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Feline Leukemia/Sarcoma Viruses and Immunodeficiency

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I. Diseases Caused by Feline Retroviruses

Feline leukemia virus (FeLV) was isolated from a lymphoma-cluster household in Glasgow, Scotland in 1964 (Jarrett *et al.*, 1964). In the ensuing 23 years, FeLV has become recognized as the principal scourge of cats in nature. It causes not only lymphomas, leukemias, and myeloproliferative diseases but also the more frequent and fatally acquired immunodeficiency syndromes, aplastic anemias, and other cytopathic diseases.

Lymphomas and leukemias, although relatively infrequent manifestations of FeLV infection, are the most frequent malignant neoplasms in the cat in nature (reviewed in Rojko and Olsen, 1984). Seventy percent of cat lymphomas have been causally associated with persistent, productive FeLV infection (Hardy *et al.*, 1976). In these cases, bone marrow origin viremia is a prelude to the emergence of virus-positive lymphoma cells (Rojko *et al.*, 1979). FeLV-positive lymphoma cells have 10–20 integrated exogenous FeLV proviruses, cytoplasmic and membrane FeLV antigens, and replicate FeLV to high titer (Casey *et al.*, 1981). More than half are mono- or oligoclonal with respect to

host/proviral junction fragments (Casey *et al.*, 1981; Neil *et al.*, 1984; Fulton *et al.*, 1987). Most FeLV-negative lymphomas have neither exogenous integrated FeLV proviruses nor viral structure antigens (Casey *et al.*, 1981). Nevertheless, FeLV may contribute to the genesis of some FeLV-negative lymphomas. Nonproducer lymphomas are more common in cats from FeLV-positive households (Hardy *et al.*, 1980a,b), and lymphomatous cats have a higher incidence of latent, reactivatable, bone marrow FeLV infection than do cats with extinguished FeLV infections (see p. 69). Furthermore, lymphomas from some virus-negative cats contain truncated FeLV sequences or viral structural proteins (Fulton *et al.*, 1987; Rezanka *et al.*, 1987).

Feline lymphomas have been classified principally by anatomic distribution, micropathology, and FeLV status (FeLV-positive versus FeLV-negative) and age of the cat (Table I; see Hardy *et al.*, 1980b). Virus-positive tumors occur in younger cats, principally in thymic, multicentric, and mesenteric lymph nodal locations. Both sexes and most breeds are equally affected. Virus-negative tumors occur in older cats and principally stem from cells in the gastric and intestinal submucosa. Again, no sex or breed is affected uniquely. Although histologically variable, most lymphoid tumors are of diffuse, small cell, noncleaved type (Valli *et al.*, 1981). Less frequent are cleaved varieties. Nodular varietal tumors and tumors comprised of large granular lymphocytes or macrophages are rare.

Classification of feline lymphomas according to immunophenotype

TABLE I

CLASSIFICATION OF FELINE LYMPHOMAS[a]

Anatomic site	Micropathology[b]	Percentage of total	FeLV status (% positive)	Age of cat[c]	
				FeLV+	FeLV−
Thymic	D, S–M, NC, TB MØ	20–38	77	2.1	3.8
Multicentric variable, usually	D, S–M, NC	43	80	3.3	5.8
Alimentary	D, S–M, NC, TB MØ	15	23	5.0	8.9
Unclassified variable		3	38	5.2	10.8

[a] Modified from Hardy *et al.* (1980b) and Valli *et al.* (1981).

[b] Classification according to Valli *et al.* (1981). D, diffuse; S, small; M, medium; NC, noncleaved; TB MØ, tingible body macrophage.

[c] In years.

and T cell receptor and immunoglobulin gene rearrangements is more recent and less complete. Thymic lymphomas induced by the Rickard strain of FeLV (FeLV-R) express terminal deoxynucleotidyl transferase (Tdt), the guinea pig erythrocyte (GPE) rosette receptor, Ia antigens, partial cortisone sensitivity, and nonspecific esterase. FeLV–R-induced tumors also contain productively rearranged TCR_{α} and TCR_{β} genes. Hence, these are clearly T cells of intermediate maturity. The majority of spontaneous FeLV-positive tumors rosette GPE and probably originate from helper T cells or null cells. They are uniformly negative for feline cytotoxic T cell antigen. Some contain rearranged TCR_{β} genes. Rarely, more mature FeLV-positive tumors produce interleukin 2-like substances or humoral moieties which induce hypercalcemia (Engelman *et al.,* 1985a). The considerable heterogeneity in their surface and cytochemical phenotype probably reflects transformation of multipotent lymphoid cells in the bone marrow and elsewhere (Engelman *et al.,* 1986; Fulton *et al.,* 1987; Rojko *et al.,* 1987).

Myeloproliferative diseases attributed to the FeLV involve cells of all hematopoietic lineages except the mast cell lineage. The particular lineage affected may be influenced by virus strain or subgroup specificity (see p. 69). Diseases reported following natural or experimental exposure include erythremic myelosis, erythroleukemia, myelogenous leukemia, eosinophilic leukemia, megakaryocytic leukemia, myelofibrosis, osteosclerosis, osteochondromatosis, and reticuloendotheliosis (Hardy, 1982).

Immunosuppression is the most frequent and the most devastating manifestation of FeLV viremia in clinical and experimental studies (Hardy, 1982; Rojko and Olsen, 1984). The hallmark of *Immunosuppression and feline acquired immunodeficiency syndrome* (FAIDS) in pet cats is an increased susceptibility to opportunistic pathogens. Viremic cats display unusual sensitivity to viral pathogens and succumb to infectious peritonitis (corona) virus (FIPV) and have chronic or recurrent herpetic rhinitis and sinusitis. Viremic cats also are uniquely susceptible to nonviral opportunists and may die of antibiotic-unresponsive enteritis, gingivitis, stomatitis (noma), dermatitis, pneumonia, or septicemia of bacterial origin. FeLV-positive cats also may develop fatal hemotropic hemobartonellosis or systemic toxoplasmosis (Cotter *et al.,* 1975). Even ostensibly healthy viremic pet cats demonstrate lymphopenia, persistent or cyclic neutropenia, and fluctuating levels of serum complement and immune complexes (Essex *et al.,* 1975; Kobilinsky *et al.,* 1979; Swenson *et al.,* 1987). Neutrophils from viremic cats fail to undergo chemiluminescence responses when

challenged with phagocytosis stimuli; this neutrophil defect may contribute to effete resistance to bacteria *in vivo* (Lewis *et al.*, 1986). Similar neutrophil defects have been described in human AIDS patients (Ras *et al.*, 1984).

In very young animals, immunosuppression may be so severe that the kitten dies of "fading kitten disease," manifest by profound cachexia, thymic atrophy, and lymphoid depletion at postmortem (Hardy, 1982). In contrast, in some young adults, marked peripheral or mandibular lymphadenophathy may be a preneoplastic phase of persistent FeLV infection. This syndrome has been given the name "distinctive peripheral lymph node hyperplasia of young cats" and most resembles angioimmunoblastic lymphadenopathy of humans and the persistent lymphadenopathy that precedes AIDS in humans (Moore *et al.*, 1986). It has been suggested that the lymphadenopathy represents excessive but ineffective stimulation of the lymphoid system in an attempt to escape persistent retrovirus infection.

The primary problem is that the T cell, particularly the helper T cell, fails to function in viremic cats. The mechanisms and consequences of FeLV-associated immunodeficiency are detailed herein. Observations also implicate another feline retrovirus, the lentivirus designated feline T lymphotropic virus, in the genesis of some feline immunodeficiency syndromes (see p. 63).

Preleukemic *anemias* are second only to immunosuppression as a frequent manifestation of FeLV viremia in naturally infected pet cats. FeLV-associated anemias are classified according to the dominant pathogenetic mechanism as hemolytic, myelodysplastic or aplastic (Mackey, 1975). Hemolytic anemias may be regenerative and transient or precede lymphoma or aplastic anemia development. Myelodysplastic and aplastic anemias are nonregenerative, progressive, and fatal. About 70% of all nonregenerative anemias in cats are attributed to productive FeLV infection (Hardy, 1982). Experimental challenge of neonatal or immunosuppressed adult cats with certain FeLV strains or subgroups (discussed herein) induces progressive erythroid aplasia and death within 10 to 30 weeks (Hoover *et al.*, 1974; Onions *et al.*, 1982). Packed cell volumes plummet to 5–10% from a normal of 25–35% as a result of ablation of infected erythroid precursors in the bone marrow (see p. 75). The mean myeloid:erythroid ratio shifts from 1.6 in normal cats to 10.4 in anemic cats (Boyce *et al.*, 1981). Serum erythropoietin levels rise markedly and transient or persistent macrocytosis may occur (Kociba *et al.*, 1983; Weiser *et al.*, 1983). Erythrocyte survival time is unchanged but plasma radioiron disappearance halftime and erythrocyte use of radioiron are markedly diminished

(Madewell and Jarrett, 1983). Erythroid aplasia usually is associated with severe paracortical lymphoid depletion, peripheral lymphopenia, thymic atrophy, and cachexia. Some anemic cats also experience pancytopenia, medullary fibrosis or osteosclerosis and hemorrhagic enteritis (Hoover *et al.,* 1974; Hardy, 1982).

An early and frequent target for FeLV replication is the myelomonocytic precursor cell (Rojko *et al.,* 1979a). The pathologic consequences are profound as FeLV replication leads to functional and numerical attrition of neutrophils. Neutrophils from cats with either productive or latent FeLV infection have depressed chemiluminescence responses to phagocytosis stimuli (Lewis *et al.,* 1986; Lafrado and Olsen, 1986). This presumably undermines the cat's resistance to opportunistic bacteria.

Transient, persistent or fluctuating neutropenias occur in both natural and laboratory FeLV infections. In one study, 15 of 19 (79%) pet cats with profound neutropenia were viremic, and the onset of neutropenic crisis often followed a stressful episode (Hardy, 1982). In another study, cats with cyclic neutropenia were found to have viremic or latent FeLV infections (Swenson *et al.,* 1987). Experimentally, the induction of persistent viremia is invariably accompanied by a 3- to 5-week period of protracted neutropenia (Rojko *et al.,* 1979a).

The anemia-causing Kawakami–Theilen strain of FeLV (FeLV-KT) induces significant macrothrombocytosis and thrombocytopenia during acute FeLV infection. Geometric mean platelet volume and plasma membrane are increased but isovolumetric shape and surface canalicular system area are unchanged. These defects may contribute to the severe decreases in platelet survival time described for FeLV-infected cats (Boyce *et al.,* 1986; Jacobs *et al.,* 1986).

A specific syndrome of FeLV-associated *enteritis* is identifiable based on histopathologic and virologic criteria (Reinacher, 1987). In a study of 218 cats, 157 (72%) were infected with FeLV. The enteric lesion of epithelial crypt degeneration, necrosis, and dilatation is similar to that described for feline parvovirus infection. However cats with FeLV-associated enteritis are negative for feline parvovirus by electron microscopic criteria and are negative for other parvovirus lesions such as panmyelophthisis, myeloid sinus hyperemia, and systemic lymphoid necrosis. In FeLV-associated enteritis, the residual viable crypt epithelium is heavily infected by FeLV and focal crypt necrosis is accompanied by ablation of infection. The pathogenesis of the enteric cytopathicity is not known.

Abortion and infertility might also occur. As observed by Cotter and her co-workers, 8 of 11 queens presented for infertility have viremic

FeLV infections (Cotter *et al.,* 1975). Experimentally, fetal death, resorption, and placental involution occur by 40 days of gestation in most viremic queens (Hoover *et al.,* 1984). Rarely, viremic queens carry litters of healthy but congenitally viremic kittens to term (63 days). The mechanism of feline embryonic death has not been elucidated. In the mouse, congenital insertional mutagenesis by Moloney MuLV fatally disrupts the α_1-collagen gene in early mouse embryos (Jähner and Jaenisch, 1985). Interestingly, cats with covert FeLV infections also sometimes transmit latent, reactivatable, FeLV infections (discussed herein) to their progeny via transplacental or transcolostral transmission.

Membranous glomerulonephritis is associated with chronic viremia in pet cats and may result in the nephrotic syndrome with progressive hypoalbuminemia, edema, and uremia (Cotter *et al.,* 1975). Also, pet cats with FeLV-related neoplasms have accompanying glomerulonephritis (Anderson and Jarrett, 1971; Glick *et al.,* 1978). Glomerulonephritis has not resulted from experimental inoculation (Glick *et al.,* 1978; Jakowski *et al.,* 1980). In some cats, diffuse, argyrophilic and glycoprotein-containing linear deposits seen histologically correspond to electron-dense subepithelial, subendothelial, and mesangial deposits seen ultrastructurally (Glick *et al.,* 1978). In other cats, thickening of Bowman's capsule is the principal lesion (Jakowski *et al.,* 1980). Immunologically, the deposits contain FeLV antigens, IgG and complement (Hardy, 1982).

Circulating immune complexes and complement consumption probably contribute to the immunosuppression seen in other viremic cats. Two further observations are cogent. Viremic cats are hypocomplementemic and have circulating immune complexes containing gp70, p15E, and anti-FeLV antibody (Kobilinsky *et al.,* 1979; Day *et al.,* 1980; Snyder *et al.,* 1982; Tuomari *et al.,* 1984).

Feline sarcoma virus (FeSV)-associated fibrosarcomas occur as multiple subcutaneous tumors on the extremities or thorax of young adult cats (usually less than 2 years old) and account for 5–12% of all cat neoplasms. Often a history of a local bite wound precedes fibrosarcoma development. At least 10 separate isolates of FeSV exist (Besmer, 1983; Besmer *et al.,* 1986). All are replication-defective and require helper FeLV for replication. Seven separate oncogenes have been transduced by helper FeLV (discussed herein) and the resultant (FeSV)FeLV pseudotypes transform fibroblasts of various species *in vitro*. Experimentally, FeSVs induce rapidly progressing mesenchymal neoplasms and death in susceptible kittens. FeSVs also are associated with intraocular lesions. Both malignant melanomas and fulminant iridocyclitis with ciliary

dysplasia have been described following intraocular or systemic FeSV inoculation (Lubin *et al.*, 1983; Niederkorn *et al.*, 1983).

A *feline lentivirus* has been isolated from a FeLV-negative, multiple cat household with a history of fatal immunodeficiency and has been designated feline T lymphotropic retrovirus (FTLV) (Pedersen *et al.*, 1987). None of the normal cats in the cattery showed serologic evidence of infection in contrast to 10 of 25 diseased cats. Experimental inoculation of specific-pathogen-free (SPF) kittens with blood or plasma from spontaneous cases caused transient leukopenia and fever and subsequent persistent, generalized lymphadenopathy. Western blot analysis demonstrated no cross-reactivity between FTLV and other lentiviruses. No evidence of cat to man transmission could be discerned.

Multiple *endogenous proviruses related to FeLV* are normal constituents of the cat genome (Soe *et al.*, 1985). Endogenous FeLVs are noninfectious, close to full-length in size, and arranged in a nontandem fashion. Subgenomic expression occurs in feline placental trophoblasts but expression has not been correlated with any disease process.

Endogenous feline retroviral sequences (*RD-114*) unrelated to FeLV but related to baboon endogenous virus are rarely expressed in adult cat tissues but are frequently expressed as subgenomic mRNA in cat placentas and tumors (Niman *et al.*, 1977). An etiologic role for RD-114 in cat disease has not been demonstrated.

Feline syncytial forming virus (FeSFV) is a cell-associated spumavirus that is present in 10–70% of pet cats (Flower *et al.*, 1985). It evokes a strong serologic response which presumably prevents its *in vivo* expression. It is readily isolated by co-culture of infected peripheral blood leukocytes with indicator fibroblasts. Its hallmark is intense cytopathicity and ballooning degeneration and syncytia formation are prominent. FeSFV has been isolated from cats with FeLV-producer and nonproducer lymphomas (Casey *et al.*, 1981) and from mononuclear cells in the joints of cats with progressive polyarthritis. Whether or not FeSFV is disease-causing remains to be established.

II. Structure of Virus

FeLV is quite similar to the better-studied murine leukemia viruses in structure and genetic map. The virus particles bud from cytoplasmic membranes into either extracellular spaces or into vacuoles. While budding, the particles appear as an inverted "c", which, when origi-

nally observed with other retroviruses, gave rise to the term c-type particles. After emergence of the budding virus and release into extracellular spaces, the electron dense cores condense but remain central within the outer shell. The particles are about 100nm in diameter and 1.15–1.16 gm/cm^3 in density.

The viral genome is present as 2 copies of single-stranded RNA about 9 kilobases in length. The virion RNA, which has the same valence as mRNA, has long terminal repeat sequences at either end as well as a cap at the 5′ end and a polyadenylated tail at the 3′ end. Three major genes are present, designated *gag, pol,* and *env.* The *gag,* which stood for "group reactive antigen" when first suggested, encodes a polyprotein that gives rise to peptides found in the cores of virus particles. The *pol* encodes those enzymes involved in virus replication, and the *env* encodes the polyprotein that gives rise to the external envelope glycoprotein and the transmembrane component.

For replication, viral reverse transcriptase transcribes the virion RNA to a linear strand of DNA. This then becomes double stranded and circularized with cellular enzymes and integrated into cellular chromosomal DNA using the *pol* gene-encoded endonuclease/integrase enzyme. In some instances the viral DNA remains in unintegrated episomal form, perhaps most often with strains of virus involved in immunosuppression (Mullins *et al.,* 1986). Ordinarily, RNAs are transcribed from the integrated proviral DNA to function as either progeny genomes or message for translation.

The viral proteins are all made from the cleavage of larger polyproteins. In the case of the *gag* gene products, this is mediated by a viral protease that is presumably encoded from sequences at the 5′ end of the *pol* gene. In the case of the *env* polyprotein, this is presumably accomplished by a host cell protease that cleaves in a lysine/arginine-rich region present at the junction of the external glycoprotein and the transmembrane protein. The *gag* gene precursor, which is about 60,000–70,000 Da, is glycosylated and myristylated at its amino terminus. Because of these posttranslational features it is also expressed at the cytoplasmic membrane of infected cells, even apart from virus budding sites. Some *gag* gene proteins are also made as part of a larger *gag–pol* "read through" precursor of approximately 180,000 Da.

At the amino terminus of the *gag* gene polyprotein is p15C, the myristylated component. Its exact location in the virus is unknown, but it is suspected to be loosely attached to the outer shell of the particle. After the p15C, going from the amino terminus is the p12 phosphoprotein, the p27 major core component, and the p10 nuclear binding protein. The p27, often called p30 in a generic sense, makes up a significant portion of the

protein mass of the virus. It is moderately immunogenic, inducing antibodies that are both group and "interspecies" specific, cross-reacting with the comparable core proteins of murine leukemia viruses and the gibbon ape leukemia virus.

Apart from the ill-defined protease, the *pol* gene encodes the reverse transcriptase of approximately 80,000 Da and the endonuclease/integrase, which is about 35,000–40,000 Da. These proteins are also occasionally immunogenic in natural infections, generating antibodies that will neutralize the functional activity of reverse transcriptase (Jacquemin *et al.*, 1978).

The primary *env* gene product is a glycosylated precursor of 85,000–90,000 Da. It has a location anchored in the cytoplasmic membrane with a cytoplasmic tail of 20–30 amino acids. This sequence is thought to function for recognition during virus assembly, to provide recognition for the virus core proteins. Two cleavage steps occur with this molecule, one to separate the external glycoprotein from the transmembrane component and one to remove the 5′ leader sequence of 15–20 residues. The envelope glycoproteins are highly immunogenic; especially the amino terminus gp70 component. Antibodies may be induced that are directed to both subgroup-specific components as well as to all FeLVs. Such antibodies may be both neutralizing for extracellular virus and cytotoxic for infected cells that express the glycoproteins at the cell surface. The transmembrane protein, p15E, is also immunogenic, but less so than gp70. It is believed to be more important for its role in immunosuppression.

New FeSVs are generated relatively frequently in cats, associated with multicentric fibrosarcomas. Presumably this occurs because cats are one of the only outbred mammalian species where a retrovirus is replicating at high levels, with provirus moving in and out of the cell chromosome. Such high level replication results in more frequent transduction of cellular DNA sequences. When such cell sequences represent a c-*onc* gene with potential for cell transformation, solid tumors occur. The newly created viral genomes are replication defective because the acquired cell genes must displace some required virus replication genes to fit in the viral genome. However because such viruses are generated in a milieu of FeLV helper virus-infected tissues, transmission of FeSV from one cell to another occurs. The result is the development of multicentric polyclonal tumors that develop and progress very rapidly. Oncogenes that have been recognized in naturally occuring feline fibrosarcomas include *fes* (Snyder and Theilen, 1969), *fms* (McDonough *et al.*, 1971), *sis* (Parodi *et al.*, 1973), *fgr* (Hardy *et al.*, 1983), *ras* (Youngren *et al.*, 1984), *kit* (Besmer *et al.*, 1986), and *abl*

(Besmer *et al.*, 1983). The *myc* oncogene has also been detected in a significant number of recombinant FeLVs that were isolated from cats with naturally occurring leukemia or lymphoma (Levy *et al.*, 1984; Mullins *et al.*, 1984; Neil *et al.*, 1984), suggesting that transduction of c-*myc* could be an important mechanism by which retroviruses induce leukemia under natural conditions.

The feline oncornavirus-associated cell membrane antigen complex (FOCMA) was initially described as an FeLV and FeSV-specific antigen complex to which effective tumor immunity was directed by immunosurveillance (Essex *et al.*, 1971a,b, 1975b). Subsequently it was recognized that a FOCMA-type antigen was expressed on both FeSV-transformed nonproducer fibroblasts, where it was designated FOCMA-S (Sliski *et al.*, 1977; Stephenson *et al.*, 1977; Sherr *et al.*, 1978a,b). FOCMA-S was recognized as the first mammalian *gag–onc* gene protein, later labelled *gag–fes* (Coffin *et al.*, 1981). The FOCMA antigen found on FeLV-immortalized lymphoblastoid cells or lymphoma cells was later designated FOCMA-L (Essex *et al.*, 1978). It was found to consist partly of FeLV-encoded information specifically from subgroup C envelope sequences (Snyder *et al.*, 1978, 1983). Since subgroup C FeLV is not normally expressed as an exogenous virus, except when rescued by FeLV-A or -B, it is logical to hypothesize that this event could also be related to the malignant alteration of lymphoid cells.

III. Replication at Cell and Host Level

FeLV has long been considered a typical noncytopathogenic, long-latency leukemia virus based on its behavior in fibroblasts *in vitro*. Recent evidence suggests that its *in vivo* behavior in critical target hemolymphatic tissues is as likely to be cytopathic as transforming. Replication in both fibroblasts and lymphocytes is initiated by the binding of FeLV gp70 to subgroup-specific cellular receptors demonstrated by interference and direct receptor assays (Sarma and Log, 1973; Hamilton *et al.*, 1987a,b). Hence, cell-surface FeLV receptors often determine the host range of FeLV isolates. As discussed herein, hypervariability and recombination in the N-terminus of gp70 are responsible for division of FeLV isolates into three subgroups: A, B, and C. On feline fibroblasts, receptors for FeLV-A and FeLV-C are equivalent in frequency and twice as common as receptors for FeLV-B. Replication of all subtypes is usually persistent and highly productive. In contrast, heterologous cells usually bind FeLV-B. Replication of

FeLV-B is persistent and productive in mink MvlLu cells and hamster E36 cells but abortive in African green monkey vero cells. Surprisingly, guinea pig cells preferentially bind FeLV-C, and replication is persistent and productive. Mouse cells neither bind nor replicate FeLV.

Of more importance is the receptor-mediated initiation of FeLV replication in cat hemolymphatic target cells. The majority of FeLV receptors on lymphocytes and bone marrow cells are for the ecotropic FeLV-A. Hence, it is not surprising that FeLV-A is the most frequent and most infectious subgroup and is transmitted contagiously (Jarrett *et al.*, 1973, 1978a,b; Hardy *et al.*, 1973). Receptors for FeLV-B are rare on cat lymphocytes. Lymphocyte receptors for FeLV-C are prominent in 75% of cats, designated high responders, and rare in 25% of cats designated low responders. Similarly, lymphocytes from high-responder cats replicate FeLV-A and FeLV-C to high titer after *in vitro* exposure whereas lymphocytes from low responders replicate FeLV-A moderately, FeLV-C poorly, and fail to replicate FeLV-B (Hamilton *et al.*, 1987b).

Following receptor-mediated entry, FeLV RNA is uncoated and reverse transcribed into DNA. This vDNA becomes circular and integrates into cellular DNA. Integration has several consequences. First, failure of integration of variant vDNA may lead to the accumulation of toxic unintegrated FeLV DNA in lymphocytes and bone marrow progenitor cells. This mechanism has been implicated in lymphoid ablation by the Colarado isolate of FeLV that causes FAIDS (FeLV–FAIDS) and is reminiscent of the cytopathic mechanism described for avian retroviruses (Weller and Temin, 1981; Mullins *et al.*, 1986). Second, successful integration provides not only the opportunity for replication and productive infection but also for latency and persistent nonproductive infection (discussed herein). Third, integration of provirus allows for insertional mutagenesis and activation and transduction of cellular oncogenes (discussed herein).

Replication of FeLV postintegration proceeds as for any long latency leukemia virus and culminates in budding of type C particles at the plasma membrane. Feline cells are known to restrict MuLV budding by means of the Bvr-1 gene product; this mechanism has not been shown to interfere with FeLV replication (O'Brien *et al.*, 1976). Both human and cat leukocyte interferons do inhibit FeLV replication late in the replication cycle (Jameson and Essex, 1983; Tompkins, 1984).

Both RD-114 and feline foamy virus are restricted for replication by cat cells *in vivo* but readily escape host control with *in vitro* culture of leukocytes or fibroblasts. The replication scheme of FTLV has not been

described. The usual route of exposure to FeLV is via oronasal contact with saliva from a viremic animal. Saliva from viremic cats generally contains 10^5–10^7 focus-forming-units (ffu) of infectious FeLV/ml; 10^5 ffu are sufficient to infect a susceptible cat (Hoover *et al.*, 1972, 1976; Francis *et al.*, 1977). FeLV probably enters the cat by adherence to gp70 receptors on tonsillar B lymphocytes and macrophages in the posterior oropharynx. The infected cells drain to the mandibular and pharyngeal lymph nodes and join the recirculating lymphocyte pool for hematogenous distribution. This cell-associated viremia is early and facilitates the bolus delivery of FeLV to the sensitive mitotic (germinal) cells of the bone marrow, systemic lymphoid tissue, and intestine within 2–14 days after experimental exposure (Rojko *et al.*, 1979a).

The first critical phase of FeLV infection ensues (Hoover *et al.*, 1976, 1977; Rojko *et al.*, 1979a). The total virus burden is dependent upon the rapidity and quantity of FeLV replication in myelomonocytic progenitors, B cells, macrophages, and intestinal crypt epithelium. Infected bone marrow precursors mature and are released into the blood as FeLV-positive neutrophils and platelets. Infectious virus is released into the plasma. This state of marrow origin, persistent viremia, in which FeLV-positive neutrophils and platelets coexist with infectious virus in the plasma, is generally regarded as the prelude to fatal FeLV-related disease. Persistent viremia is diagnosed by either fixed cell immunofluorescence for FeLV p27-positive cells or by isolation of helper FeLV in S+L− assay (Hardy *et al.*, 1976). Chronic productive infection often is accompanied by persistent antigenemia and free p27 antigen is detectable by enzyme-linked immunosorbent assay (ELISA) (Mia *et al.*, 1981).

Persistent viremia occurs in about 30% of all exposed cats. Susceptibility is generally the result of an immature or functionally compromised immune system. Virtually 100% of neonatal kittens succumb to 10^5 ffu of FeLV but only 15% of 16-week-old kittens are susceptible (Hoover *et al.*, 1976). Furthermore, age-related resistance is abrogated by immunosuppressive or cocarcinogenic treatments (see Section VIII).

Persistent viremia facilitates the distribution of marrow origin FeLV to tertiary targets in mucosal and glandular epithelia. Specifically, FeLV is replicated to high titer in the oropharynx, bladder, skin, intestine, salivary gland, and pancreas (Rojko *et al.*, 1979a). Infectious FeLV then is excreted into urine, saliva, tears, and aqueous humor to ensure contagious transmission (Francis *et al.*, 1977; Lubin *et al.*, 1983; Hawkins *et al.*, 1986).

Approximately 70% of cats are relatively resistant to FeLV. The total virus burden is overwhelmed by the rapid and effective anti-

FeLV immune response that develops (discussed herein). In the first 4–6 weeks after exposure, the cat's ability to totally eliminate FeLV depends on the number of cells with integrated FeLV proviruses that the cat acquired during primary infection. About one-half to two-thirds of all resistant cats probably eliminate all FeLV-infected cells between 16 to 18 weeks after exposure and can be said to have extinguished FeLV infections (Madewell *et al.*, 1983). The remaining cats have sustained latent infections as they retain myelomonocytic progenitors and lymphocytes with integrated FeLV proviruses (Rojko *et al.*, 1982). Even healthy cats with sustained latent infections have neutrophil dysfunction relative to unexposed cats. Severe stress can alter the host/virus relationship and allow replicating FeLV to reemerge to initiate transient or persistent viremia and disease (see p. 69).

IV. Pathogenesis of FeLV-Related Diseases

The type of FeLV-related disease that occurs and the disease-free interval probably are influenced by viral envelope proteins and glycoproteins and the consequences of proviral integration.

FeLV subgroup specificity apparently determines when and what type of disease will occur. The ecotropic FeLV-A is the most frequent subgroup found in pet cats and is transmitted contagiously (Jarrett *et al.*, 1978a,b). FeLV-A is related to endogenous rat genomic sequences and probably entered the ancestral cat population in Northern Africa one to ten million years ago. The contagious spread of FeLV-A may have been restricted by the climatic aridity of the North African desert, by the intrinsic independence of cats in nature and by the lessened viability and fertility of viremic cats. Throughout the millenia, FeLV-A and its feline host may have evolved together. FeLV-A, by itself, has minimal pathogenicity. Molecularly cloned FeLV-As induce viremia readily but rarely cause disease (Luciw *et al.*, 1986; Mullins and Hoover, 1987). As discussed previously, the majority of receptors on feline bone marrow and lymphoid cells are for FeLV-A. Thus, it is logical that FeLV-A is the most contagious subgroup in nature and the most frequent subgroup isolated from both viremic and latent infections. Cats viremic with FeLV-A alone may be healthy for years and experience transient subclinical lymphopenia only. Alternatively, these cats may by immunosuppressed due to the presence of circulating p15E and die of opportunistic infection in the preneoplastic period. Cats viremic with FeLV-A develop lymphoma or other diseases three or more years after becoming viremic. When these diseases

occur, they may be the result of second (cocarcinogenic) events like the emergence of variant or recombinant FeLVs or the activation or transduction of cat cellular genes against a background of FeLV-A-caused immunosuppression (see p. 86). Hence, FeLV-A viremia may increase the likelihood that secondary events leading to disease will occur.

A variant of FeLV-A, designated FeLV–FAIDS variant A, has been molecularly cloned from pet cats in Colarado with a spontaneous, fatal, immunodeficiency syndrome known as feline AIDS (Mullins *et al.,* 1986). Experimentally, the appearance of unintegrated FeLV–FAIDS variant A DNA in the bone marrow of affected cats is a very bad prognostic sign and may hallmark the incipient lysis of bone marrow, T cells, and B cells. The variant A viruses are defective and require exogenous helper FeLV-A for their replication. Relative to FeLV-A, variant A has an 18 bp deletion in the N-terminus of gp70 and an 18 bp addition in the C-terminus of gp70.

The emergence of FeLV-B or FeLV-C in the bone marrow, lymphoid tissues, or plasma also signals the onset of fatal hemolymphatic disease (Jarrett *et al.,* 1984; D. E. Onions, personal communication). Many divergent isolates of FeLV-B exist and have been generated by the recombination of exogenous FeLV-A with cat cellular sequences (enFeLV) highly related to endogenous MuLV *env* sequences. As a result, all FeLV-B isolates have a common Sau3a site in the N-terminus of gp70 that is related to enFeLV sequences in normal cat DNA but absent from FeLV-A, variant A, and FeLV-C isolates (Stewart *et al.,* 1986a).

Replication of monotypic FeLV-B by feline hemolymphatic cells is minimal; this likely is due to the paucity of FeLV-B receptors on these cells (Hamilton *et al.,* 1987b). FeLV-B is replicated readily when hemolymphatic cells are presented with a phenotypic mixture of FeLV-A and FeLV-B. In the presence of FeLV-A, FeLV-B increases the proportion of cats that develop persistent viremia (relative to FeLV-A alone) and may enhance immunosuppression (Jarrett *et al.,* 1987a). FeLV-B (Rickard) is implicated in accelerated thymic lymphomagenesis; this is not surprising when viewed in the light of the *env* sequence homology between FeLV-B and mouse mink cell focus-forming (MCF) viruses (Elder and Mullins, 1983). Monotypic molecularly cloned FeLV-B (Rickard) does not induce viremia *in vivo* and hence, is relatively apathogenic. FeLV-B (Glasgow GM isolate) may be important in rapid onset of myeloproliferative disease (D. E. Onions, personal communication) and molecularly cloned FeLV-B (Gardner–Arnstein isolate) induces rapid onset myelodysplastic anemia (Mullins and Hoover, 1987).

The *env* genes of FeLV-A and FeLV-C differ only slightly but probably direct marked difference in host range, cat leukocyte susceptibility to FeLV, and pathogenicity *in vivo*. FeLV-C apparently is induced *de novo in vivo* following exposure to FeLV-A; antibody to FeLV-C gp70 probably is responsible for the restriction of potential FeLV transformants *in vivo* (Essex *et al.*, 1971a,b; Vedbrat *et al.*, 1983; Jarrett *et al.*, 1978). Thus, the origin of FeLV-C (mutation? recombination?) is uncertain but important.

Sequence analysis of FeLV-C reveals marked homology with FeLV-A and marked divergence from FeLV-B (Luciw *et al.*, 1985). The principal change is a nine nucleotide deletion in FeLV-C relative to FeLV-A (bases 338–346) that is interposed between 5′ and 3′ short regions of nonhomology in the hypervariable, hydrophilic region (bases 325–355) of the N-terminus of gp70. Whether this region is responsible for receptor binding or pathogenicity has not been determined. It is, however, the same region in which the FeLV–FAIDS variant A *env* deletion has transpired (Mullins and Hoover, 1987). The observation that FeLV-A and FeLV-C have hemagglutinating activity whereas FeLV-B does not also implicates products of the *env* aminoterminus in hemagglutination (Mochizuki and Jarrett, 1985).

Replication of FeLV-C is intermediate; it is high compared to FeLV-B but low compared to FeLV-A. One third of cats have a scarcity of leukocyte receptors for FeLV-C (previously discussed); lymphocytes from these low responders replicate FeLV poorly *in vitro* (Hamilton *et al.*, 1987b). Experimentally, cats are only susceptible to the induction of persistent FeLV-C viremia if exposed immediately after birth, if simultaneously exposed to immunosuppressive corticosteroids, or if previously made viremic with FeLV-A (Onions *et al.*, 1982; Kociba *et al.*, 1982; Jarrett *et al.*, 1984; Riedel *et al.*, 1986b). In nature, FeLV-C is recovered only when FeLV-A is also present (Jarrett *et al.*, 1978). The dominant routes of FeLV-C transmission, therefore, are probably via *de novo* generation and phenotypic mixing. Importantly, once the cat begins to replicate FeLV-C in bone marrow cells, it is destined to develop fatal erythroid aplasia within 3 weeks to 3 months (Onions *et al.*, 1982; Jarrett *et al.*, 1984; Riedel *et al.*, 1986b). Recent observations indicate that the feline oncornavirus-associated cell membrane antigen (FOCMA) is not a tumor-specific antigen but rather is derived from modified FeLV-C gp70 expressed at the surface of neoplastic and nonneoplastic cells (Vedbrat *et al.*, 1983; Snyder *et al.*, 1983). In fact, FOCMA antibody lyses all cells expressing FeLV-C gp70 on their surface and neutralizes some FeLV-C virions directly. Monoclonal antibody analysis suggest that the FOCMA-related epitopes are present as defective, immature, or replication-incompetent FeLV-C

particles. The critical role for FOCMA antibody in humoral immunosurveillance is unchanged (Essex *et al.*, 1971a,b); apparently the cat recognizes a need to prevent FeLV-C from extended replication *in vivo*. By synthesizing antibody to FeLV-C gp70-related epitopes, the cat is able to protect itself against neoplastic disease (see p. 80).

P15E is a hydrophobic envelope protein that spans the plasma membrane of cells replicating FeLV and causes profound immunosuppression (Mathes *et al.*, 1978). Its effects are discussed in Section VII. P15E also is implicated in the depression of committed erythroid precursors intrinsic to the pathogenesis of fatal erythroid aplasia. Specifically, the development of the immature progenitors called BFU-E (burst-forming units-erythroid) and the more mature progenitors called CFU-E (colony-forming units-erythroid) is inhibited by p15E in *in vitro* colony-forming assays. The major core protein of FeLV, p27, is without effect (Wellman *et al.*, 1984). Inhibition requires 1 μg of p15E per 5×10^4 bone marrow cells. Inhibition occurs with smaller amounts of p15E when these are added as part of replication-competent virions (Rojko *et al.*, 1986). Presumably, FeLV gp70 receptors on the surface of bone marrow cells facilitate virion attachment and approximate virion p15E to the surface of susceptible progenitors.

Comparison by molecular sequence analysis of the LTRs of FeLV-A (Glasgow-1) and FeLV-B (Gardner–Arnstein) shows that the proviral LTRs are highly conserved and differ by only eight base substitutions and one base insertion (Stewart *et al.*, 1986). The major subgroup differences, therefore, reside principally in the envelope gene. Molecular constructs using the LTR of one subgroup linked to the envelope gene of another subgroup fail to support an influential role for the FeLV LTR in pathogenesis (Riedel *et al.*, 1986b), this is in contrast to studies in the mouse which suggest that virus competency is in part regulated by the LTR. The FeLV LTR is a strong promoter and is known to function well in heterologous cells. It specifically promotes the expression of mouse histocompatibility genes in mouse and human cells transfected with artificial constructs (Wong *et al.*, 1985).

Products of the *gag* gene, particularly p27 (p30) are known to influence productivity of MuLV infection and host range. Their role in FeLV pathogenesis has not yet been documented. Similarly, no specific role in pathogenesis has been assigned to FeLV *pol* gene products (Mullins and Hoover, 1987).

Proviral integration into host cell DNA may interrupt, inactivate, activate, or transduce normal cellular genes by a process termed insertional mutagenesis. Interruption and fatal disruption of the α1-collagen gene has been described for mouse embryos with endogen-

ized Moloney MuLV proviruses (Jähner and Jaenisch, 1985). Insertional mutagenesis by weakly transforming avian leukosis virus (ALV) isolated may lead to transcriptional activation of cellular proto-oncogene (c-*onc*) and eventual lymphomagenesis. Integration upstream to c-*onc* may generate a hybrid RNA (v-*LTR*–c–*onc*) and enhanced or constitutive expression of the *onc* gene product. Integration downstream or in retrograde orientation may lead to *onc* gene activation by viral enhancers. ALVs also frequently transduce c-*onc* from myeloid and fibroblastoid targets to generate acute transforming viruses. In the cat, activation of c-*myc* is infrequent in spontaneous lymphomas and myelogenous leukemias (Neil *et al.*, 1984; Busch *et al.*, 1983). Rearrangement of c-*myc* occurs but may be restricted to thymic lymphomas induced by the Rickard strain of FeLV (FeLV-R, subgroups A, B). Transduction of c-*myc* is relatively frequent and occurs in about 29% (8/29; see Neil *et al.*, 1984; Levy *et al.*, 1984; Mullins *et al.*, 1984) of spontaneous FeLV-positive, thymic lymphomas. Extensive testing of feline lymphomas in the NIH 3T3 transformation assay has failed to show transfer of any other activated feline oncogene (Neil *et al.*, 1984).

Sequence analysis of a cloned FeLV-*myc* provirus designated FeLV-LC shows that recombination took place in the *gag* region and resulted in a *gag–myc* fusion product (Levy *et al.*, 1984; Braun *et al.*, 1985). In another cloned FeLV-*myc* provirus, designated CT4, recombination at the *pol–env* junction generates an open reading frame whose v-*myc* gene can be expressed without fusion to proviral sequences (Stewart *et al.*, 1986b). Both the LC and CT4 v-*myc* isolates retain the full length feline c-*myc* coding sequences from exons 2 and 3, but only a short, truncated, variant of exon 1. The FeLV-LC v-*myc* coding sequence reproduces the feline c-*myc* sequence faithfully whereas FeLV CT4 v-*myc* is changed at only 3 coding positions relative to c-*myc*. The cat v-*myc* genes do not have mutations in the putative DNA binding region that are characteristic of avian v-*myc* isolates (Stewart *et al.*, 1986b). While avian v-*myc* viruses transform a wide variety of target cells (B cells, macrophages, epithelia, fibroblasts), the feline isolates transform only T cells *in vivo* and fail to transform fibroblasts *in vitro* (ibid). The relationship between the lack of mutational change and restricted transforming potential of FeLV-*myc* isolates remains to be determined.

The spontaneous thymic lymphomas from which FeLV-*myc* viruses were isolated are clonal by analysis of host/proviral junction fragments. Experimental inoculation of these viruses into newborn kittens induces rapid onset of thymic lymphomas that are oligo- or monoclonal

with respect to proviral integration patterns (D. E. Onions, personal communication). The monoclonality of proviral integration suggest that FeLV-*myc* genes alone may be insufficient for lymphomagenesis. Rather, secondary selection could influence which transformed cells would evolve into the successful tumor. A likely second event is the transduction of another cellular gene intrinsic to lymphoid maturation. Such an event has been described for one spontaneous Scottish tumor in which FeLV has separately co-opted *myc* and the β-chain of the T cell receptor (Tiβ) to generate defective separate FeLV-*myc* and FeLV-*tcr* proviruses (Fulton *et al.,* 1987). The proviral sequence is 5′LTR-*gag-pol-tcr-env*-3′LTR and the v-*tcr* insert reproduces the V-D-J-C sequence of the rearranged, joined and spliced Tiβ gene. Exactly how the transduced v-tcr contributes to lymphoma development is not known. A nonconservative change (met→ lys 324) increased the positive charge of the predicted protein in the putative transmembrane region of the T3–Tiβ complex and alters its hydrophilicity. Changes in the T3–Tiβ complex may abrogate the requirement for an $\alpha : \beta$ heterodimer in the formation of a functional surface T cell antigen receptor. Alternatively, an abnormal T3 : Tiβ complex may be activated in the absence of specific antigen and hence under autonomous control for proliferation (Fulton *et al.,* 1987).

The 5′ FeLV/*myc* junctions are unique to each isolate. At the 3′ end of transduced *myc,* an eight base pair FeLV *env* to c-*myc* homology adjoins a 6 base sequence CTCCTC that may be a 3′ recombinational hot spot for transducing FeLVs. This 3′ CTCCTC *onc*-FeLV junction is common to FeLV-*myc* (LC isolate) and FeLV-*fes* (Gardner–Arnstein (GA) and Snyder–Theilen (ST) isolates (Nunberg *et al.,* 1984; Braun *et al.,* 1985). Two other potential recombination hot spots exist. A hot spot in *gag* is identified by similar 5′ junctions in transduced FeSV isolates (Besmer *et al.,* 1986). Recently, the 5′ FeLV-*tcr* junction has been shown to be coincident with one FeLV-*myc* isolate (FTT FeLV-*myc*) and within 18 base pairs of another FeLV-*myc* isolate (CT4 FeLV-*myc*). Whether this last is a true hot spot or merely reflects the selective advantage of a proximate splice acceptor and absence of a stop codon has not been determined.

Spontaneous *onc* gene transduction resulting in acutely transforming feline sarcoma viruses is not infrequent in pet cats (reviewed in Besmer *et al.,* 1983). At least ten isolates have transduced seven oncogenes (*fes, fms, fgr, abl, kit,* ki-*ras, sis*). Oncogenes uniquely transduced by FeLV include *fes, fms, fgr,* and *kit,* c-*fes,* c-*fgr,* and c-*kit* encode tyrosine kinases. c-*fes,* transduced by GA, St, and Hardy–Zuckerman (HZ) 1 strains of FeLV, has a myristylated gene product, is

expressed by human and mouse myeloid cells, and is homologous to the avian *fps* proto-oncogene. Both c-*fes* and c-*fms* are linked to the generation of dysregulated growth signals. Cells transformed by v-*fes* may produce β-transforming growth factors (Twardzik *et al.*, 1983). The c-*fms* gene product likely is the receptor for colony stimulating factor-1 (CSF-1, Sacca *et al.*, 1986) and induces monocytoid differentiation of HL60 promyelocytic leukemia cells (Sariban *et al.*, 1985). Both c-*fes* and c-*fms* increase the rate of phosphatidyl inositol by increasing guanine nucleotide-dependent, phosphatidyl inositol 4,5-diphosphate phospholipase C. As a result, cells transformed by v-*fes* and v-*fms* have inappropriate increases in the production of diacylglycerol and inositol 1,4,5-triphosphate that may function as second messengers (Jackowski *et al.*, 1986).

Other *onc* genes (c-*myc*, c-*myb*) may be expressed in fetal hematopoietic tissues during development; no pathogenetic role has been assigned to them. The location and the nature of the transforming events specific to leukemia, lymphoma, and myeloproliferative disease have not been determined. The best understood tumors are the thymic lymphomas induced by FeLV-R. The tumors arise 7 months to 2 years after experimental inoculation, are terminal transferase-positive, have Tiβ chain rearrangements and are first apparent in the subcapsular region of the thymic cortex (Hoover *et al.*, 1973; Rojko *et al.*, 1987; Rezanka *et al.*, 1987). Tumor emergence is preceded by an increase in terminal transferase-positive cells and neoplastic lymphocytes in the bone marrow (Hoover *et al.*, 1973; Lafrado *et al.*, 1986b). Thymectomy does not preclude lymphomagenesis; rather, T cell lymphomas develop in the extrathymic tissues (Hoover *et al.*, 1975). The transforming event may be the generation of recombinant FeLVs containing subgroup B *env* genes with MCF *env* homology (Elder and Mullins, 1983). FeLV-R-induced lymphomas also show evidence of transduced or activated *myc* genes (Levy *et al.*, 1984; Mullins *et al.*, 1984; Neil *et al.*, 1984).

A subgroup B virus, FeLV-GM, has been implicated in the causation of myeloproliferative disease in Glasgow. Marrow dysplasia with abnormal myeloid progenitor development precedes frank neoplasia (Onions *et al.*, 1985). Another molecularly cloned FeLV-B isolate (GA) causes fatal myelodysplastic anemia (Mullins and Hoover, 1987). Despite the frequency of sarcoma virus involvement in avian myeloproliferative diseases, recombinant feline sarcoma viruses have not been isolated from feline myeloproliferative diseases.

FeLV-C is isolated from many spontaneous cases of FeLV-positive erythroid aplasia. Inoculation of biologically or molecularly cloned

FeLV-C reproduces the disease in neonatal cats (Onions *et al.*, 1982; Riedel *et al.*, 1986a). The principal lesion is the ablation of early (BFU-E) and late (CFU-E) erythroid progenitors (Onions *et al.*, 1982; Boyce *et al.*, 1981; Abkowitz *et al.*, 1985). The experimental disease in SPF cats mimics the disease in nature and the BFU-E are infected and disappear beginning at 10-days postinoculation (Onions *et al.*, 1982; Testa *et al.*, 1983). CFU-E inhibition occurs by 3 to 6 weeks postinoculation and progressive aplastic anemia ensues (Boyce *et al.*, 1981). In safari cats (domestic cat X Geoffroy cat), CFU-E destruction is the principal lesion and the BFU-E are preserved (Abkowitz *et al.*, 1985). *In vitro,* replication-competent FeLV and purified p15E impair both CFU-E and BFU-E development (Wellman *et al.*, 1984; Rojko *et al.*, 1986). Antibody to FeLV-C gp70 and complement may further suppress infected BFU-E early in the disease process (Kociba *et al.*, 1987). Using glucose-6-phosphate dehydrogenase heterozygotes, Abkowitz *et al.*, (1985) have determined that aplasia does not result from myelophthisis and the clonal expansion of a transformed myeloid stem cell. Interestingly, these same authors find that factors which promote BFU-E development are secreted by FeLV-infected fibroblasts (Abkowitz *et al.*, 1986).

The pathogenesis of the other FeLV-associated diseases has not been investigated in detail. The final common pathway may involve the cytopathic elimination of FeLV-replicating cells such as neutrophils, platelets, enterocytes, and fetal cells.

V. Epidemiology of the Virus Infection

Prior to the discovery of FeLV (Jarrett *et al.*, 1964), it was widely assumed that all mammalian retroviruses were acquired as genetically inherited agents, primarily because such inherited retroviruses were found so regularly in laboratory mice. Even ten years later until the mid-1970s it was widely assumed that the few reported clusters of feline leukemia were due to chance rather than to contagious transmission of FeLV.

An important development toward the resolution of the question concerning horizontal versus vertical genetic transmission of FeLV was the availability of serological tests that allowed the screening of household cats that were regularly exposed to leukemic animals. One technique involved the detection of virus structural antigens in circulating leukocytes using reference antisera in fixed cell immunofluorescence tests (Hardy *et al.*, 1969, 1973). A second test involved the

detection of antibodies to FOCMA on virus-infected reference lymphoblastoid cell lines, in this case by membrane immunofluorescence (Essex *et al.,* 1971b, 1975b). In both cases, the procedures were an accurate reflection of the presence of infectious virus in the plasma of antigen and/or antibody-positive cats. Although the transmission of FeLV is probably primarily by saliva, the presence of virus in saliva is also regularly associated with the presence of such markers in plasma or serum (Francis *et al.,* 1977).

The recognition that naturally occurring leukemic diseases in cats were regularly linked with high FeLV seroprevalence rates for both healthy contact-associated cats and cats with opportunistic infections due to immunosuppression by FeLV, was one major observation that drew attention to the likelihood of horizontal transmission (Hardy *et al.,* 1973, Essex, 1975). A second was the recognition that uninoculated littermates often seroconverted when exposed to virus-inoculated kittens (Rickard *et al.,* 1969; Essex and Snyder, 1973). A third supporting observation was the recognition that seroconversions would occur at elevated frequencies when seronegative cats were deliberately introduced to pet-cat households that already contained an infected animal (Essex *et al.,* 1977). Finally, it was demonstrated that subsequent seroconversions did not occur in uninfected cats after seropositive animals were deliberately removed from the environment (Hardy *et al.,* 1976b).

Titers of FeLV are highest in saliva (Francis *et al.,* 1977), and transmission is most efficient when cats are maintained in close contact. Presumably biting enhances the efficiency of transmission but the routine grooming that occurs in the absence of fighting is also probably important. Transmission is particularly efficient at the neonatal period, presumably due to some combination of maternal grooming and transmission of FeLV in milk. Transplacental transmission *in utero* does not appear to be as important as neonatal acquisition, despite the presence of infectious virus in blood. When transmission occurs during gestation it probably results in abortion and/or fetal resorption (Cotter *et al.,* 1975). Infection is considerably more efficient when cats are exposed at the youngest ages, but it is apparent that successful natural transmission can also occur between adults that are several years old. The induction period during which cats remain infected is usually many months or a few years. During this period the infected cats are regularly excreting infectious virus. In fact, healthy carrier cats probably excrete larger amounts of virus than leukemic animals (Francis *et al.,* 1977). They certainly play a more important role in the transmission cycle, in part because of their

mobility and in part because they are usually healthy excretors for considerably longer periods than the duration of their illness.

Little information is available on the role, if any, of breed or strain in regulating the efficiency of FeLV transmission. Although it seems logical that regulation of both immune responsiveness to FeLV and the efficiency of infection at the cellular level should, to some extent, be genetically controlled, different breeds of cats appear to experience equivalent rates of both infection and subsequent disease development (Jarrett and Russell, 1978). Subgroups B and C are not transmitted efficiently except in the presence of subgroup A FeLV. However, differing levels of overall transmission efficiency may occur in households infected with A alone as opposed to situations where both A and B are present.

The transmission of FeLV from saliva or feces contaminating inanimate surfaces is probably rare and unimportant since the virus is relatively labile (Francis *et al.*, 1979). It seems unlikely that adequate doses of infectious FeLV would be acquired from fomites, and virus maintained in extracellular environments at temperatures higher than 20°C would be rapidly inactivated. As with most viruses that bud from cytoplasmic membranes, infectivity is only maintained for prolonged periods with cell-associated material. However, since epithelial cells along the oropharyngeal surface are efficiently infected by FeLV and the cat tongue is abrasive, vigorous grooming allows efficient transmission.

Although it can occur, the contagious transmission of FeSVs is extremely rare. The FeSVs themselves are only infectious *in vitro* in the presence of excess FeLV helper virus and body fluids from FeSV-infected animals would normally contain titers that are too low for transmission. Also, cats that generate new FeSVs by the rescue of oncogenes with replicating FeLV probably live only for very brief periods of time, as opposed to FeLV carrier animals.

VI. Immune Response to FeLV

As FeLV-A is the overwhelming culprit in viremia and latency induction, it is not surprising that regression of viremia and establishment of latent infection are mediated by antibodies that neutralize FeLV-A (Russell and Jarrett, 1978a,b; Pacitti *et al.*, 1986). In natural and experimental infections, virus-neutralizing (VN) antibody to FeLV-A peaks about 6 to 8 weeks after exposure to FeLV. The delay is thought to reflect dilution by serum and consumption by FeLV-

infected cells. Virus-neutralizing antibody to FeLV-B is less common and may be delayed in its appearance for months to years, probably consequent to the relative infrequency of FeLV-B in cats that regress FeLV-A infection. It is more common in multiple cat households with a high incidence of viremia with both FeLV-A and FeLV-B (Jarrett *et al.*, 1978a) VN antibody to FeLV-C gp70 is elicited early (within 2 to 4 weeks and in response to exposure to FeLV of any subgroup). VN antibody to FeLV-A and -B gp70 only neutralizes extracellular virus. In contradistinction, VN antibody to FeLV-C not only neutralizes extracellular virus but also binds to the surface of FeLV-infected cells, fixes complement and initiates complement-mediated cytolysis (Grant *et al.*, 1983). The antibodies critical to humoral immunosurveillance and complement-mediated cytolysis of feline lymphoma cells (see p. 80) apparently recognize surface buds of immature or replication-incompetent FeLV-C related material (Vedbrat *et al.*, 1983; Snyder *et al.*, 1983).

Normal cellular antigens also serve as targets for antibody neutralization of FeLV. Cat antisera to normal feline leukocyte alloantigens specifically neutralize FeLV replicated by cells from viremic cats in an allospecific manner. Examination of a panel of alloantisera against a panel of FeLV-positive, feline lymphoma cells reveals that complement-mediated, antibody-directed cytolysis is targeted to unique and cross-reacting epitopes present on tumor cells and occasional normal splenic lymphocytes. Presumably these reactivities are evoked by FeLV budding from the surface of cat leukocytes in conjunction with feline leukocyte antigens (Grant and Michalek, 1981; Lee *et al.*, 1982). FeLV is known to bud from human lymphoblastoid cells in concert with HLA-A and HLA-B, but not HLA-DR, antigens (Azocar and Essex, 1979). Furthermore, infection with FeLV elicits a humoral response to a normal cellular protein of 105,000 M_r. It is thought that FeLV activates the normal cellular protein gene or alters the relative immunogenicity of the protein *in vivo* (Chen and Essex, 1984).

Monoclonal antibody analysis demonstrates that the neutralizing epitopes reside mainly in the aminoterminus of gp70. The hydrophilic aminoterminal domains are minimally conserved between FeLV subtypes and are thought to be involved in receptor binding and type specificity. The carboxyterminal domains are highly conserved and believed to provide structure and associate with the hydrophobic membrane anchor protein p15E. Some monoclonal antibodies recognize neutralization epitopes common to FeLV-B and -C, others specifically bind to a 14 amino acid sequence shared by all FeLV subgroup

isolates. This sequence, Met-gly-pro-asn-leu-val-leu-pro-asp-gln-lys-pro-pro-ser lies within a local peak of protein hydrophilicity midway in the gp70 molecule still within the N-terminal domain. This proline-rich region is not the receptor binding site (Hamilton *et al.*, 1987a) and may rather function as a molecular hinge in the folding of the two-domain protein. Should antibody binding interfere with protein folding, it easily could lead to the observed virus neutralization of all three subtypes (Nunberg *et al.*, 1984).

Other patterns of shared antigenic specificities are revealed by subgroup-specific cat sera from naturally infected cats. By neutralization, FeLV-A isolates are monotypic and antibodies to FeLV-A neutralize all FeLV-A isolates, and only FeLV-A isolates (Russell and Jarrett, 1978a,b; Jarrett *et al.*, 1978a). FeLV-B isolates are heterogenous reflecting their recombinatory origin, and VN antibodies to individual FeLV-B isolates recognize that isolate and may or may not react with other FeLV-B isolates. Interestingly, VN antibody to FeLV-C neutralizes not only all FeLV-C isolates but all FeLV-A isolates as well. This neutralization pattern suggests common A and C specificities and has been considered evidence for the possible recombinational or mutational derivation of FeLV-C from FeLV-A *in vivo* (Russell and Jarrett, 1978a,b).

The FeLV feline lymphoma/sarcoma model is unique in that the primary means of antitumor resistance is *humoral* and not cell-mediated immunosurveillance of neoplastic cells (Essex *et al.*, 1971a,b, 1975b). The key effectors are complement-dependent antibodies to FeLV-C related epitopes on the surface of neoplastic lymphocytes and nonneoplastic bone marrow cells. These IgG antibodies were first recognized by indirect membrane immunofluorescence (IMI) assay against feline lymphoma cells which produce all three FeLV subgroups (FL74 cells). IgG antibodies arise 2–3 weeks after exposure to FeLV of any subgroup and persist at 1–2 $\log_2$ below peak titer for years in some cats (Essex *et al.*, 1975a). Persistence is likely in response to intermittent expression by bone marrow cells in latently infected cats (see p. 69).

Difference between acute-phase and persistent anti-FeLV-C antibodies exist. Acute-phase antibodies fix rabbit complement, appear early (2–3 weeks after exposure), peak early (5 weeks after exposure), and wane rapidly (see Mathes *et al.*, 1976). Acute-phase antibodies cause rapid (4 hr) complement-dependent lysis of FL74 cells, do not correlate with IMI antibodies, and are absorbed by FeLV of any subtype. Persistent anti-FeLV-C antibodies fix cat complement to trigger slow lysis (20 hr) of FL74 cells, correlate with IMI antibodies, and are partly

absorbed by FeLV-C only (Grant *et al.*, 1977, 1978; Snyder *et al.*, 1983). These latter antibodies seemingly prevent the emergence of producer and nonproducer feline lymphoma cells *in vivo* (Grant *et al.*, 1978, 1980) and may facilitate regression of established lymphomas. Removal of circulating immune complexes by extracorporeal immunosorption of viremic cat plasma with staphylococcal protein A removes immune complexes which consist of FeLV gp70, p15E, and anti-FeLV antibody and induces remission in a small proportion of cats. Remission is associated with increases in circulating complement-dependent antibody and interferon levels (Liu *et al.*, 1984a,b).

Complement-assisted immunity is critical to protection against feline neoplasia and also is implicated in maintenance of latent FeLV infections (Kraut *et al.*, 1985, 1987). As discussed, antibody to FeLV-C gp70-related antigens fixes complement to trigger lysis of tumor cells. Experimental depletion of complement by treatment of cats with cobra-venom factor causes transient reactivation of latent FeLV infection. Upon restoration of hemolytic complement to normal levels, the cat again regresses productive FeLV infection and antibody titers to FeLV-C-related antigens show reactive increases. In contrast, experimental consumption of complement does not impair resistance in acute FeLV infection (Kraut *et al.*, 1987), and cat complement is inefficient at direct lysis of FeLV (Kobilinsky *et al.*, 1980). Also important is the observation that viremia, immune complex disease, and hypocoplementemia coexist in some cats and exacerbate FeLV-induced immunosuppression and, perhaps, lymphoma progression.

Cats acquire immune competence and most outgrow their susceptibility to viremic FeLV infection by 4 to 6 months of age (Hoover *et al.*, 1976; Klotz *et al.*, 1986). In multiple cat households, 15–30% of FeLV-exposed cats become persistently viremic (Hardy *et al.*, 1976). In the laboratory, 15% of cats older than 4 months of age develop viremia, as compared with 100% of neonatal kittens and 85% of weanling kittens (Hoover *et al.*, 1976).

Age-related increases in immune functional capacity are the result of age-related acquisition of macrophage, natural killer cell, complement and antibody production capability. The critical regulation of FeLV infection by complement-dependent antibody was discussed previously. Equally important is the relationship between FeLV and the feline macrophage (Hoover *et al.*, 1981). Kitten bone marrow and peritoneal macrophages are very susceptible to FeLV infection and produce high titers of replication-competent FeLV. Macrophages from adult cats replicate FeLV poorly and aid in viremia regression. Glucocorticoid treatment of adut cat macrophages shifts the host/virus

relationship and permits the macrophages to replicate FeLV readily. *In vivo,* glucocorticoids abrogate age-related resistance to FeLV and enhance both initial susceptibility and reactivation of latent infection (Rojko *et al.,* 1979b, 1982; Hoover *et al.,* 1981). Similarly, poisoning of adult cat macrophages with the macrophage toxin silica nullifies age-related resistance to FeLV *in vivo.*

Cats possess two types of cells with natural cytotoxic activity. Natural cytotoxic-like cells are present in peripheral blood and spleen, lyse virus-infected adherent monolayers and are not activated by viral infection. Natural killer-like cells are restricted to the spleen, lyse lymphoid tumor targets, and are maximally stimulated 6 days postviral infection (Tompkins *et al.,* 1985). Cats also possess a short-lived, nonimmune, peripheral blood cell responsible for the restriction of initial and latent FeLV infection (Rojko *et al.,* 1981, 1982). The actual role of these cells in protection from viremia has not been determined and may be minimal (Kooistra *et al.,* 1985). FeLV p15E specifically inhibits the natural lethal hit events that follow natural killer cell recognition and target-natural killer cell conjugation (Whisler *et al.,* 1987). Suppression of natural killer function does not require direct FeLV infection of effector cells and is partially nullified by (ν) interferon and IL-2 supplementation (Whisler *et al.,* 1987). The production of (γ) interferon, a known activator of natural killer cells, also is depressed by p15E (Engelman *et al.,* 1986b). The FeLV/interferon interaction is a two-edged sword; both human and cat interferons restrict late stages of FeLV replication (Jameson and Essex, 1984). The administration of interferon to anemic viremic cats occasionally lead to partial remission of viremia and recovery from anemia (Tompkins and Cummins, 1982). Lastly, interferon may regulate productivity of infection in lymphoblastoid cells (Azocar and Essex, 1979; Dubey *et al.,* 1982; Lee *et al.,* 1982). *In vitro,* transformed T cells are more susceptible to productive FeLV infection than are transformed B cells. Infection with FeLV causes B cells normally resistant to natural killing to become sensitive and causes T cells normally sensitive to natural killing to become resistant. Inhibitors of interferon synthesis decrease natural killing of FeLV-infected B cells but increase natural killing of FeLV-infected T cells. These data indicate that interferon augments natural killing of FeLV-infected B cells only and that FeLV-infected, transformed T cells resist natural killing (Dubey *et al.,* 1982). If similar mechanisms operate in viremic cats, retroviral infection may enable transformed T cells to escape immunosurveillance. This may be relevant to the T cell tropisms for transformation of certain FeLV isolates (Dubey *et al.,* 1982).

Cat K cells are principally monocytes and neutrophils and function in ADCC against chick red blood cells, and FeLV-coated chick red blood cells but do not kill autochthonous, virus-producer, fibrosarcoma cells (Kooistra *et al.*, 1985). Cats immunized with autochthonous, virus-nonproducer, transformed fibroblasts do develop cytotoxic T cells. These cytotoxic T effectors arise by 7 days postimmunization, peak at 16 days, and disappear by 35 days. Recognition and killing are in response to a combination of FeLV and self antigens on the membrane of the autochthonous transformants (McCarty *et al.*, 1983a). Whether these cytotoxic effectors express the recently described feline cytotoxic antigen defined by monoclonal antibody (Klotz *et al.*, 1986b) has not been determined. The feline histocompatibility-restricted cytotoxic effectors that arise consequent to immunization with autochthonous transformants are distinct from the nonhistocompatibility-restricted cells which arise consequent to immunization with producer allogeneic lymphoma (FL74) cells. The latter probably are a mixture of T cells sensitized to alloantigens and natural killer cells. Morphologic evidence of cell-mediated immunity important in tumor regression has been presented by Johnson *et al.* (1985). Biopsies taken from regressing sarcomas contain progressively fewer viable tumor cells and increasing numbers of lymphoid cells, histiocytes, giant cells, and normal fibroblasts.

Little is known concerning the immune responses to other feline retroviruses. Cats make IgG antibody responses to feline lenti- and spumaviruses; it is thought that these antibodies are responsible for the restricted replication and close cell association of these viruses *in vivo*.

VII. Passive Immunotherapy and Active Vaccination

Passive immunotherapy with antibodies to FeLV is highly efficacious in limiting viremia but only under certain conditions and within a narrow time frame. Colostral transfer of VN antibodies from FeLV-immune queens protects suckling kittens from immediate virulent FeLV or FeSV challenge. Active immunity is not induced and susceptibility increases parallel with the decay of antiviral antibodies and approaches that of untreated controls by 8 weeks of age (Hoover *et al.*, 1977b; Jarrett *et al.*, 1977). Passive administration of homologous and heterologous antibody to gp70 will protect weanlings from viremia induction but only if administered during the first 1–3 weeks after FeLV exposure (de Noronha *et al.*, 1978; Haley *et al.*, 1985). It

apparently prevents establishment of bone marrow infection but fails to eliminate marrow origin viremia once established. No greater protection is afforded by monoclonal antibody therapy; rather, a mixture of two VN monoclonal antibodies is not protective against viremia induction (Weijer *et al.,* 1986). Some of the failure of monoclonal antibody therapy may be attributable to the efficient cat anti-idiotype response and the rapid clearance of mouse antibodies from cats (Weijer *et al.,* 1986). Passive immunotherapy with polyclonal goat VN antibody actually may promulgate latent FeLV infection in some cats (Haley *et al.,* 1985).

The history, administration, efficacy, and contraindications of FeLV vaccines have been reviewed recently (Rojko, 1986). The ideal FeLV vaccine should induce protection against viremia and FeLV-related neoplastic and nonneoplastic diseases. It also should minimize the development and consequences of latent FeLV infections. Hence, it is vital that FeLV vaccines induce protective immunity to the envelope glycoproteins of cell-free FeLV-A, FeLV-B, and FeLV-C, particularly FeLV-A, and also to the cell-associated FeLV-C gp70-related epitopes.

Early vaccine attempts involved the administration of live versus killed FeLV or live versus killed FL74 feline lymphoma cells in conjunction with a variety of adjuvants (alum, adjuvant 65, complete or incomplete Freund's, or none). Certain clones of FL74 cells, which replicate all three subgroups and express membrane FeLV-C gp70-related antigens (Essex *et al.,* 1971a,b; Vedbrat *et al.,* 1983; Snyder *et al.,* 1983) were nonpathogenic and induced protective immunity in most cats older than 4 months of age. This resulted in the patenting of a live tumor cell vaccine in 1976 (Jarrett *et al.,* 1974, 1975, 1976). Research and development of this vaccine ceased due to aesthetic objections to the inoculation of live tumor cells and due to the observation that certain FL74 isolates were highly pathogenic, causing fatal anemias or lymphomas in neonatal kittens. Vaccines employing FL74 cells killed by formalin, heat or ultraviolet light had good immunogenicity and elicited antibodies to cell-associated FeLV-C gp70 moieties and sometimes VN antibodies. Killed tumor cell vaccines also induced protection against FeSV sarcomagenesis but evoked little defense against viremia. Vaccinated, virulent (FeSV) FeLV challenged cats died of FeLV-associated diseases (Yohn *et al.,* 1976; Olsen *et al.,* 1976; Heding *et al.,* 1976).

Subsequent vaccine protocols were designed to enhance VN antibody production and induce protection against viremia. These used killed whole FeLV or purified gp70 of bivalent or trivalent subtype (Olsen *et al.,* 1977; Salerno *et al.,* 1978). These attempts were unsuccessful. The

addition of killed FeLV to killed tumor cell vaccines did not elicit VN antibody, rather it actually nullified resistance to lymphoma and sarcoma development. This appalling result has since been attributed to the immunosuppressive effect of p15E. Purified gp70 preparations induced VN antibodies readily in guinea pigs but not in cats even when assisted by adjuvants.

The development of the soluble tumor cell antigen vaccine currently marketed as Leukocell by the Norden Laboratories, Lincoln, Nebraska (Lewis *et al.,* 1981; Beckenhauer *et al.,* 1984) is the result of clever manipulation of the conditions under which FL74 cells are propagated *in vitro* (Wolff *et al.,* 1979). FL74 cells are grown to saturation in medium containing serum and then grow in serum-free medium for several days. The supernatants are harvested, concentrated, and filtered. The soluble tumor cell antigens thus released consist of FeLV-C gp70-related antigens and a multiplicity of partly processed *env* and *gag* gene products, including p27, p15, p12, and p10 (Mastro *et al.,* 1986). The soluble tumor cell antigen preparation does not contain replication-competent FeLV and has remarkable immunogenicity *in vivo*. Immunized cats make antibodies to a protein of 70,000 M_r distinct from, but related to, FeLV gp70 (Mastro *et al.,* 1986).

In vaccine trials conducted at The Ohio State University and at Norden Laboratories, 80% of the cats receiving the soluble tumor cell antigen associated with adjuvant were protected against FeLV challenge. Unvaccinated controls were 70–100% susceptible to the identical challenge, became viremic, and died of FeLV-associated diseases. Following approval of the vaccine in January 1985, approximately two million pet cats have been vaccinated by American veterinarians. Adverse reactions are minimal. Less than 0.5% of cats have hypersensitivity with emesis, diarrhea, myxedema, erythema, or cyanosis within 48 hours following injection.

Data originating from Norden Laboratories suggests that the Leukocell vaccine may reduce the incidence of latent FeLV infections (Sharpee *et al.,* 1987). Vaccination of viremic cats is apparently without effect; neither deleterious effects nor diminution in viremia are seen. The decision whether or not to vaccinate an individual cat depends upon the cat's age, environment, and general health. Many adult cats probably do not require vaccination as their age-related, autogenous resistance will protect them from virulent FeLV exposure (Hoover *et al.,* 1976). These authors regard viremic cats, pregnant queens, blood donors, and cats housed in virtual isolation as poor candidates for vaccination.

The Leukocell vaccine works well but probably will be replaced by

future biologically or genetically engineered vaccines. Subunit vaccines which combine FeLV gp70/85 with immunostimulatory complexes (Iscoms) induce VN antibodies and protective immunity against FeLV challenge (Osterhaus *et al.,* 1985). The use of Iscoms facilitates the presentation of membrane-associated antigens as immunogens and circumvents the requirement for immunoreactive adjuvants. Other subunit vaccines have used the 14 amino acid aminoterminal *env* sequence common to all three FeLV subgroups as immunogen; this elicits good VN antibody responses in guinea pigs and mice but not in cats (Nunberg *et al.,*1986). Research involving recombinant packaging mutants, vaccinia vectors, yeast synthesized *env* proteins, and/or anti-idiotypic schemes is eagerly awaited (Luciw *et al.,* 1986).

VIII. Immunosuppression by FeLV

As mentioned previously, FeLV infection often confers a generalized susceptibility to various infectious agents and viremic cats are at greatly increased risk for the development of disease caused by opportunistic bacterial, viral or parasitic agents (Hardy, 1980). Under conditions of natural infection with FeLV, the diseases associated with immunosuppression cause more cat deaths than does leukemia or lymphoma (hardy, 1982). An early demonstration of FeLV immunosuppression at the cellular levels was the prolongation of allograft rejection in experimentally infected cats (Perryman *et al.,* 1972). Thymic atrophy (Anderson *et al.,* 1971; Hoover *et al.,* 1973), depletion of paracortical lymphoid tissues (Hoover *et al.,* 1973, 1974) and depressed peripheral blood lymphocyte counts are other manifestations of the abnormal cellular immune system associated with FeLV infection.

Most studies of FeLV immunosuppression have been directed at understanding the mechanisms that underlie T cell suppression. Possibilities have included the action of an exfoliated protein from the viral envelope denoted p15E (Mathes *et al.,* 1978), cytopathic lymphocyte infection, the formation of immune complexes and a virus-induced impairment of T helper function similar to that seen in human AIDS.

FeLV may directly alter immune cell function and a virus-induced suppressor cell has been observed. Ultraviolet-inactivated FeLV (FeLV-UV) and protein from detergent-disrupted FeLV suppress the lymphocyte blastogenic responsiveness of peripheral blood mononuclear cells from normal cats (Langweiler *et al.,* 1983). Serum from viremic cats also depresses cat lymphocyte mitogenesis (Cockerell *et*

al., 1977). FeLV-UV causes significant suppression of the mixed leukocyte reaction (Stiff and Olsen, 1983) and of lymphokine secretion (Orosz *et al.,* 1985). Specifically, FeLV-UV reversibly reduces the accumulation of IL-2 and macrophage activating factors in cultures of concanavalin A (Con A)-stimulated murine splnocytes (Orosz *et al.,* 1985). While FeL-UV does not prohibit killing by cloned murine cytotoxic T cells, it does impair the ability of human NK cells to score lethal hits against their targets (Orosz *et al.,* 1985; Whisler *et al.,* 1987). Additional immunosuppressive phenomena attributed to FeLV-UV include the suppression of cat-interferon production in response to Staphylococcal enterotoxin A, the inhibition of human lymphocyte mitogen and antigen responses, and reduction of Con A-induced cap formation by cat and human lymphocytes (Hebebrand *et al.,* 1979; Dunlap *et al.,* 1979; Engelman *et al.,* 1985b).

Many of the immunosuppressive effects caused by FeLV-UV probably are mediated by the transmembrane spike protein p15E. Related proteins have been isolated from murine and avian retroviruses and from murine and human lymphomas and are immunosuppressive, and synthetic p15E depresses lymphocyte blastogenesis (Cianciolo *et al.,* 1985). Lewis and his colleagues (1985a,b; 1986) have examined the biochemical defects induced when the hydrophobic p15E intercalates into the lipid bilayer of the lymphocyte membrane. Incorporation may interfere with the calcium/calmodulin transduction of membrane signals and the subsequent activation of adenyl cyclase in lymphocytes. This would be compatible with observations that FeLV contains calmodulin, that p15E prohibits cyclic AMP accumulation by lymphocytes and that direct activation of adenyl cyclase by forskolin can override p15E immunosuppression. Alternatively, p15E is a bulky protein and may interrupt lymphocyte function by a space-occupying mechanism alone. The reasons for the relative T cell specificity and B cell sparing have not been examined.

FeLV-UV also may directly induce *T suppressor cell function* (Langweiler *et al.,* 1985). Interestingly, though, most viremic cats have inadequate suppressor cell function (Stiff and Olsen, 1983). Such defects may facilitate the development of immune-medicated diseases such as Coomb's-positive hemolytic anemia, immune complex disease and membranous glomerulonephritis. *Immune complexes* which contain intact, infectious, virions as well as FeLV gp70, p27, p15E, and antibodies to FeLV proteins form readily in the antigen-excess state of chronic viremia (Day *et al.,* 1980; Kobilinsky *et al.,* 1979). Experimentally, immune complexes form early in the preleukemic period (Tuomari *et al.,* 1984). These small, soluble, antigen–antibody complexes

circulate, lodge in tissues like the renal glomerulus, and elicit chronic, progressive, inflammatory disease. It also is possible that immune complexes contribute to the maintenance of the viremic state and to the progression of lymphomas. Removal of circulating immune complexes from viremic cats by extracorporeal immunosorption is reported to result in the reversal of viremia and the remission of lymphoma in some cats (Jones *et al.,* 1984; Liu *et al.,* 1984a).

Direct lymphocyte infection probably is a factor in the pathogenesis of FeLV immunosuppression. All isolates of FeLV replicate in T cells, B cells, and monocyte/macrophages (Rojko *et al.,* 1978, 1979, 1981; Hoover *et al.,* 1981). FeLV-FAIDS variant A DNA is produced to excess by cat bone marrow and lymphoid cells. For unknown reasons, these excess FeLV DNA copies cannot be integrated into cat marrow or nodal chromosomal DNA, accumulate in the marrow and nodal cells, and are cytopathic. This results in ablation of marrow and nodal T cells and severe immunosuppression (Mullins *et al.,* 1986).

Noncytopathic FeLV isolates also may induce an impairment of T helper cells similar to the T helper cell dysfunction seen in human AIDS. Asymptomatic cats naturally infected with FeLV have a malfunctioning humoral immune response (Trainin *et al.,* 1983; Wernicke *et al.,* 1986). The primary and secondary responses of these cats have been studied following stimulation with a synthetic antigen, namely the multichain poly (Tyr, Glu)-poly (Ala)–poly(Lys) denoted (T,G)-A–L. After both primary and secondary administration of antigen, the nonviremic control animals responded with increased levels of antibodies within 3 days after inoculation. In the FeLV-infected cats, lower and delayed levels of antibody responses were detected after primary antigenic stimulation. Furthermore, a second challenge with antigen evoked an increase of antibody production only after 9 days. Thus, the humoral antibody response of cats naturally infected with FeLV is diminished when they are exposed to a newly encountered antigen. The suppression of the secondary response was very dramatic in several of the viremic cats.

Despite this apparent impairment in the humoral response to new antigens, the FeLV-infected cats exhibited hypergammaglobulinemia, which was twofold higher for mean levels of IgG and threefold higher for levels of IgM, compared with the uninfected controls. These findings do not necessarily indicate a normal function of the immune response and may be an expression of an intense polyclonal B cell activation leading to high serum immunoglobulin levels, similar to observations made on acquired immunodeficiency syndrome patients (Lane *et al.,* 1983). (T,G)-*A–L* is a T cell dependent antigen (Mozes and

Haimovich, 1979). The mitogenic stimulus provided by activated T helper cells is necessary for vigorous B cell proliferation, when the triggering antigen is T cell dependent. In both the primary and the secondary immune responses, the B cells from FeLV-infected cats are capable of synthesizing antibodies to (T,G)-*A–L* after a considerable delay. Thus, a defect in the production or the release of helper factor which normally would stimulate B cell proliferation can also be considered. On the other hand, the observation that uninfected animals maintain a relatively high level of antibodies against (T,G)-*A–L* for more than 8 months after the first immunization, while the FeLV-infected animals do not, is compatible with impaired B cell longevity. Similar results on the humoral response of FeLV-infected pet cats have been obtained using sheep RBCs (Hardy, 1982).

In conclusion, it seems that multiple cell types and multiple processes are involved in the development of feline retrovirus-induced immunosuppression. Although no solid evidence is available for the malfunctioning of cat T helper cells due to the paucity of T cell specific markers, the circumstantial evidence provided thus far indicates an impaired T helper function in FeLV-infected cats similar to that observed in humans infected with HIV.

Studies on the pathogenesis of FeLV-induced immunosuppression might provide a valuable mode for a better understanding and means of control of human AIDS.

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