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THE IMMUNOBIOLOGY OF THE FELINE LEUKEMIA VIRUS

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I. INTRODUCTION

Much of the notoriety attached to the feline leukemia virus (FeLV) stems from its ability to induce lymphoma and leukemia in naturally outbred cats which share the same environment with man. Leukemia and lymphoma, however, represent uncommon manifestations of FeLV infection. In its more frequent interactions with cat hemopoietic and lymphoid tissues, FeLV behaves as a tripartite agent. It acts as an infectious agent, a genetic agent, and rarely, as a disease-causing agent. Moreover, the feline host exhibits a tridimensional micro-environmental response to FeLV. Lymphoreticular and marrow cells serve as the primary targets for viral replication, as the primary effectors of viral restriction and as the primary targets for FeLV-induced cytoproliferative and cytosuppressive disease. Lastly, there are trinal macroenvironmental influences on the immunobiology of FeLV infection and FeLV-related diseases. These include evolution, feline social habits, and intercurrent pathogens.

II. BIOLOGY OF FeLV

A. FeLV as an Infectious Agent

1. Classification, morphology and proteins

FeLV is an enveloped, oncogenic RNA virus containing reverse transcriptase (RT) and is classified as an oncovirus in the family Retroviridae (Fenner, 1975). Certain viral proteins have defined contributions to the biology of FeLV. The internal core of FeLV consists of tightly coiled viral RNA packaged and protected from nucleases by hexagonally arranged core proteins of 27,000, 15,000, and 10,000 daltons, and designated p27, p15(C) and p10, respectively (Graves and Velicer, 1974; Schafer and Bolognesi, 1977). This core complex also encompasses the virion reverse transcriptase (RT) (Scolnik et al., 1970) required for synthesis of DNA complementary to the RNA of the infectious FeLV. The core complex is surrounded by acidic proteins of 12,000 daltons (p12) which constitute the inner coat. Centrifugad to the inner coat is the viral envelope whose major component is a glycosylated protein of 70,000 daltons (gp70) arranged as spheres displayed on radiating spikes derived from the minor envelope protein which masses 15,000 daltons (p15(E)) (Bolognesi et al., 1978).

2. Replication of FeLV

Following adsorption and penetration, FeLV is uncoated and the RNA is copied into a single strand of complementary DNA (cDNA) using the virion RT primed by a small tRNA molecule (reviewed in Varmus, 1982; Litvak and Araya, 1982). The de novo synthesized DNA serves as a template for the formation of the double-stranded DNA provirus which circularizes and integrates to become part of the cat genome. Synthesis and integration of the provirus occur only in those cells that undergo DNA synthesis (Temin, 1971). Viral RNA (vRNA) is transcribed from the integrated provirus by DNA-dependent RNA polymerases and translated on host ribosomes to generate precursor structural and envelope polyproteins. These polyproteins migrate to the plasma membrane to thicken the lipid bilayer and initiate the evagination known as the viral bud

(Schafer and Bolognesi, 1977; Bolognesi et al., 1978). Peripherally, the envelope precursors undergo post-translational cleavage and the minor fragment (p15(E)) spans the plasma membrane as transmembranous spikes firmly embedded in the lipid bilayer (Bolognesi et al., 1978). Protruding knobs representing the major fragment are glycosylated (gp70) and are welded to the spikes of p15(E) by disulfide linkages (Bolognesi et al., 1978). Centrally, the structural precursors are processed and the individual proteins re-associate according to their biochemical and biophysical properties to encapsidate the ribonucleoprotein core (Graves and Velicer, 1974; Schafer and Bolognesi, 1977; Okasinki and Velicer, 1977; Pinter and Fleissner, 1979).

3. Feline oncornavirus-associated cell membrane antigen (FOCMA)

The expression of all the viral structural and envelope proteins in the cytoplasm of cells with productive or partially productive FeLV infections is correlated highly with the expression of gp70 and p27 at the cellular membrane (Yoshiki et al., 1974). Expression of FeLV proteins and FeLV-induced tumor antigens, however, are discordant. FeLV-induced hemolymphatic malignancies are distinguished by a common tumor-specific antigen, the feline oncornavirus-associated cell membrane antigen (FOCMA) which is present on transformed cells from cats with highly expressed (productive) FeLV infections and from cats with minimally expressed (nonproductive) FeLV infections (Essex et al., 1971a,b; Hardy et al., 1977, 1980). FOCMA likely is present on preneoplastic lymphocytes in the bone marrow and mesenteric lymph node (Rice and Olsen, 1981) and recent investigations using monoclonal antibodies suggest that the expression of FOCMA and the partial expression of $FeLV_C$ envelope genes (env genes - see Sections II.B.2., III.C.3.) are interdependent events (Vedbrat et al., 1983). While the exact origins and functions of FOCMA remain speculative, it is clear that antibody to FOCMA determinants effects anti-tumor immunosurveillance in the cat lymphoma/FeLV model (Essex et al., 1975 and see Section III.B.4).

4. Biologic properties of FeLV proteins

The major core protein p27, known as the major group specific antigen (GSA), confers interspecies antigenic cross reactivity to FeLV (Hardy et al., 1973). Its presence in the cytoplasm of circulating neutrophils and platelets by fixed cell immunofluorescence assay (Hardy et al., 1973) has a 98% correlation with recovery of infectious virus (Fischinger et al., 1974) from plasma (Hoover et al., 1977; Jarrett et al., 1983) and defines the FeLV viremic (persistent productive - see Sections II.A.6, III.A.1.b) state. Soluble p27 in serum, generally measured by enzyme-linked immunosorbent assay (ELISA - Saxinger et al., 1980; Rice and Olsen, 1981), may manifest either viremia (68% of ELISA-positive cats) or occult FeLV infection (32% of ELISA-positive cats - Lutz et al., 1980; Saxinger et al., 1980; Rice and Olsen, 1981 and see Section III.B.9).

Three antigenically distinct forms of the major envelope glycoprotein (gp70) specify three subgroups (serotypes) of FeLV, designated $FeLV_A$, $FeLV_B$, and $FeLV_C$. These envelope gene products determine infectivity, interference, host range properties, and

pathogenicity, and as subgroup-specific antigens evoke the virus neutralizing antibody responses important in the reversal of viremia in cats that regress FeLV infection (Sarma and Log, 1973; Schaller and Olsen, 1975; Hardy et al., 1976; Jarrett and Russell, 1978; Russell and Jarrett, 1978). Virus adsorption, and hence infectivity, is dependent upon the affinity of gp70 for host cellular FeLV receptors and virus neutralizing antibody blocks initial adsorption. Dessication, heat (56°C, 3 min) and ultraviolet light detach gp 70 and inactivate FeLV.

The minor envelope protein (p15(E)) causes the profound depression of T lymphocyte function that accompanies viremia (Mathes et al., 1978, see Section III.C.1.). The binding of p15(E) to the first component of complement (Bartholomew and Esser, 1978) activates the classical pathway. This causes complement consumption and results in ineffective virolysis in cats (Kobilinsky et al., 1979, see Section III.B.8.).

5. Transmission of FeLV

Domestic cats are exposed to FeLV following prolonged contact with the saliva or urine of naturally viremic cats (Francis et al., 1977; Hoover et al., 1977). High titers of infectious FeLV are excreted by pharyngeal, salivary, bladder and intestinal epithelia but virus survival at room temperature or under conditions of dessication is less than 2 hours (Francis et al., 1977). Therefore, efficient virus transmission appears to require either direct contact between cats, transfer of saliva on hands or feeding utensils, or exposure to recently voided urine in communal litter pans.

The presumed portal of entry following contact in nature (Hardy et al., 1973; Jarrett et al., 1973) is the oronasal pharynx, and experimental oronasal challenge (Hoover et al., 1972) of cats with FeLV leads to viremia and disease or nonproductive infection and immunity (see below). Congenital transmission of FeLV has been proposed to account for the clinical observation that viremic queens may bear viremic kittens, but the question of intrauterine versus lactation-associated versus contact exposure of kittens has not been resolved (Cotter et al., 1975). Recent experiments document the transplacental passage of FeLV and its isolation from the embryonic hemolymphatic tissues of fetal kittens obtained by hysterectomy from viremic gravid queens (Hoover et al., 1983).

6. Pathogenesis of FeLV replication

Six sequential stages in the pathogenesis of FeLV replication have been identified using immunofluorescence assay for p27 in paraffin-embedded tissue sections (Table 1, see Rojko et al., 1979a). FeLV/host cell contact is initiated in the lymphoepithelia and follicular lymphocytes in the pharyngeal and palatine tonsils (Stage 1) in the first 2 days after exposure (DAE) via oronasal instillation.

The virus next is amplified in the draining lymph node and infectious FeLV is transported by lymphocytes and macrophages (Stage 2) to secondary sites (marrow, systemic and gut-associated lymphoid tissue - GALT) where massive viral amplification occurs (Stages 3, 4). This mononuclear cell-associated viremia is reminiscent of that described for other oncogenic (Epstein-Barr Virus - Epstein and Achong, 1977), and

nononcogenic viruses (Feline Panleukopenia - Carlson et al., 1978). It provides rapid, efficient distribution of FeLV to the mitotically active cells in the marrow and GALT. This is vital because cells initially infected by retroviruses must enter mitosis to permit integration of provirus and to support replication of progeny infectious virus (Temin, 1971). Cell association also protects FeLV from reticuloendothelial clearance and from inactivation by humoral lipid or protein moieties as described for feline (Welsh et al., 1975; Sherwin et al., 1978; Bartholomew and Esser, 1978), murine, and bovine retroviruses (Welsh et al., 1975; Sherwin et al., 1978; Bartholomew and Esser, 1978; de Noronha et al., 1978; Schwartz et al., 1979; Gupta and Ferrer, 1982).

Table 1. Stages of FeLV Replication in Cats

Stage	Location	Duration of Stage in:	
		Persistent Viremia	Latency & Immunity
1	Tonsil/Pharyngeal LN	2-14 DAE	2-14 DAE
2	Cell-Associated Viremia	1-14 DAE	1-14 DAE
3	Systemc Lymphoid Tissue GALT	3-12 DAE	3-12 DAE
4	Bone Marrow/GALT Systemic Lymphoid Tissue Intestinal Crypt Epithelia	7-21 DAE	7-21 DAE
5	Marrow Origin Viremia	14-28 DAE	RARE
6	Epithelia	28-56 DAE	ABSENT

Proliferation of FeLV in the spleen, lymph nodes, and GALT distant from the site of inoculation is evident in cats between 3 and 12 DAE (Stage 3). FeLV p27 is most concentrated in the rapidly dividing lymphoid cells of the germinal centers of cortical follicles. This early viral tropism for B-lymphocytes (see Section III.A.1.) mimics the proclivity of certain MuLV strains for germinal center immunoblasts early in infection (Hanna et al., 1970; Ruddle et al., 1976; Isaak et al., 1979).

The fourth stage (7-21 DAE) of widespread infection of nonlymphoid hematopoietic cells in the bone marrow overlaps the systemic lymphoid phase. Megakaryocytes accumulate large amounts of cytoplasmic viral antigen, resulting in infection of developing platelets. The majority of the marrow cells containing p27 are developing myelomonocytic precursors. The concentration of p27 increases as the cell matures. Uninfected clones of granulocytes and monocytes usually are identifiable. P27 is rare in eosinophil precursors. In erythroid series cells, intensity of FeLV replication is inversely related to cell maturation. Replication in early erythroid precursors is extinguished when hemoglobinization and nuclear pyknosis distinguish the basophilic normoblast (rubricyte). Concomitant with marrow infection is the onset of multiple foci of productive infection in the crypt (germinal) epithelium of the small and large intestines. Viral antigen is confined to the basilar mitotic cell population and is absent from the mature distal absorptive epithelium lining the villi. Retroviruses generally are not cytopathic and

replication of FeLV in the rapidly dividing cells of the marrow, lymphoid tissue, and intestine is not associated with overt cytopathic change (necrosis, polykaryocytosis, etc.). The presence of FeLV in lymphoid and marrow tissues, however, may depress normal cell turnover and initiate atrophy (see Sections III.C.1,2).

The appearance of p27-positive neutrophils and platelets in the circulation is considered the fifth stage in the evolution of progressive FeLV infection (_14-28 DAE). This phase directly reflects infection of marrow progenitors and is the onset of marrow-origin viremia (Hardy et al., 1973) which is the harbinger of persistent viremia and the induction of fatal FeLV-related disease (Hardy et al., 1973; Hoover et al., 1977; Rojko et al., 1979a).

The initial marrow release of FeLV-infected neutrophils and platelets coincides exactly with development of protracted neutropenia and lymphopenia (21 to 56 DAE - Cockerell et al., 1976b; Rojko et al., 1979a) and thrombocytopenia (Pedersen et al., 1977). These may be effected by atrophic or aplastic responses of hemolymphatic precursor cells (see Sections III.C.1,2), extravascular sequestration of FeLV-infected cells in myeloid or lymphoid tissue (see Section III.A.1), or immunologic elimination of FeLV-infected cells by the host (see Section III.B.9.). Although the onset of marrow origin viremia usually signifies the establishment of progressive FeLV infection, some cats still are able to reverse this state by clearing FeLV-infected cells and producing both virus neutralizing (VN and FOCMA antibody (Hardy et al., 1976; Rojko et al., 1979a). This latter group of cats is at particular risk for reactivatable FeLV infections (see Section III.B.9.) and p27 antigenemia (Lutz et al., 1980; Saxinger et al., 1980; Rice and Olsen, 1981; Rojko et al., 1982a).

In cats that fail to develop VN and FOCMA antibody, FeLV infection extends to multiple mucosal and glandular epithelial tissues between 28 and 42 DAE and thereafter. The earliest and most consistently infected epithelial tissues are those of the oropharynx, nasopharynx, larynx, trachea, stomach, salivary gland, pancreas, and urinary bladder. Replication begins in multiple foci in the mitotic layers and progresses to diffuse involvement of the mucosa of the bladder, oral and nasal pharynx, and trachea and the release of infectious FeLV into the secretions of persistently viremic cats.

In dramatic contrast is the tissue distribution of FeLV p27 in cats that develop immune (regressive) infection between 28 and 42 DAE and thereafter. Immune cats develop FOCMA and VN antibody by 14 to 56 DAE (Rojko et al., 1979a; Rice and Olsen, 1981; Rojko et al., 1982) and are able to abort virus production prior to widespread marrow or epithelial infection. Recent evidence indicates that immune cats remain latently infected with FeLV (Post et al., 1980; Rojko et al., 1982a; Madewell and Jarrett, 1982). Having experienced early cell-associated viremia and systemic lymphomyeloid viral replication, regressor cats must eliminate all cells with integrated ecotropic FeLV proviruses to truly ablate infection. Most do not and rather develop a persistent nonproductive infection. The evidence for and the consequences of latent FeLV infections are presented in Section III.B.9.

B. FeLV as a Genetic Agent

1. Structure of viral RNA

The structure of the ecotropic FeLV viral RNA (reviewed in Varmus, 1982; Weiss et al., 1983) is shown in Fig. 1. Ecotropic viruses are not endogenous to the genome and are acquired by infection. It is characteristic of retroviruses that two identical 35S subunits conjoin to form the viral RNA. Each has an extra cap nucleotide added at the 5' end and each is finished with a 3' tail of polyadenylic acid. Each subunit is terminated by repeated (R) sequences shared by the 5' and 3' ends and unique (U) sequences found at the 5' (U5) and 3' (U3) ends. These are regulatory domains. The initiation site for reverse transcription (induction of negative strand DNA synthesis) is located at the 3' boundary of the U5 domain. This is where the priming transfer RNA (tRNA) of host origin is attached by hydrogen bonds (Livak and Araya, 1982). Centrally located are the coding domains for the virion structural proteins and replicative enzyme RT organized 5' to 3' into gag, pol, and env regions. The group-associated gene (gag) sequences encode the polyprotein precursors of the internal core proteins (p15(C), p12, p27, p10), the polymerase (pol) region is responsible for the reverse transcriptase, and the envelope (env) sequences encode the envelope polyprotein precursor which is glycosylated and cleaved posttranslationally to yield gp70 and p15(E).

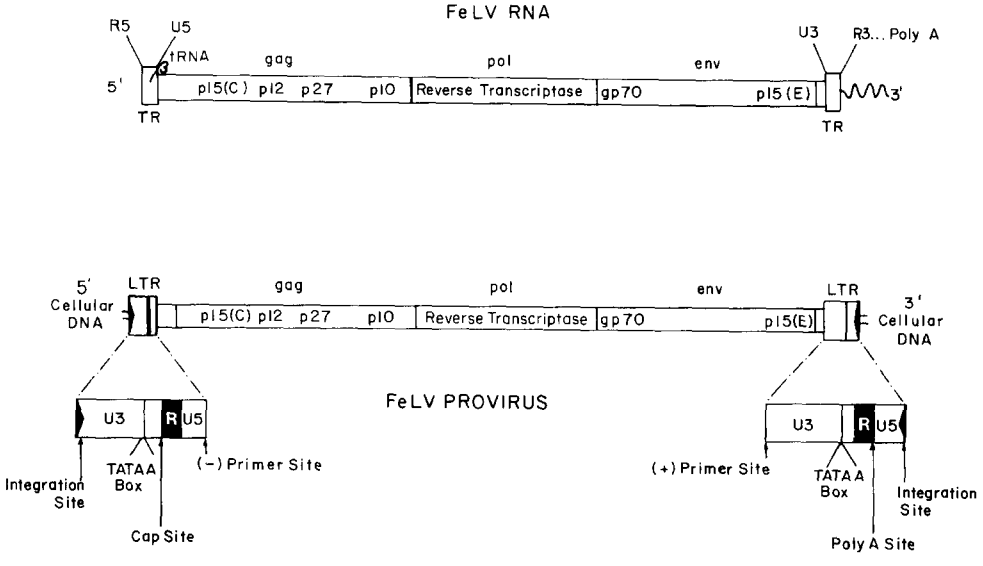

Figure 1. Structure of FeLV Viral RNA and FeLV Provirus. See Text Sections II.B.1,2 for discussion.

2. Structure, location, and significance of FeLV provirus

In the synthesis of the corresponding DNA provirus (Fig. 1), the U5 and U3 regulatory domains are duplicated and found at both the 5' and 3' ends in the order U3, R, U5 (reviewed in Varmus, 1982; Weiss et al., 1983). These redundancies are termed the long terminal repeats (LTR's) and are flanked at the 5' and 3' ends of the entire provirus by virus-specific inverted repeats of base pairs (IRs). These redundancies are critical to reverse transcription of the viral RNA, integration of the newly synthesized, circular FeLV provirus, and serve as nontranscribed regulators of expression of the integrated proviral DNA. Nucleotide sequence analysis of cloned retroviral LTR's (Hughes et al., 1978) predicts that LTR's provide functions critical to eukaryotic gene expression such as promotion, initiation, and polyadenylation of transcripts. The U3 sequence related to the "TATAA box" probably determines the initiation site for transcription.

The question whether proviruses integrate at random or into specific sites in vivo is not resolved. In a mass-infected population, proviral integration is multifocal (Hughes et al., 1978; Weiss et al., 1983). Recently, Mullins et al. (1981) have cloned exogenous (ecotropic) FeLV proviruses obtained from FeLV-infected human fibroblasts free of background retroviruses endogenous to feline DNA (see below). Their studies have revealed that replication-competent (infectious via transfection) and replication-defective (noninfectious) FeLV proviruses do not show preferred integration sites nor do they differ structurally. While provirus integration into virus-infected, nonneoplastic cells likely is random, it often is at preferred sites in neoplastic cells (Neel et al., 1981; Tschilis et al., 1983). It is thought that either the proviral insertion causes the neoplastic event or that cells with common proviral insertions are selected for during expansion of neoplastic clones. Preferred integration facilitates oncogenesis by promoter insertion in avian bursal lymphomagenesis. Using a cloned probe specific to the U3 region of the LTR of exogenous FeLV, Casey et al. (1981) have demonstrated that some FeLV-positive lymphomas have preferred integration sites and others do not. The significance of this to the leukemogenic process is commented on in Section III.C.3.

Infection of cycling cells with FeLV leads to the formation and stable integration of up to 40-50 proviruses into the cellular DNA. Integrated proviruses have been likened to a nasty genetic dowry (Bishop, 1982). These can be inserted at many sites, carry inherent potent regulatory elements in their long terminal repeats (LTRs), but also are subject to host regulatory influences (reviewed in Varmus, 1982; Weiss et al., 1983). Proviruses may act as insertional mutagens: even transcriptionally inactive (defective) forms interrupt and inactivate cellular genes which may direct the synthesis of proteins important to normal cell morphology, movement, or function. Alternatively, these may designate repressor substances responsible for the regulation of normal cell proliferation and adhesion properties.

Transcriptionally active FeLV proviruses institute viral replication and encode proteins with potential pathogenicity like the immunosuppressive p15(E) (see Section

III.C.1.). Production of sufficient infectious virus to cause viremia increases the probability of new proviral integrations and the likelihood of genetic damage. Proviruses may transduce cellular proto-oncogenes and generate replication-defective, acutely transforming viruses like the naturally occurring feline sarcoma viruses (see Section III.C.3). Alternatively, the proviral LTR regulator may evoke the expression of adjacent cellular genes. If these neighboring genes are cellular oncogenes (c-onc genes), the expression of the transforming proteins encoded may produce the transformed phenotype. This mechanism is called downstream promotion because the evoked genes usually are to the downstream (3') side of the upstream (5') viral LTR. Although not yet proven for FeLV, this is a common means by which other long latency leukemia viruses without intrinsic transforming genes cause neoplastic change (see Section III.C.3). Lastly, even transcriptionally inactive FeLV proviruses may serve as a reservoir of latent infectious FeLV. Reactivation of these genes in vivo or in vitro initiates replication and release of infectious FeLV (see Section III.B.9).

3. Endogenous feline retroviruses

The cellular DNA of the domestic cat contains two unrelated nucleic acid sequences homologous to two distinct retroviruses and represented by multiple copies (Niman et al., 1977a,b; Okabe et al., 1978; Koshy et al., 1980; Casey et al., 1981; Mullins et al., 1981; Soe et al., 1983). Eight to 10 copies of endogenous FeLV sequences per haploid genome consist of discrete, nontandemly arranged genes that differ qualitatively and quantitatively from exogenous, ectropic proviruses. The U5 LTR sequence is variable in location and copy number (up to 15 copies per cell) between cats. Each cat, however, has the same sequence organization in different tissues, indicating that the U5 pattern is inherited throughout the germ line. Cells infected with ecotropic FeLV have 10-fold increases in U5 sequences but, again, U5 patterns are characteristic to the cat, not endogenous versus exogenous sequences. In contrast, the U3 region of the LTR is shared between exogenous FeLV serotypes (see Section II.C.1) and is absent from endogenous FeLV and can be used to identify exogenously acquired FeLV sequences in neoplastic or nonneoplastic cat cells (Casey et al., 1981).

Insight into the origin and organization of endogenous FeLV elements has been provided by Soe et al. (1983) who have cloned and characterized endogenous FeLV sequences from a cat genomic library constructed from placental DNA of a specific-pathogen-free cat. Endogenous sequences reveal deletions in the gag-pol and env regions that render them incapable of encoding infectious virus and explain the subgenomic expression of endogenous FeLV in placenta and the non-inducibility of endogenous FeLVs from cat cells (Benveniste et al., 1975). It is likely that endogenous FeLVs have originated either by germ-line insertion of a complete ecotropic FeLV with subsequent deletion, or by insertion of a defective variant (Soe et al., 1983).

Other feline retroviruses, RD-114 and like viruses (McAllister et al., 1972; Benveniste et al., 1975) are unrelated to FeLV but are closely related to baboon

endogenous retrovirus, and have not been implicated in natural disease. These have xenotropic host ranges, and can be induced from, but will not infect, feline cells in vitro. RD-114 readily infects heterologous fibroblasts and was isolated originally from cat-passaged, human rhabdomyosarcoma cells (McAllister et al., 1972). Although normally repressed in vivo, RD-114 mRNA is expressed in placenta, in aging animals, and in some FeLV-positive and FeLV-negative tumors (Niman et al., 1977a,b) but expression probably is fortuitous to the transforming event. An unusual recombinant FeLV/RD-114 virus is discussed in Section II.C.1.

C. FeLV as a Disease-Causing Agent

1. Role of serotypes and viral recombinants

$FeLV_A$ is the most common subgroup isolated from naturally infected pet cats, replicates to high titers in cats, and always is present when mixtures of FeLV subgroups are isolated. $FeLV_A$ has a narrow host range in vitro (Sarma and Log, 1973), being mostly restricted to cat cells. $FeLV_B$ always is isolated in conjunction with $FeLV_A$, replicates to lower titers and $FeLV_B$ viremia is delayed in appearence relative to $FeLV_A$ viremia. $FeLV_B$ has an extended host range in vitro. $FeLV_C$ only is recovered if $FeLV_B$ and $FeLV_C$ also are present (Sarma and Log, 1973; Jarrett and Russell, 1978) and replicates in cat, human and guinea pig cells.

Evidence has accumulated suggesting that FeLV subgroups induce different types of disease. Inoculation of susceptible kittens with the Ohio State passaged Rickard strain of FeLV-FeLV-R (subgroup AB) leads to a high incidence of viremia (~85%), severe immunosuppression, and thymic lymphomas in those animals surviving 17 to 30 weeks (Hoover et al., 1972; 1973). In contrast, exposure of cats to the Glasgow passaged Rickard strain of FeLV, which contains subgroup A only, generates a low incidence of viremia, hemorrhagic enteritis and neutropenia, and occasional alimentary lymphomas or myelogenous leukemias after a very long latent period (Jarrett et al., 1971; 1973). Although not associated with a specific disease state, $FeLV_B$ is intensely cytopathic for feline marrow cells in vitro (Onions et al., 1980). Recent reports of homologous sequences shared by $FeLV_B$ and a murine retrovirus cytopathic for mink cells (mink cell focus-forming-MCF-virus) have raised speculations as to the origin and leukemogenicity of $FeLV_B$ (Elder and Mullins, 1983). MCF viruses are spontaneous env gene recombinants between ecotropic and xenotropic retroviruses with high leukemogenicity in vivo, cytopathicity and extended host range in vitro. $FeLV_B$ is the most frequent helper FeLV implicated in natural transduction of the fes proto-oncogene from cat fibroblasts and the resultant generation of acutely transforming feline sarcoma viruses (FeSVs).

The extreme delay in the appearance of free, infectious $FeLV_C$ in plasma may result from its relative defectiveness for replication in vivo. Vedbrat et al. (1983) report that even cells that produce only $FeLV_A$ or $FeLV_A$ and $FeLV_B$ as cell-free virus have many partially replicated, immature $FeLV_C$ buds embedded in the plasma membrane and suggest that the partial expression of $FeLV_C$ is equivalent to FOCMA-L expression (see Section

III.C.3). Based on its delay in appearance and defectiveness for replication of $FeLV_C$ and induction by $FeLV_C$-devoid viral stocks, many workers have considered $FeLV_C$ also a candidate recombinant virus.

Another putative recombinant virus isolated by in vitro cloning of $FeLV_A$ substrains has envelope characteristics similar to both FeLV and RD-114 (Haberman and Velicer, 1980). Further characterization of this strain, PR-8, which is partly cytopathic for feline fibroblasts in vitro and induces eosinophilic leukemia or hypereosinophilia in vivo (Lewis et al., 1983b), should enhance the understanding of the relationship between env gene products and biologic activity of retroviruses.

The Kawakami-Theilen strain of FeLV(-FeLV-KT) is a mixture of $FeLV_{ABC}$, and causes profound erythrosuppression and death within 9 weeks of inoculation of neonatal kittens but is virtually apathogenic in weanlings and adults (Hoover et al., 1974; Mackey et al., 1975; Onions et al., 1982). Passage of $FeLV_{ABC}$ through guinea pig cells in vitro selects for biologically cloned $FeLV_C$ (Sarma strain) (Sarma and Log, 1973). That this $FeLV_C$ induces viremia and erythroid aplasia in vivo and suppression of erythroid colony formation in vitro suggests a direct relationship between productive $FeLV_C$ infection and anemiagenesis (Onions et al., 1982).

2. Requirement for viremia

The induction of FeLV-related disease usually follows the onset of marrow origin viremia. Even though the leukemogenic event probably is random, high levels of viremia ensure that the damaging virus will be present when cells of the appropriate histogenesis and maturation reach a critical stage in their cycle. In the mouse, (see Section III.C.3), chronic antigenemia with MuLVgp71 incites chronic immunostimulation of uninfected T-cells and renders them susceptible to the leukemogenic event. In cats, circulating virion proteins are immunosuppressive (FeLVp15(E), see Section III.C.1), erythrosuppressive ($FeLV_C$gp70?, see Section III.C.2), and embryosuppressive (Hoover et al., 1983) and result in deaths long before leukemia can develop. Other circulating virion proteins may be complexed to antibody to trigger immune complex disease. Even transient marrow origin viremia and probably mononuclear cell-associated viremia are important. It is true that cats with these usually develop immunity. However, the heavier the original FeLV burden, the smaller the likelihood that the cat will eliminate all FeLV-infected cells, and the larger the likelihood the cat will maintain persistent poorly expressed FeLV infections and risk nonproducer disease (see Section III.B.9).

III. BIOLOGIC RESPONSES OF THE CAT TO FeLV

A. Hemolymphoreticular Cells as Targets for FeLV Replication

1. In Vivo

The pathogenesis of spontaneous viral infections often centers around a virus/host lymphomyeloid reciprocity in which certain cells are targets for productive or nonproductive (latent) infection, others are targets for cytosuppressive or cytoproliferative disease, and others are effectors of antiviral or antitumor resistance (Rouse et al., 1978;

Profitt, 1979; Huddlestone et al., 1980). A sequence of lymphoreticular/virus interactions characterizes the infections of cats with FeLV: initial virus replication in hemolymphoreticular cells (see Section II.A), establishment of persistent productive versus self-limiting (persistent nonproductive) lymphomyelopoietic infection (see Section III.B.1), and development of FeLV-related neoplasia (see Section III.C.3) or aplasia (see Sections III.C.2,3) of the lymphoid or hematopoietic system. Available evidence suggests that the histogenesis and immunologic identities of the cells involved in early virus replication, virus containment and latency, chronic (preleukemic) virus replication, and eventual oncogenesis are divergent (Table 2).

Table 2. Hemolymphoreticular targets for FeLV Infection

Type of Infection	Target Cells
Acute Productive	Circulating T lymphocytes B lymphocytes Macrophages Myeloid Series
Productive Preleukemic	B lymphocytes Macrophages Myeloid Series Neoplastic T lymphocytes
Latent	Myelomonocytic Precursors Nodal T lymphocytes Macrophages

a. Acute productive infection

Oronasal exposure of cats to FeLV-R simulates natural exposure and initiates FeLV replication in lymphoreticular cells in the tonsil, blood, germinal centers of lymphoid tissues, thymic medulla, and bone marrow, in that order (Rojko et al., 1979a). Apparently, closely related events occur in other horizontally transmitted oncogenic viral infections, e.g., primary infections with the human EBV (Epstein and Achong, 1977), murine mammary tumor virus, and Marek's disease herpesvirus. It is thought that EBV enters the oropharynx, infects tonsillar and blood lymphocytes with complement receptors and specific EBV receptors, and is disseminated via an early lymphocyte-associated viremia. The rationale for FeLV infection of specific lymphoid subsets, whether due to distribution of FeLV receptors, capacity for spontaneous DNA synthesis, and distribution or migration patterns in vivo, currently is not understood.

b. Productive preleukemic infection

In progressive FeLV infections, a persistent polyclonal infection of follicular lymphocytes and bone marrow precursors but not thymocytes in association with protracted lymphopenia and neutropenia (Rojko et al., 1979a; 1981) and limited anti-FeLV

humoral responses during preleukemia precede the emergence of neoplastic T-cells in the thymus and elsewhere (Rojko et al., 1979a; Hoover et al., 1978). The principal FeLV-infected cell in the lymph nodes of viremic cats is a nonadherent, CR-bearing B-lymphocyte (Rojko et al., 1981). P27 is diffuse in large (20-45 um) lymphoblasts with eccentric, large, round to cleaved nuclei and prominent nucleoli. Plasma cells are densely stained and are identified by eccentric round nuclei with highly condensed chromatin, dense homogeneous cytoplasm at the rounded cell periphery and a perinuclear (Golgi region) clear space. The pattern of increasing intensity of intracytoplasmic p27 staining with increasing differentiation in B-lineage cells is reminiscent of that described for myelomonocytic series cells in the bone marrow. Purified T-cells and adherent macrophages are not particularly enriched for infectious FeLV nor do they contain p27.

The functional integrity of the B-lymphocytes that replicate FeLV is unknown. In the mouse, splenic B-lymphocytes that are concurrently infected with MuLV produce less antibody to sheep erythrocytes than do uninfected spleen cells, but this is most probably due to increased T-suppressor activity (Cerny and Stiller, 1975). Naturally viremic pet cats have adequate IgM but ineffective and delayed IgG responses to a synthetic T-dependent antigen and a systemic T-helper defect has been postulated (Trainin et al., 1983). Experimentally-induced viremic cats also produce IgM but not IgG antibody in response to FeLV-associated antigens (Mathes et al., 1981). These facts may signify altered B-cell function, altered regulation by T-helper, T-suppressor or accessory cells, or a changed microenvironment (FeLV-infected lymph node versus control lymph node). Regarding the last, relative increases in nodal T-cells and FcR-cells have been reported to be simultaneous with the FeLV-associated peripheral blood lymphopenia in preleukemia (Rojko et al., 1981). Redistribution of lymphoid subpopulations also has been reported for preleukemic MuLV infections (Gillette and Fox, 1980), and increased numbers of CR- and Fc R-positive cells have been described in preneoplastic murine mammary tumor virus infections (Epstein et al., 1978). The probable mechanisms important in preleukemic immunomodulation are described further in Section III.C.1.

A critical feature that distinguishes persistent productive FeLV infection from persistent nonproductive infection is viral replication by differentiating granulocytes and macrophages in the marrow and elsewhere. Furthermore, marrow origin viremia is accepted universally as the harbinger of fatal FeLV-associated disease (Hardy et al., 1973; Hoover, et al., 1977). In contrast, in cats that regress productive marrow infection and develop latent FeLV infection (see Sections III.B.1.d, III.B.9), refractoriness of myelomonocytic series cells to FeLV replication is correlated directly with increasing maturation. Even in viremic cats which experience extensive FeLV replication in myeloid progenitor cells, differentiated granulocytes, and marrow adherent macrophages, most mature peritoneal macrophages are spared (Hoover, et al., 1981). It is speculated that these are derived from uninfected myeloid clones, or become refractory to, or abort FeLV infection during, the process of differentiation in vivo (Hoover et al., 1981; Rojko et al.,

1982a). This relationship between macrophage susceptibility to productive viral infection and disease progression also holds for mice infected with the leukemogenic Friend MuLV complex (Marcelletti and Furmanski, 1979), and was described originally for mice and monkeys infected with such non-oncogenic viruses as herpes, corona, pox and flavi viruses (Mogensen, 1979; Daughaday et al., 1981; Peiris et al., 1981; Schlesinger and Brandriss, 1981). Susceptibility versus resistance of macrophages to viral replication is central to the mechanism of age-related susceptibility versus resistance to viral persistence. This topic is amplified in Sections III.B.2,3.

c. Productive leukemic infection

FeLV replication in follicular (B) lymphocytes and myelomonocytic series cells is a constant feature of persistent productive FeLV infection. In contrast, replication in putative T-cell regions is limited to the recirculating lymphocyte pool and to the thymic medulla early in infection (Rojko et al., 1979a). The disappearance of replicating FeLV from T-cell areas during preleukemia is associated with lymphopenia (Rojko et al., 1979a; Cockerell et al., 1976a,b,c) loss of circulating T-suppressor cell (Stiff and Olsen, 1982) and T-cell mitogenic function, thymicolymphoid atrophy (Perryman et al., 1972; Hoover et al., 1973), and redistribution of latently infected T-cells to the mesenteric lymph nodes (Rojko et al., 1981), and precedes the emergence of productively infected T-lymphoma cells in the subcapsular cortical thymus and elsewhere (Cockerell et al., 1976a; Hoover et al., 1978; Rojko et al., 1979). Neoplastic lymphocytes from experimentally and naturally infected cats usually bear T-cell markers (E rosette forming capacity, surface thymocyte antigen - Cockerell et al., 1976a) but rarely do appear as SIgG-bearing B-cells or null cells (Hardy et al., 1977). The relative maturity of the transformed T-lymphoma cells is demonstrated by its lack of terminal transferase. Similar dissociation between lymphocyte tropisms for viral replication and virus-associated transformation have been observed in Moloney and AKR murine lymphomas (Lee and Ihle, 1979; Isaak and Cerny, 1983) and its implications are summarized in Section III.C.3.

d. Latent infection

Cats that regress productive marrow and lymphoid infection and become immune must either eliminate all cells with integrated FeLV proviruses or risk persistent nonproductive infection. Cells most likely to escape immune elimination are those with a long interphase as retroviral antigens are maximally expressed in mitotic cells. Based on this premise, candidate marrow and lymphoid cells would include the slow-to-cycle committed myelomonocytic precursor defined in the mouse by the in vitro spleen colony-forming unit assay (CFU_S of Till and McCulloch [1964]), memory lymphocytes, and long-lived T-lymphocytes. While none of these cells have been identified in the cat, it is known that the target cells for latent FeLV infection are compatible: marrow myelomonocytic precursor cells, macrophages, and Staphyloccocal Protein A (SPA)-reactive T-lymphocytes in the systemic lymphoid tissue. Reactivation and consequences of latent infection are considered in Sections III.B.9, III.C.3.

2. In Vitro

a. Replication of FeLV in lymphocytes

The age-related susceptibility (Hoover et al., 1976; see Section III.B.2) of kittens versus adults to progressive FeLV infection in vivo is paralleled by 50-fold and 3-5-fold increments in the susceptibility of lymphocytes (Rojko et al., 1981) and peritoneal macrophages (Hoover et al., 1981; see Section III.B.3), respectively, to productive infection in vitro. Freshly isolated, peripheral blood mononuclear leukocytes (PBML) are not permissive to productive infection with FeLV-R. Induction of viral replication is dependent upon incubation of PBML for 48 hours prior to exposure to FeLV-R. This refractoriness of freshly isolated PBML would be compatible with a requirement for unmasking or synthesizing cell surface receptors for FeLV during in vitro incubation as has been described for poliovirus infection of primary kidney cells. However, freshly isolated PBML can absorb and transfer infectious FeLV to autochthonous susceptible peritoneal macrophages (MΦ) and, therefore, are capable of passive vection or reversible receptor binding (Rojko et al., 1981).

Alternatively, differential susceptibility between freshly isolated and aged PBML could reflect alterations in the lymphocyte populations present at 0 hours versus 48 hours of incubation. Many PBML, especially unstimulated B and T lymphocytes, are short-lived in vitro. Only 40-70% of the feline PBML originally isolated are viable after 48 hours and feline T cells lose their FcR within 24 hours in vitro (Rojko et al., 1982b; Stiff and Olsen, 1982). Furthermore, the removal of FcR-positive PBML at the time of culture initiation leads to a 1325-fold enhancement of productive infection when preincubated PBML are infected with FeLV-R (Rojko et al., 1981). This suggests that naturally occurring FcR cells limit the outgrowth of FeLV-infected cells or maintain FeLV-infected cells in a virus-nonproductive state. Similar suppression of EBV-infected cells by EBV-naive T cells with FcR is known to be mediated by interferon (Thorley-Lawson, 1980). Interestingly, the preincubation prerequisite for productive infection of PBML is not applicable to mononuclear leukocytes isolated from marrow or thymus (Onions et al., 1980; Rojko et al., 1983) and feline marrow and thymus essentially are devoid of FcR-bearing, nonadherent lymphocytes (Rojko et al., 1982b).

The heterogeneity in target cell susceptibility to infectious FeLV-R has been investigated using quiescent and mitogen-activated cells from blood, spleen, lymph node, thymus and marrow cultured with lymphocyte growth factors (Rojko et al., 1981). Lymphocytes of all histogenetic origins are permissive to FeLV replication. Activation of lymphoid subsets by differential mitogenesis may lead to enhanced lymphocyte survival in vitro, the generation of more target cells, DNA synthesis, and favorable conditions for proviral integration and replication. Alternatively, residual mitogen may inactivate virus, or mitogen-activated lymphoblasts may have direct antiviral activity as has been described for PHA-stimulated human lymphoblasts exposed to coxsackie or influenza-viruses. Lastly, activated lymphoblasts may suppress the outgrowth of FeLV-infected cells as has been reported for human lymphocyte cultures exposed to EBV (Thorley-

Lawson, 1980). In the case of the FeLV/feline lymphocyte model, it appears that FeLV-infected or sham-infected lymphoblasts may be initiated spontaneously or by exposure to mitogen. Continued growth is dependent upon the inclusion of lymphocyte conditioned medium as a source of growth and viability factors only (Rojko et al., 1981). Permissiveness to productive infection, however, may be enhanced by the feline FcR and T cell mitogen, SPA, and the B cell mitogen, lipopolysaccharide (LPS). Other T cell mitogens (phytohemagglutinin-PHA, pokeweed mitogen (PWM), and concanavalin A (conA) and a B cell mitogen (dextran sulfate - DXS) have inhibitory effects on FeLV replication (Rojko et al., 1981) by currently unexplained mechanisms.

b. Replication of FeLV in macrophage

Thioglycollate-stimulated, peritoneal macrophages isolated from FeLV-naive cats have been reported to be inhospitable hosts for FeLV in vitro (Hoover et al., 1981). The susceptibility of macrophages is augmented 200-600-fold when peritoneal macrophages from FeLV-naive cats are exposed to various concentrations of hydrocortisone and is comparable to that of highly permissive embryonic cell cultures used to propagate FeLV in vitro. Glucocorticoid enhancement is specific for cells of the macrophage series: titers increase 200-600-fold in mature peritoneal macrophages, 3-4-fold in marrow adherent macrophages, and not at all in circulating monocytes or lymphocytes of variable histogenesis (spleen, node, blood, thymus). As retroviral replication normally decreases with cell maturation, macrophages should be relatively nonpermissive cells. In other cell culture systems, glucocorticoids are thought to exert their most dramatic effects in naturally nonpermissive cells. Both induction of viral receptors and induction of viral synthesis in nonproductively infected cells have been proposed as mechanisms. Although glucocorticoid receptor sites have not been determined for feline macrophages, high-affinity binding sites for glucocorticoids are present on thioglycollate-elicited peritoneal macrophages in mice (Schultz et al., 1979). In the cat macrophage, hydrocortisone treatment evokes a doubling in the DNA synthetic rate but a 200-600-fold enhancement of the viral replication rate (Hoover et al., 1981). This finding suggests that increased FeLV production is related not simply to more cells entering mitosis, but probably also is related to more progeny virus being produced per cell or more total cells replicating virus. In murine mammary tumor virus-infected cells, the triggering of messenger RNA synthesis is associated with the triggering of proviral transcription and the increased production of progeny virus (Lee et al., 1981). It is thought that a complex of hormone and host-coded receptor interacts specifically with the MMTV LTR. Similarly, corticosteroids minimally alter the replication of ecotropic MuLV in permissive mouse cells but markedly increase its replication in nonproductively infected mouse embryo cells (Dunn et al., 1975).

Glucocorticoids influence the in vivo cat/FeLV relationship remarkably. Treatment of otherwise resistant cats with prednisolone leads to abrogation of resistance and induction of persistent viremia (see Section III.B.3a). Moreover, treatment of immune cats with corticosteroids leads to reactivation of latent FeLV from marrow adherent

macrophages and myelomonocytic precursor cells (see Section III.B.9) and precipitates transient or self-perpetuating productive marrow infection. Thus, the available data concerning glucocorticoids and retrovirus expression suggest that glucocorticoids enhance susceptibility of cats by converting the macrophage/FeLV relationship from a nonpermissive state and immunity to a permissive state characterized by early viral amplification and persistent viremia.

B. Hemolymphoreticular Cells as Effectors of FeLV Restriction

1. General Remarks

Either of two host/virus relationships usually ensues in cats exposed to FeLV: persistent viremia culminating in FeLV-related disease (Hardy et al., 1976; Hoover et al., 1976; Rojko et al., 1979a) or self-limiting infection in which productive hemolymphatic infection is transient and elicits protective titers of VN and FOCMA antibodies. In a few cats infected with FeLV, viremia is present in association with moderate titers of FOCMA and/or VN antibodies.

The host/virus interactions that determine the outcome of FeLV infection occur in the lymphoreticular tissues, the blood, and in the marrow in the first 4-6 weeks after experimental exposure (Hoover et al., 1976, 1977a; Rojko et al., 1979a). This interplay is critically dependent upon the functional integrity of these tissues and the age-related, immunocompetence of the cat (Hoover et al., 1976; Schaller et al., 1978; Hoover et al., 1981; Rojko et al., 1979a,b,1981; Tarr et al., 1979). As described fully in Section II.A.6, natural (oronasal) contact with FeLV initiates sequential FeLV replication in local and systemic lymphoid tissues, blood and marrow. Complement-associated, non-immune factors in normal cat serum probably limit viremia (Bartholomew and Esser, 1978; Kobilinsky et al., 1979) and are known to be age-dependent in other systems. Age-related increases in immunocompetence usually reflect age-related increases in macrophage antiviral, antitumor, or antigen processing functions (Unanue, 1980). In addition, many viruses, including FeLV, take up permanent residence in macrophages concordant with the establishment of persistent infection and, sometimes, macrophage dysfunction (Marcelleti and Furmanski, 1979; Mogensen, 1979; Hoover et al., 1981; Peiris et al., 1981; Schlesinger and Brandriss, 1981). Natural killer activity also is age-related, and often is vital to the containment of neoplastic and/or virally infected cells (especially) in marrow and lymphoid tissue (reviewed in Herberman, 1982).

When FeLV is replicated by lymphoid and marrow cells, FeLV antigens and, probably, FOCMA are expressed at the plasma membrane in intimate association with leukocyte alloantigens (Axocar and Essex, 1979; Dubey et al., 1982; Lee et al., 1982). Moreover, leukocyte alloantigens may be incorporated into the viral envelope when the virion matures. It is well established that new or viral antigens coupled to alloantigens are potent immunogens. The FeLV-feline lymphoma model is unique in that surveillance of tumor cells is mediated primarily by complement dependent antibodies (Essex et al., 1971a,b,1975b; Grant and Michalek, 1981; Grant et al., 1977,1980a,b,1981) and not by T-

effector cells. Antibodies against envelope virion components provide protection from marrow origin viremia (VN antibody - antigp70) (Schaller and Olsen, 1975; Hardy et al., 1976; Russell and Jarrett, 1978; Lutz et al., 1980) and antibodies against virion RT are speculated to limit FeLV replication. Recent reports propose the simultaneous involvement of T-effector cells in the containment of FeLV-infected marrow cells (Rojko et al., 1982a) and FeSV-transformed, autochthonous, fibroblasts (McCarty and Grant, 1983).

It also is important to consider that inadequate or excessive immune responses may promote viremia or directly damage the host. The presence of the immunosuppressive virion components p15(E) in lymphoid tissues may render immune responses to FeLV or other antigens inadequate and may especially hamper T-helper function (see Section III.C.1). Antigen/antibody complexes may perpetuate viremia (Day et al., 1980; Jones et al., 1980; Snyder et al., 1982) and may participate in the immune-mediated disorders like immunosuppression (Cotter et al., 1975), chronic glomerulonephritis (Anderson et al., 1971; Jakowski et al., 1980) and hypocomplementemia (Kobilinsky et al., 1979) associated with persistent productive FeLV infection. Lastly, a critical concomitant of immunity to FeLV is its interdependence on persistent nonproductive (latent) infection of local lymphoid tissues and/or systemic hemolymphoreticular tissues in cats that fail to eliminate all virally-infected cells consequent to acute productive infection (see Sections III.A.1, III.B.9).

This section will review evidence for hemolymphoreticular cell restriction of FeLV infection, its consequences and present the probable stages of FeLV replication that evoke individual responses.

2. Age-related susceptibility versus resistance to FeLV viremia

By controlling the virus dose and strain, using specific-pathogen-free cats with homogenous genetic background and environment, and varying the host age at time of FeLV inoculation, cats can be segregated prospectively into those destined to develop either viremic or immune FeLV infections. Cats at risk for viremia include neonatal kittens (100% susceptible) and 8-week-old weanlings (85% susceptible) (Hoover et al., 1976a). Age-related resistance has been demonstrated for lymphomagenic FeLV-R, anemiagenic FeLV-KT, the acutely transforming FeSVs, and several FeLV strains isolated from naturally infected pet cats. Although the susceptibility of cats exposed to FeLV in nature may vary greatly, the rate of viremia conversion in adult urban cats naturally exposed to FeLV-shedding cats in multiple cat households ranges from 15-28%. Socio-environmental factors that influence susceptibility are considered in Section IV.B.

3. Macrophage function and FeLV resistance

Although the phagocytic capacities of isolated macrophages from kittens and adults are equivalent, the mean FeLV susceptibility of macrophages from kittens is five times that of macrophages from adult cats. This maturation-dependent, refractoriness to FeLV replication is overcome readily by specific enhancement by treatment with hydrocortisone in vitro (Hoover et al., 1981) or prednisolone in vivo (Rojko et al., 1979b). It also

is abrogated when macrophages are poisoned by microcrystalline silica (Hoover et al., 1981) or depleted in the marrow and lymphoid necrosis induced by the resorptive carcinogen, methynitrosourea (Schaller et al., 1978; Tarr et al., 1979).

a. Adrenal corticosteroids

Treatment with various doses of a synthetic adrenal corticosteroid, methyl prenisolone acetate, has resulted in a 7-fold augmentation of susceptibility of adult cats to persistent FeLV-R or persistent FeLV-KT viremia. These cats eventually die of FeLV-related immunosuppression, lymphoma, or anemia. Similar pretreatment of adult mice with cortisone acetate has enhanced susceptibility to oncogenesis by Moloney murine sarcoma virus (Schachat et al., 1968). The distribution of FeLV p27 in the tissues of cats exposed to concurrent FeLV and prednisolone is compatible with the hypothesis that corticosteroids enhance FeLV replication in, and impair FeLV containment by, macrophages in lymphoid and marrow tissues (Rojko et al., 1979).

In cats treated with the lower doses of prednisolone (5 mg/kg), hematologic changes specific to FeLV/corticosteroid interactions are not masked by the overwhelming neutrophilic leukocytosis that accompanies higher dose regimens in FeLV-naive or FeLV-treated cats. Induction of persistent viremia in kittens and corticosteroid-treated adults is correlated with prolonged neutropenia and lymphopenia. Conversely, virus containment in regressor adult cats is associated with transient lymphopenia only. In those FeLV-steroid-treated adults that regress infection, marrow infection is prolonged by 4-10 weeks. Eventual virus restriction follows protracted neutropenia as well as lymphopenia. Furthermore, adult cats treated with 5 mg/kg are able to respond to FOCMA. FOCMA antibody titers are minimal and transient in progressive infections in kittens and adult cats treated with high doses of steroids (mean peak titer $\geq$1:4), intermediate in progressive infections in adult cats treated with low doses of steroids (mean peak titer 1:27) and are marked and persistent (1:78) in regressive infections in adult cats.

That corticosteroids are important in regulation of FeLV replication by bone marrow myelomonocytic precursors also may be inferred from their unique capacity to reactivate latent FeLV infections (see Section III.B.9).

b. Silica

Administration of silica before virus inoculation also markedly enhances the FeLV susceptibility of adult cats. Silica is toxic for isolated macrophages but not lymphocytes in vitro, and silica produces monocytopenia and neutrophilia, delayed skin allograft rejection, and augmented FOCMA antibody responses in vivo (Hoover et al., 1981). These augmented FOCMA antibody responses are similar to those seen in the FeLV-low dose corticosteroid cats described above and probably reflect intermittent antigenemia.

c. Methylnitrosourea (MNU)

Co-exposure of adult cats to FeLV and MNU leads to a 6-fold enhancement of susceptibility to persistent viremia disease. Lymphoreticular and marrow necrosis by 2-4 DAE cause profound leukopenia and immunosuppression. MNU administered alone

prohibits allograft rejection and reduces lymphocyte blastogenic responses dramatically (Schaller et al., 1978; Tarr et al., 1979).

It is clear that macrophages participate in early virus restriction and that persistent FeLV replication in macrophages and regression of viremia are inversely correlated. A similar relationship has been reported for Friend MuLV leukemia and for murine coronaviruses and primate flaviviruses (Marcelleti and Furmanski, 1979; Mogensen, 1979; Peiris et al., 1981; Schlesinger and Brandriss, 1981). Macrophages may be refractory to viral replication by virtue of their failure to express viral receptors. If the host produces antiviral antibodies which interact with virus and generate immune complexes rather than neutralized virus, the macrophage may internalize the complex via Fc receptors. Once inside a cell, antibody with extracellular neutralizing capability is ineffectual at inhibiting virus replication (Daughaday et al., 1981). This may be a consideration in the persistence of FeLV in marrow macrophages and extracorporeal removal of immune complexes containing antibody, gp70, p27, and p15(E) from viremic cats leads to the remission of viremia.

4. Humoral immunosurveillance

First described in 1971 (Essex et al.), the concept that antibodies are protective against neoplasms induced by FeLV and FeSV has withstood 12 years of experimentation. In regressor cats exposed to FeLV or FeSV by experimental inoculation, IgG antibodies to FOCMA recognized by indirect membrane immunofluorescence (IMI) assay against virus producer, feline lymphoma (FL74) cells first appear at 2-3 WAE. and persist at 1-2 $\log_2$s below peak titer for years (Essex et al., 1975b). Persistence probably is due to intermittent stimulation by FOCMA expressed on latently infected, neoplastic or preneoplastic cells (see Section III.B.9).

Actual clearance of producer and nonproducer neoplastic cells probably is mediated by cytotoxic, complement-dependent antibody (CDA) to FOCMA (Mathes et al., 1976; Grant et al., 1977, 1980a,b, 1981). Antibodies directed against FL74 cells that bind rabbit complement appear early (2-3 WAE), peak early (5 WAE) and abate rapidly (_8-9 WAE). Rabbit complement-fixing antibodies cause rapid lysis (4 hours) of FL74 cells, do not correlate with IMI-FOCMA antibodies, and are absorbed by disrupted FeLV. Hence, these are more likely acute phase CDA to FeLV and not to FOCMA. In contrast are the antibodies to nonvirion determinants of FL74 cells that bind cat complement and initiate slow lysis (20 hr) of FL74 cells (Grant et al., 1977). These arise later and persist throughout the cat's lifespan to prevent emergence of producer or nonproducer lymphoma cells in vivo (Grant et al., 1980a,b,1981). Moreover, regressor cat serum with FOCMA activity will prevent the spontaneous reactivation of FeLV from autologous, latently infected, bone marrow cells in vitro and, presumably, functions similarly in vivo. Whether this antireactivation activity is directed against FOCMA or against normal marrow leukocyte alloantigens that intimate with FOCMA on the surface of neoplastic or preneoplastic cells (Azoar and Essex, 1979; Grant and Michalek, 1981; Dubey et al., 1981; Lee et al., 1982) deserves further study. Regarding the last, Grant and Michalek

(1981) have examined a panel of FOCMA-CDA containing alloantisera against a panel of virus producer, feline lymphoma cells. The sera identify not a single FOCMA, but rather unique and cross-reacting epitopes and react occasionally with normal splenic lymphocytes. The possibility that FOCMA is a leukocyte alloantigen or the incomplete expression of $FeLV_C$ *env* gene products at the plasma membrane is discussed further in Section III.C.3.

Mathes and Olsen (1981) have recently measured the temporal expression of IgM and IgG antibody titers to FOCMA-IMI assay in cats destined to experience progressive *versus* regressive infection. In regressors, IgM antiFOCMA appears shortly after experimental exposure and lasts 3-5 weeks. The decline in IgM antiFOCMA is accompanied by rapid increases in IgG antiFOCMA that peak at weeks 6-10 and persist at slightly decreased titers thereafter. This IgM to IgG conversion is the expected response to horizontally transmitted viral infection and is regarded as a T-helper-dependent response. The response in cats destined to become viremic is quite different. IgG antiFOCMA responses are low or absent in the face of IgM antiFOCMA responses that persist at relatively constant levels until death via FeLV-related disease. The reasons for this IgM persistence and failure of IgG conversion presently are not known. Naturally FeLV viremic pet cats are known to have ineffective IgG and adequate IgM responses to a synthetic polymer TGAL and it is postulated that this is due to defective T-helper function (Trainin et al., 1983). Cowan suggests that persistence of IgM antibody often is due to recurrent antigen stimulation (1973). In humans given yellow fever 17D live virus vaccine, the persistence of this virus in macrophages (Schlesinger and Brandriss, 1981) leads to persistent IgM responses. It is speculated, therefore, that replication of FeLV in macrophages of viremic cats and T-helper defects may contribute to effete IgG responses. The ensuing antigenemia would then trigger IgM responses continually.

As more cats die of aplastic FeLV-related diseases than die of proliferative FeLV-related diseases (Cotter et al., 1975) and as the relative risk for lymphoma increases with duration of viremia, it is obvious that most cats must limit marrow origin viremia to remain disease free. Protection from marrow origin viremia is mediated by virus neutralizing (VN) antibody directed against subgroup specific envelope gp70s (Schaller and Olsen, 1975; Hardy et al., 1976; Hoover et al., 1977; de Noronha et al., 1978; Russell and Jarrett, 1978; Lutz et al., 1980). Binding of VN antibody to gp70 prevents adsorption of FeLV to cellular FeLV gp70 receptors. Antibody to gp70 with biologic activity (neutralization or reverse interference) does not achieve high titers *in vivo* until 6-8 WAE. The delay in its appearance likely is due to dilution by serum and consumption by FeLV-infected cells. Antibody to gp70 with high VN activity *in vitro* is protective in allogeneic cats to which it is passively transferred either via the colostrum (Hoover et al., 1977) or via systemic inoculation (de Noronha et al., 1978; Cotter et al., 1980). Prevention of viremia in susceptible animals is an early event. Passive transfer of serum from cats or goats hyperimmune to FeLV gp70 or the cross-reacting MuLV gp71 must occur within the first 6 DAE of susceptible kittens to FeLV to protect against induction

of marrow origin viremia (de Noronha et al., 1978). Occasional reports suggest that passive transfer of antibody to gp70 will lead to partial remission or enhanced response to chemotherapy in lymphomatous cats (Cotter et al., 1980).

The development of ELISA and sensitive radiolabelled immunoprecipitation and fluorographic techniques has made possible the observation that viremia and antibody to gp70 can coexist (Lutz et al., 1980; Mathes and Olsen, 1981). It is not uncommon for these cats also to have persistent IgG FOCMA antibody titers and/or evidence of antibody to p27, p15(C) and p15(E). While cats with this pattern usually are considered to be healthy FeLV carriers and probably maintain antibody titers for years before succumbing to FeLV-related disease, the presence of an antibody excess, antigenemic situation may precipitate immune-mediated disorders including chronic glomerulonephritis and hypocomplementemia (Kobilinsky et al., 1979; Jones et al., 1980; Snyder et al., 1982).

5. Cell mediated responses of normal feline cells

The distribution of feline lymphocyte markers has been correlated with histogenetic origin (Taylor et al., 1975; Cockerell et al., 1976,a,b,c; Rojko et al., 1982b; Rojko et al., 1983b). The majority of feline PBML are T-cells (50% erythrocyte-rosette forming cells (E-RFC), 23% thymocyte-antigen-positive) that respond strongly to the mitogens con A (stimulation index (SI) = 80) and SPA (SI = 81), well to PWM (SI = 15) and only moderately to PHA (SI = 3). Less frequent cells are circulating B lymphocytes (34% erythrocyte-antibody complement-RFC, 30% surface-Ig-positive) that respond nicely to LPS (SI = 9) and moderately to DxS (SI = 3). On 15-20% of feline PBL, surface indicators for either T or B cells can not be demonstrated.

Observations regarding mitogen responsiveness of T lymphocytes from blood, spleen, LN, and thymus suggest that separate subpopulations of T lymphocytes are each stimulated by PHA, PWM and con A. PHA-lymphocyte activation is greatest in thymus (SI = 12) and LN (SI = 11), intermediate in spleen (SI = 6) and low in PBL (SI = 3). This identifies the feline PHA-responsive T-lymphocyte as a non-recirculating, relatively immature cell. The presence of relatively immature T-cells in feline LN manifests the unique ontogeny of the feline lymphoid system. During embryologic development, lymphocytes from fetal liver populate the peripheral nodes without prior thymic passage Ackerman, 1967).

In contrast, the widespread distribution of the PWM-responsive feline T cell allows its characterization as a recirculating cell. The relative maturity of this cell is concluded by comparison of the relative activation of T lymphocytes from spleen (SI = 36), LN (SI = 29), thymus (SI = 23) and blood (SI = 80). That con A is a potent mitogen for cells of spleen (SI = 113), blood (SI = 80), and LN (SI = 77) but not for thymus (SI = 7) facilitates the classification of the con A-responsive cell as a recirculating, mature (postthymic) lymphocyte. T cells that proliferate in response to Staphylococcal Protein A (SPA) and soluble PA bear transient $Fc_{\gamma}R$ and are present in spleen (SI = 152) and node (SI = 77), uncommon in thymus (SI = 3) and absent from marrow (SI = 0.6).

Table 3. Distribution of Feline Lymphocyte Markers in Various Lymphoid Organs

	Marker					
	E rosette[1]	Thymocyte antigen[3]	Surface Ig[3]	Complement receptor[4]	Cytoplasmic Ig[5]	Fc Receptor[6]
Lymphocyte Source						
PBL	50 (6)[7]	23 (4)	30 (3)	34 (8)	0 (0)	20 (2)
Spleen	46 (5)	35 (1)	36 (1)	34 (7)	14 (1)	32 (2)
LN	53 (13)	26 (4)	25 (1)	44 (11)	8 (1)	12 (4)
Thymus	56 (4)	32 (16)	1 (0)	6 (3)	0 (0)	2 (0)
Bone Marrow	8 (10	5 (2)	4 (1)	18 (3)	2 (0)	5 (2)

[1]By rosette assay, using AET-treated GPE.
[2]By membrane fluorescence (antifeline thymocyte serum).
[3]By membrane fluorescence (antifeline IgG and IgM).
[4]By rosette assay, using sheep erythrocytes coated with 19S antisheep erythrocytes and mouse complement.
[5]By demonstration of cytoplasmic fluorescence in methanol acetic acid-fixed, cytocentrifugelymphocyte preparations, using antifeline IgG.
[6]By rosette assay, using sheep erythrocytes coated with 7S antisheep erythrocytes.
[7]Percent positive cells, arithmetic mean (SE), n = 4 cats.
[8]Modified from Rojko et al., 1982b

6. Cell-mediated immune responses to FeLV

Cell-mediated immunity generally is regarded as subservient to humoral immunity in the control of FeLV viremia and diseases. Recent data indicate that T-cell responses and antibody-dependent, macrophage and T-cell killing function to restrict FeLV replication in myelomonocytic cells (Rojko et al., 1982a; see Section III.B.10). Furthermore, T-effector cells specifically kill cloned autochthonous testicular fibroblasts nonproductively transformed by FeSV in vitro (McCarty and Grant, 1983). Also relevant is the abrogation of T-cell responses known to be caused by FeLVp15(E) and discussed in Section III.C.1.

7. Natural killer cells and interferon

Three pieces of evidence implicate natural killer cells and/or interferon in the regulation of FeLV-infected cells (Rojko et al., 1981; Dubey et al., 1982; Tompkins and Cummins, 1982). The first is the demonstration of a short-lived mononuclear cell in the blood but not thymus or marrow of FeLV-naive cats that limits the outgrowth of FeLV-infected cells following in vitro exposure to FeLV (see Section III.A.2). Similar T-suppressor and nonT-suppressor effects described for EBV are known to be mediated by interferon (Thorley-Lawson, 1980).

The second is a clinical report that the administration of interferon to anemic, viremic cats, has led to partial remission of viremia and recovery from anemia (Tompkins and Cummins, 1982).

The third is concerned with the control of human lymphoblastoid cell lines infected with FeLV in vitro (Azocar and Essex, 1979; Dubey et al., 1982; Lee et al., 1982). Neoplastic T-lymphoblasts are more susceptible to productive FeLV infection than are

EBV-transformed, immortalized B-cells. FeLV buds from the surface of T-lymphoblasts in close association with HLA-A1,B12, but not HLA-A29,B8 determinants. When NK activity is tested against FeLV-naive and FeLV-infected human T- and B-lymphoblasts, the following pattern emerges. B-cells normally resistant to NK lysis become sensitive, and T-cells normally sensitive to NK lysis become resistant. Cycloheximide, an inhibitor of protein and interferon synthesis, decreases NK lysis of FeLV-infected B-cells but actually increases NK lysis of FeLV-infected T-cells. Tunicamycin and 2-deoxyglucose, inhibitors of virus production and glycosylation, increase the resistance to T-cell lysis conferred by productive FeLV infection. Summarized, these data indicate that interferon mediates enhanced NK lysis of FeLV-infected B cells only and that FeLV-infected, neoplastic T-cells resist NK lysis (Dubey et al., 1982). If these findings hold true for viremic cats, this may be a mechanism by which T-cells escape immunosurveillance after infection with retroviruses and may be relevant to the T-cell transformation tropism of naturally occurring retroviruses (see Section III.C.3).

8. Complement and other nonspecific humoral factors

Circulating proteins, glycoproteins, and lipoproteins that influence retrovirus replication in vivo and in vitro have been demonstrated in virus-naive and virus-exposed people and animals. Homologous suppression may be mediated by virus-induced, nonimmunoglobulin proteins such as the plasma protein present in bovine leukemia virus (BoLV)-exposed but not BoLV-naive cattle (Gupta and Ferrer, 1982). Complement components of humans, other primates, and cats but not guinea pigs lyse retroviruses directly (Bartholomew and Esser, 1978; Sherwin et al, 1978; Welsh et al., 1975; Kobilinsky et al., 1979). Binding of virion p15(E) to the C1q subunit activates the classical pathway and results in effective virolysis by human serum but ineffective virolysis by cat serum (Kobilinsky et al., 1979). Surprisingly, there is no difference in FeLV lysis by normal versus leukemic serum or by viremic versus nonviremic serum. Despite this, complement consumption in viremia is indicated by three observations: (1) viremic cats are hypocomplementemic, (2) viremic cats have circulating immune complexes containing gp70, p15(E), p27 and IgG (Jones et al., 1980; Snyder et al., 1982) and (3) viremic cats have glomerular deposits of FeLV antigens, IgG and complement. Moreover, normal cat serum contains a heat labile, antilymphoma factor thought to be complement that directs tumor regression in lymphomatous cats. Complement also mediates the antibody-dependent lysis of producer or nonproducer lymphoblasts transformed by FeLV (see Section III.B.4 above). Other inhibitors with broad spectrum activity include the very low density lipoprotein of normal mouse serum origin which inactivates ecotropic mouse and feline viruses and broadly reacting antibodies directed against retroviral glycoproteins (de Noronha and Schafer, 1978; Schwarz et al., 1979).

9. Concept of latency

Studies of the pathogenesis of regressive FeLV infection have demonstrated that in most cats exposed to the contagious feline leukemia virus (FeLV), viral replication is contained in target hemopoietic tissues and elicits humoral immunity to FeLV and to the

feline oncornavirus-associated cell membrane antigen (FOCMA) (Rojko et al., 1979a). The hypothesis that these ostensibly self-limiting infections might rather be persistent poorly expressed (latent) infections in certain hemopoietic cells could account for the protracted incubation periods, relapsing viremias (Post et al., 1980), persistently high titers of antiviral and anti-FOCMA antibodies (Essex et al., 1975b), appearance of FeLV p27 antigen in serum of otherwise nonviremic animals (Saxinger et al., 1980; Lutz et al., 1980) and occurrence of FeLV-negative but FOCMA-positive leukemias in naturally infected pet cats (Hardy et al., 1977). Recent reports have detailed the reactivation of latent FeLV from myelomonocytic and lymphoid cells of cats immune to FeLV (Table 4) (Post et al., 1980; Rojko et al., 1982a; Madewell and Jarrett, 1983), some cats bearing FeLV-negative tumors (Rojko et al., 1982a), and kittens congenitally exposed to FeLV (Rojko et al., 1982a; Hoover et al., 1983). Furthermore, the suppression of reappearance of FeLV infection by the host's immune system has been abrogogated by adrenal corticosteroid (CS) hormones in vivo and in vitro (Post et al., 1980; Rojko et al., 1982a; Madewell and Jarrett, 1983).

Regressive FeLV infection has been established in adult specific-pathogen-free cats by oral-nasal (Rojko et al., 1982a) or contact (Madewell and Jarrett, 1983) exposure to lymphomagenic FeLV-R and confirmed by serial examinations for FeLV group-specific-antigen (p27) in peripheral blood mononuclear leukocytes (PBML) and persistent FOCMA antibody responses. In the oral-nasal challenge study, freshly isolated marrow, blood and lymph node cells of adult regressors taken at 20 WAE are negative for p27 and infectious virus (VI). High titers of FeLV, however, are reactivated by in vitro propagation of marrow from 70% of cats (Table 4). Stimulation of nodal lymphocytes but not PBML by the feline T-cell mitogen SPA elicits low titers of VI. Lymphocytes activated by other mitogens do not generate FeLV. Lymphocyte and macrophage growth factors and the tumor promoter, 12-0-tetradecanoyl-phorbol-acetate, also fail to reactivate p27 or FeLV from node and PBML. Thioglycollate-induced peritoneal macrophages do not reactivate p27 or VI. Parallel cultures of marrow, lymphocytes and macrophages from FeLV-naive, SPF, cats are negative (Rojko et al., 1981).

Therefore, latent FeLV infections are characterized by the absence of infectious virus in freshly isolated marrow, node, MØ, and PBML and the reactivation of VI from cultured marrow cells and a minor subset of nodal lymphocytes. These data suggest that self-limiting (regressive) FeLV infections are actually latent (nonproductive) infections of myelomonocytic precursor cells in the bone marrow and SPA-reactive cells in the lymph nodes (Rojko et al., 1982a).

In the contact exposure study, virus has been reactivated from 9 of 16 nonviremic, antibody-positive cats considered to harbor latent FeLV. Madewell and Jarrett (1983) have found that cats infected soon after exposure to FeLV are more likely to develop latent infection than those that resist contact exposure to FeLV (by antisocial behavior?) and recover. They report no difference in serum antibody titers from cats with reactivatable versus nonreactivatable infections.

Table 4. Reactivation of Latent FeLV Infection in Immune Cats

Cell Source	Days in Culture	No. Cats Positive for VI	VI
Marrow	0	0/10	5
Marrow	6	8/10	11,794 (580-74,500)
PBML	0	0/10	5
PBML-PA	7	0/10	5
Lymph Node	0	0/5	5
Lymph Node-PA	7	5/5	155
Peritoneal MØ	0	0/3	5
Peritoneal MØ	7	0/3	5

Rojko et al., 1982a

These observations account for previous findings that healthy pet cats naturally immune to FeLV produce high levels of FOCMA antibody years after isolation from viremic animals. Furthermore, a relationship exists between persistent poorly expressed infection, protracted incubation periods, and stress-induced (relapsing) viremias. Concomitant treatment of FeLV-naive cats with FeLV-R and 5-10 mg/kg prednisolone leads to protracted, productive, marrow infection and protracted neutropenia, lymphopenia and persistent low IgG-FOCMA antibody responses and eventual progression (71%) or regression (29%) of viremia. Similarly, corticosteroid treatment of FeLV-immune cats with reactivatable marrow infections recognized by in vitro culture leads to in vivo recrudescence of marrow FeLV replication and transient profound decreases in FOCMA antibody levels. In 60% of steroid-treated, immune cats, restoration of anti-FeLV responses leads to suppression of viremia and recovery. The remaining 40% become persistently viremic and die of FeLV-related diseases (Rojko et al., 1982). That their FOCMA titers drop from those seen in regressor cats to those seen in corticosteroid-treated progressors probably indicates both in vivo consumption and suppression of adequate humoral response.

It has been speculated that corticosteroids induce reactivation by shifting the marrow macrophage/FeLV relationship from nonpermissive to permissive. Substantiating data has been provided by in vitro steroid treatment of marrow, peritoneal macrophages, node and PBML (Rojko et al., 1982a). With hydrocortisone treatment, previously nonpermissive peritoneal macrophages reactivate FeLV and marrow macrophage reactivation is enhanced 2-3-fold. Reactivation of FeLV is associated with induction of cytoplasmic p27 in 15-30% (mean 21%) of untreated regressor cat bone marrow cells and 20-32% (mean 25%) of CS-treated marrow cells. It has been concluded that the steroid amplification of viral production is due to increased viral replication in a fixed population (20-25%) of marrow cells that harbor FeLV infection.

Maternal immunity does not protect against congenital exposure to FeLV-R or FeLV-KT (Rojko et al., 1982a; Hoover et al., 1983). Reactivatable FeLV also has been found in cultured marrow and thymus of congenitally exposed kittens born to regressor queens. Passive colostral transfer of maternal FeLV-neutralizing antibody to suckling neonates (Hoover et al., 1977) likely is responsible for maintenance of the neonatal infection in its nonproductive, but reactivatable, form (Rojko et al., 1982a). The reactivation of FeLV from marrow, but not tumor cultures, of 4 of 8 cats with FeLV-negative lymphomas has been interpreted as evidence that FeLV may be a factor in nonproducer lymphomagenesis. Possible mechanisms of nonproducer lymphomagenesis are discussed in Section III.C.3.

10. Cellular and immune responses to reactivated FeLVs

A similar coupling of virus latency in vivo with viral reactivation in vitro described for the putative human herpesviral oncogen, the Epstein Barr Virus (EBV) (Thorley-Lawson, 1980) has been attributed to the presence versus absence of host immunologic containment of virally infected cells. Furthermore, viral persistence in the face of an immune response holds true for other retroviral systems, including equine infectious anemia (Crawford et al., 1978), visna virus infection in sheep (Narayan et al., 1977), enzootic bovine leukosis (Piper et al., 1979) and the recently described endemic human T-cell leukemias (Gotoh et al., 1982). The role of host antiviral defenses in the maintenance of FeLV infections in their dormant phases also has been investigated (Rojko et al., 1982). Regressor cats effect both cell-dependent and antibody-dependent killing of autochthonous marrow cells with reactivated virus but do not kill freshly isolated marrow cells. Cells from FeLV-naive cats have minimal cytotoxicity (20%) against autochthonous freshly isolated or cultured marrow cells. That the killing of FeLV-infected marrow cells by immune PBML and sera has biologic relevance is suggested by the correlation of decreases in circulating neutrophil and lymphocyte numbers with curtailment of virus replication and appearance of FOCMA antibody in regressively infected cats.

11. Replication versus restriction of FeLV in cats

The probable lymphoreticular effectors of viral restriction induced by each stage of FeLV replication are charted in Table 5.

B. Hemolymphoreticular Cells as Targets for Nonneoplastic and Neoplastic Disease

1. Immunosuppression

Many oncogenic retroviruses are associated with a dramatic loss of immunocompetence shortly after infection. In the feline system, immunosuppression accompanies induction of marrow origin viremia (see Section II.A.6), precedes detectable neoplastic transformation by months, and predisposes persistently infected cats to a variety of intercurrent, often opportunistic, pathogens. Viremic cats most commonly die of concurrent enteritis, gingivitis, pneumonia, or sepsis of bacterial origin; infectious peritonitis of coronaviral origin; or disease of hemotropic (Hemobartonella felis) or multitropic (Toxoplasma gondi) parasitic origin (Cotter et al., 1975). In other preleukemic or leukemic cats in nature, clinical manifestations of immunomodulation include

Table 5. STAGE, MECHANISMS OF RESTRICTION, AND CONSEQUENCES OF FeLV INFECTION

Stage	Sites of FeLV Replication	Induction of FOCMA	Induction of Immunity	Mechanisms of Restriction	Consequences
Prior to 1	Absent	Absent	Absent	Minimal Exposure (Antisocial Cat) or Genetic Resistance (No Receptors)	Susceptible if Exposed Resistant
1	Tonsil/Local LN	Absent (?)	Local	Local Restriction: Low doses of FeLV Macrophages? NK? Interferon? Local Ab production	Highly resistant to natural, low dose exposure May be susceptible to high dose or systemic inoculation
2,3	As in 1 plus Mononuclear CAV Systemic LN	Present (?)	Transient IgM to FOCMA-IMI Persistent IgG to FOCMA-IMI, CDA VN to gp70	CDA lysis of infected and trans-transformed cells? Complement-mediated virolysis NK restriction of infected cells Other Plasma Inhibitors (?) (Late) VN Ab to gp70	Transient viremia Highly resistant to FeLV - any dose, any route Minimal risk for FeLV reactivation, stress-induced viremia non-producer lymphoma
4	As in 3 plus Marrow, Intestine	Present in Marrow, LN-Preneoplastic Lesion	As in 2,3	As in 2,3 VN Ab to gp70 Ab to FOCMA CMI to FeLV-replicating marrow cells	Highly resistant to exogenous FeLV. Moderate risk for FeLV reactivation, etc.
5a	As in 4 plus Transient Marrow Origin Viremia	Present in Marrow, LN-Preneoplastic Lesion	As in 4 Probable nonVN antigp70, antip27, antip15(E)	As in 4	Highly resistant to exogenous FeLV. Strong risk for FeLV reactivation, etc. Intermittent release of p27, VI from marrow Risk for antibody excess, immune complex disease

(continued)

Table 5. STAGE, MECHANISMS OF RESTRICTION, AND CONSEQUENCES OF FeLV INFECTION (continued)

Stage	Sites of FeLV Replication	Induction of FOCMA	Induction of Immunity	Mechanisms of Restriction	Consequences
5b	As in 5a Protracted Marrow Origin Viremia	As in 5a	As in 5a	As in 4	As in 5a Protracted neutropenia, lymphopenia Protracted incubation period but usually regresses viremia Strong risk for reactivation Risk for immunosuppression, hypocomplementemia
6	As in 5a,5b Persistent Marrow Origin Viremia Widespread Epithelial Infection	As in 5a plus true neoplasia	Persistent IgM to FOCMA-IM No IgG to FOCMA-IMI, CDA Probable nonVN antigp70, antip27, antip15(E)	Not restricted	Persistent viremia, VI in saliva, urine, plasma. Soluble p27 in serum. Immune complexes Severe immunosuppression Death via FeLV-related disease

Abbreviations:

FeLV = feline leukemia virus
FOCMA = feline oncornavirus-associated cell membrane antigen
LN = lymph node
NK = natural killer cells
Ab = antibody
CAV = cell associated viremia
IMI = indirect membrane immunofluorescence
CDA = complement-dependent antibody
VN = virus neutralizing antibody
gp = glycoprotein
CML - cell mediated immunity
p = protein
antigp70 = antibody to gp70

peripheral lymphopenia, thymicolymphoid atrophy, circulating immune complexes, hypocomplementemia, and membranous glomerulonephritis or periglomerular fibrosis (Anderson et al., 1971; Essex et al., 1975; Kobilinsky et al., 1979; Jakowski et al., 1980; Jones et al., 1980).

Immunosuppression, therefore, is the most frequent and the most devastating effect of FeLV in its natural host. In this section, evidence for the T-cell specificity and the viral p15(E) mediation of FeLV-induced immunosuppression will be examined. Recent exciting data implicate prostaglandins, cyclic nucleotides, and interleukins as critical second messengers in the transmission of the T-cell specific lymphosuppressive signals initiated by p15(E).

a. Depression of cell-mediated immunity in viremic cats

The first clear demonstrations that viremic cats have decreased cell-mediated immunity in vivo were those of Perryman et al. (1972) and Hoover et al. (1973). Allograft rejection responses in persistently infected kittens were delayed early in preleukemia (5 WAE) and were correlated inversely with thymus weight. Severe thymic atrophy and paracortical lymphoid depletion were present by 5 WAE to FeLV and thereafter. In these and later studies, evidence for thymic or paracortical lymphocyte necrosis (Hoover et al., 1973,1976) and for FeLV replication in T-cell areas (Rojko et al., 1979a) were not found. Thymicolymphoid depletion, therefore, was attributed to altered traffic of thymocyte precursors from marrow to thymus, and altered traffic of mature thymocytes to splenic and nodal paracortex. It is unlikely that impairment of cell-mediated immunity is due only to the absence of the thymus as thymectomy of weanling kittens does not lead to alterations in skin graft rejection or lymphocyte blastogenesis (Hoover et al., 1978).

Evidence is accumulating that the immunoabrogation induced by FeLV is T-cell specific. With the onset of viremia, experimentally exposed cats also lose their capacity to respond to the T-lymphocyte mitogens concanavalin A (con A) and phytohemagglutin (PHA) and the antigen keyhole limpet hemocyanin (KLH) (Cockerell et al., 1976a,b,c). Reactivity to Staphylococcal Protein A and to the B-cell mitogen lipopolysaccharide (LPS) are comparable to nonviremic cats (Rojko et al., 1983b). Peritoneal macrophages from persistently infected cats replicate FeLV but do not differ from uninfected cat macrophages with respect to their surface FcR, phagocytosis of opsonized erythrocytes or uncoated Candida pneumotropica, killing of Staphylococcus aureus, or chemotactic responses to complement fragments (Hoover et al., 1981; Rojko et al., 1983b). Persistently infected neutrophils also display normal chemotaxis and bacterial killing.

The adduction of T-cell specificity also is supported by recent observations that naturally viremic pet cats make ineffective IgG responses to the synthetic polypeptide multichain (L-tyrosine-L-glutamic acid)-poly-DL-alanine-poly-L-lysine, denoted TGAL, as determined by enzyme-lined immunosorbent assay (ELISA) (Trainin et al., 1983). Production of IgG antibodies to TGAL is delayed and greatly reduced in viremic cats versus uninfected controls. IgM antibodies to TGAL are similar in infected and uninfected cats. TGAL is a T-dependent antigen in the rodent and it is likely that it

evokes the same general class of response in the cat. If so, production of normal IgM and reduced IgG antibody levels might indicate normal B-cell function and impaired T-helper cell function. Further support of this thesis lies in analysis of the humoral antibodies to FeLV-associated tumor antigens in viremic versus latently infected (immune regressor) cats. Latently infected cats mount strong, persistent IgG responses to FOCMA-L, defined by reactivity with surface membrane antigens of the virus-producer, feline lymphoma cell line (FL74). If viremic cats develop IgG antibodies to FOCMA-L, these antibodies are diminished in titer and delayed in appearance by 2-5 weeks relative to those of regressor cats (Mathes and Olsen, 1981). In viremic cats, however, low levels of IgM antibodies reactive with the surface of FL-74 cells arise early in, and persist throughout, preleukemia (Mathes and Olsen, 1981). Thus, a putative T-helper defect may be assigned to cats with persistent, productive FeLV infection. FeLV-induced immunosuppression may provide a model for the specific impairment of OKT-4 positive T-helper cells observed following infection of human cells with the human T-cell leukemia virus (HTLV, Miyoshi et al., 1981; Poiesz et al., 1981; Popovic et al., 1982; see Sections V.1,3).

T-cell suppression by FeLV is not limited to the T-helper population. Leukemic cats are predisposed to diseases such as Coomb's positive anemia and membranous glomerulonephritis which may be precipitated by suppressor cell dysfunction. This aspect of FeLV-related immunosuppression has been studied by evaluating suppressor cell function in vitro (Stiff and Olsen, 1982). The technique involves the elimination of T-suppressor cells by short-term incubation (24 hours) in vitro. After incubation, the lymphocytes are treated with mitogen and then cultured as a typical lymphocyte blastogenesis test. Normal feline peripheral blood mononuclear leukocytes demonstrate 4-fold increases in DNA synthesis ($p < .005$) in response to suboptimal concentrations of con A (0.1 ug/well) added at 24 hours relative to cells stimulated at 0 hours. Increased DNA synthesis by cells aged 24 hours in vitro is interpreted to mean attenuation of normal suppression consequent to preincubation. In contrast, when cells from viremic cats are incubated for 24 hours, a significant increase in con A stimulation is not observed (Stiff and Olsen, 1982). This suggests that viremic cats lack circulating suppressor cells or that these cells are not functioning as those in normal cats with normal peripheral blood mononuclear leukocytes. Interestingly, viremic cats develop depressed circulating, spontaneous suppressor cell activity concomitant with the redistribution of Fc R and T-cells to the mesenteric lymph nodes (Rojko et al., 1979a; Rojko et al., 1983b). Furthermore, naturally-occurring, short-lived cells in the peripheral blood of FeLV-naive cats are known to restrict FeLV replication under conditions of in vitro exposure (Rojko et al., 1979a; see Section III.A.1.b). Clearly, this inverse relationship between virus production and suppressor cell activity demands further attention.

b. Effects of killed FeLV and virion structural components on the immune system

The initial evidence that abrogation of immune responses might be the property of feline retroviral structural components has been provided by Schaller et al. (1977). In

these experiments, the simultaneous administration of killed FeLV and FOCMA vaccine preparations prevents the induction of effective FOCMA antibody responses. Vaccinated kittens subsequently are challenged with highly sarcomagenic FeLV-FeSV preparations or leukemogenic FeLV-R. Those kittens receiving vaccines containing killed FeLV have enhanced sarcoma engraftment (FeSV challenge) or enhanced susceptibility to FeLV viremia and disease (FeLV-R challenge) relative to kittens receiving the FOCMA vaccine alone. Kittens receiving FeLV have larger tumors and a higher percentage of progressive malignancies.

In addition to causing tumor enhancement and abrogating tumor immunity in vivo, inactivated FeLV interferes drastically with lymphocyte function in vivo. Incubation of peripheral blood mononuclear cells from FeLV-naive cats with serum from viremic cats or with ultraviolet-treated FeLV in vitro causes loss of lymphocyte reactivity to T-cell mitogens (Cockerell et al., 1976; Mathes et al., 1978; Hebebrand et al., 1979) and allogeneic leukocytes (Stiff and Olsen, 1983), and depression of lymphocyte membrane lectin receptor mobility (Dunlap et al., 1979; Lewis and Olsen, 1983). The quantity of inactivated FeLV sufficient to inhibit blastogenesis and membrane receptor mobility is not cytotoxic to lymphocytes as assessed by trypan blue dye exclusion of parallel cultures. Furthermore, interference with lectin-induced events is not due to nonspecific trapping of lectin by FeLV. Lymphocytes incubated with FeLV demonstrate reduced lectin capping. Similar membrane-related lymphocyte deficiencies accompany lymphoma in man, FeLV viremia in cats, and Friend MuLV infection in mice. Peripheral blood mononuclear leukocytes (PBML) from FeLV-naive cats undergo con A capping with a mean capping rate of 17%. In contrast, PBML from viremic cats with or without lymphoma and PBML from FeLV-naive cats treated with inactivated FeLV in vitro exhibit a mean capping rate of 7-10% (Hebebrand et al., 1979; Dunlap et al., 1979; Lewis and Olsen, 1983).

To delineate the FeLV component responsible for the immunosuppression, FeLV has been fractionated into its component proteins and each fraction tested for suppression of mitogen-induced blastogenesis. The results have identified a fraction of FeLV insoluble in aqueous buffers with suppressive properties equal to inactivated FeLV. Other FeLV proteins (i.e., gp70, p27) do not possess this biological property. Further purification of the FeLV suppressive fraction has revealed a 15,000 (FeLV p15(E)) dalton protein on polyacrylamide gel electrophoresis. Purified FeLV p15(E)) has been shown to be suppressive to the mitogen-induced LBT at a concentration of 0.2 ug/well (Mathes et al., 1978; Hebebrand et al., 1979).

In vivo administration of FeLV p15(E) reduces the cats response to FOCMA and increases the cats' susceptibility to FeLV disease. The in vivo biologic effects of FeLV p15(E) are very similar to the effects of inactivated FeLV. In addition to tumor enhancement and decreased FOCMA antibody response, administration of FeLV p15(E) interferes with the apparent helper effect of T-lymphocytes and blocks the apparent conversion of IgM to IgG FOCMA antibody. Cats given FeLV p15(E) develop persistent

IgM FOCMA antibody with only low levels of IgG (Mathes and Olsen, 1981). This profile is similar to those produced by cats that possess progressive FeLV/FeSV tumors or persistently viremic cats.

c. Effects of FeLV and/or FeLV p15(E) on lymphocyte function of heterologous hosts

Interest in the biologic effect of FeLV p15(E) on the lymphocyte functions of animal hosts other than cats has been stimulated by its resemblance to the 15,000 dalton envelope protein of murine leukemia virus (MuLV p15(E)) (Ikeda and Wright, 1975). FeLV p15(E) possesses remarkable interspecies antigenic reactivity distinct from interspecies determinants on FeLV p27, suggesting that FeLV p15(E) is a highly conserved protein and that its biologic properties should not be restricted to its homotypic host (cat). Subsequent studies revealed that FeLV p15(E) depresses blastogenesis to mitogens and antigens in PBML of nearly all normal human donors (Hebebrand et al., 1979). FeLV p15(E) also markedly inhibits con A capping in human PBML (Hebebrand et al., 1979; Dunlap et al., 1979; Lewis and Olsen, 1983). Moreover, canine and bovine but not equine PBML are permissive to FeLV repression of mitogen-induced blastogenesis. These studies clearly indicate that FeLV p15(E) can cross species barriers to abrogate lymphocyte proliferation and membrane receptor mobility.

d. Effect of p15(E) on interleukin secretion and actions

Recent reports (Copelan et al., 1983) reveal a mechanism for inactivated FeLV- and FeLV-derived, p15(E)-induced repression of lymphocyte blastogenesis. They examine factors that contribute to maximal T-cell proliferation in reaction to con A. Such factors include the production of interleukin 1 (IL-1) by accessory monocytes, the secretion of interleukin 2 (1L-2, T-cell growth factor - TGCF) by con A-activated T-cells, and the recruitment of secondary T-cell targets in response to interleukin 1 and TCGF.

Using standard assays, monocyte-depleted T-cell populations require the addition of autologous monocytes or their biologic product, interleukin 1, to attain maximal blastogenesis. Preincubation of monocytes with inactivated-FeLV does not abrogate their ability to restore blastogenesis to monocyte depleted PBML, nor does it diminish their secretion of interleukin 1 in response to lipopolysaccharide stimulation. Furthermore, the interleukin 1 produced by FeLV-treated monocytes is fully effective in initiating tritiated thymidine incorporation by human T cells or mouse thymocytes. Lastly, the addition of autologous monocytes to FeLV-suppressed lymphocytes results in partial reversal of lymphocyte suppression (Copelan et al., 1983). Whether this protective effect resides in the ability of monocytes to degrade or entrap FeLV p15(E) or to secrete factors which protect the T-cell membrane from FeLV p15(E) damage is not known.

The failure of FeLV p15(E) to depress interleukin 1 production by, and accessory function of, human monocytes in vitro is reminiscent of its failure to induce dysfunction in cat monocytes (macrophages) in vitro and in vivo (Hoover et al., 1981; Rojko et al., 1983b). Chemotactic, phagocytic and bactericidal activity of FeLV p15(E)-treated peritoneal macrophages are comparable to untreated controls. Similarly, peritoneal

macrophages from viremic cats behave exactly as macrophages from normal cats in assays for chemotaxis, opsonic and nonopsonic phagocytosis, and bacterial killing. Therefore, it is improbable that FeLV p15(E) effects T lymphosuppression by inducing monocyte (macrophage) dysfunction. This contrasts with observation that antigenically similar, 15,000 dalton, proteins derived from human and murine lymphomas, inhibit monocyte (macrophage) functions. MuLV p15(E) prohibits macrophage accumulation in response to intraperitoneal injection in mice. The 15,000 dalton protein isolated from human malignant lymphomas (Cianciolo et al., 1981) renders human monocytes immobile.

Of more relevance to the mechanism of lymphosuppression is the dramatic decrease in the secretion of, and response to, TCGF in FeLV- and p15(E)-treated PBML (Copelan et al., 1983). In these experiments, monocyte-depleted, T-cells are incubated with interleukin 1 and con A and their supernatants tested for TCGF. FeLV- and p15(E)-treated monocyte-depleted T-cells produce less than 2% of the amount of TCGF produced by untreated, monocyte-depleted T-cells. Related experiments show that FeLV and p15(E) treatment also renders T-cells unresponsive to exogenous TCGF. Copelan et al. (1983) conclude that FeLV p15(E) directly suppresses lymphocyte proliferative function through prohibition of secretion of a TCGF and prohibition of the response to TCGF.

Avian and murine retroviruses also repress lymphocyte blastogenesis in their homotypic host. Avian retrovirus p15(E) has immunosuppressive action equivalent to FeLV p15(E) and recent studies by Wainberg et al. (1983) demonstrate that blastogenesis can be restored to suppressed cultures with the addition of sufficient TCGF. The sum total of these findings is that retrovirus components, especially the p15 envelope protein, disrupt recruited lymphocyte proliferation by eliminating secretion and action of TCGF.

e. Involvement of prostaglandins and cyclic nucleotides in lymphosuppression by FeLV p15(E)

Summation of work by several investigators involving p15E inhibition of receptor capping, TCGF and erythroid burst promoting factors (see Section III.C.2) responses focuses attention to the lymphocyte surface. FeLV p15(E) is highly hydrophobic and may bind readily to the lipid of the bimolecular leaflet and thus interrupt normal membrane function. The prostaglandin and cyclic nucleotide systems are logical candidates for effectors of the T-cell suppression induced by FeLV p15(E). Both are linked closely to the immune system, cell membrane-mediated events, mobility and expression of cell surface receptors and regulation of cell proliferation. Furthermore, colchicine, a microtubule disrupting agent, reverses FeLV suppression of lectin receptor mobility. This has been interpreted to signify stabilization of microtubules by FeLV, an action likely interdependent with cyclic nucleotide metabolism. Lewis et al. (1983a) have tested the putative involvement of cyclic nucleotides and prostaglandins in FeLV-related lymphorepression. Various drugs known to influence the intermediary metabolism of prostaglandins have been evaluated for their effect on FeLV p15(E)-associated inhibition of lectin membrane receptor mobility and lectin-triggered mitogenesis. Micromolar concentrations of the exogenous prostaglandins E_1, E_2, D_2, F_2 and 6-keto-$F_{1\ n}$ and their

precursor arachidonic acid also have been examined for their direct action on normal lymphocyte blastogenesis and their conjoined actions with FeLV p15(E). The results indicate that only prostaglandins of the E series are effective depressors of blastogenesis alone (40-50% suppression) or effective depressors in conjunction with FeLV p15(E) (12% suppression). Prostaglandin E_2, however, reverses FeLV-induced capping deficits. Two known cyclo-oxygenase inhibitors, indomethacin and nordihydroguararetic acid (NDGA), reverse the p15(E) suppression of the con A receptor capping mechanism and the con A blastogenic responses. NDGA (1 uM) and indomethacin (0.1-1 uM) reverse suppression when lymphocytes are obtained from viremic donors exposed to FeLV p15(E) in vitro (Lewis and Olsen, 1983; Lewis et al., 1983a). An inhibition of prostacyclin synthetase, tranylcypromine, and an inhibitor of thromboxane synthetase, imidazole, are without effect. In studies of normal human donors and patients with immunosuppressive disorders, cyclo-oxygenase inhibitors have been shown to enhance mitogen responsiveness (Goodwin and Webb, 1980). Reversal of immunosuppression probably is due to inhibition of PGE_2 production which has been correlated positively with suppressor cell activity. Regarding the latter, Lewis et al. (1983a) noted that PGE_2 could directly suppress feline lymphocyte function or add to the depressive effect of FeLV. These results clearly implicate prostaglandins as intermediaries of the FeLV-induced abrogation of lymphocyte reactivity to lectins.

It is known that conA causes a rise in intracellular cyclic AMP levels in stimulated lymphocytes. Lewis et al. (1983a) hypothesize that the incorporation of FeLV p15(E) into the lymphocyte plasma membrane may interfere with the Ca^{++}/calmodulin transduction of the membrane signal which leads to the activation of adenylate cyclase. They have shown that treatment of lymphocytes with FeLV p15(E) has no measurable effect on cellular PGE_2 or cyclic GMP levels but that it does inhibit cyclic AMP accumulation in the presence of con A. Of greater interest is the direct activation of adenylate cyclase by forskolin which reverses FeLV suppression of lymphocyte function by raising intracellular cyclic AMP to unsuppressed levels and, hence, restores blastogenic and capping responses. Forskolin is known to bypass the GTP-binding (second) site for adenylate cyclase and activate the catalytic (third) site directly. Two other observations are relevant to the probable mechansim of FeLV p15(E) action. First, indomethacin reversal of FeLV p15(E) lymphosuppression also is associated with return of intracellular accumulations of cyclic AMP in reaction to con A (Lewis and Olsen, 1983). Second, phosphodiesterase inhibitors which promote intracellular increments in cyclic AMP by prohibiting its catabolism do not protect lymphocytes from FeLV suppression nor do they enhance intracellular cyclic AMP levels in lymphocytes co-exposed to FeLV p15(E) and con A (Lewis and Olsen, 1983). All things considered, it is highly plausible that the original action of FeLV p15(E) at or in the cell membrane is to block the triggering of the Ca^{++}/calmodulin-dependent activation of adenylate cyclase. Alternatively, the insertion of this hydrophobic bulky p15(E) may block microtubule and membrane function by a space-occupying mechanism alone. The codepressive effect of PGE_2 on FeLV

suppression of lectin responses most likely is associated with enhanced blocking at or in the membrane. Failure of generation of the second messenger cyclic AMP leads to failure of transmission of the message to proliferate or to undergo capping in response to the lectin signal.

2. Anemiagenesis

Preleukemic erythroid aplasia is second only to immunosuppression as a common manifestation of FeLV viremia in naturally infected cats (Cotter et al., 1975). Experimental inoculation of immature (Hoover et al., 1974; Mackey et al., 1975; Boyce et al., 1981; Onions et al., 1982; Testa et al., 1983) or corticosteroid-suppressed adult cats with anemiagenic subgroup C FeLVs (FeLV-KT, FeLV-Sarma) leads to progressive nonregenerative anemia and death with 10-16 weeks. Packed cell volumes of 5 to 15% reflect severe depletion of marrow erythroid precursors and a shift in the mean myeloid:erythroid (M:E) ratio from 1.6 in normal, age-matched controls to 10.4 in anemic cats (Boyce et al., 1981). Lymphopenia, severe paracortical lymphoid depletion, thymic atrophy and cachexia are invariable (Hoover et al., 1974; Mackey et al., 1975). Anemia and lymphoid depletion may be accompanied by pancytopenia, medullary fibrosis or osteosclerosis, and hemorrhagic enteritis (Hoover et al., 1974; Mackey et al., 1975). Shortening of the erythrocyte lifespan in preanemic and anemic cats may contribute to the severity of the anemia (Hoover et al., 1974), but probably is minor compared to the profound selective loss of erythroid precursors early in the preanemic period (Boyce et al., 1981; Onions et al., 1982; Testa et al., 1983).

Recently, colony-forming assays (Fig. 2) for feline marrow erythroid, myelomonocytic and fibroblastic precursors have been used to investigate the pathogenesis of this preleukemic red cell hypoplasia. Marrow cultures from $FeLV_C$-infected cats show selective depression of erythropoiesis with minimally altered granulopoiesis (Boyce et al., 1981; Onions et al., 1982; Testa et al., 1983). Relatively mature erythroid precursors, designated CFU_E (colony-forming units-erythroid) decrease by 3-5 weeks after exposure (WAE) prior to the onset of anemia (5WAE) (Boyce et al., 1981). More dramatic is the rapid fall of primitive erythroid precursors, designated BFU_E (burst-forming units-erythroid) to 25% of that in uninfected, age-matched controls by 1 WAE and to 10% by 2 WAE and thereafter (Testa et al., 1983). In $FeLV_C$-infected, preanemic and anemic cats, myelomonocytic precursors (CFU_{GM}) usually are unaffected but sometimes are increased transiently (Boyce et al., 1981). Four of 5 cats also have 3-7-fold increases in colonies of fibroblast-like, nonmacrophagic cells attached to the surface of the culture dish (Testa et al., 1983). This may be the *in vitro* equivalent of the *in vivo* capacity to develop myelofibrosis (Hoover et al., 1974).

These severe decreases in BFU_E and CFU_E following viral infection mimic those seen in human aplastic anemias of idiopathic origin and are distinct from the arrested maturation described for anemias induced by Friend and Rauscher murine leukemia viruses (MuLVs). In the mouse anemias, maturation blocks occur relatively late in erythrogenesis and are reflected in enhanced CFU_E and BFU_E numbers. Hankins (1983)

has demonstrated elegantly that both anemiagenic and polycythemiagenic strains of Friend virus directly trigger the polyclonal expansion of the EPO-positive BFU_E. In Rauscher anemias (de Both et al., 1980), the erythrocyte lifespan is reduced drastically, thereby worsening the anemia.

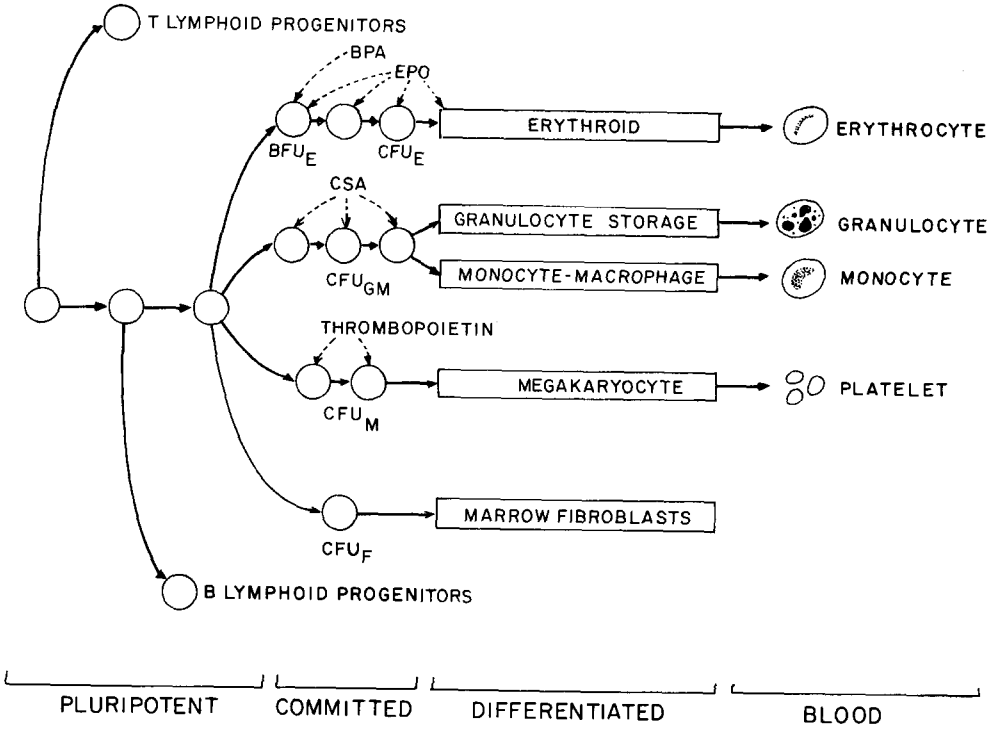

Figure 2. Proliferation, Differentiation, and Assay of Feline Marrow Cells. Single cell suspensions of bone marrow mononuclear cells are cultured in semi-solid media (usually methylcellulose) with the appropriate growth promoters erythropoietin (EPO), burst-promoting activity (BPA), or colony stimulating factor (CSF). The most primitive erythroid precursor assayable is the burst-forming unit-erythroid (BFU_E). BFU_E are large colonies with multiple subunits that arise after 7 to 10 days, and are focused around a central nurse macrophage. BFU_E probably require cell-to-cell contact for their development, are sensitive to high levels of EPO, and absolutely require BPA produced by the central marrow adherent macrophage. In the earliest phase of BFU_E generation, T-cells likely trigger the release of BPA from the marrow adherent cell. The colony-forming unit-erythroid CFU_E represents a more mature (committed) erythroid precursor. CFU_E are smaller than BFU_E, arise earlier in culture (2 to 5 days), and are sensitive to low levels of EPO. The myelomonocytic precursor assayed is a committed stem cell with the potential for granulocytic or monocytic differentiation and is designated the colony forming unit-granulocyte/macrophage (CFU_{GM}). Certain cultured cell lines, macrophages, urine, and endotoxin-activated serum serve as sources of colony stimulating factors necessary for myelomonocytic colony formation in methyl cellulose.

The mechanisms that underlie this specific erythroalteration by subgroup C FeLV are not known. In man and the mouse, culture of colony-forming hematopoietic cells has identified stem cell defects, microenvironmental disorders and lymphoreticular/hematopoietic cell interactions as pathogenetic in aplastic anemia (Camitta et al., 1982). The sustained myelomonocytic precursor activity in FeLV-infected anemic and preanemic cats implies a viable pluripotent stem cell with capacity for myelomonocytic differentiation. This argues against a FeLV-induced stem cell defect and for a committed erythroid precursor defect. A simple explanation might reside in a simple deficiency of the erythrostimulatory growth factor, erythropoietin (EPO), in the face of normal levels of colony stimulating factors (CSFs) that favor myelopoiesis. BFU_E and CFU_E require EPO for clonal expansion, maturation and hemoglobinization; and BFU_E are sensitive only to high levels of EPO. That FeLV induces a simple EPO deficiency, however, is improbable because FeLV anemic cat marrow is refractory to exogenous EPO *in vitro* and FeLV anemic cats have elevated plasma EPO levels *in vivo* (Hoover et al., 1974).

It is more plausible that impairment of feline erythroid cell growth by $FeLV_C$ may involve viral-induced inhibition of cell proliferation or host immunopathologic reactions against FeLV-infected progenitors. Evidence for a direct antiproliferative effect is found when bone marrow cells from FeLV-naive cats are exposed to various strains of FeLV *in vitro* and assayed for CFU_E, BFU_E, and CFU_{GM} in methyl cellulose (Testa et al., 1983; Rojko et al., 1983a). Exposure to $FeLV_C$ (Sarma), $FeLV_{ABC}$ (KT), or $FeLV_{AB}$ (Ohio State Rickard FeLV) but not $FeLV_A$ (Glasgow Rickard FeLV) effects 40-75% reductions in CFU_E and BFU_E. Alterations in CFU_{GM} relative to sham-inoculated controls are minor. Parallel liquid cultures assayed for viability, cell number and cell-associated incorporation of tritiated thymidine show that this specific erythrodepression is independent of any cytotoxic effect. *In vitro* suppression is correlated positively with infectivity of the input virus and with virus replication by inoculated marrow. Inactivation of FeLV by heat or ultraviolet light leads to restoration of CFU_E to sham-inoculated levels. Immunofluorescence for FeLV p27 and cytologic studies indicate that the majority of the replicating virus recovered from suppressed cultures is produced by myelomonocytic progenitor cells (Rojko et al., 1983a).

These data indicate that both anemiagenic and lymphomagenic feline retroviruses cause reductions in erythrogenesis *in vitro*. The requirement for infectious virus *in vitro* and erythrospecificity *in vivo* and *in vitro* are reminiscent of the Friend MuLV anemia/polycythemia system where infectious virus induces BFU_E and enhances erythrogenesis (Hankins, 1983). Furthermore, interaction between hemopoietic cells and infectious virus probably is prerequisite to anemia induction *in vivo*. In this regard, the early fall in BFU_E (1 WAE) in $FeLV_C$-exposed, preanemic cats exactly correlates with the onset of productive infection of marrow progenitor cells (Hoover et al., 1977; Rojko et al., 1979a; Testa et al., 1983). Similarly, erythroid progenitors produce the Friend-MuLV complex as early as 48 hours after exposure *in vivo* (Hankins, 1983).

Evidence for immunopathologic reactions against FeLV-infected progenitors is less compelling (Kociba and Wellman, 1983). In anemic cats, CFU_E formation is decreased in the absence of autologous serum and unaffected by its presence. In normal, FeLV-naive cats, neither the inclusion of pooled sera from anemic cats nor the addition of sera from cats hyperimmune to $FeLV_{ABC}$ causes suppression of CFU_E.

Lastly, when marrow from FeLV-naive cats is cultured with increased amounts of inactivated FeLV, selective decrements in CFU_E can be demonstrated. Inclusion of the purified virion component (p15(E) but not p27 in the methyl cellulose culture system also leads to depressed CFU_E (Kociba and Wellman, 1983). The question whether anemiagenesis is due to FeLV gp70, p15(E), replicating virus, or all three, deserves further study. In particular, the known specificity of p15(E) for T-cell suppressions and the observed BFU_E decrements suggest consideration of the effect of FeLV on T-cell-dependent, macrophage secretion of burst-promoting activity (Kociba and Wellman, 1983).
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3. Leukemogenesis

Many of the oncogenic retroviruses, including feline, avian, murine, bovine, gibbon ape and human T-cell leukemia viruses and the murine mammary tumor virus, induce neoplasms only after long latent periods of months to years (reviewed in Gilden and Rabin, 1982; Weiss, 1983). With the exception of the human T-cell leukemia virus (HTLV), these viruses do not immortalize cells in vitro (Yamamoto et al., 1982), nor do they appear to contain viral transforming genes (Gilden and Rabin, 1982). Often persistence of an intact provirus is not required for the induction or maintenance of the transformed state. Fully one-third of feline lymphomas are negative for FeLV antigens and negative for more sensitive indicators of FeLV proviruses (Hardy et al., 1977,1980; Koshy et al., 1980; Casey et al., 1981). The currently favored modes on oncogenesis by long latency leukemia viruses include:

(1) Activation of c-onc sequences directly by promoter insertion (Hayward et al., 1981) or activation of other transforming genes (Cooper, 1982) indirectly by insertional mutagenesis of a regulatory cellular sequence,

(2) Indirect viral effects in which the virus or viral receptors trigger a series of blastogenic responses mediated by interleukins or hormones but the virus is not the final carcinogen (McGrath et al., 1978; Lee and Ihle, 1979,1981), and

(3) The generation of highly transforming recombinant viruses with extended host range (Elder et al., 1977) or leukemia accelerating viruses (Nowinski et al., 1977).

Any proposal that these mechanisms operate in feline leukemogenesis must account for certain observations. First is the negative correlation between lymphoid targets for productive infection and targets for transformation (Cockerell et al., 1976a,b; Rojko et

al., 1979a,1981) in the face of the positive correlation between FeLV viremia and likelihood of neoplasia (see Section II.C.2). Second is the similarity between the potentially cytopathic $FeLV_B$ and cytopathic murine recombinant viruses with high leukemogenicity (Elder and Mullins, 1983). Third is the role of immunoselection versus immunosuppression in the emergence and expansion of leukemic clones. Penultimate, at present, is the identity of FOCMA and its relationship to virus producer and nonproducer neoplasia. Last is the controversial etiology of nonproducer lymphomas and leukemias.

Studies of the pathogenesis of viremic FeLV infections have established that B- but not T-cell replication of FeLV is a constant feature of preleukemia but that FeLV ultimately transforms T-cells of marrow or blood origin which migrate to the thymus (Cockerell et al., 1976a,b; Rojko et al., 1979a,1981). A similar dissociation between virus replication and transformation occurs in Moloney (Lee and Ihle, 1979; Isaak and Cerny, 1983) and AKR murine leukemias (Lee and Ihle, 1979). The transformed cell is a T-cell, but normal T-cells are not infected with MuLVs and transformation of T-cells of marrow origin follows a prolonged period of viremia and ecotropic Mo-MuLV or AKV replication by non-T-cells. Whereas the viruses isolated from early preleukemic mice are weak oncogens, viruses isolated from preneoplastic and neoplastic thymocytes of early leukemic mice are potent oncogens (Elder et al., 1977; Lee and Ihle, 1979,1981) or leukemia-accelerating viruses (Nowinski et al., 1977). Passive antibody to gp71 will protect mice from T-cell neoplasms only if administered early in preleukemia. The potent oncogens are recombinants between ecotropic AKV produced by nonthymocytes and xenotropic virus produced by thymocytes. The cell capable of generating these recombinants has not been identified but the critical role of the thymic nurse cell in leukemogenesis in vivo suggests its consideration (Haas, 1978). The recombination event usually is specific to the env region and the progeny recombinants have gp71 homologous to both xenotropic and ecotropic env gene products. It is known (see Sections II.A.3,II.C.1) that gp71 determines host range and biologic properties and is the target for VN antibody. Host range is extended to include both xenotropic and ecotropic cells (this is called amphotropism) and the newly evoked cytopathicity for mink cells in lieu of transforming gene capacity has led to the designation mink cell focus-forming (MCF) virus.

Many parallels can be drawn to FeLV leukemogenesis. Passive antibody to FeLV gp70 is protective only if administered within the first 6 DAE (de Noronha et al., 1978). Subgroup A FeLVs are long latency leukemia viruses with highly restricted host range. $FeLV_A$ replicates efficiently only in cat cells and some strains will replicate inefficiently in human and canine cells (Sarma and Log, 1973). Cats infected with known $FeLV_A$ isolates begin to replicate $FeLV_B$ late in viremia and $FeLV_B$ has an extended host range relative to $FeLV_A$. Whether in vivo cat passage actually reflects recombination or rather selection for low levels of $FeLV_B$ originally present in the thymic tumor inoculum is provoking. Naturally occurring AKV thymic tumors have only low levels of MCF viruses, in vitro passage increases the MCF component (Elder et al., 1977). In this regard, it perhaps is useful to note that the original $FeLV_A$ isolate of FeLV-Rickard is weakly

leukemogenic, that the Ohio State strain of FeLV-R contains both $FeLV_A$ and $FeLV_B$ and is highly leukemogenic (Hoover et al., 1972), and that the Glasgow strain of FeLV-R contains $FeLV_A$ only and mimics the parental FeLV-R in weak pathogenicity. Moreover, $FeLV_B$ is highly cytosuppressive to normal feline marrow and lymphoid cells in vitro (Onions et al., 1980; see Section II.C.1). Whether this cytodepressive property is due to a virion p15(E) effect or due to superinfection with unintegratable, and hence toxic, proviruses similar to that described for cytopathic avian leukemia viruses (Weller et al., 1980) is not known. The data proposing $FeLV_B$ as a MCF-like or leukemia accelerating virus are reinforced by the recent molecular evidence that the nucleotide sequence of the env gene of $FeLV_B$ reveals unique sequence homologies with a murine MCF virus (Elder and Mullins, 1983).

Although not an absolute prerequisite to lymphomagenesis, viremia increases the probability of, and decreases the latent period before, the transforming event. The leukemogenic event usually is considered to be random, however, a high replicating virus burden increases the probability of new proviral integrations into sensitive sites in sensitive cells. In avian bursal lymphomas, these are sequences upstream from the cellular homologue of avian myelocytomatosis virus, the proto-oncogene designated c-myc. Integration of proviral LTRs is sufficient to initiate expression of c-myc RNA and induce the transformed phenotype in both virus producer and virus nonproducer cells. Other lymphomas express cellular transforming genes unrelated to viral transforming genes recognized when their DNA transfected into indicator fibroblasts evokes transformation (Cooper, 1982). These genes may encode transforming (?) proteins related to the transferrin family. Their induction in bursal lymphomas probably is via downstream promotion or via insertional mutagenesis into regulatory sequences that normally function to ensure their repression. The present evidence for downstream promotion or insertional mutagenesis in FeLV lymphomagenesis is not compelling. Replication-competent proviruses from FeLV-positive lymphomas will cause viral replication but not transformation in NIH 3T3 or feline fibroblasts. Replication-defective proviruses from FeLV-positive tumors and DNA from FeLV-negative tumors cause neither productive infection nor transformation in transfection assays (Casey et al., 1981; Rosenberg et al., 1981).

An alternative explanation is that the persistent FeLV replication in non-T-cells causes immunostimulation of transformable T-cells and p15(E)-mediated suppression of regulatory T-cells and allows immunoselection and clonal expansion of putative transformants. Similar persistent ecotropic virus production by non-T-cells during preleukemia is critical to the gp71 specific, immunostimulation of uninfected T-cells in Moloney MuLV leukemogenesis in AKR and BALB/c mice (Lee and Ihle, 1979,1981). Target cells are recruited and synchronized by the monokines and lymphokines released early in the gp71-induced blastogenic event. A novel lymphokine, interleukin-3 (IL-3) (Ihle et al., 1981) promotes early T-cell differentiation by converting 20 -hydroxy steroid dehydrogenase-negative lymphocytes to the enzyme-positive state. Therefore, viral tropisms for transformation usually specify not only cell lineage (T versus B) but also stage of cell cycle

and stage of cell differentiation. Mo-MuLV lymphomas are 20 -hydroxy steroid dehydrogenase-positive. HTLV- and FeLV-induced lymphomas are terminal transferase (Tdt)-negative, indicating transformation of a differentiated T-cell and not an immature, Tdt-positive, prothymocyte. Furthermore, the density and distribution of cellular receptors for virus may depend on cell cycle or differentiation. The observation that leukemic, but not preleukemic, thymocytes in MuLV viremic mice have excess receptors specific for the inducing MuLV (McGrath et al., 1977) indicates that viral ligand/viral receptor interactions modulate not only productivity but also lymphomagenicity of MuLV infection.

A model proposing the simultaneous occurrence of viremia and proliferation and differentiation of transformable targets in FeLV-infected cats must not deny the profound antiproliferative effect of p15(E). That workers in this laboratory have been unable to demonstrate FeLV-gp70-induced blastogenesis in viremic cats may be another manifestation of p15(E)-T-cell immunodepression. However, it is improbable that all T-cell subsets are restricted equally by p15(E) (Rojko et al., 1973b). Selective depression of antiviral T-effector or T-helper cells (Trainin et al., 1983) could allow the emergence of neoplastic subsets whose proliferation is unchecked by p15(E). Furthermore, the specific failure of IgG-FOCMA humoral immunosurveillance (see Section III.B.4) would allow survival of emerging neoplastic cells. Induction of protection FeLV infection in neoplastic T-lymphoblasts by exogenous superinfection or endogenous reactivation of integrated FeLV provirus could protect the cell from NK lysis (Dubey et al., 1982).

The general consensus is that feline hemolymphoreticular and mesenchymal neoplasms are distinguished by feline oncornavirus-associated cell membrane antigens (FOCMA). FOCMA-L, expressed by the $FeLV_{ABC}$-producing, feline lymphoma line FL74, is defined by IMI using viremic or immune cat serum, and is the principal target for immunosurveillance (see Section III.B.4). Controversy regarding its identification as a nonvirion *versus* virion encoded protein and tumor-specific-antigen is rampant (see below). FOCMA-S, generated concomitant with morphologic transformation of cells by the 2 most common strains of FeSV (Snyder-Theilen [ST-Snyder and Theilen, 1969] Gardner-Arnstein [GA-Gardner et al., 1970]), is a 65,000 dalton phosphoprotein encoded by the FeSV-transforming gene, v-*fes*. Intensive molecular virology indicates that v-*fes* results when a cellular gene, c-*fes*, is co-opted consequent to a recombination event in the *gag* region of $FeLV_B$. The resultant FeLV is defective for replication having lost *pol*, *env*, and 3' *gag* sequences. Transcription of the *gag*-*fes* sequences of the integrated FeSV provirus (integration being dependent upon $FeLV_B$ help) yields a polycistronic mRNA which is translated into a precursor protein (P85-P110 *fes*) with protein kinase activity specific for tyrosine residues (Barbacid et al., 1980; Franchini et al., 1981; Hampe et al., 1982; Veronese et al., 1982). Phosphorylation of proteins in tyrosine is unique to retroviral transforming gene products, the prototype being the polyprotein pp60 *src* translational product of the v-*src* (and c-*src*) of the avian Rous sarcoma virus (Collett and Erikson, 1978). It currently is thought that transformation is due to phosphorylation of tyrosine

residues of proteins responsible for membrane-mediated regulation of cell shape, adhesion, motility and proliferation. In particular, pp60 src phosphorylates vimentin, a protein that attaches actin to the inner face of the plasma membrane to preserve the normal cytoskeleton. That pp60 src binds and phosphorylates cellular receptors for epidermal growth factor suggests that pp60 src may disturb regulation of cell proliferation. In conclusion, FOCMA-S, although heterogeneous, probably is the transforming protein of the feline sarcoma virus and is encoded by transduced cellular genes not helper FeLV sequences.

Early observations that FOCMA-S and FOCMA-L were antigenically cross-reactive have led to the hypothesis that FeLV induces a c-onc sequence in transformable cells and that the FOCMA-L thus induced was a tumor-specific antigen (Essex et al., 1971a,b; Hardy et al., 1977,1980). This has been strengthened by the apparent dissociation between expression of FOCMA-L and of FeLV structural proteins: (1) FOCMA is not present on nontransformed, FeLV-infected fibroblasts or lymphocytes (Sliski et al., 1977; Fleissner and Snyder, 1982), (2) FeLV proteins do not absorb FOCMA activity from naturally occurring viremic cat sera (Essex et al., 1971a,b; Stephenson et al., 1977), (3) VN and anti-FOCMA (IMI or CDA) activity in cat sera are not correlated (Essex et al., 1971a,b) and (4) FOCMA is expressed on the surface of nonproducer (p27-negative) feline lymphoma cells (Hardy et al., 1977,1980). Furthermore, a 70,000 dalton protein immunoprecipitated from FeLV-negative lymphoma cells has been shown to have a tryptic peptide map analogous to a 70,000 dalton protein of FL74 (FeLV-positive lymphoma) origin (Fleissner and Snyder, 1982).

More recent observations cast doubt on the relationship between FOCMA-L and FOCMA-S and consider that FOCMA-L expression correlates with expression of $FeLV_C$ gp70 epitopes and not the transformed phenotype. First are the observations that the FeSV-encoded polyproteins on nonproducer mink cells cannot be immunoprecipitated by FOCMA antibody-positive sera from either regressor or naturally viremic cats (Barbacid et al., 1980). Also, neither these sera nor reference monospecific antisera to the v-fes gene product immunoprecipitate proteins from FL74 cells with protein kinase activity and viremic cat sera with IMI FOCMA-L activity usually are negative for FOCMA-S by IMI assay (Mathes et al., 1981; Vedbrat et al., 1983). Second, FOCMA-L expression is strong on FeLV-KT ($FeLV_{ABC}$)-infected, feline embryo fibroblasts and human lymphocytes (Essex et al., 1972; Rice and Olsen, 1981). The most impressive evidence is found in recent reports by Vedbrat et al. (1983). FOCMA antibody-positive sera from naturally viremic cats react with $FeLV_C$ gp70 epitopes expressed by budding virus at the cell surface of feline lymphomas and $FeLV_C$-infected fibroblasts. The identity of those epitopes as FeLV gp70 comes from extensive analysis using monoclonal antibodies to FOCMA-L and $FeLV_C$ gp70.

The identification of FOCMA-L as the translational product of $FeLV_C$ env sequences poses an apparent dilemma. Lymphoma-inducing FeLVs usually are subgroup A or AB not C. The prototype lymphomagenic FeLV-R produced by the feline lymphoma

cell line (F422) is $FeLV_A$ and fibroblasts infected with F422 virus do not express FOCMA-L or $FeLV_C$ antigens. However, the parental F422 cells do express $FeLV_C$ gp70 on virions incompletely budded at the plasma membrane (Vedbrat et al., 1983) and, thus, truly are the model for those FOCMA-positive tumor cells which either shed infectious $FeLV_A$, $FeLV_{A,B}$ or which shed no infectious FeLV at all. Furthermore, F422 shed noninfectious $FeLV_C$ that will absorb the activity of monoclonal antibody to FOCMA-L or $FeLV_C$ gp70. Monoclonals further distinguish three antigenic determinants of the $FeLV_C$ gp70 molecule. Monoclonal 3192A recognizes antigens expressed on budding virus and cell membranes that are masked or altered in mature virions and are cross reactive with most cat antisera to FOCMA. Monoclonal B12/C3 discriminates epitopes restricted to infectious $FeLV_C$ virions, and monoclonal 2-124/2-88 identifies epitopes common to infectious and noninfectious $FeLV_C$ particles.

Even if FOCMA-L is a virally-encoded and not cell-derived antigen and even if it is expressed by $FeLV_C$-infected fibroblasts *in vitro*, it still is the antigen that evokes protective immunity to progressive FeLV-induced tumors *in vivo*. Grant and Michalek (1981) argue that FOCMA-L is not a single epitope and that complement-dependent, cytotoxic antibodies to FOCMA present in the sera of naturally infected pet cats specify unique and cross-reacting antigens on individual tumors. It will be extremely interesting to see how the epitopes identified by monoclonals to $FeLV_C$ *in vitro* compare with the epitopes identified *in vivo* by naturally infected cats. The observation that noninfectious $FeLV_C$ particles shed by F422 cells will absorb FOCMA-L activity from naturally infected pet cat sera makes it important to consider if soluble FOCMA-L known to be secreted by spent FL74 cells *in vitro* (Heding et al., 1976) and by both viremic and nonviremic cats (Lutz et al., 1980; Saxinger et al., 1980) *in vivo* (Rice et al., 1981) also is identical to noninfectious $FeLV_C$ gp70. This particularly is relevant because soluble FOCMA serves as the primary immunogen in the highly protective FeLV vaccines scheduled for commercial release in the near future (Heding et al., 1976).

The expression of $FeLV_C$ *env* genes in infected fibroblasts *in vitro* is not necessarily relevant to the expression of FOCMA-L by hemolymphoreticular cells *in vivo*. Fibroblasts are not commonly infected in cats (Rojko et al., 1979a) Since cats exposed to FeLV *in vivo* usually develop antibodies to FOCMA-L, FOCMA-L must be expressed in both nonviremic and viremic cats *in vivo*. Rice and Olsen (1982) document the emergence of a preneoplastic lesion characterized by FOCMA-L expression at 3-5 WAE to FeLV-R in the bone marrow and mesenteric lymph nodes of cats destined to develop either viremia or immunity. Determination of the epitopes present on the marrow and lymphoid cells of these cats in acute productive, preleukemic and latent infection (see Section III.A.1) should establish the nature of the preneoplastic lesion and its evolution into neoplasia.

Several lines of evidence suggest that FeLV leukemogenesis is not restricted to cats with productive FeLV infection. Virus-negative lymphomas account for 29% of all feline LSA and occur after protracted latency periods in older cats (Hardy et al., 1977,1980).

Many nonproducer leukemic cells express FOCMA. Most cats with virus-negative, FOCMA-positive lymphomas originate from FeLV-infected households (Hardy et al., 1980). Other virus-negative lymphomas have been described in FeLV-exposed kittens rendered regressors by passive immunotherapy (de Noronha et al., 1978) and complement-dependent FOCMA antibody is protective against nonproducer leukemia (Grant et al., 1980a,b). Virus-negative leukemic cats have circulating immune complexes which contain viral p27, gp70 and reverse transcriptase (Day et al., 1980; Jones et al., 1980; Snyder et al., 1982). Moreover, sixty percent of nonviremic cats with lymphoma do have low levels of serum p27 (Saxinger et al., 1982) and other nonviremic, preleukemic cats have intermittent marrow FOCMA-L expression long after regression of acute infection (Rice and Olsen, 1981). Lastly, strong evidence implicating FeLV in the genesis of nonproducer lymphoma has been provided by the demonstration that 4 of 8 cats with virus-negative lymphomas and leukemias have latent, reactivatable bone marrow infections with FeLV (Rojko et al., 1982a).

The arguments against FeLV involvement in nonproducer lymphomagenesis also are weighty. Nonproducer (NP) lymphomas are heterogeneous in morphology, tissue distribution, epidemiology, and virology. NP tumors usually are multicentric, alimentary or leukemic and not thymic in origin (Hardy et al., 1980) and a relatively high proportion bear SIg or null cell markers (Hardy et al., 1980). Also included are myelomonocytic leukemias (Madewell and Jarrett, 1983), erythemic myelosis, large granular lymphocytic leukemias, eosinophilic leukemias, systemic mastocytosis and cutaneous mycosis fungioides (Hardy et al., 1980; Rojko et al., 1983b). Nonproducer lymphomas and leukemias need not originate in cats from FeLV-endemic, multiple cat households, and often occur randomly. Furthermore, FeLV-negative tumors often carry other retroviral information, particularly foamy (feline syncytial), endogenous FeLV and RD114 proviruses (Casey et al., 1981).

Specific evidence contrary to the involvement of FeLV in nonproducer lymphomas includes the absence of cytoplasmic p27, surface gp70, and replicating FeLV from tumor and nontumor cells (Hardy et al., 1977,1980; Koshy et al., 1980; Saxinger et al., 1980; Casey et al., 1981; Rojko et al., 1982a; Madewell and Jarrett, 1983; Vedbrat et al., 1983). Four of 7 nonproducer lymphomas are negative for surface FOCMA-L and $FeLV_C$ gp70 epitopes identified by monoclonal antibodies (Vedbrat et al., 1983). Furthermore, 4 of 8 nonproducer lymphomas did not have reactivatable, marrow origin FeLV (Madewell and Jarrett, 1983; Rojko et al., 1983b). In studies employing DNA and cloned probe hybridization, NP tumor cells are negative for complementary DNA to FeLV RNA (Koshy et al., 1980) and absolutely devoid of exogenous FeLV-specific U_3 (LTR) sequences (Casey et al., 1981). Absence of exogenous U_3 sequences means that the NP cells never integrated FeLV proviruses and, therefore, never experienced horizontally transmitted infection. Furthermore, the reactivation of FeLV from NP marrows does not necessitate a role for FeLV in NP lymphomagenesis. It may be that the presence of an overwheiming

tumor burden leads to stress-induced, corticosteroid-triggered (Rojko et al., 1982a), marrow FeLV recrudescence.

If the assumption that FeLV is associated with the etiology of NP lymphoma is correct, the lack of exogenous U_3 sequences in tumor cells would suggest that intact FeLV provirus is not required for initiation of transformation. Similar "hit and run" mechanisms are described for in vitro transformation of cells by Rous sarcoma virus and Herpes Simplex Virus where progressive loss of virus genomes is accompanied by transformation (reviewed in Gilden and Rabin, 1982). Support for this premise comes from analysis of U_3 LTR sequences in tumor cells from cats with FeLV-positive lymphomas (Casey et al., 1981). Exogenously acquired proviruses in some tumors may demonstrate a simple U_3 hybridization pattern with a discrete number of single-copy provirus-host junction fragments. In these tumors, acquisition of provirus may be responsible for tumor initiation. Alternatively, cells with this pattern may be selected for during clonal expansion. In other FeLV-positive tumors, proviral integration is independent of the transforming event. The great diversity of proviral sequences has been interpreted to mean that transformation of uninfected cells precedes superinfection by exogenous FeLV (Casey et al., 1981).

IV. MACROENVIRONMENTAL INFLUENCES ON HOST VIRUS INTERACTIONS

The socioepidemiologic factors that influence the FeLV/cat relationship have been subject to thorough discussion elsewhere (Hardy et al., 1976). A facetious over-simplification of the macroenvironmental forces that have shaped the FeLV/cat relationship might state: Greed, the desire for soft living, and excessive social preoccupation with licking and grooming have caused more feline fatalities than has the much maligned feline predisposition to curiosity.

A. Evolution of FeLV

DNA hybridization studies have provided evidence that FeLV is related closely to an endogenous rat virus and analysis of melting point hybrids formed between their complementary DNAs (Benveniste et al., 1975) shows that these viruses diverged 1 to 10 million years ago. At that time, ancestral cats were confined to the arid reaches of Northern Africa and rat-to-cat transmission (transfection?) of retroviral information is thought to have been consequent to a prey-to-predator relationship. Whether the cat bit the rat or the rat bit the cat is lost to history. The prevailing climatic aridity and natural feline independence probably restricted horizontal transmission for many years. The infertility of viremic queens (Cotter et al., 1975; Hoover et al., 1983) ensured a low prevalence of congenital transmission. To this day, antisocial feral cats are protected from exposure to this readily inactivated virus (Francis et al., 1977). The incidence of exposure of free-ranging, minimally domesticated cats in India to FeLV is extremely low.

B. Socialization

Domestication and, in particular, congregation of cats in multiple cat household (MCH) has favored the cat-to-cat transmission of FeLV and its permanent residence in the MCH in lieu of FeLV test and removal programs (Hardy et al., 1976). Feline behavioral

instincts (licking, grooming, etc.) and the communal water dishes and litter pans found in MCH provide protective moisture for FeLV and allow its efficient horizontal transmission between social cats. After exposure, most cats are able to resist progression of FeLV replication by initiating local or systemic lymphoreticular containment (see Section III.B.II) but 56-80% of these immune cats will retain latent FeLV proviruses in marrow or lymphoid cells. The eventual consequence of increasing FeLV prevalence in MCH is the emergence of FeLV viremias and FeLV-related diseases. This emergence may be precipitated by overcrowding, stress, or intercurrent infection which also are concentrated in the MCH environment. It is not surprising, therefore, that the first recognition of the viral etiology of feline lymphomas was subsequent to the isolation of a cell-free, transmissible, viral agent from a cattery in Glasgow with a high frequency of alimentary and thymic lymphomas and other diseases. Other naturally occurring and experimentally stimulated MCH have provided evidence for the horizontal transmission of FeLV and the MCH influences on viremia, immunity, and producer and nonproducer FeLV-related diseases have been documented extensively. The reader is referred elsewhere for details (Hardy et al., 1976).

C. Intercurrent Infections

Much has been made of the predisposition of FeLV viremic cats to develop intercurrent viral, bacterial, and parasitic infections (Cotter et al., 1975). While the role of FeLV p15(E)-mediated immunosuppression and thymicolymphoid depletion cannot be overstated (see Section III.C.1), other factors demand consideration. MCH often are hotbeds of feline pathogen activity. Cats that attend shows or are permitted to roam return to introduce new pathogens which are assimilated readily into the relatively vulnerable populations. If the household contains kittens with incomplete immunocompetence, the pathogens may become fulminant. In particular, a virus often found in MCH with a special tropism for the lymphoreticular tissues of FeLV viremic cats is the feline infectious peritonitis coronavirus (FIPV) (Cotter et al., 1975; Horzineck and Osterhaus, 1979). It has been reported that 50% of cats with fatal FIPV infections concurrently are viremic with FeLV. The salient clinicopathologic features of progressive FIPV infections include hypergammaglobulinemia, immune complex (antigen, IgC, C3) deposition in renal glomeruli, vessels and elsewhere and indicated excessive immunostimulation (and, hence, ineffective regulatory cell activity). Whether a specific T-suppressor defect induced by FeLV allows progression of immune-mediated FIPV disease is not known. An alternative explanation for the frequent association between the two diseases is that FIPV-induced polyclonal activation of FeLV-replicating lymphoid subsets might facilitate the in vivo reactivation of FeLV from latently infected cats.

Another virus of possible relevance is the feline panleukopenia virus. Although excellent attenuated viral vaccines currently are marketed, the ramifications of routine vaccination of viremic or latently infected cats have not been studied. Regarding the latter, the association of recurrent canine distemper virus infections with exposure to

canine parvoviruses cannot be discounted (Krakowka et al., 1982). Like canine parvovirus, feline panleukopenia virus has pronounced tropism for the lymphohematopoietic tissues (Carlson et al., 1978) and may initiate transient or protracted lymphomyeloid dysfunction.

V. RELEVANCE OF FeLV MODEL TO HUMAN DISEASE

The major importance of the FeLV/cat model is as a tool to specify naturally occurring and experimentally demonstrable pathogenetic factors which independently regulate virus replication and virus-associated cytosuppressive or cytoproliferative disease. Infection with FeLV is highly reproducible and prospective studies can be conducted in animals expected to undergo acute, persistent, productive, and latent infections during a prescribed period of time.

1. Immunosuppression

The interdependence of immune responsiveness and the subsequent development of cancer is accepted generally. Whether immunosuppression precedes neoplasia or whether neoplasia causes immunosuppression remains controversial. There is little doubt, however, that the emergence and clonal expansion of neoplastic cells in vivo must be accompanied by escape from normal surveillance. In several animal models, retroviruses have been identified as the cause of lymphohematopoietic neoplasia. These viruses are associated with a dramatic loss of immunocompetence shortly after infection (see Section III.C.1). The feline system is of particular interest because immunosuppression is initiated by virus infection and precedes detectable neoplastic transformation by months (Perryman et al., 1972; Hoover et al., 1973; Cockerell et al., 1976a,b,c). In fact, the most frequent cause of death in naturally FeLV viremic cats is FeLV-associated bacterial or viral infection (Cotter et al., 1975; Essex et al., 1975b). The profound depressions in cell-mediated immunity facilitate the establishment and progression of otherwise innocuous opportunists (Perryman et al., 1972; Hoover et al., 1973; Cockerell et al., 1976a,b,c). The depression apparently is specific to T-cells and particularly T-helper (Trainin et al., 1983) and T-suppressor (Stiff and Olsen, 1982) cells. The failure of T-helper cells probably leads to ineffective humoral surveillance of FOCMA-bearing preneoplastic or neoplastic cells. Immunomodulation is favored by high levels of viremia which ensure that uninfected T-cells are exposed to the virion envelope component p15(E). This immunosuppressive virion component alters production and reception of T-cell growth regulatory factors (Copelan et al., 1983) and, in its capacity as a hydrophobic surface protein, probably inserts itself into the plasma membrane of T-cells and alters intracellular messages influenced by the cyclic nucleotide and prostaglandin systems (Lewis and Olsen, 1983; Lewis et al., 1983).

In addition to its usefulness as an animal model for the generalized lympho-suppression found in cancer, intensive study of the mechanisms of immunosuppression in viremic cats should provide information relevant to the immunosuppression that accompanies human retroviral infections. Humans in HTLV-endemic areas have a high incidence of pneumonia and filariasis. The HTLV-transformed cell has a T-helper phenotype, and HTLV probably infects only T-helper and T-suppressor cells (Miyoshi et al.,

1981; Poiesz et al., 1981; Popovic et al., 1982). Most importantly, it has been observed recently that HTLV may provoke or assist the specific T-helper loss that defines the acquired immunodeficiency syndrome (AIDS) and predisposes to death via Kaposi's sarcoma, Pneumocystis pneumonia or other opportunistic pathogens (reviewed in Francis et al., 1983).

2. Anemia

The etiologies and pathogenesis of human aplastic anemias remain obscure. The disease is characterized by selective loss of erythroid progenitors without maturation arrest in vivo and by severe decreases in BFU_E and CFU_E assayable in vitro. Considered contributory to the pathogenesis are stem cell defects, disorder microenvironments, and abnormal lymphoreticular/hematopoietic cell interactions (Camitta et al., 1982). A recent report of a human parvovirus-like virus with profound erythrosuppressive effects in vivo and in vitro adds viruses to the list of potential pathogenetic factors (Mortimer et al., 1983). The most critical cell appears to be the primitive erythroid progenitor, the BFU_E, which is dependent on high levels of erythropoietic (EPO) and on burst-promoting activity (BPA) for its proliferation, clonal expansion, and hemoglobinization. BPA is thought to be produced by marrow accessory macrophages by a T-cell dependent process.

3. Lymphoma and Leukemia

Researchers have long recognized an etiologic relationship between oncogenic retroviruses and the development of leukemias, lymphomas, and sarcomas in nonhuman species (for review, see Gilden and Rabin, 1982; Weiss et al., 1983). Two cogent implications of retroviruses in the induction of human leukemias have been provided recently: (1) Cellular genes analogous to retroviral onc genes which are present and frequently transcribed in human tumor cells can induce fibroblast transformation as demonstrated by transfection assay (Cooper, 1982) and may be activated by translocation subsequent to EBV-induced lymphoproliferation in vivo (Klein, 1983) and (2) antigenically distinct human retroviruses have been isolated from certain human T-cell leukemias. These have been named human adult T-cell leukemia viruses (HTLVs). The seroepidemiologic data obtained thus far are consistent with horizontal transmission of HTLV (Gallo, 1981) and both virus-positive patients and virus-negative contacts with HTLV-positive patients have antibody to the major core protein of the virus. The virus-replicating, transformed cell is a relatively mature, terminal trasferase-negative, T-lymphoblast. HTLV has a marked tropism for T-helper and T-suppressor lymphocytes identified by monoclonal antisera (Miyoshi et al., 1981; Poiesz et al., 1981; Popovic et al., 1982; Gotoh et al., 1982). Exposure of freshly isolated, HTLV-naive human cord blood lymphocytes to cell-free HTLV initiates persistent nonproductive HTLV infection. These latently infected cells lose their dependence upon human T-cell growth factors (TCGF, interleukin 2, see ref. 197) for continued growth and become immortalized (Miyoshi et al., 1981). Infection of adult peripheral blood lymphocytes is more readily accomplished when HTLV-immune donors or cell-associated HTLV is used.

Naturally occurring cases of human adult T-cell leukemia (HTLV) will provide materials for in vitro and retrospective in vivo studies of the critical host/virus interactions that occur between human lymphohematopoietic cells and HTLV. Prospective studies of the pathogenesis of HTLV infection and the factors that regulate productive versus latent infection in the natural host must await successful experimental transmission of HTLV to a non-human species.

The appropriateness of the FeLV model as an animal model for human leukemogenesis is remarkable. Like HTLV, FeLV is transmitted horizontally, and both virus-negative and virus-positive cats make antibody to virus or virus-associated, cell membrane antigens (See Sections I.A.5,II.B.4). Naturally occurring producer and nonproducer lymphomas and leukemias have been associated with progressive and nonproductive FeLV infections (see Sections II.B.9,III.C.3), respectively. Most FeLV-replicating transformed cells are relatively mature, terminal transferase-negative, T-lymphoblasts. Exposure of freshly isolated, adult cat or kitten lymphocytes to cell-free FeLV elicits nonproductive FeLV infection and FeLV-infected lymphoblasts have enhanced growth relative to sham-infected controls (Rojko et al., 1981).

More vital is the use of the feline model to document the humoral and cellular surveillance of productively and latently infected cells in vivo. In particular, the factors influencing oncogenic virus latency mandate further description in light of recent observations that people immune to the prospective human oncogen HTLV remain latently infected with HTLV (Gotoh et al., 1982). Data generated in the FeLV/feline lymphoma model may be generalizable to other human infections in which latent/reactivatable viruses are associated with the development of cancer.

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