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A Survey of Cats and Humans for Prevalence of Feline Leukemia–Sarcoma Virus Neutralizing Serum Antibodies[1] (37851)

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The viruses of the feline leukemia–sarcoma complex share their major internal protein antigen, termed the group-specific-1 (gs-1) antigen (1–4), but reveal similarities and differences between individual members with respect to the antigens present on their viral envelopes (5, 6).

The viruses have been classified into three subgroups, based on viral envelope antigenic characteristics demonstrable by viral neutralization and viral interference tests (5, 6). Viruses of the A subgroup are frequently found as single antigenic type, whereas, leukemia and sarcoma viruses of the B and C subgroups isolated thus far, occur as antigenic mixtures of A + B, A + C or A + B + C (5–9).

In this paper, we describe the prevalence of virus neutralizing envelope antibodies in sera of adult domestic cats with or without neoplasia. A focus neutralization test was used analogous to that we reported for avian leukosis and sarcoma viruses (10). Antibodies were found in a proportion of cats with or without neoplasia, but not in humans in veterinary practice or those working in two laboratories engaged in feline leukemia virus research.

Materials and Methods. Virus. Focus forming viruses with the genome of Harvey strain of murine sarcoma virus (H-MSV) and viral envelope of feline leukemia virus (FeLV), hereafter referred to as feline leukemia pseudotype of murine sarcoma virus H-MSV(FeLV), were prepared by trans-species rescue (11) of H-MSV genome from hamster tumor cells with purified single antigenic types of FeLV of subgroups A, B and C (6). Virus stocks containing 100- to 1000-fold excess of nontransforming helper FeLV, were prepared as clarified culture fluids of feline embryo fibroblast (FEF) cultures with confluent cell transformation effects. They were stored at −70° and the titers were determined by focus assay in FEF cultures without agar overlay (7–11). The focus count was determinced with the aid of an inverted microscope on Day 8 after virus inoculation.

Cell cultures. Primary or secondary cells of FEF derived from domestic cat embryos (taken at approximately midterm in pregnancy) were stored in liquid nitrogen in a tissue culture growth medium containing 7.5% DMSO. After removal from storage, the cells were used between passage levels of 1 to 10. Monolayer cultures were propagated in a medium consisting of Eagle's minimum essential medium (EMEM) supplemented with 10% fetal bovine serum (heated at 56° for 30 min), 2 m*M* L-glutamine and antibiotics at a concentration of penicillin, 250 units/ml; streptomycin, 250 μg/ml; Fungizone, 2.5 μg/ml; and Kantrex, 50 μg/ml. The cultures were incubated at 37° in a humidified CO_2 incubator flushed with 5% CO_2 in air. The cultures were routinely fed at 3 day intervals and serially transferred by trypsinization (12) at approximately weekly intervals.

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Cat surveyed. Sera of cats with or without neoplastic disease were tested for virus neutralizing envelope antibodies. Serum samples were collected from adult cats prior to sacrifice. An autopsy was performed. Gross and histological examinations of the internal organs was made to confirm the diagnosis. Cats with neoplasia included 36 cats with lymphoma, one with adenocarcinoma and one with melanoma. Cats not found to have neoplasia included 52 apparently normal cats, and 7 with miscellaneous diseases (2 infectious peritonitis, 1 infectious anemia, 1 pneumonitis, 1 emphysema, 1 nephritis and 1 pancreatitis).

Human sera. Sera of veterinarians engaged in small animal practice were kindly provided by Drs. R. S. Schneider and J. Riggs. Sera of workers of this laboratory and another laboratory, also engaged in feline leukemia virus research, were also included in this study. The human sera were tested undiluted (final dilution 1:2 after mixture with virus).

Experimental animal sera. Sera of experimental animals, immunized against field strains of FeLV and purified single antigenic types of FeLV (6) and a serum of a dog bearing a sarcoma induced by the GA strain of feline sarcoma virus were examined for the presence of type specific virus neutralizing antibodies.

Neutralization tests. Sera were exposed to 56° for 30 min before use. They were screened for antibodies as follows: undiluted sera or sera diluted 1:2 were mixed in 0.3 ml amounts with equal parts of virus diluted so as to contain approximately 100 focus forming units of H-MSV(FeLV)/0.2 ml of virus–serum mixtures. Replicate virus–serum mixtures were set up for each envelope antigenic type of A, B and C. Each experiment included normal cat serum control and positive serum controls (sera of immunized guinea pigs). The virus–serum mixtures were incubated at 4° for 60 min and then inoculated in 0.2 ml amounts into freshly plated cultures of FEF. The culture media were replaced on Day 3 and the cultures were examined for cell transformation effects on Day 8 after inoculation. A test serum was scored positive for virus neutralizing antibodies if it completely neutralized the cell transforming effects of the virus under the described conditions. Antibody titers were subsequently determined by testing sera at twofold dilutions up to and including 1:128.

Results. Virus neutralizing antibodies against one or more envelope antigenic types of A, B and C were detected in the sera of 9 of 38 cats with neoplasia and 13 of 59 cats without neoplasia (Table I). Of the total of 22 antibody positive sera from both groups, 18 contained antibodies to a single antigenic type of virus, whereas, 4 contained antibody mixtures in the combination of A + B, A + C, or A + B + C.

TABLE I. Prevalence of Virus Neutralizing Antibodies Against One or More Serotypes of Feline Leukemia-Sarcoma Viruses in Sera of Cats with or without Neoplastic Diseases.

Category	No. of sera	No. with antibody	H-MSV(FeLV) type(s) neutralized[a]
Cats without neoplasia	59	3	A
		6	B
		3	C
		1	A,B
Cats with neoplasia	38	2	A
		3	B
		1	C
		1	A,B
		1	A,C
		1	A,B,C
Antisera against			
GA-FSV	1	1	A,B
FL-237	1	1	A,C
FL-74	1	1	A,B,C
FeLV,A	1	1	A
FeLV,B	1	1	B
FeLV,C	3	3	C

[a] 100 focus forming units of H-MSV(FeLV) neutralized (see text).

[b] Antisera were obtained from the following tumor bearing or immunized animals: GA-FSV, serum of a dog bearing sarcoma induced by GA strain of feline sarcoma virus; FL-237, guinea pig antiserum against FL-237 strain of FeLV; FL-74, goat antiserum against FL-74 strain of FeLV; FeLV A, B, C guinea pig antisera against purified FeLV of types A, B and C.

A serum obtained from a dog bearing a sarcoma induced by the GA strain of feline sarcoma virus (a mixture of A and B types) (7, 13) neutralized both A and B subgroup viruses. A serum of a guinea pig immunized with uncloned field strain of FL-237 strain of FeLV (a mixture of A and C types) (5, 6) neutralized the A and C types. Similarly a serum of goat hyperimmunized with the FL-74 strain of FeLV (a mixture of A, B and C) (6, 14) contained antibodies to all the three described antigenic types of FeLV.

Hyperimmune guinea pig antisera against FeLV of A, B and C subgroups specifically neutralized the respective homologous H-MSV(FeLV) but not the heterologous viruses.

Virus neutralizing antibodies against the described antigenic types of virus were not detected in undiluted sera of 36 veterinary practitioners and 33 workers of two laboratories engaged in feline leukemia virus research.

A test of selected antibody positive sera for endpoints showed that the field cat sera contained virus neutralizing antibodies against one or more envelope antigenic types of H-MSV(FeLV) in titers ranging from 1:4 to 1:64 (Table II). A retest of the cat sera gave similar endpoints except that titers of two sera were twofold higher.

A goat antiserum against FL-74 strain of FeLV (a mixture of A, B and C types) (6) contained high titers of antibodies against all three described antigenic types of FeLV.

Discussion. The focus neutralization test described herein appears to be suitable as a rapid screening test for a determination of the presence of virus neutralizing antibodies in sera of cats and sera of experimental animals immunized with feline leukemia and sarcoma viruses. The reproducibility of this test was established by a repetition of the assay of antibody positive sera which gave identical endpoints.

The focus forming H-MSV(FeLV) pseudotype viruses contained 100- to 1000-fold excess of helper FeLV (11). Thus, the focus neutralization test may not provide as sensitive a test, as a test based on the neutralization of 10 to 100 infectious units of FeLV by a complement fixation antigen induction test (COCAL test) (2, 6).

Our findings on the prevalence of virus neutralizing antibodies against feline type C viruses confirms the suspected widespread distribution of these viruses in domestic cats, evidenced by the regular occurrence of lymphosarcoma and other neoplastic diseases in a portion of cats (15–17) and by our observations on the prevalence of demonstrable levels of the group-specific antigen of FeLV in a proportion of apparently normal adult and fetal cat tissues (Sarma, P. S., Gardner, M., Parks, W. and Huebner, R. J., unpublished data). We have found that cats seldom contain serum antibodies against the gs-1 antigen of FeLV, presumably as a consequence of immunological tolerance to this antigen resulting from prenatal exposure to this antigen.

TABLE II. Virus Neutralizing Serum Antibody Titers in Feline Sera and Sera of Immunized Animals Against One or More Envelope Antigenic Types of Feline Leukemia Pseudotype of Murine Sarcoma Virus.

Category	Serum no.	Antibodies present	Titer[a]
Cats with neoplasia	23428	A	4
		B	4
		C	8
	23810	A	16
		B	4
	23813	A	64
	23815	B	8
	23496	B	4
	23701	C	8
Cats without neoplasia	23848	A	8
	23741	C	8
FL-74 goat antiserum	OS 269	A	128
		B	64
		C	128
GA-FSV dog antiserum	21782	A	8
		B	32

[a] Reciprocal of highest serum dilution neutralizing 100 FFU of indicated envelope type of H-MSV (FeLV).

Our present studies suggest that cats with neoplasia as well as cats without discernible neoplastic disease are capable of responding immunologically to the viral envelope antigens of FeLV and is in agreement with similar findings made in other laboratories (J. Riggs, W. Jarrett, M. Essex, W. Hardy, Jr., personal communications).

The sero-epidemiological behavior of feline leukemia viruses reported herein is similar to that reported for avian leukosis viruses (10, 18, 22). The virus appears to be principally transmitted vertically in the covert and overt states. Horizontal transmission of the virus also occurs (1, and Hardy, W., Jr., Jarrett, W., personal communications) and presumably accounts for a proportion of naturally occurring neoplasms and virus neutralizing envelope antibodies.

The finding of natural occurrence of antibodies to more than one antigenic type of virus confirms our previous observations on the occurrence of virus mixtures, based on direct virus isolation studies (5–9). Our previous demonstration of the occurrence of A + B viruses in the GA strain of feline sarcoma virus (6, 7), A + C viruses in FL-237 strains of FeLV (5, 6) and the presence of A + B + C in the FL-74 strain of FeLV (6) was confirmed in the present studies in which we found demonstrable levels of corresponding virus neutralizing antibodies in animals experimentally immunized with these viruses.

Recent studies have shown that RD 114 virus is a representative member of a *second* family of covert endogenous type C virus of cats (19–21). In contrast to our findings on feline leukemia and sarcoma viruses, we have thus far failed to find RD 114 virus neutralizing antibodies in sera of 80 cats with or without neoplasia, suggesting that the envelope antigens of this covert endogenous cat virus are not expressed under natural conditions.

Summary. Sera of adult domestic cats and humans (veterinarians and laboratory workers) were surveyed for the presence of virus neutralizing antibodies against feline leukemia–sarcoma viruses of subgroups A, B and C. A focus neutralization test was used based on the neutralization of feline cell transforming effects of approximately 100 focus forming units of feline leukemia pseudotypes of Harvey strain of murine sarcoma virus (Harvey strain of murine sarcoma virus with the viral envelopes of the described serotypes of feline leukemia virus).

Virus neutralizing envelope antibodies against one or more envelope antigenic types were found in the sera of 13 of 59 (22%) cats without neoplasia and in 9 of 38 (23.7%) cats with neoplastic disease but not in the sera of 36 veterinarians or in 33 laboratory personnel working in two laboratories engaged in feline leukemia virus research.

The implications of these findings are discussed.

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