarcoma virus and leukemia virus progeny having the biologic, antigenic, and biophysical properties of the infecting virus.

The XC cells, a cell line originally derived from tumor cells induced in newborn Wistar albino rats by the Prague strain of Rous sarcoma virus (12), undergo cytopathic changes consisting of cell fusion and formation of syncytia when co-cultivated with mouse cells infected with murine leukemia virus (MLV) (8). This phenomenon has led to the establishment of a plaque assay for MLV by co-cultivation of MLV-infected cells and XC cells (10). It has been recently shown (6) that homogenates of infected cells as well as high concentrations of purified MLV can also cause fusion of XC cells, indicating that the virus particles are probably the activators of the fusion process. In the present study we report that the XC cells support the replication of the Moloney strain of murine sarcoma (MSV)-leukemia virus (MSV-MLV), derived from virus-releasing transformed Wistar rat (78A1) cells (3); infection of XC cells with a high multiplicity of MSV-MLV results not only in cell fusion but also in the production of progeny virus with the biologic, antigenic, and biophysical properties of the infecting virus.

The cells used in this study were as follows: XC rat cells and BALB/3T3 murine cells supplied by S. A. Aaronson and W. P. Parks, respectively, of the National Institutes of Health, and locally derived Sprague-Dawley rat embryo fibroblasts. All cells were grown in Eagle minimal essential medium containing 10% fetal calf serum for XC and BALB/3T3 cells and 20% fetal calf serum for Sprague-Dawley rat embryo fibroblasts. Virus infectivity was determined for MLV by the XC plaque assay (10) using BALB/3T3 and XC cells; for MSV, virus infectivity was determined by the focus assay described by Aaronson et al. (1) using Sprague-Dawley rat embryo fibroblasts.

Physical particles were estimated by electron microscopy using the droplet pseudoreplication method described by McCombs et al. (9). MSV-MLV preparations obtained from clarified supernatant fluids of 48-hr-old 78A1 cultures were used in the present study and had titers per ml of: 10^{10} total virus particles, 10^6 plaque-forming units (PFU) of MLV, and 10^4 focus-forming units (FFU) of MSV.

Growth curve experiments of MSV-MLV in XC cells were carried out. Twenty-hour-old XC cell monolayers (10^6 cells per 60-mm petri dish) were treated with 25 µg of diethylaminoethyl (DEAE)-dextran per ml for 1 hr (11), rinsed with growth medium, and each inoculated with 0.5 ml of undiluted virus (10^6 PFU of MLV and 10^4 FFU of MSV per ml). After 1 hr of incubation at 37 C the unadsorbed virus was removed by rinsing the cultures twice with growth medium and incubated further in the presence of 5 ml of growth medium per culture. At various times after infection the cultures were observed for cytopathic changes, and the supernatant fluids of duplicate plates were pooled and clarified at 800 × *g* for 20 min. They were kept at −90 C until assayed for infectious MLV, infectious MSV, and for virus particles.

The XC cells, which are small and round, showed a morphological change as early as 6 hr after infection. At 6 hr, areas of fused cells were present containing 5 to 10 nuclei. By 24 hr postinfection, fusion of the cells was extensive and vacuolization had begun. At 72 hr, fusion was complete and vacuolization was extensive. Thus, the undiluted MSV-MLV preparation alone was capable of inducing fusion in the XC cells; a similar observation has been noted by Johnson and co-workers (6). To determine whether the cytopathology ob-

served was paralleled by virus replication, the supernatant fluids collected from the infected XC cell cultures were assayed for infectious MSV, infectious MLV, and for virus particles as indicated above. The results of a typical growth curve experiment are presented in Fig. 1. After an eclipse of approximately 12 to 18 hr, progeny virus was detected in the supernatant fluids. The MLV population reached a peak titer of 7×10^4 PFU/ml in the 5 ml of fluid per culture at 48 hr, and the MSV portion reached a peak titer of 2×10^3 FFU/ml at 48 hr also. Such titers were equivalent to those obtained by infecting normal Sprague-Dawley or Wistar rat cells with the same strain of MSV-MLV (S. T. Trowbridge, Ph.D. dissertation, Baylor College of Medicine, 1972). After the peak titers were reached in the XC cells, however, the number of biologically active particles began decreasing, whereas the actual particle counts remained stable at 8×10^9 particles per ml up to 96 hr. This was most probably due to the fact that the XC cells had undergone extensive vacuolization and destruction by 48 hr and seemingly did not support continued virus production; thus, the virus already produced was being inactivated after prolonged incubation at 37 C.

To confirm the observation that MSV-MLV was replicating in the XC cells and new progeny virions were produced, attempts were made to isolate and characterize radioactively labeled progeny virions from XC cells. Cultures of XC cells were infected with MSV-MLV as described before except that the growth medium was supplemented with 10 μCi of ^{3}H-uridine per ml. At 48 hr post-infection, the supernatant fluid was concentrated (3) and centrifuged to equilibrium in a 20 to 25% linear sucrose density gradient. A radioactive peak in the buoyant density range of 1.14 to 1.16 g/ml, characteristic of oncornaviruses, was detected in the culture fluids of XC cells infected with MSV-MLV but not in noninfected XC cells.

To investigate whether the ^{3}H-uridine was actually incorporated into the high-molecular-weight ribonucleic acid (RNA) of MSV-MLV, the fractions showing maximal radioactivity at the buoyant density region of 1.14 to 1.16 g/cm^3 were pooled, and the RNA was isolated by the phenol m-cresol method (2). When the RNA was centrifuged in a 5 to 30% continuous sucrose density gradient for 45 min at 60,000 rev/min, two species of RNA with sedimentation coefficients of 69S and 4 to 10S respectively, were detected (Fig. 2). The
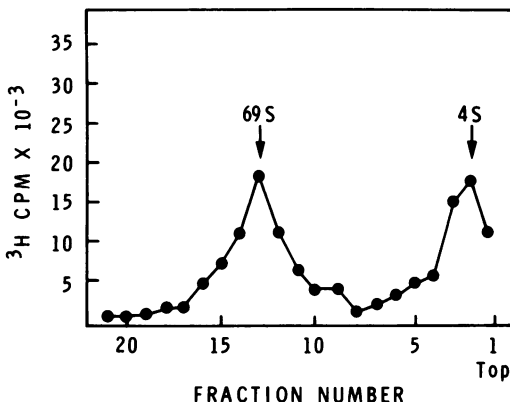
FIG. 2. Velocity sedimentation of the RNA isolated from purified virions released by XC cells infected with MSV-MLV. ^{3}H-uridine-labeled virus particles in the supernatant fluid were precipitated with 35% ammonium sulfate and banded once in a discontinuous 30 to 60% sucrose density gradient. The concentrated material was centrifuged to equilibrium on a 20 to 60% linear sucrose density gradient for 2 hr at 60,000 rev/min in a Spinco SW65 rotor. The virus band with maximum radioactivity at a buoyant density range of 1.14 to 1.16 g/cm^3 was collected, lysed, and deproteinized by the phenol m-cresol method (2); 0.25 ml of the viral RNA was layered on 5 ml of linear sucrose gradient (5 to 30%) and centrifuged for 45 min at 60,000 rev/min in a Spinco SW65 rotor. Twenty-one fractions were collected directly into scintillation vials, and radioactivity was measured using a Beckman LS25 liquid scintillation spectrometer.
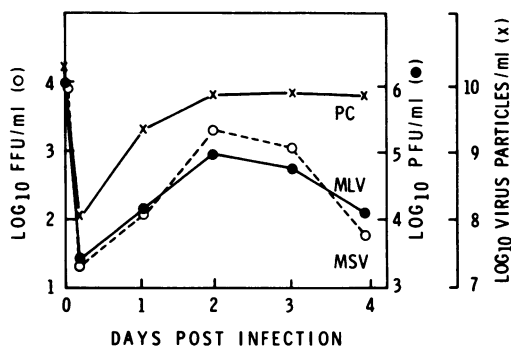
FIG. 1. Growth of MSV-MLV in XC cells. XC cell monolayers were treated with DEAE-dextran and then infected with 0.5 ml of undiluted MSV-MLV containing 10^6 PFU/ml and 10^4 FFU/ml. Cells were washed after 1 hr to remove unadsorbed virus and incubated with 5 ml of growth medium. At various times the supernatant fluid was collected and assayed for (i) total number of virus particles by electron microscopy ($\times$), (ii) MSV by focus formation on normal Sprague-Dawley rat embryo fibroblasts (O), and (iii) MLV for plaque formation on BALB/3T3 cells with XC cell overlay (●). Note the different scales for MSV and MLV.

progeny particles of the MSV-MLV-infected XC cells thus contained RNA characteristic of the input MSV-MLV (3).

Neutralization and immunofluorescence tests were performed to confirm the relatedness of the input MSV-MLV to the virus released by the infected XC cells. Both the input MSV-MLV used in the growth curve experiments and the progeny virus harvested from the XC cultures at 48 hr postinfection (Fig. 1) were tested for their neutralization in the presence of anti-MLV rabbit serum (Virgo Reagents, a Division of the Electro Nucleonic Lab., Inc., Bethesda, Md.). A constant virus-varying antiserum method (5) of neutralization was used. Equal volumes of virus, diluted to contain approximately 1,000 FFU of MSV/ml, and serial fourfold dilutions of heat-inactivated (56 C for 30 min) rabbit antiserum (or normal rabbit serum for controls) were incubated at room temperature for 30 min. Duplicate Sprague-Dawley rat embryo fibroblast cultures were then inoculated with 0.2 ml of each virus-serum mixture, and residual infectivity was determined by the focus-forming assay (1). As shown in Table 1, the normal rabbit serum had no effect on the focus-forming ability of either the parent or the progeny virus. On the other hand, with the anti-MLV rabbit serum final concentrations of 1:128 and 1:32 gave greater than 70% neutralization of the parental and progeny viruses, respectively.

For immunofluorescence, normal rat cells growing on cover slips were infected as described for the growth curve experiments with undiluted preparations of both the input and progeny viruses. At 24 and 48 hr postinfection, the cells on the cover slips were tested for the presence of MLV-specific antigens by the indirect immunofluorescence test (4), using a 1:20 dilution of anti-MLV rabbit serum (or normal rabbit serum) and goat anti-rabbit gamma globulin conjugated with fluorescein isothiocyanate (Hyland Lab., Los Angeles, Calif.) Specific cytoplasmic fluorescence was detected in the cells infected with both the input and progeny virus preparations (Table 1). Approximately 100% of the cells were positive for fluorescence with the input virus at 2 days and 4 days after infection. With the progeny virus as the inoculum, the cells showed a high percentage (50%) which fluoresced at 2 days after infection, but by 4 days 100% of the cells could be scored as positive. This difference was most likely due to the higher titer (and therefore the higher multiplicity of infection) of input virus (see Fig. 1). No reactivity was observed with the normal rabbit serum.

From the preceding results, it appears that the strain of Moloney murine sarcoma-leukemia virus used in this laboratory is sufficient to cause fusion of XC cells without requiring the aid of MLV-infected mouse cells. High concentrations of virions, however, seem to be required for the fusion since in repeat experiments dilutions of virus (10^{-1}, 10^{-2}, 10^{-3}) did not cause the cytopathic response. The XC cells alone could not be used as an assay for MLV for that reason. Both the MSV and MLV replicate in the XC cells, producing progeny which have a buoyant density of 1.14 to 1.16 g/cm^3 in sucrose, which contain 60 to 70S RNA, and which are neutralized by rabbit anti-MLV serum. All of these properties of the progeny virions are characteristic of the MSV-MLV used to infect the XC cells (3).

The replication of this strain of MSV-MLV in XC cells could be fairly unique since Klement and co-workers in 1969 (8) reported that

TABLE 1. *Neutralization and immunofluorescence tests with MSV-MLV and progeny virus of XC-infected cells with MSV-MLV*

Virus[a]	Serum (rabbit)	No. of FFU[b] per culture in the presence of:					Percent immunofluorescence of rat embryo cells at days after infection	
		Serum dilution				No serum	2	4
		1:8	1:32	1:128	1:512			
Input	Anti-MLV	0	9	78	≥200	≥200	100[c]	100
Input	Normal	≥200	≥200	≥200	≥200	≥200	0	0
Progeny	Anti-MLV	0	19	114	122	189	50	100
Progeny	Normal	117	155	164	174	168	0	0

[a] Input virus, MSV-MLV used for infection of XC cells; progeny virus, virus released by XC cells 48 hr after infection (see Fig. 1).

[b] Focus-forming units.

[c] Percent positive cells (serum dilution used 1:20).

XC cells had a very low susceptibility to several MLV strains as determined by the lack of syncytium formation or of fluorescence specific for MLV in the XC cells inoculated with the various virus strains. No growth curve experiments were reported, however. More recently, C-type virus particles were induced in these cells after treatment with 5-bromodeoxyuridine (7). The released virus had rat-specific antigenic characteristics but was not capable of replicating in normal rat cell cultures. It is unlikely that in our study infection with MSV-MLV has resulted in the activation of a similar rat C-type virus from the XC cells, especially since the progeny virus was infectious for normal rat cells.

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