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A Comparison of Cat and Rat Cultures for the Assay of Woolly Monkey Sarcoma and Related Viruses (39961)

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The presently known type C RNA viruses of primates belong to two distinct groups: (a) the *primate type C viruses* consisting of the woolly monkey sarcoma virus (WMSV) and its associated helper virus (1–3), viruses isolated from gibbons which cause lymphoma in this species (4), a virus of the Asian mouse *Mus caroli* which has been shown to be endogenous in this mouse (5), and recently described viruses antigenically related to WMSV, reported to have been isolated from humans with myelogenous leukemia (6, 7), from human cells in culture (8), and from human leukemic bone marrow cells (9); and (b) the baboon *endogenous* type C virus (10–12) which is antigenically closely related to the cat endogenous type C virus RD-114 (13, 14). We (Sarma, P. S., Foster, S., and Log, T., unpublished) and others (15) found that rat cells are more sensitive than primate cells for the *in vitro* propagation and focus assay of the WMSV. Thus, rat cells are in wide use for the propagation and assay of this virus as well as other viruses of this primate type C RNA virus group.

Recently, while attempting to prepare a feline leukemia pseudotype of WMSV, we observed that feline embryo fibroblast (FEF) cultures readily undergo morphological transformation with low doses of WMSV which failed to cause focus induction in rat embryo fibroblast (REF) cultures.

A further study of this observation, described herein, revealed that (a) cat cells are considerably more sensitive and undergo cellular morphologic transformation much earlier than rat cells after parallel infection with WMSV and HL-23 virus (6, 7); (b) cat cells are equally sensitive or more sensitive than rat cells to productive infection by the nontransforming viruses associated with these cell transforming viruses. The accompanying paper by Dodge *et al.* (16) describes similar observations.

Materials and methods. Tissue cultures. Monolayer cultures of REF were prepared by trypsinization procedure with pooled embryos of one mother taken at midterm pregnancy from Osborne-Mendel (OM) and Fischer strains of rats we obtained from the Animal Production Section of the National Institutes of Health, Bethesda, Maryland. FEF culture lines I-655, D843, and H927 from random-bred domestic cats (pooled embryos of one mother per culture) were kindly provided by Dr. Walter-Nelson Rees of the Naval Biological Laboratory, Oakland, California, through the auspices of a Virus Cancer Program supported contract. One lot each of OM and Fischer strains of rat cultures, between passage levels of 3 to 15, were used in these experiments. The FEF cell lines were used between passage levels of 8 and 45.

The cells were grown in a medium consisting of Eagle's minimum essential medium supplemented with 10% heat-inactivated (56° for 30 min) fetal bovine serum and gentamicin (50 μg/ml).

Virus stocks. Virus stocks of WMSV containing a 10- to 100-fold excess of an associated nontransforming virus, woolly monkey-associated virus (WMAV), were prepared as clarified culture fluids of a cell line of marmoset chronically infected with WMSV. This cell line HF 186 (SSV) was kindly provided by Dr. Robert Ting of BioTech Laboratories, Rockville, Maryland. Virus stocks of WMAV were similarly prepared as clarified culture fluids of a suspension culture of human lymphocytes chronically infected with the virus. This cell line, termed NC-37 SSV-1, lot No. 3139-282, was kindly provided by the John L. Smith Memorial for Cancer Research, Pfizer, Inc., Maywood, New Jersey, through the auspices of a Virus Cancer Program-supported contract. Virus stocks

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of the suspected human isolate HL-23 were prepared as clarified culture fluids of a dog thymus cell line chronically infected with the virus. This cell line, termed ASV/A7573, was kindly provided by Dr. Robert C. Gallo, Dr. Robert Gallagher, and Z. Salahuddin of the National Cancer Institute, Bethesda, Maryland. The virus stock contained a type C virus antigenically similar to the baboon endogenous virus M-7, but the presence of the virus in the stock did not affect the studies reported herein, since the M-7-like virus, which is closely related to the feline endogenous virus RD-114 (13, 14), fails to cause productive infection of feline cells (13, 14) or rat cells (11). The virus stocks were stored at −70°C.

Virus assays. Repeated parallel assays of the three viruses were carried out using at least one rat culture and one feline culture per experiment. In one experiment, two strains of rat and three strains of feline cells were used. The cell cultures used were feline cell strains I-655, D843, and H927 and rat cell cultures of the OM and Fischer strains. Cultures for virus assays were planted at a concentration of 5×10^5 to 10^6 cells in 5 ml per 60-mm plastic dish. They were inoculated within 4 hr of planting. The cultures were incubated in a humidified CO_2 incubator flushed with 5% CO_2 in air. The inoculated and control cultures were observed for cell transformation effects at regular intervals under low- and high-power magnifications of an inverted Leitz microscope. The cultures were serially propagated by cell transfers at 6- to 7-day intervals and by medium replacements once between cell transfers. On or about the 20th day after virus inoculation, clarified culture fluids were tested for virion-associated reverse transcriptase by the method described (17). Cell antigens were also collected at this time and tested for the presence of group specific antigen (gs-1 antigen) of the WMSV virus group by complement fixation (CF) test with guinea pig antiserum kindly provided by Dr. R. V. Gilden, Fort Detrick Cancer Research Center, Frederick, Maryland, through the auspices of a Virus Cancer Program-supported contract. On the 20th day a final reading on cell transformation effects, reported under Results, was taken. Unstained living cultures which failed to reveal cellular morphologic changes were washed, fixed in absolute methanol, and stained with Wright's and Giemsa stains to better visualize cellular morphologic changes.

Results. Repeated experiments showed that WMSV caused readily observable cellular morphologic transformation of cat cells at virus dilutions ranging from 10^{-1} to dilutions as high as 10^{-4}, whereas parallel inoculations of virus into cultures of OM and Fischer strains of rat cells produced cell transformation effects at dilutions of only up to 10^{-1} (Table I). Similar observations were made with HL-23 strain of primate type C virus (Table II).

Test for virus production by gs-1 antigen assay and reverse transcriptase assay revealed that cat cells are equally sensitive or more sensitive than rat cells to productive infection by the nontransforming WMAV and similar viruses present in virus stocks of WMSV and HL-23. Thus, endpoint titration results showed equal or 1 to 3 log higher titers in cat cells than in rat cells.

In some of the experiments, WMSV diluted to 10^{-1} failed to cause detectable morphologic changes in rat cell cultures. Staining of the cultures on the 20th day also failed to reveal cellular morphologic changes. In one experiment (experiment 2, OM rat culture inoculated with 10^{-1} dilution of WMSV), serial passage of clarified culture fluids of inoculated culture into cat cell cultures of D843 strain showed that, although OM rat cells did not reveal morphological changes characteristic of cell transformation, the cell transforming WMSV replicated in these cultures. Thus, cat cell culture inoculated with WMSV passaged in rat culture showed cellular transformation effects similar to those observed in cat cell cultures directly inoculated with WMSV. Rat cultures (OM strain) inoculated with a 10^{-2} dilution of virus failed to yield such a cell transforming WMSV.

Associated nontransforming viruses present in virus stocks of WMSV and HL-23 were detected by the fact that virus production was detectable by gs-1 antigen assay and reverse transcriptase assay in cultures inoculated with dilutions of 1 to 3 log immediately above those producing cell transformation effects (Tables I and II). The

TABLE I. COMPARATIVE SENSITIVITY OF RAT AND CAT CELL CULTURES TO PRODUCTIVE INFECTION AND CELL TRANSFORMATION BY THE WOOLLY MONKEY SARCOMA VIRUS AND ITS ASSOCIATED HELPER VIRUS.

Virus	Expt. No.	Cell	Highest dilution producing: RT	Highest dilution producing: gs-1	Highest dilution producing: Cell transformation[a]
Nontransforming Woolly monkey-associated virus	1	REF-OM[b]	10^{-3}	10^{-3}	$<10^{-1}$
		FEF-I-655	10^{-3}	10^{-3}	$<10^{-1}$
	2	REF-OM	10^{-3}	10^{-3}	$<10^{-1}$
		FEF-I-655	10^{-3}	10^{-3}	$<10^{-1}$
	3	REF-OM	10^{-3}	10^{-3}	$<10^{-1}$
		FEF-H927	10^{-3}	10^{-3}	$<10^{-1}$
		FEF-D843	10^{-4}	10^{-4}	$<10^{-1}$
Transforming Wooly monkey sarcoma virus	1	REF-OM	10^{-2}	10^{-2}	10^{-1}
		FEF-I-655	10^{-4}	10^{-4}	10^{-2}
	2	REF-OM	10^{-1}	10^{-1}	$<10^{-1}$[c]
		REF-F	10^{-2}	10^{-2}	10^{-1}
		FEF-D843	10^{-4}	10^{-4}	10^{-2}
	3	REF-OM	10^{-1}	10^{-1}	$<10^{-1}$
		REF-F	10^{-1}	10^{-1}	$<10^{-1}$
		FEF-I-655	10^{-4}	10^{-4}	10^{-3}
		FEF-D843	10^{-4}	10^{-4}	10^{-3}
		FEF-H927	10^{-3}	10^{-3}	10^{-3}

[a] Highest dilution shown contained between 1 and 10 foci of cell transformation.

[b] REF-OM = Osborne-Mendel strain of rat embryo fibroblasts. REF-F = Fischer strain of rat embryo fibroblasts. FEF = Feline embryo fibroblasts. Number following FEF indicates identification provided by supplier. RT = Assay for virion-associated reverse transcriptase from 2 ml of culture fluid. Culture was considered positive for virus production if the radioactive counts exceeded 2000/2 ml; control showed less than 100 counts/2 ml. gs-1 = Test for group-specific antigen p30 of primate type C viruses by complement fixation test. A positive reaction was taken to be a 3 to 4+ reaction (on a scale of 0 to 4) at a 1:2 or higher antigen dilution in the absence of anticomplementary reactions or positive reaction in control cultures.

[c] Clarified fluids of cultures from 10^{-1} and 10^{-2} dilutions were inoculated into D843 cat cultures.

TABLE II. COMPARATIVE SENSITIVITY OF RAT AND CAT CELL CULTURES TO PRODUCTIVE INFECTION AND CELL TRANSFORMATION BY THE HL-23 STRAIN OF PRIMATE TYPE C VIRUS.[a]

Virus	Expt. No.	Cell	Highest dilution positive for: Virus Replication by: RT	Highest dilution positive for: Virus Replication by: gs-1	Highest dilution positive for: Cell transformation
HL-23	1	REF-OM	10^{-3}	10^{-3}	10^{-1}
		FEF-I-655	10^{-4}	10^{-4}	10^{-4}
	2	REF-OM	10^{-3}	10^{-3}	10^{-2}
		FEF-I-655	10^{-4}	10^{-4}	10^{-4}
	3	REF-OM	10^{-3}	10^{-3}	10^{-2}
		FEF-H927	10^{-4}	10^{-4}	10^{-4}
		FEF-D843	10^{-4}	10^{-4}	10^{-4}
	4	REF-OM	10^{-3}	10^{-3}	10^{-1}
		REF-F	10^{-3}	10^{-3}	10^{-2}
		FEF-D843	10^{-5}	10^{-5}	10^{-3}
	5	REF-OM	10^{-2}	10^{-2}	$<10^{-1}$
		REF-F	10^{-3}	10^{-3}	10^{-1}
		FEF-I-655	10^{-6}	10^{-6}	10^{-3}
		FEF-D843	10^{-6}	10^{-6}	10^{-4}
		FEF-H927	10^{-4}	10^{-4}	10^{-3}

[a] Footnotes same as in Table I.

nontransforming viruses were also detected by a virus interference assay in which the respective cell transforming WMSV and HL-23 virus failed to induce cell transformation effects in these "normal-appearing," but virus-positive cultures.

A comparison of three strains of cat cell cultures, I-655, D843, and H927 (Tables I and II), suggest that there are minor variations in their relative susceptibilities to replication and focus induction by viruses. Strains D843 and I-655 appeared to be more sensitive than strain H927.

Discussion. Our results and those reported in the accompanying paper by Dodge *et al.* (16) clearly establish that cat embryo cells are superior to rat cells for the *in vitro* assay of cell transforming, as well as nontransforming, viruses of the primate type C RNA viruses we studied. Not only were the cell transformation titers higher in cat cells, but also the foci of transformed cells appeared much earlier in cat cultures. A short-term (up to 7 day) assay using agar overlay such as the one used by Dodge *et al.* (16) can thus be used in cat cultures and offers a convenient procedure for exactly quantitating the number of focus forming units of virus in a preparation. In this study, we used a fluid medium rather than agar overlay for the purpose of convenience and in view of the great delay in visualizing cell transformation effects in rat cell cultures. The results reported, although not highly quantitative, do show a 2 to 4 log difference between cat and rat cells in the titers achieved by endpoint dilution technique (highest dilution producing observable cell transformation effects).

Parallel results were obtained on the detection of gs-1 antigen of primate type C viruses and detection of virus production by reverse transcriptase assay. This observation shows that the viruses we detected were those sharing gs-1 antigen of woolly monkey sarcoma virus and not those present as contaminating type C RNA viruses in our virus stocks or induced as endogenous type C RNA viruses from rat cells (18) or cat cells (19).

Cultures of OM strain of rat cells failed to undergo cellular transformation after inoculation with a 10^{-1} dilution of virus containing between 10^3 (WMSV) and 10^5 (HL-23 virus) cell transforming units of virus. Staining of these cultures did not reveal observable cellular morphologic changes. However, in one experiment where we attempted to isolate virus by serial passage into D843 strain of cat cell cultures, WMSV was recovered from cultures inoculated with a 10^{-1} dilution but not from cultures inoculated with a 10^{-2} dilution. This suggested that high doses of WMSV inoculated into rat cultures replicated in these cultures with minimal or no observable cell transformation effects.

Since the same virus stocks of WMSV, HL-23, and WMAV were used throughout the experiments, the differences in susceptibility between rat and cat cell cultures appear to be due to a lower sensitivity of rat cell cultures to productive infection by these viruses. This could be due to a partial or complete block at the level of virus penetration into the cells or intracellular virus replication or both. The rat cell cultures underwent cell transformation effects only in those experiments where the associated nontransforming type C viruses infected the cultures at a dilution equal to or higher than 10^{-2}. It is conceivable that minor differences in sensitivity between cultures of each species we observed in these experiments could be due to experimental variations in cultural conditions.

A considerable amount of studies is now being performed on this group of primate type C virus in view of the recently reported studies implicating HL-23 and other similar viruses in human cancers (6–9). The use of cat cells should offer a convenient and sensitive method for virus isolation, seroepidemiological, and other studies to establish the etiological role of these agents in human and animal cancers.

Summary. A study was performed to determine the relative susceptibilities of rat and cat cell cultures to productive infection and cell transformation by the woolly monkey sarcoma virus, the HL-23 strain of primate type C virus, and their associated nontransforming type C viruses. Cat cells appear to be uniformly and consistently more sensitive (up to 10,000-fold) than rat cells to cell transformation effects. Cat em-

bryo cultures I-655, D843, and H927 showed such transformation effects within 3 days of virus inoculation, whereas rat embryo cell cultures from two strains of rats (Osborne–Mendel and Fischer) did not reveal cellular morphologic changes earlier than 10 days following virus inoculation. Cat cell cultures also appear to be equally susceptible or more susceptible than rat cells to productive infection by the woolly monkey sarcoma-associated helper virus and the nontransforming antigenically related primate type C virus present in virus stocks of the HL-23 virus.

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