Continuous Long-Term Replication of Feline Leukemia Virus (FeLV) in an Established Canine Cell Culture (MDCK)[1] (36129)

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(Introduced by L. K. Bustad)
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That leukemia in the cat is caused by a C-type oncornavirus has been well documented (1–8). The replication of feline leukemia virus (FeLV) in early passage monolayer cell cultures of feline (9), canine (10), and human origin (10, 11) has been reported, and a lymphoblast suspension culture that continually produces a C-type virus has been established from a leukemic cat (12).

Cell cultures derived from leukemic cats have been used to provide a standard source of FeLV (7, 12). A serious disadvantage to the use of unpurified virus from these cultures is the lack of an identical but uninfected culture for use as a control. This became especially evident to us when we were trying to distinguish between virus-specific and cell-specific reactions while attempting to develop a complement-fixation test for the detection of FeLV. Attempting to infect early passage and established cell cultures from several species, we noted that the Madin-Darby canine kidney cell line (MDCK) could support the replication of FeLV in a steady-state noncytocidal manner while providing a source of virus.

Materials and Methods. MDCK cells were grown in prescription bottles using BME-Earles medium with 10% tryptose phosphate broth and 5% fetal bovine serum. They were subcultured at 6- to 8-day intervals. A feline lymphoblast suspension culture derived in our laboratory (12) was used as the original source of FeLV. Twenty milliliters of supernatant fluid from a 72-hr-old lymphoblast suspension culture containing 2×10^6 cells/ml was centrifuged first at 1000 rpm for 10 min to remove cells, then at 5000 rpm for 20 min to remove cellular debris, and finally at 22,500 rpm for 90 min in a Beckman SW 25.2 rotor to pellet the virus. Each pellet was resuspended in 0.5 ml of media for infection of a 6-oz bottle containing about 1 million actively growing MDCK cells. Following incubation at 37° for 90 min with frequent agitation, the cells were rinsed and new media was added.

Subcultures of infected and uninfected MDCK cells were obtained by trypsinizing the monolayer and seeding new bottles with 600,000 to 800,000 cells. At 24 hr after subcultivation, and at each subsequent 24 hr interval for 12 days, cell counts of representative MDCK bottles were made. In the case of MDCK exposed to FeLV, counts were taken at the 30th subculture following infection. All counts were repeated three times at the same passage level. Culture fluids 5–7 days old were regularly saved at the time of subcultivation from uninfected MDCK and from infected MDCK at passages 5 to 56. Fifty to 150 ml samples of culture fluid saved from most passages from the 5th to 40th subcultivation of infected MDCK were temporarily frozen at −70° and later combined to make a 4-liter pool. This pool was clarified by centrifugation at 5000 rpm and then centrifuged at 30,000 rpm using a Beckman BXVI rotor with a self-forming 15–60% (w/v) gradient of sucrose in Tris buffer. Fractions collected in the 1.13 to 1.17 g/cm^3 density range, as determined with an Abbe' refractometer, were pelleted at 25,000 rpm for 90 min in a Beckman 30 rotor. After

[1] Supported in part by contract number 70-2048 within the Special Virus-Cancer Program of the National Cancer Institute, NIH, PHS.

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fixation in 2.5% glutaraldehyde, postfixation with 1% osmium tetraoxide, and staining in uranyl acetate and lead citrate, thin sections of the pellets were examined with a Phillips 200 electron microscope. Infected and uninfected MDCK cells were similarly prepared and examined with the electron microscope.

Supernatant samples from the feline lymphoblast suspension culture, the 4-liter culture fluid pool of infected MDCK, and certain individual passage levels of infected and uninfected MDCK were first clarified at 5000 rpm, then concentrated 100-fold by centrifugation at 25,000 rpm in the Beckman 30 rotor and resuspended in Veronal buffer for serological studies. The microtiter complement-fixation test described by Schmidt was used (13). The micro-Ouchterloney gel diffusion test was used with 1% agar and 4.5 mm between reagent wells. For both tests, rabbit antiserum to purified ether-treated FeLV was used. The use of this antiserum to detect FeLV antigen with the gel diffusion test has been described (14). All antigens were treated with an equal volume of chilled ethyl ether before use. Undiluted antiserum was used for the gel diffusion test, and a 1:32 dilution of serum heated at 56° for 30 min was used for the complement-fixation test.

Results. As illustrated in Fig. 1, the growth curve of FeLV infected MDCK cells at subculture 30 following infection was similar to the curve obtained with uninfected MDCK. Examination by light microscopy revealed no cytopathic effect or loss of contact inhibition in the FeLV-infected MDCK culture.

Pellet preparations obtained by ultracentrifugation of the infected MDCK culture fluid pool gave a strongly positive complement-fixation reaction at a dilution of 1512. A

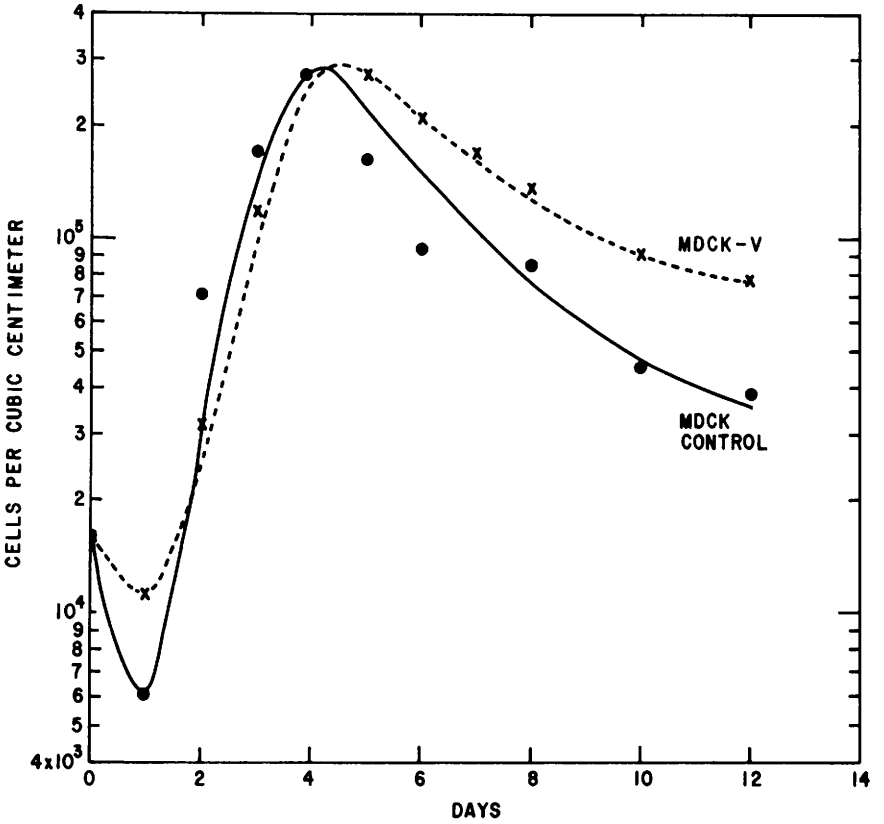

FIG. 1. Comparative growth rates of FeLV-infected MDCK and uninfected MDCK control cells: MDCK-V is FeLV-infected MDCK at the 30th subcultivation following infection. The number of cells per cubic centimeter of culture medium is plotted against the days following subcultivation.

similar preparation from an uninfected, but otherwise identical, control MDCK culture gave a negative reaction at a dilution of 1:8 (the lowest dilution tested). Similar preparations of 4- to 6-day-old culture fluids from the same number of feline lymphoblast suspension cells gave strong positive reactions at dilutions of 1:512 to 1:1024. Individual samples from infected MDCK cultures were examined by complement-fixation at passage levels 8, 12, 22, 33, and 57. All were positive at titers of 512 or 1024 (Table I).

With the use of the gel precipitation technique, the infected MDCK culture pool gave a continuous precipitation band with FeLV from the feline lymphoblast suspension culture (Fig. 2). Individual samples from the same culture at passages 5, 22, 33, and 57 were similarly positive, while samples from the uninfected MDCK control at comparable passage levels were negative (Table I).

At passage levels 8, 33, and 57, infected cells were examined on the electron microscope. As illustrated in Fig. 3, budding and mature C-type particles typical of FeLV were observed. No virus particles were seen in uninfected MDCK cells. Examination of

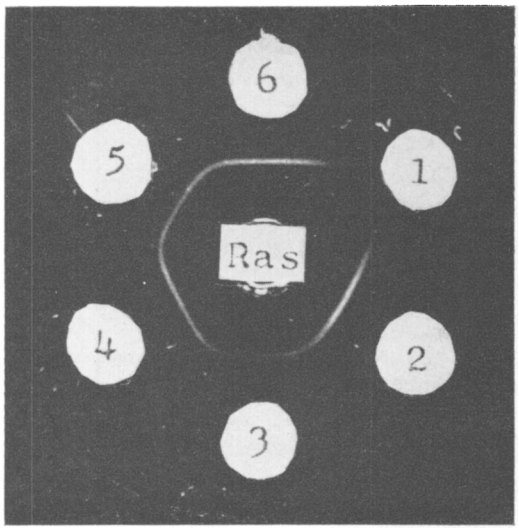

FIG. 2. Immunodiffusion test with FeLV antiserum and concentrated cell culture supernatant fluids: (Ras) rabbit antiserum to feline lymphoblast suspension culture derived FeLV; (1) concentrated cell culture fluid from uninfected MDCK; (3) concentrated FeLV-infected MDCK culture fluid pool, fraction at 1.13–1.17 g/cm^3; (5) concentrated FeLV-infected MDCK culture supernatant at passage 57; (2, 4, 6) concentrated cell culture supernatant from the feline lymphoblast suspension culture infected with FeLV.

TABLE I. Results of Complement-Fixation Test, Immunodiffusion Test, and Electron Microscopic Examination of FeLV-infected and Uninfected MDCK Cells and Culture Fluids.

Culture	Passage no.	Complement fixation FeLV antigen titer[a]	Virus detected by	
			Immunodiffusion	Electron microscopy
MDCK(FeLV)	5–56[b]	512	+	+
	5	NT[c]	+	NT
	8	512	NT	+
	12	512	NT	NT
	22	512	+	NT
	33	512	+	+
	57	1024	+	+
MDCK (control)		<8[d]	—	—
Feline lymphoblast suspension culture		1024	+	+

[a] Reciprocal of highest dilution of antigen giving complete fixation of complement.
[b] 1.13 to 1.17 g/cm^3 density band from 4-liter pool.
[c] Not tested.
[d] Lowest concentration tested.

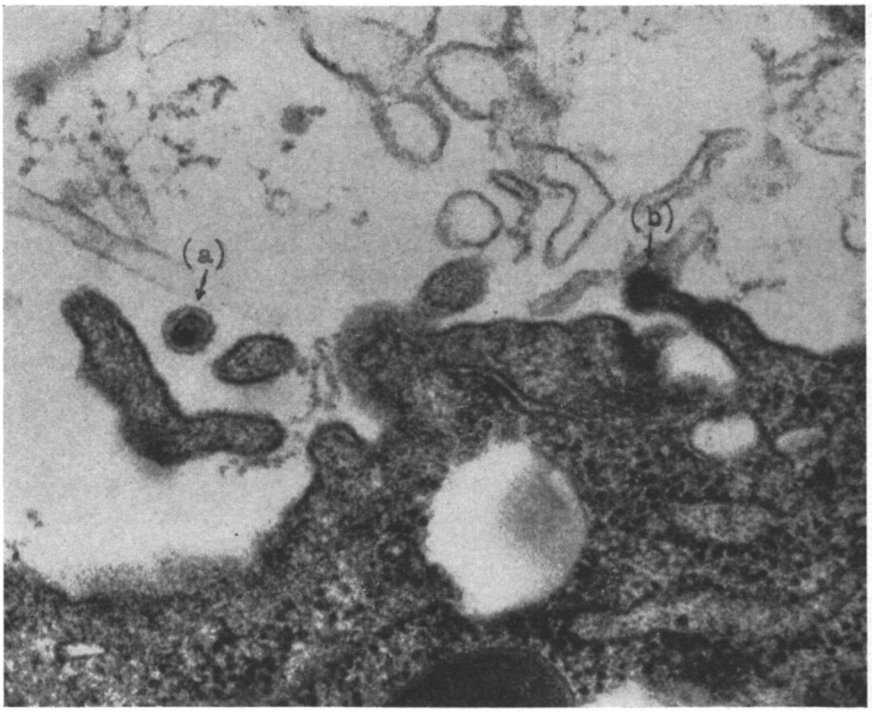

FIG. 3. Electron micrograph including (a) a mature C-type, and (b) an immature budding virus particle associated with an infected MDCK cell at passage 33; ×60,000.

the 1.13 to 1.17 g/cm^3 density fraction from the 4-liter FeLV-infected MDCK culture fluid pool also revealed many C-type virus particles (Fig. 4).

Discussion. The results conclusively demonstrate that an established cell line of canine origin, MDCK, can support the replication of FeLV in a steady-state noncytocidal manner. Virus was detected by morphologic, serologic, and biophysical means over 52 subcultures during a 1-year period.

Approximately the same amount of FeLV complement-fixing antigen was detected in MDCK and feline lymphoblast suspension cell culture supernatants when concentrates representative of the same number of cells were assayed. This suggests that cell cultures from species other than cats are capable of supporting the replication of FeLV as well as feline leukemic lymphoblast cultures.

Established cultures such as MDCK, which can maintain a continuous infection with FeLV in a noncytocidal manner, should provide a useful model for studying the long-term effects of a leukemia virus on the cell. Such a system can also be used to study the stages of leukemia virus replication, the mechanism of antigen expression in leukemia virus-infected cells, and to determine if attenuation or other alterations of the virus occur after prolonged *in vitro* cultivation. Since the induction of leukemia in dogs with FeLV has been reported (7), it may be important to compare the efficiency of canine cell-adapted FeLV to FeLV of feline origin for the induction of leukemia in the dog and cat.

Summary. An established monolayer line of Madin-Darby canine kidney (MDCK) cells was infected with FeLV. The infected culture grew in a steady-state noncytocidal manner during 57 subcultures over 1 year while continuously producing virus. Electron microscopic examination revealed virus budding from cells and in the 1.13 to 1.17 g/cm^3 density range of sucrose gradients. FeLV antigen in the culture supernatant was found by immunodiffusion and complement-fixation techniques. Approximately the same

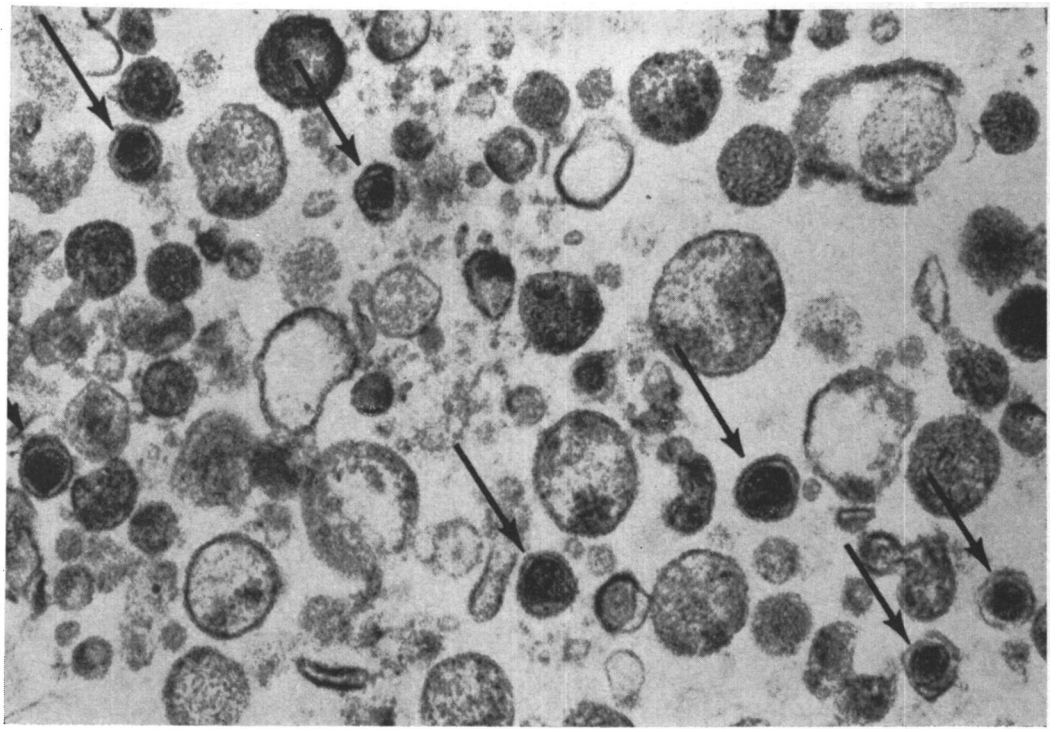

FIG. 4. Electron micrograph of a thin section prepared from the 4-liter cell culture fluid pool of FeLV-infected MDCK, fraction at 1.13–1.17 g/cm^3; ×70,000.

amount of antigen was found at subcultivations 8, 12, 22, 33, and 57 following infection.

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Received May 14, 1971. P.S.E.B.M., 1972, Vol. 139.