CHARACTERIZATIONS AND IMMUNOTHERAPY OF FELINE RETROVIRAL (FeLV) ASSOCIATED HEMATOPOIETIC MALIGNANCIES AND IMMUNODEFICIENCY: CLINICAL, IMMUNOLOGICAL AND PATHOLOGICAL STUDIES

By

ROBERT WILLIAM ENGELMAN II

Bachelor of Science
University of Kansas
Lawrence, Kansas
1978

Doctor of Veterinary Medicine
University of Missouri
Columbia, Missouri
1982

Submitted to the Faculty of the Graduate College
of the Oklahoma State University
in partial fulfillment of the requirements
for the Degree of
DOCTOR OF PHILOSOPHY
December, 1985

CHARACTERIZATIONS AND IMMUNOTHERAPY OF FELINE RETROVIRAL (FeLV) ASSOCIATED HEMATOPOIETIC MALIGNANCIES AND IMMUNODEFICIENCY: CLINICAL, IMMUNOLOGICAL AND PATHOLOGICAL STUDIES

Thesis Approved:

Thesis Adviser

Dean of the Graduate College

PREFACE

Since its identification in 1964, the feline retroviral family has been implicated as the group of etiological agents responsible for a number of clinically diverse disorders. Though historically preceded by the identification and description of avian and murine retroviruses, it represents the first retroviral family characterized for an out-bred mammalian species, and has served as an important precedent and model for the recently described retroviruses of human concern. Retroviruses have been etiologically linked with both the malignant proliferation of and the selective depletion and/or dysfunction of hematopoietic and lymphoreticular cells. These retroviral effects manifest as leukemias, lymphoma, anemia and/or immunodeficiencies. It is not clear to what extent the clinical, pathological and immunological manifestations subsequent to retroviral infection are a result of discrete genetic events between the infecting retrovirus and the host cellular DNA, or are indirectly initiated by extraneous viral debree.

My involvement in the feline-retroviral-leukemia-immunodeficiency model was initiated by an invitation to join in studies evaluating a new form of anti-malignancy therapy utilizing a purified bacterial protein. Cats with overt malignancy were to be characterized as to their presenting status, treated by way of plasma filtration over the purified bacterial protein, and monitored for

changes in their clinical condition. From this basis, and because of my curiosities concerning the extent of deficient immune function produced by retroviruses which I developed during the anti-malignancy therapy work, discrete observations were made concerning the manifestations and therapy of retroviral (FeLV) associated malignancy and immunodeficiency. These distinct findings were recorded as published manuscripts in scientific journals, and are presented in this dissertation as separate chapters.

When persistently feline leukemia virus infected cats were treated with staphylococcal protein A, the incidence of clearance of evidence of FeLV infection increased and FeLV-associated diseases regressed.

This loss of evidence of FeLV infection and regression of FeLV-associated disease occurred in some cats that first experienced an increased in plasma interferon level, followed by the appearance and increase of a cytotoxic antibody specific for the major retroviral envelope glycoprotein gp70.

The initial mean serum calcium concentration of cats that presented with hematopoietic malignancy was significantly higher than that of healthy control cats or FeLV-infected cats without malignancy, and three cases of overt hypercalcemia were identified.

During therapy with staphylococcal protein A, shifts were observed in the morphological manifestations of a case of chronic feline hematopoietic malignancy with sequential changes in the predominant cell lineage.

A new FeLV-replicating lymphoma-derived cell line was isolated from a cat presenting with anterior mediastinal lymphoma and was characterized as lacking T and B-lymphocyte surface markers.

The synthesis of gamma interferon by normal feline lymphocytes stimulated with staphylococcal enterotoxin A in vitro was reduced when UV-light inactivated FeLV was also present in culture during the period of stimulation.

In addition, immunological methods and reagents were developed which are likely to prove useful in the identification of quantitative and qualitative aberrations in specific subsets of T-lymphocytes in the cat with retroviremia, immunodeficiency and leukemia. The optimal conditions of culture and assay for identification of feline immunoglobulin secreting mononuclear cells were determined for the staphylococcal protein A - reverse hemolytic plaque assay. Initial steps were taken toward the devlopment of monoclonal antibodies which characterize T-lymphocyte subpopulations by their pattern of reactivity with cell surface antigens.

The prior publication of material appearing in this thesis includes the following manuscripts and abstracts.

Engelman, R.W., R.W. Fulton, R.A. Good, N.K. Day. Suppression of gamma interferon production by inactivated feline leukemia virus. Science 227:1368-1370, 1985.

Engelman, R.W., R.D. Tyler, W.T. Liu, L.Q. Trang. R.A. Good, N.K. Day. Clinicopathologic responses in cats with feline leukemia virus-associated leukemia-lymphoma treated with staphyloccal protein A. Am. J. Pathol. 118:367-378, 1985.

Engelman, R.W., N. Gengozian, R.A. Good, N.K. Day. Polyclonal induction of immunoglobulin synthesis by feline leukocytes as identified in a reverse hemolytic plaque assay. J. Immunol. Mthds. 81·65-71, 1985.

Engelman, R.W., R.D. Tyler, R.A. Good, N.K. Day. Hypercalcemia in cats with feline leukemia virus-associated leukemia-lymphoma. Cancer 56:777-781, 1985.

Engelman, R.W., R.A. Good, N.K. Day. Clearance of retroviremia and regression of malignancy in cats with leukemia-lymphoma during treatment with staphylococcal protein A. Cancer Detection and Prevention., 1985, in press.

Engelman, R.W., R.D. Tyler, D.A. Mosier, R.A. Good, N.K. Day. Changing manifestations of a chronic hematopoietic proliferative disease during immunotherapy with staphylococcal protein A. J. Comp. Pathol., 1985, in press.

Engelman, R.W., K. Machida, R. Longley, W.T. Liu, L.Q. Trang, R.A. Good, N.K. Day. Characterization of a newly established feline lymphoma-derived cell line (BKD) lacking T- and B-cell surface markers. In Vitro, 1985, in press.

Engelman, R.W., R.W. Fulton, R.A. Good, N.K. Day. Suppression of gamma interferon production by inactivated feline leukemia virus. In AIDS:Papers from Science, 1982-1985, ed. by R. Kulstad, 1985, in press.

Engelman, R.W. Clearance of feline leukemia virus from persistently infected cats during treatment with staphylococcal protein A. Society for Experimental Biology and Medicine, 1985.

Engelman, R.W., R.W. Fulton, R.A. Good, N.K. Day. Inactivated feline retrovirus (FeLV) suppresses gamma interferon production by normal feline lymphocytes. Fed. Proc., Abst. 3142, 1985.

Engelman, R.W., K. Machida, L.Q. Trang, O.J.M. D'Cruz, R.A. Good, N.K. Day. A new feline lymphoma cell line (BKD) lacking T- and B-cell characteristics. Fed. Proc., Abst. 7510, 1985.

ACKNOWLEDGEMENTS

This work was aided by the contributions of many kind and helpful people located on three medical campuses, the Oklahoma State University College of Veterinary Medicine, the Oklahoma Medical Research Foundation and the University of Oklahoma College of Medicine, and funded in part by grants from the American Cancer Society (IM-298), and the National Institutes of Health (CA-34103, CA-31547 and AI-22360). I appreciate both the opportunity for research training in pathology and immunology and the attention shown me in the way of assistance, instruction, criticism and encouragement during the three and one half years of my graduate program.

The guidance of my major advisor Dr. R.J. Panciera, and graduate committee members Drs. R.D. Tyler, N.K. Day, R.W. Fulton, N. Gengozian and A.W. Confer is gratefully acknowledged. These individuals were vital to the success of my training program and contributed their talents and knowledge of their specialty, use of their laboratory and diagnostic facilities, and their enthusiasm for biomedical research for which I am grateful. The circumstances of my graduate program required that they function not only as mentors, but also as negotiators; this I recognize and appreciate. Additional assistance and support was shown me by Drs. R.A. Good, W.G. Thurman, E. Koren and R.R. Badertscher for which I am grateful.

The contributions of technical and secretarial assistance by many people is also gratefully acknowledged. Thank you to Kris Pawlowski, Jill Kleinhans, Colette Moriarty, Xuan Tran, Farhaz Nahhas, Liem Trang, Larenda Burge, Karen Deatherage, Charlene Clark, Leslie Aubrey, Sandy Newsom, Erma Dulin, Monte Camp and Drs. K. Machida, O. D'Cruz, M. Yasuda, R.E. Longley, J. Yamamoto and W.T. Liu.

Thank you is extended to the many veterinarians who cooperated with and participated in the feline patient referral program. Thanks also to Blueboy, Nevada, Baker and Fred, and all of the cats who contributed maximally to this work.

Thanks to my good friend, Dr. Derek A. Mosier, who has shared with me the same office, the same occupation, and some of the same interests, and who aided not only in this work and my other professional responsibilities, but also attempted to make the whole bloody business fun.

Thanks also to Chester Sullivan, Jeffrey Marliave, Helen and Hale Fletchall, Grandpa Joe and Grandma Lucille, all of whom were instrumental in guiding me down this road.

Special loving thanks to my best friends and family, Elaine and Alex, who helped greatly in everyway with this distraction from its inception to its end. Thank you my friends for knowing and caring. Truly, I'd rather have spent the time with you. Least ways now, I've been there and done that.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

NOMENCLATURE

ADCC	antibody-dependent cell-mediated cytotoxicity
AET	aminoethylisothiourium bromide
AIDS	acquired immunodeficiency syndrome
AIHA	autoimmune hemolytic anemia
ALV	avian leukosis virus
AMP	ampicillin
ARA	aregenerative anemia
ATL	adult T-cell leukemia
BFU-e	blast forming units-erythroid
CFU-c	colony forming units-granulocyte/monocyte
CFU-e	colony forming units-erythroid
CIC	circulating immune complexes
CIg	cytoplasmic immunoglobulin
CML	cell-mediated lympholysis
c-onc	cellular (proto) oncogene
con A	concanavalin A
DMBA	dimethylbenzanthracene
DMSO	dimethylsulfoxide
DNA	deoxyribonucleic acid
E	erythrocyte
EA	erythrocyte-antibody
EAC	erythrocyte-antibody-complement
ELISA	enzyme-linked immunosorbent assay
Eos	eosinophil
env	envelope gene
EV	ex vivo
FACS	fluorescent-activated cell sorter
Fc	constant fragment
FCS	fetal calf serum
FCV	feline calici virus
FeLV	feline leukemia virus
FeLV-UV	ultraviolet-light inactivated feline leukemia virus
FeSV	feline sarcoma virus
FHV-1	feline herpes virus-type I
FIP	feline infectious peritonitis
gag	group-associated antigen gene
gp70	70,000 dalton retroviral envelope glycoprotein
GPC	guinea pig complement
GPE	guinea pig erythrocyte
HBSS	Hank's balanced salt solution
HCT	hematocrit
H&E	hematoxylin and eosin
hemob.	hemobartonellosis
HTLV-I	human T-lymphotropic virus type I

HTLV-III	human T-lymphotropic virus type III
HY-HAT	hybridoma-hypoxanthine, aminopterin, thymidine
HY-HT	hybridoma-hypoxanthine, thymidine
IFA	indirect fluorescent antibody test
IFN	interferon
Ig	immunoglobulin
IL-1	interleukin-one
IL-2	interleukin-two
Immuno Rxs	immunoadsorbent treatments
IP	intraperitoneal
IV	intraveneous
LN	lymph node
LTR	long terminal repeat
M	monocyte
Mab	monoclonal antibody
MCF	mink cell focus-forming
MCHC	mean corpuscular hemoglobin concentration
MCV	mean corpuscular volume
MDBK	Madin-Darby bovine kidney cells
M:E	myeloid:erythroid ratio
MLC	mixed lymphocyte culture
MMTV	mouse mammary tumor virus
MuLV	murine leukemia virus
MPMV	Mason-Pfizer monkey virus
mRNA	messenger ribonucleic acid
N	neuraminidase
ND	not done
NK	natural killer
NR	not reported
NRBC	nucleated red blood cell
onc	oncogene
OT	tetracycline per os
p15E	15,000 dalton retroviral envelope protein
p27	27,000 dalton retroviral protein
PBL	peripheral blood lymphocytes
PBS	phosphate buffered saline
PBM	peripheral blood mononuclear cells
PFC	plaque forming cell
PFU	plaque forming unit
Phago	phagocytic activity
PMA	phorbol-12-myristate-13-acetate
pol	polymerase gene
poly-A	polyadenylation
PWM	pokeweed mitogen
R	redundancy region
RbC	baby rabbit complement
RBC	red blood cell
RE	rat erythrocyte
Retics	reticulocytes
RFC	rosette forming cell
RHPA	reverse hemolytic plaque assay
RNA	ribonucleic acid
RSV	Rous sarcoma virus

S	surviving
SAC	Staphylococcus aureus Cowan I
SEA	staphylococcal enterotoxin A
SIg	surface immunoglobulin
SpA	staphylococcal protein A
SRBC	sheep red blood cell
T	transfussion of leukocyte-poor, washed, packed red blood cells
TCGF	T-cell growth factor
tRNA	transfer ribonucleic acid
TWBC	total white blood cells
U_3	nucleotide sequence unique to the 3' retroviral genome
U_5	nucleotide sequence unique to the 5' retroviral genome
v-onc	viral oncogene
VSV	vesicular stomatitis virus
WBC	white blood cell

CHAPTER I

BACKGROUND LITERATURE

A. Taxonomy, Terminology and Molecular Biology of Retroviridae

1. Introduction

All retroviruses share morphological, physical and chemical features which justify their inclusion in a single viral family (Table I). Their RNA genome is copied into DNA by the viral enzyme, RNA-dependent DNA polymerase and this DNA copy, called a provirus, is inserted into one of the host's chromosomes. The integrated provirus is transcribed into RNA by host enzymes, giving rise to both viral genomic RNA and viral messenger RNA.

The viral genes, gag, pol, env, encode internal-structural, enzymatic (reverse transcriptase), and envelope proteins, respectively. Additional sequences created during proviral synthesis from viral genomic RNA, flank the viral genes and are similar at each termini, forming a direct repeat. These repeated sequences found at the termini of the provirus are termed long terminal repeats (LTRs) and are made up of three segments: the U_3 region (a sequence unique to the 3' end of viral genomic RNA), a redundant region (R) found at both ends of genomic RNA, and the U_5 region (unique to the 5' genomic end). Within the LTRs are elements which enhance and

TABLE I

TAXONOMIC FEATURES OF RETROVIRIDAE*

Nucleic acid	linear positive-sense single-stranded RNA (60S-70S) composed of identical subunits (30S-35S); 5' structure $m^7G^5ppp^5NmpNp$; polyadenylated 3' end; repeated sequences at 3' and 5' ends; tRNA base-paired to genome complex
Protein	about 60% by weight; gag, internal structural proteins (4-5); pol, reverse transcriptase (1-2); env, envelope proteins (1-2)
Lipid	about 35% by weight; derived from cell membrane
Carbohydrate	about 4% by weight; associated with env proteins
Physiochemical properties	sensitive to lipid solvents, detergents, and heat inactivation (56°C, 30 min); highly resistant to UV- and X-irradiation
Morphology	spherical enveloped virions (80-120 nm diameter), variable surface projections (8 nm diameter), icosahedral capsid containing a ribonucleoprotein complex

*adapted from Teich, 1982.

promote transcriptional initiation, the initiation start site and a polyadenylation signal.

Host cellular DNA sequences which show homology to the exogenously acquired provirus are termed endogenous retroviral-related sequences and are a type of endogenous provirus. Other endogenous proviruses that lack sequence homology to the retrovirus of the species may also be present within the host cellular DNA but are activated only in heterologous cells. In addition, certain other cellular sequences which are expressed at low levels in the normal host cell, but at high levels when activated or recombined with an infecting retroviral provirus, confer on the virus cellular transforming properties both *in vitro* and *in vivo*, and have been termed oncogenes.

The retroviral genome not only encodes information required for viral synthesis but apparently may also influence cellular regulatory elements or activate cellular genes. Trans-transcriptional activating substances induced or encoded by the provirus may recognize host DNA sequences in addition to those within the proviral LTR. The downstream LTR may promote initiation of transcripts primarily comprised of host sequences, and in some cases, these sequences are frequently oncogenes. In addition, the integrating provirus may recombine with and activate host cellular DNA (either endogenous proviral or oncogenic DNA) resulting at times in the loss of proviral structural sequences, but also in the enhancement of the oncogenicity of the virus.

Evidently, it is the retroviral genome's mobility and abilities to at least recombine with, transfer, and promote the transcription

of host cellular DNA that destines the retroviral infected cell to immortality, dysfunction and/or nonviability. Whether the induced cellular changes manifest clinically as a specific form of neoplasm or as a state of deficient immunity may be a function of the isolates' cellular tropisms, their sites of DNA integration, the specificities of the transcriptional activating factors that are produced, the combination and degree to which normally quiescent cellular DNA is activated, and the products resulting from proviral recombination with the DNA of the host.

Regardless of these intracacies, retroviruses in general share common features and a mode of replication. The following summarizes these features as well as the mechanisms of proviral synthesis, integration, transcription and oncogenesis. The discussion is general, lacks species-specific information, is in no way exhaustive, and is intended only as an introduction to terminology, taxonomy and the molecular biology of retroviruses.

2. Morphology

The original formulation of the Retroviridae family was based on similarities in viral morphology revealed by electron microscopy (Bernhard, 1958, 1960). They are roughly spherical, approximately 80-120 nm in diameter, have an inner proteinacious nucleocapsid (core or nucleoid) with apparent icosohedral symetry and an outer lipid-bilayer envelope from which glycoprotein "spikes" variably project. On the basis of morphology, retroviruses are classified as either type-A, type-B, type-C or type-D particles (Figure 1).

Figure 1: Morphology of intracellular and extracellular retroviral particles. Intracellular type A particles have an electron-lucent center surrounded by a double shell. Type B, particles when budding from the plasma membrane, have doughnut-shaped cores and long envelope-associated spikes. Extracellular, mature type B particles have electron-dense, eccentrically located cores. Budding type C particles have crescent-shaped cores and short envelope-associated spikes. When mature, the extracellular type C particle has a centrally located electron-dense core and short, indistinct envelope-associated spikes. Type D particles have doughnut-shaped cores and moderately sized surface spikes when budding, while mature forms have bar-shaped cores.

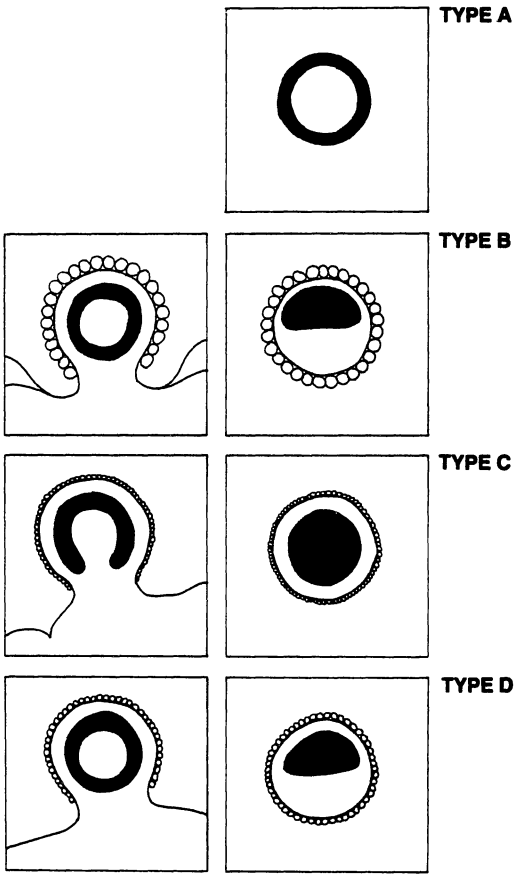


Figure 1

Type-A particles occur as intracellular, non-infectious elements with an electron-lucent center surrounded by a double shell. They are further subdivided based on their intracellular location as intracisternal and intracytoplasmic. Intracisternal type-A particles are occasionally found in cells producing type-C or type-D particles but their function is unknown. The intracytoplasmic type-A particles are apparently precursor forms of type-B virions.

Type-B particles, when budding at the plasma membrane have toroidal (doughnut-shaped) electron dense cores and long spikes at the envelope surface. After budding, mature virions contain electron-dense eccentrically-located nucleoids within the envelope. The mouse mammary tumor virus (MMTV) is the prototype member of the type-B particle group.

Most retroviruses are type-C particles, which when budding have crescent-shaped electron-dense cores. After budding, they have short ill-defined spikes and centrally-located cores that mature progressively from electron-lucent to electron-dense.

Type-D intracellular particles are ring-shaped and abundant near the plasma membrane. Budding and extracellular type-D forms have eccentrically-located, at times bar-shaped, electron-dense nucleoids and the envelope spikes are short. The Mason-Pfizer monkey virus (MPMV) is the prototype member of the type-D particle group.

3. Pathobiology

Viruses of the Retroviridae family have also been classified as members of one of three subfamilies based on their apparent *in vitro* and *in vivo* pathobiology. The subfamily Oncovirinae consists of

those retroviruses that are naturally oncogenic, and closely-related non-onocogenic isolates. The Lentivirinae or "slow" viruses are cytopathic in culture producing syncytia and cell lysis, and produce slowly progressive debilitating diseases in ungulates. The Spumavirinae or "foamy" viruses produce vacuolizations in cultured cells, and induce persistent infections but no known clincial disease.

4. Genomic Analysis

Nucleic acid hybridization and nucleotide sequence analysis have also been used to identify and classify retroviruses and are useful for proposing evolutionary relationships between apparently closely-related species of retroviruses which demonstrate polynucleotide sequence homology (Rabson and Martin, 1985). A feature which complicates these studies is the frequent presence of host cellular DNA sequences which hybridize with some proviral cDNA probes (Benveniste, et al., 1975) (Koshy, et al., 1980). These endogeneous retroviral-related sequences are homologous in part with the exogenously derived provirus, are stably integrated in the host cellular DNA as multiple, discrete, nontandem copies, are not inducible as virus and are in some cases distinquished from exogenously acquired proviruses by their incompleteness (Casey, et al., 1981). Sequence organization and location of endogeneous proviral-related sequences are the same among different tissues of an individual but unique when compared to other individuals, suggesting that inheritance is via the germ cell line. Endogenous proviral-related genes comprise an extensive multigene family. They have been variously described as entities

ancestral to infectious retroviruses, as evidence of historical retroviral exposure, and as agents encoding normal cell functions. They are not necessarily quiescent. In fact, some of the major differentiation-specific glycoproteins of the mouse are encoded by endogenous proviral sequences (Lerner, 1978).

5. Transmission

Three modes of retroviral transmission, horizontal-infectious, congenital-infectious and genetic can occur. All three modes of transmission may occur in an individual species but with differing consequences. For example, the horizontal exposure of a susceptible chick to avian leukosis virus (ALV) results in transient viremia, immunity and the rare development of leukemia, while the congenitally infected chick develops viremia, is immunologically tolerant to viral antigens and frequently develops leukemia (Rubin, et al., 1962). The congenital transmission of mouse mammary tumor virus (MMTV) particles, shed in the milk, to newborn C3H mice leads to the subsequent development of mammary tumors (Bittner, 1942a,b). Horizontally feline leukemia virus (FeLV) infected cats frequently develop a state of immunodeficiency, anemia or leukemia, but congenital infection results in abortion or fatal runting of the newborn (Jarrett, 1971). Certain in-bred strains of mice have a great numer of complete, endogenous proviruses which are naturally spread genetically and are inducible as infectious virus. Mice of the AKR strain have a great number of complete and incomplete endogenous murine leukemia viral (MuLV) proviruses and a high incidence of leukemia, and GR mice,

which experience a high incidence of mammary carcinoma, have numerous endogenous MMTVs (Chattopadhyay, et al., 1982).

6. Host Range

Host range is apparently defined in part by a retrovirus's cellular tropism. Ecotropic viruses are those which replicate in cells of the species from which they were isolated. Xenotropic viruses do not replicate well in cells of their host species, but when activated can replicate in a wide range of heterologous species. Cellular tropisms may be controlled at the genomic level by regulatory sequences or cell-specific transcriptional promotors (Lenz, et al., 1984) (Chen, et al., 1984) (Derse, et al., 1985). Alternatively or concomitantly, productive infection may be controlled at the membrane level requiring viral interaction with a specific cellular receptor (Fingeroth, et al , 1984) (Klatzmann, et al., 1984a,b). However, even cells totally resistant to infection can bind retroviruses suggesting that viral tropism for certain cells may be influenced by subsequent events such as viral penetration, and proviral synthesis, integration and transcription (Piraino, 1967).

7. Proviral Synthesis and Integration

When a retrovirus virion penetrates a susceptible cell, presumably in some form of an unenveloped core structure, viral DNA synthesis is initiated in the cytoplasm and requires only viral enzymes. When supplied only with deoxynucleoside triphosphates and appropriate salts in vitro, the virion core contains all the elements needed for complete viral DNA synthesis (Junghans, et al., 1975).

Within the virion core, genomic RNA is present as a dimer, hydrogen-bonded together via palindromic sequences, and is associated with several low molecular weight nucleic acids (Haseltine, et al., 1977) (Sawyer and Dahlberg, 1973) (Levinson, et al., 1972). One such nucleic acid is a tRNA molecule which is hydrogen-bonded to the 5' end of viral genomic RNA (Taylor and Illmensee, 1975). The particular tRNA bound is species specific; tRNA tryptophan is bound in the avian leukosis virus, and tRNA proline is bound in the murine leukemia virus (Harada, et al., 1975, 1979). This tRNA serves as a primer for RNA-dependent DNA polymerase and identifies the point of initiation for synthesis of the first viral DNA strand (Baltimore and Smoler, 1971).

The retroviral RNA genome is positive-sensed (+), that is, of same polarity as viral mRNA and capable of direct protein synthesis in in vitro translation systems. The first viral DNA strand to be synthesized then is negative-sensed (-) or of opposite polarity. Of course DNA strands are synthesized in the 5' to 3' direction, that is, deoxyribonucleotides are added to the 3' end of the growing DNA chain. Since the site of initiation for (-) strand DNA synthesis is at the 5' end of the template viral RNA strand, DNA synthesis would seem to run out of template almost immediately. There is, therefore, a strong theoretical requirement to transfer the growing (-) DNA strand to the 3' end of either the same RNA molecule or the second genomic RNA found in the virion. Though molecular details are lacking, the transfer apparently does take place and can be accomplished only because both ends of the viral genomic RNA molecule contains

regions of redundancy (R) which can align (Sutcliffe, et al., 1980) (Amer, et al., 1981) (Swanstrom, et al., 1981a,b).

Soon after the initiation and transfer of the (-) strand DNA, synthesis of the (+) strand is initiated near the 3' end of the genomic RNA using the (-) strand DNA as template (Donehower, et al., 1981). Though a specific primer for (+) strand initiation has not been identified, conjectures have been made that a 3' endonuclease activity of the RNA-dependent DNA polymerase leaves an appropriate primer when it degrades a site near the 3' end of viral genomic RNA. Following (+) strand initiation, a second transfer of the growing (-) strand occurs, this time its 3' end is transferred to and binds with the recently initiated (+) strand (Gilboa, et al., 1979). This positive strand serves as template for the completion of the (-) strand DNA which in turn serves as template for the synthesis of a majority of the (+) DNA strand.

The consequence of this series of events is a linear double-stranded DNA copy of viral genomic RNA which is known as the provirus (Figure 2). Synthesis of all linear proviruses occurs within the cytoplasm (except the Visna provirus which is made in the nucleus). The linear viral DNA then migrates from the cytoplasm to the nucleus where it serves as precursor to two forms of supercoiled circular provirus (Shank and Varmus, 1978). Proviruses join a defined pair of sites in their own DNA to a large number of different sites in the genomes of their host (Hughes, et al., 1978). Though it is not clear whether all potential proviral integration sites in the host genome are equally favored, the consequences of integration at some sites is apparently widely divergent from others.

Figure 2: The relationship of retrovirus genomic RNA, unintegrated viral DNA and the integrated provirus. The viral genomic RNA is single-stranded, positive-sensed, capped at the 5' end, has a 3' poly-A tail and is hydrogen-bonded to a tRNA primer near its 5' end. Reverse transcription of this genome is accomplished with only viral enzymes. The process includes two separate initiation events, two transcriptional-jumps or transfer events, and results in a linear double-stranded DNA copy. After migration from the cytoplasm to the nucleus, this linear copy forms one of two circular supercoiled forms as indicated. The integrated provirus contains viral genes flanked by long terminal repeat (LTR) sequences. Single-stranded RNA is denoted by a dotted bar, and the LTR regions as open bars. Cellular DNA is represented by bars with slashes. Diagrams are not to scale.

PROVIRAL SYNTHESIS AND INTEGRATION

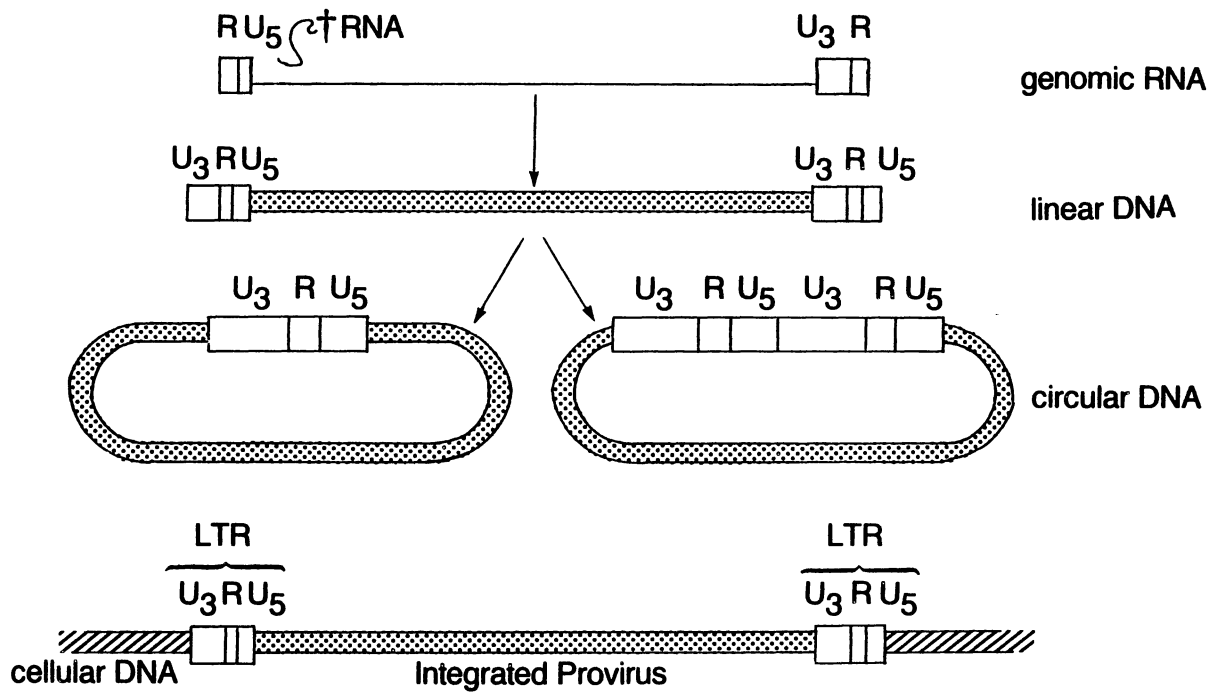


Figure 2

The integration event does not lead to the loss of host sequences, rather insertion of viral DNA is accompanied by the duplication of a small region of host cellular DNA at the integration site (Majors and Varmus, 1981). Because of this duplication, the host sequences immediately flanking the provirus form a small direct repeat ranging in size from 4-6 base pairs (Dhar, et al., 1980). In contrast, the joining of host and viral DNAs results in the loss of 2 base pairs from each end of the viral DNA (Dhar, et al., 1980) (Majors and Varmus, 1981).

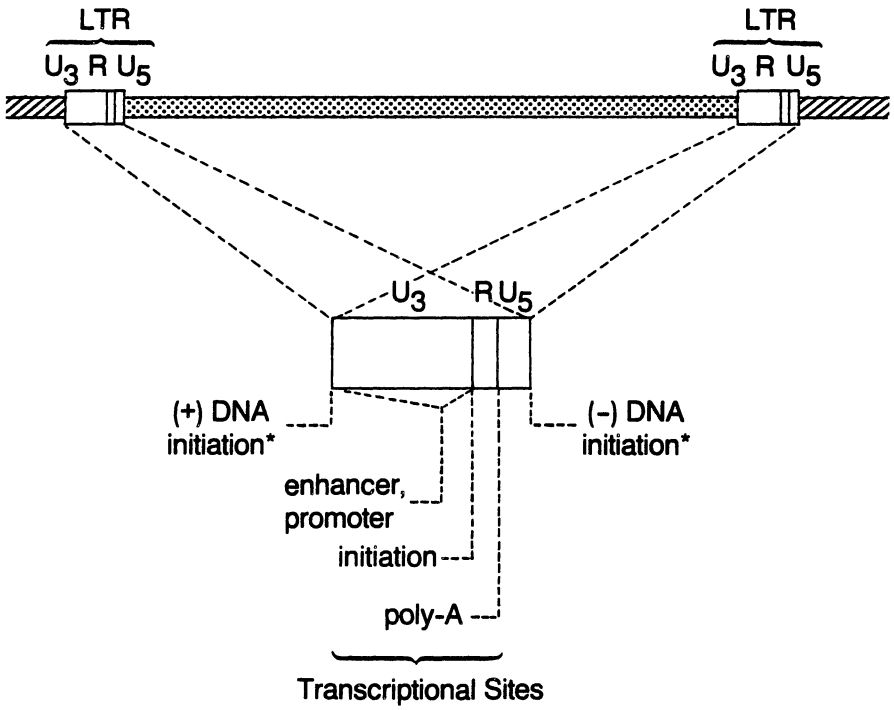
Viral genes within the provirus are flanked by a long terminal repeat (LTR) which is comprised of sequences reversely transcribed from and unique to the 3' and 5' ends of viral genomic RNA (U_3 and U_5) and a redundancy region (R) (Figure 3). The (+) and (-) strand DNA initiation sites define the extent of the LTRs, which show sequence homology among all retroviruses. Although host factors are not required for proviral synthesis, host cells must divide before integration of the provirus takes place (Varmus, et al., 1977). While the majority of the integrated proviruses are complete and contain two LTRs, integrated incomplete proviruses, some lacking internal viral sequences and others a LTR, have been demonstrated and are especially common in certain retroviral species (Martin, et al., 1979).

8. Proviral Transcription

The normal cellular transcriptional machinery transcribes the provirus and processes the mRNAs. The transcriptional start site and the polyadenylation (poly-A) site define the redundancy region

Figure 3: Functional features of the long terminal repeats (LTR). Reverse transcriptional initiation sites define the extent of the LTR. The messenger and genomic RNA transcriptional initiation site and polyadenylation signal define the extent of the redundancy (R) region.

CHARACTERISTICS OF THE PROVIRAL LTR



*Reverse Transcriptional Sites
(proviral synthesis)

Figure 3

(R) of the proviral LTR, and identifies the terminal ends of the viral genomic RNA (Figure 3) (Stoll et al., 1977). Presumably, the proximity of the start signal and the poly-A addition site prevents the poly-A site from being used efficiently until the entire viral genome has been copied. Promoter sequences (TATAA boxes, etc...) are found appropriately located within both of the LTRs near the transcriptional start site (Sutcliffe, et al., 1980). Apparently, start sites within both LTRs can be active and give rise to both viral mRNA and to transcripts of those host sequences which lie to the right of the provirus (Figure 4) (Hayward, et al., 1981) (Payne, et al., 1981, 1982). Proviral transcribed mRNAs either closely resemble mature viral genomic RNA or are spliced (Mellon and Duesberg, 1977) (Weiss, et al., 1977).

9. Molecular Mechanisms of Retroviral Oncogenesis

It is increasingly apparent that the evolution of a neoplasm involves, at some point, specific genetic alterations in individual cells whose progeny eventually predominate (Cairns, 1975, 1980). Eucaryotic transposable genetic elements, or transposons, and retroviruses share structural and functional features (Baltimore, 1985). Conceptually, retroviruses may be viewed as mobile genetic elements which integrate at any number of sites in the host genome, carry strong enhancer and promoter elements which in some cases show cellular specificity, are capable of recombination with host cellular sequences, can activate and transduce normally quiescent genes, and in some cases encode or induce the production of transcriptional activating substances (Holland, et al., 1985) (Neel, et al., 1981)

Figure 4: Transcriptional initiation from the right LTR results in downstream transcripts which include message from cellular DNA.

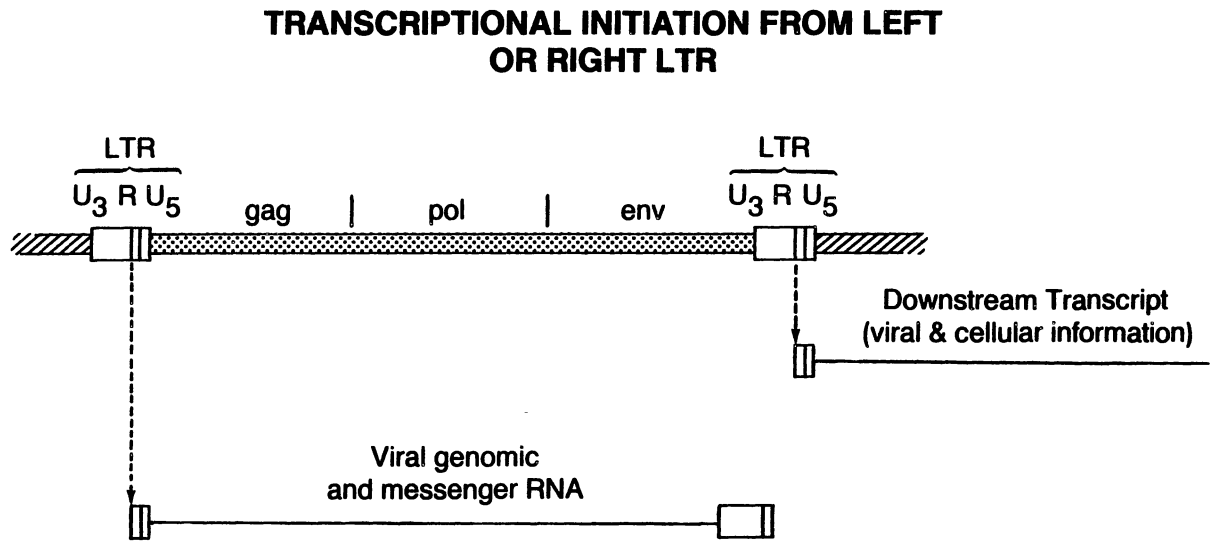

TRANSCRIPTIONAL INITIATION FROM LEFT
OR RIGHT LTR
LTR
U3 R U5
gag
pol
env
LTR
U3 R U5
Downstream Transcript
(viral & cellular information)
Viral genomic
and messenger RNA

Figure 4

(Derse, et al., 1985) (Gallo, 1985) (Hess, et al., 1985). In general, it seems likely that retroviruses possess not only an ability to infect and replicate within host cells but can potentially reshape the eucaryotic genome.

Oncogenic retroviruses can be grouped into two categories based on their biological properties. The acutely oncogenic isolates transform appropriate target cells in culture and rapidly induce neoplastic disease with high efficiency. The chronic viruses fail to transform cells in vitro and induce neoplasia with lower efficiency only after a long latency period. Elucidation of the genomic structure of both classes of oncogenic retroviruses has shown that the acutely transforming isolates are genetic recombinants that acquired DNA sequences from the host genome, typically with loss of viral genes required for replication (Rous sarcoma virus, RSV, is an exception) (Stehelin, et al., 1976) (Roussel, et al., 1979). These transduced host DNA sequences confer upon the recombinant virus the properties of cellular transformation in vitro and tumor formation in vivo and are referred to as oncogenic (onc) sequences. They are expressed in the host at high levels normally during growth and differentiation, are otherwise expressed at only low levels, and are thought to be involved in regulating development and embryogenesis (Bishop, 1985) (Bishop and Varmus, 1982). These cellular sequences (c-onc) are not viral genes or defective, endogenous proviruses since they are evolutionarily conserved, occupy a constant genetic loci in every member of a species, many have introns, and none have been found linked to endogenous proviruses (Hughes, et al., 1980) (Bishop and Varmus, 1982). By comparing the onc regions of acutely trans-

forming retroviruses (v-onc) and host DNA, over seventeen distinct cellular genes have been identified which apparently serve as progenitors of v-onc sequences (Table II) (Bishop, 1985). The proteins encoded by v-oncs are diverse and include tyrosine-specific protein kinases, nuclear binding proteins, a membrane glycoprotein which resembles the receptor for epidermal growth factor, and a homologue of platelet-derived growth factor (Hunter, 1984). Though it has been shown that the normal cellular onc is capable of in vitro transformation if experimentally expressed within the cell at high levels, and that the v-oncs are expressed at very high levels in the acutely oncogenic retroviral-infected, transformed cell, the actual role of the v-onc products in oncogenesis is largely unknown (Oskarsson, et al., 1980) (Hunter, 1984).

Though recent observations suggest that cellular oncogene transduction may occur in some chronic retroviral-induced neoplasms (Neil, et al., 1984) (Mullins, et al., 1984) (Levy, et al., 1984), in general, the weakly oncogenic viruses have been thought not to carry oncogenes. Instead, mechanisms for chronic retroviral oncogenesis that have been proposed include the selective recombination between endogenous proviral-related sequences and the integrating, exogenously acquired provirus (Evans and Cloyd, 1985) (Chattopadhyay, et al., 1982) (Lerner, 1978), the activation of a cellular oncogene by promoter insertion or through the effects of enhancer elements, both of which are contained within the LTR (Neel, et al., 1981) (Payne, et al., 1981) (Fung, et al., 1981), the alteration of transcriptional regulation or mutagenesis of gene sequences subsequent to proviral integration (Jenkins, et al., 1981) (Varmus, et al., 1981), the

TABLE II

THE PRODUCTS OF RETROVIRAL ONCOGENES*

TUMORIGENICITY	ONCOGENE	PROPERTIES OF PRODUCT
Sarcomas	src yes fps/fes ros fgr fms	TYR-specific protein kinase on/in plasma membrane
B-lymphoma	abl	
Erythroleukemias	erb-B	Membrane glycoprotein: truncated EGF receptor
Sarcomas	raf/mil	SER/THR protein kinase
Sarcomas	mos	Phosphoprotein in cytosol
Erythroleukemias and sarcomas	ras	GTP-binding protein/GTPase on/in plasma membrane
Carcinomas, sarcomas and myelocytic leukemia	myc	
Myeloblastic leukemia Osteosarcomas Sarcomas	myb fos ski	Nuclear Proteins
Sarcomas	sis	Cytoplasmic homologue of PDGF

*adapted from Bishop, 1985.

influence of retroviral encoded or induced trans-transcriptional activating substances on regulatory elements (Rosen, et al., 1985) (Derse, et al., 1985) (Felber, et al., 1985) (Sodroski, et al., 1985), the mitogenic effects of replicative gene products on a rare target cell (McGrath and Weissman, 1979), the uncoupling of the T-cell growth factor (TCGF) - TCGF receptor system (Wong-Stall, 1985), the increased risk of developing specific chromosomal translocations by expansion of the viral infected cell population (Rowley, 1983) (Erickson, et al, 1985), or, finally, the transduction and/or rearrangement of a cellular oncogene by the infecting retrovirus (Figure 5) (Neil, et al., 1984) (Mullins, et al., 1984) (Levy, et al., 1984).

Regardless of the mechanism, site-specific proviral integration is thought not to occur. Though the integrated provirus may frequently reside at a given site, this is thought to represent a process of selection, which favors the few integration events associated with neoplastic transformation (Gallo, 1985) (Neel, et al., 1981) (Hayward, et al, 1981) (Payne, et al, 1981) (Neil, et al., 1984). Depending on the neoplasm and species involved, the acquired proviral genome may commonly be complete or may be extensively deleted, yet retain a LTR and its influential promoter and enhancer elements (Yoshida, et al., 1985) (Hayward, et al., 1981). Further selective pressure in the form of immunological surveilance may then favor cells which contain only a deleted provirus acquired subsequent to retroviral infection, and which do not express viral proteins at the infected cell's surface.

Figure 5: Molecular models of retroviral oncogenesis. Some of the proposed events at the proviral level which are thought to contribute to cellular transformation are diagramatically represented here and include interactions between oncogenes (dark solid bar), newly formed viral genes (dotted bar), regulatory elements including the retroviral LTR (open bar), and cellular DNA (slashed bar). A. Recombinant-derived sarcoma viruses loose sequences encoding viral structural proteins but obtain an oncogene. B. Recombination between endogenous retroviral-related sequences and a retrovirus's structural genes may result in a retrovirus with enhanced leukemogenicity. MCF isolates of murine leukemia virus are an example. C. Enhanced transcription of downstream cellular DNA (c-onc) apparently occurs with avian leukosis virus. D. Recombination between proviral and cellular DNA, with the addition of a v-onc has been proposed for FeLV and is the general structure of the replication effective Rous sarcoma virus. E. Products encoded by extra retroviral genes or open reading frames (orf) may activate transcription not only at the LTR but also at other remote cellular regulatory sites as proposed for the human T-lymphotropic virus isolates, the bovine leukemia virus and the Visna virus of sheep.

MOLECULAR MODELS OF RETROVIRAL ONCOGENESIS

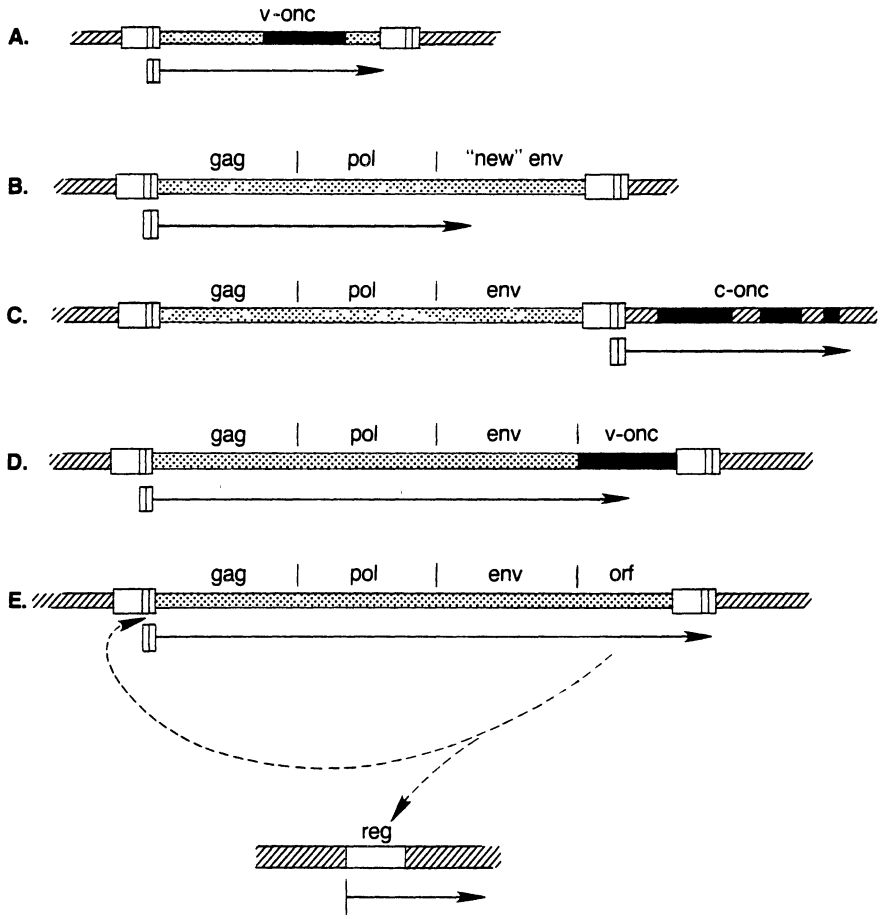


Figure 5

B. Leukemia, Anemia and Immunodeficiency-inducing Feline Retroviruses

Five different types of retroviral proviruses may be found within the feline cellular genomic DNA. The feline syncytial virus is a member of the Spumavirinae subfamily and causes inapparent infection of little clinical significance. At least two different types of endogenous proviruses exist; the RD114 and FeLV-related proviruses. In addition, two exogenous type C retroviruses of the Oncovirinae subfamily may leave integrated proviruses within the genome subsequent to viral infection. These exogenous retroviruses are classified, as described in the previous section, as acutely oncogenic or potentially oncogenic after a long period of latency, and have been named the feline sarcoma virus (FeSV) and the feline leukemia virus (FeLV), respectively.

1. RD114

The RD114 endogenous provirus encodes complete infectious virus with a xenotropic host range and was discovered following the passage of human rhabdomyosarcoma cells (the RD cell line) in the brain of a kitten (McAllister, et al., 1972). It is not infectious for feline cells and shows no detectable relationship to exogenous feline retroviruses (Livingston and Todaro, 1973). Enhanced expression of RD114 mRNA does occur in embryonic thymocytes, placenta, and some lymphomas (Niman, et al., 1977a,b).

2. FeLV-related Proviruses

Endogenous FeLV-related sequences, recognized by DNA hybridization and FeLV cDNA, are arranged as 8-12 discrete genetic elements in a nontandem fashion within normal feline DNA (Koshy, et al., 1980). Sequences homologous to the U_5 segment of the LTR are present in uninfected feline cells at much higher numbers, approximately 150 copies per cell (Casey, et al., 1981). Though exogenously acquired and endogenous proviruses show similarities in their centrally located regions (parts of gag, pol, env), the endogenous and exogenous proviruses are substantially unrelated in the U_3 region of the LTR. This divergent region may be used to identify exogenously acquired proviruses, and may be used as a probe for studying provirus number and location (Casey, et al., 1981). The transcriptional inactivity of many endogenous proviruses may be related to the absence of endogenous sequences which show homology to the U_3 segment of the LTR, where enhancer and promoter sequences for viral transcription occur (Casey, et al., 1981). In addition, endogenous sequences are variably deleted in the gag, pol and env regions, and this may render them incapable of encoding infectious virus (Benveniste, et al., 1975). The feline endogenous retroviral-related proviruses appear to behave as stable mendelian elements with the number and distribution of proviruses common in all tissues of an individual, but with evolutionary instability so that patterns vary greatly from one individual to another. It is less clear to what extent the endogenous retroviral-related proviruses of the cat are

expressed and whether their expression is essential for cellular growth and development.

3. Feline Sarcoma Viruses

The feline sarcoma viruses (FeSV) are rare genetic recombinants that have transduced cellular oncogene sequences in the feline leukemia virus (FeLV) infected cat (Snyder and Theilen, 1969) (Gardner, et al., 1970) (McDonough, et al., 1971) (Hardy, et al., 1982). At least six cellular DNA sequences, proposed as oncogenes, have been shown to recombine with the infectious FeLV (Table III). In each case, the newly acquired oncogene (v-onc) combines with the viral genome at the expense of sequences within the gag, pol or env viral structural genes (Figures 6 and 7) (Sherr, et al., 1984). Often, virtually all of the viral pol gene and much of the env gene have been deleted. Since FeSV genomes lack sequences required for replication, they can only be propagated in the presence of a "helper" virus which provides the needed functions. In natural FeLV infections where FeSV arises as a recombinant, the defective sarcoma virus genome is replicated in complex with, and packaged into virions encoded by the "helper" (FeLV) virus. Cells infected with FeSV alone acquire the provirus, express viral RNA, undergo morphological transformation apparently as a result of v-onc expression, but cannot produce infectious viral particles (Sarma and Log, 1971). The sarcoma viruses can transform fibroblasts and epithelial cells in culture, and induce multicentric subcutaneous fibrosarcomas in young animals after a 2-3 week latency period. Malignant melanomas of the skin or uvea, and rhabdomyosarcomas have rarely developed following

TABLE III

ONCOGENES OF FELINE SARCOMA VIRUSES*

Viral Oncogene	FeSV Strain	Viral Polyprotein
v-fes	Snyder-Theilen (ST)	P85gag-fes
	Gardner-Arnstein (GA)	P110gag-fes
	Hardy-Zuckerman 1 (HZ-1)	P95gag-fes
v-abl	Hardy-Zuckerman 2 (HZ-2)	P95gag-abl
v-fgr	Gardner-Rasheed (GR)	P70gag-fgr
v-fms	Susan McDonough (SM)	gP180gag-fms
v-sis	Parodi-Irgens (PI)	P75gag-sis
v-kit	Hardy-Zuckerman 4 (HZ-4)	unreported

*adapted from Sherr, 1984; Besmer, 1983.

Figure 6: Feline retrovirus proviruses and their cellular oncogenes. Relative size, in kilobases (kb), of feline proviruses including the endogenous RD114 provirus, as well as exogenously acquired feline leukemia virus proviruses and recombinants of FeLV with cellular DNA known as feline sarcoma viruses. Oncogenes are denoted by a dark solid bar, viral genes as dotted bars and the LTR as an open bar. Diagrams are adapted from restriction endonuclease maps of feline proviruses.

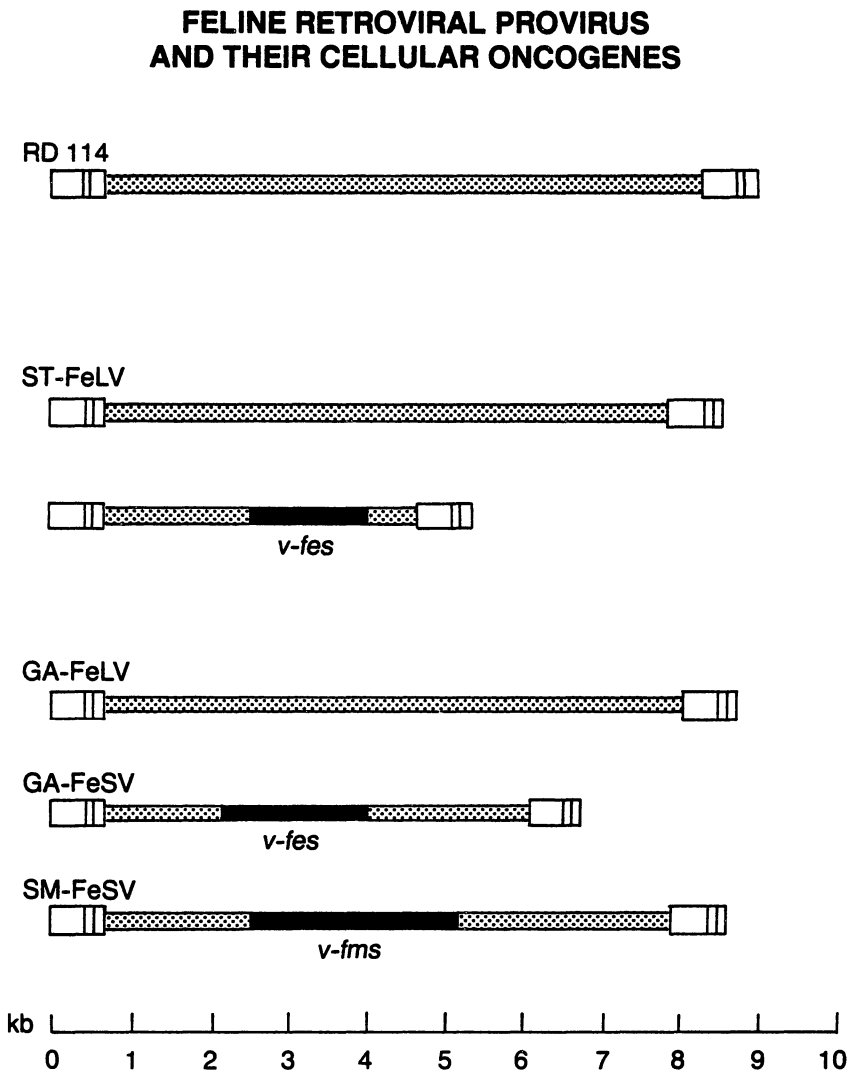

FELINE RETROVIRAL PROVIRUS
AND THEIR CELLULAR ONCOGENES
RD 114
ST-FeLV
v-fes
GA-FeLV
GA-FeSV
v-fes
SM-FeSV
v-fms
kb
0
1
2
3
4
5
6
7
8
9
10

Figure 6

Figure 7: Deletions in the exogenously acquired FeLV proviral DNA and the acceptance of new cellular DNA may result in the formation of a recombinant provirus with acutely oncogenic properties; feline sarcoma virus (FeSV).

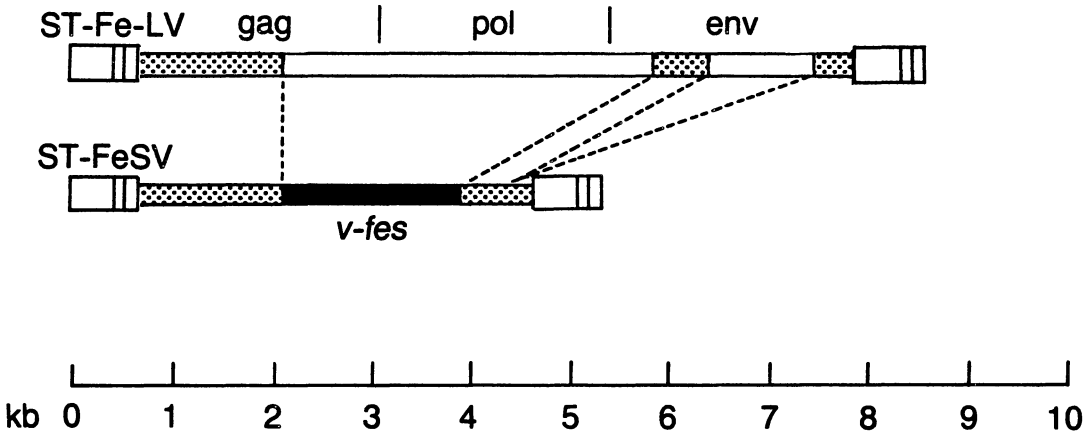


Figure 7

experimental innoculation of FeSV isolates (McCullough, et al., 1972).

4. Feline Leukemia Virus

The feline leukemia virus (FeLV) is a contagious type C retrovirus of the subfamily Oncovirinae whose spread can be reduced by eliminating contacts between infected and uninfected animals (Jarrett, et al., 1964 a,b) (Hardy, et al., 1969, 1973) (Essex et al., 1975). In about half of the natural feline population, evidence of FeLV exposure is demonstrable by the presence of anti-viral antibodies, but viremia is evident in only 1-2%. The incidence of serological evidence of virus exposure and the evidence of viremia increase to approximately 70% and 28% in multiple cat households, respectively (Essex, et al., 1975) (Hardy, 1980). Isolation of FeLV is possible from the infected cat's salivary secretions and to a much lesser extent from the urine (Hardy, 1980) (Francis, et al., 1977). Cogenital transmission is possible, but a minor mode of viral spread (Cotter, et al., 1975) (Hoover, et al., 1983).

Since the most common vehicle of transmission is saliva, FeLV initially replicates in the lymphoid tissues of the head and neck (Francis, et al., 1977) (Gardner, et al., 1971). Within a few days, viral antigen, primarily the major internal structural protein of the viral core (p27), becomes detectable by indirect immunofluorescence (IFA) within a small population of peripheral blood mononuclear cells (Figures 8 and 9) (Rojko, et al., 1979). This initial mononuclear cell-associated viremia leads to extensions of virus replication to the other lymphoid organs including the thymus, spleen, lymph nodes,

Figure 8: Diagrammatic representation of a budding feline leukemia virus. Precursor polypeptides (Pr) aggregate and are modified near the plasma membrane and give rise to virus structural proteins (p), some of which are glycosolated (gp). Proteins which play major roles in serological assays are marked with an astericks. The gp70 molecule participates in virus neutralization, interference and virus-infected cell cytotoxic events. The p27 molecule contributes in a major way to retroviral-associated antigenemias. The trans-envelope bound p15E molecule has been implicated in FeLV-mediated immunodeficiency.

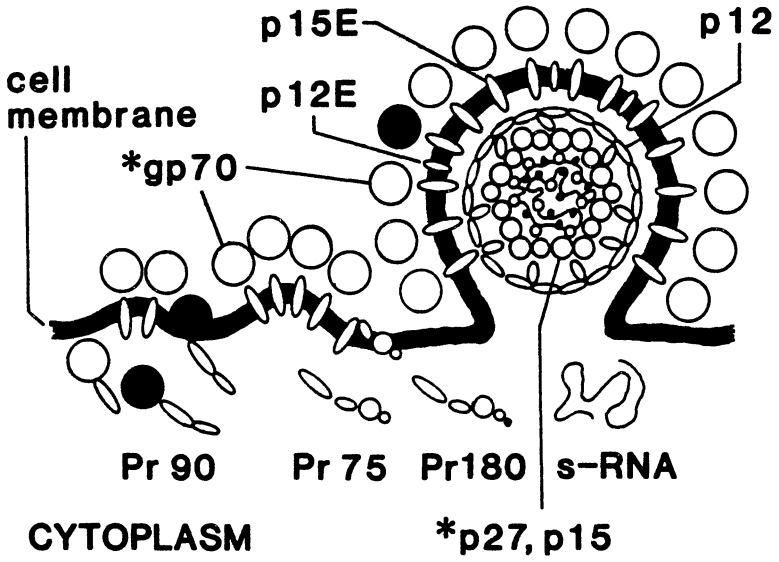

p15E
p12
cell
membrane
p12E
*gp70
Pr 90
Pr 75
Pr180
s-RNA
CYTOPLASM
*p27, p15

Figure 8

Figure 9: The systemic cycle of feline leukemia virus replication. An idealized sequence of the systemic foci of FeLV replication, if immunologically unchallenged, is diagramatically represented here. Numbers indicate days post-exposure. Pharyngeal lymphoid tissue and epithelia (1-7); draining lymph nodes, mononuclear cell-associated viremia (1-14); systemic lymphoid tissue (3-12); bone marrow, intestinal crypt epithelium (7-21); marrow-origin viremia (14-28); epithelia (28-56).

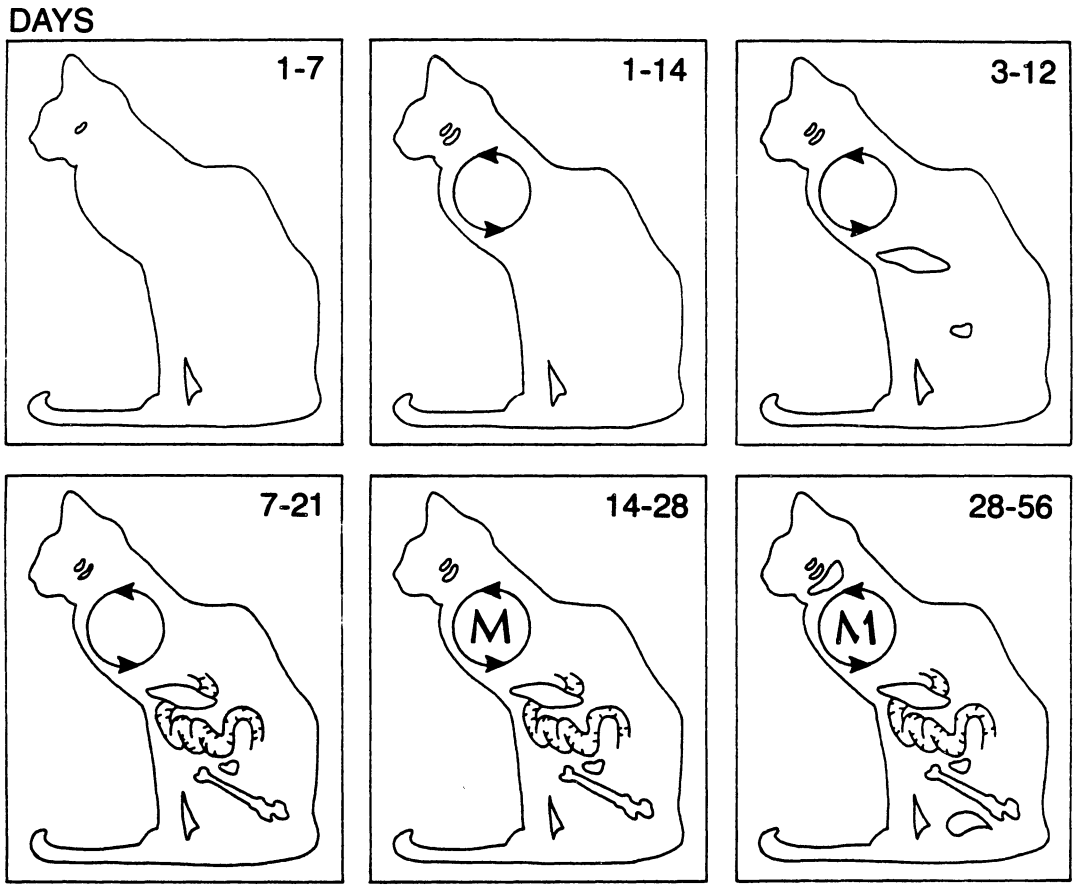


Figure 9

and Peyer's patches. In the young animal twenty-one days after exposure, the hematopoietic cells of the bone marrow and intestinal crypt epithelial cells become positive by IFA for viral antigen, and a marrow-derived primarily polymorphonuclear cell-associated viremia develops. The detection of p27 in circulating neutrophils and platelets by fixed cell IFA has a 90% correlation with recovery of infectious virus from plasma and is considered diagnostic of viremia (Hoover, et al., 1977) (Hardy, et al., 1973). Subsequent to the establishment of marrow derived viremia, multiple mucosal and glandular epithelial tissues become infected and include the oropharynx, nasopharynx, larynx, trachea, stomach, pancreas, urinary bladder and salivary glands (Figure 9).

Viral infection is often more restricted in the older cat with the initial mononuclear cell associated viremia lasting several weeks and fequently followed by the elimination of virus and virus infected cells from the bloodstream. The elimination of viremia is temporally associated with the production of antibodies directed at determinants on the major viral envelope glyproprotein, gp70. Animals that develop sufficient virus neutralizing antibody titers generally remain immune to that FeLV subgroup, though viremia and antibody to gp70 can co-exist. Different epitopes of the gp70 molecule are involved in virus interference, virus neutralization and the lysis of virus infected cells by antibody and complement (Lutz, et al., 1980) (Grant et al., 1983). Cell mediated responses may play important adjunct roles to this antiviral humoral response but to date have received little attention. Resistance to experimental virus infection is apparently influenced by the age of the animal as well

as the strain of virus innoculated, and may be attributable in part to the degree of permissiveness by macrophages and lymphocytes for FeLV replication (Hoover, et al., 1976, 1981) (Rojko, et al., 1979, 1981).

Elimination of virus infected cells and virus from the blood, as evidenced by a negative IFA test, does not necessarily terminate the exogenously acquired proviruses' influence. An enzyme-linked immunosorbent assay (ELISA) of feline plasma detects circulating p27 and correlates with viremia only 68% of the time. Reasons for these discordent serological results have been proposed, but not resolved, and include an early phase of infection, a regressive viremia, a persistent site of replication from a remote epithelial foci, integration of an incomplete provirus with continuous antigen shedding, and derepressed endogenous FeLV-related sequences (Lutz, et al., 1983). It appears likely though that in at least some cases, cells from FeLV immune cats are persistently integrated with exogenously acquired FeLV proviruses (Rojko, et al., 1982) (Madewell and Jarrett, 1983). Persistent, nonproductive, exogenously acquired proviruses could account not only for antigenemias in the absence of viremia, but also for persistently high antiviral antibody titers, relapsing viremias, and the occurrence of FeLV-negative leukemia-lymphomas (Lutz, et al., 1980) (Essex, et al., 1975) (Post, et al., 1980) (Rojko, et al., 1982).

Subcloning of FeLV field isolates has revealed the existence of three serologically distinct FeLV subgroups which are capable of replication in cultured feline embryo cells. These FeLV subgroups (A. B, C) have been identified by interference and neutralization

tests which reflect properties of the envelope glycoproteins, gp70(s) (Sarma and Log, 1973). Studies indicate that viruses of subgroup A are monotypic, always present in natural isolates, and are the most readily transmitted FeLV subgroup. In contrast, FeLV B and C viruses are present in natural isolates only in association with A, have expanded *in vitro* host ranges, and demonstrate antigenic polymorphism and cross reactivity. It is these findings, and the reported *env* gene sequence homologies between FeLV-B and recombinant MCF murine leukemia virus isolates, that have led to the proposal that B and C viruses are recombinants between FeLV-A and endogenous proviral sequences related to FeLV (Elder and Mullins, 1983). Attempts to attribute specific clinical disease to a single or a combination of FeLV subgroups have been inconclusive, but evidence suggests that FeLV-C is frequently associated with neoplasia and that immunity to this subgroup may underlie resistance to FeLV-induced tumors (Vedbrat, et al., 1983) (Grant, et al., 1983). Additionally, partial nucleotide sequence analysis of viruses classified within a single subgroup reveals significant differences among viruses (Rosenberg, et al., 1980).

Persistent viremia is recognized when sequential IFA tests of fixed leukocytes are positive over a twelve week period of time. Persistently infected cats are at a much higher risk of developing clinical disease then uninfected cats (Essex, 1982). The most frequent sequelae to persistant infection is an immunodeficient state with multiple intercurrent infections or immune-mediated diseases. Aregenerative anemia is considered the second most common sequelae, and lymphoid leukemia-lymphoma the third.

5. Induction of an Immunodeficient State

Persistently retroviremic cats often have suppressed lymphocyte blastogenic responses to T-cell mitogens (Cockerell, et al., 1976), delayed and prolonged antibody responses to a T-cell specific synthetic polypeptide (Trainin, et al., 1983), prolonged allograft rejection times (Perryman, et al., 1972), reduced lymphocyte membrane con A receptor mobility (Dunlap, et al., 1979), exhibit varying degrees of hypocomplementia, thymic atrophy and depletion of the paracortical zones of lymph nodes (Kobilinsky, et al., 1979) lack or only minimally convert from a predominantly IgM to a IgG antiviral antibody response, and have variably elevated levels of circulating immune complexes (CIC) consisting of viral antigen and anti-viral antibodies (Snyder, et al., 1982) (Day, et al., 1980). In addition, persistently viremic cats are at high risk for the development of multiple intercurrent diseases such as enteritis, gingivitis, pneumonia, bacterial sepsis, infectious peritonitis of coronaviral origin (FIP), parastitic infestations such as Hemobartonella felis or Toxoplasma gondi, glomerulonephritis and autoimmune hemolytic anemia (Cotter, et al., 1975) (Essex, 1982).

Although little is known about the way in which FeLV initiates immunosuppression, the virus may mediate immunomodulating events independent of cellular infection since UV-light inactivated FeLV (FeLV-UV) and certain FeLV structural proteins (p15E) have been shown to impair lymphocyte proliferative responses and membrane receptor capping to con A in vitro (Hebebrand, et al., 1977) (Mathes, et al., 1979). UV-inactivated FeLV has been shown to reduce the synthesis of

feline gamma-like interferon (γ-IFN) by lymphocytes from normal cats (Engelman, et al., 1985). and in murine or human systems of assay FeLV-UV and p15E have been shown to reduce the synthesis of interleukin-2 (IL-2) and responsiveness by lymphocytes to IL-2 (Orosz, et al., 1985) (Wainberg, et al, 1983, 1984) (Copelan, et al., 1983). Some studies have suggested that the unresponsiveness of lymphocytes to mitogen may be reversed with the addition of exogenously derived T-cell growth factor-like (interleukin-2-like) supplement (Wainberg, et al., 1983, 1984), but other studies disagree (Orosz, et al., 1985). Proliferation of cells in mixed lymphocyte cultures and the generation of alloantigen-induced cell-mediated lympholysis (CML) is also impaired by FeLV-UV in murine systems of assay (Orosz, et al., 1985). In addition, FeLV-UV activates the classical scheme of complement and can generate FeLV-specific suppressor cells in vitro (Langweiler and Cockerell, 1982) (Langweiler, et al., 1983).

The concept of the potential role of retroviral envelope proteins in the pathogenesis of immunosuppression is additionally supported by two observations. The transmembrane envelope protein of HTLV-I and HTLV-II, gp21E, shares significant amino acid sequence homology with p15E of murine and feline leukemia viruses as well as with the transmembrane proteins of the bovine leukemia virus and Rous sarcoma virus, and the full-length endogenous retroviral provirus of humans. Further, it has recently been reported that a peptide synthesized to correspond to a portion of this region of sequence homology inhibits the proliferation of both murine and human lymphocytes in mixed lymphocyte cultures (Cianciolo et al., 1985).

FeLV-UV and purified p15E apprently have no affect on preformed CML, interleukin-1 (IL-1), IL-2, or γ-IFN nor do they interfere with receptors for IL-1, IL-2 or γ-IFN (Orosz, et al., 1985) (Engelman, et al., 1985) (Copelan, et al., 1983). In addition, monocyte-macrophage functions including chemotaxis, phagocytic and bactericidal activities, and the synthesis of interleukin-1 are not apparently impaired by FeLV-UV or p15E in vivo or in vitro (Hoover, et al., 1981) (Copelan, et al., 1983). Cytopathic effects which contribute to immunodeficiency, as demonstrated with human T-lymphotropic virus-III (HTLV-III) isolates which selectively deplete T-lymphocytes of the helper subpopulation thereby contributing to the state of immunodeficiency in the human patient with the acquired immunodeficiency syndrome (AIDS), have not been demonstrated with any isolate of the feline leukimia virus (Klatzmann, et al., 1984) (Lane and Fauci, 1985).

Interest in the concept that inactivated FeLV or FeLV structural components could abrogate immunological responsiveness was initiated by FeLV vaccine trial studies where virus structural components were used as the immunogen (Schaller, et al., 1977). Subsequent work has focused attention on the highly hydrophobic, trans-envelope bound p15E molecule and its possible interaction with the lymphocyte surface membrane. Since the prostaglandin and cyclic nucleotide systems are both closely associated with cell membrane-mediated events and linked to the immune system (Stenson and Parker, 1980), p15E abrogated immune responsiveness may be attributable to interference with one of these systems. Increased activity of prostaglandins of the E series, abrogation of the calcium calmodulin-dependent

activation of adenylate cyclase, or interference with membrane microtubular functions are potential sequalae to cell membrane - p15E interactions and may explain in part the FeLV induced state of deficient lymphocyte responsiveness in vitro.

6. Anemiagenesis

Persistently retroviremic or experimentally FeLV-inoculated cats frequently present with aregenerative anemia with hematocrits between 5-15% and the bone marrow myeloid:erythoid (M:E) ratio shifted from the normal 1.6 to as high as 10.4 (Hoover, et al., 1974) (Onions, et al., 1982) (Boyce, et al., 1981) (Cotter, et al., 1975). The induced anemia has been described as normocytic-normochromic (Cotter, et al., 1975) or macrocytic-normochromic (Weiser and Kociba, 1983). Affected cats have normal erythrocyte survival times, but markedly diminished plasma radioiron half times (Madewell, et al., 1983). Certain subclones of FeLV subgroups, especially FeLV-C subclones, have been implicatd as etiological agents for pure red cell hypoplasias in the cat (Testa, et al., 1983) (Onions, et al., 1982) (Jarrett et al., 1984).

Colony forming assays for feline erythroid and myelomonocytic precursors have been used to investigate the pathogenesis of FeLV-induced aregenerative anemia. Primitive and more mature erythroid precursors, designated BFU-e and CFU-e (burst- and colony- forming units - erythroid) are rapidly depleted in the FeLV-innoculated cat prior to the onset of anemia, while the proportion of the myeloid precursors CFU-c (granulocyte-monocyte-colony-forming units) remains normal (Onions, et al., 1982) (Testa, et al., 1983). These

erythrosuppressive effects may be attributable to virus structural components since similar findings have been reported when inactivated FeLV or p15E are present during the period of culture (Wellman, et al., 1984).

7. Leukemogenesis

Three independent, but simultaneously reported studies recently found FeLV proviruses containing the oncogene v-myc in the DNA of a small proportion of cats with FeLV-positive lymphomas (Neil, et al., 1984) (Mullins, et al., 1984) (Levy, et al., 1984). These reports suggest that transduction of the v-myc oncogene by exogenously acquired FeLV proviruses may be possible subsequent to a recombinant event, and imply that this oncogene carrying recombinant may be transmitted contagiously between cats.

Although serological and epidermiological evidence suggests FeLV involvement in the development of the 30% of feline lymphomas which do not actively produce FeLV (Hardy, et al., 1980) (Rojko, et al., 1982), viral proteins are absent and sequences homologous to the U_3 portion of the exogenously acquired proviral LTR are not present in the tumor cells (Casey, et al., 1981).

CHAPTER II

CLINICOPATHOLOGIC RESPONSES IN CATS WITH FELINE LEUKEMIA VIRUS-ASSOCIATED LEUKEMIA-LYMPHOMA TREATED WITH STAPHYLOCOCCAL PROTEIN A

A. Summary

Purified protein A from Staphylococcus aureus Cowan I was injected intraperitoneally or was incorporated in filters ex vivo through which plasma from cats with feline leukemia virus (FeLV) associated leukemia-lymphoma was passed. Prior to treatment, 65% of the FeLV infected cats were anemic and 70% were thrombocytopenic. Concomitant infections or immune-mediated disease were common. During treatment, 50% of the cats with FeLV associated disease improved objectively with normal post-treatment hematocrits, thrombocyte and leukocyte counts, disappearance of dysplastic hematologic elements and correction of marrow dyscrasias. A 33% response to treatment occurred in cats with unequivocal manifestations of malignant disease and was characterized by reductions in tumor size and marrow and peripheral blood neoplastic cell populations. Clearance of FeLV viremia was documented in 28% of the treated cats. The several possible mechanisms by which treatment with staphylococcal protein A causes reduction in the extent of malignant disease are considered.

B. Introduction

Staphylococcal protein A (SpA), a cell wall constituent of S. aureus Cowan I (SAC), is a single polypeptide with three homologous regions which have the capacity to bind the Fc region of IgG and to combine rapidly and with great affinity with immune complexes (Kessler, 1975) (Goding, 1978). Other regions of the molecule initiate proliferative responses of T or B lymphocytes and stimulate elaboration of soluble products by lymphocytes including interferon and leukocytic migration inhibition factor (Sumija et al., 1982) (McCool et al., 1981). Extracorporeal perfusion of plasma from patients or animals with malignancy over whole SAC organisms or through filters containing purified SpA has led to reductions in tumor size and to decreased levels of circulating immune complexes (CICs) (Ray, 1982). This treatment has also been shown to induce the appearance of cytotoxic antibodies directed toward the neoplastic cells (Ray, 1982). Regression of malignancy has occurred following this treatment in patients with metastatic colon carcinoma (Bansal et al., 1978) (Ray et al., 1982a) and breast ductal cell adenocarcinoma (Terman et al., 1981), in dogs with anaplastic mammary adenocarcinoma (Terman et al., 1980), lymphoma or rectal carcinoma (Ray, 1982), and in rats with dimethylbenzanthracene (DMBA)-induced mammary adenocarcinoma (Ray et al., 1982b). However, many of the reports presented have been criticized for lack of information concerning the patients or animals with the malignancy and for inadequacy of controls. Further, they have been somewhat difficult to interpret because in

many cases other more conventional forms of anti-cancer treatment were also administered (Hellstrom & Hellstrom, 1981).

The feline leukemia virus (FeLV) is a contagious retrovirus of cats that causes leukemia-lymphoma and various non-neoplastic diseases including immunosuppression and aregenerative anemia (Hardy, 1980a). Exposed cats may develop an adequate neutralizing antibody titer to FeLV envelope antigens and may eliminate the virus (Hardy, 1980b). An inadequate anti-FeLV immunologic response occurs in about 28% of infected cats and results in a persistent viremia (Hardy, 1980b). Persistently infected cats often have suppressed blastogenic responses to T cell mitogens (Cockerell et al., 1976b), suppressed antibody responses to synthetic polypeptides (Trainin et al., 1983), prolonged allograft rejection times (Olsen et al., 1981), and they exhibit varying degrees of hypocomplementemia (Kobilinsky et al., 1979). Immunosuppression and secondary infections or immune-mediated disease is the most frequent sequelae of persistent FeLV infection and accounts for a majority of the FeLV-related deaths (Hardy, 1982a). Leukemia-lymphoma is the most common neoplasm of cats and accounts for 90% of the hematopoietic malignancies (Moulton, 1978). Feline lymphoblastic leukemia has been proposed as a model for human acute lymphoblastic leukemia (Cotter & Essex, 1977). In addition, myeloproliferative disease is a more frequent disorder in cats than in other domestic animals and is often associated with FeLV infection (Harvey, 1981).

Extracorporeal perfusion of plasma from cats with FeLV associated leukemia-lymphoma over SAC has resulted in the clearance of FeLV viremia and in clinical improvement of some of the cats (Jones et

al., 1980) (Snyder et al., 1984). However, many of these cats were exposed to a dose of whole-body irradiation prior to immunotherapy. Further, the clinicopathologic status of cats prior to treatment and consequences of treatment were inadequately characterized. Similar clearance of viremia and clinical improvement after the perfusion of plasma through filters containing SpA or after the intraperitoneal injection of SpA has occurred without adjunct therapy in cats treated in our laboratories (Day et al., 1984) (Liu et al., 1984a) (Liu et al., 1984b). It is the purpose of the present report to provide a description of the clinicopathologic status of cats prior to treatment and to record details of responses which occurred during treatment with staphylococcal protein A. In addition, the responses of healthy cats to similar manipulations with SAC, SpA columns or injections of SpA are described.

C. Material and Methods

Clinically ill, FeLV-infected cats were referred by Oklahoma veterinarians to the Department of Pathology, College of Veterinary Medicine, Oklahoma State University, and to the Oklahoma Medical Research Foundation. All cats were characterized by cytologic examination of bone marrow, lymph node, tumor and peripheral blood aspirates, and on the basis of results of the indirect immunofluorescent antibody (IFA) test for FeLV in leukocytes (Hardy et al., 1973b) (Antibodies, Inc., Davis, CA), and the enzyme-linked immunosorbent assay (ELISA) for the detection of FeLV antigen in serum (Mia et al., 1981) (Pitman-Moore, Inc., Washington Crossing, NJ). Tumors were measured in at least two diameters by ultrasonography employing

a Mark II Pulsed Echo System Model 600B (Advanced Technology Laboratories, Bellevue, WA). Thoracic and abdominal radiographs, complete blood counts, serum chemical profiles, serum protein electrophoresis and urinalyses were performed. Cytologic preparations were stained with Wright's Dip-Stat (Medi-Chem, Inc., Santa Monica, CA).

Cats were treated twice weekly with ex vivo perfusion of their plasma through filters containing purified SpA (Pharmacia Fine Chemicals, Uppsala, Sweden), or by direct intraperitoneal injection of SpA. In the case of treatment by ex vivo plasma filtration, for each cat a filter column containing SpA covalently bound to a zetachrome matrix of cellulose (AMF/Specialty Materials Group, Talcotville, CN) was used to filter plasma clarified from 10-30 ml of heparinized blood twice a week. At each treatment, 1.0 ml aliquots of the pre-filtered and post-filtered plasma were frozen for future analysis. The remaining filtered plasma and autologous cells were returned to the cat. In the case of treatment by SpA injection, cats were injected intraperitoneally with 20 µg/2.75 kg body weight of purified SpA twice a week. Pre- and post-treatment plasma samples were retained for analysis. Control cats given SpA intraperitoneally or intravenously were injected with either 100 ng, 10, 20 or 50 µg/2.75 kg body weight twice weekly. Except for six cats with bone marrow dyscrasia and/or dysplasia, FeLV infected cats without unequivocal manifestation of hematopoietic or lymphoreticular malignancy were not treated. Adjunct therapy consisted in some cases of isotonic fluids, and in cases with concomitant infections, antibiotics. In five cats transfusions of washed leukocyte-poor packed red blood cells were

given. Transfused blood was washed and filtered through cotton wool (Imugard IG500, Terumo Corp., Tokyo, Japan) prior to administration. During treatment hemograms and serologic examinations were performed weekly and serum chemistry, cytologic. sonographic and radiographic examinations were repeated every 3rd or 4th treatment. Cats were given up to 32 treatments. Cats which died or were sacrificed were subjected to thorough post-mortem examination, tissues were fixed in 10% buffered formalin, embedded in paraffin, sectioned at 8 μm, and stained with hematoxylin and eosin. Additional sections of some tissues were stained with either periodic acid-Schiff or methenamine silver.

D. Results

Forty-three cats were evaluated. Of these, 17 served as controls, 26 were FeLV infected, 10 had lymphoma, 5 leukemia, 5 myeloproliferative disorders, and 6 had other forms of abnormality which we grouped together as bone marrow dyscrasia-dysplasia. All FeLV infected cats presented with advanced disease and many had a variety of concurrent infections or immune-mediated disease. Four of the 26 FeLV infected cats (113,117.105.116) had concurrent infections with corona-virus and had titers against antigens of this virus of greater than 400. Cats of either sex ranging in age from 6 months to 7 years were represented. They were primarily domestic short- or long-haired cats. Most of the common morphologic forms of feline leukemia-lymphoma and myeloproliferative disorders were represented (Table IV). Cats given more than three treatments employing SpA are listed in Table V.

TABLE IV

FELINE LEUKEMIA VIRAL INFECTED CATS ADMINISTERED IMMUNOTHERAPY USING STAPHYLOCOCCAL PROTEIN A

Cat Classification	Treatment[a]	Concurrent Disease[b]	Adjunct Therapy[c]
Lymphoma[d]			
112 Multicentric	EVSpA(2)	ARA, pleuritis, peritonitis	-
109 Alimentary	EVSpA(1)	ARA	-
106 Mediastinal	EVSpA(10)	ARA, hemob.	OT(8-10)
111 Mediastinal	EVSpA(1)	ARA	-
Leukemia[e]			
102 Lymphoblastic	EVSpA(24)	ARA, hemob., cystitis	OT(18-24); Amp (12-15); T(12, 13,18,24)
110 Lymphoblastic	EVSpA(3)	Bronchopneumonia	
103 Lymphocytic	EVSpA(6)	ARA	

TABLE IV (Continued)

Myeloproliferative disorder[f]			
113 Erythroleukemia	EVSpA(5)	ARA	T(1,3)
117 Erythroleukemia	EVSpA(9)	FIP	T(7)
152 Erythroleukemia	IPSpA(20)	ARA	-
121 Granulocytic	EVSpA(2)	ARA, nephritis	T(0)
119 Undiff. stem cell	EVSpA(3)	ARA, myelofibrosis	T(1,2)
Other Abnormalities[g]			
101 Dyscrasia-dysplasia	EVSpA(20)	ARA	-
105 Dyscrasia-dysplasia	EVSpA(20)	-	-
116 Dyscrasia-dysplasia	EVSpA(20)	AIHA, hemob., FIP	-
153 Dyscrasia-dysplasia	IPSpA(23)	-	-
154 Dyscrasia-dysplasia	IPSpA(30)	-	-
155 Dyscrasia-dysplasia	IPSpA(32)	hemob.	-

TABLE IV (Continued)

Excluded from Table IV are 8 cats with leukemia-lymphoma that were evaluated but died prior to the administration of treatment with SpA.

[a] Purified Protein A (SpA) from Staphylococcus aureus Cowan I (SAC) was injected intraperitoneally (IP) or was incorporated in filters ex vivo (EV) through which plasma was passed. The number of treatments administered is indicated in parentheses.

[b] ARA - aregenerative anemia; hemob. - Hemobartonella felis, an eperythrocytic richettsiae; FIP - feline infectious peritonitis, a corona-viral immune-mediated disease; AIHA - autoimmune hemolytic anemia.

[c] OT - tetracycline per os; Amp - ampicillin I.M.; T - transfusion of washed leukocyte-poor, packed red blood cells. Antibiotics and transfusions were administered following the treatment indicated in parentheses.

[d] Cats with lymphoma were classified based on the anatomical location of the tumor.

[e] Cats with leukemia were classified based on the predominant cell type.

[f] Cats with myeloproliferative disorders were classified based on the predominant cell type.

[g] FeLV infected cats without frankly diagnosable malignancy but with bone marrow dyscrasia and/or dysplasia.

TABLE V

OBJECTIVE RESPONSES IN CATS GIVEN MORE THAN THREE TREATMENTS USING PROTEIN A

Cat	Time Period[a] (Days)	Treatment[b]	Reduction in[c] Malignancy	IFA/FeLV[d] Post-treatment	% Change in[e] Body Weight	Hematocrit (%)[f] Pre- and Post-Treatment
106	47	EVSpA(10)	+++	--	-29	30, 10
102	124	EVSpA(24)	++[g]	+	-33	22, 14
103	26	EVSpA(6)	+++	+	-18	23, 7
113	21	EVSpA(5)	--	+	-14	11, 7
117	46	EVSpA(9)	--	+	+17	12, 7
152	>365(S)	IPSpA(20)	+++	ND	+17	22, 35
101	>730(S)	EVSpA(20)	+++	--	+30	10, 35
105	>365(S)	EVSpA(20)	+++	+	+24	20, 37
116	248	EVSpA(20)	--	--	-5	32, 20
153	>270(S)	IPSpA(23)	++	+	+20	19, 29
154	>365(S)	IPSpA(30)	++	--	+8	24, 29
155	>330(S)	IPSpA(32)	++	--	+13	8, 20

TABLE V (Continued)

---

[a]Number of days the cat survived from the time of presentation. (S) = surviving.
[b]See Table IV.
[c]Reduction in tumor size or bone marrow neoplastic population during treatment was judged mild +, moderate ++, or marked +++. No response to treatment is indicated by --. In the case of cats with bone marrow dyscrasia-dysplasia, +, ++ and +++ indicate clearance of dysplastic cells and the correction of dyscrasias.
[d]All cats were positive by IFA for FeLV pre-treatment (except cat 113). + indicates persistent FeLV viremia post-treatment. -- indicates clearance of FeLV viremia during treatment. ND = not done.
[e]Body weight at the time of the first and last treatments were compared to determine % increase (+) or decrease (-).
[f]Hematocrit prior to the treatment period and after the last treatment.
[g]Frank and extensive lymphoid neoplasia underwent dramatic regression but was followed by successive changes in morphology of the expression of malignant disease reflecting myeloproliferative features.

1. Lymphoma

Of the 10 cats with anterior mediastinal alimentary (jejunum-illeum), or multicentric lymphoma, 6 presented with aregenerative anemia and thrombocytopenia, and 4 cats were febrile. All cats were thin and lethargic. Six cats died during the pretreatment screening period. Of the 4 cats with lymphoma that were given immunotherapy using SpA, 3 died before the 3rd treatment. Cat 106 with lymphoma was administered 10 treatments of extracorporeal perfusion of plasma through filters bearing SpA.

Cat 106 presented with dyspnea, dysphagia and an anterior mediastinal mass 4.0 x 6.5 cm, marked pleural effusion, low serum albumin (1.6 g/dl) and elevated serum calcium (12.2 mg/dl) (Figures 10,11). Aspirates of this mass consisted predominantly of large lymphoid cells with scant cytoplasm and large nuclei containing multiple nucleoli. 200 ml of a modified transudate consisting of 89% or more lymphoid cells were removed from the thorax between treatments 1 and 4. Clearance of FeLV viremia, as indicated by a negative IFA test, occurred after the 6th treatment and was documented weekly from the 6th through the 10th treatment. Following the 7th treatment, the mediastinal mass was no longer demonstrable, although a thickened mediastinum and a small amount of effusion remained in the anterior thorax (Figures 12,13). Concurrent with this reduction in tumor size, the elevated serum calcium concentration was also reduced (11.5 mg/dl). Following the 8th treatment, the cat developed a rapidly progressive aregenerative anemia. A 29% reduction in body

Figure 10: Left lateral thoracic radiograph of cat 106 with anterior mediastinal lymphoma prior to treatment. Marked pleural effusion is evident.

Figure 11: Pretreatment anterior thoracic sonogram of cat 106. A cellular mediastinal mass measures 4.0 x 6.2 cm.

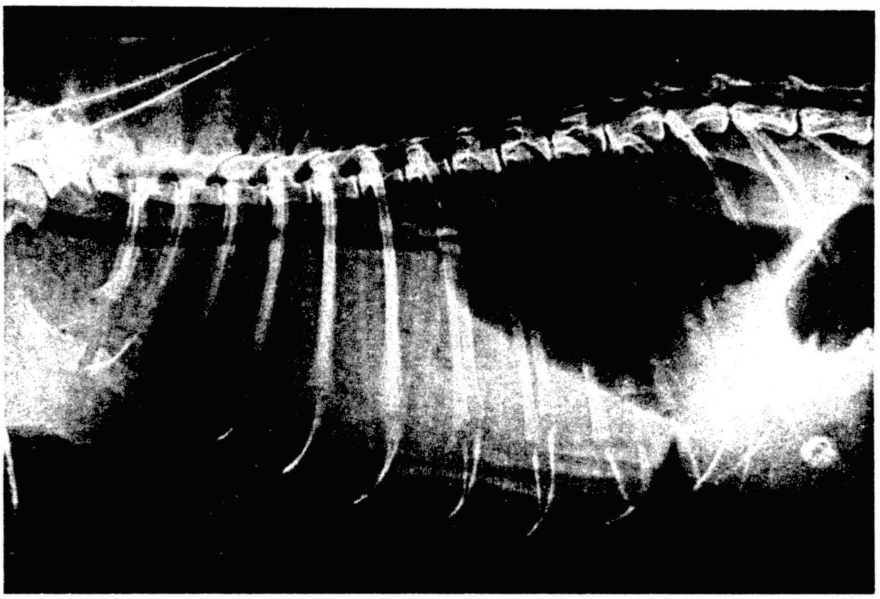


Figure 10

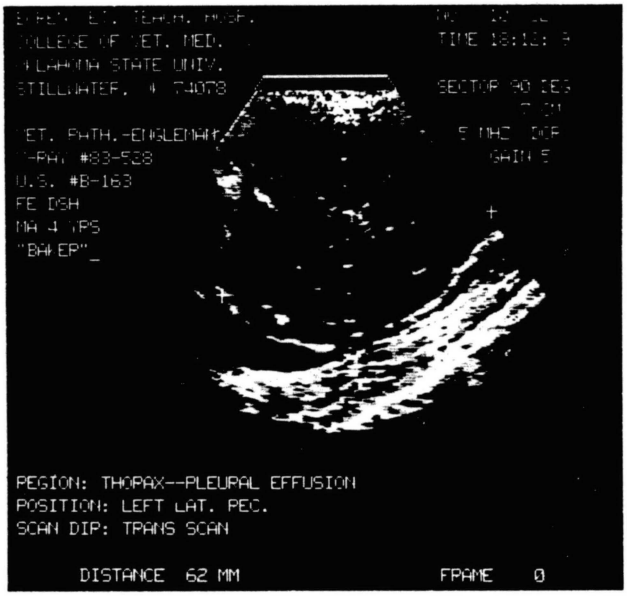


Figure 11

Figure 12: Left lateral thoracic radiograph of cat 106 after 7 treatments employing staphylococcal protein A. A small amount of increased fluid density remains in the anterior thorax.

Figure 13: Anterior thoracic sonogram of cat 106 after 7 treatments. The mediastinal mass was not identified.

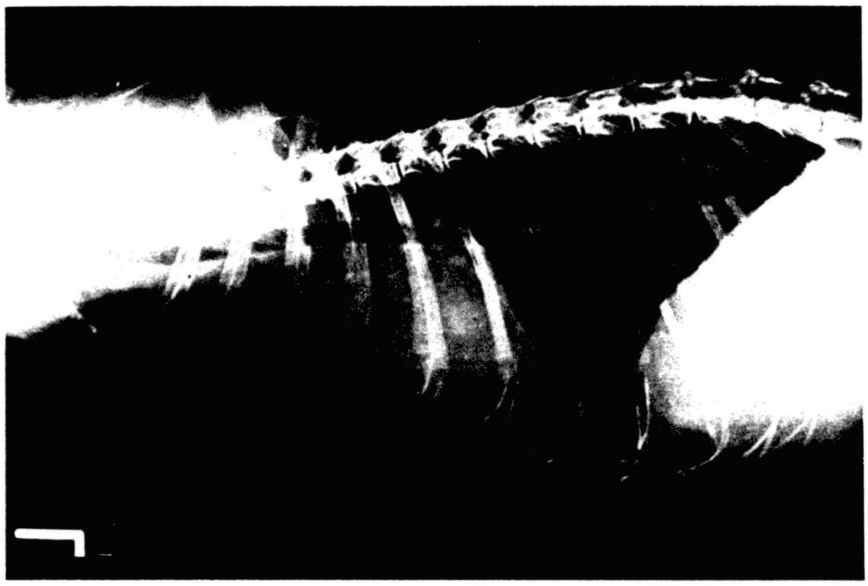


Figure 12

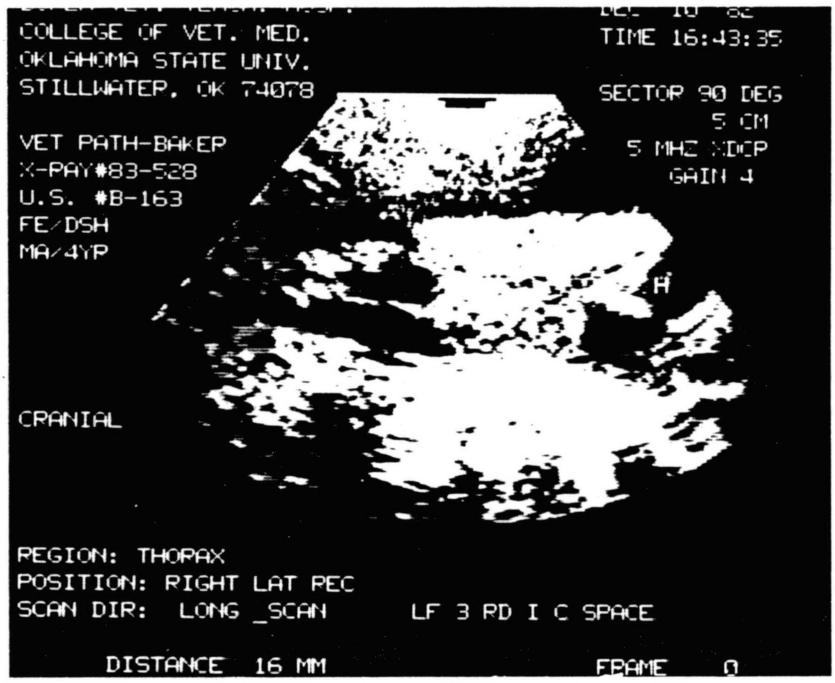


Figure 13

Figure 14: Histosection of the anterior mediastinum of cat 106 post-mortem after 10 protein A treatments. A scantly cellular thymic remnant is surrounded by an edematous connective, granulation tissue. No evidence of neoplasia was present post-mortem (H&E x64).

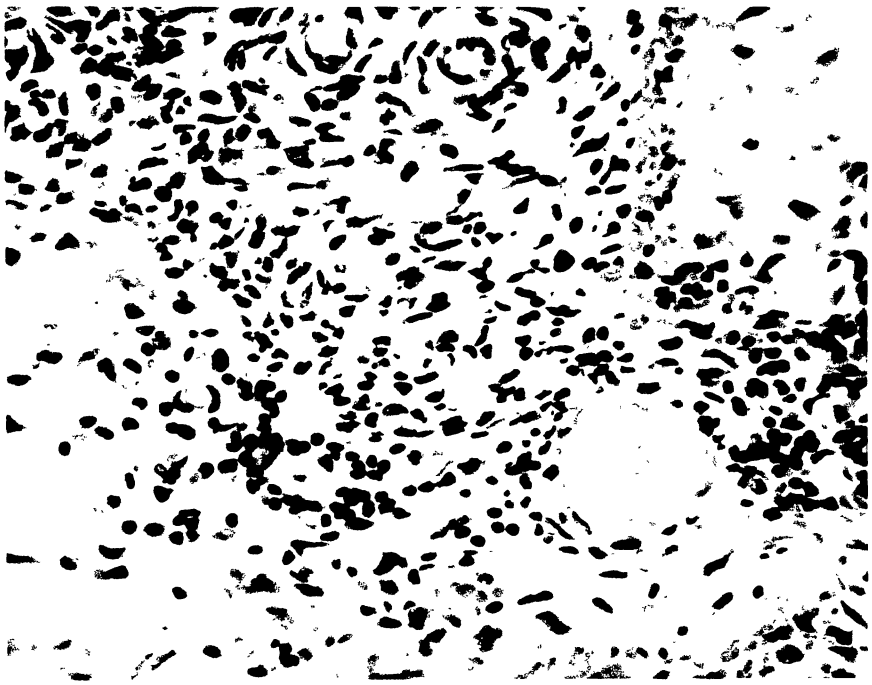


Figure 14

weight occurred during the 47-day treatment period and the cat died following the 10th treatment.

Post-mortem, the mediastinum consisted of an edematous and hemorrhagic connective, granulation tissue diffusely infiltrated with macrophages, which encompassed occasional small remnants of scantly cellular thymic tissue (Figure 14). The sinuses of hypocellular thoracic lymph nodes were filled with erythrocytes and macrophages. No recognizable evidence of neoplasia could be demonstrated in any location at post mortem.

2. Leukemia

Three of the 5 cats with lymphoblastic or lymphocytic leukemia presented with fever and anemia. Four of the 5 cats were thrombocytopenic. Two died during the pretreatment screening period. A third cat died after 3 treatments with concurrent bronchopneumonia. Cats 102 and 103 with leukemia received more than three extracorporeal SpA treatments (Table V).

Cat 103 presented with a massive lymphoid marrow population (>80%), marked lymphocytosis, 14.2 x $10^3/\mu l$ (83% of the total peripheral leukocyte count TWBC), mild aregenerative anemia and thrombocytopenia (Figure 15). Marrow myeloid and erythroid cell lines were hypoplastic and cells of these lineages were sparse. No megakaryocytes were observed. A marked reduction in peripheral blood lymphocytes to 1.7 x $10^3/\mu l$ (58% of the TWBC) occurred after two treatments, and after six treatments only 0.95 x $10^3/\mu l$ lymphocytes were present in the blood (34% of the TWBC). A marked increase in megakaryopoiesis. granulopoiesis, and erythropoiesis was present in

Figure 15: Bone marrow cytology of cat 103 with lymphocytic leukemia prior to treatment. Note the massive lymphoid infiltration, lack of megakaryocytes and sparsity of myeloid and erythroid elements (Wright's x720).

Figure 16: Bone marrow cytology of cat 103 after 6 protein A treatments. The lymphoid infiltrate is markedly reduced and myeloerythroid elements have increased from pre-treatment numbers (Wright's x720).

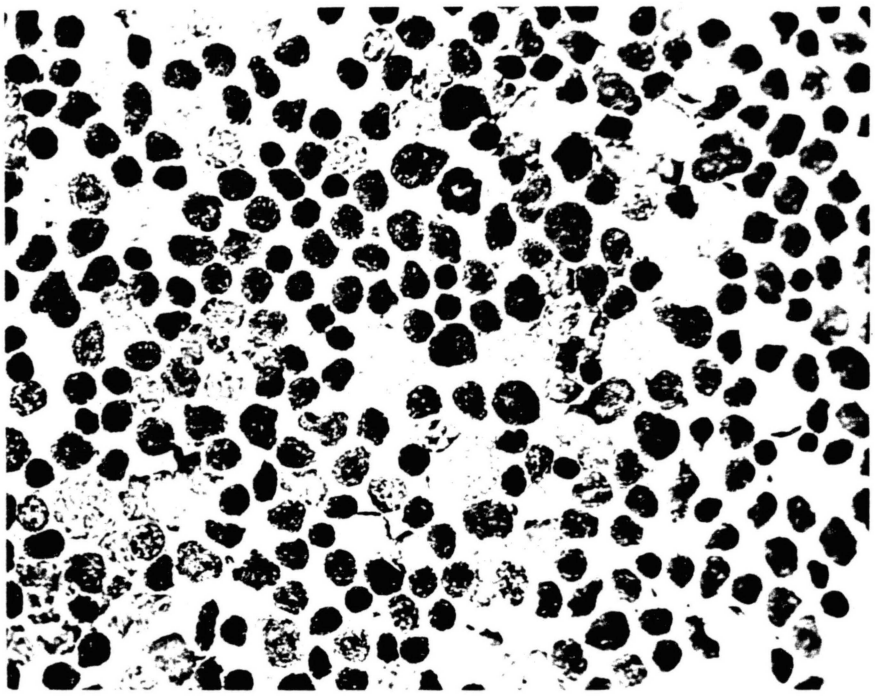


Figure 15

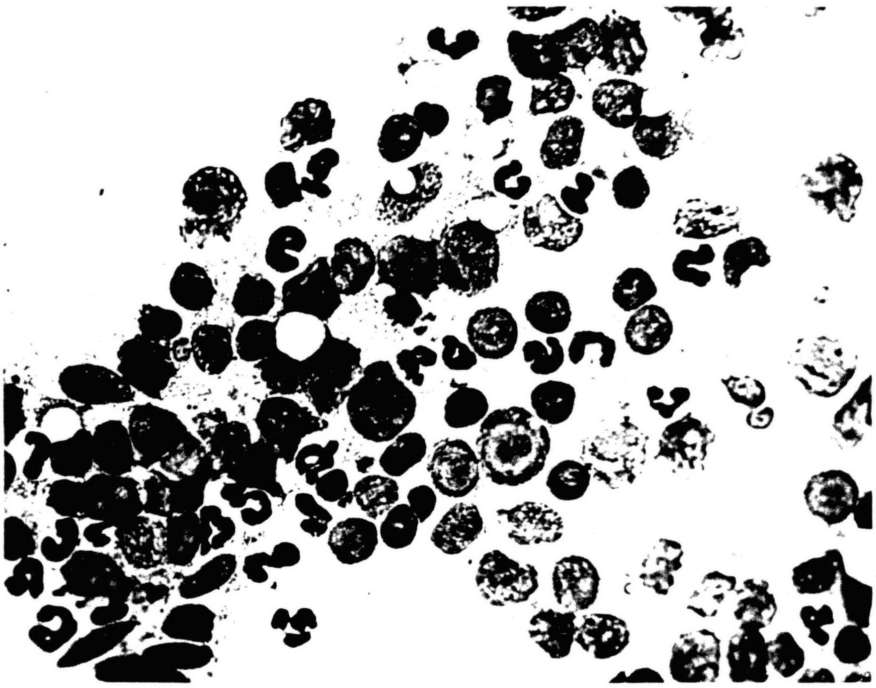


Figure 16

marrow after the 6th treatment (Figure 16). The massive lymphoid infiltrate was no longer evident (<20%). The anemia, now normocytic-hyperchromic, progressed and the cat died with a terminal hematocrit of 7%. An 18% loss in body weight occurred during the 26-day treatment period.

Cat 102 presented emaciated with mild peripheral lymph node enlargement. aregenerative anemia and bilateral intraocular lymphoma. Prior to treatment the cat was classified cytologically as having lymphoblastic leukemia based upon the morphology of blasts which represented 50% of the nucleated cells within the bone marrow aspirate. and an absolute lymphocytosis which ranged during the first 40 treatment days from 7,575-29,755/µl. Granulocyte maturation in the marrow was normal and erythroid cells were sparse. However, the pretreatment lymph node aspirate contained not only numerous lymphoid cells, but also smaller numbers of erythrocytic and granulocytic precursors and megakaryocytes. During the prolonged 124-day treatment period, successive changes in the cytological patterns of peripheral blood and bone marrow occurred (Figures 17,18,19,20,21, 22). In the marrow aspirate on day 41, erythroid cells were markedly increased in number and showed dyscrasia with excessive rubriblasts and prorubricytes and dysplasia with megaloblastoid change and erythroid gigantism. Numerous nucleated red blood cells were present within the peripheral blood. On day 67, excessive numbers of myeloblastic and progranulocytic cells were now present in the marrow and the peripheral blood showed a left shift to the level of myelocytes. On day 120, 27 days after the 24th treatment, approximately 20% of the nucleated cells within the marrow aspirate were again

Figure 17: Bone marrow cytology of cat 102 prior to treatment. Greater than 50% of the nucleated cells are blasts, most similar morphologically to lymphoblasts (Wright's x720).

Figure 18: Higher magnification of the blasts present in the bone marrow of cat 102 prior to treatment. Lymphoblastic leukemia was considered the appropriate cytologic classification for this stage of the cat's proliferative disease (Wright's x1600).

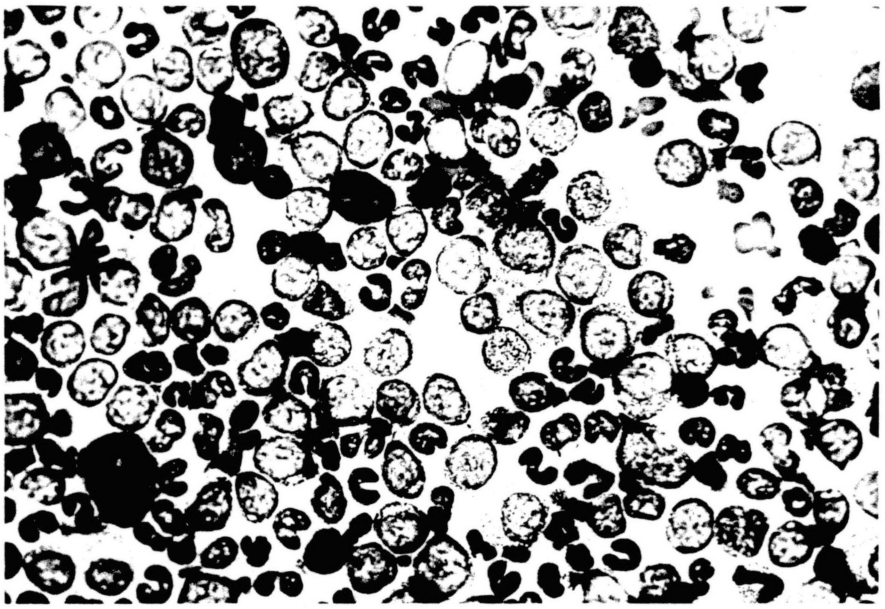


Figure 17

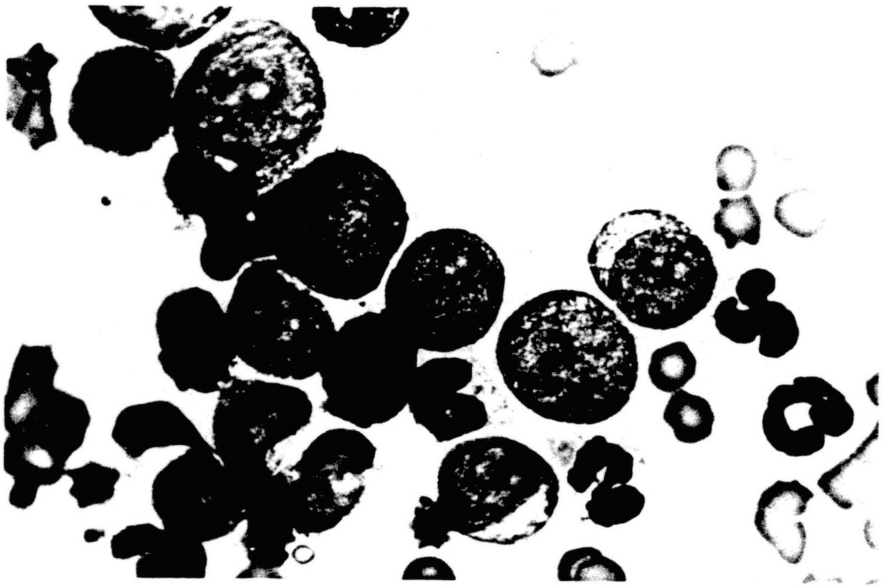


Figure 18

Figure 19: After 10 protein A treatments, a dyscrastic and dysplastic erythroid predominance was present in hypercellular marrow flecks of cat 102 (Wright's x920).

Figure 20: Higher magnification of the marrow of cat 102 after 10 treatments showing rubriblasts and prorubricytes which were present in excessive numbers (Wright's x2050).

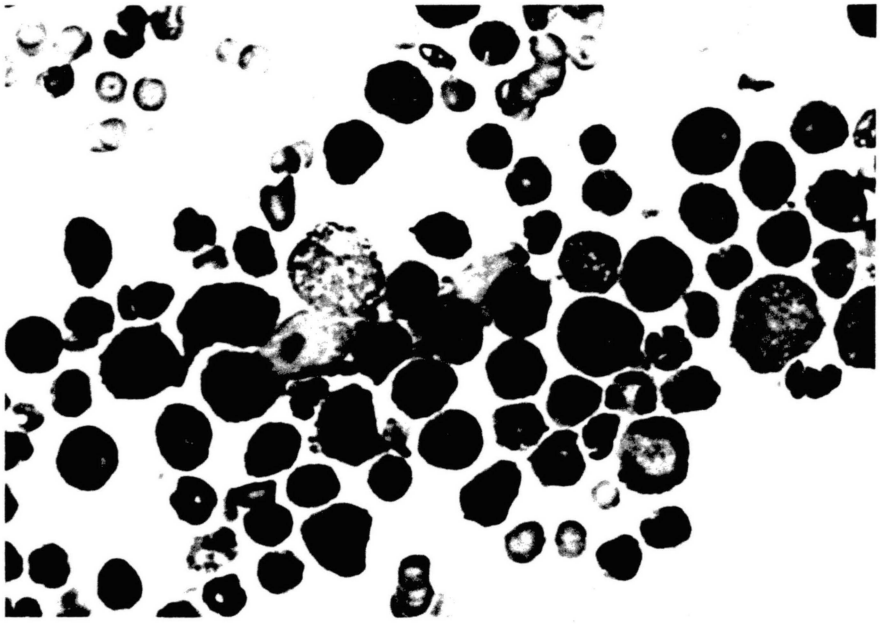


Figure 19

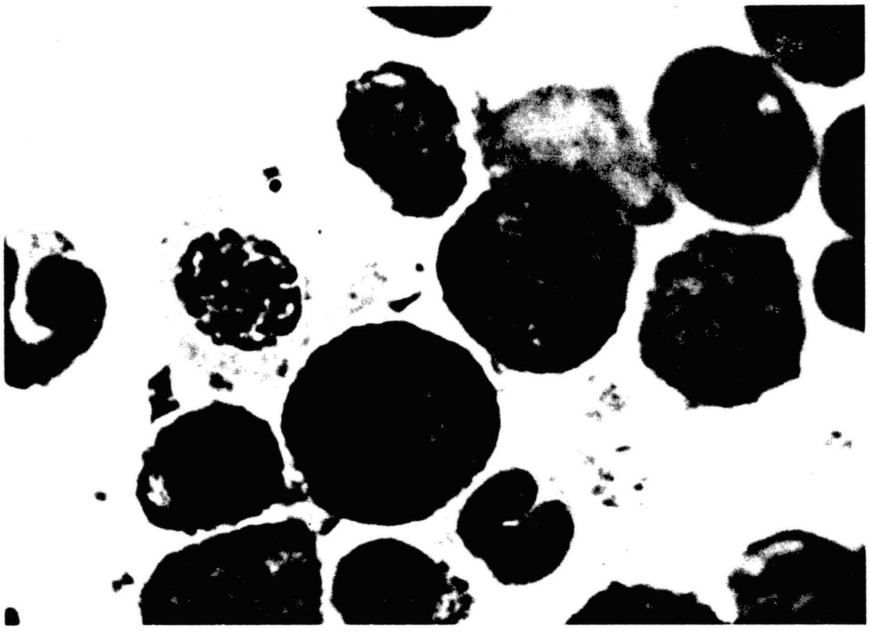


Figure 20

Figure 21: Bone marrow cytology of cat 102 after 18 treatments. Granulocytic elements predominate with excessive myeloblasts and progranulocytes (Wright's x720).

Figure 22: Higher magnification of the marrow of cat 102 after 18 treatments. This myeloid predominance was followed terminally by a lymphoblastic transformation similar morphologically to marrow cytology in Figure 17 (Wright's x1600).

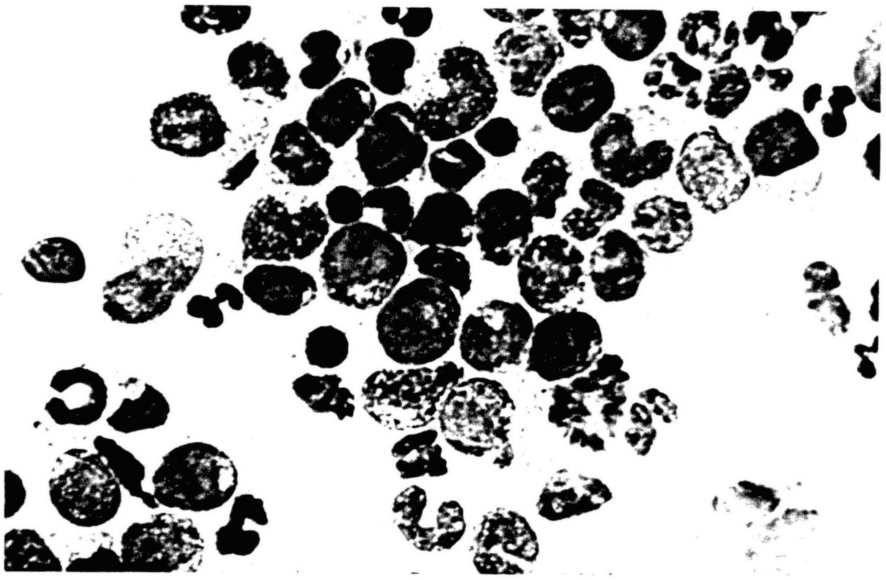


Figure 21

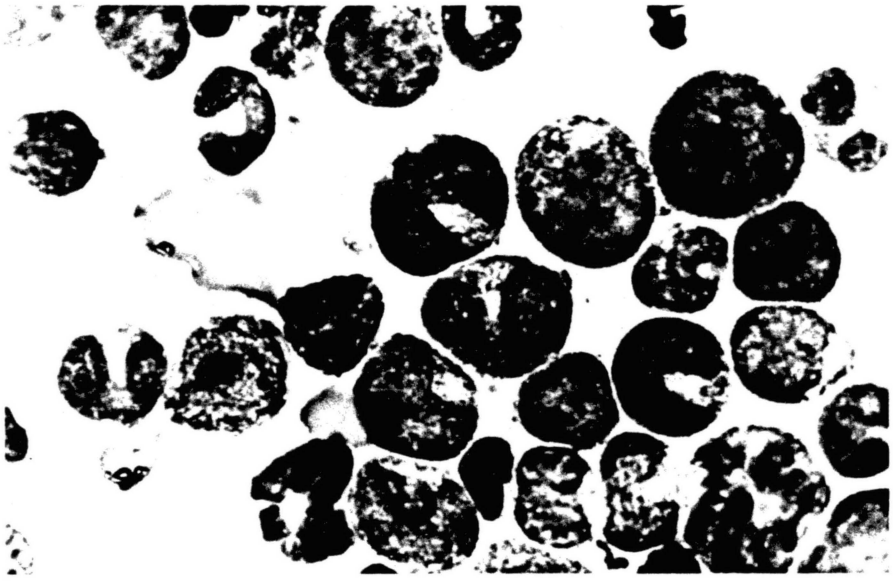


Figure 22

lymphoblasts. An absolute lymphocytosis ranged during the final 27 days from 12,833-16,728/µl. The progressive transition of this cat's hematopoietic proliferative disease from erythroid to myeloid predominance was preceded by and associated terminally with lymphoblastic transformation. Widespread organ infiltration and a large pelvic-abdominal mass of neoplastic cells were present post-mortem. The cells in this mass were predominantly lymphoid in morphology but also contained numerous megakaryocytes and both erythroid and myeloid neoplastic elements. Four red cell transfusions administered late in the treatment period did little to correct the aregenerative anemia and the cat died on day 124 with a terminal hematocrit of 14%. Weight loss during the treatment period was 33%. A membraneous glomerulonephritis and cystitis were identified post-mortem. The course of the disease according to several veterinarians who observed the cat had been much prolonged by treatment.

3. Myeloproliferative Disorders

Of the 5 cats with myeloproliferative disease, four were febrile at presentation and had aregenerative anemia and thrombocytopenia. The fifth cat presented with a responding anemia and concurrent feline infectious peritonitis (FIP). Cats 113, 117 and 152 were given more than three treatments using SpA. Cat 113 with erythroleukemia did not improve and died with a terminal hematocrit of 7% after 21 days, five treatments with SpA filters and two packed red blood cell transfusions.

Cat 117 was hypercalcemic and erythroleukemic upon presentation. Erythroid elements represented the predominant cell line prior to

treatment. Following the 8th treatment, a myeloid predominance was noted. A reduction in serum calcium occurred during treatment, from 12.7 mg/dl prior to treatment, to 8.7 and 7.4 mg/dl after treatments 7 and 9. The cat died after nine treatments with anemia and an extensive pyogranulomatous peritonitis (FIP). Weight gain during the 46-day treatment period in cat 117 was in part attributable to ascites.

Cat 152 was treated by intraperitoneal injection of SpA. Prior to treatment, the bone marrow aspirate was markedly hypercellular with excessive numbers of rubriblasts and prorubricytes (Figure 23). An appropriate increase in rubricyte and metarubricyte numbers were not present and megaloblastosis of erythroid cells was common. Numerous nucleated red blood cells, a few immature granulocytes, and an occasional atypical lymphocyte were present in the peripheral blood. The pretreatment predominance of immature erythroid cells was no longer present after 10 intraperitoneal injections with SpA (Figure 24); instead numerous dysplastic rubricytes and metarubricytes were seen. The hematocrit had decreased during the first 48 days from 22 to 13% with only slight polychromasia and anisocytosis evident. After 18 intraperitoneal SpA injections, the marrow aspirate was hypercellular, now without significant erythroid dyscrasia but with moderate megaloblastosis of rubricytes and metarubricytes. After the 20th and final treatment, significant abnormalities were not present in marrow or peripheral blood aspirates (Figure 25). All hematologic parameters were normal and the hematocrit was 35%. Unfortunately, post-treatment IFA for FeLV was not performed in this cat. After both the 19th and 20th

Figure 23: Bone marrow cytology of cat 152 with erythroleukemia prior to treatment. Excessive numbers of rubriblasts and prorubricytes and inappropriately few numbers of more mature erythrocytic precursors are present (Wright's x720).

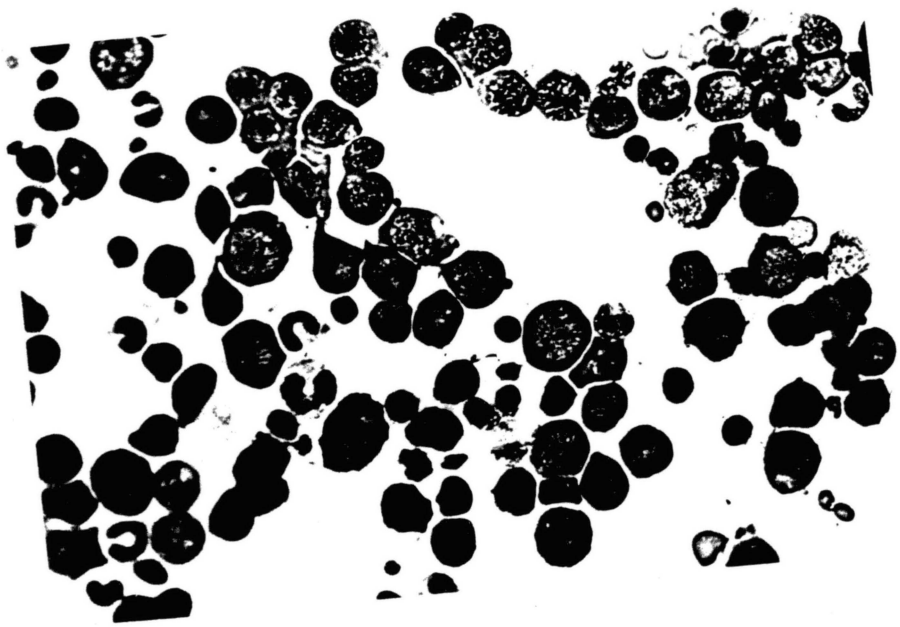


Figure 23

Figure 24: Bone marrow cytology of cat 152 after 10 intraperitoneal injections of protein A. Numerous megaloblastoid rubricytes and metarubricytes are present and the blast population is reduced from pretreatment numbers (Wright's x720).

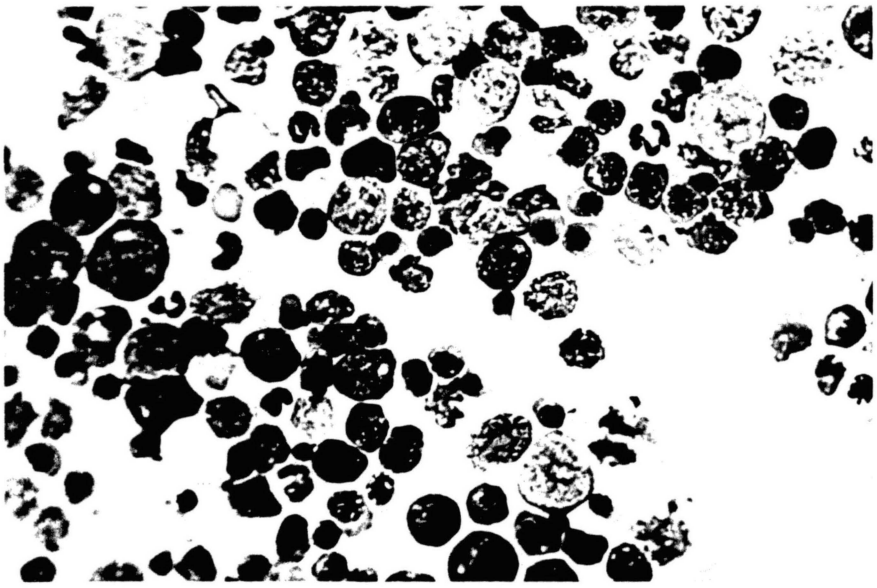


Figure 24

Figure 25: Post-treatment bone marrow cytology of cat 152. A heterogenous population of cells is present without significant abnormalities (Wright's x720).

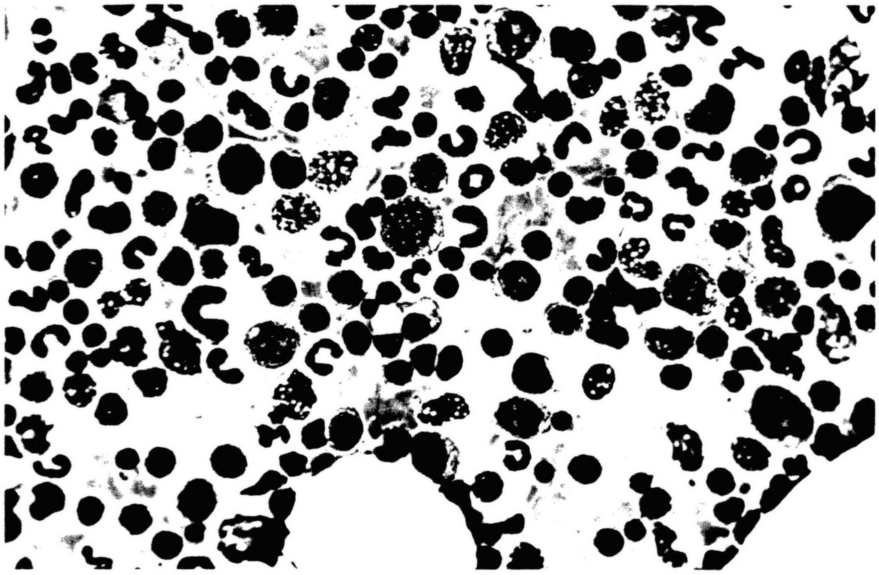


Figure 25

treatment, however, ELISA examinations for FeLV antigen in serum were negative. A 17% gain in body weight occurred during the treatment period and this cat remains healthy, alert, active and negative for FeLV by ELISA examination greater than one year after presentation.

4. Dyscrasia-Dysplasia

Six cats lacking unequivocal manifestations of frank hematopoietic or lymphoreticular neoplasia but which showed significant FeLV-related abnormalities by bone marrow examination were also administered treatment with SpA. Three (101, 105, 116) were treated by plasma perfusion through SpA filters, and three (153, 154, 155) by intraperitoneal injection of 20 µg/2.75 kg body weight of SpA. All six cats were FeLV infected and presented with, or soon after the initiation of treatment, became anemic, leukopenic and thrombocytopenic. Five of the 6 cats developed fevers during the treatment, had marked marrow myelo-erythroid dyscrasia and dysplasia characterized by erythroid hypoplasia or hyperplasia with megaloblastosis; in some cases numerous binucleate erythroid cells were present, and a moderate increase in immature granulocytic forms was seen. After treatment marrow aspirates in five of the six cats lacked significant abnormalities and all cats except 116 and 155 had normal post-treatment hemograms. Clearance of FeLV viremia based upon a negative IFA test occurred following treatment in cats 101, 116, 154 and 155. All cats except cat 116 gained weight during the treatment period and have remained alert and active to date. Cat 116 died with a 4+ indirect Coomb's autoimmune hemolytic anemia, feline infectious

peritonitis and hemobartonellosis. Cat 155 remained mildly anemic post-treatment.

Cat 101 presented with anorexia and aregenerative anemia, an initial hematocrit of 10%, and with numerous nucleated red blood cells in the peripheral blood. Pre-treatment bone marrow aspirates were mildly hypercellular with mild erythroid hyperplasia, numerous binucleate rubricytes and metarubricytes and frequent megaloblastoid erythroid cells (Figure 26). These findings prior to treatment may have represented an aleukemic state of a myeloproliferative disorder, but because of their equivocal nature the cat was grouped with other cats demonstrating varying degrees of bone marrow dyscrasia and/or dysplasia. Pre-treatment leukocyte and thrombocyte counts were normal. From the 12th through the 18th treatment, this cat was febrile ($\leq$107.6°F), leukopenic ($\geq$750/µl TWBC) with a relative lymphocytosis (88%), thrombocytopenic ($\geq$49,000/µl) and anemic ($\geq$12%). Periodic vomiting, marked anorexia, and a 23% reduction from the initial body weight were observed during this period. By the 20th treatment, the appetite had improved, the animal was normothermic, negative by IFA for FeLV, and the hematocrit and white blood cell count were normal. Dysplastic erythroid cells were no longer present on bone marrow examination. Greater than 730 days after presentation, no significant cytologic or hematologic abnormalities were present, and a 30% gain from the initial body weight had occurred. The cat is currently alert and active and has been returned to its owner.

Figure 26: Bone marrow cytology of cat 101 prior to treatment. Mild erythroid hyperplasia with numerous binucleate rubricytes and meta-rubricytes (narrow arrowhead and high magnification insets) and frequent megaloblastoid erythroid cells (broad arrowhead) are present. No significant cytologic or hematologic abnormalities were present after treatment (Wright's x450, x2000).

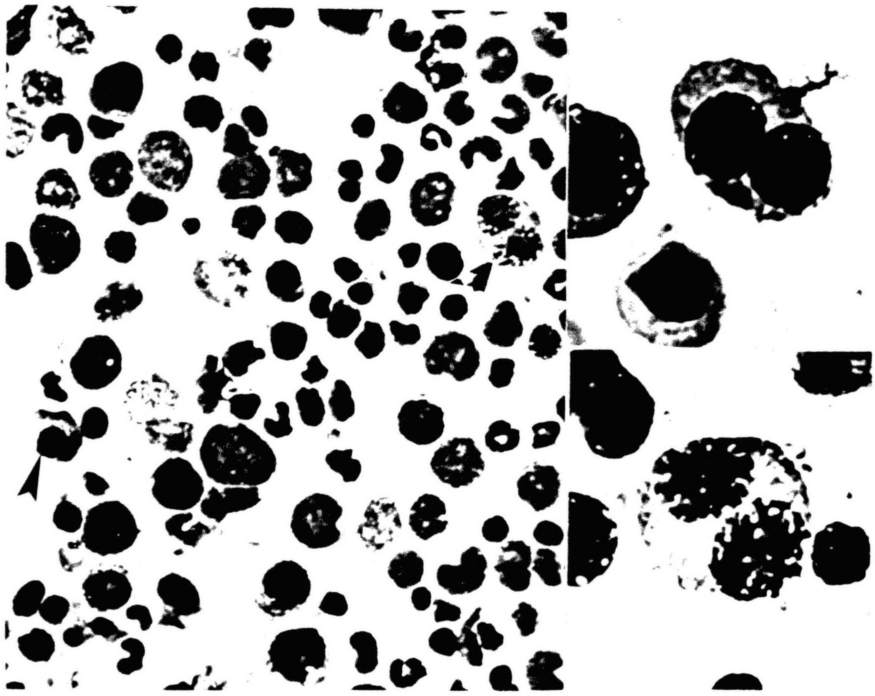


Figure 26

5. Controls

Seventeen healthy cats free of FeLV served as controls and were treated either by the perfusion of plasma over whole SAC organisms, the perfusion of plasma through filters bearing SpA, the intraperitoneal injection of 20 µg/2.75 kg body weight of SpA, the intravenous injection of 100 ng, 10, 20 or 50 µg/2.75 kg body weight of SpA, the I.V. or I.P. injection of normal saline, or were not treated but handled as treated cats with twice weekly phlebotomy. All treatments were administered twice weekly with cats receiving between 8-21 treatments. All cats, except cat 115, remained healthy and normothermic and gained an average of 12% in body weight during the treatment period. Cytologic or serologic abnormalities were never present in any of the control cats, and only cat 115 experienced a transient period of hematologic abnormality. Cat 115 was administered 21 treatments of extracorporeal plasma perfusion over whole SAC organisms and developed a transient anemia (hematocrit >12%), leukopenia and thrombocytopenia between treatments 15 and 21. Weight loss in this cat during the treatment period was 9%. Eighteen days after the 21st and final treatment of cat 115, all parameters had returned to and have remained normal.

E. Discussion

Prior reports have described the regression of malignancy in patients and animals treated by the extracorporeal perfusion of plasma over whole SAC organisms or through filters containing covalently bound SpA (Ray, 1982) (Bansal et al., 1978) (Ray et al.,

1982a,b) (Terman et al., 1980, 1981) (Jones et al., 1980) (Snyder et al., 1984) (Day et al., 1984) (Liu et al., 1984a,b). However, in many cases other forms of anti-cancer treatment had already been given or were given concomitant with immunotherapy. Also, the presenting status and clinicopathologic responses of individual patients or animals to treatment with SAC or SpA were often incompletely described. Careful characterization of FeLV associated malignancy is essential since FeLV viremic cats may remain hematologically and clinically normal for long periods of time (Essex et al., 1975a), FeLV induced marrow dyscrasia-dysplasia can occur (Maggio et al., 1978), and immunosuppression is the most frequent adverse consequence of persistent FeLV viremia (Hardy, 1982a).

In this report, only cats with advanced FeLV associated disease were treated. The severe disease status of the cats in this group is evidenced by the 31% (8 of 26) pretreatment mortality rate. Of the 18 cats with FeLV associated disease treated either by the extracorporeal perfusion of plasma through SpA filters or by the intraperitoneal injection of SpA, 9 (50%) improved objectively. During treatment, tumor regressed, marrow and peripheral blood neoplastic cell populations were reduced, dysplastic forms were no longer present, marrow dyscrasias were corrected and hematologic values became normal. Long-term remission of disease occurred in 6 of the 18 (33%) treated cats, and clearance of FeLV viremia occurred in 5 cats (28%). If the six cats given three or fewer treatments are excluded from consideration (since the treatment period in these animals was less than 10 days), the response rate to treatment would

then be 75% (9 of 12), with long-term remission in 50% of treated cats, and clearance of viremia in 42%.

FeLV associated bone marrow dyscrasia-dysplasia with anemia, leukopenia and thrombocytopenia has been proposed by some to represent a preleukemic syndrome (Maggio et al., 1978). Small numbers of neoplastic cells are thought to be present. In some cats, this syndrome eventually evolves into a lymphoproliferative or myeloproliferative disorder. It has also been suggested that this preleukemic state might be an appropriate setting for testing the hypothesis that immunotherapy is most effective when the tumor cell load is small (Pierre, 1974). Five of 6 cats (83%) with marrow dyscrasia-dysplasia treated either by the intraperitoneal injection of SpA or by the filtration of plasma over SpA experienced long-term remission and 67% (4 of 6) of these cats underwent clearance of FeLV viremia.

If, on the other hand, the cats lacking evidence of neoplasia are excluded from consideration (i.e.. cats with marrow dyscrasia-dysplasia), then 33% (4 of 12) of treated cats improved objectively, 8% (1 of 12) are in long-term remission, and 18% (2 of 11) have cleared the FeLV infection. If those cats with neoplasia that were given three or fewer treatments are also excluded, then 66% (4 of 6) of cats with neoplasia responded to treatment, 16% (1 of 6) are in long-term remission, and clearance of viremia occurred in 40% (2 of 5).

Feline hematopoietic-lymphoreticular neoplasia with concurrent aregenerative anemia was a problem in 13 of the 20 cats with frank malignancy. Aregenerative anemia in the absence of neoplasia is a

common manifestation of naturally occurring FeLV infection. It has been experimentally induced by the injection of certain FeLV isolates which produce a rapid depletion of early erythroid precursors (Hoover et al., 1974) (Onions et al., 1982), and has been described as either normocytic-normochromic (Cotter, 1975) or macrocytic-normochromic (Weiser & Kociba, 1983). Affected cats have normal erythrocytic survival times, but markedly diminished plasma radioiron half times (Madewell et al., 1983). Our observed 65% incidence of concurrent anemia with malignancy is nearly identical (68%) with previous reports (Hardy, 1980a). Most of the anemias were normocytic-normochromic and rapidly progressive. Adjunct therapy was purposefully minimal, but in those cats given leukocyte-poor packed red blood cell transfusions only a slight elevation in hematocrit occurred. 70% of the cats with malignancy were also thrombocytopenic, and one of these cats (102) showed a tendency to bleed following venipuncture. Although fever was not an uncommon finding in cats with FeLV induced disease, in only five cats was treatment associated with febrile reactions. These reactions occurred only in cats with various forms of marrow dyscrasia and/or dysplasia. None of the normal cats or cats with frank neoplasia developed fever as a consequence or manifestation of treatment.

The reported incidence of immune complex glomerulonephritis associated with FeLV-induced malignancies ranges between 14-31% (Glick et al., 1978) (Anderson & Jarrett, 1971). Light microscopic evidence of mesangial proliferation and mild hypertrophy and hyperplasia of glomerular endothelium and epithelium was present in renal sections from six cats. However, only renal lesions in one cat (102)

were considered indicative of membranous glomerulonephritis and consisted of moderate basement membrane thickening, as well as mesangial proliferation and glomerular adhesions.

The possible mechanisms by which treatment with staphylococcal protein A causes reduction in the extent of malignant disease have not been completely elucidated. Circulating immune complexes (CICs) have been implicated in the abrogation of cellular and humoral immune mechanisms (Baldwin et al., 1972) (Hellstrom et al., 1977) (Bansal et al., 1976). The immunoadsorption of CIC from plasma perfused over whole SAC organisms or through SpA-containing filters with subsequent reduction in malignancy has been reported (Snyder et al., 1982) (Day et al., 1983). In addition, certain FeLV proteins are known to be immunosuppressive and their removal along with or as part of CIC may result in the augmentation of anti-tumor and anti-viral immune mechanisms. Other immuno-reactive characteristics of SpA cannot be overlooked, however, especially in the light of our reported remissions in cats treated by the intraperitoneal injection of SpA. In vitro SpA-activated and SpA-dependent cell mediated cytotoxicity (Sumija et al., 1982), and SpA-induced lymphocyte proliferation with production of interferon and consequent generation of increased antibody-dependent cell mediated cytotoxicity (ADCC) and/or natural killer (NK) function have been reported (McCool et al., 1981) (Catalona et al., 1981). We have previously described a marked, though transient increase in circulating γ interferon levels in cats responding to treatment with SpA (Liu et al., 1984b). Remission from FeLV-induced disease and clearance of viremia in SpA treated cats was associated not only with this transient rise in γ interferon, but

also the subsequent appearance of a complement-dependent cytotoxic antibody which was shown by several analyses to be directed against the major viral envelope glycoprotein gp70, which is present on both free virus and on the membrane of virus infected cells (Liu et al., 1984a,b). γ interferon has been shown to increase human T cell-dependent antibody synthesis by B cells (Reem et al., 1983). Increased antibody synthesis along with increased cytotoxic T cell function, enhancement of ADCC, and/or increased NK activity could account for both the anti-viral and anti-tumor influences demonstrated. It seems likely to us that the injection of SpA induces the production and release of γ interferon in vivo which subsequently increases anti-viral antibody synthesis which with complement can attack and reduce the virus itself, and the number of virus-producing neoplastic cells resulting in eventual clearance of viremia, as well as regression of malignancy. A similar sequence might occur in cats treated by the extracorporeal perfusion of plasma over whole SAC organisms or through filters bearing SpA if even small amounts of SpA are present in plasma which is returned to the cats. The exact mechanisms involved in both virus clearance and in regression of the neoplasias will, however, have to be worked out by subsequent experimental studies. There exists as indicated above a number of possibilities, none of which are necessarily exclusive of the others and each of which might play a crucial role in the recovery from neoplasia and elimination of persistent virus infection.

CHAPTER III

IMMUNOLOGICAL FINDINGS IN RESPONDING CATS DURING TREATMENT WITH STAPHYLOCCAL PROTEIN A

A. Summary

When persistently FeLV infected cats are treated with staphylococcal protein A (SpA) the incidence of clearance of FeLV infection is increased and FeLV associated malignancies regress. Loss of evidence of virus infection and regression of FeLV associated disease occurs in at least some cats that first experience an increase in plasma interferon (IFN) level which is followed by the appearance and progressive increase of a complement-dependent cytotoxic antibody specific for the FeLV envelope glycoprotein gp70 which is present on both free virus and on the membrane of virus infected cells.

B. Introduction

The prognosis of persistently FeLV-infected cats is poor, with only about 2% spontaneously clearing their viremia, and more than 83% dieing within 3.5 years from a FeLV associated disease (Hardy, et al., 1976a) (McClelland, et al., 1980). A majority of persistently viremic cats die as a consequence of multiple secondary diseases which develop as a sequelae to the immunosuppressive effects of the

persistent viremia. Cats with FeLV-positive lymphoma generally die within three months of the clinical diagnosis of the malignancy (Hardy, 1980a).

Several forms of immunotherapy or multimodel chemotherapy have been unsuccessful in abrogating FeLV viremia in cats with persistent infection, and despite the occasional induction of partial or temporary remission from FeLV associated disease have not in most cases prolonged the survival of persistently infected cats. (Cotter, et al., 1980) (Squire and Bush, 1973) (Carpenter and Holzworth, 1971) (Haley, et al., 1985) (Brick, et al., 1968) (deNoronha, et al., 1978) (Langlois, et al., 1980) (Hardy, et al., 1976b) (Henness and Crow, 1977a).

Long term clinical remission occurred during treatment with SpA in 83% and the clearance of evidence of virus infection occurred in 67% of the treated cats with chronic viral infection that had FeLV-related abnormalities other than overt malignancy (Table VI). This is an approximately four-fold increase in the rate of clinical remission and more than 30 times greater clearance rate of FeLV infection compared to the incidence of remission and virus clearance in the cat receiving supportive therapy (Hardy, et al., 1976a) (McClelland, et al., 1980) (Cotter, et al., 1975, 1980). Actual clearance of viremia occurred in 28% of all cats treated. Regression of malignancy occurred in 4 of 12 (33%) treated cats demonstrated by reductions in tumor size and bone marrow neoplastic cell populations.

Immediately preceding or concurrent with these remissions from viral infection and disease, a marked though transient elevation in

TABLE VI

RATES OF REMISSION IN CATS RECEIVING PROTEIN A THERAPY

I.	All FeLV-infected cats
	50% clinical improvement
	33% long term remission
	28% clearance of viremia
II.	Leukemia-lymphoma
	33% clinical improvement
	8% long term remission
	18% clearance of viremia
III.	Non-neoplastic
	83% long term remission
	67% clearance of viremia

the plasma level of circulating gamma-like interferon occurred in some responding cats and was followed by the appearance and rising titer of a complement-dependent cytotoxic antibody which reacted with FeLV-infected cells. This IgG antibody was shown by several analyses to be specific for the major viral envelope glycoprotein gp70. These findings may provide some clue to the responsiveness of individual cats to immunotherapy with staphylococcal protein A.

C. Materials and Methods

1. Detection of Antibodies to FL74 Cells in Cat Plasma During SpA Treatment.

At each treatment, 1.0 ml aliquots of the pre-filtered and post-filtered plasma were frozen for analysis. The remaining filtered plasma and autologous cells were returned to the cat. The plasma of treated and control cats were tested for antibodies directed toward determinants on the surface membrane of cells of the established lymphoma-derived, FeLV-producing FL74 cell line as previously described (Liu, et al., 1984a,b). Briefly, a dilution of test plasma and between $2.5 \times 10^6 - 3 \times 10^7$/ml FL74 cells were coincubated at 37°C for 30 minutes. The cells were then washed and resuspended in a dilution of either guinea pig serum or fluorescein-conjugated rabbit anti-cat IgG (Cappel, Cochranville, PA). Cell viabilities were determined by vital dye exclusion and by assay for LDHase release. Immuno-fluorescent preparations were scored with a Leitz (Dialux 20) microscope under epi-illumination.

2. Characterization of Cytotoxic Antibodies to FL74 as Specific for FeLV gp70.

Several anaylses were used to show that antibodies cytotoxic for cells of the FL74 line that developed in cats during treatment with SpA were directed toward determinants of the major vital envelope glycoprotein gp70. Plasma samples positive for cytotoxic antibody to FL74 cells were absorbed with FeLV or mouse mammary tumor virus (MMTV) and evaluated for residual cytotoxic activity as described before (Liu, et al., 1984a,b). Monoclonal antibodies (MAbs) to FeLV and FL74 cells were produced and characterized by our laboratories (Wang, et al., 1983). Two of these MAbs, 73.5 and 6.5 were used in an assay for the inhibition of plasma cytotoxic activity as described before (Liu, et al., 1984a,b). MAbs 73.5 reacted with a FeLV 70,000 dalton molecular weight glycoprotein, designated gp70, as well as with viable FL74 cells. MAb 6.5 reacted with a glycoprotein (43,000 dalton molecular weight) on the surface of FL74 cells but not with FeLV. The inhibition of plasma cytotoxic activity by prior MAb treatment of FL74 cells was demonstrated by observing for the residual cytotoxicity of plasma samples using both the vital dye exclusion and LDHase release methods.

D. Results

1. Increase in Plasma Interferon Levels in Cats Responding to SpA Treatment.

Several responding cats that became FeLV seronegative as demonstrated by the IFA test, and that showed regression of FeLV associated disease had elevations in serum interferon levels as illustrated for two representative cats in Table VII. In contrast, cats that did not become virus negative had undetectable levels of IFN after SpA treatment. The plasma IFN produced by responding cats during treatment had properties of gamma interferon since only 30-40% of the antiviral activity remained after pH 2 or heat treatment of the plasma.

2. Appearance of Cytotoxic Antibody to FeLV gp70 in Cats Responding to SpA Treatment.

Prior to clinical improvement and conversion to FeLV seronegative status some responding cats developed complement-dependent FL74 directed cytotoxic antibodies as determined by both vital dye exclusion and LDHase release. The titer of cytotoxic antibody increased with additional treatments in these responding cats (Table VIII). An IFA test was used to detect IgG antibodies in responding cat plasma which reacted with the surface membrane of FL74 cells (data not shown). Absorbtion of responding cat plasma with 50 µl of FeLV reduced the cytotoxic activity of the plasma by approximately

TABLE VII

CIRCULATING PLASMA INTERFERON TITERS IN CATS RESPONDING TO TREATMENT WITH PROTEIN A

Weeks after Initial Treatment	1	2	3	4	5	6	7	8	9	10
Cat 154	<3	81	162	7	9	9	243*	9	<3	<3
Cat 155	22	71	324	72	14	18	37	<3	ND*	27

*Clearance of viral infection as indicated by weekly negative IFA tests for FeLV began during weeks 7 and 9 for cats 154 and 155 respectively.

TABLE VIII

THE DEVELOPMENT OF CIRCULATING CYTOTOXIC ANTIBODY TO FL74 CELLS DURING PROTEIN A THERAPY

	Treatment no.	Cells stained by vital dye, %	% Specific LDHase released
Cat 101	4	3	ND
	5	5	ND
	6	29	33.9
	10	49	51.6
	14	63	49.0
	16	39	45.1
	18	73	64.7

74%. No reduction in cytotoxic activity occurred after absorbtion with MMTV.

When the FL74 cells were allowed to react prior to the addition of the cytotoxic antibody with various dilutions of ascites fluid containing the MAb 73.5 that was reactive with FeLV gp70, complement-mediated lysis was almost completely abolished. MAb 6.5 failed to abolish cytotoxic activity against FL74 cells in the plasma samples. The inability of each of these two MAbs to mediate complement-dependent lysis of FL74 cells on their own was shown in separate studies. When FL74 cells were incubated with ascites fluid containing the respective MAbs (73.5 and 6.5), followed by incubation with fresh guinea pig serum as a source of complement, the percentage of cell death was found to be 6-18% by trypan blue exclusion. No difference in the percentage of cell death was observed when fresh GPS was replaced by heat-inactivated (56°C for 30 min.) GPS. These results indicate that the cytotoxic antibody developed against cat lymphoma cells (FL74) is directed toward determinants on FeLV gp70 which is also expressed on the cell surface of FL74.

E. Discussion

The mechanisms by which remissions in patients with malignancy occur during treatment with staphylococcal protein A are unknown. Tumoricidal responses have been reported in human and animal trials following either the perfussion of the patient's plasma over Staphylococcus aureus Cowan I (SAC), which contains protein A on its surface, or over immunobilized purified protein A, or following the IV or IP infusion of protein A (Harper, et al., 1985) (Bertram, et

al., 1985) (Klausner, et al., 1985) (Liu, et al., 1984a,b) (Engelman, et al., 1985d) (Terman, et al., 1980, 1981). In general, tumoricidal responses may be attributed to either the removal, reduction or redistribution of plasma factors that are thought to abrogate humoral or cell-mediated immune responses (circulating immune complexes, immunoglobulin) or to the induction or addition of substances to the patient's plasma that enhance the antitumor response. Although protein A has been suggested as the mediator of both events, infusion of plasma incubated within the Wood 46 strain of Staphylococcus aureus, which does not contain protein A, resulted in tumor reductions in one study (Gordon, et al., 1983), but not in others (Ray, et al., 1982a) (Terman, et al., 1980). In addition, treatment by perfusion of plasma over protein A did not produce or only rarely produced a tumorcidal response in some studies (Klausner, et al., 1985) (Gordon, et al., 1983).

It has been suggested that immune complexes alter immune responses and contribute to the genesis of malignancies. The removal by binding of such complexes to SAC or immobilized SpA may contribute to the induction of tumor regression. The conditions of plasma perfusion, such as volume of plasma, speed of perfusion and frequency of treatment, may be inappropriate for substantial immune complex removal. Yet, perfusion may allow for the removal of other plasma factors, including IgG. Alternatively, perfusion over SpA may generate new activity in the treated plasma which is highly mitogenic to normal lymphocytes, but not due to leakage of SpA from the filtration system (Bertram, et al., 1985).

Protein A has been shown to dissociate from SAC or from collodion charcoal during plasma perfusion (Balint and Jones, 1983) (Balint, et al., 1984). Numerous immunostimulatory activities have been attributed to SpA and include complement activation (Sjoquist and Stalenheim, 1969) (Verbrugh, et al., 1979), and the polyclonal induction of B-lymphocytes with synthesis of antibodies and the production of interferon and consequent generation of increased antibody-dependent cell mediated cytotoxicity and natural killer cell function (Smith, et al., 1983) (McCool, et al., 1981) (Catalona, et al., 1981) (Moller and Landwall, 1977). The intravenous injection of SpA alters the organ distribution of CIC and enhances their whole body elimination (Siag and Jones, 1982). The purposeful or inadvertant injection of SpA may contribute to a tumoricidal response by enhancing immunological systems and/or by the reduction or redistribution of CIC.

It has also been reported that certain commercial preparations of SpA are contaminated with Staphylococcus aureus enterotoxin A (SEA), a potent T-lymphocyte mitogen and gamma interferon inducer (Smith, et al., 1983). It may be this contamination of SpA by SEA that is responsible for the conflicting reports that SpA behaves as a T-lymphocyte mitogen and induces gamma interferon. Even minor contamination of SpA would influence evaluations of its biological or clinical effects. It is possible that the SpA preparation used in this study was impure since the IFN generated in the plasma of treated cats was sensitive to pH2 and heat treatment.

A final note of clarification should be made concerning the studies described above where cats with FeLV-associated disease were

treated with SpA. In no instance did the immunological findings, i.e. transient elevation in plasma interferon level folowed by the appearance and increasing titer of antibodies directed to gp70, correlate with clinical remission from malignancy in the SpA treated cat. Instead, these immunological findings were described in cats with myelodysplasias and dyscrasias, multiple hematological abnormalities, intercurrent infections and persistent FeLV viremia. Regardless, these rates of remission from FeLV-associated disease and clearance of retroviremia are much higher than similar rates in the supportively treated cat. In addition, the SpA therapy may have played a role in preventing the development of malignancy in these cats, since their clinical condition may have represented a state of preleukemia. The anti-gp70 antibodies, apparently of FeLV-C subgroup specificity (Liu, et al., 1984a,b), which developed in these cats has been shown to have a protective nature against the development of neoplasia (Essex, 1982). Other immunological means must be investigated in order to explain the obvious reductions and, in some cases, complete remissions from malignancy during treatment with SpA which are described above.

CHAPTER IV

HYPERCALCEMIA IN CATS WITH FELINE LEUKEMIA VIRUS ASSOCIATED LEUKEMIA-LYMPHOMA

A. Summary

Three cases of hypercalcemia were recognized among eleven cats presenting with leukemia-lymphoma for ex vivo immunoadsorption therapy using staphylococcal protein A coated filters. In addition, the initial mean serum calcium concentration of cats with leukemia-lymphoma was significantly higher ($p < 0.005$) than that of healthy control cats or feline leukemia virus infected cats without malignancy. During immunotherapy of the hypercalcemic cats, objective reduction in the extent of the malignancies was associated with a reduction in the serum calcium concentrations. This response to treatment, the lack of skeletal metastasis, and absence of renal and parathyroid pathology imply that humorally-mediated mechanisms may have been responsible for the production of the hypercalcemia.

B. Introduction

Hypercalcemia is a common complication in patients with certain carcinomas (breast, lung, kidney, pancreas, colon) or hematopoietic and lymphoreticular neoplasms (Mundy et al., 1974a). It occurs most frequently with overt skeletal metastasis and localized osteolysis

produced possibly by the presence of the tumor cells or by humoral products of the metastasis which stimulate osteoclastic resorbtion (Tashjian, 1978). However, a significant number of hypercalcemic malignancies lack boney metastasis. In such cases, removal of the tumor often leads to remission of the hypercalcemia and recurrence of tumor to the reappearance of hypercalcemia (Tashjian, 1978) (Metuen et al., 1981). Several humoral tumor products have been proposed as mediators of these hypercalcemic malignancies. Compounds implicated include parathyroid hormone-like peptides (Sherwood et al., 1967), osteoclast activating factor (Mundy et al., 1974a,b), prostaglandin E_2 (Tashjian, 1978) (Seyberth et al., 1975), and provitamin D_3-like sterols (Gordon et al., 1966). The relative importance and frequency of occurrence of each of these substances is not known.

Hypercalcemic malignancy has been documented in dogs with lymphoma, testicular interstitial cell tumor, multiple myeloma and carcinoma of the mammary gland, stomach, lung or anal sac apocrine glands (MacEwen & Siegel, 1977) (Chew & Meuten, 1982) (Meuten et al., 1982a). It occurs with the HSDM murine fibrosarcoma, the VX carcinoma in the rabbit and rarely with equine gastric carcinoma (Tashjian, 1978) (Meuten et al., 1978). In most cases, cause of the hypercalcemia is unknown. Bone resorbing compounds secreted by tumor cells are suspected in those cases which lack skeletal metastasis, parathyroid hyperactivity or renal insufficiency (Meuten et al., 1981) (MacEwen & Siegel, 1977) (Meuten et al., 1982a).

Lymphoma-leukemia is the most common neoplasm of cats, accounting for 90% of all feline hematopoietic malignancies. It occurs most frequently in feline leukemia virus (FeLV) infected cats (Hardy,

1980a,b). Hypercalcemic malignancy in the cat has been reported only twice, in one case associated with mediastinal lymphoma (Chew et al., 1975), and one aleukemic granulocytic leukemia with myelofibrosis (Zenoble & Rowland, 1979). Both animals were infected with FeLV. In a previous review of 27 cats with FeLV associated leukemia-lymphoma, none were found to have elevated serum calcium concentrations (Wilkins & Hurvitz, 1975).

In the present study, cats receiving ex vivo immunoadsorption were characterized clinicopathologically prior to and during treatment. Three cases of hypercalcemia were recognized among 11 cats presenting with leukemia-lymphoma. During immunotherapy with staphylococcal protein A, objective reduction in the extent of the malignancy was associated with a reduction in the serum calcium concentration. In addition, the presenting mean serum calcium concentration of cats with leukemia-lymphoma was significantly higher than that of either healthy control cats or FeLV infected cats without malignancy.

C. Material and Methods

Clinically ill, FeLV infected cats were referred by Oklahoma veterinarians. Controls were conventionally maintained, clinically healthy cats free of FeLV. All cats were characterized based on the cytologic examination of bone marrow, lymph node, tumor and peripheral blood aspirates, and on the results of the indirect immunofluorescent antibody (IFA) test for FeLV in leukocytes (Hardy et al., 1973b) (Antibodies, Inc., Davis, CA) and the enzyme-linked immunosorbent assay (ELISA) for the detection of FeLV antigen in

serum (Mia et al., 1981) (Pitman-Moore, Inc., Washington Crossing, NJ). Tumors were measured in at least two diameters by ultrasonography employing a Mark II Pulsed Echo System Model 600B (Advanced Technology Laboratories, Bellevue, WA). Thoracic and abdominal radiographs, complete blood counts, serum protein electrophoresis and urinalysis were performed. Serum chemical profiles included albumin, blood urea nitrogen, calcium, chloride, creatinine, glucose, phosphorus, potassium, alkaline phosphatase, sodium and alanine aminotransferase. Serum calcium was determined by a fluorometric titration method employing a Corning Calcium Analyzer 940 (Corning Scientific Instruments, Medfield, MA). Albumin (Coulter Diagnostics, Hialeah, FL) and phosphorus (Worthington Diagnostic Systems, Inc., Freehold, NJ) were determined by a colorimetric method and read on a spectrophotometer model 24 (Beckman Instruments, Fullerton, CA). Cytologic preparations were stained with Wright's Dip-Stat (Medi-Chem, Inc., Santa Monica, CA).

Cats with malignancy and control cats were treated twice weekly with ex vivo immunoadsorption employing protein A (SpA) (Pharmacia Fine Chemicals, Uppsala, Sweden) purified from Staphylococcus aureus Cowan I as previously described (Day et al., 1984). For each cat, a filter column containing SpA covalently bound to a zetachrome matrix of cellulose (AMF/Specialty Materials Group, Talcotville, CN) was used to filter plasma clarified from 10-30 ml of heparinized blood twice a week. Filtered plasma and the autologous cells were returned to the cat. FeLV infected cats without unequivocal manifestation of hematopoietic or lymphoreticular malignancy were not treated. Adjunct therapy consisted only of isotonic fluids and, in cases of

concomitant infections, antibiotics. During treatment hemograms and serologic examinations were performed weekly and serum chemistry, cytologic, sonographic and radiographic examinations were repeated every third or fourth treatment. Cats were given up to 24 treatments. All cats were examined macroscopically at euthanasia or upon death and all treated cats were also examined microscopically. Tissues from control and leukemia-lymphoma cats were fixed in 10% buffered formalin, embedded in paraffin, cut at 8 μm, and stained with hematoxylin and eosin. Tissues examined microscopically included tumor, bone marrow, lymph node, kidney, liver, spleen, lung, heart, brain, intestine and urinary bladder.

D. Results

Thirty-one cats were evaluated. Of these, 8 served as controls. Twenty-three were FeLV infected, 11 had leukemia-lymphoma, and 12 had a variety of infections or immune-mediated disease. The 11 with malignancy represented cats of either sex. They ranged from 6 months to 7 years in age. They were primarily domestic, short- or long-haired cats. Most of the common morphologic forms of feline leukemia-lymphoma were represented (Table IX). Approximately 1/3 of these cats showed significant response to treatment.

Prior to ex vivo immunoadsorption, the mean serum calcium concentration of cats with leukemia-lymphoma was significantly greater ($p < 0.005$) than the mean serum calcium concentration of either FeLV infected or control cats (Table X). Mean serum phosphorus and albumin concentrations of cats with malignancy were less than those of the other two groups, but were not significantly

TABLE IX

CLINICAL STATUS OF CATS UPON PRESENTATION

Cat	Age	Sex	Clinical diagnosis	IFA/ELISA
128	4	M	Multicentric lymphoma	+/+
109	2	M	Alimentary lymphoma	+/+
106	4	M	Mediastinal lymphoma	+/+
111	2	M	Mediastinal lymphoma	+/+
131	2	F	Mediastinal lymphoma	+/+
102	7	F	Lymphoblastic leukemia	+/+
110	0.5	F	Lymphoblastic leukemia	+/+
127	-	M	Lymphoblastic leukemia	+/+
103	-	M	Lymphoblastic leukemia	+/+
113	4	M	Erythroleukemia	-/+
117	2	M	Erythroleukemia	+/+

TABLE X

SERUM CALCIUM, PHOSPHORUS AND ALBUMIN CONCENTRATIONS PRIOR TO SpA IMMUNOADSORPTION TREATMENT IN CATS WITH FeLV VIREMIA OR FeLV ASSOCIATED LEUKEMIA-LYMPHOMA AND IN CLINICALLY NORMAL CATS

A. LEUKEMIA-LYMPHOMA

Cat	128	109	106	111	131	102	110	127	103	113	117	Mean
Calcium mg/dl	9.8	8.9	12.2	9.8	9.7	9.4	9.5	8.9	10.9	8.4	12.7	10.01 (±1.37)
Phosphorus mg/dl	4.4	3.9	5.3	3.6	4.5	6.8	6.5	4.6	3.8	4.2	5.8	4.85 (±1.10)
Albumin g/dl	-	1.9	1.6	2.4	2.9	1.8	2.1	2.7	3.0	2.0	2.2	2.26 (±0.48)

B. FeLV VIREMIC

Cat	133	134	135	136	104	137	138	139	118	123	140	141	Mean
Calcium mg/dl	9.8	8.9	9.3	8.0	8.4	8.5	8.7	7.6	8.1	9.2	8.5	8.5	8.63 (±0.61)
Phosphorus mg/dl	6.7	5.9	6.9	5.3	4.1	4.6	5.5	5.0	6.5	5.5	5.8	4.5	5.52 (±0.89)
Albumin g/dl	2.4	2.2	-	1.5	3.4	3.3	2.3	1.7	2.1	2.8	2.5	3.0	2.47 (±0.61)

C. CONTROL

Cat	107	122	114	120	108	132	142	143	Mean
Calcium mg/dl	8.5	8.9	7.7	8.3	8.1	8.7	8.7	8.4	8.41 (±0.38)
Phosphorus mg/dl	6.0	6.3	4.4	5.3	4.2	6.1	5.3	5.3	5.36 (±0.77)
Albumin g/dl	2.1	2.3	2.6	2.7	2.5	3.3	2.7	2.8	2.63 (±0.36)

different. All FeLV-infected and control cat calcium levels were within the normal reference range (6.2-10.2 mg/dl). Three cases of hypercalcemic malignancy were recognized (cats 106, 103, 117).

Cat 106 presented with an anterior mediastinal lymphoma 4.0 x 6.2 cm, marked pleural effusion, low serum albumin (1.6 g/dl) and elevated serum calcium (12.2 mg/dl). 200 ml of a modified transudate were removed from the thorax between treatments 1 and 4. Following the 7th treatment, the mediastinal mass was no longer identifiable but the thickened mediastinum measured 1.0 x 1.6 cm. Concurrent with this reduction in tumor size, the serum calcium concentration was mildly reduced (Table XI). After the 9th treatment, the calcium concentration was even lower. Serum chemical evaluation was not repeated and the cat died after 10 treatments.

Cat 103 presented with a massive lymphoid marrow population (>80%) and marked lymphocytosis, 14.2 x $10^3/\mu l$ (83% of the total peripheral leukocyte count TWBC). Marrow myeloid and erythroid cell lines were hypoplastic and cells of these lineages were sparce. No megakaryocytes were present. A marked reduction in peripheral blood lymphocytes to 1.7 x $10^3/\mu l$ (58% of the TWBC) occurred after two treatments, and after 6 treatments only 0.95 x $10^3/\mu l$ lymphocytes were present (34% of the TWBC). A marked increase is megakaryopoiesis, granulopoiesis and erythropoiesis was present in marrow after the 6th treatment. The massive lymphoid infiltrate was no longer evident (<20%). Biochemical evaluation was performed pre-treatment and following the 3rd treatment. The calcium concentration was mildly lower post-treatment (Table XI). The cat died after treatment #6.

TABLE XI

CHANGES IN SERUM CALCIUM, PHOSPHORUS AND ALBUMIN CONCENTRATIONS IN THREE CATS WITH LEUKEMIA-LYMPHOMA AND TWO CONTROL CATS DURING SpA IMMUNOADSORPTION TREATMENT

	LEUKEMIA-LYMPHOMA							
Cat	106			103		117		
SpA treatments	0	5	9	0	3	0	7	9
Calcium mg/dl	12.2	11.9	11.5	10.9	10.0	12.7	8.7	7.4
Phosphorus mg/dl	5.3	5.5	4.0	3.8	6.0	5.8	4.9	5.0
Albumin g/dl	1.6	1.2	1.9	3.0	3.6	2.2	1.7	2.1
Adjusted calcium (mg/dl)	14.1		13.1	11.4	10.1	14.0		8.8

	CONTROL					
Cat	122			115		
SpA treatments	0	4	8	0	10	17
Calcium mg/dl	8 9	9.3	9.7	7.7	8.6	8.2
Phosphorus mg/dl	6.3	4.0	6.5	4.4	4.4	5.1
Albumin g/dl	2.3	2.3	2.5	2.6	2.5	2.3

Cat 117 was erythroleukemic upon presentation with marked marrow myeloerythroid dyscrasia-dysplasia characterized by excessive immature forms and megaloblastosis. Erythroid was the predominant cell line prior to treatment. Following the 8th treatment, a myeloid predominance was noted. A reduction in serum calcium occurred during treatment, from 12.7 mg/dl prior to treatment to 8.7 and 7.4 mg/dl after treatments #7 and #9 (Table XI). Concurrent feline infectious peritonitis was diagnosed post-mortem when the cat died after 9 treatments.

Alkaline phosphatase, blood urea nitrogen and creatinine values were within normal ranges for all cats. Urinalyses were normal except in cat 102 which had cystitis. Survey radiographs of cats with leukemia-lymphoma failed to detect osteolytic lesions. Parathyroid glands were uniformly small and macroscopically unremarkable. Skeletal metastasis was not evident in any cat, though neoplastic marrow populations were present in cats with leukemia. Soft tissue mineralization was not present post-mortem in any cat.

E. Discussion

Elevated levels of parathyroid hormone-like peptides have been demonstrated in plasma and tumor tissue of patients with non-endocrine neoplasms (Sherwood et al., 1967). Osteoclast activating factor secreted by cultured normal leukocytes in response to antigen or mitogen stimulation is indistinguishable from substances produced by tumors of lymphoid or myeloma cell origin (Mundy et al., 1974b). Prostaglandin E_2 acts systemically to enhance bone resorbtion in the

HSDM murine fibrosarcoma and VX carcinoma of the rabbit (Tashjian, 1978).

Although disorders in feline mineral metabolism are rarely reported, hypercalcemia and hypercalcemic malignancy are considered much less common in cats than in man or dogs (Chew & Meuten, 1982). By analogy to proposed mechanisms in man, serum calicum may become elevated in cats with leukemia-lymphoma as a result of skeletal metastasis and localized osteolysis, diffuse osteolysis caused by humoral products derived from the tumor, concurrent parathyroid hyperactivity or renal insufficiency. In addition, hypercalcemia has been documented in numerous cases of lymphoma associated with human T-cell leukemia-lymphoma virus (HTLV-I) (Blayney et al., 1983) (Bunn et al., 1983) (Yunoki et al., 1982). Similar etiologic mechanisms may play a role in the induction of hypercalcemia in both the feline and human retroviral-induced lymphomas. To our knowledge, feline primary hyperparathyroidism has not been reported. Skeletal metastasis is considered to be less commonly associated with tumors in small animals than in man (Chew & Meuten, 1982) (Meuten et al., 1982a), and in the absence of renal insufficiency, it appears that the most likely cause of hypercalcemic malignancy in small animals is the production by the neoplasm of bone-resorbing compounds.

All three cases of hypercalcemic malignancy reported here responded when the neoplasm was reduced by a form of immunotherapy. This reduction in serum calcium during treatment of malignancies did not occur in treated control cats which were not hypercalcemic (Table XI). The lack of skeletal metastasis and absence of overt parathyroid or renal pathology in cat 106 with mediastinal lymphoma, and the

simultaneous reduction in serum calcium and tumor size imply that the tumor in this cat may have initiated a humorally-mediated mechanism for the elevation in serum calcium. Cat 103 also responded to treatment with simultaneous reduction in both malignancy and serum calcium. This cat also lacked evidence of other causes of hypercalcemia, but presented with advanced leukemia and a massive lymphoid infiltration of the marrow. In this case, localized osteolysis resulting from the presence of the neoplastic marrow cells or a product of these cells may have contributed to the hypercalcemia. The change from erythroid to myeloid predominance in the myeloproliferative disorder of cat 117 may have selected against calcemia-producing neoplastic cells. The possible means by which ex vivo immunoadsorption employing staphylococcal protein A caused reduction in the extent of the malignant disease of these cats have been discussed elsewhere (Day et al., 1984) (Liu et al., 1984a,b).

Approximately 50% of the measurable serum calcium is protein-bound, primarily to albumin (90%) (Meuten et al., 1982a). Hypoalbuminemia can decrease the measured calcium. Correction formulas for calcium values have been derived for use in dogs that take into account changes in serum protein and albumin (Meuten et al., 1982b). The elevated mean serum calcium concentration in the cats with leukemia-lymphoma and the number of hypercalcemic malignancies in this study would have been even greater if the low serum albumin concentrations could have been considered. In the three cats which had elevated calcium levels and responded to treatment from our analyses application of correction factors derived from studies in dogs suggest that the calcium levels in each instance were almost

certainly pathologically elevated and declined with effective treatment of the disease (Table XI).

Although cases of feline hypercalcemic malignancy are rarely reported (Chew et al., 1975) (Zenoble & Rowland, 1979) (Wilkins & Hurvitz, 1975), the incidence of frank hypercalcemia in cats with malignancy in this study (3/11; 27%) was significantly greater ($p < 0.05$ by Fisher's exact test) than that in cats without malignancy (0/20). In addition, cats with FeLV-associated leukemia-lymphoma in this study did, as a group, have a significantly greater mean serum calcium concentration than normal or FeLV-infected cats without malignancy. Our observations indicate that hypercalcemia does occur with feline malignancy and can respond to ex vivo immunoadsorption treatment with SpA. In addition, it appears that cats with leukemia-lymphoma are more likely to present with elevated calcium levels that fall within the upper range of normal or are only slightly elevated. These findings suggest that future studies should be carried out to identify cats in which frank hypercalcemia is associated with malignancy. Definitive analyses must then be made, both in terms of the response to treatment and the identification in more precise terms of the basis of hypercalcemia.

CHAPTER V

CHANGING MANIFESTATIONS OF A CHRONIC FELINE HEMATOPOIETIC PROLIFERATIVE DISEASE DURING IMMUNOTHERAPY WITH STAPHYLOCOCCAL PROTEIN A

A. Summary

A cat presenting with feline leukemia virus associated malignant disease was treated by ex vivo immunoadsorption using staphylococcal protein A coated filters. During the twelve-week course of treatment, the morphological manifestations of the hematopoietic disease exhibited a progressive transition from erythroid to myeloerythroid to myeloid predominance, and was preceded by and associated initially and terminally with a blast transformation of lymphoblastic morphology. Necropsy revealed massive meningio-cerebral, as well as hepatic, renal, myeloid, lymphoid, peritoneal and pelvic infiltrations largely consisting of lymphoblastic cells. Yet evidence of myeloid and erythroid differentiation was present in all the infiltrates. The several possible bases for this shifting of morphological expression are considered.

B. Introduction

The unifying concept of myeloproliferative disease as those proliferative disorders involving one, several or all of the bone

marrow cell lines is based on the premise of neoplastic stem cell clones which are capable of at least some degree of differentiation (Quesenberry & Levitt, 1979). In most cases, these neoplastic clones appear to arise from a pluripotential stem cell and differentiate into one of several cell lines, granulocytic, monocytic, erythrocytic or megakaryocytic (Figure 27) (Harvey, 1981). In some cases either an earlier stem cell common to the pluripotential and lymphoid stem cells, or a more committed stem cell common only to granulocytic and monocytic cell lines may be involved (Harvey, 1981) (Quesenberry & Levitt, 1979 (Schalm & Theilen, 1970). Classification of these disorders has been based on which cell line(s) predominate, presumed maturity of the cell type, and whether neoplastic cells are present in the blood or are confined to the hematopoietic tissue (Harvey, 1981). Attempts to separate myeloproliferative disorders into distinct entities based upon morphologic features may be artificial since neoplastic stem cells appear to be able to differentiate into any of several cell types, manifestation of the disorder may vary with time, only rarely is a single marrow cell line affected, and disorders may often evolve from one morphologic form into another (Harvey et al., 1978) (Harvey, 1981) (Quesenberry & Levitt, 1979) (Schalm & Theilen, 1970) (Schalm et al., 1975) (Schalm, 1975).

Myeloproliferative disease is a more frequent disorder in cats than in other domestic animals (Harvey, 1981). It may manifest as a proliferation of cells with predominantly granulocytic (Fraser et al., 1974), monocytic (Henness et al., 1977b) (Tsujimoto et al., 1981), eosinophilic (Silverman, 1971) (Simon & Holzworth, 1967), megakaryocytic (Michel et al., 1976), or erythrocytic ((Harvey, 1981)

Figure 27: The relationships between hematopoietic stem cells and mature blood cells. Used with permission, P.W. Kincade and R.A. Phillips, Fed. Proc. 44:2874-2881, 1985.

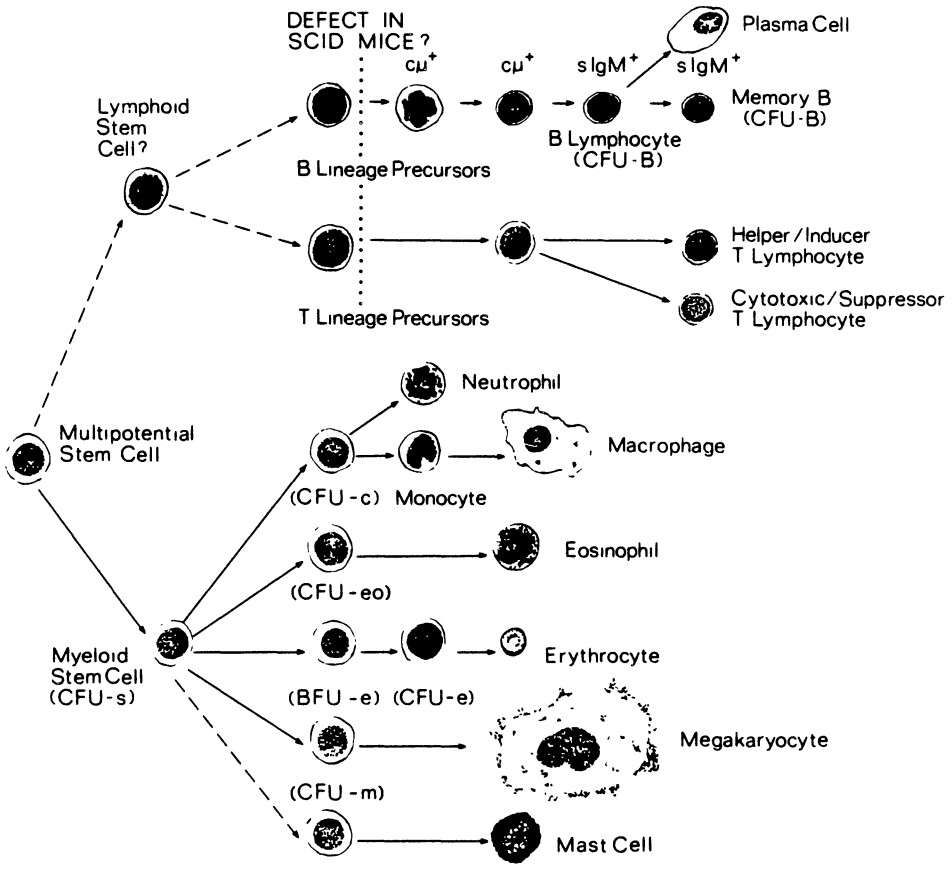

DEFECT IN
SCID MICE ?
Plasma Cell
cμ+
cμ+
sIgM+
sIgM+
Lymphoid
Stem
Cell?
Memory B
(CFU-B)
B Lymphocyte
(CFU-B)
B Lineage Precursors
Helper / Inducer
T Lymphocyte
Cytotoxic / Suppressor
T Lymphocyte
T Lineage Precursors
Neutrophil
Multipotential
Stem Cell
Macrophage
(CFU-c) Monocyte
Eosinophil
(CFU-eo)
Myeloid
Stem Cell
(CFU-s)
Erythrocyte
(BFU-e) (CFU-e)
Megakaryocyte
(CFU-m)
Mast Cell

Figure 27

(Schalm et al., 1975) (Ward et al., 1969) (Zawidzka et al., 1964) characteristics. Simultaneous proliferation of more than one cell line may occur (Maede & Murata, 1980) (Stann, 1979), poorly differentiated cells may be encountered (Crow et al., 1977), and the transition from a disorder of predominantly one cell type into a disorder involving another cell type has been recognized (Harvey et al., 1978) (Harvey, 1981) (Schalm & Theilen, 1970) (Schalm, 1975) (Schalm et al., 1975) (Ward et al., 1969). In the present analysis, we consider a 7-year-old mixed breed female cat with hematopoietic proliferative disease which was referred to the Oklahoma State University, Department of Veterinary Pathology, for anti-cancer immunoadsorption treatment. This experimental therapy employed staphylococcal protein A filtration columns (Day et al., 1984). Sequential evaluation of bone marrow, lymph node and peripheral blood cytology during the course of treatment revealed changes in the morphological manifestations of the proliferative disease and involved sequential changes in the predominant cell lineage. The observations support the view that neoplastic proliferation may involve multipotential stem cells and suggest that in some cases such clones may arise from a primitive pluripotential stem cell common to both the lymphoid and the myelo-erythroid cell lines.

C. Material and Methods

The cat which is reported herein was one of a series of pet cats referred to the Oklahoma State University Department of Veterinary Pathology and selected for treatment by ex vivo immunoadsorption employing staphylococcal protein A filters (AMF/Specialty Materials

Group, Talcotville, CN) after cytologic and radiographic examination had revealed hematopoietic neoplasia (Day et al., 1984). Viral status for feline leukemia virus (FeLV) was determined using a fixed cell indirect immunofluorescent antibody test (IFA) and an enzyme-linked immunosorbent assay (ELISA) as described (Hardy et al., 1973b) (Mia et al., 1981). This cat received ex vivo immunoadsorption treatment as previously described for 12 weeks (Day et al., 1984). Physical examination and body temperature were determined daily. Body weight, hemograms and serologic tests were performed weekly and bone marrow and lymph node cytology were analyzed at every 5th treatment. Periodic urinalysis and evaluation of serum chemistries and protein electrophoresis were also performed. Adjunct therapy was minimal and consisted only of I.V. or S.Q. fluids and antibiotics. Four transfusions of washed leukocyte-poor packed red blood cells were given late in the course of the disease. Cytologic preparations were stained with Wright's Dip-Stat (Medi-Chem, Inc., Santa Monica, CA). Post-mortem, tissues were fixed in 10% buffered formalin, embedded in paraffin, cut at 6-8 μm, and stained with hematoxylin and eosin. Additional sections of some tissues were stained with either periodic acid-Schiff or methenamine silver.

D. Results

The cat studied presented with moderate peripheral lymphadenopathy, blindness resulting from ocular neoplastic infiltrates, and was infected with feline leukemia virus (FeLV). Approximately 40% of the cells in the pretreatment cytologic preps of the lymph node were lymphoblasts with scant, moderately blue cytoplasm, large, oval to

angular nuclei with smudged chromatin and one or more irregular nucleoli. A small number of megakaryocytes, occasional rubricyte, metarubricyte, and metamyelocyte and moderate numbers of bands and segmented granulocytes were also present. Flecks of bone marrow obtained by aspiration showed hypercellularity and abundant blasts, similar morphologically to those present in lymph node aspirates. These blast forms comprised approximately 50% of the nucleated cells in the marrow (Figure 28). Erythroid cells were few in number and proportion. Granulocytes were normal or slightly decreased but appeared to be maturing normally. Peripheral blood examination showed evidence of a mild, nonregenerative anemia, absolute leukocytosis, neutrophilia, monocytosis and lymphocytosis with numerous blast forms (Table XII).

Although a diagnosis of myeloproliferative disease was considered, the animal was classified cytologically as having multicentric lymphoma with lymphoblastic leukemia based upon the morphology of the blast forms within the marrow and lymph node. Supporting this conclusion was the absolute lymphocytosis which ranged during the first 40 days from 7,575-29,755/µl with a mean of 10,438/µl, the apparent normal maturation of marrow granulocytes, and the sparcity of erythroid cells in the marrow. The absolute neutrophilia and monocytosis were attributed to an abscess present on the ventral perineum, from which Streptococcus canis was cultured. The animal was treated with I.M. ampicillin, 10 mg/kg b.i.d. from days 40-56.

Experimental ex vivo immunoadsorption treatments for the animal's hematopoietic proliferative disease were administered twice weekly for the next 12 weeks. During this time, the animal became

Figure 28: Pretreatment marrow cytology. Prior to immunotherapy, lymphoblastic leukemia was considered the appropriate cytological classification for the cat's hematopoietic proliferative disease. Greater than 50% of the nucleated cells in the pretreatment bone marrow were lymphoblasts (x720, x1600).

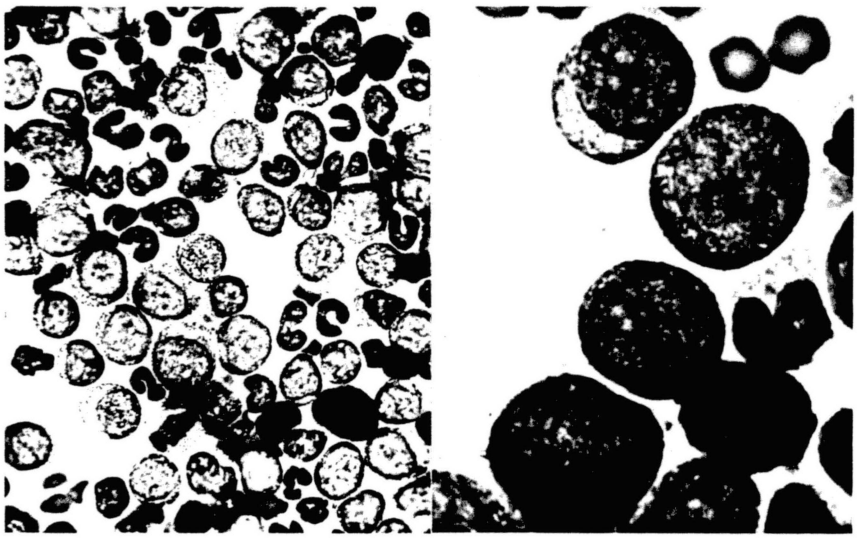


Figure 28

TABLE XII

HEMATOLOGIC TRENDS OF A CAT TREATED BY EX VIVO IMMUNOADSORPTION USING STAPHYLOCOCCAL PROTEIN A

	HCT[a]	MCV	MCHC	Retics	NRBC	WBC	Seg	Band	Lymph	Mono	Eos	Meta	Myelo	Pros	Thromb	
DAY	%	μ^3	%	%	100 WBC	x10^3/µl	µl	µl	µl	µl	µl	µl	µl	µl	x10^3/µl	Rxs
	24-45	39-55	30-36		0	5.5-19.5	2500-12,500	0-300	1500-7000	0-800	0-1500	0	0	0	300-700	
1	22	39	38.2	0	1	30.3	19,695	0	7575	1818	1212	0	0	0	651	0
18	23	41	42.1	0.7	0	54.1	22,181	0	29,755	1623	541	0	0	0	300	4
35	12	41	42.5	0	2	25.8	14,190	0	8772	2322	516	0	0	0	189	9
53	22	45	34.6	0.1	11	28.5	18,810	1425	5130	3135	0	0	0	0	290	14
67	17	60	25.9	0.4	14	57.7	32,889	8655	5770	0	0	8655	1731	0	76	18
77	12	44	41.7	0.3	5	30.0	22,200	900	4200	300	600	900	300	300	226	20
97	12	46	35.0	0.2	17	31.3	12,500	4069	12,833	313	933	626	0	0	740	24
124	14	43	36.4	0	2	24.6	3936	2460	16,728	984	492	738	0	0	970	24

progressively more anemic with a hematocrit as low as 11 and with increasing numbers of nucleated red blood cells in the peripheral blood (Table XII). Peripheral lymph nodes became progressively smaller and could no longer be palpated easily after day 20. Lymph node cytology was not re-evaluated. Bone marrow flecks on day 41 were markedly hypercellular with increased numbers of erythroid cells which showed dyscrastic (excessive rubriblasts and prorubricytes) and dysplastic (megaloblastoid change, and gigantism) features (Figure 29). Granulocytic cells were moderately increased, but without apparent dyscrasia. Approximately 20% of the nucleated cells in the marrow at this juncture were lymphoblasts.

The identification of megaloblastoid change, gigantism and preponderance of rubriblasts and prorubricytes suggested that the erythroid proliferation was of a neoplastic nature. A diagnosis of myeloproliferative disease with erythroid prominence and marrow infiltration by lymphoblasts was made.

Washed leukocyte-poor packed red blood cell transfusions administered on days 46, 49, 67 and 93 caused only mild improvement in hematocrit. On day 67, a granulocytic left shift to the myelocyte level was recognized (Table XII). Marrow flecks at this stage showed marked hypercellularity, an increase of granulocytic cells with excessive numbers of myeloblasts and progranulocytes. Numerous rubriblasts and frequent megaloblastoid cells were also present. Erythroleukemia was considered to be the most appropriate cytologic classification for this stage of the animal's proliferative disorder.

Figure 29: Mid-treatment marrow cytology. On day 41 after 10 immunoadsorption treatments, a dyscrastic and dysplastic erythroproliferative predominance with excessive numbers of rubriblasts and prorubricytes were present in hypercellular marrow flecks (x920, x2050).

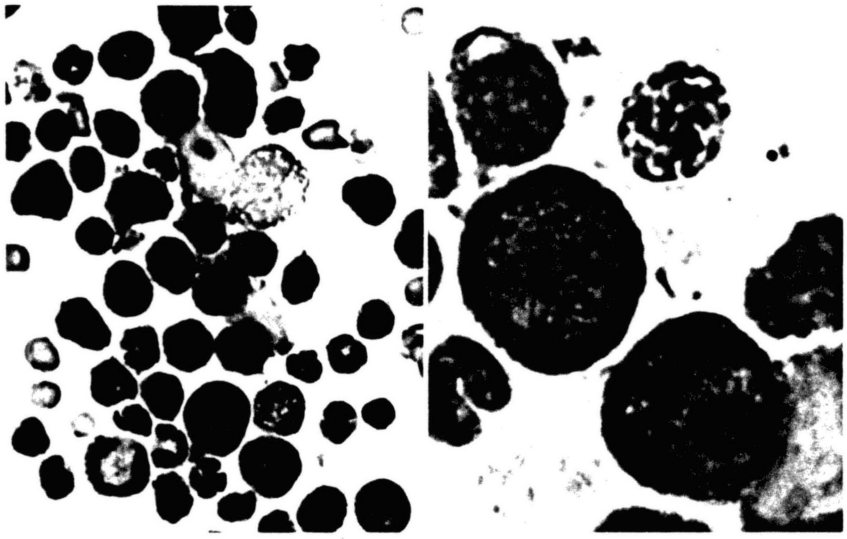


Figure 29

Other clinical problems included the identification of hemobartonellosis on day 67, which was treated with oral tetracyclines until day 93, and abnormalities indicative of inflammation and glomerulotubular damage present on urinalysis on days 65 and 76.

On day 77 the left shift in the neutrophil series extended to the promyelocyte level. Markedly increased numbers of granulocytes with an excess of myeloblasts were present in hypercellular marrow flecks (Figure 30). At this stage, erythroid cells were present without dyscrasia and in approximately normal numbers, but megaloblastic forms were still readily identified.

On day 120, 27 days after the 24th and final ex vivo immunoadsorption treatment, the marrow was again populated (approximately 20% of the nucleated cells) by lymphoblasts as previously described. Erythroid cells were moderately decreased in number and granulocytes were increased with an excessive proportion of myeloblasts. An absolute lymphocytosis of the peripheral blood which was present since the 24th treatment was recognized. The descriptive cytologic classification used for this stage of the animal's proliferative disease was granulocytic leukemia with lymphoblastic marrow infiltration. The sequential cytologic classifications of this hematopoietic proliferative disease and their basis are presented in Table XIII.

The animal died on day 124 after a weight loss of 1.12 kg with a non-regenerative anemia, marked absolute thrombocytosis, and FeLV infection. Complete serum chemistry and electrophoretic evaluations on days 14, 29, 43, 56, 69, 77 and 93 did not identify significant or consistent abnormalities.

Figure 30: Post-treatment marrow cytology. Hypercellular bone marrow aspirates on day 77 after 20 immunoadsorption treatments consisted of excessive numbers of myeloblasts and progranulocytes. At this stage of the disease, a left shift in the neutrophil series extended to the promyelocyte level (x720, x1600).

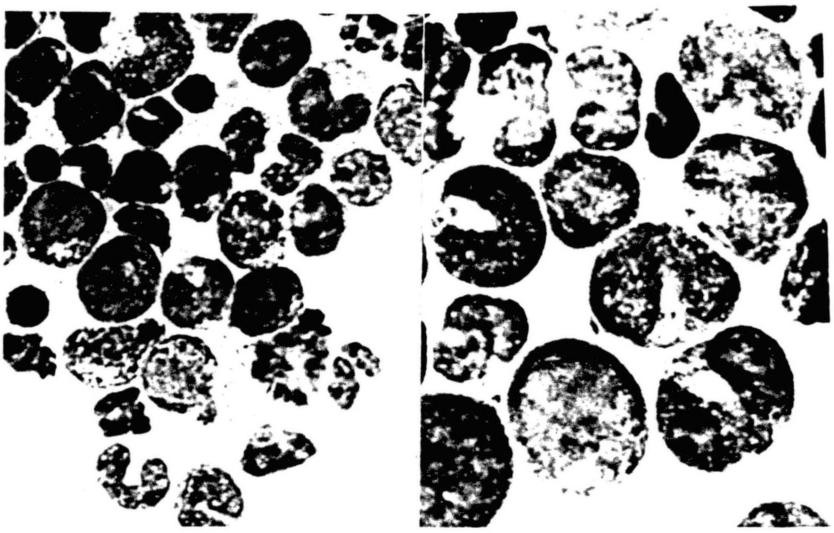


Figure 30

TABLE XIII

SEQUENTIAL CYTOLOGIC CLASSIFICATIONS OF A CAT'S HEMATOPOIETIC PROLIFERATIVE DISORDER AND THEIR BASIS

Day	Immuno Rxs	Classification	Bone Marrow[a]	Peripheral Blood[b]
0	0	Lymphoblastic leukemia	>50% lymphoblasts	Lymphocytosis (7575-29,755/μl)
41	10	Erythemic myelosis	Dyscrastic, dysplastic, erythroid predominance	NRBC's
67	18	Erythroleukemia	Dyscrastic, dysplastic, myeloerythroid proliferation	Left shift to myelocyte, NRBC's
77	20	Granulocytic leukemia	Dyscrastic, dysplastic, myeloid proliferation	Left shift to promyelocyte, NRBC's
120	24	Lymphoblastic crisis with granulocytic leukemia	>20% lymphoblasts with dyscrastic, dysplastic, myeloid proliferation	Lymphocytosis (12,833-16,728/μl)

[a]All bone marrow aspirates were markedly hypercellular.

[b]All total blood leukocyte counts were elevated.

At necropsy, the animal was emaciated. Neoplastic infiltrates were identified in the meninges, ependyma, eyes and extending into the brain, lungs, and present multifocally in the liver and kidneys. The neoplastic material also formed a large caudal abdominal and pelvic mass which surrounded both kidneys, ureters, the urinary bladder and filled the pelvic cavity. Microscopically, similar cells comprised the pelvic-abdominal mass and the parenchymal infiltrates. Most of the cells were lymphoblasts. Mitotic figures were common. Numerous other cells were also present and appeared to represent all stages of myeloid and erythroid development. Megakaryocytes were frequently encountered. Large, extracapsular densely cellular aggregates were frequently encountered in lymph nodes, spleen and kidney. The bone marrow was markedly hypercellular with similar cells. A mild membranous glomerulonephritis was recognized using periodic acid-Schiff and methenamine silver stains.

E. Discussion

A number of hematopoietic proliferative disorders appear to represent neoplastic transformation of stem cells at one of three levels. Potentially neoplastic clones could be derived from a primitive pluripotential stem cell with the capacity to differentiate to all blood cell types including lymphocytes, or from a pluripotential stem cell capable of producing myeloid, erythroid and megakaryocytic cells, or from a granulocyte-monocyte stem cell (Figure 27) (Harvey, 1981) (Quesenberry & Levitt, 1979). Evidence for this concept in human myeloproliferative disorders has been obtained by demonstrating the presence of shared chromosomal abnormalities or the presence of a

single isoenzyme of X-linked glucose-6 phosphate dehydrogenase within the proliferative, heterogenous cell types (Quesenberry & Levitt, 1979). These shared characteristics suggest the presence of a single progenitor cell for a morphologically heterogeneous population of cells. In addition, some disorders appear to evolve or change manifestation with time. Although in humans the intermediate, myeloerythroid pluripotential stem cell is thought to be most frequently involved, several lines of evidence has been presented suggesting that in some cases of chronic myelogenous leukemia, the neoplastic transformation may involve a more primitive stem cell common to both the pluripotential stem cell and the lymphoid system (Quesenberry & Levitt, 1979). In addition, approximately 20% of humans with chronic myelogenous leukemia undergo blast transformation with blasts which have distinct lymphoid morphology, as well as certain characteristics of cells of the T-lymphocyte lineage (Peterson et al., 1976). In cases of myelomonocytic leukemia, the affected cell may be a more differentiated granulocyte-monocyte stem cell.

Myeloproliferative disease occurs relatively frequently in the cat, manifests as an abnormal proliferation of granulocytes, monocytes, eosinophils, megakaryocytes or erythrocytes, or as a simultaneous proliteration of more than one cell type (Schalm et al., 1975). In some cases, disease manifestation may change dramatically from a disorder predominantly of one cell type to another (Gilmore et al., 1964) (Quesenberry & Levitt, 1979) (Schalm & Theilen, 1970) (Schalm, 1975) (Schalm et al., 1975) (Ward et al., 1969). Electron microscopic observations of type-C viral particles budding from marrow cells of cats with myeloproliferative disease (Herz et al., 1970),

and the high incidence of feline leukemia virus (FeLV) infection in affected cats imply a causal relationship between FeLV and myeloproliferative disorders (Harvey, 1981). Only once, however, has a FeLV isolate produced this disorder experimentally (Jarrett et al., 1971).

Cats with myeloproliferative disease generally present with hepatic, splenic and sometimes lymph node enlargement and often have nonregenerative anemias which are either normocytic, normochromic or macrocytic, normochromic. Nucleated red blood cells are frequently present in circulation. Leukocyte, platelet and erythrocyte numbers and morphology vary depending upon the cytologic classification of the disorder (Harvey, 1981) (Schalm et al., 1975). The proliferating cell lines are variably distributed in the bone marrow, liver, spleen, and lymph nodes and are usually confined to vascular and lymphatic space, although in more aggressive proliferations neoplastic cells (erythroid, myeloid and/or megakaryocytes) can be found throughout most tissues (Gilmore et al., 1964) (Moulton, 1978) (Ward et al., 1969).

The progressive transition of hematopoietic proliferative disease in this cat from erythroid to myeloerythroid to myeloid predominance was preceded by and associated terminally with a blast transformation which had lymphoblastic morphology. Widespread organ infiltration and a large pelvic-abdominal mass of neoplastic cells were present at post-mortem examination. Malignant infiltrates were not confined to vascular, lymphatic space and frequently formed variably sized nodular masses with parenchyma and outside capsules. Central nervous system, ocular and urinary tract infiltrates were

most extensive. Extensive tissue infiltration and central nervous system involvement are common in the 20% of humans with chronic myelogenous leukemia that undergo blast transformation with blasts of lymphoid morphology (Peterson et al., 1976) (Quesenberry & Levitt, 1979).

Malignant transformation of the primitive pluripotential or possibly the myelo-erythroid pluripotential stem cell represent potential origins for this cat's proliferative disease. Lesions of the magnitude and variability recognized in this cat have not been reported previously. With declining clinical condition, most cats with myeloproliferative disorder are euthanized. The experimental ex vivo immunoadsorption therapy using staphylococcal protein A filtration columns employed in this study has produced regression of breast cancer in dogs (Terman et al., 1980) and humans (Terman et al., 1981), as well as regressions of FeLV-induced neoplasia and even to a virus-negative state in a few cats (Day et al., 1984) (Jones et al., 1980) (Liu et al., 1984a,b) (Snyder et al., 1984). The possible mechanisms by which this experimental immunotherapy causes reduction in the extent of FeLV-induced malignant disease have not been completely elucidated, but have been discussed elsewhere (Liu et al., 1984a,b) (Snyder et al., 1984). These treatments may have played a role in selectively reducing more susceptible blood cell populations, thereby contributing to the successive changes in manifestation of disease in this cat. It is also possible that these changes in manifestation were observed because the survival of the cat was prolonged.

If treatment with staphyloccocal protein A can indeed participate in altering the morphologic expression of lympho-myeloproliferative disease in cats, and/or prolonging the survival of cats with this disease, future studies may permit opportunity to analyze definitively whether these proliferative disorders are attributable to several clones of malignant cells derived from a common progenitor stem cell, or alternatively, represent a single malignant clone whose morphologic expression is influenced by protein A immunotherapy. Important will be initial chromosomal and/or enzymatic analyses of the malignant cells and successive similarly detailed analyses of the cells that predominate at different stages of response.

CHAPTER VI

SUPPRESSION OF GAMMA INTERFERON PRODUCTION BY INACTIVATED FELINE LEUKEMIA VIRUS

A. Summary

Supernates from cultures of normal feline lymphocytes stimulated with Staphylococcus enterotoxin A (SEA) effected antiviral activity characterized as a gamma-like interferon. With the addition of inactivated feline leukemia virus (FeLV), markedly less interferon was produced. This reduction in interferon production was not attributable to either lowered lymphocyte viability or reduced SEA mitogenic properties and appears to be a direct retroviral effect. This finding may reflect clinically relevant events which may contribute to the development of either the feline (FeLV-induced) or the human (HTLV-III-induced) states of acquired immunodeficiency.

B. Introduction

The feline leukemia virus (FeLV), a contagious retrovirus which is transmitted primarily via salivary secretions, causes either neoplastic or non-neoplastic diseases in the infected cat including a state of immunodeficiency characterized by diminution of both cellular and humoral immunity. Retrovirus-induced immunosuppression, which is the most frequent sequelae of persistent FeLV viremia,

predisposes the animal to secondary illness of infectious or auto-immune origin and accounts for a majority of the FeLV-related deaths (Hardy, 1980a,b) (Hardy, 1982). Viremic cats have suppressed blastogenic responses to T-cell mitogens, reduced lymphocyte membrane con A receptor mobility, suppressed antibody responses to synthetic polypeptides, prolonged allograft rejection times, and exhibit varying degrees of hypocomplementemia, thymic atrophy and depletion of the paracortical zones of lymph nodes (Cockerell et al., 1976b) (Dunlap et al., 1979) (Trainin et al., 1983) (Olsen et al., 1981) (Kobilinsky et al., 1979). In addition, our earlier investigations demonstrated that peripheral blood and splenic lymphocytes from FeLV infected cats when stimulated with T-cell mitogens could not be induced to produce or when induced produced only low titers of interferon (IFN) (Liu et al., 1984b). Although little is known about the way in which FeLV initiates immunosuppression, the virus may mediate immuno-modulating events independent of cellular infection since UV-light inactivated FeLV and certain FeLV structural proteins have been shown to impair lymphocyte proliferative responses and membrane receptor capping to con A *in vitro* (Hebebrand et al., 1977) (Mathes et al., 1979). Further studies suggest that this inability of lymphocytes to respond to mitogenic stimuli during co-incubation with inactivated virus may be due to decreased elaboration of T-cell growth factor (possibly interleukin-2) activity (Wainberg et al., 1983). In this report, we show that the *in vitro* synthesis of a gamma-like interferon by normal feline lymphocytes which have been stimulated with *Staphylococcus* enterotoxin A (SEA) is reduced when

UV-light inactivated FeLV is also present in culture during the period of stimulation.

C. Material and Methods

Seven healthy domestic cats free of FeLV maintained in our laboratory served as donors of peripheral blood lymphocytes (PBL). These cells were isolated from defibrinated whole blood by Ficoll-hypaque density centrifugation. Cell viabilities were determined by vital dye exclusion. Five million lymphocytes per milliliter in complete medium supplemented with 5 x 10^{-5} M 2-mercaptoethanol were placed in 16mm diameter flat-bottom microplate wells and cultured either in the absence of mitogen, in the presence of 1.0 µg/ml SEA (Microbial Biochemistry Branch, Division of Microbiology, Food and Drug Administration, Cincinnati, OH), or with both 1.0 µg/ml SEA and between 10 and 625 µg of UV-inactivated KT-FeLV. KT-FeLV was purified and UV-inactivated as described (Mathes et al., 1979). Final culture volume in all cases was 1.0 ml. Microplates were placed in culture boxes, incubated at 37°C in a humidified atmosphere containing 10% CO_2 and rocked at 8 cycles/min for 72 hrs. Cultures were then harvested, cells pelleted by slow centrifugation and supernates collected and stored at 4°C. The antiviral activity of supernatant fluids from these cultures were titrated for ability to inhibit the cytopathic effects of approximately 40 plaque-forming units (PFU) of vesicular stomatitis virus (VSV) on a monolayer of feline lung cells (FL, American Type Culture Collection, Rockville, MD), using a plaque reduction method. All titrations were carried out in duplicate. IFN units/ml were determined as the reciprocal of

the dilution which reduced the number of PFU by 50%. A laboratory feline IFN reference sample was used to monitor the assays.

To determine whether the inactivated FeLV we were using suppressed mitogen-induced lymphocyte proliferation, four separate lymphocyte blastogenesis assays were performed using the methodology previously reported (Mathes et al., 1979), except that between 2.5-10.0 µg con A/well or 0.05-0.5 µg SEA/well served as mitogens and 18.75 µg of inactivated FeLV was used in test wells. Under these conditions, the inactivated virus to lymphocyte ratio was 18 x 10^{-5} µg/cell and the percent residual proliferative response ranged between 22.4% and 78.5% which is comparable to the reported determinations.

D. Results

Two, three or four separate culture series were established using lymphocytes from each of the seven cats. The induced antiviral activity of the supernate is presented as an average of 2-4 determinations in Table XIV. Supernate from wells containing only lymphocytes and complete medium demonstrated no antiviral activity (except for 2 of the 4 cultures using cells from cat 248). Cultures containing SEA-stimulated lymphocytes produced between 47 and 768 U/ml IFN. When 625 µg of inactivated FeLV was co-incubated with SEA in lymphocyte cultures, the supernate demonstrated no antiviral effect. With the addition of 400 µg of virus to SEA-stimulated lymphocyte cultures, the induced antiviral activity of the supernate was markedly reduced as compared to the positive control. Under these conditions, the virus to lymphocyte ratio was 8 x 10^{-5} µg/cell

TABLE XIV

TITER OF INTERFERON GENERATED AND PERCENT VIABILITY OF PERIPHERAL BLOOD LYMPHOCYTES AFTER 72 HOURS OF CULTURE

			IFN (U/ml)							
				SEA (1.0 µg/ml)						
				UV-inactivated FeLV (µg/ml)						
Cat	# of Exps.	No Mitogen		625	400	300	200	100	50	10
301	2	<10	214	ND	19	52	61	134	150	196
249	3	<10	168	ND	39	67	124	150	141	378
1225	2	<10	47	<10	<10	<10	17	ND	ND	25
1264	2	<10	200	ND	39	43	99	112	83	ND
302	2	<10	289	ND	35	125	44	119	150	130
248	4	367	768	ND	409	89	734	980	875	815
000	2	<10	287	<10	ND	ND	160	367	ND	ND
				Viability (post-culture)						
# Samples tested		15	28	ND	14	11	13	11	6	8
% Viable		90±10	93±4	ND	86±11	90±6	89±6	92±7	95±2	96±2

and the percent residual antiviral activity ranged between 8.8% and 53.2%. With further dilution of the virus, the induced antiviral activity of the supernate increased. In some cases, when small amounts of inactivated FeLV were incorporated in cultures the resultant antiviral effect exceeded the positive control (cats 249, 248).

To characterize the antiviral activity of supernates, in separate tests supernates were dialyzed at pH 2, heat-treated at 56°C for 30 min, or submitted to ultracentrifugation at 110,000 x g for 90 min. No antiviral activity remained in samples dialyzed to pH 2 or subjected to heat treatment, while ultracentrifugation did not reduce the antiviral effect (Table XV). Supernates that effected plaque reduction when VSV was used as the challenge virus also demonstrated this protective antiviral effect when feline calicivirus (FCV, F-9) was substituted as challenge virus, showing that the antiviral substance inhibited effectively the replication of other viruses. Feline herpesvirus (FHV-1, C27) was insensitive to any antiviral activity in any of the tested supernates. Variation in the sensitivity of different viruses to interferon has previously been described. Samples that demonstrated antiviral activity in feline lung cell monolayers showed no activity in a heterologous system of IFN assay using MDBK bovine kidney cell monolayers (American Type Culture Collection, Rockville, MD), and VSV as a challenge virus. Based on these findings, the antiviral effect resulting from the treatment of feline lung cell monolayers with supernates from cultures containing SEA-stimulated lymphocytes was attributable to an

TABLE XV

CHARACTERIZATION OF THE ANTIVIRAL ACTIVITY OF CULTURE SUPERNATES AS A GAMMA-LIKE INTERFERON

IFN (U/ml)

A. Treatment at pH 2

Cat	pH 7.2	pH 2
248	767	<10
249	339	<10
1264	246	<10

B. Treatment at 56°C/30 min

Cat	Control	56°
1276	44	<10
1264	246	<10

C. Activity against Other Viruses

Cat	VSV	FCV	FHV-1
301	150	267	<10

D. Heterologous Cells

Cat	FL	MDBK
302	200	<10
301	150	<10

interferon. This interferon was a gamma-like (immune) interferon based on pH 2 susceptibility.

The reduction in IFN production in lymphocyte cultures where cells were co-incubated with virus and SEA was not solely attributable to a reduction in the number of cultured viable lymphocytes. By resuspending individual cell pellets in 1.0 ml of complete medium after harvesting the supernates, average lymphocyte viability after 72 hrs of incubation was determined in 106 cultures from three experiments involving 4 of the cats (Table XIV). Post-culture lymphocyte viabilities differed only slightly, regardless of the conditions of culture.

To show that the addition of virus did not neutralize the mitogenic properties of SEA, 1250 µg of inactivated FeLV and 50 µg of SEA were incubated together at 37°C for 18 hr in 4.0 ml of complete medium. The virus was then removed by ultracentrifugation at 100,000 x g for 90 min. The remaining SEA-containing supernate, used in place of stock SEA for the stimulation of lymphocytes, induced comparable IFN titers when compared to stock SEA (Table XVI). To determine whether IFN was produced but subsequently neutralized by the presence of inactivated virus, 400 µg of FeLV was added to SEA-stimulated lymphocyte cultures for the final two hours of incubation. Subsequent testing of these supernates showed no reduction in antiviral activity (Table XVI), suggesting that the FeLV-induced impairment of anti-viral activity was a result of reduced IFN synthesis and not reduced IFN effectiveness caused by the presence of FeLV.

TABLE XVI

FeLV DOES NOT ALTER THE MITOGENIC PROPERTIES OF SEA OR THE ANTIVIRAL ACTIVITY OF SEA-STIMULATED LYMPHOCYTE SUPERNATES

IFN (U/ml)

A. Treatment of SEA

Cat	Stock SEA	SEA after Incubation with FeLV
1276	550	727
249	150	139

B. Treatment of Culture Supernate

Cat	No treatment	Addition of 400 µg FeLV
301	123	80
301	150	150
301	138	143

E. Discussion

FeLV-mediated reduction in interferon synthesis in vitro may reflect clinically relevant impaired lymphocyte function in cats persistently infected with FeLV. Gamma interferon appears to occupy a pivotal regulatory position in cellular immune responses and has been shown to enhance human macrophage antimicrobial activity, as well as to augment cytotoxic and natural-killer cell activities and the expression of interleukin-2 receptors on T-cells (Kawase et al., 1983) (Nathan et al., 1983) (Johnson & Farrar, 19838). Deficient synthesis of this lymphokine may be clinically relevant to the pathogenesis of the state of immunodeficiency of FeLV infected cats. This finding is especially provocative in light of the proposed retroviral etiology (HTLV-III) of the human acquired immunodeficiency syndrome (AIDS) (Marx, 1984) (Popovic et al., 1984) (Gallo et al., 1984) (Schupbach et al., 1984) (Jaffe et al., 1984). A recent study of 16 patients with AIDS who had opportunistic infections showed that mononuclear cells from 11 of the 16 produced sub-normal amounts of gamma interferon in response to mitogen (Murray et al., 1984). Although this sub-normal synthesis of interferon may reflect a quantitative deficiency in the principal T-cell subset responsible for interferon synthesis, impaired interferon synthesis in the 11 patients and substantial interferon synthesis in the other 5 could not necessarily be attributed with assurance to alterations in lymphocyte numbers. It is possible that in addition to or independent of a reduction in IFN-synthesizing cells, the presence of immunosuppressive quantities of retrovirus at the cellular level may

markedly impair the synthesis of IFN and, perhaps, other lymphokines *in vivo*. The immunologic amplification attributable to these mediators would then be significantly reduced resulting in a diminution of functional immunity and an increased susceptibility of the patient to serious secondary illness.

CHAPTER VII

POLYCLONAL INDUCTION OF IMMUNOGLOBULIN SYNTHESIS BY FELINE LEUKOCYTES AS IDENTIFIED IN A REVERSE HEMOLYTIC PLAQUE ASSAY

A. Summary

Optimal conditions of culture and assay for identification of feline immunoglobulin secreting mononuclear cells were determined for the staphylococcal protein A-reverse hemolytic plaque assay (SpA-RHPA). Hemolytic plaques were most distinct and numerous when peripheral blood mononuclear cells were stimulated with 6.9 g/ml pokeweed mitogen for seven days. Immunoglobulin secreting cells were identified morphologically within a zone of hemolysis utilizing a 1:5 dilution of rabbit anti-cat IgG and a 1:30 dilution of guinea pig complement as developing reagents. The SpA-RHPA system should contribute to an understanding of normal feline T and B-lymphocyte interactions and will likely aid in the identification and understanding of immune cell dysfunctions associated with chronic feline leukemia virus infection.

B. Introduction

Cells secreting immunoglobulin (Ig) can be identified as hemolytic plaque forming cells (PFC) in assays in which focal lysis in

the vicinity of the Ig-synthesizing cell is revealed in a field of erythrocytes after incubation with complement. A direct hemolytic PFC secretes IgM which either reacts specifically with determinants of naturally occuring sheep erythrocytic membrane antigens (Jerne & Nordin, 1963), or with a highly haptenated sheep erythrocytic membrane surface (Nespoli et al., 1979). Cells secreting low efficiency hemolysin (primarily IgG) are identified in the presence of optimal dilutions of anti-immunoglobulin and are termed indirect PFC (Dresser & Wortis, 1965; Fauci et al., 1980a).

Cells secreting immunoglobulin of unknown specificity can be identified in a reverse hemolytic plaque assay (RHPA) in which the target erythrocyte is directly coated with anti-Ig by a chromium chloride method (Gold & Fudenberg, 1967; Molinaro & Dray, 1974; Eby et al., 1975). In the RHPA system, total or class specific immunoglobulin production against multiple specificities can be measured by coating the target erythrocyte with an appropriate anti-immunoglobulin reagent which binds immunoglobulin secreted by the PFC. A modification of the RHPA utilizes staphylococcal protein A (SpA), coupled to the erythrocytic membrane by chromium chloride treatment, which binds the constant fragment (Fc) of the anti-Ig which in turn binds the immunoglobulin secreted by the PFC (Gronowicz et al, 1976). In each assay, after incubation in the presence of complement, PFC in a field of erythrocytes are identified morphologically within a zone of hemolysis.

The <u>in vitro</u> production of Ig subsequent to pokeweed mitogen (PWM) induced polyclonal lymphocyte activation is a function of opposing T-lymphocyte helper and suppressor influences (Fauci et al.,

1980b; Pryjma et al., 1980a) and, under appropriate culture conditions which limit cell to cell interactions, is apparently monocyte dependent (Rosenberg & Lipsky, 1979). These cellular interactions which regulate the generation of immunoglobulin can be evaluated using the protein A-reverse hemolytic plaque assay (SpA-RHPA).

Domestic cats transmit horizontally a type C retrovirus (feline leukemia virus, FeLV) which can induce leukemia-lymphoma or a state of immunodeficiency in the chronically infected cat (Hardy, 1980a,b). Some FeLV-induced diseases have been proposed to serve as experimental models of preleukemia, aplastic anemia and acute lymphoblastic leukemia of humans (Cotter & Essex, 1977; Maggio et al., 1978). A means of evaluating feline immune cell interactions has been needed, since understanding of retroviral-induced states of immunodeficiency and hematopoietic-lymphoreticular neoplasms neccessitates definition of helper and suppressor T-lymphocyte influences as well as functions of B-lymphocytes.

Previous efforts in cats using the SpA-RHPA system failed to identify feline plaque forming cells using a one-step procedure where all developing reagents were added simultaneously (Langweiler, 1983). The present report describes for the first time the optimal culture and assay conditions for the demonstration of feline PFC using a two-step SpA-RHPA procedure where developing antiserum, SpA-SRBC and mononuclear cells are mixed, plated then bathed in complement.

C. Material and Methods

1. Culture Conditions

Defibrinated whole blood was obtained by external jugular venipuncture from normal adult male and female cats ranging in age from 2-6 years. Cats used were shown to be free of FeLV as demonstrated by the indirect immunofluorescent antibody test (IFA) for FeLV in leukocytes (Hardy et al., 1973b) (Antibodies Inc., Davis, CA) and the enzyme-linked immunosorbent assay (ELISA) for the detection of FeLV antigen in serum (Mia et al., 1981) (Pitman-Moore Inc., Washington Crossing, NJ). Mononuclear cell suspensions were derived from defibrinated blood by Ficoll-hypaque (Histopaque 1077; Sigma, St. Louis, MO) density centrifugation and washed three times in Hank's balanced salt solution without Ca^{++} and Mg^{++} (incomplete HBSS; Gibco, Grand Island, NY). Cells were then adjusted to 1 X 10^6/ml in Complete Medium (RPMI-1640 with L-glutamine (Gibco) supplemented with 10% heat inactivated and SRBC-absorbed fetal calf serum (FCS), 100 U/ml penicillin and 100 µg/ml streptomycin).

Stock pokeweed mitogen (Gibco) was suspended at 1450 µg/ml, aliquoted in 500 µl quantities and frozen at -20°C. On the day of culture, stock PWM was diluted 1:5, 1:10 or 1:15 in Complete Medium. 50 µl of the diluted PWM was placed at the bottom of 12 X 76 mm plastic culture tubes and followed by 1.0 ml of mononuclear cell suspension. Final PWM concentrations were 4.6 µg/ml, 6.9 µg/ml and 13.8 µg/ml. Tubes were loosely capped and incubated at 37°C in a 10% CO_2 humidified atmosphere for 3-8 days.

2. Coupling of SpA to SRBC

Sheep blood was collected in citrate-phosphate-dextrose-adenine solution collection bags (Fenwal Labs, Deerfield, IL), kept at 4°C and used within 2 weeks. SRBC were washed five times with phosphate buffered saline (PBS; Gibco) and pelleted. One milliliter of staphylococcal protein A (SpA; Pharmacia, Piscataway, NJ) stock solution at 1.0 mg/ml was added to 4.0 ml of PBS and used to resuspend each milliliter of washed, packed SRBC. To this suspension, 5.0 ml of 6.0 µg/ml $CrCl_3$ solution (J.T. Baker Co., Phillipsburg, NJ) at pH 5 was slowly added. The cellular suspension was then incubated in a 30°C water bath for 60 minutes with gentle inversion every 10 minutes. The labeled cells were washed twice with PBS, once with complete HBSS (with Ca^{++} and Mg^{++}) and resuspended to a 35-40% suspension in complete HBSS. Labeled erythrocytes (SpA-SRBC) were kept at 4°C and used within 5 days.

3. Assay for PFC

After 3-8 days of incubation, supernate from each PWM-stimulated mononuclear cell culture tube was removed using a Pasteur pipet and discarded. The cellular pellet was suspended and washed twice with 250 µl of complete HBSS. Supernate was decanted following the second wash leaving the cells suspended in 100 µl of complete HBSS. SpA-SRBC were washed once and suspended to 35-40% in complete HBSS. 250 mg of agarose (Sigma) in 50 ml of complete HBSS was brought to a boil with constant stirring. 500 µl of this agarose solution was added to individual 12 X 76 mm glass tubes in a 46°C water bath and allowed to

equilibrate. The IgG fraction of either goat anti-cat serum, goat anti-cat IgG, rabbit anti-cat serum or rabbit anti-cat IgG (Cappel, Cochranville, PA) was diluted 1:2, 1:5 or 1:10 in complete HBSS or used undiluted.

Within a minimum amount of time, 25 µl of antiserum, 50 µl of 35-40% SpA-SRBC and 100 µl of mononuclear cell suspension were added to a tube containing 500 µl of agarose kept at 46°C. The mixture was then spread over an agarose-coated slide and allowed to harden at room temperature. Slides were inverted onto a developing tray and flooded with complement diluted in complete HBSS. Complement sources tested included a 1:15, 1:20, 1:30, 1:40 or 1:80 dilution of SRBC-absorbed guinea pig complement (GPC; Gibco), a 1:4, 1:8 or 1:16 dilution of baby rabbit complement (RbC; Pel Freeze, Rogers, AR), or a 1:10, 1:20 or 1:40 dilution of fresh cat serum.

Trays of slides were kept at 37°C until plaques were macroscopically visible, approximately 3-4 hours. The number of plaques per slide were counted at 10-40X magnification using a dissecting microscope. The presence of a mononuclear cell (PFC) located centrally within an individual plaque was confirmed periodically using light microscopy. The number of plaques per slide are reported as PFC/10^6 originally cultured mononuclear cells.

In addition to performance in the SpA-RHPA system, the efficacy of different antisera and sources of complement to induce SpA-SRBC lysis was evaluated using 100 µl of a 1% SpA-SRBC suspension and 50 µl of 1.0 mg/ml cat IgG (Cappel) incubated 20 minutes at 37°C in glass tubes. Following two washes in complete HBSS, 100 µl of antiserum was added to the cat IgG-SpA-SRBC suspension and incubated at

37°C for 15 minutes. The supernate was then decanted to a 50 µl volume and 50 µl of complement was added. Cat IgG-SpA-SRBC suspensions were observed for agglutination and/or hemolysis.

D. Results

Optimal dilutions of appropriate developing reagents (antiserum and complement) were selected after testing for hemolysis of cat IgG-SpA-SRBC cells in glass tubes and after evaluating for ability to demonstrate hemolytic PFC in the RHPA system (Table XVII). Lysis of cat IgG-sensitized SpA-SRBC was complete when the IgG fraction of rabbit anti-cat IgG serum and guinea pig complement were used as developing reagents. Other reagents produced only slight to mild lysis or were unable to lyse cat IgG-sensitized SpA-SRBC. A 1:30 dilution of SRBC-absorbed guinea pig complement exhibited the least background hemolytic activity to washed SpA-SRBC and still aided in the complete hemolysis of cat IgG-sensitized SpA-SRBC as well as in the development of complete hemolytic plaques when used with rabbit anti-cat IgG serum in the RHPA system (Table XVII). The IgG fraction of rabbit anti-cat IgG serum diluted 1:5 was selected as the optimal antiserum based on similar criteria. Other dilutions and other complement sources either showed moderate to marked background hemolytic activity to SpA-SRBC, were unable to lyse cat IgG-SpA-SRBC, or led to development of incompletely hemolyzed plaques in the RHPA system.

In order to determine the optimal PWM concentration and time of mononuclear cell culture for the demonstration of PFC, cells from three cats were cultured in the presence of either 4.6, 6.9 or 13.8

TABLE XVII

EFFICACY OF SpA-RHPA DEVELOPING REAGENTS

Method of evaluation	Complement source	Source and specificity of antiserum (IgG fraction)			
		goat anti-cat serum	goat anti-cat IgG	rabbit anti-cat serum	rabbit anti-cat IgG
Lysis of cat IgG-sensitized SpA-SRBC (tube method)*	guinea pig	++	+	++	++++
	baby rabbit	-	-	-	-
	cat serum	-	-	-	+
Development of hemolytic plaques in RHPA system‡	guinea pig	++	-	-	++++

*Goat anti-cat serum or IgG, rabbit anti-cat serum or IgG and three sources of complement, guinea pig, baby rabbit and fresh cat serum, were evaluated for ability to lyse cat IgG-treated SpA-SRBC. Hemolysis was judged slight (+) to complete (++++). - indicates no hemolysis.

‡The four antisera were evaluated for ability to develop hemolytic plaques with a 1:30 dilution of guinea pig complement in the SpA-RHPA system. Plaque development was judged slight (+) to complete (++++). - indicates no hemolytic plaque development.

µg/ml PWM for 3-8 days. PFC were not demonstrated in any animal after 3 or 4 days of incubation and rarely on day 8. Hemolytic plaques were most distinct and numerous, and varied least in number when developed in the RHPA system after 7 days of culture (Table XVIII). When mononuclear cells were stimulated with 4.6 µg/ml PWM, the mean number of PFC identified increased slightly. Yet, when using PWM at this concentration, variability in the number of developed PFC also increased as evident in the larger standard deviation values (Table XVIII). Separate paired t-tests were used to compare the mean number of PFC produced when mononuclear cells were stimulated with either 4.6 or 6.9 µg/ml PWM. No significant difference in the number of PFC produced at either of these two PWM concentrations after 5, 6 or 7 days of culture was identified. 6.9 µg/ml PWM was selected then as the optimal mitogen concentration since the number of PFC identified was relatively more consistent.

Mononuclear cells from 16 cats were cultured in the presence of optimal (6.9 µg/ml) and suboptimal (13.8 µg/ml) PWM concentrations for 7 days. PFC were demonstrated using mononuclear cells from 15 of the 16 cats with considerable individual variation. The results from seven representative animals are listed in Table XIX.

E. Discussion

The protein A-reverse hemolytic plaque assay provides a means of evaluating both the capacity of B-lymphocytes to differentiate into immunoglobulin secreting cells and the roles of T-lymphocyte helper or suppressor influences on the induction of immunoglobulin synthesis and release (Fauci et al., 1980b: Pryjma et al., 1980a). Herein we

TABLE XVIII

PLAQUE FORMING CELLS IDENTIFIED AFTER 5, 6 OR 7 DAYS OF CULTURE*

	PWM µg/ml	Cat #1225	Cat #1279	Cat #1265
	13.8	137 ± 91	ND	ND
Day 5	6.9	179 ± 45	79 ± 23	112 ± 9
	4.6	224 ± 53	74 ± 25	128 ± 33
	13.8	155 ± 26	ND	ND
Day 6	6.9	318 ± 68	128 ± 23	269 ± 31
	4.6	367 ± 39	146 ± 52	297 ± 41
	13.8	156 ± 31	ND	ND
Day 7	6.9	386 ± 50	133 ± 24	276 ± 25
	4.6	415 ± 91	160 ± 52	274 ± 61

*Values represent the mean number of PFC/10^6 originally cultured mononuclear cells ± one standard deviation identified in four separate determinations.
ND = not done.

TABLE XIX

PLAQUE FORMING CELLS IDENTIFIED AFTER 7 DAYS OF CULTURE‡

	PWM µg/ml	
Cat	6.9	13.8
901	8 ± 7	10 ± 13
902	256 ± 102	219 ± 83
904	288 ± 98	187 ± 24
905	204 ± 27	34 ± 23
1276	800 ± 146	543 ± 165
1278	49 ± 10	34 ± 24
1284	0	0
1480	183 ± 11	78 ± 7

‡Values represent the mean number of PFC/10^6 originally cultured mononuclear cells ± one standard deviation identified in 4-6 separate determinations.

describe for the first time the optimal conditions of culture and assay for the feline SpA-RHPA system. Plaque forming cells were identified in cultures of 10^6 mononuclear cells stimulated with PWM after 5-7 days of incubation using the IgG fraction of rabbit anti-cat IgG serum diluted 1:5 and guinea pig complement diluted 1:30 as developing reagents. No plaques were observed when mononuclear cells were assayed for PFC in the absence of antiserum. Considerable individual dispersion of the number of PFC is expected and has been demonstrated in the human SpA-RHPA system (Tauris, 1983a). Individual PFC-nonresponsiveness (as in cat 1284) has in cultured human mononuclear cells been attributed to a high level of radiosensitive T-lymphocyte suppressor cell activity (Tauris, 1983b).

In addition to being more sensitive than direct hemolytic plaque assays (Lanzavecchia et al., 1979), the SpA-RHPA system has the advantage of being able to enumerate and evaluate the function of cells that secrete a given class of Ig (Pryjma et al., 1980b) by using immunoglobulin class specific developing antiserum. It has also been used to demonstrate excessive suppressor cell activity in patients with primary humoral immunodeficiencies (Herrod & Buckley, 1979), and deficient helper T-cell activity in patients with chronic graft-versus-host disease following HLA-identical bone marrow transplantation (Lum et al., 1981).

The feline SpA-RHPA system should prove useful in evaluating normal feline cellular interactions that culminate in the release of immunoglobulin. In addition, it should aid in the identification and understanding of immune cell dysfunctions associated with chronic

retroviral (FeLV) infection that have been partially described using other methodologies (Hardy, 1982).

CHAPTER VIII

CHARACTERIZATION OF A NEWLY ESTABLISHED FELINE LYMPHOMA-DERIVED CELL LINE (BKD) LACKING T AND B CELL SURFACE MARKERS

A. Summary

A new feline lymphoma-derived cell line, designated BKD, was isolated from an anterior mediastinal tumor. Cells of this line were characterized as lymphoid based on morphology, the lack of intracellular esterase and peroxidase activity and absence of phagocytic function. In contrast with other established feline lymphoma-derived cell lines, cells of the BKD line lack characteristics of both feline T-cells and B-cells in that they neither form rosettes with guinea pig erythrocytes or have demonstrable surface or cytoplasmic immunoglobulin. Approximately one third of BKD cells form EAC rosettes, a significant number of rosette forming cells ($p = 0.0001$) when compared to background sheep E-rosetting activity. In addition, a consistently titratable level of interleukin-2-like activity was produced when BKD cells were co-incubated with concanavalin A and phorbol-12-myristate-13-acetate. Chromosome analysis showed that a majority of BKD cells are diploid. This new cell line has been continuously replicating in culture for over one year and produces feline leukemia virus as demonstrated by several analyses.

B. Introduction

The feline leukemia virus (FeLV), a contagious retrovirus transmitted primarily via salivary secretions, causes either neoplastic or non-neoplastic diseases in the chronically infected cat (Hardy, 1980a). FeLV-associated disease includes leukemia-lymphoma, the most common feline neoplasm (Moulton, 1978), and a state of immunodeficiency characterized by diminution of both cellular and humoral immunity (Hardy, 1982).

Major feline lymphoid populations are defined by a set of surface markers and functional attributes. T-cells form spontaneaous rosettes with guinea pig erythrocytes (GPE), lack surface membrane and cytoplasmic immunoglobulin (SIg and CIg), and exhibit mitogenic responses to certain plant lectins, while B-cells synthesize immunoglobulin, do not form GPE-rosettes, and may bear receptors for complement or the constant fragment of IgG (Hardy, 1982) (Cockerell et al., 1976a) (Taylor et al., 1975a) (Mackey et al., 1975) (Rojko et al., 1982b). Yet when utilizing these methodologies, previously reported determinations have left between 20-64% of normal feline peripheral blood lymphocytes unclassified. Feline leukemia-lymphomas have been primarily classified based on the anatomical distribution of the tumor(s). Characterization of the neoplasms' cytoidentity has been limited to the identification of surface membrane markers (Hardy et al., 1977). 64% of all forms of feline lymphoma, and 73% of the more common multicentric and anterior mediastinal forms are comprised of cells that exhibit T-cell surface characteristics (Hardy et al., 1977). In addition, all readily available feline lymphoma-derived

cell lines possess T-cell markers (Theilen et al., 1969) (Rickard et al., 1969) (Neil et al., 1984) (Grant et al., 1984) (Grant & Michalek, 1981).

Feline lymphoma-derived cell lines have served as a source of FeLV and FeLV-infected transformed lymphoid cells for detection and characterization studies of FeLV-specified antigens and proposed transformation-associated cellular antigens (Theilen et al., 1969) (Rickard et al., 1969). Further, such cell lines have been invaluable in sero-epidemiological studies of the role of FeLV in development of neoplastic and non-neoplastic disease (Grant & Michalek, 1981) (Hardy, 1980b) (Grant et al., 1980), in studies of therapeutic development for assessing induction of anti-viral and anti-tumor activity in the treated cat (Liu et al., 1984a,b), and for analysis of molecular mechanisms involved in retroviral leukomogenesis (Neil et al., 1984). They have also served as a source of immunogen for development of monoclonal antibodies directed against viral derived antigens (Vedbrat et al., 1980) (Wang et al., 1983).

Nearly all feline anterior mediastinal lymphomas are comprised of cells with T-cell surface characteristics and are thought to arise from T-cells of the thymus (Hardy, 1980a) (Hardy et al., 1977). Herein we describe a new feline lymphoma-derived cell line designated BKD which was isolated from an anterior mediastinal tumor. This cell line is comprised of lymphoid cells which actively replicate FeLV and which lack characteristics of both T-cells and B-cells.

C. Material and Methods

1. Animals

Clinically healthy kittens and adult cats free of FeLV as determined by the indirect immunoflourescent antibody (IFA) test for FeLV in leukocytes (Hardy et al., 1973b) (Antibodies Inc., Davis, CA) and the enzyme-linked immunosorbent assay (ELISA) for the detection of FeLV antigen in serum (Mia et al., 1981) (Pitman-Moore Inc, Washington Crossing, NJ) served as donors of normal peripheral blood mononuclear cells (PBM), thymocytes, and lymph node leukocytes.

2. Cellular Preparations

PBM were obtained by centrifugation of defibrinated whole blood over Ficoll-hypaque gradients (Sigma, St. Louis, MO). Thymuses and lymph nodes were collected aseptically, minced, and passed through a stainless steel screen to obtain single cell suspensions. The established feline lymphoma-derived T-cell line FL-74 (Theilen et al., 1969) was maintained at 37°C in a 10% CO_2 humidified atmosphere, in McCoy's 5A medium supplemented with 15% fetal calf serum (FCS), 4 mM L-glutamine, 100 U/ml penicillin, and 100 µg/ml streptomycin (Gibco, Grand Island, NY). Culture medium was changed every 3 or 4 days based on microscopic evidence of cellular proliferation. All cells were washed and adjusted to concentrations appropriate for assays as described below.

3. Establishment of the BKD Cell Line

A two year old female mixed breed domestic cat with a clinical history of anorexia, dyspnea and FeLV infection died soon after presentation at the Oklahoma Medical Research Foundation on January 20, 1984. At post mortem, a 3.5 x 4.0 x 6.5 cm tumor occupied the anterior thoracic cavity and was adherent to the pericardial sac and anterior lung lobes. A portion of the tumor was immediately removed and placed in RPMI-1640 medium (Gibco), minced and gently pushed through a stainless steel screen. The suspended cells were washed twice, adjusted to 1 X 10^6/ml in RPMI-1640 supplemented with 15% FCS, 4 mM L-glutamine, 100 U/ml penicillin, 100 µg/ml streptomycin, 250 ng/ml fungizone (Gibco) and 5 X 10^{-5}M 2-mercaptoethanol (Eastman-Kodak, Rochester, NY), and maintained at 37°C in a 10% CO_2 humidified atmosphere. Counts of total cells cultured and the percent viable were determined by the vital dye exclusion method. Culture medium was changed every 3 or 4 days based on microscopic evidence of cellular proliferation. FeLV infection of the cultured cells was periodically documented using the IFA test. Twofold dilutions of the culture supernatant were periodically screened for FeLV antigens using the ELISA procedure.

4. E-Rosette Assays

Heparinized guinea pig or rat blood was collected by cardiac puncture. Sheep blood was collected in citrate-phosphate-dextrose-adenine solution collection bags (Fenwal Laboratories, Deerfield, IL) by external jugular venipuncture. Erythrocytes were packed, washed

three times with phosphate buffered saline (PBS; Gibco), once with RPMI-1640 (Gibco), and resuspended to 5%. 0.2 ml of Vibrio cholerae-derived neuraminadase (Calbiochem-Behring Corp., La Jolla, CA) at 50 units/ml was added for each ml of the 5% erythrocyte suspension and incubated at 37°C for 60 minutes with inversion once every 15 minutes. The erythrocytes were then washed twice in RPMI-1640, once in RPMI-1640 supplemented with 10% FCS, and adjusted to 1%. Neuraminadase-treated erythrocytes were stored at 4°C and used within 2 days.

Guinea pig erythrocytes (GPE) were also treated separately with 0.143 M aminoethylisothiourium bromide (AET; Sigma, St. Louis, MO) according to the methods previously described (Rojko et al., 1982b) (Kaplan & Clark, 1974) with minor modification. After AET treatment and incubation, the erythrocytes were resuspended in RPMI-1640 supplemented with 10% FCS and adjusted to 1%.

For E-rosette assays, thymic, peripheral blood, FL-74 and BKD cells were washed twice in RPMI-1640, once in 10% FCS supplemented RPMI-1640 and adjusted to 2.5 X 10^6/ml. 100 µl of a 1% suspension of either neuraminadase or AET treated guinea pig erythrocytes (GPE-N, GPE-AET), rat erythrocytes (RE-N), or untreated sheep erythrocytes (SRBC) were added to 100 µl of cells in 10 X 75 mm glass tubes (Fischer Scientific, Pittsburg, PA), centrifuged at 200 X g for 5 minutes and left on ice for 60 minutes. In the case of SRBC, cellular mixtures were handled as in EA and EAC rosetting assays, were kept in unsupplemented RPMI-1640 and were incubated at 37°C for 30 minutes (see below). 150 µl of the supernate was then removed and the cellular pellet gently resuspended. 150 µl of 0.01% Gentian

violet in 0.15 M NaCl (J.T. Baker Co., Phillipsburg, NJ) was added and a minimum of 200 viable cells were counted. A rosette forming cell (RFC) was defined as a viable mononuclear cell with three or more attached erythrocytes.

5. EA-rosette Assays

Equal volumes of washed SRBC adjusted to 5% in RPMI-1640 and either the 7s(IgG) or 19s(IgM) fraction of anti-sheep erythrocyte serum (Cordis Labs, Miami, FL) diluted to the maximum subagglutinating titer (1:2560 for 7s, and 1:160 for 19s) were mixed and incubated at 37°C for 30 minutes. Following incubation the SRBC were washed three times, adjusted to a 1% suspension in RPMI-1640, stored at 4°C and used within 2 days.

Cells were washed three times and adjusted to 4.0 X 10^6/ml in RPMI-1640. 100 µl of 7sEA or 19sEA suspension were added to 100 µl of cells, pelleted at 200 X g for 5 minutes, incubated at 37°C for 30 minutes, gently resuspended, stained and quantitated.

6. EAC-rosette Assays

Equal volumes of sheep 19sEA cells adjusted to 5% in RPMI-1640 and a 1:10 dilution of fresh mouse serum were mixed, incubated at 37°C for 30 minutes, then washed three times and adjusted to a 1% suspension. EAC complexed cells were then mixed with cells to be tested, and evaluated as in the EA-rosette assay above.

7. Demonstration of Surface or Cytoplasmic Immunoglobulin

For the demonstration of SIg, cells were washed three times and adjusted to 8.0 X 10^6/ml in Hank's balanced salt solution (HBSS; Gibco). 50 µl of the cellular suspensions were added to individual wells of a 96-microwell plate and mixed with 50 µl of the IgG fraction of a rabbit anti-cat IgG serum (Cappel Labs, Cochranville, PA) diluted 1:20 in HBSS. Microwell plates were then left on ice for 30 minutes. Cellular pellets were washed three times with 250 µl of FA buffer (Difco, Detroit, MI), then mixed with 50 µl of flourescein-conjugated goat anti-rabbit immunoglobulin (Cappel) diluted 1:20 in HBSS. Plates were left on ice for 30 minutes. Pellets were then washed three times with FA buffer, resuspended and evaluated for membrane flourescence.

For the demonstration of cytoplasmic immunoglobulin, cytocentrifuge cellular preparations were fixed in 5% glacial acetic acid-95% methanol at -20°C for 5 minutes, washed three times in PBS, and stained with the reagents used to demonstrate surface membrane immunoglobulin.

8. Assay for Phagocytic Activity

Cells were washed three times and adjusted to 2.0 X 10^6/ml in HBSS. 100 µl of 0.81 µm diameter latex beads (Difco) diluted 1:50 was added to 1.0 ml of cellular suspension. The mixture was incubated at 37°C for 60 minutes with continuous rotation (Multipurpose rotator, Scientific Industries, Springfield, MA). Cells were washed three times with HBSS, then evaluated with the aid of a hemocyto-

meter. Cells ingesting three or more latex beads were considered positive. Contaminating monocytes in PBM preparations were used as control.

9. Cytochemical Analysis

Cytocentrifuge cellular preparations were evaluated for intracellular chloracetate esterase, nonspecific esterase (with and without sodium fluoride), peroxidase, and acid phosphatase enzymatic sites using described methods (Yam et al., 1977) (Wachstein & Wolf, 1977) (Bennett, 1977). Additional cytocentrifuge preparations were stained with periodic acid Schiff and Wright's-Giemsa stains.

Electron micrographs of FL-74 and BKD cells were kindly provided by S.H. Chang at the University of Maryland School of Medicine, Department of Pathology.

10. Chromosome Analysis

Chromosome analysis of the BKD cell line was performed using previously described methods with minor modification (Moorhead et al., 1960). Ten milliliters of BKD cells were adjusted to 1.0 X 10^7/ml. 0.4 µg of colchicine (Sigma) was added per milliliter of cellular suspension and incubated for three hours at 37°C. Cells were then pelleted, the supernate discarded, and cells were resuspended in 5 ml of 0.075 M KCl solution. After twenty minutes of incubation at 37°C, the cells were again pelleted and resuspended in 1.0 ml of 0.075 M KCl. A fixative solution consisting of one volume glacial acetic acid and three volumes of methanol was then added dropwise to a final volume of 5 ml. After 15 minutes of incubation

at room temperature, the process of fixation was repeated this time to a final volume of 2 ml. Smears of this suspension were heat fixed and stained with Giemsa. Approximately 100 groups of spread chromosomes were evaluated for number and morphology, and compared to the normal feline karyotype (Hsu & Rearden, 1965).

11. Induction and Assay for Interleukin-2-like Activity

The induction and analysis for interleukin-2-like (IL-2-like) activity by BKD cells was performed using previously described methods (Farrar et al., 1980). FL-74 and BKD cells were adjusted to a concentration of 2.5 X 10^6/ml and cultured in 16 mm wells with 4, 8 or 16 µg/ml concanavalin A (con A; Pharmacia, Piscataway, NJ) or with con A and 10 ng/ml phorbol-12-myristate-13-acetate (PMA; P.L. Biochemicals, Milwaukee, WI) added. Cell free supernates were collected after 24 hour incubation at 37°C and filtered through 0.45 µm filters. Supernates were assayed for IL-2-like activity using the IL-2 dependent cell line, HT-2 (Watson, 1979). Serial twofold dilutions of supernates, or a murine IL-2 standard (123 U), were added to triplicate wells containing 5 X 10^4 HT-2 cells. Following incubation at 37°C for twenty hours, 1.0 µCi of ^{3}H-thymidine was added to each well and incubation continued for an additional four hours. Cells were harvested and the incorporation of ^{3}H-thymidine determined by liquid scintilation counting.

12. Induction and Assay for Interferon

The induction and analysis for antiviral activity in supernatant fluids from cultures of BKD cells stimulated with Staphylococcous

enterotoxin A were performed using previously described methods without modification (Engelman et al., 1985c) (Liu et al., 1984b).

D. Results

During the first month of isolation, cultured BKD cells varied in viability between 60-90%. When viability was less than 60%, dead cells were removed by centrifugation of cultured cellular suspensions over ficoll-hypaque gradients. Four months following the initial isolation, BKD cell viabilities exceeded 90%. Periodically, ampules containing between 1.0×10^7 - 2.0×10^7 cells/ml were stored in liquid nitrogen after being frozen in 10% dimethylsulfoxide and 90% FCS. When ampules were thawed at 37°C, BKD cells were readily re-established in culture. Under our culture conditions, cell doubling time for the FL-74 line ranged between 36-72 hours, while cultured BKD cells doubled in number after 72-120 hours of incubation. Both cell lines were always positive for FeLV by IFA and ELISA.

1. Morphological and Cytochemical Analysis

Morphologically, the FL-74 cells were typically lymphoid. BKD cells grew singly or in aggregates, measured approximately 12-16 µm in diameter and possessed an oval to deeply-indented nucleus and one or more prominent nucleoli (Figure 31). Cytochemical stains for intracellular enzymatic sites were all negative in both BKD and FL-74 cell preparations, except for a moderate, diffuse cytoplasmic positive reaction for acid phosphatase

Figure 31: Light photomicrograph of lymphoid cells of the BKD cell line showing the variation in nuclear morphology from oval to deeply indented. One or more prominent nucleoli are present within each cell nucleus (Wright-Giemsa, X2000).

Figure 32 Electron photomicrograph of type-C virus particles budding from and neighboring the plasma membrane of two BKD cells (X17000).

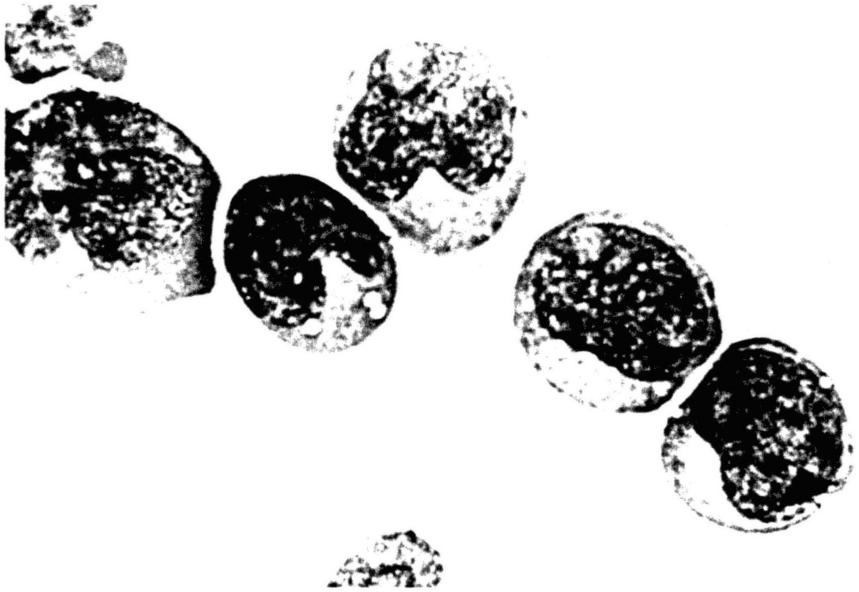


Figure 31

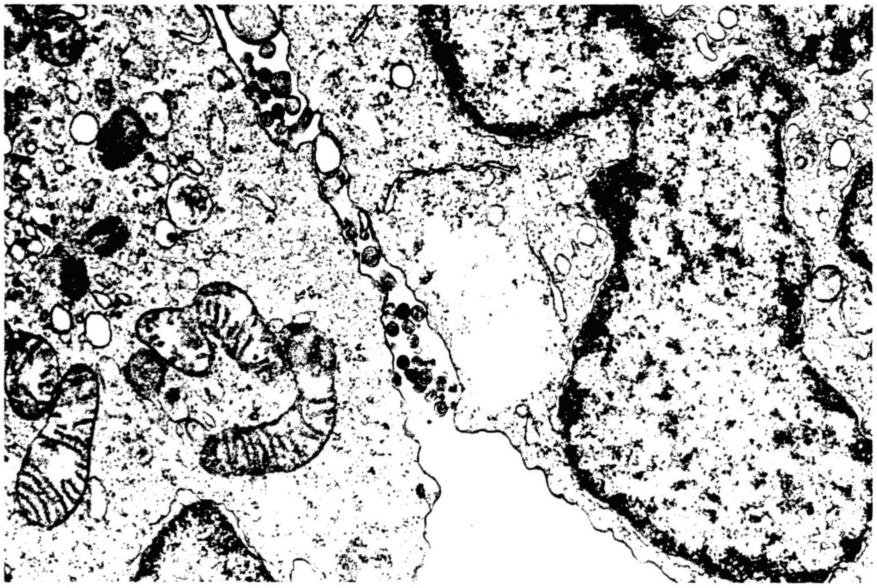


Figure 32

Figure 33: Chromosome analysis of the BKD cells showed that 88% possess a karyotype of 36+XX; diploid for this species.

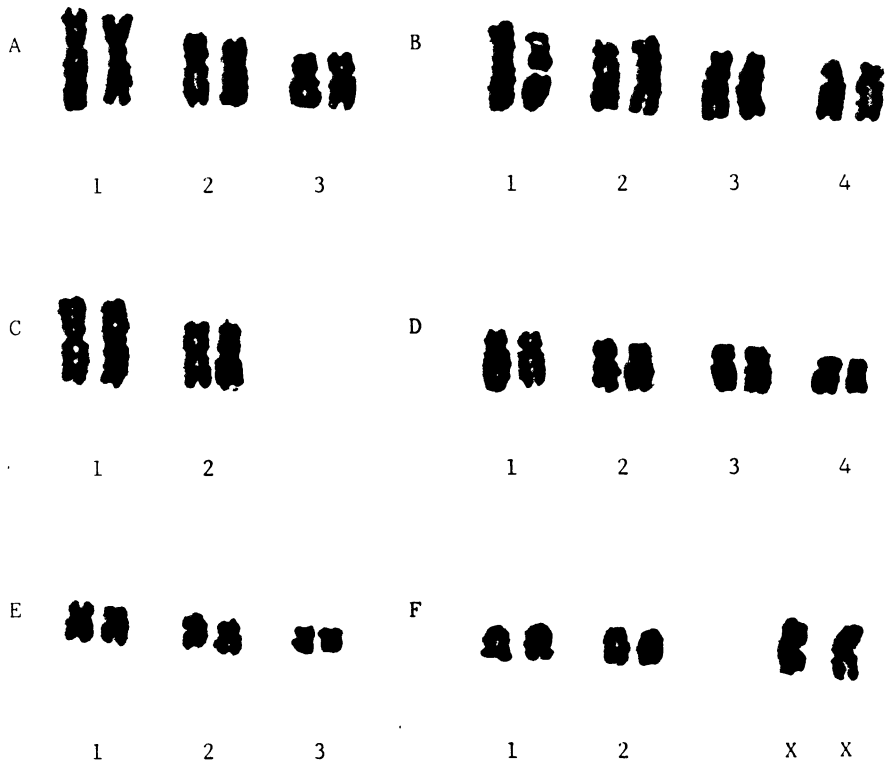


Figure 33

Electron micrographs of BKD cells taken on the second day of culture demonstrated type-C virus particles adjacent to and budding from the plasma membrane of the cultured cells (Figure 32). Greater numbers of virus particles were evident in electron micrographs of cells of the established FL-74 cell line.

2. Chromosomal Analysis

The number of BKD cellular chromosomes ranged from 31+XX to 37+XX, with 88% of the evaluated cells possessing 36+XX chromosomes (Figure 33). Thus, the majority of cells of this line are diploid.

3. Induction and Assay for Interleukin-2-like Activity

The induced production of interleukin-2-like activity was studied using both FL-74 and BKD cells. IL-2-like activity, measured by the IL-2 dependent proliferation of HT-2 cells, was not detectable when either cell line was cultured with con A alone. Induced IL-2-like activity, however, was enhanced only with BKD cells when the cells of this line had been stimulated with both con A and PMA (Table XX). Although the induced HT-2 proliferation achieved with supernatants of BKD cells resulted in an approximately 10-fold less stimulation than was achieved with a standard supernatant obtained from con A stimulated murine splenic leukocytes, this BKD-induced IL-2-like activity was consistently titratable (on eight seperate occasions). By contrast, FL-74 cells did not produce any IL-2-like activity after comparable stimulation.

TABLE XX

INDUCED INTERLEUKIN-2-LIKE ACTIVITY IN BKD CELL CULTURE SUPERNATANTS

Dilution of Supernatant	HT-2 Cell Proliferation (cpm) BKD Cell Cultures (2.5×10^6/ml)				
	PMA	Con A (μg/ml)			
	10 ng/ml	0	4	8	16
1:2	-	450	498	240	684
1:4	-	352	420	286	400
1:8	-	400	314	450	421
1:2	+	426	6052	5700	3838
1:4	+	412	2452	1975	1072
1:8	+	485	290	410	358

4. Induction and Assay for Interferon

Supernatant fluids from separate cultures of PBM and BKD cells stimulated with Staphylococcous enterotoxin A were evaluated for antiviral activity on two occasions. Although interferon activity was present in supernates from PBM cultures, no antiviral activity was induced in cultures of BKD cells (data not shown).

5. E-Rosette Formation

Normal feline thymocytes, peripheral blood mononuclear cells, and cells from the FL-74 and BKD cell lines were next evaluated for ability to form E-rosettes with either neuraminadase-treated or AET-treated guinea pig or rat erythrocytes. As shown in Table XXI, approximately 76% of thymocytes, 24% of PBM, and 79% of FL-74 cells formed spontaneous rosettes with GPE-N. These values are consistent with previously reported determinations. Fewer rosettes were formed with each of the cellular preparations when GPE-AET or RE-N were used. In no instance did cells from the BKD cell line form rosettes with GPE-N, GPE-AET or RE-N. During the rosetting assay, BKD cells did show a tendency to aggregate in small clumps, the size and number of which increased moderately when medium with increased FCS concentration was used. These clumps occasionally included small numbers of erythrocytes. True BKD E-rosettes were never identified.

TABLE XXI

CELL MARKER AND FUNCTIONAL ASSAYS

	Thymocytes	PBM	FL-74	BKD
GPE-N	76 ± 5	24 ± 6	79 ± 5	0.0
GPE-AET	ND	14 ± 3	30 ± 4	0.0
RE-N	45 ± 2	15 ± 1	0.0	0.0
SIg	<1	24 ± 4	<1	<1
CIg	ND	+(LN)*	<1	<1
Phago	ND	+(M)‡	0.0	0.0
Acid Phos	ND	ND	+	+
IFA-FeLV	—	—	+	+
ELISA-FeLV	—	—	+	+

Normal feline thymocytes and peripheral blood mononuclear cells (PBM) and cells of the FL-74 and BKD cell lines were evaluated for E-rosette forming cells using neuraminadase or AET-treated guinea pig erthrocytes (GPE-N, GPE-AET) or neuraminadase-treated rat erythrocytes (RE-N), for surface and cytoplasmic immunoglobulin (SIg, CIg) for phagocytic function (Phago), for acid phosphatase enzymatic activity (Acid Phos), and for the presence of feline leukemia virus (FeLV). Values represent the mean ± one standard deviation of 4-8 separate determinations.

*Cytocentrifuge lymph node (LN) leukocytic preparations served as positive control for cytoplasmic immunoglobulin assays.

‡Contaminating monocytes (M) in PBM preparations served as positive control for phagocytic functional assays.

TABLE XXII

ASSAYS FOR CELL Fc AND COMPLEMENT RECEPTORS

	Thymocytes	PBM	FL-74	BKD
SRBC	ND	0.0	10 ± 3	10 ± 4
7sEA	3 ± 1	17 ± 6	14 ± 7	21 ± 6
19sEA	0.0	1 ± 1	14 ± 5	13 ± 1
EAC	2 ± 1	21 ± 5	16 ± 6	35 ± 11

Normal feline thymocytes and peripheral blood mononuclear cells (PBM) and cells of the FL-74 and BKD cell lines were evaluated for E-rosette forming cells using sheep erythrocytes (SRBC), for 7sEA and 19sEA-rosette forming cells, and for EAC-rosette forming cells. Values represent the mean ± one standard deviation of 4-12 separate determinations.

6. Studies of Surface Immunoglobulin and Phagocytic Activity

The results of determinations for SIg, and CIg and phagocytic function are also presented in Table XXI. Cells from both the FL-74 and BKD lines were negative for SIg, CIg, and the ability to ingest latex particles.

7. EA and EAC-Rosette Formation

Table XXII presents data concerning 7sEA, 19sEA and EAC rosette forming cells in thymocyte, PBM, FL-74 and BKD cellular preparations. Untreated, washed SRBC were handled in an identical manner as EA and EAC complexed cells with incubation at 37°C for 30 minutes prior to resuspension and evaluation. As shown in Table XXII, washed SRBC did not form rosettes with normal PBM but did form rosettes with approximately 10% of either FL-74 or BKD cells. Little variation was noted from this background E-rosetting activity when FL-74 cells were incubated with 7sEA, 19sEA or EAC complexed erythrocytes. In contrast, a significant three fold increase ($p = 0.0001$) in rosette forming cells from background E-rosetting activity was seen when BKD cells were mixed with EAC complexed cells in twelve experiments. When SRBC in Alsever's solution (Gibco) were used in two experiments, however, no E, EA or EAC rosette forming cells were demonstrated.

E. Discussion

Two well established feline lymphoma-derived cell lines, FL-74 and F422 (Theilen et al., 1969) (Rickard et al., 1969), and other incompletely described feline lymphoma cell isolates that are presum-

ably maintained as continuously replicating cell lines (3272, 3281, 79-14940, 3191, 3201B and T3) (Neil et al., 1984) (Grant et al., 1984) (Grant & Michalek, 1981) serve as resource for FeLV and FeLV-infected transformed cells, and are all comprised of lymphoid cells bearing T-cell surface markers. To the best of our knowledge, the BKD cell line represents the first FeLV-producing feline lyphoma-derived cell line that lacks both demonstrable surface or cytoplasmic immunoglobulin and ability to form rosettes with guinea pig or rat erythrocytes. Approximately 6% of feline lymphomas are comprised of cells lacking both T and B-cell characteristics (Hardy et al., 1977).

The possibility that the BKD cell line was derived from myeloid or monocytic cells was investigated because of the BKD cell's heterogenous nuclear morphology (oval to deeply-indented). The lack of phagocytic activity and absence of chloracetate esterase, non-specific esterase and peroxidase enzymatic activity, and the negative results with periodic acid Schiff stain suggests that the BKD cell line was derived from lineages other than the myelo-monocytic.

Approximately 100 groups of spread chromosomes from BKD cells were evaluated for number and morphology. 88% of the BKD cells evaluated possessed a karyotype of 36+XX chromosomes which is consistent with the normal feline karyotype (Hsu & Rearden, 1965). Thus, a majority of the cells of this new line are diploid.

Several reports have described murine, primate or human leukemias which when exposed to mitogens secrete interleukin-2 in high titer (Grant et al., 1984) (Friedman et al., 1982) (Rabin et al., 1981). None of the feline lymphoma cell lines previously tested

secrete IL-2 after addition of mitogen (Grant et al., 1984). Consistently titratable amounts of IL-2-like activity was produced only by BKD cells when coincubated with PMA and con A suggesting that at least a small subpopulation of BKD cells are IL-2 producers or that cells of this line can produce small amounts of IL-2. An alternative possibility is that feline IL-2 is produced by cells of this line in larger amounts than is apparent in the assay, but that IL-2 of feline origin is less effective than either mouse or human IL-2 in promoting proliferation of HT-2 cells. Observations with normal feline PBM which also produced relatively low IL-2-like activity indicates that the latter possibility is likely the correct one. Further subcloning of these cells in order to establish a subline for use in the production and characterization of higher titer feline IL-2 is currently underway in this laboratory.

Less than 1% of normal feline PBM form rosettes with sheep erythrocytes (Taylor et al., 1975a). In a previous report, cells of the FL-74 cell line did not form EAC rosettes (Cockerell et al., 1976a). It seems likely that the observed rosette formation between FL-74 and SRBC, EA or EAC cells seen in our study represents a nonspecific interaction between cells since the percent of FL-74 cells that formed rosettes in each assay was approximately 10-15%. However, the threefold increase in BKD rosette forming cells when EAC are substituted for SRBC may indicate the presence of a membrane receptor for complement on BKD cells. This data was evaluated using an analysis of variance with Duncan's multiple comparison test. The mean number of BKD rosette forming cells identified with EAC was

significantly greater ($p = 0.0001$) than the BKD background sheep E-rosetting activity.

Further characterizations of this surface immunoglobulin-negative and E-rosette-negative lymphoma-derived cell line are currently underway but beyond the scope of this paper. In addition to a usefulness in feline leukemia-lymphoma and feline leukemia virus research, this new cell line may prove valuable in defining that portion of feline lymphocytes and lymphomas which as yet remain undefined and unclassified.

CHAPTER IX

PRELIMINARY DEVELOPMENT OF MONOCLONAL ANTIBODIES WHICH DEFINE FELINE T-LYMPHOCYTE HETEROGENEITY

A. Introduction

A family of human retroviruses, the human T-lymphotropic viruses (HTLV), which share a tropism for T-lymphocytes and impair T-cell function have been identified during the last seven years (Gallo, 1984) (Popovic, et al., 1984). Though viruses of this group have common morphological and genomic properties, they have been linked to two diseases of opposite manifestation (Wong-Stall and Gallo, 1985). Apparently, HTLV-I is the etiologic agent of adult T-cell leukemia (ATL) and HTLV-III is the etiologic agent of the acquired immunodeficiency syndrome (AIDS) (Gallo, 1984) (Popovic, et al., 1984) (Wong-Stall and Gallo, 1985).

Numerous animal retroviruses have served as precedents for the HTLV family. Though retroviruses of other species are considered more closely homologous in genomic structure and virion morphology to the HTLV isolates, the leukemia immunodeficiency inducing viruses of the cat (feline leukemia viruses, FeLV) are uniquely appropriate for study as a clinical model of HTLV associated leukemia and immunodeficiency (Derse et al., 1985) (Gonda et al., 1985). Like HTLV, FeLV is transmitted horizontally and is responsible for inducing either the

malignant proliferation of lymphocytes or the development of lymphocytic abnormalities and immunodeficiency (Hardy, 1980). Isolates of the FeLV family have been attributed with lymphoproliferative, cytopathic, lymphosuppressive and anemigenic effects (Rojko and Olsen, 1984). The feline retroviral model is considered a useful clinical reflection of human acute lymphoblastic leukemia (Cotter and Essex, 1977), aplastic anemia, states of immunodeficiency and preleukemia (Maggio, et al., 1978) (Sarngadharan, et al., 1984). It has served in the evaluation of new forms of cancer immunotherapy (Engelman, et al., 1985d) (Liu, et al., 1984a,b) (Snyder, et al., 1984) and has contributed to an understanding of the molecular mechanisms of retroviral induced leukomogenesis (Neil, et al., 1984).

A major impairment of the elucidation of leukomogenesis in the cat has been the lack of understanding of feline immune cell regulatory and effector mechanisms due to an inability to define normal feline lymphoid cell populations. Although heteroantisera, Fc receptor binding and erythrocyte rosetting have been used to describe major feline lymphocyte populations, precise delineation of feline immunoregulatory and effector cells has not yet been possible (Rojko, et al., 1982) (Taylor, et al., 1975). In addition, feline leukemia-lymphomas have been classified based only on the anatomical distribution of the tumor(s) and on the predominant cellular morphology present (Hardy, 1980).

It has been suggested that a state of immunodeficiency, though a frequent sequelae of retroviremia independent of the development of malignancy, may also precede and allow for the rare development of

detectable lymphoid malignancies in the FeLV infected cat, and that this preiod of deficient immunity is T-cell specific (Rojko and Olsen, 1984). However, because of the absence of appropriate technology the nature of the T-cell defect as well as the cytoidentity of the subsequent neoplasms have yet to be clearly defined. Cats with retroviremia manifest delays in skin allograft rejection decreased in vitro lymphocyte proliferation to mitogen and antigen (Cockerell et al., 1976) and reduced antibody responses to a T-cell specific synthetic polypeptide (Trainin, et al., 1983). Additionally, purified UV-inactivated FeLV and certain FeLV envelope proteins (p15E) have been shown to impair normal lymphocyte in vitro proliferation to mitogen (Mathes, et al., 1979), the synthesis of gamma interferon (Engelman, et al., 1985c), and in human and murine systems of assay have been shown to suppress the generation of interleukin-2 (IL-2) (Yorosz, et al., 1985) (Wainberg, et al., 1983). Whether these abnormalities are due to alterations in cell number within a given lymphocyte subset, or to qualitative functional abnormalities of the subset, or are related to the multiple intercurrent infectious and immune mediated diseases that develop in the cat with retroviremia is not known.

In order to aid in the identification of quantitative and qualitative aberrations in specific subsets of T-lymphocytes in the cat with retroviremia, immunodeficiency, and leukemia, monoclonal antibodies which will characterize T-cell subpopulations by their pattern of reactivity with lymphoid cell surface antigens will be developed. A panel of monoclonal antibodies which describe T-cell surface phenotype heterogeneity will aid in the identification of thymocytic

differential and maturational stages. Coupled with appropriate immunological assays, monoclonal antibodies of this specificity will contribute to the definition of T-cell functional subsets, and provide the foundation for defining and subclassifying feline lymphocyte disorders such as leukemia, lymphoma, autoimmunity and immunosuppression. Accurate identification of the cell lineage and stage of differentiation of leukemia cells will also be possible.

B. Material and Methods

1. Animals

Clinically healthy kittens and adult cats free of FeLV as determined by the indirect immunofluorescent antibody (IFA) test for FeLV in leukocytes (Hardy, et al., 1973) (Antibodies Inc., Davis, CA) and the enzyme linked immunosorbent assay (ELISA) for the detection of FeLV antigen in serum (Mia, et al., 1981) (Pitmann-Moore, Inc., Washington Crossing, NJ) served as donors of normal peripheral blood lymphocytes (PBL), thymocytes, bone marrow, splenic and lymph node leukocytes. Five to six week old Balb/c mice served as hosts for the immunogens.

2. Cells and cell lines

Single cell suspensions were obtained by the centrifugation of defibrinated whole blood over Ficoll-hypaque gradients (Sigma, St. Louis Mo), and by the mincing and passage of lymphoid parenchymal tissues through a stainless steel screen. Counts of total cells and

the percent viable were determined by the vital dye exclusion method.

3. Immunization

Balb/c mice were injected intraperitoneally (IP) with 2 x 10^7 feline thymocytes or nylon-wool filtered PBL. Two to five immunizations, 2-3 weeks apart were administered. Two days following the last immunization, mice were selected based on their plasma level of anti-cat thymocyte antibodies as detected in a micro-well IFA test. Immunofluorescent preparations were scored with a Leitz (Dialux 20) microscope under epi-illumination.

4. Fusion

Spleenocyte-myeloma fusion protocols originally described by Kohler and Milstein were followed (1976). Spleens from selected immunized mice were collected aseptically and dispersed into single cell suspensions. Spleenocytes were suspended in maintenance medium consisting of Dulbecco's Modified Eagle's Medium with glucose and L-glutamine (Gibco, Grand Island, NY) and supplemented with 20% heat inactivated fetal calf serum (FCS), 100 mM sodium pyruvate, 200 mM L-glutamine and 10,000 U/ml penicillin-streptomycin. Prior to fusion, sp2/0 myeloma cells were grown in maintenance medium with 0.13 mM 8-azaguanine.

For fusion, 10^8 spleenocytes were mixed with 2 x 10^7 sp2/0 cells in the presence of 45% polyethylene glycol 1000 (Sigma). After washing, the cells were resuspended in medium containing 10% NCTC 109 medium (M.A. Bioproducts), 0.15 mg/ml oxaloacetate, 0.2 U/ml bovine

insulin, 13.6 µg/ml hypoxanthine and 3.78 µg/ml thymidine (referred to as hybridoma medium HY-HT). Approximately 2 x 10^5 cells in 50 µl were placed in flat-bottom wells of 96 well plates. After 24 hours, the cells received 150 µl of selection medium (HY-HAT) containing 0.8 uM aminopterin. Medium was aspirated and replaced with fresh HY-HAT medium on several occasions over a 2-4 week period prior to screening hybridoma supernatants for anti-cat thymocyte antibody.

5. Screening by FACS analysis

4 x 10^5 cells were incubated with 50 µl of supernatant for 30 minutes on ice. After two washes with RPMI-1640 (Gibco) with 5% FCS, the cells were treated with 10 µl of fluorescein-conjugated goat anti-mouse kappa light chain or a fluorescein-conjugated goat anti-mouse IgG, M, A heavy chains sera and incubated on ice for 30 minutes. After three washes, positive staining were assessed on a EPICS V (Coulter, Hialeah, FL) by comparing cells treated with culture supernatant or rabbit anti-cat thymocyte serum and second antibody, or second antibody alone.

6. Establishment of hybridoma clones

Hybridomas secreting antibodies which bind to feline T-lineage lymphocytes were gradually adapted to larger culture volumes in maintenance medium. Hybridoma lines were subcloned by limiting dilution and retested by flow microfluometry. Clones and subclones were frozen in 90% FCS and 10% dimethylsulfoxide (DMSO, Sigma) and stored in liquid nitrogen.

7. Complement mediated microcytotoxity

2.5 x 10^5 cells and 25 ul of supernatant were incubated on ice for 30 minutes. After washing, 50 ul of 1:15 diluted guinea pig complement (Gibco) was added and the cellular mixture incubated at 37° C for 45 minutes. Cell viability was determined by the exclusion of vital dye.

8. Preparation of monoclonal antibodies

Supernatants were concentrated by precipitating overnight at 4° C with 40% saturated ammonium sulfate solution. Precipitates were pelleted at 48,000 x g for 30 minutes, resuspended and dialyzed extensively with 0.1 M PBS, and stored at -70° C. Hybridoma-derived ascites were produced by inoculating 5-10 x 10^6 cells IP into Balb/c mice primed 10 days to 3 weeks previously with 0.5 ml of pristane (Aldrich Chemical Inc.). The ascites fluid was drained, clarified and stored at -70° C.

C. Results

Six fusions of sp2/0 myeloma cells and spleenocytes from immunized mice resulting in 866 hybridomas have thus far been completed. Two spleens were from mice immunized first with nylon-wool filtered PBL and second with thymocytes. The other four spleens were derived from mice receiving 2-5 IP injections of thymocytes. Spleenocytes from mice immunized by the second immunization protocol, when fused with sp2/0 myeloma cells resulted in the greatest number of hybridomas. Supernatant fluids from these 866 hybridomas were

screened for reactivity with thymocytes by indirect immunofluorescense and flow microfluorometry and some have been evaluated for thymocytolytic activity. 120, or 13% of the 866 supernatant fluids from wells with growth reacted with between 10% and 96% thymocytes with all levels of reactivity represented. Thymocytolytic activity has been demonstrated in some of the supernatants with between 10% and 46% of thymocytes being killed as determined by the inclusion of vital dye. In addition, the supernatant fluids from the 866 hybridomas were screened for reactivity with peripheral blood lymphocytes by indirect immunofluorescence and flow microfluometry. 97, or 11% of the 866 supernatant fluids from wells with growth reacted with between 14% and 95% peripheral blood lymphocytes with all levels of reactivity represented.

D. Discussion

Monoclonal antibodies to feline T-lymphocytes would serve as invaluable tools in defining and following aberrations in cellular number or function within T-lymphocyte subsets. These antibodies could be used to document improvements in immunoregulatory and effector functions during experimental treatment of viral infected and leukemic cats. New forms of anti-leukemia and anti-immunodeficiency therapy could be more precisely assessed. In addition, these monoclonal antibodies may be evaluated for their therapeutic potential in the treatment of retroviral associated clinical diseases.

All studies of this sort, utilizing the feline retroviral model, will aid not only in the characterization and treatment of feline leukemia, preleukemia, immunodeficiency and retroviremia but since

these are well accepted reflections of similar human conditions, should almost certainly contribute to the understanding, diagnosis and treatment of human disease.

LITERATURE CITED

Amer, C.A., J.T. Parsons, A.J. Faras. Direct proof of the 5' to 3' transcriptional jump during reverse transcriptase of the avian retrovirus genome by DNA sequencing. J. Virol. 38:398-402, 1981.

Anderson, L.J., W.F.H. Jarrett. Membranous glomerulonephritis associated with leukemia in cats. Research in Vet. Science, 12:179-180, 1971.

Balint, J., F.R. Jones. Perfusion of canine serum over Staphylococcus aureus Cowan I: evidence for release of protein A and changes in specific antibody activity. Immunol. Comm. 12:573-591, 1983.

Balint J., Y. Ikeda, J.J. Langone. Tumoricidal response following perfussion over immobilized protein A:identification of immunoglobulin oligomers in serum after perfusion and their partial characterization. Cancer Res. 44:734-743, 1984.

Baldwin R.A., M.R. Price, R.A. Robins. Blocking of lymphocyte-mediated cytotoxicity for rat hepatoma cells by specific antigen-antibody complexes. Nature New Biol 238:185-187, 1972.

Baltimore, D., D. Smoler. Primer requirement and template specificity of the DNA polymerase of RNA tumor viruses. Proc. Natl. Acad. Sci. USA 68:1507-1511, 1971.

Baltimore, D. Retroviruses and retrotransposons: the role of reverse transcription in shaping the eucaryotic genome. Cell 40:481-482, 1985.

Bansal, S.C., B.R. Bansal, J.P. Boland. Blocking and unblocking serum factors in neoplasia. Curr. Topics Microbiol. Immunol. 75:45-76, 1976.

Bansal, S.C., B.R. Bansal, H.L. Thomas, P.O. Siegel, J.E. Rhoads, D.R. Cooper, D.S. Terman, R. Mark. Ex vivo removal of serum IgG in a patient with colon carcinoma. Cancer 42:1-18, 1978.

Bennett, J.M. Correlations of morphology, cytochemistry and therapy. Ann. Intern. Med. 18:747-749, 1977.

Benveniste, R.E., C.J. Sherr, G.J. Todaro. Evolution of type C viral genes: origin of feline leukemia virus. Science 190:886-888, 1975.

Bernhard, W. Electron microscopy of tumor cells and tumor viruses. A review. Cancer Res. 18:491-509, 1958.

Bernhard, W. The detection and study of tumor viruses with the electron microscope. Cancer Res. 20:712-727, 1960.

Bertram, J.H., S.M. Grunberg, I. Shulman, M.L.J. Apuzzo, D. Boquiren, L. Kunkel, J.C.D. Hengst, J. Nelson, W.J. Waugh, D. Plotkin, M.S. Mitchell. Staphylococcal protein A column: correlation of mitogenicity of perfused plasma with clinical response. Cancer Res. 45:4486-4494, 1985.

Besmer, P. Acute Transforming feline retroviruses. Curr. Top. Microbiol. Immunol. 107:1-27, 1983.

Bishop, J.M., H. Varmus. Functions and origins of retroviral transforming genes. In, RNA Tumor Viruses, R. Weiss, N. Teich, H. Varmus, J. Coffin, eds. Cold Spring Harbor, pg. 999-1108, 1982.

Bishop, J.M. Viruses, genes, and cancer. II. Retroviruses and cancer genes. Cancer 55:2329-2333, 1985.

Bittner, J.J. Possible relationship of the estrogenic hormones, genetic susceptibility, and milk influence in the production of mammary cancer in mice. Cancer Res. 2:710-721, 1942a.

Bittner, J.J. The milk influence of breast tumors in mice. Science 95:462-463, 1942b.

Blayney D.W., E.S. Jaffe, R.I. Fisher, et al. The human T-cell leukemia/lymphoma virus (HTLV), lymphoma, lytic bone lesions and hypercalcemia. Ann. Intern. Med. 98:144-151, 1983.

Boyce, J.T., E.A. Hoover, G.J. Kociba, R.G. Olsen. Feline leukemia virus induced erythroid aplasia: in vitro hemopoietic culture studies. Exptl. Hematol. 9:990-1001, 1981.

Brick, J.O., W.J. Roenigk, G.P. Wilson. Chemotherapy of malignant lymphoma in dogs and cats. J. Am. Vet. Med. Assoc. 153:47-52, 1968.

Bunn, P.A. Jr., G.P. Schecter, E.S. Jaffe, et al. Clinical course of retrovirus-associated adult T-cell lymphoma in the United States. N. Engl. J. Med. 309:257-264, 1983.

Cairns, J. Mutation selection and the natural history of cancer. Nature 255:197-200, 1975.

Cairns, J. The origins of human cancers. Nature 289:353-357, 1980.

Carpenter, J.L., J. Holzworth. Treatment of leukemia in the cat. J. Am. Vet. Med. Assoc. 158:1130, 1971.

Casey, J.W., A. Roach, J.I. Mullins, K.B. Burck, M.O. Nicolson, M.B. Gardner, N. Davidson. The U_3 portion of feline leukemia virus DNA identifies horizontally acquired proviruses in leukemic cats. Proc. Natl. Acad. Sci. USA 78:7778-7782, 1981.

Catalona, W.J., Ratliff, T.L., McCool, R.E.: Gamma interferon induced by S. aureus Protein A augments natural killing and ADCC. Nature 291:77-79, 1981.

Chattopadhyay, S.K., M.W. Cloyd, D.L. Linemeyer, M.R. Lander, E. Rands, D.R. Lowy. Cellular origin and role of mink cell focus-forming viruses in murine thymic lymphomas. Nature 295:25-31, 1982.

Chen, I.S.Y., J. McLaughlin, D.W. Golde. Long terminal repeats of human T-cell leukemia virus II genome determine target cell specificity. Nature 309:276-279, 1984.

Chew, D.J., D.J. Meuten. Disorders of calcium and phosphorus metabolism. Vet. Clin. North Am. 12(3):411-438, 1982.

Chew D.J., M. Schaer, S.K. Liu, J. Owens. Pseudohyperparathyroidism in a cat. J. Am. Animal Hosp. Assoc. 11:46-52, 1975.

Cianciolo, G.J., T.D. Copeland, S. Oroszlan, R. Snyderman. Inhibition of lymphocyte proliferation by a synthetic peptide homologous to retroviral envelope proteins. Science 230:453-455, 1985.

Cockerell, G.L., S. Krakowka, E.A. Hoover, R.G. Olsen, D.S. Yohn. Characterization of feline T and B lymphocytes and identification of an experimentally induced T-cell neoplasm in the cat. J. Natl. Cancer Inst. 57:907-911, 1976a.

Cockerell, G.L., E.A. Hoover, S. Krakowka, R.G. Olsen, D.S. Yohn. Lymphocyte mitogen reactivity and enumeration of circulating B and T cells during feline leukemia virus infection in the cat. J. Natl. Can. Inst. 57:1095-1099, 1976b.

Copelan, E.A., J.J. Rinehart, M. Lewis, L. Mathes, R. Olsen, A. Sagone. The mechanism of retrovirus suppression of human T-cell proliferation in vitro. J. Immunol. 131:2017-2020, 1983.

Cotter, S.M. Anemia associated with feline leukemia virus infection. J. Am. Vet. Med. Assoc. 175:1191-1194, 1975.

Cotter, S.M., W.D. Hardy, M. Essex. Association of feline leukemia virus with lymphosarcoma and other disorders in the cat. J. Am. Vet. Med. Assoc. 166:449-454, 1975.

Cotter, S.M., M. Essex. Animal model of human disease: acute lymphoblastic leukemia, aplastic anemia. Am. J. Pathol. 87:265-268, 1977.

Cotter S.M., M. Essex, M.F. McLane, C.K. Grant, W.D. Hardy Jr. Chemotherapy and passive immunotherapy in naturally occurring feline mediastinal lymphoma. In: Hardy W.D. Jr., M. Essex, A.J. McClelland, ed. Feline Leukemia Virus. New York: Elsevier/North Holland, 1980:219-225.

Crow, S.E., B.R. Madewell, A.M. Henness. Feline reticuloendotheliosis: a report of four cases. J. Am. Vet. Med. Assoc. 170:1329-1332, 1977.

Day, N.K., C.O. Felice, W.D. Hardy, R.A. Good, S.S. Witkin. Circulating immune complexes associated with naturally occurring lymphosarcoma in pet cats. J. Immunol. 126:2363-2366, 1980.

Day, N.K., R.D. Tyler, R.W. Engelman, K. Hou, R. Glaser, C.Y. Wang, J. Yamamoto, K. Machida, F. Nahhas, X. Tranx, R.A. Good. Immunological and bone marrow changes associated with remission of leukemia in cats following treatment with AMF Protein A filter. Fed. Proc. 42(5):1194 (abstr.), 1983.

Day, N.K., R.W. Engelman, W.T. Liu, L.Q. Trang, R.A. Good. Remission of lymphoma-leukemia in cats following ex vivo immunosorption therapy using Staphylococcus protein A. J. Biol. Resp. Mod. 3:278-285, 1984.

deNoronha, F., W. Schafer, M. Essex, D.P. Bolognesi. Influence of antisera to oncornavirus glycoprotein (gp71) on infections of cats with feline leukemia virus. Virol. 85:617-621, 1978.

Derse, D., S.J. Caradonna, J.W. Casey. Bovine leukemia virus long terminal repeat: A cell type-specific promoter. Science 227:317-320, 1985.

Dhar, R., W.L. McClements, L.W. Enquist, G.F. VandeWoude. Nucleotide sequences of integrated Moloney sarcoma provirus long terminal repeats and their host and viral junctions. Proc. Natl. Acad. Sci. USA 77:3937-3941, 1980.

Donehower, L.A., A.L. Huang, G.L. Hager. Regulatory and coding potential of the mouse mammary tumor virus long terminal redundancy. J. Virol. 37:226-238, 1981.

Dresser, D.W., H.H. Wortis. Use of an antiglobulin serum to detect cells producing antibody with low hemolytic efficiency. Nature 208:859, 1965.

Dunlap, J.E., W.S. Nichols, L.C. Hebebrand, L.E. Mathes, R.G. Olsen. Mobility of lymphocyte surface membrane concanavalin A receptors of normal and feline leukemia virus-infected viremic felines. Cancer Res. 39:956-958, 1979.

Eby, W.C., C. Chong, S. Dray, G.A. Molinaro. Enumerating immunoglobulin-secreting cells among peripheral human lymphocytes. A

hemolytic plaque assay for a B-cell function. J. Immunol. 115:1700, 1975.

Elder, J., J.I. Mullins. Nucleotide sequence of the envelope gene of Gardner-Arnstein feline leukemia virus B reveals unique sequence homologies with a murine MCF virus. J. Virol. 46:871-880, 1983.

Engelman, R.W., N. Gengozian, R.A. Good, N.K. Day. Polyclonal induction of immunoglobulin synthesis by feline leukocytes as identified in a reverse hemolytic plaque assay. J. Immunol. Mthds. 81:65-71, 1985a.

Engelman, R.W., R.D. Tyler, R.A. Good, N.K. Day. Hypercalcemia in cats with feline leukemia virus-associated leukemia-lymphoma. Cancer, 56:777-781, 1985b.

Engelman, R.W., R.W. Fulton, R.A. Good, N.K. Day. Suppression of gamma interferon production by inactivated feline leukemia virus. Science 227:1368-1370, 1985c.

Engelman, R.W., R.D. Tyler, W.T. Liu, L.Q. Trang, R.A. Good, N.K. Day. Clinicopathologic responses in cats with feline leukemia virus-associated leukemia-lymphoma treated with staphyloccal protein A. Am. J. Pathol. 118:367-378, 1985d.

Erikson, J., D.L. Williams, J. Finan, P.C. Nowell, C.M. Crose. Locus of the alpha-chain of the T-cell receptor is split by chromosome translocation in T-cell leukemias. Science 229:784-786, 1985.

Essex, M., W.D. Hardy, S.M. Cotter, R.M. Jakowski, A. Sliski. Naturally occurring persistent feline oncornavirus infections in the absence of disease. Infect. and Immun., 11:470-475, 1975a.

Essex, M., A. Sliski, S.M. Cotter. Immunosurveillance of naturally occurring feline leukemia. Science 190:790-792, 1975b.

Essex M. Feline leukemia: A naturally occurring cancer of infectious origin. Epid. Rev. 4:189-203, 1982.

Evans, L.H., M.W. Cloyd. Friend and Moloney murine leukemia viruses specifically recombine with different endogenous retroviral sequences to generate mink cell focus-forming viruses. Proc. Natl. Acad. Sci. USA 82:459-463, 1985.

Farrar, J.J., J. Farrar-Fuller, P.L. Simon, M.L. Hilfiker, B.M. Stadler, W.L. Farrar. Thymona production of T-cell growth factor (Interleukin-2). J. Immunol. 125:2555-2558, 1980.

Fauci, A.S., G. Whalen, C. Burch. Activation of human B-lymphocytes. XV Spontaneously occurring and mitogen-induced indirect anti-sheep red blood cell plaque-forming cells in normal human peripheral blood. J. Immunol. 124:2410, 1980a.

Fauci, A.S., G. Whalen, C. Burch. Activation of human B-lymphocytes. XVI Cellular requirements, interactions, and immunoregulation of pokeweed mitogen-induced total-immunoglobulin producing plaque-forming cells in peripheral blood. Cell. Immunol. 54:230, 1980b.

Felber, B.K., H. Paskalis, C. Kleinman-Ewing, F. Wong-Staal, G.N. Pavlakis. The pX protein of HTLV-I is a transcriptional activator of its long terminal repeats. Science 229:675-679, 1985.

Fingeroth, J.D., J.J. Weis, T.F. Tedder, J.L. Strominger, P.A. Biro, D.T. Fearon. Epstein-Barr Virus receptor of human B lymphocytes is the C3d receptor CR2. Proc. Natl. Acad. Sci. USA 81:4510-4514, 1984.

Francis, D.P., M. Essex, W.D. Hardy. Excretion of feline leukemia virus by naturally infected pet cats. Nature 269:252-254, 1977.

Fraser, C.J., G.N. Joiner, J.H. Jardine, C.A. Gleiser. Acute granulocytic leukemia in cats. J. Am. Vet. Med. Assoc. 165:355-359, 1974.

Friedman, S.M., G. Thompson, J.P. Halper, D.M. Knowles. OT-CLL: A human T-cell chronic lymphocytic leukemia that produces IL-2 in high titer. J. Immunol. 128:935-940, 1982.

Fung, Y.T., A.M. Fadly, L.B. Crittenden, H.J. Kung. On the mechanism of retrovirus-induced avian lymphoid leukosis: deletion and integration of the proviruses. Proc. Natl. Acad. Sci. USA 78:3418-3422, 1981.

Gallo, R.C. Human T-cell leukemia-lymphoma virus and T-cell malignancies in adults. In: Cancer Surveys; J. Wyke, R. Weiss (eds.), Oxford University, England, 3:113, 1984.

Gallo, R.C., S.Z. Salahuddin, M. Popovic, G.M. Shearer, M. Kaplan, B.F. Haynes, T.J. Palker, R. redfield, J. Oleske, B. Safai, G. White, P. Foster, P.D. Markham. Frequent detection and isolation of cytopathic retroviruses (HTLV-III) from patients with AIDS and at risk for AIDS. Science 224:500-503, 1984.

Gallo, R.C. Human T-cell leukemia (lymphotropic) retroviruses and their causative role in T-cell malignancies and acquired immune deficiency syndrome. Cancer 55:2317-2323, 1985.

Gardner, M.B., R.W. Rongey, P. Arnstein, J.D. Estes, P. Sarma, R.J. Huebner, C.G. Richard. Experimental transmission of feline fibrosarcoma to cats and dogs. Nature 226:807-809, 1970.

Gardner, M.B., R.W. Rongey, E.Y. Johnson, R. DeJournett, R.J. Huebner. C-type virus particles in salivary tissue of domestic cats. J. Natl. Can. Inst. 47:561-565, 1971.

Gilboa, E., S.W. Mitra, S. Goff, D. Baltimore. A detailed model of reverse transcription and tests of crucial aspects. Cell 18:93-100, 1979.

Gilmore, C.E., V.H. Gilmore, T.C. Jones. Reticuloendotheliosis, a myeloproliferative disorder of cats: a comparison with lymphocytic leukemia. Path. Vet. 1:161-183, 1964.

Glick, A.D., R.G. Horn, M. Holscher. Characterization of feline glomerulonephritis associated with viral-induced hematopoietic neoplasms. Amer. J. Pathol. 92(2):321-332, 1978.

Goding, J.W. Use of Staphylococcal Protein A as an immunological reagent. J. Immunol. Methods 20:241-253, 1978.

Gold, E.R., H.H. Fudenberg. Chromic chloride: A coupling reagent for passive hemagglutination reactions. J. Immunol. 99:858, 1967.

Gonda, M.A., F. Wong-Staal, R.C. Gallo, J.E. Clements, O. Naryan, R.V. Gilden. Sequence homology and morphologic similarity of HTLV-III and Visna virus, a pathogenic Lentivirus. Science 227:173-177, 1985.

Gordon, B.R., R.E. Matus, S.D. Saal, E.G. MacEwen, A.I. Hurvitz, K.H. Stenzel, A.L. Rubin. Protein A independent tumorcidal responses following extracorporeal perfusion of plasma over Staphylococcus aureus. J. Natl. Cancer Inst. 70:1127-1133, 1983.

Gordon G.S., T.J. Cantino, L. Erhardt, J. Hansen, W. Lubich. Osteolytic sterol in human breast cancer. Science 151:1226-1228, 1966.

Grant, C.K., D. Weissman, S. Cotter, E.E. Zuckerman, W.D. Hardy, M. Essex. Mechanisms of feline leukemia escape from antibody-mediated immunity. In Feline Leukemia Virus, eds. W.D. Hardy, M. Essex, A.J. McClelland. Elsevier/North Holland, 1980: 171-178.

Grant, C.K., M.T. Michalek. Feline leukemia-unique and cross-reacting antigens on individual virus producing tumors identified by complement dependent antibody. Int. J. Cancer 28:209-217, 1981.

Grant, C.K., B.J. Ernisse, O. Jarrett, F.R. Jones. Feline leukemia virus envelope gp70 of subgroups B and C defined by monoclonal antibodies with cytotoxic and neutralizing functions. J. Immunol. 131:3042-3048, 1983.

Grant, C.K., B.J. Ernisse, R. Pontefract. Comparison of Feline Leukemia virus-infected and normal cat T-cell lines in interleukin-2 conditioned medium. Cancer Res. 44:498-502, 1984.

Gronowicz, E., A. Coutinho, F. Melchers. A plaque assay for cells secreting Ig of a given type or class. Eur. J. Immunol. 6:588, 1976.

Haley, P.J., E.A. Hoover, S.L. Quackenbush, P.W. Gasper, D.W. Macy. Influence of antibody infusion on pathogenesis of experimental feline leukemia virus infection. J. Natl. Cancer Inst. 74:821-827, 1985.

Harada, F., G.G. Peters, J.E. Dahlberg. A primer ribonucleic acid for initiation of in vitro Rous sarcoma virus deoxyribonucleic acid synthesis. Nucleotide sequence and amino acid acceptor activity J. Biol. Chem. 250:3487-3497, 1975.

Harada, F., G.G. Peters, J.E. Dahlberg. The primer tRNA for Moloney murine leukemia virus DNA synthesis. Nucleotide sequence and aminoacylation of tRNA. J. Biol. Chem. 254:10979-10985, 1979.

Hardy, W.D., G. Geering, L.J. Old, E. Deharven, K.S. Brody, S. McDonough. Feline leukemia virus: occurence of viral antigen in the tissues of cats with lymphosarcoma and other diseases. Science 166:1019-1021, 1969.

Hardy, W.D., L.J. Old, P.W. Hess. Horizontal transmission of feline leukemia virus. Nature 244:266-269, 1973a.

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

Hardy W.D. Jr., P.W. Hess, E.G. MacEwen, A.J. McClelland, E.E. Zuckerman, M. Essex, S.M. Cotter, O. Jarrett. Biology of the feline leukemia virus in the natural environment. Cancer Res. 36:582-584, 1976a.

Hardy, W.D., P.W. Hess, E.G. MacEwen, A.A. Hayes, R.L. Kassel, N.K. Day, L.J. Old. Treatment of feline lymphosarcoma with feline blood constituents. Comp. Leuk. Res. 43:518-521, 1976b.

Hardy, W.D., E.E. Zuckerman, E.G. MacEwen, A.A. Hayes, M. Essex. A feline leukemia virus and sarcoma virus-induced tumor-specific antigen. Nature (London) 270:249-251, 1977.

Hardy W.D. Feline leukemia virus diseases. In: W.D. Hardy, M. Essex, A.J. McClelland, eds. Feline Leukemia Virus. Elsevier/North-Holland, 1980a:3-31.

Hardy W.D. The virology, immunology and epidemiology of the feline leukemia virus. In: W.D. Hardy, M. Essex, A.J. McClelland, eds. Feline Leukemia Virus. Elsevier/North-Holland, 1980b:33-78.

Hardy, W.D. Immunopathology induced by the feline leukemia virus. Springer Semin. Immunopathol. 5:75-106, 1982.

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

Harper, H.D., J. Sjoquist, W.D. Hardy, F.R. Jones. Antitumor Activity of Protein A administered intravenously to pet cats with leukemia or lymphosarcoma. Cancer 55:1863-1867, 1985.

Harvey, J.W., R.P. Shields, J.M. Gaskin. Feline myeloproliferative disease, changing manifestations in the peripheral blood. Vet. Path. 15:437-448, 1978.

Harvey, J.W. Myeloproliferative disorders in dogs and cats. Vet. Clin. North Am. 11(2):349-381, 1981.

Haseltine, W., A.M. Maxam, W. Gilbert. Rous Sarcoma virus genome is terminally redundant: The 5' sequence. Proc. Natl. Acad. Sci. USA 74:989-993, 1977.

Hayward, W.S., B.G. Neel, S. Astrin. Activation of a cellular onc gene by promoter insertion in ALV-induced lymphoid leukosis. Nature 290:475-480, 1981.

Hebebrand L.C., L.E. Mathes, R.G. Olsen. Inhibition of concanavalin A stimulation of feline lymphocytes by inactivated feline leukemia virus. Cancer Res. 37:4532-4533, 1977.

Hellstrom, K.E., I. Hellstrom, J.T. Nepom. Specific blocking factors, are they important? Biochemica. et Biophysica. Acta. 473:121-148, 1977.

Hellstrom, K.E., I. Hellstrom. Does perfusion with treated plasma cure cancer? N. Engl. J. Med. 305:1215-1216, 1981.

Henness, A.M., S.E. Crow. Treatment of feline myelogenous leukemia: four case reports. J. Am. Vet. Med. Assoc. 171:263-266, 1977.

Henness, A.M., S.E. Crow, B.C. Anderson. Monocytic leukemia in three cats. J. Am. Vet. Med. Assoc. 170:1325-1328, 1977.

Herrod, H.G., R.H. Buckley. Use of a human plaque-forming cell assay to study peripheral blood bursa-equivalent cell activation and excessive suppressor cell activity in humoral immunodeficiency. J. Clin. Invest. 63:868, 1979.

Herz, A., G.H. Theilen, O.W. Schalm, R.J. Munn. C-type virus in bone marrow cells of cats with myeloproliferative disorders. J. Natl. Can. Inst. 44:339-348, 1970.

Hess, J.L., J.E. Clements, O. Naryan. Cis and transacting transcriptional regulation of visna virus. Science 229:482-485, 1985.

Holland, C.A., J.W. Hartley, W.P. Rowe, N. Hopkins. At least four viral genes contribute to the leukemogenicity of murine retrovirus MCF 247 in AKR mice. J. Virol. 53:158-165, 1985.

Hoover, E.A., G.J. Kociba, W.D. Hardy, D.S. Yohn. Erythroid hypoplasia in cats inoculated with feline leukemia virus. J. Natl. Cancer Inst. 53:1271-1276, 1974.

Hoover, E.A., R.G. Olsen, W.D. Hardy, J.P. Schaller, L.E. Mathes. Feline leukemia virus infection: age-related variation in response of cats to experimental infection. J. Natl. Cancer Inst. 57:365-369, 1976.

Hoover, E.A., R.G. Olsen, L.E. Mathes, J.P. Schaller. Relationship between feline leukemia virus antigen expression in blood and bone marrow and viral infectivity in blood, bone marrow, and saliva of cats. Cancer Res. 37:3707-3710, 1977.

Hoover, E.A., J.L. Rojko, P.L. Wilson, R.G. Olsen. Determinants of susceptibility and resistance to feline leukemia virus. I. Role of macrophages. J. Natl. Cancer Inst. 67:889-898, 1981.

Hoover, E.A., J.L. Rojko, S.L. Quackenbush. Congenital feline leukemia virus infection. Comp. Leuk. Res. 11:7-8, 1983.

Hsu, T.C., H.H. Rearden. Further karyological studies on felidae. Chromosoma (Berlin) 16:365-371, 1965.

Hughes, S.H., P.R. Shank, D.H. Spector, H.J. Kung, J.M. Bishop, H.E. Varmus, P.K. Vogt, M.L. Breitman. Provirus of avian sarcoma virus are terminally redundant co-extensive with unintegrated linear DNA and integrated at many sites. Cell 15:1397-1410, 1978.

Hughes, S.H., P.K. Vogt, E. Stubblefield, H. Robinson, J.M. Bishop, H.E. Varmus. Organization of endogenous and exogenous viral and linked nonviral sequences. Cold Spring Harbor Symp. Quant. Biol. 44:1077-1089, 1980.

Hunter, T. The proteins of oncogenes. Sci. Amer. 251:70-79, 1984.

Jaffe, H.W., D.P. Francis, M.F. McLane, C. Cabradilla, J.W. Curran, B.W. Kilbourne, D.N. Lawrence, H.W. Haverkos, T.J. Spira, R.Y. Dodd, J. Gold, D. Armstrong, A. Ley, J. Groopman, J. Mullins, T.H. Lee, M. Essex. Transfussion-associated AIDS: Serological evidence of human T-cell leukemia virus infection of donors. Science 223:1309-1312, 1984.

Jarrett O., M.C. Golder, S. Toth, D.E. Onions, M.F. Stewart. Interaction between feline leukemia virus subgroups in the patho-

genesis of erythroid hypoplasia. Int. J. Cancer 34:283-288, 1984.

Jarrett, W.F.H., E. M. Crawford, W.B. Martin, F. Davey. Leukemia in the cat. A virus-like particle associated with leukemia. Nature 202:567-568, 1964a.

Jarrett, W.F.H., W.B. Martin, G.W. Crighton, R.G. Dacton, M.F. Stewart. Transmission experiments with leukemia. Nature 202:566-567, 1964b.

Jarrett, W.F.H. Feline leukemia. Int. Rev. Exp. Pathol. 10:243-263, 1971.

Jarrett, W.F.H., L.J. Anderson, O. Jarrett, H.M. Laird, M.F. Stewart. Myeloid leukemia in a cat produced experimentally by feline leukemia virus. Res. in Vet. Sci. 12:385-387, 1971.

Jenkins, N.A., N.G. Copeland, B.A. Taylor, B.K. Lee. Dilute (d) coat color mutation of DBA/2J mice is associated with the site of integration of an ecotropic MuLV genome. Nature 293:370-374, 1981.

Jerne, N.K., A.A. Nordin. Plaque formation in agar by single antibody-producing cells. Science 140:405, 1963.

Johnson H.M., W.L. Farrar. The role of a gamma interferon-like lymphokine in the activation of T-cells for expression of interleukin-2 receptors. Cell Immunol. 75:154-159, 1983.

Jones, F.R., L.H. Yoshida, W.C. Ladiges, M.A. Kenny. Treatment of feline leukemia and reversal of FeLV by ex vivo removal of IgG. Cancer 46:675-684, 1980.

Junghans, R.P., P.H. Duesberg, C.A. Knight. In vitro synthesis of full-length DNA transcripts of Rous sarcoma virus RNA by viral DNA polymerase. Proc. Natl. Acad. Sci. USA 72:4895-4899, 1975.

Kaplan, M.E., C. Clark. An improved rosetting assay for detection of human T lymphocytes. J. Immunol. Methods 5:131-135, 1974.

Kawase I., C.G. Brooks, K. Kuribayashi, S. Olabuenaga, W. Newman, S. Gillis, C.S. Henney. Interleukin-2 induces gamma interferon production: participation of macrophages and NK-like cells. J. Immunol. 131:288-292, 1983.

Kessler, S.W. Rapid isolation of antigens from cells with a Staphylococcal Protein A - antibody absorbent: parameters of the interaction of antibody-antigen complexes with Protein A. J. Immunol. 115:1617-1624, 1975.

Klatzmann, D., E. Champagne, S. Chamaret, J. Gruest, D. Guetard, T. Hercend, J.C. Gluckman, L. Montagnier. T-lymphocyte T4 molecule

behaves as the receptor for human retrovirus LAV. Nature 312:767-768, 1984a.

Klatzmann, D., F. Barre-Sinoussi, M.T. Nugeyre, C. Dauguet, E. Vilmer, C. Griscelli, F. Brun-Vezinet, C. Rouzioux, J.C. Gluckman, J.C. Chermann, L. Montagnier. Selective tropism of lymphadenopathy associated virus (LAV) for helper-inducer T-lymphocytes. Science 225:59-63, 1984b.

Klausner, J.S., W.J. Miller, T.D. O'Brien, R.F. Branda. Effects of plasma treatment with purified protein A and Staphylococcus aureus Cowan I on spontaneous animal neoplasms. Cancer Res. 45:1263-1269, 1985.

Kobilinsky L., W.D. Hardy, N.K. Day. Hypocomplementemia associated with naturally occurring lymphosarcoma in pet cats. J. Immunol. 122:2139-2142, 1979.

Kohler, G., C. Milstein. Derivation of specific antibody-producing tissue culture and tumor lines by cell fusion. Eur. J. Immunol. 6:511, 1976.

Koshy, R., R.C. Gallo, F. Wong-Staal. Characterization of the endogenous feline leukemia virus-related DNA Sequences in cats and attempts to identify exogenous viral sequences in tissues of virus-negative leukemic animals. Virology 103:434-445, 1980.

Lane, H.C., A.S. Fauci. Immunologic abnormalities in the acquired immunodeficiency syndrome. Ann. Rev. Immunol. 3:477-500, 1985.

Langlois, A., T. Matthews, J. Thiel, D. Bolognesi. Interaction of antibody with host effector cells during serum immunotherapy. In: Feline Leukemia Virus, W.D. Hardy, M. Essex, A.J. McClelland, eds., Elsevier/North Holland, New York. 1980: 261-269.

Langweiler, M., G.L. Cockerell. Generation of concanavalin A induced suppressor cells in the cat. Int. Archs. Allergy Appl. Immunol. 69:148-155, 1982.

Langweiler, M., G.L. Cockerell, F. deNoronha. Role of suppressor cells in feline leukemia virus-associated immunosuppression. Cancer Res. 43:1957-1960, 1983.

Langweiler, M.H. Feline mitogen-induced suppressor cells and their role in FeLV-associated immunosuppression. Ph.D. thesis, Cornell University. Dissertation Abstracts International 43/12-B:3914, 1983.

Lanzavecchia, A., G. Sacchi, R. Maccario, A. Vitiello, L. Nespoli, A.G. Ugazio. Plaque assay with protein A-coated erythrocytes for the evaluation of human immunoglobulin-secreting cells induced by pokeweed mitogen. Scand. J. Immunol. 10:297, 1979.

Lenz, J., D. Celander, R.L. Crowther, R. Patarca, D.W. Perkins, W.A. Haseltine. Determination of the leukemogenicity of a murine retrovirus by sequences within the long terminal repeat. Nature 308:467-470, 1984.

Lerner, R.A. Recombinant origins of leukemogenic murine viruses. Amer. J. Pathol. 93:10-20, 1978.

Levinson, W.E., H.E. Varmus, A. Garapin, J.M. Bishop. DNA of Rous sarcoma virus: its nature and significance. Science 175:76-78, 1972.

Levy, L.S., M.B. Gardner, J.W. Casey. Isolation of a feline leukemia provirus containing the oncogene myc from a feline lymphosarcoma. Nature 308:853-856, 1984.

Liu, W.T., R.W. Engelman, L.Q. Trang, K. Hou, R.A. Good, N.K. Day. Appearance of cytotoxic antibody to viral gp70 on feline lymphoma cells (FL-74) in cats during ex vivo immunoadsorption therapy: quantitation characterization and association with remission of disease and disappearance of viremia. Proc. Natl. Acad. Sci. 81:3516-3520, 1984a.

Liu, W.T., R.A. Good, L.Q. Trang, R.W. Engelman, N.K. Day. Remission of leukemia and loss of feline leukemia virus in cats injected with Staphylococcus protein A: association with elevated levels of circulating gamma interferon and complement-dependent cytotoxic antibody. Proc. Natl. Acad. Sci. USA 81:6471-6475, 1984b.

Livingston, D.M., G.J. Todaro. Endogenous type-C virus from a cat cell clone with properties distinct from previously described feline type C virus. Virology 53:142-151, 1973.

Lum, L.G., M.C. Seigneuret, R.F. Storb, R.P. Witherspoon, E.D. Thomas. In vitro regulation of immunoglobulin synthesis after marrow transplantation. I. T-cell and B-cell deficiencies in patients with and without chronic graft-versus-host disease. Blood 58:431, 1981.

Lutz, H., N. Pederson, J. Higgins, V. Hubscher, F.A. Troy, G.H. Theilen. Humoral immune reactivity to feline leukemia virus and associated antigens in cats naturally infected with feline leukemia virus. Cancer Res. 40:3642-3651, 1980.

Lutz, H., N.C. Pederson, G.H. Theilen. Course of feline leukemia virus infection and its detection by enzyme-linked immunosorbent assay and monoclonal antibodies. Am. J. Vet. Res. 44:2054-2059, 1983.

MacEwen E.G., S.D. Siegal. Hypercalcemia: a paraneoplastic disease. Vet. Clin. North Am. 7(1):187-194, 1977.

Mackey, L.J., W.F. Jarrett, R.R. Coombs. Two populations of lymphocytes in the cat. Vet. Rec. 96:41-43, 1975.

Madewell, B.L. and O. Jarrett. Recovery of feline leukemia virus from non-viraemic cats. Vet. Rec. 112:339-342, 1983.

Madwell, B.R., P.H. Holmes, D.E. Onions. Ferrokinetic and erythrocyte survival studies in healthy and anemic cats. Am. J. Vet. Res. 44:424-427, 1983.

Maede, Y., H. Murat. Erythroleukemia in a cat with special reference to the fine structure of primitive cells in its peripheral blood. Japanese J. Vet. Sci. 42:531-541, 1980.

Maggio, L., R. Hoffman, S.M. Cotter, N. Dainiak, S., Mooney, L.A. Maffei. Feline preleukemia: an animal model of human disease. The Yale J. of Biol. and Med. 51:469-476, 1978.

Majors, J., H.E. Varmus. Nucleotide sequences at host-proviral junctions for mouse mammary tumor virus. Nature 289:253-258, 1981.

Martin, G.S., K. Radke, S. Hughes, N. Quintrell, J.M. Bishop, H.E. Varmus. Mutants of Rous sarcoma virus with extensive deletions of the viral genome. Virology 96:530-536, 1979.

Marx, J.L. A strong new candidate for AIDS agent. Science 224:475, 1984.

Mathes, L.E., D.S. Yohn, R.G. Olsen. Purification of infectious feline leukemia virus from large volumes of tissue culture fluids. J. Clin. Microbiol. 5:372, 1977.

Mathes L.E., R.G. Olsen, L.C. Hebebrand, E.A. Hoover, J.P. Schaller, P.W. Adams, W.S. Nichols. Immunosuppressive properties of a virion polypeptide, a 15,000-dalton protein, from feline leukemia virus. Cancer Res. 39:950-955, 1979.

McAllister, R.M., M. Nicolson, M.B. Gardner, R.W. Rongey, S. Rasheed, P.S. Sarma, R.J. Heubner, M. Hatanaka, S. Oroszlan, R.V. Gilden, A. Kabigting, L. Vernon. C-type virus released from cultured human rhabomyosarcoma cells. Nature New Biol. 235:3-6, 1972.

McClelland A.J., W.D. Hardy Jr., E.E. Zuckerman. Prognosis of healthy feline leukemia virus infected cats. In: W.D. Hardy Jr., M. Essex, S.J. McClelland, eds. Feline Leukemia Virus. New York: Elsevier/North Holland, 1980:121-126.

McCool, R.E., W.J. Catalona, M.P. Langford, T.L. Ratliff. Induction of human gamma interferon by Protein A from Staphylococcus aureus. J. Interferon Res. 1(4):473-481, 1981.

McCullough, B., J. Schaller, J.A. Shadduck, D.S. Yohn. Induction of malignant melanomas associated with fibrosarcomas in gnotobiotic cats inoculated with Gardner feline fibrosarcoma virus. J. Natl. Canc. Inst. 48:1893-1896, 1972.

McDonough, S.K., S. Larsen, R.S. Brodey, N.D. Stock, W.D. Hardy. A transmissible feline fibrosarcoma of viral origin. Cancer Res. 31:953-956, 1971.

McGrath, M.S., I.L. Weissman. AKR leukemogenesis: Identification and biological significance of thymic lymphoma receptors for AKR retroviruses. Cell 17:65-75, 1979.

Mellon, P., P.H. Duesburg. Subgenomic, cellular Rous sarcoma virus RNAs contain oligonucleotides from the 3' half and the 5' terminus of virion RNA. Nature 270:631-634, 1977.

Meuten D.J., S.M. Price, R.M. Seiler, L. Krook. Gastric carcinoma with pseudohyperparathyroidism in a horse. Cornell Vet. 68:179-195, 1978.

Meuten D.J., B.J. Cooper, C.C. Capen, D.J. Chew, G.J. Kociba. Hypercalcemia associated with an adenocarcinoma derived from the apocrine glands of the anal sac. Vet. Path. 18:454-471, 1981.

Meuten D.J., C.C. Capen, G.J. Kociba, B.J. Cooper. Hypercalcemia associated with an adenocarcinoma of the apocrine glands of the anal sac. Am. J. Pathol. 108:366-370, 1982a.

Meuten D.J., D.J. Chew, C.C. Capen, G.J. Kociba. Relationship of calcium to albumin and total proteins in dogs. J. Am. Vet. Med. Assoc. 180:63-67, 1982b.

Mia A.S., D.E. Kah, M.M. Tierney, J.E. Post. A micro-enzyme-linked immunosorbent assay (ELISA) test for the detection of feline leukemia virus in cats. Comp. Immunol. Microbiol. Infect. Dis. 4:111-117, 1981.

Michel, R.L., P. O'Handley, A.W. Dade. Megakaryocytic myelosis in a cat. J. Am. Vet. Med. Assoc. 168(11):1021-1024, 1976.

Molinaro, G.A., S. Dray. Antibody coated erythrocytes as a manifold probe for antigens. Naure 248:515, 1974.

Moller, G., P. Landwall. The polyclonal B-cell-activating property of protein A is not due to its interaction with the Fc part of immunoglobulin receptors. Scand. J. Immunol. 6:357-366, 1977.

Moorhead, P.S., P.C. Nowell, W.J. Mellman, D.M. Battips, D.A. Hungerrford. Chromosome preparations of leukocytes cultured from human peripheral blood. Exp. Cell. Res. 20:613-616, 1960.

Moulton, J.E.: Tumors in domestic animals. U. of Calif. Press, Berkely, Calif., 1978, 193-195.

Mullins, J.I., D.S. Brody, R.C. Binari, S.M. Cotter. Viral transduction of c-myc gene in naturally occurring feline leukemias. Nature 308:856-858, 1984.

Mundy, G.R., R.A. Luben, L.G. Raisz, J.J. Oppenheim, D.N. Buell. Bone-resorbing activity in supernatants from lymphoid cell lines. N. Engl. J. Med. 290:867-871, 1974a.

Mundy G.R., L.G. Raisz, R.A. Cooper, G.P. Schechter, S.E. Salmon. Evidence for the secretion of an osteoclast stimulating factor in myeloma. N. Engl. J. Med. 291:1041-1046, 1974b.

Murray, H.W., B.Y. Rubin, H. Masur, R.B. Roberts. Impaired production of lymphokines and immune (gamma) interferon in the Acquired Immunodeficiency Syndrome. N. Engl. J. Med. 310:883, 1984.

Nathan, C.F., H.W. Murray, M.E. Wiebe, B.Y. Rubin. Identification of gamma interferon as the lymphokine that activates human macrophage oxidative metabolism and antimicrobial activity. J. Exp. Med. 158:670-689, 1983.

Neel, B.G., W.S. Hayward, H.L. Robinson, J. Fang, S.M. Astrin. Avian leukosis virus-induced tumors have common proviral integration sites and synthesize discrete new RNAs: oncogenesis by promoter insertion. Cell 23:323-334, 1981.

Neil, J.C., D. Hughes, R. McFarlane, N.M. Wilkie, D.E. Onions, G. Lees, O. Jarrett. Transduction and rearrangement of the myc gene by feline leukemia virus in naturally occurring T-cell leukemias. Nature 308:814-820, 1984.

Nespoli, L., A. Vitiello, R. Maccario, A. Lanzavecchia, A.G. Ugazio. Polyclonal activation of human B lymphocytes in vitro by pokeweed mitogen: a simple technique for the simultaneous assessment of cell proliferation, generation of plasma cells, plaque-forming cells and immunoglobulin production. Scand. J. Immunol. 8:489, 1979.

Niman, H.L., M. Akhari, M.B. Gardner, J.R. Stephenson, P. Roy-Burman. RD114 and feline leukemia virus genome expression in natural lymphomas of domestic cats. Nature (London) 266:357-360, 1977a.

Niman, H.L., M.B. Gardner, J.R. Stephenson, P. Roy-Burman. Endogenous RD114 virus genome expression in malignant tissues of domestic cats. J. Virol. 23:578-586, 1977b.

Olsen, R.G., L.E. Mathes, S.W. Nichols. FeLV-related immunosuppression. In, Feline Leukemia ed. R.G. Olsen. CRC Press, Boca Raton, Fla., 1981, 149-165.

Onions, D., O. Jarrett, N. Testa, F. Frassoni, S. Toth. Selective effect of feline leukemia virus on early erythroid precursors. Nature 296:156-158, 1982.

Orosz, C.G., N.E. Zinn, R.G. Olsen, L.E. Mathes. Retrovirus mediated immunosuppression. I. FeLV-UV and specific FeLV proteins alter T-lymphocyte behavior by inducing hyporesponsiveness to lymphokines. J. Immunol. 134:3396-3403, 1985.

Oskarsson, M., W.L. McClements, D.G. Blair, J.V. Maizel, G.F. Vande Woude. Properties of a normal mouse cell DNA sequence (sarc) homologous to src sequences of Moloney sarcoma virus. Science 207:1222-1224, 1980.

Payne, G.S., S.A. Courtneidge, L.B. Crittenden, A.M. Fadly, J.M. Bishop, H.E. Varmus. Analysis of avian leukosis virus DNA and RNA in bursal tumors: viral gene expression is not required for maintenance of the tumor state. Cell 23:311-322, 1981.

Payne, G.S., J.M. Bishop, H.E. Varmus. Multiple arrangements of viral DNA and an activated host oncogene (c-myc) in bursal lymphomas. Nature 295:209-213, 1982.

Perryman, L.E., E.A. Hoover, D.S. Yohn. Immunological reactivity of the cat: immunosuppression in experimental feline leukemia. J. Natl. Cancer Inst. 49:1357-1359, 1972.

Peterson, L.C., C.D. Bloomfield, R.D. Brunning. Blast crisis as an initial or terminal manifestation of chronic myeloid leukemia. Am. J. Med. 60:209-220, 1976.

Pierre, R.V. Preleukemic states. Semin. Hematol. 11:73-92, 1974.

Piraino, F. The mechanisms of genetic resistance of chick embryo cells to infection by Rous sarcoma virus - Bryan strain (BS-RSV). Virology 32:700-707, 1967.

Popovic, M., M.G. Sarngadharan, E. Read, R.C. Gallo. Detection, isolation, and continuous production of cytopathic retroviruses (HTLV-III) from patients with AIDS and Pre-AIDS. Science 224:497-500, 1984.

Post, J.E. and L. Warren. Reactivation of latent feline leukemia virus. Developments in Cancer Res. 151-158, 1980.

Pryjma, J., J. Munoz, R.M. Galbraith, H.H. Fudenberg, G. Virella. Induction and suppression of immunoglobulin synthesis in cultures of human lymphocytes: effects of pokeweed mitogen and Staphylococcus aureus Cowan I. J. Immunol. 124:656, 1980a.

Pryjma, J., J. Munoz, G. Virella, H.H. Fudenberg. Evaluation of IgM, IgG, IgA, IgD and IgE secretion by human peripheral blood lymphocytes in cultures stimulated with pokeweed mitogen and Staphylococcus aureus Cowan I. Cell. Immunol. 50:115, 1980b.

Quesenberry, P., L. Levitt. Hematopoietic stem cells. N. Engl. J. Med. 301:755-760, 1979.

Rabin, H., R.F. Hopkins, F.W. Ruscetti, R.H. Neubauer, R.L. Brown, R.G. Kawakami. Spontaneous release of a factor with properties of T-cell growth factor from a continuous line of primate tumor cells. J. Immunol. 127:1852-1856, 1981.

Rabson, A.B., M.A. Martin. Molecular organization of the AIDS retrovirus. Cell 40:477-480, 1985.

Ray, P.K. Suppressor control as a modality of cancer treatment: perspectives and prospects in the immunotherapy of malignant disease. Plasma Ther. Transfus. Technol. 3:101-121, 1982.

Ray, P.K., A. Idiculla, R. Mark, J.R. Rhoads, H. Thomas, J.G. Bassett, D.R. Cooper. Extracorporeal immunoadsorption of plasma from a metastatic colon carcinoma patient by Protein A-containing non-viable Staphylococcus aureus. Clinical, biochemical, serological and histological evaluation of the patient's response. Cancer 49:1800-1809, 1982a.

Ray, P.K., S. Raychaudhuri, P. Allen. Mechanism of regression of adenocarcinomas in rats following plasma adsorption over Protein A-containing Staphylococcus aureus. Cancer Res. 42:4970-4974, 1982b.

Reem G.H., L.A. Cook, J. Vilcek. Gamma interferon synthesis by human thymocytes and T lymphocytes inhibited by cyclosporin A. Science 221:63-65, 1983.

Rickard, C.G., J.E. Post, F. Noronha, L.M. Barr. A transmissible virus-induced lymphocytic leukemia of the cat. J. Natl. Cancer Inst. 42:987-996, 1969.

Rojko, J.L., E.A. Hoover, L.E. Mathes, S. Krakowka, R.G. Olsen. Influence of adrenal corticosteroids on the susceptibility of cats to feline leukemia virus infection. Cancer Res. 39:3789-3791, 1979a.

Rojko, J.L., E.A. Hoover, L.E. Mathes, R.G. Olsen, J.P. Schaller. Pathogenesis of experimental feline leukemia virus infection. J. Natl. Can. Inst. 63:759-768, 1979b.

Rojko, J.L., E.A. Hoover, B.L. Finn, R.G. Olsen. Determinants of susceptibility and resistance to feline leukemia virus infection. II. Susceptibility of feline lymphocytes to productive feline leukemia infection. J. Natl. Can. Inst. 67:899-910, 1981.

Rojko, J.L., E.A. Hoover, S.L. Quackenbush, R.G. Olsen. Reactivation of latent feline leukemia virus infection. Nature 298:385-388, 1982a.

Rojko, J.L., E.A. Hoover, B.L. Finn, R.G. Olsen. Characterization and mitogenesis of feline lymphocyte populations. Int. Arch. Allergy Appl. Immunol. 68:226-232, 1982b.

Rojko, J.K., R.G. Olsen. The immunobiology of the feline leukemia virus. Veterinary Immunology and Immunopathology 6:107, 1984.

Rosen, C.A., J.G. Sodroski, R. Kettman, A. Burny, W.A. Haseltine. Trans activation of the bovine leukemia virus long terminal repeat in BLV-infected cells. Science 227:320-322, 1985.

Rosenberg, S.A., P.E. Lipsky. Monocyte dependence of pokeweed mitogen-induced differentiation of immunoglobulin-secreting cells from human peripheral blood mononuclear cells. J. Immunol. 122:926, 1979.

Rosenberg, Z.F., F.S. Pederson, W.A. Haseltine. Comparative analysis of the genomes of feline leukemia viruses. J. Virol. 34:542-546, 1980.

Roussel, M., S. Saule, C. Lagrou, C. Rommens, H. Beug, T. Graf, D. Stehelin. Three new types of viral oncogene of cellular origin specific for hematopoietic cell transformation. Nature 281:452-455, 1979.

Rowley, J.D. Human oncogene locations and chromosome aberrations. Nature 301:290-291, 1983.

Rubin, H., L. Fanshier, A. Cornelius, W.F. Hughes. Tolerance and immunity in chickens after congenital and contact infection with an avian leukosis virus. Virology 17:143-156, 1962.

Sarma, P.S., T. Log. Viral interference in feline leukemia-sarcoma complex. Virology 44:352-358, 1971.

Sarma, P.S., T. Log. Subgroup classification of feline leukemia and sarcoma viruses by viral interference and neutralization tests. Virology 54:160-169, 1973.

Sarngadharan, M.G., M. Popovic, L. Bruch, J. Schupbach, R.C. Gallo. Antibodies reactive with human T-lymphotropic retroviruses (HTLV-III) in the serum of patients with AIDS. Science 224:506, 1984.

Sawyer, R.C., J.E. Dahlberg. Small RNAs of Rous sarcoma virus: characterization by two-dimensional polyacrylamide gel electrophoresis and fingerprint analysis. J. Virol. 12:1226-1237, 1973.

Schaller, J.P., E.A. Hoover, R.G. Olsen. Active and passive immunization of cats with inactivated feline oncornaviruses. J. Natl. Cancer Inst. 59:1441-1450, 1977.

Schalm, O.W., Theilen, G.H. Myeloproliferative disease in the cat associated with C-type leukovirus particles in bone marrow. J. Am. Vet. Assoc. 157(11):1686-1696, 1975.

Schalm, O.W. Myeloproliferative disorders in the cat, progression from erythroleukemia into granulocytic leukemia. Fel. Prac. 5(6):31-33, 1975.

Schalm, O.W., N.C. Jain, E.J. Carroll. Veterinary Hematology, Lea & Febiger, Philadelphia, pp. 573-591, 1975.

Schupbach, J., M. Popovic, R.V. Gilden, M.A. Gonda, M.G. Sarngadharan, R.C. Gallo. Serological analysis of a subgroup of human T-lymphotropic retroviruses (HTLV-III) associated with AIDS. Science 224:503-505, 1984.

Seyberth, H.W., G.V. Segre, J.L. Morgan, B.J. Sweetman, J.T. Potts, J.A. Oates. Prostaglandins as mediators of hypercalcemia associated with certain types of cancer. N. Engl. J. Med. 293:1278-1283, 1975.

Shank, P.R., H.E. Varmus. Virus-specific DNA in the cytoplasm of avian sarcoma virus infected cells is a precursor to covalently closed circular viral DNA in the nucleus. J. Virol. 25:104-114, 1978.

Sherr, C.J. Transformation by feline retroviruses. Pharmac. Ther. 26:45-58, 1984.

Sherwood, L.M., J.L. O'Rioradan, G.D. Aurbach, J.T. Potts. Production of parathyroid hormone by nonparathyroid tumors. J. Clin. Endocr. & Metabolism 27:140-146, 1967.

Siag, W.M., J.M. Jones. Alteration by protein A of the distribution of immune complexes containing antigen of retrovirus. Clin. Immunol. Immunopathol. 24:186-193, 1982.

Silverman, J. Eosinophilic leukemia in a cat. J. Am. Vet. Med. Assoc. 158(2):199, 1971.

Simon, N., Holzworth, J. Eosinophilic leukemia in a cat. Cornell Vet. 67:579-597, 1967.

Sjoquist, J., G. Stalenheim. Protein A from Staphylococcus aureus: IX. Complement-fixing activity of protein A-IgG complexes. J. Immunol. 103:467-475, 1969.

Smith, E.M., H.M. Johnson, J.E. Blalock. Staphylococcus aureus protein A induces the production of interferon-gamma in human lymphocytes and interferon-alpha/beta in mouse spleen cells. J. Immunol. 130:773-776, 1983.

Snyder, S.P., G.H. Theilen. Transmissible feline fibrosarcoma. Nature 221:1074-1075, 1969.

Snyder, H.W., W.D. Hardy, E.E. Zuckerman, E. Fleissner. Characterization of a tumor-specific antigen on the surface of feline lymphosarcoma cells. Nature 275:656-658, 1978.

Snyder, H.W., F.R. Jones, N.K. Day, W.D. Hardy. Isolation and characterization of circulating feline leukemia virus-immune complexes from plasma of persistently infected pet cats removed by ex vivo immunosorption. J. Immunol. 128:2726-2730, 1982.

Snyder H.W., M.C. Singhal, W.D. Hardy, F.R. Jones. 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. 132:1538-1543, 1984.

Sodroski, J., C. Rosen, F. Wong-Staal, S.Z. Salahuddin, M. Popovic, S. Arya, R.C. Gallo, W.A. Haseltine. Transacting transcriptional regulation of human T-cell leukemia virus type III long terminal repeat. Science 227:171-173, 1985.

Squire R.A., M. Bush. The therapy of canine and feline lymphosarcoma. In: R.M. Dutcher, L. Chieco-Bianchi, eds. Unifying Concepts of Leukemia. Basal: Karger, 1973:189-197.

Stann, S.E. Myelomonocytic leukemia in a cat. J. Am. Vet. Med. Assoc. 174(7):722-725, 1979.

Stehelin, D., H.E. Varmus, J.M. Bishop, P.K. Vogt. DNA related to the transforming gene(s) of avian sarcoma viruses is present in normal avian DNA. Nature 260:170-173, 1976.

Stenson, W.F., C.W. Parker. Prostaglandins, macrophages and immunity. J. Immunol. 125:1-5, 1980.

Stiff, M.I., R.G. Olsen. Effects of retrovirus protein on the feline one-way mixed leukocyte reaction. J. Gen. Virol. 64:957, 1983.

Stoll, E., M.A. Billeter, A. Palmenberg, J. Weissmann. Avian myeloblastosis virus RNA is terminally redundant: implications for the mechanism of retrovirus replication. Cell 12:57-72, 1977.

Sumija, M., S. Kano, F. Takaku. Induction of cytotoxic lymphocytes by Protein A derived from Staphylococcus aureus. Int. Arch. Allergy Appl. Immunol. 67:66-73, 1982.

Sutcliffe, J.G., T.M. Shimnick, I.M. Verma, R.A. Lerner. Nucleotide sequence of Moloney leukemia virus: 3'end reveals details of replication, analogy to bacterial transposons, and an unexpected gene. Proc. Natl. Acad. Sci. USA 77:3302-3306, 1980.

Swanstrom, R., H.E. Varmus, J.M. Bishop. The terminal redundancy of the retrovirus genome facilitates chain elongation by reverse transcriptase. J. Biol. Chem. 256:115-121, 1981a.

Swanstrom, R., W.J. DeLorbe, J.M. Bishop, H.E. Varmus. Nucleotide sequence of cloned unitegrated avian sarcoma virus DNA: viral DNA contains direct and inverted repeats similar to those in transposable elements. Proc. Natl. Acad. Sci. USA 78:124-128, 1981b.

Tashjian A.H. Role of prostaglandins in the production of hypercalcemia by tumors. Can. Res. 38:4138-4141, 1978.

Tauris, P. Plaque-forming cells in man. I. Basic technical results obtained with the protein A technique. Scand. J. Immunol. 18:241, 1983a.

Tauris, P. Plaque-forming cells in man. II. Evidence of the existance of active suppressor cells in peripheral blood of normal plaque-forming cell non-responders. Scand. J. Immunol. 18:249, 1983b.

Taylor, D., Y. Hokama, S.F. Perri. Differentiating feline T and B lymphocytes by rosette formation. J. Immunol. 115:862-865, 1975.

Taylor, J.M., R. Illmensee. Site on the RNA of an avian sarcoma virus at which primer is bound. J. Virol. 16:553-558, 1975.

Teich, N. Taxonomy of retroviruses. In, RNA Tumor Viruses, ed. by R. Weiss, N. Teich, H. Varmus, J. Coffin. Cold Spring Harbor Press, 1984 pp. 25-208.

Terman, D.S., T. Yamamoto, M. Mattioli, G. Cook, R. Tillquist, J. Henry, R. Poser, Y. Daskal. Extensive necrosis of spontaneous canine mammary adenocarcinoma after extracorporeal perfusion over Staphylococcus aureus Cowan I. J. Immunol. 124:795-805, 1980.

Terman, D.S., J.B. Young, W.T. Shearer, C. Ayus, D. Lehane, C. Mattioli, R. Espada, J.F. Howell, T. Yamamoto, H.I. Zaleski, L. Miller, P. Frommer, L. Feldman, J.F. Henry, R. Tillquist, G. Cook, Y. Daskal. Preliminary observations of the effects on breast adenocarcinoma of plasma perfused over immobilized protein A. N. Engl. J. Med. 305:1195-1200, 1981.

Testa, N.G., D. Onions, O. Jarrett, F. Frassoni, J.F. Eliason. Hemopoietic colony formation (BFU-E, GM-CFU) during the development of pure red cell hypoplasia induced in the cat by feline leukemia virus. Leuk. Res 7:103-116, 1983.

Theilen, G.H., T.G. Kawakami, J.D. Rush, J.J. Munn. Replication of cat leukemia virus in cell suspension cultures. Nature 222:589-590, 1969.

Trainin, Z., D. Wernicke, H. Ungar-Waron, M. Essex. Suppression of the humoral antibody response in natural retrovirus infections. Science 220:858-859, 1983.

Tsujimoto, H., K. Shirota, T. Hayashi, A. Hasegawa, I. Tomoda, K. Fujiwara. Monocytic leukemia in a cat. Japanese J. Vet. Sci. 43:957-961, 1981.

Varmus, H.E., T. Padgett, S. Heasley, G. Simon, J.M. Bishop. Cellular functions are required for the synthesis and integration of avian sarcoma virus-specific DNA. Cell 11:307-319, 1977.

Varmus, H.E., N. Quintrell, J. Wyke. Revertants of an ASV-transformed rat cell line have lost the complete provirus or sustained mutations in src. Virology 108:28-46, 1981.

Vedbrat, S., M. Evans-McCalla, H. Lutz, W. Prensky. Monoclonal antibodies to FOCMA: binding to FeLV-C infected cells. In Feline Leukemia Virus, eds. W.D. Hardy, M. Essex, A.J. McClelland. Elsevier/North Holland, 457-470, 1980.

Vedbrat, S., S. Rasheed, H. Lutz, M.A. Gonda, S. Ruscetti, M.B. Gardner, W. Prensky. Feline oncornavirus-associated cell membrane antigen: A viral and not a cellularly coded transformation-specific antigen of cat lymphomas. Virology 124:445-461, 1983.

Verbrugh, H.A., C.V. Van Dijk, R. Peters, M.E. Van Der Tol, J. Verhoef. The role of Staphylococcus aureus cell-wall peptidoglycan, teichoic acid, and protein A in the process of complement activation and opsonization. Immunol. 37:615-621, 1979.

Wachstein, M., G. Wolf. The histochemical demonstration of esterase activity in human blood and bone marrow smears. J. Histochem. Cytochem. 6:457-469, 1977.

Wainberg, M.A., S. Vydelingum, R.G. Margolese. Viral inhibition of lymphocyte mitogenesis: interference with the synthesis of functionally active T cell growth factor activity and reversal of inhibition by the addition of same. J. Immunol. 130:2372-2378, 1983.

Wainberg, M.A., S. Vydelingum, M. Boushira, J. Legace-Simard, R.G. Margolese, B. Spira, J. Mendelson. Reversible interference with TCGF activity by virus particles. Clin. Exp. Immunol. 57:663-670, 1984.

Wang, C.Y., Y. Bushkin, R.W. Engelman, R.A. Good, N.K. Day. Monoclonal antibodies to feline leukemia virus (FeLV) and a feline leukemic cell line FL74: application in feline leukemia. Abstract, 5th International Congress of Immunology, Kyoto, Japan, 1983.

Ward, J.M., C.H. Sodikoff, O.W. Schalm. Myeloproliferative disease and abnormal erythrogenesis in the cat. J. Am. Vet. Med. Assoc. 155(6):879-888, 1969.

Watson, J. Continuous proliferation of murine antigen specific helper T-lymphocytes in culture. J. Exp. Med. 150:1510-1519, 1979.

Weiser, M.G., G.J. Kociba. Erythrocyte macrocytosis in feline leukemia virus associated anemia. Vet. Pathol. 20:687-697, 1983.

Weiss, S.R., H.E. Varmus, J.M. Bishop. The size and genetic composition of virus-specific RNAs in the cytoplasm of cells producing avian sarcoma-leukemia viruses. Cell 12:983-992, 1977.

Wellman, M.L., G.J. Kociba, M.G. Lewis, L.E. Mathes, R.G. Olsen. Inhibition of erythroid colony-forming cells by a M15,000 protein of feline leukemia virus. Cancer Res. 44:1527-1529, 1984.

Wilkins, R.J., A.I. Hurvitz. Chemical profiles of feline diseases. J. Am. Animal Hosp. Assoc. 11:29-41, 1975.

Wong-Staal, F. Some perspectives on the molecular mechanisms of in vitro transformation and in vivo leukemogenesis by HTLV. Curr. Top. Micro. Immunol. 115:211-220, 1985.

Wong-Staal, F., R.C. Gallo. The family of human T-lymphotropic leukemia viruses: HTLV-I as the cause of adult T-cell leukemia and HTLV-III as the cause of acquired immunodeficiency syndrome. Blood 65:253, 1985.

Yam, L.T., C.Y. Li, W.H. Crosby. Cytochemical identification of monocytes and granulocytes. Am. J. Clin. Pathol. 55:283-290, 1977.

Yoshida, M., S. Hattori, M. Seiki. Molecular biology of human T-cell leukemia virus associated with adult T-cell leukemia. Curr. Top. Micro. Immunol. 115:157-175, 1985.

Yunoki, K., M. Matsumoto, T. Matsumoto, et al. Adult T-cell leukemia in Kagoshima: Its clinical features and skin lesions. Gann. Monogr. Cancer Res. 28:151-163, 1982.

Zawidzka, Z.Z., R. Janzen, H.C. Grice. Erythemic myelosis in a cat. Path. Vet. 1:530-541, 1964.

Zenoble, R.D., G.N. Rowland. Hypercalcemia and proliferative, myelosclerotic bone reaction associated with feline leukovirus infection in a cat. J. Am. Vet. Med. Assoc. 175:591-595, 1979.

VITA

Robert William Engelman

Cadidate for the Degree of

Doctor of Philosophy

Thesis: CHARACTERIZATIONS AND IMMUNOTHERAPY OF FELINE RETROVIRAL (FeLV) ASSOCIATED HEMATOPOIETIC MALIGNANCIES AND IMMUNODEFICIENCY: CLINICAL, IMMUNOLOGICAL AND PATHOLOGICAL STUDIES

Major Field: Veterinary Pathology

Biographical:

Personal Data: Born in Ann Arbor, Michigan, September 6, 1954, the son of Joseph A. and Lucille T. Engelman. Married to Elaine M. Dunagin on September 5, 1981. Elaine and Bob have one son, Alexander Sean, born May 16, 1983.

Education: Received the Bachelor of Science degree with Honors and Distinction in Biology from the University of Kansas in May, 1978; received the Doctor of Veterinary Medicine degree from the University of Missouri, College of Veterinary Medicine in May, 1982; completed requirements for the Doctor of Philosophy degree at the Oklahoma State University, College of Veterinary Medicine in December, 1985.

Professional Experience: Resident, Department of Veterinary Pathology, College of Veterinary Medicine, Oklahoma State University, June, 1982 to June, 1984; Research Associate, Cancer Research Program, Oklahoma Medical Research Foundation, June, 1984 to present; Assistant Professor, Department of Pathology, College of Medicine, University of Oklahoma Health Sciences Center, June, 1985 to present.