Isolation of a T-Lymphotropic Virus from Domestic Cats with an Immunodeficiency-Like Syndrome

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A highly T-lymphotropic virus was isolated from cats in a cattery in which all the animals were seronegative for feline leukemia virus. A number of cats in one pen had died and several had an immunodeficiency-like syndrome. Only 1 of 18 normal cats in the cattery showed serologic evidence of infection with this new virus, whereas 10 of 25 cats with signs of ill health were seropositive for the virus. Tentatively designated feline T-lymphotropic lentivirus, this new feline retrovirus appears to be antigenically distinct from human immunodeficiency virus. There is no evidence for cat-to-human transmission of the agent. Kittens experimentally infected by way of blood or plasma from naturally infected animals developed generalized lymphadenopathy several weeks later, became transiently febrile and leukopenic, and continued to show a generalized lymphadenopathy 5 months after infection.

DOMESTIC CATS MAY BECOME INfected with several retroviruses, including feline leukemia virus (FeLV) (*1, 2*), feline sarcoma virus (FeSV) (*3, 4*), endogenous type C oncornavirus (RD-114) (*5*), and feline syncytium-forming virus (FeSFV) (*6*). The first three of these retroviruses have been classified as Oncornavirinae, while FeSFV belongs to the subfamily Spumavirinae (*7*). Feline leukemia virus is the most important pathogen in the group, causing a large number of diverse disease syndromes including lymphoreticular and myeloid neoplasms, anemias, immune-mediated disorders, and an immunodeficiency syndrome somewhat similar to human acquired immune deficiency syndrome (AIDS) (*8*).

Because of its immunosuppressive properties, FeLV infection has been compared to human AIDS and is often referred to as feline AIDS or "FAIDS" (*8*). Recently, however, the immunosuppressive properties of FeLV have been attributed to a replication-defective FeLV mutant that arises during the natural course of infection with replication-competent FeLV (*9*). This defective FeLV has been referred to as "FeLV-FAIDS."

We describe here the isolation of another retrovirus from cats with a chronic AIDS-like syndrome. This virus, which is unlike FeLV, endogenous RD-114 virus, FeLV-FAIDS, or FeSFV, is highly T lymphotropic and is morphologically similar but antigenically distinct from the human immunodeficiency virus (HIV).

This new feline lentivirus was brought to our attention by a peculiar outbreak of disease in a large colony of pet cats. The colony consisted of 43 animals ranging from 1/2 to 13 years of age (Table 1). The animals were homeless prior to entering the colony and some were feral. Many of them were neutered at the time of entry and were given routine vaccinations for feline panleukopenia (parvovirus) and for viral infections of the upper respiratory tract.

All animals had been routinely tested for FeLV infection by an indirect immunofluorescence assay (IFA) or an enzyme-linked immunosorbent assay (ELISA) since the mid-1970s. Cats that tested positive for FeLV infection were not allowed onto the premises and retesting was periodically conducted on animals in the group to reconfirm the FeLV-free status of the cattery. The 43 animals were placed in one of five outdoor wire pens (pens A to E) or in the home. Once the animals were assigned to one of these areas they were seldom moved from one pen to another. Disease problems within the cattery were low during the period between 1968 and 1982, and very few animals died. Diseases that did occur during this time were of the type normally associated with a cattery of this size: mild transient diarrheas and upper respiratory infections, ear mites, ringworm, intestinal parasites, fleas, abscesses, oral cavity disease, feline urologic syndrome, and a few FeLV-related disorders prior to the institution of FeLV control measures in the mid-1970s.

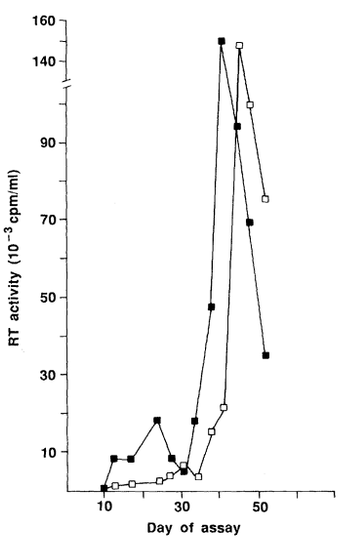

Fig. 1. Reverse transcriptase (RT) activity detected in infected PBL from cats 2428 (□) and 2429 (■). The cultures were supplemented with normal donor lymphocytes (T-cell enriched) when the viable cell count fell below 1×10^5 cells per milliliter. Culture medium contained RPMI 1640, 10% fetal bovine serum, human rIL-2 (100 U/ml) (*12*), Polybrene (2 μg/ml), penicillin (100 U/ml) and streptomycin (100 μg/ml).

A significant change in the pattern and severity of disease in this cattery emerged in 1982. The new problems seemed largely limited to the cats in pen D and occurred after the introduction of a 4-month-old female kitten (cat Cy) to the enclosure (Table 2). This cat began having intermittent bouts of diarrhea at 7 months of age, developed a persistent mucopurulent rhinitis and conjunctivitis, and aborted a litter of kittens. This state of ill health persisted for the next 2 years. In the third year, the cat became very thin and anemic, and began to demonstrate abnormal neurologic behavior consisting of compulsive roaming and frequent movements of the mouth and tongue. Her gums were badly infected and many teeth had been lost from severe periodontal disease. Several blood transfusions were of temporary benefit but ultimately the emaciation, chronic infections, and anemia worsened and the cat died. Between 1982 and 1986, nine other cats from pen D developed a similar type of illness and died (see Table 2). Only one cat fom other pens (pen B) died during this period.

Given the possible infectious nature of the disease syndrome, an attempt was made to serially transmit the disease from affected cattery cats to specific pathogen-free (SPF) kittens. Whole blood (1 ml) from cat FL was inoculated intraperitoneally into SPF kitten 2428. A second SPF kitten (2429) was inoculated intraperitoneally with 5 ml of filtered (0.22 μ*M*) pooled plasma from cats SA and TC. Plasma from these donor cats was used instead of blood because both of them tested positive for FeSFV infection. FeSFV is highly cell-associated and very little virus occurs free in plasma (*6*). Plasma, buffy coat preparations, and bone marrow samples from cats FL and SA were also cocultivated with Fc9, Fcwf-4, and Crfk feline fibroblasts. Cultures were monitored for cytopathic effect (CPE), Mg^{2+}- and Mn^{2+}-dependent reverse transcriptase (RT) activity (*10*), and FeLV-p27 antigen expression by ELISA (*11*). The cocultures with plasma and buffy coat cells were discarded after they were negative for 6 weeks. Bone

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marrow cultures from cat SA yielded FeSFV after several passages but were negative when examined by transmission electron microscopy for other agents.

Kittens 2428 and 2429 were monitored daily for clinical signs of illness and complete blood counts were taken weekly. A generalized enlargement of peripheral lymph nodes was noticed beginning 4 weeks after inoculation. A low-grade fever appeared 2 weeks later and was associated with a precipitous drop in the total leukocyte count and absolute numbers of polymorphonuclear neutrophils. Platelet and red blood cell counts remained normal, however. Cat 2429 developed a severe bacterial cellulitis at the site of ear tagging, septicemia, and high fever within a week of the time that the white cell count decreased. The infection and high fever disappeared after broad spectrum antibiotic therapy was instituted. The leukopenia and low-grade fever disappeared after 2 to 4 weeks, but the generalized lymphadenopathy has persisted (5 months after inoculation).

Buffy coats were prepared from blood samples collected from the two experimentally inoculated kittens (2428 and 2429) during the leukopenic phase of their illness. The buffy coats were cocultured with stimulated peripheral blood lymphocytes (PBL) from normal SPF cat donors. The donor lymphocytes were harvested from fresh whole blood by means of Ficoll-Hypaque gradients and were stimulated with concanavalin A (Con A; 5 μg/ml). Three days after Con A stimulation, nonadherent blood leukocytes were transferred to fresh flasks and further stimulated with recombinant human interleukin-2 (IL-2; 100 U/ml) (*12*). Cells examined after 7 days in culture were more than 95% T lymphocytes as determined by an IFA with the use of rabbit antiserum to feline theta, immunoglobulin G (IgG), and immunoglobulin M (IgM) antigens. Cultures were replenished with fresh Con A and IL-2–stimulated PBL every 5 to 7 days. A cytopathic effect (CPE) consisting of ballooning degeneration, increased cell death, and giant cell formation was noted in cultures of cells from cat 2429 within 14 to 21 days of cocultivation. A similar CPE was seen in cultures from cat 2428 after 4 to 6 weeks. Cytopathic changes were associated with the appearance of RT activity (Fig. 1) that was totally Mg^{2+}-dependent; the optimum conditions for reactivity resembled those of the HIV enzyme and differed from those of the RT of FeLV (Fig. 2). Lymphoid cell cultures showing CPE and RT activity were negative for FeLV-p27 antigen by ELISA and for FeSFV by IFA. Transmission electron micrographs of RT and CPE-positive cultures

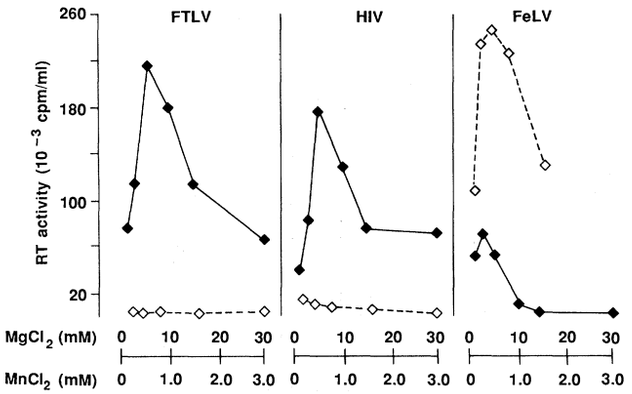


Fig. 2. Mg^{2+}- (◆) and Mn^{2+} (◇) -dependent RT activity in culture fluids containing the new feline retrovirus (FTLV), HIV, or FeLV. Culture fluids from FTLV-infected primary cat PBL, FeLV-producer line FL-74, and HIV-infected H9 cells, were clarified by centrifugation at 3000 rpm for 2 hours. Portions of cell-free fluids (1 ml) were centrifuged at 17,000 rpm for 1 hour and the virus pellets were assayed for RT activity. Reverse transcriptase was measured with poly(rA)-oligo(dT_{12-18}) template primer, four different deoxyribonucleoside triphosphates, and 20 m*M* KCl for Mg^{2+} and 60 m*M* NaCl for Mn^{2+} assays. Five microcuries of [^{3}H]TTP-labeled deoxyribonucleoside triphosphate was used for each sample.

Table 1. The age, sex, and, health status of the 43 cats in the cattery. The exposure of the cats to FeLV, FeSFV, feline infectious peritonitis virus (FIPV), and the new retrovirus was also determined. This table excludes cats in Table 2 that died before the serological survey was conducted.

Cat number	Pen designation	Sex	Age (years)	Health status	Virological status*			
					FeLV	FeSFV	FIPV	FTLV
1	C	M	4	Healthy	–	–	–	–
2	C	M	4	Healthy	–	–	–	–
3	C	M	4	Healthy	–	–	–	–
4	C	M	4	Healthy	–	–	+	–
5	B	M	7	Sickly	–	+	+	–
6	B	M	4	Healthy	–	+	+	–
7	B	M	6	Sickly	–	–	+	+
8	B	F	1	Healthy	–	–	+	–
9	A	F	5	Sickly	–	+	+	–
10	A	F	7	Healthy	–	+	+	–
11	A	F	5	Sickly	–	–	+	–
12	A	F	6	Sickly	–	–	+	–
13	A	F	6	Sickly	–	–	+	–
14	A	F	Aged	Sickly	–	–	+	–
15	Home	M	1/2	Healthy	–	–	+	–
16	Home	F	1	Healthy	–	–	+	–
17	Home	F	1	Healthy	–	–	+	–
18	Home	F	1/2	Healthy	–	–	+	–
19	Home	F	1/2	Healthy	–	–	+	–
20	Home	F	1/2	Healthy	–	–	+	–
21	E	F	2	Healthy	–	–	+	–
22	E	F	1/2	Healthy	–	–	+	–
23	E	F	7	Sickly	–	–	–	+
24	E	F	Aged	Sickly	–	+	–	–
25	E	F	Aged	Sickly	–	–	–	–
26	E	M	2	Healthy	–	–	+	–
27	E	M	11	Sickly	–	+	+	–
28	D	F	10	Sickly	–	+	–	+
29	D	F	5	Healthy	–	+	+	+
30	D	F	5	Sickly	–	+	–	+
31	D	M	6	Sickly	–	+	+	+
32	D	M	6	Sickly	–	–	+	–
33	D	M	9	Sickly	–	–	+	+
34	D	F	13	Sickly	–	+	+	–
35	D	F	13	Sickly	–	–	+	–
36	D	F	13	Sickly	–	+	+	–
37	D	F	7	Sickly	–	+	+	–
38	D	F	5	Sickly	–	–	+	+
39	D	F	9	Sickly	–	+	+	+
40	D	M	5	Sickly	–	+	+	–
41	D	M	9	Healthy	–	–	–	–
42†	D	F	7	Sickly	–	+	+	+
43	D	F	7	Sickly	–	+	+	+

*FeLV infection status determined by detection of p27 core protein in serum by ELISA. FeSFV, FIPV, and FTLV exposure status determined by measuring serum antibodies by IFA at a serum dilution of 1:10. †Died since survey was conducted.

Table 2. Disease course and clinical signs observed in ten cats from pen D that died between 1982 and 1986.

Cat name	Duration of illness	Clinical signs
Cy	3 years	Chronic rhinitis, conjunctivitis, diarrhea, abortion, vague neurologic abnormalities, periodontitis, gingivitis, anemia, emaciation
GK	7 months	Chronic rhinitis, diarrhea, periodontitis, gingivitis, anemia, emaciation
FL*	9 months	Diarrhea, hematochezia, periodontitis, gingivitis, alopecia, seborrheic dermatitis, emaciation
SA*	7 months	Found extremely dehydrated, depressed, and near death. Responded well to treatment but fell into a pattern of depression, dehydration, and weight loss. Severe diarrhea for the last 2 weeks of life
TC*	6 months	Found near death and died 5 hours later. Appeared healthy the night before. History of periodontitis and gingivitis for several months prior to death. Evidence of severe chronic and acute enteritis at necropsy
CY	hours	Found dead. Appeared healthy the night before. Possible acute enteritis
RU	hours	Found dead. Appeared healthy the night before. Possible acute enteritis
BL	1 month	Chronic diarrhea, anorexia, dehydration, emaciation
CH†	1 month	Thin, seborrheic dermatitis, chronic rhinitis, anemia
CL	5 months	Found depressed and hypothermic with terminal hysteria and rage

*The new retrovirus was isolated from blood of cat FL and from pooled plasma from cats SA and TC. †Tested positive for FTLV prior to death. All others cats died prior to testing for the new virus.

revealed mature, immature, and budding particles typical of lentiviruses (Fig. 3). The particles were slightly smaller and more ellipsoid than HIV and had more prominent envelope spikes. Particles with the morphology of type C or D oncornaviruses or FeSFV were not observed. Virus purified from feline T-lymphoid cultures banded at a density of 1.15 g/cm^3 in continuous sucrose gradients (Fig. 4).

Reverse transcriptase levels in culture supernatants increased progressively after each serial passage of the virus in fresh PBL and peaked after day 40 to 45 (Fig. 1). After exposure of fresh PBL with infected cell-free culture supernatants, the RT level in the culture increased progressively and peaked by day 7. Infectivity for T-lymphocyte enriched PBL could be readily demonstrated with both filtered tissue culture fluid and cellular inocula. The virus failed to replicate, however, in several feline fibroblastic cell lines, including Fc9, Fcwf-4, and Crfk. Infectivity studies with long-term human T-lymphoid cell lines (H9, HUT 78) and with primary cultures of human PBL stimulated with phytohemagglutinin and IL-2 have been negative to this point.

The serologic relation between this new feline virus and HIV was examined. Serum samples from all 43 cats were uniformly negative against HIV when tested by IFA and Western blotting. Western blots, prepared from gradient-purified virus from cat 2429 or from virus-infected cell lysates, reacted with sera from experimentally infected cat 2429 and with several cats from the cattery, but not with pooled human sera positive for HIV (Fig. 5). Three humans who had persistent and close contact with cats in the cattery had no antibodies to HIV or to the new feline virus by IFA and Western blotting.

A serologic survey of the cattery was conducted by using an IFA with infected feline lymphocytes as the substrate. As shown in Table 1, most of the infected animals were confined to pen D; only two infected animals were found in other pens. A thorough physical examination of all 43 cats in the cattery showed that 18 were healthy and 25 had various ailments (Table 1). Unhealthy cats were either very thin and rough-coated or had one or more of a number of chronic infections including gingivitis, periodontitis, pustular dermatitis, ear infections, chronic rhinitis, chronic conjunctivitis, or diarrhea. Only 1 of the 18 (5.6%) healthy cats was seropositive for the new virus, whereas 10 of the 25 (40%) unhealthy cats had antibodies to the new virus (Table 1). None of the cats were infected with FeLV; however, several were seropositive for FeSFV and feline infectious peritonitis virus (FIPV) (Table 1).

Clinical signs observed in the ten cats that were seropositive for the new virus included chronic rhinitis, excessive thinness, and anemia (Table 3). One of these animals had a recurrent bacterial cystitis that is uncommon

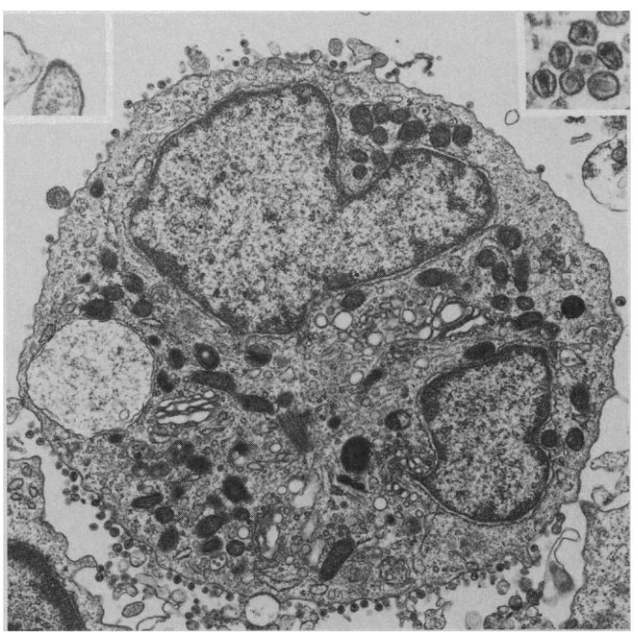


Fig. 3. Transmission electron micrograph of a lymphocyte culture infected with FTLV. Extracellular viral particles surrounding a T lymphocyte (center, ×25,000), and a budding particle from T lymphocyte (left inset, ×80,000). Mature virions were ellipsoid (120 by 150 nm) in shape with typical lentivirus-type nucleocapsids (right inset, ×80,000).

Table 3. Clinical signs of illness seen in the ten cats that were seropositive for the new virus and were still alive in December 1986.

Cat number	Pen	Clinical signs
7	B	Recurrent cystitis
23	E	Chronic periodontitis, chronic rhinitis, premature loss of teeth
28	D	Chronic proliferative gingivitis and stomatitis
29	D	Healthy
30	D	Extremely thin, mild chronic gingivitis
31	D	Chronic periodontitis, chronic rhinitis, extremely thin, rough hair coat
33	D	Chronic intermittent diarrhea, thickened bowel loops on palpation, chronic otitis externa, recurrent aural hematomas
38	D	Chronic severe pustular dermatitis
39	D	Chronic severe pustular dermatitis
43	D	Chronic rhinitis, extremely thin, anemia

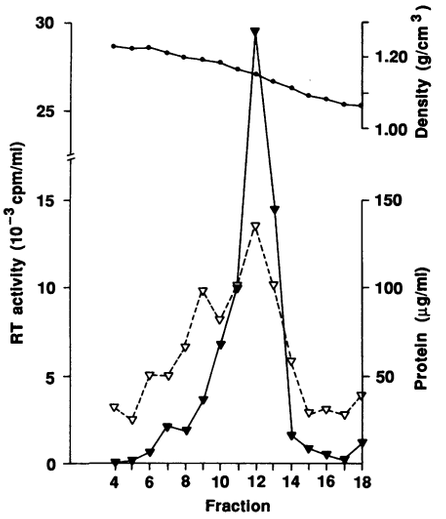
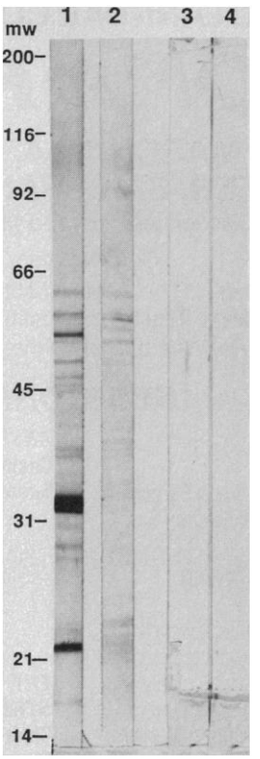


Fig. 4 (**left**). Sucrose density gradient banding of FTLV. Virus was concentrated from T-lymphocyte culture supernatants by low speed centrifugation to remove subcellular debris and ultracentrifugation to pellet virion particles. Pelleted virions were then layered on a 10/50% (W/V) continuous sucrose gradient in tris-base (*p*H 7.4) containing 0.1*M* NaCl and 1 m*M* EDTA, and centrifuged in a Beckman SW41 rotor for 3 hours at 36,000 rpm. Fractions (0.5 ml) were collected from the bottom of the gradient and assayed for RT activity (▼), protein concentration (▽), and density (●). This purification procedure yielded 1 mg of FTLV per liter. **Fig. 5** (**right**). Western blot of FTLV-infected cell lysate with the use of serum samples from 1, cat 2429; 2, antibody-negative SPF cat; 3, HIV-positive humans (pooled samples); and 4, HIV-negative humans (pooled samples). Although antigenic comparison was not made, the position of these bands may correspond to the major core protein p24, *gag* precursor protein p55, and endonuclease protein p32 of HIV.



in cats. Periodontitis, stomatitis, gingivitis, miliary pustular dermatitis, bacterial otitis externa, and aural hematomas were recognized in both seropositive and seronegative cats. These diseases were more common, however, among seropositive than seronegative animals. Neurologic abnormalities were not observed in any of the surviving seronegative or seropositive cats; they were recognized, however, in two cats in pen D that had died prior to the institution of testing for the new virus (Table 2).

A disease identical to that transmitted to SPF kittens 2428 and 2429 inoculated with whole blood or plasma was subsequently transmitted to other SPF kittens by means of purified virus that had been propagated in tissue culture. The cultured virus was then reisolated from the blood of these kittens several weeks later. An identical virus was also repeatedly reisolated from cats 2428 and 2429 over a period of 4 months or more, thus indicating the persistence of the infection. A limited serologic survey of cats presented to the Veterinary Medical Teaching Hospital of the School of Veterinary Medicine has confirmed the existence of the new virus in diseased cats from many different geographic areas of Northern California.

We have tentatively designated this new virus "feline T-lymphotropic lentivirus," abbreviated to FTLV. Its morphology and the metal requirements of its RT resemble those of the lentivirus subfamily and HIV. HIV also shows some similarities to animal lentiviruses and, like this new feline agent, is highly T lymphotropic. Alternative names, such as feline lentivirus (FLV) or feline immunodeficiency virus (FIV), were considered, but posed problems. FLV is often used interchangeably with FeLV. FeLV is also a well-recognized cause of acquired immunodeficiency, and the designation FIV for the new retrovirus would be somewhat presumptuous at this time. The name FTLV is consistent with accepted guidelines for the nomenclature of retroviruses (*13*).

The isolation of FTLV from cats has obvious implications to both veterinary and human medicine. The virus does not appear to be antigenically related to HIV of humans and there is no evidence for cat-to-human or human-to-cat infection. The antigenic dissimilarity of FTLV and HIV was expected. To date, none of the animal lentiviruses (caprine arthritis encephalitis virus, Maedi/visna, equine infectious anemia virus) show great antigenic similarity to HIV or to each other. We assume, therefore, that FTLV is a highly species-adapted lentivirus that has existed in cats for some time. The exact relation of FTLV to human and animal retroviruses must await more detailed antigenic and genetic comparisons.

The importance of FTLV infection in cats is unknown. Preliminary studies on cats from unrelated households suggests that the infection is widespread. If the disease-causing potential of the virus in general cat populations is as great as it is in the cattery described in this report, its clinical importance will be substantial. Many FeLV negative cats have disease syndromes characterized by anemias, neurologic signs, and chronic infections. Aborted kittens and neonatal deaths are also common in many FeLV negative catteries. The current understanding of FTLV infection resembles that of FeLV in 1973, the time when FeLV diagnostic tests were first applied in a clinical setting (*14*). As a result of routine FeLV testing, a myriad of different disease syndromes of unknown and poorly understood etiology were found to be related directly or indirectly to FeLV infection (*2, 8*).

FTLV infection of cats has important implications for the study of human AIDS. The virus is more closely related to HIV in terms of disease pathogenesis than it is to the ungulate or equine lentiviruses, which are not T lymphotropic and do not induce chronic immunosuppression (*15–20*). In terms of T lymphotropism and immunosuppressive properties, FTLV is more closely related to the primate T-lymphotropic retroviruses (*21–24*). Since primates are difficult to obtain, the naturally occurring FTLV in cats may provide a useful model for HIV research.

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