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Development of virus non-producer lymphosarcomas in pet cats exposed to FeLV

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Naturally occurring oncoviruses of several species[1–6] are transmitted contagiously and cause lymphosarcoma (LSA) or leukaemia in their hosts[7]. All naturally occurring oncoviruses replicate *in vivo* in the tumours they induce or, as with bovine leukaemia virus, can be isolated from tumour cells grown in short-term cell culture[7,8]. However, we have shown that feline leukaemia virus (FeLV) is not present in a significant minority of pet cats that develop LSA[9–11]. Unlike experimentally induced virus-negative leukaemias and sarcomas of other species, LSA cells from FeLV-negative LSA cats lack any FeLV proteins, including p15 or p12, and complete functional copies of FeLV provirus and thus do not produce FeLV when grown in cell culture[4,9–19]. Thus, except for FeLV, the naturally occurring animal leukaemogenic oncoviruses seem to induce only virus-producing lymphoid tumours. Our earlier findings prompted a study to determine the frequency of occurrence of FeLV non-producer (NP) LSA in pet cats and whether NP LSAs develop in cats exposed to FeLV. We report here epidemiological data which indicate that development of NP LSAs in pet cats is associated with exposure to FeLV and suggest that FeLV may be the aetiological agent for FeLV NP feline LSAs. Thus, feline NP LSAs may be suitable for studying the potential viral aetiology and mechanism of leukaemogenesis of human lymphoid tumours in which no oncoviruses have, as yet, been proved to cause the disease.

The FeLV status of most cats with LSA was determined by the immunofluorescent antibody (IFA) test for FeLV performed on peripheral blood leukocytes using a multivalent rabbit anti-FeLV serum which detects FeLV gp70, p30, p15, and p12 (ref. 17); but an immunodiffusion test[9], tissue culture isolation[9], and radioimmunoassays (RIAs)[18] were also done to determine the FeLV status of some cats. The presence of RD114 viral proteins was determined by RIAs of LSA cell lysates[19]. In general, the results of the IFA test for FeLV correlated well with all other immunological tests and with tissue culture isolation (Table 1 and refs 9, 11, 13, 17, 19 and 20). We compared the results of the IFA test from 274 cats with our ability to recover infectious FeLV in tissue culture from these cats. We could isolate FeLV from only three of the 153 IFA test-negative cats, a 98% concordance between the two methods of FeLV detection. Conversely, FeLV was isolated from 118 of 121 IFA test-positive cats, a 97.5% concordance. Similar results were obtained when we studied cats with LSA. Of the 57 IFA test-positive LSA cats, 56 (98.3% concordance) were found to have FeLV by tissue culture isolation and of the 33 IFA test-negative LSA cats 31 (93.9% concordance) had no FeLV. These results indicate that detection of FeLV antigens by the IFA test is equivalent to the detection of infectious FeLV. Furthermore, the IFA test can detect even the partial FeLV expression that

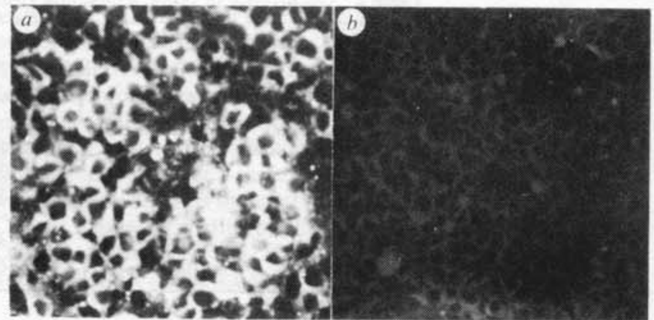

Fig. 1 Examination of feline lymphosarcoma cells for FeLV antigens by the fixed-cell indirect IFA test. The test was done with a multivalent rabbit antiserum against disrupted FeLV that had known reactivity to FeLV gp70, p30, p15 and p12 antigens. *a*, Strong cytoplasmic fluorescence indicates presence of FeLV, but *b*, no such fluorescence is seen in NP LSA cells. Thus the NP LSA cells are devoid of replicating FeLV and FeLV gp70, p30, p15 and p12 expression.

Table 1 Expression of feline leukaemia and RD114 viral antigens and the feline oncornavirus-associated cell membrane antigen (FOCMA) in normal and lymphosarcoma tissues of pet cats

Diagnosis	No. of cats tested	FeLV* status	FOCMA† status	RIA competition assay‡: average viral antigen expression (ng competitor per mg total protein) FeLV antigen expression gp70	p30	p15	p12	RD114 antigen expression gp70	p15
Normal lymphoid cells	4	−	−	<10	<5	<5	<5	<10	<5
Normal lymphoid cells	2	+	−	ND	1084	259	321	<10	<5
Lymphosarcoma (FeLV non-producers)	9	−	+	<10	<5	<5	<5	21	34
Lymphosarcoma (FeLV producers)	9	+	+	483	859	304	463	22	36

* Determined by the fixed-cell IFA test[17].
† Determined by the viable cell membrane IFA test[13,19].
‡ RIA competition assays were performed as described in ref. 30 with 5 ng of ^{125}I-labelled purified viral protein and a limiting amount of specific antibody diluted to the point of ~50% precipitation of the labelled protein. Competition with protein from detergent-lysed cells was quantified by comparison with a standard competition curve with known amounts of purified unlabelled viral protein corresponding to the ^{125}I-labelled protein and expressed as the amount required to give 50% reduction in labelled protein precipitation[30].

Table 2 Occurrence of feline leukaemia virus in naturally occurring lymphosarcomas of pet cats

Gross form of lymphosarcoma	No. of cats tested	Per cent of cases	No. FeLV positive	Per cent FeLV-positive LSA	No. FeLV-negative	Per cent FeLV non-producer LSA
Multicentric	198	39.1	159	80.3	39	19.7
Thymic	174	34.3	134	77.0	40	23.0
Alimentary	69	13.6	16	23.2	53	76.8
Unclassified	13	2.6	5	38.5	8	61.5
Form unknown	53	10.4	46	86.8	7	13.2
Total	507*	100	360	71.0	147	29.0

* All pet cats with lymphosarcoma were histologically confirmed clinical cases and were examined by W.D.H. or E.G.M. at the Henry Bergh Memorial Hospital of the ASPCA or the Animal Medical Center in New York City.

Table 3 Occurrence of lymphosarcoma in FeLV-exposed and unexposed pet cats

Cat exposure history	Observation years based on:	No. of cats	FeLV status	Observation years Total	Observation years Average per cat	Observed cases of lymphosarcoma FeLV+	Observed cases of lymphosarcoma FeLV−	No. of LSAs developing per cat observation year
Never exposed	Ages of cats	1,074	1,074−	3,235	3.1	0	0†	0
Exposed	From time of FeLV exposure*	538	149+ 389−	2,334	4.3	30	11†	0.018
	Total cats	1,612						

* The observation periods for both FeLV-unexposed control cats and FeLV-infected and -uninfected exposed cats were determined in the following ways. For the unexposed FeLV-uninfected control cats the observation period was the age of the cat on the date of its last FeLV test. The observation periods for the exposed cats was the period between the date of the first positive FeLV test of any cat in the household and the termination of this study. The total number of cat observation years for the unexposed and exposed cats was calculated by adding the individual observation periods of all cats in each group. The observation periods for the exposed and unexposed groups of cats were not the same as the exposed cats because the exposed cats had not been exposed for their entire life. To ensure that the difference in the observation periods did not bias our results, we also calculated the observation period for exposed cats in the same way as we did for the unexposed cats—according to their age. In this analysis there was a total of 558 cats, the additional 20 cats were cats that died before our study began and had not been tested for FeLV. The 558 cats were observed for their entire lives for a total observation period of 3,199 yr (an average of 5.7 yr—range 1–24 yr). We found 56 cases of LSA among these 558 exposed cats (30 FeLV-positive, 11 NP LSA and 15 whose status was unknown). The development of LSA per cat observation year was found to be the same (0.018 case) when the observation period was calculated from either (1) the time of FeLV exposure (41 cases of LSA per 2,334 cat observation years = 0.018), or (2) the ages of the cats (56 cases of LSA per 3,199 cat observation years = 0.017). Thus, the difference between the two methods of determining the observation period of the unexposed and exposed tested cats did not alter the conclusion of this study.

† $P<0.001$.

occurs in FeSV-transformed NP mink cells which do not replicate FeLV or FeSV but which express only FeLV p15 and p12 (ref. 21).

We tested 507 LSA cats for the presence of FeLV and 14 LSA cats for the presence of RD114 viral proteins. We found that 360 of the 507 cats tested (71.0%) had FeLV-positive LSAs and 147 (29.0%) had NP LSAs (Table 2). NP LSAs occurred most frequently in cats with the alimentary form of LSA (Table 2). As reported previously, we found that cats with NP LSA were markedly older than cats which developed FeLV-positive LSAs[14]. The mean age of cats with NP LSAs was 6.7 yr; and the median age was 7 yr. In contrast, the mean age of the cats with FeLV-positive LSA was 2.9 yr; and their median age was only 2 yr. Nine FeLV-positive LSAs were tested by RIA and were found to contain large quantities of FeLV structural proteins (Table 1), whereas nine NP LSAs were found to have no detectable FeLV gp70, p30, p15, or p12. However, as reported previously[14], both the FeLV-positive and NP LSAs had low levels of RD114 gp70 (21–22 ng per mg protein) and p15 (34–36 ng per mg protein) whereas FeLV-positive and -negative normal lymphoid tissues had no detectable RD114 antigens (Table 1).

A total of 1,612 FeLV-exposed and unexposed pet cats from all regions of the US were observed for the development of LSA (Table 3). A cat was classified as unexposed if the cat was FeLV-negative and if, according to the owner, the cat had not lived with any FeLV test-positive cat or with any cat which had an FeLV disease. The FeLV status of all cats was determined by the IFA test for FeLV antigens[17]. Of the 1,612 cats, 1,074 living in 96 households had never been exposed to FeLV and were uninfected with FeLV. These cats served as controls and were observed for a total of 3,235 cat observation years (an average of 3.1 yr per cat). None of the unexposed control cats developed LSA during this study. In contrast, the remaining 538 pet cats living in 23 households had been exposed to an FeLV-infected cat (Table 3). All of these exposed cats were tested for FeLV and 389 were found to be uninfected whereas 149 were infected. These cats were observed for a total of 2,334 cat observation years (an average of 4.3 yr per cat with a range of 1 to 6 yr) and 41 of these cats developed LSA; 30 of these LSAs were FeLV-positive and 11 were NP LSAs. The difference in the occurrence of NP LSA between the unexposed control cats and the exposed cats is highly significant by the χ^2 test, $P<0.001$. In one household where there were four unrelated cats, one cat was

infected with FeLV and died of FeLV-induced anaemia. What made the household unique was that all three uninfected cats died of NP alimentary LSA.

As infectious FeLV particles, FeLV antigens, and complete copies of FeLV provirus cannot be detected in feline NP LSAs by electron microscopy[9], tissue culture isolation[4,9,17], immunofluorescence (Fig. 1)[4,17], RIA[10,14,19], or by nucleic acid hybridization[14,15,16], the aetiological role of FeLV in these tumours has been uncertain. In contrast to FeLV NP LSA cells, virtually all other oncovirus-transformed cells including FeSV- and MSV-transformed NP fibroblasts, Abelson MuLV and the acute avian leukaemia virus-transformed fibroblasts or leukaemia cells and bovine leukaemia cells are positive for at least the N-terminal viral gag protein by RIA even though they are negative for replicating virus[22]. We have recently reported that the FeLV-associated tumour-specific antigen, the feline oncornavirus-associated cell membrane antigen (FOCMA), is present on the surface of all FeLV- and FeSV-transformed, virus-producing cat cells, but not on non-transformed virus-producing cat cells. We found that FOCMA is also present on FeLV NP LSA cells suggesting that FeLV may play a role in the transformation of normal lymphocytes into NP LSA cells[13,22,23]. Our finding that there is a comparable epidemiological association between FeLV exposure and the development of both NP- and FeLV-positive LSAs (Koch's postulates have been fulfilled for the FeLV-positive LSAs)[24] supports the hypothesis that FeLV may be the aetiological agent for most feline LSAs regardless of whether or not they express FeLV.

At least three possible hypotheses explain the mechanism by which a replicating oncovirus such as FeLV causes NP feline LSAs[25]. First, FeLV may be involved in the generation of a recombinant replication-defective leukaemogenic virus from cat cellular genes and FeLV provirus, similar to the Abelson MuLV, AK-T8 mink cell focus-inducing virus and several avian oncoviruses, although in these NP tumours some viral gag gene expression is present[26–28]. Second, only a fragment of the FeLV genome may be present as provirus in leukaemic cell chromosomes. If such a fragment was present, it would presumably be unable to code for the production of any detectable viral structural proteins, but may be able to initiate transformation[29]. Third, a 'hit and run' phenomenon might occur in which FeLV alters, but does not stably integrate into the chromosomes of the leukaemic cell.

Feline NP LSA is the only known example of a naturally occurring lymphoid tumour which develops after exposure to a leukaemogenic virus but in which no evidence of that leukaemogenic virus remains. NP LSAs may thus be valuable for the study of the basic mechanism by which viruses, chemicals or radiation induce transformation.

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