In Vitro Transformation of Canine Embryo Cells by Murine Sarcoma Virus (Kirsten)[1] (37223)

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The Kirsten strain of murine sarcoma virus (Ki-MSV), a MSV isolate from a rat passaged murine erythroblastosis virus (1), shares not only the group-specific (gs) complement fixing (CF) antigen of the murine leukemia-sarcoma virus group (2) but also many *in vivo* properties with the Moloney (3) and Harvey (4) strains of MSV. Murine sarcoma virus is capable of transforming mouse, rat, hamster, bovine, human, and guinea pig cells *in vitro* (5–12). The present communication reports results of experiments showing that canine embryo cells can be transformed morphologically *in vitro* by Ki-MSV and that these morphologically altered cells contain both infectious virus and gs antigen.

Materials and Methods. Cell cultures and media. Canine embryo cells were obtained from Microbiological Associates, Inc. Cells were grown and maintained in Eagle's minimum essential medium with 10% fetal bovine serum, 2 m*M* glutamine, 100 μg/ml of penicillin and 100 mg/ml of streptomycin (EMEM + 10% FBS).

Virus. Ki-MSV stock [supernatant fluids from a Ki-MSV-transformed rat cell line No. 58967 (11)] was kindly supplied by Dr. V. Klement, Children's Hospital, Los Angeles, CA. Its infectivity titer was 1.2×10^4 FFU/ml in NRK cells.

Virus assay. The replication of virus in infected cultures was determined by the following methods: (*1*) examination for the presence of morphological alteration, (*2*) assay for CF antigen reactive with murine-leukemia-virus-group-reactive rat serum (*2*); (*3*) assay for RNA-dependent DNA polymerase activity (13, 14).

Transformation assay. A 16-oz bottle culture of primary canine embryo cells was infected with virus stock and was incubated for 2 hr at 37°, then washed once with medium. Cells were then trypsinized, centrifuged, resuspended in fresh media, seeded on 60-mm Falcon plastic petri dishes (4×10^5 cells/plate), and incubated at 37° under 5% CO_2 in air. Control cultures consisted of the same preparation of the cells in EMEM + 10% FBS, but were not inoculated with virus. Both inoculated and control cultures were fed with fresh media 24 hr later, and they were thereafter refed at 3–4-day intervals. Two weeks after inoculation, some cultures were subdivided by trypsin treatment and continued as serial cell lines.

Complement fixation. Complement fixation (CF) tests were carried out in the microtiter technique described for tumor antigen studies (15). Titers were recorded as reciprocals of the highest dilution giving 3+ to 4+ fixation of 1.8 units of complement.

Preparation of CF antigens. Cell pack preparations for CF testing were made as previously described (2).

Antiviral sera. Mouse sarcoma virus antisera used in the CF test were obtained from Fischer rats carrying transplanted sarcomas induced by the Moloney strain of MSV (M-MSV) (2).

Preparation and examination of cells by electron microscopy. Cells were spun at 500 rpm at 4° for 10 min. The pellet was fixed in 2% glutaraldehyde in 0.1 *M* sodium cacodylate buffer, sectioned into 1-mm cubes and postfixed in Dalton's chrome-osmium (16).

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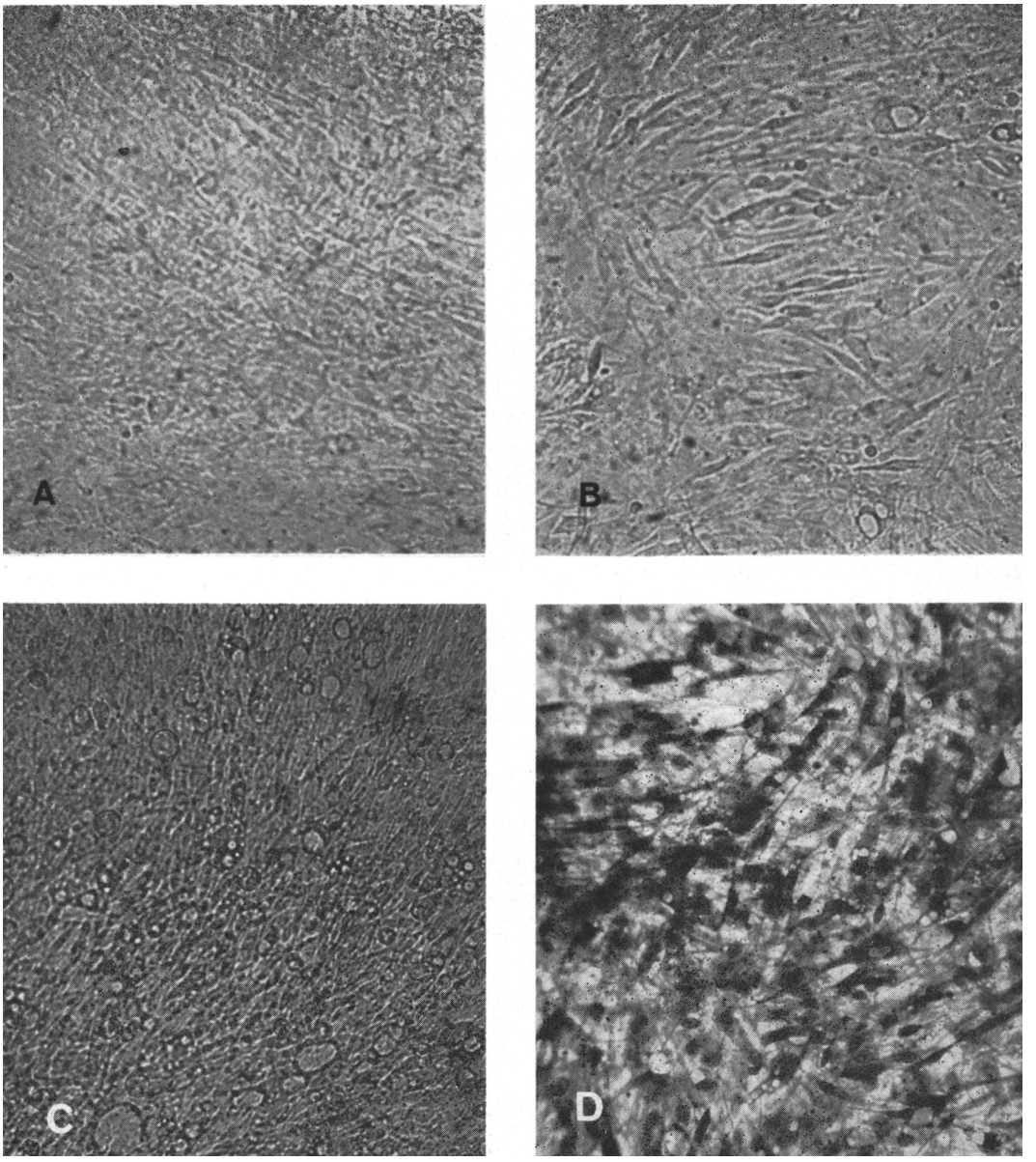

FIG. 1. Cultures of canine embryo cells (×70). (A) Uninfected control, second passage. (B) A transformed focus appearing on canine embryo cells at 18 days after infection, second passage. (C) A more advanced focus seen on canine embryo cells at 28 days after infection, second passage. (D) The same as C. Giemsa stain.

The cells were then dehydrated in methyl alcohol and propylene oxide and embedded in Epon-Araldite. Ultrathin sections were obtained with an LKB Ultratome and stained with uranyl acetate and lead citrate. A Hitachi HU 11 F electron microscope was used for examination.

Reverse transcriptase assay. Supernatant fluids from the infected cultures were clarified by centrifugation at 10,000 rpm for 10 min; then viruses were pelleted at 30,000 rpm for 2 hr. Pellets were resuspended in 0.01 *M* Tris buffer, pH 7.4, usually in 1/100th of the original volume. Assay mixtures

contained 0.04 ml of virus suspension (disrupted by 0.01 ml of 0.5% Nonidet P-40 and preincubated for 1 hr at 4° just before use), 0.01 ml of reaction mixture [5 ml of 1 *M* glycine-NaOH buffer (pH 8.3), 0.1 ml of 1 *M* $MnCl_2$, 3.75 ml of 4 *M* NaCl, 2.0 ml of 1 *M* DTT, adjusted to 20 ml with distilled water], 0.01 ml of poly rA:oligo dT (25 μg/ml), and 0.01 ml of ^{3}H-deoxythymidine triphosphate (50 μ*M*, 1 Ci/mmole). After the incubation of the mixtures at 37° for 30 min, aliquots were removed, and materials insoluble in cold 6% trichloroacetic acid were collected on Millipore filters. Radioactivity was determined by a Beckman LS 250 scintillation system.

Results and Discussion. Approximately 18–24 days after infection, foci consisting of fusiform cells mixed with round cells began to appear in the infected cultures. These foci increased in size during the next week. After one transfer, quite distinct foci were seen in the infected cultures, but none were observed in the uninfected controls (Fig. 1). The transformed foci were similar to those obtained with Ki-MSV in mouse and rat embryo cells, and they contained many cytoplasmic vacuoles. The foci gradually increased in size and showed a pronounced proliferative effect with multilayered cell growth (Fig. 1C and D), however, the foci were rather diffuse, making it difficult to count the exact number. Characteristics of a Ki-MSV transformed cell line are shown in Table I. The transformed cells were found to release virus continuously. Cell-free preparations of supernatant fluid from the *in vitro* transformed cultures produced similar altered foci in NRK, guinea pig, and canine embryo cells. It should be noted here that, in some experiments, foci in canine embryo cells were not readily visible upon inoculation. However, after one or two transfers distinct foci were seen in the infected culture. This may be due to low titers of the virus preparation. The transformed cells contained high titers of gs antigen characteristic of the viruses of murine leukemia-sarcoma complex (2, 17).

Virus replication in the infected cultures was evidenced by the presence of reverse transcriptase activity (Table I). The time of appearance of RNA-dependent DNA polymerase activity in supernatant fluids of infected cultures was correlated with the time course of appearance of morphological alterations in the infected cultures (Table I).

The transformed cells produced type C virus particles. Electron microscopy revealed budding and free extracellular particles possessing the characteristic type C morphology (Fig. 2).

Studies are in progress to determine whether the transformed cells will produce tumors when inoculated into homologous hosts and also to further characterize the transformed

TABLE I. Characteristics of Ki-MSV Transformed Canine Embryo Cell Line (R-892-A).

Subculture level	Cumulative no. of days after infection	CF titers[a] vs MSV rat serum	Examination of culture supernatant fractions: Reverse transcriptase (cpm)	Induction of foci after inoculation: NRK	guinea pig embryo	canine embryo
Primary	6	NT[b]	0[d]	NT	NT	NT
	9	NT	0	NT	NT	NT
	13	NT	432	NT	NT	NT
	20	NT	2898	NT	NT	NT
1	21	>32	53203	+(6)[c]	+(6)	+(16)
2	25	>4	98438	NT	NT	NT
3	31	>8	65738	+(6)	+(6)	+(14)

[a] Reciprocal of CF titer.
[b] Not tested.
[c] Days after infection.
[d] Subtracted background.

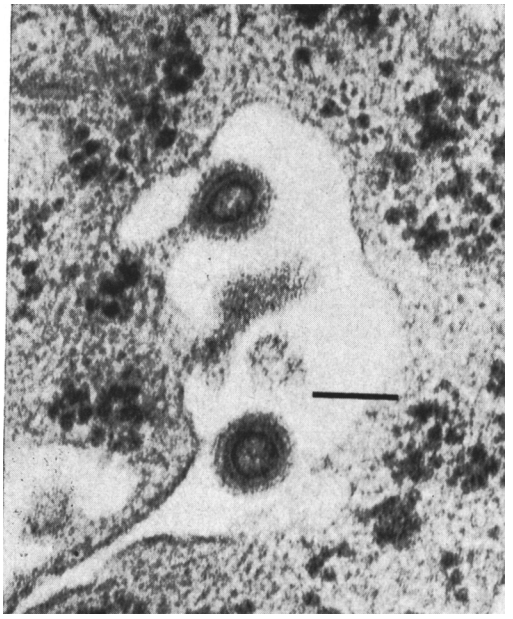

FIG. 2. Typical budding type C virus particles (×110,000); bar = 100 nm.

cells. Since the canine cell-growth virus transformed canine embryo cells readily (14–16 days after inoculation), whereas the original virus did not do it readily, the virus may have acquired new properties (Table I.) Focal areas of transformed cells were isolated by cloning. After a few transfers, morphologically altered cells could still be seen. These cultures still contained high titers of gs antigen and had RNA-dependent DNA polymerase activity.

The susceptibility of canine embryo cells to the Moloney and Harvey isolates of MSV were carried out. No transformation and virus growth have yet been observed with these viruses in canine embryo cells by the methods described above.

The present results and the earlier finding that Ki-MSV can replicate also in cells of guinea pig origin (12) indicate that members of the murine sarcoma-leukemia virus complex, particularly Ki-MSV, exhibit a broader host range than was hitherto believed.

Summary. Canine embryo cells were transformed *in vitro* by Kirsten murine sarcoma virus (Ki-MSV). The transformed cells contained group-specific complement-fixing antigen characteristic of the murine leukemia-sarcoma virus complex. Cell-free supernatant fluids from the transformed cultures were infectious for rat, guinea pig, and canine embryo cells. RNA-dependent DNA polymerase activity was demonstrated in the transformed cells. Electron microscope observation showed that the transformed cells produced type C virus particles. The present results indicate that members of the murine sarcoma-leukemia virus complex, particularly Ki-MSV, exhibit a broader host range than was hitherto believed.

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