

The SM Strain of Feline Sarcoma Virus. Biologic and Antigenic Characterization of Virus¹ (36675)

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Naturally occurring neoplasms of the domestic cat such as fibrosarcoma and osteosarcoma are frequently associated with C-type virus particles and/or species-specific group-specific (gs-1) antigen of the viruses of feline leukemia-sarcoma complex (1-4). Feline sarcoma viruses (FSV) presumed to be the etiologic agents of such neoplasms have been isolated from feline fibrosarcoma by *in vivo* techniques employing the inoculation of cell-containing or cell-free tumor preparations into newborn (1-3) or unborn kittens (2). Direct *in vitro* isolation of the Gardner-Arnstein strain of FSV (hereafter designated GA-FSV) from feline fibrosarcoma was facilitated by coinfection of feline cultures with a helper feline leukemia virus (FeLV) (5).

This report describes the *in vitro* biologic and antigenic characteristics of a new strain of FSV isolated from a naturally occurring feline fibrosarcoma of an 18-month old cat (3). The characteristics of the virus (hereafter referred to as SM-FSV), including the capacity to morphologically transform and replicate in heterologous host cells, were similar to those of Snyder-Theilen FSV (hereafter designated ST-FSV) and GA-FSV previously described (1, 2, 5, 6). Cellular transformation of feline cells followed one-hit kinetics. Virus stocks contained an excess of an associated noncytopathogenic feline C-type virus of antigenic subgroup A as well as B.

Methods and results. Tissue culture. Procedures for the preparation of tissue cultures have been described previously (4-10). The cultures were propagated in a medium

consisting of Eagles' minimum essential medium supplemented with 10% fetal bovine serum and antibiotics.

Virus stock. The initial isolation of the SM-FSV described herein was done by *in vivo* transplantation of tumor in newborn kittens as described (3). In the present studies, a clarified homogenate of a third kitten transplant tumor was inoculated in FEF cultures. Virus stocks were prepared as clarified culture fluids of cultures which showed confluent cell transformation effects. Similar virus stocks were also prepared at various serial passages of SM-FSV in FEF cultures. Some of the tissue culture virus preparations were inoculated subcutaneously into newborn kittens and the resulting tumors were prepared into virus stocks as clarified 20% tumor homogenates.

Focus assay. The kinetics of cell transformation (11) were determined in FEF cultures. Immediately prior to preparation of virus dilutions, the stock viruses were passed through Millipore 0.45 μ filters to eliminate virus aggregates. Twofold virus dilutions were prepared and inoculated into freshly seeded cultures. To prevent the local virus spread and development of secondary foci, the liquid culture media were replaced at 18-20 hours after virus infection with a growth medium containing 0.8% Noble agar. The foci were counted with an inverted microscope, 7 days after infection.

COCAL test. The replication of SM-FSV and the associated nontransforming feline C-type virus (herein designated as SM-FeLV) in various tissue cultures was demonstrated by the immunologic detection in complement fixation (CF) test of the gs-1 antigen of the viruses of the feline leukemia-sarcoma group.

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TABLE I. Detection of the Presence of an Excess of Noncytopathogenic Feline C-type Virus in SM-Feline Sarcoma Virus.

Virus dilution	Foci/dish FEF ^a	Feline leukemia-sarcoma, group-specific CF viral antigen in inoculated FEF ^b (the COCAL test)	Release of [³ H] uridine-labelled virus into culture medium
10 ⁻²	confluent	>8	+
10 ⁻³	65	>8	+
10 ⁻⁴	4	>8	+
10 ⁻⁵	0	>8	+
10 ⁻⁶	0	<2	—
control FEF	0	<2	—

^a Average number of foci in two feline embryo fibroblast (FEF) culture dishes.

^b Reciprocal of CF antigen titer of culture antigens against four CF antibody units of feline sarcoma dog antibodies (5).

This test, the COCAL test, was performed by described procedures (4, 8).

Transformation and virus replication in feline cells. SM-FSV preparation induced morphological transformation of FEF cells within 3–5 days after inoculation of virus. The foci of transformed cells consisted predominantly of refractile fibroblasts in parallel and criss-cross orientations. In cultures maintained by serial cell transfers, a few refractile round cells were also regularly observed. Stock tumor and tissue culture virus preparations contained 10³–10⁵ focus-forming units (FFU) of virus per milliliter.

Detection of an associated noncytopathogenic virus. In virus assay experiments in FEF cultures, the gs-1 antigen of the feline C-type viruses was regularly present in cultures showing cell transformation effects. In addition, the CF antigen was also present in cultures inoculated with one-decimal dilutions beyond those producing cell transformation effect, thus suggesting the presence of an associated noncytopathogenic C-type virus (4–6, 11) (Table I). Serial passage of clarified culture fluids of transformed cultures regularly induced morphological transformation in FEF cultures. On the other hand, similar serial passage of fluids of cultures presumed to contain a noncytopathogenic virus resulted in the isolation of this virus with properties identical to FeLV. We designated this virus as SM-FeLV.

Antigenic characteristics as determined by viral interference and neutralization tests. Viral interference in feline C-type viruses is mediated by type-specific viral envelope antigens involved in the induction of virus-neutralizing antibodies (5, 6, 10, 13). We have recognized the existence of three subgroups of feline C-type viruses which contain the major type-specific envelope antigens of *a*, *b*, and *c* respectively. We found that many field strains of FeLV occur in nature as antigenic mixtures containing a A subgroup virus as one of the components. The ST and GA strains of feline sarcoma viruses were also found to be mixtures of A and B viruses (5, 6, 10, 13). Our present studies showed that the SM-FSV described herein is also a mixture of antigenic types of A and B subgroup viruses.

Viral interference tests in FEF cultures (10) showed that the capacity of SM-FSV to cause cellular morphologic transformation of feline cells was markedly inhibited or abolished by prior infection of the cultures with the SM-FSV associated noncytopathogenic C-type virus, SM-FeLV, described above. The antigenic similarity between SM-FSV and the other strains of FSV and the presence of viruses of A and B subgroups in virus stocks of SM-FSV was initially suggested by results of viral interference tests. Thus, FEF cultures productively infected with SM-FeLV were also resistant to challenge with 10³ FFU

each of ST-FSV, GA-FSV and feline leukemia pseudotypes of murine sarcoma virus [MSV(FeLV)] of subgroups A and B (10). The cultures were susceptible to a similar challenge dose of MSV(FeLV) of subgroup C.

The antigenic relationship between SM-FSV and other FSV strains was further verified by viral neutralization tests. A potent dog antiserum against the GA-FSV (2, 5) which neutralized high titers of the A and B subgroup viruses (13) completely abolished the infectivity of 10^3 FFU each of the SM-FSV, GA-FSV and ST-FSV as well as MSV(FeLV) of subgroups A and B. This antiserum failed to neutralize a similar dose of MSV(FeLV) of subgroup C.

Isolation of a subgroup A FeLV from SM-FeLV. The presence of a subgroup A and B viruses in SM-FSV virus stocks was further confirmed by the isolation of a subgroup A noncytopathogenic feline C-type virus from virus stocks of the SM-FSV associated virus. The SM-FeLV was serially passed in FEF cultures at the highest decimal dilution capable of inducing the FeLV gs antigen. After three consecutive passages at intervals of 20–22 days (time required for one COCAL test) (4, 8), the virus present in the culture inoculated with the highest virus dilution, was characterized. It was found to induce

viral interference against the cell-transforming capacity of 10^3 FFU of the A subgroup MSV(FeLV) but not against the B subgroup MSV(FeLV). We are presently attempting to isolate the B subgroup component by treatment of virus stock with antiserum against FeLV of the A subgroup.

One-hit focus titration in feline cultures. Focus assays of tissue culture derived FSV as well as virus derived from kitten tumors did not reveal a defect in the ability of the virus to transform FEF cells. The number of foci was proportional to the virus-dilution factor (Fig. 1). Focus titers were not enhanced by coinfection of cultures with 10^4 – 10^5 infectious units of FeLV. The focus titers were not diminished by the addition of a potent virus-neutralizing dog antiserum or control normal dog serum to FEF cultures, 24 hr after virus infection. The growth of foci thus appeared to be due to the growth of transformed cells rather than through transformation of adjoining normal cells by local spread of FSV.

Infectivity of tissue culture propagated FSV for kittens. The FEF culture propagated SM-FSV retained its pathogenicity for newborn kittens. Cell-free, as well as cell-containing culture fluid preparations of a seventh tissue-passage virus induced sarcomas in kittens within 30–40 days after subcutaneous inoculation.

Transformation of heterologous host cells. SM-FSV was found to be similar to GA-FSV and ST-FSV in its ability to propagate and cause cellular morphologic transformation of heterologous mammalian host cells of human, canine, and porcine origin. The gs-1 antigen of the feline C-type viruses was detected in high titers in cultures productively infected with the virus.

Discussion. The SM-FSV described in this report was found to have antigenic and biologic characteristics similar to those of the previously described GA and ST stains of feline sarcoma viruses (1, 2, 5, 6). The presence of an associated noncytopathogenic feline C-type virus suggested that the sarcoma virus may be dependent on this virus for virus replication. Focus assay in feline cells followed one-hit kinetics suggesting that a single virus particle is sufficient to induce cell

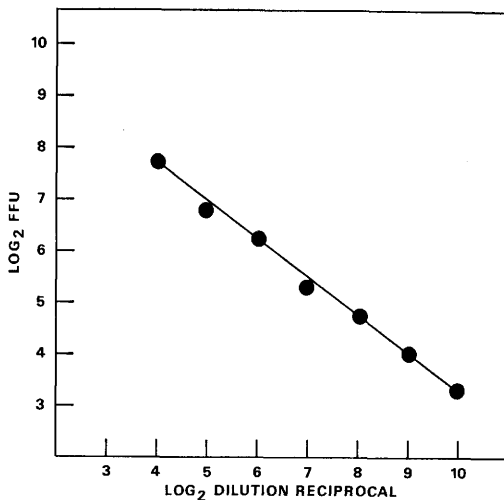


FIG. 1. Focus assay of SM-FSV in feline embryo fibroblast cultures. The number of foci is proportional to the virus dilution factor.

transformation, *i.e.*, cell transformation can be initiated without a helper FELV. However, it was not possible to determine if replication of infectious sarcoma virus required the presence of the associated noncytopathogenic virus. Our attempts to produce cell clones of feline cells singly infected with feline sarcoma virus alone have so far been unsuccessful.

The presence of more than one antigenic type of virus in field isolates of FeLV (13) and in virus stocks of SM-FSV as well as other feline sarcoma viruses (5, 6, 13) is intriguing, in as much as it suggests that the feline C-type viruses occur in nature as antigenic mixtures.

The capacity of SM-FSV to infect, morphologically transform, and propagate in human, canine, and other host cells further substantiates the previously made observations that feline C-type viruses have a remarkable capacity to cross species barriers and readily infect certain heterologous host cells (7, 14, 15). Despite this fact, our limited seroepidemiological studies of humans in close association with cats have not shown evidence of human infections with the feline viruses in field and laboratory personnel (unpublished observation).

Summary. The SM strain of feline sarcoma virus (SM-FSV) isolated by *in vivo* methods from a naturally occurring case of feline fibrosarcoma was characterized *in vitro*. The virus propagated and induced morphologic transformation of homologous feline host cells as well as heterologous host cells of human, canine and porcine origin. The virus propagated in feline cultures retained its oncogenic potential for newborn cats.

The SM-FSV was found to contain an associated nontransforming feline C-type virus at a concentration of approximately ten fold higher level than the cell transforming virus. The sarcoma virus transformed feline cells with one-hit kinetics suggesting that this associated nontransforming virus is not re-

quired for the initiation of the cell transforming event.

Viral interference tests and viral neutralization tests with type-specific antisera suggested that the SM-FSV consisted of a mixture of antigenic types of the feline leukemia-sarcoma virus subgroups A and B. Isolation of a A subgroup nontransforming virus from virus stocks of the nontransforming SM-FSV associated virus was achieved by virus cloning techniques.

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