Feline Leukemia Virus

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The feline leukemia virus (FeLV) is the causative agent of the most important fatal infectious disease complex of American domestic cats today. It is a horizontally transmitted (i.e., contagious) ribonucleic acid (RNA) virus belonging to the family Retroviridae. Retroviruses causing lymphoid tumors have been identified in a number of animal species, including cats, cattle, domestic fowl, certain nonhuman primates, and rodents. Recently a new and unique retrovirus of human beings, the human T-cell leukemia virus (HTLV), has been isolated from a number of people with particularly aggressive lymphoid malignancies; it remains to be determined whether HTLV is indeed the causative agent of these tumors.

The oncogenic (cancer-causing) retroviruses are commonly referred to as the RNA tumor viruses, or oncornaviruses (oncogenic RNA viruses). In addition, there are other retroviruses that are associated with nonmalignant disease processes, such as progressive pneumonia of sheep, arthritis-encephalitis of goats, and infectious anemia of horses. Retroviruses also exist that have not as yet been associated with any recognized disease process, such as the syncytium-forming viruses that have been isolated from a number of species, including the cat.

Replication-competent retroviruses carry with them a unique and fascinating enzyme called reverse transcriptase. This enzyme is capable of producing a deoxyribonucleic acid (DNA) copy of the retroviral RNA, which then is inserted into the chromosomal DNA of the infected cell. This alien intruder, known as a provirus, or proviral DNA, then divides whenever the host cell divides and can serve as a template for the intracellular production of new virus particles. The new viruses are assembled in the cytoplasm of the cell and are released at the cell membrane, where they acquire a lipid envelope and surface receptors known as pexpomers.

At present there is no practical method of removing, or excising, integrated proviruses from infected cells of living animals. A cell infected with a retrovirus is infected essentially for the length of its lifetime, as are all of its daughter cells. This is of great significance in latent FeLV infections and in the persistence of intracellular FeLV in the face of an active immune response.

Because a portion of their genetic material becomes a part of the total genetic information of the cells they infect, retroviruses are among the most intimate parasites known in nature.

Feline Retroviruses

Three members of the Retroviridae family are of importance in this discussion: FeLV, the feline sarcoma virus (FeSV), and the virus known as RD-114. Of these only FeLV is a truly exogenous agent, that is, infection is spread from cat to cat as a contagion, so that unexposed cats are free of FeLV proviral DNA. FeSV, a replication-defective mutant of FeLV, apparently arises within individual cats by a recombinational event in which a small piece of chromosomal DNA is incorporated into an FeLV provirus. The resulting FeSV is unable to replicate without "assistance" from replication-competent FeLV ("helper" virus), because a portion of its genetic information is lost during recombination. In nature FeSV recombination events apparently occur infrequently, and the recombinant viruses are probably not contagious per se. The virus known as RD-114 is a true endogenous virus. That is, multiple RD-114 proviruses are present within the chromosomal DNA of all cells of all domestic cats and are transmitted vertically through the germ line. However, production of virus is usually repressed, so that the agent is not contagious. The RD-114 retrovirus does not appear to be related to FeLV/FeSV and has not been proven to cause any recognized disease in cats.

Origin of feline retroviruses. Recent scientific studies suggest that neither FeLV/FeSV nor RD-114 is indigenous to cats. Rather, these agents appear to have been acquired at some time in the distant past from other species of animals. Sophisticated molecular studies have demonstrated relationships between FeLV/FeSV and some retroviruses of rodents, and between RD-114 and certain primate retroviruses. The results of these and other studies suggest that an ancient rodent retrovirus, transferred in some manner to an ancestor of the domestic cat, may have served as the origin of FeLV/FeSV millions of years ago, while the RD-114 virus may have been transmitted from ancestors of today's Old World monkeys.

Host range. In nature FeLV/FeSV infections appear to be restricted to members of the cat family, including domestic breeds as well as certain small exotic cats—sand cats, European wild cats, jungle cats, and possibly leopard cats. In addition a retrovirus closely related to RD-114 has been isolated from a European wild cat.

Mechanisms of Tumor Induction

The precise biochemical mechanisms by which normal, healthy cells are transformed into malignant cells unresponsive to cellular control mechanisms are not yet known. However, studies in recent years using oncornaviruses from a number of animal species have begun to raise the curtain of darkness and yield faint but tantalizing glimpses into the complex molecular interactions responsible for induction and maintenance of malignancy.

It has been appreciated for some time that the incubation period (the time interval between infection and tumor production) may be quite prolonged (months to years) for some oncornaviruses, such as FeLV, but may be quite short (weeks) for others, such as FeSV. The vast majority of oncornaviruses with short incubation periods are replication defective (such as FeSV); those with prolonged incubation periods are fully capable of replication without the assistance of "helper" viruses. All of the defective oncornaviruses that have been closely examined have a common feature: the replacement of genetic information necessary for replication by "foreign" genetic sequences coding for the production of a protein unnecessary for replication but essential for induction and maintenance of the malignant state. In many cases these sequences, gener-
ally referred to as viral onc genes, appear to be closely related to sequences of chromosomal DNA from cells that the virus has infected (cellular onc genes). It has thus been proposed that onc genes present in defective oncornaviruses such as FeSV have been acquired by aberrant recombination between a nondefective oncornavirus (such as FeLV) and host chromosomal DNA. The function (or functions) of cellular onc genes in uninfected, healthy cells is not known, but it is suspected that they are involved in normal cellular growth processes and in the control of cellular differentiation. Acquisition of these sequences by oncornaviruses during recombination confers on these viruses the ability to disrupt normal cellular proliferation and differentiation—a recognized characteristic of the disorders we collectively refer to as cancer. Tumors induced by defective oncornaviruses (such as FeSV) have short incubation periods, because these viruses carry viral onc genes within their RNA and can thus initiate the cascade of events leading to malignancy soon after they infect a cell. The mechanisms by which nondefective oncornaviruses with prolonged incubation periods (such as FeLV) induce malignancy are less clear, but it may be that the location of the proviral DNA is inserted is critical. Thus insertion of proviral DNA near a cellular onc gene may "activate" that gene in some manner leading to malignant transformation of the infected cell. This may explain the lengthy incubation period, since it may require months to years of random proviral insertions in many cells before activation of a critical cellular onc gene takes place.

**Immunogenic Importance of FeLV Structural Components**

Individual particles of FeLV consist of two distinct morphologic components: a dense inner core, or nucleoid, and an outer envelope containing an immunologically important protein known as gp70. This protein is the principal antigen present in the virus surface receptors, which are responsible for attachment of the virus to cells during infection. Virus-neutralizing antibody (VNA) directed against gp70 is an essential component of a successful immunologic response to FeLV, and its presence in the blood is an indication of past FeLV exposure. Most persistently viremic cats (cats in which FeLV circulates in the bloodstream for a prolonged period of time) produce little or no VNA. In addition, most cats in the general feline population do not have levels of VNA that are protective against infection, probably because they have not been exposed to an infective dose of FeLV. On the other hand, about 40 to 50 percent of healthy cats in FeLV-infected multicat households have protective VNA titers. These cats are generally believed to be resistant to subsequent FeLV infection, and most will not become persistently viremic.

The second major antigen of the FeLV particle is the protein p27, which is a structural component of the inner viral core. This protein can be found in great abundance in the cytoplasm of infected white blood cells and platelets, and in soluble form in plasma and serum of viremic cats. The significant of the immunologic response of the cat to p27 is at present uncertain, since antibodies directed against it apparently are not protective, preventing neither viremia nor FeLV-related disease. Indeed, some studies suggest that immune-complex disease can result from an unbalanced immunologic response to this protein in persistently viremic cats. The primary importance of p27 lies in its being the major FeLV antigen detected by the two "FeLV tests"—the slide test and the kit test—commonly used in veterinary clinical practice today.

Suppression of normal protective immunologic responses is one of the most important consequences of persistent infection with FeLV. Both the humoral (antibody-forming) and cellular arms of the immune system are affected by the virus. A major cause of FeLV-induced immunosuppression appears to be a specific FeLV structural protein, p15(E), that is associated with the viral envelope. Both intact and disrupted (inactivated) virus particles retain immunosuppressive capabilities. The significance of FeLV-induced immunosuppression is especially apparent when considering the array of secondary diseases associated with FeLV infection. In addition, immunosuppression induced by p15(E)—even p15(E) from "inactivated" virus—is an important consideration in the design of an effective FeLV vaccine.

**Pathogenesis of FeLV Infection**

Recent scientific studies have succeeded in identifying some of the early host-virus interactions of FeLV infection. After infection of lymphoid tissues surrounding the site of initial virus penetration, a low-grade viremia involving small numbers of certain white blood cells occurs within two weeks of exposure. In this way the virus is transported to other regions of the body, especially to systemic lymphoid tissue, intestinal tissue, and bone marrow. These areas contain populations of rapidly dividing cells, where FeLV replication can be enhanced. Infection of white blood cell and platelet precursors in the bone marrow and the subsequent release of infected cells into the circulation result in a second, more profound viremia (persistent viremia). In those cats that resist widespread infection and replication of FeLV, virus containment occurs in the early lymphoid stage of infection, after transient viremia. In those animals destined to become persistently viremic, infection proceeds to extensive involvement of the bone marrow, pharynx,
can also be spread in blood transfusions. Therefore, "asocial" cats appear to be less readily infected than more "outgoing" cats. Virus with other persistently viremic cats.

Virus that kittens may become infected either in colostrum are also known to occur, so that kittens may become infected through an infected queen or close contact with other persistently viremic cats. Persistent close contact (days to weeks) between cats is usually sufficient for effective transmission of FeLV. Thus "asocial" cats appear to be less readily infected than more "outgoing" cats. Virus can also be spread in blood transfusions from viremic cats and possibly also by the bites of bloodsucking insects, such as fleas. The time period between initial exposure to an infective dose of FeLV and the development of either viremia or immunity is quite variable and may be dependent in part on the route of virus transmission.

Studies have demonstrated that age at the time of infection and the amount and strain of the infective dose are important determinants of the outcome of FeLV exposure. Whereas most kittens exposed to FeLV become persistently viremic, most cats over six months of age resist persistent viremia, suggesting that age-related maturation of the immune system is involved. Evidence indicates that these changes occur in cats between two and four months of age. However, it does appear that some older animals may become persistently viremic if the time of exposure to the virus is lengthy (years).

In common with a number of other enveloped RNA viruses, FeLV is extremely labile once outside the cat and is rapidly inactivated by alcohol and most common household detergents and disinfectants. The infectivity of virus in saliva left to dry at room temperature has been shown to decline to inconsequential levels within three or four hours. However, the infectivity of FeLV suspended in liquid at room temperature may persist for several days and for even longer periods at refrigerator temperature.

Transmission of FeLV
All persistently viremic FeLV cats are shedders of infectious virus and probably remain so for the rest of their lives. They thus serve as a source of infection for healthy, uninfected, susceptible cats with which they come into contact. Cats that develop immunity experience an initial transient viremia lasting from one to two days up to eight weeks, during which time they too can shed infectious FeLV. Excretion of FeLV occurs primarily in salivary secretions, although virus may also be present in respiratory secretions, feces, and urine. Thus the social grooming habits of cats—licking and biting—sneezing, and the urban practice of sharing litter boxes probably represent the major modes of spread of FeLV among pet cats. In addition, in utero transfer of virus across the placenta and excretion of FeLV in colostrum are also known to occur, so that kittens may become infected through an infected queen or close contact with other persistently viremic cats. Prolonged close contact (days to weeks) between cats is usually required for effective transmission of FeLV. Thus "asocial" cats appear to be less readily infected than more "outgoing" cats. Virus can also be spread in blood transfusions from viremic cats and possibly also by the bites of bloodsucking insects, such as fleas. The time period between initial exposure to an infective dose of FeLV and the development of either viremia or immunity is quite variable and may be dependent in part on the route of virus transmission.

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The FeLV-associated Diseases
Persistently viremic FeLV cats are subject to development of a number of disease entities that are either directly or indirectly caused by FeLV. Those directly caused by FeLV include lymphoid malignancies, a number of myeloproliferative disorders, several types of anemia, the panleukopenia-like and thymic atrophy syndromes, at least one form of kidney disease, certain reproductive disorders, and several miscellaneous conditions. Diseases indirectly caused by FeLV include a myriad of conditions that develop secondary to FeLV-induced immunosuppression. The prognosis for survival of persistently viremic cats is poor; approximately 50 percent die within six months of infection, and over 80 percent die within three years of infection.

Lymphoid malignancies. Lymphosarcoma (LSA) and its leukemic counterpart are the most common tumors of American domestic cats. Several forms of LSA have been identified, and their classification is based most commonly on their anatomic distribution. The tumors consist primarily of solid masses of proliferating lymphocytes and constitute the majority of malignancies caused by FeLV.

The alimentary form is characterized by tumor cell infiltration of the gastrointestinal tract and other organs, for example, the gastrointestinal lymph nodes, liver, kidneys, and spleen. Common presenting signs include inappetence, weight loss, vomiting, diarrhea, bloody stool, and jaundice. Occlusion of the bowel lumen by the proliferating tumor results in constipation or obstruction.

The thymic, or mediastinal, form is characterized by the presence of a large tumor mass (or masses) infiltrating the thymus gland and spreading to regional lymphoid tissue and sometimes to extrathoracic structures. Clinical signs are a reflection of pressure effects of the mass and the severe intrathoracic fluid accumulation that frequently accompanies the tumor. Physical examination thus may reveal difficult respiration, cyanosis, muffled heart sounds, coughing, difficult swallowing, and incompressibility of the chest wall.

The multicentric form is characterized by primary involvement of many lymphoid tissues of the body and additional involvement of other structures, such as the liver, bone marrow, kidneys, spleen, and lungs. Presenting signs are variable and depend on the precise anatomic distribution of the tumor, but often include painless swelling of peripheral lymph nodes and enlargement of the spleen and liver and often of the intestinal lymph nodes.

Atypical forms of LSA also occur and consist usually of solitary tumor masses involving primary sites of origin in nonintestinal, nonlymphoid structures. These include the kidneys, central nervous system, and eyes,
and, rarely, the skin or bones. Presenting signs vary according to the location of the tumor.

Lymphocytic leukemia is characterized by the presence of circulating cancerous lymphocytes in the blood and bone marrow. Lymphocytic leukemia may precede the development of LSA, or it may be associated secondarily with LSA. Presenting signs usually consist of nonspecific inappetence, depression, and weight loss. More-specific signs that may be seen include anemia, fever, jaundice, and enlargement of the liver, spleen, and lymph nodes.

Lymphoid tumors occur in cats of all ages, but certain age-related tendencies have been observed. Thus the thymic form of LSA and lymphocytic leukemia occur most commonly in younger cats, the multicentric form of LSA occurs most commonly in middle-aged animals, and the alimentary form of LSA occurs most commonly in middle-aged and older cats.

FeLV-negative lymphoid malignancies. During the course of investigations into the biology of FeLV, it has become apparent that 10 to 50 percent of lymphoid malignancies in cats are negative for FeLV-related material, such as virus particles, viral structural antigens, or proviral DNA. Most of these FeLV-negative tumors are of the alimentary type and occur proportionally more often in older age groups than in younger age groups. The cause of FeLV-negative tumors is unknown, but it is suspected, for a number of reasons, that FeLV is ultimately responsible for their development.

Myeloproliferative disorders. Myeloproliferative disorders are a group of primary bone marrow disorders characterized by abnormal proliferation of one or more hemopoietic (blood-forming) cell lines. Granulocytic (or myelogenous) leukemia, erythroleukemia, erythremic myelosis, megakaryocytic leukemia, polycythemia rubra vera, and reticuloendotheliosis are all terms that have been applied to various forms of these disorders. Classification depends on the cell lines of origin. Clinico-pathologic differentiation between different forms is sometimes difficult, if not impossible, however, because more than one hemopoietic cell line may be involved, either sequentially or simultaneously. Presenting signs often include inappetence, depression, weight loss, relentless and progressive anemia, fever, jaundice, peripheral lymph node enlargement, and enlargement of the liver and spleen secondary to massive infiltration by abnormally proliferating cells.

Nonregenerative anemia. Nonregenerative anemia (NRA) is probably one of the most common manifestations of FeLV infection. This type of anemia, also known as hypoplastic, aplastic, or depression, anemia, is characterized by a severe reduction in the number of red cell precursors in the bone marrow, resulting in failure to produce an adequate number of circulating red cells. Sometimes there may be a pancytopenia, in which red cell, white cell, and platelet precursors are all affected. NRA may occur alone or may be associated with LSA or myeloproliferative disease, or it may precede the development of an FeLV-induced malignancy. Since many severely ill cats with NRA are put to sleep, the true incidence of subsequent malignancy cannot be accurately determined. Unfortunately, clinical signs are usually not detected until the anemia is well advanced. Common signs include inappetence, depression, weight loss, respiratory difficulty, pallor of the mucous membranes, and increased heart rate. Coinfection of such cats with Hemobartonella felis, the parasite causing feline infectious anemia, may contribute to the severity of the anemia.

Other anemias. In addition to NRA, other types of anemia may occur in cats in association with FeLV infection. These include (1) leukopenic/hypoplastic anemia, characterized by the simultaneous presence of circulating nucleated (immature) red blood cells in numbers out of proportion to the severity of the anemia, and of certain immature white cells (this type of anemia has been observed in some cases of LSA); (2) megaloblastic anemia (similar to the anemia of vitamin B12/folate deficiency), sometimes seen in cats with myeloproliferative disorders; (3) hemolytic anemia, characterized by premature destruction of circulating red blood cells by an immunologic process; (4) anemia of chronic disease, due to the ineffective reutilization of iron for hemoglobin synthesis.

Panleukopenialike syndrome. A syndrome somewhat similar to panleukopenia (feline "distemper") has been observed in some FeLV-infected cats known to be properly immunized against panleukopenia. Presenting signs often include inappetence, depression, dehydration, weight loss, fever, vomiting, diarrhea (which may be bloody), and a profound reduction in the number of circulating white blood cells. Anemia may also be present. Although affected cats may respond transiently to supportive therapy, the disease is progressive and usually fatal.

Thyric atrophy syndrome. Kittens born to persistently viremic queens often develop a syndrome of lethargy, inappetence, wasting, stunted growth, atrophy of the thymus gland and other lymphoid structures, and enhanced susceptibility to infection with other disease-causing agents ("fading kitten syndrome"). The degree of thyric atrophy may be severe, amounting to virtual disappearance of the organ in some cases. Such kittens do not gain weight and often do not nurse vigorously. Many die from secondary bacterial or viral infections within the first few weeks of life. Those that survive are carriers of FeLV and thus capable of transmitting the virus to other susceptible cats. The syndrome may also precede the development of an FeLV-induced malignancy.

Chronic kidney disease. Glomerulonephritis, a type of kidney disease, has been described in cats in association with LSA, lymphocytic leukemia, and granulocytic leukemia. In addition, glomerular disease in the absence of malignancy has been reported in FeLV-infected cats. In one study the leading cause of death in an FeLV-infected household of 134 cats over a five and a half year period was glomerulonephritis. Other studies suggest that immune-complex disease, ranging from subclinical microscopic lesions to the nephrotic syndrome, may be caused by formation of antigen-antibody complexes that accumulate in kidney glomeruli. The primary antigen against which the antibodies are directed appears to be the p27 core protein of FeLV. A mild to moderately severe glomerulonephritis has also been reported in some cats suffering from chronic progressive polyarthritis, a symmetrical polyarthritis with similarities to rheumatoid arthritis of humans. Although the cause of this condition is unknown, there is some evidence that coinfection of cats with FeLV and with the feline syncytium-forming virus may be associated with the disease in some cats.

Reproductive disorders. Queens infected with FeLV may experience one or more reproductive disorders, including fetal resorption, abortion, infertility, endometritis, and birth of fading kittens. Abortions characteristically occur late in gestation and are more frequent in high-density multiple-cat FeLV households than in solitary-cat households or multiple-cat households free of FeLV. It has been reported that nearly 75 percent of FeLV-infected queens experience abortions and/or fetal resorptions.

Miscellaneous disorders. Infection with FeLV has been associated with several other miscellaneous disorders, including osteochondromatosis (aberrant growth of cartilage-capped bony protrusions from certain long bones) and persistent pupillary dilation (probably induced by paralysis of ciliary nerves in the eye).

Diseases secondary to immunosuppression. The array of secondary disease entities associated with FeLV-induced immunosuppression is one of the most important manifestations of FeLV infection. It has been estimated that nearly 50 percent of all cats with severe bacterial infections and infectious anemia, and 75 percent of cats with toxoplasmosis, have an underlying FeLV infection. In addition to these disorders, FeLV-induced immunosuppression has been associated with chronic mouth and gum infections, poorly healing or recurrent abscesses, deep skin infections, chronic respiratory infections, acute colitis, severe ear infections, and infectious peritonitis. (All of these problems, of course, may also be seen in cats not infected with FeLV.) FeLV-induced immunosuppression proba-
by contributes also to the development of FeLV-induced malignancies.

The FeSV-associated Diseases
FeSV is the causative agent of some fibrosarcomas (tumors of connective tissue cells) and malignant melanomas (tumors of pigment-producing cells) in cats. Multiple fibrosarcomas arising in the skin of younger cats (generally less than five years of age) are usually associated with FeSV/FeLV infection, whereas solitary fibrosarcomas found in older cats usually are not. FeSV-induced malignancies occur only rarely in cats, however, and thus are of relatively minor clinical significance when compared to the array of problems associated with persistent FeLV infection.

Treatment of Selected FeLV-associated Diseases
The therapeutic goals of the veterinarian in treating many of the FeLV-associated diseases are to provide palliative relief from clinical signs and to prolong life. However, therapy should be advocated only if there is the possibility of maintaining a good quality of life for the prospective patient. In addition, ethical questions regarding prolonged treatment of persistently viremic cats shedding an oncogenic virus into their environment must also be addressed by both the veterinarian and cat owner.

Lymphoid malignancies. Several therapeutic methods are currently available for treatment of lymphoid malignancies, including chemotherapy, surgery, and radiation therapy. In general, lymphoid malignancies are quite responsive to radiation therapy, but their widespread anatomic distribution usually dictates other methods of treatment. Consequently, chemotherapy has become the treatment of choice for these tumors. Combination chemotherapy involving simultaneous administration of several drugs generally will enhance chances for obtaining a clinical remission. The most common therapeutic agents used in treating lymphoid malignancies in cats include prednisolone, vincristine (Leukeran®), cyclophosphamide, and cytosine arabinoside (Cytosar®). Alternative drugs that can be used initially or substituted following a clinical relapse include vinblastine (Velban®), cyclophosphamide, and cytosine arabinoside. The results of therapy of myeloproliferative disorders in cats, however, have been generally disappointing.

Nonregenerative anemia. Similarly, administration of fresh whole blood to cats with NRA is imperative when red blood cell counts fall below acceptable levels. Transfusions also are often supplemented with corticosteroid therapy (prednisolone) and sometimes with additional agents such as cyclophosphamide. NRA is generally a relentless, progressive condition, however, and repeated transfusions are frequently required to maintain adequate numbers of circulating red blood cells. Recently, some success in treating NRA has been reported by using bovine interferon as an experimental therapeutic agent.

Diagnostic Aids for FeLV Infection
Currently there are three basic laboratory procedures to assist the veterinarian in determining the FeLV and FeLV-immune status of an animal: (1) detection of viral antigens; (2) detection of virus-neutralizing antibody; and (3) detection of antibodies to the feline oncornavirus cell membrane-associated antigen (FOCMA).

Detection of viral antigens. The FeLV p27 core protein provides the major antigenic basis for the detection of FeLV in both the indirect immunofluorescence assay (IFA), also known as the slide test, or Hardy test, and the enzyme-linked immunosorbent assay (ELISA), also known as the kit test (Leukassay F, Pitman-Moore, Inc.). A positive test for FeLV by IFA indicates the presence of FeLV-infected blood cells in a cat at the time the sample was taken. Additionally, a positive IFA test implies that a cat is shedding FeLV and is a potential health hazard to uninfected susceptible cats, especially kittens and cats on immunosuppressive drug therapy. A positive test does not diagnose an FeLV-associated disease. Approximately 97 percent of cats that test positive by IFA remain positive for life. A negative IFA test indicates that no detectable infected blood cells are present. It does not exclude the possibility that a cat is incubating FeLV at the time of testing, nor does it imply that a cat has developed immunity to FeLV.

A positive test by ELISA indicates the presence of circulating FeLV p27 in the blood fraction (plasma, serum, whole blood) tested at the time of sampling. Most, but not all, cats positive by ELISA are actively shedding infectious FeLV. A negative ELISA test indicates that no detectable FeLV p27 is present, but, as in the IFA test, does not exclude the possibility of incubation of virus and is not an indication of immunity to FeLV.

Transiently viremic cats (cats undergoing the initial infection) characteristically test positive and then revert to negative status within about eight weeks. It is thus important that FeLV tests be repeated in six to eight weeks to determine whether the viremia is transient or persistent. Virtually all cats positive by IFA are persistently viremic. Both transiently and persistently viremic cats can shed infectious FeLV for the duration of the viremia.

Recently, comparison studies of FeLV test methods have identified some cats that remain positive by ELISA but negative by IFA or by virus isolation for many months, or even for years. As many as 30 percent of cats positive by ELISA may be negative by one or both of the other methods. Retests performed one to three months after initial testing show that the FeLV status of most of these cats remains unchanged. The cellular source of the FeLV antigen causing the persistent positive ELISA results has not yet been identified; it may be bone marrow and/or lymph nodes. The most recent studies published indicate that persistently ELISA-positive IFA-negative cats, unlike their persistently IFA-positive counterparts, do not give birth to infected kittens, and that in general, these animals do not appear to be shedding infectious FeLV into their environment. The following cautious recommendation may therefore be made: Until further research should prove otherwise, cats remaining ELISA positive and IFA negative for a period of at least three months may be considered free of infectious FeLV. However, studies have not progressed for a long enough period to determine whether these cats are still at risk to develop one or more of the FeLV-associated diseases.

Detection of virus-neutralizing antibody. Cats with protective levels of virus-neutralizing antibody (VNA) IFA (≥ 1:10 by focus inhibition assay) have resisted widespread FeLV infection and in most cases are protected against subsequent development of per-
sistent viremia. Thus most cats with protective levels of VNA will not develop any of the FeLV-induced diseases. However, because a DNA copy of the genetic information of FeLV stably integrates into the host cell chromosomal DNA during viral infection and replication, latent FeLV proviral infection resulting in malignant transformation at some time in the future cannot always be excluded in cats with VNA. Previously exposed FeLV-negative cats treated with corticosteroids can experience a reactivation of their infection, presumably due to the continued presence of integrated FeLV proviral DNA (See "The Problem of Latency").

Detection of FOCMA antibody. A certain percentage of cats exposed to FeLV develop antibody against FOCMA, a tumor-specific antigen complex found on the surface of FeLV-infected cells that have undergone malignant transformation. Antibody titers to FOCMA tend to be higher in cats that have resisted widespread FeLV infection and in persistently viremic but healthy cats, and lower in cats with FeLV-induced malignancies. In general, the higher the FOCMA antibody titer, the greater is the probability that a cat is protected against the oncogenic effects of FeLV. Titers of ≥1:32 are probably protective for most cats in the general population. However, recent research has suggested that a more complex situation may exist, involving a constellation of specific antibodies constituting the anti-FOCMA immune response.

Control of FeLV Infections

Elimination of FeLV from an infected household can be achieved by implementation of an FeLV test-and-removal program using the IFA test. This program has been highly effective in removing FeLV from infected multiple-cat households. In a survey of forty-five households from which 159 FeLV-positive cats were removed, 561 of 564 (99.5 percent) FeLV-negative cats remained negative on retesting. Multiple-cat households in which FeLV test and removal has not been implemented may experience infection rates over forty times greater than those experienced by households in which the program has been successfully introduced.

FeLV-positive households. All cats in the household should be tested by IFA, regardless of age or condition. All cats found positive should be removed, and the household premises cleaned with a commercial detergent or disinfectant. All litter boxes and food and water bowls should be replaced. Cats that initially tested negative should be retested several times over a period of eight to twelve months, in case they were infected just before the first test, before the onset of detectable viremia, or are cycling in their level of detectable viremia. The time period between exposure and viremia is extremely variable, and an infected cat that tested negative initially may be positive when tested again later. During the testing period new cats should be allowed to enter the household. If any FeLV-positive cats are found on subsequent testing, they should be removed and another period of quarantine and testing imposed. All cats in the household should test negative for FeLV in two tests at least three months apart for the household to be considered "free" of infectious FeLV.

FeLV-negative households. All new cats entering an FeLV-negative household should be tested before entry. Any positive cats should be excluded from entering the household. Cats that test negative should be quarantined in separate quarters for three to five months and retested negative one or two times before being allowed to intermix with the established FeLV-negative household population. Ideally new cats should be obtained only from other households or catteries practicing FeLV test and removal.

Routine yearly or twice-yearly testing for FeLV is suggested for cats in catteries, because of the variable incubation period of infection. Persistently viremic cats should never be used for breeding purposes, in part because infected queens will transmit the virus to their viable offspring.

If an FeLV-positive cat is removed from a single-cat household, a waiting period (up to ninety days if possible) should be observed before repopulating with one or more FeLV-negative cats. The litter box and feeding dishes should be replaced, and the premises thoroughly cleansed.

Certain modifications of the test-and-removal program may be made for households in which both FeLV-negative and FeLV-positive cats are kept. The positive cats in these households should be isolated from contact with all other cats. This will not only prevent the spread of infectious FeLV to susceptible cats but will also decrease exposure of immunosuppressed viremic cats to other infectious agents to which they may have a heightened susceptibility. No new cats should be introduced at any time, and the FeLV-positive cats should not be allowed to breed. Separate litter boxes and feeding dishes should be maintained for positive and negative cats. Cleanliness and proper hygiene should be observed at all times, and it has been suggested that separate clothing be kept for contact with FeLV-positive cats, to minimize mechanical transmission of the virus. However, FeLV is relatively labile in the environment, and the degree of virus transmission possible under these circumstances is uncertain but probably minimal.

The Problem of Latency

The persistence of the integrated provirus in infected cells and in their offspring is an important aspect of the replication cycle of retroviruses. Cells so infected frequently persist in the face of an active immunologic response against the infecting retrovirus, a phenomenon well recognized in progressive pneumonia of sheep, infectious anemia of horses, and bovine leukosis virus infection. Only recently, however, has the full significance of latent proviral infection of cats with FeLV begun to be appreciated.

It has been known for some time that the majority of cats exposed to FeLV in nature develop an initial transient viremia lasting for a short but variable period of time, during which an active immunologic response to the virus develops that results in the disappearance of virus from the bloodstream and in apparent recovery from infection. Thus apparently only a minority of cats (estimated to be about 30 percent) fail to overcome the infection and proceed to develop persistent viremia. However, important studies have now shown that most (if not all) cats exposed to an infective dose of FeLV develop a persistent infection of certain white blood cell precursors in the bone marrow and of a smaller number of white blood cells in lymph nodes. The implication of this finding is that many cats that have "recovered" from FeLV infection by developing an immunologic response and eliminating the virus from the bloodstream are nevertheless persistently infected, harboring integrated FeLV proviral DNA in bone marrow and lymph node cells. Thus, "FeLV-negative" cats are not necessarily free of FeLV. Healthy FeLV-negative cats that lack both VNA and FOCMA antibody have probably never been exposed to an infective dose of FeLV and are thus truly free of the virus. VNA and/or FOCMA antibody in healthy FeLV-negative cats, however, are telltale "footprints" of previous FeLV exposure and, in the light of the new data, probably indicate in most cases the existence of latent FeLV proviral infection. In addition, some cats with FeLV-negative tumors have been shown to harbor latent FeLV infections in the bone marrow.

Cats latently infected with FeLV are not viremic and thus do not shed infectious FeLV into their environment. However, administration of corticosteroids (such as prednisolone) can reactivate latent infections, resulting in reemergence of FeLV into the bloodstream (i.e., reversion to FeLV-positive status). Blood smears from such cats usually contain FeLV-infected cells as determined by IFA, but plasma samples may be negative by ELISA, suggesting that most extracellular virus in the bloodstream is complexed with VNA and not available for ELISA detection. Because corticosteroid release from the adrenal glands is a natural physiological response of animals to stress, it seems reasonable to suspect that certain stressful life situations in the day-to-day existence of cats, such as overcrowding, movement to new quarters, territorial conflicts, pregnancy and lactation, improper nutrition, and
perhaps intercurrent disease, may serve to reactivate latent FeLV infections in nature. This could explain the occasionally observed instances in which a cat with a long history of negative FeLV tests in a closed cattery apparently free of FeLV suddenly becomes FeLV positive. The virus may not have penetrated from the outside; it may have been present in the cat all along.

Further research is currently in progress on this important problem. In the next several years it is hoped that a deeper understanding of latent FeLV proviral infections will develop, especially regarding the potential of cats with reactivated infections to shed FeLV to other cats, and the long-term risks (if any) of latently infected cats to develop one or more of the FeLV-associated diseases.

Immunization against FeLV
Research into development of an effective vaccine for the prevention of FeLV infection has progressed slowly over the past several years. Despite an increase in knowledge of the biological behavior of FeLV and pathogenesis of infection, development of a safe protective vaccine has been elusive. Several strategies for FeLV immunization have been investigated.

Inactivated ("killed") virus vaccines. Because of the immunosuppressive properties of the pl15(E) protein of FeLV and/or because of the recognized age-related susceptibility of cats to widespread FeLV infection, inactivated virus vaccines have been unsuccessful in producing effective immunity in young kittens. In fact, vaccinated kittens are more susceptible to infection than are unvaccinated kittens.

Envelope protein (gp70) vaccine. A subunit vaccine composed of the gp70 envelope protein, although theoretically appealing, has been demonstrated to be only poorly immunogenic in vaccinated cats.

Killed tumor cell vaccines. Inoculation with killed tumor cells carrying FOCMA usually induces protective levels of FOCMA antibody in both kittens and adult cats, thus protecting them against the development of most lymphoid malignancies. Many of these vaccines do not produce protective levels of VNA, so that vaccinated animals are still susceptible to FeLV infection, persistent viremia and shedding of infectious virus, and noncancerous FeLV-associated diseases. However, recent studies indicate that certain killed tumor cell vaccines may additionally protect against viremia, depending on the method by which the cells are treated.

Dual killed vaccine. A vaccine containing both inactivated FeLV and killed tumor cells carrying FOCMA has been shown to produce no VNA and to induce FOCMA antibody levels strikingly lower than those produced by killed tumor cell vaccines without inactivated FeLV. Vaccinated cats are correspondingly more susceptible to tumor induction than nonvaccinated cats. Once again, immunosuppressive pl15(E) present in the inactivated FeLV fraction is the most likely explanation for these results.

Live virus vaccines. Studies of live virus vaccines have shown that immunization is most effective when performed at twelve to sixteen weeks of age or later. Vaccination of younger kittens is ineffective, resulting instead in viremia and malignancy. Advantages of such vaccines include greater effectiveness in producing high titers of VNA than inactivated virus vaccines, and a lower frequency of revaccination. The major disadvantages, of course, are the potential for latent proviral infection and the possible reversion to virulence of vaccine strain-FeLV once it is "released" into the vaccinated population.

Live tumor cell vaccines. Inoculation with live tumor cells carrying FOCMA and actively producing FeLV induces protective levels of VNA and FOCMA antibody. These vaccines, however, have the same major disadvantages as do live virus vaccines.

Soluble tumor cell antigen vaccine. Only recently developed, the soluble tumor cell antigen vaccine (STAV) is a unique subunit vaccine that contains neither tumor cells nor FeLV but is composed only of immunogenically important antigens naturally released from lymphoid tumor cells grown in the laboratory. Studies have shown that adult cats as well as kittens vaccinated with STAV produce protective VNA and FOCMA antibody, and that most are protected against the development of lymphoid malignancies. In addition, although STAV contains the pl15(E) protein, its immunosuppressive action is apparently not exerted in vaccinated animals. Work is currently in progress to further evaluate this unusual and highly promising immunization approach.

Public Health Aspects of FeLV
The public health significance of FeLV, most importantly the question of oncogenic potential for human beings, is still largely unknown. Surveys designed to determine the prevalence of circulating FeLV and/or antibody to FeLV in human serum have produced conflicting results over the years. However, most surveys have failed to find evidence of FeLV infection of human beings, including many with lymphoid (and other) malignancies. However, most cats with FeLV-induced malignancies have little or no circulating VNA, and a variable number of such cases (10 to 50 percent) are FeLV-negative. Until a more complete understanding of the public health implications of FeLV can be obtained, the author considers it prudent to restrict as much as possible human exposure to persistently viremic cats. It must be emphasized, however, that as of this writing there is no conclusive evidence that any human illness (including cancer) has ever been caused by a feline retrovirus.

Summary
1. There are three currently recognized feline retroviruses: FeLV, FeSV, and RD-114. Of these only FeLV appears to be of great clinical significance.
2. In cats FeLV is the direct cause of lymphoid malignancies, a number of myeloproliferative disorders, several types of anemia, the panleukopenialike and thymic atrophy syndromes, at least one type of kidney disease, certain reproductive disorders, and several miscellaneous conditions. Diseases indirectly caused by FeLV include a myriad of conditions that develop secondary to FeLV-induced immunosuppression.
3. The majority of cats exposed to FeLV in nature develop an initial transient viremia, during which an active immunologic response to the virus develops that results in disappearance of virus from the bloodstream and apparent recovery from infection. Thus only a minority (about 30 percent) of cats fail to overcome widespread infection and proceed to develop persistent viremia. However, recent studies have shown that most (if not all) cats exposed to an infective dose of FeLV develop a persistent infection of certain white blood cell precursors in the bone marrow and of a smaller number of white blood cells in lymph nodes.
4. Excretion of FeLV occurs primarily in salivary secretions, although virus may also be present in respiratory secretions, feces, and urine. In addition, in utero transfer of virus across the placenta and excretion in colostrum are also known to occur. Virus can also be spread by blood transfusions from viremic cats and possibly also by the bites of bloodsucking insects, such as fleas. FeLV is extremely liable outside the cat and is rapidly inactivated by alcohol and most common household detergents and disinfectants.
5. Elimination of FeLV from an infected household can be achieved by implementation of an FeLV test-and-removal program using the IFA test. Multiple-cat households in which this program has not been implemented have experienced infection rates forty times greater than those experienced by households in which the program has been successfully introduced. When available, however, vaccination will probably be the method of choice for the control of FeLV infection.
6. In nature, FeLV/FeSV infections appear to be restricted to members of the cat family. As of this writing there is no conclusive evidence that any human illness (including cancer) has ever been caused by a feline retrovirus.
Selected General References


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About the Cornell Feline Health Center

The ultimate purpose of the Cornell Feline Health Center is to improve the health of cats by developing methods to prevent or cure feline diseases and by providing continuing education to veterinarians and cat owners. The Cornell Feline Health Center is a nonprofit organization supported largely by private tax-deductible contributions. Correspondence may be directed to:

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