Characterization of Mouse Mammary Tumor Viruses Propagated in Heterologous Cells

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SUMMARY

Mouse mammary tumor viruses (MMTV) from three different strains of mice have been used to establish productive infections in feline and mink cell lines. The virions that are released by these cells compete completely in a radioimmunoassay for the major virion surface glycoprotein of MMTV (gp52), thus demonstrating that antigenic determinants of gp52 are viral coded. Competitive molecular hybridization studies have shown that the 60 to 70 S RNA's of MMTV's propagated in feline cells contain all the nucleic acid sequences found in 60 to 70 S RNA from MMTV synthesized by murine cells. The virion buoyant densities in sucrose and cesium chloride, virion sedimentation coefficient, divalent cation requirement of the virion DNA polymerase, and morphology of MMTV's synthesized in heterologous cells are similar to those of MMTV's grown in murine cells. Cultures of MMTV-infected feline cells have continuously released between 0.1 and 1.0 μg of virus per 10^6 cells (75-cm² flask) per day during the 60-week observation period. No detectable feline or murine type C viruses were produced by these cultures.

INTRODUCTION

MMTV's are the only retroviruses known to induce carcinomas. The susceptibility of a given mouse to MMTV infection has been shown to be, in part, dependent on the donor virus; i.e., mice resistant to an MMTV from one strain are susceptible to virus from another strain (19). An understanding of the mechanism by which the virus causes mammary carcinoma and of the biology of the virus has been hampered by the lack of a suitable in vitro system in which to study these phenomena. Lasfargues et al. (11–13, 27) recently successfully infected established cell lines of feline and mink origin with MMTV purified from the milk of RIII mice. These cell lines were shown to release a virus with a typical type B virion morphology and containing a magnesium-prefering virion-associated DNA polymerase. The cells also contained MMTV-related antigens at the plasma membrane as determined by immunofluorescence.

In this report we have confirmed and extended these studies. We have infected feline and mink cells with MMTV's from several mouse strains; several cultures of feline cells have produced MMTV at consistent levels during an observation period of more than 60 weeks. The viruses released by MMTV-infected feline cells competed completely in a radioimmunoassay specific for MMTV gp52. Radioactively labeled 60 to 70 S RNA from purified viruses released by MMTV-infected feline cells has been utilized in molecular hybridization studies to characterize further the MMTV's grown in heterologous cells.

MATERIALS AND METHODS

Cells. The mink cell line Mv1Lu, derived from the lungs of nearly full-term mixed-sex fetuses of the Aleutian mink (ATCC, CCL-64), was obtained from Dr. G. Todaro, NCI, Bethesda, Md. The feline embryo kidney cell line CrFK was obtained from Dr. A. Hackett, Naval Biomedical Research Laboratories, Oakland, Calif. These cell lines were grown at 37°C in Dulbecco's modified Eagle's medium containing 10% heat-inactivated fetal calf serum; penicillin, 10 units/ml; and streptomycin, 10 μg/ml. Both cell lines have a generation time of 20 to 25 hr when grown in this medium.

The mouse mammary tumor cell line Mm5mt/c, (21), which was derived from a spontaneous mammary tumor of a C3H mouse, was obtained from Dr. D. Fine, Frederick Cancer Research Center, Frederick, Md.; it was grown in Dulbecco's modified Eagle's medium containing 10% heat-inactivated fetal calf serum; penicillin, 10 units/ml; streptomycin, 10 μg/ml; bovine insulin (Sigma Chemical Co., St. Louis, Mo.), 10 μg/ml; and dexamethasone, 2 μg/ml.

All cell lines were tested monthly for the presence of Mycoplasma. The cells were also tested for species of origin by Dr. C. S. Stublberg, Child Research Center of Michigan, through the Office of Resources and Logistics, Virus Cancer Program, NCI.

Viruses. MMTV(C3H) virus was obtained from culture fluids of the C3H mammary tumor cell line Mm5mt/c, and concentrated as described below. MMTV(RII) and MMTV derived from GR mice were isolated from freshly collected milk from RII and GR mice, respectively. Milk was collected from mice at the 3rd or higher parity. Avian myeloblastosis virus was isolated from the plasma of leukemic chickens.
MMTV’s Propagated in Heterologous Cells

was obtained from culture fluids of MPV-infected NC-37 mice (University Laboratories, Highland Park, N. J.). MPV cells and concentrated as described for MMTV(C3H) virus. FeLV was obtained from the Rickard-422 leukemic feline thymus cell line. The endogenous feline virus, RD-114, was obtained from culture fluids of the RD-114 cell line (16). The above viruses were obtained through the Office of Resources and Logistics of the Virus Cancer Program, NCI.

Preparation of Virus Inocula and Inoculation of Cells. The source of virus for infection of cells was freshly collected milk from mice at the 3rd or higher parity or supernatant fluids from the Mm5mt/c, cell line. When virus was purified from fresh milk, the milk was first clarified of cells and debris by centrifugation at 3000 and 8000 × g, each for 10 min. MMTV(C3H) in culture fluids from Mm5mt/c, cells was concentrated 1000-fold as described previously (7).

Clarified milk or freshly concentrated virus from Mm5mt/c, cells was layered onto a 15 to 65% (w/w) sucrose gradient in TNE and centrifuged at 95,100 × g for 3 hr at 4° in a Beckman SW27 rotor. Fractions were collected by bottom puncture of the tube, and the fractions corresponding to densities from 1.15 to 1.20 g/ml were pooled. The pooled fractions were diluted 4-fold with TNE and pelleted through 2.5 ml of 20% (v/v) glycerol in TNE at 195,700 × g for 60 min in a Beckman SW41 rotor. The virus-containing pellets were resuspended in phosphate-buffered saline (170 mM sodium chloride, 3.4 mM potassium chloride, 10 mM disodium phosphate, and 2 mM potassium dihydrogen phosphate [pH 7.4]) overnight at 4°. The viral suspensions were then brought up to a volume equal to the original volume of milk or the original volume of 1000-fold-concentrated virus from cell culture fluids (see above) in MEM containing 10% heat-inactivated fetal calf serum; penicillin, 10 units/ml; streptomycin, 10 µg/ml; bovine insulin, 10 µg/ml; and polybrene, 4 µg/ml (MEM-polybrene). The inoculum was filtered through a 0.45-µm Millipore membrane precoated with polyvinylpyrrolidone (12) to remove any contaminating murine cells and bacteria. Cells were then infected with MMTV’s essentially as described previously (11-13).

Typically, 1 × 10⁶ cells, suspended in 1 ml of MEM-polybrene, were mixed with 4 ml of virus inoculum and incubated with occasional shaking at 37° for 45 min. The cell suspension was diluted in 20 ml of the same medium, and 5-ml aliquots were plated in 25-cm² plastic tissue culture flasks (Costar, Cambridge, Mass.)

Two days later, the medium was changed to growth medium without polybrene. Seven days after infection, dexamethasone was added to a final concentration of 2 µg/ml. From this point on, cells were routinely grown in this medium (MEM-dexamethasone). Twenty-four-hr culture medium collections were assayed at weekly intervals for the presence of virion-associated DNA polymerase activity using oligo(dG) (poly(rC)) (P-L Biochemicals, Milwaukee, Wis.) as described previously (18). Results of greater than 1000 cpm above zero time background per aliquot at 60 min were scored as positive.

Molecular Hybridization. DNA was extracted from nuclei isolated from cell pellets as described by Colcher et al. (4), and all DNA’s used had A₂⁶₀/A₂⁸₀ of >1.8. Approximately 1,500 cpm of high-molecular-weight viral RNA was annealed to 500 µg of denatured (2 min, 100°) nuclear DNA at a concentration of 3 mg/ml to a C₅₀ of 35,000 (corrected to a standard of 120 mM sodium phosphate buffer (11) at 68°. The hybrids formed were assayed for the acquisition of RNase resistance as previously described (4). The percentage of hybridization was corrected for the inherent 2 to 3% double strandedness of the viral RNA’s.

For competitive molecular hybridization studies, each 100-µl hybridization reaction contained approximately 2.2 × 10⁻³ µg of MMTV(C3H) 60 to 70 S RNA (2400 cpm), 100 µg denatured DNA fragments from Mm5mt/c,(C3H) mammary tumor cells, 400 mM sodium phosphate (pH 6.8), 0.05% sodium dodecyl sulfate, and various amounts of unlabeled viral 60 to 70 S RNA up to a maximum of 5 µg. After incubation at 68° for 66 hr (to a C₅₀ of 5200), hybrids were assayed for RNase resistance and the percentage of competition was determined as described previously (4).

Radioimmunoassays. Competition radioimmunoassay for the detection of MMTV gp52 was carried out as described by Cardiff et al. (2, 3). Briefly, competing antigen was added to a dilution of goat anti-MMTV(RIII) gp52 (kindly
provided by Dr. Wade Parks, NCI) which has a 50% end point titer of 1:10,000. After incubation at 37°C for 1 hr, 20 ng of MMTV(RIII) virions externally labeled with 125I (approximately 10,000 cpm) were added, and the new mixture was incubated for an additional hr. Finally, porcine anti-goat IgG was added followed by incubation at 37°C for 1 hr and at 4°C overnight. The samples were centrifuged at 15,000 rpm for 1.5 min in a Beckman microfuge 152. The percentage of 125I-labeled MMTV(RIII) bound was calculated following counting of both supernatant and pellet fractions in a Beckman γ counter. Greater than 90% of the 125I-labeled MMTV virions has been shown previously (26), and in our own studies, to be associated with gp52.

**Immunodiffusion.** Double diffusion (Ouchterlony) tests were performed in 2% Noble agar slides (Immunoplate Pattern C; Hyland Laboratories, Costa Mesa, Calif.). Purified MMTV(RIII), 100 μg, grown in feline cells, was disrupted with 0.5% Triton X-100 for 15 min at 37°C and reacted with 20 to 40 μl of antisera prepared against Tween ether-disrupted RD-114 virus, baboon (M7) virus, FeLV, Moloney and Gross MuLV's, MPV, and MMTV, as well as antisera prepared against fetal calf serum proteins. Anti-MPV and anti-fetal calf serum were raised in rabbits following multiple injections of the appropriate antigens. All antisera were preabsorbed with fetal calf serum if necessary. The slides were incubated at 37°C in a humidified chamber. Precipitin lines usually appeared within 24 hr and were observed for an additional 48 to 72 hr.

**Electron Microscopy.** Cell monolayers in 25-sq cm flasks were fixed in situ as previously described (18) except that postfixation was performed using 1% osmium tetroxide in place of Dalton's chromium-osmium. Micrographs were taken at a magnification of ×10,000 to ×40,000 in a Siemens-Elmiskop 1A electron microscope.

**Species Verification of Cell Lines.** All infected and uninfected feline (CrFK) and mink (Mv1Lu) cells were shown to be associated with gp52 formed a line of identity in immunodiffusion with homologous antisera. Monospecific antisera to MMTV(RIII) grown in feline cells, was identical with that obtained using purified MMTV(RIII). The competition curve obtained with MMTV(RIII)Fel or MMTV(C3H)Fel preparations was also tested by radioimmunoassay (kindly performed by Dr. C. Sherr and Dr. G. Todaro) and/or immunodiffusion for the presence of the following type C viruses: AD-114, FeLV, Moloney, Gross MuLV, and MMTV, respectively. None of the cultures showed any evidence for the presence of murine, canine, or simian cells when tested by immunofluorescence. Of the metaphases scanned for ploidy, 85% from uninfected CrFK cells and 97% from uninfected Mv1Lu cells were in the diploid chromosome range (2N = 38 and 30, respectively). Scanning of metaphases from MMTV-infected CrFK and Mv1Lu cells revealed 88 and 97%, respectively, in the diploid chromosome range. Chromosome banding techniques presented no evidence for the presence of other cell species.

**RESULTS**

**Radioimmunoassays.** The major external protein of MMTV is a glycoprotein of 52,000 daltons (gp52). Purified MMTV propagated in feline cells competed completely for the binding of anti-MMTV(RIII) gp52 serum to 125I-labeled MMTV(RIII) (Chart 1). No competition was seen with fetal calf serum proteins or murine C-type (AKR) virus. The slope of the competition curve generated by adding increasing amounts of MMTV(RIII)Fel, i.e., MMTV(RIII) grown in feline cells, was identical with that obtained using purified MMTV(RIII). The competition curve obtained with MMTV(RIII)Fel virus was slightly displaced from the MMTV(RIII) standard curve, indicating either differences in the degree of purity of the viruses, a lesser amount of competing antigen per MMTV(RIII)FeI virion, or altered host cell-specific glycosylation. The average yield of purified MMTV(RIII)Fel virus was calculated from radioimmunoassays to be approximately 1.0 μg/10^7 cells (75-sq cm tissue culture flask) per 24 hr.

**Chart 1.** Competition radioimmunoassay for MMTV gp52. MMTV(RIII), grown in feline cells, was concentrated and assayed by competition radioimmunoassay for the gp52 of MMTV(RIII). Competing antigen was added to goat anti-MMTV(RIII), followed 1 hr later by 125I-labeled MMTV(RIII) virions, and the immune complex was precipitated by further addition of porcine anti-goat IgG. After centrifugation, the percentage of 125I-labeled MMTV(RIII) bound by the antisera was calculated following counting of pellet and supernatant fractions. Percentages were normalized to the amount of 125I-labeled MMTV(RIII) bound by antibody in the absence of competing antigen (approximately 50%). ○, MMTV(RIII) from mouse milk; □, MMTV(RIII) grown in feline cells; △, murine type C (Gross) virus; Δ, fetal calf serum.

**Competitive Molecular Hybridization.** Competitive molecular hybridization studies were undertaken to determine whether the virus grown in feline cells, i.e., MMTV(RIII)Fel, contains the same nucleic acid sequences in its 60 to 70 S RNA as does the infecting virus. We have previously shown (17) that, with the technique of competitive molecular hybridization, there is at least 95% sequence homology among the MMTV's of RIII, C3H, and GR mice. This is also shown in Chart 2, in which the 60 to 70 S RNA's of

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The text continues with a detailed description of the experiments and results, including electron microscopy, species verification, and competitive molecular hybridization. The text is rich with scientific details and references, typical of a research paper in the field of virology.
MMTV's Propagated in Heterologous Cells

Chart 2. Nucleic acid sequence homology between MMTVs grown in murine and feline cells. High-molecular-weight viral 60 to 70 S RNA's were purified as described in "Materials and Methods." The following competitor 60 to 70 S RNA's were added to a hybridization reaction between 60 to 70 S MMTV(RIII) [3H]RNA and C3H mammary tumor cell DNA: •, MMTV(C3H); ○, MMTV(RIII); △, MMTV(RIII) grown in feline cells; ∆, avian myeloblastosis virus; □, MuLV (Rauscher).

MMTV(C3H) and MMTV(RIII) compete completely in the hybridization between MMTV(C3H) 60 to 70 S [3H]RNA and C3H mammary tumor DNA. As shown in Chart 2, MMTV(RIII)Fel 60 to 70 S RNA also competes completely in this assay, thus demonstrating that it contains most, if not all, of the sequences found in the virus used to infect the feline cells.

Divalent Cation Preference of Virion DNA Polymerase. It has been shown previously (6, 18) that MMTV's grown in mouse cells have a DNA polymerase with a strict Mg²⁺-divalent cation preference, while murine type C viruses will utilize both Mg²⁺ and Mn²⁺ with a preference for Mn²⁺. MMTV's grown in feline cells contain a DNA polymerase activity that exhibits a strict divalent cation preference for magnesium over manganese when titrated over a 2,000-fold range (Chart 3; Table 1). Very little activity was seen when manganese was used as divalent cation, and the ratios of activities with magnesium to those with manganese were usually greater than 20:1. These ratios are indicative of the presence of type B virions, because both murine and feline type C viruses do not have DNA polymerase activities exhibiting a strict preference for magnesium (Table 1).

Optimal DNA polymerase activity when oligo(dG)⋅poly(rC) was used as primer-template was found in the presence of 8 mM magnesium (Chart 3). This optimum was similar to that of the infecting virus.

Production of MMTV by Infected Cells. Three cultures of MMTV-infected feline cells continually released virus during the 14-, 13-, and 10-month observation periods, respectively. Virus production, as determined by assaying for the presence of virion-associated DNA polymerase activity in culture fluids, is shown for one of the chronically producing feline culture in Chart 4A. During the entire observation period, no Mn²⁺-preferring DNA polymerase activity was observed. In 3 separate experiments with MMTV-infected mink cells, however, cultures produced virus until 80 to 100 days after infection, at which time virus production ceased (Chart 4B).

The RIII milk samples, from which the infecting MMTV was derived, contained varying amounts of virus, ranging from 0.2 to 2.2 mg/inoculum as determined by radioimmu-

Chart 3. Titration of divalent cation preference of virion-associated DNA polymerase of MMTV(RIII) grown in feline (A) and murine (B) cells. Virus was obtained from culture fluids and assayed for the presence of viral DNA polymerase activity using oligo(dG)⋅poly(rC) primer-template as described in "Materials and Methods." The assays were performed in the presence of various concentrations of magnesium ion (■) or manganese ion (□) as indicated.

noassay (Table 2). There was no obvious correlation seen between the amount of virus used to infect the cells and the time at which progeny virus was first detected after infection. In 3 of 4 experiments in which different inocula were diluted 100-fold before infection of the cells, this final dilution failed to lead to a productive infection (Table 2). The estimated multiplicity of infection required to establish a productive infection was thus calculated to range from 6 × 10⁴ to 5 × 10⁵ particles/cell in 3 separate experiments: in 1
Table 1

Divalent cation preference of DNA polymerase of MMTV's grown in heterologous cells

MMTV's were concentrated from culture fluids, and the DNA polymerase activities were assayed in the presence of magnesium and manganese as described in “Materials and Methods.” Results are expressed as the ratio of activity in the presence of 20 mM magnesium to that in the presence of 0.2 mM manganese.

<table>
<thead>
<tr>
<th>Original source</th>
<th>Cells propagated in</th>
<th>Mg$^{2+}$/Mn$^{2+}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>RIII</td>
<td>Mouse</td>
<td>21.7</td>
</tr>
<tr>
<td>C3H</td>
<td>Mouse</td>
<td>82.8</td>
</tr>
<tr>
<td>GR</td>
<td>Mouse</td>
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</tr>
<tr>
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<td>Mink</td>
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</tr>
<tr>
<td>C3H</td>
<td>Mink</td>
<td>47.0</td>
</tr>
<tr>
<td>GR</td>
<td>Mink</td>
<td>19.1</td>
</tr>
<tr>
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<tr>
<td>FeLV</td>
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</tr>
<tr>
<td>RD-114</td>
<td></td>
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Table 2

Infection of feline cells with MMTV

<table>
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<th>Experiment</th>
<th>mg viral protein</th>
<th>mg total protein</th>
<th>Days after infection when virus release first detected$^a$ at following dilution of inoculum</th>
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<tr>
<td>1</td>
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<td>6.1</td>
<td>46</td>
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<tr>
<td>2</td>
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</tr>
<tr>
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<td>0.9</td>
<td>40</td>
</tr>
<tr>
<td>5</td>
<td>0.7</td>
<td>1.0</td>
<td>11</td>
</tr>
<tr>
<td>6</td>
<td>0.6</td>
<td>1.6</td>
<td>19</td>
</tr>
</tbody>
</table>

$^a$ MOI, lowest multiplicity of infection (particles per cell) required to establish a productive infection. This was determined by the highest dilution of inoculum tested that resulted in a productive infection. Calculations for virus content were as previously described (26, 28); Neg, negative; NT, not tested.

$^b$ MMTV content of inoculum per 10$^6$ cells was determined by radioimmunoassay for MMTV gp52 as described in “Materials and Methods.” MMTV was derived from RIII mouse milk.

Total protein content of inoculum per 10$^6$ cells was determined by the method of Lowry et al. (14).

$^c$ Virus detected by assaying culture fluids at weekly intervals for the presence of virion-associated DNA polymerase activity using the synthetic primer-template oligo-(dG)$_{15-18}$-poly(rC) as described in “Materials and Methods.” All results of lower than 1000 cpm above background at 60 min were scored as negative. No assays were performed after Day 70.
MMTV's Propagated in Heterologous Cells

The higher level of hybridization to mammary tumor DNA than to liver DNA is consistent with hybridizations using the infecting viruses, i.e., MMTV(RIII) and MMTV(C3H), and with previous reports (7, 17).

Electron Microscopy. Electron micrographs of MMTV(RIII)-infected feline and mink cells taken 6 to 8 months after infection are shown in Figs. 1 to 6. Virus particles with surface "spikes" could be seen budding from the ends of microvilli (Figs. 3 and 4), and there were numerous extracellular virions with typical type B acenriic cores (Fig. 6). Type B particles can be readily distinguished morphologically from type C particles in thin sections by the presence of long spikes on the surface of the virion, by the acenriic location of the nucleoid in mature extracellular particles, and by the presence of an intermediate layer between the core and the viral envelope (5, 22, 23, 25). The virions seen in infected feline and mink cells fulfill all of these criteria for type B particles, and no typical type C particles have been observed.

The cells also contained numerous intracytoplasmic type A particles (Fig. 5): frequently, numerous type A particles were seen along the inner edge of the cytoplasmic membrane with relatively few virus particles actively budding (Fig. 2).

DNA-RNA Hybridization Studies. High-molecular-weight RNA obtained from virions released by MMTV-infected feline cells had a sedimentation coefficient of 60 to 70 S as determined by sedimentation centrifugation in glycerol gradients (Chart 7). MMTV(C3H)Fel and MMTV(RIII)Fel 60 to 70 S RNA's were hybridized to nuclear DNA from various feline and murine cells. As shown in Table 3, the 60 to 70 S [3H]RNA from the MMTV's grown in feline cells hybridized extensively to DNA from feline cells in which the viruses were grown. The lack of hybridization to normal feline liver DNA and uninfected CrFK cellular DNA indicates the presence of little, if any, RD-114 viral RNA or feline-specific RNA in the MMTV(C3H)Fel and MMTV(RIII)Fel preparations. The feline-grown MMTV 60 to 70 S [3H]RNA's hybridized to a greater extent to nuclear DNA from murine mammary tumors than that from livers (Table 3). The higher level of hybridization to mammary tumor DNA than to liver DNA is consistent with hybridizations using the infecting viruses, i.e., MMTV(RIII) and MMTV(C3H), and with previous reports (7, 17).
DISCUSSION

The data presented here demonstrate that the viruses released by MMTV-infected feline and mink cells exhibit many of the properties of the infecting murine mammary tumor viruses. The complete inhibition in the radioimmunoassay by MMTV(RIII)Fel virus indicates that it contains surface antigenic determinants identical with those of MMTV released from murine cells. Since the radioimmunoassay used is specific for the major external MMTV virion glycoprotein gp52 (2, 3, 10, 26), this is a demonstration that the gp52 antigen is viral coded.

The 60 to 70 S RNA from MMTV(RIII)Fel virus has been shown by competition hybridization studies to contain all the sequences of MMTV(C3H). Additional competitive molecular hybridization experiments, moreover, indicate that hybrid formation between MMTV(RIII)Fel 60 to 70 S [3H]RNA and MMTV(RIII)Fel DNA can be reduced by at least 95% by the addition of unlabeled MMTV(RIII) 60 to 70 S RNA. One cannot rule out the possibility, however, that variant viruses are present as a minor component of the MMTV populations grown in feline cells. Since the radioimmunoassay used is specific for the major external MMTV virion glycoprotein gp52 (2, 3, 10, 26), this is a demonstration that the gp52 antigen is viral coded.

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The demonstration of the growth of MMTV in heterologous cells was important enough to warrant a thorough examination of the virus-producing cells to ensure that they did not contain contaminating murine cells. Cytogenetic and isoenzyme analyses presented no evidence for the presence of murine cells or cells of species other than feline or mink. Immunofluorescence studies failed to reveal the presence of any cells that reacted with antibody to murine cells. The various virus preparations grown in feline and mink cells also did not contain detectable levels of contaminating type C viruses as determined immunologically, by electron microscopy, or by the magnesium preference of their DNA polymerase activity.

The variation in the multiplicities of infection required to establish a productive infection by MMTV from RIII mouse milk may be due to differences in the various virus preparations used, such as percentage of intact virions or the possible presence of defective interfering particles. The explanation for this variation is currently under investigation.

Electron micrographs indicated that the majority of cells contained intracytoplasmic A particles but that in many cases, although they reached the plasma membrane, only a small proportion were seen to be actively budding into the extracellular space. This may be indicative of an inhibition in the process of virus release in these cells.

Since MMTV from 3 different mouse strains has been shown to infect heterologous cells, this indicates that the ability to infect heterologous cells is not unique to MMTV from RIII mouse milk but may be a general property of most or all MMTV's. The demonstration here of the synthesis of MMTV by heterologous cells provides a source of MMTV free of contaminating murine cellular nucleic acids and antigens, and these viruses should prove to be valuable reagents for immunological and molecular biological studies.
Table 3
Hybridization of 60 to 70 S [3H]RNA's of MMTV's grown in heterologous cells to various cellular RNA's

| Source of 60-70 S [3H]RNA | Source of DNA | % hybridization
|--------------------------|---------------|----------------
| MMTV(C3H) | C3H spontaneous mammary tumor | 62 |
| MmSm/c, (C3H) mammary tumor cell line | 70 |
| C3H liver | 36 |
| MMTV(C3H)-infected feline | CrFK cells | 68 |
| Uninfected CrFK cells | 3 |
| Feline liver | 4 |
| Bovine thymus | 0 |
| MMTV(C3H)Feli | C3H spontaneous mammary tumor | 46 |
| MmSm/c, (C3H) mammary tumor cell line | 70 |
| C3H liver | 28 |
| MMTV(C3H)-infected feline | CrFK cells | 78 |
| Uninfected CrFK cells | 1 |
| Feline liver | 0 |
| Bovine thymus | 0 |
| MMTV(RIII) | RIII mammary tumor | 47 |
| RIII liver | 28 |
| MMTV(RIII)-infected feline | CrFK cells | 47 |
| Uninfected CrFK cells | 1 |
| Feline liver | 2 |
| Bovine thymus | 1 |
| MMTV(RIII)Feli | RIII mammary tumor | 53 |
| RIII liver | 38 |
| MMTV(RIII)-infected feline | CrFK cells | 59 |
| Uninfected CrFK cells | 3 |
| Feline liver | 3 |
| Bovine thymus | 1 |

* Hybridizations were taken to a C14 value of 35,000 as described in "Materials and Methods."
* MMTV(C3H)Feli, MMTV(C3H) propagated in feline cells after experimental infection.

ACKNOWLEDGMENTS

The authors are grateful to Susan Reed-Chambers, Donna Venexky, and Jeanne Mitchell for excellent assistance. We also thank G. Schidlovsky for helpful comments.

REFERENCES


Figs. 1 to 6. Electron micrographs of thin sections of MMTV(RIII)-infected heterologous cells. Electron micrographs of thin sections of MMTV(RIII)-infected feline and mink cells were taken 6 to 8 months after infection. Typical type B virions with membrane "spikes" can be seen budding from microvilli in MMTV(RIII)-infected feline (Figs. 1 and 3) and mink (Fig. 4) cells. The preparations also contained virions with acenitic nucleoids (Fig. 6) and numerous intracytoplasmic type A particles (Fig. 5). Frequently, particles can be seen along the cytoplasmic membrane but with few actively budding particles in evidence (Fig. 2). Fig. 1, X 15,000; Fig. 2, X 64,000; Fig. 3, X 140,000; Fig. 4, X 140,000; Fig. 5, X 49,000; Fig. 6, X 105,000.
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