ST Feline Sarcoma Virus. Biological Characteristics and *in vitro* Propagation[1] (35807)

PADMAN S. SARMA, T. LOG, AND GORDON H. THEILEN

National Cancer Institute and Microbiological Associates, Bethesda, Maryland 20014; and the School of Veterinary Medicine, Davis, California 95616

The sarcoma viruses of chickens and mice have similar properties such as size, morphology, composition, mode of replication in infected tissues, the ability to infect homologous host cells with the production of sarcomas *in vivo*, and the ability to induce morphological alteration of monolayer culture cells *in vitro* (1–6). Feline sarcoma viruses (FSV) isolated from naturally occurring feline fibrosarcoma (7–9) have been found to have many properties similar to those of the avian and murine sarcoma viruses. The Snyder-Theilen strain of FSV (ST-FSV) was isolated from a naturally occurring fibrosarcoma of a 2-year old cat. Our studies of ST-FSV described herein indicate that this strain of FSV has many properties similar to those of the Gardner-Arnstein strain of FSV (GA-FSV) which we described recently (10). Properties such as "one-hit" focus titration patterns in feline embryo fibroblast (FEF) cultures, the presence of an excess of an associated noncytopathogenic C-type virus, and the ability to infect and propagate in heterologous host cultures were similar, whereas, the focus morphology and growth characteristics in FEF cultures were dissimilar.

Methods and Results. Procedures for the propagation of FEF and other mammalian cultures, preparation of virus stocks and focus assays have been previously described (10–15). To establish focus titration patterns, culture media were replaced at 18–20 hr after virus infection with a medium containing 0.8% Noble agar. The CF antigen induction test (COCAL test) and viral interference test for feline leukemia and sarcoma viruses were performed as described (14, 15).

Cell transformation in FEF cultures. Partially purified concentrates (16, 17) of experimentally induced ST-FSV sarcoma as well as virus stocks consisting of clarified fluids or of clarified fluids of disrupted FEF cells and culture fluids, induced morphologic transformation of FEF cells in dilutions as high as 10^{-4} to 10^{-5}. The foci tended to be diffuse and consisted of a mixture of refractile fibroblasts and round cells (Fig. 1). Such foci were easily identified and counted with the aid of a Leitz inverted microscope.

The normal development of foci in FEF cultures was not inhibited by the presence in the culture medium of a 1% concentration of virus-neutralizing dog antiserum or control normal dog serum. These sera were added to cultures 24 hr after virus inoculation and continuously maintained in the culture. This indicated that focus development in FEF cultures did not require the growth and local spread of virus through culture medium to adjoining cells (6).

Propagation of virus in FEF cultures. The cell transformation effects were reproducibly induced in FEF cultures by the serial passage of clarified culture fluids of infected FEF cultures. Such serial propagation *in vitro* did not result in a diminution of the focus titers of the virus. On the other hand, similar serial propagation of GA-FSV, previously reported (10), resulted in a gradual reduction of the focus titer of the virus.

Detection of the presence of an associated C-type virus. In routine focus assays of ST-FSV in FEF cultures, examination of the inoculated cultures for the feline C-type virus

[1] This work was supported by Contract No. NIH70-2068C of the Special Virus Cancer Program of the National Cancer Institute, Bethesda, Maryland 20014.

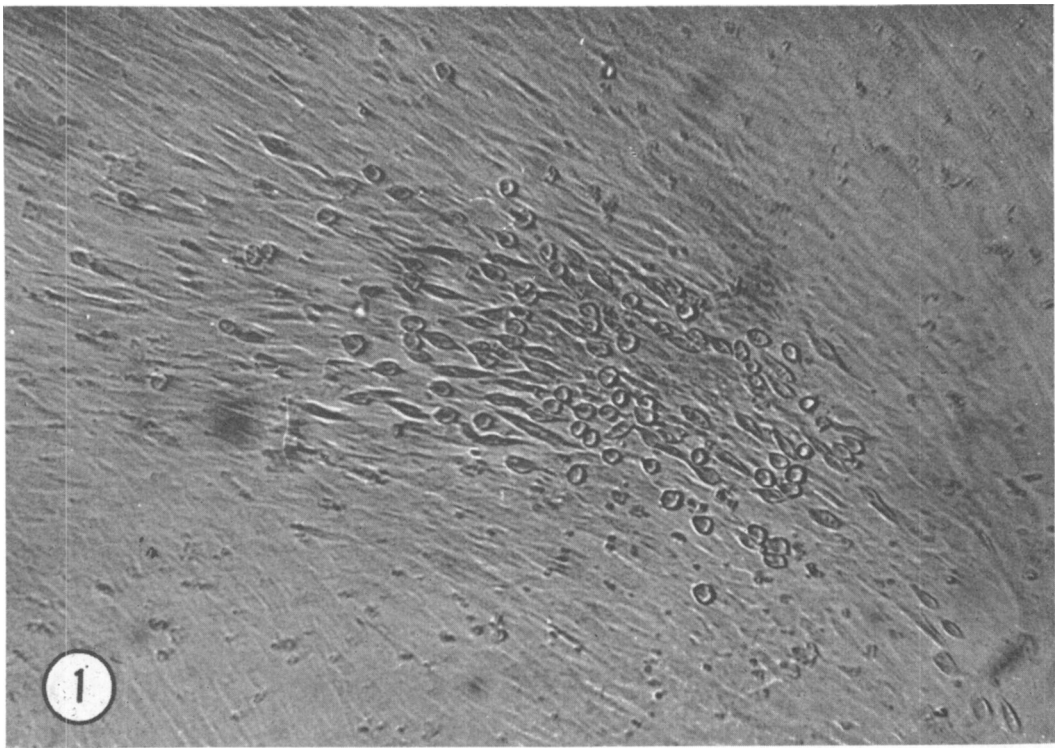

FIG. 1. Morphological transformation of feline embryo cells by ST feline sarcoma virus. A diffuse focus of refractile round and fibroblastic cells; unstained culture; magnification ×60.

by techniques such as the complement-fixing (CF) antigen induction test for the feline leukemia-sarcoma viruses (COCAL test) (14) and the tritiated uridine labeling of viral RNA (11) showed that the virus stocks contained a 1 to 3 $\log_{10}$ excess of a noncytopathogenic virus over the cell transforming FSV (Table I). Serial passage in FEF cultures of clarified fluids of transformed cultures consistently resulted in the induction of similar cell alterations. Similar serial passage of fluids of cultures containing CF viral anti-

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

Virus dilution	Foci/dish FEF[a]	Feline leukemia-sarcoma, group-specific CF viral antigen in inoculated FEF[b]	Release of ^{3}H uridine labeled virus into culture medium
10^{-3}	176	>8	+
10^{-4}	16	>8	+
10^{-5}	2	>8	+
10^{-6}	0	>8	+
10^{-7}	0	<2	—
10^{-8}	0	<2	—
Control FEF	0	<2	—

[a] Average number of foci in 2 feline embryo fibroblast culture dishes.

[b] Reciprocal of CF antigen titer of culture antigens against 4 CF antibody units of feline sarcoma dog antibodies (14).

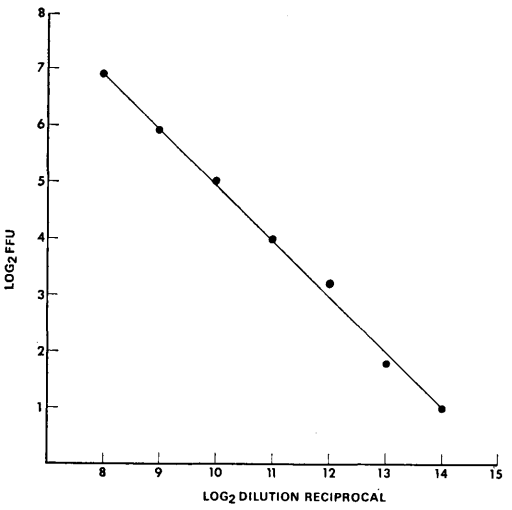

FIG. 2. Focus assay of ST-FSV in feline embryo fibroblast cultures. The number of foci is proportional to the virus dilution factor.

gens and no cellular morphological changes, resulted in the isolation of a feline C-type virus with properties identical to the noncytopathogenic FeLV. Tissue culture virus stocks of this virus, designated herein as ST-FeLV (prepared as described above) contained 10^5 to 10^6 infectious units/0.2 ml of culture fluid as determined by the CF antigen induction test (COCAL test) (14).

Focus titration patterns in FEF cultures. Focus assay in FEF cultures using 2-fold dilutions of tissue culture propagated virus gave one-hit titration patterns showing that the number of foci was proportional to the virus dilution factor (Fig. 2). Focus development was not enhanced by co-infection of cultures with 10^4 to 10^5 infectious units of feline leukemia virus.

Viral envelope characteristics as determined by viral neutralization and interference tests. A potent dog antiserum against the Gardner-Arnstein strain of FSV (GA-FSV) (8) containing a high titer of virus neutralizing antibodies against GA-FSV ($\log_{10}$ neutralization index ≥ 4), neutralized up to 10^3 FFU of ST-FSV. Thus, the ST-FSV appeared to be antigenically related to the GA-FSV, a member of the subgroup B of the feline leukemia sarcoma complex (15). This classification of the feline C-type viruses, described in detail elsewhere (15), is based on similarities and differences in viral envelope antigens demonstrable in the viral interference tests (15) in FEF cultures against challenge with the focus-forming FSV and FeLV pseudotypes of murine sarcoma virus (MSV-FeLV) (*i.e.*, murine sarcoma viruses with the viral envelopes of FeLV) (13). The ST-FSV and ST-FeLV demonstrated the same pattern of viral interference in FEF cultures as the presently known members of the subgroup B viruses. Thus, FEF cultures productively infected with ST-FeLV were resistant to challenge with ST-FSV as well as focus-forming viruses of subgroups B and A, but were susceptible to challenge with MSV(FeLV) of subgroup C. The ST-FSV produced foci in FEF cultures productively infected with FeLV of subgroups A and C, but not in those infected with FeLV of subgroup B. Preliminary studies have shown that the presently known viruses of subgroups B and C contain an A subgroup virus as a component which explains the nonreciprocal viral interference patterns between the ST strain viruses and subgroup A viruses described above (18).

Pathogenicity of tissue culture propagated virus for kittens. Intramuscular inoculation of the culture fluid of the fourth FEF tissue culture passage of ST-FSV into five 1-day-old kittens resulted in the production of progressively growing and fatal fibrosarcomas in all inoculated kittens within 2 to 3 weeks after inoculation. ST-FSV was recovered in high titers *in vitro* from such experimentally induced tumors.

Infectivity for heterologous host cells. ST-FSV was similar to GA-FSV in its ability to transform heterologous mammalian cells. The ST-FSV propagated in FEF culture, replicated and induced morphologic transformation of secondary whole embryo cultures of human, canine, and porcine origin.

Discussion. The antigenic and biological properties of ST-FSV were similar to those of GA-FSV, previously described (10), except for the following differences: The GA-FSV had a far greater excess of the associated, noncytopathogenic C-type virus than the ST-FSV (3 to 4 $\log_{10}$ versus ≤ 2 $\log_{10}$). The GA-

FSV produced indistinct foci of refractile spindle cells in FEF cultures and replicated to low titers; whereas, the ST-FSV produced large foci of refractile fibroblasts and round cells and could be relatively easily assayed and propagated in feline cultures with the maintenance of infectivity for feline cells *in vitro* and *in vivo*. The ST-FSV has a wide experimental host range and induces fibrosarcoma in cats, dogs, rabbits, ovine fetuses, and different species of monkeys (19–21). Limited studies of the GA-FSV indicates that this strain may not have such a wide experimental host range (22).

As described elsewhere (15) the feline leukemia and sarcoma viruses can be divided into three subgroups of A, B, and C, based on the patterns of viral interference shown in FEF cultures against challenge with focus-forming feline sarcoma viruses and FeLV pseudotypes of murine sarcoma virus. Our preliminary studies have indicated that presently known FeLV strains of B and C subgroups also contain an A subgroup virus as a component (18). Based on the viral interference against B and A subgroup viruses, we have found that the ST-FeLV and ST-FSV are members of the presently known subgroup B viruses. The Gardner-Arnstein FSV (8, 10) as well as the S. McDonough FSV (9, 18) were also found to belong to this subgroup.

These studies indicate that the ST-FSV is similar to other known strains of feline, avian, and murine sarcoma viruses in most of its basic biological properties and *in vitro* behavior in cultures of homologous host cells.

Studies in progress are designed to determine the possible dependence of ST-FSV and GA-FSV on the associated, noncytopathogenic, feline, C-type virus for the synthesis of their viral coats.

Summary. The biological properties and *in vitro* growth behavior of the Snyder-Theilen strain of feline sarcoma virus (ST-FSV) were determined. The virus replicated and induced cell transformation in feline embryo fibroblast cultures as well as in heterologous host cultures of human, canine, and porcine origin. Virus stocks were found to contain an excess of an associated, noncytopathogenic virus identical with the feline leukemia virus. Focus assays in feline embryo cultures gave "nondefective" "one-hit" titration patterns. The viral envelope characteristics established by viral neutralization and viral interference tests indicated that the ST-FSV is similar or identical with the Gardner-Arnstein strain of feline sarcoma virus and is a member of the subgroup B of the feline leukemia-sarcoma viruses.

1. Vogt, P. K., Advan. Virus Res. **2**, 293 (1965).

2. Huebner, R. J., Proc. Nat. Acad. Sci. **58**, 835 (1967).

3. Huebner, R. J., Nat. Cancer Inst. Monogr. **29**, 319 (1968).

4. Manaker, R. A., and Groupe, V., Virology **4**, 432 (1956).

5. Rubin, H., and Vogt, P. K., Virology **17**, 184 (1962).

6. Hartley, J. W., and Rowe, W. P., Proc. Nat. Acad. Sci. **55**, 780 (1966).

7. Snyder, S. P., and Theilen, G. H., Nature (London) **221**, 1074 (1969).

8. Gardner, M. B., Arnstein, P., Rongey, R. W., Estes, J. D., Sarma, P. S., Rickard, C. G., and Huebner, R. J., Nature (London) **226**, 807 (1970).

9. McDonough, S. K., Larsen, S., Brodey, R. S., Stock, N. D., and Hardy, W. D., Jr., Cancer Res. **31**, 953 (1971).

10. Sarma, P. S., Huebner, R. J., Gilden, R. V., Baskar, J., and Gardner, M. B., Proc. Soc. Exp. Biol. Med. **137**, 1333 (1971).

11. Sarma, P. S., Baskar, J. F., Gilden, R. V., Toni, R., Vernon, L., and Huebner, R. J., Virology **41**, 377 (1970).

12. Sarma, P. S., Huebner, R. J., Baskar, J. F., Vernon, L., and Gilden, R. V., Science **168**, 1098 (1970).

13. Sarma, P. S., Log, T., and Huebner, R. J., Proc. Nat. Acad. Sci. **65**, 81 (1970).

14. Sarma, P. S., Gilden, R. V., and Huebner, R. J., Virology, **44**, 137 (1971).

15. Sarma, P. S., and Log, T., Virology, **44**, 352 (1971).

16. Moloney, J. B., J. Nat. Cancer Inst. **16**, 877 (1956).

17. Huebner, R. J., Hartley, J. W., Rowe, W. P., Lane, W. T., and Capps, W. I., Proc. Nat. Acad. Sci. U.S.A. **56**, 1164 (1966).

18. Sarma, P. S., Log, T., and Kabigting, A., unpublished data.

19. Theilen, G. H., Synder, S. P., Wolfe, L. G., and Landon, J. C., Proc. 4th Int. Symp. Comp. Leuk. Res., Biblio. Hematol., **36**, 393 (1970).

20. Theilen, G. H., J. Amer. Vet. Med. Ass., **158**,

1040 (1971).

21. Deinhardt, F., Wolfe, L. G., Theilen, G. H., and Snyder, S. P., Science **167**, 881 (1970).

22. Gardner, M. B., Arnstein, P., Johnson, E., Rongey, R. W., Charman, H. P., and Huebner, R. J., J. Amer. Vet. Med. Ass., **158**, 1046 (1971).

Received Apr. 8, 1971. P.S.E.B.M., 1971, Vol. 137.