CHAPTER 2

Feline Oncoretroviruses

WILLIAM D. HARDY, JR.

Elucidation of the biology of feline oncoretroviruses over the past 20 years has changed the concepts of retroviruses in all animals, including humans. This chapter considers the history, virology, epidemiology, immune responses, pathology, and vaccination of feline oncoretroviruses.

1. HISTORY

Three major groups, formerly considered subfamilies of Retrovirinae exist in cats (Table I): (1) Oncovirinae, (2) Lentivirinae, and (3) Spumavirinae (Teich, 1982; Riggs, *et al.*, 1969). Domestic and wild large cats are infected with members of all three groups. Recently a more extensive classification of the retrovirus family has been recommended (see *Volume* 1, Chapter 2). The feline leukemia virus (FeLV), an oncovirus, was first isolated from a cat that had developed lymphosarcoma (LSA) while living in a multiple cat household in Scotland in 1964 (Jarrett *et al.*, 1964). Several cats in this household had previously also developed LSA (cluster household). At that time all retroviruses were thought to be endogenous viruses that were only transmitted genetically (vertically) (Huebner and Todaro, 1969). However, observations of pet cats (Hardy, *et al.*, 1969, 1973a; Brodey *et al.*, 1970; Essex *et al.*, 1977a), and later laboratory experiments (Jarrett *et al.*, 1973a), demonstrated that FeLV is an exogenous retrovirus that is transmitted contagiously among cats. This observation was the first conclusive proof that any retrovirus was transmissible by contagious means, and this finding changed the prevailing concepts on these viruses. It is now known that all disease-inducing retroviruses

WILLIAM D. HARDY, Jr. • Center for Infectious Diseases, Bronx Lebanon Hospital Center, and Albert Einstein School of Medicine, Bronx, New York 10456, and Infectious Disease Service, Memorial Sloan Kettering Cancer Center, New York, New York 10021.

J. A. Levy (ed.), *The Retroviridae*

TABLE I. Feline Retroviruses

1. Oncoviruses	Genetically transmitted
A. Endogenous viruses	
(1) RD-114	Xenotropic—does not productively infect cat cells; no known disease association
(2) FeLV-related sequences	Full length and shorter sequences Cannot be induced to replicate Recombines with exogenous FeLV-A to form FeLV-B and FeLV-C
(3) MAC-1	Cannot be induced to replicate in cat cells
B. Exogenous viruses	Spread contagiously
Chronic leukemia viruses	
(1) FeLVs	
Subgroup A	Ecotropic—found in all infected cats
Subgroup B	Polytropic—found in 50% of infected cats
Subgroup C	Polytropic—found rarely, less than 1%
(2) Defective FeLV-FAIDS	Experimentally induces FAIDS
(3) FeLV-GM1	Defective virus, induces myeloid leukemia
Acute leukemia viruses	
(1) Defective FeLV-*myc*	Found in 14% of FeLV-infected thymic LSAs Recombinant proviruses
(2) Defective FeLV-*tcr*	Found in 1 LSA along with FeLV-*myc*
(3) FeSVs: 11 well-characterized isolates	Recombinants between FeLV and cellular oncogenes
2. Lentiviruses	Induces AIDS syndrome
A. FIV (feline immunodeficiency virus)	
3. Spumaviruses	Causes no known disease
A. FeSFV (feline syncytium-forming virus)	

Xenotropic: Grows in heterologous (noncat) cells only.
Ecotropic: Grows in homologous (cat) cells only.
Polytropic: Grows in homologous and heterologous cells.

of animals and humans, except some found in inbred laboratory mice, are exogenous and are contagiously transmitted among members of their species (Teich, 1982; Hardy, 1983a,b). Seroepidemiological studies have found that about 2%, or more than 1 million of the estimated 60 million pet cats in the United States, are infected with FeLV (Hardy, 1981b). Most of these cats are healthy carriers of the virus.

Elucidation of the unique biology of feline retroviruses has often presented new directions and concepts for the study of retroviruses of all animals including humans. For example, as noted above, FeLV was the first naturally occurring retrovirus to be shown to be commonly transmitted in a contagious manner (Hardy *et al.*, 1969, 1973a; Brodey *et al.*, 1970; Jarrett *et al.*, 1973a; Essex *et al.*, 1977a), and now all disease-inducing retroviruses, including human retroviruses, are known to be spread in a similar manner. FeLV was among the first retroviruses shown to have arisen, most likely, from transspecies transmission of retroviruses (Todaro *et al.*, 1974; Benveniste *et al.*, 1975; Todaro, 1980). FeLV was the first "oncogenic" retrovirus to be shown not only to cause cancer, but more often to cause degenerative diseases such as

aplastic anemia and immunosuppressive diseases now resembling AIDS (Hardy *et al.*, 1969, 1973a, 1976a; Anderson *et al.*, 1971; Perryman *et al.*, 1972; Hoover *et al.*, 1974, 1976; Cotter *et al.*, 1975; Mackey *et al.*, 1975; Hardy, 1981a). Many retroviruses are presently known to cause similar diseases in animals and humans. FeLV was the first retrovirus for which a simple and practical diagnostic test for viral infection, an immunofluorescent antibody (IFA) test for detection of FeLV antigens in blood leukocytes, was developed (Hardy *et al.*, 1973b, 1974, 1976b). This accomplishment demonstrated that accurate and practical IFA, enzyme-linked immunosorbent assay (ELISA), and immunoblot (Western blot) tests for detection of retrovirus infections in animals and humans were possible. FeLV was the first retrovirus where an effective program to control and prevent the spread of a retrovirus was developed and used (Hardy *et al.*, 1974, 1976b; Weijer and Daams, 1978). Similar programs are now in effect worldwide to prevent the spread of bovine, equine, and human retroviruses (Hardy, 1983a,b; Wong-Staal and Gallo, 1985). Moreover, the concept that contagiously transmitted retroviruses could occur naturally in a latent state was first demonstrated by Post and Warren (1980); this observation was later confirmed by Rojko *et al.* (1982) in FeLV-infected cats that had rejected the virus. Finally, FeLV was the first retrovirus for which a vaccine was developed (Olsen *et al.*, 1976, 1980) and it could be a model for vaccines for other animal retroviral vaccines as well as the human immunodeficiency virus (HIV).

The understanding of the biology and basic virology of FeLV and the bovine leukemia virus (BLV), during the 1960s and 1970s, laid much of the groundwork that would prove so relevant for the discovery of human retroviruses (Poiesz *et al.*, 1980; Gallo *et al.*, 1984; Wong-Staal and Gallo, 1985). Studies of "virus-negative" leukemias of cats and cows helped to develop the concepts and techniques that were eventually so important in the isolation of the first human pathogenic retrovirus, the human T-lymphotropic virus (HTLV-I) (Poiesz *et al.*, 1980).

Following the discovery of FeLV, three feline sarcoma viruses (FeSVs) were found in the late 1960s and early 1970s in pet cats with multiple fibrosarcomas. The first two of these viruses were later shown to possess the *fes* oncogene (Snyder and Theilen, 1969; Gardner *et al.*, 1971a) and the third FeSV has a unique oncogene, v-*fms* (McDonough *et al.*, 1971). Since then eight new FeSV strains have been found to possess five different oncogenes, two of which are unique to FeSVs.

Current investigations of feline retroviruses include molecular characterization of defective *myc* and *tcr* containing FeLVs; search for and characterization of new oncogenes in new isolates of feline sarcoma viruses; studies of the recombination of exogenous FeLV-A with endogenous FeLV-related sequences that generate highly pathogenic viruses; and studies of the viral determinants of pathogenicity. Feline oncoretroviral studies may lead to elucidation of the general mechanisms by which retroviruses induce cancer, immune deficiency, and cytoreductive diseases and thus may contribute significantly to the treatment and prevention of human cancers and AIDS.

II. VIROLOGY

In general, endogenous retroviruses are DNA sequences that are integrated in the chromosomes of both germ and somatic cells of their hosts (Table II). Endogenous retroviruses of one species have been transferred to other species where they have become contagious pathogenic retroviruses (Table III) (Todaro, 1980). In the case of the cat, they are present in multiple copies per haploid genome, do not replicate in cats, and are not known themselves to induce disease (Todaro, 1980). However, RD-114 virus can be isolated from cat cells in tissue culture (Todaro *et al.*, 1973; Levy, 1978). In contrast, exogenous retroviruses are not found as unexpressed viral DNA and are maintained in their host species by contagious spread among individual members of that species.

Exogenous feline retroviruses can be divided into chronic and acute viruses (Hardy, 1983a). Chronic retroviruses are replication competent, induce disease after long latent periods, and do not possess a transforming gene or oncogene. Acute transforming retroviruses are usually replication defective, induce disease after long latent periods, and possess transforming genes or oncogenes derived from cellular sequences (Tables I, IV, and V). In this regard, the feline system resembles the avian and murine oncoretroviruses (see Chapters 6 and 7).

A. Endogenous Feline Oncoretroviruses

At least three distinct sets of endogenous retroviral sequences are in the cellular DNA of all domestic cats: (1) RD-114 virus, (2) MAC-1 virus, and

TABLE II. Endogenous Retrovirus Sequences in Cats (Family Felidae)

Genus	Cellular DNA homology (%)	RD-114	MAC-1	enFeLV
Domestic cat	100	+	+	+
European wildcat	>95	+	+	+
Sand cat	>95	+	+	+
Jungle cat	>95	+	+	+
Golden cat	>95	−	+	−
Serval	>95	−	+	−
Leopard cat	>95	−	+	−
Geoffroy's cat	>95	−	+	−
Bobcat	>95	−	+	−
Lion	92	−	+	−
Snow leopard	91	−	+	−
Jaguar	90	−	+	−

From Todaro, 1977, 1980.

TABLE III. Origin of Feline Oncoretroviruses

Virus	Possible source or donor of virus	Spread of virus among cats
Exogenous		
FeLV	Rat ancestor	Contagiously
FeSV	FeLV/proto-oncogenes	*de novo*
Endogenous		
enFeLV	Mouse ancestor	Genetically
RD-114	Old World monkey	Genetically

(3) endogenous FeLV-related (enFeLV) sequences (Todaro, 1977, 1980) (Table II).

1. RD-114 Virus

The RD-114 virus is an endogenous oncoretrovirus of domestic cats that is only distantly related to FeLV (Fischinger *et al.*, 1973; Livingston and Todaro, 1973; McAllister *et al.*, 1973). It was originally discovered after passage of a human tumor through newborn kittens. While initially believed to be a virus of human origin, it was eventually identified as a feline endogenous retrovirus. RD-114 is a xenotropic virus: that is, it does not productively infect (replicate) in cat cells but only replicates in nonfeline cells such as human and dog cells. Even though the virus does not actively replicate in cats, RD-114 viral mRNA and p30 are expressed in stimulated leukemic and nonleukemic lymphoid tissues regardless of their FeLV status (Niman *et al.*, 1977). However, RD-114-like viruses have been recovered from feline embryo cells in culture (Todaro *et al.*, 1973). Multiple complete copies of the RD-114 viral genomes are found in all domestic cat cells. Sera from healthy

TABLE IV. Cellular Genes Transduced by Feline Oncoretroviruses[a]

Cellular gene	Number of isolates	Disease association
myc	8	Lymphosarcoma
fes	3	Fibrosarcoma
fms	2	Fibrosarcoma
abl	1	Fibrosarcoma
sis	1	Fibrosarcoma
fgr-actin	1	Fibrosarcoma
kit	1	Fibrosarcoma
K-*ras*	1	Fibrosarcoma
tcr	1	Lymphosarcoma

[a] Adapted from Neil *et al.*, 1987; Hardy, 1990.

TABLE V. Feline Sarcoma Viruses

Oncogene	FeSV isolate		Protein product	Oncogene type
fes				
	Snyder-Theilen	ST-FeSV	P85$^{gag\text{-}fes}$	Tyrosine kinase
	Gardner-Arnstein	GA-FeSV	P95$^{gag\text{-}fes}$	Tyrosine kinase
	Hardy-Zuckerman 1	HZ1-FeSV	P96$^{gag\text{-}fes}$	Tyrosine kinase
fms				
	Susan-McDonough	SM-FeSV	gp1700$^{gag\text{-}fms}$	CSF-1 receptor
	Hardy-Zuckerman 5	HZ5-FeSV	ND	CSF-1 receptor[a]
sis				
	Parodi-Irgens	PI-FeSV	P76$^{gag\text{-}sis}$	Platelet-derived growth factor
fgr				
	Gardner-Rasheed	GR-FeSV	P70$^{gag\text{-}actin\text{-}fgr}$	Tyrosine kinase/actin
	Theilen-Pedersen 1	TP1-FeSV	P83$^{gag\text{-}fgr}$	Tyrosine kinase/actin[a]
abl				
	Hardy-Zuckerman 2	HZ2-FeSV	P98$^{gag\text{-}abl}$	Tyrosine kinase
Ki-*ras*				
	Noronha-Youngren	NY-FeSV	ND	Adenyl cyclase G protein[a]
kit				
	Hardy-Zuckerman 4	HZ4-FeSV	P80$^{gag\text{-}kit}$	Receptor for *kit* ligand (KL)

ND, not determined.
[a] Assumed although not yet determined.

pet cats and cats with various diseases do not contain antibodies to RD-114 viral proteins, which indicates that these antigens are rarely expressed and/or that cats are immunologically tolerant to RD-114 proteins (Mandel *et al.*, 1979).

The baboon endogenous virus (BaEV) is closely related to the RD-114 virus, and it is likely that they originated together (Todaro, 1980). The progenitor of these viruses possibly arose as a result of horizontal transmission from one species to the other or via horizontal transmission from a third species into ancestral cats and baboons at approximately the same time (Table II) (Benveniste *et al.*, 1975). Genes related to the nucleic acid of the endogenous RD-114 domestic cat virus are found in the cellular DNA of anthropoid primates, but, surprisingly, they are absent from many members (wild cats) of the cat family Felidae (Todaro, 1977, 1980) (Table II). The absence of RD-114-related sequences in most wild cats is consistent with the acquisition of this virus relatively recently, 3 million to 10 million years ago, in feline evolution. The related species of the genus *Felis*, including the domestic cat, have RD-114 sequences, but only those cats from North Africa and the Middle East, not the species that live in Asia, North America, and South America. It appears that the RD-114 virus was acquired only by those cats that had contact with African primates, most probably with baboons or one of their close relatives (Todaro, 1977, 1980).

RD-114 is not known to cause any feline disease, and there is no evidence that these endogenous sequences recombine with exogenous FeLVs or FIV to produce recombinant retroviruses.

2. MAC-1 Virus

In addition and distinct from the RD-114 sequences, viral gene sequences related to the primate oncoretrovirus MAC-1 are found in the cellular DNAs of various carnivores including cats (Todaro *et al.*, 1978; Bonner and Todaro, 1979; Todaro, 1980). MAC-1 is an endogenous primate oncoretrovirus isolated from a macaque (*Macaca arctoides*) cell line (Todaro *et al.*, 1978). MAC-1 sequences are not related to the baboon endogenous virus (BaEV) but are found in the cellular DNAs of related primates. The MAC-1 transcripts hybridize to the DNA of cats at significant levels, as compared with various other mammalian cellular DNAs. The MAC-1-related sequences occur in members of the family Felidae (Table II) that have RD-114 and enFeLV sequences and also to those cats lacking these sequences. Related sequences have been found in lions, leopards, and jaguars, as well as in all members of the genus *Felis*. The MAC-1 sequences are not related to the RD-114 or enFeLV sequences. The MAC-1 sequences are found in a number of carnivores, and this indicates that they have been present for several million years. It has been calculated that the MAC-1 sequences have been present in primates for at least 30 million years, far longer than the RD-114 and enFeLV sequences have been present in cats (Todaro, 1980). It has not yet been determined whether carnivores acquired the MAC-1 sequences from ancestral primates or vice versa or if both families acquired them from a third source. In the cat, the MAC-1 sequences do not give rise to infectious replicating virus particles.

3. Endogenous FeLV-Related Sequences (enFeLV)

Healthy FeLV-uninfected domestic cats and their close *Felis* relatives, but not their more distantly related *Felis* species, possess cellular DNA sequences that are not related to RD-114 or MAC-1 sequences but are partially homologous to the RNA of exogenous horizontally transmitted FeLVs (Table II) (Baluda and Roy-Burman, 1973; Benveniste and Todaro, 1973; Okabe *et al.*, 1976, 1978; Bolognesi *et al.*, 1978; Koshy *et al.*, 1979). However, a portion of the exogenous FeLV genome is not endogenous to uninfected cat cells (Okabe *et al.*, 1978). Endogenous FeLV-related sequences (enFeLVs) are found in specific pathogen-free domestic cats (*Felis catus*), and in closely related Felidae including the jungle cat (*Felis chaus*), the European wildcat (*Felis sylvestris*), and the sand cat (*Felis margarita*), all originating from the Mediterranean basin (Benveniste *et al.*, 1975). Other species of *Felis* from sub-Saharan Africa, Southwest Asia, and North and South America lack enFeLV.

Only the cellular DNA of rodents, and in particular rats, contains related retrovirus gene sequences (Benveniste *et al.*, 1975). This presence of related sequences in rodents and the lack of enFeLV in most Felidae suggests that enFeLVs were acquired by cats via transspecies infection with a virus of rodent origin. This transspecies transmission occurred subsequent to the initial Felidae divergence but prior to the divergence of the four positive *Felis* species. It should be noted that those *Felis* species that possess sequences related to RD-114 virus also possess enFeLV sequences. This finding indicates that

transspecies transmission of retroviruses to the *Felis* species was not uncommon since each grouping of viral genes was derived from a distinctly different group of animals: primates and rodents.

The cellular control of enFeLV virogenes is quite different from that of the RD-114 virogene (Benveniste *et al.*, 1975). The enFeLV virogenes have not been induced from cat cells in culture whereas the RD-114 virogenes can be readily induced from normal "virus-free" cat cells but are generally restricted from replicating to high levels in cat cells (Livingston and Todaro, 1973).

The enFeLV sequences are present in multiple copies (8–12) per haploid genome, and are arranged as discrete copies in a nontandem fashion. They are conserved among the tissues of the same cat but vary among different cats (Koshy *et al.*, 1979). Full-length and truncated enFeLV sequences exist in cat cells, and not even the full-length sequences are inducible as infectious virus (Benveniste *et al.*, 1975; Bolognesi *et al.*, 1978; Okabe *et al.*, 1978; Soe *et al.*, 1985). The U3 region of the long terminal repeats (LTRs) of the enFeLV and exogenous FeLV are different, and this difference can be used to distinguish these genomes (Casey *et al.*, 1981; Soe *et al.*, 1983). Even though some full-length enFeLV sequences exist they are not infectious. Most enFeLV sequences are shorter than the exogenous, cloned, infectious FeLVs, which are 8.5 to 8.7 kilobase pairs (kbp) in length (Soe *et al.*, 1983, 1985). The truncated enFeLV sequences can have a large deletion (3.3 to 3.6 kbp) in the *gag-pol* region and a 0.7 to 1.0 kbp deletion in the *env* region.

Sequence analysis of the genomes of the three subgroups of exogenous infectious FeLV (FeLV-A, -B, and -C) has shown that FeLV-B and -C arise through recombination of FeLV-A with enFeLV *env* sequences to form *env* recombinant FeLVs (Baluda and Roy-Burman, 1973; Mullins *et al.*, 1981; Stewart *et al.*, 1986b; Overbaugh *et al.*, 1988a,b; Mullins and Hoover, 1990). In this regard, they resemble the murine polytropic mink-cell focus forming (MCF) viruses (see Chapter 5). This recombination will be discussed later in this chapter.

B. Exogenous Infectious Oncoretroviruses (FeLVs)

As was mentioned previously, feline exogenous retroviruses can be divided into chronic and acute types (Table I). The contagiously transmitted FeLVs are replication-competent chronic leukemia viruses that do not possess an oncogene and induce monoclonal lymphosarcomas after a long latent period. These exogenous FeLVs probably originated through cross-species infection of an endogenous rodent retrovirus into ancestors of the pet cat (Table III) (Todaro *et al.*, 1974; Benveniste *et al.*, 1975; Todaro, 1980). The exogenous FeLVs can recombine with enFeLV to give rise to highly pathogenic retroviruses. In addition, exogenous chronic feline retroviruses can transduce cellular genes and form acute transforming feline retroviruses (Tables I, IV, and V) (See Section B.2).

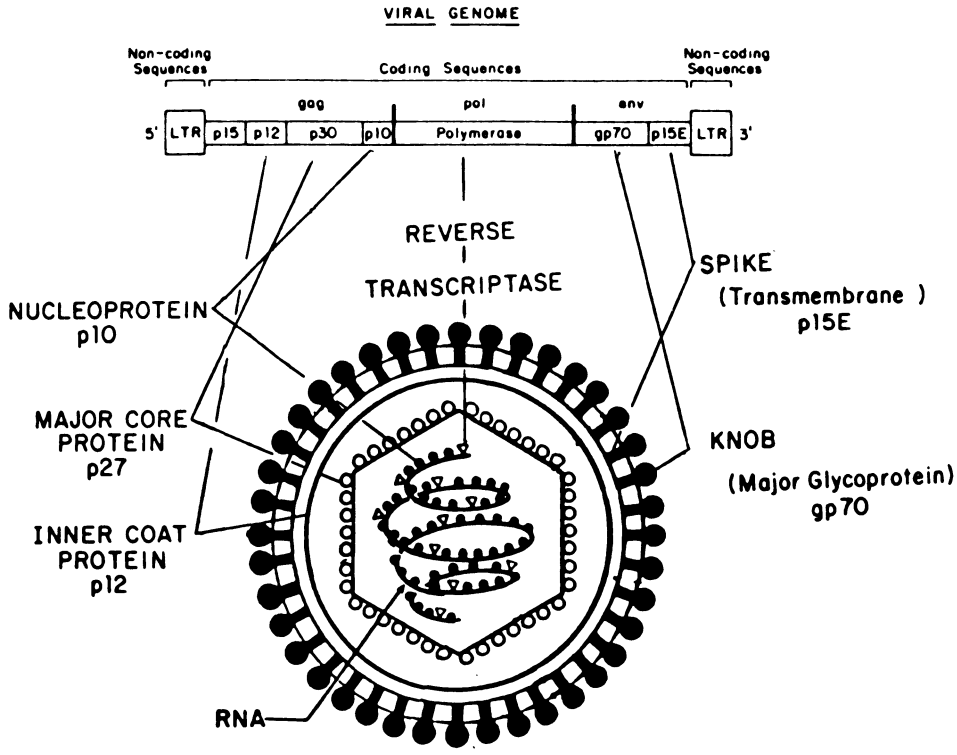

FIGURE 1. The FeLV genome and structure of the virus.

1. Chronic Leukemia Viruses

a. FeLV Genome

FeLV is a replication-competent chronic leukemia virus that possesses only the viral genes 5′-*gag-pol-env*-3′ necessary for replication (Fig. 1). For more details on retroviral genomes and the replicative process, see Chapter 5.

b. FeLV Proteins

Nine proteins are encoded by the FeLV genome (Table VI) and include: (1) the *gag* gene internal viral structural proteins p15 (matrix protein, MA), p12 (unknown) function, p27 (capsid protein, CA), and p10 (nucleocapsid protein, NC); (2) the *pol* gene enzymes: p14 (protease, PR), p80 (reverse transcriptase, RT), p46 (integration protein, IN); and (3) the *env* gene envelope proteins gp70 (surface protein, SU) and p15E (transmembrane protein, TM) (Teich, 1982; Hardy, 1983a,b; Leis *et al.*, 1988).

The FeLV structural proteins are produced in great excess in the cell membrane and the cytoplasm of infected cells and free viral proteins are

TABLE VI. Functions and Locations of Feline Leukemia Virus Proteins

Viral gene	Protein	Protein two-letter designation	Function or enzyme activity or location in virus
gag 5′	p15	MA	Matrix protein
	p12	?[a]	Unknown
	p27	CA	Capsid protein
3′	p10	NC	Nucleocapsid protein
pol 5′	p14[b]	PR	Protease
	p80	RT	Reverse transcriptase
3′	p46[b]	IN	Integration protein
env 5′	gp70	SU	Surface protein
3′	p15E	TM	Transmembrane protein

[a] Unknown function; no name yet given.
[b] Assumed from analogous MuLV protein; not yet identified for FeLV.

released into the plasma and tissue fluids of infected cats after the cells die (Hardy *et al.*, 1973b; Yoshiki *et al.*, 1974).

The study of the occurrence and control of FeLV in pet cats has been accomplished by detection of FeLV antigens in the cytoplasm of peripheral blood leukocytes by indirect immunofluorescent antibody (IFA) tests or by detection of soluble antigens in the plasma by enzyme-linked immunosorbent assays (ELISA) (Hardy *et al.*, 1969, 1973a,b, 1974, 1976a,b; Weijer and Damms, 1978; Kahn *et al.*, 1980; Gwalter, 1981). All of the FeLV biology and control methods were elucidated using the IFA test for FeLV during the 1970s (Hardy *et al.*, 1973b, 1976a). A positive IFA test correlates 98% of the time with the ability to isolate FeLV from the blood (Hardy, 1981b, 1990; Hardy *et al.*, 1973a,b; Hardy and Zuckerman, 1991a,b) and indicates persistent, usually lifelong (in 90–97% of IFA positive cats) viremia and shedding of the virus in the saliva (Hardy *et al.*, 1973a; Francis *et al.*, 1977). However, many positive FeLV ELISA tests cannot be confirmed by IFA and thus represent false-positive tests (Hardy, 1990).

c. Feline Oncornavirus-Associated Cell Membrane Antigen (FOCMA)

There has been much controversy about the origin and function of the FOCMA molecule. FOCMA was first defined by an IFA test using antibody present in pet cat sera. It was detected as an antigen on cell membranes of feline FL74 cultured lymphosarcoma cells, which produce all three subgroups of FeLV (Essex *et al.*, 1971; Theilen *et al.*, 1969). High titers of FOCMA antibody protect cats against FeSV-induced fibrosarcomas and FeLV-induced LSAs (Hardy *et al.*, 1976a; Essex *et al.*, 1971, 1975, 1977b). FOCMA antibody and FeLV neutralizing (VN) antibody are discordant in many cats (Hardy *et al.*, 1976a; Snyder *et al.*, 1978, 1980, 1983). This finding initially suggested that FOCMA was not an FeLV structural antigen, at least not an FeLV-A or -B structural antigen.

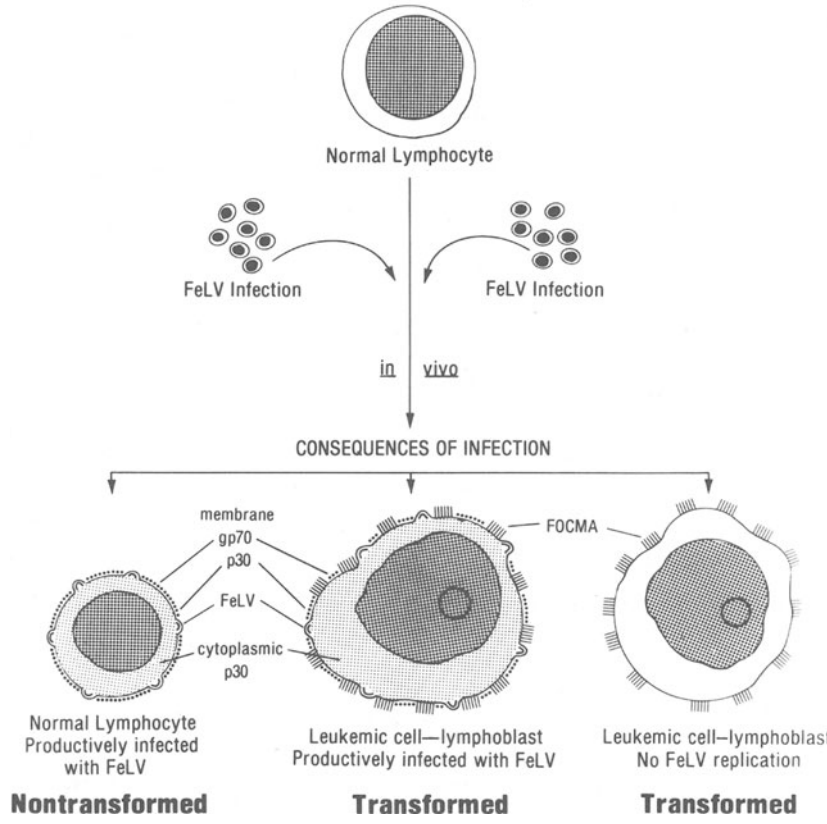

FIGURE 2. The consequences of feline lymphocytes infected *in vivo* with FeLV and the expression of FOCMA on the FeLV-transformed lymphosarcoma cells. (Reprinted with permission from Cold Spring Harbor Laboratory, Cold Spring Harbor, NY) (Hardy *et al.*, 1978).

FOCMA is expressed on the cell membranes of all feline T- and B-cell LSA cells (Fig. 2) irrespective of their FeLV status, on FeSV-induced fibrosarcoma cells, and on FeLV-infected erythroid and myeloid leukemic cells (Hardy *et al.*, 1977, 1978, 1980a,b; Snyder *et al.*, 1978, 1980, 1983; Francis *et al.*, 1979). In contrast, FOCMA is not found on normal feline lymphocytes, even those productively infected with FeLV-A and -B, but it was reported to be present on normal cultured FeLV-infected fibroblasts (Rice *et al.*, 1981). Taken together these data suggest that FOCMA is an FeLV- and FeSV-induced tumor-specific antigen. The finding of FOCMA on LSA cells not expressing FeLV suggests that FOCMA is a marker of FeLV leukemogenesis.

FOCMA was isolated and characterized from FeLV-positive and FeLV-negative LSA cells and was found to be a 70-kDa protein, which, by peptide mapping, was different from the gp70 molecules of FeLV-A and -B (Snyder *et al.*, 1978, 1980, 1983). Later, FOCMA was reported to be identical to the gp70 of FeLV-C, the FeLV subgroup that is rarely (less than 1%) found in infected pet cats (Vedbrat *et al.*, 1983). However, further studies found that FOCMA is related to, but is distinguishable from, FeLV-C gp70 (Snyder *et al.*, 1983).

The degree of concordance in the occurrence of FOCMA antibody and of FeLV-C neutralizing antibody in the sera of 409 cats was studied to clarify further the relationship between FOCMA and FeLV-C gp70. Only 66 of 96 (68.8%) cats with FOCMA antibody had neutralizing antibody to FeLV-C. Similarly, 66 of 86 (76.7%) cats with neutralizing antibody to FeLV-C had antibody to FOCMA (Hardy, 1985). These serological data suggest that FOCMA is related to, but is serologically distinguishable from, FeLV-C gp70.

As noted above, neutralizing antibodies to FeLV-C are present in many cats that are viremic with FeLV-A even though FeLV-C cannot be isolated from these cats (Hardy *et al.*, 1976a; Russell and Jarrett, 1978). These findings suggested that FeLV-A transforms some lymphocytes and may recombine with enFeLV sequences to produce the FeLV-C gp70-FOCMA recombinant molecule. FOCMA may be a recombinant FeLV-C gp70 molecule with additional endogenous cellular determinants. These conflicting FOCMA findings remain unresolved but do not alter the previous conclusion that FOCMA is an FeLV- or FeSV-induced tumor-specific antigen. Moreover, none of the new studies refute the tumor-protective effect of FOCMA antibody.

d. FeLV Subgroups

Three subgroups of FeLV (FeLV-A, -B, and -C) have been identified by their envelope gp70 molecules (Tables I, VII) (Jarrett *et al.*, 1973b; Sarma and Log, 1973) (see also Chapter 1). An 85% sequence homology exists among the genomes of the three FeLV subgroups (Levin *et al.*, 1976) although more divergence has been noted by oligonucleotide analysis (Rosenberg *et al.*, 1980).

FeLV-A is ecotropic and has the most restricted host range (Table VII). It grows almost exclusively in cat cells. FeLV-A has been found in all infected

TABLE VII. Host Range of FeLV Subgroups

Species origin of cells	Replication of FeLV subgroups		
	FeLV-A	FeLV-B	FeLV-C
Cat	+	+	+
Dog	−	+	+
Human	−	+	+
Bovine	−	+	+
Mink	−	+	+
Hamster	−	+	−
Pig	−	+	−
Monkey (Vero)	−	+	−
Guinea pig	−	−	+
Mouse	−	−	−
Rat	−	−	−

From Sarma and Log, 1973; Jarrett *et al.*, 1973b; Hardy, 1981b.

pet cats either alone (50%), in combination with FeLV-B (49%), or together with FeLV-B and FeLV-C (1%) (Hardy *et al.*, 1976a; Jarrett *et al.*, 1978). FeLV-B is polytropic, with the widest host range (Table VII), including replication in cat, dog, mink, hamster, pig, bovine, monkey cells, but only poorly in human cells. FeLV-C is also polytropic, but has an intermediate host range between those of FeLV-A and FeLV-B and can replicate in cat, dog, mink, guinea pig, and human cells (Jarrett *et al.*, 1973b; Sarma and Log, 1973).

FeLV-C occurs only rarely in pet cats, less than 1%, even though many cats have antibodies to the gp70 of FeLV-C. This finding suggests that FeLV-C is formed by recombination with FeLV-A and is immunogenic in these cats (Snyder *et al.*, 1982). The reason why FeLV-C is not found often in infected cats may be due to suppression of viral replication by the neutralizing antibody or because the recombinational events with FeLV-A are rare and FeLV-C is not commonly formed.

No clear association of any subgroup of FeLV with any specific naturally occurring disease has been demonstrated although experimental evidence exists for subgroup disease associations. Under experimental conditions, the Rickard strain of FeLV-A induces mainly thymic lymphosarcomas (LSAs); a variant of FeLV-A causes the feline acquired immune deficiency syndrome (FAIDS); and several isolates of FeLV-C induce erythroid hypoplasia (aplastic anemia) (Hoover *et al.*, 1974, 1976; Mackey *et al.*, 1975; Onions *et al.*, 1982; Testa *et al.*, 1983; Mullins *et al.*, 1986; Overbaugh *et al.*, 1988a,b; Riedel *et al.*, 1986, 1988).

e. FeLV-FAIDS Viruses

More pet cats die of FeLV-induced immunodeficiency than die from lymphoid tumors. A specific FeLV variant, termed FeLV-FAIDS, has been isolated and shown to induce feline AIDS (FAIDS) in 100% of specific pathogen-free (SPF) cats (Mullins *et al.*, 1986; Overbaugh *et al.*, 1988a; Hoover *et al.*, 1989). The FeLV-FAIDS variant is replication defective and is associated with replication-competent FeLVs. Recently, mutations in the *env* gene have been shown to lead to the generation of highly pathogenic, immunodeficiency disease-inducing FeLVs (Overbaugh *et al.*, 1988b; Poss *et al.*, 1989). The mutation alters the gp70 molecule and the mechanism of T-cell killing involves a delay or failure to protect against superinfection interference in already infected T cells.

The Duplan murine leukemia virus (MuLV) induces a severe immunodeficiency disease in C57BL/6 mice and has recently been shown, like the FeLV-FAIDS isolate, to be defective (Aziz *et al.*, 1989). Unlike the FeLV-FAIDS virus, which harbors the determinant of pathogenicity in its *env* sequences, the Duplan MuLV DNA has complete deletions of the *pol* and *env* genes with a completely conserved *gag* gene remaining. The *gag* gene has a novel sequence encoding a unique p12 protein, which probably is the pathogenic determinant of this virus. These similar observations in cats and mice suggest

that there may be other, as yet undiscovered, defective immunosuppressive oncoretroviruses present in other species.

2. Acute Transforming Feline Retroviruses

Acute transforming retroviruses possess viral oncogenes derived from cellular genes and induce both lymphosarcomas and sarcomas after a short period of time. Chickens and cats are the species with the most frequently occurring acute transforming retroviruses.

*a. FeLV-*myc *Retroviruses*

Defective FeLV proviruses containing the *myc* oncogene have been found in the DNA of 14% of pet cats with naturally occurring FeLV-positive thymic LSAs (Neil *et al.*, 1984; Mullins *et al.*, 1984; Levy *et al.*, 1984; Stewart *et al.*, 1986a). In addition, a c-*myc* rearrangement was found in one FeLV-positive thymic LSA and a c-*myc* amplification was found in an FeLV-negative B-cell alimentary LSA (Neil *et al.*, 1984). The novel *myc* sequences were found to be encapsidated in functional viruses, which suggests that FeLVs possessing the *myc* oncogene may be transmitted contagiously among pet cats and may induce some forms of thymic LSAs. Defective FeLV-*myc* viruses have been rescued by replicating FeLV and found to induce lymphosarcoma rapidly when inoculated into kittens (Onions *et al.*, 1987; Forrest *et al.*, 1987; Levy *et al.*, 1988) and to partially transform feline fibroblasts in culture (Bonham *et al.*, 1987). The significance of these acute transforming FeLVs as leukemogenic agents in nature is not known since more than 75% of all feline lymphosarcomas do not contain such viruses.

*b. FeLV-*tcr *Retrovirus*

In addition to the FeLV-*myc*-containing viruses isolated from LSAs, an FeLV (FeLV-T17) carrying a T-cell receptor gene, v-*tcr*, has been isolated from an LSA (Neil *et al.*, 1988). This is the first example of retroviral transduction of an immunological effector molecule, and it has led to the reconsideration of the importance of cell surface receptors of the immune system in leukemia development.

c. Feline Sarcoma Viruses (FeSVs)

Other than chickens, pet cats have the greatest number of acute transforming sarcoma retroviruses of any species. Feline sarcoma viruses (FeSVs) are replication-defective, acute transforming viruses that possess transforming genes, viral oncogenes (v-*oncs*), acquired by recombination of the replication-competent FeLV genome with single-copy cellular c-*onc* (proto-oncogenes) genes (Frankel *et al.*, 1979; Bishop, 1981; Besmer, 1983). FeSVs arise *de novo* in individual infected cats, and they do not appear to be transmitted contagiously. They induce sarcomas with a short latent period in ani-

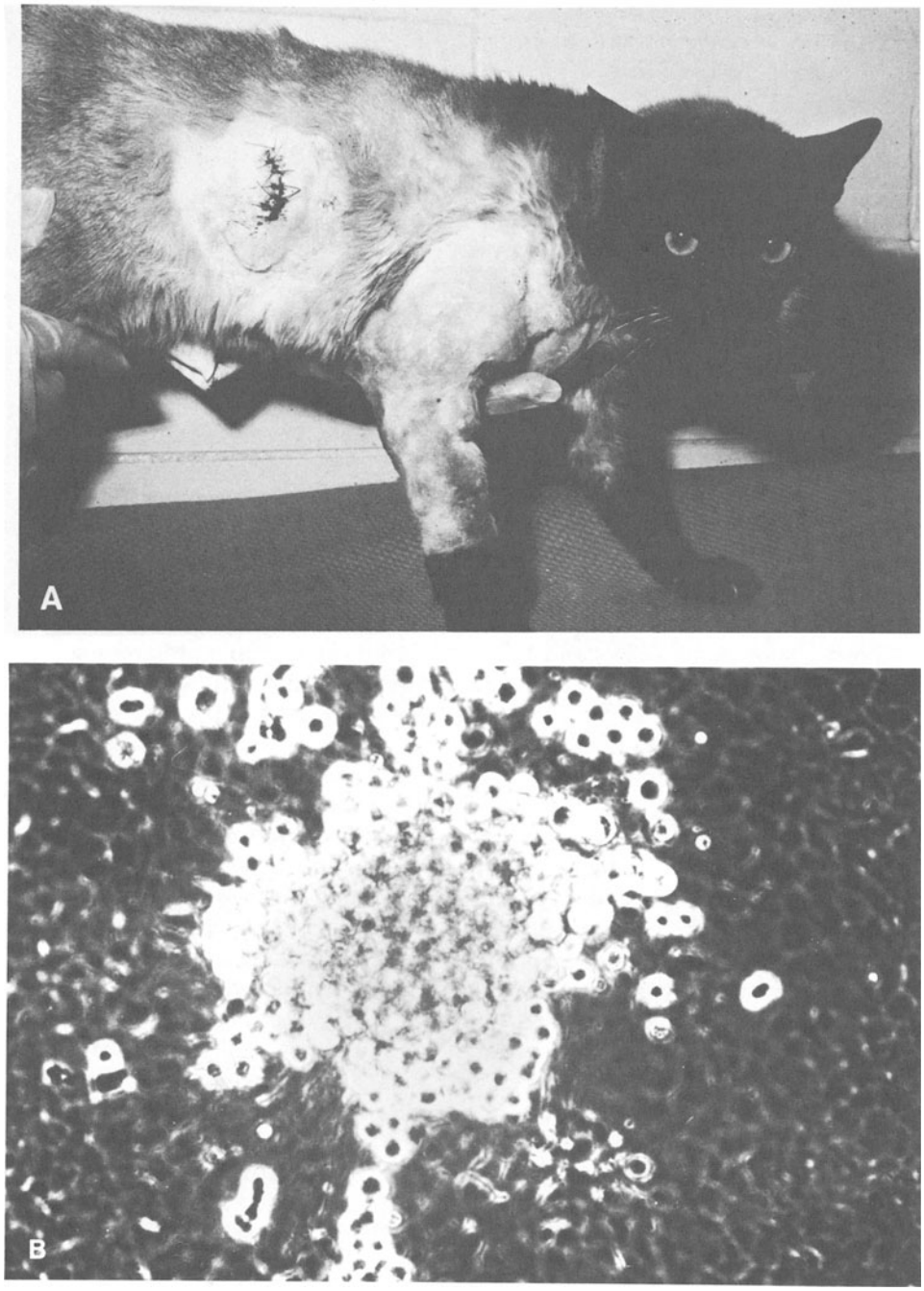

FIGURE 3. A. A pet cat with an FeSV-induced multicentric fibrosarcoma involving the right foreleg, head, and right thorax. The Hardy-Zuckerman 2 FeSV (HZ2 FeSV) containing the *abl* oncogene was isolated from this cat. (Reprinted with permission from *J. Am. Anim. Hosp. Assoc.* (Hardy, 1981c). B. A focus of mink fibroblasts transformed by HZ1 FeSV. The cells in the transformed focus pile up because they have lost contact inhibition. Nontransformed cells grow in a normal orderly monolayer around the focus. (Reprinted with permission from Churchill Livingstone, Inc.) (Rojko and Hardy, 1989).

mals and rapidly transform tissue culture cells (Fig. 3) of various species including human cells (Frankel *et al.*, 1979; Hardy, 1981c; Besmer, 1983).

Pet cats are a plentiful source of oncogene-containing acute transforming sarcoma retroviruses because over 1 million healthy pet cats are chronically viremic with the replication-competent FeLV. Eleven naturally occurring FeSVs, possessing seven different oncogenes, have already been isolated from pet cats with fibrosarcomas (Table V). The study of oncogenes appears to offer great promise to our understanding of the cause of cancer and insight into normal cell growth and regulation.

The 11 well-characterized replication-defective FeSV isolates and their oncogenes are listed in Table V. All FeSV-infected cats are also infected with replication-competent helper FeLV, which allows the replication-defective FeSV to replicate its genome *in vivo* (Hardy, 1980b, 1981c; Besmer, 1983).

FeSVs, like FeLVs, appear to be able to experimentally induce "virus-negative" tumors in cats and in heterologous species such as monkeys, sheep, and dogs (Deinhardt *et al.*, 1970; Gardner *et al.*, 1970, 1971a; Theilen *et al.*, 1970, 1971; Pearson *et al.*, 1973; Hardy, 1980b, 1981c). *In vitro* some FeSV-transformed cells can retain their FeSV provirus but revert to a normal morphology (Donner *et al.*, 1980). In experimentally inoculated cats in which sarcomas have regressed, FeSV can persist in the blood for up to 3 months before all infectious FeLV and FeSV disappear from the blood and all tissues. In about 6% of these FeSV- and FeLV-negative cats, recurrent sarcomas develop that contain integrated FeSV proviruses (Alrdich and Pedersen, 1974; Noronha *et al.*, 1983; Pedersen *et al.*, 1984b). The occurrence of fibrosarcomas not expressing FeSV in cats that were once infected with FeSV is similar to the FeLV-negative lymphosarcomas that develop in FeLV-negative cats previously infected with FeLV. Sarcoma cell cultures derived from these FeSV-free cats, who have developed recurrent fibrosarcomas, do not express FeSV or FeLV antigens during initial passages but, after several passages, infectious FeSV and FeLV are detected (Pedersen *et al.*, 1984b). These observations suggest that infectious FeLV/FeSV production is suppressed *in vivo*, possibly by an antiviral immunity but that the antisarcoma immunity is not as competent. This differential immune responsiveness allows recurrent virus-negative sarcomas to develop in cats, monkeys, and dogs possessing integrated FeSV v-*onc* genes but who are immune to the FeSV-helper FeLV. Thus the expression of the v-*onc* gene induces the malignancy. Thus far there is no evidence that FeSV induces virus-negative fibrosarcomas in FeSV-exposed uninfected pet cats (Hardy, 1981c).

i. v-fes FeSVs. The most prevalent acute transforming retroviral oncogene is the *fes* oncogene, which has been found in three FeSVs and five Fujinami avian sarcoma viruses (ASVs) (Besmer, 1983). The Snyder-Theilen (ST)-FeSV, Gardner-Arnstein (GA)-FeSV, and Hardy-Zuckerman-1 (HZI)-FeSV possess the *fes* oncogene (Snyder and Theilen, 1969; Gardner *et al.*, 1970; Frankel *et al.*, 1979; Hardy *et al.*, 1982; Snyder *et al.*, 1984a). The Fujinami avian sarcoma virus (ASV) *fps* oncogene was found to be homologous with the *fes* oncogene of the FeSVs (Fujinami and Inamoto, 1914; Shibuya *et al.*, 1980).

This was the first observation that acute transforming retroviruses, isolated from different species, are able to transduce homologous c-*oncs*. The *gag-fes* product exhibits a tyrosine-specific protein kinase activity (H. W. Snyder, 1982).

The ST-FeSV can transform murine pre-B cells (Pierce and Aaronson, 1983) and, in addition to inducing fibrosarcomas in experimentally inoculated kittens, the GA-FeSV can induce melanomas when inoculated into the eye or intradermally (McCullough *et al.*, 1973; Niederkorn *et al.*, 1980; Shadduck *et al.*, 1981). Thus the *fes* oncogene, and probably other oncogenes, can transform cells of different germ line origin.

ii. v-fms *FeSVs.* The Susan McDonough (SM) FeSV and the Hardy-Zuckerman-5 (HZ5)-FeSV possess the v-*fms* oncogene and display indistinguishable *in vitro* transformed cell focus morphology (McDonough *et al.*, 1971; Besmer *et al.*, 1986a). The protein products of the SM-FeSV genome are somewhat unusual and consist of a primary translation 155,000-dalton *gag-fms* protein that is glycosylated to yield a gp170 *gag-fms* protein. This viral gene product is then cleaved to yield gp120 and gp140 *fms* proteins and a p60 *gag* protein (Barbacid *et al.*, 1980; Reynolds *et al.*, 1981; Anderson *et al.*, 1982). Unlike other acute retroviral transforming proteins, the v-*fms* protein products are extensively glycosylated (Anderson *et al.*, 1982). The genome organization of the 8.6 kb HZ5-FeSV provirus is 5′-*gag-fms-pol-env*-3′ (Besmer *et al.*, 1986a).

The proto-oncogene c-*fms* protein product is the receptor for monocyte-macrophage proliferation, which is designated monocyte colony-stimulating factor-1 (M-CSF-1) (Sherr *et al.*, 1985; Sariban *et al.*, 1985; Sacca *et al.*, 1986). The c-*fms* is expressed during macrophage differentiation in normal cats, mice, and humans, and normal fibroblasts produce M-CFS-1 (Sariban *et al.*, 1985; Sacca *et al.*, 1986). Since v-*fms* contains the entire outer domain of the M-CSF-1 receptor, SM- and HZ-5-FeSV-infected fibroblasts become transformed possibly because cells infected by these viruses acquire the ability to overrespond to M-CSF-1 ligand by producing the v-*fms* encoded CSF-1 receptor. The v-*fms*-expressing cells then bind M-CSF-1, which may continually stimulate these cells to proliferate via an autocrine loop. Recent experimental results support this hypothesis. Recombinant human CSF-1, produced in a yeast expression system, can stimulate the growth of v-*fms* transformed rat fibroblasts (Lyman *et al.*, 1988). These data show that the altered CSF-1 receptor encoded by the v-*fms* oncogene retains a capacity to bind and be stimulated by CSF-1.

iii. v-abl *FeSV.* The v-*onc* of the Hardy-Zuckerman-2 (HZ2)-FeSV (Fig. 3A) is homologous to the v-*abl* of the Abelson murine leukemia virus (A-MuLV) (Hardy *et al.*, 1982; Besmer *et al.*, 1983b). The A-MuLV can transform B, T, and myeloid cells but has not induced fibrosarcomas in mice, whereas the HZ2-FeSV can induce multicentric fibrosarcomas in kittens (Abelson and Rabstein, 1970; Goff and Baltimore, 1982; Besmer *et al.*, 1983b).

The HZ2-FeSV$^{gag\text{-}abl}$ 98,000-dalton polyprotein has a protein kinase activ-

ity and is found with the cytosol and membrane fractions of transformed cells (Besmer *et al.*, 1983b; Lederman *et al.*, 1985; Bergold *et al.*, 1987). Most of the 98k polyprotein is found on the cytoplasmic aspect of the plasma cell membranes and has properties of an integral membrane protein (Besmer *et al.*, 1983b; Lederman *et al.*, 1985).

iv. v-sis *FeSV.* The Parodi-Irgens (PI)-FeSV v-*onc* sequences are homologous to the v-*sis* sequences of the Woolly monkey simian sarcoma virus (SSV) (Besmer *et al.*, 1983a; Irgens *et al.*, 1973). Both viruses display indistinguishable transformed cell focus morphology even though the v-*sis* sequences in these viruses are expressed with different strategies. The SSV genome structure is 5′-*gag-pol-env-sis-env*-3′ whereas the PI-FeSV genome structure is 5′-*gag-sis-env*-3′.

The PI-FeSV v-*sis* 76k protein product does not exhibit protein kinase activity (Doolittle *et al.*, 1983). However, it has extensive homology with platelet-derived growth factor (PDGF), a growth factor produced in platelets and which stimulates fibroblast proliferation in wound healing (Robbins *et al.*, 1983; Waterfield *et al.*, 1983). Thus, an overproduction of PDGF by *sis*-transformed fibroblasts might lead to excessive proliferation of cells with PDGF receptors, such as fibroblasts, and result in an autocrine stimulation and neoplastic growth.

v. v-fgr *FeSV.* The Gardner-Rasheed (GR)-FeSV and the Theilen-Pedersen-1 (TP1)-FeSV possess two unique cellular genes, gamma actin and c-*fgr* (Rasheed *et al.*, 1982; Naharro *et al.*, 1983; Ziemiecki *et al.*, 1984). The translation product, $P70^{gag\text{-}actin\text{-}fgr}$, is a protein-tyrosine kinase structurally unique among retrovirus-transforming proteins (Rasheed *et al.*, 1982; Naharro *et al.*, 1983, 1984; Sugita *et al.*, 1989). In recent studies of the transforming activities of the v-*fgr* and v-*actin* components of GR-FeSV it was found that v-*fgr* is responsible for the transforming potential of the virus whereas the v-*actin* sequences inhibit protein-tyrosine kinase and transforming activity of the virus. Thus, the *actin* domain is not necessary or sufficient for transforming activity. The GR-FeSV can transform a large variety of mammalian cell lines.

vi. v-kit *FeSV.* The Hardy-Zuckerman-4 (HZ4)-FeSV has a unique oncogene that has been designated v-*kit* (Besmer *et al.*, 1986b). To date v-*kit* has been found only in the HZ4-FeSV. The HZ4-FeSV genome is 5′-*gag-kit-pol-env*-3′, which encodes a $P80^{gag\text{-}kit}$ polyprotein with protein kinase activity (Majumder *et al.*, 1990). There is a 58% homology of the v-*kit* and v-*fms* sequences. The 1.1 kb v-*kit* segment corresponds to inner cellular domain of the 2.9-kb-long v-*fms* sequences.

Thus, like c-*fms*, c-*kit* was thought to encode a transmembrane receptor that is involved in signal transduction (Besmer *et al.*, 1986b). Recent findings have confirmed this hypothesis and have shown that the ligand for the c-*kit* receptor, named the *kit* ligand (KL), is the product of the murine *steel* gene (Qiu *et al.*, 1990; Flanagan and Leder, 1990). In addition, it was found that the *kit* gene is identical to the *W* gene, which was known to govern the development of murine pigment cells, mast cells, germ cells, and blood cells (Huang

TABLE VIII. Prevalence of FeLV in Healthy Cats

Environment/FeLV exposure	Percent FeLV-infected
Multiple cat households	
Exposed to FeLV	28
Not exposed to FeLV	0
Shelter-adopted cats	
Unknown FeLV exposure	2
Single-cat households	
Not exposed to FeLV	1
Stray cats	
Unknown FeLV exposure	1

From Hardy *et al.*, 1976a.

et al., 1990; Anderson *et al.*, 1990). The KL appears to have an unusually early role in the development of essential blood and immune cells by stimulating primitive bone marrow cells to develop into vital elements of the blood and immune systems.

Thus the KL signaling system operates in bone marrow, germ cells, mast cells, and pigment cells. The *kit* gene in the primitive bone marrow cell provides a message for the production of the *kit* receptor that is embedded in the cell membrane of these cells. Other bone marrow cells use the message of the *steel* gene to produce KL, which acts as a growth factor to stimulate cell growth and development of cells carrying the *kit* receptor. It is possible that the KL ligand of the *kit* oncogene of the HZ4-FeSV may someday facilitate bone marrow transplantation and may be used to counter the immune suppression associated with human and animal retroviruses and chemotherapy.

vii. v-K-ras FeSV. The Noronha-Youngren (NY)-FeSV v-*onc* is homologous to the v-*ras* of the Kirsten murine sarcoma virus (Youngren and deNoronha, 1983; Hardy, 1985). No studies of the *gag-onc* protein or the biology of this virus have yet been reported.

The oncogenes *src, ros,* and *yes* have been isolated only from retroviruses of birds and have not been found in any of several dozen mammalian retroviruses, whereas the *fms, kit,* and *fgr* oncogenes have been found only in feline retroviruses (Besmer *et al.*, 1986b). Viral recombinogenic sequences, functional constraints for the expression of transforming proteins, or different tissue tropisms of feline and avian retroviruses may be factors for this apparent species specificity. However, a more likely explanation is the lack of a vigorous search for acute transforming retroviruses in most species.

The domestic pet cat is an excellent animal from which to isolate oncogene-containing acute transforming viruses because there are many healthy pet cats that are chronically viremic with the replication-competent chronic leukemia virus, FeLV. The continual replication of FeLV in cat cells for long periods of time (3–5 years) increases the chances that the rare transduction

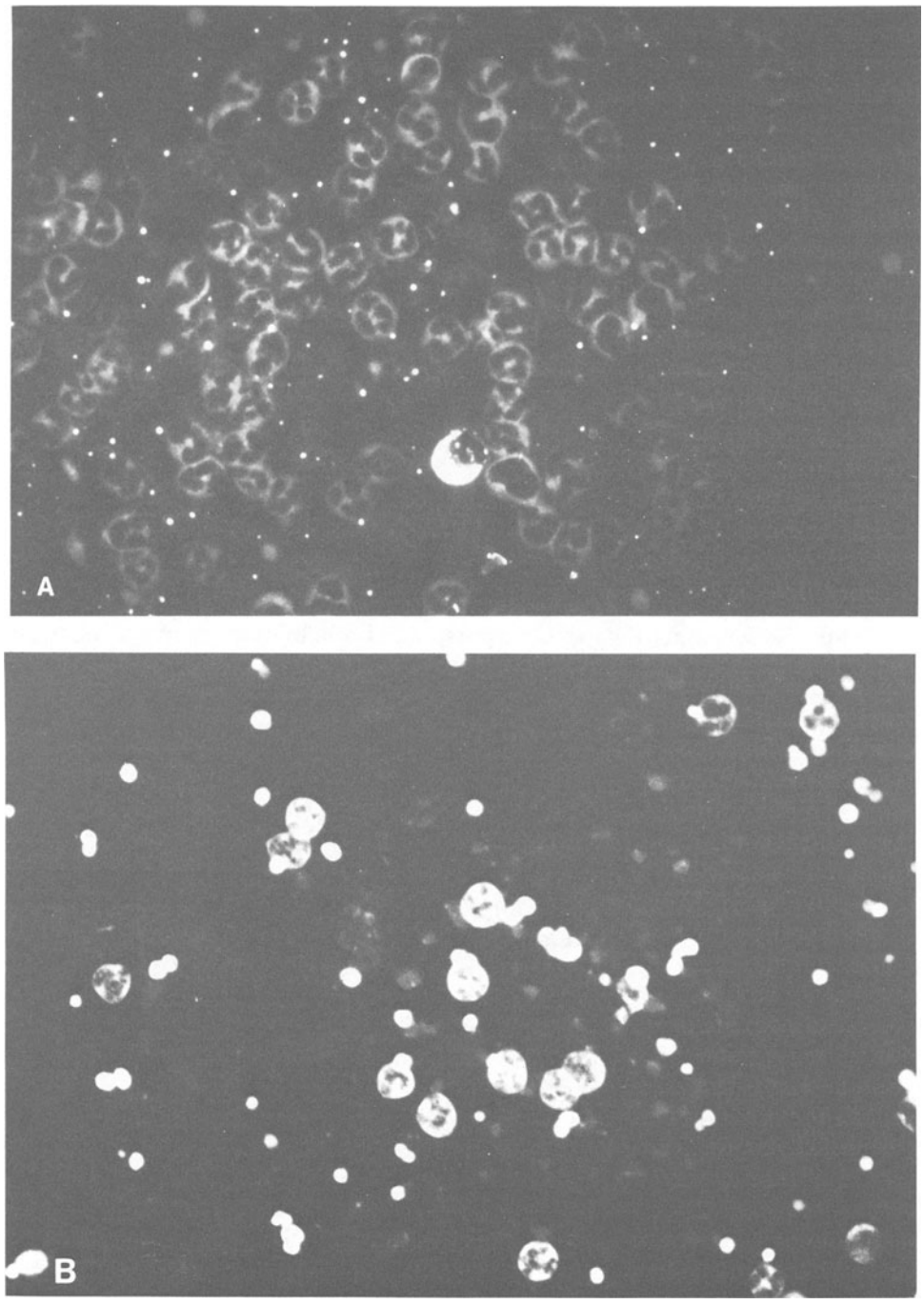

FIGURE 4. The immunofluorescent antibody (IFA) test for detection of FeLV in the peripheral blood leukocytes of cats. A. An IFA-negative blood smear. No FeLV antigens are present in neutrophils and lymphocytes. B. An IFA-positive normal blood smear. FeLV antigens are present in neutrophils, lymphocytes, and platelets. C. An IFA-positive blood smear from a cat with a leukemic blood profile. FeLV antigens are present in neutrophils, platelets, and neoplastic lymphoid cells.

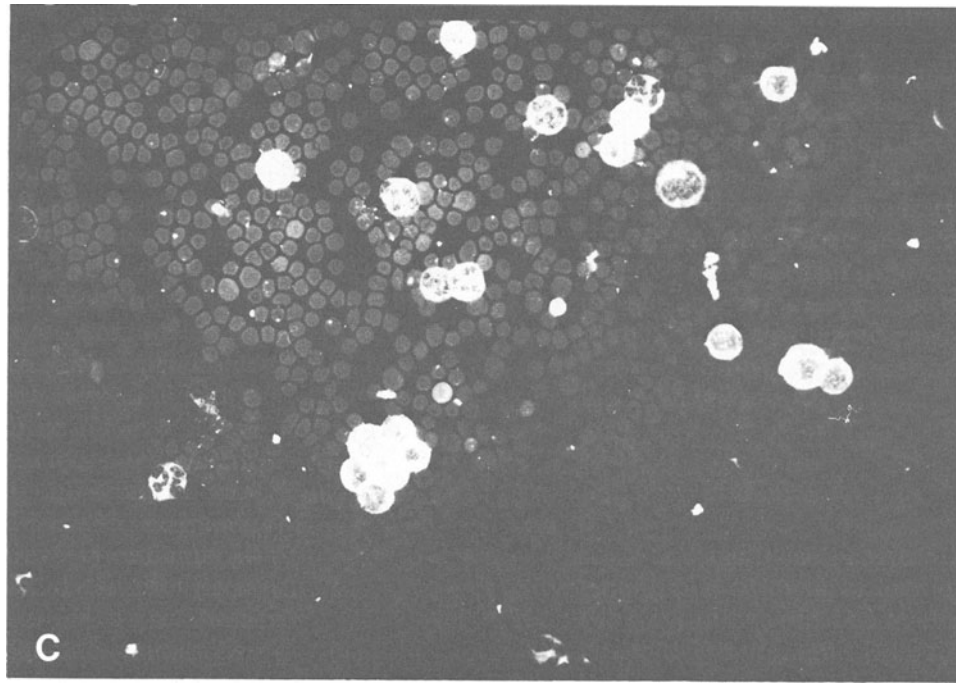

FIGURE 4. (*Continued*)

of a c-*onc* and the subsequent generation of acute transforming viruses will occur.

III. EPIDEMIOLOGY

Both the epidemiology and the occurrence of FeLV (Table VIII) in pet cats are determined by several factors including the FeLV genome, FeLV proteins, and the cat's immune response to these proteins.

A. Occurrence of FeLV in Healthy Cats

Numerous studies have examined the occurrence of FeLV in different cat populations. In 1970 an immunofluorescent antibody test (IFA) was developed for large epidemiological surveys of FeLV infection in the United States and Europe (Hardy *et al.*, 1973b, 1974, 1976a,b; Cotter *et al.*, 1975; Francis *et al.*, 1977; Gardner *et al.*, 1977; Weijer and Damms, 1978; Lutz *et al.*, 1980; Gwalter, 1981; Jarrett *et al.*, 1982). Twenty-eight percent of healthy FeLV-exposed cats living in multiple cat households were found to be persistently infected, whereas only 1% to 2% of household pet cats with no known exposure were found to be infected (Table VIII). Less than 1% of stray cats and 2% of shelter cats have been found infected with FeLV (Hardy, 1990).

B. Outcome of Persistent FeLV Infection

A healthy cat's expected life span is significantly shortened by persistent FeLV infection. In a prospective study we found that 83% of healthy FeLV-infected pet cats die within 3.5 years compared to only 16% of FeLV-uninfected cats living in the same households (McClelland *et al.*, 1980). Most of these deaths were caused by FeLV-induced immune deficiency and the development of FAIDS. Thus five times more FeLV-infected cats died in these households than did FeLV-uninfected cats.

C. Methods for Detection of FeLV Infection

An IFA test for detection of FeLV antigens in leukocytes in the peripheral blood (Fig. 4) was introduced into veterinary medicine in 1973 (Hardy *et al.*, 1973a,b). The epidemiology of FeLV and the definition of all of the FeLV diseases were elucidated using the IFA test. A positive test indicates FeLV

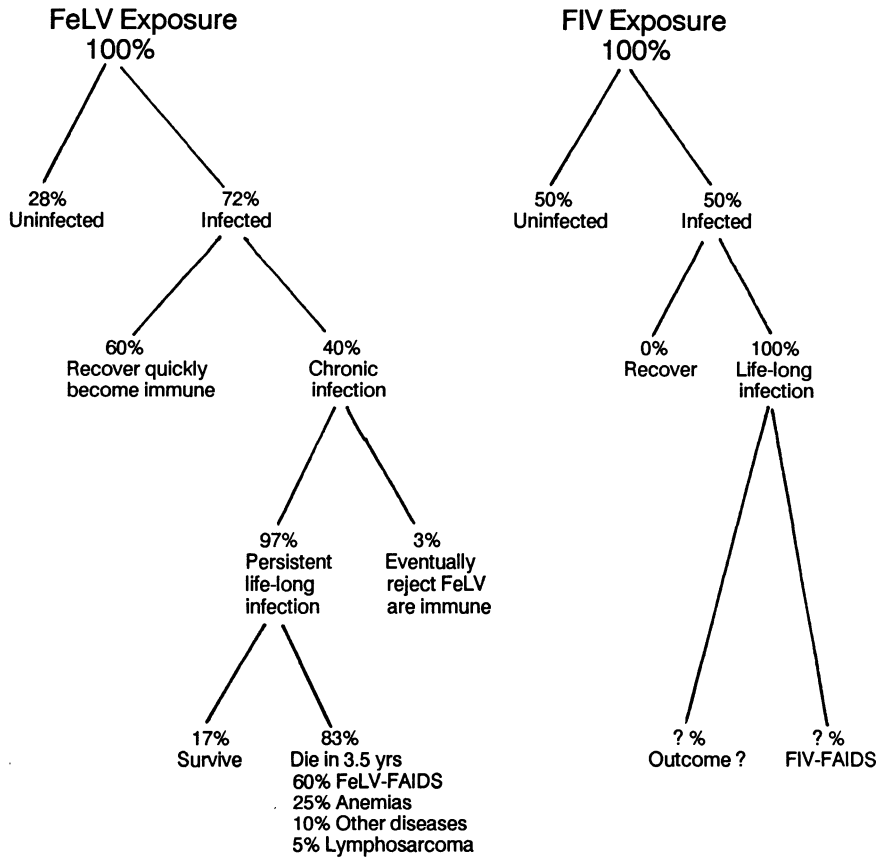

FIGURE 5. Consequences of exposure and infection with feline onco- and lentiretroviruses (see also Chapter 3).

infection but is not diagnostic of any disease. As noted before, there is an excellent correlation (97.5%) between a positive IFA test result and the ability to isolate FeLV from IFA-positive cats. In addition, an IFA-positive cat is shedding the virus in its saliva (Hardy *et al.*, 1973a,b, 1980a; Hardy, 1991; Hardy and Zuckerman, 1991a,b). Ninety percent to 97% of IFA-positive cats remain infected for life, whereas 3–10% are able to reject the virus, become virus free, and immune (Fig. 5). FeLV cannot be isolated from 98% of IFA-test-negative cats. Thus, in about 2% of the IFA and tissue culture isolation comparative tests, there were disagreements in the results. This indicates that either the IFA or tissue culture isolation tests were incorrect 2% of the time.

In-hospital enzyme-linked immunosorbent assays (ELISAs) for FeLV were introduced into veterinary medicine in the 1980s and comparative studies of the IFA and ELISA tests have been performed (Kahn *et al.*, 1980; Hardy, 1981b; Jarrett *et al.*, 1982; Rojko and Hardy, 1989; Hardy and Zuckerman, 1991a,b). In general, there is fairly good agreement, 86.7%, with negative results between the ELISA and IFA tests. However, in one large study consisting of over 20,000 comparative tests, there was only a 40.8% overall agreement between ELISA-positive test results and the IFA test (Hardy, 1990). Thus, as with a positive HIV-1 test (Handsfield, 1985; Nuttall *et al.*, 1986), all ELISA-positive FeLV tests should be confirmed by a more specific test, such as the IFA test.

IV. IMMUNE RESPONSES

A. Immune Response to FeLV Infection

1. Natural Immune Response to FeLV

Exposure to FeLV does not invariably result in persistent infection and development of an FeLV disease. A rapid and vigorous immunological response to FeLV after exposure can lead to resistance to persistent infection. Both humoral and cellular immune responses are critical for this virus control. Macrophages are essential in the initial defense against FeLV infection and, experimentally, agents that depress macrophage responses make cats more susceptible to infection with the virus (Hoover *et al.*, 1981). Antibody to the viral envelope gp70 early after infection will result in neutralization of the virus and clearance from the body (Hardy *et al.*, 1976a).

In persistently infected cats, an inappropriate antibody response against the FeLV *gag* antigens p15, p12, p27, p10 are not beneficial and, in some cats, may lead to the formation of immune complexes, immune deficiency, the development of feline AIDS (FAIDS), and immune complex glomerulonephritis (Weksler *et al.*, 1975; Day *et al.*, 1980; Jakowski *et al.*, 1980; Hardy, 1981b, 1982; Snyder *et al.*, 1982).

The humoral immune response to the FeLV envelope gp70 molecule, virus neutralizing (VN) antibody, and to the FeLV-induced tumor specific antigen (FOCMA) govern the outcome of an FeLV-infected cat (Hardy *et al.*,

1976a; Essex *et al.*, 1975; Russell and Jarrett, 1978; Essex, 1980). A sufficient VN antibody titer to the FeLV gp70 is required to render a cat protected against both FeLV infection and FeLV-induced diseases, except FeLV-negative LSAs. In cats who develop FeLV-negative LSAs, FeLV infection of lymphocytes probably occurs before the VN antibody develops. After FeLV integrates into lymphocytes the VN antibody response develops and the virus is rejected, leaving the FeLV genetic-induced changes to cause transformation later in life. The FeLV provirus does not exist in the FeLV-negative LSA cells, but the FeLV provirus can be detected in some bone marrow cells, which suggests a probable indirect mechanism for this transformation.

Exposed pet cats with protective titers (1:10 or greater) of VN antibody have rejected the virus and are resistant to subsequent viral infection (Hardy *et al.*, 1976a; Russell and Jarrett, 1978). An appropriate cellular immune response is also probably necessary and is found concordant with VN antibody in immune cats. However, specific studies of the cellular immune responses to FeLV in immune cats have not been done.

Seroepidemiological studies have shown that unexposed pet cats do not have VN antibodies to FeLV subgroups A and B. However, about 42% of exposed household pet cats but only 1% to 4% of uninfected stray cats (unknown exposure to FeLV) have VN antibodies to FeLV-A and -B (Hardy *et al.*, 1976a; Russell and Jarrett, 1978; Hardy, 1981b). No persistently FeLV-infected cats have protective VN antibody to FeLV-A and -B. There is a high prevalence of VN antibodies to FeLV-C in all populations of cats, especially in FeLV-A and -B viremic cats (45%), most of whom (98%) have no VN antibodies to FeLV-A and -B. The reason for this unusual discordant immune response to FeLV-C in these cats in not known.

2. Immune Response to the Feline Oncornavirus-Associated Cell Membrane Antigen (FOCMA)

FeLV-infected cats with protective titers of FOCMA antibody, without protective titers of VN antibody, are resistant to the development of LSA or other FeLV-induced tumors. However, they are not resistant to FeLV infection and the development of nonneoplastic FeLV diseases such as FeLV-FAIDS and anemias (Essex *et al.*, 1971, 1975; Hardy *et al.*, 1976a, 1978). In fact, a recent study found that FeLV-uninfected cats with FOCMA antibody of 1:16 or greater had a significantly higher prevalence of a history of certain diseases than did cats without FOCMA antibody (Swenson *et al.*, 1990). Diseases in these cats included upper respiratory tract infections, abscesses, ear infections, lower urinary tract infections, gastrointestinal disease, pneumonia, uterine infection, lymphadenopathy, fever of unknown origin, and bacterial infections. Thus, there is compelling evidence for a retrovirus-mediated transient or prolonged immune deficiency after exposure to FeLV in FeLV-uninfected cats.

FOCMA antibody is found in FeLV-negative cats exposed to FeLV (38.4%) but it is not found in SPF cats (Essex *et al.*, 1971, 1975; Hardy *et al.*, 1976a; Hardy, 1981b; Swenson *et al.*, 1990). Twenty-five percent of FeLV-

TABLE IX. Consequences of Exposure to FeLV

Permanent FeLV status	Exposure to FeLV	FeLV immune response	Percentage of cats[a]
Not infected	Not exposed	Not immune	30
Not infected	Exposed	Immune	42[b]
Infected	Exposed	Not immune	28

[a] From Hardy *et al.*, 1976a. Cats classified as "exposure to FeLV" were cats who lived in the same household with at least one FeLV-infected cat. As a result of this household exposure, some cats were classified as "not exposed" since they were neither infected with nor immune to FeLV. These cats were not even transiently infected and thus were probably never exposed by contact to infected cats in the household.
[b] Were transiently infected.

infected cats have protective titers of FOCMA antibody. FOCMA antibody has even been used therapeutically, by infusion of small amounts of cat serum containing high titers of FOCMA antibody, to induce remission of LSAs in pet cats (Hardy *et al.*, 1980c).

B. Consequences of FeLV Exposure

Studies of the spread of FeLV demonstrated that 28% of cats exposed to FeLV become persistently infected and 42% become immune to the virus, whereas the remaining 30% become neither immune nor infected (Fig. 5) (Table IX) (Hardy *et al.*, 1976a; Hardy, 1981b). Thus, it is apparent that about 70% of FeLV-exposed cats become immune or are not exposed enough to be infected or immunized. Of the 28% of cats that become persistently infected, 83% will die of an FeLV-induced disease within 3.5 years (McClelland *et al.*, 1980). Only about 10% of these cats develop LSA, whereas more than 50% die of the immunosuppressive effects of the virus (Hardy *et al.*, 1973a, 1976a; Cotter *et al.*, 1975; Essex *et al.*, 1975; Gardner *et al.*, 1977; Hardy, 1981b, 1982).

C. Latent FeLV

Latent, unexpressed, nonreplicating FeLV occurs in a small number of mononuclear cells of the bone marrow of some cats that have rejected the contagiously transmitted FeLV infection and become immune (Post and Warren, 1980; Rojko *et al.*, 1982). Latent FeLV is different from endogenous FeLV and other retrovirus sequences of cats and other species in that the latent FeLV provirus genomes are acquired by infectious transmission and persists in only a few cells after all replicating FeLV has been rejected by the immune response of the cat. In contrast, endogenous retroviruses of cats and other species are transmitted genetically to uninfected cells as cellular sequences and are thus found in all cells.

Latent FeLV can be reactivated from the bone marrow cells of cats by treatment with corticosteroids or by stimulating bone marrow cultures with

corticosteroids or *Staphylococcus aureus* Cowans I (Rojko *et al.*, 1982). Latent FeLV can apparently be rarely reactivated in some pregnant mothers and can be transmitted to their fetuses, who are then subsequently born and become viremic (Pacitti *et al.*, 1986). Latent virus is usually extinguished and can no longer be reactivated from most cats several months after suppression of replicating FeLV (Pedersen *et al.*, 1984a). Under natural conditions, reactivation of latent virus does not occur often in pet cats.

Recent reports suggest that latent FeLV is associated with persistent immune deficiency in FeLV nonviremic cats who produce neutralizing antibody and a strong cellular (lymphocyte) immune response to the virus (Lafrado *et al.*, 1989). The apparent chronic immune dysfunction occurs in the neutrophils of these cats.

V. PATHOGENESIS

A. Spread of FeLV in an Infected Cat

The saliva of naturally infected pet cats has as much as 2×10^6 infectious FeLV per ml. The virus is mainly transmitted contagiously by intimate and prolonged, direct contact through the saliva to the mucous membranes of the head of uninfected cats (Hardy *et al.*, 1969; Gardner *et al.*, 1971b; Francis *et al.*, 1977; Rojko *et al.*, 1979). The pathogenesis of the stages of FeLV infection has been elucidated by use of the IFA test in experimentally inoculated SPF cats (Rojko *et al.*, 1979; Hoover, 1984). After contact infection, the virus replicates initially in lymphocytes of the local lymph nodes of the head and neck (Fig. 6A). Most infected cats reject the virus at this early stage, become virus free, and immune (Hardy *et al.*, 1976a; Rojko *et al.*, 1979; Hardy 1981b). In this regard, studies of the spread of FeLV demonstrated that 28% of cats exposed to FeLV become persistently infected, 42% become immune to the virus, whereas the remaining 30% become neither immune nor infected (see Section I. Consequences of FeLV Exposure).

In those cats that are unable to reject the virus in this early stage FeLV spreads to the bone marrow (Fig. 6B) where it replicates to high titers in all nucleated myeloid and erythroid cells. The virus spreads throughout the cat's body in infected leukocytes and platelets released from the infected bone marrow, or as whole virus in the plasma (10^5 infectious FeLV per ml). Within 6 to 8 weeks the virus infects cells of the salivary glands (Fig. 6C), oral mucosa, and respiratory epithelium from where it is shed. FeLV is also transmitted *in utero* to unborn fetuses and through the milk of infected mothers (Hardy *et al.*, 1969; Hardy, 1981b). The period of time from FeLV infection to disease development is highly variable, but 83% of infected healthy cats die within 3.5 years from FeLV-induced diseases (McClelland *et al.*, 1980).

Most cats (97%) that have widespread replication of FeLV in their bone marrow remain persistently infected (Fig. 5). Thus 3% of infected cats can reject FeLV infection and rid themselves of all virus-replicating cells (Hardy *et al.*, 1976a; Hardy, 1981b). This observation could have important implica-

tions for humans infected with retroviruses; some people may be able to reject HIV or HTLV infections. Cats that reject FeLV develop high titers of neutralizing antibody. In humans, however, neutralizing antibody and retroviruses coexist, and no person has yet been shown to have a natural protective immunity to any human retrovirus. Cats persistently infected with FeLVs do not produce detectable neutralizing (gp70) antibody to the oncoretrovirus with which they are infected (Hardy *et al.*, 1976a). However, some infected cats do produce antibody to the gp70 molecule of the FeLV envelope, which becomes complexed with FeLV, but which does not neutralize the virus. Like humans infected with HIV, neutralizing antibody coexists in cats infected with the feline lentivirus, FIV (Hardy and Zuckerman, unpublished data).

B. Mechanisms of FeLV-Induced Leukemogenesis

The molecular (genetic) mechanisms by which most naturally occurring oncogenic retroviruses induce leukemia are not fully understood. However, four reviews on the mechanisms of retroviral-induced leukemogenesis by Hardy (1983a,b), Neil *et al.* (1987), and Onions and Jarrett (1987) suggest several possibilities.

The best understood example of the mechanism by which a chronic leukemia virus causes tumors occurs in mice where polytropic MCF-MuLVs are generated as recombinants between ecotropic MuLV and endogenous *env* sequences somewhat related to xenotropic viruses (Hartley *et al.*, 1977; Elder *et al.*, 1977; Chattopadhyay *et al.*, 1982). The generation of MCF viruses appears to be the proximal event in the induction of lymphomas in AKR mice (see Chapter 7).

The feline system appears to be quite analogous to the murine system in that subgroup FeLV-B and a Moloney virus-derived MuLV MCF virus show a striking homology in the nucleotide sequences of their envelope genes (Elder and Mullins, 1983). The homologies are in the substituted (presumably xenotropic-like) portion of the MuLV MCF envelope genes. Since FeLV-B and -C arise from recombination of FeLV-A with enFeLV *env* sequences these recombinant viruses, like the MCF MuLVs, may play a proximal role in the formation of lymphoid tumors in cats (Stewart *et al.*, 1986b; Elder and Mullins, 1983; Overbaugh *et al.*, 1988b).

Acute transforming oncogene-containing FeLVs have been discovered in some cats with T-cell LSAs. These defective FeLV-*myc* containing viruses are recombinant viruses derived from FeLV and c-*myc* (Neil *et al.*, 1984, 1987; Mullins *et al.*, 1984; Levy *et al.*, 1984). However, more than 75% of naturally occurring LSAs in cats do not have acute transforming defective FeLV-*myc* viruses. Thus there must be several ways by which FeLV can induce LSAs.

In addition to the FeLV-*myc*-containing viruses isolated from LSAs, an FeLV (FeLV-T17) carrying a T-cell receptor gene, v-*tcr*, has been discovered (Neil *et al.*, 1987, 1988; Onions and Jarrett, 1987). This is the first example of retroviral transduction of an immunological effector molecule and has led to the reconsideration of the importance of cell surface receptors of the immune

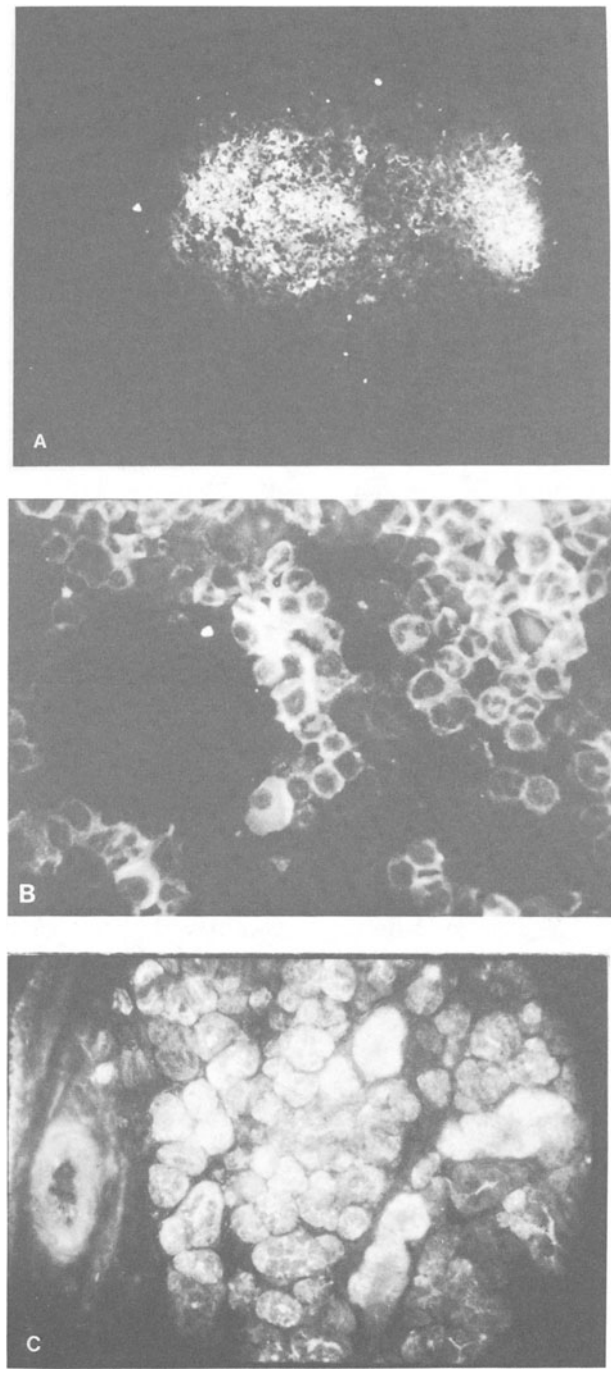

FIGURE 6. Pathogenesis of FeLV infection. Demonstration of FeLV infection by the IFA technique. A. Early stage, 2 to 4 days, post-FeLV infection. Submandibular lymph node of the head with FeLV antigens in the germinal centers. Most exposed cats reject the virus at this stage and become immune. The virus spreads to the bone marrow in those cats that do not reject the virus at this stage. (Reprinted with permission *J. Natl. Cancer Inst.*) (Rojko *et al.*, 1978.) B. Middle

system in leukemia development. Antigen receptors of lymphoid cells bind external ligands and are crucial in the control of immune specificities and cellular proliferation and differentiation. Thus, the discovery of the FeLV-T17 virus suggests that autocrine stimulation in leukemogenesis may operate through antigen receptors via ligand/receptor interactions (Neil *et al.*, 1988).

Recently a locus termed *flvi-1* has been found in some feline LSAs, which may represent a common integration domain of FeLV in a subset of naturally occurring LSAs (Levesque *et al.*, 1990). The LSAs with FeLV integrated at *flvi-1* were non-T-cell LSAs and had an average of only three proviruses compared to an average of eight proviruses for other types of LSAs. The small number of proviral integration events in tumors of this subgroup suggests that an early proviral integration event into the *flvi-1* locus may induce leukemogenesis.

VI. PATHOLOGY

FeLV-induced diseases were collectively the leading cause of death among pet cats from infectious causes before the introduction of the first FeLV vaccine in the mid-1980s (Hardy, *et al.*, 1969, 1973a; Cotter *et al.*, 1975; Essex, 1980; Hardy, 1980a, 1981b,d, 1982). It is presently not known if the vaccine has significantly lowered the occurrence of FeLV diseases (see Section VII).

Rapidly dividing lymphoid, myeloid, mucosal, and epithelial cells support FeLV replication best, and the virus can induce proliferative (neoplastic) and degenerative (blastopenic) diseases in these cells (Fig. 4) (Table X) (Hardy *et al.*, 1973b; Rojko *et al.*, 1979; Hardy, 1981b). Lymphosarcoma (LSA) and lymphoid hyperplasia (lymphadenopathy syndrome) are the proliferative diseases of lymphocytes caused by FeLV, whereas thymic atrophy and general lymphoid depletion are the degenerative diseases of lymphocytes (Anderson *et al.*, 1971; Hoover *et al.*, 1973, 1976; Hardy *et al.*, 1973a; Hardy, 1982). FeLV proliferative diseases of erythroid cells are erythremic myelosis and erythroleukemia, whereas the degenerative erythroid disease, aplastic anemia (erythroblastopenia), occurs far more often in pet cats than do the proliferative diseases (Hardy, *et al.*, 1969, 1973a; Hoover *et al.*, 1973, 1974; Mackey *et al.*, 1975; Hardy, 1981d; Onions *et al.*, 1982; Overbaugh *et al.*, 1988a).

Overall, the most frequent clinical manifestation of FeLV infection is severe immune deficiency (50% of FeLV diseases), which results in the development of secondary opportunistic infections and death (Essex *et al.*, 1975;

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stage, 7 to 21 days, post-FeLV infection. FeLV is present in all nucleated bone marrow cells. The virus replicates to high titers in the bone marrow and then spreads to the intestines, bladder, and epithelial cells of the pharynx, oral mucosa, and salivary glands. C. Late stage, 28 to 56 days, post-FeLV infection. Replication of FeLV in the salivary epithelial cells allows shedding of the virus into the oral cavity. The virus is then transmitted via intimate social contact mainly by mutual grooming.

TABLE X. Diseases Induced by the Feline Leukemia Virus

Cell type	Proliferative diseases (neoplastic)	Degenerative diseases (blastopenic)
Bone marrow cells		
Primitive mesenchymal cell	Reticuloendotheliosis	—
Erythroblast	Erythremic myelosis	Erythroblastosis (regenerative anemia)
	Erythroleukemia	Erythroblastopenia (aplastic anemia)
		Pancytopenia
Myeloblast	Granulocytic leukemia	Myeloblastopenia-enteritis syndrome
Megakaryocyte	Megakaryocytic leukemia	Thrombocytopenia
Fibroblast	Myelofibrosis	—
Osteoblast	Medullary osteosclerosis	—
	Osteochondromatosis	—
Lymphocytes	Lymphosarcoma	Thymic atrophy
	Lymphadenopathy syndrome (distinctive peripheral lymph node hyperplasia)	Lymphopenias
		Feline acquired immune deficiency syndrome (FAIDS)
Intestine	—	Enteritis
Kidney	—	FeLV immune complex Glomerulonephritis
Uterus	—	Abortions and resorptions
Fibroblasts–skin	FeSV-induced multicentric fibrosarcomas	—

Hardy, 1981a, 1982; Hardy and Essex, 1986). This syndrome is called the FeLV-induced feline acquired immune deficiency syndrome or FeLV-FAIDS and is very similar to human AIDS (Hardy and Essex, 1986).

A. Lymphocyte Diseases

1. Proliferative (neoplastic) Lymphocyte Diseases

a. Lymphosarcoma (LSA)

Two hundred cases of LSA occur annually per 100,000 cats at risk, which is the highest incidence of spontaneous LSA of any animal (Dorn *et al.*, 1968; Hardy, 1980a, 1981b). Cats are prone to development of hematopoietic tumors (one-third of all tumors) and 90% of these are lymphoid tumors (Dorn *et al.*, 1968). Lymphosarcoma occurs in four gross anatomic forms in cats. The most common form is multicentric LSA where the tumor localizes in internal organs and lymph nodes (Fig. 7) (Table XI). Approximately 30% of the cats

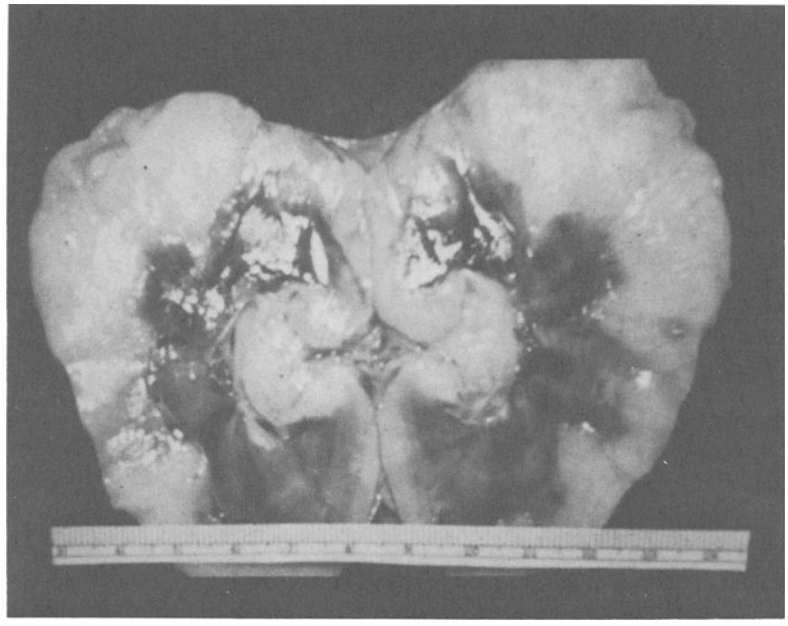

FIGURE 7. Multicentric form of feline lymphosarcoma. Lymphosarcoma cell infiltration of the renal cortex in an FeLV-infected cat. (Reprinted with permission from *J. Am. Anim. Hosp.Assoc.*) (Hardy, 1981d.)

with LSA have leukemic blood profiles, and these animals are usually classified as having multicentric LSA (Crighton, 1969; Hardy, 1980a, 1981d). Thymic LSA is the second most common form where the tumor localizes mainly in the thymus gland or anterior mediastinal lymph nodes of young cats (Fig. 8). Alimentary LSA, where the tumor localizes in the gastrointestinal tract, is usually FeLV-negative and is the third most common form (Fig. 9) (Crighton, 1969; Hardy *et al.*, 1977, 1980a; Hardy, 1981d). Unclassified LSA, where the tumor localizes in an unusual site, is the least common form (Fig. 10).

Most feline LSAs are T-cell tumors although B-cell LSAs occur frequently in the gastrointestinal tract (Tables XI, XII) (Hardy *et al.*, 1977, 1980a,b). Leukemic cells in the peripheral blood are uncommon in cats with lymphoid

TABLE XI. Lymphosarcomas in Pet Cats

Anatomic site	Percent of total	Percent FeLV positive	Average age (yr) of cats FeLV+/FeLV−	Cell or origin (%)			
				T	B	Null	Mixed
Multicentric	44	80	3.3/5.8	54	13	4	29
Thymic	38	77	2.1/3.8	93	0	0	7
Alimentary	15	23	5.0/8.9	20	60	0	20
Unclassified	3	38	5	0	0	0	100[a]

[a] Only one tumor studied for cell of origin.

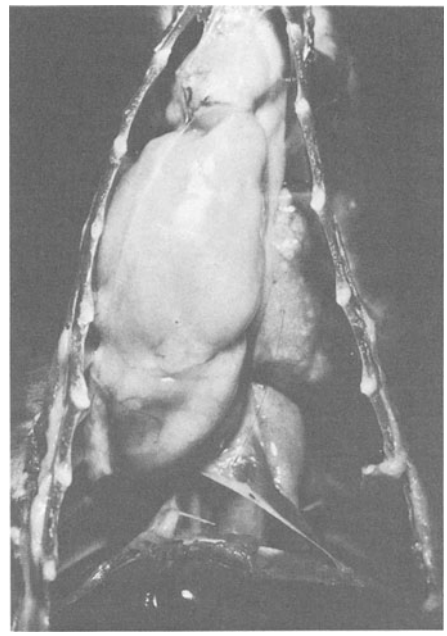

FIGURE 8. Thymic form of feline lymphosarcoma. Massive thymic lymphosarcoma in the anterior mediastinal area of a young FeLV-infected cat.

tumors, and about two-thirds of cats with LSA have nonregenerative anemias; almost all of these cats are FeLV-positive (Crighton, 1969; Hardy, 1981d). The anemia in these cats is induced by the virus and is not secondary to the LSA.

Seventy percent of cats with LSA have the virus in their tumors (Fig. 11A), but FeLV-negative LSA occurs in 30% of all cats that develop LSA (Fig. 11B) (Tables XI, XII). No FeLV antigens can be detected nor can infectious FeLV be isolated from these "virus-negative" tumors (Hardy *et al.*, 1977, 1978, 1980a; Koshy *et al.*, 1979). However, the FeLV-induced FOCMA is present on the membranes of both FeLV-positive and FeLV-negative LSA cells and strongly indicates a role for FeLV as the cause of these tumors (Hardy *et al.*, 1980a,b). Cats with FeLV-negative LSAs are usually old, over 7 years of age, and often have alimentary B-cell LSAs, whereas cats with FeLV-positive LSAs are usually younger, less than 7 years old, and have T-cell multicentric or thymic LSAs (Tables XI, XII) (Hardy *et al.*, 1977, 1980a,b; Francis *et al.*, 1979).

FeLV-negative LSAs develop often in cats living in households where FeLV-infected cats live (Table XII) (Hardy *et al.*, 1969, 1980a,b; Brodey *et al.*, 1970; Hardy, 1981b; Francis *et al.*, 1979). In a large epidemiological study we found that cats who developed FeLV-negative LSAs were exposed to FeLV as often as cats who developed FeLV-positive LSAs (Hardy *et al.*, 1977, 1980a). No FeLV proviral sequences, above the level of endogenous FeLV sequences present in all uninfected cat cells, are present in the FeLV-negative LSA tumor

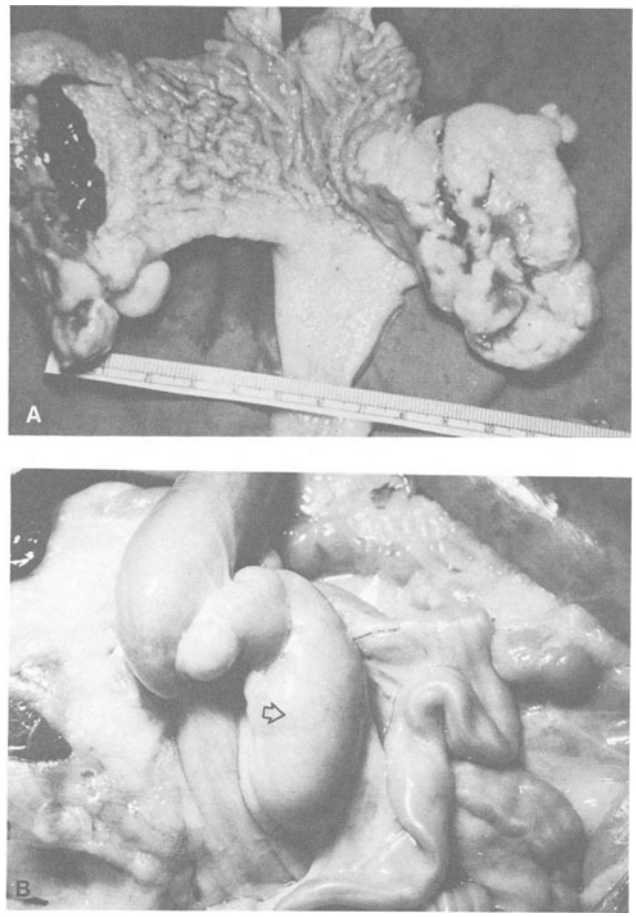

FIGURE 9. Alimentary form of feline lymphosarcoma. A. Gastric lymphosarcoma mass in the pyloric portion of the stomach in an old FeLV-negative cat. B. Cecal and mesenteric lymph node lymphosarcoma (arrow) in an old FeLV-negative cat. (Reprinted with permission from *J. Am. Anim. Hosp. Assoc.*) (Hardy, 1981d).

tissues, which suggests an indirect mechanism of leukemogenesis in these cases (Koshy *et al.*, 1979) (see Section V). Additional FeLV sequences can be found in non-LSA tissues, most often bone marrow cells, in 60% of these cats. This finding indicates that cats with FeLV-negative LSAs were previously infected with FeLV and that integrated exogenous FeLV sequences exist in some non-LSA tissues. Latent FeLV can be reactivated from the bone marrow, but not from the FeLV-negative LSA cells, of 80% of cats with FeLV-negative LSAs (Rojko *et al.*, 1982; Post and Warren, 1980). Thus, it is apparent that FeLV induces both FeLV-positive and FeLV-negative LSAs in cats and that the virus is not required to maintain the neoplastic state. After this observation, a similar conclusion was made in some Abelson MuLV experimentally induced mouse lymphomas in which the virus was eliminated but the malignancy persisted (Grunwald *et al.*, 1982) (see *Volume* 1, Chapter 7).

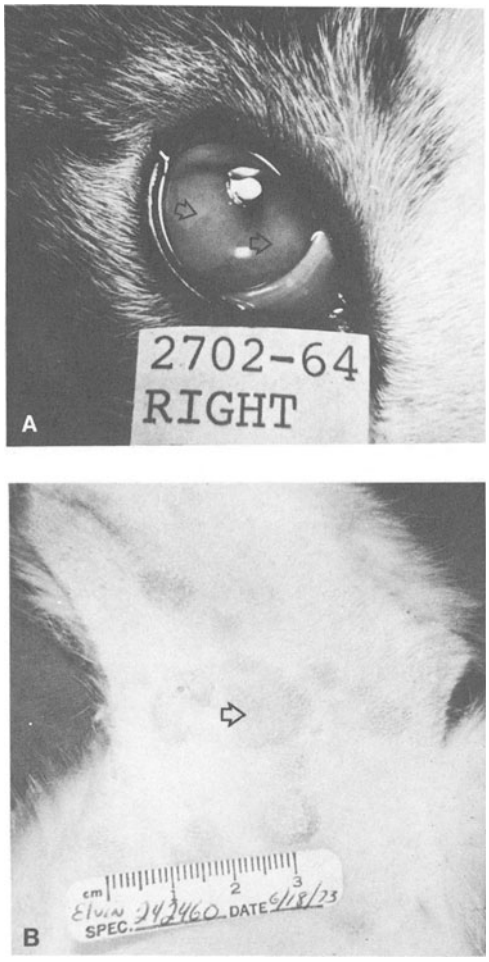

FIGURE 10. Unclassified form of feline lymphosarcoma. A. Ocular lymphosarcoma. Lymphosarcoma cells infiltrated the iris and anterior chamber of the eye in an FeLV-infected cat. B. Skin lymphosarcoma. Lymphosarcoma masses (arrow) localized in the skin of an old FeLV-negative cat. (Reprinted with permission from *J. Am. Anim. Hosp. Assoc.*) (Hardy, 1981d.)

Experimentally, FeLV can also induce FeLV-negative LSAs in dogs (Rickard *et al.*, 1973). Puppies inoculated with FeLV *in utero* developed FeLV-positive LSAs whereas those inoculated with FeLV during the first day of life developed FeLV-negative LSAs. FeLV is capable of altering the genome of lymphocytes to induce transformation but then is not required to persist in the cellular genome to maintain the transformed state. These observations in pet cats, where a commonly occurring leukemogenic retrovirus can induce virus-negative lymphoid tumors, suggest that similar lymphoid tumors may also occur in humans and the search for these viruses should include nontumor tissues such as bone marrow.

b. Lymphadenopathy Syndrome (LAS)

The lymphadenopathy syndrome (LAS), also called distinctive peripheral lymph node hyperplasia, occurs in FeLV-infected young cats 6 months to 2

TABLE XII. Comparison of FeLV-Positive and FeLV-Negative Feline Lymphosarcomas

Category	FeLV-positive LSAs	FeLV-negative LSAs
Exposure to FeLV	Exposed	Exposed
Infectious FeLV	Present	Absent from LSA cells Reactivatable form: bone marrow cells not from LSA cells[a]
Exogenous FeLV genome in LSAs	Present	Absent
FeLV antigens	Present	Absent
FOCMA	Present	Present
Anatomical form	Multicentric form Thymic form	Alimentary or skin form
Lymphocyte origin	Usually T cell	Usually B cell
Age	Young—under 7 yrs	Old—over 7 yrs

[a] *In vivo* after steroid treatment of *in vitro* after culture and steroid treatment (Rojko *et al.*, 1982).

years of age and is characterized by peripheral lymph node enlargement (Fig. 12) (Hardy, 1981a, 1982; Moore *et al.*, 1986). Feline LAS is very similar to the lymphadenopathy syndrome that precedes the development of AIDS in humans (Hardy, 1984). Half of the cats with LAS appear normal while the others develop fever, lethargy, anorexia, and hepatosplenomegaly. The mandibular lymph nodes are commonly involved, but popliteal, visceral, and other nodes can also be affected. Histologically, the architecture of the lymph nodes is distorted and the sinuses and follicles are difficult to discern. Macrophages, lymphocytes, immunoblasts, and plasma cells fill the paracortex, which causes distortion of the lymphoid follicles.

This syndrome has a variable clinical outcome. In some cats the lymph nodes return to normal size whereas in other cats the syndrome progresses to LSA years later. Most cats with LAS progress to develop FeLV-FAIDS. The pathogenesis of LAS is not fully understood, but inappropriate stimulation by replicating FeLV in follicular germinal centers probably causes lysis of lymphocytes and immunosuppressive disease.

2. Degenerative Lymphoid Diseases

The most frequent and most devastating consequence of FeLV infection is the induction of a profound immune deficiency syndrome. FeLV replicates to high titers in lymphoid cells and often induces severe depletion and dysfunctions of these cells (Table XIII). Although the following diseases are described as separate entities, they in fact probably represent different aspects of FeLV-FAIDS.

a. Thymic Atrophy

Thymic atrophy is an FeLV-induced degenerative lymphoid disease of T lymphocytes of the thymus gland that occurs in young cats (Anderson *et al.*,

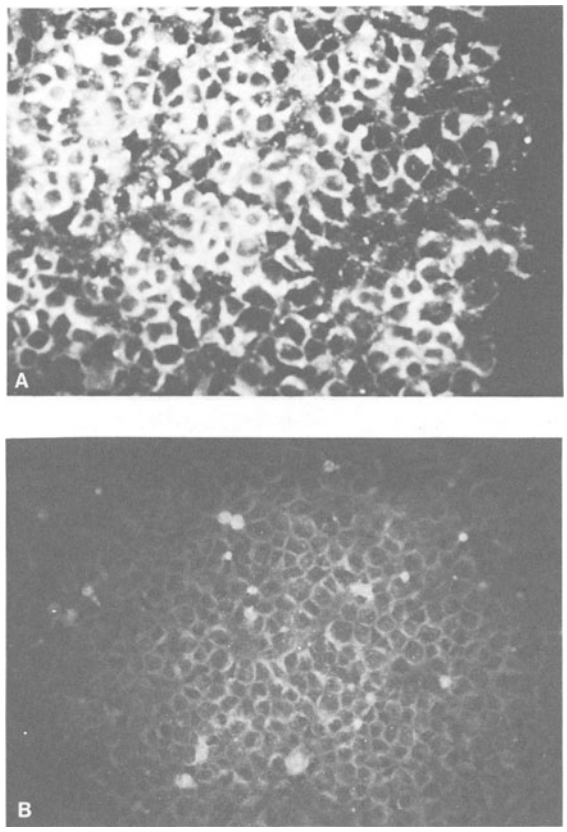

FIGURE 11. Expression of FeLV in feline lymphosarcomas. A. IFA FeLV-positive thymic lymphosarcoma. Cytoplasmic FeLV antigens are expressed in all of the tumor cells. B. IFA FeLV-negative alimentary lymphosarcoma. None of the lymphosarcoma cells express FeLV antigens in their cytoplasms. (Reprinted with permission from *J. Am. Anim. Hosp. Assoc.*) (Hardy, 1981d).

1971; Hardy, 1981a, 1982). Thymic lymphocytes and lymphocytes in other lymphoid organs are depleted, which results in a deficient cell-mediated immune response and renders infected kittens susceptible to opportunistic infectious microorganisms (Fig. 13). Many FeLV-infected kittens with thymic atrophy become cachectic, develop bronchopneumonia or enteritis, and usually die of these diseases in the first 3 months of life.

b. Lymphoid Atrophy

Generalized peripheral lymphoid hyperplasia occurs early in most FeLV-infected cats but usually progresses to generalized lymphoid atrophy, lymphopenia, and death from opportunistic infections (Anderson *et al.*, 1971; Hoover *et al.*, 1973; Hardy, 1982).

c. FeLV-Induced Feline Acquired Immune Deficiency Syndrome (FeLV-FAIDS)

Two feline retroviruses, FeLV and the feline immunodeficiency lentivirus (FIV), can induce almost identical immunodeficiency syndromes, feline AIDS or FAIDS (see Chapter 3) (Pedersen *et al.*, 1987; Hardy, 1988). In FeLV-induced FAIDS, the virus replicates in cells of the immune system, lymphoid

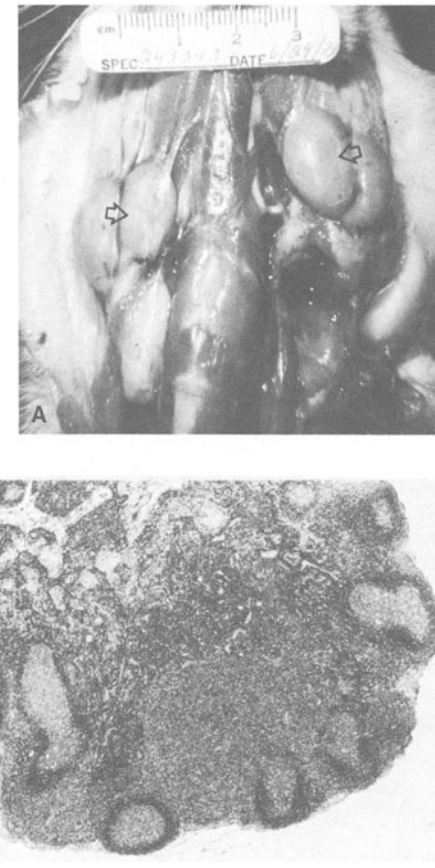

FIGURE 12. Feline lymphadenopathy syndrome. A. Severe mandibular lymphadenopathy (arrows), termed distinctive peripheral lymph node hyperplasia, in an FeLV-infected cat. B. Histology of distinctive peripheral lymph node hyperplasia in an FeLV-infected young cat. Paracortical hyperplasia and unusually shaped lymphoid follicles distort the architecture of the lymph node. (Reprinted with permission from Churchill Livingstone, Inc.) (Rojko and Hardy, 1989).

and myeloid cells, and can cause degenerative blastopenic diseases, lymphopenias and neutropenias, involving these cells (Hardy *et al.*, 1973b; Essex *et al.*, 1975; Hardy, 1980a, 1981a,b, 1982; Hoover *et al.*, 1980). FeLV-induced immune cell deficiencies, characterized by drastic reductions in lymphocyte and neutrophil numbers (Hoover *et al.*, 1980; Hardy, 1981b, 1982), and immune cell dysfunctions consisting of reduced T-cell blastogenic responsiveness (Cockerell *et al.*, 1976; Cockerell and Hoover, 1977), depressed helper T-cell function as evidenced by impaired antibody production (Hardy, 1982; Trainin *et al.*, 1983), cutaneous anergy (Perryman *et al.*, 1972), and neutrophil dysfunctions (Table XIII) (Hardy, 1982; Lafrado and Olsen, 1986; Lafrado *et al.*, 1989; Dezzutti *et al.*, 1990). Experimentally, a defective variant of FeLV, named FeLV-FAIDS, with point mutations, deletions and duplications in the *env* gene, has been shown to induce a severe immunodeficiency syndrome.

This FeLV-FAIDS variant FeLV was experimentally shown to deplete T-lymphocyte colony-forming cells drastically, which prefigures the clinical onset of FAIDS (Quackenbush *et al.*, 1989). In addition, the peripheral blood lymphocytes of 67% of FeLV-infected cats with FAIDS failed to produce detectable levels of interleukin-2 when stimulated with concanavalin A com-

TABLE XIII. Immune Disorders in Cats with FeLV-FAIDS

I. Immune cell dysfunctions
A. Deficient cell-mediated immune response
1. Cutaneous anergy—decreased allograft rejection
B. Deficient antibody-mediated immune response
1. To threshold antigen stimulation
C. Deficient neutrophil functions
II. Immune cell deficiencies
A. Lymphoid depletions
1. Thymic atrophy-kittens
2. General lymphoid depletion—adults
B. Myeloid depletion
1. Neutropenias—myeloblastopenia syndrome
III. Pathogenic antibody immune-mediated disease
A. Immune complex glomerulonephritis
IV. Complement deficiency

pared to 21% of uninfected healthy cats and 41% of FeLV-infected asymptomatic cats (Tompkins *et al.*, 1989). These data indicate that T-lymphocyte dysfunction precedes the development of clinical disease.

B-cell dysfunctions also occur in FeLV-infected cats. Infected cats are less able (fourfold) to produce antibody to threshold doses of antigens than are uninfected cats (Hardy, 1982; Trainin *et al.*, 1983).

Many more pet cats die from FeLV-induced FAIDS than die from FeLV-induced neoplastic diseases (Hardy, 1981a, 1982). FeLV infection often underlies the following secondary diseases (Table XIV) in adult cats: feline infectious peritonitis (feline coronavirus), chronic oral inflammation, gingivitis (Fig. 14); necrotizing stomatitis, chronic upper respiratory disease (Fig. 15); and pneumonia, chronic generalized infections (septicemias and pyothorax), chronic cutaneous or deep dermal abscesses and nonhealing lesions of the skin (Fig. 16). In addition, 87% of kittens with thymic atrophy are infected with FeLV (Anderson *et al.*, 1971; Hardy, 1981a, 1982).

Many FeLV immunosuppressed cats have unremitting spirochete, coliform, staphylococcal, and streptococcal infections. Common secondary infections include: (1) viruses: feline herpesvirus (rhinotracheitis), feline coronavirus (feline infectious peritonitis virus), FIV; (2) pathogenic fungal infections: Candida, Cryptococcus, and Aspergillus; and (3) protozoa: Toxoplasma and Haemobartonella (Hardy, 1981a, 1982; Rojko and Hardy, 1989).

FeLV has been shown to be a potentiating co-factor for the primary and secondary stages of experimentally induced FIV infection (Pedersen *et al.*, 1990) (see Chapter 10). Preexistent FeLV infection greatly potentiates the severity of the transient primary and chronic secondary stages of FIV infection. However, a retrospective review of the FeLV and FIV status of several thousand healthy and sick pet cats did not reveal a similar potentiating effect (Hardy and Zuckerman, unpublished data).

The mechanism by which FeLV induces immune deficiency may be by one or a combination of the following: (1) viral replication and budding from

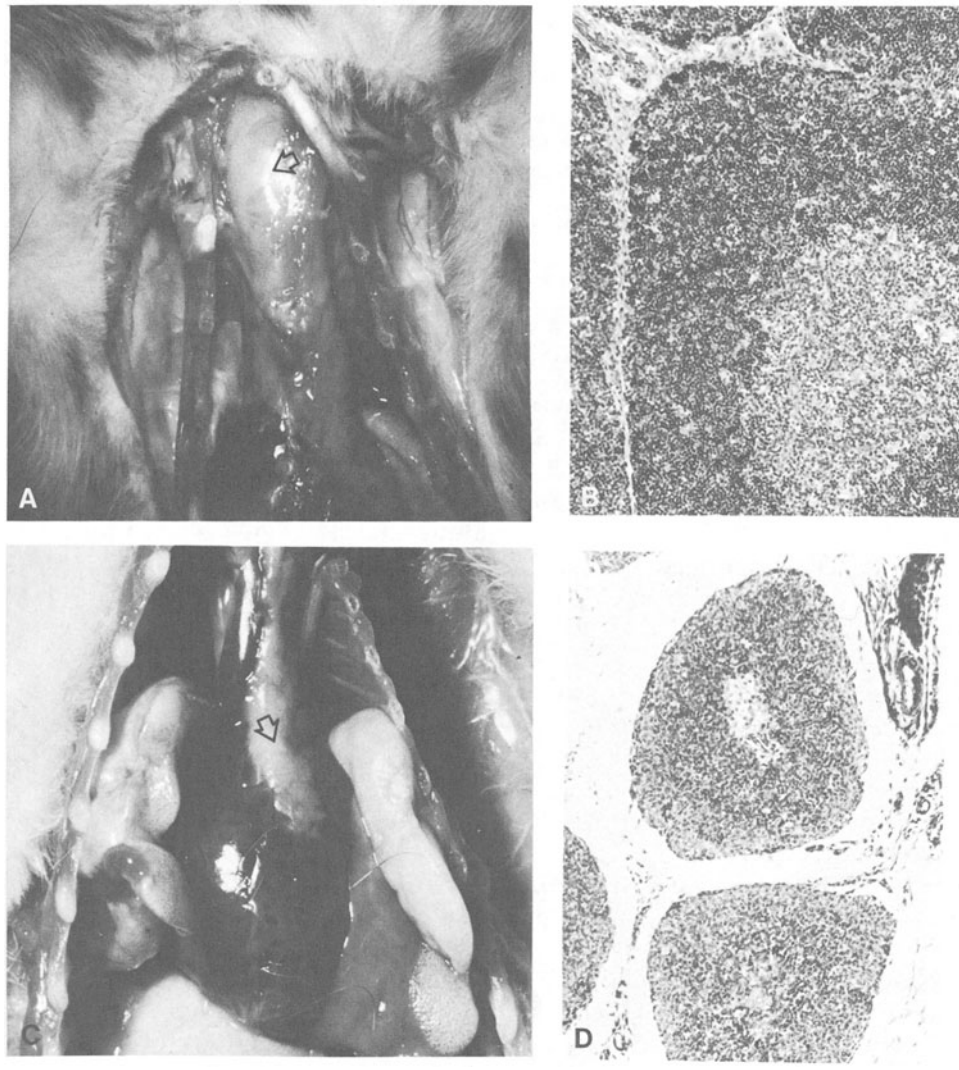

FIGURE 13. FeLV-induced thymic atrophy. A. Normal thymus in the anterior thorax (arrow) of an FeLV-negative kitten. (Reprinted with permission from *J. Am. Anim. Hosp. Assoc.*) (Hardy, 1981a). B. Histology of a normal thymus from an FeLV-negative kitten. The cortical and medullary regions of a thymic lobule are clearly distinguishable. (Courtesy E.A. Hoover. Reprinted with permission from Cancer Research and Springer-Verlag [Hardy, 1982]). C. Atrophic small thymus (arrow) in the anterior thorax of an FeLV-infected kitten with thymic atrophy. (Reprinted with permission from *J. Am. Anim. Hosp. Assoc.*) (Hardy, 1981a). D. Histology of an atrophic thymus from an FeLV-infected kitten. The thymus is atrophic with depletion of the cortical thymocytes. (Courtesy E.A. Hoover. Reprinted with permission from Cancer Research and Springer-Verlag [Hardy, 1982].)

TABLE XIV. Opportunistic Infectious Diseases in FeLV-FAIDS Cats

Disease	Percent FeLV-infected
Thymic atrophy-kittens	87
Chronic generalized infections	57
Upper respiratory disease and pneumonias	55
Chronic stomatitis and gingivitis	48
Viral infections	
Feline infectious peritonitis	46
FIV infection	8
Skin sores and recurrent abscesses	34

lymphocyte and neutrophil cell membranes may cause lysis or sensitize cells to cell-mediated immune destruction; (2) FeLV soluble circulating antigens by themselves or as constituents of immune complexes may cause immune deficiency. Purified p15E has been reported to decrease *in vitro* blast transformation by 45% to 92% (Cockerell and Hoover, 1977; Mathes *et al.*, 1978; Ruegg *et al.*, 1989). (3) Circulating immune complexes (CICs) are immunosuppressive, and CICs composed of whole infectious FeLV, FeLV gp70, p27, p15, and p15E occur in FeLV-infected pet cats (Fig. 17) (Day *et al.*, 1980; Snyder *et al.*, 1982; Hardy, 1982). In this regard, when FeLV CICs were therapeutically removed by *ex vivo* immunosorption on *Staphylococcus aureus* Cowans I columns, several cats with FeLV-FAIDS have shown clinical improvement (Jones *et al.*, 1980; Snyder *et al.*, 1984b). (4) The complement system is also affected in cats infected with FeLV. In one study all FeLV-infected cats with LSA and 50% of FeLV-infected healthy cats were hypocomplementemic (Kobilinsky *et al.*, 1979). The hypocomplementemia observed in FeLV-infected cats probably contributes to the generalized immune deficiency. (5) Recent studies of a molecularly cloned FeLV-FAIDS isolate have shown that small changes in the gp70 molecule (*env* gene mutants) were able to convert minimally pathogenic viruses into highly immunosuppressive viruses. This mechanism apparently entails the delay or prevention on superinfection interference and the accumulation of unintegrated viral DNA in lymphoid cells and a delay in the processing of the FeLV glycoprotein in the infected cells (Poss *et al.*, 1990). In this regard, a delay in superinfection interference and accumulation of unintegrated avian retrovirus DNA in avian cells has been shown to induce cell death (Weller *et al.*, 1980; Temin, 1988).

Neutrophils and neutrophil progenitors are always infected in cats with persistent FeLV infection (Figs. 4B, 6B) (Hardy *et al.*, 1973b). The consequences of neutrophil infection are effector inhibition and reduction of their numbers. Many FeLV-infected cats have persistent, transient, and cyclic neutropenias, which can progress to FeLV-FAIDS (Hardy *et al.*, 1973a; Cotter *et al.*, 1975; Pedersen *et al.*, 1977; Hardy, 1981a, 1982). Several studies have found dysfunctions in neutrophils from FeLV-infected and nonviremic FeLV exposed cats (Dezzutti *et al.*, 1990; Lafrado and Olsen, 1986). One functional

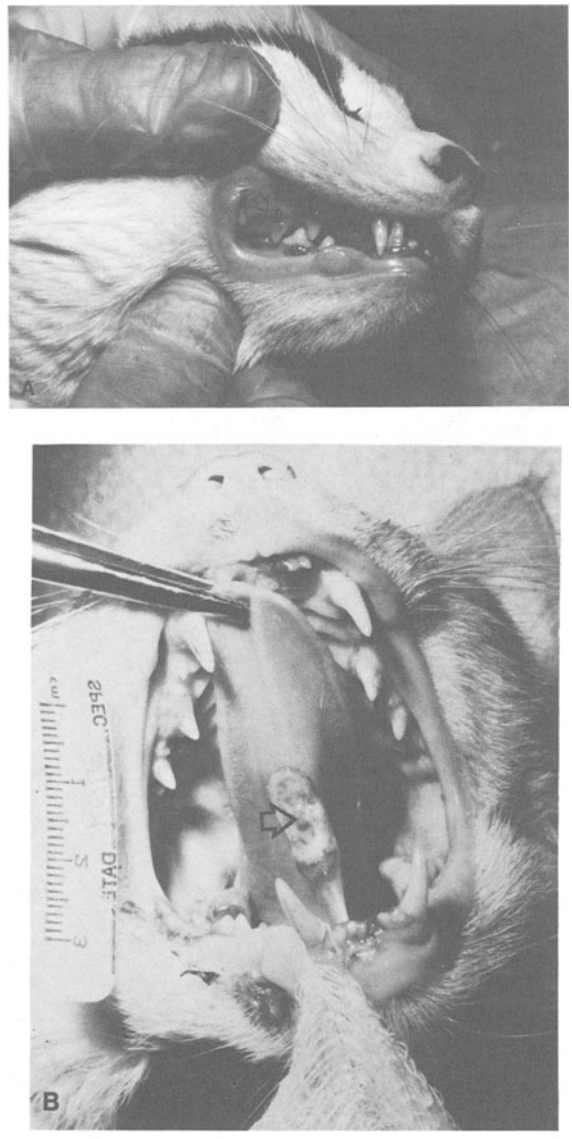

FIGURE 14. Feline acquired immunodeficiency syndrome (FAIDS). A. Chronic proliferative gingivitis in an FeLV-infected cat with FAIDS. (Reprinted with permission from Churchill Livingstone, Inc.) (Rojko and Hardy, 1989). B. Chronic oral ulcer of the tongue, probably due to feline herpesvirus, in an FeLV-infected cat with FAIDS.

study of SPF cat neutrophils found that FeLV-infected neutrophils had a suppressed TPA-induced chemiluminescent (CL) response compared to neutrophils of uninfected cats. The suppression is apparently due to an intracellular mechanism as *in vitro* exposure of neutrophils to virus or viral components did not suppress the CL response (Dezzutti *et al.*, 1990). This study supports the clinical impressions that FeLV-infected cat neutrophils are functionally suppressed (Hardy, 1982).

Many similarities exist between human AIDS and FeLV-induced FAIDS in pet cats (Hardy, 1984; Hardy and Essex, 1986). In both species the syndromes are characterized by lymphopenias, reduced lymphocyte blastogenesis, cutaneous anergy, reduced numbers of T cells, impaired antibody re-

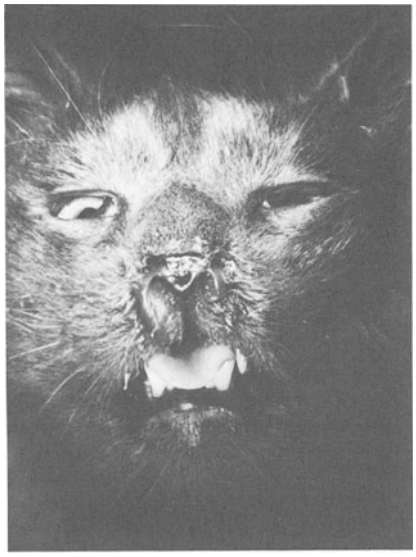

FIGURE 15. Feline acquired immunodeficiency syndrome (FAIDS). Chronic upper respiratory disease, rhinitis, with mucopurulent nasal exudate in an FeLV-infected cat with FAIDS. (Reprinted with permission from *J. Am. Anim. Hosp. Assoc.*) (Hardy, 1981a).

sponse, and the occurrence of secondary infectious diseases (Tables XV, XVI) (Gottlieb *et al.*, 1981; Masur *et al.*, 1981; Hardy and Essex, 1986). However, significant differences exist between FeLV and HIVs, and it is now apparent that the new feline lentiretrovirus, FIV, is a more appropriate viral model for human AIDS (Table XVII) (Pedersen *et al.*, 1987; Chapter 3).

B. Erythroid Diseases

In the bone marrow, FeLV replicates in all nucleated erythroid cells and can induce erythroid neoplastic or blastopenic diseases (Fig. 6B) (Table X) (Hardy, 1980a, 1981a,b).

1. Erythroid Neoplastic Diseases

Erythroid progenitor cells, which still have nuclei, support FeLV replication, but as these erythroid cells mature the nuclei are extruded and thus not only the FeLV provirus but the ability of FeLV to replicate in these cells is also

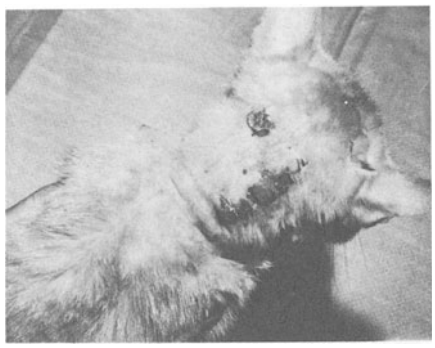

FIGURE 16. Feline acquired immunodeficiency syndrome (FAIDS). Chronic nonhealing skin lesions on the dorsal aspect of the neck of an FeLV-infected cat with FAIDS.

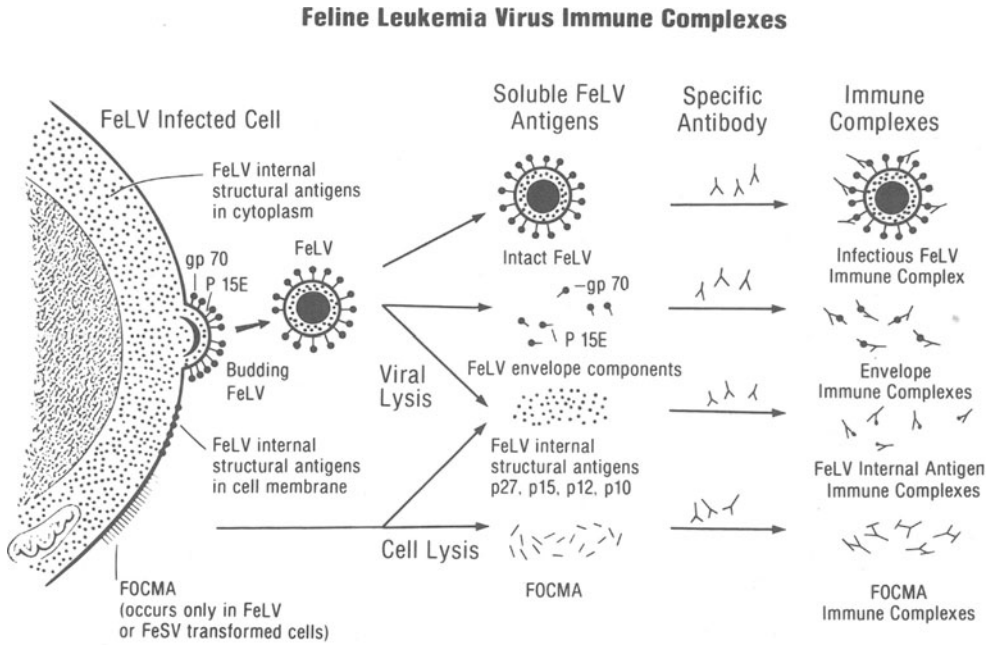

FIGURE 17. FeLV immune complexes. A schematic representation of the generation of circulating immune complexes composed of FeLV, FeLV antigens, FOCMA, and antibodies to these antigens. (Reprinted with permission Springer-Verlag [Hardy, 1982]).

lost (Fig. 6B) (Hardy, 1981a,d). Feline erythroid neoplasms are similar to those that occur in chickens and in mice infected with oncogene-containing acute transforming retroviruses (Roussel *et al.*, 1979; Scolnick, 1982). However, FeLV rarely induces erythremic myelosis and erythroleukemia in infected cats, and no acute transforming oncogene-containing feline retroviruses have been found in these diseases.

a. Reticuloendotheliosis

FeLV-induced reticuloendotheliosis, the proliferation of primitive mesenchymal pluripotent stem cells, occurs very rarely in pet cats (Hardy, 1981d). Cats with reticuloendotheliosis are usually anemic, and the neoplastic cells appear to be closely related to both erythroid and granulocytic myeloid precursor cells.

b. Erythremic Myelosis

Erythremic myelosis is characterized by abnormally high numbers of proliferating nucleated erythroid cells without significant concurrent proliferation of granulocytes (Hardy, 1981d). The disease does not occur commonly in pet cats, and clinically there is a severe nonregenerative anemia and marked variations in the numbers and morphology of the nucleated erythrocytes. Even though there are nucleated erythrocytes, there is a normal or

TABLE XV. Comparison of Immune Parameters of Cats and Humans with AIDS

FeLV-FAIDS	AIDS
I. Humoral immunity	I. Humoral immunity
A. Impaired antibody	A. Impaired antibody response to
1. Sheep RBCs-low dose	1. New antigens
2. Synthetic polypeptide antigen	2. Have antibody to infecting microorganisms.
II. Cellular immunity	II. Cellular immunity
A. Reduction in numbers and functions of T lymphocytes	A. Reduction of helper (CD4) T lymphocytes but normal numbers of suppressor (CD8) T lymphocytes.
B. Lymphopenias	B. Lymphopenias
C. Reduced lymphocytes	C. Reduced lymphocyte blastogenesis
D. Cutaneous anergy	D. Cutaneous anergy

reduced number of reticulocytes indicating a block in maturation from the early nucleated erythrocyte to the reticulocyte.

c. Erythroleukemia

No clear distinction exists between erythremic myelosis and erythroleukemia (Dameshek, 1951). For the purpose of classifying myeloproliferative diseases, however, the distinction between the two diseases is based on the presence of myeloblasts (granulocytic leukocyte precursors) along with abnormal nucleated erythrocytes in the peripheral blood of cats with erythroleukemia, whereas only neoplastic erythroid cells are present in cats with

TABLE XVI. Secondary Intercurrent Immune Deficiency Disorders of Cats and Humans with AIDS

Cats FeLV-FAIDS	Human AIDS
I. Viral diseases	
A. Herpesvirus	A. Herpesvirus
B. Coronavirus (FIP)	B. Cytomegalovirus
C. Feline immunodeficiency virus (FIV)	C. Hepatitis B virus
	D. HTLV-I/HTLV-II
II. Parasitic, fungal, protozoal	
A. Toxoplasmosis	A. Toxoplasmosis
B. Candida	B. Mucosal candida
C. Cryptococcosis	C. Cryptococcosis
III. Lymph nodes	
A. Lymphadenopathy	A. Lymphadenopathy
IV. Respiratory	
A. Pneumonia and upper respiratory diseases	A. Pneumonia—Pneumocystis carinii
V. Skin	
A. Skin sores and infections	A. Kaposi's sarcoma

TABLE XVII. Comparison of FeLV and FIV

FeLV	FIV
1. Oncovirinae	1. Lentivirinae
2. Pancytotropic—bone marrow	2. Helper T-lymphocyte tropic and lymphoid cells
3. Diseases	3. Diseases
a. FAIDS	a. FAIDS
b. Anemia (severe)	b. Anemia (mild)
c. Cancers	c. Cancers
d. Neurologic disorders	d. Neurologic disorders
4. Morphology: oval nucleoid	4. Morphology: rod-shaped nucleoid
5. Reverse transcriptase Mg + dependent	5. Reverse transcriptase Mn + dependent
6. Spread via saliva: licking	6. Spread via saliva: biting
7. Moderately contagious	7. Poorly contagious
8. Does not coexist with antibody	8. Coexists with antibody
9. Detected by finding viral antigens	9. Detected by finding viral antibodies

erythremic myelosis (Dameshek, 1951; Hardy, 1981d). Most cats with erythroleukemia have a profound nonregenerative anemia and, even though nucleated erythrocytes are present, there is a normal or low reticulocyte count indicating a block in the process of erythroid cell maturation. Erythroid and myeloid neoplastic cells are found in the blood, bone marrow, and in various organs such as the spleen, liver, and lymph nodes.

2. Erythroid Blastopenic Diseases

Blastopenic diseases of erythroid cells occur far more often in FeLV-infected pet cats than do neoplastic diseases (Hardy, 1980a, 1981a,d). FeLV induces three types of anemias: (1) erythroblastosis (regenerative anemia), (2) erythroblastopenia (aplastic or nonregenerative anemia), and (3) pancytopenia (Mackey *et al.*, 1975; Hardy, 1980a, 1981a). Experimentally, FeLV-A has been shown to induce nonfatal transient erythroblastosis, whereas several FeLV-C isolates have induced fatal aplastic anemias (erythroblastopenias) (Mackey *et al.*, 1975; Onions *et al.*, 1982; Dornsife *et al.*, 1989). The number of platelets can also be severely reduced in some infected cats.

The mechanism by which FeLV causes degenerative bone marrow diseases is unknown, but three possibilities exist: (1) FeLV may cause lysis by damaging the infected cell's membrane, possibly by the process of budding; (2) FeLV may antigenically alter the cell membrane, by budding or by inducing FOCMA, and the altered membrane may then be lysed by antibody to FeLV or to FOCMA or lysed by killer lymphocytes; and (3) FeLV may affect effector cells such as erythropoietin-producing cells, which then may not produce sufficient erythropoietin, resulting in erythroid depletion. This last possibility seems unlikely, however, since erythropoietin levels were found to be increased in most cats with erythroid aplasia (Kociba *et al.*, 1983).

Recent molecular studies have shown that the pathogenic determinant for anemogenesis of one molecularly cloned isolate of FeLV-C is confined to an 886-base pair region that encodes for 73 amino acids at the 3′ end of the *pol* gene and 241 amino acids of the N-terminal portion of the extracellular *env* gene gp70 (Riedel *et al.*, 1986, 1988; Dornsife *et al.*, 1989). The biological mechanism by which this determinant induces anemias in cats is still not known.

Cats are more susceptible to anemias than most other species because their erythrocytes have a shorter life span, 70–80 days, compared to the erythrocyte life span of approximately 120 days for most other species. The three distinct types of primary FeLV-induced anemias are: (1) FeLV erythroblastosis (regenerative anemia); (2) FeLV erythroblastopenia (aplastic or nonregenerative anemia); and (3) FeLV pancytopenia. The three anemias often develop in sequence in FeLV-infected cats beginning with erythroblastosis, which leads to erythroblastopenia, and finally to a pancytopenia and death. These phases indicate that FeLV has an initial stimulatory effect on erythroid cells followed by a degenerative effect.

a. FeLV Erythroblastosis (Regenerative Anemia)

FeLV-induced regenerative anemias—erythroblastosis—occurs less often than FeLV-erythroblastopenia or FeLV-pancytopenia. However, this may be due to the fact that during this early stage of the anemia crisis cats appear healthy and are not taken in for veterinary care. Only about 15% of FeLV-infected anemic pet cats have regenerative anemias (Cotter *et al.*, 1975; Hardy, 1981a). In response to the FeLV-induced anemia, immature erythrocytes are released from the bone marrow and there is an increase in the number of reticulocytes and nucleated red blood cells in the blood and bone marrow. There is also extramedullary hematopoiesis in the spleen and liver. Cats with non-FeLV-induced regenerative anemias usually have a good prognosis whereas FeLV-infected cats with regenerative anemias do not have good prognoses since many of them will progress and eventually develop the fatal erythroblastopenia, or pancytopenia syndromes. Some cats will also progress to develop myeloproliferative disease or lymphosarcoma. Experimentally, FeLV-A causes regenerative anemias but not the more common and lethal nonregenerative anemias (Mackey *et al.*, 1975).

b. FeLV Erythroblastopenia (Aplastic or Nonregenerative Anemia)

The most common form of FeLV-induced anemia is FeLV erythroblastopenia, also known as pure red cell aplasia, erythroid aplasia, or aplastic anemia. This disease occurs more often than any FeLV-induced neoplasm (Hardy, 1981d). Sixty-eight percent of cats with this type of anemia are FeLV-positive (Hardy, 1981b,d; Cotter *et al.*, 1975). Thus, the cause of aplastic anemias in the other 32% of cats is not FeLV. Alternatively, FeLV may also be responsible for inducing these "FeLV-negative anemias" much like it induces the "FeLV-negative LSA" and the "FeLV-negative neutrophil dysfunction"

syndromes. FeLV aplastic anemia is a progressive and fatal degenerative disease involving only the erythroid cells. Hypoplasia of the bone marrow erythroid elements occurs, and a normocytic and normochromic anemia develops.

Several groups have shown that FeLV-C can induce aplastic anemias in SPF kittens (Hoover *et al.*, 1974; 1976; Onions *et al.*, 1982; Riedel *et al.*, 1986, 1988; Dornsife *et al.*, 1989). However, since all pet cats that develop nonregenerative anemias are infected with FeLV-A and since FeLV-A recombines with enFeLV sequences and other non-enFeLV endogenous cellular sequences to form FeLV-C, it is likely that FeLV-A is responsible for all forms of FeLV-induced anemias that develop in pet cats (Dornsife *et al.*, 1989; Mullins and Hoover, 1990).

Ferrokinetic data show decreased erythropoiesis in cats with FeLV-C-induced aplastic anemias even though serum erythropoietin concentrations are high, 10 to 20 times normal values (Kociba *et al.*, 1983; Wardrop *et al.*, 1986). Bone marrow culture studies of these cats revealed low numbers of BFU-E and CFU-E, but normal numbers of granulocyte-macrophage progenitors remained (Onions *et al.*, 1982; Wardrop *et al.*, 1986). FeLV-C infection apparently impairs the ability of the bone marrow to respond physiologically to the anemia. Intra-bone-marrow inoculation of cells infected with molecularly cloned FeLV-C causes irreversible depletion of BFU-E and induces rapidly fatal aplastic anemias (Dornsife *et al.*, 1989). In another study, bone marrow fibroblast colony-forming units (CFU-F) were decreased in cats experimentally infected with FeLV who developed anemia (Wellman *et al.*, 1988).

c. FeLV Pancytopenia

FeLV pancytopenia is the second most common form of primary FeLV myelodegenerative anemia. All hematopoietic cells—erythroid, myeloid, and megakaryocytes—are involved and there is a normocytic, normochromic nonregenerative anemia, leukopenia, and decreased numbers of platelets. These cats have gone through the erythroblastosis and erythroblastopenia stages of FeLV-induced erythroid changes, and they often have recurrent secondary immunosuppressive diseases due to their low leukocyte counts (Hardy, 1981a). In some cats, FeLV pancytopenia can lead to end stage myelofibrosis and/or FAIDS before death.

d. Pathogenesis of FeLV Anemias

The different subgroups of FeLV cause different types of anemias when inoculated into cats. FeLV-A and FeLV-AB induce transient nonfatal macrocytic, normochromic anemias (erythroblastosis) with extensive splenic extramedullary hematopoiesis (Hoover *et al.*, 1974; Mackey *et al.*, 1975), whereas FeLV-C induces a fatal aplastic or nonregenerative normocytic, normochromic anemia in SPF cats (Hoover *et al.*, 1974, 1976; Onions *et al.*, 1982; Riedel *et al.*, 1986, 1988). FeLV-C has been routinely isolated from pet cats with aplastic anemia, which may indicate that this once thought to be rarely occur-

ring subgroup has clinical significance in nature (Rojko and Hardy, 1989). No anemias developed in kittens inoculated with FeLV-B alone. Thus two subgroups, FeLV-A and FeLV-C, of the three FeLV subgroups have been found to be capable of inducing different types of anemias.

FeLV-C infection destroys two classes of erythroid progenitor cells: colony forming units-erythroid (CFU-E) and burst forming units-erythroid (BFU-E) cells. Ten days after exposure to FeLV-C the BFU-E are infected and begin to disappear from the bone marrow (Onions *et al.*, 1982; Testa *et al.*, 1983). CFU-E inhibition occurs 3 to 6 weeks after exposure and marks the initiation of the fatal anemia (Hoover and Kociba, 1974; Testa *et al.*, 1983; Abkowitz *et al.*, 1987). The FeLV envelope spike transmembrane (TM) p15E protein can suppress lymphocyte functions and also affects BFU-E and CFU-E adversely. The development of BFU-E and CFU-E is inhibited when uninfected cat bone marrow cells are exposed, *in vitro*, to infectious FeLV, to inactivated FeLV, or to the purified TM (p15E) (Wellman *et al.*, 1984; Rojko *et al.*, 1986).

The molecular pathogenesis of aplastic anemia is determined by the two FeLV envelope proteins, gp70 and p15E (Wellman *et al.*, 1984; Rojko *et al.*, 1986; Dornsife *et al.*, 1989). The gp70 external knob glycoproteins are responsible for subgroup FeLV-A, -B, and -C specificities, host ranges, and disease determinants. As was discussed previously, FeLV-A, the contagiously transmitted subgroup, gives rise by recombination with endogenous FeLV-C *env* sequences and additional non-enFeLV sequences to the anemiogenic FeLV-C subgroup, which then induces fatal anemias in some cats.

C. Myeloid Diseases

FeLV replicates in all precursor and differentiated myeloid cells in the bone marrow and can induce proliferative or degenerative diseases of these cells (Fig. 18A) (Table X) (Hardy *et al.*, 1973b; Hardy, 1981a).

1. Myeloid Proliferative (Myeloproliferative) Diseases

FeLV can induce myeloproliferative disease, reticuloendotheliosis, myelogenous (granulocytic—usually neutrophilic) leukemia, megakaryocytic leukemia, myelofibrosis, and osteosclerosis in pet cats (Hardy, 1981d; Herz *et al.*, 1970). These diseases are similar to those induced by acute transforming oncogene-containing retroviruses of chickens but they occur relatively rarely in cats.

The term "myeloproliferative" was first used in 1951 to indicate abnormal proliferation of a variety of bone marrow cells that leads to severe anemia and which often terminates in granulocytic leukemia (Dameshek, 1951). Myeloproliferative diseases (MPD) are a group of primary bone marrow neoplastic disorders that may involve any one or a combination of two or more cell types originating in the bone marrow. The primitive mesenchymal cell of the bone marrow gives rise to erythroblasts, myeloblasts, megakaryoblasts, osteo-

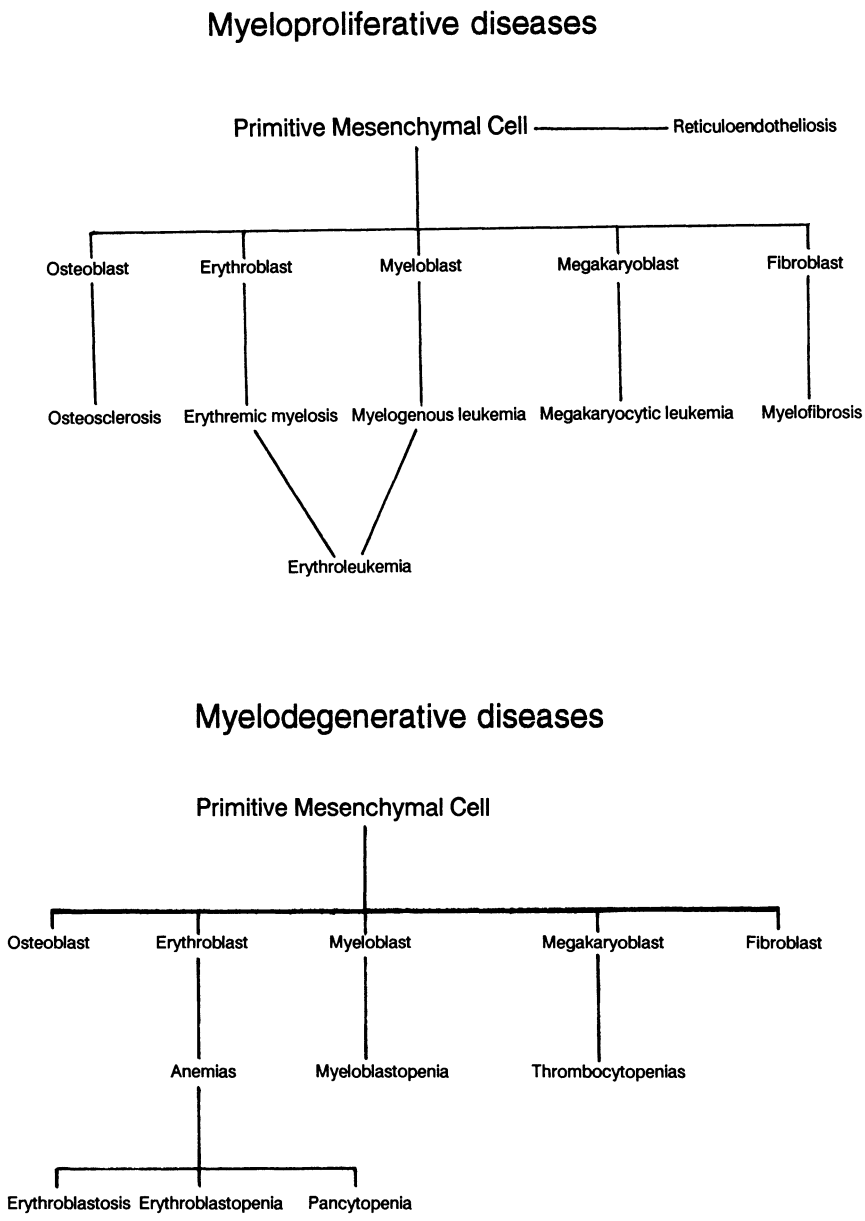

FIGURE 18. FeLV-induced diseases of the bone marrow.

blasts, and fibroblasts (Fig. 18). FeLV can replicate in all of these nucleated cells and can apparently transform all of these cell types, except eosinophils (Hardy *et al.*, 1973b; Hardy, 1981d).

The four stages of MPDs (Table X) in cats are: stage 1, erythremic myelosis is characterized by a marked hyperplasia of erythroid cells of the bone marrow; stage 2, characterized by a mixed erythroid and granulocytic precursor proliferation called erythroleukemia; stage 3, the major proliferative cell is the myeloblast; and stage 4, characterized by the presence of erythroid and granulocytic leukocyte precursor cells in the blood and spleen together with a proliferation of cancellous bone and/or fibrous tissues in the bone marrow resulting in medullary osteosclerosis or myelofibrosis. Stage 4 represents the terminal stage of feline MPD, in which the bone marrow is replaced with cancellous bone and fibrous tissue.

Cats with MPD usually have profound normocytic, normochromic, nonregenerative anemias with either neoplastic erythroid or granulocytic myeloid precursor cells, or a mixture of both types of cells, in the blood and bone marrow (Dameshek, 1951). Extramedullary hematopoiesis in the spleen, liver, and lymph nodes—which if severe enough causes splenomegaly, hepatomegaly, or lymphadenopathy—usually occurs. Platelet numbers are often reduced, resulting in bleeding disorders, and occasionally giant, abnormal platelets are present.

a. Myelogenous (Granulocytic) Leukemias

FeLV can replicate in normal and neoplastic neutrophils and in normal eosinophils (Hardy *et al.*, 1980a). Granulocytic leukocytes (neutrophils and basophils) can be transformed by FeLV, but myelogenous leukemias occur far less commonly in cats than do lymphoid malignancies.

Neutrophilic leukemia occurs rarely in cats. The leukocyte numbers are usually increased in neutrophilic leukemia, and neutrophilic peroxidase-positive myelocytes, progranulocytes, and myeloblasts are present in the blood (Case, 1970; Fraser *et al.*, 1974). Splenomegaly, hepatomegaly, and variable lymphadenopathy also usually occur along with a severe anemia even though large numbers of nucleated erythrocytes are present. Recently the GM1 strain of FeLV was isolated from a naturally occurring case of myeloid leukemia, and this isolate induces severe hematopoietic abnormalities including myeloid leukemia (Tzavaras *et al.*, 1990). The GM1 FeLV is replication defective with an *env* gene phenotype of subgroup B and does not possess an oncogene.

Eosinophilic leukemia is the only MPD of cats not known to be induced by FeLV. The disease occurs very rarely in pet cats and is characterized by an overproduction of eosinophils with immature forms present in the blood and various tissues. However, it is possible that this is an FeLV-induced "virus-negative" form of MPD since the virus replicates in eosinophilic precursors and is found in mature normal eosinophils in the peripheral blood of infected cats.

Megakaryocytic leukemia is also extremely rare in cats (Hardy *et al.*, 1978). The disease is characterized by large numbers of bizarre platelets in the peripheral blood, by increased numbers of megakaryocytes in the bone marrow, liver, and spleen (hepatosplenomegaly), and by a severe anemia.

b. Myelofibrosis

Myelofibrosis represents the end stage of MPD, but most FeLV-infected cats with MPD die before they develop myelofibrosis. Myelofibrosis is characterized by severe anemia, by extensive replacement of the bone marrow with fibrous tissue (fibroblasts) with very few remaining erythroid or myeloid cells in the marrow, and by fibrosis of the liver (Hardy, 1981d; Blue, 1988).

c. Osteochondromatosis and Medullary Osteosclerosis

Osteochondromatosis or multiple cartilaginous exostoses is a benign proliferative disease of bone that occurs in humans, dogs, horses, and cats (Pool and Harris, 1975). The disease appears to have a hereditary basis in humans, dogs, and horses, but not in cats. Multiple osteochondromas are usually

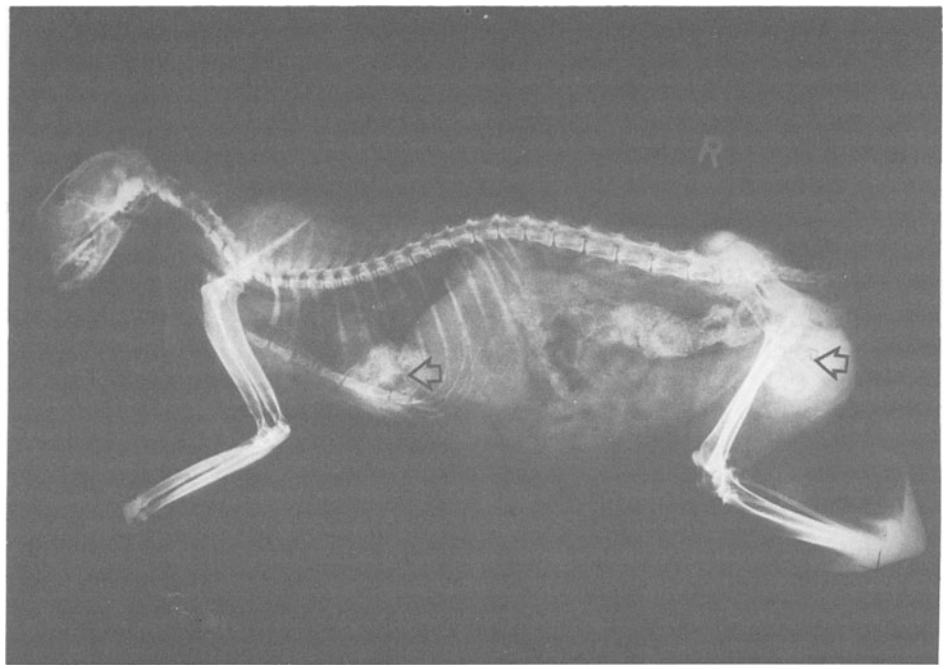

FIGURE 19. Osteochondromatosis, multiple cartilaginous exostoses (arrows) of the sternum, ribs, and pelvis, in an FeLV-infected cat. (Reprinted with permission Churchill and Livingstone [Rojko and Hardy, 1989]).

found both in the metaphyseal regions of long bones and in flat bones such as the scapulae, ribs, pelvis, and vertebrae. The growths usually cease when the growth plates close in young adult people, dogs, and horses.

In cats, osteochondromatosis appears to be different since the disease occurs in mature cats about 2 years old whose growth plates have already closed and affects mainly the flat bones (Fig. 19), the scapulae, pelvis, ribs, and skull, rather than the long bones. In one study, medullary osteosclerosis developed in 12 out of 13 kittens who became anemic after experimental infection with FeLV (Hoover and Kociba, 1974). C-type viral particles were seen in osteocytes, osteoblasts, and megakaryocytes, and FeLV antigens were present in the peripheral blood leukocytes of these cats.

Osteochondromatosis and medullary osteosclerosis may be induced by FeLV stimulation or transformation of periosteal fibroblasts or medullary osteocytes and osteoblasts resulting in excess cartilage, bone, or fibrous tissue proliferation. Medullary osteosclerosis and myelofibrosis represent the final stages of reactive bone marrow cells in FeLV-induced MPD.

2. Myeloid Blastopenic Diseases

Blastopenic myeloid diseases occur more commonly than do myeloproliferative diseases in FeLV-infected cats (Fig. 18B) (Table X).

a. FeLV-Myeloblastopenia-Enteritis Syndrome (MES) (Panleukopenia-like Syndrome)

The FeLV-myeloblastopenia-enteritis syndrome (MES) (panleukopenia-like syndrome) was previously termed FeLV myeloblastopenia syndrome without the notation of enteritis (Hardy, 1980a, 1981a; Reinacher, 1987). However, a syndrome of FeLV-induced enteritis without the concomitant panleukopenia has been described that appears different from this syndrome. Severe panleukopenia and enteritis with dysentery, which can lead to a severe anemia and prostration due to the rapid GI blood loss, characterize MES. There is erosion of the epithelium of the tips of the small intestinal villi that causes bleeding and permits opportunistic infections to enter, resulting in septicemia and death (Hardy, 1980a, 1981a; Hardy and Essex, 1986). Replicating virus and FeLV antigens are present in the intestinal epithelial cells and lymphocytes in the lamina propria (Rojko *et al.*, 1979). This syndrome probably should be classified as part of FeLV-FAIDS.

Although MES resembles panleukopenia (feline distemper, feline parvovirus) it often occurs in FeLV-infected cats that are immune to the panleukopenia virus. FeLV-infected healthy cats that are stressed by such things as cat fights or hospitalization often develop this syndrome 2–3 weeks after the stress. Hypoplasia of the granulocytic leukocytes in the bone marrow causes a severe leukopenia. There is lymphoid depletion, and hemorrhagic necrosis (Fig. 20) occurs in the mesenteric, cecal, colonic, and sublumbar lymph nodes.

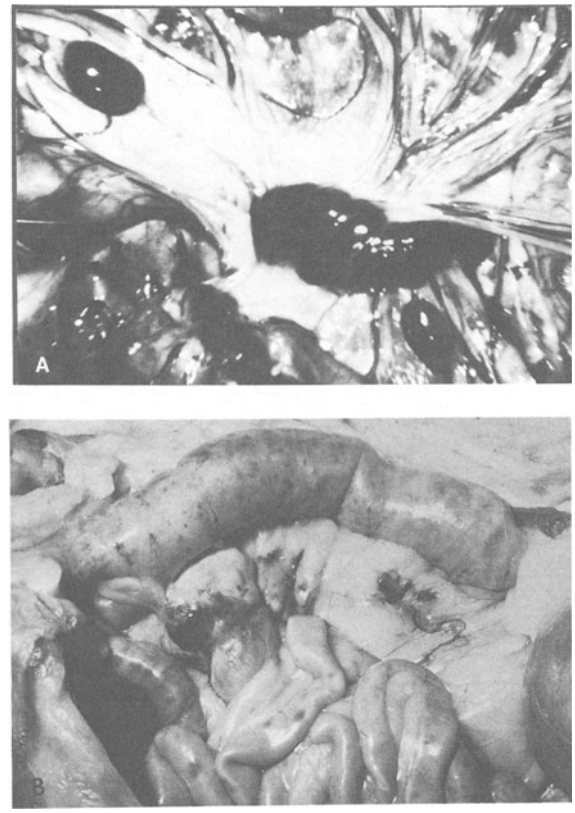

FIGURE 20. FeLV-myeloblastopenia (panleukopenia-like) syndrome. A. Hemorrhagic lymphadenopathy of the mesenteric lymph nodes in an FeLV-infected cat. (Reproduced with permission of Springer-Verlag [Hardy, 1982]). B. Enteritis with petechial hemorrhages of the serosal surface of the large intestine indicating underlying hemorrhagic enteritis in an FeLV-infected cat. (Reprinted with permission Churchill and Livingstone [Rojko and Hardy, 1989]).

3. Megakaryocyte Disease

Megakaryocytes are the first cells in the bone marrow to become infected with FeLV after the virus reaches the marrow (Hardy *et al.*, 1973b). Infected megakaryocytes bud off platelets that contain FeLV antigens, and budding virus has been observed in their membranes. FeLV infection of the platelets is not without consequences. The infected platelets may be too large (macrothrombocytosis), may have an abnormal morphology, may be reduced in numbers (thrombocytopenia), or have decreased function and decreased life spans (Boyce *et al.*, 1986; Jacobs *et al.*, 1986). The mean platelet half-life is reduced in infected cats by about 50%, from 21.5 hours for platelets from uninfected cats to 11.9 hours for FeLV-infected cats. However, bleeding disorders occur only rarely even though many FeLV-infected cats have abnormal platelets.

D. Other FeLV Diseases

1. Abortions and Resorption Syndromes

FeLV has been detected in two-thirds of pet cats with a history of abortions or fetal resorptions (Hardy, 1980a, 1981a). Experimentally, FeLV has

been shown to induce fetal death, resorption, placental involution, and abortion in the middle trimester (Hoover *et al.*, 1983; Hoover, 1984). Infected queens can give birth to live infected kittens even after having aborted previous litters (Hardy, 1981a). Transmission of the virus to fetal or newborn kittens is transplacental and transcolostral (Hardy *et al.*, 1969; Hoover *et al.*, 1983; Hoover, 1984).

Queens with latent FeLV infections will occasionally bear kittens with congenital or perinatal FeLV infections (Rojko *et al.*, 1982; Pacitti *et al.*, 1986). In these latently infected queens focal mammary reactivation of latent FeLV and secretion of infectious virus in the milk occurs (Pacitti *et al.*, 1986).

Although the pathogenesis of FeLV-induced abortions and resorptions in cats is unknown, the pathogenesis of a similar syndrome in mice has been elucidated. Infection of pregnant mice with MuLV leads to fetal death (Jahner and Haenisch, 1985). In these mice the MuLV provirus inserts itself into the alpha 1 collagen gene in early mouse embryos, which prevents the synthesis of collagen. The fetal blood vessels and connective tissues are weakened, which cause the fetuses to hemorrhage fatally. Experimentally, FeLV has also been shown to infect and reduce the viability of hamster fetuses (Chapman *et al.*, 1974). Thus, FeLV may cause feline fetal mortality in cats by a similar mechanism.

2. FeLV Neurologic Syndrome

A neurologic syndrome occurs in FeLV-infected pet cats and is characterized by progressive fore and hind leg weakness leading to eventual paralysis (Hardy, 1980a, 1981a). However, FeLV has not yet been proven to cause this syndrome under experimental conditions. This syndrome is very similar to the neurologic syndrome observed in MuLV-infected wild mice (Gardner *et al.*, 1973). In addition, a similar neurologic syndrome characterized by paresis progressing to paralysis also occurs in humans infected with HTLV-I and has been named HTLV-associated myelopathy (HAM) or tropical spastic paraparesis (TSP) (Osame *et al.*, 1986; Vernant *et al.*, 1987). A neurologic disorder is also associated with HIV-1 infections in humans and is characterized by encephalopathy and dementia (Snider *et al.*, 1983).

3. FeLV Immune Complex Glomerulonephritis

Pet cats persistently infected with FeLV have a lifelong viremia that is ideal for the formation and deposition of immune complexes in glomeruli and the induction of glomerulonephritis (Hardy, 1980a, 1982; Day *et al.*, 1980; Jakowski *et al.*, 1980; Snyder *et al.*, 1982). FeLV replication produces a continuous supply of soluble viral antigens over a long period of time, and these antigens occur in excess of antibody concentrations, thus encouraging the formation of the more nephrotoxic small immune complexes (Hardy *et al.*, 1969, 1976a). The continual formation and circulation of immune complexes is necessary for disease production, and the proportion of antigen and

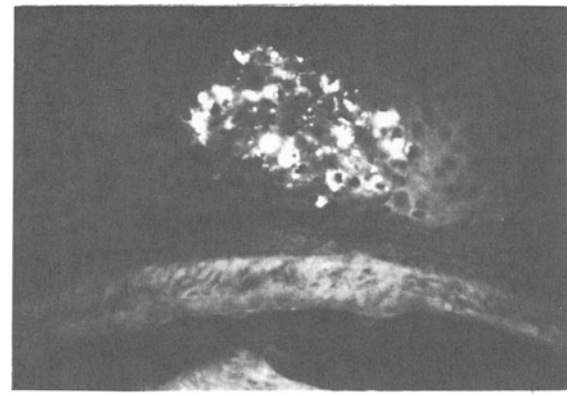

FIGURE 21. FeLV-induced glomerulonephritis. Deposition of IgG, FeLV proteins, and complement in a glomerulus of an FeLV-infected cat. (Reproduced with permission of Springer-Verlag [Hardy, 1982]).

antibody in immune complexes influences their deposition in glomerular capillaries (Fig. 21) (Weksler *et al.*, 1975).

Circulating immune complexes consisting of whole infectious FeLV complexed with cat IgG occur in 50% of FeLV-infected cats (Hardy, 1982; Hardy and Essex, 1986). FeLV-antigens, antibody (IgG), and complement deposited in the glomeruli have been found in 25% of healthy FeLV-infected cats, but none of these cats had glomerulonephritis (Weksler *et al.*, 1975; Glick *et al.*, 1978; Hardy, 1982; Hardy and Essex, 1986). Clinical glomerulonephritis is not a frequent outcome of FeLV infection even though there are many chronically viremic pet cats.

4. Enteritis

FeLV-induced enteritis is a recently described syndrome that appears to be distinct from the previously described FeLV-myeloblastopenia (panleukopenia-like) syndrome (Hardy, 1981a, 1982; Hardy and Essex, 1986; Reinacher, 1987). In the FeLV enteritis syndrome, hemorrhagic vomitus, diarrhea, anemia, and lymphoid hyperplasia are prominent. Cats with FeLV-enteritis have intestinal lesions in the crypts, not the tips of the villi as occurs in the FeLV-myeloblastopenia enteritis syndrome, and they are most often not panleukopenic and do not have hemorrhagic lymphadenopathy. The distinctions between these two syndromes are slight, however, and may only represent different stages of the same disease.

5. Cachexia

Many FeLV-infected cats fail to thrive and become cachectic with as much as 50% to 60% loss in body weight (Rojko and Hardy, 1989). Lesions include severe muscle atrophy, especially in the temporal muscles, and serous atrophy (myxomatous degeneration) of pleural and peritoneal fat stores. Although the pathogenesis of cachexia is not known, anorexia and depression certainly contribute to the malnourished state. Cachexia also occurs in hu-

mans with AIDS and is called "slims disease" in many parts of Africa. Humans with AIDS often have deficiencies of selenium and other trace elements and develop increases in tumor necrosis factor or cachetin (Dworkin *et al.*, 1986). The nutritional status of FeLV-infected cats with cachexia has not been studied.

E. FeSV-Induced Tumors of Pet Cats

Fibrosarcomas account for 6% to 12% of all cat tumors (Hardy, 1980b, 1981c). Most feline fibrosarcomas are single tumors that occur in old cats, average age of 10 years, and are not induced by FeSVs. In contrast, FeSVs induce multicentric fibrosarcomas of young cats, average age of 3 years (Hardy, 1980b, 1981c). FeSV-induced fibrosarcomas are usually poorly differentiated, are more invasive, and grow more rapidly than do the non-FeSV-induced fibrosarcomas (Fig. 3).

A histological difference exists between the FeSV-induced multicentric fibrosarcomas of young cats and the FeSV-negative solitary fibrosarcomas of older cats (Snyder and Theilen, 1969; Gardner *et al.*, 1970; McDonough *et al.*, 1971; Hardy, 1980b). Solitary fibrosarcomas of old cats are usually compact, well-differentiated, slowly invasive tumors that contain considerable amounts of collagen and reticulum. Mitotic activity is usually modest and thus the tumors are slow growing and can reach considerable size. In contrast, the multicentric FeSV-induced fibrosarcomas are less compact (multiple lesions), less well differentiated, more invasive tumors that contain less collagen and reticulum. The fibrosarcoma cells are often invasive into surrounding tissues and grow rapidly. The tumors are usually pleomorphic, containing fusiform, polygonal, and giant fibroblasts with numerous mitotic figures. Frozen sections of these tumors contain FeLV-FeSV antigens in the tumor cell cytoplasm.

As was discussed previously, FeLV transduces a cellular proto-oncogene to form an FeSV *de novo*. Although it is clear that FeLV is spread contagiously among cats, no evidence shows that FeSV is also transmitted contagiously.

VII. PREVENTION AND VACCINE DEVELOPMENT

A. Prevention

1. FeLV Test and Removal Program

The FeLV IFA test and removal program (Fig. 4) has been used successfully in veterinary medicine for almost 20 years (Hardy *et al.*, 1974, 1976b; Weijer and Daams, 1978; Hardy, 1981b) to prevent the spread of the virus in cats living in multiple cat households. In this program all cats in the household are tested for FeLV, and any infected cats are removed or isolated. After the infected cats have been removed, all uninfected animals should be vacci-

nated against FeLV (see next section) and should be quarantined in the household and retested 3 months later. The 3-month retest is needed because the incubation period of FeLV infection can be as long as 3 months. If any of the initially uninfected cats are found to be FeLV positive in the second FeLV test, they should be removed or isolated and a third test is done 3 months later. When all cats test negative in two consecutive tests, done 3 months apart, the cats in the household are free of FeLV infection. Using this program the spread of FeLV has been efficiently halted in numerous households (Hardy *et al.*, 1976b; Weijer and Daams, 1978).

B. Vaccine Development

2. FeLV Vaccines

Since FeLV-A is the only subgroup of FeLV that is transmitted contagiously among cats an FeLV vaccine need only produce protection against this FeLV-A to protect cats from all three FeLV subgroups (see Section IIB, 1d). Several types of FeLV vaccines have been studied (Table XVIII), which include: (1) low-dose live virus vaccine (Hardy, 1981b, 1985; Pedersen *et al.*, 1979), (2) several killed virus vaccines (Jarrett *et al.*, 1974; Olsen *et al.*, 1976; Pedersen *et al.*, 1979), (3) a recombinant FeLV-vaccinia virus vaccine (Gilbert *et al.*, 1987), (4) a gp70 *env* gene recombinant vaccine expressed in *E. coli*, (5) an immunostimulating complex (ISCOM) FeLV vaccine (Osterhaus *et al.*, 1985, 1989a; Akerblom *et al.*, 1989), (6) an anti-idiotype FeLV vaccine (Os-

TABLE XVIII. Types of FeLV Vaccines

Vaccine type	Efficacy and comments
1. Live attenuated FeLV	Too dangerous Induced FeLV-negative lymphosarcoma
2. Inactivated (killed) whole virus	Thought p15E was immunosuppressive Recent killed vaccines effective and safe
3. Subunit-envelope gp70 or gp85 (gp70 & p15E)	
a. Recombinant *env* gp70	Effective and safe
b. Purified gp70 or gp85 from culture	
(1) Adjuvants-gp70	Some did not protect Choice of adjuvant very important
(2) ISCOMS-gp85	Very effective
c. FeLV cell culture supernatant-inactivated	First commercial vaccine approved Numerous disputes as to efficacy Some studies showed no protection Contains: cell proteins, whole FeLV, all FeLV proteins, and FOCMA
4. Live recombinant virus vaccine	First vaccine did not protect
a. Vaccinia virus	
b. Herpes virus	None tested yet

terhaus *et al.*, 1989b), and a recombinant feline herpesvirus expressing FeLV envelope and internal core *gag* proteins (Cole *et al.*, 1990).

One live virus vaccine produced extremely high VN antibody titers after transient viremia followed by rejection of the virus in nine cats. However, one of the nine vaccinated cats developed a virus-negative LSA several years after vaccination (Hardy, 1981b). One of the killed FeLV vaccines enhanced FeLV infection rather than protecting cats from infection while the other was marketed but withdrawn after only several months. The recombinant FeLV-vaccinia vaccine was not immunogenic in cats (Olsen *et al.*, 1976; Gilbert *et al.*, 1987). The recombinant feline herpesviruses vaccine expressing FeLV *env* and *gag* proteins was able to express these proteins in cells in a form similar to that found in native FeLV (Cole *et al.*, 1990). Future studies of this expression system and vaccine trials in cats are warranted.

The first retrovirus vaccine employed in any animal was developed for protection of cats against FeLV infection and is a "subunit" vaccine (Olsen *et al.*, 1976, 1980). By manipulating the growth conditions of FL74 LSA cells, which produce all three subgroups of FeLV, -A, -B, and -C, the viral antigens and FOCMA are solubilized and released into the supernatant fluids of the culture medium (Mastro *et al.*, 1986). The vaccine preparation is inactivated to ensure that there is no infectious FeLV in the product. Clinical trials of the efficacy of the vaccine, performed by the manufacturer, demonstrated an 80% protection against virus challenge. Another study showed that vaccinated cats were three times more resistant to contact infection than were placebo-inoculated controls (Pollock and Scarlett, 1990). However, other studies of the immunogenicity and efficacy of this vaccine reported that there was little or no protection (Pedersen and Ott, 1985; Legendre *et al.*, 1990). Only 50% of vaccinated cats developed good antibody to FeLV gp70-related antigens. The response to virus challenge was disappointing in one study (Pedersen and Ott, 1985) and in another no protection was observed (Legendre *et al.*, 1990). Recently a 2-dose regime vaccine has replaced this 3-dose vaccine (Haffer *et al.*, 1990). Evaluation of this new vaccine by the manufacturer showed that 72% of the vaccinated cats versus 40% of the unvaccinated cats were protected from persistent viremia by challenge virus. In addition to the 28% that became persistently viremic, another 36% of the vaccinates became transiently viremic. Only 36% of the vaccinates were completely protected from transient or persistent viremia.

Recently an FeLV-A gp70 *env* gene recombinant vaccine expressed in *E. coli* has been marketed in the United States. Preliminary vaccine efficacy trials have shown this vaccine to be safe and effective (Kensil *et al.*, 1991; Clark, *et al.*, 1991). The vaccinated cats produced neutralizing antibody to FeLV-A and were protected from challenge with FeLV-A, FeLV-B, and FeLV-C. Thus, his vaccine appears to be the preferred immunogen.

Immunostimulating complexes (iscoms) have been prepared from FeLV envelope proteins, the transmembrane p15E, and external glycoprotein gp70 (Akerblom *et al.*, 1989; Osterhaus *et al.*, 1985, 1989a). In FeLV, the gp70 is disulfide-linked to the protein p15E, which contains a hydrophobic region that anchors it in the virion lipid bilayer (Bolognesi *et al.*, 1978). The resulting

gp85 should be an excellent immunogen for an envelope subunit FeLV vaccine (Osterhaus *et al.*, 1985; Hammar *et al.*, 1989). To present the gp85 molecule in the proper antigenic configuration it should be anchored in a carrier structure. Such a carrier structure is the immunostimulating complex, or ISCOM. The matrix of the iscom is the glycoside Quil A, which in micelle form has regions accessible for hydrophobic interaction with membrane proteins and thus complexes are formed (Osterhaus *et al.*, 1985). FeLV-gp85 iscom vaccine produced an immune response to the gp70 molecule in mice and cats and an immune response that protected cats from challenge with FeLV (Osterhaus *et al.*, 1985). ISCOMS containing FeLV envelope components may be a safe and effective vaccine of the future.

FeLV vaccines will not reverse FeLV infection in a viremic cat. It is recommended that all cats that are to be vaccinated be tested for FeLV at the time of the first dose of vaccine and those found to be infected should be removed from the household or strictly isolated away from uninfected cats. Only FeLV-uninfected cats should be vaccinated since vaccination of viremic cats who have not produced antibodies against FeLV has not been of any benefit. Employing the FeLV test and removal program along with vaccination should significantly reduce the spread of FeLV among pet cats.

VIII. CONCLUSIONS

Ever since the discovery of FeLV in 1964 in Scotland much of the general biology of retroviruses has been learned by studying this virus. FeLV was the first retrovirus that was shown to be transmitted contagiously (horizontally) in nature. Until that time all retroviruses were thought to be transmitted by hereditary routes. The first practical immunodetection test for routine detection of any animal retrovirus infection, the immunofluorescent antibody test, was developed for detection of FeLV in cat blood cells. The discovery of FeLV disease was a forerunner of similar diseases in other species and included anemias, immunodeficiency syndromes, virus-negative lymphoid tumors, myeloproliferative diseases, and multiple fibrosarcomas. The FeLV-induced feline immunodeficiency syndrome was especially important in this regard as it stimulated those working on the etiology of human AIDS in the early 1980s to search for a retrovirus as its cause.

A molecular understanding of the recombinational events between a contagious exogenous FeLV-A subgroup and endogenous FeLV-related sequences to form highly pathogenic FeLV is important conceptually for all retrovirus-induced diseases. FeLV continues to capture oncogenes in pet cats as feline sarcoma viruses that help in our understanding of the mechanisms of transformation and normal cell growth and differentiation. Finally, the first retrovirus vaccine was developed against FeLV in 1986, which gives reason for optimism for similar vaccines against HIV-I, HIV-II, HTLV-I, and HTLV-II of humans.

Although much has been learned about FeLV much still remains to be discovered. Do the pathogenic recombinant transforming FeLVs occur natu-

rally in pet cats as has been found in experimentally infected cats? What are the mechanisms of FeLV transformation of lymphoid and myeloid cells? Exactly what is the mechanism of the severe immune deficiency induced by FeLV? How does FeLV induce FeLV-negative lymphosarcomas? Can FeLV be successfully eliminated from the pet cat population through the FeLV test and removal program and vaccination? Finally, FeLV offers an excellent system in which to develop antiretroviral compounds. It has already contributed in this area when it was shown that AZT was effective *in vitro* and *in vivo* to greatly reduce FeLV replication (Hardy *et al.*, 1985). This finding helped speed up the testing of AZT in humans and accelerated the drugs eventual approval as the first antiretroviral therapy.

ACKNOWLEDGMENTS. The author thanks E. E. Zuckerman for her excellent and devoted technical help during the past 18 years, which enabled the collection of much of the data cited in this chapter. I also thank my colleagues and friends L. J. Old, M. Essex, R. Gallo, F. Wong-Staal, H. Snyder, Jr., G. MacEwen, P. Hess, H. Nauts, J. Wolfe, E. Hoover, J. Rojko, O. Jarrett, F. deNoronha, D. Armstrong, J. Gold, J. Whitehead, G. Robinson, and the many veterinarians for their help. I also thank the National Institutes of Health, Leukemia Society of America, Cancer Research Institute, New York State Department of Health, and the Society of Memorial Sloan Kettering Cancer for support of our research.

IX. REFERENCES

Abelson, H. T., and Rabstein, L. S., 1970, Lymphosarcoma, virus-induced thymic independent disease in mice, *Cancer Res.* **30**:2213.

Abkowitz, J. L., Holly, R. D., and Adamson, J. W., 1987, Retrovirus-induced feline pure red cell aplasia: The kinetics of marrow failure, *J. Cell Physiol.* **132**:571.

Akerblom, L., Stromstedt, K., Hoglund, S., Osterhaus, A., and Morein, B., 1989, Formation and characterization of FeLV ISCOMS, *Vaccine* **7**:142.

Aldrich, C. D., and Pedersen, N. C. 1974, Persistent viremia after regression of primary virus-induced feline fibrosarcomas, *Am. J. Vet. Res.* **35**:1383.

Anderson, L. J., Jarrett, W. F. H., Jarrett, O., and Laird, H. M., 1971, Feline leukemia-virus infection of kittens: Mortality associated with atrophy of the thymus and lymphoid depletion, *J. Natl. Cancer Inst.* **47**:807.

Anderson, S. J., Furth, M., Wolff, L., Ruscetti, and S. K., and Sherr, C. J., 1982, Monoclonal antibodies to the transformation specific glycoprotein encoded by the feline retroviral oncogene v-*fms*. *J. Virol.* **44**:696.

Anderson, D. M., Lyman, S. D., Baird, A., Wignall, J. M., Eisenman, J., Rauch, C., March, C. J., Boswell, H. S., Gimpel, S. D., Cosman, D., and Williams, D. E., 1990, Molecular cloning of mast cell growth factor, a hematopoietin that is active in both membrane bound and soluble forms. *Cell* **63**:235.

Aziz, D. C., Hanna, Z., and Jolicoeur, P., 1989, Severe immunodeficiency disease induced by a defective murine leuaemia virus, *Nature* **338**:505.

Baluda, M. A., and Roy-Burman, P., 1973, Partial characterization of RD-114 virus by DNA-RNA hybridization studies, *Nature* **244**:59.

Barbacid, M., Lauver, A. V., and Devare, S. G., 1980, Biochemical and immunological characterization of polyproteins coded for by the McDonough, Gardner-Arnstein, and Snyder-Theilen strains of feline sarcoma virus, *J. Virol.* **33**:196.

Benveniste, R. E., and Todaro, G. J., 1973, Homology between type-G viruses of various species as determined by molecular hybridization, *Proc. Natl. Acad. Sci. USA* **70**:3316.

Benveniste, R. E., Sherr, C. J., and Todaro, G. J., 1975, Evolution of type C viral genes: Origin of feline leukemia virus, *Science* **190**:886.

Bergold, P. J., Wang, J. Y. J., Hardy, W. D., Jr., Littau, V., Johnson, E., and Besmer, P., 1987, Structure and origins of the HZ2-feline sarcoma virus, *Virology* **158**:320.

Besmer, P., 1983, Acute transforming feline retroviruses, *Curr. Top. Microbiol. Immunol.* **107**:1.

Besmer, P., Snyder, H. W., Jr., Murphy, J. E., Hardy, W. D., Jr., and Parodi, A., 1983a, The Parodi-Irgens feline sarcoma virus and simian sarcoma virus have homologous oncogenes, but in different contexts of the viral genomes, *J. Virol.* **46**:606.

Besmer, P., Hardy, W. D., Jr., Zuckerman, E. E., Bergold, P., Lederman, L., and Snyder, H. W., Jr., 1983b, The Hardy-Zuckerman 2-FeSV, a new feline retrovirus with oncogene homology to Abelson-MuLV, *Nature* **303**:825.

Besmer, P., Lader, E., George, P., Bergold, P. J., Qui, F-H., Zuckerman, E. E., and Hardy, W. D., Jr., 1986a, A new acute transforming feline retrovirus with *fms* homology specifies a c-terminally truncated version of the *c-fms* protein that is different from SM-feline sarcoma virus *v-fms* protein, *J. Virol.* **60**:194.

Besmer, P., Murphy, J. E., George, P. C., Qui, F. H., Bergold, P. J., Snyder, H. W., Jr., Brodeur, D., Zuckerman, E. E., and Hardy, W. D., Jr., 1986b, A new acute transforming feline retrovirus and relationship of its oncogene *kit* with the protein kinase gene family, *Nature* **320**:415.

Bishop, J. M., 1981, Enemies within: The genesis of retrovirus oncogenes, *Cell* **23**:5.

Blue, J. T., 1988, Myelofibrosis in cats with myelodysplastic syndrome and acute myelogenous leukemia, *Vet. Pathol.* **25**:154.

Bolognesi, D. P., Montelaro, R. C., Frank, H., and Schafer, W., 1978, Assembly of type C oncornaviruses: A model, *Science* **199**:183.

Bonham, L., Lobelle-Rich, P. A., Henderson, L. A., and Levy, L. S., 1987, Transforming potential of a *myc*-containing variant of feline leukemia virus in vitro in early-passage feline cells, *J. Virol.* **61**:3072.

Bonner, T. I., and Todaro, G. J., 1979, Carnivores have sequences in their cellular DNA distantly related to the primate endogenous virus, *Virology* **94**:224.

Boyce, J. T., Kociba, G. J., Jacobs, R. M., and Weiser, M. G., 1986, Feline leukemia virus-induced thrombocytopenia and macrothrombocytosis in cats, *Vet. Pathol.* **23**:16.

Brodey, R. S., McDonough, S., Frye, F. L., and Hardy, W. D., Jr., 1970, Epidemiology of feline leukemia, in: *Comparative Leukemia Research* (R. M. Dutcher ed.), pp. 333–342, Karger, Basel.

Case, M. T., 1970, A case of myelogenous leukemia in the cat, *Zentralbl Veterinarmed* **17**:273.

Casey, J. W., Roach, A., Mullins, J. T., Burck, K. B., Nicolson, M. O., Gardner, M. B., and Davidson, N., 1981, The U3 portion of the feline leukemia virus identifies horizontally acquired proviruses in leukemic cats, *Proc. Natl. Acad. Sci. USA* **78**:7778.

Chapman, A. L., Weitlauf, H. M., and Bopp, W., 1974, Effect of feline leukemia virus on transferred hamster fetuses, *J. Natl. Cancer Inst.* **52**:583.

Chattopadhyay, S. K., Cloyd, M. W., Linemeyer, D. L., Lander, M. R., Rands, E., and Lowy, D. R., 1982, Cellular origin and role of mink cell focus-forming viruses in murine thymic lymphomas, *Nature* **295**:25.

Clark, N., Kushner, N. N., Barrett, C., Kensil, C. R., Salsbury, D., and Cotter, S., 1991, Efficacy and safety field trials of a recombinant DNA vaccine against feline leukemia virus infection, *J. Am. Vet. Med. Assoc.* **199**:1433.

Cockerell, G. L., and Hoover, E. A., 1977, Inhibition of normal lymphocyte mitogenic reactivity by serum from feline leukemia virus-infected cats, *Cancer Res.* **37**:3985.

Cockerell, G. L., Hoover, E. A., Krakowka, S., Olsen, R. G., and Yohn, D. S., 1976, Lymphocyte mitogen reactivity and enumeration of circulating B- and T-cells during feline leukemia virus infection in the cat, *J. Natl. Cancer Inst.* **57**:1095.

Cole, G. E., Stacy-Phipps, S., and Nunberg, J. H., 1990, Recombinant feline herpesviruses expressing feline leukemia virus envelope and *gag* proteins, *J. Virol.* **64**:4930.

Cotter, S. M., Hardy, W. D., Jr., and Essex, M., 1975, The association of the feline leukemia virus with lymphosarcoma and other disorders, *J. Am. Vet. Med. Assoc.* **166**:449.

Crighton, G. W., 1969, Lymphosarcoma in the cat, *Vet. Rec.* **84**:329.

Dameshek, W., 1951, Some speculations on the myeloproliferative syndromes, *Blood* **6**:372.

Day, N. K., O'Reilly-Felice, C., Hardy, W. D., Jr., Good, R. A., and Witken, S. S., 1980, Circulating immune complexes associated with naturally occurring lymphosarcoma in pet cats, *J. Immunol.* **126**:2363.

Deinhardt, F., Wolfe, L. G., Theilen, G. H., and Snyder, S. P., 1970, ST-feline fibrosarcoma virus: Induction of tumors in marmoset monkeys, *Science* **167**:881.

Dezzutti, C. S., Lafrado, L. J., Lewis, M. G., and Olsen, R. G., 1990, Inhibition of phorbol ester-induced neutrophil chemiluminescence by FeLV, *Arch. Virol.* **111**:75.

Donner, L., Turek, L. P., Ruscetti, S. K., Fedele, L. A., and Sherr, C. J., 1980, Transformation defective mutants of feline sarcoma virus which express a product of the viral *src* gene, *J. Virol.* **35**:129.

Doolittle, R. F., Hunkapiller, M. W., Hood, L. E., Devare, S. G., Robbins, K. C., Aaronson, S. A., and Antoniades, H. N., 1983, Simian sarcoma virus *onc* gene, *v-sis*, is derived from the gene (or genes) encoding a platelet-derived growth factor, *Science* **221**:275.

Dorn, C. R., Taylor, D. O. N., Schneider, R., Hibbard, H. H., and Klauber, M. R., 1968, Survey of animal neoplasms in Alameda and Contra Costa Counties, California. II: Cancer morbidity in dogs and cats from Alameda County, *J. Natl. Cancer Inst.* **40**:307.

Dornsife, R. E., Gasper, P. W., Mullins, J. I., and Hoover, E. A., 1989, Induction of aplastic anemia by intra-bone marrow inoculation of a molecularly cloned feline retrovirus, 1989, *Leuk. Res.* **13**:745.

Dworkin, B. M., Rosenthal, W. S., Wormser, G. P., and Weiss, L., 1986, Selenium deficiency in the acquired immunodeficiency syndrome, *J. Parenter. Nutr.* **10**:405.

Elder, J. H., and Mullins, J. T., 1983, Nucleotide sequence of the envelope gene of Gardner-Arnstein feline leukemia virus B reveals unique sequence homologies with a murine mink cell focus-forming virus, *J. Virol.* **46**:871.

Elder, J. H., Gautsch, J. W., Jensen, F. C., Lerner, R. A., Hartley, J. W., and Rowe, W. P., 1977, Biochemical evidence that MCF murine leukemia viruses are envelope (*env*) gene recombinants, *Proc. Natl. Acad. Sci. USA* **74**:4676.

Essex, M., 1980, Feline leukemia and sarcoma viruses, in: *Viral Oncology* (G. Klein, ed.), pp. 205–229, Raven Press, New York.

Essex, M., Klein, G., Snyder, S. P., and Harrold, J. B., 1971, Correlation between humoral antibody and regression of tumours induced by feline sarcoma virus, *Nature* **233**:195.

Essex, M., Sliski, A., Cotter, S. M., Jakowski, R. M., and Hardy, W. D., Jr., 1975, Immunosurveillance of naturally occurring feline leukemia, *Science* **190**:790.

Essex, M., Cotter, S. M., Sliski, A. H., Hardy, W. D., Jr., Stephenson, J. R., Aaronson, S. A., and Jarrett, O., 1977a, Horizontal transmission of feline leukemia virus under natural conditions in a feline leukemia cluster household, *Int. J. Cancer*, **19**:90.

Essex, M., Cotter, S. M., Stephenson, J. R., Aaronson, S. A., and Hardy, W. D., Jr., 1977b, Leukemia, lymphoma and fibrosarcoma of cats as models for similar diseases of man, in: *Origins of Human Cancer* (H. W. Hiatt, J. D. Watson, and J. A. Winston eds.), pp. 1197–1214, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Fischinger, P. G., Pebbles, P. T., Nomura, S., and Haapala, D. K., 1973, Isolation of RD-114-like oncornavirus from cat cell lines, *J. Virol.* **11**:978.

Flanagan, J. G., and Leder, P., 1990, The *kit* ligand: A cell surface molecule altered in steel mutant fibroblasts, *Cell* **63**:185.

Forrest, D., Onions, D., Lees, G., and Neil, J. C., 1987, Altered structure and expression of *c-myc* in feline T-cell tumors, *Virology* **158**:194.

Francis, D. P., Essex, M., and Hardy, W. D., Jr., 1977, Excretion of feline leukemia virus by naturally infected pet cats, *Nature* **269**:252.

Francis, D. P., Cotter, S. M., Hardy, W. D., Jr., and Essex, M., 1979, Comparison of virus-positive and virus-negative cases of feline leukemia and lymphoma, *Cancer Res.* **39**:3866.

Frankel, A. E., Gilbert, J. H., Porzig, F. J., Scolnick, E. M., and Aaronson, S. A., 1979, Nature and distribution of feline sarcoma virus nucleotide sequences, *J. Virol.* **30**:821.

Fraser, C. J., Joiner, G. N., Jardine, J. H., and Gleiser, C. A., 1974, Acute granulocytic leukemia in cats, *J. Am. Vet. Med. Assoc.* **165**:355.

Fujinami, A., and Inamoto, K., 1914, Ueber geschwulste bei japanischen haushuhnern insbesondere uber einen transplantablen tumor, *Z. Krebsforsch* **14**:94.

Gallo, R. C., Salahuddin, S. Z., Popovic, M., Shearer, G. M., Kaplan, M., Haynes, B. F., Palker, T. J., Redfield, R., Oleske, J., Safai, B., White, G., Foster, P., and Markham, P. D., 1984, Frequent detection and isolation of cytopathic retroviruses (HTLV-III) from patients with AIDS and at risk for AIDS, *Science* **224**:500.

Gardner, M. B., Arnstein, P., Rongey, R. W., Estes, J. D., Sarma, P. S., Rickard, C. F., and Huebner, R. J., 1970, Experimental transmission of feline fibrosarcoma to cats and dogs, *Nature* **226**:807.

Gardner, M. B., Arnstein, P., Johnson, E., Rongey, R. W., and Charman, H. P., 1971a, Feline sarcoma virus tumor induction in cats and dogs, *J. Am. Vet. Med. Assoc.* **158**:1046.

Gardner, M. B., Rongey, R. W., Johnson, F. V., De Journett, R., and Huebner, R. J., 1971b, C-type virus particles in salivary tissue of domestic cats, *J. Natl. Cancer Inst.* **47**:561.

Gardner, M. B., Henderson, B. E., Officer, J. E., Rongey, R. W., Parker, J. C., Oliver, C., Estes, J. D., and Huebner, R. J., 1973, A spontaneous lower motor neuron disease apparently caused by indigenous type C RNA virus in wild mice, *J. Natl. Cancer Inst.* **51**:1243.

Gardner, M. B., Brown, J. C., Charman, H. P., Stephenson, J. R., Rongey, R. W., Hauser, D. E., Diegmann, F., Howard, E., Dworsky, R., Gilden, R. V., and Huebner, R. J., 1977, FeLV epidemiology in Los Angeles cats: Appraisal of detection methods, *Int. J. Cancer* **19**:581.

Gilbert, J. H., Pedersen, N. C., and Nunberg, J. H., 1987, Feline leukemia virus envelope protein expression encoded by a recombinant vaccinia virus: Apparent lack of immunogenicity in vaccinated animals, *Virus Res.* **7**:49.

Glick, A. D., Horn, R. G., and Holscher, M., 1978, Characterization of feline glomerulonephritis associated with viral-induced hematopoietic neoplasms, *Am. J. Pathol.* **92**:321.

Goff, S. P., and Baltimore, D., 1982, The cellular oncogene of the Abelson murine leukemia virus genome, in: *Advances in Viral Oncology* (G. Klein, ed.), pp. 127–139, Raven Press, New York.

Gottlieb, M., Schroff, R., Schanker, H. M., Weisman, J. D., Fan, P. T., Wolf, R. A., and Saxon, A., 1981, Pneumocystis carinii pneumonia and mucosal candidiasis in previously healthy homosexual men, *N. Engl. J. Med.* **305**:1425.

Grundwald, D. J., Dale, B., Dudley, J., Lamph, W., Sugden, B., Ozanne, B., and Risser, R., 1982, Loss of viral gene expression and retention of tumorigenicity by Abelson lymphoma cells. *J. Virol.* **43**:92.

Gwalter, R. H., 1981, FeLV-testmethoden, IFA order ELISA, *Kleintier Praxis* **26**:23.

Haffer, K. N., Koertje, W. D., Derr, J. T. and Beckenhauer, W. H., 1990, Evaluation of immunosuppressive effect and efficacy of an improved-potency feline leukemia vaccine, *Vaccine* **8**:12.

Hammar, L., Eriksson, S., Malm, K., and Morein, B., 1989, Concentration and purification of feline leukaemia virus (FeLV) and its outer envelope protein gp70 by aqueous two-phase systems, *J. Virol. Methods* **24**:91.

Handsfield, H. H., 1985, Screening for HTLV-III antibody, *N. Engl. J. Med.* **313**:888.

Hardy, W. D., Jr., 1980a, Feline leukemia virus disease, in: *Feline Leukemia Virus* (W. D. Hardy, Jr., M. Essex, and A. J. McClelland, eds.), pp. 3–31, Elsevier, New York.

Hardy, W. D., Jr., 1980b, The biology and virology of the feline sarcoma viruses, in: *Feline Leukemia Virus* (W. D. Hardy, Jr., M. Essex, and A. J. McClelland eds.), pp. 79–118, Elsevier, New York.

Hardy, W. D., Jr., 1981a, Feline leukemia virus non-neoplastic diseases, *J. Am. Anim. Hosp. Assoc.* **17**:941.

Hardy, W. D., Jr., 1981b, The feline leukemia virus, *J. Am. Anim. Hosp. Assoc.* **17**:951.

Hardy, W. D., Jr., 1981c, The feline sarcoma viruses, *J. Am. Anim. Hosp. Assoc.* **17**:981.

Hardy, W. D., Jr., 1981d, Hematopoietic tumors of cats, *J. Am. Anim. Hosp. Assoc.* **17**:921.

Hardy, W. D., Jr., 1982, Immunopathology induced by the feline leukemia virus, in: *Springer Semin. Immunopathol* (G. Klein, ed.), pp. 75–105, Springer-Verlag, New York.

Hardy, W. D., Jr., 1983a, Naturally occurring retroviruses (RNA tumor viruses). I: *Cancer Invest.* **1**:67.

Hardy, W. D., Jr., 1983b, Naturally occurring retroviruses (RNA tumor viruses). II: *Cancer Invest.* **1**:163.

Hardy, W. D., Jr., 1984, Feline leukemia virus as an animal retrovirus model for the human T-cell leukemia virus, in: *Human T-Cell Leukemia/Lymphoma Viruses* (R. C. Gallo, M. Essex, and L. Gross, eds.), pp. 35–43, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Hardy, W. D., Jr., 1985, Feline retroviruses, in: *Advances in Viral Oncology*, Vol. 5. (G. Klein, ed.), pp. 1–34, Raven Press, New York.

Hardy, W. D., Jr., 1988, Feline T-lymphotropic lentivirus: Retrovirus-induced immunosuppression in cats, *J. Am. Anim. Hosp. Assoc.* **24**:241.

Hardy, W. D., Jr., 1990, Biology of feline retroviruses, in: *Retrovirus Biology and Human Disease* (R. C. Gallo and F. Wong-Staal, eds.), pp. 33–86, Marcel Dekker, New York.

Hardy, W. D., Jr., 1991, General principles of retrovirus immunodetection tests, *J. Am. Vet. Med. Assoc.* **199**:1282.

Hardy, W. D., Jr., and Essex, M., 1986, FeLV-Induced feline acquired immune deficiency syndrome (FAIDS): A model for human AIDS, in: *Acquired Immune Deficiency Syndrome, Progress in Allergy* 37 (E. Klein, ed.), pp. 353–376, Karger, Basel.

Hardy, W. D., Jr., and Zuckerman, E. E., 1991a, Development of the immunofluorescent antibody test for detection of feline leukemia virus infection in cats, *J. Am. Vet. Med. Assoc.* **199**:1327.

Hardy, W. D., Jr., and Zuckerman, E. E., 1991b, Ten-year study comparing enzyme-linked immunosorbent assay with the immunofluorescent antibody test for detection of feline leukemia virus infection in cats, *J. Am. Vet. Med. Assoc.* **199**:1365.

Hardy, W. D., Jr., Geering, G., Old, L. J., deHarven, E., Brodey, R. S., and McDonough, S., 1969, Feline leukemia virus; occurrence of viral antigen in the tissues of cats with lymphosarcoma and other diseases, *Science* **166**:1019.

Hardy, W. D., Jr., Old, L. J., Hess, P. W., Essex, M., and Cotter, S., 1973a, Horizontal transmission of feline leukaemia virus, *Nature* **244**:266.

Hardy, W. D., Jr., Hirshaut, Y., and Hess, P., 1973b, Detection of the feline leukemia virus and other mammalian oncornaviruses by immunofluorescence, in: *Unifying Concepts of Leukemia* (R. M. Dutcher and L. Chieco-Bianchi, eds.), pp. 778–799, Karger, Basel.

Hardy, W. D., Jr., McClelland, A. J., Hess, P. W., and MacEwen, G., 1974, Veterinarians and the control of feline leukemia virus, *J. Am. Anim. Hosp. Assoc.* **10**:367.

Hardy, W. D., Jr., Hess, P. W., MacEwen, E. G., McClelland, A. J., Zuckerman, E. E., Essex, M., Cotter, S. M., and Jarrett, O., 1976a, Biology of feline leukemia virus in the natural environment, *Cancer Res.* **36**:582.

Hardy, W. D., Jr., McClelland, A. J., Zuckerman, E. E., Hess, P. W., Essex, M., Cotter, S. M., MacEwen, E. G., and Hayes, A. A., 1976b, Prevention of the contagious spread of feline leukaemia virus and the development of leukaemia in pet cats, *Nature* **263**:326.

Hardy, W. D., Jr., Zuckerman, E. E., MacEwen, E. G., Hayes, A. A., and Essex, M., 1977, A feline leukaemia virus- and sarcoma virus-induced tumour-specific antigen, *Nature* **270**:249.

Hardy, W. D., Jr., Zuckerman, E. E., Essex, M., MacEwen, E. G., and Hayes, A. A., 1978, Feline oncornavirus-associated cell-membrane antigen: An FeLV- and FeSV-induced tumor-specific antigen, in: *Differentiation of Normal and Neoplastic Hematopoietic Cells* (B. Clarkson, P. A. Marks, and J. E. Till, eds.), pp. 601–623, Cold Spring Laboratory, Cold Spring Harbor, NY.

Hardy, W. D., Jr., McClelland, A. J., Zuckerman, E. E., Snyder, H. W., Jr., MacEwen, E. G., Francis, D., and Essex, M., 1980a, Development of virus non-producer lymphosarcomas in pet cats exposed to FeLV, *Nature* **288**:90.

Hardy, W. D., Jr., McClelland, A. J., Zuckerman, E. E., Snyder, H. W., MacEwen, E. G., Francis, D. P., and Essex, M., 1980b, Immunology and epidemiology of feline leukemia virus nonproducer lymphosarcomas, in: *Viruses in Naturally Occurring Cancers* (M. Essex, G. Todaro, and H. ZurHausen, eds.), pp. 677–697, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Hardy, W. D., Jr., MacEwen, E. G., Hayes, A. A., and Zuckerman, E. E., 1980c, FOCMA antibody as specific immunotherapy for lymphosarcoma of pet cats, in: *Feline Leukemia Virus* (W. D. Hardy, Jr., M. Essex, and A. J. McClelland, eds.), pp. 227–233, Elsevier, New York.

Hardy, W. D., Jr., Zuckerman, E., Markovich, R., Besmer, P., and Snyder, H. W., 1982, Isolation of feline sarcoma viruses from pet cats with multicentric fibrosarcomas, in: *Advances in Comparative Leukemia Research 1981* (D. S. Yohn and J. R. Blakeslee, eds.), pp. 205–206, Elsevier, North-Holland, New York.

Hardy, W. D., Jr., Zuckerman, E. E., Lehrman, S. N., and Barry, D. W., 1985, Anti-viral effects of BW A509U (AZT) against a naturally occurring feline acquired immunodeficiency syndrome (FAIDS)-inducing retrovirus, in: *Abstracts of the Twenty-fifth Antimicrobial Agents and Chemotherapy*, p. 171.

Hartley, J. W., Wolford, N. K., Old, L. J., and Rowe, W. P., 1977, A new class of murine leukemia virus associated with development of spontaneous lymphomas, *Proc. Natl. Acad. Sci. USA* **74**:789.

Herz, A., Theilen, G. H., and Schalm, O. W., 1970, C-type virus in bone marrow cells of cats with myeloproliferative disorders, *J. Natl. Cancer Inst.* **44**:339.

Hoover, E. A., 1984, Experimental studies of the pathogenesis of feline leukemia virus infection, in: *RNA Tumor Viruses, Oncogenes, Human Cancer and AIDS: On the Frontiers of Understanding* (P. Furmanski, J. C. Hager, and M. A. Rich, eds.), pp. 267–288, Martinus Nijhoff, Boston.

Hoover, E. A., and Kociba, G. J., 1974, Bone lesions in cats with anemia induced by feline leukemia virus, *J. Natl. Cancer Inst.* **53**:1277.

Hoover, E. A., Perryman, L. E., and Kociba, G. J., 1973, Early lesions in cats inoculated with feline leukemia virus, *Cancer Res.* **33**:145.

Hoover, E. A., Kociba, G. J., Hardy, W. D., Jr., and Yohn, D. S., 1974, Erythroid hypoplasis in cats inoculated with feline leukemia virus, *J. Natl. Cancer Inst.* **53**:1271.

Hoover, E. A., Olsen, R. G., Hardy, W. D., Jr., Schaller, J. P., and Mathes, L. E., 1976, Feline leukemia virus infection: Age related variation in response of cats to experimental infection, *J. Natl. Cancer Inst.* **57**:365.

Hoover, E. A., Rojko, J. L., and Olsen, R. G., 1980, Host-virus interactions in progressive versus regressive feline leukemia virus infection in cats, in: *Viruses in Naturally Occurring Cancers* (M. Essex, G. Todaro, and H. zurHausen, eds.), pp. 635–651, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Hoover, E. A., Rojko, J. L., Wilson, P. L., and Olsen, R. G., 1981, Determinants of susceptibility and resistance to feline leukemia virus infection. I: Role of macrophages, *J. Natl. Cancer Inst.* **67**:889.

Hoover, E. A., Rojko, J. L., and Quackenbush, S. L., 1983, Congenital feline leukemia virus infection, *Leuk. Rev. Intl.* **1**:7.

Hoover, E. A., Zeidner, N. S., Perigo, N. A., Quackenbush, S. L., Strobel, J. D., Hill, D. L., and Mullins, J. I., 1989, Feline leukemia virus-induced immunodeficiency syndrome in cats as a model for evaluation of antiretroviral therapy, *Intervirology* **30**(Suppl. 1):12.

Huang, E., Nocka, K., Beier, D. R., Chu, T-Y., Buck, J., Lahm, H-W., Wellner, D., Leder, P., and Besmer, P., 1990, The hematopoietic growth factor KL is encoded by the SI locus and is the ligand of the c-*kit* receptor, the gene product of the *W* locus, *Cell* **63**:225.

Huebner, R. J., and Todaro, G. J., 1969, Oncogenesis of RNA tumor viruses as determinants of cancer, *Proc. Natl. Acad. Sci. USA* **64**:1087.

Irgens, K., Wyers, M., Moraillon, A., Parodi, A., and Fortuny, V., 1973, Isolement d'un virus sarcomatogene feline a partir d'un fibrosarcome spontane du chat, etude du pouvoir sarcomatogene *in vivo*, *C. R. Acad. Sci. (Paris)* **276**:1783.

Jacobs, R. M., Boyce, J. T., and Kociba, G. J., 1986, Flow cytometric and radioisotopic determinations of platelet survival time in normal cats and feline leukemia virus-infected cats, *Cytometry* **7**:64.

Jahner, P., and Haenisch, R., 1985, Chromosomal position and specific demethylation in enhancer sequences of germ-line transmitted retroviral genomes during mouse development, *Mol. Cell Biol.* **5**:2212.

Jakowski, R. M., Essex, M., Hardy, W. D., Jr., Stephenson, J. R., and Cotter, S. M., 1980, Membranous glomerulonephritis in a household of cats persistently viremic with feline leukemia virus, in: *Feline Leukemia Virus* (W. D. Hardy, Jr., M. Essex, and A. J. McClelland, eds.), pp. 141–149, Elsevier, New York.

Jarrett, W. F. H., Crawford, E. M., Martin, W. B., and Davie, F., 1964, A virus-like particle associated with leukemia (lymphosarcoma), *Nature* **202**:567.

Jarrett, W. F. H., Jarrett, O., Mackey, L., Laird, H., Hardy, W. D., Jr., and Essex, M., 1973a, Horizontal transmission of leukemia virus and leukemia in the cat. *J. Natl. Cancer Inst.* **51**:833.

Jarrett, O., Laird, H. M., and Hay, D., 1973b, Determinants of the host range of feline leukaemia viruses, *J. Gen. Virol.* **20**:169.

Jarrett, W., Mackey, L., Jarrett, O., Laird, H., and Hood, C., 1974, Antibody response and virus survival in cats vaccinated against feline leukemia, *Nature* **248**:230.

Jarrett, O., Hardy, W. D., Jr., Golder, M. C., and Hay, D., 1978, The frequency of occurrence of feline leukemia virus subgroups in cats, *Int. J. Cancer* **21**:334.

Jarrett, O., Golder, M. C., and Weijer, K., 1982, A comparison of three methods of feline leukaemia virus diagnosis, *Vet. Rec.* **110**:325.

Jones, F. R., Yoshida, L. H., Ladiges, W. C., and Kenny, M. A., 1980, Treatment of feline leukemia and reversal of FeLV by *ex vivo* removal of IgG, *Cancer* **46**:675.

Kahn, D. E., Mia, A. S., and Tierney, M. M., 1980, Field evaluation of Leukassay F, an FeLV detection test kit, *Feline Pract.* **10**:41.

Kensil, C. R., Barrett, C., Kushner, N., Beltz, G., Storey, J., Patel, U., Aubert, A., and Marciani, D., 1991, Development of a genetically engineered vaccine against feline leukemia virus infection, *J. Am. Vet. Med. Assoc.* **199**:1423.

Kobilinsky, L., Hardy, W. D., Jr., and Day, N. K., 1979, Hypocomplementemia associated with naturally occurring lymphosarcoma in pet cats, *J. Immunol.* **122**:2139.

Kociba, G. J., Lange, R. D., Dunn, C. D. R., and Hoover, E. A., 1983, Serum erythropoietin changes with feline leukemia virus-induced erythroid aplasia, *Vet. Pathol.* **20**:548.

Koshy, R., Wong-Staal, F., Gallo, R. C., Hardy, W. D., Jr., and Essex, M., 1979, Distribution of feline leukemia virus DNA sequences in tissues of normal and leukemic domestic cats, *Virology* **99**:135.

Lafrado, L. J., and Olsen, R. G., 1986, Demonstration of depressed polymorphonuclear leukocyte function in non-viremic FeLV-infected cats, *Cancer Invest.* **4**:297.

Lafrado, L. J., Dezzutti, C. S., Lewis, M. G., and Olsen, R. G., 1989, Immunodeficiency in latent feline leukemia virus infections, *Vet. Immunol. Immunopathol.* **21**:39.

Lederman, L., Singhal, M. C., Besmer, P., Zuckerman, E. E., Hardy, W. D., Jr., and Snyder, H. W., Jr., 1985, Immunological and biochemical characterization of HZ2 feline sarcoma virus and Abelson murine leukemia virus translation products, *J. Gen. Virol.* **66**:2057.

Legendre, A. M., Mitchener, K. L., and Potgieter, L. N., 1990, Efficacy of a feline leukemia virus vaccine in a natural exposure challenge, *J. Vet. Intern. Med.* **4**:92.

Leis, J., Baltimore, D., Bishop, J. M., Coffin, J., Fleissner, E., Goff, S. P., Oroszlan, S., Robinson, H., Skalka, A. M., Temin, H. M., and Vogt, V., 1988, Standardized and simplified nomenclature for proteins common to all retroviruses, *J. Virol.* **62**:1808.

Levesque, K. S., Bonham, L., and Levy, L. S., 1990, Flvi-1, a common integration domain of feline leukemia virus in naturally occurring lymphomas of a particular type, *J. Virol.* **64**:3455.

Levin, R., Ruscetti, S. F., Parks, W. P., and Scolnick, E. M., 1976, Expression of feline type-C virus in normal and tumor tissues of the domestic cat, *Int. J. Cancer* **18**:661.

Levy, J. A., 1978, Xenotropic type-C viruses, *Curr. Top. Microbiol. Immunol.* **79**:111.

Levy, L. S., Gardner, M. B., and Casey, J. W., 1984, Isolation of a feline leukaemia provirus containing the oncogene *myc* from a feline lymphosarcoma, *Nature* **308**:853.

Levy, L. S., Fish, R. E., and Baskin, G. B., 1988, Tumorigenic potential of a *myc*-containing strain of feline leukemia virus in vivo in domestic cats, *J. Virol.* **62**:4770.

Livingston, D. M., and Todaro, G. J., 1973, Endogenous type-C virus from a cat cell clone with properties distinct from previously described feline viruses, *Virology* **53**:142.

Lutz, H., Pedersen, N. C., Harris, C. W., Higgins, B. S., and Theilen, G. H., 1980, Detection of feline leukemia virus infection, *Feline Pract.* **10**:13.

Lyman, S. D., Park, L., and Rohrschneider, L. R., 1988, Colony stimulating factor-1 induced growth stimulation of *v-fms* transformed fibroblasts, *Oncogene* **3**:391.

Mackey, L. J., Jarrett, W., Jarrett, O., and Laird, H., 1975, Anemia associated with feline leukemia virus infection in cats, *J. Natl. Cancer Inst.* **54**:209.

Majumder, S., Ray, P., and Besmer, P., 1990, Tyrosine protein kinase activity of the HZ-4-feline sarcoma virus $P80_{gag\text{-}kit}$-transforming protein, *Oncogene Res.* **5**:329.

Mandel, M. P., Stephenson, J. R., Hardy, W. D., Jr., and Essex, M., 1979, Endogenous RD-114 virus of cats: Absence of antibodies to RD-114 envelope antigens in cats naturally exposed to the feline leukemia virus, *Infect. Immunol.* **24**:282.

Mastro, J. M., Lewis, M., Mathes, L. E., Sharpee, R., Tarr, M. J., and Olsen, R. G., 1986, Feline leukemia vaccine: Efficacy, contents and probable mechanism, *Vet. Immunol. Immunopathol.* **11**:205.

Masur, H., Michelis, M. A., Greene, J. B., Onorato, I., Vande Stouwe, R. A., Holzman, R. S., Wormser, G., Brettman, L., Lange, M., Murray, H. W., and Cunningham-Rundles, S., 1981, An outbreak of community-acquired pneumocystis carinii pneumonia. Initial manifestation of cellular immune dysfunction, *N. Engl. J. Med.* **305**:1431.

Mathes, L. E., Olsen, R. G., Hebebrand, L. C., Hoover, E. A., and Schaller, J. P., 1978, Abrogation of lymphocyte blastogenesis by a feline leukemia virus protein, *Nature* **274**:687.

McAllister, R. M., Nicolson, M., Gardner, M. B., Rongey, R. W., Rasheed, S., Sarma, P. S., Huebner, R. J., Hatanaka, M., Oroszlan, S., Gilden, R. V., Kabigting, A., and Vernon, L., 1973, RD-114 comparison with feline and murine type C viruses released from RD cells, *Nature* **242**:75.

McClelland, A. J., Hardy, W. D., Jr., and Zuckerman, E. E., 1980, Prognosis of healthy feline leukemia virus infected cats, in: *Feline Leukemia Virus* (W. D. Hardy, Jr., M. Essex, and A. J. McClelland, eds.), pp. 121–126, Elsevier, New York.

McCullough, B., Schaller, J., Shadduck, J. A., and Yohn, D. S., 1973, Induction of malignant melanomas associated with fibrosarcoma in gnotobiotic cats inoculated with Gardner feline fibrosarcoma virus, *J. Natl. Cancer Inst.* **48**:1893.

McDonough, S. K., Larsen, S., Brodey, R. S., Stock, N. D., and Hardy, W. D., Jr., 1971, A transmissible feline fibrosarcoma of viral origin, *Cancer Res.* **31**:953.

Moore, F. M., Emerson, W. E., Cotter, S. M., and DeLellis, R. A., 1986, Distinctive peripheral lymph node hyperplasia of young cats, *Vet. Pathol.* **23**:386.

Mullins, J. I., and Hoover, E. A., 1990, Molecular aspects of feline leukemia virus pathogenesis, in: *Retrovirus Biology: An Emerging Role in Human Disease* (R. C. Gallo and F. Wong-Staal, eds), Marcel Dekker, New York.

Mullins, J. T., Casey, J. W., Nicolson, M. D., Burck, K. B., and Davidson, N., 1981, Sequence arrangement and biological activity of cloned feline leukemia proviruses from a virus productive human cell line, *J. Virol.* **38**:688.

Mullins, J. T., Brody, D. S., Binari, R. C., Jr., and Cotter, S. M., 1984, Viral transduction of c-*myc* gene in naturally occurring feline leukaemias, *Nature* **308**:856.

Mullins, J. T., Chen, C. S., and Hoover, E. A., 1986, Disease-specific and tissue-specific production of unintegrated feline leukemia virus variant DNA in feline AIDS, *Nature* **319**:332.

Naharro, G., Dunn, C. Y., and Robbins, K. C., 1983, Analysis of the primary translational product and integrated DNA of a new feline sarcoma virus, GR-FeSV, *Virology* **125**:502.

Naharro, G., Robbins, K. C., and Reddy, E. P., 1984, Gene product of *v- fgr onc:* Hybrid protein containing a portion of actin and a Tyrosine-specific protein kinase, *Science* **223**:63.

Neil, J. C., Hughes, D., McFarlane, R., Wilkie, N. M., Onions, D. E., Lees, G., and Jarrett, O., 1984, Transduction and rearrangement of the *myc* gene by feline leukaemia virus in naturally occurring T-cell leukaemias, *Nature* **308**:814.

Neil, J. C., Forrest, D., Doggett, D. L., and Mullins, J. I., 1987, The role of feline leukaemia virus in naturally occurring leukaemias, *Cancer Surv.* **6**:117.

Neil, J. C., Fulton, R., McFarlane, R., Rigby, M., Stewart, M., Terry, A., and Tzavaras, T., 1988, Receptor-mediated leukaemogenesis: Hypothesis revisited, *Br. J. Cancer Suppl.* **9**:76.

Niederkorn, J. Y., Shadduck, J. A., Albert, D., and Essex, M., 1980, Humoral antibody response to FOCMA in feline sarcoma virus-induced ocular melanoma, in: *Feline Leukemia Virus* (W. D. Hardy, Jr., M. Essex, and A. J. McClelland, eds.), pp. 181–185, Elsevier, New York.

Niman, H. L., Stephenson, J. R., Gardner, M. B., and Roy-Burman, P., 1977, RD-114 and feline leukemia virus genome expression in natural lymphomas of domestic cats, *Nature* **226**:357.

Noronha, F., Grant, C. K., Lutz, H., and Keyes, A., 1983, Circulating levels of feline leukemia and sarcoma viruses and fibrosarcoma regression in persistently viremic cats, *Cancer Res.* **43**:1663.

Nuttall, P., Pratt, R., Nuttall, L., and Daly, C., 1986, False-positive results with HIV ELISA kits, *Lancet* **ii**:512.

Okabe, H., Twiddy, E., Gilden, R. V., Hatanaka, M., Hoover, E. A., and Olsen, R. G., 1976, FeLV-related sequences in DNA from a FeLV-free cat colony, *Virology* **69**:798.

Okabe, H., DuBuy, J., Gilden, R. V., and Gardner, M. B., 1978, A portion of the feline leukemia virus genome is not endogenous in cat cells, *Int. J. Cancer* **22**:70.

Olsen, R. G., Hoover, E. A., Mathes, L. E., Heding, L. D., and Schaller, J. P., 1976, Immunization against feline oncornavirus disease using a killed tumor cell vaccine, *Cancer Res.* **36**:3642.

Olsen, R. G., Lewis, M., Mathes, L. E., and Hause, W., 1980, Feline leukemia vaccine, efficacy testing in a large multicat household, *Feline Pract.* **10**:13.

Onions, D. E., and Jarrett, O., 1987, Viral oncogenesis: Lessons from naturally occurring animal viruses, *Cancer Surv.* **6**:161.

Onions, D., Jarrett, O., Testa, N., Frassoni, F., and Toth, S., 1982, Selective effect of feline leukaemia virus on early erythroid precursors, *Nature* **296**:156.

Onions, D. E., Lees, G., Forrest, D., and Neil, J. C., 1987, Recombinant feline viruses containing the *myc* gene rapidly produce clonal tumors expressing T-cell antigen receptor gene transcripts, *Int. J. Cancer* **40**:40.

Osame, M., Usuku, K., Izumo, S., Ijichi, N., Amitani, H., Igata, A., Matsumoto, M., and Tara, M., 1986, HTLV-I associated myelopathy, a new clinical entity, *Lancet* **i**:1031.

Osterhaus, A., Weijer, K., Uytdehaag, F., Jarrett, O., Sundquist, B., and Morein, B., 1985, Induction of protective immune response in cats by vaccination with feline leukemia virus ISCOM, *J. Immunol.* **135**:591.

Osterhaus, A., Weijer, K., UytdeHaag, F., Knell, P., Jarrett, O., Akerblom, L., and Morein, B., 1989a, Serological responses in cats vaccinated with FeLV ISCOM and an inactivated FeLV vaccine, *Vaccine* **7**:137.

Osterhaus, A. D., Rimmelzwaan, G. F., Weijer, K., and Uytdehaag, A. G., 1989b, Induction of antiviral immune response by immunization with monoclonal anti-idiotype antibodies directed to private idiotopes, *Viral Immunol.* **2**:255.

Overbaugh, J., Donahue, P. R., Quackenbush, S. L., Hoover, E. A., and Mullins, J. I., 1988a, Molecular cloning of a feline leukemia virus that induces fatal immunodeficiency disease in cats, *Science* **239**:906.

Overbaugh, J., Riedel, N., Hoover, E. A., and Mullins, J. I., 1988b, Transduction of endogenous envelope genes by feline leukemia virus *in vitro*, *Nature* **332**:731.

Pacitti, A. M., Jarrett, O., and Hay, D., 1986, Transmission of feline leukemia virus in the milk of a non-viraemic cat, *Vet. Rec.* **118**:381.

Pearson, L. D., Snyder, S. P., and Aldrich, C. D., 1973, Oncogenic activity of feline firbosarcoma virus in newborn pigs, *Am. J. Vet. Res.* **34**:405.

Pedersen, N. C., and Ott, R. L., 1985, Evaluation of a commercial feline leukemia virus vaccine for immunogenicity and efficacy, *Feline Pract.* **15**:7.

Pedersen, N. C., Theilen, G., Keane, M. A., Fairbanks, L., Mason, T., Orser, B., Chen, D-H., and Allison, C., 1977, Studies of naturally transmitted feline leukemia virus infection, *Am. J. Vet. Res.* **38**:1523.

Pedersen, N. C., Theilen, G. H., and Werner, L. L., 1979, Safety and efficacy studies of live- and killed-feline leukemia virus vaccines, *Am. J. Vet. Res.* **40**:1120.

Pedersen, N. C., Meric, S. M., Ho, E., Johnson, L., Plucker, S., and Theilen, G. H., 1984a, The clinical significance of latent feline leukemia virus infections in cats, *Feline Pract.* **14**:328.

Pedersen, N. C., Johnson, L., and Theilen, G. H., 1984b, Biological behavior of tumors and associated retroviremia in cats inoculated with Snyder-Theilen fibrosarcoma virus and the phenomenon of tumor recurrence after primary regression, *Infect. Immun.* **43**:631.

Pedersen, N. C., Ho, E. W., Brown, M. L., and Yamamoto, J. K., 1987, Isolation of a T-lymphotropic virus from domestic cats with an immunodeficiency-like syndrome, *Science* **235**:790.

Pedersen, N. C., Torten, M., Rideout, B., Sparger, E., Tonachini, T., Luciw, P. A., Ackley, C., Levy, N., and Yamamoto, J., 1990, Feline leukemia virus infection as a potentiating cofactor for the primary and secondary stages of experimentally induced feline immunodeficiency virus infection, *J. Virol.* **64**:598.

Perryman, L. E., Hoover, E. A., and Yohn, D. S., 1972, Immunologic reactivity of the cat: Immunosuppression in experimental feline leukemia, *J. Natl. Cancer Inst.* **49**:1357.

Pierce, J. H., and Aaronson, S. A., 1983, In vitro transformation of murine pre-B lymphoid cells by Snyder-Theilen feline sarcoma virus, *J. Virol.* **46**:993.

Poiesz, B. G., Ruscetti, F. W., Gazdar, A. F., Bunn, P. A., Minna, J. D., and Gallo, R. C., 1980, Detection and isolation of type C retrovirus particles from fresh and cultured lymphocytes of a patient with cutaneous T-cell lymphoma, *Proc. Natl. Acad. Sci. USA* **77**:7415.

Pollock, R. V., and Scarlett, J. M., 1990, Randomized blind trial of a commercial FeLV vaccine, *J. Am. Vet. Med. Assoc.* **196**:611.

Pool, R. R., and Harris, J. M., 1975, Feline osteochondromatosis, 1975, *Feline Pract.* **5**:24.

Poss, M. L., Mullins, J. I., and Hoover, E. A., 1989, Posttranslational modifications distinguish the envelope glycoprotein of the immunodeficiency disease-inducing feline leukemia virus retrovirus, *J. Virol.* **63**:198.

Poss, M. L., Quackenbush, S. L., Mullins, J. I., and Hoover, E. A., 1990, Characterization and significance of delayed processing of the feline leukemia virus FeLV-FAIDS envelope glycoprotein, *J. Virol.* **64**:4338.

Post, J. E., and Warren, L., 1980, Reactivation of latent feline leukemia virus, in: *Feline Leukemia Virus* (W. D. Hardy, Jr., M. Essex, and A. J. McClelland, eds.), pp. 151–155, Elsevier, New York.

Quackenbush, S. L., Mullins, J. I., and Hoover, E. A., 1989, Colony forming T lymphocyte deficit in the development of feline retrovirus induced immunodeficiency syndrome, *Blood* **73**:509.

Qiu, F., Ray, P., Brown, K., Barker, P. E., Jhanwar, S., Ruddle, F. H., and Besmer, P., 1990, Primary structure of c-*kit*: Relationship with the CSF-1/PDGF receptor kinase family-oncogenic activation of v-*kit* involves deletion of extracellular domain and C terminus, *EMBO J.* **7**:1003.

Rasheed, S., Barbacid, M., Aaronson, S., and Gardner, M. B., 1982, Origin and biological properties of a new feline sarcoma virus, *Virology* **117**:238.

Reinacher, M., 1987, Feline leukemia virus-associated enteritis: A condition with features of feline panleukopenia, *Vet. Pathol.* **24**:1.

Reynolds, F. H., Van de Ven, W. J. M., Blomberg, J., and Stephenson, J. R., 1981, Differences in mechanism of transformation by independent feline sarcoma virus isolates, *J. Virol.* **38**:1084.

Rice, J. B., Schaller, J. P., Lewis, M. G., Mathes, L. E., Hoover, E. A., and Olsen, R. G., 1981, Infection of feline embryo adherent cells with feline leukemia virus: Feline oncornavirus-associated cell membrane antigen expression and morphologic transformation, *J. Natl. Cancer Inst.* **66**:89.

Rickard, C. G., Post, J. E., Noronha, F., and Barr, L. M., 1973, Interspecies infection by feline leukemia virus: Serial cell-free transmission in dogs of malignant lymphomas induced by feline leukemia virus, in: *Unifying Concepts of Leukemia* (R. M. Dutcher, and L. Chieco-Bianchi, eds.), pp. 102–112, Karger, Basel.

Riedel, N., Hoover, E. A., Gasper, P. W., Nicolson, M. O., and Mullins, J. I., 1986, Molecular analysis and pathogenesis of the feline aplastic anemia retrovirus, feline leukemia virus C-SARMA, *J. Virol.* **60**:242.

Riedel, N., Hoover, E. A., Dornsife, R. E., and Mullins, J. I., 1988, Pathogenic and host range determinants of the feline aplastic anemia retrovirus, *Proc. Natl. Acad. Sci. USA* **85**:2758.

Riggs, I. L., Oshiro, L. S., Taylor, D. O. N., and Lennette, E. H., 1969, Syncytium-forming agent isolated from domestic cats. *Nature* **222**:1190.

Robbins, K. C., Antoniades, H. N., Devare, S. G., Hunkapiller, M. W., and Aaronson, S. A., 1983, Structural and immunological similarities between simian sarcoma virus gene product(s) and human platelet-derived growth factor, *Nature* **305**:605.

Rojko, J. L., and Hardy, W. D., Jr., 1989, Feline leukemia virus and other retroviruses, in: *Diseases of the Cat: Diagnosis and Management* (R. G. Sherding, ed.), pp. 229–332, Churchill Livingstone, New York.

Rojko, J. L., Hoover, E. A., Mathes, L. E., Hause, W. R., Schaller, J. P., and Olsen, R. G., 1978, Detection of feline leukemia virus in tissues of cats by a paraffin embedding immunofluorescence procedure, *J. Natl. Cancer Inst.* **61**:1315.

Rojko, J. L., Hoover, E. A., Mathes, L. E., Olsen, R. G., and Schaller, J. P., 1979, Pathogenesis of experimental feline leukemia virus infection, *J. Natl. Cancer Inst.* **63**:759.

Rojko, J. L., Hoover, E. A., Quackenbush, S. L., and Olsen, R. G., 1982, Reactivation of latent feline leukaemia virus infection, *Nature* **298**:385.

Rojko, J. L., Cheney, C. M., Gasper, P. W., Hamilton, K. L., Hoover, E. A., Mathes, L. F., and Kociba, G. J., 1986, Infectious feline leukemia virus is erythrosuppression *in vitro*, *Leukemia Res.* **10**:1193.

Rosenberg, Z., Pedersen, F. S., and Haseltine, W. A., 1980, Comparative analysis of the genomes of feline leukemia viruses, *J. Virol.* **35**:542.

Roussel, M., Saule, S., Lagrou, C., Rommens, C., Beug, H., Graf, T., and Stehelin, D., 1979, Three new types of viral oncogenes of cellular origin specific for haematopoietic cell transformation, *Nature* **281**:452.

Ruegg, C. L., Monell, C. R., and Strand, M., 1989, Identification, using synthetic peptides, of the minimum amino acid sequence from the retroviral transmembrane protein p15E required for inhibition of lymphoproliferation and its similarity to gp21 of human T-lymphotropic virus types I and II, *J. Virol.* **63**:3250.

Russell, P. H., and Jarrett, O., 1978, The occurrence of feline leukemia virus neutralizing antibodies in cats, *Int. J. Cancer* **22**:351.

Sacca, R., Stanley, E. R., Sherr, C. J., and Rettenmier, C. W., 1986, Specific binding of the mononuclear phagocyte colony-stimulating factor CSF-1 to the product of the v-*fms* oncogene, *Proc. Natl. Acad. Sci. USA* **83**:3331.

Sariban, E., Mitchell, T., and Kufe, D., 1985, Expression of the c-*fms* proto-oncogene during human monocyte differentiation, *Nature* **316**:164.

Sarma, P. S., and Log, T., 1973, Subgroup classification of feline leukemia and sarcoma viruses by viral interference and neutralization tests, *Virology* **54**:160.

Scolnick, E. M., 1982, Hyperplastic and neoplastic erythroproliferative diseases induced by oncogenic murine retroviruses, *Acta Biochem. Biophys.* **651**:273.

Shadduck, J. A., Albert, D. M., and Niederkorn, J. Y., 1981, Feline uveal melanomas induced with feline sarcoma virus: Potential model of the human counterpart, *J. Natl. Cancer Inst.* **67**:619.

Sherr, C. J., Rettenmier, C. W., Sacca, R., Roussel, M. F., Look, A. T., and Stanley, D. R., 1985, The c-*fms* proto-oncogene product is related to the receptor for the mononuclear phagocyte growth factor, CSF-1, *Cell* **41**:665.

Shibuya, M., Hanafusa, T., Hanafusa, H., and Stephenson, J. R., 1980, Homology exists among the transforming sequences of avian and feline sarcoma viruses, *Proc. Natl. Acad. Sci. USA* **77**:6536.

Snider, W. B., Simpson, D. M., Kielsen, S., Gold, J. W. M., Metroka, C. E., and Posner, J. B., 1983, Neurological complications of acquired immune deficiency syndrome: Analysis of 50 patients, *Ann. Neurol.* **14**:403.

Snyder, H. W., Jr., 1982, Biochemical characterization of protein kinase activities associated with transforming gene products of the Snyder-Theilen and Gardner-Arnstein strains of feline sarcoma virus, *Virology* **117**:165.

Snyder, S. P., and Theilen, G. H., 1969, Transmissible feline fibrosarcoma, *Nature* **221**:1074.

Snyder, H. W., Jr., Hardy, W. D., Jr., Zuckerman, E. E., and Fleissner, E., 1978, Characterization of a tumour-specific antigen on the surface of feline lymphosarcoma cells, *Nature* **275**:656.

Snyder, H. W., Jr., Phillips, K. J., Hardy, W. D., Jr., Zuckerman, E. E., Essex, M., Sliski, A. H., and Rhim, J., 1980, Isolation and characterization of proteins carrying the feline oncornavirus-associated cell-membrane antigen, *Cold Spring Harb. Symp. Quant. Biol.* **44**:787.

Snyder, H. W., Jr., Jones, F. R., Day, N. K., and Hardy, W. D., Jr., 1982, Isolation and characterization of circulating feline leukemia virus-immune complexes from plasma of persistently infected pet cats removed by *ex vivo* immunosorption, *J. Immunol.* **128**:2726.

Snyder, H. W., Jr., Singhal, M. C., Zuckerman, E. E., Jones, F. R., and Hardy, W. D., Jr., 1983, The feline oncornavirus-associated cell membrane antigen (FOCMA) is related to, but distinguishable from, FeLV-C gp70, *Virology* **131**:315.

Snyder, H. W., Jr., Singhal, M. C., Zuckerman, E. E., and Hardy, W. D., Jr., 1984a, Isolation of a new feline sarcoma virus (HZI-FeSV), Biochemical and immunological characterization of its translation product, *Virology* **132**:205.

Snyder, H. W., Jr., Singhal, M. C., Hardy, W. D., Jr., and Jones, F. R., 1984b, Clearance of feline leukemia virus from persistently infected pet cats treated by extracorporeal immunoadsorption is correlated with an enhanced antibody response to FeLV gp70, *J. Immunol.* **132**:1538.

Soe, L. H., Devi, B. C., Mullins, J. I., and Roy-Burman, P., 1983, Molecular cloning and characterization of endogenous feline leukemia virus sequences from a cat genomic library, *J. Virol.* **46**:829.

Soe, L. H., Shimizu, R. W., Landolph, J. R., and Roy-Burman, P., 1985, Molecular analysis of several classes of endogenous feline leukemia virus elements, *J. Virol.* **56**:701.

Stewart, M. A., Forrest, D., McFarlane, R., Onions, D., Wilkie, N., and Neil, J. C., 1986a, Conservation of the c-*myc* coding sequence in transduced feline v-*myc* genes, *Virology* **154**:121.

Stewart, M. A., Warnock, M., Wheeler, A., Wilkie, N., Mullins, J. I., Onions, D. E., and Neil, J. C., 1986b, Nucleotide sequences of a feline leukemia virus subgroup A envelope gene and long terminal repeat and evidence for the recombinational origin of subgroup B viruses, *J. Virol.* **58**:825.

Sugita, K., Gutkind, J. S., Katamine, S., Kawakami, T., and Robbins, K. C., 1989, The actin domain of Gardner-Rasheed feline sarcoma virus inhibits kinase and transforming activities, *J. Virol.* **63**:1715.

Swenson, C. L., Kociba, G. J., Mathes, L. E., Hand, P. J., Neer, C. A., Hayes, K. A., and Olsen, R. G., 1990, Prevalence of disease in nonviremic cats previously exposed to feline leukemia virus, *J. Am. Vet. Med. Assoc.* **196**:1049.

Teich, N., 1982, Taxonomy of retroviruses, in: *RNA Tumor Viruses* (R. Weiss, N. Teich, H. Varmus, and J. Coffin, eds.), pp. 25–207, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Temin, H. M., 1988, Mechanisms of cell killing/cytopathic effects by nonhuman retroviruses, *Rev. Infect. Dis.* **10**:399.

Testa, N. G., Onions, D., Jarrett, O., Frassoni, F., and Eliason, J. F., 1983, Haematopoietic colony formation (BFU-E, GM-CFC) during the development of pure red cell hypoplasia induced in the cat by feline leukemia virus, *Leuk. Res.* **7**:103.

Theilen, G. H., 1971, Continuing studies with transmissible feline fibrosarcoma virus in fetal and newborn sheep, *J. Am. Vet. Med. Assoc.* **158**:1040.

Theilen, G. H., Kawakami, T. G., Rush, J. D., and Munn, R. J., 1969, Replication of cat leukaemia virus in cell suspension cultures, *Nature* **222**:589.

Theilen, G. H., Snyder, S. P., Wolfe, L. G., and Landon, J. L., 1970, Biological studies with viral induced fibrosarcomas in cats, dogs, rabbits, and nonhuman primates, in: *Comparative Leukemia Research 1969* (R. M. Dutcher, ed.), pp. 393–400, Karger, Basel.

Todaro, G. J., 1977, RNA tumor virus genes (virogenes) and the transforming genes (oncogenes): Genetic transmission, infectious spread, and modes of expression, in: *Origins of Human Cancer* (H. H. Hiatt, J. D. Watson, and J. A. Winsten, eds.), pp. 1169–1196, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Todaro, G. J., 1980, Interspecies transmission of mammalian retroviruses, in: *Viral Oncology* (G. Klein, ed.), pp. 291–309, Raven Press, New York.

Todaro, G. J., Benveniste, R. E., Lieber, M. M., and Livingston, D. M., 1973, Infectious type-C viruses released by normal cat embryo cells, *Virology* **55**:506.

Todaro, G. J., Tevethia, S., and Melnick, J., 1974, Isolation of an RD-114 related type-C from feline sarcoma virus-transformed baboon cells, *Intervirology* **1**:399.

Todaro, G. J., Benveniste, R. E., Sherwin, S. A., and Sherr, C. J., 1978, MAC-1, a new genetically transmitted type-C virus of primate: Low frequency activation from stumptail monkey cell cultures, *Cell* **13**:775.

Tompkins, M. B., Ogilvie, G. K., Gast, A. M., Franklin, R., Weigel, R., and Tompkins, W. A., 1989, Interleukin-2 suppression in cats naturally infected with feline leukemia virus, *J. Biol. Response Mod.* **8**:86.

Trainin, Z., Wernicke, D., Ungar-Waron, H., and Essex, M., 1983, Suppression of the humoral antibody response in natural retrovirus infections, *Science* **220**:858.

Tzavaras, T., Stewart, M., McDougall, A., Fulton, R., Testa, N., Onions, D. E., and Neil, J. C., 1990, Molecular cloning and characterization of a defective recombinant feline leukaemia virus associated with myeloid leukaemia, *J. Gen. Virol.* **71**:343.

Vedbrat, S. S., Rasbeed, S., Lutz, H., Gonda, M. A., Ruscetti, S., Gardner, M. B., and Prensky, W., 1983, Feline oncornavirus-associated cell membrane antigen: A viral and not a cellularly coded transformation-specific antigen of cat lymphomas, *Virology* **124**:445.

Vernant, J. C., Maurs, L., Gessain, A., Barin, F., Gout, O., Delaporte, J. M., Sanhadji, K., Buisson, G., and de-The, G., 1987, Endemic tropical spastic paraparesis associated with human T-lymphotropic virus type I: A clinical and seroepidemiological study of 25 cases, *Ann. Neurol.* **21**:123.

Wardrop, K. J., Kramer, J. W., Abkowitz, J. L., Clemons, G., and Adamson, J. W., 1986, Quantitative studies of erythropoiesis in the clinically normal, phlebotomized, and feline leukemia virus-infected cat, *Am. J. Vet. Res.* **47**:2274.

Waterfield, M. D., Scrace, G. T., Whittle, N., Stroobant, P., Johnsson, A., Wasteson, A., Westermark, B., Heldin, C-H., Huang, J. S., and Deuel, T. F., 1983, Platelet-derived growth factor is structurally related to the putative transforming protein $p28^{sis}$ of simian sarcoma virus, *Nature* **304**:35.

Weijer, K., and Daams, J. H., 1978, The control of lymphosarcoma/leukaemia and feline leukaemia virus, *J. Small Anim. Pract.* **19**:631.

Weksler, M. E., Ryning, F. W., and Hardy, W. D., Jr., 1975, Immune complex disease in cancer, *Clinical Bull.* **5**:109.

Weller, S. K., Joy, A. E., and Temin, H. M., 1980, Correlation between cell killing and massive second-round superinfection by members of some subgroups of avian leukosis virus, *J. Virol.* **33**:494.

Wellman, M. L., Kociba, G. J., Lewis, M. G., Mathes, L. E., and Olsen, R. G., 1984, Inhibition of erythroid colony-forming cells by a 15,000 dalton protein of feline leukemia virus, *Cancer Res.* **44**:1527.

Wellman, M. L., Kociba, G. J., Mathes, L. E., and Olsen, R. G., 1988, Suppression of feline bone marrow fibroblast colony-forming units by feline leukemia virus, *Am. J. Vet. Res.* **49**:227.

Wong-Staal, F., and Gallo, R. C., 1985, Human T-lymphotropic retroviruses, *Nature* **317**:395.

Yoshiki, T., Mellors, R. C., Hardy, W. D., Jr., and Fleissner, E., 1974, Common cell surface antigen associated with mammalian C-type RNA viruses, *J. Exp. Med.* **139**:925.

Youngren, S. D., and deNoronha, F., 1983, A transmissible feline sarcoma virus containing sequences related to the K-*ras* onc gene, in: *RNA Tumor Viruses* (E. Scolnick and N. Hopkins eds.), p. 33, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Ziemiecki, A., Hennig, D., Gardner, L., Ferdinand, F. J., Friis, R. R., Bauer, H., Pedersen, N. C., Johnson, L., and Theilen, G. H., 1984, Biological and biochemical characterization of a new isolate of feline sarcoma virus: Theilen-Pedersen (TP1-FeSV), *Virology* **138**:324.