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Haematological disorders associated with feline retrovirus infections

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Feline leukaemia virus (FeLV) and feline immunodeficiency virus (FIV) are major contagious pathogens in the outbred domestic cat population. Persistent viraemia with FeLV is associated with a greater than 80% mortality rate over 3 to 4 years, most cats dying from the immunosuppressive complications of the virus. FeLV has a wide in vivo host cell range, including haematopoietic cells, lymphoid cells, and accessory cells within the haematopoietic microenvironment, and a significant number of infected cats suffer from cytopenias and/or proliferative disorders. Thus, studies of the interactions between feline cells and FeLV provide insights into the cellular, biochemical, molecular, and genetic events involved in retroviral-mediated deregulation of haematopoiesis and leukaemogenesis.

FIV, a lentivirus that is biologically similar to human immunodeficiency virus (HIV), is found predominantly in older, free-roaming domestic cats. Clinical, seroepidemiological, and experimental infection studies have confirmed that chronic FIV infection is causally related to an immunodeficiency syndrome characterized by progressive CD4 T-lymphocytopenia. In addition, cats with chronic FIV infection, or experimentally induced acute FIV infection, develop peripheral blood cytopenias and marrow morphological abnormalities that are similar to those seen in patients with late-stage or primary HIV infection. Thus, FIV provides a valuable large animal model to study the mechanisms of the marrow suppression that is associated with the acquired immunodeficiency syndrome (AIDS).

CLASSIFICATION OF FELINE RETROVIRUSES

Domestic cats are natural hosts of representatives of each retrovirus subfamily, including FeLV (subfamily Oncornavirinae), FIV (subfamily Lentivirinae), and feline syncytia-forming virus (FeSFV, subfamily Spumavirinae) (Table 1). Although FeSFV induces cytopathic changes in feline cells in vitro and is highly prevalent among some cat populations, it has not been related to any feline illness (Scott, 1971; Shroyer and Refaat Shalaby, 1978).

Table 1. Classification and characterization of feline retroviruses.

Classification	Characterization
Subfamily: Oncornavirinae	
Exogenous feline leukaemia virus (FeLV)	
Replication-competent FeLVs	
Subgroup A	Ubiquitous; highly contagious; low to moderate pathogenicity
Subgroup B	Often isolated from ill cats; arises from recombination of FeLV-A with enFeLV *env* gene sequences
Subgroup C	Rarely isolated; associated with pure red cell aplasia
Replication-defective FeLVs	
Mutant FeLV variants	Some pathogenic variants identified
Recombinant FeLVs	Isolated from lymphomas and fibrosarcomas (FeSVs); viral genome contains transduced *c-onc* sequences
Endogenous feline leukaemia virus	
enFeLV	Germ line FeLV-like sequences; recombines with exogenous FeLV; expressed in lymphoid tumours
Endogenous RD-114 virus	
	Replication is restricted in most feline cells; unknown role in disease
Subfamily: Lentivirinae	
Feline immunodeficiency virus (FIV)	Horizontally transmitted; associated with long latency immunodeficiency syndrome
Subfamily: Spumavirinae	
Feline syncytia-forming virus	Cytopathic in vitro; no disease association in vivo

Exogenous FeLVs are classified based on the ability of the provirus to replicate and form infectious virions (replication-competent) or on the requirement for a 'helper' virus (replication-defective) (Table 1). Replication-defective FeLVs contain mutations, deletions, insertions, and/or transduced cellular gene sequences which interfere with the production, assembly, and/or packaging of infectious viral particles. Although replication-defective FeLV genes may be actively expressed and the proviral genome fully transcribed, transmission of the defective virus genome requires packaging in a pseudotype virion produced by a coinfecting replication-competent helper FeLV.

Replication-competent FeLVs are subclassified into subgroups A, B, or C, based on differences within regions of the viral surface envelope glycoprotein that define the host cell range, the in vitro interference pattern, and susceptibility to neutralizing antibodies (Sarma and Log, 1971; Sarma et al, 1975; Jarrett, 1980) (Table 1). Subgroup A virus is found in all FeLV-infected cats and is isolated alone in two-thirds of healthy viraemic cats. Thus, FeLV-A appears to represent the prototypic, highly contagious FeLV with low to moderate pathogenicity (Jarrett et al, 1978). Subgroup B FeLVs are always isolated with FeLV-A, and a majority of viraemic cats with

clinical illnesses harbour FeLV-B (Jarrett et al, 1978). These observations suggest that FeLV-B is more highly pathogenic and/or that the virus arises in vivo during long-standing infection with FeLV-A. Subgroup C FeLV also is always associated with FeLV-A; however, FeLV-C is only rarely isolated in naturally infected cats (approximately 1% of cases) (Jarrett et al, 1978), and subgroup C strains are specifically associated with pure red cell aplasia (Hoover et al, 1974; Onions et al, 1982).

Endogenous retroviruses are identified in the uninfected domestic cat genome. These include sequences homologous to FeLV (enFeLV) and regions genetically unrelated to FeLV (RD-114 viruses) (Table 1). The enFeLV sequences, present at 7–12 copies per haploid genome, consist of full-length and subgenomic regions that are dissimilar from exogenous FeLV in variable regions of the *env* gene and in the U3 portion of the long terminal repeat (Niman et al, 1980; Casey et al, 1981; Soe et al, 1985; Berry et al, 1988; Kumar et al, 1989). There is no evidence that full-length enFeLV proviruses produce infectious virus; however, these sequences may play a role in disease. For example, enFeLV genes are expressed in both FeLV-positive and FeLV-negative lymphomas (Niman et al, 1977a; Neil et al, 1990), and these gene products may be responsible for the associated antibodies to an FeLV-related putative tumour-specific antigen (the feline oncornavirus-associated cell membrane antigen; FOCMA) (Snyder et al, 1983). In addition, exogenous subgroup A FeLV can recombine with enFeLV *env* gene sequences to generate FeLV subgroup B variants (Stewart et al, 1986; Overbaugh et al, 1988a) which could have increased pathogenicity. The RD-114 sequences are expressed in lymphoid and non-lymphoid tumours of FeLV-positive or negative cats (Niman et al, 1977a,b). However, cats do not mount a serological response to RD-114 products and there is no evidence that RD-114 sequences are involved in recombination events with exogenous FeLV. Furthermore, a replication-competent strain of endogenous RD-114 is generally considered to be noninfectious in cat cells (Dunn et al, 1993), suggesting that RD-114 virus cannot be readily transmitted in vivo. Therefore, a pathogenic role of RD-114 remains undefined.

FELINE LEUKAEMIA VIRUS

Viral structure, genetic organization and FeLV gene products

Viral C-type particles were recognized by Jarrett and colleagues in 1964 as the transmissible agent that induced lymphosarcomas in kittens inoculated with a primary tumour cell homogenate (Jarrett et al, 1964a,b). These observations were the first to characterize an oncogenic retrovirus in an outbred mammalian species.

The virions of infectious FeLV are 110–120 nm in diameter (Figure 1). Virions bind to susceptible host cells through interactions of the surface envelope glycoprotein (SU) with subgroup-specific cell membrane receptors. After viral entry and provirus integration, viral genes are expressed and SU is
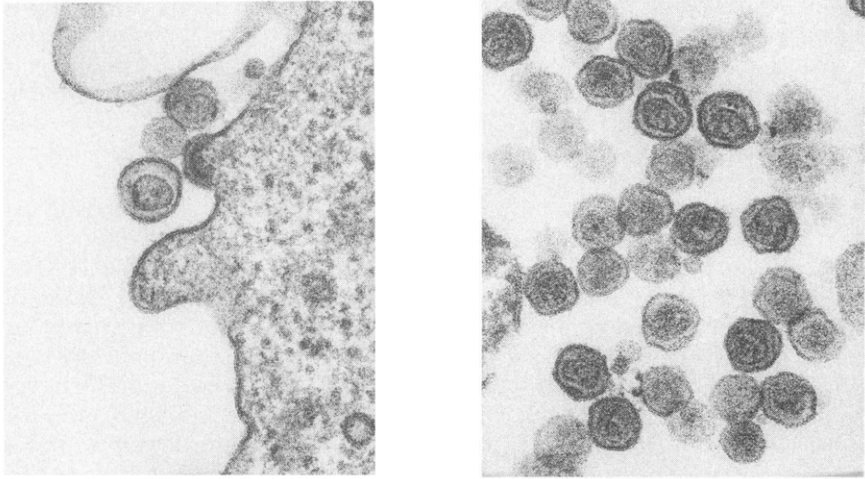
Figure 1. Electron micrographs of FeLV virions budding from the membrane of an infected cell (left) and extracellular mature C-type FeLV particles (right). The mature virions are 110–120 nm in diameter and contain typical round nucleocapsids. Photographs courtesy of Dr Niels Pedersen and Robert J. Munn.

displayed on the host cell membrane. The display of viral SU prevents superinfection of the host cell by FeLVs of the same subgroup (i.e. the concept of viral interference). Specific cell membrane receptors for subgroup A and subgroup B FeLVs have been identified by ligand–receptor biochemical studies and receptor cross-interference assays, respectively (Ghosh et al, 1992; Takeuchi et al, 1992). However, more recent studies have revealed that heterogenous SU molecules from closely related strains of FeLV can compete for binding to a common cell membrane receptor but that superinfection interference may still not be established (Reinhart et al, 1993). These observations suggest that FeLV either binds to a family of closely related, but distinct, primary cell surface receptors, or that secondary cell surface receptors and/or additional membrane-related factors are involved in virus entry and establishment of interference.

Replication-competent FeLV proviral DNA is roughly 8.4 kb in length and consists of a 5' long terminal repeat (LTR), *gag, pol,* and *env* open reading frames, and a 3' LTR (Neil and Onions, 1985; Donahue et al, 1988) (Figure 2). Each LTR contains redundant (R) sequences flanked by 5' and 3' unique regions (U5 and U3, respectively). There are no identified accessory genes encoding regulatory proteins in FeLV, as there are in FIV and other lentiviruses, or in bovine, simian, and human oncornaviruses. Proviral gene expression is regulated by *cis*-acting promoter and enhancer sequences in the U3 region of the LTR. This region contains a cluster of consensus sequences that encode putative binding sites for nuclear proteins (Figure 1) (Fulton et al, 1990; Plumb et al, 1991). The two major transcription products of proviral DNA are a full-length RNA, which is either incorporated as infectious virion RNA or used as the template for trans-
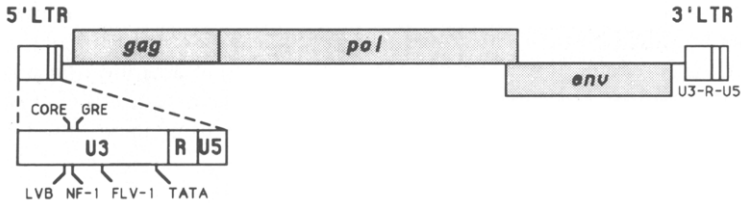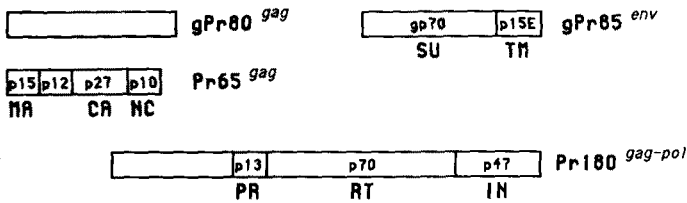
FeLV PROVIRAL DNA

PROTEIN PRODUCTS

Figure 2. Genomic organization of a typical FeLV provirus (upper figure) and FeLV protein products (lower figures). Replication-competent proviral DNA is roughly 8.4 kb in length and contains *gag*, *pol*, and *env* structural genes without identified accessory genes encoding regulatory proteins. The U3 region of the LTR contains consensus sequences encoding binding motifs. Binding motifs identified in the U3 region of FeLV-A/Glasgow-1 are depicted (derived from data in Fulton et al (1990)). These include sequences homologous to LVB and nuclear factor 1 (NF1) binding sites, the SV 40 core enhancer sequence (CORE), a glucocorticoid response element (GRE) sequence, and a novel FeLV-specific binding site (FLV-1). The FeLV precursor proteins and the known and deduced mature proteins are discussed in detail in the text.

lation of the *gag* or *gag-pol* precursor proteins, and a spliced 3.0 kb *env* mRNA (Mullins and Hoover, 1990).

Precursor and mature FeLV protein products have been characterized, in part, by peptide sequence data, known and deduced molecular weights, and functional activity. These protein products, designated by standard nomenclature (Leis et al, 1988), are illustrated in Figure 1 (Neil and Onions, 1985; Mullins and Hoover, 1990). The primary *gag* and *gag-pol* gene products include: gPr80 *gag*, a glycosylated protein that does not undergo further modifications; Pr65 *gag*, a precursor that is cleaved to yield the core viral matrix (MA; p15), capsid (CA; p27), nucleocapsid (NC; p10) (Copeland et al, 1984), and p12 (unclassified) proteins; and Pr180 *gag-pol* which is ultimately processed into the reverse transcriptase (RT; p70), protease (PR; 13) (Yoshinaka et al, 1985), and putative integration protein (IN; p47). The spliced *env* mRNA is translated and processed to an 80–85 kDa glycosylated precursor (Poss et al, 1989), gPr 85 *env*, which is cleaved to form the mature surface glycoprotein (SU; gp70) and transmembrane protein (TM; p15E).

Viral protein synthesis and virion production usually occur at a high rate

in cells infected with replication-competent FeLV. In particular, cells produce an excess of the Pr65 *gag* precursor protein. This excess cytoplasmic and extracellular (plasma) *gag* antigen can be readily detected by immuno-assays of clinical blood samples from infected cats (Hardy and Zuckerman, 1991a). As the detection of intracellular *gag* antigen in peripheral blood cells correlates highly with the ability to isolate virus (Hardy and Zuckerman, 1991b; Jarrett et al, 1991), such assays have been widely used in veterinary practice to diagnose persistent FeLV infection.

Transmission of FeLV and host immune response

High titres of infectious, cell-free FeLV are found in the saliva, nasal secretions, and plasma of cats with chronic viraemia (Francis et al, 1977). The virus is horizontally transmitted by repeated, close social contact (mutual grooming, sneezing, shared feeding), and can be vertically trans-mitted from queens to fetuses (Hardy et al, 1973, 1976; Jarrett et al, 1973).

The ultimate consequences of exposure to FeLV depend upon the host immune responses. Within 2 to 12 days after oronasal transmission, FeLV replicates in tonsillar lymphocytes and macrophages, lymph node follicular centre cells, the thymus, spleen, and gut-associated lymphoid tissue (Rojko et al, 1979). Serum γ-interferon activity increases within 3 days of experi-mental FeLV infection, but α-interferon levels do not increase (Rojko and Kociba, 1991). At 2–6 weeks after exposure, marrow nucleated cells become infected, virus is detected in the plasma (*gag* antigenaemia) and in circulat-ing leukocytes, and both cell mediated and humoral immune responses develop (Rojko et al, 1979; Charreyre and Pedersen, 1991). In 60% of exposed cats, the virus neutralizing antibody and cell mediated immune responses eradicate detectable plasma and cell associated FeLV (regressor infection). However, one-third to one-half of regressor cats transiently harbour latent virus in marrow myelomonocytic cells (Rojko et al, 1982; Madewell and Jarrett, 1983; Pedersen et al, 1984) and stromal fibroblast cells (Linenberger, 1990), as viral replication can be reactivated by culturing these cells in the presence of corticosteroids. Although latent FeLV infection has been experimentally reactivated in regressor cats by exogenous corticosteroid administration, the frequency of spontaneous reactivation in nature is likely to be extremely low.

Persistent viraemia with FeLV occurs in roughly 30% of exposed cats. These animals fail to mount an adequate immune response by 8 weeks after exposure and develop chronic productive infection involving the marrow, lymph node, epithelial, and glandular tissue cells (Rojko et al, 1979). Chronic infection is associated with a greater than 80% mortality rate at 3–4 years, mostly due to immunosuppressive and/or cytopenic complications (McClelland et al, 1980).

Approximately 10% of cats with natural infection, and 25% of cats with experimental infection, mount an incomplete immune response to virus challenge resulting in an atypical or sequestered, active FeLV infection (Hoover and Mullins, 1991; Hayes et al, 1992). These animals have non-protective virus neutralizing antibody titres, no detectable (or only intermit-

tent) antigenaemia or virus in the blood, but evidence of productively infected cells (i.e. intracytoplasmic *gag* antigen expression) in histological sections of lymphoid, mammary gland, and occasionally intestinal tissues. This condition is relevant to other observations that FeLV-exposed, but antigen-negative, cats have higher rates of infection and illness than nonexposed cats (Swenson et al, 1990), suggesting that the subclinical expression of sequestered virus in some cases may play a role in chronic immune dysfunction.

Cytopenic and immunosuppressive disorders associated with FeLV

The acute stage of infection with FeLV (i.e. early viraemia) may be associated with transient haematological abnormalities. For example, early viraemia induced by disease-associated strains of FeLV is often accompanied by mild to moderate pancytopenia with marrow hypocellularity (Pedersen et al, 1977) and $CD4^+$ T-lymphocytopenia (Tompkins et al, 1991). These cytopenias usually resolve within a few weeks. In addition, inoculation of neonatal kittens with an acute immunodeficiency-inducing strain of FeLV (FeLV-FAIDS) induces lymphopenia and anaemia associated with rapid weight loss, diarrhoea, and opportunistic infections (Hoover et al, 1987). In comparison, acute infection with a minimally pathogenic strain of subgroup A FeLV (FeLV-A/61E) is not associated with peripheral blood cytopenias (Linenberger, unpublished observation). Thus, the mechanisms responsible for the haematological abnormalities induced by acute infection with FeLV appear to be related to specific viral strains, and they may also be related to factors such as host age, immune response, and viral load.

Long-term, persistent viraemia with FeLV results in chronic degenerative haematolymphatic disorders and/or neoplastic diseases that are frequently accompanied by anaemia. Over half of cats with non-neoplastic FeLV-related fatal illnesses, and two-thirds with lymphomas (without marrow involvement), have a hypoproliferative anaemia (i.e. with a low reticulocyte count) (Mackey et al, 1975; Hardy, 1980; Reinacher, 1989). These anaemias may be macrocytic yet unresponsive to vitamin B_{12} or folate therapy (Ward et al, 1969; Weiser and Kociba, 1983). Furthermore, FeLV-induced chronic anaemias may be associated with normal iron levels, increased marrow myeloid-erythroid (M/E) ratio, and improvement after treatment of co-existing infections (Cotter, 1979; Wardrop et al, 1986). Thus, one likely mechanism for these anaemias is an inflammatory block of iron utilization secondary to the FeLV-associated malignancies and/or oportunistic infections. Some cases of anaemia are associated with leukopenia, thrombocytopenia, and increased numbers of circulating nucleated erythrocytes or immature myeloid cells (Mackey et al, 1975; Cotter, 1979). Such cases may be associated with marrow cell megaloblastic maturation and increased blasts, suggesting that the ineffective haematopoiesis is due to an FeLV-induced myelodysplastic syndrome (Maggio et al, 1978; Madewell et al, 1979; Blue et al, 1988). Studies have not been carried out on such animals to determine if FeLV-associated myelodysplasia is due to a clonal stem cell process, as it is in man.

Severe pure red cell aplasia occurs in less than 1% of viraemic cats and is specifically associated with subgroup C FeLV. This association has been confirmed by experimental infection studies using biological and molecular clones of FeLV-C (Jarrett et al, 1984; Abkowitz et al, 1985; Riedel et al, 1986). The pathophysiology and pathogenic viral determinants involved in FeLV-C-induced pure red cell aplasia are discussed below.

A minority of FeLV infected cats (15–20%) suffer from a hyperproliferative anaemia characterized by a high reticulocyte count, decreased marrow M/E ratio, and splenic extramedullary haematopoiesis (Cotter, 1979; Hardy, 1981a). Hyperproliferative anaemias may be related to blood loss or may be due to haemolysis secondary to coinfection with *Hemobartonella felis*, or, rarely, anti-erythrocyte antibodies (Scott et al, 1973) or oxidant stress (Christopher, 1989). In addition, FeLV-associated hyperproliferative anaemia has been noted to evolve to pancytopenia and leukaemia, suggesting that this disorder may represent a myelodysplastic process.

Isolated neutropenia is much less common than anaemia in cats with persistent FeLV infection. However, a panleukopenia-like syndrome (similar to that caused by feline parvovirus—see Chapter 3), characterized by severe leukopenia (with normal platelet and erythrocyte counts), enteritis, and dysentery, has been reported as the cause of death in up to 9% of FeLV-infected cats (Hardy et al, 1973; Hardy, 1981a, Reinacher, 1989). FeLV is assumed to be the causal agent, as this disorder has occurred in cats vaccinated against feline panleukopenia virus, and necrotic intestinal tissues contain epithelial and lymphoid cells expressing FeLV antigen. However, recent studies of an outbreak of panleukopenia-like disease in a specific-pathogen-free (SPF) cat colony inoculated with FeLV-A/Glasgow revealed the presence of panleukopenia virus antigen in intestinal tissue extracts (Lutz et al, 1993), suggesting that low levels of this parvovirus may indeed participate in the pathogenesis of this syndrome. Cyclic neutropenia and isolated chronic neutropenia have been rarely diagnosed in FeLV-infected cats, and some cases have been successfully treated with corticosteroids (Gabbert, 1984; Willard, 1985). More commonly, neutropenia is found associated with anaemia and thrombocytopenia. In addition to quantitative changes, neutrophils from viraemic cats demonstrate functional abnormalities, including impaired chemotaxis and ability to generate an oxidative metabolic burst (Lewis et al, 1986; Kiehl et al, 1987).

Chronic infection with FeLV is commonly associated with a slow decline in circulating and tissue lymphocyte populations, qualitative defects in T- and B-lymphocyte functions, and eventual clinical immunodeficiency (Olsen et al, 1987; Tompkins et al, 1989). It is unclear whether this natural history is related to intrinsic viral features, or pathogenic features acquired during in vivo viral evolution. Recently, an FeLV strain that induces an acute immunodeficiency syndrome (FeLV-FAIDS) has been isolated, and a number of replication-defective FeLV variants have been directly cloned from tissues of cats infected with FeLV-FAIDS (Overbaugh et al, 1988b). Experimental infection with the FeLV-FAIDS isolate, the major variant molecular clone (FeLV-61C), or chimeric molecular clones, causes signifi-

cant T-lymphopenia, functional lymphocyte defects, and a fatal immuno-deficiency syndrome (wasting, diarrhoea, and opportunistic infections) (Hoover et al, 1987; Overbaugh et al, 1988b; Quackenbush et al, 1989, 1990; Diehl and Hoover, 1992). High levels of unintegrated viral DNA have been detected in marrow cells of cats infected with the FeLV-FAIDS strain, suggesting that host cell death in vivo is related to a lack of superinfection interference by the FeLV-FAIDS variant (Mullins et al, 1986). This hypothesis has been supported by in vitro studies that revealed that FeLV-FAIDS variant-induced cytopathicity in a T-lymphocyte cell line is related to defective post-translational processing of the *env* precursor protein (Poss et al, 1989, 1990), failure to establish superinfection interference, and accumulation of unintegrated viral DNA (Donahue et al, 1991). Molecular studies have indicated that these phenotypic features, common to multiple FeLV-FAIDS-derived variant and chimeric clones, are mediated by determinants of the *env* gene encoding the 34 amino acid C-terminal segment of SU (Quackenbush et al, 1990; Donahue et al, 1991; Overbaugh et al, 1992). Furthermore, the differences in the rapidity and severity of in vivo disease, or in vitro cytopathicity, between individual variant clones appear to be mediated by determinants of *env* encoding other regions of SU and/or TM (Thomas and Overbaugh, 1993). Thus, alterations in FeLV *env* are likely to be important in FeLV-associated immunopathogenesis. It is unknown whether similar mechanisms of host cell cytopathicity occur in the marrow haematopoietic cells and whether such events may play a causal role in the hypoproliferative anaemia associated with FeLV-FAIDS.

Proliferative haematological disorders associated with FeLV infection

Lymphomas account for 90% of haematological malignancies in cats. Roughly two-thirds of lymphomas occur in FeLV-infected animals (Cotter et al, 1975). In addition, many of the FeLV-negative lymphomas occur in cats with significant prior exposure to FeLV or with latent virus in marrow nucleated cells, suggesting an indirect, or 'hit-and-run' role of the virus in these cases (Hardy et al, 1980; Francis et al, 1981).

The majority of FeLV-associated lymphomas present as metastatic thymic tumours or as a multicentric disease involving multiple nodal and extranodal sites (Hardy, 1981b; Hoover and Mullins, 1991). These tumours consist of a clonal population of malignant lymphocytes, as identified by clonal retroviral integration sites, histochemical staining patterns, cell surface antigen expression, and, in some cases, rearrangement of the T-cell receptor β-chain gene (Casey et al, 1981; Rojko et al, 1989). However, thymic lymphomas induced by the Rickard strain of FeLV may consist of cells bearing either mature T-lineage membrane antigens (e.g. CD4 and/or CD8) (Nelson et al, 1993), prothymocyte features (terminal transferase and Ia antigens, without CD4 or CD8), or monocyte/null cell features (non-specific esterase and Ia antigens, but without any lymphoid markers) (Rojko et al, 1989; Nelson et al, 1993), suggesting that the transformed host target cell may be, in some cases, a monocytic precursor or a multilineage precursor. A group of FeLV-associated splenic lymphomas have also been

described which contain malignant cells that are not T-lymphocytes (i.e. no rearrangement of the T-cell receptor gene) or B-lymphocytes (i.e. no rearrangement of the immunoglobulin heavy chain gene), further indicating that FeLV has the potential to induce transformation of a wide range of host cell phenotypes within lymphoid organs (Levesque et al, 1990). The least common type of FeLV-associated lymphoma is alimentary lymphoma, which affects predominantly the intestines, mesenteric lymph nodes, and visceral abdominal organs; these tumours consist of either malignant T-lymphocytes or B-lymphocytes.

Primary neoplastic disorders involving the marrow occur less frequently than lymphomas in FeLV-infected cats. These disorders include myelo-dysplastic syndromes, acute nonlymphocytic leukaemias (ANLL), and acute lymphocytic leukaemias (ALL). Roughly 75% of cats with myelo-dysplastic syndromes, 90% with ANLL and 70% with ALL are infected with FeLV, suggesting that FeLV plays a causal role in those disorders (Francis et al, 1979; Blue et al, 1988).

The myelodysplastic syndromes include haematological disorders associ-ated with marrow hypercellularity, megaloblastic maturation abnormalities, increased reticulin fibrosis and increased numbers of immature cells and blasts (5–30% of nucleated cells) (Maggio et al, 1978; Madewell et al, 1979; Hardy, 1980; Blue, 1988; Blue et al, 1988). Peripheral blood abnormalities include severe hypoproliferative anaemia, erythrocyte macrocytosis, occasional pancytopenia, and increased numbers of circulating immature erythroid cells. Extramedullary haematopoiesis occurs in the liver and spleen of these animals, resulting in organomegaly. These disorders may evolve to frank acute leukaemia or persist with morbidity and mortality due to severe anaemia, infections, and haemorrhage. An experimental model of myelodysplasia and myeloid leukaemia induced by the strain FeLV-AB/GM1 has been characterized (Toth et al, 1986; Testa et al, 1988) and is discussed below.

Acute nonlymphocytic leukaemia is diagnosed in FeLV-infected cats with high numbers of circulating myeloid or erythroid blasts or, more commonly, in cats with peripheral blood cytopenias and infrequent numbers of circulat-ing blasts, but with ≥30% blasts in the marrow (Blue et al, 1988). Hepato-splenomegaly with extramedullary leukaemic infiltration is frequently found. The leukaemia cells, characterized by morphological and histo-chemical features, are most commonly myeloblasts; they are less commonly erythroblasts and only rarely consistent with myelomonocytic, basophilic, eosinophilic, or megakaryocytic lineages (Fraser et al, 1974; Cotter et al, 1975; Stann, 1979; Hardy, 1980; Toth et al, 1986; Facklam and Kociba, 1986; Blue et al, 1988). The incidence of ANLL among FeLV-infected cats is thought to be low (10% of all haematological malignancies), however, this may be an underestimation, as a careful marrow evaluation is frequently required, but often not pursued, to confirm the diagnosis.

ALL occurs in 8–40% of all FeLV-infected cats diagnosed with lymphoid malignancies (Cotter and Essex, 1977; Hardy, 1980). In comparison, metastatic involvement of the marrow with malignant lymphocytes occurs in one-third of FeLV-infected cats with lymphoma. Primary ALL infrequently

presents with leukocytosis or lymphocytosis (only in 10 or 40% of cases, respectively) (Cotter and Essex, 1977), and therefore it is most often diagnosed after marrow studies are done to evaluate severe neutropenia and/or hypoproliferative anaemia. Marrow ALL blasts are recognized by positive staining for acid phosphate and periodic acid Schiff (Facklam and Kociba, 1986) but the diagnosis usually is made based on morphology of Wright–Giemsa stained specimens, the absence of cytochemical staining for myeloid markers, and the absence of associated dysmyelopoietic features.

Abnormal proliferation of marrow mesenchymal cells in FeLV-infected cats results in myelofibrosis or osteosclerosis. Increased reticulin staining and/or fibrosis have been noted in marrow biopsies from one-third to one-half of cats with myelodysplastic syndromes or ANLL (Blue, 1988). As FeLV infects a high frequency of fibroblast precursor cells (Linenberger and Abkowitz, 1992), this process could result from direct effects of the virus on marrow fibroblast cell growth. Alternatively, myelofibrosis could represent a reactive process induced by altered cytokine production by infected cells within the marrow microenvironment. Medullary osteosclerosis, character-ized by an overgrowth of bony trabeculae into the marrow cavity of the long bones, is associated with subgroup C FeLV infections (Hoover and Kociba, 1974). It is undetermined whether the abnormal osteocyte proliferation is a direct effect of viral infection or a response to the local effects of FeLV-C and/or cytokines.

Pathogenesis of FeLV-induced pure red cell aplasia

FeLV-C, although isolated infrequently from community cats, is epidemio-logically associated with severe anaemia (Onions et al, 1982). When such isolates are inoculated into newborn kittens, the anaemia rapidly develops (Mackey et al, 1975; Onions et al, 1982), suggesting that a multistep pathogenic process is not required. Similarly, in vivo passaged, in vitro passaged, and molecularly cloned FeLV-C/Sarma induce a hypoprolifera-tive anaemia in experimentally infected cats (Hoover et al, 1974; Testa et al, 1983; Abkowitz et al, 1985; Riedel et al, 1986; Dornsife et al, 1989). This feline disorder satisfies all criteria for human pure red cell aplasia (PRCA) (Ammus and Yunis, 1987); profound anaemia (haematocrits range from 4–15%), an absence or near-absence of reticulocytes in the blood, and the marrow lacks haemoglobinized cells. The erythropoietin level is high and ferrokinetic studies confirm that erythropoiesis is severely diminished, but that red cell survival is normal (Kociba et al, 1983; Madewell et al, 1983; Wardrop et al, 1986). Occasionally, cats with late-stage disease will develop associated mild granulocytopenia, thrombocytopenia, and/or osteosclerosis (Hoover and Kociba, 1974).

All cats that are chronically viraemic with subgroup C FeLV develop PRCA. The onset of anaemia in neonatal and immunocompromised cats is faster than that in adult animals (Boyce et al, 1981; Abkowitz et al, 1985). These data suggest that the PRCA results directly from viral replication and not as a consequence of a host immune response. Consistent with this, in vitro studies have failed to demonstrate aberrant populations of T-cells or

autoantibodies that recognize erythropoietin or antigenic determinants on normal or infected erythroid progenitor cells in infected cats (Abkowitz et al, 1987a). Similarly, feline PRCA fails to respond to immunosuppressive therapies such as prednisone, antithymocyte globulin (Zack and Kociba, 1988), cyclosporine, or cyclophosphamide. Other studies have demonstrated that FeLV-C/Sarma-induced PRCA is not a clonal or neoplastic disorder (Abkowitz et al, 1985).

In longitudinal studies of experimentally infected cats, we have characterized FeLV-C/Sarma-induced PRCA from a physiological perspective. Within a few weeks of the onset of anaemia, CFU-E decrease in number while BFU-E are preserved (Abkowitz et al, 1987b; Abkowitz, 1991). Because BFU-E are able to develop into erythroid bursts in vitro, the conditions of the methylcellulose culture permit full erythroid maturation. We therefore reasoned that the CFU-E were no longer present in the cat and that PRCA reflected an in vivo block in the ability of BFU-E to mature to CFU-E. Thus, FeLV-C/Sarma has a lineage (erythroid) and stage-specific (BFU-E to CFU-E) effect. Other investigators have observed extremely low frequencies (or absence) of BFU-E within a few weeks of viral inoculation (Boyce et al, 1981; Testa et al, 1983). It is possible that this discrepancy reflects a higher quantity of viral inoculum, the use of specific pathogen-free versus immunologically intact cats, the infection of younger (versus adult) animals, or the use of different conditions for in vitro BFU-E growth. As lymphoid cells, granulocytic cells, megakaryocytes, CFU-GM, and marrow fibroblasts are also infected with FeLV-C/Sarma in viraemic animals, this specificity is not the result of restricted range of target cells (Hoover et al, 1974; Abkowitz et al, 1987c; Dean et al, 1992; Linenberger and Abkowitz, 1992a).

To determine the viral components required for PRCA, Mullins and colleagues constructed chimeric retroviruses containing regions of FeLV-A/ 61E and C/Sarma viruses (Riedel et al, 1988; Brojatsch et al, 1992). Although most env sequence is conserved among FeLVs, the nucleotide and predicted amino acid sequences of the SU glycoprotein of FeLV-C/Sarma (and of FeLV subgroup B viruses) differ from all FeLV-A isolates in four discrete regions (designated variable regions 1–4) (VR1–4). Viral constructs which contain a 723 base pair region that encodes the N-terminal 241 amino acids of SU of FeLV-C/Sarma (which include VR1–3) induce PRCA when inoculated into neonatal cats. No anaemia is seen when comparable animals are inoculated with viruses which contain the FeLV-A sequences in the region of env. Using a similar experimental strategy, Rigby et al (1992) demonstrated that the sequences of env encoding the 30 amino acid VR1 region of FeLV-C/Sarma SU contain the required determinants for anaemia. Of major interest, FeLV-A recombinant viruses which contain VR-1 from FeLV-C/Sarma retained the ability to infect guinea pig cells in culture (Riedel et al, 1988; Brojatsch et al, 1992; Rigby et al, 1992), a unique feature of subgroup C viruses. In contrast, viruses lacking the FeLV-C/ Sarma VR-1 could not infect guinea pig cells. Thus, it appears that the 30 amino acid region of SU which is required for anaemia is also necessary to infect target cells. These observations suggest the possibility that the cell surface receptor for FeLV-C/Sarma is necessary for normal erythroid

differentiation (i.e. is essential for BFU-E maturation to CFU-E). Theoretically, excess FeLV-C/Sarma SU glycoprotein could impair the cell surface expression of this receptor and/or its ability to bind its physiological ligand (i.e. the same mechanisms involved in viral superinfection interference) and thus inhibit erythropoiesis. In additional studies, the SU encoding *env* gene regions of three additional natural isolates of FeLV-C (FZ215, FA27, FS246) were recently sequenced (Brojatsch et al, 1992). The VR1 sequence of each clone was unique and distinct from the conserved pattern of FeLV-A viruses, suggesting that different structural changes in this region can result in a convergent phenotype.

Does the SU glycoprotein of FeLV-C/Sarma directly impair BFU-E differentiation, as implied above? The behaviour of BFU-E, but not CFU-GM, from cats with FeLV-C/Sarma-induced PRCA is abnormal in vitro. The percentage of BFU-E in the S phase of the cell cycle is two times the controls, BFU-E from these cats are usually sensitive to in vitro lysis after exposure to complement, and progenitors are poorly responsive to the haematopoietic growth factor, kit ligand (Abkowitz et al, 1987b,c, Abkowitz, 1991; Abkowitz, unpublished observation). In addition, highly enriched populations of BFU-E fail to differentiate in the presence of FeLV-C/Sarma in suspension cultures (Abkowitz, unpublished observation).

Could feline PRCA be a non-immune consequence of the effects of FeLV-C/Sarma on accessory cells within the marrow microenvironment? Recent studies of Kahn et al (1993) suggest that TNF release by FeLV-C/Sarma-infected monocytes might play a role in PRCA. This is an unlikely explanation for the specific and complete inhibition of erythropoiesis, as monocytes infected with FeLV-A also induce TNF (though perhaps at a lower titre) (Khan et al, 1993), and many other retroviruses, including FIV, induce TNF expression in vivo (see later discussion). However, additional effects of the SU glycoprotein and/or other viral components (e.g. the LTR (Rojko et al, 1992)) on microenvironmental and lymphoid target cells could influence disease severity.

Pathogenesis of FeLV-induced lymphomas

Several virus and host cell mechanisms are likely to be involved in the multistep pathogenesis of FeLV-induced lymphomas. Studies of DNA sequences from lymphoid tumours have revealed some recurrent patterns of viral genotypic features and host genetic alterations with putative direct and indirect roles in tumorigenesis. First, thymic lymphomas that developed in cats inoculated with a minimally pathogenic molecular clone, FeLV-A/61E, were found to contain proviral variants with *env* gene deletions, truncation mutations, and *env* insertion mutations (Rohn et al, 1994). Such *env* gene mutations may contribute to viral pathogenicity in general by affecting the processing and display of SU (perhaps altering superinfection interference) or they could contribute more directly to lymphomagenesis by affecting cell membrane signalling events and altering cell growth.

Second, some thymic tumours associated with FeLV-A/61E (Rohn et al,

1994) or the Rickard strain of FeLV (FeLV-A/R) (Fulton et al, 1990; Neil et al, 1991) and some spontaneous FeLV-associated lymphoid (and myeloid) malignancies (Matsumoto et al, 1992) are linked to proviruses that contain direct repeats of enhancer motifs in the U3 region of the LTR. These LTR repeats likely augment the enhancer and promoter functions of the viral LTR; and they would increase the expression of nearby rearranged host proto-oncogenes.

Third, integration of FeLV provirus DNA next to specific host proto-oncogene sequences may be an important step in the pathogenesis of spontaneous and experimentally induced lymphomas. Insertional mutagenesis of the *c-myc* proto-oncogene has been noted in 7–38% of spontaneous FeLV-associated lymphomas and in up to 50–75% of thymic lymphomas experimentally induced by FeLV-A/R (Neil et al, 1984; Forrest et al, 1987; Miura et al, 1987; Neil et al, 1987; Mullins and Hoover, 1990; Rezanka et al, 1992; Levy et al, 1993a). In cases of FeLV-A/R-induced lymphomas, the provirus is usually integrated upstream and in the opposite orientation of *c-myc*, suggesting that the enhancer function of the LTR drives *c-myc* overexpression. Recently, a unique common integration site of FeLV proviruses, named *flvi-2*, was identified in spontaneous thymic lymphomas and in experimentally induced T-cell lymphomas (Levy and Lobelle-Rich, 1992; Levy et al, 1993a). The *flvi-2* locus has been identified as the feline homologue of the murine *bmi-1* gene (Levy et al, 1993b). Murine *bmi-1* encodes a nuclear protein, and rearrangement of this proto-oncogene accelerates the development of B-cell lymphomas in $E\mu$-*myc* transgenic mice infected with Moloney murine leukaemia virus (Haupt et al, 1991; Van Lohuizen et al, 1991). In FeLV-positive lymphomas, provirus is generally integrated downstream of *bmi-1*, suggesting that the LTR enhancer activates the *bmi-1* promoter, stimulating an increase in *bmi-1* expression. Additional studies have revealed a 54% co-occurrence of rearranged, or overexpressed, *c-myc* among 13 natural or experimentally induced FeLV-positive tumours with rearranged *flvi-2* (Levy et al, 1993a). Thus, proto-oncogene cooperativity likely plays a major role in the development of these T-cell lymphomas. Additional unique common integration sites, designated *flvi-1* (Levesque et al, 1990), and *fit-1* (Tsujimoto et al, 1993), have been identified in four of seven naturally occurring FeLV-associated splenic non-T-cell lymphomas and in T-cell tumours induced by recombinant FeLV proviruses containing a transduced *v-myc* gene, respectively. Although the *flvi-1* gene locus is highly conserved among mammals (Levesque et al, 1991), the identity of this putative proto-oncogene and its role in leukaemogenesis remain undefined. Similarly, the nucleotide sequence of the *fit-1* region does not show any close match to identified proto-oncogene sequences (Tsujimoto et al, 1993).

Finally, recombinant FeLV proviruses containing either transduced enFeLV sequences or cellular proto-oncogene sequences likely play an important role in some lymphomas. Recombinant proviral variants containing *env* gene sequences originating from enFeLV are detected in three-fourths of FeLV-positive spontaneous thymic and alimentary lymphomas and in one-third of multicentric lymphomas (Sheets et al, 1993). Some of

these recombinants contain mutations affecting a region of *env* that encodes an important immunogenic epitope of SU, suggesting that these variants might contribute to lymphomagenesis by facilitating escape from immune surveillance, enhancing the host cell range of the viruses, and/or by increasing host cell superinfection and the chance of a mutagenic event.

Recombinant FeLV variants containing a transduced *c-myc* gene (FeLV-*myc* isolates) have also been characterized (Levy et al, 1984; Mullins et al, 1984; Neil et al, 1984; Rezanka et al, 1992). In all isolates tested, infection of tissue culture cells or inoculation of neonatal kittens with FeLV-*myc* (along with a helper FeLV) does not result in neoplastic transformation in vitro or the acute induction of multiclonal tumours in vivo (Bonham et al, 1987; Onions et al, 1987; Levy et al, 1988; Doggett et al, 1989). Rather, these recombinants induce shorter-latency, clonal T-cell lymphomas that apparently arise after acquisition of additional de novo mutagenic events. Experimental evidence for this hypothesis came from observations that coinfection of cells with FeLV-*myc* and a *ras*-expressing retrovirus resulted in in vitro transformation (Doggett et al, 1989). Evidence that additional mutagenic events cooperate with FeLV-*myc* in vivo came with the discovery in an FeLV-*myc*-containing tumour of a second recombinant provirus containing a transduced portion of the T-cell antigen receptor β-chain gene (Fulton et al, 1987). This observation, and the finding of rearrangements of both *c-myc* and *flvi-2* in individual thymic lymphomas (Levy et al, 1993a) support the hypothesis that FeLV-induced lymphomas arise as a result of a multistep process.

Pathogenesis of FeLV-induced myeloid leukaemia

The FeLV-AB/GM1 strain induces acute myelodysplasia and myeloid leukaemia in neonatal kittens. Inoculated animals develop trilineage marrow morphological abnormalities within 2 weeks, pancytopenia and increased marrow blasts at 3–5 weeks, and frank myeloblastic or myelo-monocytic leukaemia after 7 weeks (Toth et al, 1986; Testa et al, 1988). Marrow culture studies performed on inoculated cats reveal an early polyclonal expansion of CFU-GM (starting at week 2), with an apparent enhanced sensitivity of progenitor cells to low levels of colony-stimulating activity in serum (Testa et al, 1988). This pattern is followed by a decrease in CFU-GM frequencies and an increase in the growth of small cell clusters correlating with increased marrow blasts. Long-term marrow cultures from cats with myelodysplasia induced by FeLV-AB/GM1 maintain CFU-GM poorly, consistent with an additional effect of the virus on the marrow microenvironment (Testa et al, 1988). Inoculation of kittens with a mixture of molecular clones of subgroup A and subgroup B viruses derived from fibroblasts infected with FeLV-AB/GM-1 induced early expansion of the marrow CFU-GM population followed by a later appearance of small cell clusters in clony-forming assays (Tzavaras et al, 1990). However, marrow morphological and peripheral blood changes did not occur in the majority of these animals, and only FeLV-A was isolated in the one cat that developed myeloid leukaemia.

These observations suggest that specific strains of FeLV may directly alter the growth of marrow progenitors and the function of marrow accessory cells, promoting rapid evolution to myeloid leukaemia. The causal role of the FeLV-B variants in this experimental model of myeloid leukaemia has not been defined, and therefore it is unclear whether enFeLV sequences may be important. Furthermore, additional molecular studies are needed to determine the roles of viral factors such as mutations of *env* or other genes, LTR duplications, or transduced host cell proto-oncogene sequences in the pathogenesis of FeLV-induced myeloid leukaemias. Genes important in myeloid cell growth and differentiation may be deregulated by FeLV insertional mutagenesis or transduction events, analogous to the findings with FeLV-associated lymphomas.

Effects of FeLV within the marrow microenvironment

Accessory cells within the marrow microenvironment provide the structural framework, cytoadhesive molecules, and growth-regulatory cytokines necessary for normal haematopoiesis. As marrow macrophages, T-lymphocytes, and stromal fibroblasts are known targets of FeLV in vivo, alteration of the haematopoietic-supporting function of these accessory cells could play a role in FeLV-induced haematological diseases.

Infection of feline embryonic fibroblasts with subgroup A or subgroup C FeLV induces the secretion of soluble multilineage colony-stimulating activity (CSA) (Abkowitz et al, 1986). Theoretically, such an effect on accessory cells in the marrow microenvironment in vivo could indirectly promote leukaemogenesis by stimulating the proliferation and outgrowth of a neoplastic haematopoietic cell clone. Heterogenous long-term marrow culture stromal cells infected with FeLV support the generation of two- to three-fold greater numbers of nonadherent CFU-GM and total non-adherent cells, compared to uninfected stromal cells (Linenberger and Abkowitz, 1992b). Further studies, however, have revealed that FeLV infection down-modulates the production of soluble progenitor growth-inhibitory activity by heterogenous marrow stromal cells or marrow fibro-blasts, without altering production of soluble CSA by these stromal cells (Linenberger and Abkowitz et al, 1992c).

Some studies have revealed impaired in vitro growth of marrow stromal cells from cats infected with highly pathogenic strains of FeLV. For example, fibroblast colony-forming units (CFU-F) or long-term marrow culture stromal cells from cats viraemic with the anemogenic Kawakami–Theilen (KT) strain of FeLV (Wellman et al, 1988) or the leukaemogenic strain FeLV-AB/GM1 (Testa et al, 1988) grow poorly in culture, suggesting that FeLV can adversely affect the proliferation and viability of marrow microenvironmental cells. Furthermore, in vitro incubation of normal feline bone marrow cells with purified TM (p15E) protein significantly decreases CFU-F-derived colony formation (Wellman et al, 1988).

Together, these observations indicate that FeLV and/or viral proteins can affect marrow accessory cell viability, growth, and/or production of haematopoietic progenitor growth-regulating substances. Furthermore,

these effects appear to occur in a virus strain-specific and host cell lineage-specific manner. In addition, infected marrow microenvironmental cells provide a reservoir of latent virus (Rojko et al, 1982; Madewell and Jarrett 1983; Pedersen et al, 1984; Linenberger, 1990), which likely plays a role in the ultimate incidence and severity of disease in nonviraemic, FeLV-exposed cats (Francis et al, 1981; Swenson et al, 1990). Thus, complex interactions occur between accessory cells, accessory cell products, virus, viral products, and haematopoietic progenitor cells ultimately resulting in the cytopenic or proliferative haematological diseases associated with FeLV.

FELINE IMMUNODEFICIENCY VIRUS

Viral structure, genetic organization and FIV gene products

Feline immunodeficiency virus was initially isolated and characterized in 1987 (Pedersen et al, 1987). The index cases were communal FeLV-negative cats suffering from emaciation, chronic diarrhoea, oral infections, and skin lesions. The transmissible agent in these cases was a T-lymphotropic retrovirus that expressed Mg^{2+}-dependent RT activity. The infectious virions were 120–150 nm in size and contained typical bar-shaped lentivirus nucleocapsids (Figure 3). Subsequent serological surveys, immunoassays, and proviral DNA sequence analyses showed that this feline lentivirus is antigenically and genetically distinct from primate and ungulate lenti-
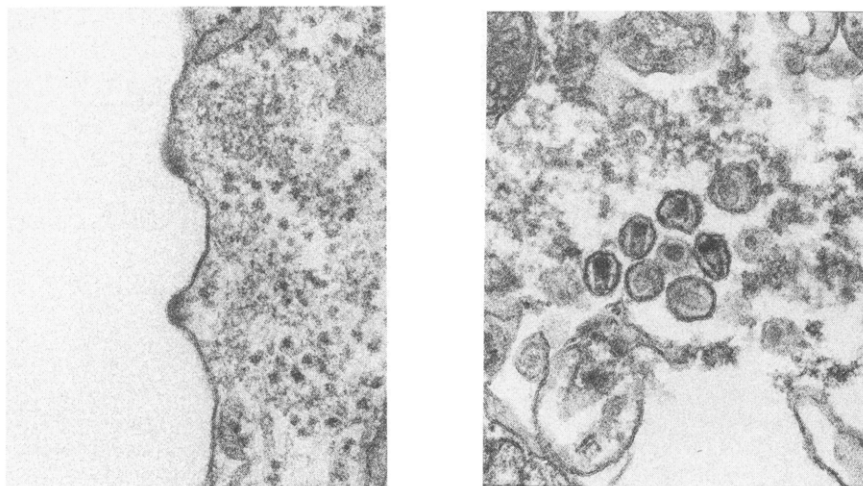

Figure 3. Electron micrographs of FIV virions budding from the membrane of an infected cell (left) and extracellular mature particles (right). Mature FIV virions are 120–150 nm in size and contain bar-shaped nucleocapsids typical of lentiviruses. Photographs courtesy of Robert J. Munn; original photograph (right) from Pedersen et al (1987), © 1987 by the AAAS, reprinted with permission.

viruses, and is causally associated with an immunodeficiency-like illness (reviewed in Pedersen et al (1989) and Sparger et al (1989)).

Numerous proviral genomes of FIV have been molecularly cloned (Olmsted et al, 1989; Talbott et al, 1989; Miyazawa et al, 1991; Morikawa et al, 1991; Maki et al, 1992; Siebelink et al, 1992). The typical FIV genome is about 9500 bases, including open reading frames (ORFs) encoding known and putative regulatory proteins, in addition to *gag*, *pol*, and *env* genes (Figure 4). Although FIV is biologically and virologically similar to HIV, some genetic features are more closely related to other nonprimate lentiviruses. For example, the FIV *pol* gene contains sequences encoding
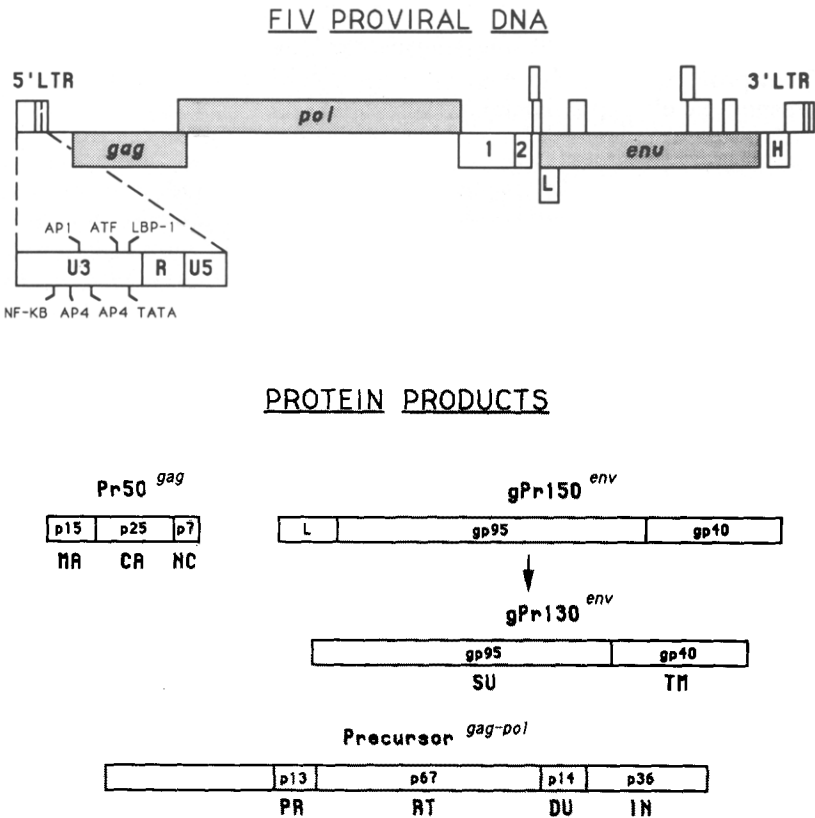
FIV PROVIRAL DNA

PROTEIN PRODUCTS

Figure 4. Genomic organization of the FIV molecular clone pF34 (upper figure) and FIV protein products (lower figures). The FIV genome contains open reading frames encoding putative and characterized regulatory proteins (designated by white boxes), in addition to the *gag*, *pol*, and *env* structural genes (see text). The U3 region of the pF34 LTR contains sequences encoding putative functional binding domains (described in the text). The precursor *env* protein product gPr150[env] is processed to gPr130[env] by cleavage of the leader (L) region. The *gag-pol* precursor protein has not been defined. The other precursor and mature protein products are described in the text. Genomic organization of pF34 was derived from data in Talbott et al (1989) and adapted from figures in Sparger et al (1992), with permission of the author and Academic Press.

deoxyuridine triphosphatase (DU), an enzyme that converts deoxyuridine triphosphate (dUTP) to a monophosphate (dUMP) (Wagaman et al, 1993). This feature is similar to DU-encoding regions of *pol* in the ungulate lentiviruses (i.e. equine infectious anaemia virus (EIAV); caprine arthritis-encephalitis virus (CAEV); and visna virus), but different from *pol* in the primate lentiviruses (i.e. HIV; and simian immunodeficiency virus (SIV)) or the type-C oncornaviruses) (Elder et al, 1992). In addition, FIV does not appear to encode a gene product with transcriptional transactivation function (i.e. analogous to the *tat* protein produced by HIV), suggesting that FIV gene expression may be regulated by mechanisms similar to that of the visna virus (Sparger et al, 1992). FIV does contain a *rev* gene, which is encoded by ORF L and ORF H (exon 1 and exon 2, respectively, of *rev*) (Kiyomasu et al, 1991; Phillips et al, 1992). The FIV *rev* gene product most likely functions like the HIV *rev* protein, as it has been localized to the nucleoli of cells, and putative *rev*-responsive elements (RRE) have been identified in sequences in the 3' end of *env* (Phillips et al, 1992). The FIV ORF 1 appears to encode the *vif* gene, as the primary gene transcript is a singly spliced mRNA species similar to the HIV *vif* transcript, and cell-free virions generated from ORF 1 mutants have significantly impaired infectivity (Tomonaga et al, 1992). The role of ORF 2 in FIV gene regulation has not been defined, however studies with frameshift mutants suggest that this region is required for efficient viral replication in T-lymphocytes (Tomonaga et al, 1993).

The FIV LTR is approximately 360 bases in length. Sequence analyses of molecular clones described to date have identified multiple putative functional domains (see Figure 4) (Kawaguchi et al, 1992; Sparger et al, 1992). Studies utilizing plasmids containing FIV LTR or LTR mutants linked to the bacterial chloramphenicol acetyltransferase (CAT) gene have revealed a high basal promoter activity in some transfected feline and human cell lines (Miyazawa et al, 1992; Sparger et al, 1992). Constitutive LTR-CAT activity is altered by mutation of putative ATF, AP-1, 5' AP-4 or NF-κB sites in some cell lines but not in others (Sparger et al, 1992). These observations suggest that multiple transcription factors, with perhaps overlapping activities, provide sufficient signals for basal FIV replication. Other studies have revealed that AP-1 and AP-4 sites mediate transcription activation of LTR-CAT in feline T-cells stimulated with phytohaemagglutinin and dibutyryl c-AMP, respectively, suggesting that these sequences are important in the inducible expression of FIV genes (Miyazawa et al, 1993). Host cell infection with feline herpesvirus type 1 (Kawaguchi et al, 1991), but not FeLV (Sparger et al, 1992), enhances the promoter activity of a transfected LTR-CAT construct. In comparison, only modest enhancement of FIV LTR-CAT activity is noted in some cell lines cotransfected with full-length FIV molecular clones (Sparger et al, 1992). Thus, the FIV LTR is a strong basal promoter that is activated by host cell factors responding to exogenous stimulation, or by herpesvirus infection; however, LTR activity is only modestly affected by FIV proviral transactivation. FIV replication and gene expression may be most dependent on host cell rather than viral regulatory mechanisms.

The FIV gene products are depicted in Figure 4. The sizes of the

precursor and mature proteins have been estimated by DNA sequence analysis or determined by immunological methods, isolation of purified protein products, and by ion spray mass spectrometry (Egberink et al, 1990; North et al, 1990; Steinman et al, 1990; Stephens et al, 1991; Elder et al, 1993). Similar to FeLV, the FIV *gag* proteins are produced in great excess compared to other gene products (Elder et al, 1993). The FIV CA protein (p25) appears to share immunogenic epitopes with CA proteins of EIAV, SIV, and HIV, as determined by radioimmunoprecipitation assay, Western blot assays, and epitope mapping studies (Egberink et al, 1990; Steinman et al, 1990; Matsuo et al, 1992). Thus, lentivirus *gag* gene products are related and highly conserved among species.

The FIV *pol* gene products, generated from a putative *gag-pol* precursor protein, include: a PR protein (p13) that cleaves a family of substrate peptides, similar to the function of HIV-1 PR (Farmerie et al, 1991); an RT protein (p67) with RNase H activity that is inhibited by drugs that inhibit the RT of other lentiviruses (Cronn et al, 1992); a DU protein (p14) that likely functions to limit misincorporation of uracil into proviral DNA (by keeping the level of dUTP low) (Wagaman et al, 1993); and a putative IN protein that has not been characterized.

The primary *env* gene precursor protein (gPr 150 *env*) is rapidly processed to a secondary precursor (gPr 130 *env*) which is ultimately cleaved to generate SU (gp 95) and TM (gp 40) (Stephens et al, 1991; Verschoor et al, 1993). The FIV *env* gene contains multiple variable regions with sequences that are divergent among the clones described to date (Morikawa et al, 1991; Pancino et al, 1993a). These variable regions encode immunodominant epitopes in SU and TM (Avrameas et al, 1992; Lombardi et al, 1993; Pancino et al, 1993b; Siebelink et al, 1993), and therefore they may be important in the escape of FIV from immune surveillance. In addition, FIV *env* gene variable regions may encode determinants that are important in host cell tropism, infectivity, and pathogenic phenotypic features, analogous to HIV. Evidence that *env* gene products may play a role in host cell cytopathicity, at least in a host cell lineage-specific fashion, come from one study that showed a correlation between the induction of syncytia and cell death with an altered SU glycosylation pattern (Poss et al, 1992).

Transmission of FIV and host immune response

FIV, unlike FeLV, is not commonly transmitted by infrequent social contact. Chronic asymptomatic FIV infection is associated with a very low number of infected circulating mononuclear cells and low titres of cell-free virus in serum, plasma, saliva, and cerebrospinal fluid. In comparison, virus is readily recovered from peripheral blood cells and bodily fluids of sick cats with late-stage disease (Yamamoto et al, 1989; Sparger, 1993). Experimental transmission studies and demographic data of infection rates among field cats (Hosie et al, 1989; Ishida et al, 1989; Shelton et al, 1989a; Yamamoto et al, 1989; O'Connor et al, 1991) indicate that horizontal FIV transmission occurs primarily by biting and fighting. However, proviral DNA and FIV RNA have been detected in peripheral blood and marrow

cells of seronegative cats chronically exposed to docile infected cats (Dandekar et al, 1992), and therefore social contact transmission may occur under certain circumstances. Venereal transmission of FIV has not been documented. FIV can be transmitted to kittens via maternal milk from queens experimentally infected either during gestation (Wasmoen et al, 1992) or immediately post-partum (Sellon et al, 1994). Vertical transmission to kittens does not appear to occur from queens in the asymptomatic, chronic stage of infection.

Chronic, active FIV infection is associated with a sustained humoral immune response that fails to eradicate virus-infected cells. Thus, chronic FIV infection, like HIV infection, is most easily diagnosed by identifying serum anti-FIV antibodies using Western blot and/or enzyme immunoassay methods (Barr et al, 1991; Egberink et al, 1991). Utilizing such techniques, large seroepidemiological surveys in the USA, UK, Italy, and Japan have revealed FIV infection prevalence rates of 1.2–11% in healthy domestic cats and 9–89% in sick cats (Hosie et al, 1989; Shelton et al, 1989a; Yamamoto et al, 1989; O'Connor et al, 1991; Bandecchi et al, 1992). Most studies have found no association between FIV seropositivity and FeLV antigenaemia; the peak incidence of FIV infection occurs in outdoor, free-roaming male cats at 5–10 years of age, whereas the peak incidence of FeLV infection occurs in 1–5-year-old cats. In contrast, FIV infection has been highly associated with FeSFV coinfection (Yamamoto et al, 1989; Bandecchi et al, 1992).

Virus-host cell interactions and host immune responses have been characterized during experimental primary infection with FIV. Within 4 weeks after inoculation, lymphadenopathy develops, plasma viraemia is detectable, circulating total and $CD4^+$ T-lymphocyte numbers decrease, and FIV-infected peripheral blood $CD4^+$ T-lymphocytes, $CD8^+$ T-lymphocytes, and B-lymphocytes become readily detectable (Yamamoto et al, 1988; Tompkins et al, 1991; English et al, 1993). Significantly decreased responses of peripheral blood mononuclear cells (PBMC), spleen cells, and lymph node cells to mitogens such as conconavalin A (Con A), phyto-haemagglutinin (PHA), and pokeweed mitogen (PWM) are noted within the first 3 weeks post-inoculation, indicating that functional abnormalities rapidly develop in T-lymphocytes during primary infection (Lawrence et al, 1992). However, interleukin-2 (IL-2) generation by these cells does not appear to be significantly altered. Anti-FIV antibodies, against *gag* and *env* gene products and FIV RT, often appear by week 3–4 post-inoculation, however seroconversion may be delayed for many weeks in cats inoculated with a low infectious dose of FIV (Hosie and Jarrett, 1990; Fevereiro et al, 1991; Egberink et al, 1992).

From weeks 4–20 post-FIV inoculation, generalized lymphadenopathy persists, systemic symptoms (fever, diarrhoea, depression) develop, and neutropenia occurs (Yamamoto et al, 1988). During this time the $CD4^+$ T-lymphocyte count recovers and the $CD8^+$ T-lymphocyte numbers increase, resulting in a persistently decreased CD4/CD8 ratio (Barlough et al, 1991; Willett et al, 1993). In some experimentally infected cats, particularly neonatal kittens and cats aged 7–12 years, $CD4^+$ lymphopenia persists

(George et al, 1993). The increased CD8$^+$ population includes cells that display a low level of CD8 antigens and a relatively high level of major histocompatibility (MHC) class II antigens, suggesting that this population could include natural killer cells (Willett et al, 1993). PBMC of cats at week 7 post-inoculation and beyond, demonstrate MHC class I-restricted, CD8$^+$ T-cell mediated cytolytic activity against FIV-infected target cells (Song et al, 1992). Cell mediated immunity is likely important in the abatement of the high viral replicative activity during acute infection. By 12 weeks post-inoculation, increased numbers of FIV-infected B lymphocytes, and relatively fewer infected T-lymphocytes, are detected in peripheral blood (English et al, 1993). In addition, cats inoculated with acutely immuno-suppressive, macrophage-tropic strains of FIV, but not apathogenic strains, have increased numbers of provirus-containing monocytes in their circu-lation (Dow et al, 1993). Defective responses of peripheral blood B-lymphocytes to mitogenic stimulation with lipopolysaccharide (LPS), and of T-lymphocytes to recall antigens have been noted at 8–20 weeks post-inoculation (Barlough et al, 1991). Plasma TNF has been detected at high levels in some cats (Lawrence et al, 1992) but not others (Lehmann et al, 1992) with acute FIV infection, thus, the role of inflammatory mediators in the pathophysiology of acute FIV infection remains unclear.

Clinical signs and symptoms associated with experimentally induced acute FIV infection usually resolve by week 20 post-inoculation, but generalized lymphadenopathy may persist for 9 to 12 months. As with HIV infection, primary infection with FIV is associated with a significant viral burden in the lymphoid tissue. Immunohistochemical studies of FIV *gag* antigen expres-sion (Toyosaki et al, 1993), or in situ hybridization assays for viral RNA (Beebe et al, 1994), have revealed infected T-lymphocytes, macrophages, and undefined paracortical cells (possibly dendritic cells) in the lymph nodes of cats necropsied at 3–20 weeks after experimental infection. It is yet to be demonstrated whether there is a relationship between the viral load during primary infection and the subsequent rapidity and severity of the immuno-pathogenesis during late-stage chronic FIV infection (after an asymptomatic stage of many months to many years).

Late stages of natural or experimental chronic FIV infection are character-ized by weight loss, opportunistic infections, lymphoid depletion (occasion-ally following a recurrence of persistent generalized lymphadenopathy), and haematological abnormalities (Sparger et al, 1989; Yamamoto et al, 1989; Pedersen and Barlough, 1991; Ishida et al, 1992). Additional clinical disorders noted in field cats include neurological/behavioural abnormalities, ocular disorders, renal disease, haematological malignancies, and squamous cell carcinoma of the mouth and skin (Pedersen and Barlough, 1991). These clinical disorders are associated with a progressive decrease in the number of circulating CD4$^+$ T-lymphocytes (Ackley et al, 1990; Novotney et al, 1990; Barlough et al, 1991; Hoffmann-Fezer et al, 1992). Concomitant abnor-malities are detected in the in vitro proliferative responses of PBMC to B-cell mitogens (LPS), T-cell mitogens (Con A and IL-2), a T-cell-dependent B-cell mitogen (PWM), and a T-cell antigen (keyhole limpet haemocyanin) (Lin et al, 1990; Siebelink et al, 1990; Barlough et al, 1991;

Torten et al, 1991; Bishop et al, 1992). The production of IL-2 by Con A-stimulated PBMC from cats with long-standing experimental FIV infection is also decreased (Siebelink et al, 1990). Functional T-cell defects are demonstrable in vivo, as the antibody response to a T-cell-dependent immunogen is significantly impaired in cats beyond 2 years post-FIV inoculation. Polymerase chain reaction (PCR) assays of purified peripheral blood lymphocyte populations from cats with chronic experimental or natural FIV infection have revealed that B-lymphocytes are the most frequently infected circulating host cell, with lower infection rates among $CD4^+$ and $CD8^+$ T-lymphocytes (English et al, 1993). As the numbers of peripheral blood B-lymphocytes and $CD8^+$ T-lymphocytes do not significantly change during chronic infection, these host cells are apparently not susceptible to the cytopathic mechanisms involved in CD4 T-lymphocytopenia (Bishop et al, 1993).

Haematological disorders associated with FIV

Peripheral blood cytopenias and marrow morphological abnormalities frequently occur during experimental acute FIV infection and during the late, symptomatic stages of chronic infection. The characteristics and frequency of these disorders closely resemble the haematological manifestations of HIV infection, suggesting that the pathogenesis of the marrow suppression associated with both of these related lentiviruses is similar.

Neutropenia (i.e. neutrophil count $< 2500/\mu l$) develops in a majority of cats inoculated with in vivo and in vitro passaged FIV isolates (Yamamoto et al, 1988; Mandell et al, 1992; Callanan et al, 1992b; Moraillon et al, 1992; George et al, 1993; Linenberger et al, 1994). Neutropenia usually occurs within 4–8 weeks of inoculation and may persist for 2–18 weeks, with nadir counts ranging from 200–2000 cells/μl. Neutropenia is severe (i.e. < 500 cells/μl) in FIV-inoculated neonatal kittens (George et al, 1993) and in cats with pre-existing asymptomatic FeLV infection (Pedersen et al, 1990). Dually infected cats have more severe clinical signs of primary FIV infection and a higher rate of fatal infections and/or gastrointestinal complications. Significant decreases in the mean circulating eosinophil count have been noted at weeks 6–13 post-inoculation with FIV Petaluma (Mandell et al, 1992); however, peripheral blood monocyte counts, platelet counts, and haematocrits are not significantly altered during experimental primary FIV infection (Yamamoto et al, 1988; Mandell et al, 1992; George et al, 1993).

Histological marrow evaluations during the neutropenic phase of experimental acute FIV infection occasionally reveal myeloid hyperplasia with a shift to immature precursors, lymphoid follicles, and/or marrow lymphocytosis (Pedersen et al, 1990; Beebe et al, 1992; Callanan et al, 1992b; Mandell et al, 1992). However, serial marrow aspirates on a cohort of inoculated cats failed to detect significantly increased frequencies of marrow lymphocytes, plasma cells, or eosinophils when compared to uninfected control cats (Mandell et al, 1992).

Infected marrow mononuclear cells and megakaryocytes are detectable by in situ hybridization assays for FIV nucleic acids in histological marrow

sections from cats with experimental acute FIV infection (Beebe et al, 1992; Linenberger et al, 1995). Double-labelling studies have identified some of these mononuclear cells as infected T-lymphocytes and monocyte/macrophages (Beebe et al, 1993). High frequencies of infected cells have also been detected in cats with experimental acute FIV infection and pre-existing FeLV infection (Beebe et al, 1992). As maturing marrow myeloid and erythroid precursors in these sections do not appear to harbour proviral DNA, these lineages apparently are either not major targets of FIV or infected progenitors do not survive to give rise to infected progeny. Marrow buffy coat cells from cats at weeks 4–12 post-FIV inoculation produce infectious cell-free virus in culture (Linenberger et al, 1994). Furthermore, indirect immunofluorescence assays for intracytoplasmic FIV CA protein or in situ hybridization assays for FIV nucleic acids identify infected mononuclear and macrophage cells in these cultures. These observations support the hypothesis that marrow accessory cells (monocyte/macrophages and T-lymphocytes) are targets of FIV during acute infection and that these cells, and/or their extracellular products, may play a role in the pathogenesis of neutropenia.

The asymptomatic stage of chronic FIV infection lasts from many months to many years and is not usually associated with peripheral blood cytopenias (Shelton et al, 1990a). The marrow during this stage may contain increased numbers of lymphocytes, plasma cells, or eosinophils, but the myeloid, erythroid, and megakaryocytic lineages mature normally (Shelton et al, 1990a). Similarly, marrow culture studies on asymptomatic, FIV-seropositive cats with normal peripheral blood counts demonstrate normal frequencies of CFU-GM, BFU-E, and CFU-E, with normal progenitor cell cycle kinetics and in vitro growth responsiveness to haematopoietic growth factors (Linenberger et al, 1991). In situ hybridization studies of marrow sections and soluble FIV CA antigen assays of marrow buffy coat cell cultures often fail to identify virus-producing cells during this stage of infection (Beebe et al, 1992; Linenberger, unpublished observation). Thus, haematopoiesis is unperturbed during asymptomatic infection and marrow cell viral replication occurs at only a very low level, if at all. Drug-induced haematological complications may occur more frequently in these animals. For example, severe neutropenia has been reproducibly induced in asymptomatic FIV-seropositive cats but not in seronegative animals by exposure to griseofulvin (Shelton et al, 1990b). This observation is analogous to the increased sensitivity of HIV-infected individuals to the cytopenic adverse effects of certain drugs. The griseofulvin-induced neutropenia in FIV-infected cats appears to be mediated by binding of antibody or immune complexes to circulating neutrophils, facilitating their clearance by the reticuloendothelial system (Abkowitz, unpublished observation).

Cats suffering from late-stage FIV-related symptoms, including fever, weight loss, persistent generalized lymphadenopathy, neurological disorders, diarrhoea, opportunistic infections, and malignancies, frequently have peripheral blood cytopenias (Hosie et al, 1989; Ishida et al, 1989; Yamamoto et al, 1989; Shelton et al, 1990a; Fleming et al, 1991; Sparkes et al, 1993). Anaemia, neutropenia, and thrombocytopenia are found in

roughly 30, 25, and 6–16%, respectively, of clinically ill FIV-seropositive cats. Anaemia and thrombocytopenia are more common in FIV-infected cats with more severe clinical illness. Coinfection with FeLV produces similar cytopenias but with more frequency than that seen with FIV infection alone (Shelton et al, 1990a). As these abnormalities occur in cats without coexistent infection with FeLV, *Hemobartonella felis*, *Toxoplasma gondii*, and feline coronavirus, FIV and/or factors related to profound late-stage immune dysfunction likely play a direct causal role in marrow suppression. An increased neutrophil count has been noted in 23–35% of FIV-infected cats in some studies, often in association with purulent bacterial infections (Yamamoto et al, 1989; Fleming et al, 1991; Sparkes et al, 1993). Thus, FIV-infected animals can mount an appropriate leukaemoid response under some conditions. Similarly, haemolytic anaemia due to *Hemobartonella felis* infection has been noted in some FIV-infected cats (Sparkes et al, 1993), demonstrating that appropriate compensatory erythropoiesis can also occur.

Marrow morphological abnormalities are found in 72% of cats with symptomatic FIV-associated illnesses: myeloid and erythroid cell hyperplasia; erythroid megaloblastic maturation abnormalities; infiltration with reactive lymphocytes and plasma cells; and, less commonly, infiltration with neoplastic lymphoid or myeloid cells (most commonly in cats coinfected with FeLV) (Shelton et al, 1990a). In situ hybridization studies have identified FIV-infected marrow cells in cats with late-stage terminal illnesses, and double-labelling studies have further identified cells as predominantly monocyte/macrophages (Gluckstern et al, 1993). Late-stage FIV infection therefore appears to be associated with increased virus replication in marrow accessory cells, ineffective haematopoiesis with dysplastic marrow cell maturation, and peripheral blood cytopenias.

Haematological malignancies associated with FIV

Chronic infection with FIV is associated with an increased risk of developing lymphoma. A retrospective serological survey revealed an overall adjusted 5.1-fold relative risk of lymphoma in cats infected with FIV, compared to uninfected cats matched for age, sex, geographic location, and time of study (Shelton et al, 1990c). The relative risks of developing lymphoma in cats infected with FeLV alone, or coinfected with FIV and FeLV were 62.1-fold greater, or 77.3-fold greater, respectively, than controls. The tumours associated with FIV infection were predominately multicentric nodal or extranodal. FIV-associated lymphomas develop in cats at a mean age of 8.7 years old, compared to mean ages of 3.8 or 6.3 years old in cats with lymphomas associated with FeLV infection, or FIV and FeLV coinfection, respectively (Shelton et al, 1990c). These observations agree with other reports of lymphomas among retrospective surveys of cats with spontaneous FIV infection (Hosie et al, 1989; Ishida et al, 1989; Yamamoto et al, 1989).

FIV-associated lymphomas differ from FeLV-induced tumours in the anatomical locations involved and the phenotype of the malignant cells. Extranodal sites of tumour involvement are common in FIV seropositive

cats. These sites include the liver, kidneys, spleen, and periorbital tissues (Shelton et al, 1990c; Hutson et al, 1991; Callanan et al, 1993). Similar lymphomas have occurred, albeit rarely, in cats experimentally infected with FIV (Callanan et al, 1992a, 1993). Histological, immunophenotypic, and molecular analyses of spontaneous and experimental FIV-associated lymphomas have revealed clonal populations of immunoblastic B-cells (Callanan et al, 1993). These tumour cells do not harbour FIV provirus, and they do not contain rearrangements of c-myc. In comparison, one tumour in a cat coinfected with FeLV consisted of neoplastic T-cells containing FeLV proviral DNA (Callanan et al, 1993). Thus, FIV infection appears to pre-dispose cats to clonal outgrowths of malignant B-lymphocytes, similar to the association of lymphomas and HIV infection in man. The pathogenic mechanisms involved in FIV-associated tumorigenesis remain undeter-mined; impairment of immune surveillance and dysregulation of B-lymphopoiesis likely play permissive roles.

Myeloid leukaemia has been rarely diagnosed in FIV seropositive cats (Yamamoto et al, 1989; Hutson et al, 1991; Sparkes et al, 1993). There has been one report of myeloid leukaemia developing within 2 months after inoculation of a kitten with FIV Petaluma (Yamamoto et al, 1988). Other reports of myeloproliferative disorders in FIV seropositive cats involved animals coinfected with FeLV. Collectively, these observations do not strongly support an association between FIV infection and nonlymphoid haematological malignancies. Long-term prospective studies of large cohorts of FIV-infected cats will be required to identify such an association.

Potential mechanisms of FIV-associated haematological disorders

Productively-infected marrow T-lymphocytes, monocytic/macrophages, and megakaryocytes are detected during primary and late-stages of FIV infection. In contrast, myeloid and erythroid precursors cells do not appear to be major targets of FIV in vivo. This pattern of host cell range is similar to that of HIV-1, and suggests that FIV-associated marrow suppression is mediated by mechanisms similar to those implicated by studies with HIV. Such mechanisms include suppression of haematopoietic progenitors by marrow accessory cells, accessory cell products, extracellular virus, and/or viral products (Scadden et al, 1989; Louache et al, 1992; Zauli et al, 1992). However, FIV differs from HIV-1 in some respects, and these differences could relate to additional or alternative mechanisms of marrow suppression. For example, FIV infects a significant number of B-lymphocytes and $CD8^+$ T-lymphocytes in vivo, whereas these host cell phenotypes do not appear to be major targets of HIV in vivo. Thus, these cells may be more directly involved in the pathogenesis of marrow suppression associated with FIV. Other susceptible or infected host cells in the marrow microenvironment could also be important. The broader in vivo host cell range of FIV, compared to HIV, is explained by the recent observations that the major receptor for FIV is not feline CD4 (Norimine et al, 1993) but rather a 24 kDa protein (Willett et al, 1994). The putative FIV receptor is constitutively expressed on susceptible cell lines, neutrophils, macrophages, and astro-

cytes, and it is inducible on T-lymphocytes by Con A and IL-2 exposure (Hosie et al, 1993). Although this receptor is expressed on neutrophils, these cells are not infected with FIV, suggesting that additional factors likely are involved in virus entry and/or integration. Receptor-bearing cells in the marrow could be affected by interactions with extracellular FIV or the SU protein, without virus entry and integration, analogous to the effect of HIV *env* proteins on human marrow progenitor and accessory cells (Sugiura et al, 1992; Zauli et al, 1992).

FIV-induced marrow suppression could be related to dysregulation of cytokine production in the marrow microenvironment. Peritoneal macrophages from FIV-infected cats have an impaired ability to produce interleukin-1 following LPS stimulation (Lin and Bowman, 1992), suggesting that the haematopoietic-supporting function of these cells in the marrow could also be compromised. Some studies have detected increased levels of plasma TNFα in cats with experimental acute FIV infection (Lawrence et al, 1992) and increased levels of plasma interleukin-6-like activity in cats with chronic natural FIV infection (Ohashi et al, 1992). In vitro, TNFα inhibits the growth of feline haematopoietic progenitors (Khan et al, 1992). TNFα also enhances apoptotic death of FIV-infected fibroblastic cells in culture (Ohno et al, 1993). Thus, inflammatory mediators may contribute to marrow suppression by adversely affecting progenitor survival or growth or by enhancing the cytocidal effects of FIV in infected marrow accessory cells.

Studies of haematopoiesis during the asymptomatic stage of natural FIV infection reveal no abnormalities in haematopoietic progenitor frequencies, cell cycle kinetics, and in vitro growth characteristics. In addition, sera from these cats do not contain progenitor growth inhibitory activity or complement-fixing antibodies that recognize progenitors (Linenberger et al, 1991). These observations concur with others (see above) that suggest that progenitors are not major targets of FIV and that factors associated with high levels of virus expression and/or progressive immune dysfunction likely play significant roles in marrow suppression during primary or late-stage infections. Long-term follow-up studies of a cohort of naturally FIV infected cats showed that peripheral neutrophil and lymphocyte counts gradually decrease over time and that episodes of transient neutropenia are occasionally seen (Shelton et al, 1994). In marrow culture studies on these cats, the frequencies of BFU-E were significantly decreased with long-term asymptomatic infection, but the in vitro growth response of the BFU-E to exogenous colony-stimulating activity, or to autologous or normal cat sera, was unperturbed. These resemble the decreased erythroid progenitor frequencies seen in asymptomatic HIV seropositive patients (Bagnara et al, 1990), and suggest that these progenitors are more susceptible to the marrow suppressive mechanisms associated with chronic lentivirus infection.

Comprehensive studies of haematopoiesis in cats with symptomatic late-stage natural FIV infection have not been performed. One cat with chronic FIV infection, weight loss, severe lymphopenia, and persistent neutropenia was found to have an inhibitory activity in the serum that suppressed the in vitro growth of autologous CFU-GM, but not erythroid progenitors (Shelton et al, 1989b). This activity was not mediated by an antibody, and it

only weakly inhibited the growth of normal cat CFU-GM. Because frequency of progenitors were not altered in the marrow of this cat, this inhibitory activity likely impaired the proliferation or differentiation of the CFU-GM progeny.

A number of marrow abnormalities occur during the period of peripheral blood neutropenia and high levels of virus replication in cats with experimentally induced acute FIV infection (Linenberger et al, 1994). Frequencies of marrow CFU-E, BFU-E, and CFU-GM are significantly increased at weeks 1.5–4 post-inoculation but uniformly depressed at weeks 6–12, when neutropenia occurs. In addition, the percentages of progenitors in the DNA-synthetic phase of the cell cycle are frequently high at weeks 6–12 post-inoculation. Sera from cats at weeks 4–12 post-inoculation frequently contain activity that inhibits the in vitro growth of autologous CFU-GM, whereas progenitor inhibitory activity is not found in sera of cats at weeks 1.5 or 3 post-inoculation. These progenitor and serum changes correlate with the appearance of productively FIV-infected accessory cells in the marrow and peak levels of viraemia. Thus, virus, viral antigens, accessory cells, and/or accessory cell products may mediate the suppression of progenitor growth and/or viability, resulting in the transient neutropenia. Anaemia is not seen in this setting, probably because of the long life-span of erythrocytes and the absence of factors that shorten red cell survival, like haemolysis and haemorrhage. As these abnormalities mimic those seen in late stages of FIV and also HIV infections (when cytopenias and high levels of virus replication also occur), further studies of haematopoiesis during acute FIV infection could provide insights to the viral and host cell factors important in marrow suppression associated with chronic lentivirus infection.

SUMMARY

Feline oncornavirus and lentivirus infections have provided useful models to characterize the virus and host cell factors involved in a variety of marrow suppressive disorders and haematological malignancies. Exciting recent progress has been made in the characterization of the viral genotypic features involved in FeLV-associated diseases. Molecular studies have clearly defined the causal role of variant FeLV *env* gene determinants in two disorders: the T-lymphocyte cytopathicity and the clinical acute immunosuppression induced by the FeLV-FAIDS variant and the pure red cell aplasia induced by FeLV-C/Sarma. Variant or enFeLV *env* sequences also appear to play a role in FeLV-associated lymphomas. Additional studies are required to determine the host cell processes that are perturbed by these variant *env* gene products. In the case of the FeLV-FAIDS variant, the aberrant *env* gene products appear to impair superinfection interference, resulting in accumulation of unintegrated viral DNA and cell death. In other cases it is likely that the viral *env* proteins interact with host products that are important in cell viability and/or proliferation. Understanding of these mechanisms will therefore provide insights to factors involved in normal

lymphohaematopoiesis. Similarly, studies of FeLV-induced haematological neoplasms should reveal recombination or rearrangement events involving as yet unidentified host gene sequences that encode products involved in normal cell growth regulation. These sequences may include novel proto-oncogenes or sequences homologous to genes implicated in human haematological malignancies.

The haematological consequences of FIV are quite similar to those associated with HIV. As with HIV, FIV does not appear to directly infect myeloid or erythroid precursors, and the mechanisms of marrow suppression likely involve virus, viral antigen, and/or infected accessory cells in the marrow microenvironment. Studies using in vitro experimental models are required to define the effects of each of these microenvironmental elements on haematopoietic progenitors. As little is known about the molecular mechanisms of FIV pathogenesis, additional studies of disease-inducing FIV strains are needed to identify the genotypic features that correlate with virulent phenotypic features. Finally, experimental FIV infection in cats provides the opportunity to correlate in vivo virological and haematological changes with in vitro observations in a large animal model that closely mimics HIV infection in man.

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