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Comparative Pathobiology of Viral Diseases

Volume II

Edited by
Richard G. Olsen
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FOREWORD

Pathobiology has been introduced as a new expression in medical terminology within the last 3 decades. It reflects the expansion of pathology into related basic sciences (microbiology, immunology, biochemistry, molecular genetics etc.) in order to fulfill one of its traditional missions of redefining the ever changing concepts and principles of disease. Virchow, introducing the concepts of cellular pathology in the last century, already remarked in his keynote address to the Pathology Congress in Berlin that, unless methods are developed to reveal the function of the cellular structures discovered on the microscope, all our work will be in vain. These methods are now available. Modern pathologists (pathobiologists) employ a multitude of methods, ranging from gross observation to molecular genetics, to investigate disease-related problems.

A group of scientists from the Department of Veterinary Pathobiology, Ohio State University (a department combining pathology, microbiology, parasitology, and immunology) presents in 21 chapters of these volumes the current state of knowledge of a selected group of viral diseases in animals to which these authors made substantial contributions over the past decade. This publication reflects several years of cooperative research of a successful team of investigators trained in different disciplines but sharing common research interests. The book does not attempt to cover the whole field of virology but discusses selected viral diseases of broad interest to both veterinary and human medicine. It will fulfill an important need of all investigators involved in the study of viral diseases by providing them (in two volumes) with valuable information presently scattered in many national and international journals.

The title of the volumes, *Comparative Pathobiology of Viral Diseases* appropriately reflects the interdisciplinary team work of the authors. The range of interest is broader than the title indicates since most of the diseases treated in the volumes are excellent models of comparable human diseases. This two-volume set is greatly welcomed at this time.

Adalbert Koestner, D.V.M., Ph.D.
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PREFACE

The pathobiology of viral diseases encompasses areas of knowledge of virology and the physiology of the host. These various parameters culminate in the discipline of "biology of viral disease". It was our intent in this treatise to select a few viral diseases of animal species to identify the various virus-host parameters that may be the basis of viral pathobiology. We feel that this level of understanding of viral diseases will be the basis of more effective control of viral diseases by prophylaxis and preventive medicine. Moreover, this approach may be the basis of understanding and developing innate disease resistance in animals of economic importance.

Richard G. Olsen
Steven Krakowka
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Dr. Blakeslee and Dr. Olsen had the distinct pleasure of working with Dr. Herold Cox during his tenure as chairman of the Department of Viral Oncology at Roswell Park Memorial Institute. They are indeed grateful to have been associated with this outstanding virologist, outdoorsman, and gentleman.

THE EDITORS

Richard G. Olsen, Ph.D., is currently Professor of Virology and Immunology in the Department of Veterinary Pathobiology, College of Veterinary Medicine, Microbiology, Department of Biological Sciences, and the Comprehensive Cancer Center, The Ohio State University. Professor Olsen is a native of Independence, Missouri and graduated with a B.A. from the University of Missouri and Kansas City. He obtained a M.S. degree from Atlanta University and a Ph.D. in Virology from the State University of New York (Roswell Park Memorial Cancer Institute Division), Buffalo, New York.

Professor Olsen joined the faculty at Ohio State University in 1969 and since then has developed a graduate program in pathobiology of viral diseases. He holds grants from the National Institutes of Health and the Department of Defense. He has published with his colleagues and graduate students over 200 papers in the fields of virology, immunology, immunopharmacology, and immunopathology. Professor Olsen has patented a unique procedure for the production of a feline leukemia vaccine. This patent reflects the pathobiologic approach of the immunotoxic effects of feline leukemia disease and indentifies the essential viral factor that cats immunologically recognize to resist disease. He is a member of many national and international associations. His current research interests are delineation of the mechanism of the retrovirus-induced acquired immune deficiency in cats, characterization of the preneoplastic events of retroviral disease, delineation of the biochemical mechanism of hydrazine-induced suppressor cell defects, and characterization of lichen planus dermatopathy as a presquamous cell carcinoma.

Steven Krakowka, D.V.M., Ph.D., received his D.V.M. degree from Washington State University in 1971 and a Ph.D. from the Ohio State University in 1974. He is currently a professor in the Department of Veterinary Pathobiology, College of Veterinary Medicine, The Ohio State University. His research interests are neuropathology, virology, and immunology. He is a co-author with Dr. Richard G. Olsen of a previous book entitled *Immunology and Immunopathology of Domestic Animals.*

James R. Blakeslee, Jr., Ph.D., received his B.S. in bacteriology in 1962 from the University of Pittsburgh and his M.S. and Ph.D. in Microbiology from the Roswell Park Memorial Institute Division of Microbiology of the State University of New York at Buffalo in 1971.

Dr. Blakeslee joined the faculty of the Department of Veterinary Pathobiology at the Ohio State University in 1973 and is an Associate Professor of virology and immunology in that department, the Department of Microbiology, and the Ohio State University Comprehensive Cancer Research Center. His research interests are mainly concerned with the interactions and effects of environmental chemicals on virus-induced neoplasias. Current research efforts are directed towards investigation of human and nonhuman primate T-cell lymphotropic viruses and co-factors that may interact with the infected host resulting in frank T-cell leukemias.

Dr. Blakeslee was a National Cancer Institute pre-doctoral fellow and, recently, an International Fellow of the Japan Society for the Promotion of Science while a Visiting Professor at the Institute for Virus Research at Kyoto University, Japan. He is a member of several national and international associations and has been co-editor of several books on comparative leukemia research and a contributor to a text on feline leukemia. He has over 50 publications on the subject of oncogenic viruses, modulators of virus expression, and host immunity.

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TABLE OF CONTENTS

Volume II

Chapter 1
Feline Leukemia 1
James R. Blakeslee, Jr. and Jennifer L. Rojko

Chapter 2
Bovine Lymphoma 21
Robert M. Jacobs

Chapter 3
Jaagsiekte: An Infectious Pulmonary Adenomatosis of Sheep 53
D. W. Verwoerd, R. C. Tustin, and A. Payne

Chapter 4
Canine Parainfluenza Virus 77
Wolfgang Baumgartner

Chapter 5
The Pathobiology of Feline Caliciviruses 85
R. Charles Povey

Chapter 6
Bovine Respiratory Synctial Virus 105
Lawrence E. Mathes and Michael K. Axthelm

Chapter 7
Feline Infectious Peritonitis 115
Niels C. Pedersen

Chapter 8
Canine Distemper Virus 137
Steven Krakowka, Michael K. Axthelm, and Gayle C. Johnson

Chapter 9
Rabies 165
H Fred Clark and Bellur S. Prabhakar

Index 215

Chapter 1

FELINE LEUKEMIA

James R. Blakeslee, Jr. and Jennifer L. Rojko

TABLE OF CONTENTS

I. Historical Perspectives 2

II. Feline Leukemia Virus 2
- A. Nomenclature and Structure of FeLV 2
- B. Molecular Biology of FeLV 3
- C. Virus Replication 3
- D. Biologic Properties of Virus Proteins 3
- E. FOCMA 4
- F. FeLV Subgroups 4
- G. Pathogenesis of FeLV Infection 5
- H. Acute, Persistent, and Latent Infection 7
- I. Age-Related Susceptibility to FeLV Infection 8

III. Immunobiology of FeLV 9
- A. Humoral Antibody 9
- B. Cell-Mediated Immunity (CMI) 9
- C. Natural Killer Cells and Interferon 10
- D. Complement and other Humoral Factors and FeLV 10
- E. Immunosuppression and FeLV 10
- F. Immunoprophylaxis 12
- G. FeLV as a Model for Human Disease 13

References 13

I. HISTORICAL PERSPECTIVES

Feline leukemia has been the subject of intense research over the last decade following its recognition as an infectious disease, and its potential significance as a model for the study of human disease. Feline leukemia provides a model of an outbred animal in close association with the human population which presents a unique opportunity to study aspects of neoplastic disease from a vantage point not possible with other animal systems. The evidence in favor of a causal relationship between a virus and the disease is in the epidemiologic data obtained in FeLV-positive leukemia cluster households.[1,2] One important finding was the identification of virus negative lymphomas in the cat. Although no virus was found in these cats, the virus was believed to be latent, in that feline oncornavirus-associated cell membrane antigen (FOCMA) was detected on the cell surface in these cats.[1,3-6] Under natural conditions cats become infected with the FeLV predominantly by contact with salivary and nasal secretions of cats with persistent, active FeLV infection. Within 6 weeks after infection of cats with FeLV, either of two major host-virus relationships usually develops: (1) persistent active infection, which is a progressive infection or (2) a selflimiting regressive infection. The cats may develop anemia, be immunosuppressed leading to other types of disorders, or they may develop leukemia. Thus, it is possible to divide diseases related to FeLV infection into two categories: neoplastic and non-neoplastic disease.

When FeLV infection results in neoplastic disease, classification is based on the cell type that has undergone malignant transformation. Subclassification is further based on the location of the primary lesion. The clinical manifestations are determined by the histologic type and location of the lesions.

A broad spectrum of non-neoplastic diseases has made diagnosis of many of these disorders quite difficult. The non-neoplastic diseases have been commonly confused with other feline diseases. Non-neoplastic diseases may include a nonregenerative anemia, a panleukopenia-like syndrome, thymic atrophy, reproductive disorders, glomerulonephritis, hemolytic anemia, and immunosuppression (acquired immune deficiency). In the case of neoplastic diseases, the spectrum includes lymphoproliferative disorders, including lymphocytic leukemia, lymphosarcoma, and/or myeloproliferative diseases such as myelosclerosis, granulocytic anemia, erythroleukemia, erythremic myelosis, myelofibrosis, and reticuloendotheliosis. Thus, the broad spectrum of neoplastic and non-neoplastic diseases induced by FeLV are manifold.

The FeLV model of non-neoplastic and neoplastic disease has taken on greater importance with the finding of human retroviruses that are involved in adult T cell leukemia/lymphoma and the acquired immunodeficiency syndrome (AIDS). Thus, it appears that the investigation of FeLV in cats and the responsible mechanism of leukemogenesis may be of great importance in the study of the pathogenesis of certain types of leukemia in man.

II. FELINE LEUKEMIA VIRUS

A. Nomenclature and Structure of FeLV

FeLV is an enveloped, oncogenic RNA virus containing a single-stranded 70s RNA composed of two identical 35s subunits and is classified in the family Retroviridae, subfamily Oncovirinae.[7] The FeLV core consists of tightly coiled single-stranded viral RNA surrounded by core proteins of 27,000, 15,000, and 10,000 daltons, and designated p27, p15(C), and p10, respectively,[8,9] and the RNA dependent DNA polymerase (RDDP).[10] The core complex is surrounded by acidic proteins of 12,000 daltons (p12) which constitute the inner coat. The viral envelope consists of a glycosylated protein

of 70,000 daltons (gp70) arranged as spheres displayed on radiating spikes derived from the transmembrane 15,000 dalton protein designated p15(E).[11]

B. Molecular Biology of FeLV

The single-stranded viral RNA is composed of two identical 35s subunits. Each subunit has an extra cap nucleotide added at the 5′ end; 3′ end contains a tail of polyadenylic acid. Unique regulatory domains are located at the 5′ (U5) and 3′ (U3) ends, and each subunit is terminated by repeated (R) sequences shared by the 5′ and 3′ ends. The initiation site for reverse transcription is located at the 3′ boundary of the U5 domain where primer transfer RNA (tRNA) of host origin is attached by hydrogen bonds.[12] The coding domains for the virion structural proteins and the RDDP are organized from the 5′ to 3′ end in the following order: gag, pol, and env regions. The group-associated gene (gag) sequences encode the polyprotein precursors of the internal core proteins (p15(C), p12, p27, p10, the polymerase (pol) region codes for the RDDP, and the envelope (env) sequences encode the envelope polyprotein precursor which is glycosylated and cleaved post-translationally to yield gp70 and pl5(E).

C. Virus Replication

Following adsorption and penetration to susceptible cells, FeLV is uncoated and the RNA is copied into a single strand of complementary DNA (cDNA) by the virion RDDP which is primed by a small tRNA molecule.[12,13] The *de novo* synthesized DNA serves as a template for the formation of the double-stranded DNA provirus which circularizes and integrates to become part of the cell genome. Viral RNA (vRNA) is transcribed from the integrated provirus by DNA-dependent RNA polymerases (DDRP) and translated on host ribosomes to generate precursor structural and envelope polyproteins. These polyproteins migrate to the plasma membrane where "budding" occurs.[11,14] Peripherally, the envelope precursors undergo post-translational cleavage and the minor fragment (p15[E]) spans the plasma membrane as transmembranous spikes firmly embedded in the lipid bilayer.[11] Protruding knobs representing the major fragment are glycosylated (gp70) and are welded to the spikes of p15(E) by disulfide linkages.[11] Centrally, the structural precursors are processed and the individual proteins re-associate according to their biochemical and biophysical properties to encapsulate the ribonucleoprotein core.[8,14,15,16]

D. Biologic Properties of FeLV Proteins

The major group-specific antigen (GSA) which confers interspecies antigenic cross-reactivity to FeLV resides in the major core protein p17.[16] p17 Can be detected in the cytoplasm of circulating neutrophils and platelets by fixed cell immunofluorescence assay[76] and shows a high correlation with recovery of infectious virus[17] from plasma.[18] Serum from FeLV-infected cats contains soluble p27 generally detected by an enzyme-linked immunosorbent assay[19,20] which detects both viremic cats or occult FeLV infection.[19,29,21]

Three antigenically distinct forms of the major envelope glycoprotein (gp70) specify three subgroups (serotypes) of FeLV, designated $FeLV_A$, $FeLV_B$, and $FeLV_C$. These envelope gene products determine infectivity, interference, host range properties, and pathogenicity, and as subgroup-specific antigens evoke the virus neutralizing antibody responses important in the reversal of viremia in cats that regress FeLV infection.[22-26] Virus adsorption, and hence infectivity, is dependent upon the affinity of gp70 for host cellular FeLV receptors; virus neutralizing antibody blocks initial adsorption. Dessication, heat (56°C, 3 min), and ultraviolet (UV) light detach gp70 and inactivate FeLV.

The minor envelope protein (p15[E]) depresses T lymphocyte function that accompanies viremia.[27] The binding of p15(E) to the first component of complement[28] acti-

vates the classical pathway which causes complement depletion and results in ineffective virolysis in cats.[29]

E. FOCMA

An apparent virus-directed antigen on the surface of cat cells transformed by feline sarcoma virus was detected by membrane immunofluorescence using antisera from resistant cats.[5,30,31] This antigen was subsequently named Feline oncornavirus-associated cell membrane antigen (FOCMA). It was subsequently found on plasma membranes of cells infected in vitro with FeSV and on tumor cell surfaces from cats with fibrosarcomas, leukemias, and lymphomas.[5,32,33] An inverse relationship was noted in that antibody to FOCMA correlated inversely with tumor progression[5,32] and was therefore thought to be associated with the development of anti-FeLV/FeSV-induced tumor immunity, and has since been observed in vaccine studies.[33-35] FOCMA was shown to evoke a complement-dependent antibody response effective in prevention of tumor development.[32,36-44] While the exact origins and functions of FOCMA remain speculative, it is clear that antibody to FOCMA determinants effects antitumor immunosurveillance in these tumor-bearing or leukemia-infected animals. Several lines of evidence suggest a nonvirion nature of FOCMA. These include: (1) the inability to remove FOCMA-specific antibodies from appropriate sera by absorption with disrupted FeLV-purified gp70 or p27 proteins;[45] (2) the lack of correlation between the presence of antibody to FOCMA and antibody to gp70 or p27 in the sera of a large number of FeLV or FeSV exposed cats, as determined by membrane immunofluorescence and radioimmunoprecipitation;[46] (3) the failure to correlate the presence of anti-gp70 antibody with FOCMA antibody;[38,42,47,48] and (4) the presence of FOCMA in nonproducer mink cells transformed by FeSV.[49]

Further, FOCMA is present on preneoplastic lymphocytes in the bone marrow and mesentery lymph nodes.[20] Recently, an FeLV vaccine was developed using FOCMA and gp70. The details of this vaccine will be discussed later in this chapter.

FeLV-induced malignancies are distinguished by a common tumor-specific antigen, the feline oncornavirus-associated cell membrane antigen (FOCMA) which is present on transformed cells from cats with highly expressed (productive) FeLV infections and from cats with minimally expressed (nonproductive) FeLV infections.[50-53] Recent studies with monoclonal antibodies suggest that the expression of FOCMA and the partial expression of $FeLV_C$ envelope genes are interdependent events.[54]

F. FeLV Subgroups

$FeLV_A$ is the most common subgroup isolated from naturally infected pet cats. It replicates to high titers in infected cats, and always is present when mixtures of FeLV subgroups are isolated. $FeLV_A$ has a narrow host range in vitro,[22] being mostly restricted to cat cells. $FeLV_B$ is isolated always in conjunction with $FeLV_A$, replicates to lower titers, and $FeLV_B$ viremia is delayed in appearance relative to $FeLV_A$ viremia. $FeLV_B$ has an extended host range in vitro. $FeLV_C$ is only recovered if $FeLV_B$ and $FeLV_C$ also are present[22,25] and replicates in cat, human, and guinea pig cells.

Evidence has accumulated suggesting that FeLV subgroups induce different types of disease. Inoculation of susceptible kittens with the Rickard strain of FeLV-FeLV-R (subgroup AB) leads to a high incidence of viremia ($\geq$ 85%), severe immunosuppression, and thymic lymphomas in those animals surviving 17 to 30 weeks.[55,56] In contrast, exposure of cats to the Glasgow passaged Rickard strain of FeLV, which contains subgroup A only, generates a low incidence of viremia, hemorrhagic enteritis, and neutropenia, and occasional alimentary lymphomas or myelogenous leukemias after a very long latent period.[47,57] Although not associated with a specific disease state, $FeLV_B$ is intensely cytopathic for feline marrow cells in vitro.[58] Recent reports of homologous sequences shared by $FeLV_B$ and a murine retrovirus cytopathic for mink cells

(mink cell focus-forming-MCF-virus) have raised speculations as to the origin and leukemogenicity of $FeLV_B$.[59] MCF viruses are spontaneous env gene recombinants between ecotropic and xenotropic retroviruses with high leukemogenicity in vivo, cytopathicity, and extended host range in vitro. $FeLV_B$ is the most frequent helper FeLV implicated in natural transduction of the fes proto-oncogene from cat fibroblasts and the resultant generation of acutely transforming feline sarcoma viruses (FeSVs).

The delay in the appearance of free, infectious $FeLV_C$ in plasma may result from its relative defectiveness for replication in vivo. Vedbrat et al.[54] report that even cells that produce only $FeLV_A$ or $FeLV_A$ and $FeLV_B$ as cell-free virus have many partially replicated, immature $FeLV_C$ buds embedded in the plasma membrane and suggest that the partial expression of $FeLV_C$ is equivalent to FOCMA-L expression. Based on its delay in appearance and defectiveness for replication of $FeLV_C$ and induction by $FeLV_C$-devoid viral stocks, many workers have considered $FeLV_C$ also a candidate recombinant virus.

The Kawakami-Theilen strain of FeLV(-FeLV-KT) is a mixture of $FeLV_{ABC}$, and causes profound erythrosuppression and death within 9 weeks of inoculation of neonatal kittens but is virtually apathogenic in weanlings and adults.[60,61,62] Passage of $FeLV_{ABC}$ through guinea pig cells in vitro selects for biologically cloned $FeLV_C$ (Sarma strain).[22] That this $FeLV_C$ induces viremia and erythroid aplasia in vivo and suppression of erythroid colony formation in vitro suggests a direct relationship between productive $FeLV_C$ infection and anemiagenesis.[62]

G. Pathogenesis of FeLV Infection

Domestic cats are exposed to FeLV following prolonged contact with the saliva or urine of naturally viremic cats.[18,63] High titers of infectious FeLV are excreted by pharyngeal, salivary, bladder, and intestinal epithelia but virus survival at room temperature or under conditions of dessication is less than 2 hr.[63] Therefore, efficient virus transmission appears to require either direct contact between cats, transfer of saliva on hands or feeding utensils, or exposure to recently voided urine in communal litter pans.

The presumed portal of entry following contact in nature[47,64] is the oronasal pharynx. Experimental oronasal challenge of cats with FeLV leads to viremia and disease or nonproductive infection and immunity (see below). Congenital transmission of FeLV has been proposed to account for the clinical observation that viremic queens may bear viremic kittens, but the question of intrauterine vs. lactation-associated vs. contact exposure of kittens has not been resolved.[65] Recent experiments document the transplacental passage of FeLV and its isolation from the embryonic hemolymphatic tissues of fetal kittens obtained by hysterectomy from viremic gravid queens.[66]

The induction of FeLV-related disease usually follows the onset of marrow origin viremia. Even though the leukemogenic event probably is random, high levels of viremia ensure that the damaging virus will be present when cells of the appropriate histogenesis and maturation reach a critical stage in their cycle. In the mouse, chronic antigenemia with MuLV gp71 incites chronic immunostimulation of uninfected T cells and renders them susceptible to the leukemogenic event. In cats, circulating virion proteins are immunosuppressive, erythrosuppressive, and embryosuppressive.[66] Other circulating virion proteins may be complexed to antibody to trigger immune complex disease. Even transient marrow origin viremia and probably mononuclear cell-associated viremia are important. It is true that cats with these usually develop immunity. However, the heavier the original FeLV burden, the smaller the likelihood that the cat will eliminate all FeLV-infected cells, and the larger the likelihood the cat will maintain persistent poorly expressed FeLV infections and risk nonproductive disease.

The pathogenesis of FeLV replication has six identifiable stages.[67] FeLV/host cell contact is initiated in the lymphoepithelia and follicular lymphocytes in the pharyngeal

and palatine tonsils (stage 1) in the first 2 days following oronasal viral instillation.

Virus amplification occurs in the draining lymph node and infectious FeLV is transported by lymphocytes and macrophages (stage 2) to secondary sites (marrow, systemic and gut-associated lymphoid tissue (GALT) where massive viral amplification occurs (stages 3 and 4). The secondary virus amplification provides rapid, efficient distribution of FeLV to mitotically active cells in the bone marrow and GALT. Viral integration also protects FeLV from reticuloendothelial clearance and from inactivation by humoral lipid or protein moieties as described for feline, murine, and bovine retroviruses.[28,68-72]

Proliferation of FeLV in the spleen, lymph nodes, and GALT distant from the site of inoculation is evident in cats between 3 and 12 days postinfection (stage 3). FeLV p27 is most concentrated in the rapidly dividing lymphoid cells of the germinal centers of cortical follicles. Early viral tropism for B lymphocytes has been shown for certain MuLV strains early in infection.[73-75]

In the fourth stage (7 to 21 days postinfection), widespread infection of nonlymphoid hematopoietic cells in the bone marrow overlaps the systemic lymphoid phase. Megakaryocytes accumulate large amounts of cytoplasmic viral antigen, resulting in infection of developing platelets. The majority of the marrow cells containing p27 are developing myelomonocytic precursors. The concentration of p27 increases as the cell matures. However, p27 is rare in eosinophil precursors. In erythroid maturation, the intensity of FeLV replication is inversely related to cell maturation. Concomitant with marrow infection is the onset of multiple foci of productive infection in the crypt germinal epithelium of the small and large intestines. Viral antigen is confined to the basilar mitotic cell population and is absent from the mature distal absorptive epithelium lining the villi. Retroviruses generally are not cytopathic and replication of FeLV in the rapidly dividing cells of the marrow, lymphoid tissue, and intestine is not associated with overt cytopathic change (necrosis, polykaryocytosis, etc.). However, the presence of FeLV in lymphoid and marrow tissues may depress normal cell turnover and initiate atrophy.

p27-Positive neutrophils and platelets next appear in the circulation and are considered the fifth stage in the evolution of progressive FeLV infection. This phase directly reflects infection of marrow protenitors and is the onset of marrow-origin viremia[76] and the induction of fatal FeLV-related disease.[18,67,76]

The initial marrow release of FeLV-infected neutrophils and platelets coincides exactly with development of protracted neutropenia, lymphopenia, and thrombocytopenia 21 to 56 days postinfection.[67,77,78] These may be affected by atrophic or aplastic responses of hemolymphatic precursor cells, extravascular sequestration of FeLV-infected cells in myeloid or lymphoid tissue, or immunologic elimination of FeLV-infected cells by the host. Although the onset of marrow origin viremia usually signifies the establishment of progressive FeLV infection, some cats still are able to reverse this state by clearing FeLV-infected cells and producing both virus neutralizing (VN) and FOCMA antibody. This latter group of cats is at particular risk for reactivatable FeLV infections and p27 antigenemia.[19-21,78,79]

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

In contrast is the tissue distribution of FeLV p27 in cats that develop immune (re-

gressive) infection between 28 and 42 days postinfection. Immune cats develop FOCMA and VN antibody by 14 to 56 days[20,67] and are able to abort virus production prior to widespread marrow or epithelial infection. Recent evidence indicates that immune cats remain latently infected with FeLV.[78-81] The early cell-associated viremia and systemic lymphomyeloid viral replication must be eliminated in all cells with integrated FeLV proviruses. Most do not and rather develop a persistent nonproductive infection.

The pathogenesis of spontaneous viral infections often centers around a virus/host lymphomyeloid reciprocity in which certain cells are targets for productive or nonproductive (latent) infection, others are targets for cytosuppressive or cytoproliferative disease, and others are effectors of antiviral or antitumor resistance.[82-84] A sequence of lymphoreticular/virus interactions characterizes the infections of cats with FeLV: initial virus replication in hemolymphoreticular cells, and development of FeLV-related neoplasia or aplasia of the lymphoid or hematopoietic system. Available evidence suggests that the histogenesis and immunologic identities of the cells involved in early virus replication, virus containment and latency, chronic (preleukemic) virus replication, and eventual oncogenesis are divergent.

H. Acute, Persistent, and Latent FeLV Infection

Oronasal exposure of cats to the Rickard strain of FeLV simulates natural exposure and initiates FeLV replication in lymphoreticular cells in the tonsil, blood, germinal centers of lymphoid tissues, thymic medulla, and bone marrow, in that order.[67] Apparently, closely related events occur in other horizontally transmitted oncogenic viral infections, e.g., primary infections with the human EBV,[85] murine mammary tumor virus, and Marek's disease herpesvirus in chickens. It is believed that EBV enters the oropharynx, infects tonsillar and blood lymphocytes with complement receptors and specific EBV receptors, and is disseminated via an early lymphocyte-associated viremia. The rationale for FeLV infection of specific lymphoid subsets, whether due to distribution of FeLV receptors, capacity for spontaneous DNA synthesis, and distribution or migration patterns in vivo, currently is not understood.

In progressive FeLV infections, a persistent polyclonal infection of follicular lymphocytes and bone marrow precursors but not thymocytes in association with protracted lymphopenia and neutropenia[67,86] and limited anti-FeLV humoral responses during preleukemia precede the emergence of neoplastic T cells in the thymus and elsewhere.[67,87] The principal FeLV-infected cell in the lymph nodes of viremic cats is a nonadherent, CR-bearing B lymphocyte.[86] p27 Is diffuse in large (20 to 45 μm) lymphoblasts with eccentric, large, round to cleaved nuclei and prominent nucleoli. Plasma cells are densely stained and are identified by eccentric round nuclei with highly condensed chromatin, dense homogeneous cytoplasm at the rounded cell periphery, and a perinuclear (Golgi region) clear space. The pattern of increasing intensity of intracytoplasmic p27 staining with increasing differentiation in B-lineage cells is reminiscent of that described for myelomonocytic series cells in the bone marrow. Purified T cells and adherent macrophages are not particularly enriched for infectious FeLV nor do they contain p27.

The functional integrity of the B lymphocytes that replicate FeLV is unknown. In the mouse, splenic B lymphocytes that are concurrently infected with MuLV produce less antibody to sheep erythrocytes than do uninfected spleen cells, but this is most probably due to increased T-suppressor activity.[88] Naturally viremic pet cats have adequate IgM but ineffective and delayed IgG responses to a synthetic T-dependent antigen and a systemic T-helper defect has been postulated.[89] Experimentally induced viremic cats also produce IgM but not IgG antibody in response to FeLV-associated antigens.[90] These facts may signify altered B-cell function, altered regulation by T

helper, T suppressor or accessory cells, or a changed microenvironment (FeLV-infected lymph node vs. control lymph node). Regarding the last, relative increases in nodal T cells and FcR cells have been reported to be simultaneous with the FeLV-associated peripheral blood lymphopenia in preleukemia.[86] Redistribution of lymphoid subpopulations also has been reported for preleukemic MuLV infections,[91] and increased numbers of CR- and Fc R-positive cells have been described in preneoplastic murine mammary tumor virus infections.[92]

A critical feature that distinguishes persistent productive FeLV infection from persistent nonproductive infection is viral replication by differentiating granulocytes and macrophages in the marrow and elsewhere. Furthermore, marrow origin viremia is accepted universally as the harbinger of fatal FeLV-associated disease.[18,64] In contrast, in cats that regress productive marrow infection and develop latent FeLV infection, refractoriness of myelomonocytic series cells to FeLV replication is correlated directly with increasing maturation. Even in viremic cats which experience extensive FeLV replication in myeloid progenitor cells, differentiated granulocytes, and marrow adherent macrophages, most mature peritoneal macrophages are spared.[93] It is speculated that these are derived from uninfected myeloid clones, or become refractory to, or abort FeLV infection during the process of differentiation in vivo.[79,93] This relationship between macrophage susceptibility to productive viral infection and disease progression also holds for mice infected with the leukemogenic Friend MuLV complex,[94] and was described originally for mice and monkeys infected with such nononcogenic viruses as herpes, corona, pox, and flavi viruses.[95-98] Susceptibility vs. resistance of macrophages to viral replication is central to the mechanism of age-related susceptibility vs. resistance to viral persistence.

FeLV replication in follicular (B) lymphocytes and myelomonocytic series cells is a constant feature of persistent productive FeLV infection. In contrast, replication in putative T-cell regions is limited to the recirculating lymphocyte pool and to the thymic medulla early in infection.[67] The disappearance of replicating FeLV from T-cell areas during preleukemia is associated with lymphopenia,[67,77,101,102] loss of circulating T-suppressor cell,[103] and T-cell mitogenic function, thymicolymphoid atrophy,[56,104] and redistribution of latently infected T cells to the mesenteric lymph nodes,[86] and precedes the emergence of productively infected T-lymphoma cells in the subcapsular cortical thymus and elsewhere.[67,87,101] Neoplastic lymphocytes from experimentally and naturally infected cats usually bear T-cell markers (E rosette forming capacity, surface thymocyte antigen[101]) but rarely do appear as SIg-bearing B cells or null cells.[52] The relative maturity of the transformed T-lymphoma cells is demonstrated by its lack of terminal deoxynucleotidyl transferase (TdT). Similar dissociation between lymphocyte tropisms for viral replication and virus-associated transformation have been observed in Moloney and AKR murine lymphomas.[105,106]

Cats that regress productive marrow and lymphoid infection and become immune must either eliminate all cells with integrated FeLV proviruses or risk persistent nonproductive infection. Cells most likely to escape immune elimination are those with a long interphase as retroviral antigens maximally expressed in mitotic cells. Based on this premise, candidate marrow and lymphoid cells would include the slow-to-cycle committed myelomonocytic precursor defined in the mouse by the in vitro spleen colony-forming unit assay (CFU_s of Till and McCulloch[107]), memory lymphocytes, and long-lived T lymphocytes. While none of these cells has been identified in the cat, it is known that the target cells for latent FeLV infection are compatible: marrow myelomonocytic precursor cells, macrophages, and Staphylococcal Protein A (SPA)-reactive T lymphocytes in the systemic lymphoid tissue.

I. Age-Related Susceptibility to FeLV Infection

Control of the FeLV dose and strain, coupled with variation of the host age at time

of FeLV inoculation, will result in populations of cats destined to develop either viremic or immune FeLV infections. Even though the susceptibility of cats exposed to FeLV in nature may vary greatly, the rate of viremic disease in adults naturally exposed to FeLV in multi-cat households ranges from 15 to 28%. Experimentally, cats at risk for viremia include neonatal kittens (100% susceptible) and 8-week old weanlings (85% susceptible).[99]

Heightened susceptibility to FeLV in the neonate may be macrophage dependent in that macrophages from kittens are five times more sensitive to in vitro infection than macrophages from adults. This maturation-dependent event is abrogated by treatment with hydrocortisone[93] or prednisolone.[100]

Treatment with various doses of prednisolone acetate results in a sevenfold increase in susceptibility of adult cats to FeLV viremia. These animals eventually die of FeLV-related disease.

That corticosteroids are important in regulation of FeLV replication by bone marrow myelomonocytic precursors also may be inferred from their capacity to reactivate latent FeLV.

III. IMMUNOBIOLOGY OF FeLV

A. Humoral Antibody

Essex and colleagues were the first to promote the concept that antibodies are protective against neoplasms induced by feline retroviruses.[51] Protection is correlated to the development of IgG antibodies to FOCMA recognized by indirect membrane immunofluorescence (IMI) assay.

Actual clearance of virus-infected transformed cells probably is mediated by cytotoxic, complement-dependent antibody to both virion antigens and also to FOCMA.[108-112] In contrast, antibodies to nonvirion determinants of FL-74 cells (e.g., FOCMA) which activate feline complement arise later and persist throughout the cat's lifespan. It is likely that these antibodies serve to prevent emergence of producer or nonproducer lymphoma cells in vivo.[110-112] These same antibodies also inhibit the in vitro reactivation phenomenon.

The temporal expression of IgM vs. IgG antibody titers to FOCMA also has prognostic value.[90] In regressors, IgM anti-FOCMA appears soon after exposure and lasts 3 to 5 weeks. The decline in IgM anti-FOCMA is accompanied by a rapid rise in IgG anti-FOCMA. This IgM to IgG conversion is expected and is regarded as a T-helper-dependent response. Cats destined to become viremic differ in that this IgG anti-FOCMA response is low or absent, whereas the IgM anti-FOCMA persists at constant levels until death. The reasons for IgM persistence and failure of IgG conversion presently are not known, though it is postulated that this is due to defective T-helper function,[89] due to constant and recurrent antigen stimulation,[113] or to virus persistence in macrophages.

Limiting marrow-origin viremia in cats is essential. This appears to be mediated by virus neutralizing (VN) antibody to subgroup-specific envelope gp70.[18,21-24,26,70] Antibody to gp70 with high VN in vitro is passively protective in cats when transferred by colostrum[114] or systemic inoculation[70,115] if done early in the course of infection.[70] There is some suggestion that passive transfer of anti-gp70 induces partial remission or enhanced responses to chemotherapy in lymphoma.[115]

B. Cell-Mediated Immunity (CMI)

The first demonstrations that viremic cats have decreased CMI in vivo were those of Perryman et al.[104] and Hoover et al.[56] Allograft rejection responses in persistently infected kittens were delayed and this was correlated inversely with severe thymic atrophy

and paracortical lymphoid depletion. In later studies, thymic depletion was attributed to altered traffic of thymocyte precursors from marrow to thymus, and/or altered exit of mature thymocytes to splenic and nodal paracortex.

Abrogation of immunity is T-cell specific. With the onset of viremia, cats also lose their capacity to respond to the T-lymphocyte mitogens and to the antigen keyhole limpet hemocyanin, whereas reactivity to Staphylococcal Protein A and to the B-cell mitogen lipopolysaccharide (LPS) are comparable to uninfected cats.[77,101,102,116]

T-cell specificity also is supported by the fact that viremic cats make ineffective IgG responses to a synthetic multichain T-cell specific polypeptide.[89] IgM antibodies were similar in infected and uninfected cats. This peptide indicates normal B-cell function and impaired T-helper cell function. FeLV-induced immunosuppression may provide a model for the specific impairment of OKT-4 positive T-helper cells observed following infection of human cells with the human T-cell leukemia virus (HTLV).[117-119]

T-cell suppression by FeLV is not, however, limited to the T-helper population. FeLV-related immunosuppression has been studied by evaluating suppressor cell function in vitro.[103] Viremic cats lack circulating suppressor cells or these cells are not functioning as those in normal cats.

C. Natural Killer Cells and Interferon

The evidence implicating natural killer (NK) cells and/or interferon in the biology of FeLV infection is fragmentary. Clinically, it has been reported that the administration of interferon to anemic, viremic cats has led to partial remission of viremia and recovery from anemia.[120-122] In vitro neoplastic T lymphoblasts are more susceptible to productive FeLV infection than are EBV-transformed lymphoblasts. FeLV buds from the surface of T lymphoblasts with HLA-A1, B12, but not HLA-A29, B8 determinants. Cycloheximide, an inhibitor of interferon synthesis, decreases NK lysis of FeLV-infected B cells but increases NK lysis of FeLV-infected T cells, indicating that interferon mediates enhanced NK lysis of FeLV-infected B cells only and that FeLV-infected, neoplastic T cells resist NK lysis.[123]

D. Complement and other Humoral Factors and FeLV

Circulating substances including proteins, glycoproteins, and lipoproteins are known to influence retrovirus replication. Complement components of humans, other primates, and cats but not guinea pigs, lyse retroviruses directly.[28,29,68,69] It appears that virion p15(E) binds to C1q thereby activating the classical pathway and thus virolysis. Surprisingly, there is no difference in FeLV lysis by normal vs. leukemic serum or by viremic vs. nonviremic serum.[29] Despite this, complement consumption in viremia is indicated by the facts that viremic cats are hypocomplementemic, have circulating immune complexes containing gp70, p15(E), p27 and IgG,[123,124] and have deposits of FeLV, IgG and complement in renal glomeruli. Complement also mediates the antibody-dependent lysis of producer or nonproducer lymphoblasts transformed by FeLV. Other inhibitors with broad spectrum activity include the very low density lipoprotein of normal mouse serum origin which inactivates ecotropic mouse and feline viruses and broadly reacting antibodies directed against retroviral glycoproteins.[70,71]

E. Immunosuppression and FeLV

Many oncogenic retroviruses are associated with a rapid and sustained decline in immunocompetence soon after infection. In FeLV-infected cats, immunosuppression accompanies induction of marrow origin viremia, precedes detectable neoplastic transformation by months, and predisposes persistently infected cats to a variety of intercurrent, often opportunistic, pathogens. Viremic cats most commonly die of concurrent enteritis, gingivitis, pneumonia, or sepsis of bacterial origin; infectious peritonitis

of coronaviral origin; or disease of hemotropic (*Hemobartonella felis)* or parasitic origin.[65] Clinical manifestations of immunomodulation include peripheral lymphopenia, thymicolymphoid atrophy, circulating immune complexes, hypocomplementemia, and membranous glomerulonephritis or periglomerular fibrosis.[29,36,123,125,126] Immunosuppression, therefore, is the most frequent and the most devastating, thus the most biologically prominent effect of FeLV in its natural host.

Early killed virus vaccine experiments suggested that immunosuppression did not necessarily require live virus.[33] Inactivated FeLV also interferes drastically with lymphocyte function in vitro. Incubation of feline leukocytes with serum from viremic cats or with UV-treated FeLV causes in vitro loss of lymphocyte reactivity to T-cell mitogens,[27,102,127] allogeneic leukocytes,[129] and depression of lymphocyte membrane lectin receptor mobility.[129,130] Similar membrane-related lymphocyte deficiencies accompany lymphoma in man, FeLV viremia in cats, and Friend MuLV infection in mice.

To delineate the FeLV component responsible for the immunosuppression, FeLV has been fractionated into its component polypeptides and each fraction was tested for suppression of mitogen-induced blastogenesis. Purification of the FeLV suppressive fraction has revealed a 15,000 dalton protein on polyacrylamide gel electrophoresis (FeLV p15(E)). Purified FeLV p15(E) has been shown to be suppressive to the mitogen-induced LBT at low concentrations.[27,127] Administration of FeLV p15(E) to cats reduces the subsequent response to FOCMA and also increases susceptibility to FeLV disease. The in vivo biologic effects of FeLV p15(E) are very similar to the effects of inactivated FeLV. In addition to tumor enhancement and decreased FOCMA antibody response, administration of FeLV p15(E) interferes with the apparent helper effect of T-lymphocytes and blocks the apparent conversion of IgM to IgG FOCMA antibody. Cats given FeLV p15(E) develop persistent IgM FOCMA antibody with only low levels of IgG.[99] This profile is similar to that observed in viremic cats.

FeLV-origin p15(E) does not appear to affect production of interleukin 1 (IL-1) by feline monocytes in vitro.[131] This contrasts with observation that antigenically similar, 15,000 dalton proteins derived from human and murine lymphomas inhibit monocyte (macrophage) functions. The mechanism of suppression is more likely related to the dramatic decrease in the secretion of IL-2 (T-cell growth factor) in FeLV- and p15(E)-treated leukocyte.[131] Similarly, avian retrovirus p15(E) has immunosuppressive action equivalent to FeLV p15(E) and recent studies by Wainberg et al.[132] demonstrate that blastogenesis can be restored to suppressed cultures with the addition of sufficient IL-2. Thus, retrovirus components, especially p15 envelope protein, disrupt recruited lymphocyte proliferation by eliminating secretion and action of IL-2.

FeLV p15(E) is highly hydrophobic and may bind readily to cell membrane lipids, thus interrupting normal membrane functions. The prostaglandin and cyclic nucleotide systems are logical candidates for effectors of the T-cell suppression induced by FeLV p15(E). Both are linked closely to the immune system, cell membrane-mediated events, mobility and expression of cell surface receptors and regulation of cell proliferation. Furthermore, colchicine, a microtubule disrupting agent, reverses FeLV suppression of lectin receptor mobility. Lewis et al.[133] have tested the putative involvement of cyclic nucleotides and prostaglandins in FeLV-related lymphorepression and have shown that only prostaglandins of the E series depress lymphagic blastogenesis alone or in conjunction with FeLV p15(E).

It is known that conA causes a rise in intracellular cyclic AMP levels in stimulated lymphocytes. Incorporation of FeLV p15(E) into the lymphocyte plasma membrane may interfere with the Ca^{+} transduction of the membrane signal which leads to the activation of adenylate cyclase.[133] Treatment of lymphocytes with FeLV p15(E) has no effect on cellular cyclic GMP but does inhibit cyclic AMP accumulation in the presence of mitogen. Indirect activation of adenylate cyclase by forskolin reverses FeLV

suppression of lymphocyte function by raising intracellular cyclic AMP to normal levels. From this it is likely that the initial action of p15(E) at the cell membrane is to block the activation of adenylate cyclase. Failure to generate the second messenger cyclic AMP leads to failure of transmission of the message to proliferate or to undergo capping in response to the lectin signal.

F. Immunoprophylaxis

The basic virology of feline leukemia and the pathogenesis of the feline leukemia disease is well characterized and it is reasonable to assume that an appropriate vaccine to block this disease is possible. As discussed earlier, feline leukemia virus is the confirmed etiologic agent of feline leukemia disease and that this rough species of virus is responsible for the acquired immunodeficiency syndrome, lymphosarcoma, leukemia, thymic lymphoma, fibrosarcoma, nonregenerative anemia, and fetal absorption in cats. Not only are all these diseases thought to be induced by a single species of feline leukemia virus, but the neoantigen (FOCMA) is also common to all feline retrovirus diseases. Though the serotype of feline retrovirus may be associated with more than one particular form of feline leukemia disease, the feline retrovirus envelope antigen that elicits virus-neutralizing antibody has been more characterized. Most important, it is known that the pathogenesis of the feline leukemia virus diseases is initiated with the productive virus infection as a result of horizontal transmission of the feline leukemia agent. The ensuing disease culminates in the formation of the neoplastic disease.

The immune mechanisms that have been demonstrated to play a role in the protection from these diseases include the production of virus-neutralizing antibody to subgroup specific components that prevent viremia of the virus-envelope, and the induction of antibody towards FOCMA. Antibody to FOCMA has been demonstrated to bestow resistance to feline leukemia infected diseases. Thus, resistance to and recovery from feline leukemia disease, depends upon the development of at least two separate immunologic responses: one towards the infecting virus and one towards the surface antigen of the neoplastic cell. Because of the FeLV-gp70 (71,000 dalton glycoprotein), a feline leukemia virus is antigenically distinct from FOCMA. A feline vaccine would theoretically contain one or both of these components. Earlier attempts to develop a feline leukemia vaccine focused on the attempt to induce antiviral immunity as a means of prevention of feline leukemia disease. Vaccines composed of an activated feline leukemia virus-induced adequate virus neutralizing antibody in adult cats and, in fact, kittens born to dams immunized with said virus were apparently protected from disease. By contrast, kittens failed to produce significant virus neutralizing antibody to feline leukemia and these studies show that kittens less than 1 month of age lacked a vigorous immune response to feline leukemia and, in fact, were more susceptible to disease than nonimmunized cats. The relevance of these findings is still to be determined.

These studies and others suggest that conceivably the cat lacks the genetic determinant for complete response to many of the epitopes associated with FeLV-gp70; furthermore, it can be questioned whether the immune response to the glycosylated proteins with the feline leukemia envelope played the dominant role in protection against disease.

Recently, a subunit vaccine to feline leukemia has been developed and has been shown to be efficacious against FeLV disease. Investigators at Ohio State University developed a method of recovering FOCMA and other immunogens from spent-cell culture media from cells persistently infected with FeLV. It was found that actively growing FeLV infected cells when placed in serum free media maintain high cellular viability up to 96 hr of incubation. Moreover, 75% of these cells express FOCMA. Using a FOCMA-specific cytotoxicity inhibition assay and immunoblotting tech-

niques, soluble FOCMA was detected in serum free media from cells grown and maintained in this incomplete media. The release of FOCMA and virion antigens from FOCMA-positive cells is not unexpected. It was found that synchronized cells expressed maximum FOCMA during the G1S phase of cell cycle. The amount of FOCMA diminished as the cell cycle passed through the S and G2 phases. It was logical to assume that membrane FOCMA and FeLV antigens were released into the media as the cells proceeded to the cell cycle.

Evaluation of soluble FOCMA and virion antigens as a vaccine demonstrated them to be a potent immunogen and cats immunized with these soluble factors demonstrated nearly complete protection against FeLV disease.

Western blot analysis demonstrated that all envelope and gag proteins, as well as a FOCMA-like substance were found in the soluble tumor vaccine, the difference being that many of these components were found in high molecular weight, and it's speculated that FOCMA and envelope and gag proteins may be associated with cell membrane components that influence their immunogenicity. It's interesting to speculate that conceivably these factors could be associated with Class I and Class II histocompatibility antigens which may influence both their immunogenicity as well as influencing the cell-mediated cytotoxicity response.

G. FeLV as a Model for Human Disease

FeLV is a unique retrovirus in that it is a horizontally transmitted infectious agent which infects an outbred population of cats. Recently, a family of T-lymphotropic human retroviruses designated human T cell leukemia/lymphoma viruses (HTLV) has been isolated from several lymphoproliferative diseases. HTLV-I virus originally was isolated from two patients with cutaneous T-cell lymphoma and leukemia (CTLC) after initiation of T-cell lines and recovery of the virus from the cell lines.[118,134] Subsequently, a new subgroup, designated HTLV-II virus, was isolated from cultured T-cells of a hairy cell leukemia[135] and recently a third subgroup, designated HTLV-III, was isolated from patients with acquired immunodeficiency syndrome (AIDS).[136,137] Both FeLV and HTLV viruses are T lymphotropic and in the human, the $OKT4^+$ subset ("helper") is the target cell.

Immunodeficiency in many FeLV infected cats is characterized by lymphopenia and neutropenia, cutaneous anergy, impaired macrophage function, impaired blastogenic responses to mitogens and antigens, and impaired humoral antibody responses.[93,102,104] Clinically infected cats may present with lymphadenopathy, pneumonias, gingivitis, skin sores, and susceptibility to viral diseases. Similarly, human AIDS patients also present with lymphadenopathy, particularly in the pre-AIDS period. The disease is manifested by opportunistic infection, predominantly *pneumocystis carinii* pneumonia, in addition to toxoplasmosis, candida and cryptococcus infections, and Kaposi's sarcoma.[136] Intercurrent viral infections are also found in AIDS. Thus, there are striking similarities between FeLV and the HTLV family of viruses.

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