In Vitro Isolation and Characterization of the GA Strain of Feline Sarcoma Virus[1] (35783)

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The naturally-occurring neoplasms of the domestic cat, such as the feline lymphosarcoma (leukemia) and feline sarcoma are frequently associated with C-type RNA tumor viruses which share common morphological, biophysical, and immunological properties (1–4). The feline leukemia viruses (FeLV) replicate in cultures of homologous hosts without causing visible changes, whereas the feline sarcoma virus (FSV) induces observable foci of cell transformation (5–10). Studies described herein concern the direct *in vitro* isolation of the Gardner-Arnstein (GA) strain of FSV (GA-FSV) from a naturally-occurring fibrosarcoma of a Siamese cat (9) and the characterization of the biological behavior of this virus *in vitro*. The initial isolation of the focus-forming FSV from the naturally-occurring fibrosarcoma in feline embryo fibroblast (FEF) cultures was successful only when an *exogenous* helper FeLV was added to cultures to promote focus induction by the FSV.

Methods and Results. Secondary and tertiary monolayer cultures of FEF were propagated in disposable plastic Falcon dishes in a humidified CO_2 incubator as previously described (6, 7, 12–14). The original, naturally-occurring fibrosarcoma, as well as tumors experimentally induced by GA-FSV in cats and dogs (9), were used in infectivity studies. These tumors were prepared as clarified 10% tissue homogenates and as partially purified tumor concentrates (15, 16).

Isolation of FSV and accompanying FeLV in tissue cultures. In addition to the *in vivo* procedures used for isolation of the GA-FSV, previously described (9), a partially purified concentrate (15, 16) of the primary tumor was inoculated in 0.2-ml amounts into freshly planted cultures of FEF. One set of parallel cultures was similarly inoculated and immediately co-infected with 10^4 infectious units [determined by the COCAL test (7)] of FeLV. The infected and parallel control cultures were propagated by medium replacements at 3-day intervals and by cell transfers at approximately weekly intervals. The cultures were periodically examined microscopically for cell transformation. Culture antigens were also collected and tested for the presence of feline leukemia-sarcoma, group-specific antigens, by the complement-fixation test (COCAL test) (7).

Cultures inoculated with the tumor concentrate and FeLV showed focal changes, characteristic of cell transformation. These changes were observed 2 weeks after inoculation. Cell transformation effects were reproducibly induced in normal FEF cultures by serial transfer of clarified, cell-free culture fluids from transformed cultures. Similar techniques failed to reveal the presence of a cell-transforming virus in cultures inoculated without FeLV. On the other hand, the CF antigen induction test (COCAL test) revealed the presence of a noncytopathogenic C-type virus in these cultures, suggesting that the FeLV thus isolated was present in the original tumor as a FSV-associated virus.

Focus induction in feline and canine cultures. The FSV isolated in FEF cultures with a helper FeLV as well as the virus isolated by inoculation of kittens *in utero* without an exogenous helper FeLV (9), induced cell transformation in feline and canine embryo

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TABLE I. Detection of the Noncytopathogenic Feline C-Type Virus Present in Virus Stocks of GA-Strain of Feline Sarcoma Virus.

Virus dilution	Cell transformation			C-Type particles			Release of ^{3}H uridine labeled virus			Presence of COCAL[a] antigen		
	Feline	Canine	Human	Feline	Canine	Human	Feline	Canine	Human	Feline	Canine	Human
10^{-1}	+	+	+	+	+	+	+	+	+	+	+	+
10^{-2}	+	+	+	+	+	+	+	+	+	+	+	+
10^{-3}	+	0	0	+	+	+	+	+	+	+	+	+
10^{-4}	0	0	0	+	+	+	+	+	+	+	+	+
10^{-5}	0	0	0	+	+	+	+	+	+	+	+	+
10^{-6}	0	0	0	+	+	+	+	+	+	+	+	+
10^{-7}	0	0	0	0	0	0	0	0	0	0	0	0
Control culture	0	0	0	0	0	0	0	0	0	0	0	0

[a] Complement-fixation test for feline leukemia virus (7).

fibroblast cultures. Cell transformation and virus replication also occurred in human embryo fibroblast cultures (14). In the feline and canine cells, the focus of cell transformation consisted predominantly of refractile fibroblasts in parallel and sometimes criss-cross orientation. Very few round cells or giant cells were observed. The continuous presence in the FEF and beagle embryo tissue culture fluids of a 1% concentration of a potent GA-FSV neutralizing dog antiserum or control normal dog serum commencing 24 hr after virus inoculation did not result in a suppression of virus transformation effects or the growth of foci. This suggested that growth and local spread of virus were not necessary for the production of detectable foci as described for focus induction in mouse cultures by the Moloney strain of murine sarcoma virus (18).

Growth characteristics of GA-FSV in vitro. Focus titers of GA-FSV in different culture lots of FEF varied over a 10-fold range. Serial propagation of the virus in sensitive FEF cultures was accompanied by a gradual reduction in the focus titer of the virus. This low yield of the tissue culture propagated GA-FSV (titer $\leq 10^2$ FFU/ml) contrasted with our observation on virus yields from experimentally-induced GA-FSV sarcomas in newborn kittens (9) (titer $>10^3$ FFU/ml), and with the higher focus titers and virus yields in FEF cultures infected with ST-FSV (19) and SM-FSV (20) strains of FSV (titers, $>10^3$ FFU/ml).

Detection of an associated, noncytopathogenic, feline C-type virus. Focus assays in feline, canine as well as human embryo cultures, revealed the presence in virus stocks of a great excess of a feline, C-type virus incapable of causing morphological changes. As shown in Table I, a 4-log excess of this noncytopathogenic virus over the cell-transforming FSV was readily demonstrated by electron microscopy, by CF antigen induction tests (COCAL test) and by ^{3}H uridine labeling technique (6). Virus stocks of FSV propagated in FEF prepared as clarified fluids of disrupted cells and culture fluids, contained 10^2 to 10^3 focus-forming units (FFU)/ml and a 100- to 1000-fold excess of

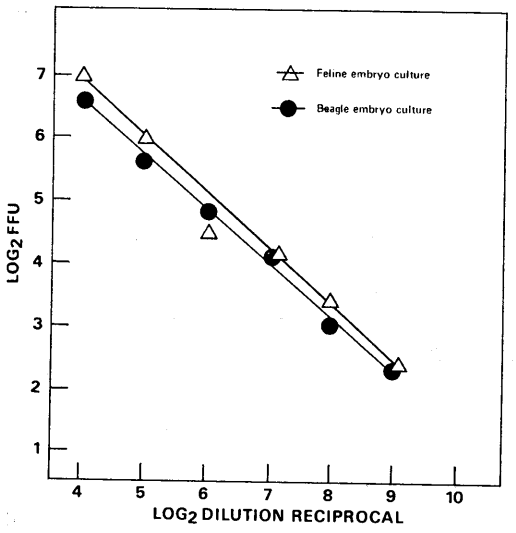

FIG. 1. Focus assay of GA-FSV in feline and beagle embryo fibroblast cultures. The number of foci is proportional to the virus dilution factor.

the associated, noncytopathogenic, feline C-type virus.

"One-hit" focus titration pattern. Focus assays in feline and canine embryo cultures, using 2-fold dilutions, revealed that the numbers of foci were proportional to the dilution factor (Fig. 1). The addition of 10^4 to 10^5 infectious units of "helper" FeLV at each virus dilution, did not enhance the focus titers. These observations suggested that under the conditions of these experiments, the FSV strain did not require the help of an *exogenous* FeLV for focus induction in feline and canine embryo cultures (18). Whether the great excess of *endogenous* FeLV present in these virus stocks provided this helper function remains to be determined.

Viral envelope characteristics. Viral interference tests using the focus-forming GA-FSV and FeLV pseudotypes of MSV (12, 13) showed that the noncytopathogenic, C-type virus accompanying the GA-FSV, designated herein as GA-FeLV, could be assayed by a viral interference technique (12). Focus induction by the GA-FSV was interfered with by prior infection of FEF cultures with GA-FeLV and FeLV strains of the subgroup B, but not by FeLV of the subgroups A and C of the feline leukemia-sarcoma viruses (12). This subgrouping of the feline C-type viruses described in detail elsewhere (12) was based on similarities and differences in viral envelope antigens demonstrable in viral interference tests.

GA-FSV appeared to be related to ST-FSV (8) and SM-FSV (12) of FSV by viral neutralization tests as well as by viral interference patterns. The serum of an adult dog immunized with GA-FSV, containing a high titer of virus neutralizing antibodies against GA-FSV ($\geqq 4$ $\log_{10}$ neutralization index), was found to neutralize 10^3 FFU each of ST-FSV and SM-FSV. The three strains of FSV were classified as members of subgroup B of the feline leukemia-sarcoma complex (12).

Discussion. We have described the *in vitro* isolation and biological characterization of the GA-FSV. The original tumor was found to contain FeLV since the FSV present in the original tumor required the help of *exogenous* FeLV for focus induction in FEF cultures, it is presumed that the FeLV was present in this tumor in insufficient quantities to provide the helper function. The great excess of noncytopathogenic FeLV found in virus stocks of *in vivo* propagated GA-FSV (9) was thus derived from the original naturally-occurring sarcoma and not recovered as a passenger virus in the serial *in vivo* passage of the GA-FSV (9).

The FSV present in the original tumor was isolated without the use of a helper virus by the inoculation, *in utero,* into kittens as previously reported (9). This transplantation of the tumor into kittens prior to birth allowed the growth of the tumor as well as the sarcoma and leukemia viruses to the point where cell-free preparations alone yielded both the FSV and the associated FeLV.

Focus induction in feline and canine cultures was not enhanced by addition of *exogenous* FeLV although the FSV present in the original tumor required this help. Whether or not GA-FSV requires the help of the large excess of the associated, noncytopathogenic C-type virus (GA-FeLV) for the synthesis of its coat protein remains to be determined.

The morphology of feline cells transformed by GA-FSV differed from those transformed by other strains of FSV, the ST-FSV (19)

and the SM-FSV (20). The GA-FSV induced indistinct foci of refractile fibroblasts. The SM-FSV foci were also predominantly fibroblastic, whereas the ST-FSV foci were diffuse and consisted of refractile, round, and fibroblastic cells. The three strains of FSV were found to share common antigenic and biologic properties, including the possession of an associated, noncytopathogenic C-type virus and the capacity to infect and propagate in heterologous mammalian host cells (19, 20).

Summary. The Gardner-Arnstein strain of feline sarcoma virus (GA-FSV) present in a naturally-occurring feline fibrosarcoma, was isolated in feline embryo fibroblast cultures. Virus isolation was successfully accomplished by co-infecting the feline embryo cultures with helper feline leukemia virus. Cultures inoculated with tumor concentrate alone contained a nontransforming feline-C-type virus which was identified as a GA-FSV associated virus (GA-FeLV). Both the GA-FSV as well as the accompanying GA-FeLV were serially propagated in feline and canine embryo cultures. The foci of cell transformation induced by GA-FSV consisted of aggregates of refractile fibroblasts in parallel and crisscross orientations. Evidence was obtained that the development of foci in feline and canine embryo cultures depended on the multiplication of transformed cells rather than in the local spread of virus to adjoining cells. GA-FSV stocks containing high titers of the associated feline leukemia virus gave "one-hit" focus titration patterns in feline and canine embryo cultures. *Exogenous* "helper" FeLV failed to enhance the focus titers in feline and canine embryo cultures. The GA-FSV and GA-FeLV were classified as members of the subgroup B of the feline leukemia-sarcoma complex.

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