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"Nonproductive" Mouse Sarcoma Virus Transformed Gorilla Cells which Contain Noninfectious Type C Virus Particles[1] (40193)

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We have previously shown that whereas the Moloney and Harvey strains of murine sarcoma virus (MSV) have a limited host range among the various mammalian cells tested, the Kirsten strain of MSV (Ki-MSV), derived from chronically infected and transformed rat cells, has an extremely wide host range, being able to infect and transform human, guinea pig, canine, rabbit, porcine, bovine and feline cells (1, 2). We have recently extended the host range study of Ki-MSV in various simian cell lines in order to investigate the possibility that certain simian cells could be used for studies on transformation by Ki-MSV. Further attempts have been made to characterize transformed cell lines in an effort to isolate nonproducer (NP) cells. Such NP cells may be useful not only in permitting the generation of pseudotype sarcoma viruses with various helper viruses, but also in isolating an endogenous simian virus. During the study we found that "nonproductive" transformation could be produced with Ki-MSV in gorilla cells. Gorilla NP cells were obtained by infecting gorilla cells with Ki-MSV and subculturing them. These altered NP cells did not produce infectious virus or murine leukemia virus (MuLV) gs antigen, but contained a rescuable MSV genome and also noninfectious type C virus particles.

Materials and methods. The gorilla spleen (passage 4) line was a gift from Drs. C. J. Gibbs, Jr., and D. C. Gadjusek, Laboratory of Central Nervous System Studies, National Institute of Neurological Diseases and Stroke, NIH, Bethesda, MD. The horse skin [E. Derm, NBL-6 (CCL-57), passage 15] line was obtained from the American Type Culture Collection, Rockville, MD. Primary cultures of NIH Swiss mouse embryo (NIH-ME) and Fischer rat embryo (RE) were obtained from Microbiological Associates, Inc., Bethesda, MD. The human embryonic skin and muscle (HESM) cell line was obtained from Flow Laboratories, Inc., Rockville, MD. Cells were grown and maintained in Eagle's minimum essential medium with 10% fetal bovine serum, 2 mM glutamine, 100 units of penicillin and 100 μg of streptomycin per ml (EMEM + 10% FBS).

The Ki-MSV stock used was a rat cell-grown virus (1). MSV pseudotype with baboon placenta (BP) virus (3) was produced in human S^+L^- cells (4) by cocultivation with baboon virus releasing human osteosarcoma line.

A combination of the following procedures were used to detect the replication of the virus in cultures as described previously in detail (2): (a) examination for the presence of morphological alteration; (b) assay for CF antigen reactive with specific type-C leukemia virus gs antiserum; (c) assay for RNA-dependent DNA polymerase activity.

CF test was carried out by the microtiter technique described for tumor antigen studies (5). Titers were recorded as reciprocals of the highest dilution giving 3+ to 4+ fixation of 1.8 units of complement.

The procedure of assaying supernatant viral RNA-dependent DNA polymerase has been described in detail (2). A synthetic template, poly rA: oligo dT, was used to enhance enzyme detection.

Transformation assay. A suspension of freshly trypsinized gorilla spleen cells was centrifuged, resuspended in fresh media, seeded on 60 mm Falcon plastic dishes (2 × 10^5 cells per plate) and incubated at 37° under 5% CO_2 in air. After 24 hr the DEAE-dextran (25 μg/ml for 30 min) pretreated

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cultures were fed with fresh media and infected with virus and returned to the incubator. Control cultures consisted of the same preparation of cells in EMEM + 10% FBS but were not inoculated with viruses. Both inoculated and control cultures were fed with fresh media 24 hr later and they were thereafter refed at 3- to 4-day intervals. Three weeks after inoculation, some cultures were subdivided by trypsin treatment and continued as serial cell lines.

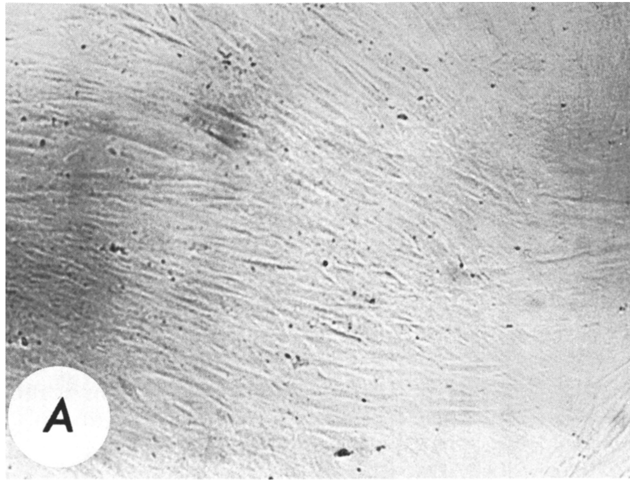
Results. Within 5 days after infection with Ki-MSV gorilla cells showed distinct foci of morphologically transformed cells; these foci consisted of fusiform cells mixed with round cells. These foci increased in size during the next week and became quite distinct from the surrounding field of nontransformed gorilla cells (Fig. 1A, B). The transformed foci were similar to those obtained with Ki-MSV in horse (6) and human cells (7). Focus formation by Ki-MSV on the gorilla line was "one-hit" and was approximately 100-fold less efficient than on the horse cell line (6).

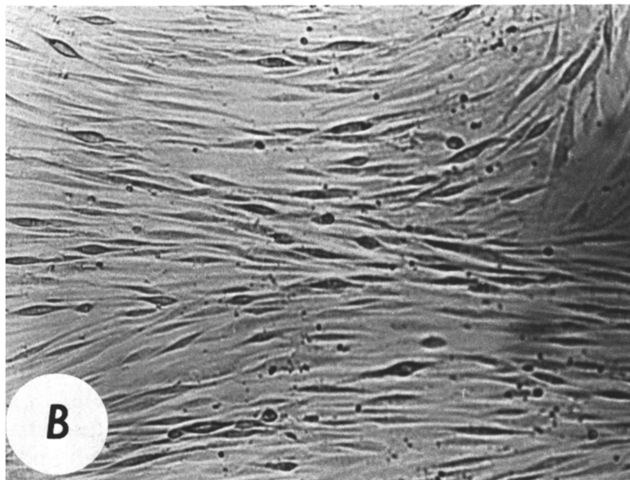
Three weeks after infection, some cultures were subdivided by trypsin treatment and established as serial cell lines. Characteristics of a Ki-MSV transformed line were also studied (Table I). The Ki-MSV transformed line was found to be "nonproducer". The transformed line contained no MuLV CF antigen or detectable infectious virus although cellular alterations were clearly observed. Culture fluids from the NP line were negative when tested for supernatant reverse transcriptase activities and for focus formation on mouse, rat, horse and gorilla cells. In other experiments, NP cells were cocultivated with equal numbers of various cells (NIH-ME, Fischer rat, horse, gorilla, chimp, and human cells) and passed twice over a period of 4 weeks. Supernatants from these cultures taken at 2 and 4 weeks were assayed for viral enzyme; the results were negative. However, examination of NP cells (passage 14) by electron microscopy showed a few budding type C particles (Fig. 2); whereas uninfected, control gorilla cells did not exhibit any virus-like particles. Sucrose gradient banding of the supernatant fluid of NP cells by ^{3}H-uridine labeling showed a peak of radioactivity at a bouyant density of 1.16 g/ml.

The presence of the sarcoma genome in NP cells could be readily detected by cocultivation with cells carrying helper type-C viruses (Table II). NP cells were cocultivated with equal numbers of "helper" RD-114 or woolly leukemia virus (WoLV) releasing cells (5×10^5 cells/flask). Supernatants from these cultures taken at 14 days were passed through a 0.45 HA filter, inoculated into fresh gorilla, horse and human HESM cultures and examined for the presence of foci. The MSV rescued by RD-114, MSV (RD-114), and by WoLV, MSV (WoLV), produced foci readily in gorilla, horse and human cells (Table II).

The susceptibility of gorilla cells to MSV (BP) was also examined. The transformed foci in gorilla cells were seen within 5 days and were morphologically similar to those obtained with Ki-MSV (Fig. 1B and C). Three weeks after inoculation, some cultures were subdivided by trypsin treatment and continued as serial cell lines. Characteristics of a MSV (BP) transformed line were studied (Table I). The transformed cells were found to release virus continuously. Cell free preparations of supernatant fluids from the *in vitro* transformed cultures produced similar altered foci in Fischer RE, horse and gorilla cells but not in NIH-ME cells. The transformed cells contained viral reverse transcriptase. Cell suspensions prepared from MSV (BP) transformed gorilla cells were positive for both baboon endogenous virus gs antigen and RD-114 virus gs antigen and negative for MuLV gs antigen (Table I). As previously reported (8) cell pack preparation of MSV (RD-114) contained the gs antigen of both RD-114 and baboon endogenous virus. Focal areas of transformed cells were isolated by the cloning cylinder technique and propagated; these clones continued to show the altered morphology and contained high titers of baboon virus and RD-114 gs antigens and reverse transcriptase activity.

There has been no evidence of expression of the endogenous gorilla type-C viral genome in studies carried out so far. Polymerase activity of uninfected gorilla cultures have been consistently low despite more than one year of continuous cultivation. One-day-old cultures of NP cells were exposed to 100 μg/ml of 5-bromodeoxyuridine (BrdU). After treatment of BrdU for 24 hr, cells were washed and fresh medium was added. At various intervals, the medium was collected and were examined for the presence of re-





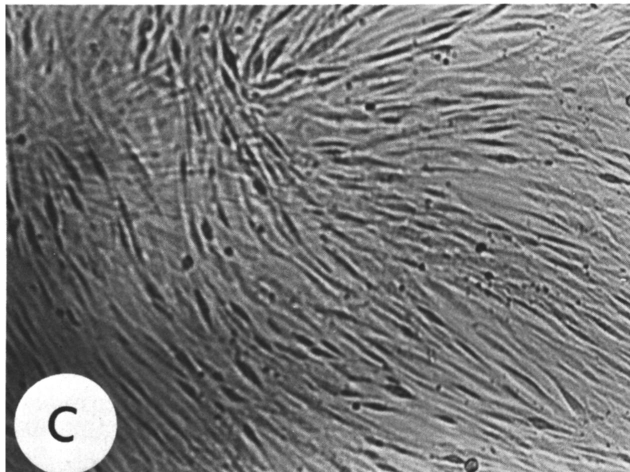


FIG. 1. Gorilla spleen cells, unstained (A–C) (× 30). A: Uninfected control. B: A transformed focus seen on gorilla spleen cells at 9 days after infection with Ki-MSV. C: A focus seen on gorilla spleen cells at 9 days after infection with MSV (BP).

TABLE I. CHARACTERISTICS OF Ki-MSV AND MSV (BP) TRANSFORMED GORILLA CELL LINES.

Type-C viruses	Sub-culture level	Cumulative No. of days after infection	Examination of culture supernate fractions					CF titers vs antiserum		
			Poly-merase[a] activity (cpm)	Induction of foci after inoculation						
				Gor-illa	Fisher RE	Horse	NIH-ME	MuLV	RD-114	BP
K-MSV	2	27	130	–[b]	–	–	–	0	0	0
	4	47	215	–	–	–	–	0	0	0
	5	54	318	–	–	–	–	0	0	0
	10	103	135	–	–	–	–	0	0	0
	14	153	200	–	–	–	–	0	0	NT
MSV (BP)	2	27	1150	NT	NT	NT	NT	0	1:4[c]	1:2
	4	47	4751	+	+	+	–	NT	NT	NT
	5	54	32,335	NT	NT	NT	NT	0	16	NT

[a] RNA-dependent DNA polymerase activity, cpm incorporated ^{3}H-TMP/ml: poly rA: oligo dT was used as the synthetic template.
[b] Negative means no foci in two petri dishes inoculated with undiluted filtrates.
[c] Reciprocal of CF titer. 0 = indicates a titer <1:2. NT = not tested.

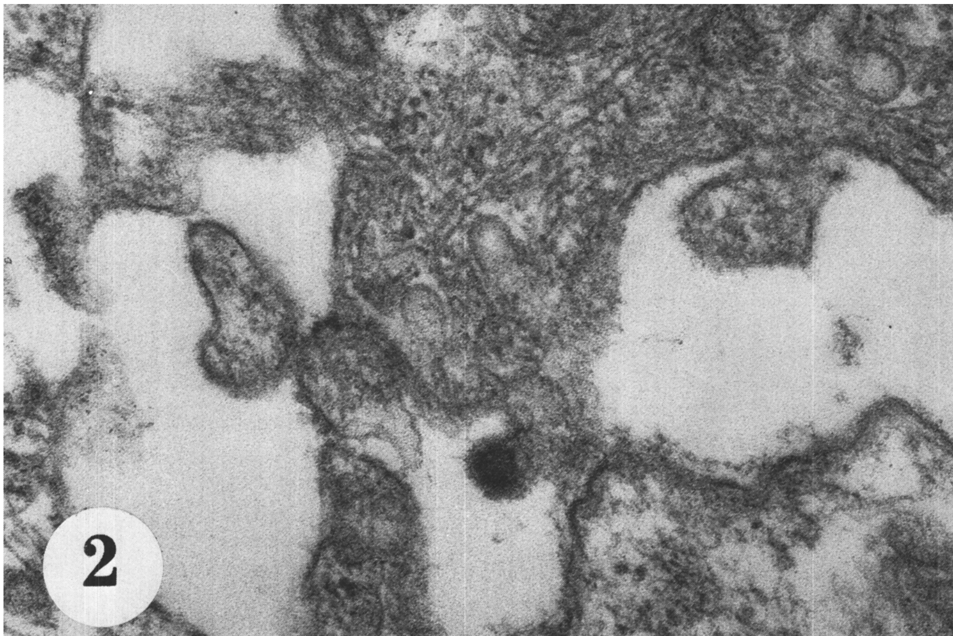


FIG. 2. A budding particle possessing characteristic type-C morphology × 82,500.

verse transcriptase activity. There was no elevation of polymerase activity in supernatants of treated NP cultures. Prolonged cultivation in the BrdU (25 μg/ml) containing medium for 3 weeks also failed to show an elevation of polymerase activity. Further, supernatants from these gorilla NP cells have been used to infect rat, horse, HESM cells; there have been no detectable foci of transformed cells.

Discussion. This study is of special interest for at least three reasons: (a) productive and "nonproductive" transformation of gorilla cells by type-C sarcoma viruses; (b) the induction of NP cells by simple infection of gorilla cells with Ki-MSV; and (c) the detection of noninfectious type-C virus particles without viral antigen in the gorilla NP cells.

The present results show that Ki-MSV and MSV pseudotype of the endogenous baboon

TABLE II. RESCUE OF MSV GENOME FROM GORILLA NP CELLS.

	Induction of foci after inoculation into cells		
Rescue method	Gorilla	Horse	HESM
Co-cultivation[a]			
RD-114/MA-160 cells	0[b]	0	0
RD-114/MA 160 cells + gorilla NP cells	38	55	6
Gorilla NP cells	0	0	0
WoLV/HOS	0	0	0
WoLV/HOS + Gorilla NP cells	6	32	4
	ND		
Gorilla NP cells	20	0	0

[a] Gorilla NP cells were co-cultivated with equal number of "helper" RD-114 or woolly leukemia virus (WoLV) releasing cells. Supernatants from these cultures were taken at 14 days, passed through a 0.45 μ HA filter, inoculated into gorilla, horse and human embryonic skin and muscle (HESM) cells and examined for the presence of foci.

[b] Average No. of foci/plate; 0 indicates no foci in two petri dishes inoculated with undiluted virus. RD-114/MA-160 cells = RD-114 virus releasing human benign prostate (MA-160) line. WoLV/HOS = Woolly leukemia virus releasing human osteosarcoma (TE-85, Clone F-5) line.

virus can readily transform gorilla cells. A baboon pseudotype has recently been demonstrated to be capable of inducing tumors in simians (Kalter *et al.,* unpublished). The *in vitro* transformed MSV (BP) gorilla cells were producer, however, the Ki-MSV transformed gorilla cells were nonproducer. These NP cells did not release any infectious virus or MuLV gs antigen but contained a rescuable sarcoma virus genome and noninfectious type-C virus particles with a density of 1.16 g/ml. Thus the data indicate that productive and "nonproductive" transformation can be produced with type-C sarcoma viruses in gorilla cells. Indeed, to our knowledge, this is the first report of NP transformed cell line made with Ki-MSV in simian cells. Previous NP transformants of MSV have been made in hamster (9), mouse (10), rat (11), guinea pig (12), mink (13) and human cells (14). In addition, it is interesting to note that NP cells can be isolated from transformed cells by the means of simple virus infection and subcultivation. Most of the reported MSV NP transformants have been isolated by the techniques of either cylinder cloning (10–13) or agar suspension cultures (15), using endpoint dilution technique.

Evidence is presented here to show that the NP gorilla cells described represents a new class of transformants; gorilla NP cells containing noninfectious type-C virus particles without viral antigen (Table III). As previously reported, MSV has been shown to produce three classes of transformed cells. These are: (a) MSV producer cells that result from coinfection by MSV and MuLV (16); (b) MSV nonproducer cells which are transformed by MSV in the absence of MuLV and lack any evidence of virus production or MuLV antigen (9–14). The sarcoma genome in such cells can be rescued by superinfection with MuLV; (c) sarcoma-positive, leukemia-negative (S^+L^-) cells which do not release infectious sarcoma virus except upon superinfection with MuLV (15, 17). S^+L^- cells do release noninfectious virus particles (18) and contain MuLV antigen (15). These latter properties distinguish them from MSV NP cells. S^+L^- transformants have been made in mouse (15) and human cells (4), however, S^+L^- human cells are different from S^+L^- mouse cells in that the S^+L^- human cells do not release detectable type-C like particles (19). Gorilla NP cells contain noninfectious type-C virus particles like S^+L^- mouse cells but do not contain MuLV antigen like mouse NP cells.

Chemical induction of focus-forming virus from NP cells transformed by MSV has been described in the mouse and rat cell system (11, 20). The host range and antigenic characteristics of the chemically induced MSV appeared to be conferred upon it by an endogenous helper leukemia virus of host cell rather than by the helper virus of the original transforming virus stock. It has already been shown that mouse and rat cells contain the genetic information of helper virus production in chemical induction of murine and rat type C viruses from virus-free clonal lines (21–23). Thus, activation of the sarcoma genome from MSV NP cells probably occurred secondarily to induction of this endogenous helper virus. We have attempted to rescue the genome of focus-forming virus from gorilla NP cells by BrdU. Our BrdU genome rescue

TABLE III. PROPERTIES OF MSV TRANSFORMED MAMMALIAN CELLS

Transformed types	Morphology	Infectious virus	MuLV antigen	Type-C virus particles	Presence of sarcoma genome	Cell types
Producer	+	+	+	+	NA[a]	mouse, rat, hamster guinea pig, bovine, horse, human and others
Nonproducer	+	−	−	−	+	mouse, rat, hamster, guinea pig, mink, human
	+	−	−	+	+	gorilla
S^+L^-	+	−	+	+	+	mouse
	+	−	+	−	+	human

[a] NA = Not applicable.

attempts yielded negative results. We have also attempted to induce the endogenous type-C virus from gorilla cells by BrdU treatment: the BrdU induction attempts also yielded negative results.

Summary. Gorilla spleen cells can be readily transformed morphologically *in vitro* by Kirsten murine sarcoma virus (Ki-MSV) and the MSV pseudotype of baboon placenta virus-MSV (BP). Gorilla spleen cells transformed *in vitro* by the MSV (BP) were found to be virus producers; whereas Ki-MSV transformed gorilla spleen cells were found to be nonproducer (NP). These NP cells were obtained by simply infecting gorilla cells with Ki-MSV and subculturing them. These morphologically altered NP cells did not produce infectious virus or murine leukemia virus gs antigen, but contained a rescuable MSV genome and also noninfectious type-C virus particles.

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