Enhancement of Murine Leukemia Virus Replication in Rat Tumor Cells Containing Rat C-Type Virus

W. TURNER, G. PEARSON, AND LESLIE R. BASS

Viral Biology Branch, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20014

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The yield of Maloney leukemia virus (MLV) from MLV-infected rat cells was shown to be enhanced in rat cells containing rat C-type virus. The MLV produced in these cells was shown to be identical to murine-derived MLV and devoid of properties related to rat C-type virus.

The detection of C-type virus particles biologically and antigenically distinguishable from murine C-type virus has been reported in both spontaneously and chemically induced rat mammary carcinoma (3, 4), transformed cells originating spontaneously in tissue culture from rat embryo cells (7; V. V. Bergs et al., Int. J. Cancer, in press), as well as transformed cells derived from a tumor induced in rats by Maloney-murine sarcoma virus (M-MSV; 1). These C-type virus isolates have been shown to replicate in rat cells in vitro without inducing cytopathological changes or transformation. However, a rat C-type virus (WF-1 virus) isolated from Wistar-Furth rat embryo fibroblast cells spontaneously transformed in vitro has been shown to induce changes suggestive of transformation in embryonic lung cells derived from Sprague-Dawley rats (V. V. Bergs et al., in press). None of these C-type virus isolates has been shown to be oncogenic in rats, mice, or hamsters (7). Thus, the etiological significance of these virus particles in the induction of rat neoplasms is still unknown.

Rat cells infected with rat C-type virus have been shown to contain a virus-specific antigen(s) associated with the membrane of the infected cell which can be detected by the indirect immunofluorescence test (G. Pearson et al., Int. J. Cancer, in press). The membrane fluorescence technique has been used for monitoring replication, as well as quantitation, of rat C-type viruses in vitro (G. Pearson et al., in press). In addition, rat C-type virus isolated from M-MSV(O) stocks have been shown to act as helper virus for M-MSV(O) in vitro (1). This report concerns the enhanced yields of Maloney leukemia virus (MLV) from rat C-type virus containing rat tumor cells after superinfection with MLV.

Cell lines employed in these studies were as follows: (i) W/Fu/Em, a continuous line of normal, virus-free rat embryo fibroblast (REF) cells derived from embryos of rats of the Wistar-Furth strain (V. V. Bergs et al., in press); (ii) WF-1, a virus-positive tumor cell line established from W/Fu rat embryonic cells that transformed spontaneously in vitro (V. V. Bergs et al., in press); (iii) R-35, a virus-positive tumor cell line derived from a mammary carcinoma occurring spontaneously in a Sprague-Dawley rat (4); (iv) normal, virus-free REF cells derived from embryos of Fisher rats purchased from Microbiological Associates, Inc. (MAI), Bethesda, Md.; (v) a continuous line of normal mammary tissue cells derived from a Sprague-Dawley rat (NRMTC); (vi) Dunning tumor, a continuous line of virus-negative tumor cells derived from a chemically induced tumor of a Fisher rat (6); (vii) mouse embryo fibroblast cells (MEF) derived from 14-day-old embryos from National Institutes of Health Swiss mice purchased from MAI; and (viii) XC cells, a cell line derived from a tumor induced in a Wistar rat by the Prague strain of Rous sarcoma virus.

All of the cell lines except MEF and XC cells were grown in RPMI 1640 medium (Gibco, Grand Island, N.Y.) supplemented with 10% fetal calf serum (FCS), 100 units of penicillin per ml, and 100 μg of streptomycin per ml. MEF cells and XC cells were grown in Eagle minimal essential medium (Gibco, Grand Island, N.Y.) supplemented with 10% FCS, 1% glutamine, 100 units of penicillin per ml, and 100 μg of streptomycin per ml.
The murine leukemia virus (MuLV) employed in these studies was MLV. This virus was in its 45th passage in MEF cells and had a titer of $10^{6.7}$ plaque-forming units (PFU)/ml as determined by the XC plaque assay described previously (8). Cells to be employed in these experiments were plated at a density of $3.5 \times 10^8$ cells/4.0 ml in 60-mm plastic petri dishes. Cultures were incubated for 24 hr at 37°C in a humidified atmosphere containing 5% CO₂. These cultures were treated with diethylaminoethyl-dextran (50 μg/plate/4.0 ml) and infected with MLV at a multiplicity of infection of 0.6 PFU/cell as previously described (9). Cells and supernatant fluids of infected cultures were collected and processed for virus assay on the third and sixth day after MLV infection, as previously described (9).

The virus content of infected cultures was determined by the semi-micro XC assay for MLV (2) employing MEF cells, and titer was expressed as the mean infective dose (ID₉₀) per milliliter. The presence of rat C-type virus in rat cells employed in these experiments was determined by the membrane immunofluorescence and electron microscope procedures described, respectively, by Pearson et al. (in press) and Bergs et al. (in press).

Data indicate that the MLV titer of MLV-infected rat cells containing rat C-type virus was greater than the MLV titer of MLV-infected rat cells free from rat C-type virus (control cells) 3 and 6 days after MLV infection (Table 1). The MLV titer of MLV-infected WF-1 cells was shown to be $10^{3.1}$ and $10^{2.7}$ ID₉₀/ml greater, respectively, on the third and sixth day after infection than the MLV titer of MLV-infected control rat cells (W/Fu-Em).

Similarly, MLV-infected R-35 cell cultures showed a MLV titer $10^{4.4}$ and $10^{2.8}$ ID₉₀/ml greater, respectively, on the third and sixth day after MLV infection than control cells (NRMTC). In contrast, the MLV titer after infection of the rat C-type virus-free Dunning tumor cell cultures was $10^{3.6}$ and $10^{2.7}$ ID₉₀/ml lower, respectively, on the third and sixth day after MLV infection than the MLV titer of infected control cells (REF cells).

The data clearly show that the replication of MLV in rat tumor cells infected with rat C-type virus was enhanced. The absence of enhanced replication of MLV in rat C-type virus-free Dunning tumor cells supported the hypothesis that enhanced MLV replication observed in WF-1 and R-35 cells was related to the presence of rat C-type virus in these cells rather than being solely related to the enhanced metabolic activity of these tumor cells.

Investigation of progeny MLV from MLV-infected rat tumor cells and murine cell-derived MLV had identical replication patterns in MEF cells. In addition, the rat tumor cell-derived MLV was capable of inducing leukemia in mice with histopathological characteristics identical to mouse leukemia induced by murine cell-derived MLV.

Observation of MLV-infected rat tumor cells carrying rat C-type virus by the membrane immunofluorescence technique employing a specific antiserum to rat C-type virus derived from W/Fu-1 cells which cross-reacts with rat C-type virus derived from R-35 cells indicated

**Table 1. Replication of murine leukemia virus (MLV) in rat cells infected with rat C-type virus.**

| Cell line            | Rat strain from which cell lines were derived | Detection of rat⁴ C-type virus in cell lines determined by Log₉₀ titer (ID₉₀/ml) of MLV in cultures of rat cell lines after infection with MLV |
|---------------------|---------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------|
| EM                  | W/Fu                                        | EM IF 3 days 6 days                                                                                                             |
| W/Fu-EM             | W/Fu                                        | – – 1.5 2.1                                                                                                                     |
| WF-1                | W/Fu                                        | + (20-35%) 4.6 4.8                                                                                                               |
| NRMTC               | S-D                                         | – + (5-15%) 4.0 4.3                                                                                                              |
| R-35                | S-D                                         | – + (5-15%) 4.0 4.3                                                                                                              |
| REFF                | Fisher                                      | – – 2.0 2.4                                                                                                                     |
| Dunning tumor cells | Fisher                                      | – – 1.2 1.5                                                                                                                     |

* W/Fu, Wistar-Furth strain; S-D, Sprague-Dawley strain.

⁴ The indirect immunofluorescence (IF) technique as described by Pearson et al. (in press) was used in these studies and employed a pooled serum from rats bearing WF-I tumors as the source of antibodies. This serum had previously been shown to contain antibodies reactive with the membranes of cells infected with WF-I or R-35 viruses (Pearson et al., in press). Cells were prepared for electron microscopy (EM) studies by the method described by Berg et al. (in press). (+) Presence of budding C-type viral particles or immunofluorescence; (−) absence of budding C-type viral particles or immunofluorescence.
that the rat C-type virus content of the rat tumor lines was not increased or decreased by MLV infection. Attempts to detect in vitro the presence of rat C-type virus in MLV pools derived from MLV-infected rat C-type virus-positive rat tumor cells by the membrane immunofluorescence technique were negative.

These data clearly show that the yields of MLV from rat tumor cells infected with rat C-type virus is greater than the yields of MLV from MLV-infected rat cells free from the rat C-type virus. The data strongly suggest that presence of the rat C-type virus in rat cells is associated with the increased yields of superinfecting MLV. However, the exact mechanism by which the observed viral enhancement was induced is not known.

Since the ability of rat C-type to enhance MLV replication in rat cells may be an additional biological property of rat C-type viruses and therefore useful in the detection or assay, or both, of rat C-type virus in vitro, further investigation concerning the enhanced replication of MLV in rat C-type virus-positive rat cells is underway in this laboratory.

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