INFECTION AND IMMUNITY, Mar. 1975, p. 470-475

Vol. 11, No. 3
Printed in U.S.A.

Naturally Occurring Persistent Feline Oncornavirus Infections in the Absence of Disease

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Healthy feline leukemia virus (FeLV)-infected cats from leukemia cluster environments were followed for up to 23 months for development of disease and evidence of alteration in the hemogram. The incidence of disease development in FeLV-positive cats was more than fivefold higher than the incidence for FeLV-negative cats. Ten cases of leukemia developed in 69 infected cats, whereas one case of leukemia occurred in 59 uninfected cats. The incidence for development of diseases other than leukemia was 30.4% for FeLV-infected cats as opposed to 6.8% for uninfected cats. This could be a result of the immunosuppressive effects of FeLV. FeLV-infected cats had no evidence of subclinical anemia. Mean packed cell volumes and total leukocyte counts were about the same for infected and uninfected animals. The only variation seen in healthy FeLV-infected cats was a decreased mean lymphocyte count. The difference between mean lymphocyte count for FeLV-infected and uninfected animals was significant at the 0.999 level. These findings suggest that the incubation period for feline leukemia may be very prolonged under natural conditions and that an increased susceptibility to unrelated infectious diseases exists during this period. This increased susceptibility was apparently not associated with anemia or depressed total leukocyte counts.

Horizontally transmitted oncornaviruses cause lymphatic leukemia and various forms of lymphosarcoma in outbred domestic cats (5, 6, 7, 13, 16, 18, 20). After experimental inoculation of newborn kittens with feline leukemia virus (FeLV), the incubation period for these diseases can be as short as a few weeks (21, 25, 30) or as long as several months to several years (17, 19, 23).

One of the earliest and most consistent clinical signs associated with lymphoproliferative malignancies of cats is nonregenerative anemia (12). Additionally, many cats with severe or fatal anemia (in the absence of leukemia) are FeLV positive (M. Essex, S. M. Cotter, W. D. Hardy, Jr., P. Hess, W. Jarrett, O. Jarrett, L. Mackey, H. Laird, L. Perryman, R. G. Olsen, and D. S. Yohn, submitted for publication; 16). Anemia was observed to develop in 7.5% of 148 healthy FeLV-positive cats in a mean period of 5.3 months (16). One possible explanation for this observation is that certain strains of FeLV cause anemia as a specific disease entity. Strains of oncornaviruses have been described that appear to do this in mice (28, 29). Another possibility is that typical strains of FeLV induce such an early and severe anemia that death sometimes occurs before clinical leukemia is observed.

Healthy cats from either laboratory colonies or disease-free pet household environments are very rarely FeLV positive (M. Essex, S. M. Cotter, J. L. Carpenter, W. D. Hardy, Jr., P. Hess, W. Jarrett, J. Schaller, and D. S. Yohn, J. Nat. Cancer Inst., in press; 16). Conversely, up to 50% of the healthy cats from households with a history of feline leukemia are FeLV positive (M. Essex, R. M. Jakowski, W. D. Hardy, Jr., S. M. Cotter, P. Hess, and A. Sliski, J. Nat. Cancer Inst., in press; 16). Many virus-positive cats remain clinically healthy for at least several months, whereas many others develop unrelated infectious diseases. It is not known whether subclinical anemia and other alterations from normal hematologic values are an immediate result of the establishment of persistent FeLV infections or if FeLV-positive cats that appear to remain clinically healthy also maintain normal blood values. This study was undertaken as an approach to this question. Total leukocyte counts, lymphocyte counts, total packed cell volumes, and the presence or absence of circulating atypical lymphocytes were determined for cats having

persistent FeLV infections for various intervals. These values were compared to those obtained for uninfected cats living in the same environments.

MATERIALS AND METHODS

Serology. The test for FeLV antigen using peripheral blood smears was described in detail previously (14). Briefly, the smears are fixed in acetone, incubated with rabbit anti-FeLV group-specific antigen serum which was absorbed in vivo in a young cat, washed, incubated with fluorescein-conjugated goat anti-rabbit immunoglobulin G, washed again, and examined. Detection of FeLV group-specific antigens in this manner is regularly associated with the presence of infectious virus in peripheral blood, or viremia (16).

Hematology. The microhematocrit procedure was used to determine packed cell volume values (2). Total leukocyte counts were determined with a fresh sample of whole blood in a tube coated with ethylenediaminetetraacetic acid and counted in a standardized hemacytometer. Differential leukocyte counts were done on the same blood smears that were collected for FeLV determination. The Wright-Giemsa stain was used, and at least 100 cells were counted. Atypical lymphocytes were defined as those with dark, clumped, nuclear chromatin, basophilic cytoplasm, vacuolization, or unusual size or shape.

Cats. All cats involved in the study were from two households where multiple cases of leukemia had been confirmed before the present study began. Neither FeLV nor other cats were introduced into either house during the course of the study, so all infections and diseases were naturally acquired. The cats represented three breeds: Abyssinian, Burmese, and domestic. Inbreeding was not practiced, and no evidence existed for significant differences between cats in different breeds or families for rate of infection. Further biographical details of the cats are published elsewhere (M. Essex, R. M. Jakowski, W. D. Hardy, Jr., S. M. Cotter, P. Hess, and A. Sliski, in press; 1, 2).

Blood samples were collected at 3- to 5-month intervals for a total period varying from 3 to 23 months. In all but two cases, cats that were found to be FeLV positive remained positive for subsequent tests. The former two were not included in the study. Several virus-negative cats converted to positive. The minimal period for maintenance of persistent infection was based on serial tests for virus positivity. Since 71% of the virus-positive healthy cats were positive at first testing, it is likely that most positive cats were viremic for periods much longer than the "minimal period" used for inclusion of cats in the study. Final diagnosis of disease was based on gross and microscopic examination of all spontaneous deaths which occurred during the period of study.

RESULTS

Cats infected with FeLV in the leukemia "cluster" environments had a greatly increased risk for leukemia development (Table 1). Ten cases of leukemia occurred in 69 virus-positive cats during the study period. Only one case of leukemia was seen in 59 virus-negative cats from the same environment that were followed for the same period. Two cases of anemia were also found in the FeLV-positive cats, whereas none were observed in the FeLV-negative cats. Surprisingly, however, the incidence of all diseases was 44.9% in the FeLV-positive group as opposed to 8.5% in the uninfected group. The incidence of infectious diseases other than leukemia was four to five times higher in the FeLV-infected group.

The normal packed cell volume for healthy cats ranges from 24 to 45%, with an average of 37% (22). Of the packed cell volumes we observed for 48 healthy uninfected cats, only 4 fell outside this range, and the mean was 37.2% (Table 2). The range for normal total leukocyte counts is 8,000 to 25,000 with a mean of 17,000 (22). The mean we observed for healthy uninfected cats was somewhat lower (13,631). Five of 48 were slightly outside this range. The range for normal lymphocyte counts for healthy cats is listed as 1,600 to 13,750, and the mean is 5,440

TABLE 1. *Development of various diseases in FeLV-infected and uninfected cats from leukemia cluster environments*

FeLV status	No. examined	Disease developed		
		Types	No.	%
Positive	69	All	31	44.9
		Leukemia	10	14.5
		Granulomatous disease	3	4.4
		Enteritis	3	4.4
		Membranous glomerulonephritis	3	4.4
		Bacterial pneumonia	2	2.9
		Infectious peritonitis	2	2.9
		No diagnosis	2	2.9
		Hepatitis	1	1.5
		Anemia	1	1.5
		Pulmonary medial arterial hyperplasia	1	1.5
		Colitis	1	1.5
		Granulomatous disease and bacterial pneumonia	1	1.5
		Membranous glomerulonephritis and anemia	1	1.5
Negative	59	All	5	8.5
		Leukemia	1	1.7
		Granulomatous disease	1	1.7
		Infectious peritonitis	1	1.7
		Membranous glomerulonephritis	1	1.7
		Pyothorax	1	1.7

TABLE 2. *Mean blood values for healthy FeLV-infected and uninfected cats from leukemia cluster environments*

Category	No. of cats	Packed cell vol[a]	Total leukocyte count[b]	Total lymphocyte count[b]
FeLV infected	31	36.3 ± 1.1	14,338 ± 1,122	2,120 ± 213
Uninfected	48	37.2 ± 0.8	13,631 ± 640	3,197 ± 203

[a] Expressed as cubic centimeters per 100 cubic centimeters; mean and standard error.
[b] Expressed as cells per cubic millimeter; mean and standard error.

(22). Of 48 healthy uninfected cats, only 6 were below 1,600 and none were above 13,750, even though the mean was only 3,197. About 50% of the uninfected cats had some atypical lymphocytes, but most had less than 10%, which is not unusual for normal healthy cats.

The mean packed cell volume for healthy FeLV-infected cats was similar to that for both the standard and the healthy uninfected cats from these cluster households. Only 3 of 31 fell outside the normal range of 24 to 45. The percentage of cats with 5% or more atypical lymphocytes was 38.7%.

The mean lymphocyte count for uninfected cats was about 50% higher than the comparable mean for infected cats from the same environment. This difference is significant at the 0.999 level using Student's *t* test. Eleven of 31 FeLV-infected cats had lymphocyte counts well below the range considered normal. Mean total leukocyte counts were about the same for infected and uninfected cats.

The ages for healthy and sick infected and uninfected cats are compared in Table 3. No significant differences were seen in either the age distribution or mean for cats in the four categories. None of the cats in any of the categories was less than a year of age, and only one was more than 9 years old.

Healthy cats with persistant FeLV infections were categorized according to the duration of infection, and mean blood values were compared (Table 4). No significant change in packed cell volume, total leukocyte count, or lymphocyte count was seen with an increase in time after infection.

Since the period listed as "known duration of FeLV infection" represented the minimum period, many of the cats listed in each category may have been infected for much longer periods. For this reason, cats known to have converted during the observation period of 245 to 671 days were compared to those that were positive at first observation and remained so for the duration of the experiment (Table 5). Six of 10 (60.0%) "newly positive" cats had lymphocyte counts below normal, as opposed to 8 of 27 (29.6%) "constantly positive" cats. The mean lymphocyte count for newly positive cats was also below the mean for constantly positive cats, but this difference was only significant at the 0.93 level. None of the newly positive cats developed leukemia during the observation period.

DISCUSSION

Within the same leukemia "cluster" environments, healthy cats that were FeLV positive were more likely to develop leukemia than FeLV-negative cats. This is in agreement with a previous study that showed a high risk for leukemia development in FeLV-positive cats (16). More than half of the FeLV-positive cats remained healthy for prolonged periods. Forty-five percent of the positive cats remained healthy and positive for at least 245 days with a mean "minimum duration of infection" of 496 days. Since only 29% of the positive cats converted from FeLV negative to positive during the study period, we must conclude that many were FeLV positive for periods considerably longer than those listed.

The experimental induction of leukemia in kittens includes instances where a high percentage develop leukemia in a few weeks or months after the administration of large doses of concentrated virus (21, 25). Other reports, using more natural inoculation routes and/or lower, less pure virus doses, describe a lower efficiency of tumor induction and prolonged incubation periods (17, 19, 23). Our results suggest that the incubation period for leukemia under natural conditions may be variable and prolonged.

Infected cats also had an increased risk for development of diseases other than leukemia, since 30.4% of the FeLV-infected cats developed other diseases as opposed to only 6.8% of the uninfected group. Although two cases of fatal anemia, which may be directly related to FeLV infection, occurred in the infected group, 17 confirmed cases of other unrelated infectious diseases were also observed. One possible explanation for the increased risk for development of infectious diseases other than leukemia in infected animals is FeLV-mediated immunosuppression. Immunosuppression with murine on-

TABLE 3. *Age distribution for FeLV-infected and uninfected healthy and sick cats*

Category	No. of cats	Age (years)[a]						Mean age
		1-2	3-4	5-6	7-8	9-10	Over 10	
Healthy								
All	79	25 (31.6)	24 (30.4)	20 (25.3)	7 (8.9)	2 (2.5)	1 (1.3)	4.1
Uninfected	48	17 (35.5)	12 (25.0)	13 (27.1)	6 (12.5)	0	0	4.0
Infected	31	8 (25.8)	12 (38.8)	7 (22.6)	1 (3.2)	2 (6.5)	1 (3.2)	4.3
Sick								
All	36	12 (33.3)	8 (22.2)	15 (41.7)	1 (2.8)	0	0	3.8
Uninfected	5	2 (40.0)	0	2 (40.0)	1 (20.0)	0	0	4.2
Infected	31	10 (32.2)	8 (25.8)	13 (41.9)	0	0	0	3.7

[a] Numbers in parentheses are the percentage of total.

TABLE 4. *Mean blood values for FeLV-infected cats according to known duration of infection*

Duration of infection (days)	No. of animals	Packed cell vol[a]	Total leukocyte count[a]	Lymphocyte count[a]
0	48	37.2 ± 0.8	13,631 ± 640	3,197 ± 203
1-100	6	41.5 ± 1.7	10,433 ± 811	2,218 ± 681
101-200	3	35.3 ± 6.7	ND[b]	ND
201-300	7	39.9 ± 3.9	12,047 ± 1,368	1,759 ± 406
301-400	10	37.5 ± 1.1	12,298 ± 1,761	2,173 ± 340
401-500	14	34.6 ± 1.6	12,606 ± 1,526	2,168 ± 351
501-600	10	37.4 ± 2.0	16,797 ± 2,577	1,784 ± 301
601-700	7	34.9 ± 2.1	13,117 ± 773	2,384 ± 483

[a] Expressed as in footnotes to Table 2.
[b] ND, Not done.

cornaviruses is a well-documented observation (4), and FeLV-mediated immunosuppression has been described (24). An increase in the incidence of infectious peritonitis in cats from leukemia cluster environments has been found previously (2). This increased risk for certain types of unrelated infectious diseases could be due to a differential suppression of those elements of the immune response most essential for defense against the given disease.

A higher than expected frequency of either serologically detectable FeLV (15) or type C virus presumed to be FeLV (26) has been reported previously for cats with non-neoplastic diseases. The suggested explanation for these observations was activation of "latent" FeLV by the non-neoplastic disease agents. Our current results indicate that FeLV-mediated immunosuppression, as a predisposing factor to non-neoplastic disease development, must also be considered as a possible explanation.

No evidence was found for the development of slowly progressive anemia concurrent with or soon after FeLV infection. Healthy cats infected with FeLV for prolonged periods had mean packed cell volumes that were essentially the same as those for uninfected cats. Although anemia is a frequent sign in leukemia, these results indicate that it is not a regular direct result of FeLV infection.

TABLE 5. *Comparison of mean lymphocyte counts for cats known to convert to FeLV positive during the study period to counts for cats that were FeLV positive when first tested*

Group	No. tested	Lymphocyte count[a]	
		Range	Mean
Newly positive	10	103-5,034	1,818 ± 446
Constantly positive	27	713-4,481	2,266 ± 230

[a] See footnote to Table 2 for description.

A decreased mean lymphocyte count was the only evidence found for an alteration from the normal blood picture in FeLV-infected healthy cats. Considerable variation was seen in lymphocyte counts for individual infected cats, indicating that this test was of little or no value for predicting infection of healthy animals with FeLV. The difference between means for infected and uninfected groups was, however, highly significant. This is of particular interest because lymphopenia is a frequent clinical finding in feline leukemia (3, 27). Cats known to convert from FeLV negative to positive within a

few months of the sample date had lower lymphocyte counts than cats with long established infections. It is possible that a "crisis" period develops soon after infection—a period when susceptibility to clinical deterioration is high. Cats surviving such a crisis may remain healthy for prolonged periods. Most infected cats are virus excretors (20), and healthy FeLV-positive cats should be regarded as "carriers" capable of infecting others by contact exposure (10, 13). The correlation between relative lymphopenia or disease development and FeLV positivity could not be explained on the basis of age, because no significant age differences were observed for cats in the various categories.

The reason that many cats remain healthy while infected with FeLV remains unknown. The presence of avirulent strains of virus in disease-free cats is a possibility, but less likely in view of the high incidence of leukemia in others from the same environment. The explanation we favor is a more efficient immune response against tumor cells rather than virus. We have previously demonstrated such a role for tumor immunity in cats with virus-induced fibrosarcoma (8, 9, 11) and demonstrated a lack of humoral antibodies in cats with naturally occurring leukemia (M. Essex, S. M. Cotter, W. D. Hardy, Jr., P. Hess, W. Jarrett, O. Jarrett, L. Mackey, H. Laird, L. Perryman, R. G. Olsen, and D. S. Yohn, submitted for publication). Future studies with FeLV-infected healthy cats may help clarify these issues.

ACKNOWLEDGMENTS

We thank Merideth Dodd, Linda Taylor, Laura Mahoney, and Wajeed Khan for excellent technical assistance.

The research was supported by grants from the Anna Fuller Fund, the Jane Coffin Childs Fund for Medical Research, National and Massachusetts Branches of the American Cancer Society, Cancer Research Institute, the Oliver S. and Jennie R. Donaldson Charitable Trust, and a Public Health Service grant from the National Cancer Institute, CA-15579. M. E. and W. D. H. are scholars of the Leukemia Society of America.

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