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Cell Line Derived from a Murine Sarcoma Virus (Moloney Pseudotype)-Induced Tumor: Cultural, Antigenic, and Virological Properties

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A cell line derived from a murine sarcoma virus (Moloney pseudotype)-induced tumor has been established. It retains oncogenicity, releases both sarcoma and leukemia viruses, and has virus-induced cellular antigens.

In the course of our studies on immunity to murine sarcoma virus (MSV), we established an in vitro line of cells derived from a virus-induced tumor for use in in vitro cytotoxic immunity tests. Primary MSV-Moloney pseudotype (MSV-M)-induced tumors are difficult to dissociate into single cell suspensions for use in such tests, and transplantable tumors bearing the related antigens must be adapted to ascitic form and characterized for antigenic and virological properties before use in in vitro immune systems. An in vitro cultivated line provides a readily available source of single cell suspension with defined characteristics for use in cytotoxic humoral and cellular immune reactions. In addition, the cells produce useful stocks of virus free from contaminating agents. The present communication describes the cultural, antigenic, and virological characteristics of such a line of tissue culture cells, designated MSC.

MATERIALS AND METHODS

Animals. Male BALB/c mice, 4 to 6 weeks of age, were used for most experiments; suckling BALB/c mice were inoculated within 72 hr of birth in several experiments.

Virus. MSV-M was obtained from the Program Resources and Logistics Segment, Special Virus Cancer Program, National Cancer Institute, as pool SVRP 215, 1 g-equivalent per ml of tumor homogenate. The titer by focus assay on secondary mouse embryo cell culture (MEC) was 2×10^7 focus-forming units (FFU)/ml; the ratio of sarcoma virus (focus assay) to leukemia virus (X-C assay) was 1:2.

Tissue culture. MSV-M-induced tumors were aseptically removed from the legs of 10 adult BALB/c mice 9 days after intramuscular (im) inoculation of 0.2 ml of MSV-M tumor homogenate containing 10^5 FFU. The tissue was finely minced with scissors and washed with Hanks balanced salt solution (HBSS). The mince was then forced through a stainless-steel mesh tissue press to obtain a single cell suspension. The cells were washed twice with HBSS and suspended in Eagle's minimal essential medium, Earle's base (MEM) with 10% fetal calf serum (FCS) at a concentration of 5×10^5 cells/ml. A 30-ml amount was seeded into 250-ml plastic T flasks. After 1 week, the medium was changed to MEM with 20% FCS and 15% NCTC109 medium. The cells are currently passed weekly by scraping into fresh medium and splitting 1:3. A 12-ml amount of cell suspension was seeded to 250-ml plastic T flasks. Medium was replaced 3 to 4 days after seeding.

X-C cells. The X-C cell line of Svoboda et al. (10) was obtained from Willie Turner of Viral Biology Branch, National Cancer Institute. The cultures were trypsinized once weekly and seeded in MEM with 10% FCS at 2.5×10^5 cells per 250-ml plastic T flasks. They were fed at 3 to 4 days.

Virus titration. MSV-M virus was assayed for FFU on secondary MEC (National Institutes of Health Swiss). Primary monolayer cultures were trypsinized and seeded at 10^6 cells per 4 ml into 60-mm petri plates. A 0.2-ml amount of virus inoculum was added immediately. The cultures were incubated at 37 C in 5% CO_2 in air atmosphere and were fed at 3 days. Foci were counted 6 days after infection.

Murine leukemia virus was assayed by the X-C cell assay of Rowe et al. (8); dilutions were started at the focus assay end point. A 0.1-ml amount of serial two-fold dilutions was added to 24-hr MEC cultures after treatment with diethylaminoethyl dextran for 1 hr. Six days later, the cultures were irradiated at a distance of 27 cm from a GE germicidal lamp for 40 sec. We added, per plate, 10^6 X-C cells in MEM with 10% FCS. Four days later, the cultures were fixed and stained. Focal areas of giant cells containing ≥ 8 nuclei were counted with the aid of a dissecting microscope.

Fluorescent-antibody staining. The technique for fluorescent-antibody staining used in this laboratory has been described (11). The serum used was produced in Fisher rats bearing MSV-M-induced tumors. The conjugate was goat anti-rat gamma globulin conjugated with fluorescein isothiocyanate. Both reagents

FIG. 1. (*a*) *MSC cells 3 days after passage. Note colony of fibroblastic cells associated with round cells. Five months after initiation of cultures.* × *600, bright field.* (*b*) *MSC cells 5 days after passage. Note both fibroblastic and round cell types and disoriented growth without contact inhibition. Five months after initiation of cultures.* × *600, bright field.*

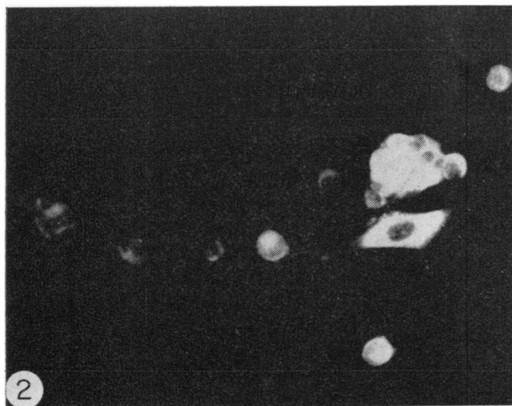

FIG. 2. *Fluorescent-antibody staining of MSC cells 3 days after passage. Acetone-fixed culture. Note cytoplasmic staining of both fibroblastoid and round cells. Five months after initiation of cultures.* × *240.*

were supplied by R. Wilsnack, Huntington Research Laboratories, Inc., Baltimore, Md., under contract 69-54 to the Special Virus Cancer Program, National Cancer Institute.

^{51}Cr release cytotoxicity tests. MSC cells were labeled with ^{51}Cr (2, 6) for determination of immune cytotoxicity. MSC cells (2×10^7 to 4×10^7) in 2 ml of HBSS were incubated with 250 μCi of ^{51}Cr (sodium chromate, specific activity, 200 to 400 μCi/μg of Cr) for 45 min at 37 C with gentle agitation on a rocker platform. The cells were spun at 1,000 rev/min for 10 min in a refrigerated PR-2 centrifuge and were washed twice with 40 ml of HBSS. The labeled cells were resuspended to 10^6 cells/ml in MEM with 10% FCS and allowed to stand overnight at 4 C. They were then washed twice with 40 ml of MEM with 10% FCS and resuspended to a concentration of 10^6 cells/ml.

A modification of the technique of Sanderson (9) was used for cytotoxic antibody determinations. Briefly, 10^5 MSC target cells labeled with ^{51}Cr in 0.1 ml were incubated with 0.1 ml of a 1:10 dilution of antiserum and 0.1 ml of a 1:3 dilution of guinea pig complement for 60 min in a water bath at 37 C. Controls were included which contained guinea pig serum heated for 30 min at 56 C instead of active complement. Maximum release of label was determined by lysing 10^5 cells with distilled water. After incubation, 0.3 ml of medium was added and the tubes were spun in the cold in a PR-2 centrifuge at 2,500 rev/min for 10 min. A 0.3-ml amount of fluid was removed and counted in a Packard Tri-Carb gamma counter. Less than 10% of the label was spontaneously released from control cells incubated with complement alone. The per cent release was calculated as: per cent release = (counts per minute released from serum-treated cells − counts per minute released from complement control cells)/counts per minute released with distilled water.

RESULTS

Cultural and antigenic characters. The MSC cell line has been in continuous culture for 10 months and over 45 passages. Two cell types have been present in approximately equal numbers: fibroblastoid cells, which are attached to the surface, and rounded cells which are only loosely attached and are also found floating free in the medium (Fig. 1). The fibroblastoid cells grew in a disoriented manner, were not contact-inhibited, and formed colonies readily. The round cells were always found in association with the fibroblastoid ones but were not firmly attached to the surface and hence did not readily form colonies. It is not yet clear whether the round cells were formed from the fibroblastoid type or were a separate cell type. Both cell types contained the gs-1 murine leukemia virus antigen and Moloney type-specific membrane fluorescent antigen (Fig. 2). MSV-M type-specific cytotoxic antibody caused release of ^{51}Cr from MSC cells in the presence of guinea pig or rabbit complement but not in the presence of heated guinea pig or rabbit serum. ^{51}Cr was also released from the MSC cells in the presence of spleen cells obtained from BALB/c mice which had regressed MSV-M virus-induced tumors (D. B. Schwartz, *unpublished data*).

Release of infectious virus. The cultures released defective MSV-M and a Moloney-type leukemia virus. The ratio of focus titer to X-C titer decreased from 1:250 after 3 months in culture to 1:6 at 8 months (Table 1). The virus complex was oncogenic for both adult and suckling BALB/c mice (Table 2).

The leukemia virus component replicating in MSC cells was isolated by diluting the virus harvested at 3 months eightfold beyond the focus end point and inoculating this dilution into MEC. No foci were observed, and the cells and fluid were harvested on day 7. This "helper" pool contained 10^4 X-C units/ml and was neutralized in vitro by anti-Moloney leukemia virus serum

TABLE 1. *In vitro virological parameters of sarcoma and leukemia viruses released from MSC cells*

Virus source	FFU/ml	X-C plaque forming units/ml	Hartley-Rowe pattern
MSV-M-SVRP 215, tumor homogenate	10^7	2×10^7	Defective
MSC harvest,[a] 3 months in culture	2.3×10^4	5.1×10^6	Defective
MSC harvest,[a] 5 months in culture	2.0×10^4	10.2×10^4	Defective
MSC harvest,[a] 8 months in culture	1.4×10^6	6.3×10^6	Defective

[a] Supernatant fluid from cultures containing 10^7 cells.

TABLE 2. *Bioassays of cell-free supernatant fluids of MSC cultures*

Virus	Dose (FFU/ml)	Tumor incidence (%)	Range of tumor appearance (days)	Regressions (%)	MST[a] (days)
MSV-M-SVRP 215, tumor homogenate	10^5	90	7–25	11	63
	10^4	90	14–32	55	>80
	10^3	0			
	10^2	0			
MSC harvest,[b] 3 months in culture	10^4	80	60–92	0	123
MSC harvest,[b] 5 months in culture	10^4	60	7–14	8	70
	10^3	60	11–25	58	>80
	10^2	0			

[a] Median survival time.
[b] Supernatant fluid from cultures containing 10^7 cells.

(University Laboratories, Inc., Highland Park, N.J.) The "helper" virus was inoculated into newborn BALB/c mice to determine its oncogenic potential. No tumors developed during a 16-week observation period. This component of the virus complex being produced by the MSC cells is thus apparently nononcogenic per se. Its immunizing potential is currently under investigation.

Tumorigenic potential of MSC cells. MSC cells were iniected im into BALB/c mice to determine the oncogenic capacity. The cells maintained the capacity to form tumors during 8 months in culture. Cells (10^3) formed tumors in 50% of inoculated animals with a median survival time of 43 to 56 days when tested at 3 months in vitro cultivation. At 8 months, the 50% tumor dose was 10^4 cells; 20% of the tumors regressed.

DISCUSSION

Tissue culture cell lines derived from tumors have been valuable tools in determining immune mechanisms in many tumor systems (1, 3–5, 7). However, details of the in vitro characteristics of MSV-M-induced tumor cells have not been described. The MSC line described in this report provided a well characterized source of materials for the study of the pathogenesis and immunity of MSV-M-induced tumors. The cells are easily grown in vitro and have retained oncogenicity when introduced into animals. The viruses of the sarcoma-leukemia complex are continuously produced, and the sarcoma component is oncogenic in BALB/c mice. Since the antigens involved in humoral and cellular cytotoxic reactions in vitro are present and the cells are readily labeled with ^{51}Cr with little spontaneous release, they make a useful system for the in vitro study of immune mechanisms. The isolation of the nononcogenic "helper" component may prove useful in immunization studies.

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