Infectious Agents and Pathogenesis

In vivo Models of HIV Disease and Control

Edited by
HERMAN FRIEDMAN
STEVEN SPECTER
MAURO BENDINELLI

In vivo Models of HIV Disease and Control

INFECTIOUS DISEASES AND PATHOGENESIS

Recent volumes in this series:

IN VIVO MODELS OF HIV DISEASE AND CONTROL
Edited by Herman Friedman, Steven Specter, and Mauro Bendinelli

INFECTIOUS DISEASES AND SUBSTANCE ABUSE
Edited by Herman Friedman, Catherine Newton, and Thomas W. Klein

CHLAMYDIA PNEUMONIAE INFECTION AND DISEASE
Edited by Herman Friedman, Yoshimasa Yamamoto, and Mauro Bendinelli

DNA TUMOR VIRUSES
Oncogenic Mechanisms
Edited by Giuseppe Barbanti-Brodano, Mauro Bendinelli, and Herman Friedman

ENTERIC INFECTIONS AND IMMUNITY
Edited by Lois J. Paradise, Mauro Bendinelli, and Herman Friedman

HELICOBACTER PYLORI INFECTION AND IMMUNITY
Edited by Yoshimasa Yamamoto, Herman Friedman, and Paul S. Hoffman

HERPES VIRUSES AND IMMUNITY
Edited by Peter G. Medveczky, Herman Friedman, and Mauro Bendinelli

HUMAN RETROVIRAL INFECTIONS
Immunological and Therapeutic Control
Edited by Kenneth E. Ugen, Mauro Bendinelli, and Herman Friedman

MICROORGANISMS AND AUTOIMMUNE DISEASES
Edited by Herman Friedman, Noel R. Rose, and Mauro Bendinelli

OPPORTUNISTIC INTRACELLULAR BACTERIA AND IMMUNITY
Edited by Lois J. Paradise, Herman Friedman, and Mauro Bendinelli

In vivo Models of HIV Disease and Control

Edited by

Herman Friedman
University of South Florida
Tampa, FL, USA

Steven Specter
University of South Florida
Tampa, FL, USA

Mauro Bendinelli
University of Pisa
Pisa, Italy

Springer

Herman Friedman
Department of Medical Microbiology
and Immunology
University of South Florida
College of Medicine
Tampa, FL 33612
USA

Steven Specter
Department of Medical Microbiology
and Immunology
University of South Florida
College of Medicine
Tampa, FL 33612
USA

Mauro Bendinelli
Department of Experimental
Pathology and Retrovirus Center
University of Pisa
Via S. Zeno, 37
Pisa, I-56127
Italy

Library of Congress Control Number: 2005924712

ISBN-10: 0-387-25740-3 e-ISBN 0-387-25741-1
ISBN-13: 978-0387-25740-2

Printed on acid-free paper.

Printed in Singapore. (BS/KYO)

9 8 7 6 5 4 3 2 1

springeronline.com

Contributors

ERIC R. ANDERSON • Department of Microbiology and Specialized Neurosciences Program, University of Puerto Rico Medical Sciences Campus, San Juan, Puerto Rico 00936-5067

JUDITH M. BALL • Texas A and M University, Department Veterinary Pathobiology, College Station, TX 77843-4467

MARGARET C. BARR • College of Veterinary Medicine, Western University of Health Sciences, Pomona, CA 91766-1854

FILIPPO BELARDELLI • Department of Cell Biology and Neuroscience, Istituto Superiore di Sanità, Rome, Italy, 00161

WILLIAM P. CHEEVERS • Department of Veterinary Microbiology and Pathology, Washington State University, Pullman, WA 99164-7040

STEPHEN DUNHAM • Retrovirus Research Laboratory, Institute of Comparative Medicine, University of Glasgow, Faculty of Veterinary Medicine, Bearsden Road, Glasgow G61 1QH, UK

FREDERICK J. FULLER • Department of Microbiology, Pathology and Parasitology, College of Veterinary Medicine, North Carolina State University, Raleigh, NC 27650

MURRAY B. GARDNER • Center for Comparative Medicine, University of California Davis School of Medicine, Davis, CA 95616

HOWARD E. GENDELMAN • Department of Pharmacology and Experimental Neuroscience and Internal Medicine Center for Neurovirology and Neurodegenerative Diseases, Omaha, NE 68198-5880

KATRIN HARTMANN • Medizinische Kleintierklinik, Ludwig-Maximillians-Universität, 80539 München, GERMANY

ISIDRO HÖTZEL • Department of Veterinary Microbiology and Pathology, Washington State University, Pullman, WA 99164-7040

OSWALD JARRETT • Retrovirus Research Laboratory, Institute of Comparative Medicine, University of Glasgow, Faculty of Veterinary Medicine, Bearsden Road, Glasgow G61 1QH, UK

CATERINA LAPENTA • Department of Cell Biology and Neuroscience, Istituto Superiore di Sanità, Rome, Italy 00161

WAH-SENG LIM • Texas A and M University, Department Veterinary Pathobiology, College Station, TX 77843-4467

MARTA L. MARTHAS • California National Primate Research Center, University of California, Davis, CA 95616

LOYDA MELENDEZ • Departments of Microbiology and Specialized NeuroSciences Program, University of Puerto Rico Medical Sciences Campus, San Juan, PR 00936-5067

DAVID B. MORTON • Centre for Biomedical Ethics, Department of General Practice and Primary Care, Primary Care Clinical Sciences Building University of Birmingham Edgbaston, Birmingham B15 2TT, UK

SUSAN L. PAYNE • Texas A and M University, Department Veterinary Pathobiology, College Station, TX 77843-4467

TOM R. PHILLIPS • College of Veterinary Medicine, Western University of Health Sciences, Pomona, CA 91766-1854

MAURO PISTELLO • Department of Experimental Pathology, University of Pisa, Pisa, ITALY 56127

STEFANO M. SANTINI • Department of Cell Biology and Neuroscience, Istituto Superiore di Sanità, Rome, Italy, 00161

ULRIKE SAUERMANN • German Primate Center, Göttingen, GERMANY

RAMAN P. SINGH • California National Primate Research Center, University of California, Davis, CA 95616

SIEGHART SOPPER • German Primate Center, Göttingen, GERMANY

MASSIMO SPADA • Department of Cell Biology and Neuroscience, Istituto Superiore di Sanità, Rome, Italy, 00161

ELLEN E. SPARGER • Department of Medicine and Epidemiology, School of Veterinary Medicine, University of California, Davis, Davis, CA 95616

CHRISTIANE STENGEL • Medizinische Kleintierklinik, Ludwig-Maximillians-Universität, 80539 München, GERMANY

MARIA PIA TESTA • College of Veterinary Medicine, Western University of Health Sciences, Pomona, CA 91766-1854

MARY B. TOMPKINS • Immunology Program, North Carolina State University College of Veterinary Medicine, Raleigh, NC 27606

WAYNE A.F. TOMPKINS • Immunology Program, North Carolina State University College of Veterinary Medicine, Raleigh, NC 27606

THOMAS W. VAHLENKAMP • Federal Research Institute for Animal Health, Boddenblick 5a, 17493 Greifswald-Insel Riems, Germany

KOEN K.A. VAN ROMPAY • California National Primate Research Center, University of California, Davis, CA 95616

HUANGUI XIONG • Department of Pharmacology and Experimental Neuroscience Center for Neurovirology and Neurodegenerative Diseases, Omaha, NE 68198-5880

JIALIN ZHENG • Department of Pharmacology and Experimental Neuroscience and Pathology and Microbiology Center for Neurovirology and Neurodegenerative Diseases, Omaha, NE 68198-5880

Preface to the Series

The mechanisms of disease production by infectious agents are presently the focus of an unprecedented flowering of studies. The field has undoubtedly received impetus from the considerable advances recently made in the understanding of the structure, biochemistry, and biology of viruses, bacteria, fungi, and other parasites. Another contributing factor is our improved knowledge of immune responses and other adaptive or constitutive mechanisms by which hosts react to infection. Furthermore, recombinant DNA technology, monoclonal antibodies, and other newer methodologies have provided the technical tools for examining questions previously considered too complex to be successfully tackled. Probably the most important incentive of all is the regenerated idea that infection might be the initiating event in many clinical entities presently classified as idiopathic or of uncertain origin.

Infectious pathogenesis research holds great promise. As more information is uncovered, it is becoming increasingly apparent that our present knowledge of the pathogenic potential of infectious agents is often limited to the most noticeable effects, which sometimes represent only the tip of the iceberg. For example, it is now well appreciated that pathologic processes caused by infectious agents may emerge clinically after an incubation of decades and may result from genetic, immunologic, and other indirect routes more than from the infecting agents themselves. Thus, there is a general expectation that continued investigation will lead to the isolation of new agents of infection, the identification of hitherto unsuspected etiologic correlations, and, eventually, more effective approaches to prevention and therapy.

Studies on the mechanisms of disease caused by infectious agents demand a breadth of understanding across many specialized areas, as well as much cooperation between clinicians and experimentalists. The series *Infectious Agents and Pathogenesis* is intended not only to document the state of the art in this fascinating and challenging field but also to help lay bridges among diverse areas and people.

M. Bendinelli
H. Friedman

Preface

This volume, *In vivo Models of HIV Disease and Control*, is very appropriate, we believe, for this continuing series on infectious agents and pathogenesis. This book describes accumulated and recent knowledge about animal model systems for studying acquired immunodeficiency caused by viruses, especially retroviruses and lentiviruses. It is widely acknowledged that studies on animal retrovirus infections have been a major contributor to the collective understanding of the biology and mechanisms of the diseases caused by such viruses. In particular, the onset of the acquired immunodeficiency syndrome (AIDS) pandemic in the early 1980s due to infection by the human immunodeficiency virus (HIV) infection resulted in an urgent need to understand mechanisms regarding how the immune system is altered. The resulting collapse of the immune system leading to opportunistic infections has provided a unique perspective on host-microbe interactions. As a result, during the past few decades there has been an explosion of knowledge concerning the nature and function of normal immunity, both humoral and cellular, and especially the role of soluble factors (i.e., cytokines). It was soon recognized that HIV infection preferentially compromises cells of the adaptive as well as innate immune systems and results in marked immunosuppression so an infected individual becomes highly susceptible to opportunistic microbes, including other viruses as well as intracellular opportunistic bacteria and fungi. Studies of animal retroviruses, including lentiviruses, which cause immunodeficiency states in rodents, nonhuman primates, and even cats, and so forth, increased as models. It is widely acknowledged that an understanding of the nature and mechanism whereby animal studies of immunodeficiency virus infection, especially ones concerning the nature and mechanism how such viruses suppress immune resistance, provide important information applicable to the human AIDS pandemic. In this volume chapters concerning the simian AIDS model, feline immunodeficiency virus (FIV), and other animal lentiviral infections are examined as model for AIDS.

The first chapter in the book is a historical perspective by Dr. Murray Gardner, University of California, who pioneered development of the feline retrovirus model, as well as the simian AIDS model. He presents an his-

torical perspective of studies concerning animal retrovirus infection directly related to AIDS. He reviews the early oncogenic virus program at the National Institutes of Health and worldwide of the late 1960s and 1970s, which provided early knowledge concerning how RNA tumor viruses in mice, cats, chickens, cows, and even reptiles, result in malignancy and collapse of the immune system. In particular, studies with murine retroviruses resulted in discovery of reverse transcriptase and thus a better understanding of the molecular biology of how these viruses replicate, resulting in infection of host cells. This chapter spans immunosuppressive retrovirus research in the murine system and in larger animals such as cow, horses, goats, and sheep, as well as the simian models. These studies provided the foundation for understanding the pathogenesis of lentivirus immunodeficiency infection.

Subsequent chapters describe in detail the SIV model, including a chapter concerning anti-AIDS drug studies in SIV infected monkeys. Other chapters describe the FIV model, including pathogenesis of the disease, FIV as a model for HIV treatment with antiviral drugs, protective FIV vaccines in infected cats, and the FIV model for studies of drug abuse. Additional chapters review equine infectious anemia virus as a lentiviral model and caprine arthritis-encephalitis, also as a model for AIDS. Use of animal models for direct infection with the AIDS virus is described in a chapter concerning severe combined immunodeficiency in mice. A chapter concerning newer information about the pathogenesis of HIV infection and the role of chemokines, and a final chapter on future perspectives using animals for research directly applicable to humans, especially pathogenesis, chemotherapeutic treatment, and vaccine development for human diseases follow.

It is widely acknowledged that immunodeficiency virus studies have already and will in the future provide numerous beneficial insights for both basic science and applied medicine. The editors of this volume, as well as the authors of individual chapters, are encouraged by the many recent molecular biologic, immunologic, and pathologic studies, which provide a more thorough understanding of the nature and mechanism whereby immunodeficiency viruses cause disease. We believe continuing interest in animal models of HIV disease and control will contribute significantly to advances in the prevention and treatment for immunodeficiency disease states in humans and domestic animals.

The editors express their gratitude to Ms. Ilona Friedman, who served as an outstanding editorial assistant for this volume, as for all books in this series.

Herman Friedman
Steven Specter
Mauro Bendinelli

Contents

1

Historical Perspective

MURRAY B. GARDNER

1. INTRODUCTION

Research on animal retrovirus infections has contributed mightily to our understanding of biology and disease over the past century. Until the 1980s most attention was directed at the cancer-inducing role of the oncogenic retroviruses and, in particular, their genetic transmission in inbred mice as endogenous proviral DNA. Only since the onset of the AIDS pandemic in 1981 has interest centered on the nononcogenic, particularly immunosuppressive properties of animal and human retroviruses and their epigenetic transmission as infectious RNA viruses. The history of animal oncogenic retrovirus research leading up to the discovery of the first human oncogenic retrovirus, the human T-cell leukemia virus (HTLV), has been extensively reviewed.[1,2] However, certain aspects of this history bear repeating here because of the insight they provide into the search for the cause of AIDS and identification of relevant animal models. In this retrospective overview I will mention, from my 35 years experience, some of the major lessons learned and "misadventures" experienced, intertwined with major events in recent retrovirus history (Table I).

2. THE VIRUS CANCER PROGRAM (VCP) ERA

1968–1981: Role of Oncogenic Retroviruses and Endogenous Virogenes

During the late 1960s and 1970s the VCP, an NCI-sponsored program, part of the Nixon era's War on Cancer, orchestrated a major interdisciplinary effort toward understanding the natural history of oncogenic retroviruses in animals and to seek their presence and significance in humans, particularly as possible causes of cancer.[3] The VCP was probably the earliest example of "big science," meaning a coordinated effort of many scien-

MURRAY B. GARDNER • Center for Comparative Medicine, University of California, Davis, CA 95616

TABLE I
Major Discoveries in the History of Retroviruses

1904	Equine infectious anemia virus, i.e., filterable agent
	Valle, Carre
1908–1911	Avian leukosis and sarcoma viruses
	Ellerman, Bang, Rous
1944	Murine mammary tumor virus
	Andervont, Bryan, Bittner
1950	Murine leukemia and sarcoma viruses
	Gross, Moloney, Friend, Rauscher, Kaplan, Kirsten, Harvey, and others
1950s	Lentivirus of sheep
	Sigurdsson, Palsson, Clements, Cheevers, Narayan, Haase, Nathanson
1965–1975	Feline leukemia and sarcoma viruses
	Jarrett, Rickard, Thielen, Kawakami, Gardner, Hardy, and others
1969	Bovine leukemia virus
	Miller, Olson
	Gibbon ape leukemia virus
	Kawakami
	Mason-Pfizer monkey virus
	Chopra, Mason
	Lentiviruses of goats and cows
	Hadlow, Crawford, Cork, VanDer Maaten, Gonda, and others
1970s	Endogenous retroviruses in cats, mice and nonhuman primates
	McAllister, Todaro, Benveniste, Bentzvelsen, Aaronson, Rowe, and others
1980–1982	Human and simian T-cell leukemia viruses
	Poiesz, Ruscetti, Gallo, Hinuma, Yoshida, Miyoshi
1983–1984	Type D retroviruses and simian AIDS
	Daniel, Letvin, Desrosiers, Marx, Gardner and others
	Human immunodeficiency virus (HIV-1) and human AIDS
	Barre-Sinoussi, Chermann, Montagnier, Gallo, Levy
1985	Simian immunodeficiency virus (SIVmac) and simian AIDS
	Daniel, Letvin, Desrosiers, and others
1986	Human immunodeficiency virus (HIV-2)
	Clavel, Montagnier
1987	Feline immunodeficiency virus and feline AIDS
	Pedersen, Yamamoto

tists and institutions toward a common goal. It established precedence in that sense for the much larger genome projects of recent times. However, the VCP was largely unencumbered by the proprietary concerns that govern much of current day biotechnology research. Knowledge in the mid-1960s that the then so-called RNA tumor viruses existed in many domestic chickens, lab mice strains, and domestic cats and a strong suspicion of similar agents in cows and even reptiles called attention to their widespread occurrence in nature and their etiologic association with lymphoma, sarcoma, breast cancer, and other hematological malignancies.

The isolation, in lab mice, of the mammary tumor Type B retroviruses (MMTV) in the early 1940s and, especially the murine leukemia Type C retroviruses (MuLV) in the early 1950s, and demonstration of their vertical (i.e., maternal-fetal) transmission, rekindled enthusiasm for seeking such agents in other mammals including, of course, humans. Especially mind-boggling was the realization, in the late 1960s, that some of the chicken and mice oncogenic retroviruses were apparently transmitted vertically; not just by exogenous means, but also by inheritance in the germ cell chromosomal DNA as so-called endogenous virogenes.

The almost universal detection of Type C retroviral core antigen in chicken and mouse embryos, even in the absence of complete viruses, and the immunological tolerance of adult chickens and mice to this antigen first suggested to Huebner and colleagues that these retrovirus genes might be inherited and expressed during embryonic development.[4] The ability of these RNA tumors viruses to exist not only as exogenous virus but also as DNA proviral genes integrated into cellular DNA was established in 1970 with the discovery by Temin and Baltimore of the viral reverse transcriptase (RT) enzyme (which prompted the renaming of these agents as retroviruses) and later by techniques of molecular hybridization and transfection of cellular DNA. Temin, in his DNA provirus hypothesis, suspected that the retroviruses were generated de novo by reverse transcription of cellular RNA to DNA using cellular RT (i.e., polymerase (pol) enzyme); this process then prompted the "illegitimate" recombination of endogenous retroviral genes (e.g., LTR, core, pol and env), which become expressed as RNA containing virus particles. Independently, this process might also induce malignant cell transformation via mutations in chromosomal DNA.[5] Huebner and Todaro, in their viral oncogene hypothesis, favored the concept that endogenous retrovirus genes, including the so-called oncogenes, were inherited over evolutionary periods, the result of ancient infections and, that their activation, postnatally, triggered cancer.[6] Both of these ideas were partly correct and together they provided much of the scientific rationale for the VCP effort of the 1970s. The viral oncogene hypothesis was prescient in highlighting the inheritance of virogenes over evolutionary time periods but wrong in considering the oncogene an integral component of the virus. The protovirus hypothesis was perceptive in predicting a function for cellular RT enzymes in normal cell biology (e.g., pseudogene formation and gene duplication), but probably overstated the importance of RT in causing mutations leading to cancer or creating oncogenes de novo. However, RT containing retrotransposons, which make up a major portion of repetitive DNA in the human genome, are now suspected of speeding up the natural mutagenesis process tremendously by facilitating genetic shuffling.[7] Another major contribution of the avian and murine Type C retroviruses was, of course, as a source of RT enzyme, which has been essential for making complementary DNA from RNA transcripts

in vitro, a vital step for recombinant DNA technology and modern gene expression assays.

Support for the importance of endogenous retrovirus genes was initially gained from inbred AKR laboratory mice in which thymic lymphoma resulted universally by about 1 year of age from the activation and later recombination of inherited Type C retrovirus genes.[8] Evidence around 1970 suggested that similar endogenous virogenes were present and potentially activatable in the genomes of all mammals, including feral mice and other species such as feral jungle fowl. It was also then known that some sarcomas, arising spontaneously in chickens, mice, or cats, or experimentally induced in rats by MuLV, contained a mixture of replication competent Type C leukemia viruses and replication-defective but rapid cell-transforming sarcoma viruses.[9] In the prototype Rous sarcoma virus (RSV) of chickens the highly oncogenic component was called the sarc gene. The counterpart sarc gene of the Moloney sarcoma virus (MSV) of lab mice could be "rescued" in vitro or in vivo by the competent "helper" MuLV and even by leukemia viruses of unrelated species (e.g., FeLV). Quite logically, this "moveable oncogene" was at first considered a component of the retrovirus genome. However, the RSV sarc oncogene was later shown by molecular hybridization (1976) to be a normal cellular gene that had been transduced by the Type C avian leukemia virus (ALV). Similar oncogenes, representing cell genes acting at various levels of the normal growth signal-promoting cascade, were then found in oncogenic retroviruses isolated from mouse mammary tumors and from sarcomas or other highly malignant hematopoietic cancers in chickens, turkeys, lab mice, domestic cats, and a woolly monkey. Naturally occurring feline sarcoma viruses were a particularly rich source of transduced cellular oncogenes. The same oncogenes were occasionally transduced by sarcoma viruses of different species (e.g., chickens, cats, mice). Animal Type C sarcoma viruses were thus the source of the initially characterized cellular oncogenes, about 30 in number. After 1980 it became apparent that the more common and slowly acting animal leukemia viruses were free of transduced oncogenes and caused their type of cancer by transforming specific target cells via activation of cellular oncogenes (e.g., myc) from the nearby insertion of exogenous proviral DNA containing promoter elements in the LTR.[9] In humans, the cellular oncogenes were activated in the neoplastic process, not by retroviral transduction or proviral insertional activation, but by mutational activity via chromosomal aberrations such as translocation, amplification, or point mutation.

The VCP research emphasis on the role of endogenous retrovirogenes now seems unrealistic in retrospect, but it certainly paid off with the discovery of cellular oncogenes. The growth industry of signal transduction research continues vigorously to this day and has led to several successful anticancer drugs directed at oncogene products. In summary, apart from

the inbred lab mouse, the naturally occurring leukemia viruses in chickens, wild mice, domestic cats, gibbon apes, and cows, all discovered before 1980, are all transmitted exogenously via body secretions, although recombination with related endogenous virogenes may enhance their pathogenicity. Reduction of virus load by active or passive immunization, or especially inbreeding of resistance genes, often helps to prevent the associated disease. In contrast to the animal lentiviruses, described below, the genetic stability and low pathogenicity of most animal oncoretroviruses make them much more amenable to vaccine control measures.

The discovery of HTLV in 1980[10,11] proved that, as anticipated, humans are not exempt from retrovirus infection. However, HTLV causes only a small fraction (<1%) of total human leukemias, mostly occurring outside the United States (e.g., southern Japan), a fact that helps explain why it took a decade of intensive search to find this first human retrovirus.[12] Like the animal leukemia viruses, HTLV is transmitted exogenously (i.e., not inherited), and like MMTV and the wild mouse MuLV, mainly by milk. In its relative genetic stability, failure to recombine with endogenous virogenes or oncogenes, and its ability to induce neurologic disease as well as chronic leukemia, HTLV resembles the wild mouse MuLV discovered a decade earlier.[13] Milk transmission of either wild mouse MuLV or HTLV can be largely controlled by foster nursing, and this results in a decreased incidence of the associated diseases. Rather than promoting cell transformation by proviral insertional activation of cellular oncogenes, as seen with the animal leukemia viruses, HTLV carries an extra viral gene called TAX that transactivates (rather than cis-activates) cellular IL-2 or its receptor, thus initiating the neoplastic process.[14] In its viral genome organization and pathogenesis HTLV resembles the bovine leukemia virus (BoLV), an interesting development insofar as bovine leukemia–prone cowherds were suspected of harboring a causative retrovirus long before isolation of BoLV in 1969. Soon following the isolation of HTLV, numerous closely related STLVs were isolated from African and Asian nonhuman primates and apes.[15] Phylogenetic analysis suggested the HTLV strains originated by cross-species spread of STLV from monkeys and apes to humans in distant evolutionary times. With sequencing of the human genome now complete it is clear that endogenous retrovirus elements, mostly LTR, gag, and pol sequences, make up a major portion of human repetitive DNA. However, these sequences are largely inert, and even when expressed as RNA transcripts or protein products they have not been proven to be linked to any known beneficial or harmful biologic effects, many suggestions not withstanding.[16] No wonder, then, that so much effort has been made in vain to uncover a role for endogenous virogenes in human cancer or other diseases. Regardless, the VCP provided the opportunity for many scientists, including new investigators like myself, to work and learn together about retroviruses. This was a truly exciting endeavor, and it furnished the exper-

tise, reagents, and technology that later proved so vital to the discovery and understanding of the immunosuppressive retroviruses.

The AIDS Era: 1981–Present: Immunosuppressive Retroviruses

Following the first recognition of AIDS in young homosexual men in 1980, it was 3 years before the isolation of the causative retrovirus. During this interval oncogenic Type C or HTLV-1 related retroviruses were strongly suspected and searched for based on the knowledge that retroviruses could induce nonneoplastic degenerative and immunosuppressive diseases in animals (Table II) and man. Certain strains of ALV, MuLV, and FeLV had long been known to induce a generalized immunosuppression in chickens, lab mice, and domestic cats, respectively. Their immunosuppressive effects were thought to probably involve defects in cytokine production affecting normal T- and B-cell maturation and function or the presence of suppressor T-cells.[17] Certain MuLV strains in wild and lab mice were found causative of a degenerative neurologic disease, not too unsimilar to HIV encephalopathy, accompanied in some instances by thymic depletion and

TABLE II
Animal Models of Retrovirus Infection and Immunosuppression

Animal	Retrovirus	Genus	Pathogenesis
Chickens	ALV, REV, SNV	Type C	Lysis of T-cells; suppression of T+B cell production
Mice	MuLV	Type C	Suppression of T+B cell production; super antigen driven T+B cell proliferation from defective MuLV (MAIDS)
Cats	FeLV	Type C	Suppression of T+B cell proliferation. T-cell lyses by defective FeLV (FAIDS)
Cats	FIV	Lentivirus	Similar to AIDS; selective depression of CD4+ T-cells
Macaques	SRV	Type D	Suppression of T+B cell proliferation, possibly by viral interference with cell receptor function
Macaques	SIV	Lentivirus	Similar to AIDS; selective depression of CD4+ T-cells
Macaques	HIV-2	Lentivirus	Similar to AIDS; selective depression of CD4+ T-cells
Macaques	SHIVs	SIV/HIV Recombinants	Similar to AIDS; selective depression of CDT4+ T-cells
Baboon	HIV-2	Lentivirus	Some AIDS-like features but not consistently reproducible

immunosuppression.[18,19] One particular strain of MuLV (Dupan-Laterjet strain) was later touted as a murine model for AIDS, called MAIDS.[20] Similarly, a strain of FeLV caused an acute immunosuppressive disease experimentally in domestic cats that was called feline AIDS or FAIDS.[21] Both MAIDS and FAIDS resemble the acute leukemia and sarcoma viruses of chicken and mice in that they are mixtures of defective and competent helper viruses. The defective component of the MAIDS virus is responsible for the polyclonal activation of both T- and B-cells, preempting their normal function and leading to secondary immune dysregulation. The FAIDS virus is a highly T-cell cytotropic and cytopathic defective virus whose cytopathicity is attributed to specific mutations in the envelope glycoprotein. In both of these models the helper virus can cause leukemia after a longer incubation period. As putative models for AIDS all of the oncogenic animal retroviruses have the disadvantage of not causing a selective depletion of CD4+ T-helper cells, the hallmark of AIDS. Moreover, the lentiviruses are not thought to involve mixtures of pathogenic defective and helper leukemogenic viruses nor do lentiviruses activate cellular oncogenes, as far as we know. An extensive search for lentiviruses in wild mice (*Mus musculus domesticus*) in southern California, Africa, and Europe, using serologic and PCR probes, proved unsuccessful (unpublished data). Therefore, for AIDS pathogenesis, vaccine and drug therapy research, the small animal oncogenic retrovirus models have largely been replaced by the large animal lentivirus models.

3. DISCOVERY OF HIV

Based primarily on the FeLV model of immunosuppression it was strongly suspected, at first, that the new disease, AIDS, was caused by an immunosuppressive variant of the existing HTLV family of human retroviruses; thus the initial nomenclature HTLV III. Supporting this hypothesis was the detection in AIDS patients of RT activity and serologic cross-reactivity with HTLV-1, but these results were later proven to be nonspecific. Because oncogenic retroviruses do not lyse infected cells, some investigators thought it unlikely that a retrovirus of that type could cause a destruction of CD4+ T lymphocytes as seen in AIDS. The discovery of the bona fide causative retrovirus in the cultured lymph node cells from an AIDS patient took place at the Pasteur Institute in 1983.[22] The secrets to success were the detection of RT activity in culture media after a much shorter incubation period (~1 week) than characteristic of HTLV (~3 weeks), and the disappearance of RT activity coincident with loss of CD4 T-cells in the culture, in contrast to the immortalization of CD4+ T-cells as seen with HTLV. The virus, at first called LAV for lymphadenopathy virus, could only be propagated by the repeated addition of fresh peripheral

blood mononuclear cells (PBMCs) containing the target T-cells. Most telling was the immunologic cross-reaction in gel diffusion of LAV antigens with the equine lentivirus (EIAV) and the electron microscopic appearance characteristic of lentivirus particles. Ironically, the very first animal disease to be assigned a viral etiology (i.e., filterable agent) in 1904 was equine infectious anemia. However, it was 1976 before EIAV was classified as a lentivirus. Despite considerable skepticism, these findings were soon proven correct, and further research, done at the Pasteur Institute, National Cancer Institute (NCI), and elsewhere, established this lentivirus, renamed HIV-1, as the cause of the AIDS epidemic and began to elucidate its pathogenesis.[23] The NCI's initial AIDS virus isolate, HTLV-III, was later shown to be identical to LAV, whereas the first bona fide U.S. AIDS virus isolate became known in 1984 from a San Francisco gay male.[24] A second, less pathogenic, strain of HIV (HIV-2) was isolated in 1986 by Institute Pasteur scientists.[25] Based on historical precedence, the AIDS pandemic and its causative lentivirus were totally unexpected. No animal oncogenic retrovirus models even remotely resembling AIDS in epidemiologic or clinical pathologic features had ever been observed. Furthermore, lentiviruses had never before been found in humans, so the recognition of HIV as a lentivirus was a huge surprise.

4. SHEEP, GOAT, HORSE, AND COW LENTIVIRUSES

Since the mid-1950s the lentivirus genus of retroviruses had been recognized as the cause of slowly progressive degenerative diseases of certain farm animals, namely sheep—visna virus (VV), goats—caprine arthritis encephalitis virus (CAEV), horses—equine infectious anemia virus (EIAV), and cows—bovine immunodeficiency virus (BIV). Indeed, visna virus was the prototype "slow virus," responsible in the 1950s for wiping out all of the sheep in Iceland; the inbred sheep had become infected through introduction of a few carrier rams from Germany.[26] However, these slow virus diseases had a lower priority in the VCP agenda because this type of retrovirus was not directly associated with cancer. Ironically, the natural history of the large animal lentivirus diseases proved to be a remarkable harbinger of what was to come with HIV infection of humans. In common with HIV infection, monocyte/macrophages are a major target cell for lentivirus infection in these animals, and disease manifestations reflect the antiviral immune response directed at tissue sites where the infected macrophages congregate (e.g., brain, lung, joints).[27] Most ominously, also in line with HIV, the immune defenses of these animals are unable to eliminate the virus, primarily because of the frequent mutations in the viral genes and the existence of latent proviral DNA, out of sight of the immune defenses. Not surprisingly, early attempts to vaccinate sheep, goats, and horses

against their lentivirus usually failed and sometimes enhanced the infection. A major difference from AIDS-inducing lentiviruses, discovered later, is that the farm animal lentiviruses do not infect and kill CD4+ T lymphocytes and, therefore, do not cause immunosuppression; instead they cause a lifelong chronic infection resulting in an often fatal, immunopathogenic inflammatory disease.

5. ANIMAL MODELS OF HIV INFECTION

Soon after the discovery of HIV-1 in 1983, extensive efforts were made to infect small animals and nonhuman primates with representative HIV-1 isolates. Rodents and rabbits were not susceptible to infection. Efforts to create a HIV-1/AIDS susceptible transgenic mouse strain by insertion of HIV-1, CD4, and chemokine coreceptor genes has not yet been achieved. Among macaque species, rhesus monkeys were not susceptible and pig-tailed macaques (*M. nemestrina*) were only transiently infected with HIV-1. The only animals susceptible to persistent infection with HIV-1 were gibbon apes and chimpanzees, but only the latter were available in sufficient number for adequate testing. With the exception of one HIV-1 chimpanzee that developed AIDS-like symptoms 10 years after inoculation, none of the few infected gibbons or over 100 persistently infected chimpanzees developed any disease over many years of observation. These animals appeared able to control the virus, although not eliminate it, by virtue of a strong humoral and cellular immune response. Therefore, as animal models for HIV-1–induced AIDS, none of the small animals or nonhuman primates tested appear suitable.[28]

However, it has been proven possible to infect human T-cells with HIV-1, leading to characteristic cytopathology in immunotolerant SCID mice bearing human transplants of thymus, liver, or PBMCs.[29,30] These SCID/hu mouse models have been useful for studying virus–T-cell interactions and the effects of antiviral therapy but not for investigating the immunopathologic responses that are so instrumental in the pathogenesis of AIDS. SCID/hu mice bearing replication competent HIV-1 transgenic leukocytes provide a new system for characterizing reservoirs of HIV-1 and evaluating therapeutic strategies.[31]

By contrast, certain strains of HIV-2 (e.g., strain 287) have been found to cause persistent infection and typical AIDS in pig-tailed macaques;[32] this represents an excellent animal model for AIDS pathogenesis, vaccine, and treatment research. Baboons are also susceptible to persistent infection with some strains of HIV-2 (e.g., UC2) and some of the animals develop AIDS like symptoms.[33] However, the experimental induction of full-blown AIDS with HIV-2 is less reproducible in baboons than in pig-tailed macaques.

6. MACAQUE RETROVIRUS MODELS OF AIDS

Macaque species are susceptible to two different retroviral-induced fatal immunosuppressive disease syndromes, both of which have been called simian AIDS or SAIDS.[34] Historically, the first of these syndromes described occurs naturally in captive macaques and is caused by a nononcogenic Type D retrovirus, now called Simian retrovirus (SRV), which is indigenous (not endogenous) to macaque species. The second syndrome is caused by the lentivirus SIV, which is not indigenous in macaques, so is purely an experimental disease. Because SIV is most closely related to HIV-1 and even more closely related to HIV-2, and because the pathogenesis of SIV induced SAIDS appears so similar to that of HIV-1–induced AIDS, the term Simian AIDS (SAIDS) is now reserved for the SIV experimentally induced disease.

7. SRV: TYPE D VIRUS

In 1981–1983, after recognition of AIDS in humans but before the isolation of the causative lentivirus, a Type D retrovirus was independently discovered, almost simultaneously, as the cause of outbreaks of a fatal wasting, immunosuppressive syndrome resembling AIDS that had been occurring for several years in rhesus macaques at the New England (NERPRC) and California Regional Primate Centers (CRPRC).[35] This discovery focused further attention on the likely etiologic role of a retrovirus in the newly observed AIDS outbreak. The virus strain, SRV-1, isolated at both centers, was found closely related, not to HIV-1, but instead to the prototype Type D virus called Mason Pfizer monkey virus (MPMV). MPMV had been isolated in 1970 from a spontaneous mammary carcinoma of a rhesus monkey. Efforts, at that time, to transmit cancer to recipient rhesus monkeys with this Type D virus were unsuccessful, much to the disappointment of the VCP. Instead, the virus induced thymic atrophy and a fatal immunosuppressive disease in newborn macaques,[36] an observation that did not attract much attention because AIDS had not yet been recognized in humans. In the mid-1980s MPMV was reisolated from a frozen stored sample of the original rhesus breast tumor and reinoculated into rhesus macaques in which it induced the same fatal immunosuppressive disease as was then occurring naturally, caused by the related but distinct SRV-1 strain.[37] Several other SRV strains were subsequently recovered from various macaque species with the immunosuppressive syndrome at other primate centers. At the Washington and Oregon Regional Primate Centers the SRV-2 strain, more distantly related to SRV-1 and MPMV (SRV-3), was associated, particularly in pig-tailed macaques, with not only fatal immunosuppression, but also with a unique vascular and mesenclymal cell proliferation, called

retroperitoneal fibromatosis (RF), that exhibited many features in common with Kaposi's sarcoma. In recent years a herpes virus closely related to the Kaposi sarcoma herpes virus has been demonstrated in association with RF.[38]

The clinical-pathologic features of end-stage SRV disease resemble terminal AIDS in that both syndromes feature profound lymphoid depletion of lymphoid organs and similar opportunistic infections. SRV differs from HIV/SIV by having a wider cell tropism including CD4+ and CD8+ T-cells, B-cells, macrophages and epithelial cells in the gastrointestinal tract, salivary gland, and choroid plexus. SRV thus induces a more generalized immunosuppression without selective depletion of CD4+ T-cells. The pathogenic mechanism may involve an antiproliferative effect on lymphoid cells as a result of SRV occupying a cell surface receptor normally functioning in neutral amino acid transport. SRV also differs from HIV/SIV by causing a lesser degree of immune activation in earlier stages of the infectious process and by the absence of associated lymphomas.

SRV is spread primarily via saliva during fighting and biting. Compared to HIV/SIV, SRV is a much more stable virus with minimal genetic variation within each strain or outbreak. Accordingly, the macaque immune system can often contain and occasionally eliminate the virus by development of neutralizing antibodies and other immune defenses (e.g., cytotoxic T-lymphocytes). In its natural history, SRV infection of macaques rather resembles FeLV infection of cats. Vaccines made of whole killed SRV or recombinant vaccinia virus expressing SRV envelope antigen protect against the homologous virus by the induction of neutralizing antibody.

Because of its high morbidity and mortality SRV remains a serious problem for investigators working with macaques and for macaque-breeding facilities. SRV infection accounts for over 99% of the naturally occurring AIDS-like syndrome in macaque colonies at various primate facilities. Undetected infection with SRV can certainly confound all types of other research using macaques. Fortunately, highly sensitive and specific serologic and PCR assays are available in reference labs to eliminate SRV-infected monkeys. Despite the differences between SRV and SIV/HIV, the highly reproducible and rapid transmission of SRV disease, along with the short turnaround time (7–10 days) for in vitro assays makes this an attractive primate model for studying the mechanism of immune suppression from an acquired retrovirus.

8. SIV: LENTIVIRUS

The history of the SIV macaque model, like that of the SRV macaque model, reveals that unrecognized outbreaks of SIV-induced AIDS occurred in rhesus monkeys more than a decade before human AIDS was recog-

nized.[35] Several years after the CRPRC was founded in the early 1960s, African sooty mangabeys (*Cercocebus atys*) were cohoused in outdoor corrals with Asian rhesus macaques (*Macaca mulatta*). In retrospect, we now know, based on seroepidemologic and virologic evidence from stored material, that some of these sooty mangabeys were naturally infected with SIVsm, which was spread to the rhesus macaques, presumably by fighting. Between 1969 and 1974 the colony of SIV-infected macaques developed what would now be called Simian AIDS or SAIDS. They had a high incidence of B-cell lymphomas, which in the mid-1970s, prompted a retrospective fruitless search in frozen stored tissue for a Type C retrovirus. It was recognized at that time that the monkeys were also immunosuppressed and suffered from many opportunistic infections. However, the immune impairment was thought to probably have an environmental etiology, such as toxic chemicals in the soil or nutritional deficiencies such as too little zinc in the diet. Although difficult to believe now, especially in view of the clustering of disease in this one colony, an infectious cause for the immune impairment was not investigated at that time. Indeed, in the 1970s there was relatively little academic interest in infectious diseases and no virus isolation facilities were available at this primate center. No one at that time could have foreseen that in the next 30 years about 35 new infectious diseases would burst onto the scene and that the U.S. death rate from infectious disease, which dropped in the first part of the 20th century, would double what it was in 1980. In 1970, a few surviving rhesus monkeys, at least one that we now know was a healthy carrier of SIVsm, were shipped to the NEPRC, which had never housed sooty mangabeys. SIVsm was thereby introduced into rhesus monkeys at the New England Center in the early 1970s, and it was subsequently traced to the individual monkey from whom the prototype SIVmac was first isolated in 1985.[39] Interestingly, until this time (1985), almost all (95%) of the monkeys with an AIDS-like immunosuppressive syndrome at the NEPRC had been attributed to SRV-1 infections. SIVmac was isolated from one of the very few immunosuppressed monkeys that was free of SRV infection, but surprisingly, was seropositive against HIV-1.[40] SIV was then quickly shown to be a lentivirus closely related to HIV and capable of reproducing in macaques a disease syndrome closely resembling AIDS.[41]

This scenario was repeated in the late 1970s when a colony of stumptailed macaques (*M. arctoides*) at the CRPRC developed a high incidence of avian tuberculosis infection and occasional lymphomas. Again, not recognized as an infectious outbreak, several surviving carrier monkeys were sent in 1978 to the Yerkes Regional Primate Research Center (YRPRC). A decade later, SIV Stm was isolated at the CRPRC from a 10-year frozen lymph node of one of the stumptailed macaques, and the virus experimentally induced SAIDS in rhesus monkeys.[42] At the YRPRC a spontaneous outbreak of SAIDS in rhesus monkeys in the late 1980s was found to be

caused by the same SIV Stm strain that had apparently remained dormant there since arriving in the carrier monkeys about a decade before.[43] Therefore, the CRPRC can rightly claim to be the "Home of Simian AIDS," although this claim is rather hollow insofar as these events were inadvertent and unrecognized at the time of their first occurrence. Soon after the discovery of SIVmac, seroepidemiologic surveys and virus isolation results on captive and feral monkeys established that SIV was indigenous, highly prevalent, and genetically quite divergent among at least 30 different species of African nonhuman primates. SIVcpz was also found in low prevalence (~1%) in chimpanzees in Africa, and SIVcpz was later shown to be the source of HIV-1 by cross-species infection of humans in central Africa, presumably around 1930 via the bush meat trade.[44] Remarkably, the natural African simian hosts were found unaffected by the virus, presumably because they had adapted over eons of harboring these agents. Similarly, SIVsm was the source of SIVmac, as described above, and also the source of HIV-2 by cross-species spread to humans in West Africa. With little opportunity for natural adaptation to HIV-1 and HIV-2 or SIV the native humans and macaques have proven super-susceptible to infection and resultant AIDS, as we have unfortunately witnessed over the past 20 years.

The experimental induction of simian AIDS with SIVmac and related SIV strains has become the premier animal model for AIDS. Like HIV-1, SIV uses the homologous primary CD4 receptor and secondary R5 and X4 chemokine coreceptors and thus has a similar cell tropism. The pathogenesis of SIV-induced simian AIDS in macaques appears remarkably similar to that of HIV-1 induced human AIDS.[34] The past decade has witnessed the construction of numerous recombinants between SIV and HIV, so-called SHIVs, many of which are also pathogenic in macaques. Most of these SHIVs are constructed from the SIVmac core and HIV-1 env genes. The induction of fatal SAIDS with uncloned or molecularly cloned SIV or SHIV strains, within a few months to several years following systemic or mucosal routes of infection, presents a convenient time frame for investigators to examine various aspects of pathogenesis, treatment, and vaccination. Results of several hundred vaccine trials in the SIV or SHIV macaque model have illustrated the limitations that are likely to be faced by HIV-1 vaccination of humans. Under ideal conditions, using a homologous virus immunization and challenge systems, several vaccine strategies can dramatically lower the virus load and prolong survival. Most effective have been the genetically modified live SIV vaccines, but none of these are safe enough for modeling of a homologous HIV-1 vaccine. However, with any of the SIV vaccine strategies, sterilizing immunity is seldom achieved, and the residual virus continues to replicate, mutate, escape from immune surveillance, and eventually cause AIDS. Vaccine protection against heterologous SIV or SHIV strains, similar to the diversity of global HIV-1 strains, remains an experimental goal yet to be obtained. On the other hand, the

more virulent disease course of SIV or SHIV infection in macaques compared to HIV-1 infection of humans may present a worse-case scenario, perhaps setting an unrealistically high hurdle to be overcome by various antiviral strategies.

One might well suspect that valuable insight into the pathogenesis of AIDS might be gained by a comparison of host and viral parameters in the SIV-infected asymptomatic natural host (i.e., African monkey species and chimpanzees) with the pathogenic SIV-infected experimental host (i.e., Asian macaques). Such a comparison has failed to pinpoint any dramatic difference in virus load, tissue distribution, or virus mutation rates. The most obvious difference between natural and experimental hosts is the lack of any evidence of immune activation in the natural hosts, an observation that emphasizes the critical importance of untoward immune stimulation in the loss of CD4+ T-cells.

The U.S. Regional Primate Research Centers, initially seven in number, were founded 40–50 years ago for the purpose of providing nonhuman primates as models for human disease. The founding fathers could never have foreseen a human pandemic as devastating as AIDS nor could they have possibly appreciated how valuable, indeed indispensable, the animal resource provided by these facilities would be for the scientific battle against AIDS.

9. FIV: LENTIVIRUS

The discovery of FIV in 1987[45] followed the same map that had facilitated the isolation of SIV from SRV seronegative but immunosuppressed rhesus macaques 2 years previously. Investigators at the University of California Davis School of Veterinary Medicine were aware of a cat household in which many cats, particularly in one backyard pen, were nonthriving and immunosuppressed. Surprisingly, these cats all tested negative for FeLV antigenemia, raising the possibility, to both the astute owner and alert investigators, that a lentivirus such as HIV/SIV might be responsible. A blood transfusion from an ill cat to a naïve recipient fulfilled this expectation by inducing fever and lymphoadenopathy and FIV was isolated from the cultured PBMCs. Now, 15 years later, we know that FIV, like SIV, is widespread in nature; at least 17 of 36 nondomestic feline species are naturally infected. Lions and pumas have an especially high prevalence of FIV infection. The wide extent of genetic variation among FIV isolates within and between species indicates that, like SIV in African monkeys, FIV has been present for eons in nondomestic felidae. Also, like the natural simian hosts of SIV, the natural nondomestic feline hosts of FIV are also completely asymptomatic, presumably because of longtime adaptation. However, in contrast to SIV infection of macaques, an experimental artifact, FIV infection of domestic cats occurs naturally. The extent of genetic variation

among FIV isolates of domestic cats also indicates that, like FIV in exotic cats, the virus has been present for millions of years, probably ever since domestic cats evolved from their feral predecessors.

Since FIV has been indigenous in domestic cats for a long time a measure of host adaptation has had a chance to take place. FIV infection of domestic cats is therefore much less virulent than SIV infection of macaques. In domestic cats the incubation period before FIV infection induces an AIDS-like terminal stage is about 7–9 years, intermediate between SIV in macaques (2 years) and HIV-1 in humans (10 years). Earlier stages of FIV disease closely resemble the corresponding stages of HIV infection.[46] Because of the relatively long incubation period before end-stage disease and the need for environmental cofactors to enhance the pathogenesis, domestic cats with typical feline AIDS are much more commonly seen in veterinary clinics than in the experimental laboratory. In the laboratory environment investigators must use earlier signs of infection such as fever, lympadenopathy, and CD4 T-cell suppression to measure the effect of antiviral vaccines or drug therapy. The disease is most common in older male feral cats (>5 years) who roam about and fight, spreading the virus through saliva and blood. FIV primarily uses the X4 chemokine receptor and not CD4 on T-cells. Nevertheless, it induces the same selective depletion of CD4 T-cells as seen with HIV-1 and SIV. In its more indolent disease course FIV infection of domestic cats more closely resembles HIV infection of humans, particularly HIV-2 infection, than it does the super-virulent SIV macaque model. However, because FIV is genetically more distant to HIV-1 (~35% similarity), and because the AIDS disease endpoint is much more quickly reached with SIV than FIV, the SIV-macaque model has captured most of the scientific attention and research funding. Regardless, the FIV model is also highly informative, practical, and relevant to HIV. Because of its greater practicality the FIV model is being used worldwide and does not require expensive monkeys and special primate facilities. Most remarkably, a commercial FIV vaccine, made of inactivated whole virus from two genetically distant FIV clades, is now available worldwide.[47] Who would have thought, 20 years ago, that in the massive effort to make an apparently efficacious, safe commercially available global AIDS vaccine, the cat model would win the race? Both neutralizing antibodies and cytotoxic T lymphocytes (CTLs) contribute to the FIV vaccine protection. However, it remains to be seen how efficacious this vaccine will be under natural conditions outside the controlled laboratory environment.

10. SUMMARY

Insofar as HIV-1 does not produce AIDS in any animal, there exists no perfect model for this pandemic virus. Nevertheless, nature has provided us with three animal lentivirus models that are excellent surrogates for HIV-

1 infected humans and AIDS: (1) SIV/macaque, (2) HIV-2/macaques, and (3) FIV/cats. Each of these models, although differing in incubation period and virulence, is characterized by a selective decline in CD4+ T-helper cells, the major characteristic of HIV/AIDS. In each of these models, the ability of the virus to persist in face of a strong immune response, to evolve by frequent mutation and to ultimately cause fatal immunosuppression, closely resembles the pathogenesis of HIV-l.[48] The FIV/cat model is the most underutilized; its relatively low pathogenicity and long incubation period are disadvantages outweighed by the advantages of its practicality and currently available specific reagents and assays. The SIV and HIV-2 macaque models will, of course, continue to be the gold standard, but they suffer the disadvantage of greater expense and less availability. The HIV-1 chimpanzee model is no longer much studied because of the absence of disease and its impracticality. The SCID/hu mice will continue to provide useful knowledge concerning the interaction of HIV-1 with target lymphoid cells and for testing antiviral strategies, but not as a complete model for AIDS. The Type C oncoretrovirus models of immunosuppressive disease in chickens, mice, and cats and the Type D retrovirus-induced immunosuppressive disease of macaques could provide further insight into the molecular pathogenesis of retrovirus-induced immune system impairment and destruction; such mechanisms might share some steps in common with lentivirus-induced AIDS. Similarly, the farm animal lentivirus models give us further insight into lentivirus pathogenesis in the presence of an intact immune system. In view of the unabated AIDS pandemic and the scientific, not to mention political, hurdles yet to be overcome, we are fortunate indeed to have access to these animal models and to have acquired such a wealth of knowledge, reagents, and expertise concerning retroviruses in general. Without questions the AIDS animal model systems discussed in this volume will continue to be essential resources in the battle against HIV and AIDS.

REFERENCES

1. R. Weiss, N. Teich, H. Varmus, et al., Origins of contemporary RNA tumor virus research, pp. 1–24, in *RNA Tumor Viruses*, edited by R. Weiss, N. Teich, H. Varmus and J. Coffin (Cold Spring Harbor, New York, 1982).
2. M. B. Gardner, Historical background, in *Molecular Biology of RNA Tumor Viruses*, edited by J. R. Stephenson, pp. 1–46 (Academic Press, New York, 1980).
3. M. B. Gardner, Special topic overview. The virus cancer program of the 1970s: a personal and retrospective view, Lab. An. Sci. 44(2), 101–113 (1994).
4. R. J. Huebner, G. J. Kelloff, P. S. Sarma, et al., Group-specific antigen expression during embryogenesis of the genome of the C-type RNA tumor virus: implications for ontogenesis and oncogenesis, Proc. Natl. Acad. Sci. USA 67, 366–376 (1970).
5. H. M. Temin, The DNA provirus hypothesis. The establishment and implications of RNA-directed DNA synthesis, Science 192, 1075–1080 (1976).

6. R. J. Huebner and G. J. Todaro, Oncogenes of RNA tumor viruses as determinants of cancer, Proc. Natl. Acad. Sci. USA 64, 1087–1094 (1969).
7. W. Makalowski, Not junk after all, Science 300, 1246–1247 (2003).
8. J. W. Hartley, N. K. Wolford, L. J. Old, et al., A new class of murine leukemia virus associated with development of spontaneous lymphomas, Proc. Natl. Acad. Sci. USA 74, 789–792 (1977).
9. H. Fan, Retroviruses and their role in cancer, in *The Retroviridae*, vol. 3, edited by Jay A. Levy, pp. 313–362 (Plenum Press, New York, 1994).
10. B. J. Poiesz, F. W. Ruscetti, A. F. Gazdar, et al., Detection and isolation of Type C retrovirus particles from fresh and cultured lymphocytes of a patient with cutaneous T-cell lymphoma, Proc. Natl. Acad. Sci. USA 77, 7415–7419 (1980).
11. M. Yoshida, I. Miyoshi, and Y. Hunuma, Isolation and characterization of retrovirus (ATLV) from cell lines of human adult T-cell leukemia and its implication I the disease, Proc. Natl. Acad. Sci. USA 79, 2031–2035 (1981).
12. M. B. Gardner, S. Rasheed, S. Simizu, et al., Search for RNA tumor virus in humans, in *Cold Spring Harbor Conferences on Cell Proliferation*, vol. 4, edited by H. H. Hiatt, J. D. Watson and J. A. Winsten, pp. 1235–1251 (Cold Spring Harbor Laboratory, New York, 1972).
13. M. B. Gardner, Naturally occurring leukemia viruses in wild mice: how good a model for humans?, in *Cancer Surveys*, vol. 6, edited by D. E. Onions and O. Jarrett, pp. 55–71 (Oxford: Oxford University Press, 1987).
14. P. L. Green and I. S. Y. Chen, Molecular features of the human T-cell leukemia virus. Mechanisms of transformation and leukemogenicity, in *The Retroviridae*, vol. 3, edited by Jay A. Levy, pp. 277–311 (Plenum Press, New York, 1994).
15. P. N. Fultz, Simian T-lymphotropic virus type I, in *The Retroviridae*, vol. 3, edited by Jay A. Levy, pp. 111–131 (Plenum Press, New York, 1994).
16. D. A. Wilkinson, D. L. Mager, and J. C. Leong, Endogenous human retroviruses, in *The Retroviridae*, vol. 3, edited by Jay A. Levy, pp. 465–535 (Plenum Press, New York, 1994).
17. M. D. Bendinelli, D. Matteucci, and H. Friedman, Retrovirus induced acquired immunodeficiencies, Adv. Cancer Res. 45, 125–181 (1985).
18. M. B. Gardner, Retroviral spongiform polioencephalomyelopathy, Rev. Infect. Dis. 7, 99–110 (1985).
19. P. K. Y. Wong, G. Prasad, J. Hansen, et al., TS-1, a mutant of Moloney murine leukemia virus-TB causes both immunodeficiency and neurologic disorders in Balb/c mice, Virology 170, 450–459 (1989).
20. D. E. Mosier, R. A. Yetter, and H. C. Morse III, Retroviral induction of acute lymphoproliferative disease and profound immunosuppression in adult C57B1/6 mice. J. Exp. Med. 161, 766–784 (1985).
21. J. I. Mullins, C. S. Chen, and E. A. Hoover, Tissue- and disease-specific production of unintegrated feline leukemia virus variant DNA in feline AIDS, Nature 319, 333–336 (1986).
22. F. Barre-Sinoussi, J. C. Chermann, F. Rey, et al., Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immunodeficiency syndrome (AIDS), Science 220, 868–871 (1983).
23. R. G. Gallo, S. Z. Salahuddin, M. Popovic, et al., Frequent detection and isolation of cytopathic retroviruses (HTLV-III) from patients with AIDS and at risk of AIDS, Science 224, 500–503 (1984).
24. J. A. Levy, A. D. Hoffman, S. M. Kramer, et al., Isolation of lymphadenopathic retroviruses from San Francisco patients with AIDS, Science 225, 840–842 (1984).
25. F. Clavel, M. Guyader, D. Guetard, et al., Molecular cloning and polymorphism of the human immunodeficiency virus type 2, Nature 324, 691–695 (1986).
26. B. Sigurdsson, Maedi, a slow progressive pneumonia of sheep: an epizootiological and pathologic study, Br. Vet. J. 110, 255–270 (1954).
27. A. T. Haase, Pathogenesis of lentivirus infections, Nature 322, 130 (1986).
28. M. B. Gardner and P. A. Luciw, Animal models of AIDS, FASEB J. 3, 2593 (1989).

29. J. M. McCune, R. Namikawa, H. Kanashima, et al., The SCID-hu mouse: murine model for the analysis of human hematolymphoid differentiation and function, Science 241, 1632–1638 (1988).
30. D. E. Mosier, R. J. Gulizia, S. M. Baird, et al., Transfer of a functional human immune system to mice with severe combined immunodeficiency, Nature 335, 256–259 (1988).
31. E. J. Wang, M. Pettoello-Mantovani, C. M. Anderson, et al., Development of a novel transgenic mouse/SCID-hu mouse system to characterize the in vivo behavior of reservoirs of human immunodeficiency virus type 1-infected cells, J. Infect. Dis. 186(10), 1412–1421 (2002).
32. J. McClure, A. M. Schmidt, M. A. Rey-Cuille, et al., Derivation and characterization of a highly pathogenic isolate of human immunodeficiency virus type 2 that causes rapid CD4+ cell depletion in Macaca nemestrina, J. Med. Primatol. 29:114–116 (2000).
33. S. W. Barnett, K. K. Murthy, IlG. Herndier, et al., An AIDS-like condition induced in baboons by HIV-2, Science 226, 642–646 (1994).
34. M. B. Gardner, M. Endres, and P. Barry, The Simian retroviruses SIV and SRV, in *The Retroviridae*, vol. 3, edited by Jay A. Levy, pp. 133–276 (Plenum Press, New York, 1994).
35. M. B. Gardner, The history of simian AIDS, J. Med. Primatol. 25, 148–157 (1996).
36. D. L. Fine, J. C. Landon, R. J. Pienta, et al., Responses of infant rhesus monkeys to inoculation with Mason-Pfizer monkey virus materials, J. Natl. Cancer Inst. 54, 651–658 (1975).
37. M. L. Bryant, M. B. Gardner, P. A. Marx, et al., Immunodeficiency in rhesus monkeys associated with the original Mason-Pfizer monkey virus, J. Natl. Cancer Inst. 77, 957 (1986).
38. M. L. Bosch, E. Harper, A. Schmidt, et al., Activation in vivo of retroperitoneal fibromatosis-associated herpesvirus, a simian homologue of human herpesvirus-8, J. Gen. Virol. 80, 467–475 (1999).
39. K. G. Mansfield, N. W. Lerche, M. B. Gardner, et al., Origins of simian immunodeficiency virus infection in macaques at the New England Regional Primate Research Center, J. Med. Primatol. 24, 116–122 (1995).
40. M. D. Daniel, N. L. Letvin, R. D. Hunt, et al., A new type D retrovirus isolated from macaques with an immunodeficiency syndrome, Science 223, 602–605 (1984).
41. N. L. Letvin, M. D. Daniel, P. K. Sehgal, et al., Induction of AIDS-like disease in macaque monkeys with T-cell tropic retrovirus STLV-III, Science 230, 71–73 (1985).
42. L. J. Lowenstine, M. W. Lerche, J. L. Yee, et al., Evidence for a lentiviral etiology in an epizootic of immune deficiency and lymphoma in stump-tailed macaques (Macaca arctoides), J. Med. Primatol. 21, 1–14 (1992).
43. F. J. Novembre, V. M. Hirsch, H. M. McClure, et al., SIV from stump-tailed macaques: molecular characterization of a highly transmissible primate lentivirus, Virology 186, 783–787 (1992).
44. B. H. Hahn, G. M. Shaw, K. M. De Cock, et al., AIDS as a zoonosis: scientific and public health implications, Science 287, 607–617 (2000).
45. N. C. Pedersen, E. Ho, M. C. Brown, et al., Isolation of a T-lymphotropic virus from domestic cats with an immunodeficiency-like syndrome, Science 235, 790–797 (1987).
46. J. K. Yamamoto, E. Sparger, E. W. Ho, et al., The pathogenesis of experimentally induced feline immunodeficiency virus (FIV) infection in cats, Am. J. Vet. Res. 49, 1246–1258 (1988b).
47. R. Pu, J. Coleman, M. Omori, et al., Dual-subtype FIV vaccine protects cats against in vivo swarms of both homologous and heterologous subtype FIV isolates, AIDS 15, 1225–1237 (2001).
48. J. A. Levy, The human immunodeficiency viruses: detection and pathogenesis, in AIDS Pathogenesis and Treatment, edited by J. A. Levy, pp. 159–229 (Marcel Dekker, New York, 1989).

2

Animal Model Systems of HIV-Diseases

ERIC R. ANDERSON, HUANGUI XIONG, and HOWARD E. GENDELMAN

1. INTRODUCTION

Human immunodeficiency virus (HIV) infection is arguably the most significant global health problem of the modern era. Infection has all but devastated third-world countries and continues to threaten public health in developed nations. With the numbers of deaths approaching tens of millions each year, the greatest imperative for world health is the realization of an effective preventative vaccine. The major obstacles prohibiting this goal include a better understanding of protective immunity in the natural host of the virus. In working toward this objective, animal model systems were developed to recapitulate disease processes and viral diversity as it occurs in natural infections of man. Nonetheless, HIV is species specific and is difficult to study in animal systems. Transgenic animals have been developed expressing human receptors in order to overcome some of these limitations; but an animal model that can be progressively infected by HIV remains elusive. Thus, a number of animal models have been established that utilize "other" lentiviruses that mimic HIV infection in specific ways and provide the means to mirror natural infection in its human host. Alternatively, relevant animal models replicate aspects of human disease through the engraftment of infected human cells. Ultimately, these animal model systems of HIV disease provide insight into specific disease processes and serve to elucidate underlying mechanisms of infection and subsequent disease.

ERIC R. ANDERSON • Department of Microbiology and Specialized NeuroSciences Program, University of Puerto Rico Medical Sciences Campus, San Juan, Puerto Rico 00936-5067 HUANGUI XIONG • Center for Neurovirology and Neurodegenerative Disorders, Department of Pharmacology and Experimental Neuroscience, University of Nebraska Medical Center, Omara, NE 68198-5880, and HOWARD E. GENDELMAN • Center for Neurovirology and Neurodegenerative Disorders, Departments of Pharmacology and Experimental Neuroscience and Internal Medicine, University of Nebraska Medical Center, Omaha, NE 68198-5880

Arguably, the most significant epidemic of the modern age is that of HIV infection. In endemic regions such as sub-Saharan Africa, HIV is the leading cause of death in adults.[36] Case numbers in North America have exceeded 1 million as of 2004.[126] HIV infection leads over time to progressive immunosuppression heralded by a specific loss of CD4+ T lymphocytes, a wasting syndrome and a wide range of opportunistic infections. Disease course is debilitating and inevitably fatal. Although highly active anti-retroviral therapy (HAART) can attenuate disease by lowering viral load, increasing CD4+ T lymphocyte numbers and limiting secondary infection, these drugs are rarely available in the developing world. Global economic and political events have further accelerated the rates of HIV infection worldwide. The paucity of preventative measures in affected regions has further affected any measures to control the epidemic. In December 2003, 40 million people in the world were currently living with AIDS,[126] rendering the need for eradication of this infection clear and immediate. Efforts are under way to reverse the trends, but they remain planned rather than realized.

Although intensive biomedical research efforts have been under way for more than two decades, a safe and effective vaccine has not yet been realized. Studies have pinpointed the diversity of viral strains and antigen recognition by induced humoral and cellular immune responses and the ability of the host to control viral growth is being unraveled. HIV is separated into two distinct types: HIV-1 and 2 that are clinically and biologically distinct.[25,49,125] The majority of HIV research focuses on Type 1, as Type 2 is primarily seen in remote world regions and is more attenuated in disease outcome.[34,58,73,81] HIV-1 can be divided into three groups: major (M), outlier (O), and new (N). The majority of HIV-1 isolates to date are derivatives of M. Both groups O and N are geographically restricted.[21,47,115] The HIV-1 M group is further fragmented into 10 distinct clades (A through J) based upon viral sequence variation.[91] Clade variations affect the molecular structure of important viral molecules including the envelope glycoproteins (Env), which are commonly targeted in vaccine strategies. Clades A, B, and C account for approximately 90% of all HIV-1 infections worldwide with clade C being responsible for 48% of infections.[82] Thus, preventative vaccine research is primarily aimed at clades and designed to take advantage of ways to affect antigen expression and recognition and viral diversity.[119] Unique aspects of disease pathogenesis, spread, and dissemination also remain critical avenues of current research pursuits.

Clearly, the need for animal models of human disease is mandatory if effective preventative measures are to be realized. One major factor hampering research progress is the fact that HIV only infects human cells and produces significant disease only in humans. Simian immunodeficiency virus (SIV) can infect rhesus macaques among other nonhuman primates,

but pathogenic, molecular, biochemical, and structural dissimilarities between this virus and its human counterpart are operative. Feline and bovine immunodeficiency viruses (FIV and BIV, respectively) target animals that are readily available and easily maintained but are even more diverse than SIV in affecting disease. Simian-human immunodeficiency virus (SHIV) infections provide HIV/SIV chimeras, but differences in disease course and pathogenesis abound. Visna-Maedi, caprine arthritis encephalitis virus (CAEV) and equine infectious anemia virus (EIA) are the prototypic lentiviruses but produce only modest immunosuppression and nearly exclusively infect cells of monocyte-macrophage lineage. Mouse-human chimeras have been produced utilizing nonobese diabetic severe combined immunodeficient mice and can mimic a number of primary and secondary aspects of human disease. However, human cells reconstituted into mice still can affect graft versus host disease, leading to a hyper-activation of the reconstituted human cells. Moreover, differences between mouse and human innate and adaptive immune responses are frequently observed. Transgenic or knockout animals either expressing viral proteins or receptors have aided much in HIV studies but remain developmental especially in regard to disease mechanisms and drug or vaccine testing. This certainly also includes studies of mouse leukemia virus and other oncogenic retroviruses. The intent of this chapter is to provide the reader with a broad review of animal model systems of HIV disease. It is only intended as an introduction to the field for future reference in other chapters of the current book, not as a comprehensive review.

2. HIV-1 PATHOGENESIS

To best evaluate the potential relevance of an animal model for human disease, events in disease pathogenesis need to be best understood. As analogous events are observed in species-specific disease processes, animal model with full potential to mirror human disease may be fully realized. Through recapitulation of human pathobiology in animal systems, scientists will have a readily available tool with which to study potential therapeutic and drug treatments.

HIV-1 Transmission

HIV infection occurs through an exchange of body fluids. Such transmission can occur by direct inoculation of infected materials into the bloodstream as with a needle stick,[54] transplanted infected tissue,[93,100,108,116] or blood transfusions.[31,93] Additionally, HIV may enter into a host through open wounds or sores, mucous membranes, through receptive anal and

vaginal intercourse,[2,52,134] or perinatal transmission.[41,74] The virus infects cells primarily through the CD4 receptor but also uses the coreceptors CXCR4 and CCR5. This principally involves CD4+ T lymphocytes and mononuclear phagocytes (MP; monocytes, tissue macrophages, dendritic cells, and microglia). These infected cells transport the virus through the bloodstream and lymph nodes where it can further spread to tissue.

The Acute Seroconversion Reaction

Often presenting as a transient symptomatic or asymptomatic illness, acute HIV-1 infection heralds a seroconversion reaction. In many cases illness manifests as an infectious mononucleosis[27] syndrome accompanied by a plethora of clinical signs and symptoms such as fever, fatigue, weakness, and rash. This reaction follows extensive viral replication without adaptive humoral and cellular immune responses. Importantly, at this time, several steps in the disease transpire. First, as CD4+ T lymphocytes decline, virus preferentially infects virus-specific CD4+ T-cells.[5,37,38,104] This allows for the development of concurrent infections.[46,129] CD4+ T lymphocyte counts typically rebound following resolution of the primary infection but rarely rise to baseline numbers without effective antiretroviral drugs. Second, it is during the initial infection that seeding of the virus into tissues occurs, including the establishment of persistent HIV-1 infection in lymphoid organs. An expansive viremia eventually declines coincident with the appearance of antiviral-specific CD8+ T lymphocytes.[11,17,63] These CD8+ T-cells typically remove infected cells directly by MHC I-mediated cytolysis, or indirectly through secreted factors, effectively culling the blood of progeny virus.[128,136]

Humoral and Cellular Immune Responses

Humoral and cellular immune responses serve to control viral infection. Following acute HIV-1 infection, cytotoxic T lymphocytes (CTL) mount an HIV-1–specific adaptive immune response against infected cells. These cells will leave a pool of HIV-1 specific CD8 memory cells. The gradual decline of the CD8+ T lymphocyte cellular immune response during progressive HIV-1 infection is inevitable due to viral mutation.[137]

An HIV-infected individual shows continuous HIV RNA, antigens, and progeny virus in blood and other body tissues throughout the course of disease. Within days to weeks, the host immune system will produce antibodies to the genetic structure of virus particles. The majority of antibodies target free-floating virions, although some may ultimately assist in the destruction of infected cells. As with the CD8 responses to HIV-1 infection, the effectiveness of the humoral response also gradually declines, due, in large measure, to viral mutation.

Subclinical Period of Viral Infection

In the plasma, the levels of HIV-1 antigen typically drop after the acute seroconversion reaction by immune system clearance, or the establishment of a steady-state between progressive viral infection and the production of available CD4+ T lymphocyte host cells.[97] HIV titers drop significantly after primary infection and remain relatively stable for months or even years. This period is typically and on average 10 years.[9,69,78] An average drop of 100 CD4+ T lymphocytes/mm^3/year occurs starting from an average of 1000 cell/mm^3. The human host is commonly asymptomatic until the CD4+ T lymphocytes drop to less than 200 cells/mm^3, when the incidence of opportunistic infections increases substantially.

Clinical Disease

Advanced HIV disease is typically marked by a reduction in CD4+ T-cells to below 200 cells/mm^3, an increase in levels of plasma HIV RNA, and coincident opportunistic infections and disease manifestations. Common symptoms include wasting and anemia accompanied by episodes of secondary infections. Clinical manifestations that typically occur in advanced HIV disease include, but are not limited to, HIV-1-associated dementia (HAD), peripheral neuropathy, Kaposi's sarcoma, renal disease, diarrhea, weight loss, interstitial pulmonary disease, cardiac injury, and bacterial, fungal, parasitic, and opportunistic viral infections. Opportunistic infections and malignancies are common as the host immune system is severely weakened. When the CD4+ T lymphocyte counts drop to below 50 cells/mm^3, mycobacterial and opportunistic viral infections typically occur, and damage to the central nervous system (CNS) is common. Ultimately, death results, likely due to electrolyte abnormalities, circulatory failure, and nervous system damage.

3. ANIMAL MODELS FOR HIV-1 INFECTION: OVERVIEW

The central challenge toward controlling or inevitably eradicating HIV-1 is to find ways to appropriately treat or prevent infection. The greatest test is to prevent primary infection through effective vaccination. Viral mutation and escape from immune surveillance and the emergence of reservoirs for persistent viral infection make a vaccination success difficult to realize. Furthermore, HIV and its clinical manifestations cannot be easily studied because of the dynamics between the virus, the host cell, and the immune system. In answer to this conundrum, a number of animal models have been developed in which human disease pathogenesis is paralleled.

None are optimal but each has inherent strengths and weaknesses. These are cited below.

Ideally, studies of HIV infection and disease pathogenesis would be conducted in an animal system that would manifest all aspects of human disease. However, since no one animal model has been able to exactly replicate human illness, several model systems were developed in different species utilizing a broad range of viral strains. In order for the model to be relevant, it must present HIV-associated pathological and clinical manifestations and be used successfully to test vaccine and therapeutic regimens.

In efforts to drive HIV research the World Health Organization (WHO) outlined what would be considered an ideal animal model.[133] Characteristics were defined that include availability, expense, and utility for HIV research. The first consideration was whether the animal model should make use of HIV. This has not easily been realized. Although several easily available animal models can utilize immunodeficiency or leukemia viruses and provide some insight into human disease, limitations abound, and WHO suggested that the animal model be easily acquired and maintained and that the genetics, immunology, and metabolism be well known and appreciated. Optimally, the target cells affected by the virus should also be the ones affected in human disease, namely CD4+ T lymphocytes and MP. Similarly, the target organs affected would include blood, lymphoid, lung, and brain tissues. The transmission of the virus would also mimic that of HIV including perinatal transmission. Infection of the animal should be possible with both virus-infected cells and free virus. The induced disease should have a short incubation and resemble human AIDS. Table I presents a summary.

Despite the lack of a single animal model that fills all of these criteria several recapitulate specific and critical aspects of human disease. The two primary paradigms employed include infection by HIV in a relevant animal model or alternatively infection with other viruses that induce an AIDS-like

TABLE I
Ideal Animal Model Recommendations by the World Health Organization

Makes use of HIV
Is a small animal model
Established knowledge of the genetics, immunology, and metabolism
Target cells are $CD4^+$ T lymphocytes and mononuclear phagocytes
Target organs include blood, lymphoid, lung, and brain
Transmission mimics that of HIV, including perinatal transmission
Infection is possible with both virus-infected cells and free virus
Induced disease has a short incubation period
Induced disease resembles AIDS

illness. Both of these have been used to great effect in understanding viral pathogenesis and for vaccine testing. Several animal model systems derived from these paradigms are summarized below.

4. MOUSE MODELS

Rodents are a common and assessable option in the pursuit of any model of human disease. Mice and rats are easily obtainable, relatively inexpensive to maintain, and their immunology, genetics, and metabolism are well known and appreciated. As mice and rats are not naturally susceptible to HIV infection, and there is no known naturally occurring murine lentivirus, the effects of HIV and AIDS had to be studied through immune chimeras, heterologous viruses or genetic approaches.

Murine Leukemia Virus (MuLV)

A limited number of murine AIDS models utilize well-recognized retroviruses. These models have primarily made use of oncoviruses. Select laboratory strains of MuLV can induce AIDS-like effects including suppression of humoral and cellular immunity. The MuLVs are closely related and include the Friend, Moloney, and Raucher strains.[13] The Friend and Raucher viral strains affect immunosuppresion by infecting precursor B, T, and macrophage-lineage cells. The Moloney strain, on the other hand, is not immunosuppressive but is transferred from the mother to its offspring, thereby providing a model for transplacental viral passage. While primarily utilized in studying drug treatments, there are mutant strains of the Moloney MuLV that are immunosuppressive, such as the ts-mutant.[135] Due to the specific immunosuppressive nature of the ts-mutant, it can model human disease including virus-induced neurodegeneration.[24,72]

Murine AIDS (MAIDS) was described in C57/Bl6 mice that had been infected with a radiation-induced strain of MuLV.[77] Commonly referred to as the LP-BM5 model, this form of MuLV is ecotropic, recombinant mink cell focus-forming, and expresses an aberrant gag-encoded polyprotein.[22] The strain induces polyclonal B-cell proliferation, lymphadenopathy, splenomegaly, and hypergammaglobulinemia. Such processes result in significant immunosuppression and death.[24,92,132]

Transgenic (CCR5/CD4, gp120, tat, rev, nef, and Full-Length Proviral DNA)

The widespread use of genetic manipulation has produced transgenic rodent models for modeling HIV disease. Of these transgenic mouse models, the most popular are mice that have incorporated viral transgenes,

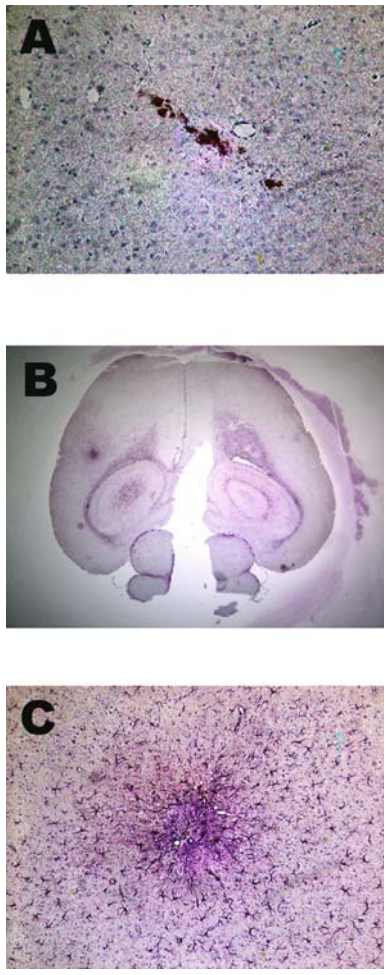

FIGURE 1. Pathobiology of HIV encephalitis in SCID mice. Seven days following injection of HIV-1 infected MDM into the basal ganglia of SCID mice, pathological features of human HIVE are observed readily. Panel A illustrates the presence of human vimentin antigen positive MDM in the subcortex of recipient mice. Panel B shows widespread astrogliosis by GFAP immunopositive cells present around the site of cell implantation (Panels A and C, 100×, B, 10×).

those that are genetically modified through the incorporation of specific cellular receptors and coreceptors for viral entry into the cell, and the genetically modified mice that allow for the engraftment of infected cells or tissue (Figure 1).[92] Other transgenic rodent models use autoimmunity to study AIDS.

The transgenic models that have incorporated the virus into its genome have been useful in modeling select pathogenic manifestations of chronic HIV-1 diseases, for example, the HIV-1 transgenic rat that contains full-length proviral DNA with expression in virus target organs.[56,65] These

affected tissues, including the lymph nodes, thymus, liver, kidney, and spleen, showed significant pathology consistent with clinical manifestations of disease.[61,101] Likewise, transgenic murine models of HIV also express the HIV-1 transgene in immune cells, resulting in an AIDS-like disease.[33a,62]

Human-Mouse Chimeras

Finally, transgenic rodent models have allowed for the engraftment of infected tissues or cells. The transgenic mouse that serves as the core for this area of animal research is the severe combined immunodeficient (SCID) mouse. These models are typically engrafted with human immune cells or target organs that are associated with immune function. Such organs include the fetal liver, thymus, lymph nodes, peripheral blood, and bone marrow.[1,16,20] These immune-competent human tissues implanted into SCID mice allow for the immune cells derived from the implanted tissue to reconstitute the immune system of the mice. With the advent of these models, human disease may be simulated in part, and resultant disease pathology can be examined (Figure 2).

Rodent models that have been genetically altered in such a way that human receptors and coreceptors are incorporated brought the reality of a small animal model of HIV-1 closer. Furthermore, by understanding the interplay between the virus and the cell, viral transmission and pathology can now be studied. The addition of the human CD4 and CCR5 to rats,[59,60] mice,[18] and rabbits[118] permits the development of transgenic animals that are susceptible to HIV-1 infection.[121] Later, with the addition of the human protein cyclin T1, the infected cells produce viral gene products.[60] However, while these animal cells are able to produce CD4, CCR5, and cyclin T1, they do not elicit persistent infection, indicating that there still remain missing pieces to the puzzle of viral entry and replication. Nonetheless, with continuous research, the hope of creating a small animal model that can be productively infected with HIV can be realized.

5. UNGULATES

Lentiviral infection of sheep, goats, cattle, and horses are models for persistent infection. While ungulate lentiviral infections are not generally immunosuppressive, they share similarities with HIV. These include persistent infection, a prolonged incubation period, integration of proviral DNA and its restricted expression, macrophage tropism, and viral mutation or antigenic drift. Moreover, because these lentiviruses pose no threat for human infection and disease, they are quite safe and economical to utilize for studies of disease pathogenesis and to evaluate antiviral therapies or vaccine candidates.

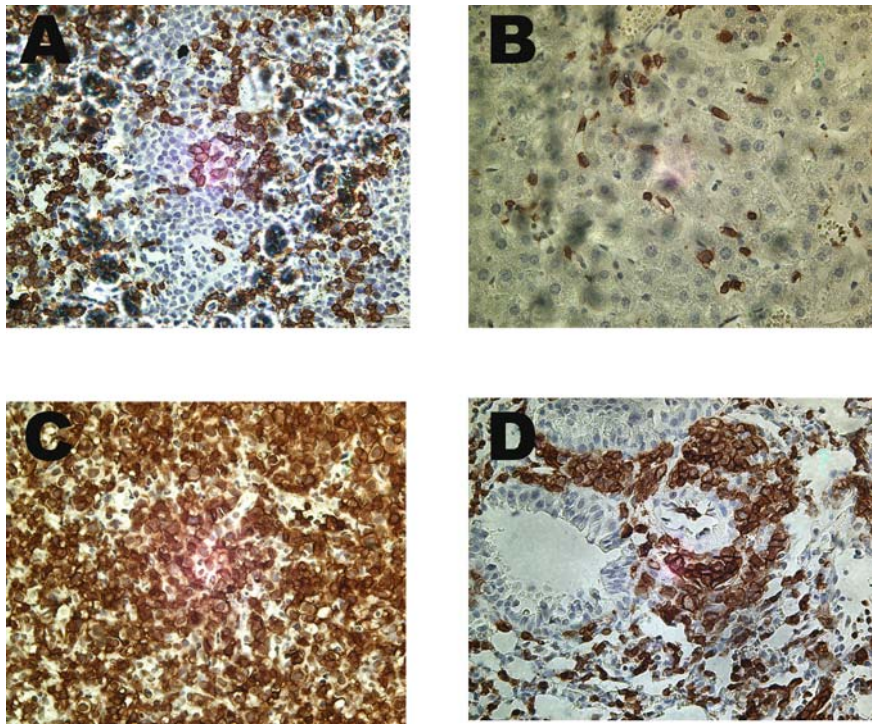

FIGURE 2. Human lymphocyte reconstitution of SCID mice. Human blood lymphocytes are injected intraperitoneally then observed in a variety of issues in the reconstituted SCID mice. Mice were sacrificed after 21 days and tissue sections immunostained with antibodies for human CD45. Panels show human cells in spleen (A), liver (B), lymph node (C), and lung (D) of the reconstituted animals. Magnification ×100.

Caprine Arthritis Encephalitis Virus (CAEV)

CAEV is a nosologic disease that was first described in 1974.[30] CAEV is a retrovirus that is antigenically related to lentiviruses of sheep. This disease was found to be long-established and widespread in dairies, transmitted primarily through colostrum and milk, and also passed perinatally.[105] Persistent viral infection is intimately associated with the macrophage[80] and microglia.[12] Disease follows a lengthy incubation period and causes joint degeneration and encephalitis. The latter is common in children less than six months of age, which may progress to paralysis within a few months.[29,96] Although the joints are the most notably affected tissue, viral transcripts have been identified in inflamed areas of the brain, spinal cord, lung, and mammary gland.[139] The encephalitis and resultant brain inflammation induced by CAEV, combined with its tropism for macrophages and microglia, make it suitable for studies of human HAD.

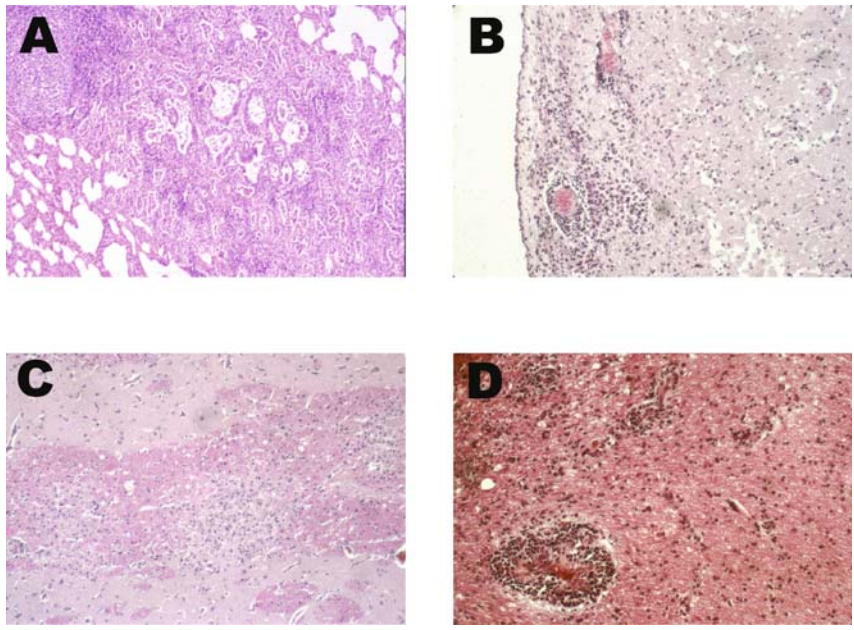

FIGURE 3. Pathobiology of interstitial pneumonitis and encephalitis of visna infected sheep. All panels depict tissue samples taken from sheep infected with visna virus. Panel A shows productive viral replication occurs in the macrophages inciting an adaptive immune response and significant T cell lung infiltration. Panel B shows subependymal encephalitis as a product of progressive viral infection in brain macrophages. Panel C and D depict an advanced form of leukoencephalitis. All slides are at 20X magnification and counterstained with hematoxylin. Contributed by Dr. Opendra Narayan.

Visna Maedi

Visna Maedi is a retrovirus of sheep inducing an interstitial pulmonary disease as well as a wasting degenerative process that includes lameness and neurodegeneration. Commonly, a progressive pneumonia precedes and often accompanies other aspects of disease (Figure 3).[45,83,84] Like other lentiviruses, it replicates principally in cells of monocyte/macrophage lineage.[33] Lymphocytes are not infected, indicating that the mechanism of viral tropism, while unknown, is not through the CD4 receptor.[32] Visna Maedi is primarily transmitted through aerosolized nasal discharge, leading to primary infection of free and sessile macrophages in the respiratory tract.[28,44] It is thought that trafficking monocytes and monocyte-derived macrophages provide the vehicle from which to disseminate the virus throughout the body. Postmortem findings typically identify massive amounts of lymphocytes in the lungs infected with Visna Maedi.[19] Study of this retrovirus has allowed for parallels in viral structure and functions to

be drawn to HIV, including genomic organization, regulatory and structural proteins, and viral mutation.[51,130] These analogies have resulted in a sheep model system for the testing of antiretroviral drugs that may be used in humans.[107]

Equine Infectious Anemia Virus (EIAV)

EIAV is a naturally occurring lentivirus that can infect horses, mules, and donkeys worldwide. EIAV primarily resides in the tissue macrophages of its host, rather than the peripheral circulating monocytes.[111] Nevertheless, the transmission of the disease is through blood by insects, needles, and surgical instruments.[53] Vertical transmission is also known to occur.[124] The disease typically presents in its host in acute, chronic, or unapparent infection.[110] Hosts with acute EIAV typically suffer from high fever, anemia, and thrombocytopenia.[26] A chronic EIAV infection is manifest by recurrent fever, weight loss, severe anemia, and edema. Those who have an unapparent infection seem healthy but may lapse into acute or chronic stages at any time.[23] EIAV was the first virus shown to be related to HIV through antigenic and molecular tests.[75] Indeed, the two viruses share many structural and biochemical similarities. EIAV is believed to be a useful model for studies of HIV control and persistence.[76]

6. IMMUNODEFICIENCY LENTIVIRUSES

Lentiviral infections are species-specific and can induce an AIDS-like illness reminiscent of what occurs in man. The majority of these viruses show similarities in molecular structure and in causing disease. Through the study of these immunodefiency causing lentiviruses much has been learned regarding underlying mechanisms of viral infection, tissue reservoirs, and therapeutics and vaccines.

Feline Immunodeficiency Virus (FIV)

FIV was first discovered in 1987 and was found to be quite common in pet and feral cats worldwide.[89] FIV is a lentivirus that closely resembles HIV and SIV in its tropism, protein composition, and morphology.[90] FIV has been cloned and sequenced in its entirety that confirmed its lentiviral structure and organization. It is distinguished from other lentiviruses, implying that it did not arise from a common origin.[85,86,123] FIV has been found to be transmitted between cats primarily through saliva and blood during fights and by vertical[3,4] and horizontal transmission.[57] Pathological manifestations of disease include infections of the oral cavity, upper respiratory track, intestine, conjunctiva, and nervous system.[14] Histological

studies have localized the presence of virally infected cells in the bone marrow, lymph node, thymus, mucosal-associated lymphoid tissue, and spleen, but few cells in the liver and none in the kidney or brain.[103] Although cats seroconvert following infection, they do not succumb to the immunodeficiency disease until after several years of viral incubation.

This animal model has been utilized primarily as a vehicle with which to test antiviral drugs but also as a model of human disease. Specifically, the FIV model has been utilized to examine virus-induced neurodegeneration. Similarities between HIV-1 associated cognitive deficits and FIV induced neurological deficits have supported the use of FIV for the study of human HAD (Figure 4).[39,88,114,138] Furthermore, the FIV model can examine the range of disease affected by drugs of abuse.[10,42] Because FIV represents a lentivirus model that progressively infects its host then induces immunodeficiency, it serves as a very useful system to study AIDS in man.

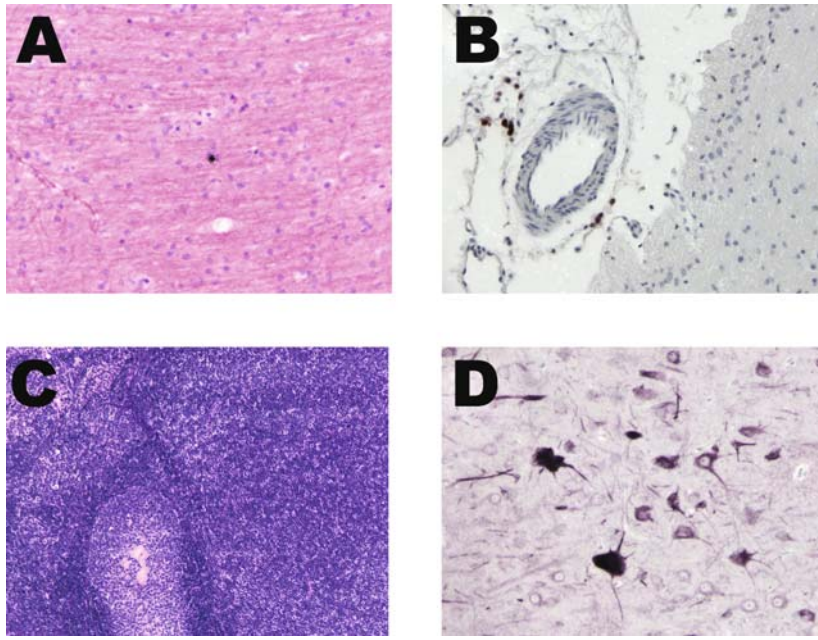

FIGURE 4. Pathobiology of FIV. Cats infected with FIV show rare brain pathology. Panel A shows rare brain cells immunostained for FIV antigens. Panel B demonstrates inflammatory macrophages in the meninges. Panel C illustrates a hypertrophic lymph node with a large germinal center that has lost some of its architecture. Panel D is an immunochemical stain for nonphosphorylated neurofilaments showing increases in cortical neurons in FIV infected cats. All slides are at 20X magnification and were counterstained with hemotoxylin and eosin. Contributed by Dr. Howard Fox.

Bovine Immunodeficiency Virus (BIV)

BIV is a lentivirus that shares antigenic and genetic homology with HIV-1. Although the disease is not generally immunosuppressive,[7,40,120] BIV host cells and tissue tropisms correspond to affected human organs, in particular the spleen.[122] BIV is also distinct from HIV in regards to tat transcriptional activities and, unlike other lentiviruses, BIV does not require specific cellular cofactors to complete its life cycle.[15] This variation in tat has provoked further study in the cellular mechanics involved in BIV with the hope to better understand the viral mechanics in human disease.

Simian Immunodeficiency Virus (SIV)

As far as animal models are concerned, the system that bears the most resemblance to that of human biology is the nonhuman primates. Nonhuman primates have been used extensively in animal research and their utility has been quite beneficial to HIV research. Chimpanzees and gibbon apes in particular are the only nonhuman primates that are susceptible to infection with HIV-1. Scientists have achieved infection with HIV-1 producing both measurable viral loads and significant amounts of antibodies to viral coat proteins.[8,71,79] Regrettably, HIV-1 infections in these primate models have led generally to asymptomatic infections.[81a,87]

Although HIV-2 is specifically relevant to a focused region in Africa, this strain is used in AIDS studies through its abilities to infect nonhuman primates. HIV-2 is not as debilitating as HIV-1, but is utilized in animal model systems because the study permits insights into viral processes or mechanics. HIV-2 is similar to SIV and has been speculated to represent a rare crossover from nonhuman primates to humans. HIV-2 has been shown to progressively infect and seroconvert macaques[35,99] and baboons.[68] Although HIV-2 infection of simians has not been studied as extensively as HIV-1 or SIV, there have been instances of AIDS-like disorders occurring in these animals,[67] supporting their utility as an animal model for studying the pathogenesis of HIV-2 and for their potential for testing vaccine strategies or treatment regimens.

SIV is a nonhuman primate strain that has been found in certain populations of primates. It has several derivatives, which include SIVagm, SIVmac, and SIVsm among others. Different isolates of SIV have been acquired from several different regions of the world.[6] The SIVs are the closest relatives to HIV and can infect nonhuman primates. SIV infection of nonhuman primates is arguably the most widely accepted animal model for HIV.

Importantly, SIV has been shown to be genetically similar to HIV and utilizes the typical pathway of infection in nonhuman primates as does in humans.[127] Similarly, researchers isolated specific viral proteins that operate

in a similar manner to HIV proteins.[70] SIV relies on specific chemokine receptors in order to infect different cell types. The receptor type that remains constant in the HIV and SIV strains is CCR5.[127] HIV-2 bears remarkable genetic similarity to SIV and shares the same route of infectivity through receptors such as Bonzo and BOB that are not utilized by HIV-1.[98,131] HIV-2 is speculated to represent an intermediary virus that stemmed from nonhuman primates and crossed over to the human population.[112] However, due to the distinct sequence dissimilarity between HIV-1 and any of the SIVs, the origins of HIV-1 remain uncertain.

Interestingly, while the pathogenesis of SIV does parallel that of the human virus, the detrimental manifestations observed in HIV-1 infection in humans do not typically occur in nonhuman primates infected with SIV. However, the resultant symptoms observed following the injection of specific strains (SIVmac) into macaques parallel human clinical disease and pathology. Such efforts have yielded macaque models for AIDS associated neurodegeneration,[64,95] AIDS-associated non-Hodgkin's lymphoma,[48] AIDS-related gastrointestinal complications,[50,117] and AIDS pathogenesis (Figure 5).[92,102,109]

Simian-Human (SHIV)

While SIV and its various strains may provide some insight into mechanisms commonly shared by lentiviruses that may be attributable to HIV-1, specific treatments generated to combat the human disease must be established utilizing HIV-1 or its viral components. In order to address this idea, a transgenic virus was used in simians to replicate some aspects of HIV-1. This chimeric virus is a form of SIV with the gp120 coat of HIV-1.[66,106,113] This virus was developed in order to establish a primate system for the evaluation of viral envelope targeting vaccines, anti–HIV-1 envelope glycoprotein antiserum, monoclonal antibodies, and anti–HIV-1 drugs designed to inhibit the functions of the tat, rev, and env viral proteins. Additionally, a simian model system intended to recapitulate viral drug resistance has also been created utilizing a chimeric virus.[140] Simian models utilizing this transgenic virus have received increasing attention in hopes that this may lead to the formulation of preventative treatment strategies.

7. CONCLUSION

Most lentiviruses are species specific, and disease manifestations do not necessarily parallel HIV infection in its human host. This suggests that HIV infections of humans need be studied to fully realize the goal of effective vaccine prevention of viral infection. Nonetheless, HIV does not infect animals easily including nonhuman primates. Although several other

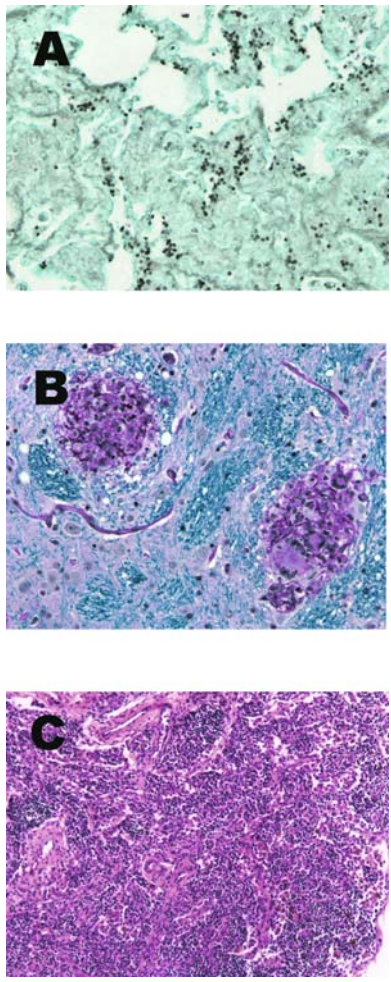

FIGURE 5. SIV Pathology. Tissues derived from monkeys with SIV show disease consequences. Panel A illustrates the presence of *pneumocystis carinii*, a common opportunistic infection in lung during advanced HIV and SIV infections. Panel B depicts an SIV encephalitic brain with a multinucleated giant cells encephalitis and astrogliosis. Lung and brain tissue were stained with Luxol Fast Blue and is at 20X. Panels C shows disorganized architecture of the lymph node at 10X magnification and stained with hemotoxylin and eosin. Contributed by Dr. Howard Fox.

lentiviruses, similar in many respects to HIV, have been discovered, including SIV and FIV; where correlations in disease pathogenesis are manifest, these species-specific viruses are genetically distinct.

Presently, no animal system exists that is completely representative of the human disease process. However, each animal model can recapitulate some manifestations of human disease. In order to construct a model of the human lentiviral infection, an animal would have to be genetically altered so that the virus could affect it in a manner similar to what is found in man. Progress has been made in this direction, though it is by no means complete, and currently no "completely relevant" model of AIDS have been produced.

In summary, the ideal animal model system would involve the study of HIV-1 and be based in a relevant small animal model. However, this is currently not possible. Animal models remain the sole means to vigorously examine the effects of disease-preventative regimens that would have a profound effect on reversing the suffering manifest worldwide as a result of HIV disease.

Acknowledgments. We thank Nell Ingrahan and Robin Taylor for outstanding administrative and graphic support. This work was supported, in part by National Institutes of Health Research Grants 2 R37 NS36126, P01 NS31492; P01 NS43985; U54 NS43011-01; 2 R01 NS034239; P20 RR15635.

REFERENCES

1. Aldrovandi, G. M., Feuer, G., Gao, L., Jamieson, B., Kristeva, M., Chen, I. S., and Zack, J. A., 1993, The SCID-hu mouse as a model for HIV-1 infection, *Nature.* 363:732–736.
2. Alexander, N. J., 1990, Sexual transmission of human immunodeficiency virus: virus entry into the male and female genital tract. World Health Organization, Global Programme on Acquired Immune Deficiency Syndrome, *Fertil Steril.* 54:1–18.
3. Allison, R. W., and Hoover, E. A., 2003, Covert vertical transmission of feline immunodeficiency virus, *AIDS Res Hum Retroviruses.* 19:421–434.
4. Allison, R. W., and Hoover, E. A., 2003, Feline immunodeficiency virus is concentrated in milk early in lactation, *AIDS Res Hum Retroviruses.* 19:245–253.
5. Altfeld, M., Rosenberg, E. S., Shankarappa, R., Mukherjee, J. S., Hecht, F. M., Eldridge, R. L., Addo, M. M., Poon, S. H., Phillips, M. N., Robbins, G. K., Sax, P. E., Boswell, S., Kahn, J. O., Brander, C., Goulder, P. J., Levy, J. A., Mullins, J. I., and Walker, B. D., 2001, Cellular immune responses and viral diversity in individuals treated during acute and early HIV-1 infection, *J Exp Med.* 193:169–180.
6. Apetrei, C., Robertson, D. L., and Marx, P. A., 2004, The history of SIVS and AIDS: epidemiology, phylogeny and biology of isolates from naturally SIV infected non-human primates (NHP) in Africa, *Front Biosci.* 9:225–254.
7. Archambault, D., Nadin-Davis, S., Lutze-Wallace, C., and Bouillant, A. M., 1993, The bovine immunodeficiency virus: 1990–1992 update, *Vet Res.* 24:179–187.
8. Arthur, L. O., Bess, J. W., Jr., Waters, D. J., Pyle, S. W., Kelliher, J. C., Nara, P. L., Krohn, K., Robey, W. G., Langlois, A. J., Gallo, R. C., and et al., 1989, Challenge of chimpanzees (Pan troglodytes) immunized with human immunodeficiency virus envelope glycoprotein gp120, *J Virol.* 63:5046–5053.
9. Bacchetti, P., and Moss, A. R., 1989, Incubation period of AIDS in San Francisco, *Nature.* 338:251–253.
10. Barr, M. C., Billaud, J. N., Selway, D. R., Huitron-Resendiz, S., Osborn, K. G., Henriksen, S. J., and Phillips, T. R., 2000, Effects of multiple acute morphine exposures on feline immunodeficiency virus disease progression, *J Infect Dis.* 182:725–732.
11. Barry, S. M., Johnson, M. A., and Janossy, G., 2003, Increased proportions of activated and proliferating memory CD8+ T lymphocytes in both blood and lung are associated with blood HIV viral load, *J Acquir Immune Defic Syndr.* 34:351–357.
12. Baszler, T. V., Harwood, W. G., Lester, K. L., Davis, W. C., and Knowles, D. P., 1994, Characterization of caprine microglial cells and in vitro infection with caprine arthritis-encephalitis lentivirus, *Lab Invest.* 70:933–943.

13. Bendinelli, M., Matteucci, D., and Friedman, H., 1985, Retrovirus-induced acquired immunodeficiencies, *Adv Cancer Res.* 45:125–181.
14. Bendinelli, M., Pistello, M., Lombardi, S., Poli, A., Garzelli, C., Matteucci, D., Ceccherini-Nelli, L., Malvaldi, G., and Tozzini, F., 1995, Feline immunodeficiency virus: an interesting model for AIDS studies and an important cat pathogen, *Clin Microbiol Rev.* 8:87–112.
15. Bogerd, H. P., Wiegand, H. L., Bieniasz, P. D., and Cullen, B. R., 2000, Functional differences between human and bovine immunodeficiency virus tat transcription factors, *J Virol.* 74:4666–4671.
16. Bonyhadi, M. L., and Kaneshima, H., 1997, The SCID-hu mouse: an in vivo model for HIV-1 infection in humans, *Mol Med Today.* 3:246–253.
17. Borrow, P., Lewicki, H., Hahn, B. H., Shaw, G. M., and Oldstone, M. B., 1994, Virus-specific CD8+ cytotoxic T-lymphocyte activity associated with control of viremia in primary human immunodeficiency virus type 1 infection, *J Virol.* 68:6103–6110.
18. Browning, J., Horner, J. W., Pettoello-Mantovani, M., Raker, C., Yurasov, S., DePinho, R. A., and Goldstein, H., 1997, Mice transgenic for human CD4 and CCR5 are susceptible to HIV infection, *Proc Natl Acad Sci USA.* 94:14637–14641.
19. Cadore, J. L., Greenland, T., Cordier, G., Guiguen, F., and Mornex, J. F., 1996, Histogenesis of the pulmonary lesions in the course of visna maedi virus-induced pneumonia, *Vet Res.* 27:419–426.
20. Chargui, J., Dye, D., Blomberg, J., Desgranges, C., and Touraine, J. L., 1995, The humanized severe combined immunodeficient mouse as a model for primary human humoral response against HIV1 peptides, *J Immunol Methods.* 181:91–100.
21. Charneau, P., Borman, A. M., Quillent, C., Guetard, D., Chamaret, S., Cohen, J., Remy, G., Montagnier, L., and Clavel, F., 1994, Isolation and envelope sequence of a highly divergent HIV-1 isolate: definition of a new HIV-1 group, *Virology.* 205:247–253.
22. Chattopadhyay, S. K., Morse, H. C., III, Makino, M., Ruscetti, S. K., and Hartley, J. W., 1989, Defective virus is associated with induction of murine retrovirus-induced immunodeficiency syndrome, *Proc Natl Acad Sci USA.* 86:3862–3866.
23. Cheevers, W. P., and McGuire, T. C., 1985, Equine infectious anemia virus: immunopathogenesis and persistence, *Rev Infect Dis.* 7:83–88.
24. Clark, S., Duggan, J., and Chakraborty, J., 2001, Tsl and LP-BM5: a comparison of two murine retrovirus models for HIV, *Viral Immunol.* 14:95–109.
25. Clavel, F., Guyader, M., Guetard, D., Salle, M., Montagnier, L., and Alizon, M., 1986, Molecular cloning and polymorphism of the human immune deficiency virus type 2, *Nature.* 324:691–695.
26. Cohen, N. D., and Carter, G. K., 1991, Persistent thrombocytopenia in a case of equine infectious anemia, *J Am Vet Med Assoc.* 199:750–752.
27. Cooper, D. A., Gold, J., Maclean, P., Donovan, B., Finlayson, R., Barnes, T. G., Michelmore, H. M., Brooke, P., and Penny, R., 1985, Acute AIDS retrovirus infection. Definition of a clinical illness associated with seroconversion, *Lancet.* 1:537–540.
28. Cordier, G., Cozon, G., Greenland, T., Rocher, F., Guiguen, F., Guerret, S., Brune, J., and Mornex, J. F., 1990, In vivo activation of alveolar macrophages in ovine lentivirus infection, *Clin Immunol Immunopathol.* 55:355–367.
29. Cork, L. C., Hadlow, W. J., Crawford, T. B., Gorham, J. R., and Piper, R. C., 1974, Infectious leukoencephalomyelitis of young goats, *J Infect Dis.* 129:134–141.
30. Cork, L. C., Hadlow, W. J., Gorham, J. R., Piper, R. C., and Crawford, T. B., 1974, Pathology of viral leukoencephalomyelitis of goats, *Acta Neuropathol (Berl).* 29:281–292.
31. Curran, J. W., Lawrence, D. N., Jaffe, H., Kaplan, J. E., Zyla, L. D., Chamberland, M., Weinstein, R., Lui, K. J., Schonberger, L. B., Spira, T. J., et al., 1984, Acquired immunodeficiency syndrome (AIDS) associated with transfusions, *N Engl J Med.* 310: 69–75.

32. Dalziel, R. G., Hopkins, J., Watt, N. J., Dutia, B. M., Clarke, H. A., and McConnell, I., 1991, Identification of a putative cellular receptor for the lentivirus visna virus, *J Gen Virol.* 72 (Pt 8):1905–1911.
33. Dawson, M., 1987, Pathogenesis of maedi-visna, *Vet Rec.* 120:451–454.
33a. Dickie, P., Felser, J., Eckhaus, M., Bryant, J., Silver, J., Marinos, N., and Notkins, A. L., 1991, HIV-associated nephropathy in transgenic mice expressing HIV-1 genes, *Virology.* 185:109–119.
34. Donnelly, C., Leisenring, W., Kanki, P., Awerbuch, T., and Sandberg, S., 1993, Comparison of transmission rates of HIV-1 and HIV-2 in a cohort of prostitutes in Senegal, *Bull Math Biol.* 55:731–743.
35. Dormont, D., Livartowski, J., Chamaret, S., Guetard, D., Henin, D., Levagueresse, R., van de Moortelle, P. F., Larke, B., Gourmelon, P., Vazeux, R., et al., 1989, HIV-2 in rhesus monkeys: serological, virological and clinical results, *Intervirology.* 30 Suppl 1:59–65.
36. Dorrington, R., Bourne, D., Bradshaw, D., Laubscher, R., and Timaeus, I., 2001, Medical Research Council, Tygerber, pp. 1–58.
37. Douek, D. C., 2003, Disrupting T-cell homeostasis: how HIV-1 infection causes disease, *AIDS Rev.* 5:172–177.
38. Douek, D. C., Brenchley, J. M., Betts, M. R., Ambrozak, D. R., Hill, B. J., Okamoto, Y., Casazza, J. P., Kuruppu, J., Kunstman, K., Wolinsky, S., Grossman, Z., Dybul, M., Oxenius, A., Price, D. A., Connors, M., and Koup, R. A., 2002, HIV preferentially infects HIV-specific CD4+ T cells, *Nature.* 417:95–98.
39. Dow, S. W., Dreitz, M. J., and Hoover, E. A., 1992, Feline immunodeficiency virus neurotropism: evidence that astrocytes and microglia are the primary target cells, *Vet Immunol Immunopathol.* 35:23–35.
40. Flaming, K., van der Maaten, M., Whetstone, C., Carpenter, S., Frank, D., and Roth, J., 1993, Effect of bovine immunodeficiency-like virus infection on immune function in experimentally infected cattle, *Vet Immunol Immunopathol.* 36:91–105.
41. Gaillard, P., Melis, R., Mwanyumba, F., Claeys, P., Muigai, E., Mandaliya, K., Bwayo, J., and Temmerman, M., 2002, Vulnerability of women in an African setting: lessons for mother-to-child HIV transmission prevention programmes, *Aids.* 16:937–939.
42. Gavrilin, M. A., Mathes, L. E., and Podell, M., 2002, Methamphetamine enhances cell-associated feline immunodeficiency virus replication in astrocytes, *J Neurovirol.* 8:240–249.
43. Gazzinelli, R. T., Hartley, J. W., Fredrickson, T. N., Chattopadhyay, S. K., Sher, A., and Morse, H. C., III, 1992, Opportunistic infections and retrovirus-induced immunodeficiency: studies of acute and chronic infections with Toxoplasma gondii in mice infected with LP-BM5 murine leukemia viruses, *Infect Immun.* 60:4394–4401.
44. Gendelman, H. E., Narayan, O., Molineaux, S., Clements, J. E., and Ghotbi, Z., 1985, Slow, persistent replication of lentiviruses: role of tissue macrophages and macrophage precursors in bone marrow, *Proc Natl Acad Sci USA.* 82:7086–7090.
45. Georgsson, G., Martin, J. R., Palsson, P. A., Nathanson, N., Benediktsdottir, E., and Petursson, G., 1979, An ultrastructural study of the cerebrospinal fluid in visna, *Acta Neuropathol (Berl).* 48:39–43.
46. Gupta, K. K., 1993, Acute immunosuppression with HIV seroconversion, *N Engl J Med.* 328:288–289.
47. Gurtler, L. G., Hauser, P. H., Eberle, J., von Brunn, A., Knapp, S., Zekeng, L., Tsague, J. M., and Kaptue, L., 1994, A new subtype of human immunodeficiency virus type 1 (MVP-5180) from Cameroon, *J Virol.* 68:1581–1585.
48. Habis, A., Baskin, G. B., Murphey-Corb, M., and Levy, L. S., 1999, Simian AIDS-associated lymphoma in rhesus and cynomolgus monkeys recapitulates the primary pathobiological features of AIDS-associated non-Hodgkin's lymphoma, *AIDS Res Hum Retroviruses.* 15:1389–1398.

49. Hart, A. R., and Cloyd, M. W., 1990, Interference patterns of human immunodeficiency viruses HIV-1 and HIV-2, *Virology.* 177:1–10.
50. Heise, C., Vogel, P., Miller, C. J., Halsted, C. H., and Dandekar, S., 1993, Simian immunodeficiency virus infection of the gastrointestinal tract of rhesus macaques. Functional, pathological, and morphological changes, *Am J Pathol.* 142:1759–1771.
51. Hotzel, I., and Cheevers, W. P., 2003, Caprine arthritis-encephalitis virus envelope surface glycoprotein regions interacting with the transmembrane glycoprotein: structural and functional parallels with human immunodeficiency virus type 1 gp120, *J Virol.* 77:11578–11587.
52. Ilaria, G., Jacobs, J. L., Polsky, B., Koll, B., Baron, P., MacLow, C., Armstrong, D., and Schlegel, P. N., 1992, Detection of HIV-1 DNA sequences in pre-ejaculatory fluid, *Lancet.* 340:1469.
53. Issel, C. J., McManus, J. M., Hagius, S. D., Foil, L. D., Adams, W. V., Jr., and Montelaro, R. C., 1990, Equine infectious anemia: prospects for control, *Dev Biol Stand.* 72:49–57.
54. Jagger, J., Hunt, E. H., Brand-Elnaggar, J., and Pearson, R. D., 1988, Rates of needlestick injury caused by various devices in a university hospital, *N Engl J Med.* 319:284–288.
55. Johnson, P. R., and Hirsch, V. M., 1992, SIV infection of macaques as a model for AIDS pathogenesis, *Int Rev Immunol.* 8:55–63.
56. Jolicoeur, P., Laperriere, A., and Beaulieu, N., 1992, Efficient production of human immunodeficiency virus proteins in transgenic mice, *J Virol.* 66:3904–3908.
57. Jordan, H. L., Howard, J. G., Bucci, J. G., Butterworth, J. L., English, R., Kennedy-Stoskopf, S., Tompkins, M. B., and Tompkins, W. A., 1998, Horizontal transmission of feline immunodeficiency virus with semen from seropositive cats, *J Reprod Immunol.* 41:341–357.
58. Kanki, P. J., Travers, K. U., S, M. B., Hsieh, C. C., Marlink, R. G., Gueye, N. A., Siby, T., Thior, I., Hernandez-Avila, M., Sankale, J. L., et al., 1994, Slower heterosexual spread of HIV-2 than HIV-1, *Lancet.* 343:943–946.
59. Keppler, O. T., Welte, F. J., Ngo, T. A., Chin, P. S., Patton, K. S., Tsou, C. L., Abbey, N. W., Sharkey, M. E., Grant, R. M., You, Y., Scarborough, J. D., Ellmeier, W., Littman, D. R., Stevenson, M., Charo, I. F., Herndier, B. G., Speck, R. F., and Goldsmith, M. A., 2002, Progress toward a human CD4/CCR5 transgenic rat model for de novo infection by human immunodeficiency virus type 1, *J Exp Med.* 195:719–736.
60. Keppler, O. T., Yonemoto, W., Welte, F. J., Patton, K. S., Iacovides, D., Atchison, R. E., Ngo, T., Hirschberg, D. L., Speck, R. F., and Goldsmith, M. A., 2001, Susceptibility of rat-derived cells to replication by human immunodeficiency virus type 1, *J Virol.* 75:8063–8073.
61. Klotman, P. E., and Notkins, A. L., 1996, Transgenic models of human immunodeficiency virus type-1, *Curr Top Microbiol Immunol.* 206:197–222.
62. Klotman, P. E., Rappaport, J., Ray, P., Kopp, J. B., Franks, R., Bruggeman, L. A., and Notkins, A. L., 1995, Transgenic models of HIV-1, *Aids.* 9:313–324.
63. Koup, R. A., Safrit, J. T., Cao, Y., Andrews, C. A., McLeod, G., Borkowsky, W., Farthing, C., and Ho, D. D., 1994, Temporal association of cellular immune responses with the initial control of viremia in primary human immunodeficiency virus type 1 syndrome, *J Virol.* 68:4650–4655.
64. Koutsilieri, E., Sopper, S., Scheller, C., ter Meulen, V., and Riederer, P., 2002, Parkinsonism in HIV dementia, *J Neural Transm.* 109:767–775.
65. Leonard, J. M., Abramczuk, J. W., Pezen, D. S., Rutledge, R., Belcher, J. H., Hakim, F., Shearer, G., Lamperth, L., Travis, W., Fredrickson, T., et al., 1988, Development of disease and virus recovery in transgenic mice containing HIV proviral DNA, *Science.* 242:1665–1670.
66. Li, J., Lord, C. I., Haseltine, W., Letvin, N. L., and Sodroski, J., 1992, Infection of cynomolgus monkeys with a chimeric HIV-1/SIVmac virus that expresses the HIV-1 envelope glycoproteins, *J Acquir Immune Defic Syndr.* 5:639–646.

67. Livartowski, J., Dormont, D., Boussin, F., Chamaret, S., Guetard, D., Vazeux, R., Lebon, P., Metivier, H., and Montagnier, L., 1992, Clinical and virological aspects of HIV2 infection in rhesus monkeys, *Cancer Detect Prev.* 16:341–345.
68. Locher, C. P., Witt, S. A., Herndier, B. G., Tenner-Racz, K., Racz, P., and Levy, J. A., 2001, Baboons as an animal model for human immunodeficiency virus pathogenesis and vaccine development, *Immunol Rev.* 183:127–140.
69. Longini, I. M., Jr., Clark, W. S., Byers, R. H., Ward, J. W., Darrow, W. W., Lemp, G. F., and Hethcote, H. W., 1989, Statistical analysis of the stages of HIV infection using a Markov model, *Stat Med.* 8:831–843.
70. Luciw, P. A., Shaw, K. E., Unger, R. E., Planelles, V., Stout, M. W., Lackner, J. E., Pratt-Lowe, E., Leung, N. J., Banapour, B., and Marthas, M. L., 1992, Genetic and biological comparisons of pathogenic and nonpathogenic molecular clones of simian immunodeficiency virus (SIVmac), *AIDS Res Hum Retroviruses.* 8:395–402.
71. Lusso, P., Markham, P. D., Ranki, A., Earl, P., Moss, B., Dorner, F., Gallo, R. C., and Krohn, K. J., 1988, Cell-mediated immune response toward viral envelope and core antigens in gibbon apes (Hylobates lar) chronically infected with human immunodeficiency virus-1, *J Immunol.* 141:2467–2473.
72. Lynn, W. S., and Wong, P. K., 1998, Neuroimmunopathogenesis of ts1 MoMuLV viral infection, *Neuroimmunomodulation.* 5:248–260.
73. Marlink, R., Kanki, P., Thior, I., Travers, K., Eisen, G., Siby, T., Traore, I., Hsieh, C. C., Dia, M. C., Gueye, E. H., et al., 1994, Reduced rate of disease development after HIV-2 infection as compared to HIV-1, *Science.* 265:1587–1590.
74. McIntyre, J., and Gray, G., 2002, What can we do to reduce mother to child transmission of HIV?, *Brit Med J.* 324:218–221.
75. Montagnier, L., 1985, Lymphadenopathy-associated virus:from molecular biology to pathogenicity, *Ann Intern Med.* 103:689–693.
76. Montelaro, R. C., Ball, J. M., and Issel, C. J., 1990, Characterization of EIAV immunogenicity during persistent infections: humoral responses and antigen targets, *Dev Biol Stand.* 72:19–30.
77. Mosier, D. E., Yetter, R. A., and Morse, H. C., III 1985, Retroviral induction of acute lymphoproliferative disease and profound immunosuppression in adult C57BL/6 mice, *J Exp Med.* 161:766–784.
78. Munoz, A., Wang, M. C., Bass, S., Taylor, J. M., Kingsley, L. A., Chmiel, J. S., and Polk, B. F., 1989, Acquired immunodeficiency syndrome (AIDS)-free time after human immunodeficiency virus type 1 (HIV-1) seroconversion in homosexual men. Multicenter AIDS Cohort Study Group, *Am J Epidemiol.* 130:530–539.
79. Nara, P. L., Robey, W. G., Arthur, L. O., Asher, D. M., Wolff, A. V., Gibbs, C. J., Jr., Gajdusek, D. C., and Fischinger, P. J., 1987, Persistent infection of chimpanzees with human immunodeficiency virus: serological responses and properties of reisolated viruses, *J Virol.* 61:3173–3180.
80. Narayan, O., Wolinsky, J. S., Clements, J. E., Strandberg, J. D., Griffin, D. E., and Cork, L. C., 1982, Slow virus replication: the role of macrophages in the persistence and expression of visna viruses of sheep and goats, *J Gen Virol.* 59:345–356.
81. Ng, H., 2000, AIDS in Africa: a regional overview, *Harv AIDS Rev.* 2–5.
81a. Novembre, F. J., Saucier, M., Anderson, D. C., Klumpp, S. A., O'Neil, S. P., Brown, C. R., 2nd, Hart, C. E., Guenthner, P. C., Swenson, R. B., and McClure, H. M., 1997, Development of AIDS in a chimpanzee infected with human immunodeficiency virus type 1, *J Virol.* 71:4086–4091.
82. Novitsky, V. A., Montano, M. A., McLane, M. F., Renjifo, B., Vannberg, F., Foley, B. T., Ndung'u, T. P., Rahman, M., Makhema, M. J., Marlink, R., and Essex, M., 1999, Molecular cloning and phylogenetic analysis of human immunodeficiency virus type 1 subtype C: a set of 23 full-length clones from Botswana, *J Virol.* 73:4427–4432.

83. Oliver, R. E., Gorham, J. R., Parish, S. F., Hadlow, W. J., and Narayan, O., 1981, Ovine progressive pneumonia: pathologic and virologic studies on the naturally occurring disease, *Am J Vet Res.* 42:1554–1559.
84. Oliver, R. E., Gorham, J. R., Perryman, L. E., and Spencer, G. R., 1981, Ovine progressive pneumonia: experimental intrathoracic, intracerebral, and intra-articular infections, *Am J Vet Res.* 42:1560–1564.
85. Olmsted, R. A., Barnes, A. K., Yamamoto, J. K., Hirsch, V. M., Purcell, R. H., and Johnson, P. R., 1989, Molecular cloning of feline immunodeficiency virus, *Proc Natl Acad Sci USA.* 86:2448–2452.
86. Olmsted, R. A., Hirsch, V. M., Purcell, R. H., and Johnson, P. R., 1989, Nucleotide sequence analysis of feline immunodeficiency virus: genome organization and relationship to other lentiviruses, *Proc Natl Acad Sci USA.* 86:8088–8092.
87. O'Neil, S. P., Novembre, F. J., Hill, A. B., Suwyn, C., Hart, C. E., Evans-Strickfaden, T., Anderson, D. C., deRosayro, J., Herndon, J. G., Saucier, M., and McClure, H. M., 2000, Progressive infection in a subset of HIV-1-positive chimpanzees, *J Infect Dis.* 182:1051–1062.
88. Patrick, M. K., Johnston, J. B., and Power, C., 2002, Lentiviral neuropathogenesis: comparative neuroinvasion, neurotropism, neurovirulence, and host neurosusceptibility, *J Virol.* 76:7923–7931.
89. Pedersen, N. C., Ho, E. W., Brown, M. L., and Yamamoto, J. K., 1987, Isolation of a T-lymphotropic virus from domestic cats with an immunodeficiency-like syndrome, *Science.* 235:790–793.
90. Pedersen, N. C., Yamamoto, J. K., Ishida, T., and Hansen, H., 1989, Feline immunodeficiency virus infection, *Vet Immunol Immunopathol.* 21:111–129.
91. Perrin, L., Kaiser, L., and Yerly, S., 2003, Travel and the spread of HIV-1 genetic variants, *Lancet Infect Dis.* 3:22–27.
92. Persidsky, Y., Limoges, J., McComb, R., Bock, P., Baldwin, T., Tyor, W., Patil, A., Nottet, H. S., Epstein, L., Gelbard, H., Flanagan, E., Reinhard, J., Pirruccello, S. J., and Gendelman, H. E., 1996, Human immunodeficiency virus encephalitis in SCID mice, *Am J Pathol.* 149:1027–1053.
93. Peterman, T. A., Jaffe, H. W., Feorino, P. M., Getchell, J. P., Warfield, D. T., Haverkos, H. W., Stoneburner, R. L., and Curran, J. W., 1985, Transfusion-associated acquired immunodeficiency syndrome in the United States, *Jama.* 254:2913–2917.
94. Petersen, L. R., Simonds, R. J., and Koistinen, J., 1993, HIV transmission through blood, tissues, and organs, *Aids.* 7 Suppl 1:S99–107.
95. Petry, H., and Luke, W., 1997, Infection of macaque monkeys with simian immunodeficiency virus: an animal model for neuro-AIDS, *Intervirology.* 40:112–121.
96. Phelps, S. L., and Smith, M. C., 1993, Caprine arthritis-encephalitis virus infection, *J Am Vet Med Assoc.* 203:1663–1666.
97. Phillips, A. N., 1996, Reduction of HIV concentration during acute infection: independence from a specific immune response, *Science.* 271:497–499.
98. Pohlmann, S., Lee, B., Meister, S., Krumbiegel, M., Leslie, G., Doms, R. W., and Kirchhoff, F., 2000, Simian immunodeficiency virus utilizes human and sooty mangabey but not rhesus macaque STRL33 for efficient entry, *J Virol.* 74:5075–5082.
99. Putkonen, P., Bottiger, B., Warstedt, K., Thorstensson, R., Albert, J., and Biberfeld, G., 1989, Experimental infection of cynomolgus monkeys (Macaca fascicularis) with HIV-2, *J Acquir Immune Defic Syndr.* 2:366–373.
100. Quarto, M., Germinario, C., Fontana, A., and Barbuti, S., 1989, HIV transmission through kidney transplantation from a living related donor, *N Engl J Med.* 320:1754.
101. Reid, W., Sadowska, M., Denaro, F., Rao, S., Foulke, J., Jr., Hayes, N., Jones, O., Doodnauth, D., Davis, H., Sill, A., O'Driscoll, P., Huso, D., Fouts, T., Lewis, G., Hill, M.,

Kamin-Lewis, R., Wei, C., Ray, P., Gallo, R. C., Reitz, M., and Bryant, J., 2001, An HIV-1 transgenic rat that develops HIV-related pathology and immunologic dysfunction, *Proc Natl Acad Sci USA.* 98:9271–9276.

102. Reinhart, T. A., Rogan, M. J., Huddleston, D., Rausch, D. M., Eiden, L. E., and Haase, A. T., 1997, Simian immunodeficiency virus burden in tissues and cellular compartments during clinical latency and AIDS, *J Infect Dis.* 176:1198–1208.
103. Rogers, A. B., Mathiason, C. K., and Hoover, E. A., 2002, Immunohistochemical localization of feline immunodeficiency virus using native species antibodies, *Am J Pathol.* 161:1143–1151.
104. Rosenberg, E. S., Billingsley, J. M., Caliendo, A. M., Boswell, S. L., Sax, P. E., Kalams, S. A., and Walker, B. D., 1997, Vigorous HIV-1-specific CD4+ T cell responses associated with control of viremia, *Science.* 278:1447–1450.
105. Rowe, J. D., and East, N. E., 1997, Risk factors for transmission and methods for control of caprine arthritis-encephalitis virus infection, *Vet Clin North Am Food Anim Pract.* 13:35–53.
106. Sakuragi, S., Shibata, R., Mukai, R., Komatsu, T., Fukasawa, M., Sakai, H., Sakuragi, J., Kawamura, M., Ibuki, K., Hayami, M., et al., 1992, Infection of macaque monkeys with a chimeric human and simian immunodeficiency virus, *J Gen Virol.* 73 (Pt 11):2983–2987.
107. Salvatori, D., Vincenzetti, S., Maury, G., Gosselin, G., Gaubert, G., and Vita, A., 2001, Maedi-visna virus, a model for in vitro testing of potential anti-HIV drugs, *Comp Immunol Microbiol Infect Dis.* 24:113–122.
108. Samuel, D., Castaing, D., Adam, R., Saliba, F., Chamaret, S., Misset, J. L., Montagnier, L., and Bismuth, H., 1988, Fatal acute HIV infection with aplastic anaemia, transmitted by liver graft, *Lancet.* 1:1221–1222.
109. Sauermann, U., 2001, Making the animal model for AIDS research more precise: the impact of major histocompatibility complex (MHC) genes on pathogenesis and disease progression in SIV-infected monkeys, *Curr Mol Med.* 1:515–122.
110. Sellon, D. C., 1993, Equine infectious anemia, *Vet Clin North Am Equine Pract.* 9:321–336.
111. Sellon, D. C., Perry, S. T., Coggins, L., and Fuller, F. J., 1992, Wild-type equine infectious anemia virus replicates in vivo predominantly in tissue macrophages, not in peripheral blood monocytes, *J Virol.* 66:5906–5913.
112. Sharp, P. M., Robertson, D. L., and Hahn, B. H., 1995, Cross-species transmission and recombination of "AIDS" viruses, *Philos Trans R Soc Lond B Biol Sci.* 349:41–47.
113. Shibata, R., Kawamura, M., Sakai, H., Hayami, M., Ishimoto, A., and Adachi, A., 1991, Generation of a chimeric human and simian immunodeficiency virus infectious to monkey peripheral blood mononuclear cells, *J Virol.* 65:3514–3520.
114. Silvotti, L., Corradi, A., Brandi, G., Cabassi, A., Bendinelli, M., Magnani, M., and Piedimonte, G., 1997, FIV induced encephalopathy: early brain lesions in the absence of viral replication in monocyte/macrophages. A pathogenetic model, *Vet Immunol Immunopathol.* 55:263–271.
115. Simon, F., Mauclere, P., Roques, P., Loussert-Ajaka, I., Muller-Trutwin, M. C., Saragosti, S., Georges-Courbot, M. C., Barre-Sinoussi, F., and Brun-Vezinet, F., 1998, Identification of a new human immunodeficiency virus type 1 distinct from group M and group O, *Nat Med.* 4:1032–1037.
116. Simonds, R. J., 1993, HIV transmission by organ and tissue transplantation, *Aids.* 7 Suppl 2:S35–38.
117. Smit-McBride, Z., Mattapallil, J. J., McChesney, M., Ferrick, D., and Dandekar, S., 1998, Gastrointestinal T lymphocytes retain high potential for cytokine responses but have severe CD4(+) T-cell depletion at all stages of simian immunodeficiency virus infection compared to peripheral lymphocytes, *J Virol.* 72:6646–6656.
118. Speck, R. F., Penn, M. L., Wimmer, J., Esser, U., Hague, B. F., Kindt, T. J., Atchison, R. E., and Goldsmith, M. A., 1998, Rabbit cells expressing human CD4 and human CCR5

are highly permissive for human immunodeficiency virus type 1 infection, *J Virol.* 72:5728–5734.
119. Spira, S., Wainberg, M. A., Loemba, H., Turner, D., and Brenner, B. G., 2003, Impact of clade diversity on HIV-1 virulence, antiretroviral drug sensitivity and drug resistance, *J Antimicrob Chemother.* 51:229–240.
120. Straub, O. C., and Levy, D., 1999, Bovine immunodeficiency virus and analogies with human immunodeficiency virus, *Leukemia.* 13 Suppl 1:S106–109.
121. Sutton, R. E., Coskun, A. K., and Nguyen, V., 2003, Development of a mouse model for HIV/AIDS, *Res Initiat Treat Action.* 8:22–24.
122. Tajima, M., Sato, N., Kirisawa, R., Onuma, M., and Maede, Y., 1997, Distribution of bovine immunodeficiency virus in the organs of experimentally infected cows, *Jpn J Vet Res.* 45:163–167.
123. Talbott, R. L., Sparger, E. E., Lovelace, K. M., Fitch, W. M., Pedersen, N. C., Luciw, P. A., and Elder, J. H., 1989, Nucleotide sequence and genomic organization of feline immunodeficiency virus, *Proc Natl Acad Sci USA.* 86:5743–5747.
124. Tashjian, R. J., 1984, Transmission and clinical evaluation of an equine infectious anemia herd and their offspring over a 13-year period, *J Am Vet Med Assoc.* 184:282–288.
125. Tong-Starksen, S. E., Welsh, T. M., and Peterlin, B. M., 1990, Differences in transcriptional enhancers of HIV-1 and HIV-2. Response to T cell activation signals, *J Immunol.* 145:4348–4354.
126. UNAIDS/WHO, 2003, in *Trop Doct*, Geneva, pp. 1–48.
127. Unutmaz, D., KewalRamani, V. N., and Littman, D. R., 1998, G protein-coupled receptors in HIV and SIV entry: new perspectives on lentivirus-host interactions and on the utility of animal models, *Semin Immunol.* 10:225–236.
128. Vella, C., and Daniels, R. S., 2003, CD8+ T-cell-mediated non-cytolytic suppression of human immuno-deficiency viruses, *Curr Drug Targets Infect Disord.* 3:97–113.
129. Vento, S., Di Perri, G., Garofano, T., Concia, E., and Bassetti, D., 1993, Pneumocystis carinii pneumonia during primary HIV-1 infection, *Lancet.* 342:24–25.
130. Villet, S., Bouzar, B. A., Morin, T., Verdier, G., Legras, C., and Chebloune, Y., 2003, Maedivisna virus and caprine arthritis encephalitis virus genomes encode a Vpr-like but no Tat protein, *J Virol.* 77:9632–9638.
131. Vodros, D., Thorstensson, R., Biberfeld, G., Schols, D., De Clercq, E., and Fenyo, E. M., 2001, Coreceptor usage of sequential isolates from cynomolgus monkeys experimentally infected with simian immunodeficiency virus (SIVsm), *Virology.* 291:12–21.
132. Watson, R. R., 1989, Murine models for acquired immune deficiency syndrome, *Life Sci.* 44:iii–xv.
133. WHO, 1990, Report of a WHO informal consultation on animal models for evaluation of drugs and vaccines for HIV infection and AIDS. W.H.O., Geneva, 14–15 September 1989, *Biologicals.* 18:225–233.
134. Wofsy, C. B., Cohen, J. B., Hauer, L. B., Padian, N. S., Michaelis, B. A., Evans, L. A., and Levy, J. A., 1986, Isolation of AIDS-associated retrovirus from genital secretions of women with antibodies to the virus, *Lancet.* 1:527–529.
135. Wong, P. K., Prasad, G., Hansen, J., and Yuen, P. H., 1989, ts1, a mutant of Moloney murine leukemia virus-TB, causes both immunodeficiency and neurologic disorders in BALB/c mice, *Virology.* 170:450–459.
136. Yang, O. O., Kalams, S. A., Trocha, A., Cao, H., Luster, A., Johnson, R. P., and Walker, B. D., 1997, Suppression of human immunodeficiency virus type 1 replication by CD8+ cells: evidence for HLA class I-restricted triggering of cytolytic and noncytolytic mechanisms, *J Virol.* 71:3120–3128.
137. Yokomaku, Y., Miura, H., Tomiyama, H., Kawana-Tachikawa, A., Takiguchi, M., Kojima, A., Nagai, Y., Iwamoto, A., Matsuda, Z., and Ariyoshi, K., 2004, Impaired processing and presentation of cytotoxic-T-lymphocyte (CTL) epitopes are major escape mechanisms

from CTL immune pressure in human immunodeficiency virus type 1 infection, *J Virol.* 78:1324–1332.
138. Zenger, E., Tiffany-Castiglioni, E., and Collisson, E. W., 1997, Cellular mechanisms of feline immunodeficiency virus (FIV)-induced neuropathogenesis, *Front Biosci.* 2:d527–537.
139. Zink, M. C., Yager, J. A., and Myers, J. D., 1990, Pathogenesis of caprine arthritis encephalitis virus. Cellular localization of viral transcripts in tissues of infected goats, *Am J Pathol.* 136:843–854.
140. Zuber, B., Bottiger, D., Benthin, R., ten Haaft, P., Heeney, J., Wahren, B., and Oberg, B., 2001, An in vivo model for HIV resistance development, *AIDS Res Hum Retroviruses.* 17:631–635.

3

Chemokines and Their Receptors and the Neuropathogenesis of HIV-1 Infection

HOWARD E. GENDELMAN, ERIC R. ANDERSON, LOYDA MELENDEZ, and JIALIN ZHENG

1. INTRODUCTION

Infection of the central nervous system (CNS) by the human immunodeficiency virus (HIV) causes a broad spectrum of behavioral, motor, and cognitive dysfunctions. In its most severe form, HIV-1-associated dementia or HAD, occurs late in viral infection, often associated with profound immunosuppression. Disease is perpetuated by cellular and viral neurotoxins produced from brain mononuclear phagocytes (MP; macrophages and microglia) without direct infection of neurons. Such MP neurotoxins are, in measure, homeostatic immune products that negatively affect neuronal function when produced in abundance. Induction of disease through metabolic events perpetrated by pro-inflammatory cytokines, chemokines, platelet-activating factor, arachidonic acid and its metabolites, nitric oxide, quinolinc acid, glutamate viral structural, and regulatory proteins makes the neuropathogenesis of HIV infection unique. Indeed, neuronal damage

HOWARD E. GENDELMAN • Center for Neurovirology and Neurodegenerative Disorders, Departments of Pharmacology and Experimental Neuroscience Pathology and Microbiology and Internal Medicine, University of Nebraska Medical Center, Omaha, NE 68198-5880 ERIC R. ANDERSON • Department of Microbiology and Specialized NeuroSciences Program, University of Puerto Rico Medical Sciences Campus, San Juan, Puerto Rico 00936-5067 LOYDA MELENDEZ • Department of Microbology and Specialized NeuroSciences Program, University of Puerto Rico Medical Sciences Campus, San Juan, Puerto Rico 00936-5067 JIALIN ZHENG • Center for Neurovirology and Neurodegenerative Disorders, Departments of Pharmacology and Experimental NeuroScience and Pathology and Microbiology, and Pharmacology, University of Nebraska Medical Center, Omaha, NE 68198-5880

is induced directly by the toxins engaging specific receptors or indirectly through widespread MP inflammatory activities. Chemokines play a central role in such a metabolic encephalopathy. A chemokine gradient established from brain MP and astrocytes initiate monocyte transendothelial migration, increases the viral reservoir, and provides cell sources for inflammatory products. Chemokines and viral products cause neuronal dysfunction by engaging neural chemokine receptors then activating pathways that alter synaptic transmission. The elucidation of mechanisms involved in chemokine-mediated neural compromise will likely provide unique insights into HAD pathogenesis, but also to a wide range of neurodegenerative disorders.

The state of "immune privilege" within the CNS is likely an evolutionary adaptation (Streilein, 1995). Support for such CNS privilege comes from multiple sources, including the prolonged survival of allografts (Rao et al., 1989; Poltorak and Freed, 1991) in the brain and the unique blood brain barrier (BBB). Certainly, the maintenance of nervous system function requires protection from a variety of insults occurring during inflammatory activities, such as HIV-1 encephalitis (HIVE). Interplay between the peripheral and CNS immune systems is operative and leads to HIV penetration into the brain from blood and the development of HIV-1 encephalitis HAD (Garden, 2002; Anderson et al., 2002).

HAD manifests during the later stages of viral infection and virus-induced immunosuppression as a spectrum of neurological and psychiatric symptoms (Marder et al., 1996; Kieburtz and Schiffer, 1989; Navia et al., 1986a; Gendelman et al., 1994). Behavioral, motor, and sensory impairments are present at varying degrees, but deficits in cognition predominate. The clinical syndrome usually occurs late in the course of disease and rapidly evolves from subtle cognitive changes (forgetfulness and apathy) and physical slowing, to florid memory loss, behavioral abnormalities, incontinence, hallucinations, seizures, coma, and in the very latest stages death (Gelbard and Epstein, 1995; Janssen et al., 1991; Navia et al., 1986a). Although the incidence of HAD has dropped dramatically after the introduction of active antiretroviral therapy, from 20% adults and 50% children, to less than 10% of all infected subjects (Sacktor et al., 2001), neurological dysfunction remains prevalent at or above previous levels. Clearly, resistance to antiretroviral therapy continues to grow with viral strain mutations that parallel the impaired ability of drugs to penetrate the BBB. This suggests that HAD will continue to be a significant complication of advanced HIV-1 disease (Carpenter et al., 2000; McArthur et al., 1999; Krebs et al., 2000).

HAD is commonly associated pathologically with a multinucleated giant cell HIVE (Navia et al., 1986a, 1986b). Importantly, it occurs in most, but not all, cases of dementia related to HIV-1 infection. Immune activation and productive viral replication of mono- and multinuclear phagocytes

(MP; perivascular and parenchymal brain macrophage and microglia) with the formation of microglial nodules, reactive astrocytosis, myelin pallor, and neuronal dropout characterize viral encephalitis. Moreover, brain inflammation produced as a consequence of MP infection produces a chemotactic gradient resulting in continuous monocyte transendothelial migration and BBB compromise during disease (Anderson et al., 2002; Cho and Miller, 2002; Boven et al., 2000; Williams and Hickey, 2002).

The principal productively infected cells in the brain are MP. Astrocytes and brain endothelial cells are rarely infected, if at all (Tornatore et al., 1991; Moses et al., 1993; Ranki et al., 1995; Nath et al., 1995; Ma et al., 1994). MP neurotoxic products include cellular and viral proteins produced as a consequence of productive viral infection and immune activation (Aquaro et al., 2000; Conant et al., 1998; Gabuzda et al., 1998; Gendelman, 1997; Glass et al., 1995; Koenig et al., 1986; Nath and Geiger, 1998; Perno et al., 1994; Strizki et al., 1996; Wiley et al., 1986; Zheng and Gendelman, 1997). A significant astrogliosis is demonstrated in brain areas with axonal and dendritic damage. The operative mechanisms by which MP become activated and cause neuronal damage are incompletely understood. This chapter describes what is known about MP activation and neuronal dysfunction with a focus on chemokines.

Why the focus on chemokines and why do they play such an integral role in the pathogenesis of HAD? Chemokines (chemotactic cytokines) and chemokine receptors are an important part of the immune response that affect cell migration, activation, and tissue homeostasis (Shields and Adams, 2002; Richard et al., 2002). Functionally, following local production, chemokines induce leukocyte cytoskeletal changes, for example actin polymerization, affecting cell migration to areas of microbial infection or degeneration. Chemokines also induce leukocyte differentiation from stem cells in bone marrow, thymic differentiation of T lymphocytes, as well as being involved in angiogenesis and tumor development (Shields and Adams, 2002; Richard et al., 2002).

Within the past several years the links between chemokines, chemokine receptors, and HIV pathogenesis have become both clear and significant (Feng et al., 1996; Cocchi et al., 1995; Dragic et al., 1996; Deng et al., 1996; Michael, 2002; Rizzardi and Pantaleo, 2002; O'Brien et al., 2002). Chemokine receptors play a critical role in the early stages of HIV cell entry including protection against HIV infection. Chemokine receptors CCR5 and CXCR4 are the major coreceptors for viral entry into CD4+ cells (Feng et al., 1996; Cocchi et al., 1995; Dragic et al., 1996; Deng et al., 1996). These observations have elicited intense interest into chemokine biology. In a parallel series of investigations, chemokines and chemokine receptors have been shown to play a critical role in the development of the nervous system and in disease (Cho and Miller, 2002). Indeed, the inflammatory response manifested in the brain during HIV-1 infection of the

nervous system leads to the development of a chemo attractant gradient, resulting in the formation of a MGC in HIVE (Williams and Hickey, 2002). This enables inflammatory monocyte-derived macrophages (MDM) to enter the brain, become infected, and expand the sources of neurotoxic secretory factors that lead to the pathological and clinical aspects of disease (Gendelman et al., 1997). Chemokine receptors are critical for infection in perivascular macrophages and microglia. It has also been shown that chemokines and their receptors play a more direct role in the neuropathogenesis of HIV-1 infection. It is now clear that neurons and glia express chemokine receptors, and the interactions of HIV-1 gp120 with neuronal chemokine receptors can lead to apoptosis of neurons (Cho and Miller, 2002; Cotter et al., 2002; Ryan et al., 2002). These effects may be regulated by chemokines that act on the same receptors. The presence of chemokine receptors on neural cells also supports the notion that chemokines regulate neuronal physiological functions. These ideas in the context of the neuropathogenesis of HIV infection will be the focus of this chapter in the greater context of HIV infection of the nervous system.

2. HIV NEUROPATHOGENESIS

The pathogenesis of HAD revolves around the numbers of virus-infected and immune-competent brain MP (Glass et al., 1995) and the resultant secretion of a plethora of neurotoxins. Viral infection and subsequent immune activation of MP act as a catalyst for the secretion and amplification of factors that cause neuronal damage and subsequent cognitive dysfunction. Although the importance of MP activation in HAD pathogenesis has been widely recognized, the process by which it occurs remains undefined. One likely scenario is that HIV-1 infection predisposes MP to immune activation through intracellular events. These events lower the cytosolic signal transduction threshold necessary for macrophage activation and subsequent production of secretory factors (Genis et al., 1992; Nottet and Gendelman, 1995; Persidsky et al., 1996; Cotter et al., 1999b). In this way, HIV "primes" the cell to become readily activated upon future immune stimulation. HIV-1 proteins, Tat and gp120, can engage MP receptors to induce intracellular events, which either alter the threshold for, or induce, activation *de novo* (Jones et al., 1998; Nath et al., 1999; Lipton, 1994; Conant et al., 1998; New et al., 1998; Perry et al., 1998).

Once infected, it is also possible that MP become activated in response to a peripheral immune challenge. This activation results in secretion of chemokines and cytokines, which may impair BBB function and subsequently cause widespread inflammation in the brain (Fiala et al., 1998; Nottet et al., 1996; Persidsky et al., 1997, 1999). Such events, combined with changes in cellular migratory capabilities and upregulated adhesion

molecules, augment leukocyte transendothelial migration into the CNS and amplify the cellular sources of toxic molecules including virus and viral proteins in the nervous system (Ghorpade et al., 2001; McManus et al., 2000; Nottet et al., 1996; Weiss et al., 1999; Wu et al., 2000). These observations clearly underline the importance of chemokines in their abilities to affect recruitment and activation of monocytes and macrophages during HAD.

Activated MP secrete an abundance of cellular and viral neurotoxins. These include glutamate (Jiang et al., 2001), arachidonic acid (Nottet et al., 1995), platelet activating factor (PAF) (Gelbard et al., 1994), tumor necrosis factor alpha (TNF-α) (Gelbard et al., 1993; Talley et al., 1995), interleukin-1 beta (IL-1β), quinolinic acid (Heyes et al., 1991; Kerr et al., 1998), NTox (Giulian et al., 1996), and nitric oxide (NO) (Adamson et al., 1996). Viral neurotoxic proteins include HIV-1 gp120 (Brenneman et al., 1988), gp41 (Adamson et al., 1996) and Tat (Ma and Nath, 1997; Magnuson et al., 1995; Nath et al., 1996; New et al., 1997, 1998; Shi et al., 1998). Secreted excitatory amino acids (EAA) can induce excitotoxicity in neurons. Induced cytotoxicity results in excessive calcium influx and the overstimulation of glutamate receptors, resulting in NO and superoxide anion (free radical) formation and subsequent apoptosis. It is believed that multiple effector mechanisms, from both microglia and astrocytes, are operative in disease and explain the diffuse compromise of neural function characteristic of HAD (Gendelman et al., 1997; Lipton and Gendelman, 1995; Williams and Hickey, 2002; Garden, 2002).

HIV enters the brain early after viral exposure (Price, 1993). In experimental simian immunodeficiency virus (SIV) infection, viral entry into the brain occurs between a few days to a few weeks after viral inoculation (Chakrabarti et al., 1991; Davis et al., 1992; Lackner et al., 1994). However, the development of clinical neurological disease follows after years of progressive viral infection, often coinciding with profound immunosuppression (Price, 2000). This suggests that viral replication is *necessary* but not *sufficient* to induce clinical neurological injury. The mechanism by which the virus actually enters the brain is not exactly defined. Several possibilities include the idea that a free virus infects the brain endothelial cells or that a virus gains entry to the CNS directly by infecting intermediate cells. Alternatively, the virus is carried into the brain through infected and immune competent leukocytes (Williams and Hickey, 2002). While most patients with HAD and advanced immunosuppression have high levels of virus in their brains (Wiley and Achim, 1994; Achim et al., 1994), absolute levels of virus in the brain or cerebral spinal fluid (CSF) do not directly correlate with the presence or severity of neurologic disease (Wiley and Achim, 1994; Kure et al., 1990). This suggests that both viral and cellular factors amplify the production of one another, and play prominent roles in the disease onset and progression. Additionally, there is still no clear

explanation why only a subset of patients develop HAD and others remain unaffected. Whether this revolves around host or viral factors, or both, remains unanswered. Viral replication likely precipitates neuroimmune activation that occurs in the face of a profound CD4+ T-cell depletion (Garden, 2002). Such processes instigate, in some individuals, brain macrophage activation with secretory activities, affecting the transendothelial migration of monocytes. Ultimately, an expanded viral reservoir in the brain is produced. Reseeding of the brain by HIV-1 late in the disease with a profound infiltration of MP into the CNS likely heralds an expansion in viral load and subsequent immunological activation. Nonetheless, the quantity of HIV gene expression in the brain does not always correlate with similar degrees of neurological impairments. As HIV disease progresses there are inherent changes in both the secretory and migratory capabilities of macrophages, in the periphery and the brain, that allow such processes to continue unabated (Anderson et al., 2002; Boven et al., 2000). In support of this theory, recent works have demonstrated the emergence of specific monocyte subsets in patients with HAD. Identified by their cell surface markers, CD14/CD16 and CD14/CD69, positive monocytes may exhibit enhanced migratory and neurotoxic potential (Pulliam et al., 1997; Williams et al., 2001; Fischer-Smith et al., 2001). These characteristics may make them crucial to disease pathogenesis. The potential importance of peripheral immune activity in HAD pathogenesis is supported by evidence of enhanced monocyte trafficking during late-stage HIV disease. Chung and colleagues (2002) demonstrated that potassium channels, expressed in macrophages, are vital for MP movement through the BBB. This study also demonstrated that monocyte/macrophage migration could be inhibited by voltage- and calcium-activated potassium channel blockers. The importance of these findings are that alterations in cell volume, differentiation, and shape represent pivotal factors in macrophage migration into and throughout the brain, and may be affected by the immune environment that occurs during progressive HIVE/HAD. HIV-1 gp120 activates multiple ionic macrophage signaling responses mediated by CCR5 and CXCR4 (Liu et al., 2000). The upregulation of soluble markers of monocytes and monocyte activation in the plasma of infected patients with cognitive dysfunction and brain atrophy further supports the importance of peripheral monocyte activation in cell trafficking and, in general, HAD pathogenesis (Ryan et al., 2001b).

Mononuclear Phagocytes

As clearly described above, MP are the principal target cells for HIV infection in the brain and a significant source of neurotoxins during disease (Genis et al., 1992; Koenig et al., 1986; Gabuzda et al., 1986). However, under steady-state conditions, MP function as scavenger cells to eliminate microbial pathogens and other foreign material through phago-

cytosis and intracellular killing, as well as adaptive immune mechanisms. They can serve an important innate immune function by their secretion of trophic factors required for tissue homeostasis. In the brain, macrophages nurture neural cells, secreting neurotrophins, which provide the necessary environment for neurons to function normally and thrive (Gendelman and Folks, 1999). For example, MPs secrete brain derived neurotrophic factor, nerve growth factor, neurotrophin-3, glial-derived neurotrophic factor, and basic fibroblast growth factor (Batchelor et al., 1999; Caroleo et al., 2001; Kullander et al., 1997; Elkabes et al., 1996; Heese et al., 1998; Zheng et al., 2001c; Rapalino et al., 1998; Lazarov-Spiegler et al., 1996, 1998a, 1998b; Zeev-Brann et al., 1998). This normal innate immune function of MP has been used to therapeutic advantage during neurodegenerative disorders (Rapalino et al., 1998). It remains unclear, however, how the macrophage evolves from a neurotrophic cell to a neurotoxic cell.

Recent works suggest specific signaling pathways arise following MP immune activation, which can regulate protective or destructive innate immune function of macrophages (Shibata et al., 2003). Indeed, during HIVE and HAD, viral infection and immune activation of MP could serve to change a protective cell to a destructive one, leading to neuronal injury and ultimately cell death. Perturbations in cell signaling pathways may underlie such molecular events. This is highlighted by the fact that neurotrophins engage Trk receptors, expressed on neural cells, then activate signaling pathways by phosphorylation of cytoplasmic tyrosine residues. Trk converts Ras to an active conformation, inducing the MAP kinase cascade (ERK1/2), which is involved in cellular transcription and growth (Segal and Greenberg, 1996; Shi et al., 1996; Soontornniyomkij et al., 1998; Zheng et al., 2001c).

Three distinct MP are found in the brain parenchyma and are targets for virus. They include ramified "resting" microglia, activated "amoeboid" microglia, and perivascular macrophages. Microglia represent up to 10% of the parenchymal brain cell population in some regions. Neighboring elliptical microglia contact each other in series and in parallel, forming a neural network. Unlike microglia, parenchymal and perivascular macrophages usually appear amoeboid and, in morphology and function, these cells more closely resemble tissue macrophages in other organs. Recent studies, by several groups, support the idea that perivascular macrophages are preferentially infected and pose the greatest threat as vehicles of dissemination of virus and sources of neurotoxic activities (Pulliam et al., 1997; Rappaport et al., 2001; Williams et al., 2001; Williams and Hickey, 2002).

Multiple mechanisms have been proposed for the initiation of brain inflammatory responses during HAD (Anderson et al., 2002; Cotter et al., 2002; Zheng and Gendelman, 1997). Monocytes express the TNF-family activation receptor CD40 and activated T-cells express CD40 ligand (CD40L). Binding of soluble CD40L activates infected and uninfected

monocyte lineage cells. Decline in the CD4+ T lymphocytes allows macrophages to express a metabolically active, tissue-destructive phenotype. Alternatively, circulating pro-inflammatory cytokines trigger monocyte activation independent of lymphocytes. Indeed, late-stage HIV-1 patients with dementia show elevated serum TNF receptors (Ryan et al., 2001a). Whatever the mechanism, when activated, brain macrophages secrete a variety of neurotoxic immune and viral factors. Functionally, changes in macrophage immunity lead to gp120-mediated cell fusion, MGC formation, disruption of neuronal homeostasis, and ultimately disease. However, the role of macrophages in disease persistence and pathogenesis is not limited to the brain or CNS. Submucosal macrophages in the gut and cervix are thought to be the first cells infected by the virus (Zhu et al., 1996). It is likely that these cells sustain the viral population throughout the course of the disease and are a common link for viral dissemination in the lymph nodes and inevitably to the brain.

Astrocytes

In prior years, astrocytes were believed to lack functional excitability, having no role in signal integration. Their role was considered solely as supporters of nerve cell structure and function. Certainly, during steady state conditions astrocytes contribute to the structural scaffolding of both neurons and glia and participate in neuronal sustenance (Haydon, 2001). However, recent works also demonstrate that astrocytes participate in synaptic integration by releasing glutamate through calcium regulated exocytosis-like processes. This process may follow activation of CXCR4 through the stromal cell-derived factor 1 (SDF-1) (Bezzi et al., 2001). In contrast, astrocyte impairment may result in the release of excessive glutamate, resulting in neuronal injury. Altered glutamate transport, by activated astrocytes, results in inhibition of glutamate uptake and to its enhanced secretion. Such events may lead to neuronal excitotoxicity and apoptosis (Bezzi et al., 2001). They may play a prominent role in the disease by virtue of being the most abundant cell type in the CNS, through their homeostatic functions and their close juxtaposition to neurons. After exposure of astrocytes with TNF-α, IL-1β, or phorbol esters, latent infection may become reactived (Tornatore et al., 1994). Potentially, but not yet proven, astrocytes may provide HIV with a sanctuary protecting the virus against antiretroviral therapy. Astrocytes, along with MP, may also serve as reservoirs for HIV within the CNS. They are infected, albeit at restricted levels, and are capable of transferring infection to MP (Brack-Werner, 1999).

Astrocyte infection by HIV is CD4-independent (Sabri et al., 1999). Expression of CCR5, CCR3, and CXCR4 are, however, found on astrocytes (Boutet et al., 2001). Nonetheless, astrocyte cell lines can be infected independent of both CD4 and CXCR4 (Schweighardt et al., 2001). Astrocytes

produce a number of chemokines, including interleukin-8 (IL-8), monocyte chemotactic protein-1 (MCP-1), and regulated upon activation normal T-cell expressed and secreted (RANTES) in response to the pro-inflammatory cytokines IL-1β and TNF-α (Hesselgesser and Horuk, 1999) that may exacerbate disease pathogenesis. These chemokines can attract HIV-infected peripheral blood lymphocytes and monocytes to the brain. It has been reported that viral gene products, such as Tat, can induce MCP-1 production by the astrocytes, facilitating monocyte transmigration across the blood-brain barrier (Chauhan et al., 2003).

It is not known, however, whether alterations in astrocyte phenotype and function are beneficial or detrimental in regards to neuronal function. The cumulative excitotoxic effects perpetuated by activated astrocytes may substantially increase the amount of neuronal damage caused by HIV infection. The production of chemokines, such as MCP-1, by endothelial cells and astrocytes, affects the migration of leukcoytes across the BBB and is critical to HAD pathogenesis. MCP-1 is a chemoattractant that clearly accelerates monocyte entry into the brain. Astrocytes are the major source of MCP-1, whose levels may be induced after glial exposure to cellular (TNF-α, IFN-γ and IL-1β) or viral (for example Tat) neurotoxins (Weiss et al., 1998).

Secretion of other soluble neurotoxic factors by astrocytes can lead to paracrine neuroimmune activation, an upregulation of apoptotic factors such as Fas and Fas ligand (FasL), and, ultimately, increased neuronal cell death by distinct mechanisms. Indeed, increased FasL expression has been observed in lymphocytes, monocytes, and CSF in HIV-1 infected patients. It is not yet known whether FasL-mediated neurotoxicity is part of the pathogenic process of neural damage during HIVE/HAD (Ghorpade and Gendelman, 2003).

Blood Brain Barrier (BBB)

Under steady-state conditions, the BBB serves to regulate passage of immune cells and immune products. During the disease, disruption of the BBB occurs through the upregulation of adhesion molecules on the surface of the brain's microvascular endothelial cells (BMVEC), as well as by the alteration of cell morphology, permitting entry of cells and immune factors that would normally be excluded. Increased expression of intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule (VCAM), among others, as a consequence of brain inflammation, instigate the passage of monocytes into the brain parenchyma (Nottet et al., 1996). Despite the considerable immune activation that occurs during advanced HIV-1 infection throughout most of the course of viral infection, disruption of the BBB remains minimal. It is only very late in the disease that the BBB integrity and function break down.

A prominent neuropathological finding with HIV infection is perivascular infiltration of monocytes across the BBB (Price et al., 1998). Postmortem studies of HIVE patients have revealed that the integrity of the BBB was damaged, as demonstrated by fibrinogen leakage (Petito and Cash, 1992). A likely scenario involves the "priming" of macrophages by viral infection and lowering of the signaling threshold necessary for activation (Genis et al., 1992; Nottet et al., 1995), facilitating BBB migration. Indeed, MP activation results not only in the secretion of cytokines and chemokines that can impair the BBB but also in the upregulation of adhesion molecules, which facilitate cell trafficking into the brain (Nottet et al., 1996).

Upon entry into tissue and differentiation into macrophages, the cells become targets for viral infection. Undifferentiated peripheral monocytes are infected at low frequency and are present in the circulation for limited time periods, usually measured in hours for lentiviral infections (Gendelman and Gendelman, 1992; Gendelman et al., 1984, 1985, 1986). Virus is carried into the brain by MDM or CD4+ T lymphocytes during disease. Free progeny virus can cross the BBB and infect MDM in the perivascular space. Endothelial activation during progressive HIV-1 infection has been postulated to allow migration of MDM into the CNS. During disease, a principal component for how virus-infected monocytes and macrophages penetrate the BBB is through their abilities to change shape and volume. The ability of cells to swell then restore their cell volume to normal upon exposure to hyposmicity is achieved by the efflux of potassium and chloride channels with consequent loss of water. Such ion channels appear to be regulated by immune activation and viral infection and facilitate the movement of infected cells across the BBB in disease. Regardless of whether infection is established in endothelia, HIV-1 may cross the BBB as a free progeny virus. This may be accomplished through the ability of HIV-1 gp120 to induce viral adsorptive endocytosis and transcytosis in brain endothelia.

Chemokines appear to be key regulators of monocyte recruitment during HAD. Endothelial cells, microglia, and astrocytes are major cellular sources of β-chemokines (for example, macrophage chemotactic factor-1 or MCP-1) in the CNS, whose production during HIVE sets up an inflammatory chemo-attractant gradient. β-chemokines bind to chemokine receptors on monocytes, regulating cell transendothelial migration into the brain. One brain chemokine, fractalkine, can promote adhesion and migration of leukocytes across the BBB (Imai et al., 1999; Tong et al., 2000). Neurons, astrocytes, and endothelial cells express fractalkine (Cotter et al., 2002; Tong et al., 2000) and are likely to also play a role in the recruitment of monocytes into the brain.

Several questions remain about how HIV-1 infected MDM enter the brain, including: (1) the exact identification and relative abundance of chemokines produced by macrophages, astrocytes, and other inflammatory

cells; (2) the pathobiological conditions under which chemokines are produced; (3) paracrine and autocrine regulation of chemokines and pro-inflammatory cytokines in microglia, astrocytes, endothelial cells, and neurons; and (4) the role of chemokines in glial activation and HIVE.

Infected monocytes, macrophages, and perhaps CD4+ T lymphocytes act as vehicles through which a virus enters the brain. Microglia and astrocytes produce chemokines and control monocyte migration across the BBB (Persidsky et al., 1999). Activated microglia, and to a lesser extent astrocytes, express MHC class I and II antigens, adhesion molecules, secrete cytokines, and reactive oxygen intermediates. All are shown to be important factors that contribute to HAD (Lipton and Gendelman, 1995). Although the event(s) that triggers monocyte invasion into the nervous system remains unknown, it likely involves the secretion of macrophage attractant chemokines and the upregulation of adhesion molecules on activated endothelial and immune cells. α and β chemokines, such as IL-8, interferon gamma inducible protein 10 (IP-10), growth-related oncogene α (GRO-α), macrophage inflammatory protein 1α (MIP-1α), MIP-1β, RANTES, and MCP-1, are induced by pro-inflammatory factors and are found in infected brain tissue. These factors may also participate directly in the disease process. The entry of virus and activated macrophages into the brain is likely precipitated by BBB damage heralded by activation of brain MP. Neuronal damage as well as alterations in the integrity of tight junction or regulation of its immune function occur as a consequence of viral and cellular secretory products. These events are crucial to HIV-1 brain transport.

Endothelial cells that comprise the BBB can secrete a variety of chemokines themselves upon stimulation by the pro-inflammatory cytokines, including MCP-1, MIP-1α, RANTES, MGSA, IP-10, MIG, and fractalkine or neurotactin. Endothelial cells also express CXCR4 and CCR5, whose expression may change dependent upon the cytokine environment. For example, pro-inflammatory cytokines upregulate chemokines and their receptors by endothelial cells, facilitating the entry of HIV into the brain. All together, leukocyte migration from the blood to the brain is a complex process involving rolling and tight adhesion and transendothelial migration. Depending on the stage of brain endothelial cell activation and interactions with cell factors produced as a consequence of leukocyte secretory activities, monocytes and some lymphocytes enter the brain parenchyma during disease.

3. CHEMOKINES: AN OVERVIEW

*Chemo*tactic cyto*kines*, or chemokines, is a large family of structurally related proteins that affect numerous biological functions. They are small (8–10 kd) proteins that are produced by a variety of different cell types,

including blood leukocytes, endothelial cells, glia, and neurons. A number of factors affect their regulation, including bacterial lipopolyaccharide (LPS), pro-inflammatory cytokines, and viruses. Chemokines are classified into four families, based on the number and positions of the N-terminal-conserved cysteine residues (Shields and Adams, 2002; Richard et al., 2002). Indeed, their classification depends on the relative position of cysteine residues within the protein. They exert their effects by activating a family of seven transmembrane G-protein coupled receptors (GPCR). These receptors are also divided into four groups: α-chemokine receptors (such as CXCR2 and CXCR4); β-chemokine receptors (such as CCR5, CCR4, CCR3, and CCR2); γ-chemokine receptor (XCR1); and δ-chemokine receptors (CX3CR1) (Gabuzda et al., 1998; Hesselgesser and Horuk, 1999; Klein et al., 1999; Miller and Meucci, 1999; van der Meer et al., 2000). Produced copiously in all tissues including the brain (Kutsch et al., 2000), these factors regulate the migration, recruitment, accumulation, and activation of leukocytes (Wu et al., 2000).

Since the identification of IL-8, nearly 15 years ago, over 40 chemokines have been identified (Shields and Adams, 2002). Certainly, chemokines are part of a large family of molecules with important physiologic functions, ranging from development and cell growth to angiogenesis and neoplasia, cellular migration, inflammation, and in interactions with microglial pathogens (Baggiolini et al., 1997). Chemokines are composed of 92–125 amino acids with four conserved cysteines linked by disulfide bonds. Four subfamilies are designated as CC, CXC, C, and CX3C. The prefix CC designates the chemokines with two adjacent cysteines, while CXC designates chemokines that contain two cystines separated by one amino acid. The human chemokine genes are clustered on chromosomes 4 (CXC chemokines) and 17 (CC chemokines). The CXC chemokines include IL-8, MGSA, IP-10, and SDF-1. The CC class includes RANTES, MCP-1, and MIP-1αβ. Fractalkine (FKN) is part of the CX3C group. The group of C chemokines includes lymphotactin. Each of the chemokines recognizes a particular subset of leukocytes. IL-8 and MGSA attract neutrophils, SDF-1 and IP-10 attract T lymphocytes, eotaxin attracts eosinophils, and RANTES and MCP-1 attract T-cells and monocytes (Schall and Bacon, 1994). The chemokines act on their target cells by binding to cell surface chemokine receptors. Chemokine receptors belong to the superfamily of receptors that signal through the GTP-binding, seven transmembrane proteins (Baggiolini et al., 1997). Table I provides a summary of Chemokines.

Chemokine receptors are categorized into four different groups: shared, specific, promiscuous, and viral (Premack and Schall, 1996). Most of the chemokine receptors can bind more than one chemokine ligand belonging to the same chemokine subfamily (shared group). The promiscuous receptors bind to a chemokine ligand from different subfamilies.

TABLE I
Chemokines and Their Receptors in the Brain

Cell Type	Chemokines	Receptors	Reference
Monocytes/ macrophages	MCP-1 MIP-1α/β RANTES IL-8, IP-10	CCR2, CCR3, CCR4, CCR5, CXCR4, CX3CR1	(Bernasconi et al., 1996, Cinque et al., 1998, Kelder et al., 1998, Collman et al., 2000, Gabuzda et al., 2002)
Microglia	MIP-1α/β RANTES MCP-1,3 IL-8, IP-10	CCR2, CCR3, CCR4, CCR5, CXCR3, CXCR4, CX3CR1	(Bernasconi et al., 1996, Cinque et al., 1998, Kelder et al., 1998)
Astrocytes	MCP-1, MIP-1α/β RANTES, SDF-1α, IL-8, IP-10, Fractalkine	CCR2, CCR3, CCR4, CCR5, CXCR4	(Conant et al., 1998, Zheng et al., 1999b, 2000)
Neurons	Fractalkine, MCP-1	CXCR4, CX3CR1, CXCR2, CCR5, DARC	(Coughlan et al., 2000, Zheng et al., 2000)
Endothelium	IL-8, Fractalkine, IP-10, MGSA, MIG, MCP-1, 3, 4	DARC, CXCR4, CCR5, DARC	(Gabzuda et al., 2002)

Two viruses encode chemokine receptors: CMV (US28) and herpes virus saimiri (ECRF3).

Chemokines and HIV Coreceptors

HIV-1 enters CD4+ T lymphocytes by fusion at the plasma membrane after interaction with CD4 and a coreceptor. The identity of this coreceptor was prompted by studies that showed that the β-chemokines, RANTES, MIP-1α, and MIP-1β, could block viral infectivity in vitro (Cocchi et al., 1995). However, these chemokines were only able to block viral infectivity mediated by macrophage tropic (M-tropic) viruses and did not affect entry of T-cell tropic strains of a virus. It was demonstrated that the chemokine receptor CXCR4, initially called Fusin or Lestr, blocked the entry of T-tropic strains (Feng et al., 1996). The biological function of this receptor is to bind SDF-1 (Bleul et al., 1996a, 1996b; Oberlin et al., 1996). All HIV strains studied to date use one or both of these molecules for viral entry (Edinger et al., 1999; Berger and Major, 1999). The genetic polymorphism in the HIV envelope glycoprotein gp120 determines which coreceptor is used and thus determines the type of host cell that is preferentially infected.

HIV-1 is neuroinvasive and enters the CNS early in infection. However, defining the precise molecular and biological bases for viral neurotropism still eludes investigators. The principal issue remains the ability to separate M-tropic from neuro-tropic signatures (Lipton and Gendelman, 1995; Gabuzda and Wang, 1999). Indeed, M-tropic virus can be isolated from brain tissue throughout HIV disease (Brew et al., 1990, 1996a, 1996b; Brew and Miller, 1996). Such isolates replicate well in cultured human microglia from both adult or fetal tissue (Strizki et al., 1996; Ghorpade et al., 1998a) and induce neurotoxic responses (Genis et al., 1992). Nonetheless, HIV isolates that propagate in MDM also infect microglia. Such dual tropism is consistent with previous molecular analyses for HIV-1 envelope, where determinants for MDM and microglial tropism map to identical regions of the genome (Sharpless et al., 1992). The tropism is largely determined by the V3 hypervariable region of gp120, a viral surface protein. The V3 function is distinct from the gp120–CD4 requirement and more associated with post-CD4 binding interactions, such as, proteolytic cleavage and fusion.

The importance of the V3 loop sequences determining tropism has also been controversial. The residues thought to be critical for neurovirulence were also found in the majority of nondemented patients (Reddy et al., 1996). The proline determinant at position 305 that was associated with neurovirulence was absent in brain-derived isolates (Di Stefano et al., 1996). The V1/V2 region may also contribute independently or in concert with V3. Phylogenetic studies done on the V3 envelope of strains derived from all brain regions indicated that there is an independent regional evolution of HIV quasispecies after it crosses into the brain, and there are sequences associated with regional neuropathology (Chang et al., 1998; Di Stefano et al., 1996).

T-tropic strains are associated with the α-chemokine receptor CXCR4. However, T-cells may be infected by either M- or T-tropic strains. For MDM and microglia the pathways for viral infection are similar if not identical. Microglia express CD4 and a number of chemokine receptors, including CCR5, CCR3, CXCR4 (Lavi et al., 1997; Vallat et al., 1998). However, they are most susceptible to infection by R5 viruses (Lavi et al., 1997; Shieh et al., 1998). There may be qualitative differences between MDM and microglia regarding the use of chemokine receptors. Experiments showing lack of HIV inhibition by monoclonal antibodies to CCR5 and CCR3 suggest that additional coreceptors may also be used by microglia (Ghorpade et al., 1998b). Macrophage tropic strains alone may not be sufficient to cause clinical neurologic impairment. Because HIV-1 relies on chemokine receptors for entry into MP, chemokine receptor expression by brain macrophages and microglia may influence viral evolution within the brain reservoir, leading to neurovirulence.

The importance of CCR5 for HIV-1 transmission was underscored by the observation that certain individuals who had been repeatedly exposed

to HIV-1, but remained uninfected, had a defect in CCR5 expression (Liu et al., 1996a). CD4+ T lymphocytes from these individuals were highly resistant to in vitro infection of primary macrophage tropic HIV, but were readily infectable with viruses adapted to grow in transformed T-cell lines (Liu et al., 1996b). These noninfectable individuals were found to be homozygous for a defective CCR5 allele that contains an internal 32-base pair deletion (CCR5 δ32). The truncated protein encoded by this gene is apparently not expressed at the cell surface. CCR5 δ32 homozygous individuals comprise 1% of the Caucasian population, and heterozygous individuals comprise 20%. Individual who are heterozygous for the deletion have been shown to progress more slowly to AIDS than wild-type homozygous individuals, suggesting that CCR5 expression may be altered in these individuals, and that this affects HIV-1 replication in vivo. Additional mutations associated with HIV resistance include chemokine receptor, CCR2b, and the ligand SDF-1 (Smith, 1991; Smith and Hale, 1997; Winkler et al., 1998).

Lastly, at least nine other chemokine receptors, of structurally related molecules, have also been described as supporting HIV-1 envelope-mediated membrane fusion or viral entry in vitro. These include CCR2b (Doranz et al., 1996), CCR3 (Bazan et al., 1998), BOB/GPR15 (Farzan et al., 1997a, 1997b), BONZO/STRL33/TYMSTR (Farzan et al., 1997a, 1997b), GPR1 (Farzan et al., 1997a, 1997b), CCR8 (Horuk et al., 1998), V28/CX3CR1 (Rucker et al., 1997), and APJ (Choe et al., 1998a, 1998b). Several studies have compared coreceptor usage by primary HIV-1 isolates of several genetic subtypes, but the coreceptor usage analysis has shown that most HIV strains use CCR5 or CXCR4 efficiently (Dooms et al., 2000).

Chemokines and Neural Function

The importance of chemokine receptors in HIV neuropathogenesis was brought to the forefront when they were found expressed on neurons. It is now clear that most brain cells express chemokine receptors (Table I) and the interactions of virus with these receptors may initiate apoptotic death of neurons. This outcome may be modified by chemokines that act at the same receptors. Moerover, chemokine receptor expression on neurons suggests that these molecules affect neuronal homeostatic function (Miller and Meucci, 1999). Cultured human fetal neurons express CXCR2, CXCR4, CCR1, and CCR5 (Hesselgesser et al., 1997). This finding presents a scenario in which HIV-1 could damage neurons directly. Although HIV-1 cannot infect neurons, HIV-1 gp120 may bind to neuronal-expressed CXCR4, initiating signal transduction pathway(s), leading to cellular dysfunction or apoptosis (Hesselgesser et al., 1998a; Zheng et al., 1999a, 1999b, 1999c). This idea is supported by studies in which an inhibitor of chemokine receptors can inhibit HIV-1 gp120-induced

damage. The resultant neuronal damage may then elicit the secretion of brain derived chemokines in order to recruit macrophages to the site of injury (Erichsen et al., 2003; Cotter et al., 2002). In this way, the neurons themselves serve as a means to perpetuate the damage brought about by macrophage-produced neurotoxins.

Some chemokines such as SDF-1α and FKN are constitutively produced in the brain and likely play an important role in CNS homeostasis. Others, such as MIP-1α and MIP-1β, MCP-1, and RANTES, are induced by inflammatory stimuli. These chemokines are likely involved in the pathogenesis of a variety of neurodegenerative diseases where inflammation plays a role in pathogenesis, such as Alzheimer's disease, stroke, and HAD (Letendre et al., 1999; Minami and Satoh, 2000; Sanders et al., 1998).

During HAD, chemokines are produced at high levels by MP, astrocytes, or neurons and affect cell migration, neural signaling, apoptosis, and by inciting cascades of neuroinflammatory reactions that regulate viral replication (Albright et al., 1999; Broder and Collman, 1997; Cotter et al., 2001; Endres et al., 1996; Ghorpade et al., 1998b; He et al., 1997; Kitai et al., 2000; Lavi et al., 1997; Luster, 1998; Mackay, 1996; Shieh et al., 1998; Vallat et al., 1998; Vicenzi et al., 2000; Zheng et al., 1999b). Brain MP, astrocytes, and neurons may also directly participate as effector cells in disease pathogenesis independent of peripheral immune responses. This possibility is supported by data demonstrating that α and β chemokines, such as IL-8, IP-10, GRO-α, MIP-1α, MIP-1β, RANTES, and MCP-1, are produced in infected brain tissue by HIV-1-infected and immune-activated MP and astrocytes (Conant et al., 1998; Cotter et al., 1999a; Desbaillets et al., 1994; Kornbluth et al., 1998; Persidsky, 1999). Neuronal injury, induced by HIV-1 viral proteins or cytokines, may itself trigger chemokine secretion. Neuronal chemokines may serve as a damage signal to recruit macrophages and microglia to the site of injury, serving to stimulate brain inflammation independent of peripheral immune responses (Figure 1) (Jung et al., 2000; Tong et al., 2000; Zheng et al., 2001a; Zujovic et al., 2000; Erichsen et al., 2003; Cotter et al., 2002).

MP activation and neuronal loss lead to alterations in dendritic arbor and decreased synaptic density and are neuropathological signatures of HAD. Much work has been done in recent years to explore the mechanisms by which HIV-1 infected macrophages induce neuronal injury (Gabuzda et al., 1998; Gelbard et al., 1994; Gendelman, 1997; Lipton and Gendelman, 1995; Nath and Geiger, 1998; New et al., 1998; Shi et al., 1996, 1998). A number of questions remain: First, although altered dendritic arbor and synaptic density are major features of neuronal injury in HAD (Everall et al., 1994; Wiley et al., 1991), how they are linked to specific MP secretory activities and affected by antiretroviral and adjunctive therapies remains unclear (Aquaro et al., 1997, 1998, 2000; Fox et al., 2000; Gendelman et al., 1998; Limoges et al., 2000; Perno et al., 1988, 1994,

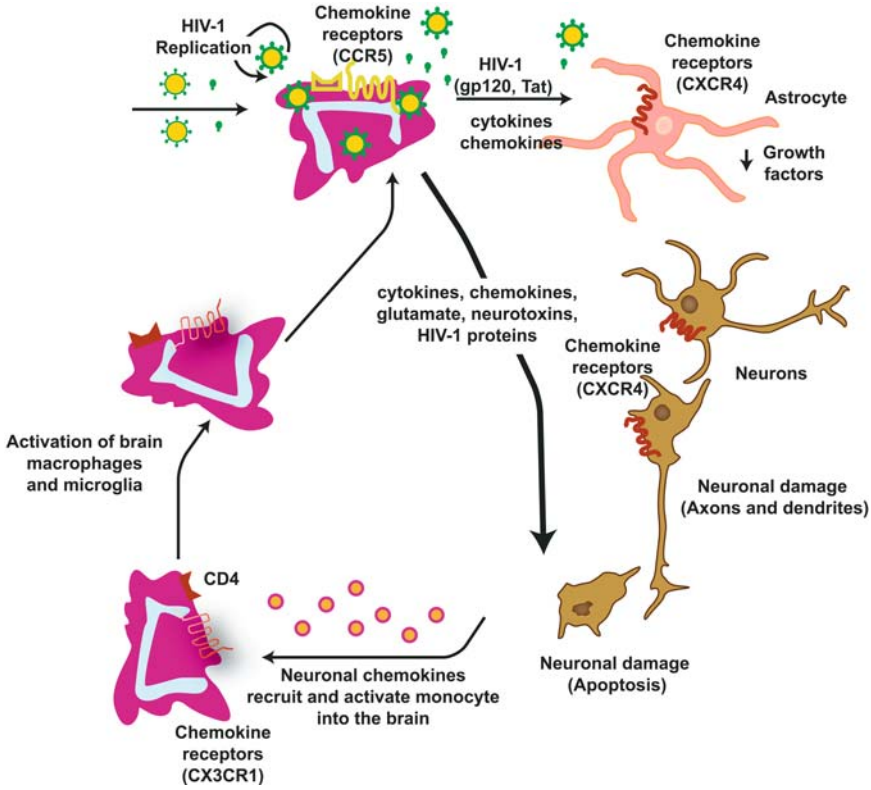

FIGURE 1. A proposed pathophysiological mechanism for how chemokines and their receptors influence the neuropathogenesis of HIV-1 infection. Chemokines are produced from activated microglia and astrocytes and affect the transendothelial migration of monocytes into the nervous system. During cell maturation, macrophages acquire the ability to sustain productive HIV-1 infection. Release of progeny virion leads to infection of resident microglia. Virus-infected MP become immune-activated by a process that remains incompletely understood, but likely involves peripheral cytokines. Activated HIV-1-infected brain macrophages secrete a variety of factors that affect neuronal function and CNS inflammation and including cytokines and α- and β- chemokines. Chemokines, HIV-gp120, Tat, and whole virions may interact with neuronal receptors to induce intracellular signal transduction alterations, the final results of which are neuronal compromise. Paradoxically, some chemokines act to affect neuronal survival, while others induce apoptosis.

1998). Second, how the normal protective functions of MP are altered or reversed following immune activation remains unclear (Lazarov-Spiegler et al., 1998a; Wiley et al., 2000; Zeev-Brann et al., 1998; Zheng et al., 2001c). Third, how mutual receptor interactions occur between HIV gp120 and chemokines could be significant in the ultimate outcome of neuronal loss versus cellular protection. Fourth, how activation of chemokine receptors

occurs in brain inflammation is critical. The process affects specific signaling events such as calcium mobilization and influx, both critical for neuronal homeostasis.

In order to address these questions and further define the relationship among HIV-1 infection, MP activation, chemokine production and neuronal demise, laboratory assays were developed to mimic HIV-1 associated neuronal injury. The effects of virus-infected or immune-activated MP secretory products on aspects of neuronal morphology were quantitatively assessed. In these assays, rat cortical, hippocampal, and human cortical neurons were exposed to secretory products from HIV-1-infected and activated human MDM. Assays for alterations in neuronal dendritic arbor and cell loss included the quantification of neurofilament (NF), neuron-specific enolase (NSE), and microtubule associated protein-2 (MAP-2) by ELISA, which demonstrated that MP produce both neurotrophic and toxic factors (Lazarov-Spiegler et al., 1998a; Wiley et al., 2000; Zeev-Brann et al., 1998; Zheng et al., 2001c; Zheng et al., 2001d). MDM conditioned media (MCM) enhanced neuronal survival and differentiation. Several of the neurotrophic factors made by MDM are beginning to be defined (Shibata et al., 2003). In contrast, MCM obtained from MDM infected with HIV-1_{ADA} and activated by LPS induced neuronal cell death, characterized by neuronal apoptosis (Zheng et al., 2001d), altered dendritic arbor and decreased neuronal density (Zheng et al., 2001c). Utilizing a spectrum of HIV-1 strains to infect human MDM, productive viral replication was shown to be necessary, but not sufficient, for MP induction of neuronal injury. Neuronal demise was also induced by virion-free HIV-1-infected and immune-activated MDM supernatants. Similar responses were observed with MCM from human fetal microglia, further supporting the role of HIV-1-infected and immune-activated brain MP in overall neurotoxic responses (Zheng et al., 2001c). Further, alterations in glutamate-mediated neuronal signaling were observed from neurons treated with secretory products from both HIV-1-infected and immune-activated MDM (Zheng et al., 2001c). An inhibition of long-term potentiation was also found in rat hippocampal slices (Xiong et al., 1999a, 1999b) exposed to secretory products from both HIV-1-infected and immune-activated MDM. HIV-1-infected and immune-activated MCM-mediated neuronal injury was also found to be partially blocked by the *N*-methyl *D*-aspartate (NMDA) receptor antagonist, MK 801 (Jiang et al., 2001; Zheng et al., 2001d; Xiong et al., 2003). Taken together, these data support a primary role for immune-activation and HIV-1 infection in MP-mediated neuronal dysfunction.

Chemokines and HIV-1 Neuropathogenesis

A number of α-chemokine receptors are expressed on leukocytes, astrocytes, and neurons. For example, CXCR4 is found on neurons,

microglia and astrocytes (Albright et al., 1999; Banisadr et al., 2000; Broder and Collman, 1997; Coughlan et al., 2000; Endres et al., 1996; Ghorpade et al., 1998b; He et al., 1997; Horuk et al., 1997; Lavi et al., 1997; Luster, 1998; Mackay, 1996; Vallat et al., 1998). CXCR4 has been shown to play a substantive role in receptor-mediated apoptosis and cell function (Hesselgesser et al., 1998b; Zeng et al., 2000; Zheng et al., 1999a, 1999b; Zou et al., 1998). Neurons also express CXCR2 (Coughlan et al., 2000; Horuk et al., 1997) and CXCR5 (Kouba et al., 1993). Preliminary data, from others and our laboratory, suggest that CXCR5 is expressed predominantly on human neuronal dendrites. CXCR2 and CXCR4 are expressed diffusely throughout the cell (Coughlan et al., 2000; Zheng et al., 2000).

As HIV-1 gp120 can instigate signal transduction by binding to chemokine receptors (Davis et al., 1997; Guntermann et al., 1999; Weissman et al., 1997), the identification of neuronal chemokines are linked to tissue homeostasis and disease. Activation of neuron-expressed chemokine receptors, through virion or endogenous ligand, may alter intracellular signaling events and cause apoptosis (Ohagen et al., 1999; Zheng et al., 1999a). Ligands that stimulate neuron-expressed CXCR4, including HIV-1 gp120, and the endogenous CXCR4 ligand, SDF-1α, can both elicit neuronal dysfunction and apoptosis (Hesselgesser et al., 1998b; Pandey and Bolsover, 2000; Sanders et al., 2000; Zeng et al., 2000; Zheng et al., 1998; Zheng et al., 1999b).

Activated MP secrete β-chemokines (Cotter et al., 1999a, 2001; Conant et al., 1998; Desbaillets et al., 1994; Kelder et al., 1998; Kornbluth et al., 1998; McManus et al., 2000; Persidsky, 1999). The receptors for β-chemokines include CCR2, CCR3, CCR4, CCR5, and CCR8. Macrophages and microglia express CCR3 and CCR5 (Albright et al., 1999; Ghorpade et al., 1998b; He et al., 1997; Lavi et al., 1997; Vallat et al., 1998) and affect the regulation of HIV-1 infection (Cotter et al., 2001; Kitai et al., 2000). Human neurons express CCR4. Unlike α-chemokine receptors, the major expression of β-chemokine receptors in the brain has been reported in macrophages, microglia, and astrocytes. Importantly, it has recently been suggested that differential utilization of CCR5 and CXCR4 by brain X4, R5, and R5X4 viruses for macrophages and microglia infection may underlie neurovirulence of HIV-1 and induce disease (Gorry et al., 2001; Wang et al., 2001).

The most recently discovered receptor CX_3CR_1 is highly expressed on neurons and MP (Boehme et al., 2000; Chapman et al., 2000a, 2000b; Dorf et al., 2000; Harrison et al., 1998; Imai et al., 1997; Meucci et al., 2000; Tong et al., 2000). FKN, the ligand for CX_3CR_1, is expressed on the neuronal cell surface. Unlike other chemokine types, the polypeptide chain of the human CX_3C motif is predicted to be part of a 373 amino acid protein that carries the chemokine domain on top of an extended mucin-like stalk

(Bazan et al., 1997). To date, human FKN is the only known chemokine that engages the CX_3CR_1 receptor. FKN exists as a membrane-bound protein with adhesion properties. When cleaved as a soluble protein, FKN is chemotactic for monocytes and lymphocytes (Chapman et al., 2000b; Imai et al., 1997; Tong et al., 2000). Studies show that neuronal FKN RNA is not responsive to glutamate stimulation. However, at the protein level, FKN is detected in supernatants from cultured neurons treated with glutamate. This response is prevented by matrix metalloprotease inhibitors (Chapman et al., 2000a). These findings suggest that elevated FKN might be due to protein cleaved from injured neurons (Chapman et al., 2000a; Zheng et al., 2001a). Thus, FKN induces chemotaxis by providing a chemotactic gradient to direct cell migration. However, it is not certain if this mechanism requires signal transduction or receptor mediated G protein activation (Chapman et al., 2000b; Haskell et al., 1999, 2000; Shiraishi et al., 2000).

Since MP activation is associated with neuronal injury in HAD, we have proposed that FKN regulates MP effector function. In support of this idea, it was reported that FKN is upregulated in the brain tissue and CSF of patients with HAD (Meucci et al., 2000; Tong et al., 2000; Zheng et al., 2001a). HIV-1 progeny virions (IIIB and ADA), gp120, and TNF-α induced neuronal apoptosis in human neuronal cultures coincident with an increase in FKN production (Meucci et al., 2000; Tong et al., 2000; Zheng et al., 2001a; Erichsen et al., 2003). Moreover, FKN can effect chemotaxis of primary monocytes across an artificial BBB and is neuroprotective to cultured neurons (Meucci et al., 2000; Tong et al., 2000; Zheng et al., 2001a). These results, taken together, demonstrate potential roles for FKN in MP migration and activation, both critical events in HAD pathogenesis.

Chemokines and Neuroprotection

While several chemokines have been proposed to play an active role in neuronal injury, some may be neuroprotective. Several reports have demonstrated that IL-8 and FKN are neuroprotective (Kaul and Lipton, 1999; Meucci et al., 1998, 2000; Tong et al., 2000; Zheng et al., 2001b). IL-8, an endogenous ligand for CXCR2, is secreted in high levels by HIV-1-infected lymphocytes and macrophages (Zheng et al., 2000). It has been shown that HIV-1 infection and immune activation induce MDM to produce high levels of IL-8 (Zheng et al., 2000). Immune activators such as LPS and CD40L potentiate IL-8 secretion from HIV-1-infected and uninfected MP (Zheng et al., 2000). A similar observation was obtained from human fetal microglia cultures (Zheng et al., 2001b). Further, IL-8 levels are higher in the CSF of HAD patients than in infected subjects without neurological disease, supporting the importance of this factor in disease pathogenesis (Zheng et al., 2001b).

Others have explored how chemokines induce neuronal injury and protection. These experiments demonstrated that engagement of CXCR2 or CXCR4 stimulates several distinct neuronal intracellular signaling cascades. Binding of IL-8 to CXCR2 or SDF-1α to CXCR4 causes a transient increase in cytosolic (Ca^{2+}) in cultured neurons (Zheng et al., 1999b, 2000). IL-8 and SDF-1α binding inhibits neuronal adenylate cyclase activity, leading to decreased cytosolic levels (Bajetto et al., 1999; Zheng et al., 1999b, 2000). IL-8 and SDF-1α stimulate MAP kinase phosphorylation (Lazarini et al., 2000). From a biological perspective, binding of neuron-expressed chemokine receptors can result in diverse outcomes. When applied to neuronal cultures, SDF-1α and recombinant gp120 can induce neuronal apoptosis through CXCR4 binding (Zheng et al., 1999b). Surprisingly, and in stark contrast, activation of CXCR2 by IL-8 seems to induce neuronal protection against injury (Zheng et al., 2001b). Presently, it is not known what predominant effect chemokines exert on neuronal function in HIVE. Certainly, there are several potential pathways through which chemokines, HIV-1 gp120, or whole virions can interact with chemokine receptors to trigger neuronal apoptosis.

4. CONCLUSION

The neuropathogenesis of HIV-1 infection revolves around inflammatory factors secreted from virus-infected and immune competent brain MP. One principal inflammatory factor is chemokines. Chemokines and their chemokine receptors are expressed in the nervous system, and their engagement affects neuronal and glial function. MP, astrocyte, and neuronal chemokines, together with HIV-1 gp120, affect neuronal signaling pathways by binding to chemokine receptors, contributing to neuronal injury and repair. A chemokine gradient produced during HAD influences the transendothelial migration of monocytes into the brain during disease. This leads to an expanded reservoir for HIV and an increase in brain inflammation; both influencing neuronal destruction. Indeed, HIV-1 infection and immune activation work in synergy to stimulate chemokine secretion. Overall, it is likely that elucidation of the mechanisms for how chemokines are regulated and affect neuronal injury in HAD will provide novel means for therapeutic interventions as well as have broad applicability in other neurodegenerative disorders.

Acknowledgments. We thank Nell Ingraham and Robin Taylor for outstanding administrative and graphic support. This work was supported, in part, by National Institutes of Health research grants 2 R37 NS36126, PO1 NS31492, 2R01 NS134239, P20RR 15635, P01A1050244, P30

A142845, 1R01 NSA136127, 1 R01 NS 41858, U54 NS 43011-01, 1RO1 NS41862, and 1RO1 NS 43113.

REFERENCES

Achim, C. L., Wang, R., Miners, D. K., and Wiley, C. A., 1994, Brain viral burden in HIV infection, *J Neuropathol Exp Neurol.* 53:284–294.

Adamson, D. C., Wildemann, B., Sasaki, M., Glass, J. D., McArthur, J. C., Christov, V. I., Dawson, T. M., and Dawson, V. L., 1996, Immunologic NO synthase: Elevation in severe AIDS dementia and induction by HIV-1 gp41, *Science.* 274:1917–1926.

Albright, A. V., Shieh, J. T., Itoh, T., Lee, B., Pleasure, D., O'Connor, M. J., Doms, R. W., and Gonzalez-Scarano, F., 1999, Microglia express CCR5, CXCR4, and CCR3, but of these, CCR5 is the principal coreceptor for human immunodeficiency virus type 1 dementia isolates, *J Virol.* 73:205–213.

Anderson, E., Zink, W., Xiong, H., and Gendelman, H. E., 2002, HIV-1-associated dementia: a metabolic encephalopathy perpetrated by virus-infected and immune-competent mononuclear phagocytes, *J Acquir Immune Defic Syndr.* 31 Suppl 2:S43–54.

Aquaro, S., Balestra, E., Cenci, A., Francesconi, M., Calio, R., and Perno, C. F., 1997, HIV infection in macrophage: role of long-lived cells and related therapeutical strategies, *J Biol Regul Homeost Agents.* 11:69–73.

Aquaro, S., Calio, R., Balestra, E., Bagnarelli, P., Cenci, A., Bertoli, A., Tavazzi, B., Di Pierro, D., Francesconi, M., Abdelahad, D., and Perno, C. F., 1998, Clinical implications of HIV dynamics and drug resistance in macrophages, *J Biol Regul Homeost Agents.* 12:23–27.

Aquaro, S., Panti, S., Caroleo, M. C., Balestra, E., Cenci, A., Forbici, F., Ippolito, G., Mastino, A., Testi, R., Mollace, V., Calio, R., and Perno, C. F., 2000, Primary macrophages infected by human immunodeficiency virus trigger CD95-mediated apoptosis of uninfected astrocytes, *J Leukoc Biol.* 68:429–435.

Baggiolini, M., Dewald, B., and Moser, B., 1997, Human chemokines: an update, *Annu Rev Immunol.* 15:675–705.

Bajetto, A., Bonavia, R., Barbero, S., Piccioli, P., Costa, A., Florio, T., and Schettini, G., 1999, Glial and neuronal cells express functional chemokine receptor CXCR4 and its natural ligand stromal cell-derived factor 1, *J Neurochem.* 73:2348–2357.

Banisadr, G., Dicou, E., Berbar, T., Rostene, W., Lombet, A., and Haour, F., 2000, Characterization and visualization of [125I] stromal cell-derived factor-1alpha binding to CXCR4 receptors in rat brain and human neuroblastoma cells, *J Neuroimmunol.* 110:151–160.

Batchelor, P. E., Liberatore, G. T., Wong, J. Y., Porritt, M. J., Frerichs, F., Donnan, G. A., and Howells, D. W., 1999, Activated macrophages and microglia induce dopaminergic sprouting in the injured striatum and express brain-derived neurotrophic factor and glial cell line-derived neurotrophic factor, *J Neurosci.* 19:1708–1716.

Bazan, H. A., Alkhatib, G., Broder, C. C., and Berger, E. A., 1998, Patterns of CCR5, CXCR4, and CCR3 usage by envelope glycoproteins from human immunodeficiency virus type 1 primary isolates, *J Virol.* 72:4485–4491.

Bazan, J., Bacon, K., Hardiman, G., Wang, W., Soo, K., Rossi, D., Greaves, D., Zlotnik, A., and Schall, T., 1997, A new class of membrane-bound chemokine with a CX3C motif, *Nature.* 385:640–644.

Berger, J. R., and Major, E. O., 1999, Progressive multifocal leukoencephalopathy, *Semin Neurol.* 19:193–200.

Bernasconi, S., Cinque, P., Peri, G., Sozzani, S., Crociati, A., Torri, W., Vicenzi, E., Vago, L., Lazzarin, A., Poli, G., and Mantovani, A., 1996, Selective elevation of monocyte chemo-

tactic protein-1 in the cerebrospinal fluid of AIDS patients with cytomegalovirus encephalitis, *J Infect Dis.* 174:1098–1101.

Bezzi, P., Domercq, M., Brambilla, L., Galli, R., Schols, D., De Clercq, E., Vescovi, A., Bagetta, G., Kollias, G., Meldolesi, J., and Volterra, A., 2001, CXCR4-activated astrocyte glutamate release via TNFalpha: amplification by microglia triggers neurotoxicity, *Nat Neurosci.* 4:702–710.

Bleul, C. C., Farzan, M., Choe, h., Parolin, C., Clark-Lewis, I., Sodroski, J., and Springer, T. A., 1996a, The lymphocyte chemoattractant SDF-1 is a ligand for LESTR/fusin and blocks HIV-1 entry, *Nature.* 382:829–833.

Bleul, C. C., Fuhlbrigge, R. C., Casasnovas, J. M., Aiuti, A., and Springer, T. A., 1996b, A highly efficacious lymphocyte chemoattractant, stromal cell-derived factor 1 (SDF-1), *J Exp Med.* 184:1101–1109.

Boehme, S. A., Lio, F. M., Maciejewski-Lenoir, D., Bacon, K. B., and Conlon, P. J., 2000, The chemokine fractalkine inhibits Fas-mediated cell death of brain microglia, *J Immunol.* 165:397–403.

Boutet, A., Salim, H., Leclerc, P., and Tardieu, M., 2001, Cellular expression of functional chemokine receptor CCR5 and CXCR4 in human embryonic neurons, *Neurosci Lett.* 311:105–108.

Boven, L. A., Middel, J., Breij, E. C., Schotte, D., Verhoef, J., Soderland, C., and Nottet, H. S., 2000, Interactions between HIV-infected monocyte-derived macrophages and human brain microvascular endothelial cells result in increased expression of CC chemokines, *J Neurovirol.* 6:382–389.

Brack-Werner, R., 1999, Astrocytes: HIV cellular reservoirs and important participants in neuropathogenesis, *Aids.* 13:1–22.

Brenneman, D. E., Westbrook, G. L., Fitzgerald, S. P., Ennist, D. L., Elkins, K. L., Ruff, M. R., and Pert, C. B., 1988, Neuronal cell killing by the envelope protein of HIV and its prevention by vasoactive intestinal peptide, *Nature.* 335:639–642.

Brew, B., Bhalla, R., Paul, M., et al., 1990, Cerebrospinal fluid neopterin in human immunodeficiency virus type 1 infection, *Ann Neurol.* 28:556–560.

Brew, B. J., Evans, L., Byrne, C., Pemberton, L., and Hurren, L., 1996a, The relationship between AIDS dementia complex and the presence of macrophage tropic and nonsyncytium inducing isolates of human immunodeficiency virus type 1 in the cerebrospinal fluid, *J Neurovirol.* 2:152–157.

Brew, B. J., and Miller, J., 1996, Human immunodeficiency virus type 1-related transient neurological deficits, *Am J Med.* 101:257–261.

Brew, B. J., Wesselingh, S. L., Gonzales, M., Heyes, M. P., and Price, R. W., 1996b, How HIV leads to neurological disease, *Med J Aust.* 164:233–234.

Broder, C. C., and Collman, R. G., 1997, Chemokine receptors and HIV, *J Leuk Biol.* 62:20–29.

Caroleo, M. C., Costa, N., Bracci-Laudiero, L., and Aloe, L., 2001, Human monocyte/macrophages activate by exposure to LPS overexpress NGF and NGF receptors, *J Neuroimmunol.* 113:193–201.

Carpenter, C. C., Cooper, D. A., Fischl, M. A., Gatell, J. M., Gazzard, B. G., Hammer, S. M., Hirsch, M. S., Jacobsen, D. M., Katzenstein, D. A., Montaner, J. S., Richman, D. D., Saag, M. S., Schechter, M., Schooley, R. T., Thompson, M. A., Vella, S., Yeni, P. G., and Volberding, P. A., 2000, Antiretroviral therapy in adults: updated recommendations of the International AIDS Society-USA Panel, *JAMA.* 283:381–390.

Chakrabarti, L., Hurtrel, M., Maire, M. A., Vazeux, R., Dormont, D., Montagnier, L., and Hurtrel, B., 1991, Early viral replication in the brain of SIV-infected rhesus monkeys, *Am J Pathol.* 139:1273–1280.

Chang, J., Jozwiak, R., Wang, B., Ng, T., Ge, Y. C., Bolton, W., Dwyer, D. E., Handle, C., Osborn, R., Cunnlngham, A. C., and Saksena, N. D., 1998, Unique HIV type 1 V3 region sequences

derived from six different regions of brain: region-specific evolution within host-determined quasispecics, *AIDS Res Hum Retroviruses.* 14:25–30.

Chapman, G. A., Moores, K., Harrison, D., Campbell, C. A., Stewart, B. R., and Strijbos, P. J., 2000a, Fractalkine cleavage from neuronal membranes represents an acute event in the inflammatory response to excitotoxic brain damage, *J Neurosci (Online).* 20:RC87.

Chapman, G. A., Moores, K. E., Gohil, J., Berkhout, T. A., Patel, L., Green, P., Macphee, C. H., and Stewart, B. R., 2000b, The role of fractalkine in the recruitment of monocytes to the endothelium, *Eur J Pharmacol.* 392:189–195.

Chauhan, A., Turchan, J., Pocernich, C., Bruce-Keller, A., Roth, S., Butterfield, D. A., Major, E., and Nath, A., 2003, Intracellular human immunodeficency virus tat expression in astrocytes promotes astrocyte survival but induces potent neurotoxicity at distant sites via axonal transport, *J Biol Chem.* 278:13512–13519.

Cho, C., and Miller, R. J., 2002, Chemokine receptors and neural function, *J Neurovirol.* 8:573–584.

Choe, H., Farzan, M., Konkel, M., Martin, K., Sun, Y., Marcon, L., Cayabyab, M., Berman, M., Dorf, M. E., Gerard, N., Gerard, C., and Sodroski, J., 1998a, The orphan seven-transmembrane receptor apj supports the entry of primary T-cell-line-tropic and dual-tropic human immunodeficiency virus type 1, *J Virol.* 72:6113–6118.

Choe, H., Martin, K. A., Farzan, M., Sodroski, J., Gerard, N. P., and Gerard, C., 1998b, Structural interactions between chemokine receptors, gp120 Env and CD4, *Semin Immunol.* 10:249–257.

Chung, I., Zelivyanskaya, M., and Gendelman, H. E., 2002, Mononuclear phagocyte biophysiology influences brain transendothelial and tissue migration: implication for HIV-1-associated dementia, *J Neuroimmunol.* 122:40–54.

Cinque, P., Vago, L., Mengozzi, M., Torri, V., Ceresa, D., Vicenzi, E., Transidico, P., Vagani, A., Sozzani, S., Mantovani, A., Lazzarin, A., and Poli, G., 1998, Elevated cerebrospinal fluid levels of monocyte chemotactic protein-1 correlate with HIV-1 encephalitis and local viral replication, *Aids.* 12:1327–1332.

Cocchi, F., DeVico, A. L., Garzino-Demo, A., Arya, S. K., Gallo, R. C., and Lusso, P., 1995, Identification of RANTES, MIP-1alpha, and MIP-1beta as the major HIV-suppressive factors produced by CD8+ T-cells, *Science.* 270:1811–1815.

Collman, R. G., Yi, Y., Liu, Q. H., and Freedman, B. D., 2000, Chemokine signaling and HIV-1 fusion mediated by macrophage CXCR4: implications for target cell tropism, *J Leukoc Biol.* 68:318–323.

Conant, K., Garzino-Demo, A., Nath, A., McArthur, J. C., Halliday, W., Power, C., Gallo, R. C., and Major, E. O., 1998, Induction of monocyte chemoattractant protein-1 in HIV-1 Tat-stimulated astrocytes and elevation in AIDS dementia, *Proc Natl Acad Sci USA.* 95:3117–3121.

Cotter, R., Williams, C., Ryan, L., Erichsen, D., Lopez, A., Peng, H., and Zheng, J., 2002, Fractalkine (CX3CL1) and brain inflammation: implications for HIV-1-associated dementia, *J Neurovirol.* 8:585–598.

Cotter, R., Zheng, J., Che, M., Niemann, D., Liu, Y., He, J., Thomas, E., and Gendelman, H. E., 2001, Regulation of HIV-1 infection, b-chemokine production, and CCR5 expression in CD40L-stimulated macrophages: immune control of viral entry, *J Virol* 75:4308–4320.

Cotter, R., Zheng, J., Niemann, D., Thomas, E., and Gendelman, H., 1999a, in XIth International Congress of Virology International Union of Microbiological Societies, Sydney, Australia, (VP33.14).

Cotter, R. L., Burke, W. J., Thomas, V. S., Potter, J. F., Zheng, J., and Gendelman, H. E., 1999b, Insights into the neurodegenerative process of Alzheimer's disease: a role for mononuclear phagocyte-associated inflammation and neurotoxicity, *J Leukoc Biol.* 65:416–427.

Coughlan, C. M., McManus, C. M., Sharron, M., Gao, Z., Murphy, D., Jaffer, S., Choe, W., Chen, W., Hesselgesser, J., Gaylord, H., Kalyuzhny, A., Lee, V. M., Wolf, B., Doms, R. W., and Kolson, D. L., 2000, Expression of multiple functional chemokine receptors and monocyte chemoattractant protein-1 in human neurons, *Neuroscience.* 97:591–600.

Davis, C. B., Dikic, I., Unutmaz, D., Hill, C. M., Arthos, J., Siani, M. A., Thompson, D. A., Schlessinger, J., and Littman, D. R., 1997, Signal transduction due to HIV-1 envelope interactions with chemokine receptors CXCR4 or CCR5, *J Exp Med.* 186:1793–1798.

Davis, L. E., Hjelle, B. L., Miller, V. E., Palmer, D. L., Llewellyn, A. L., Merlin, T. L., Young, S. A., Mills, R. G., Wachsman, W., and Wiley, C. A., 1992, Early viral brain invasion in iatrogenic human immunodeficiency virus infection, *Neurology.* 42:1736–1739.

Deng, H., Liu, R., Ellmeier, W., Choe, S., Unutmaz, D., Burkhart, M., Di Marzio, P., Marmon, S., Sutton, R. E., Hill, C. M., Davis, C. B., Peiper, S. C., Schall, T. J., Littman, D. R., and Landau, N. R., 1996, Identification of a major co-receptor for primary isolates of HIV-1, *Nature.* 381:661–666.

Desbaillets, I., Tada, M., de Tribolet, N., Diserens, A. C., Hamou, M. F., and Van Meir, E. G., 1994, Human astrocytomas and glioblastomas express monocyte chemoattractant protein-1 (MCP-1) in vivo and in vitro, *Int J Cancer.* 58:240–247.

Di Stefano, M., Wilt, S., Gray, F., Dubois-Dalcq, M., and Chiodi, F., 1996, HIV type 1 V3 sequences and the development of dementia during AIDS, *AIDS Res Hum Retrovir.* 12:471–482.

Dooms, H., Van Belle, T., Desmedt, M., Rottiers, P., and Grooten, J., 2000, Interleukin-15 redirects the outcome of a tolerizing T-cell stimulus from apoptosis to anergy, *Blood.* 96:1006–1012.

Doranz, B. J., Rucker, J., Yi, Y., Smyth, R. J., Samson, M., Peiper, S. C., Parmentier, M., Collman, R. G., and Dome, R. W., 1996, A dual-tropic primary HIV-1 isolate that use fusin and the b chemokine receptor CKR5, CKR3, and CKR-2b as fusion cofactors, *Cell.* 85:1149–1158.

Dorf, M. E., Berman, M. A., Tanabe, S., Heesen, M., and Luo, Y., 2000, Astrocytes express functional chemokine receptors, *J Neuroimmunol.* 111:109–121.

Dragic, T., Litwin, V., Allaway, G. P., Martin, S. R., Huang, Y., Nagashima, K. A., Cayanan, C., Maddon, P. J., Koup, R. A., Moore, J. P., and Paxton, W. A., 1996, HIV-1 entry into CD4+ cells is mediated by the chemokines receptor CC-CKR-5, *Nature.* 381:667–673.

Edinger, A. L., Blanpain, C., Kunstman, K. J., Wolinsky, S. M., Parmentier, M., and Doms, R. W., 1999, Functional dissection of CCR5 coreceptor function through the use of CD4-independent simian immunodeficiency virus strains, *J Virol.* 73:4062–4073.

Elkabes, S., DiCicco-Bloom, E. M., and Black, I. B., 1996, Brain microglia/macrophages express neurotrophins that selectively regulate microglial proliferation and function, *J Neurosci.* 16:2508–2521.

Endres, M. J., Clapham, P. R., Marsh, M., Ahuja, M., Turner, J. D., McKnight, A., Thomas, J. F., Stoebenau-Haggarty, B., Choe, S., Vance, P. J., Wells, T. N. C., Powers, C. A., Sutterwala, S. S., Doms, R. W., Landau, N. R., and Hoxie, J. A., 1996, CD4-independent infection by HIV-2 is mediated by fusin/CXCR4, *Cell.* 87:745–756.

Erichsen, D., Lopez, A. L., Peng, H., Bauer, M., Williams, D. A., Morgello, S., Cotter, R. L., Ryan, L. A., Ghorpade, A., Gendelman, H. E., and Zheng, J., 2003, Neuronal injury regulates fractalkine production: relevance to HIV-1 associated dementia, *J. Neuroimmunol.* 138:144–156.

Everall, I. P., Glass, J. D., McArthur, J., Spargo, E., and Lantos, P., 1994, Neuronal density in the superior frontal and temporal gyri does not correlate with the degree of human immunodeficiency virus-associated dementia, *Acta Neuropathol.* 88:538–544.

Farzan, M., Choe, H., Martin, K., Marcon, L., Hofmann, W., Karlsson, G., Sun, Y., Barrett, P., Marchand, N., Sullivan, N., Gerard, N., Gerard, C., and Sodroski, J., 1997a, Two orphan seven transmembrane segment receptors which are expressed in CD4-positive cells support simian immunodeficiency virus infection, *J Exp Med.* 186:405–411.

Farzan, M., Choe, H., Martin, K. A., Sun, Y., Sidelko, M., Mackay, C. R., Gerard, N. P., Sodroski, J., and Gerard, C., 1997b, HIV-1 entry and macrophage inflammatory protein-1beta-mediated signaling are independent functions of the chemokine receptor CCR5, *J Biol Chem.* 272:6854–6857.

Feng, Y., Broder, C. C., Kennedy, P. E., and Berger, E. A., 1996, HIV-1 entry cofactor: functional cDNA cloning of a seven-transmembrane, G protein-coupled receptor, *Science.* 272:872–877.

Fiala, M., Gan, X. H., Zhang, L., House, S. D., Newton, T., Graves, M. C., Shapshak, P., Stins, M., Kim, K. S., Witte, M., and Chang, S. L., 1998, Cocaine enhances monocyte migration across the blood-brain barrier. Cocaine's connection to AIDS dementia and vasculitis, *Adv Exp Med Biol.* 437:199–205.

Fischer-Smith, T., Croul, S., Sverstiuk, A. E., Capini, C., L'Heureux, D., Regulier, E. G., Richardson, M. W., Amini, S., Morgello, S., Khalili, K., and Rappaport, J., 2001, CNS invasion by CD14 +/CD16+ peripheral blood-derived monocytes in HIV dementia: perivascular accumulation and reservoir of HIV infection, *J Neurovirol.* 7:528–541.

Fox, H. S., Weed, M. R., Huitron-Resendiz, S., Baig, J., Horn, T. F., Dailey, P. J., Bischofberger, N., and Henriksen, S. J., 2000, Antiviral treatment normalizes neurophysiological but not movement abnormalities in simian immunodeficiency virus-infected monkeys, *J Clin Invest.* 106:37–45.

Gabuzda, D., He, J., Ohagen, A., and Vallat, A., 1998, Chemokine receptors in HIV-1 infection of the central nervous system, *Immunology.* 10:203–213.

Gabuzda, D., and Wang, J., 1999, Chemokine receptors and virus entry in the central nervous system, *J Neurovirol.* 5:643–658.

Gabuzda, D., Wang, J., and Gorry, P., 2002, HIV-1-associated dementia, In: *Chemokines and the nervous system*, Eds., R. M. Ransohoff, K. Suzuki, A. E. I. Proudfoot, W. F. Hickey, and J. K. Harrison. Amsterdam: Elsevier Science, pp. 345–360.

Gabuzda, D. H., Ho, D. D., Monte, M. S. D. L., Rota, T. R., and Sobel, R. A., 1986, Immunohistochemical identification of HTLV-III antigen in brains of patients with AIDS, *Ann Neurol.* 20:289–295.

Garden, G. A., 2002, Microglia in human immunodeficiency virus-associated neurodegeneration, *Glia.* 40:240–251.

Gelbard, H., Nottet, H., Dzenko, K., Jett, M., Genis, P., White, R., Wang, L., Choi, Y.-B., Zhang, D., Lipton, S., Swindells, S., Epstein, L., and Gendelman, H., 1994, Platelet-activating factor: a candidate human immunodeficiency virus type-1 infection neurotoxin, *J Virol.* 68:4628–4635.

Gelbard, H. A., Dzenko, K., Diloreto, D., Cerro, C. D., Cerro, M. D., and Epstein, L. G., 1993, Neurotoxic effects of tumor necrosis factor in primary human neuronal cultures are mediated by activation of the glutamate AMPA recetor subtypes implications for AIDS neuropathogenesis, *Dev. Neurosci.* 15:417–422.

Gelbard, H. A., and Epstein, L. G., 1995, HIV-1 encephalopathy in children, *Curr Opin Pediatr.* 7:655–662.

Gendelman, H., and Gendelman, S., 1992, Neurological aspects of human immunodeficiency virus infection, In: *Neuropathogenic Viruses and Immunity*, Ed., S. Specter, et al. New York: Plenum Press, pp. 229–254.

Gendelman, H. E., 1997, The neuropathogenesis of HIV-1-dementia, In: *The neurology of AIDS*, Eds., H. E. Gendelman, S. A. Lipton, L. G. Epstein, and S. Swindells. New York: Chapman and Hall, pp. 1–10.

Gendelman, H. E., and Folks, D. G., 1999, Innate and acquired immunity in neurodegenerative disorders, *J Leuk Biol.* 65:407–409.

Gendelman, H. E., Genis, P., Jett, M., Zhai, Q. H., and Nottet, H. S., 1994, An experimental model system for HIV-1-induced brain injury, *Adv Neuroimmunol.* 4:189–193.

Gendelman, H. E., Narayan, O., Kennedy-Stoskopf, S., Clements, J. E., and Pezeshkpour, G. H., 1984, Slow virus-macrophage interactions. Characterization of a transformed cell

line of sheep alveolar macrophages that express a marker for susceptibility to ovine-caprine lentivirus infections, *Lab Invest.* 51:547–555.

Gendelman, H. E., Narayan, O., Kennedy-Stoskopf, S., Kennedy, P. G., Ghotbi, Z., Clements, J. E., Stanley, J., and Pezeshkpour, G., 1986, Tropism of sheep lentiviruses for monocytes: susceptibility to infection and virus gene expression increase during maturation of monocytes to macrophages, *J Virol.* 58:67–74.

Gendelman, H. E., Narayan, O., Molineaux, S., Clements, J. E., and Ghotbi, Z., 1985, Slow, persistent replication of lentiviruses: role of tissue macrophages and macrophage precursors in bone marrow, *Proc Natl Acad Sci USA.* 82:7086–7090.

Gendelman, H. E., Persidsky, Y., Ghorpade, A., Limoges, J., Stins, M., Fiala, M., and Morrisett, R., 1997, The neuropathogenesis of the AIDS dementia complex, *Aids.* 11:S35–45.

Gendelman, H. E., Zheng, J., Coulter, C. L., Ghorpade, A., Che, M., Thylin, M., Rubocki, R., Persidsky, Y., Hahn, F., Reinhard, J., Jr., and Swindells, S., 1998, Suppression of inflammatory neurotoxins by highly active antiretroviral therapy in human immunodeficiency virus-associated dementia, *J Infect Dis.* 178:1000–1007.

Genis, P., Jett, M., Bernton, E., Boyle, T., Gelbard, H., Dzenko, K., Keane, R., Resnick, L., Mizrachi, Y., Volsky, D., Epstein, L., and Gendelman, H., 1992, Cytokines and arachidonic metabolites produced during human immunodeficiency virus (HIV)-infected macrophage-astroglia interactions: implications for the neuropathogenesis of HIV disease, *J Exp Med.* 176:1703–1718.

Ghorpade, A., and Gendelman, H. E., 2003, Non-neuronal cells and their interactions in AIDS-associated dementia. In: *Non-neuronal cells in the nervous system: function and dysfunction., in press.*

Ghorpade, A., Nukuna, A., Che, M., Haggerty, S., Persidsky, Y., Carter, E., Carhart, L., Shafer, L., and Gendelman, H. E., 1998a, Human immunodeficiency virus neurotropism: an analysis of viral replication and cytopathicity for divergent strains in monocytes and microglia, *J Virol.* 72:3340–3350.

Ghorpade, A., Persidskaia, R., Suryadevara, R., Che, M., Liu, X., Persidsky, Y., and Gendelman, H. E., 2001, Mononuclear phagocyte differentiation, activation and viral infection regulate matrix metalloproteinase expression: implications for HIV-1-associated dementia, *J Virol.* 75:6572–6583.

Ghorpade, A., Xia, M. Q., Hyman, B. T., Persidsky, Y., Nukuna, A., Bock, P., Che, M., Limoges, J., Gendelman, H. E., and Mackay, C. R., 1998b, Role of the b-chemokine receptors CCR3 and CCR5 in human immunodeficiency virus type 1 infection of monocytes and microglia, *J Virol.* 72:3351–3361.

Giulian, D., Yu, J., Xia, L., Tom, D., Li, J., Lin, S. N., Schwarz, R., and Noonan, C., 1996, Study of receptor-mediated neurotoxins released by HIV-1 infected mononuclear phagocytes found in human brain, *J Neurosci.* 16:3139–3153.

Glass, J. D., Fedor, H., Wesselingh, S. L., and McArthur, J. C., 1995, Immunocytochemical quantitation of human immunodeficiency virus in the brain: correlations with dementia, *Ann Neurol.* 38:755–762.

Gorry, P., Ohagen, A., Holm, G., Birch, C., Bell, J., Kunstman, K., Wolinsky, S., and Gabuzda, D., 2001, In 8th Conference on Retroviruses and Opportunistic Infections Foundation for Retrovirology and Human Health, Chicago.

Guntermann, C., Murphy, B. J., Zheng, R., Qureshi, A., Eagles, P. A., and Nye, K. E., 1999, Human immunodeficiency virus-1 infection requires pertussis toxin sensitive G-protein-coupled signalling and mediates cAMP downregulation, *Biochem Biophys Res Commun.* 256:429–435.

Harrison, J. K., Jiang, Y., Chen, S., Xia, Y., Maciejewski, D., McNamara, R. K., Streit, W. J., Salafranca, M. N., Adhikari, S., Thompson, D. A., Botti, P., Bacon, K. B., and Feng, L., 1998, Role for neuronally derived fractalkine in mediating interactions between neurons and CX3CR1-expressing microglia, *Proc Natl Acad Sci.* 95:10896–10901.

Haskell, C. A., Cleary, M. D., and Charo, I. F., 1999, Molecular uncoupling of fractalkine-mediated cell adhesion and signal transduction. Rapid flow arrest of CX3CR1-expressing cells is independent of G-protein activation, *J Biol Chem.* 274:10053–10058.

Haskell, C. A., Cleary, M. D., and Charo, I. F., 2000, Unique role of the chemokine domain of fractalkine in cell capture. Kinetics of receptor dissociation correlate with cell adhesion, *J Biol Chem.* 275:34183–34189.

Haydon, P. G., 2001, GLIA: listening and talking to the synapse, *Nat Rev Neurosci.* 2:185–193.

He, J., Chen, Y., Farzan, M., Choe, H., Ohagen, A., Gartner, S., Busciglio, J., Yang, X., Hofmann, W., Newman, W., Mackay, C. R., Sodroski, J., and Gabuzda, D., 1997, CCR3 and CCR5 are co-receptors for HIV-1 infection of microglia, *Nature.* 385:645–649.

Heese, K., Hock, C., and Otten, U., 1998, Inflammatory signals induce neurotrophin expression in human microglial cells, *J Neurochem.* 70:699–707.

Hesselgesser, J., Halks-Miller, M., DelVecchio, V., Peiper, S. C., Hoxie, J., Kolson, D. L., Taub, D., and Horuk, R., 1997, CD4-independent association between HIV-1 gp120 and CXCR4: functional chemokine receptors are expressed in human neurons, *Curr Biol.* 7:112–121.

Hesselgesser, J., and Horuk, R., 1999, Chemokine and chemokine receptor expression in the central nervous system, *J Neurovirol.* 5:13–26.

Hesselgesser, J., Liang, M., Hoxie, J., Greenberg, M., Brass, L. F., Orsini, M. J., Taub, D., and Horuk, R., 1998a, Identification and characterization of the CXCR4 chemokine receptor in human T-cell lines: ligand binding, biological activity, and HIV-1 infectivity, *J Immunol.* 160:877–883.

Hesselgesser, J., Taub, D., Baskar, P., Greenberg, M., Hoxie, J., Kolson, D. L., and Horuk, R., 1998b, Neuronal apoptosis induced by HIV-1 gp120 and the chemokine SDF-1alpha mediated by the chemokine receptor CXCR4, *Curr Biol.* 8:595–598.

Heyes, M. P., Brew, B. B., Martin, A., Price, R. W., Salazar, A., Sidtis, J. J., Yergey, J. A., Mouradian, M. M., Sadler, A. E., Keilp, J., Rubinow, R., and Markey, S. P., 1991, Quinolinic acid in cerebrospinal fluid and serum in HIV-1 infection: relationship to clinical and neurological status, *Ann Neurol.* 29:202–209.

Horuk, R., Hesselgesser, J., Zhou, Y., Faulds, D., Halks-Miller, M., Harvey, S., Taub, D., Samson, M., Parmentier, M., Rucker, J., Doranz, B. J., and Doms, R. W., 1998, The CC chemokine I-309 inhibits CCR8-dependent infection by diverse HIV- 1 strains, *J Biol Chem.* 273: 386–391.

Horuk, R., Martin, A. W., Wang, Z., Schweitzer, L., Gerassimides, A., Guo, H., Lu, Z., Hesselgesser, J., Perez, H. D., Kim, J., Parker, J., Hadley, T. J., and Peiper, S. C., 1997, Expression of chemokine receptors by subsets of neurons in the central nervous system, *J Immunol.* 158:2882–2890.

Imai, T., Hieshima, K., Haskell, C., Baba, M., Nagira, M., Nishimura, M., Kakizaki, M., Takagi, S., Nomiyama, H., Schall, T. J., and Yoshie, O., 1997, Identification and molecular characterization of fractalkine receptor CX3CR1, which mediates both leukocyte migration and adhesion, *Cell.* 91:521–530.

Imai, Y., Kimura, T., Murakami, A., Yajima, N., Sakamaki, K., and Yonehara, S., 1999, The CED-4-homologous protein FLASH is involved in Fas-mediated activation of caspase-8 during apoptosis, *Nature.* 398:777–785.

Janssen, R., Cornblath, D., Epstein, L., Foa, R., McArthur, J., Price, R., Asbury, A., Beckett, A., Benson, D., Bridge, T., Levanthal, C., Satz, P., Saykin, A., Sidtis, J., and Tross, S., 1991, Nomenclature and research case definitions for neurological manifestations of human immunodeficiency virus type 1 (HIV-1) infection, *Neurology.* 41:778–785.

Jiang, Z., Piggee, C., Heyes, M. P., Murphy, C., Quearry, B., Bauer, M., Zheng, J., Gendelman, H. E., and Markey, S. P., 2001, Glutamate is a mediator of neurotoxicity in secretions of activated HIV- 1-infected macrophages, *J Neuroimmunol.* 117:97–107.

Jones, M., Olafson, K., Del Bigio, M. R., Peeling, J., and Nath, A., 1998, Intraventricular injection of human immunodeficiency virus type 1 (HIV- 1) Tat protein causes inflammation, gliosis, apoptosis, and ventricular enlargement, *J Neuropathol Exp Neurol.* 57:563–570.

Jung, S., Aliberti, J., Graemmel, P., Sunshine, M. J., Kreutzberg, G. W., Sher, A., and Littman, D. R., 2000, Analysis of fractalkine receptor CX(3)CR1 function by targeted deletion and green fluorescent protein reporter gene insertion, *Mol Cell Biol.* 20:4106–4114.

Kaul, M., and Lipton, S. A., 1999, Chemokines and activated macrophages in HIV gp120-induced neuronal apoptosis, *Proc Natl Acad Sci USA.* 96:8212–8216.

Kelder, W., McArthur, J. C., Nance-Sproson, T., McClernon, D., and Griffin, D. E., 1998, B-chemokines MCP-1 and RANTES are selectively increased in cerebrospinal fluid of patients with human immunodeficiency virus-associated dementia, *Ann Neurol.* 44: 831–835.

Kerr, S. J., Armati, P. J., Guillemin, G. J., and Brew, B. J., 1998, Chronic exposure of human neurons to quinolinic acid results in neuronal changes consistent with AIDS dementia complex, *AIDS.* 12:355–363.

Kieburtz, K., and Schiffer, R., 1989, Neurologic manifestations of human immunodeficiency virus infections, *Neurol Clin.* 7:447–468.

Kitai, R., Zhao, M. L., Zhang, N., Hua, L. L., and Lee, S. C., 2000, Role of MIP-1beta and RANTES in HIV-1 infection of microglia: inhibition of infection and induction by IFNbeta, *J Neuroimmunol.* 110:230–239.

Klein, R., Williams, K., Alvarez-Hernandez, X., Westmoreland, S., Force, T., Lackner, A., and Luster, A., 1999, Chemokine receptor expression and signaling in macaque and human fetal neurons and astrocytes: implications for the neuropathogenesis of AIDS, *J Immunol.* 163:1636–1646.

Koenig, S., Gendelman, H. E., Orenstein, J. M., Canto, M. C. D., Pezeshkpour, G. H., Yungbluth, M., Janotta, F., Aksamit, A., Martin, M. A., and Fauci, A. S., 1986, Detection of AIDS virus in macrophages in brain tissue from AIDS patients with encephalopathy, *Science.* 233:1089–1093.

Kornbluth, R. S., Kee, K., and Richman, D. D., 1998, CD40 ligand (CD154) stimulation of macrophages to produce HIV-1-suppressive beta-chemokines, *Proc Natl Acad Sci USA.* 95:5205–5210.

Kouba, M., Vanetti, M., Wang, X., Schafer, M., and Hollt, V., 1993, Cloning of a novel putative G-protein-coupled receptor (NLR) which is expressed in neuronal and lymphatic tissue, *FEBS Lett.* 321:173–178.

Krebs, F. C., Ross, H., McAllister, J., and Wigdahl, B., 2000, HIV-1-associated central nervous system dysfunction, *Adv Pharmacol.* 49:315–385.

Kullander, K., Kylberg, A., and Ebendal, T., 1997, Specificity of neurotrophin-3 determined by loss-of-function mutagenesis, *J Neurosci Res.* 50:496–503.

Kure, K., Weidenheim, K. M., Lyman, W. D., and Dickson, D. W., 1990, Morphology and distribution of HIV-1 gp41-positive microglia in subacute AIDS encephalitis. Pattern of involvement resembling a multisystem degeneration, *Acta Neuropathol.* 80:393–400.

Kutsch, O., Oh, J., Nath, A., and Benveniste, E. N., 2000, Induction of the chemokines interleukin-8 and IP-10 by human immunodeficiency virus type 1 tat in astrocytes, *J Virol.* 74:9214–9221.

Lackner, A. A., Vogel, P., Rames, R. A., Kluge, D., and Marthas, M., 1994, Early events in tissues during infection with pathogenic (SIVmac239) and nonpathogenic (SIVmac1A11) molecular clones of simian immunodeficiency virus, *Am J Pathol.* 145:428–439.

Lavi, E., Strizki, J. M., Ulrich, A. M., Zhang, W., Fu, L., Wang, Q., O'Connor, M., Hoxie, J. A., and Gonzalez-Scarano, F., 1997, CXCR-4 (fusin), a co-receptor for the type 1 human immunodeficiency virus (HIV-1) is expressed in the human brain in a variety of cell types, including microglia and neurons, *Am J Pathol.* 151:1035–1042.

Lazarini, F., Casanova, P., Tham, T. N., De Clercq, E., Arenzana-Seisdedos, F., Baleux, F., and Dubois-Dalcq, M., 2000, Differential signalling of the chemokine receptor CXCR4 by stromal cell-derived factor 1 and the HIV glycoprotein in rat neurons and astrocytes, *Eur J Neurosci.* 12:117–125.

Lazarov-Spiegler, O., Rapalino, O., Agranov, G., and Schwartz, M., 1998a, Restricted inflammatory reaction in the CNS: a key impediment to axonal regeneration?, *Mol Med Today.* 4:337–342.

Lazarov-Spiegler, O., Solomon, A. S., and Schwartz, M., 1998b, Peripheral nerve-stimulated macrophages simulate a peripheral nerve-like regenerative response in rat transected optic nerve, *Glia.* 24:329–337.

Lazarov-Spiegler, O., Solomon, A. S., Zeev-Brann, A. B., Hirschberg, D. L., Lavie, V., and Schwartz, M., 1996, Transplantation of activated macrophages overcomes central nervous system regrowth failure, *Faseb J.* 10:1296–1302.

Letendre, S. L., Lanier, E. R., and McCutchan, J. A., 1999, Cerebrospinal fluid beta chemokine concentrations in neurocognitively impaired individuals infected with human immunodeficiency virus type 1, *J Infect Dis.* 180:310–319.

Limoges, J., Persidsky, Y., Poluektova, L., Rasmussen, J., Ratanasuwan, W., Zelivyanskaia, M., McClernon, D., Lanier, E., and Gendelman, H., 2000, Evaluation of antiretroviral drug efficacy for HIV-1 encephalitis in SCID mice, *Neurol.* 54:379–389.

Lipton, S., 1994, HIV coat protein gp120 induces soluble neurotoxins in culture medium, *Neurosci Res Commun.* 15:31–37.

Lipton, S. A., and Gendelman, H. E., 1995, Dementia associated with the acquired immunodeficiency syndrome, *New Engl J Med.* 16:934–940.

Liu, R., Paxton, W. A., Choe, S., Ceradini, D., Martin, S. R., Horuk, R., MacDonald, M. E., Stuhlmann, H., Koup, R. A., and Landau, N. R., 1996a, Homozygous defect in HIV-1 coreceptor accounts for resistance of some multiply-exposed individuals to HIV-1 infection, *Cell.* 86:367–377.

Liu, X., Kim, C. N., Yang, J., Jemmerson, R., and Wang, X., 1996b, Induction of apoptotic program in cell-free extracts: requirement for dATP and cytochrome C, *Cell.* 86:147–157.

Liu, Y., Tang, X. P., McArthur, J. C., Scott, J., and Gartner, S., 2000, Analysis of human immunodeficiency virus type 1 gp160 sequences from a patient with HIV dementia: evidence for monocyte trafficking into brain, *J Neurovirol.* 6 Suppl 1:S70–81.

Luster, A. D., 1998, Chemokines-chemotactic cytokines that mediate inflammation, *N Eng J Med.* 338:436–445.

Ma, M., Geiger, J. D., and Nath, A., 1994, Characterization of a novel binding site for the human immunodeficiency virus type 1 envelope protein gp120 on human fetal astrocytes, *J Virol.* 68:6824–6828.

Ma, M., and Nath, A., 1997, Molecular determinants for cellular uptake of tat protein of human immunodeficiency virus type 1 in brain cells, *J Virol.* 71:2495–2499.

Mackay, C. R., 1996, Chemokine receptors and T-cell chemotaxis, *J Exp Med.* 184:799–802.

Magnuson, D. S., Knudsen, B. E., Geiger, J. D., Brownstone, R. M., and Nath, A., 1995, Human immunodeficiency virus type 1 tat activates non-N-methyl-D-aspartate excitatory amino acid receptors and causes neurotoxicity, *Ann Neurol.* 37:373–380.

Marder, K., Albert, S., Dooneief, G., Stern, Y., Ramachandran, G., and Epstein, L., 1996, Clinical confirmation of the American Academy of Neurology algorithm for HIV-1-associated cognitive/motor disorder, *Neurol.* 47:1247–1253.

McArthur, J. C., Sacktor, N., and Selnes, O., 1999, Human immunodeficiency virus-associated dementia, *Semin Neurol.* 19:129–150.

McManus, C. M., Weidenheim, K., Woodman, S. E., Nunez, J., Hesselgesser, J., Nath, A., and Berman, J. W., 2000, Chemokine and chemokine-receptor expression in human glial elements: induction by the HIV protein, tat, and chemokine autoregulation, *Am J Pathol.* 156:1441–1453.

Meucci, O., Fatatis, A., Simen, A. A., Bushell, T. J., Gray, P. W., and Miller, R. J., 1998, Chemokines regulate hippocampal neuronal signaling and gp120 neurotoxicity, *Proc Natl Acad Sci.* 95:14500–14505.

Meucci, O., Fatatis, A., Simen, A. A., and Miller, R. J., 2000, Expression of CX3CR1 chemokine receptors on neurons and their role in neuronal survival, *Proc Natl Acad Sci USA.* 97:8075–8080.

Michael, N. L., 2002, Chomokine receptors as HIV-1 coreceptors. In: *chemokine receptors and AIDS,* Ed., T. R. O'Brien. New York: Marcel Dekker, pp. 75–91.

Miller, R. J., and Meucci, O., 1999, AIDS and the brain: is there a chemokine connection?, *Trends Neurosci.* 22:471–479.

Minami, M., and Satoh, M., 2000, [Chemokines as mediators for intercellular communication in the brain], *Nippon Yakurigaku Zasshi.* 115:193–200.

Moses, A. V., Bloom, F. E., Pauza, C. D., and Nelson, J. A., 1993, HIV infection of human brain capillary endothelial cells occurs via a CD4/galactosylceramide-independent mechanism, *Proc Natl Acad Sci USA.* 90:10474–10478.

Nath, A., Conant, K., Chen, P., Scott, C., and Major, E. O., 1999, Transient exposure to HIV-1 tat protein results in cytokine production in macrophages and astrocytes. A hit and run phenomenon, *J Biol Chem.* 274:17098–17102.

Nath, A., and Geiger, J., 1998, Neurobiological aspects of human immunodeficiency virus infection: neurotoxic mechanisms, *Prog Neurobiol.* 54:19–33.

Nath, A., Hartloper, V., Furer, M., and Fowke, K. R., 1995, Infection of human fetal astrocytes with HIV-1: viral tropism and the role of cell to cell contact in viral transmission, *J Neuropathol Exp Neurol.* 54:320–330.

Nath, A., Psooy, K., Martin, C., Knudsen, B., Magnuson, D. S., Haughey, N., and Geiger, J. D., 1996, Identification of a human immunodeficiency virus type 1 tat epitope that is neuroexcitatory and neurotoxic, *J Virol.* 70:1475–1480.

Navia, B. A., Cho, E. S., Petito, C. K., and Price, R. W., 1986a, The AIDS dementia complex: II. neuropathology, *Ann Neurol.* 19:525–535.

Navia, B. A., Jordan, B. D., and Price, R. W., 1986b, The AIDS dementia complex: I. clinical features, *Ann Neurol.* 19:517–524.

New, D. R., Ma, M., Epstein, L. G., Nath, A., and Gelbard, H. A., 1997, Human immunodeficiency virus type-1 tat protein induces death by apoptosis in primary human neuron cultures, *J Neurovirol.* 3:168–173.

New, D. R., Maggirwar, S. B., Epstein, L. G., Dewhurst, S., and Gelbard, H. A., 1998, HIV-1 tat induces neuronal death via tumor necrosis factor alpha and activation of non-NMDA receptors by a NFkB-independent mechanism, *J Biol Chem.* 273:17852–17858.

Nottet, H., and Gendelman, H., 1995, Unraveling the neuroimmune mechanisms for the HIV-1-associated cognitive/motor complex, *Immunol Today.* 16:441–448.

Nottet, H. S., Jett, M., Flanagan, C. R., Zhai, Q. H., Persidsky, Y., Rizzino, A., Bernton, E. W., Genis, P., Baldwin, T., Schwartz, J. H., LaBenz, C. J., and Gendelman, H. E., 1995, A regulatory role for astrocytes in HIV-1 encephalitis. An overexpression of eicosanoids, platelet-activating factor, and tumor necrosis factor-alpha by activated HIV-1-infected monocytes is attenuated by primary human astrocytes, *J Immunol.* 154:3567–3581.

Nottet, H. S., Persidsky, Y., Sasseville, V. G., Nukuna, A. N., Bock, P., Zhai, Q. H., Sharer, L. R., McComb, R. D., Swindells, S., Soderland, C., and Gendelman, H. E., 1996, Mechanisms for the transendothelial migration of HIV-1-infected monocytes into brain, *J Immunol.* 156:1284–1295.

Oberlin, E., Amara, A., Bachelerie, F., Bessia, C., Virelizier, J.-L., Arenzana-Seisdedos, F., Schwartz, O., Heard, J.-M., Clark-Lewis, I., Legler, D. F., Loetscher, M., Baggiolini, M., and Moser, B., 1996, The CXC chemokine SDF-1 is the ligand for LESTR/fusin and prevents infection by T-cell-line-adapted HIV-1, *Nature.* 382:833–835.

O'Brien, T. R., Michael, N. L., Sheppard, H. W., and Buchbinder, S., 2002, HIV-1 infection in patients with the CCR5-D32 homozygous genotype. In: *Chemokine receptors and AIDS*, Ed., T. R. O'Brien. New York: Marcel Dekker, pp. 215–225.

Ohagen, A., Ghosh, S., He, J., Huang, K., Chen, Y., Yuan, M., Osathanondh, R., Gartner, S., Shi, B., Shaw, G., and Gabuzda, D., 1999, Apoptosis induced by infection of primary brain cultures with diverse human immunodeficiency virus type 1 isolates: evidence for a role of the envelope, *J Virol.* 73:897–906.

Pandey, V., and Bolsover, S. R., 2000, Immediate and neurotoxic effects of HIV protein gp120 act through CXCR4 receptor, *Biochem Biophys Res Commun.* 274:212–215.

Perno, C. F., Aquaro, S., Rosenwirth, B., Balestra, E., Peichl, P., Billich, A., Villani, N., and Calio, R., 1994, In vitro activity of inhibitors of late stages of the replication of HIV in chronically infected macrophages, *J Leukoc Biol.* 56:381–386.

Perno, C. F., Newcomb, F. M., Davis, D. A., Aquaro, S., Humphrey, R. W., Calio, R., and Yarchoan, R., 1998, Relative potency of protease inhibitors in monocytes/macrophages acutely and chronically infected with human immunodeficiency virus, *J Infect Dis.* 178:413–422.

Perno, C. F., Yarchoan, R., Cooney, D. A., Hartman, N. R., Gartner, S., Popovic, M., Hao, Z., Gerrard, T. L., Wilson, Y. A., Johns, D. G. et al., 1988, Inhibition of human immunodeficiency virus (HIV-1/HTLV-IIIBa-L) replication in fresh and cultured human peripheral blood monocytes/macrophages by azidothymidine and related 2′,3′-dideoxynucleosides, *J Exp Med.* 168:1111–1125.

Perry, S. W., Hamilton, J. A., Tjoelker, L. W., Dbaibo, G., Dzenko, K. A., Epstein, L. G., Hannun, Y., Whittaker, J. S., Dewhurst, S., and Gelbard, H. A., 1998, Platelet-activating factor receptor activation. An initiator step in HIV-1 neuropathogenesis, *J Biol Chem.* 273:17660–17664.

Persidsky, Y., 1999, Model systems for studies of leukocyte migration across the blood-brain barrier, *J Neurovirol.* 5:579–590.

Persidsky, Y., Ghorpade, A., Rasmussen, J., Limoges, J., Liu, X. J., Stins, M., Fiala, M., Way, D., Kim, K. S., Witte, M. H., Weinand, M., Carhart, L., and Gendelman, H. E., 1999, Microglial and astrocyte chemokines regulate monocyte migration through the blood-brain barrier in human immunodeficiency virus-1 encephalitis, *Am J Pathol.* 155: 1599–1611.

Persidsky, Y., Limoges, J., McComb, R., Bock, P., Baldwin, T., Tyor, W., Patil, A., Nottet, H. S., Epstein, L., Gelbard, H., Flanagan, E., Reinhard, J., Pirruccello, S. J., and Gendelman, H. E., 1996, Human immunodeficiency virus encephalitis in SCID mice, *Am J Pathol.* 149:1027–1053.

Persidsky, Y., Stins, M., Way, D., and et al., 1997, A model for monocyte migration through the blood-brain barrier during HIV-1 encephalitis, *J Immunol.* 158:499–510.

Petito, C. K., and Cash, K. S., 1992, Blood-brain barrier abnormalities in the acquired immunodeficiency syndrome: immunohistochemical localization of serum proteins in postmortem brain, *Ann. Neurol.* 32:658–666.

Poltorak, M., and Freed, W. J., 1991, BN rats do not reject F344 brain allografts even after systemic sensitization, *Ann Neurol.* 29:377–388.

Premack, B. A., and Schall, T. J., 1996, Chemokine receptors: gateways to inflammation and infection, *Nat Med.* 2:1174–1178.

Price, D. L., Sisodia, S. S., and Borchelt, D. R., 1998, Genetic neurodegenerative diseases: the human illness and transgenic models, *Science.* 282:1079–1083.

Price, J., 1993, Organizing the cerebrum, *Nature.* 362:590–591.

Price, R. W., 2000, The two faces of HIV infection of cerebrospinal fluid, *Trends Microbiol.* 8:387–391.

Pulliam, L., Gascon, R., Stubblebine, M., Mcguire, D., and McGrath, M. S., 1997, Unique monocyte subset in patients with AIDS dementia, *Lancet.* 349:692–695.

Ranki, A., Nyberg, M., Ovod, V., Haltia, M., Elovaara, I., Raininko, R., Haapasalo, H., and Krohn, K., 1995, Abundant expression of HIV Nef and Rev proteins in brain astrocytes in vivo is associated with dementia, *AIDS*. 9:1001–1008.

Rao, K., Lund, R. D., Kunz, H. W., and Gill, T. J., III, 1989, The role of MHC and non-MHC antigens in the rejection of intracerebral allogeneic neural grafts, *Transplantation*. 48:1018–1021.

Rapalino, O., Lazarov-Spiegler, O., Agranov, E., Velan, G. J., Yoles, E., Fraidakis, M., Solomon, A., Gepstein, R., Katz, A., Belkin, M., Hadani, M., and Schwartz, M., 1998, Implantation of stimulated homologous macrophages results in partial recovery of paraplegic rats, *Nature Med.* 4:814–821.

Rappaport, A., Shaked, M., Landau, M., and Dolev, E., 2001, Sweet's syndrome in association with Crohn's disease: report of a case and review of the literature, *Dis Colon Rectum*. 44:1526–1529.

Reddy, R. T., Achim, C. L., Sirko, D. A., Tehranchi, S., and Group, H. N. R., 1996, Sequence analysis of the V3 loop in brain and spleen of patients with HIV encephalitis, *AIDS Res Hum Retro*. 12:477–482.

Richard, R. M., Snyderman and Haribabu, B., 2002, Chemokine receptor expression and regulatory mechanisms. In: *Chemokine receptors and AIDS*, Ed., T. R. O'Brien. New York: Marcel Dekker, pp. 31–51.

Rizzardi, G. P., and Pantaleo, G., 2002, Pathogenesis of HIV-1 infection. In: *Chemokine Receptors and AIDS*, Ed., T. R. O'Brien. New York: Marcel Dekker, pp. 51–75.

Rucker, J., Edinger, A. L., Sharron, M., Samson, M., Lee, B., Berson, J. F., Yi, Y., Margulies, B., Collman, R. G., Doranz, B. J., Parmentier, M., and Doms, R. W., 1997, Utilization of chemokine receptors, orphan receptors, and herpesvirus-encoded receptors by diverse human and simian immunodeficiency viruses, *J Virol.* 71:8999–9007.

Ryan, L., Zheng, J., Brester, M., Bohac, D., Hahn, F., Gendelman, H. E., and Swindells, S., 2001a, In: 8th Conference on Retroviruses and Opportunistic Infections. Foundation for Retrovirology and Human Health, Chicago.

Ryan, L. A., Cotter, R. L., Zink, W. E., Gendelman, H. E., and Zheng, J., 2002, Macrophages, chemokines and neuronal injury in HIV-1 associated dementia, *Cell Molec Biol.* 48:137–150.

Ryan, L. A., Zheng, J., Brester, M., Bohac, D., Hahn, F., Anderson, J., Ratanasuwan, W., Gendelman, H. E., and Swindells, S., 2001b, Plasma levels of soluble cd14 and tumor necrosis factor-alpha type II receptor correlate with cognitive dysfunction during human immunodeficiency virus type 1 infection, *J Infect Dis.* 184:699–706.

Sabri, F., Tresoldi, E., Di Stefano, M., Polo, S., Monaco, M. C., Verani, A., Fiore, J. R., Lusso, P., Major, E., Chiodi, F., and Scarlatti, G., 1999, Nonproductive human immunodeficiency virus type 1 infection of human fetal astrocytes: independence from CD4 and major chemokine receptors, *Virology*. 264:370–384.

Sacktor, N., Lyles, R. H., Skolasky, R., Kleeberger, C., Selnes, O. A., Miller, E. N., Becker, J. T., Cohen, B., and McArthur, J. C., 2001, HIV-associated neurologic disease incidence changes: multicenter AIDS cohort study, 1990–1998, *Neurology*. 56:257–260.

Sanders, V. J., Everall, I. P., Johnson, R. W., and Masliah, E., 2000, Fibroblast growth factor modulates HIV coreceptor CXCR4 expression by neural cells. HNRC group, *J Neurosci Res.* 59:671–679.

Sanders, V. J., Pittman, C. A., White, M. G., Wang, G., Wiley, C. A., and Achim, C. L., 1998, Chemokines and receptors in HIV encephalitis, *AIDS*. 12:1021–1026.

Schall, T. J., and Bacon, K. B., 1994, Chemokines, leukocyte trafficking, and inflammation, *Curr Opin Immunol.* 6:865–873.

Schweighardt, B., Shieh, J. T., and Atwood, W. J., 2001, CD4/CXCR4-independent infection of human astrocytes by a T-tropic strain of HIV-1, *J Neurovirol.* 7:155–162.

Segal, R. A., and Greenberg, M. E., 1996, Intracellular signaling pathways activated by neurotrophic factors, *Annu Rev Neurosci.* 19:463–489.

Sharpless, N. E., Verdin, E., Kufta, C. V., Chen, I. S. Y., and Dubois-Dalcq, M., 1992, Human immunodeficiency virus type 1 tropism for brain microglial cells is determined by a region of the env glycoprotein that controls macrophage tropism, *Virol.* 66:2588–2593.

Shi, B., Girolami, U. D., He, J., Wang, S., Lorenzo, A., Busciglio, J., and Gabuzda, D., 1996, Apoptosis induced by HIV-1 infection of the central nervous system, *J Clini Investi.* 98:1979–1990.

Shi, B., Rainha, J., Lorenzo, A., Busciglio, J., and Gabuzda, D., 1998, Neuronal apoptosis induced by HIV-1 tat protein and TNF-A: potentiation of neurotoxicity mediated by oxidative stress and implications for HIV-1 dementia, *J Neurovirol.* 4:281–290.

Shibata, A., Zelivyanskaya, M., Limoges, J., Carlson, K. A., Gorantla, S., Branecki, C., Bishu, S., Xiong, H., and Gendelman, H. E., 2003, Peripheral nerve induces macrophage neurotrophic activities: regulation of neuronal process outgrowth, intracellular singnaling and synaptic function. *J Neuroimmunol.* 142:112–129.

Shieh, J. T. C., Albright, A. V., Sharron, M., Gartner, S., Strizki, J., Doms, R. W., and Gonzalez-Scarano, F., 1998, Chemokine receptor utilization by human immundeficiency virus type 1 isolates that replicate in microglia, *J Virol.* 72:4243–4249.

Shields, P. L., and Adams, D. H., 2002, Chemokines and chemokine receptor interactions and functions. In: *Chemokine receptors and AIDS*, Ed., T. R. O'Brien. New York: Marcel Dekker, pp. 1–31.

Shiraishi, K., Fukuda, S., Mori, T., Matsuda, K., Yamaguchi, T., Tanikawa, C., Ogawa, M., Nakamura, Y., and Arakawa, H., 2000, Identification of fractalkine, a CX3C-type chemokine, as a direct target of p53, *Cancer Res.* 60:3722–3726.

Smith, A., 1991, *Symbol modalities test.* Los Angeles: Western Psychological Services.

Smith, G. M., and Hale, J. H., 1997, Macrophage/microglia regulation of astrocytic tenascin: synergistic action of transforming growth factor-beta and basic fibroblast growth factor, *J Neurosci.* 17:9624–9633.

Soontornniyomkij, V., Wang, G., Pittman, C. A., Wiley, C. A., and Achim, C. L., 1998, Expression of brain-derived neurotrophic factor protein in activated microglia of human immunodeficiency virus type 1 encephalitis, *Neuropathol Appl Neurobiol.* 24:453–460.

Streilein, J. W., 1995, Unraveling immune privilege, *Science.* 270:1158–1159.

Strizki, J. M., Albright, A. V., Sheng, H., O'Connor, M., Perrin, L., and Gonzalez-Scarano, F., 1996, Infection of primary human microglia and monocyte-derived macrophages with human immunodeficiency virus type 1 isolates: evidence of differential tropism, *J Virol.* 70:7654–7662.

Talley, A. K., Dewhurst, S., Perry, S. W., Dollard, S. C., Gummuluru, S., Fine, S. M., New, D., Epstein, L. G., Gendelman, H. E., and Gelbard, H. A., 1995, Tumor necrosis factor alpha-induced apoptosis in human neuronal cells: protection by the antioxidant N-acetylcysteine and the genes bcl-2 and crmA, *Mol Cell Biol.* 15:2359–2366.

Tong, N., Perry, S. W., Zhang, Q., James, H. J., Guo, H., Brooks, A., Bal, H., Kinnear, S. A., Fine, S., Epstein, L. G., Dairaghi, D., Schall, T. J., Gendelman, H. E., Dewhurst, S., Sharer, L. R., and Gelbard, H. A., 2000, Neuronal fractalkine expression in HIV-1 encephalitis: roles for macrophage recruitment and neuroprotection in the central nervous system, *J Immunol.* 164:1333–1339.

Tornatore, C., Meyers, K., Atwood, W., Conant, K., and Major, E., 1994, Temporal patterns of human immunodeficiency virus type 1 transcripts in human fetal astrocytes, *J Virol.* 68:93–102.

Tornatore, C., Nath, A., Amemiya, K., and Major, E. O., 1991, Persistent human immunodeficiency virus type 1 infection in human fetal glial cells reactivated by T-cell factor(s) or by the cytokines tumor necrosis factor alpha and interleukin-1 beta, *J Virol.* 65:6094–6100.

Vallat, A.-V., Girolami, U. D., He, J., Mhashikar, A., Marasco, W., Shi, B., Gray, F., Bell, J., Keohane, C., Smith, T. W., and Gabuzda, D., 1998, Localization of HIV-1 co-receptors

CCR5 and CXCR4 in the brain of children with AIDS, *Am J Path.* 152:167–178.

van der Meer, P., Ulrich, A. M., Gonzalez-Scarano, F., and Lavi, E., 2000, Immunohistochemical analysis of CCR2, CCR3, CCR5, and CXCR4 in the human brain: potential mechanisms for HIV dementia, *Exp Mol Pathol.* 69:192–201.

Vicenzi, E., Alfano, M., Ghezzi, S., Gatti, A., Veglia, F., Lazzarin, A., Sozzani, S., Mantovani, A., and Poli, G., 2000, Divergent regulation of HIV-1 replication in PBMC of infected individuals by CC chemokines: suppression by RANTES, MIP-1 alpha, and MCP-3, and enhancement by MCP-1, *J Leukoc Biol.* 68:405–412.

Wang, J., Crawford, K., and Gabuzda, D., 2001, In: 8th Conference on Retroviruses and Opportunistic Infections Foundation for Retrovirology and Human Health, Chicago.

Weiss, J., Downie, S., Lyman, W., and Berman, J., 1998, Astrocyte-drived monocyte-chemoattractant protein-1 directs the transmigration of leukocytes across a model of the human blood-brain barrier, *J Immunol.* 161:6896–6903.

Weiss, J. M., Nath, A., Major, E. O., and Berman, J. W., 1999, HIV-1 tat induces monocyte chemoattractant protein-1-mediated monocyte transmigration across a model of the human blood-brain barrier and up- regulates CCR5 expression on human monocytes, *J Immunol.* 163:2953–2959.

Weissman, D., Rabin, R., Arthos, J., Rubbert, A., Dybul, M., Swofford, R., Venkatesan, S., Farber, J., and Fauci, A., 1997, Macrophage-tropic HIV and SIV envelope proteins induce a signal through the CCR5 chemokine receptor, *Nature.* 389:981–985.

Wiley, C. A., and Achim, C., 1994, Human immunodeficiency virus encephalitis is the pathological correlate of dementia in acquired immunodeficiency syndrome, *Ann Neurol.* 36:673–676.

Wiley, C. A., Achim, C. L., Hammond, R., Love, S., Masliah, E., Radhakrishnan, L., Sanders, V., and Wang, G., 2000, Damage and repair of DNA in HIV encephalitis, *J Neuropathol Exp Neurol.* 59:955–965.

Wiley, C. A., Masliah, E., Morey, M., Lemere, C., Teresa, R., Grafe, M., Hansen, L., and Terry, R., 1991, Neocortical damage during HIV infection, *Ann Neurol.* 29:651–657.

Wiley, C. A., Schrier, R. D., Nelson, J. A., Lampert, P. W., and Oldstone, M. B. A., 1986, Cellular localization of human immunodeficiency virus infection within the brains of acquired immune deficiency syndrome patients, *Proc Natl Acad Sci USA.* 83:7089–7093.

Williams, K. C., Corey, S., Westmoreland, S. V., Pauley, D., Knight, H., deBakker, C., Alvarez, X., and Lackner, A. A., 2001, Perivascular macrophages are the primary cell type productively infected by simian immunodeficiency virus in the brains of macaques: implications for the neuropathogenesis of AIDS, *J Exp Med.* 193:905–915.

Williams, K. C., and Hickey, W. F., 2002, Central nervous system damage, monocytes and macrophages, and neurological disorders in AIDS, *Annu Rev Neurosci.* 25:537–562.

Winkler, C., Modi, W., Smith, M. W., Nelson, G., Wu, X., Carrington, M., Dean, M., Honjo, T., Tashiro, K., Yabe, D., Buchbinder, S., Vittinghoff, E., Goedert, J. J., O'Brien, T., Jacobson, L. P., Detels, R., Donfield, S., Willoughby, A., Vlahov, E. G. D., and Phair, J., 1998, Genetic restriction of AIDS pathogenesis by an SDF-1 chemokine gene variant, *Science.* 279:389–393.

Wu, D. T., Woodman, S. E., Weiss, J. M., McManus, C. M., D'Aversa, T. G., Hesselgesser, J., Major, E. O., Nath, A., and Berman, J. W., 2000, Mechanisms of leukocyte trafficking into the CNS, *J Neurovirol.* 6 Suppl 1:S82–85.

Xiong, H., Boyle, J., Winkelbauer, M., Gorantla, S., Zheng, J., Ghorpade, A., Persidsky, Y., Carlson, K. A., and Gendelman, H. E., 2003, Inhibition of long-term potentiation by interleukin-8: implications for human immunodeficiency virus-1-associated dementia, *J Neurosci Res.* 71:600–607.

Xiong, H., Zeng, Y. C., Zheng, J., Thylin, M., and Gendelman, H. E., 1999a, Soluble HIV-1 infected macrophage secretory products mediate blockade of long-term potentiation: a mechanism for cognitive dysfunction in HIV-1-associated dementia, *J Neuro Virol.* 5:519–528.

Xiong, H., Zheng, J., Thylin, M., and Gendelman, H. E., 1999b, Unraveling the mechanisms for neurotoxicity in HIV-1-associated dementia: inhibition of neuronal synaptic transmission by macrophage secretory products, *AIDS Res Hum Retroviruses.* 15:57–63.

Zeev-Brann, A. B., Lazarov-Spiegler, O., Brenner, T., and Schwartz, M., 1998, Differential effects of central and peripheral nerves on macrophages and microglia, *Glia.* 23:181–190.

Zeng, Y. C., Zheng, J., Gendelman, H. E., and Xiong, H., 2000, In: 7th Conference on Retroviruses and Opportunistic Infections Foundation for Retrovirology and Human Health, San Francisco.

Zheng, J., and Gendelman, H. E., 1997, The HIV-1 associated dementia complex: a metabolic encephalopathy fueled by viral replication in mononuclear phagocytes, *Curr Opin Neurol.* 10:319–325.

Zheng, J., Ghorpade, A., Niemann, D., Cotter, R. L., Thylin, M. R., Epstein, L., Swartz, J. M., Shepard, R. B., Liu, X., Nukuna, A., and Gendelman, H. E., 1999a, Lymphotropic virions affect chemokine receptor-mediated neural signaling and apoptosis: implications for human immunodeficiency virus type 1-associated dementia, *J Virol.* 73:8256–8267.

Zheng, J., Lopez, A., Erichsen, D., Bauer, M., Cotter, R. L., Ryan, L. A., Williams, C., Ghorpade, A., Morgello, S., and Gendelman, H. E., 2001a, In: 8th Conference on Retroviruses and Opportunistic Infections Foundation for Retrovirology and Human Health, Chicago.

Zheng, J., Niemann, D., Bauer, M., Leisman, G. B., Cotter, R. L., Ryan, L. A., Lopez, A., Williams, C., Ghorpade, A., and Gendelman, H. E., 2000, In: 7th Conference on Retroviruses and Opportunistic Infections Foundation for Retrovirology and Human Health, San Francisco.

Zheng, J., Niemann, D., Bauer, M., Williams, C., Lopez, A., Erichsen, D., Ryan, L. A., Cotter, R. L., Ghorpade, A., Swindells, S., and Gendelman, H. E., 2001b, In: 8th Conference on Retroviruses and Opportunistic Infections Foundation for Retrovirology and Human Health, Chicago.

Zheng, J., Thylin, M., Ghorpade, A., Cotter, R., Persidsky, Y., and Gendelman, H. E., 1998, CXCR4 mediates neuronal dysfunction by HIV-1 infected macrophage secretory product: importance for HIV-1 associated dementia, *Soc Neurosci Abs.* 24:776.6.

Zheng, J., Thylin, M., Ghorpade, A., Xiong, H., Persidsky, Y., Cotter, R., Niemann, D., Che, M., Zeng, Y., Gelbard, H., Shepard, R., Swartz, J., and Gendelman, H., 1999b, Intracellular CXCR4 signaling, neuronal apoptosis and neuropathogenic mechanisms of HIV-1-associated dementia, *J Neuroimmunol.* 98:185–200.

Zheng, J., Thylin, M. R., Cotter, R. L., Lopez, A. L., Ghorpade, A., Persidsky, Y., Xiong, H., Leisman, G. B., Che, M. H., and Gendelman, H. E., 2001c, HIV-1 infected and immune competent mononuclear phagocytes induce quantitative alterations in neuronal dendritic arbor: relevance for HIV-1-associated dementia, *Neurotox. Res.* 3:443–459.

Zheng, J., Thylin, M. R., Ghorpade, A., Xiong, H., Persidsky, Y., Cotter, R., Niemann, D., Che, M., Zeng, Y. C., Gelbard, H. A., Shepard, R. B., Swartz, J. M., and Gendelman, H. E., 1999c, Intracellular CXCR4 signaling, neuronal apoptosis and neuropathogenic mechanisms of HIV-1-associated dementia, *J Neuroimmunol.* 98:185–200.

Zheng, J., Thylin, M. R., Persidsky, Y., Williams, C. E., Cotter, R. L., Zink, W., Ryan, L., Ghorpade, A., Lewis, K., and Gendelman, H. E., 2001d, HIV-1 infected immune competent mononuclear phagocytes influence the pathways to neuronal demise, *Neurotox Res.* 3:461–484.

Zhu, S., Cerutis, D., Anderson, J., and Toews, M., 1996, Regulation of hamster alpha 1 B-adrenoceptors expressed in CHO cells, *Eur J Pharmacol.* 299:205–212.

Zou, Y. R., Kottmann, A. H., Kuroda, M., Taniuchi, I., and Littman, D. R., 1998, Function of the chemokine receptor CXCR4 in haematopoiesis and in cerebellar development, *Nature.* 393:595–599.

Zujovic, V., Benavides, J., Vige, X., Carter, C., and Taupin, V., 2000, Fractalkine modulates TNF-alpha secretion and neurotoxicity induced by microglial activation, *Glia.* 29:305–315.

4

SCID Mice Transplanted With Human Cells as Small Animal Models in AIDS Research

CATERINA LAPENTA, STEFANO M. SANTINI, MASSIMO SPADA, and FILIPPO BELARDELLI

1. INTRODUCTION

The progress in AIDS research has been hampered by the lack of a small and practical animal model susceptible to HIV infection, suitable for a rapid testing of antiviral compounds and vaccines. Over the past 20 years, different attempts have been made to develop various models for AIDS in mice. An ensemble of studies carried out in several laboratories during the past decade by using SCID mice transplanted with human cells and infected with HIV have revealed that these chimeric human-mouse models can indeed represent valuable animal systems for addressing a variety of important issues in AIDS research.

SCID (severe combined immunodeficient) mice are characterized by a genetic defect in their DNA repair system which does not allow gene rearrangement of both immunoglobulin and T-cell receptor genes; these mice are profoundly deficient in both humoral and cellular immunity due to the lack of mature B- and T-cells, thus allowing the transplantation of allogeneic and xenogeneic tissues or cells without rejection (Bosma and Carroll, 1991). Both normal and neoplastic human cells can be efficiently engrafted into immunodeficient mice and this specific feature has rendered feasible the in vivo investigation of selected aspects of HIV infection and AIDS. Two major chimeric human/SCID mouse models have proved to be of special interest in AIDS research: (1) the so-called hu-PBL-SCID mouse model, originally developed by Mosier and coworkers (Mosier et al.,

CATERINA LAPENTA, STEFANO M. SANTINI, MASSIMO SPADA, and FILIPPO BELARDELLI • Department of Cell Biology and Neuroscience, Istituto Superiore di Sanità, Rome, Italy, 00161

1988), which has allowed investigation of HIV infection of mature lymphoid cells; (2) the SCID-hu model, described by McCune and colleagues (McCune et al., 1988), which has allowed dissection of HIV infection of fetal hematopoietic cells and tissues, immature as well as mature lymphoid cells. Both animal models have been widely used over the past decade for studies on the pathogenesis and therapy of HIV-1 infection.

In 1988, Mosier and colleagues first reported that human peripheral lymphocytes can stably be engrafted in SCID mice (Mosier et al., 1988). Human peripheral lymphocytes were injected intraperitoneally in SCID mice and persisted in the peritoneal cavity and organs for several months, converting them in human-mouse xenochimeric animals called hu-PBL-SCID mice. Since its description, the hu-PBL-SCID mouse model has been widely used to study selected aspects of human immune response, autoimmunity, and infectious diseases (Mosier, 1996; Duchosal et al., 1990; Saeki et al., 1992; Mima et al., 1995). Xenochimeric mice grafted with human PBLs can be successfully infected with HIV as originally reported by Mosier and collegues (Mosier et al, 1991), and several immunological and viral parameters can be easily monitored in these virus-infected chimeric animals. In the SCID-hu model, human hematopoietic progenitors contained in fetal liver or bone marrow tissue grafts are coimplanted with fetal thymus tissue to create a chimeric animal (McCune et al., 1988). This chimeric model has been widely used in AIDS research for virologic, pathologic, and therapeutic studies.

In this chapter, we will review the main studies performed by using the chimeric human SCID mouse models in AIDS research. We will discuss the importance of these models and the contribution of the studies carried out in these practical animal systems to the development of new concepts on the AIDS pathogenesis and to the evaluation of strategies for the treatment and prevention of HIV infection. Major attention will be given to the hu-PBL-SCID mouse model and to the multiple modified versions of this model, which have been widely utilized by our group over the years.

2. THE HU-PBL-SCID MOUSE MODEL: MAJOR TECHNICAL ASPECTS

Hu-PBL-SCID mouse xenochimeras are obtained by injecting 20–40 $\times$ 10^6 purified human peripheral blood mononuclear cells into the peritoneum of 3- to 5-week-old SCID mice (higher numbers may result in a lethal graft *versus* host disease). Human cells rapidly spread from the peritoneal cavity, setting in the liver, lymph nodes, the white pulp of the spleen, and bone marrow. However, 1–2 months later, the peritoneal cavity remains the major source of human cells surviving to mouse reaction. At this time, the majority of the cells rescued from the peritoneal environment by peri-

toneal washing are represented by human T lymphocytes and they are stained by monoclonal antibodies recognizing CD45, CD3, or CD4 and CD8 human surface markers. Notably, SCID mouse spleens are smaller as compared to those of immunocompetent mice, and these organs occasionally reach abnormal size upon reconstitution. However, few human cells can be recovered from spleen disruption, very often accounting for less than 1% of the total splenic cell population. On the other hand, lymph nodes are not easily detectable, since they are very small in size and enclosed in mouse fat. However, lymph nodes can be pooled and human cells from mouse organs can be enriched by several methods.

In experiments of HIV-1 infection of hu-PBL-SCID-mice, spleen and lymph nodes can generally be used for PCR analysis or cocultivation assays with human PHA-stimulated PBLs in order to monitor p24 release and recover infectious virus. Since human cells become activated early by injection in the murine environment, as confirmed by detection of lymphocyte activation markers and production of large quantities of human antibodies, they represent suitable viral targets and easily support HIV replication. After a few weeks, the human immunologic repertoire is shaped by a murine environment, narrowing the potential responses by human lymphocytes and finally culminating in anergy and unresponsiveness to mitogenic stimuli (Tary-Lehmann and Saxon, 1992; Tary-Lehmann et al., 1995). This phenomenon should be taken into account when interpreting the results obtained in this xenochimeric model or when designing experiments, as the lymphocyte status can markedly influence the outcome of HIV infection. Of note, in the model originally developed by Mosier and co-workers, hu-PBL-SCID mice were challenged with HIV 2 weeks after transplantation of human PBLs (Mosier et al., 1991). In this regard, it should be considered that human T lymphocytes turn into CD45RO memory cells within 2 to 3 weeks after transplantation and become mostly anergic (Tary-Lehmann and Saxon, 1992; Tary-Lehmann et al., 1995). In contrast, at early times after reconstitution, human T-cells are highly activated, expressing the surface activation marker CD69, followed by HLA-DR and CD25 expression (Rizza et al., 1996).

In the SCID-hu thy/liv model, thymus and fetal liver fragments from human embryos are coimplanted under the renal capsule of SCID mice. Fetal liver supplies myeloid and lymphoid precursors, whereas thymus microenvironment supports T-cell differentiation. This model does not allow the generation of B lymphocytes, and the transplanted fetal tissues reach maximum size within 6 months; then, the spilling of human T-cells in peripheral circulation of xenochimeric mice progressively reduces and stops, presumably due to exhaustion of precursors. Some modifications of the model can be performed to increase T-cell number in peripheral circulation, such as implantation of several pieces of thymuses under both kidney capsules.

Further studies have been performed in alternative SCID mice models, combining implantation of human tissues and transfer of human PBLs onto SCID mice or by engrafting immunodeficient mice with cell lines permissive to HIV replication. Virus and cells have been injected by several routes. A novel system has been recently developed by combining SCID mice implanted with thymic and liver tissues from human fetuses with transgenic mouse technology. Hu-SCID mice are injected with leukocytes from transgenic carrying full-lengh provirus, which provide replication competent HIV-1 able to infect thymic implants (Wang et al., 2002).

3. HIV INFECTION IN THE HU-PBL-SCID MOUSE MODEL

Infection of Hu-PBL-SCID Mice

HIV infection of hu-PBL-SCID mice leads to infection and depletion of human CD4 T-cells as a primary consequence. The rate of cell loss depends on distinct factors such as the timing of infection from reconstitution of SCID mice and the viral strain used for the infection of xenochimeric mice. Infection with different viral isolates results in different rates of viral replication and T-cell depletion; virus cell tropism has a key role in determining the fate of CD4 T-cells.

Activation of human lymphocytes in SCID mice has been postulated to mimic the in vivo state of activation of the immune system, which has been recognized as an important cofactor in AIDS pathogenesis (Stevenson et al., 1990). It has become apparent that the hu-PBL-SCID mouse model can reproduce several aspects of virus-cell interaction occurring in HIV-infected patients, not easily detectable under in vitro conditions.

Of note, macrophage-tropic nonsyncytial-inducing (NSI) HIV isolates have been shown to mediate and sustain primary infection of humans, whereas lymphotropic syncytial-inducing (SI) HIV isolates arise at late stages of the disease and have been shown to account for the rapid T-cell depletion. The first evidence showing that the in vivo behavior of distinct HIV-1 isolates cannot be predictable by their in vitro characteristics came from an elegant study by Mosier and colleagues (Mosier et al, 1993), who found that macrophage-tropic clones induced a more rapid loss of human CD4 T-cells as compared to lympho-tropic HIV clones previously found to retain high cytopathicity and syncytial-inducing capacity. In a subsequent study with recombinant viruses carrying reciprocal substitutions in the V3 loop of the gp120 envelope glycoprotein, it has been found that envelope proteins, which are important in determining cell-tropism, play a key role in determining T-cell depletion rates in hu-PBL-SCID mice (Gulizia et al., 1996). Further aspects of HIV-induced pathogenesis and its relationship to cell tropism were found to depend on cellular activation of human target

cells and have been investigated by infecting xenochimeric mice both shortly and at a 2-week time after reconstitution with human PBLs (Fais et al., 1999). In fact, infection of SCID mice performed at 2 hours after intraperitoneal injection of human PBLs has been previously demonstrated to result in a more severe outcome of the infection with respect to mice infected at the 2-week reconstitution time, as a consequence of the special in vivo activation of the human cells at the time of the challenge with lymphotropic HIV strains (Rizza et al., 1996). Infection of highly activated cells at an early time after reconstitution (i.e., 2 hours) showed that infection with either a T-cell-tropic (X4) or a macrophage tropic viral strain (R5) resulted in a comparable level of infection, whereas at 2 weeks after reconstitution the R5 strain proved to be superior in inducing T-cell depletion and higher levels of viral replication, which were associated with a dramatic drop of human immunoglobulin M in mouse sera.

Experiments performed with recombinant clones, carrying reciprocal substitutions in the V3 loop, pointed out the importance of the state of activation/differentiation of human CD4 cells and gp120-coreceptor interactions at the time of primary infection in hu-PBL-SCID model (Fais et al., 1999). Of interest, while the percentage of CXCR4-expressing cells did not change significantly upon injection into SCID mice, CCR5 expression was increased at an early time (24 hours) after injection. Within 1 week, more than 50% of the CD4 cells expressed CCR5, while CXCR4 only showed a slight reduction in its intensity, suggesting a marked susceptibility to R5 strains at later times after engraftment. However, infection of hu-PBL-SCID with R5 strains mice was shown to induce a dramatic depletion of CD4 cells and profound impairment of immune parameters, independently from the state of activation of human cells, whereas infection with X4 strains was deeply affected by the timing of viral challenge and the activation/differentiation of target cells. These results emphasize the major role played by the env gene in determining the pathogenic potential of distinct HIV-1 strains in the hu-PBL-SCID model (Fais et al., 1999). It is of interest to consider that this phenomenon was consistent with that observed in SHIV-infected macaques (Karlsson et al., 1998). In this model, the rate of T-cell depletion was determined by single aminoacid substitutions in the env ectodomain, which conferred increased chemokine receptor binding and enhanced membrane fusogenic activity.

There are several examples of reported differences in the outcome of infection when HIV was used to infect human cells cultured in vitro with respect to when the virus is injected in SCID mice transplanted with human cells. For example, it can be considered paradigmatic that the SCID mouse environment could alter the cell phenotype of human CEM cells after their injection, rendering this cell line, which is not permissive to infection with M-tropic strains in vitro, fully susceptible to the SF162 R5 strains, as a result of an in vivo occurring modulation of the phenotype of the transplanted

cells (Lapenta et al., 1998). In particular, we showed that subcutaneous injection of CEM cells into SCID mice resulted in the acquisition of a memory-like phenotype, upregulating CD45RO, CCR2, CCR3, and CCR5, whose expression is associated with a high susceptibility to the in vivo infection with the HIV-1 monocytotropic strain SF162 and with a series of monocytotropic clinical isolates, which cannot infect CEM cells in vitro (Lapenta et al., 1998). The susceptibility of CEM cells transplanted into SCID mice to HIV infection was shown to be selectively inhibited by the β-chemokines such as RANTES, Mip-1α, and Mip-1β (the natural CCR5 ligands), In this simplified hu-SCID mouse model, the susceptibility to Fas/Fas-L-mediated suicide, upon HIV infection, was modulated by a murine environment and progressively lost upon culture of ex-vivo CEM cells. Of note, when the ability of distinct HIV-1 variants to establish infection in cultured PBLs or in the hu-PBL-SCID mice was compared, the selective or preferential transmission of NSI variants and a pattern of restriction in viral transmission similar to that observed in patients was observed in hu-PBL-SCID mice. NSI variants appeared to infect the xenochimeras with higher efficiency than SI variants, resulting in the more rapid decline in CD4 T-cells (Markham et al., 1996). Perhaps, massive cell activation in cell culture after mitogenic stimulation may explain the flattening of pathogenetic effects exerted by different viral strains or isolates. Of interest, R5 strains proved to be less pathogenic for fetal thymus graft in SCID-hu model (Berkowitz et al., 1999), and the pathogenic differences were predominantly linked to the differences in viral tropism, coreceptor use, and, consequently, to the altered tropism for cell subpopulations within the Thy/Liv organ (Berkowitz et al., 2000). Moreover, distinct R5 HIV-1 isolates taken from a patient prior the onset of AIDS and at AIDS diagnosis have been shown to infect SCID-hu mice with different efficiencies. The AIDS-associated clone showed higher replication rate and efficiently depleted CD4 cells in human thymus (Scoggins et al., 2000). The characteristics of the type of human-mouse SCID model used for infection studies must be taken into account; for instance, in the hu-SCID model, thymocytes express CXCR4 during most stages of maturation and only low levels of CCR5 (Berkowitz et al., 1998) and this can explain the preferential infection with SI strains in this model.

HIV Infection in Modified Models of Hu-PBL-SCID Mice

The hu-PBL-SCID mouse model originally developed by Mosier (Mosier et al., 1988), consisting in the infection of xenochimeric mice 2 weeks after intraperitoneal reconstitution with hu-PBLs, has sometimes been modified and adapted to address specific issues in AIDS research.

As mentioned above, studies carried out in the hu-PBL-SCID mouse model have revealed that HIV cell tropism and the timing of virus chal-

lenge of hu-PBL-SCID mice are tightly linked aspects affecting the outcome of infection. We have compared the infection of hu-PBL-SCID mice shortly or at a later time point after reconstitution (Rizza et al., 1996). As early as 2 hours after reconstitution, a significant number of human T-cells become activated and upregulate the expression of the surface CD69 marker. However, at that time a large number of CD4 cells are still naïve CD45RA+ cells, expressing very low levels of CCR5, which is increased only at later times after PBL engraftment. Of interest, when hu-PBL-SCID mice were infected with the X4 laboratory strain IIIB, a marked CD4+ T-cell depletion and a significant reduction in IgA, IgM, and sIL-2R serum levels and sICAM-1 in peritoneal washings were observed in the xenochimeras infected at 2 hours, while the immune functions of hu-PBL-SCID mice infected at 2 weeks were almost comparable to that of uninfected controls. Of note, the generalized impairment of immune functions detected in the xenochimeras infected at early times was accompanied by the selective upregulation of the expression of some cytokines (IL-4, IL-5, and IL-10) as revealed by RT-PCR. This was reminiscent of the Th2 profile observed in HIV patient experiencing rapid progression of the disease and a worse prognosis. The switch of Th1 into Th2 profile of cytokine production has also been suggested to account for the higher rate of CD4+ T-cell apotosis seen in these patients (Clerici et al., 1994). Comparable levels of infection were found challenging hu-PBL-SCID mice with either the R5 SF162 or the X4 IIIB HIV-1 strains at 2 hours after reconstitution. However, infection with the R5 strain resulted in high levels of virus replication and in a marked CD4 T-cell depletion together with a significant drop in IgM and soluble factors levels at both 2 hours and 2 weeks after human cell grafting. In contrast, the X4 virus induced a very low rate of CD4 depletion and viral replication, with limited viral spread to mouse spleen and lymph nodes, when injected 2 weeks after reconstitution. These results obtained in the hu-PBL-SCID mouse model (Fais et al., 1999) are consistent with the concept that the state of activation of immune cells can markedly affect the rate of HIV replication and disease progression in HIV-infected individuals (Stevenson et al., 1990; Bentwich et al., 1995; Sousa et al., 2002). In particular, it has been argued that the chronic state of immune activation (due to the parasitic and bacterial diseases) is the major cause of the impressive prevalence and incidence of HIV infection in Africa as well as of the rapid progression to AIDS in the infected patients (Bentwich et al., 1995). This is also consistent with data obtained in SIV-infected macaques, where hyperimmune activation has been shown to accelerate disease progression (Folks et al., 1997). Thus, the ensemble of experimental data obtained in our modified model of HIV-1 infection in hu-PBL-SCID mice support the concept of the important role of acute or chronic immune activation in natural infection in rendering the host more receptive to HIV and more sensitive to its pathologic effects.

The investigation of particular aspects of HIV infection or pathogenesis in hu-PBL-SCID mice has been hampered by the intrinsic limitations of this xenochimeric model, such as the lack of physiologic replenishment of T-cells, human microenvironment, and lymph nodes. However, new approaches are currently being tested to circumvent some of these limitations. Selected aspects of HIV research have been investigated in xenochimeric models with major or minor adaptation or modification of the classical hu-PBL-SCID model and considering different infection routes. Modalities of mucosal and sexual transmission of HIV have been investigated in modified hu-PBL-SCID mice models. Administration of IL-4 to hu-PBL-NOD/SCID mice has been shown to favor CD4 and CD8 T-cell migration in spleen and peritoneal tissues as a modification of the SCID model described by Bombil and coworkers (Bombil et al, 1996). However, it was found that even high concentrations of cell-free R5 and X4 viruses failed to infect xenochimeric mice by oral transmission with or without trauma, suggesting that some constraints, such as lack of normal tonsillar tissues or presence of aspecific inhibitors, may limit viral passage to CD4 T-cells through oral mucosa. In this setting, the transmission of HIV-1 infection by cell-associated virus was not evaluated (Nakao et al., 2003). Of interest, Di Fabio and colleagues (Di Fabio et al, 2001) have developed a modified Hu-SCID model for the investigation of vaginal HIV-1 transmission by infecting progesteron-treated hu-PBL-SCID mice by vaginal route with virus-infected cells. In this model, progesteron pretreatment was necessary to modify the vaginal ephitelium, allowing the transepithelial migration of HIV-infected PBLs to regional lymph nodes. This model has been proposed for the testing of prophylactic microbicides inhibiting cell-associated HIV-1 transmission. A similar Hu-PBL-SCID model of vaginal HIV-1 transmission proved useful for studying the effects of an agent interfering with migration of HIV-1-infected cells and testing its potential efficacy in preventing sexual HIV-1 transmission (Khanna et al., 2002). A more complex model has been developed by Kish and colleagues (Kish et al., 2001), who implanted vaginal tissue within the subcutaneous space on the back of SCID-NOD mice, whose growth was supported by a coimplanted estrogen pellet. Mice are injected intraperitoneally with PBLs and have been shown to be useful for studies on HSV and HPV infection as well as for vaginal transmission of HIV-1. This is not a completely novel approach as combined engraftment of SCID mice has been sometimes performed to investigate selected aspects of HIV infection.

A novel system for studying viral reservoirs, transmission, and therapy has been recently developed by combining SCID mice implanted with thymic and liver tissues from human fetuses with transgenic mouse technology. Hu-SCID mice were injected with leukocytes from transgenic animals carrying full-lengh provirus, which provide replication competent HIV-1 able to infect thymic implants (Wang et al., 2002).

Some researchers have attempted to adapt xenochimeric mouse models for investigating neurodegenerative disorders associated with HIV infection. An example of a modified model in hu-NOD-SCID mice was obtained by injecting HIV-infected macrophages into mouse brains (Poluektova et al., 2002). This model can recapitulate several aspects of human HIV-1 encephalitis, highlighting the importance of cell-cell interactions between brain macrophages and lymphocytes and cell migration. Poluektova and colleagues reported that several features of natural infection could be reproduced in this xenochimeric model, including the viral production in both brain and blood, CD4 T-cell depletion, and the generation of a CTL response against infected macrophages restricting viral replication.

Infiltration of the perivascular region of the brain with HIV-1-infected cells was obtained by LPS treatment of hu-PBL-NOD-SCID mice in order to investigate in vivo the role of apoptosis in HIV-associated dementia (Miura et al., 2003). Miura and coworkers suggested a causative role of TRAIL in the neuronal apoptosis in HIV-associated dementia. In fact, the neurons neighboring TRAIL-expressing human-infected macrophages were frequently apoptotic. The administration of neutralizing antibodies against TRAIL blocked neuronal apoptosis. Of note, the same authors had also previously utilized the hu-PBL-NOD-SCID mouse model for investigating the mechanism of T-cell depletion (Miura et al., 2001), finding that TRAIL is primarily responsible for apoptosis of bystander CD4 T-cells in the lymphoid organs of infected xenochimeric mice, as evidenced by TUNEL staining.

4. THERAPY AND IMMUNIZATION STUDIES

Testing of Antiviral Agents

An antiviral molecule, once tested in vitro, needs to be tested in an animal model before its possible clinical use, in order to determine toxicity, metabolism, bioavailability, clearance, and antiviral activity. Xenochimeric SCID mice (especially the SCID-hu model) have been used to test antiviral substances (McCune et al., 1990). In spite of the remarkable advantages of these models for studies on therapy of HIV infection, there are still two important limitations: (1) the restriction of HIV to a subset of the transplanted human cells; (2) the relatively short persistence of viral replication. In an attempt to circumvent some of these problems, we have developed a modified model consisting of the efficient engraftment of the U937 cell line into SCID mice; virus infection of these hu-SCID mice permitted high levels of viremia and long lasting HIV-1 infection (Lapenta et al., 1997). Of note, xenochimeric SCID models are even useful

to dissect some specific aspects linked to antiviral therapy, as pointed out by Picchio and colleagues (Picchio et al., 2000), who suggested that studies in SCID mice could reflect some in vivo constraints on virus spread; in particular, the characteristics of the hu-PBL-SCID mice may accentuate minor differences in viral kinetics and spread, which are flattened under in vitro conditions. HIV-1 isolates from patients who failed protease inhibitor-based therapy were shown to exhibit diminished replication capacity in xenochimeric mice, accounting for a possible contribution to CD4+ cell preservation in the presence of virologic failure. Difference in kinetics of plasma viremia was shown by virologic monitoring, demonstrating a decrease in relative viral fitness associated with resistance-conferring mutations.

Recently, the hu-PBL-SCID mouse model has also been used to investigate the in vivo activity of a monoclonal antibody against nerve growth factor (NGF), which proved to be capable of inhibiting HIV infection occurring after injection of virus-infected macrophages (Garaci et al., 2003); it was suggested that the antibody acted by inducing apoptosis of HIV-1-infected macrophages as previously reported in vitro studies with virus-infected cells.

Evaluation of the Anti-HIV Activity of Cytokines: Lessons on Type-I IFN

Chimeric models of SCID mice transplanted with human cells can be useful for the evaluation of the in vivo anti-HIV activity of cytokines and Type-I IFN is one of the cytokines extensively tested in these models. Although several in vitro studies had shown an inhibitory activity on HIV-1 replication, the role of Type-I IFN in the pathogenesis of AIDS was still a matter of debate as both beneficial and adverse effects on the immune system have been considered. Some studies performed in the hu-PBL-SCID mouse model have addressed this issue by using different approaches. Vieillard and colleagues (Vieillard et al., 1999) utilized a modified model, which consisted of the periodic inoculation of activated CD4 T-cells to maintain high levels of human cell engraftment. Injection of hu-PBL-SCID mice with IFN-β-transduced CD4 T-cells significantly reduced HIV-1 levels in preexisting infections and resulted in an improved survival of human CD4 T-cells. In 40% of treated mice, no virus could be detected in vivo or after cocultivation of cells from the xenochimeric mice with activated T-cells. Furthermore, an enhancement of the expression of Th1 cytokine transcripts, such as IL-12 and IFNγ, could be detected in the spleen of the hu-PBL-SCID mice receiving IFN-β-transduced CD4 T-cells. By using a different experimental setting, we demonstrated that the daily treatment of hu-PBL-SCID mice with Type-I IFN inhibited HIV-1 replication even more efficiently than AZT treatment, preserving human CD4 T-cells. Notably IFN-treated mice showed a marked increase in serum human IgM levels

(Lapenta et al., 1999). The data obtained in this model are somehow consistent with those reported by Vieillard and colleagues and suggest that IFN neither suppress immune response nor affect CD4 T-cell viability, but it may rather enhance the generation of a protective immune response against the virus.

Evaluation of the Immune Response to HIV

Today, increasing attention is given to the research efforts of developing immunity-based strategies for the therapy and prevention of HIV-1 infection. In fact, although the course of AIDS has profoundly changed following the introduction of highly active antiviral treatment (HAART), numerous shortcomings are emerging. Despite the clinical benefits, antiviral treatments are limited by side toxicity and cost of lifelong administration, while virus burst often occurs upon discontinuation of therapy, which results in containment of infection but not in HIV eradication.

While hu-PBL-SCID mice have been extensively used in testing antiviral drugs, the usefulness of using these xenochimeric animal models for studies of immunotherapy of HIV infection as well as in vaccine research is still a matter of debate. It is generally thought that the shaping of human immune repertoire, the lack of physiologic lymphocyte replenishment by lymphoid organs, and the loss of professional antigen-processing cells may hamper the generation of specific response to primary antigens, even though a humoral response to recall antigens has been repeatedly demonstrated (Somasundaram et al., 1995; Albert et al., 1997). Undoubtedly, the ability to induce anti-HIV immunity in a small and practical animal model using human cells, which can also be infected with the same virus naturally infecting AIDS patients, would offer the unique opportunity to test immunogens, immunization procedures, and adjuvants, as well as to monitor viral and immunologic parameters in vivo. In the following two sections, we will review some studies showing that the hu-PBL-SCID model can successfully adapted to be used for studies of both passive and active immunity against HIV.

Studies of Passive Immunity in Hu-PBL-SCID Mice

Several investigators have bypassed difficulties in obtaining a primary immune response in hu-PBL-SCID mice by focusing their research efforts on passive administration of antibodies or cellular effectors in an attempt to protect xenochimeric mice from HIV infection. Early reports suggested that passive administration of high doses of neutralizing antibodies could protect xenochimeric mice from HIV challenge (Safrit et al., 1993). Interestingly, antibody half-life in mouse blood was similar to that of mouse immunoglobulins. Mice were protected at an antibody concentration

attainable in human blood (Gauduin et al., 1995). Passive antibody administration protected hu-PBL-SCID mice against primary virus challenge. A high dose of a potent neutralizing human monoclonal antibody has been shown to confer protection against HIV even when given several hours after viral challenge, supporting the concept that postexposure antibody prophylaxis could contribute to an effective strategy of immune prevention. (Gauduin et al., 1997). However, some drawbacks connected to passive immunization with mAbs have also been highlighted in the hu-PBL-SCID mouse model, as administration of an mAb toward the V3 loop of HIV-1 as preinfection prophylaxis resulted in rapid emergence of escape mutants resistant to neutralization, despite the fact that 50% of the challenged xenochimeric mice were protected from infection. Notably, postinfection treatment was shown ineffective, unless given soon after virus infection (Andrus et al., 1998). While feasibility and opportunity to perform large-scale administration of neutralizing antibodies are regarded with skepticism, a possible potential application of antibodies might be restricted to selected cases, such as blocking of maternal-fetal transmission as a post-exposure prophylaxis of primary HIV-1 infection. This application has been postulated by the work by Wang and colleagues (Wang et al.), as intravenous infusion of a monoclonal antibody directed to HIV receptor complex has been shown to confer pre- and postexposure protection against HIV infection in both hu-PBL-SCID mice and chimpanzees. However, cross-reactivity with distinct viral isolates should be a requirement for any antibody or antibody cocktail to be used in clinics, as none of the antibodies proposed so far have been demonstrated to confer a wide protection against divergent strains. Of note, a CD4-immunoglobulin fusion protein has been tested in hu-PBL-SCID mice and found to neutralize a broad range of primary isolates (Gauguin et al., 1998). On the other hand, neutralizing antibodies have been shown to retain limited effects on the control of established HIV-1 infection in hu-PBL-SCID mice (Poignard et al., 1999). From studies in animal models it might be argued that both arms of the immune response (neutralizing antibodies and cellular immunity) are important and complementary. Of interest, in infected hu-PBL-SCID mice, HIV-1 gag-specific CTL clones have been shown to exert antiviral activity in vivo (McKinney et al., 1999), reducing plasma viral load more effectively than the infusion of large quantities of neutralizing antibodies (Poignard et al., 1999). However, CTL clones proved to be short-lived, as they rapidly disappeared upon encounter with infected targets (McKinney et al., 1999). Intriguingly, infusion of either HIV-specific or irrelevant CTL clones in hu-PBL-SCID mice led to a similar protection extent, suggesting that protection could be due to a HLA-unrestricted mechanism (Van Kuyk et al., 1994).

Hu-PBL-SCID mice have been used for evaluating the immune state of HIV-infected patients or vaccinated individuals and for testing their pro-

tection against HIV. In 1993, Mosier and coworkers (Mosier et al., 1993) used the PBLs from donors vaccinated with the HIV-1 gp160 protein to engraft SCID mice to test the efficacy of a candidate vaccine. Hu-PBL-SCID mice made a secondary antibody response to HIV and showed resistance to HIV infection; however, there was no correlation between antibody detection and resistance to infection, which was linked with the T-cell proliferative response. In general, it has been suggested that cellular immunity is very important for containment of HIV infection in HIV-infected and exposed individuals. In this regard, engraftment of PBLs from HIV-infected donors in SCID mice has been shown to result in the burst of viral replication and dramatic CD4 depletion; HIV could be detected in the spleen, peritoneal washings, and blood of these mice (Boyle et al., 1995). However, the engraftment with PBLs from long-term nonprogressors (LTNPs) resulted in a marked resistance of the hu-PBL-SCID mice to HIV-1 challenge. Resistance was specifically mediated by CD8 T-cells and associated with proliferative response to p24 antigen (de Quiros et al., 2000). In addition, SCID/beige mice reconstituted with PBLs from HIV-exposed seronegative donors have been shown to resist HIV-1 challenge (Zhang et al., 1996). Of note, depletion of human CD8 cells conferred susceptibility to HIV-1 infection, indicating that protective immunity in HIV-exposed individuals was CD8-dependent.

Immunization Studies in Hu-PBL-SCID Mice

As reported above, this model has been shown to retain some constraints and intrinsic limitations regarding the generation of a human primary immune response. However, a suitable microenvironment and APCs can be provided in order to permit the response to primary antigens. To this purpose, Delhem and colleagues (Delhem et al., 1998) engrafted human skin onto hu-PBL-SCID, thus providing both tissue resident APCs and environment. In fact, intradermal vaccination with recombinant canarypox virus expressing HIV-1 gp160 was shown to result in the generation of specific CD4+ cell lines exhibiting Th1 cytokine profile and MHC class II–restricted cytotoxic activity against gp160 antigens; both CD4 and CD8 infiltrating cells could be detected in the skin graft. Of note, in this model no antibody response was detected. This report suggested that it was possible to supply some necessary components for mounting a human primary immune response into xenochimeric SCID mice. Since the strategy of grafting SCID mice with human tissues is complex, laborious, and time-consuming, we decided to use a different approach for immunizing hu-PBL-SCID mice, consisting in supplying suitable numbers of antigen-pulsed dendritic cells (DCs), that is, the crucial APCs for mounting a primary immune response. DCs were injected intraperitoneally, at very early times after reconstitution with human PBLs, when the immune reper-

toire is still intact. In our experiments, DCs were generated from autologous monocytes cultured the presence of GM/CSF and IFN-α, according to a procedure developed in our laboratory (Santini et al., 2000), which permits the rapid generation of functionally competent DCs, thus allowing the infusion of highly active antigen-pulsed APCs as early as 3 days after reconstitution of SCID mice with human PBLs. Immunizations consisted in intraperitoneal injection of DCs pulsed with inactivated HIV-1, at 7-day intervals, followed by a single injection of free inactivated virus. Immunized xenochimeras exhibited increasing levels of circulating specific human antibodies. High levels of human antibodies directed to virtually all HIV-1 proteins were detected at the time of sacrifice, as demonstrated by western blot analysis. Mouse sera were found to exert inhibitory activity against in vitro infection of activated T-cells by the homologous HIV-1 viral strain. Furthermore, it was possible to isolate human HIV-specific IFNγ-producing CD8+ cells from both peritoneum and spleens of vaccinated mice, as detected by ELISPOT assay. Interestingly, in the present study, the induction of the anti-HIV-1 immune response was associated with inhibition of HIV-1 infection/replication, when the animals were challenged with the homologous SF-162 viral strain, with absent or very low levels of virus in mouse peritoneum or proviral DNA in mouse organs (Lapenta et al., 2003). The data obtained in this model represent the first "proof of concept" on the potential in vivo efficacy of human DC-based anti-HIV vaccines. Although our experiments have been designed to evaluate the efficacy of DC-based vaccines in a prophylactic context because of the characteristics of the hu-PBL-SCID mouse model, the ensemble of results may be more relevant to the design of therapeutic vaccination strategies, as this potentially highly effective vaccination strategy is not practicable for large-scale preventive immunization.

5. FINAL REMARKS

Studies performed by several groups, including ours, during the past decade have revealed that the hu-PBL-SCID mouse model originally developed by Mosier and co-workers in 1988 (Mosier et al., 1988) can be successfully used and, under specific experimental needs, selectively modified in order to address a variety of issues of considerable importance in AIDS research, regarding aspects of pathogenesis, therapy, and immunity of HIV-1 infection. In several cases, it was possible to demonstrate that results obtained in these animal models can recapitulate events and mechanisms occurring in AIDS patients. As mentioned above, the transplantation of SCID with human cells has led to other models, which may have advantages for specific applications in AIDS research. Even though chimeric models of human SCID mice exhibit some obvious limitations with respect

to studies performed in other animal models for AIDS, they exhibit some unique advantages such as the possibility to study in vivo the interaction between HIV and human cells; moreover, these models are small, practical, and poorly expensive as compared to large animal models and can be easily implemented to address specific issues by further manipulation, cell injections, and treatments. It is reasonable to assume that these small animal models will be used to address an increasing number of issues in AIDS research in the following years, spanning from pathogenesis to therapy and immune prevention and immunotherapy of HIV infection, in a complementary manner with respect to the use of other valuable animal models for AIDS.

Acknowledgments. We are grateful to Cinzia Gasparrini and Anna Ferrigno for excellent secretarial assistance. This work was supported in part by grants from the Italian Project on AIDS (Istituto Superiore di Sanità-Ministry of Health) and from the special project on Cytokines as Vaccine Adjuvants (Italian Ministry of Health).

REFERENCES

Albert, S. E., McKerlie, C., Pester, A., Edgell, B. J., Carlyle, J., Petric, M., and Chamberlain, J. W., 1997, Time-dependent induction of protective anti-influenza immune responses in human peripheral blood lymphocyte/SCID mice. *Immunology.* 159:1393.

Andrus, L., Prince, A. M., Bernal, I., McCormack, P., Lee, D. H., Gorny, M. K., and Zolla-Pazner, S., 1998, Passive immunization with a human immunodeficiency virus type 1-neutralizing monoclonal antibody in Hu-PBL-SCID mice: isolation of a neutralization escape variant. *J Infect Dis.* 177:889.

Bentwich, Z., Kalinkovich, A., and Weisman, Z., 1995, Immune activation is a dominant factor in the pathogenesis of African AIDS. *Immunol Today.* 16:187.

Berkowitz, R. D., Alexander, S., and McCune, J. M., 2000, Causal relationships between HIV-1 coreceptor utilization, tropism, and pathogenesis in human thymus. *AIDS Res Hum Retroviruses.* 16:1039.

Berkowitz, R. D., Beckerman, K. P., Schall, T. J., and McCune, J. M., 1998, CXCR4 and CCR5 expression delineates targets for HIV-1 disruption of T-cell differentiation. *J Immunol.* 161:3702.

Berkowitz, R. D., van't Wout, A. B., Kootstra, N. A., Moreno, M. E., Linquist-Stepps, V. D., Bare, C., Stoddart, C. A., Schuitemaker, H., and McCune, J. M., 1999, R5 strains of human immunodeficiency virus type 1 from rapid progressors lacking X4 strains do not possess X4 type pathogenicity in human thymus. *J Virol.* 73:7817.

Bombil, F., Latinne, D., Kints, J. P., and Bazin, H., 1996, Human recombinant interleukin-4 (HurIL-4) improves SCID mouse reconstitution with human peripheral blood lymphocytes. *Immunobiology.* 196:437.

Bosma, M. J., and Carroll, A. M., 1991, The SCID mouse mutant: definition, characterization, and potential uses. *Ann Rev Immunol.* 9:323.

Boyle, M. J., Connors, M., Flanigan, M. E., Geiger, S. P., Ford, H. Jr., Baseler, M., Adelsberger, J., Davey, R. T. Jr., and Lane, H. C., 1995, The human HIV/peripheral blood lymphocyte

(PBL)-SCID mouse. A modified human PBL-SCID model for the study of HIV pathogenesis and therapy. *J Immunol.* 154:6612.

Clerici, M., Sarin, A., Coffman, R. L., Wynn, T. A., Blatt, S. P., Hendrix, C. W., Wolf, S. F., Shearer, G. M., and Henkart, P. A., 1994, Type 1/type 2 cytokine modulation of T-cell programmed cell death as a model for human immunodeficiency virus pathogenesis. *Proc Natl Acad Sci USA.* 91:11811.

de Quiros, J. C., Shupert, W. L., McNeil, A. C., Gea-Banacloche, J. C., Flanigan, M., Savage, A., Martino, L., Weiskopf, E. E., Imamichi, H., Zhang, Y. M., Adelsburger, J., Stevens, R., Murphy, P. M., Zimmerman, P. A., Hallahan, C. W., Davey, R. T. Jr., and Connors, M., 2000, Resistance to Replication of Human Immunodeficiency Virus Challenge in SCID-Hu Mice Engrafted with Peripheral Blood Mononuclear Cells of Nonprogressors Is Mediated by CD8+ T-Cells and Associated with a Proliferative Response to p24 Antigen. *J Virol.* 74:2023.

Delhem, N., Hadida, F., Gorochov, G., Carpentier, F., de Cavel, J. P., Andreani, J. F., Autran, B., and Cesbron, J. Y., 1998, Primary Th1 cell immunization against HIVgp160 in SCID-hu mice coengrafted with peripheral blood lymphocytes and skin. *J Immunol.* 161:2060.

Di Fabio, S., Giannini, G., Lapenta, C., Spada, M., Binelli, A., Germinario, E., Sestili, P., Belardelli, F., Proietti, E., and Vella, S., 2001, Vaginal transmission of HIV-1 in hu-SCID mice:a new model for the evaluation of vaginal microbicides. *AIDS.* 15:2231.

Duchosal, M. A., McConahey, P. J., Robinson, C. A., and Dixon, F. J., 1990, Transfer of human systemic lupus erythematosus in severe combined immunodeficient (SCID) mice. *J Exp Med.* 172:985.

Fais, S., Lapenta, C., Santini, S. M., Spada, M., Parlato, S., Logozzi, M., Rizza, P., and Belardelli, F., 1999, Human immunodeficiency virus type 1 strains R5 and X4 induce different pathogenic effects in hu-PBL-SCID mice, depending on the state of activation/differentiation of human target cells at the time of primary infection. *J Virol.* 73:6453.

Folks, T., Rowe, T., Villinger, F., Parekh, B., Mayne, A., Anderson, D., McClure, H., and Ansari, A. A., 1997, Immune stimulation may contribute to enhanced progression of SIV induced disease in rhesus macaques. *J Med Primatol.* 26:181.

Garaci, E., Aquaro, S., Lapenta, C., Amendola, A., Spada, M., Covaceuszach, S., Perno, C. F., and Belardelli, F., 2003, Anti-nerve growth factor Ab abrogates macrophage-mediated HIV-1 infection and depletion of CD4+ T lymphocytes in hu-SCID mice. *Proc Natl Acad Sci USA.* 100:8927.

Gauduin, M. C., Allaway, G. P., Olson, W. C., Weir, R., Maddon, P. J., and Koup, R. A., 1998, CD4-immunoglobulin G2 protects Hu-PBL-SCID mice against challenge by primary human immunodeficiency virus type 1 isolates. *J Virol.* 72:3475.

Gauduin, M. C., Parren, P. W., Weir, R., Barbas, C. F., Burton, D. R., and Koup, R. A., 1997, Passive immunization with a human monoclonal antibody protects hu-PBL-SCID mice against challenge by primary isolates of HIV-1. *Nat Med.* 3:1389.

Gauduin, M. C., Safrit, J. T., Weir, R., Fung, M. S., and Koup, R. A., 1995, Pre- and post-exposure protection against human immunodeficiency virus type 1 infection mediated by a monoclonal antibody. *J Infect Dis.* 171:1203.

Gulizia, R. J., Levy, J. A., and Mosier, D. E., 1996, The envelope gp120 gene of human immunodeficiency virus type 1 determines therate of CD4-positive T-cell depletion in SCID mice engrafted with human peripheral blood leukocytes. *J Virol.* 70:4184.

Karlsson, G. B., Halloran, M., Schenten, D., Lee, J., Racz, P., Tenner-Racz, K., Manola, J., Gelman, R., Etemad-Moghadam, B., Desjardins, E., Wyatt, R., Gerard, N. P., Marcon, L., Margolin, D., Fanton, J., Axthelm, M. K., Letvin, N. L., and Sodroski, J., 1998, The envelope glycoprotein ectodomains determine the efficiency of CD4+ T lymphocyte depletion in simian-human immunodeficiency virus-infected macaques. *J Exp Med.* 188:1159.

Khanna, K. V., Whaley, K. J., Zeitlin, L., Moench, T. R., Mehrazar, K., Cone, R. A., Liao, Z., Hildreth, J. E., Hoen, T. E., Shultz, L., and Markham, R. B., 2002, Vaginal transmission

of cell-associated HIV-1 in the mouse is blocked by a topical, membrane-modifying agent. *J Clin Invest.* Jan 109:205.

Kish, T. M., Budgeon, L. R., Welsh, P. A., and Howett, M. K., 2001, Immunological characterization of human vaginal xenografts in immunocompromised mice: development of a small animal model for the study of human immunodeficiency virus-1 infection. *Am J Pathol.* 159:2331.

Lapenta, C., Fais, S., Rizza, P., Spada, M., Logozzi, M. A., Parlato, S., Santini, S. M., Pirillo, M., Belardelli, F., and Proietti, E., 1997, U937-SCID mouse xenografts: a new model for acute in vivo HIV-1 infection suitable to test antiviral strategies. *Antiviral Res.* 36:81.

Lapenta, C., Parlato, S., Spada, M., Santini, S. M., Rizza, P., Logozzi, M., Proietti, E., Belardelli, F., and Fais, S., 1998, Human lymphoblastoid CD4(+) T-cells become permissive to macrophage-tropicstrains of human immunodeficiency virus type 1 after passage into severe combined immunodeficient mice through in vivo upregulation of CCR5: in vivo dynamics of CD4(+) T-cell differentiation in pathogenesis of AIDS. *J Virol.* 72:10323.

Lapenta, C., Santini, S. M., Logozzi, M., Spada, M., Andreotti, M., Di Pucchio, T., Parlato, S., and Belardelli, F., 2003, Potent Immune Response against HIV-1 and Protection from Virus Challenge in hu-PBL-SCID Mice Immunized with Inactivated Virus-pulsed Dendritic Cells Generated in the Presence of IFN-α. *J Exp Med.* 198:361.

Lapenta, C., Santini, S. M., Proietti, E., Rizza, P., Logozzi, M., Spada, M., Parlato, S., Fais, S., Pitha, P. M., and Belardelli, F., 1999, Type I interferon is a powerful inhibitor of in vivo HIV-1 infection and preserves human CD4(+) T-cells from virus-induced depletion in SCID mice transplanted with human cells. *Virology.* 263:78.

Markham, R. B., Schwartz, D. H., Templeton, A., Margolick, J. B., Farzadegan, H., Vlahov, D., and Yu, X. F., 1996, Selective transmission of human immunodeficiency virus type 1 variants to SCID mice reconstituted with human peripheral blood monoclonal cells. *J Virol.* 70:6947.

McCune, J. M., Namikawa, R., Kaneshima, H., Shultz, L. D., Lieberman, M., and Weissman, I. L., 1988, The SCID-hu mouse: murine model for the analysis of human hematolymphoid differentiation and function. *Science.* 241:1632.

McCune, J. M., Namikawa, R., Shih, C. C., Rabin, L., and Kaneshima, H., 1990, Suppression of HIV infection in AZT-treated SCID-hu mice. *Science.* 247:564.

McKinney, D. M., Lewinsohn, D. A., Riddell, S. R., Greenberg, P. D., and Mosier, D. E., 1999, The antiviral activity of HIV-specific CD8+ CTL clones is limited by elimination due to encounter with HIV-infected targets. *J Immunol.* 163:861.

Mima, T., Saeki, Y., Ohshima, S., Nishimoto, N., Matsushita, M., Shimizu, M., Kobayashi, Y., Nomura, T., and Kishimoto, T., 1995, Transfer of rheumatoid arthritis into severe combined immunodeficient mice. The pathogenetic implications of T-cell populations oligoclonally expanding in the rheumatoid joints. *J Clin Invest.* 96:1746.

Miura, Y., Misawa, N., Kawano, Y., Okada, H., Inagaki, Y., Yamamoto, N., Ito, M., Yagita, H., Okumura, K., Mizusawa, H., and Koyanagi, Y., 2003, Tumor necrosis factor-related apoptosis-inducing ligand induces neuronal death in a murine model of HIV central nervous system infection. *Proc Natl Acad Sci USA.* 100:2777.

Miura, Y., Misawa, N., Maeda, N., Inagaki, Y., Tanaka, Y., Ito, M., Kayagaki, N., Yamamoto, N., Yagita, H., Mizusawa, H., and Koyanagi, Y., 2001, Critical contribution of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) to apoptosis of human CD4+ T-cells in HIV-1-infected hu-PBL-NOD-SCID mice. *J Exp Med.* 193:651.

Mosier, D. E., 1996, Humanizing the mouse. *Semin Immunol.* 8:185.

Mosier, D. E., Gulizia, R. J., Baird, S. M., and Wilson, D. B., 1988, Transfer of a functional human immune system to mice with severe combined immunodeficiency. *Nature.* 335:256.

Mosier, D. E., Gulizia, R. J., Baird, S. M., Wilson, D. B., Spector, D. H., and Spector, S. A., 1991, Human immunodeficiency virus infection of human-PBL-SCID mice. *Science.* 251:791.

Mosier, D. E., Gulizia, R. J., MacIsaac, P. D., Corey, L., and Greenberg, P. D., 1993, Resistance to human immunodeficiency virus 1 infection of SCID mice reconstituted with peripheral blood leukocytes from donors vaccinated with vaccinia gp160 and recombinant gp160. *Proc Natl Acad Sci USA.* 90:2443.

Mosier, D. E., Gulizia, R. J., MacIsaac, P. D., Torbett, B. E., and Levy, J. A., 1993, Rapid loss of CD4+ T-cells in human-PBL-SCID mice by noncytopathic HIV isolates. *Science.* 260: 689.

Nakao, R., Hanada, N., Asano, T., Hara, T., Abdus Salam, M., Matin, K., Shimazu, Y., Nakasone, T., Horibata, S., Aoba, T., Honda, M., Amagasa, T., and Senpuku, H., 2003, Assessment of oral transmission using cell-free human immunodeficiency virus-1 in mice reconstituted with human peripheral blood leucocyte. *Immunology.* 109:271.

Picchio, G. R., Valdez, H., Sabbe, R., Landay, A. L., Kuritzkes, D. R., Lederman, M. M., and Mosier, D. E., 2000, Altered viral fitness of HIV-1 following failure of protease inhibitor-based therapy. *J Acquir Immune Defic Syndr.* 25:289.

Poignard, P., Sabbe, R., Picchio, G. R., Wang, M., Gulizia, R. J., Katinger, H., Parren, P. W., Mosier, D. E., and Burton, D. R., 1999, Neutralizing antibodies have limited effects on the control of established HIV-1 infection in vivo. *Immunity.* 10:431.

Poluektova, L. Y., Munn, D. H., Persidsky, Y., and Gendelman, H. E., 2002, Generation of cytotoxic T-cells against virus-infected human brain macrophages in a murine model of HIV-1 encephalitis. *J Immunol.* 168:3941.

Rizza, P., Santini, S. M., Logozzi, M. A., Lapenta, C., Sestili, P., Gherardi, G., Lande, R., Spada, M., Parlato, S., Belardelli, F., and Fais, S., 1996, T-cell dysfunction in hu-PBL-SCID mice infected with Human Immunodeficiency Virus (HIV) shortly after reconstitution: in vivo effects of HIV on highly activated human immune cells. *J Virol.* 70:7958.

Saeki, Y., Mima, T., Sakoda, S., Fujimura, H., Arita, N., Nomura, T., and Kishimoto, T., 1992, Transfer of multiple sclerosis into severe combined immunodeficiency mice by mononuclear cells from cerebrospinal fluid of the patients. *Proc Natl Acad Sci USA.* 89:6157.

Safrit, J. T., Fung, M. S., Andrews, C. A., Braun, D. G., Sun, W. N., Chang, T. W., and Koup, R. A., 1993. Hu-PBL-SCID mice can be protected from HIV-1 infection by passive transfer of monoclonal antibody to the principal neutralizing determinant of envelope gp120. *AIDS.* 7:15.

Santini, S. M., Lapenta, C., Logozzi, M., Parlato, S., Spada, M., Di Pucchio, T., and Belardelli, F., 2000, Type I interferon as a powerful adjuvant for monocyte-derived dendritic cell development and activity in vitro and in Hu-PBL-SCID mice. *J Exp Med.* 191:1777.

Scoggins, R. M., Taylor, J. R. Jr., Patrie, J., van't Wout, A. B., Schuitemaker, H., and Camerini, D., 2000, Pathogenesis of primary R5 human immunodeficiency virus type 1 clones in SCID-hu mice. *J Virol.* 74:3205.

Somasundaram, R., Jacob, L., and Herlyn, D., 1995, Tetanus toxoid-specific T-cell responses in severe combined immunodeficiency (SCID) mice reconstituted with human peripheral blood lymphocytes. *Clin Exp Immunol.* 101:94.

Sousa, A. E., Carneiro, J., Meier-Schellersheim, M., Grossman, Z., and Victorino, R. M., 2002, CD4 T-cell depletion is linked directly to immune activation in the pathogenesis of HIV-1 and HIV-2 but only indirectly to the viral load. *J Immunol.* 169:3400.

Stevenson, M., Stanwick, T. L., Dempsey, M. P., and Lamonica, C. A., 1990, HIV-1 replication is controlled at the level of T-cell activation and proviral integration. *EMBO J.* 9:1551.

Tary-Lehmann, M., and Saxon, A., 1992, Human mature T-cells that are anergic in vivo prevail in SCID mice reconstituted with human peripheral blood. *J Exp Med.* 175:503.

Tary-Lehmann, M., Saxon, A., and Lehmann, P. V., 1995, The human immune system in hu-PBL-SCID mice. *Immunol Today.* 16:529.

Van Kuyk, R., Torbett, B. E., Gulizia, R. J., Leath, S., Mosier, D. E., and Koenig, S., 1994, Cloned human CD8+ cytotoxic T lymphocytes protect human peripheral blood leukocyte-severe

combined immunodeficient mice from HIV-1 infection by an HLA-unrestricted mechanism. *J Immunol.* 153:4826.

Vieillard, V., Jouveshomme, S., Leflour, N., Jean-Pierre, E., Debre, P., De Maeyer, E., and Autran, B., 1999, Transfer of human CD4(+) T lymphocytes producing beta interferon in Hu-PBL-SCID mice controls human immunodeficiency virus infection. *J Virol.* 73:10281.

Wang, C. Y., Sawyer, L. S., Murthy, K. K., Fang, X., Walfield, A. M., Ye, J., Wang, J. J., Chen, P. D., Li, M. L., Salas, M. T., Shen, M., Gauduin, M. C., Boyle, R. W., Koup, R. A., Montefiori, D. C., Mascola, J. R., Koff, W. C., and Hanson, C. V., 1999, Postexposure immunoprophylaxis of primary isolates by an antibody to HIV receptor complex. *Proc Natl Acad Sci USA.* 96:10367.

Wang, E. J., Pettoello-Mantovani, M., Anderson, C. M., Osiecki, K., and Moskowitz, D., 2002, Goldstein, H., Development of a novel transgenic mouse/SCID-hu mouse system to characterize the in vivo behavior of reservoirs of human immunodeficiency virus type1-infected cells. *J Infect Dis.* 186:1412.

Wang, E. J., Pettoello-Mantovani, M., Anderson, C. M., Osiecki, K., Moskowitz, D., and Goldstein, H., 2002, Development of a novel transgenic mouse/SCID-hu mouse system to characterize the in vivo behavior of reservoirs of human immunodeficiency virus type 1-infected cells *J Infect Dis.* 186:1412.

Zhang, C., Cui, Y., Houston, S., and Chang, L. J., 1996, Protective immunity to HIV-1 in SCID/beige mice reconstituted with peripheral blood lymphocytes of exposed but uninfected individuals. *Proc Natl Acad Sci USA.* 93:14720.

5

SIV as a Model for AIDS Pathogenesis Studies

ULRIKE SAUERMANN and SIEGHART SOPPER

1. INTRODUCTION

HIV vaccine trials are conducted in nonhuman primate models. Macaques infected with the simian immunodeficiency virus (SIV) and its genetically engineered derivatives, such as chimeras between HIV-1 and SIV (SHIV), are thus indispensable for the proof-of-concept testing and the definition of potential correlates of immune protection in HIV-vaccine design. SIV-infected macaques are also the animal model system of choice to perform etiopathological investigations. In contrast to humans, the monkeys are selected for age, sex, and provenance. They are infected under controlled experimental conditions with a pretitrated dose of a well-characterized viral isolate or a viral clone. The macaque model of AIDS is therefore most suited to study virus dissemination and host responses during the acute phase of infection, which is very difficult to study in HIV-infected humans. This phase is especially important since its outcome determines the disease course in HIV-1 infected humans and SIV-infected monkeys. Furthermore, organs usually not accessible for investigations in humans can be analyzed at any point during the infection. This animal model also provides the opportunity to intervene experimentally with the disease process. For example, much information has been gathered by depleting various lymphocyte subsets or by labeling lymphocytes in order to monitor their turnover. In addition, by selecting specific viral mutants or animals according to predefined criteria it is possible to decipher important viral and host factors influencing the course of the disease.

For the macaque model of AIDS, however, several monkey species as well as different SIV isolates and different routes of infection are used. We will review here the characteristics of different monkeys models for AIDS

ULRIKE SAUERMANN • German Primate Center, 37077 Göttingen, Germany
SIEGHART SOPPER • Bayerische Julius-Maximilians-Universität, 97078 Würzburg, Germany

pathogenesis research, give an overview on the natural course of the experimental SIV infection, and review some aspects in more detail such as organ-specific diseases and immune responses, coreceptor usage, T- and B-cell turnover in comparison to HIV infection.

2. VIRUSES

SIV represent a large family of primate lentiviruses. They are prevalent in many African primate species such a chimpanzees, African green monkeys, mandrills, and sooty mangabeys but do not induce any disease. The reasons for the lack of pathogenicity of SIV in its African hosts are not yet known, but it may well be that each species has evolved its own means to cope with the infection. Whereas chimpanzees are, for example, able to clear the virus efficiently, African green monkeys and sooty mangabeys maintain high viral loads without any disease symptoms.

The ability of SIV to induce AIDS in Asian macaques was detected by serendipity. The observation of enhanced death rates of macaques in the New England Regional Primate Center in the United States led to the identification of a virus isolate termed SIVmac to indicate the monkey species susceptible to the pathogenic virus.[8] Meanwhile, it is conceivable that the enhanced death rates were due to transmission of the virus from juvenile SIV-infected African to Asian macaques kept in the same enclosure. Later, SIV was isolated from stump-tailed macaques (SIVstm), a pig-tailed macaque (SIVmne), and sooty mangabeys (SIVsm).[20]

The most commonly used SIV isolates are derivatives of SIVmac251 and SIVmac239 as well as some SIVsm isolates. However, for particular experimental purposes, virus isolates like macrophage-specific viruses, and viruses with differing pathogenicity and SHIVs are available. These different viral strains are described in more detail in another chapter of this book, and will not be reviewed here.

3. MONKEY SPECIES

The most commonly used monkey species for experimental AIDS research is the rhesus macaque (*Macaca mulatta*). However, due to the shortage of rhesus macaques of Indian origin, especially in Europe, cynomolgus macaques (*Macaca fascicularis*) are also used. In addition, several research groups work with pig-tailed macaques (*Macaca nemestrina*). Other monkey species play a negligible role for AIDS research.

Meanwhile, it is acknowledged that different species present a differential susceptibility to SIV infection and subsequent disease course (Table I). Most of the comparative studies have been performed for the Western

TABLE I
Pathogenicity of Some SIV Isolates and HIV-2 in Macaques

Virus	Rhesus	Cynomolgus	Pigtailed
HIV-1	–	–	(+)
SIVmac239/251	+++	+++	++
SIVmne	++	++	+++
SIVsmE660	+++	+++	+++
SIVagm/ver90	–	–	++
HIV-2	+	+	+–++[1]

+++ = persistent infection, highly pathogenic; ++ = persistent infection, moderate pathogenic; + = persistent infection, no disease; (+) = minimal infectious but not pathogenic
[1] a pathogenic HIV-2 strain is described for this species[2]

(Indian) and Eastern (Chinese) type of rhesus macaques. These two subspecies have probably been separated during the glacial time 2.5 million years ago. The Eastern-type macaques have been probably further separated into subspecies due to habitat fragmentation. All available genetic data indicate that Chinese macaques are genetically much more diverse than, and partially even more different from, Indian origin rhesus macaques. However, there is consentaneous evidence that rhesus macaques originating from China have a lower viral load and, subsequently, a slower disease course than rhesus macaques of Indian origin.

Cynomolgus macaques are phylogenetically the closest relative of the rhesus macaque, and the species might even hybridize today. Sequence analysis of cynomolgus *Mhc-DRB* alleles revealed that about 10% of the exon 2 sequences are identical with rhesus macaque *DRB*-sequences.[50] For infecting cynomolgus macaques, usually the same viral stocks are used as for rhesus macaques (e.g., SIVmac251). The reports on disease progression in SIV-infected cynomolgus macaques are conflicting, but the differential results may also stem from subspecies differences within cynomolgus macaques.

The least frequently used macaque species for AIDS-research, which seems to be the most susceptible to SIV infection, is the pig-tailed macaque.[20] This species can even be infected with strains of HIV-1. Since HIV replicates only at very low levels and induces no AIDS-like symptoms, this model is not suitable for AIDS research. However, some rhesus-adapted SIV strains (SIVmac239) are less pathogenic in pig-tailed macaques. The reasons for species-specific differences are again unknown.

In this chapter we will mainly concentrate on infection of rhesus monkeys with different strains of SIVmac, as most of our knowledge on the pathogenesis has been derived from this animal model.

4. NATURAL COURSE OF SIV INFECTION

Upon infection with pathogenic SIV, macaques develop a disease with pathological and clinical manifestations remarkably similar to those found in human AIDS patients, although AIDS-defining symptoms occur much faster in macaques than in HIV-infected humans.

The primary or acute infection is characterized by a strong viremia with peak viral levels of 10^6 to more than 10^8 virus particles per ml plasma around 7–10 days postinfection. During this time adaptive immune responses are initiated. Strong cytotoxic T-cell responses are detected by 3 weeks postinfection, correlating temporally with a decline of viral load.[31] Seroconversion and early SIV–neutralizing antibodies are observed 4 weeks postinfection. The titers and reactivity, especially of the neutralizing antibodies, rise and broaden over a time period of several months. The extent of reduction of the peak viremia during primary infection, probably related to the quality and strength of CTL responses, is directly related to subsequent survival time.[25] An involvement of CTL in curtailing primary viremia has been demonstrated in experiments where $CD8^+$ T-cells were depleted for several weeks by administration of antibodies.[38] The effects of $CD8^+$ T-cell depletion in later stages of the disease are less marked. Interestingly, reduction of primary viremia by transient antiretroviral treatment can also facilitate long-term control of viral replication, leading to prolonged survival.[26] All these studies underline the importance of the initial events after infection for the subsequent course. Eight to 16 weeks postinfection a viral set point is reached, prognostic of the disease course. However, in some monkeys plasma viremia rises again at later times, probably due to escape mutants. Whereas neutralizing antibodies are less important for resolving primary viremia, high neutralizing antibody titers may correlate with lower plasma virus during the postacute phase of infection.[39]

Different Types of Disease Progression After Pathogenic SIV Infection

After infection with pathogenic strains of SIVmac, macaques display a varying course of disease. Monkeys that display very high viral plasma RNA levels (10^6–10^9 viral RNA copies per ml plasma) and die within 8 to about 28 weeks postinfection with clinical symptoms of AIDS are termed rapid progressors. Rapid disease progression is a well-known disease pattern and may affect up to 25% of Indian origin rhesus monkeys.[11] This particular type of disease course is associated with a selective loss of memory ($CD29^{hi}$) $CD4^+$, and $CD8^+$ cells.[37] Also, early B-cell abnormalities have been described. Consequently, these monkeys fail to mount an appropriate humoral and cellular immune response.[44] Moreover, a significant fraction of the rapid progressors develop neurological symptoms.[44] In most cases

the rhesus monkeys carry *Mhc* class I genes not known to mount an efficient CTL response or carry *Mhc* class I genes selecting for rapid escape.[30] In addition, particular *Mhc* class II genes may predispose to this type of disease.[37] However, unknown host genes are clearly also involved since some monkeys carrying protective *Mhc* genes such as *Mamu-A*01* may also become rapid progressors. HIV-1 infected humans displaying a rapid disease course are only rarely diagnosed. Rapid disease progression appears thus to be a special type of disease course mainly confined to SIV-infected macaques.

Monkeys able to mount an immune response become intermediate progressors with survival of up to 3 years. These monkeys show for some time no clinical symptoms, although they exhibit lymphadenopathy and gradually declining numbers of $CD4^+$ cells. At some time point, their condition deteriorates and signs of AIDS such as opportunistic infections or tumors develop. This switch from the asymptomatic to the symptomatic stage is usually accompanied by an increase in urinary neopterin levels.[21]

Long-term survivors or long-term nonprogressors are able to control viral replication most efficiently and can survive an infection for more than 8 years. The long-term survivors, representing only a fraction (1%) of rhesus monkeys of Indian origin infected with pathogenic SIV, are particularly interesting. They can control viral replication for many years at or below the limit of detection. Strong CTL or T-helper cell responses are usually not detected probably due to the lack of antigenic stimuli. These monkeys are resistant to super infection and immune activation, and their CTLs may secrete high amounts of a cellular antiviral factor termed $CD8^+$ T-cell antiviral factor.[24] However, in most of these monkeys the immune system becomes eventually "exhausted," resulting in the development of AIDS-like symptoms.

AIDS in humans and SIV-infected macaques are characterized by a number of clinical complications. The hallmark of pathogenic HIV and SIV infection is generalized immunosuppression due to both a loss and functional impairment of $CD4^+$ T-cells. Later in infection, the functions of most other immune cells such as CTL, B-cells, and natural killer cells are likewise impaired. The clinical complications can be considered either as SIV-associated lesions without opportunistic infectious agents or pathomorphological changes in combination with numerous opportunistic infections. These complications are represented by opportunistic infections (e.g., cryptosporidiosis, CMV, *Pneumocystis carinii*), primary immunodeficiency associated lesions (e.g., arteriopathy, encephalopathy, enteropathy, malignancies like non-Hodgkin lymphomas), and hematological disorders (e.g., thrombocytopenia, anemia). The disorders are neither statistically distributed in HIV-1 infected humans nor in SIV-infected macaques and are in many cases not directly related to viral load.

Different Routes of Infection Do Not Influence Pathogenicity but Infectability

HIV infection can occur by needle-sticks, homosexual and heterosexual contact, birth, and breast feeding. To mimic the different settings in the monkey model of AIDS, different routes of infection have been evaluated, such as intravenous, rectal, vaginal infection, and oral infection via the tonsils. However, neither in humans nor in macaques have any spectacular differences of the disease course depended on the route of infection. Furthermore, no model exists for the typical infection of heterosexual males.

The main differences between these types of infection models refer to the different doses needed for infection and the reliability of infection, since the mucosa represents an appreciable barrier the virus has to transverse. To infect all monkeys by the intravenous route, about 30 $TCID_{50}$ are needed, whereas approximately at least a 10- to 100-fold higher dose is needed for the mucosal routes of infection. Furthermore, failures of infection have been reported for the rectal and oral route of infection, indicative of great interindividual differences, which have not been elucidated yet. The monkey model has revealed some interesting details on the impact of the hormone levels changing during the menstrual cycle. In contrast to progesterone, high estrogen levels appear to be protective against vaginal infection.[41] The differences probably reflect the differential influence of these hormones on the cervicovaginal epithelium, which seems to be thickened by estrogen in humans and monkeys.

Organ-Specific Manifestations

Infection with HIV and SIV not only induces immunosuppression, leading to opportunistic infections and tumors, but also directly causes specific disease entities in some organs. These organ-specific manifestations comprise among others an enteropathy of the gastrointestinal tract, encephalitis of the central nervous system (CNS) leading to HIV-dementia, and peripheral neuropathy. Active viral replication in the respective organs is a prerequisite for the development of these disorders. Nevertheless, these syndromes cannot be ascribed to direct cytopathic effects of the virus, as those cells that mediate the tissue-specific functions, like epithelial cells in the gut and neurons in the brain, are not infected. Rather, indirect mechanisms such as changes in microenvironment induced by the infection or the activation of components of the immune system in these organs have been implicated in the pathogenesis of these diseases.[49]

The poor accessibility of organs such as the gut or the brain for investigations in humans provides an additional rationale to utilize animal models.

Enteropathy

During the course of HIV infection, the gastrointestinal tract is affected by opportunistic infections and malignancies. However, also in the absence of secondary infections, many HIV-infected patients develop gastrointestinal symptoms, termed HIV enteropathy, histologically characterized by villous atrophy (crypt hyperplasia, villus blunting, inflammatory infiltrates). The clinical manifestations include diarrhea and weight loss. The morphological and functional changes of the intestinal mucosa have been attributed to local replication of HIV or immunological changes in the mucosa.[49] For example, decreased CD4/CD8 ratios in the intestinal mucosa were consistently described in HIV-infected patients. As the gut harbors a large lymphocyte population, such alterations induced by the infection have also had an important impact on the total T-cell pool and the virus-specific immune response. Again, the rhesus monkey animal model provides the opportunity to study the kinetics of events, when secondary infections have not yet developed and gastrointestinal symptoms are still lacking. SIV-infected macaques display similar gastrointestinal disturbances as HIV-infected patients.[49] Several studies with SIV-infected rhesus monkeys have demonstrated that the acute state of infection is characterized by high viral replication, severe $CD4^+$ T-cell decline, and $CD8^+$ cell expansion in the gut associated lymphoid tissue (GALT). The gut is thus an important organ for viral replication and persistence.

The percentage of $CD4^+$ T-cells declines steeper in the lamina propria than in blood and lymphoid organs already during primary viremia.[47,49] A likely explanation for this fact is that, similar to other nonlymphoid organs, T-cells in the gut display an activated phenotype. For example, a higher proportion of T-helper cells expresses the chemokine receptor CCR5, the major coreceptor for HIV and SIV, and are therefore more susceptible to infection. In a recent study, despite a marked reduction in the circulation CD4/CD8 ratio, the absolute number of $CD4^+$ T-lymphocytes in the intestinal mucosa of SIV-infected macaques in the asymptomatic phase was not decreased.[43] Still it remains reasonable that $CD4^+$ T-cells are continuously depleted at such sites of increased activation but can be replenished during the asymptomatic stage. Only in late stages do $CD4^+$ T-cells also decline in absolute numbers in the lamina propria. This discrepancy might provide a clue for the hyperregenerative villous atrophy found in the early phase of SIV infection, contrasting the hypoproliferation found in late stages of

HIV infection, as a functional interrelation exists between the gut epithelium and the mucosal immune system.[49]

Encephalopathy

One of the most debilitating manifestation of HIV infection is a neurological syndrome described as AIDS dementia complex or HIV-dementia.[34] Clinically, HIV-dementia presents as a subcortical dementia and the symptoms range from subclinical motor/cognitive deficits already in the asymptomatic phase to full dementia with paraplegia in late stages of AIDS, affecting about 20% of AIDS patients. The histological correlate, HIV-encephalitis, is characterized by multinucleated giant cells, microglia nodules, and local viral replication.[2] HIV-infection of brain cells—mainly microglia—is a necessary prerequisite for the development of HIV-dementia. The pathogenesis, however, has not been clarified in detail, but it is clear that indirect mechanisms like production of neurotoxic substances by infected cells or immunoactivated cells are involved. The annual incidence of dementia is about 7% and this figure has not changed much since the introduction of highly active antiretroviral therapy, although the absolute numbers have of course dropped, at least in the industrialized countries.

The high incidence and devastating consequences of HIV-dementia have resulted in a great research effort to understand the pathogenesis and to develop therapeutical approaches. Therefore, several animal models have been established. Among these, SIV infection of macaques is considered to be the most relevant animal model, as it mirrors most of the features found in HIV-infected patients.[42] The histopathological picture in late stages of the disease is very similar. SIV infected macaques, like HIV-infected humans, develop cognitive and motor impairment, as demonstrated by sophisticated behavioral and electrophysiological test systems.[15,35] Overt neurological signs have been found in a small number of animals and include lethargy, ataxia, blindness, seizure, and paresis.[44]

Hallmarks of the so-called SIV-encephalitis are microglia nodules and multinucleated giant cells with abundant expression of viral antigens. As in HIV infection, only cells of the mononuclear phagocyte lineage, such as microglia and perivascular macrophages, are productively infected. On rare occasions, viral antigen is detected in $CD4^+$ lymphocytes, especially in the early phase of the infection.[3] Infection of other neural cells has not yet been reported. Other histopathological alterations often found in AIDS patients such as myelin pallor and vacuolar myelopathy are less frequent and only found in focal areas surrounding areas with inflammation and SIV expression. Reduced dendritic arborization, axonal degeneration, and ultimately neuronal loss have been demonstrated both with imaging tech-

niques and morphometric analyses. In addition alterations in several neurotransmitter systems have been described.[22]

This animal model is exquisitely suited to study kinetics of viral invasion, intrathecal immune response, and neurological functions. The first infected cells can be detected in the CNS as early as 1 week postinfection,[3,6] and the viral load in the cerebrospinal fluid (CSF) follows the kinetics of the one in the blood.[9] It seems that the virus enters the brain in a cell-associated form as the viremia is still very low at that time point and perivascular macrophages and infiltrating mononuclear phagocytes are the major cell types productively infected during the first few weeks. Later in the course of the infection, also resident parenchymal microglia become productively infected and carry most of the viral burden in the brain.[5,9] Activation of microglia can be found at early stages of the disease when viral load in the CNS is still very low,[3] possibly contributing to the neurophysiological and neurochemical changes found already in the asymptomatic phase.[15,22]

In animals with AIDS, the occurrence of neurological signs correlates with intrathecal viral load and histopathological lesions.[9] The level of viral replication in the brain is influenced by viral and host factors. A strong capacity of the virus to grow in cells of the monocyte/macrophage lineage is a prerequisite to replicate in brain cells.[9] However, only the lack of a strong antiviral immune response as found in rapid progressors permits unrestricted viral growth,[44] and consequently neurological signs can develop.

Until now, not many therapeutic studies concerning the neurological consequences of immunodeficiency virus infection have been conducted in this animal model. Antiretroviral treatment regimens with reverse transcriptase inhibitors have succeeded in reducing viral load in CSF and normalizing some but not all neurophysiological abnormalities.[15,35] In a neuropharmacological approach with the dopaminergic drug selegiline, which had been used in human preclinical trials on HIV-dementia patients, neurochemical deficiencies in SIV-infected macaques could be restored.[6,22]

5. SELECTED ASPECTS OF SIV PATHOGENICITY

MHC-Genes, CTL, and Antibody Response(s) Shape Viral Genotype, Viral Replication Rate, and the Host's Survival Time

Although SIV-infected monkeys as well as HIV-infected humans are able to generate strong humoral and cellular immune responses, they are unable to control viral replication in the long term. One reason is the ability of the virus to escape the immune response due to its high mutation and replication rate. The precise contribution of viral escape mechanisms to

immune failure is not yet clear, since not all viral CTL epitopes develop escape mutants and the steadily growing dysfunction of CTL, $CD4^+$ T-cells, and natural killer cells may be equally important. However, the monkey model of AIDS offers the opportunity to study the early events after infection under well-controlled conditions. Furthermore, all available data suggest that the findings in macaques are similar to human HIV infection.

During the acute phase of infection in most macaques, a broad, multiepitopic CTL response, interestingly directed mainly against the small regulatory proteins (tat, rev, nef, vif, vpr, vpx) is generated.[29] There are great interindividual differences concerning the magnitude, breadth, and identities of the particular CTL epitopes. These differences can be largely explained by the individual major histocompatibility complex genotype. MHC molecules present peptides (e.g., viral fragments) to T-cells. Each MHC molecule can bind and present only a limited set of peptides. Since the MHC region is highly polymorphic, in general, each individual has a different MHC genotype and thus a different capacity to present antigenic peptides. However, the immunological pressure exerted on the virus in concert with the error-prone reverse transcriptase and the high replication rate, generating several billions of viruses each day, favor the emergence of escape mutants. These mutant viruses have changes in the amino acid positions responsible for anchoring the antigenic peptides in the binding pockets of the MHC molecule, at T-cell receptor recognition sites or close to them. CTL escape mutants can arise as early as 4 weeks after infection during the acute phase of infection.[31] There may be a relationship between the functional avidity (T-cell responsiveness to a peptide) of a CTL response as a measure of the intensity of the selection pressure and time of appearance of escape mutants.[31] The early escape from vigorous CTL responses might aid acquiring the chronic state of infection. Later in infection, CTL responses against structural proteins become dominant, however, escape mutants of these CTL responses also appear. The relation between *Mhc* class I alleles, CTL responses, viral escape, and survival time has also been documented in HIV-1 infected humans. However, due to obvious constraints, such as lack of the exact knowledge of the nucleotide sequence of the infecting virus and in most cases lack of knowledge of the exact infection time point, comparable studies are hard to conduct in HIV-infected individuals.

Relating *Mhc* class I genes to survival time and viral load revealed that some alleles are associated with longer survival. Mamu-A∗01 turned out repeatedly to be associated with longer survival. During the acute phase, a Tat-derived peptide presented by this molecule elicits a strong CTL response. These CTL responses disappear due to escape. However, during the chronic phase of infection an immunodominant response against a gag-derived peptide is elicited by Mamu-A∗01. Possibly because of the ability of Mamu-A∗01 to elicit dominant immune responses during the acute and

chronic phase of infection, it is associated with a more benign disease course. Furthermore, the presence of more than one MHC class I molecules able to mount a significant CTL response seems to be associated with even longer survival.[30,32] In these animals viral CTL escape mutants appear more slowly compared to other animals, possibly because of a lesser replication rate of the virus due to an improved containment of the virus. However, the presence of several protective *Mhc* class I alleles is certainly not sufficient to promote the status of long-term survival, indicating that other cellular antiviral factors such as $CD8^+$ T-cell antiviral factor or defensins may be important in controlling viral replication just as in humans.

Similar to HIV-infected humans, selection pressure by neutralizing antibodies can be observed by the appearance of antibody escape mutants. Neutralizing antibodies are important, since CD4-independent SIV and primary HIV strains are particularly neutralization sensitive. Moreover, high titers of neutralizing antibodies can confer protection against infection.[13] However, neutralization insensitive viruses emerge fast in HIV- and SIV-infected individuals, and the commonly used SIV239 strain is neutralization insensitive. Although neutralizing antibodies may prevent the emergence of CD4-independent viruses, which display a broader tropism, their exact contribution to disease progression in HIV- and SIV-infected individuals is a matter of debate.

T-Cell Turnover

For many years, HIV infection was considered a slow disease process with low-level replication and few infected cells being destroyed by the virus (Figure 1B). Alternatively, the gradual decline of $CD4^+$ T-cells was thought to be the result of a block in the generation of new cells imposed by the virus.[19,27] (Figure 1C). However, more recent results have revealed a more dynamic picture of viral replication and T-cell turnover, and it became clear that the impact of HIV infection on the homeostasis of T-cells is central to the pathogenesis of AIDS (Figure 1).[10,16,27] In humans, parameters influencing T-cell turnover, like production of new cells and deathrates, can only be examined in the blood, which accounts for only 2% of the total lymphocyte pool. Therefore, much of our knowledge on this issue is derived from the SIV/macaque animal model. By labeling dividing cells with 5′-bromo-2′-deoxyuridine several groups have established that the peripheral proliferation and deathrates are increased for both $CD4^+$ and $CD8^+$ T-cell subsets in SIV-infected monkeys.[28,36] In longitudinal studies, a sharp increase in proliferation rates of $CD8^+$ T-cells could be demonstrated during peak viremia, reflecting the strong reaction of this subset to the infection.[45] In contrast, the peripheral proliferation rate of $CD4^+$ T-cells is depressed, suggesting a selective depletion of dividing cells by the massive viral replication during this early time point. Later, proliferation rates of

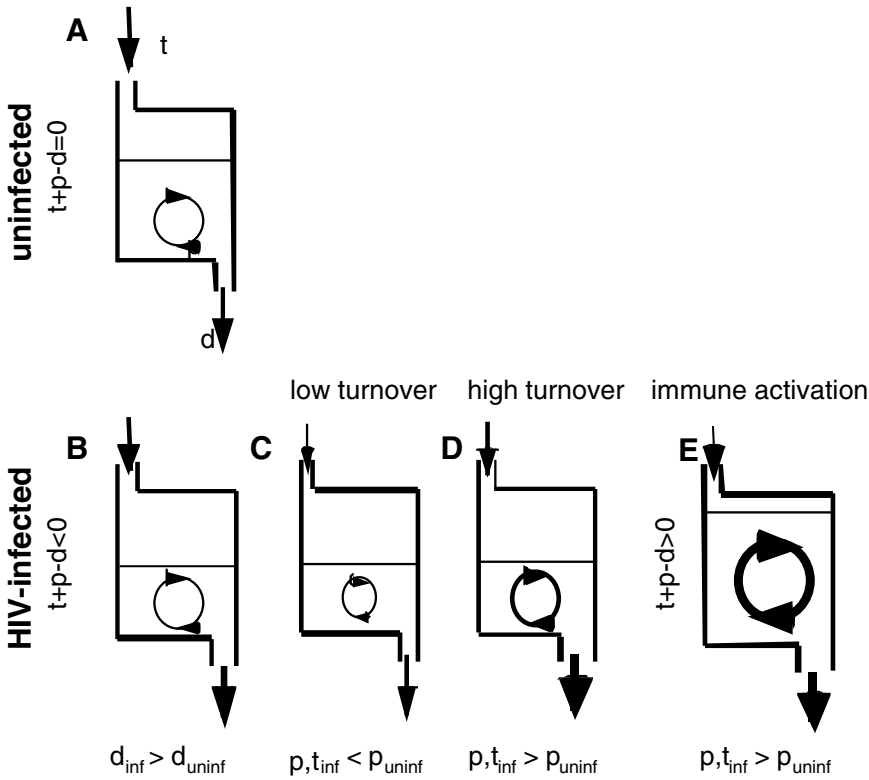

FIGURE 1. Models of T-cell turnover **A**: In uninfected individuals, death (d) and renewal of lymphocytes either by thymic production (t) or by peripheral proliferation (p) is tightly balanced by homeostatic mechanisms with the result that the total number of T-cells, the T-cell pool remains constant. **B**: The simplest explanation for the lower CD4 cell counts in HIV-infected patients would be an increased deathrate of infected cells. **C**: In the low turnover model, the $CD4^+$ T-cell numbers decrease because the virus interferes with the renewal of CD4-cells either at the level of the thymus or the peripheral proliferation. **D**: The increased proliferation rates in HIV patients are explained in the high turnover or open tap/open drain model by homeostatic mechanisms trying to balance the loss of CD4 cells caused by the infection by increasing the production of new cells. But because the deathrate is also strongly increased these mechanisms ultimately fail. **E**: According to the immune activation model, the increased proliferation rates are the result of an immunologic response to the infection. Such a model is consistent with an increased total $CD4^+$ T-cell pool found in the asymptomatic phase SIV/macaque animal model, which cannot be explained by the other models.

$CD4^+$ T-cells increase above preinfection levels in those animals that are able to curtail viral load.[45] These and other findings in humans have led to the high turnover concept that the drain on $CD4^+$ T-cells imposed by the infection results in increased proliferation of T-cells in order to replace lost $CD4^+$ T-cells (Figure 1D). However, as the deathrate of the $CD4^+$ T-cells,

caused either directly by the virus or by immune mediated mechanisms, by which possibly even uninfected cells are destroyed, is also increased, these homeostatic efforts of the immune system fall short of replenishing the $CD4^+$ T-cell pool. More recently, immune activation has been proposed as the driving force of the increased proliferation rates of both $CD4^+$ and $CD8^+$ T-cells.[16,18] With this model it is easier to explain some of the findings, such as the high deathrate of $CD8^+$ T-cells, which are not infected by the virus. Moreover, chronic immune activation may even lead to a transient increase of absolute numbers of $CD4^+$ T-cells above normal levels, as revealed during the asymptomatic phase of the SIV-macaque animal model, where the lymphocyte subsets were enumerated in many different organs[43] (Figure 1E). Since an increased size of the total T-cell pool cannot be the result of homeostatic mechanisms, supposedly only trying to replenish it, these findings corroborate the hypothesis of immune activation as the major cause for the increased T-cell turnover found in HIV patients. The increased deathrates of both $CD4^+$ and $CD8^+$ T-cell subsets would then possibly be the result of homeostatic mechanisms to resolve the increased numbers of lymphocytes.

The mechanisms leading to increased immune activation are less well understood. It could be either a direct response to the ongoing viral replication or the result of a deviation in the cytokine balance induced by the infection. Immune activation usually correlates with viral load in HIV-infected individuals and pathogenic SIV models[23,28] and is a predictor for faster disease progression in HIV patients.[23] In contrast, in the apathogenic African monkey SIV models, immune activation is only slightly increased despite a comparably high viral load.[40] Therefore, it has been hypothesized that the chronically increased immune activation ultimately leads to exhaustion of the regenerative potential of the immune system. More detailed studies on parameters of T-cell turnover and their correlation with disease progression are needed to finally resolve this issue.

Coreceptor Usage and Viral Evolution in the Host

To enter targets cells, HIV and SIV envelope proteins bind to the CD4 protein and, after a conformational change, to a coreceptor, which is usually a chemokine receptor. CCR5 seems to be the main coreceptor for SIV just as for the infecting HIV. Since both viruses use CCR5 as a coreceptor and since the macaque and human CCR5 are highly similar, drugs aiming to block the coreceptor binding site of immunodeficiency viruses can be tested in the macaque model of AIDS. However, an additional seven transmembrane-spanning G-protein-coupled chemokine receptors such as CCR8, CCR3, GPR15, GPR1, and APJ are used.[4]

Approximately 50% of the HIV-1 infected humans acquire a virus that utilizes only CXCR4 or both CXCR4 and CCR5. This coreceptor switch

often marks the onset of AIDS in HIV-1 infected humans. Although HIV and SIV in general use the same coreceptors (CCR5, CCR3, CCR8), remarkably, SIV usually does not use CXCR4 as its coreceptor. However, in rhesus macaques infected with a SHIV virus containing the env from an HIV-X4 (CXCR4) strain, the $CD4^+$ cells rapidly declined in accordance with viral tropism.

Although during the HIV-1 infection both types of viruses are transmitted, only the CCR5 utilizing viruses are infectious. So far, it remains poorly understood where and how selection for the R5 (CCR5) virus occurs. To elucidate the basis for early dominance of R5 viruses in HIV infection, rhesus macaques were coinfected with SHIV-R5 and -X4 strains. Initially both viral isolates replicated effectively.[17] After onset of specific immune responses, the R5 isolate dominated in most monkeys, which could be partially reversed by $CD8^+$ cell depletion. It was concluded that the X4 isolates are better controlled by the immune system, particularly by the CTLs, since they mainly infect $CD4^+$ lymphocytes. In contrast, R5 viruses infect long-lived macrophages, representing viral reservoirs. Transferring the results to HIV-infected humans could mean that the emergence of X4 variants is not the reason, but the consequence, of immune failure. However, this does not readily explain why in severely immune compromised monkeys no X4 viruses emerge.

The monkey model allowed further interesting insights into virus evolution in the host. Using SIVsm isolates, two groups showed that these viruses also evolve in the infected macaque. A surprising consequence, the coreceptor usage of late stage viruses, is more restricted compared to early stage viruses, although they replicate more efficiently.[14,48] Viruses isolated from the early stages of infection used a number of coreceptors such as CCR5, GPR15, CXCR6, or CCR8, and could also fuse with indicator cells independent of CD4. Late stage variants of SIV nearly uniformly utilized CCR5 as coreceptor. These findings can be partially explained by the fact that the potentially more pathogenic CD4-independent strains are sensitive to neutralizing antibodies and persist only in rapid progressors, which are unable to mount an efficient immune response. In addition, one might speculate that the changing availability of coreceptors, of which the expression is known to be modulated by immunodeficiency lentiviruses, also influences viral evolution. However, the link between coreceptor expression, in particular on dendritic cells important in transmitting immunodeficiency viruses to T-cells and macrophages, and viral coreceptor use needs to be established in SIV-infected macaques.

Nef: A Viral Gene-Enhancing Pathogenicity

The immunodeficiency lentiviruses encode, in addition to their structural proteins, six small regulatory proteins that are essential for structured viral replication and assembly. Furthermore, they manipulate a number of

host factors to promote viral replication and survival and are therefore essential for viral pathogenicity. Among them Nef has gained significant attention. Initially it was thought that Nef is a negative factor of replication. However, in vivo studies showed that deletions in *nef* are associated with a significant prolonged AIDS-free time.[7] Moreover, *nef*-deleted viruses confer potent and long-lasting protection against infection with wild-type virus, and were therefore proposed as live-attenuated vaccines. Meanwhile, it is clear that SIV- and HIV-infected individuals with nef-deleted viruses will eventually progress to AIDS.

Nef is a small multifunctional protein.[1,12,33] The Nef protein of SIV and HIV-2 is about 260 amino acid in length, whereas the HIV-1 Nef is slightly smaller with about 106 amino acids. HIV and SIV Nef enhance viral replication, infectivity, and ensure viral survival through a combination of different effects. To interfere with T-cell activation signals, Nef interacts with a number of different cellular proteins such as AP-1, AP-2 clathrin receptors, TCR zeta chain tyrosine kinases, Pak2, CD4, and MHC I proteins. Many of the cellular proteins bind to different amino acid sequence motifs of Nef. Two of the numerous pathogenic effects of HIV- and SIV-Nef, refer to their ability to down regulate CD4 and MHC class I molecules. Both functions are not related to a direct repression of the transcription. Instead, HIV- and SIV-Nef interfere with endosome trafficking pathways, triggering the endocytosis of CD4 from the cell surface and targeting CD4 molecules into the lysosomal compartment for degradation. The degradation of cell-surface CD4 is thought to prevent superinfection and to impair the immune function of CD4 cells. Down-modulation of MHC class I molecules is achieved by inhibiting their transport to the cell surface and by redirecting MHC class A and B molecules from the cell membrane into the transgolgi network and subsequently into the lysosomes for degradation. Although efficient down-regulation of MHC class I molecules confers a selective advantage in SIV-infected macaques, the exact contribution of nef-mediated CTL-impairment is not clear, since strong CTL immune responses can be elicited in HIV- and SIV-infected individuals. Other functions of SIVmac-Nef comprise its ability to down-regulate the TCR-CD3 complex and CD28.

Although, the full spectrum of Nef function is not fully investigated, it appears that most of the Nef functions are conserved between SIV and HIV Nef in spite of differences in their primary structure.

6. CONCLUSION AND PERSPECTIVE

The SIV/macaque animal model for AIDS has proven extremely useful not only to assess vaccine efficacy or antiretroviral treatment protocols, but also to study the pathogenesis of this devastating disease. The overall disease pattern and many of the specific disease manifestations are in prin-

ciple the same in humans and macaques. Much information has been gathered in this animal model about viral and host factors important for disease progression. In this chapter, however, we have concentrated on particularly interesting aspects of AIDS pathogenesis. Other topics, for example, the complete spectrum of opportunistic infections and AIDS-associated tumors, could not be covered.

As outlined above, different species present a differential susceptibility to SIV infection and subsequent disease course. Therefore, a more detailed knowledge of species-dependent differential susceptibility to SIV might be important in this respect. Systematic investigations into the genetic and physiological differences upon SIV infection are needed to decide which animal species is most suited for the investigation of a particular aspect of HIV infection and to identify resistance and susceptibility factors at the subspecies or individual level. Such research efforts will be boosted as more genetic information is being gathered.

The ultimate rationale for elucidating the pathogenesis must be to identify novel targets for therapy and novel strategies for vaccine development. HIV and SIV utilize a vast number of host factors to establish infection and complete replication.[46] In addition, the viruses have developed intricate means to evade innate and adaptive antiviral defense pathways such as the inactivation of APOBEC3G by vif and the downregulation of MHC class I. It is conceivable that differences in the host factors or regulatory sequences account for the differential susceptibility to immunodeficiency lentivirus infection. Therefore, a detailed characterization of the genetic background will help to identify these factors, providing the basis for targeted antiretroviral therapies. Unfortunately, most experiments in this model were done with small groups of arbitrarily selected animals for which no pedigree information is available. In order to fully tap the potential of this valuable animal model, in the future, more systematic collaborative efforts are needed.

REFERENCES

1. J. L. Anderson and T. J. Hope, Recent insights into HIV accessory proteins, *Curr. Infect. Dis. Rep.* 5, 439–450 (2003).
2. H. Budka, Neuropathology of human immunodeficiency virus infection, *Brain Pathol.* 1, 163–175 (1991).
3. L. Chakrabarti, M. Hurtrel, M. A. Maire, R. Vazeux, D. Dormont, L. Montagnier, and B. Hurtrel, Early viral replication in the brain of SIV-infected rhesus monkeys, *Am. J. Pathol.* 139, 1273–1280 (1991).
4. P. R. Clapham and A. McKnight, Cell surface receptors, virus entry and tropism of primate lentiviruses, *J. Gen. Virol.* 83, 1809–1829 (2002).

5. M. A. Cosenza, M. L. Zhao, Q. Si, and S. C. Lee, Human brain parenchymal microglia express CD14 and CD45 and are productively infected by HIV-1 in HIV-1 encephalitis, *Brain Pathol.* 12, 442–455 (2002).
6. S. Czub, E. Koutsilieri, S. Sopper, M. Czub, C. Stahl-Hennig, J. G. Muller, V. Pedersen, W. Gsell, J. L. Heeney, M. Gerlach, G. Gosztonyi, P. Riederer, and V. ter Meulen, Enhancement of central nervous system pathology in early simian immunodeficiency virus infection by dopaminergic drugs, *Acta Neuropathol. (Berl)* 101, 85–91 (2001).
7. M. D. Daniel, F. Kirchhoff, S. C. Czajak, P. K. Sehgal, and R. C. Desrosiers, Protective effects of a live attenuated SIV vaccine with a deletion in the nef gene, *Science* 258, 1938–1941 (1992).
8. M. D. Daniel, N. L. Letvin, N. W. King, M. Kannagi, P. K. Sehgal, R. D. Hunt, P. J. Kanki, M. Essex, and R. C. Desrosiers, Isolation of T-cell tropic HTLV-III-like retrovirus from macaques, *Science* 228, 1201–1204 (1985).
9. M. Demuth, S. Czub, U. Sauer, E. Koutsilieri, P. Haaft, J. Heeney, C. Stahl-Hennig, V. ter Meulen, and S. Sopper, Relationship between viral load in blood, cerebrospinal fluid, brain tissue and isolated microglia with neurological disease in macaques infected with different strains of SIV, *J. Neurovirol.* 6, 187–201 (2000).
10. D. C. Douek, L. J. Picker, and R. A. Koup, T-cell dynamics in HIV-1 infection, *Annu. Rev. Immunol.* 21, 265–304 (2003).
11. M. Dykhuizen, J. L. Mitchen, D. C. Montefiori, J. Thomson, L. Acker, H. Lardy, and C. D. Pauza, Determinants of disease in the simian immunodeficiency virus-infected rhesus macaque: characterizing animals with low antibody responses and rapid progression, *J. Gen. Virol.* 79 (Pt 10), 2461–2467 (1998).
12. O. T. Fackler and A. S. Baur, Live and let die: Nef functions beyond HIV replication, *Immunity.* 16, 493–497 (2002).
13. F. Ferrantelli, R. A. Rasmussen, R. Hofmann-Lehmann, W. Xu, H. M. McClure, and R. M. Ruprecht, Do not underestimate the power of antibodies—lessons from adoptive transfer of antibodies against HIV, *Vaccine* 20 Suppl 4, A61–A65 (2002).
14. S. Forte, M. E. Harmon, M. J. Pineda, and J. Overbaugh, Early- and intermediate-stage variants of simian immunodeficiency virus replicate efficiently in cells lacking CCR5, *J. Virol.* 77, 9723–9727 (2003).
15. H. S. Fox, M. R. Weed, S. Huitron-Resendiz, J. Baig, T. F. Horn, P. J. Dailey, N. Bischofberger, and S. J. Henriksen, Antiviral treatment normalizes neurophysiological but not movement abnormalities in simian immunodeficiency virus-infected monkeys, *J. Clin. Invest* 106, 37–45 (2000).
16. Z. Grossman, M. Meier-Schellersheim, A. E. Sousa, R. M. Victorino, and W. E. Paul, CD4+ T-cell depletion in HIV infection: are we closer to understanding the cause?, *Nat. Med.* 8, 319–323 (2002).
17. J. M. Harouse, C. Buckner, A. Gettie, R. Fuller, R. Bohm, J. Blanchard, and C. Cheng-Mayer, CD8+ T-cell-mediated CXC chemokine receptor 4-simian/human immunodeficiency virus suppression in dually infected rhesus macaques, *Proc. Natl. Acad. Sci. U.S.A.* 100, 10977–10982 (2003).
18. M. D. Hazenberg, J. W. Stuart, S. A. Otto, J. C. Borleffs, C. A. Boucher, R. J. de Boer, F. Miedema, and D. Hamann, T-cell division in human immunodeficiency virus (HIV)-1 infection is mainly due to immune activation: a longitudinal analysis in patients before and during highly active antiretroviral therapy (HAART), *Blood* 95, 249–255 (2000).
19. M. K. Hellerstein and J. M. McCune, T-cell turnover in HIV-1 disease, *Immunity.* 7, 583–589 (1997).
20. V. M. Hirsch and J. D. Lifson, Simian immunodeficiency virus infection of monkeys as a model system for the study of AIDS pathogenesis, treatment, and prevention, *Adv. Pharmacol.* 49, 437–477 (2000).

21. J. Hoch, S. M. Lang, M. Weeger, C. Stahl-Hennig, C. Coulibaly, U. Dittmer, G. Hunsmann, D. Fuchs, J. Muller, S. Sopper, et al., Vpr deletion mutant of simian immunodeficiency virus induces AIDS in rhesus monkeys, *J. Virol.* 69, 4807–4813 (1995).
22. E. Koutsilieri, C. Scheller, S. Sopper, M. E. Gotz, M. Gerlach, V. ter Meulen, and P. Riederer, Selegiline completely restores choline acetyltransferase activity deficits in simian immunodeficiency infection, *Eur. J. Pharmacol.* 411, R1–R2 (2001).
23. S. D. Lawn, S. T. Butera, and T. M. Folks, Contribution of immune activation to the pathogenesis and transmission of human immunodeficiency virus type 1 infection, *Clin. Microbiol. Rev.* 14, 753–777, table (2001).
24. J. A. Levy, The search for the CD8+ cell anti-HIV factor (CAF), *Trends Immunol.* 24, 628–632 (2003).
25. J. D. Lifson, M. A. Nowak, S. Goldstein, J. L. Rossio, A. Kinter, G. Vasquez, T. A. Wiltrout, C. Brown, D. Schneider, L. Wahl, A. L. Lloyd, J. Williams, W. R. Elkins, A. S. Fauci, and V. M. Hirsch, The extent of early viral replication is a critical determinant of the natural history of simian immunodeficiency virus infection, *J. Virol.* 71, 9508–9514 (1997).
26. L. N. Martin, M. Murphey-Corb, K. F. Soike, B. Davison-Fairburn, and G. B. Baskin, Effects of initiation of 3′-azido,3′-deoxythymidine (zidovudine) treatment at different times after infection of rhesus monkeys with simian immunodeficiency virus, *J. Infect. Dis.* 168, 825–835 (1993).
27. J. M. McCune, The dynamics of CD4+ T-cell depletion in HIV disease, *Nature* 410, 974–979 (2001).
28. H. Mohri, S. Bonhoeffer, S. Monard, A. S. Perelson, and D. D. Ho, Rapid turnover of T lymphocytes in SIV-infected rhesus macaques, *Science* 279, 1223–1227 (1998).
29. B. R. Mothe, H. Horton, D. K. Carter, T. M. Allen, M. E. Liebl, P. Skinner, T. U. Vogel, S. Fuenger, K. Vielhuber, W. Rehrauer, N. Wilson, G. Franchini, J. D. Altman, A. Haase, L. J. Picker, D. B. Allison, and D. I. Watkins, Dominance of CD8 responses specific for epitopes bound by a single major histocompatibility complex class I molecule during the acute phase of viral infection, *J. Virol.* 76, 875–884 (2002).
30. T. Muhl, M. Krawczak, P. ten Haaft, G. Hunsmann, and U. Sauermann, MHC class I alleles influence set-point viral load and survival time in simian immunodeficiency virus-infected rhesus monkeys, *J. Immunol.* 169, 3438–3446 (2002).
31. D. H. O'Connor, T. M. Allen, T. U. Vogel, P. Jing, I. P. DeSouza, E. Dodds, E. J. Dunphy, C. Melsaether, B. Mothe, H. Yamamoto, H. Horton, N. Wilson, A. L. Hughes, and D. I. Watkins, Acute phase cytotoxic T lymphocyte escape is a hallmark of simian immunodeficiency virus infection, *Nat. Med.* 8, 493–499 (2002).
32. D. H. O'Connor, B. R. Mothe, J. T. Weinfurter, S. Fuenger, W. M. Rehrauer, P. Jing, R. R. Rudersdorf, M. E. Liebl, K. Krebs, J. Vasquez, E. Dodds, J. Loffredo, S. Martin, A. B. McDermott, T. M. Allen, C. Wang, G. G. Doxiadis, D. C. Montefiori, A. Hughes, D. R. Burton, D. B. Allison, S. M. Wolinsky, R. Bontrop, L. J. Picker, and D. I. Watkins, Major histocompatibility complex class I alleles associated with slow simian immunodeficiency virus disease progression bind epitopes recognized by dominant acute-phase cytotoxic-T-lymphocyte responses, *J. Virol.* 77, 9029–9040 (2003).
33. V. Piguet and D. Trono, The Nef protein of primate lentiviruses, *Rev. Med. Virol.* 9, 111–120 (1999).
34. R. W. Price, B. Brew, J. Sidtis, M. Rosenblum, A. C. Scheck, and P. Cleary, The brain in AIDS: central nervous system HIV-1 infection and AIDS dementia complex, *Science* 239, 586–592 (1988).
35. D. M. Rausch, M. P. Heyes, E. A. Murray, and L. E. Eiden, Zidovudine treatment prolongs survival and decreases virus load in the central nervous system of rhesus macaques infected perinatally with simian immunodeficiency virus, *J. Infect. Dis.* 172, 59–69 (1995).

36. M. Rosenzweig, M. A. DeMaria, D. M. Harper, S. Friedrich, R. K. Jain, and R. P. Johnson, Increased rates of CD4(+) and CD8(+) T lymphocyte turnover in simian immunodeficiency virus-infected macaques, *Proc. Natl. Acad. Sci. U.S.A.* 95, 6388–6393 (1998).
37. U. Sauermann, C. Stahl-Hennig, N. Stolte, T. Muhl, M. Krawczak, M. Spring, D. Fuchs, F. J. Kaup, G. Hunsmann, and S. Sopper, Homozygosity for a conserved Mhc class II DQ-DRB haplotype is associated with rapid disease progression in simian immunodeficiency virus-infected macaques: results from a prospective study, *J. Infect. Dis.* 182, 716–724 (2000).
38. J. E. Schmitz, M. J. Kuroda, S. Santra, V. G. Sasseville, M. A. Simon, M. A. Lifton, P. Racz, K. Tenner-Racz, M. Dalesandro, B. J. Scallon, J. Ghrayeb, M. A. Forman, D. C. Montefiori, E. P. Rieber, N. L. Letvin, and K. A. Reimann, Control of viremia in simian immunodeficiency virus infection by CD8+ lymphocytes, *Science* 283, 857–860 (1999).
39. J. E. Schmitz, M. J. Kuroda, S. Santra, M. A. Simon, M. A. Lifton, W. Lin, R. Khunkhun, M. Piatak, J. D. Lifson, G. Grosschupff, R. S. Gelman, P. Racz, K. Tenner-Racz, K. A. Mansfield, N. L. Letvin, D. C. Montefiori, and K. A. Reimann, Effect of humoral immune responses on controlling viremia during primary infection of rhesus monkeys with simian immunodeficiency virus, *J. Virol.* 77, 2165–2173 (2003).
40. G. Silvestri, D. L. Sodora, R. A. Koup, M. Paiardini, S. P. O'Neil, H. M. McClure, S. I. Staprans, and M. B. Feinberg, Nonpathogenic SIV infection of sooty mangabeys is characterized by limited bystander immunopathology despite chronic high-level viremia, *Immunity.* 18, 441–452 (2003).
41. S. M. Smith, G. B. Baskin, and P. A. Marx, Estrogen protects against vaginal transmission of simian immunodeficiency virus, *J. Infect. Dis.* 182, 708–715 (2000).
42. S. Sopper, E. Koutsilieri, C. Scheller, S. Czub, P. Riederer, and M. ter Meulen V., Macaque animal model for HIV-induced neurological disease, *J. Neural Transm.* 109, 747–766 (2002).
43. S. Sopper, D. Nierwetberg, A. Halbach, U. Sauer, C. Scheller, C. Stahl-Hennig, K. Matz-Rensing, F. Schafer, T. Schneider, M. ter Meullen V. and J. G. Muller, Impact of simian immunodeficiency virus (SIV) infection on lymphocyte numbers and T-cell turnover in different organs of rhesus monkeys, *Blood* 101, 1213–1219 (2003).
44. S. Sopper, U. Sauer, S. Hemm, M. Demuth, J. Muller, C. Stahl-Hennig, G. Hunsmann, M. ter Meulen V., and R. Dorries, Protective role of the virus-specific immune response for development of severe neurologic signs in simian immunodeficiency virus-infected macaques, *J. Virol.* 72, 9940–9947 (1998).
45. S. Sopper, U. Sauer, J. G. Muller, C. Stahl-Hennig, and M. ter Meulen V., Early activation and proliferation of T-cells in simian immunodeficiency virus-infected rhesus monkeys, *AIDS Res. Hum. Retroviruses* 16, 689–697 (2000).
46. M. Stevenson, HIV-1 pathogenesis, *Nat. Med.* 9, 853–860 (2003).
47. R. S. Veazey, M. DeMaria, L. V. Chalifoux, D. E. Shvetz, D. R. Pauley, H. L. Knight, M. Rosenzweig, R. P. Johnson, R. C. Desrosiers, and A. A. Lackner, Gastrointestinal tract as a major site of CD4+ T-cell depletion and viral replication in SIV infection, *Science* 280, 427–431 (1998).
48. D. Vodros, R. Thorstensson, R. W. Doms, E. M. Fenyo, and J. D. Reeves, Evolution of co-receptor use and CD4-independence in envelope clones derived from SIVsm-infected macaques, *Virology* 316, 17–28 (2003).
49. M. Zeitz, R. Ullrich, T. Schneider, S. Kewenig, K. Hohloch, and E. O. Riecken, HIV/SIV enteropathy, *Ann. N.Y. Acad. Sci.* 859, 139–148 (1998).
50. N. Leuchte, N. Berry, B. Köhler, N. Almond, R. LeGrand, R. Thorstensson, F. Titti, and U. Sauermann, *MhcDRB* sequences from cynomolgus macaques (*Macaca fascicularis*) of different origin, *Tissue Antigens* 63, 529–537 (2004).

6

SIV Infection of Macaques as a Model for AIDS Drug Studies

KOEN K. A. VAN ROMPAY, RAMAN P. SINGH, and MARTA L. MARTHAS

1. INTRODUCTION: THE NEED FOR AN ANIMAL MODEL

An increasing arsenal of anti-HIV drugs is currently being used, and many new candidates are continuously being developed.[1] Based on their viral targets during the viral replication cycle, the main antiviral drugs that have been approved or are being developed belong to several groups: inhibitors of attachment, fusion, reverse transcriptase (RT), integrase or protease (Figure 1). During recent years, combination therapy of these compounds, so-called highly active antiretroviral therapy (HAART), has led to major improvement in the clinical management of HIV-infected people in the developed countries.[2] Despite this considerable success, there is still much room for improvement, as not everyone can experience the desired long-term benefits. Once established, HIV infection can probably never be cured,[3] so long-term administration of these drugs is usually required for the majority of individuals, and problems of toxicity, compliance, drug resistance, and costs become very relevant. In addition, due to the current costs of antiviral drugs and the need for expertise monitoring of viral and immunological parameters and toxicity, current drug regimens are beyond the reach of the majority of HIV-infected people who live in developing countries.

Accordingly, the quest for better antiviral drugs and drug regimens continues. The "ideal" antiviral drug strategy would be one that induces strong and persistent suppression of virus replication, gives prolonged immunological and clinical benefits at the lowest toxicity, can be adminis-

KOEN K. A. VAN ROMPAY, RAMAN P. SINGH, and MARTA L. MARTHAS • California National Primate Research Center, University of California, Davis, CA 95616

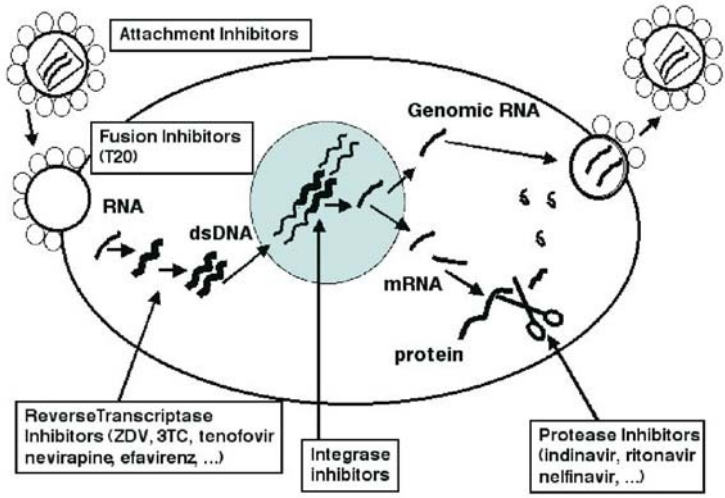

FIGURE 1. Overview of HIV life cycle and main groups of antiviral drugs.

tered at infrequent dosage intervals, is affordable and easy to store, and can thus benefit the greatest number of HIV-infected people worldwide.

While many new compounds are continuously being developed, the pipeline that drugs need to cross between the first demonstration of in vitro antiviral effects and approval for clinical use is time consuming, very expensive, and tedious. Many compounds that inhibit virus replication in vitro are never further developed (due to lack of resources), or they fail in preclinical testing or clinical trials, due to unfavorable pharmacokinetics, toxicity or lack of sufficient antiviral activity in vivo.

Another confounding problem is that many drugs have already been approved for HIV-infected patients. Because it is considered unethical to treat "control" groups with anything less than the currently available "gold" standard of combination therapy, clinical trials to prove the efficacy of novel antiviral drugs or drug combinations become more complicated. Therefore, the efficacy of new drugs is now often evaluated in patients failing currently available HAART regimens, who usually have many existing drug resistance mutations, low CD4+ cell counts, and poor immune responses, and thus the response in this "worst-case scenario" may underestimate the potency of the drug for treatment-naive patients with better health status.

These dilemmas underscore the need for a reappraisal of the role of animal models in the drug development process. Appropriate animal models that allow rapid evaluation of the efficacy and the toxicity of antiviral compounds would be very useful, as they allow sorting out those drugs that are promising and deserve to enter human clinical trials from those

drugs that should probably be discarded. A number of animal models are available for rapid evaluation of anti-HIV compounds (reviewed elsewhere in this book). While murine and feline models are very appropriate for initial screening, further testing is best done in nonhuman primate models that better resemble HIV infection of humans.

2. SIV INFECTION OF MACAQUES: ANIMAL MODEL OF HIV AND AIDS

Nonhuman primates are phylogenetically the closest to humans. The similarities in physiology (including drug metabolism, placentation, fetal and infant development, etc.) and immunology allow a more reliable extrapolation of results obtained in nonhuman primate models to clinical applications for the human population. Infection of macaques with virulent simian immunodeficiency virus (SIV) isolates or SIV-HIV chimeric viruses (SHIVs) results in a disease that resembles human AIDS, including opportunistic infections, weight loss, wasting, and central nervous system disorders.[4,5] Compared to HIV infection of humans, infection of macaques with virulent SIV or SHIV isolates results in an accelerated course, as most animals develop clinical disease within 3–30 months (compared to 7–10 years for humans). Infection of macaques with SIV or SHIV is not necessarily fatal, as there are many attenuated or nonpathogenic virus isolates that give transient or low-level viremia and very slow or no disease; accordingly, investigators can select the virus isolate or clone that is most appropriate for their study design. A pediatric AIDS animal model has been developed using newborn and infant macaques (0–4 weeks of age); similar to observations in HIV-infected human infants, the disease course in newborn macaques following inoculation with virulent SIV strains is usually accelerated.[6,7] This pediatric animal model has been proven very useful for antiviral drug testing (see further).

Because of the many similarities between the primate models and HIV infection of humans, the role of the primate models in HIV/AIDS research is multiple. In addition to allowing investigators to unravel the virus-host interactions during disease pathogenesis, the SIV-macaque model has also been used to investigate the pharmacokinetics, toxicity, and antiviral efficacy of antiviral drugs. It is the balance among all these in vivo interactions that eventually determines the long-term clinical usefulness of the antiviral drug (Figure 2).

Besides being a test system for preclinical screening of the efficacy of novel drugs, a good animal model can also be used to test hypotheses that are difficult or impossible to study in humans. Investigators use different study designs (by manipulating variables such as initiation of drug treatment relative to virus inoculation, the duration of treatment, the age of the

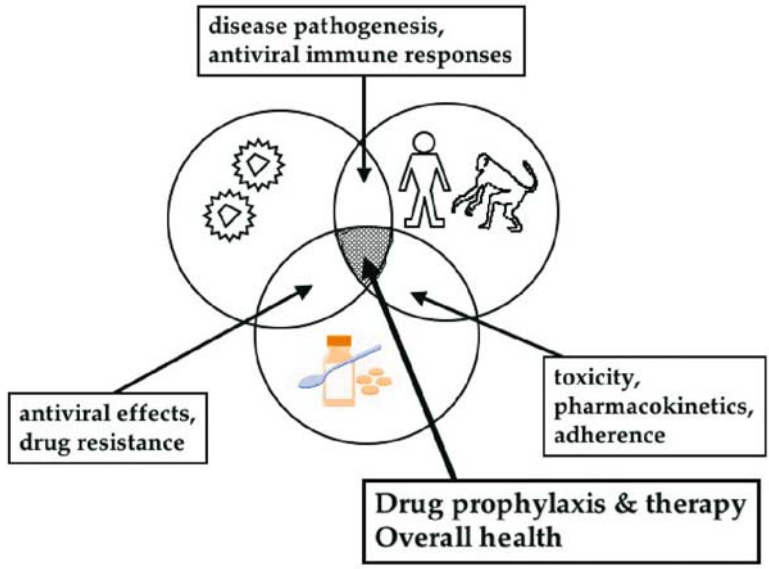

FIGURE 2. Many interactions between the virus, host, and antiviral drugs determine the overall health outcome of the host.

animals, the virulence and drug susceptibility of the virus inoculum, the status of the immune system, etc.), to address very specific questions directly relevant to treatment of human HIV infection. Examples of this are studies aimed at determining the pre- and postexposure prophylactic efficacy of antiviral drugs, or studies aimed at determining the in vivo virulence and clinical implications of drug-resistant viral mutants.

3. MACAQUE SPECIES AND VIRUS ISOLATES USED IN ANTIVIRAL DRUG STUDIES

Antiviral drug studies generally used rhesus macaques (*Macaca mulatta*) or cynomolgus macaques (*M. fascicularis*). Most drug studies in macaques have used SIV isolates belonging to a few groups, in particular SIVmac, SIVsmm, and SIVmne. Because the polymerase region of SIV has about 60% amino acid homology to HIV-1,[5] SIV is susceptible to many of the same inhibitors, such as the nucleoside reverse transcriptase inhibitors [NRTI; e.g., zidovudine (AZT), didanosine (ddI), zalcitabine (ddC), stavudine (d4T), and abacavir (1592U89)], the nucleotide RT inhibitor tenofovir (PMPA), and inhibitors of protease enzymes.[8,9] Some compounds, especially non-nucleoside RT inhibitors (NNRTI) such as nevirapine and efavirenz are active only against HIV-1, and not against HIV-2 or SIV.[10] To

evaluate such RT inhibitors in the macaque model, the construction of infectious SHIVs has been proven useful. SHIVs are constructed by replacing specific genes of SIVmac with their counterparts of HIV-1, and many of them are infectious and can cause disease following inoculation of macaques. The availability of an infectious RT-SHIV clone, which contains the RT of HIV-1 in an SIVmac background, now enables investigators to use this animal model to study the efficacy and the emergence of drug resistance for NNRTI.[11–14]

4. DEVELOPMENT OF THE ANIMAL MODEL: INITIAL OBSTACLES AND SOLUTIONS

Because of the many similarities in physiology to humans, the macaque model has been proven very useful to study toxicity and pharmacokinetics of antiviral drugs, including the assessment of drug interactions, the effect of pregnancy, and transplacental drug transfer.[15–29]

During the initial years of the HIV pandemic, however, the role of nonhuman primate models in testing antiviral efficacy was rather limited. Although SIV is susceptible to many anti-HIV drugs in vitro, many initial drug studies in macaques were not very effective. One of the main reasons for the less-than-spectacular results of the primate studies was that most drugs that were available at that time were not highly potent in suppressing viremia. Nonhuman primate models at that time were also not practical to reliably evaluate the efficacy of prolonged drug therapy of chronic infection, due to a number of problems, especially with regard to the detection of antiviral effects and the administration of drugs.

The time course of SIV disease progression in juvenile and adult macaques is highly variable, as the asymptomatic period can range from months to years, and depends on host factors such as the strength of the initial immune response. It was therefore often hard to determine whether a small difference in clinical outcome was due to host factors or to the drug treatment, especially because only relatively small numbers of animals can be used in each study.[30] There were also no adequately sensitive assays to detect antiviral effects of the drug during the asymptomatic stage by monitoring surrogate markers, such as virus levels in blood and tissues.[31] Most SIV-infected juvenile/adult macaques have no detectable p27 antigenemia or infectious virus in plasma during the asymptomatic stage of disease, and the technology to measure viral RNA in plasma was not available at that time. Accordingly, testing the efficacy of an antiviral drug was mainly limited to determining its potential to prevent infection or reduce/delay the acute viremia (see below). In recent years (since about 1997), sensitive assays have been developed to monitor virus replication in SIV-infected macaques, including quantitative viral RNA assays to detect virus in plasma

TABLE I
Key Parameters to Monitor Infection and Response to Treatment

Viral Parameters:
— Virus levels in blood and lymphoid tissues (PCR, RT-PCR, bDNA, quantitative virus isolation, . . .)
— Drug resistance (phenotypic, genotypic)
— Viral diversity (heteroduplex mobility assay, sequencing, . . .)
— In vitro replication kinetics, cell tropism, co-receptor usage, etc.
Immune Parameters:
— Antibody responses (ELISA, western blot, neutralizing antibodies, . . .)
— Cell-mediated immune responses (cytotoxic T lymphocytes, ELISPOT, proliferation, intracellular cytokine flow cytometry, tetramer staining, NK cell activity, . . .)
— Lymphocyte subpopulations (flow cytometry)
— Cytokine and chemokine levels (ELISA, RT-PCR, immunohistochemistry, . . .)
Clinical Parameters:
— Weight gain
— Opportunistic infections
— Disease-free survival

or tissues.[32–34] In addition, the development of a growing number of immunological assays has provided us a better understanding of disease pathogenesis (Table I). All these assays are very similar to those used to monitor human patients and enable us to better monitor the effects of drug therapy. This growing arsenal of tools has spurred the use of macaques with chronic SIV infection for antiviral drug studies.

As discussed elsewhere,[35,36] the use of juvenile and adult macaques in drug studies is often complicated by difficulties in administering drugs for a prolonged period of time. This problem becomes more pronounced for drugs with a short half-life, which require frequent administration to maintain sufficient blood levels. In addition, the amount of drug that is needed can lead to considerable costs, especially for compounds that are still in preclinical stages of development and for which test quantities are usually very expensive to manufacture. Many of these problems can be overcome by using newborn and infant macaques, which are easier to handle for drug administration and require less drug.[35] Another important feature of this pediatric animal model is that the disease course following inoculation with highly virulent SIV isolates (such as SIVmac251) is more uniform than in older animals, and is characterized by persistently high viremia, rapid immunosuppression, and development of clinical disease within about 2–4 months.[7] Because of this predictably rapid disease course, effects of drug therapy on disease progression can be evaluated more reliably and quickly in newborn macaques than with older animals, and with fewer animals.[30,35,37] These aspects of the infant macaque model can be especially important if a test compound has limited antiviral effects but may have immunomodulatory effects that prolong disease-free survival. The demon-

stration of clinical benefits in a "worst-case scenario" of high viremia and rapid disease is the best indicator of the potency of any drug strategy. Accordingly, information obtained in a pediatric animal model of AIDS applies directly to older SIV-infected macaques and HIV infection of human infants as well as adults.

5. OVERVIEW OF DRUG STUDIES IN NONHUMAN PRIMATES

Prophylaxis: Prevention of Infection

Many studies in nonhuman primates have focused on testing whether drug administration, starting around the time of virus inoculation, was effective in preventing infection. While prevention of infection is traditionally considered as the complete absence of any viral or immunological evidence of infection, the development of more sensitive techniques (including DNA PCR, viral RNA quantitation) has sometimes resulted in transient detection of low-level signs of infection, usually within the first months after virus inoculation.[38,39] Accordingly, for the purposes of this review, prophylaxis is defined as protection against persistent infection, with persistent infection being defined as persistent viremia or persistently detectable virus-specific immune responses.

Early studies, which mostly used zidovudine (AZT), were not highly effective in preventing infection, but a likely reason for this was the high dose of virus used in these experiments.[40–43] In subsequent studies (Table II), when a lower dose of SIV was used to inoculate animals, administration of several drugs [including AZT, adefovir (PMEA), tenofovir (PMPA), and 3′-fluorothymidine (FLT)] starting prior to, or at the time of virus inoculation, prevented SIV infection.[39,44–48] Nevirapine treatment starting prior to virus inoculation prevented HIV-1 infection of chimpanzees.[38] Very few compounds have been shown to prevent infection when treatment was started after virus inoculation: tenofovir, 2′,3′-dideoxy-3′-hydroxymethyl cytidine (BEA-005) and GW420867.[13,44,49–51] Of these three compounds, only tenofovir has so far been approved for use in humans. Recently it was found that a 1- or 2-dose regimen of tenofovir was sufficient to protect newborn macaques against oral SIV infection.[45] Tenofovir was also partially effective to protect newborn macaques against infection following inoculation with a mutant SIV isolate that has reduced in vitro susceptibility to tenofovir.[52]

Macaques have also been used to test the efficacy of antiviral compounds administered as topical microbicides to prevent mucosal SIV or SHIV infection. While topical administration of high-dose tenofovir, 3-hydroxyphthaloyl-β-lactoglobulin, dextrin-2-sulphate, PRO 2000, benzalkonium chloride, and cellulose acetate phtalate in gel format protected adult

TABLE II
Examples of Macaque Studies with Antiviral Drugs Started Prior to or Very Early (<5 days) After Virus Inoculation

Author, year[(citation)]	Virus	Inoculum Size & Route	Start of Treatment Relative to Virus	Drug, Daily Dose, Route, Duration	Outcome
McClure, 1990[43]	SIVsmmPBJ14	20 $TCID_{50}$, IV	1–72 h post	ZDV, 3 × 33 mg/kg SQ for 14 d	Protection against acute disease
Balzarini, 1991[126]	SIVmacBK28	IV	1 day pre	Adefovir, 2 × (5 or 10) mg/kg IM for 29 d	Delayed seroconversion
Böttiger, 1992[46]	SIVsm/HIV-2	2–10 AID_{50}, IV	8 h post	3′-fluorothymidine, 3 × 5 mg/kg, SQ for 10 d	Partial protection
Van Rompay, 1992[87]	SIVmac251	1–10 AID_{50}, IV	2 h pre	ZDV, 3 × 50 mg/kg oral for 6 weeks	Protection against infection
Martin, 1993[68]	SIVdeltaB670	10 AID_{50}, IV	1–72 h post	ZDV, 4 × 25 mg/kg, SQ for 28 d	Reduced viremia, antigenemia
Le Grand, 1994[71]	SIVmac251	4 AID_{50}, IV	7 days pre	ZDV, 3 × 15 mg/kg, SQ for 35 d	Reduced viremia
Tsai, 1994[48]	SIVmne	10^3 $TCID_{50}$, IV	48 h pre	PMEA, 10 or 20 mg/kg/day, SQ for 28 d	Protection against infection
Van Rompay, 1995[30]	SIVmac251	10–100 AID_{50}, IV	2 h pre to 7 d post	ZDV, 3 × 25 mg/kg oral for >15 months	Reduced viremia
Rausch, 1995[75]	SIVdeltaB670	10 AID_{50}, IV	2 h pre	ZDV, continuous or intermittent IV	Reduced antigenemia
Tsai, 1995[49]	SIVmne	10 AID_{50}, IV	48 h pre to 24 h post	Tenofovir, 20 or 30 mg/kg SQ for 4 weeks	Protection against infection
Überla, 1995[11]	RT-SHIV	200–3000 $TCID_{50}$, IV	8 hr pre	ZDV, 3 × 15 mg/kg or LY300046-Hcl, 3 × 5 mg/kg SQ for 5 d	LY300046-HCl delayed antigenemia
Joag, 1997[70]	SIVmac239	2000 $TCID_{50}$, IV	1 day pre	PMEA, 20 mg/kg, SQ for 30 d	Reduced viremia

Böttiger, 1997[50]	SIVsm/HIV-2	10–1000 AID_{50}, IV or intrarectal	8 h pre to 6 d post	BEA-005, 3 × (0.05–10) mg SQ for 1–10 d	Protection or delayed viremia
Lori, 1997[127]	SIVsmmPBJ14	10 units IV	24 h pre	Didanosine, 5 mg/kg IV and/or hydroxyurea, 100 mg/kg IV	Reduced viremia in didanosine-treated groups
Watson, 1997[74]	HIV-2_{287}	25 TCID50, IV	Immediately post	Stavudine, orally for 16 weeks	Reduced viremia
Van Rompay, 1998[45]	SIVmac251	10^5 $TCID_{50}$, oral	4 h pre	Tenofovir, 30 mg/kg SQ at −4 h, +20 h	Protection against infection
Hodge, 1999[78]	SIVsmmPBj	1 $TCID_{50}$, IV	3 or 5 days post	Tenofovir, 30 mg/kg SQ until d 14	Reduced viremia and protection against acute disease
Mori, 2000[13]	RT-SHIV	100 $TCID_{50}$, IV	8 or 24 h post	GW420867, 30 mg/kg for 28 days	Protection against infection or reduced viremia
Le Grand, 2000[72]	SHIV-89.6P	50 MID_{50}, IV	4 h or 72 h post	ZDV (4.5 mg/kg) + lamivudine (2.5 mg/kg) + indinavir (20 mg/kg) oral until d 28	Reduced viremia and protection against acute CD4+ T-cell loss
Lifson, 2000[79]	SIVsmE660	20 AID_{50}, IV	24 or 72 h post	Tenofovir 30 mg/kg SQ for 3, 28 or 63 d	Persistently reduced viremia in most animals
Otten, 2000[51]	HIV-2 GB122	10^5 $TCID_{50}$, vaginal	12, 36 or 72 h post	Tenofovir, 30 mg/kg SQ for 28 d	Protection against infection in most animals
Van Rompay, 2001[39]	SIVmac251	10^5 $TCID_{50}$, oral	4 h pre or 1 h post	Tenofovir, 4 or 30 mg/kg SQ, 1–2 doses	Protection against persistent infection

$TCID_{50}$ = tissue culture infectious doses 50%; AID_{50} = animal infectious dose 50%; IV = intravenously; SQ = subcutaneously; d = days; ZDV = zidovudine (AZT)

macaques in varying degrees of efficacy against intravaginal SIV or SHIV infection, topical low-dose tenofovir was not protective against the oral route of virus inoculation.[53–57]

The demonstration that systemic levels of antiviral drugs can prevent infection in macaques has preceded the demonstration of such effects in humans following exposure to HIV in several clinical settings. The first one is the use of antiviral drugs to prevent infection following needle-stick accidents of health care workers. AZT administration following percutaneous HIV exposure was found to reduce the risk of infection by 80%.[58] Postexposure drug prophylaxis is now also offered following sexual exposure.[59] In recent years, as transmission of HIV often involves drug-resistant virus, postexposure prophylaxis regimens typically consist of a combination of drugs. Another clinical area where antiviral drugs have been very useful is in the prevention of mother-to-infant transmission of HIV. While vertical transmission can also occur in utero or postnatally (through breastfeeding), evidence suggests that a majority of HIV infections occur shortly before or during birth (potentially through contact with maternal blood and fluids).[60] The demonstration that AZT administration to HIV-infected pregnant women beginning at mid- to late gestation, and continuing to their newborns during the first 6 weeks of life, reduced the rate of vertical HIV transmission by two thirds has been a major advancement in the combat against pediatric HIV infection in developed countries.[61] Since then, studies have demonstrated partial efficacy of shorter AZT regimens, alone or in combination with lamivudine.[62–65] Currently the simplest and most affordable regimen for developing countries is the two-dose HIVNET-012 nevirapine (NVP) regimen, with one dose given to the mother at the onset of delivery and the second dose of NVP to the newborn shortly after birth.[66] To counteract potential problems with drug resistance mutations that are induced by this simple NVP regimen in a proportion of the women,[67] the promising data with tenofovir in the macaque model[39] have spurred interest to test the feasibility of a two-dose tenofovir regimen to reduce perinatal HIV transmission in future years, and a phase I human trial (PACTG-394) is currently ongoing as a first step.

Therapy: Treatment of Infection

Numerous drug studies in the macaque model have demonstrated that even when infection was not prevented, early drug treatment delayed or reduced the peak of rapid virus replication that occurs during the first weeks of infection, enhanced antiviral immune responses, and delayed disease progression (Table III).[11,13,30,40,42,43,47,68–79] This information applies directly to drug treatment of humans during the primary viremia stage. Indeed, in recent years there is increasing evidence that early drug intervention offers strong benefits for HIV-infected patients to achieve

TABLE III
Examples of Macaque Studies With Antiviral Drugs Started ≥5 Days After Virus Inoculation, When Systemic Infection Has Occurred

Author, year[(citation)]	Virus	Start of Treatment Relative to Virus Inoculation	Drug, Daily Dose, Route, Duration	Outcome
Böttiger, 1992[31]	SIVsm	111–174 d post	ZDV, 20 mg/kg SQ for 9 weeks; 3′-fluorothymidine for 4 or 9 weeks	No reduction in viral antigen levels in lymph nodes
Van Rompay, 1992[87]	SIVmac251	6 weeks post	ZDV, 3 × 50 mg/kg oral for 10 weeks	No reduction of infectious viremia
Tsai, 1995[90]	SIVmne	chronic infection	Adefovir, 20–30 mg/kg SQ for 4–8 weeks	Reduced viremia
Van Rompay, 1996[37]	SIVmac251	3 weeks post	Tenofovir, 30 mg/kg, SQ for >1–7 years	Reduced viremia, prolonged survival
Tsai, 1997[89]	SIVmne	>19 weeks post	Tenofovir, 30 or 75 mg/kg, SQ for 28 days	Reduced viremia
Van Rompay, 1999[73]	SIVmac251	5 days post	Tenofovir, 30 mg/kg SQ for 14 or 60 days	Reduced viremia and delayed disease
Silvera, 2000[92]	SIVmac251	18 weeks post	Adefovir or tenofovir, 20 mg/kg SQ for 28 days	Reduced viremia in most animals
Smith, 2000[77]	$SHIV_{KU}$	1 week post	Tenofovir, 30 mg/kg, SQ for 12 weeks	Reduced viremia; protection against acute CD4+ T decline
Rosenwirth, 2000[76]	RT-SHIV	1 or 2 weeks post	Tenofovir, 30 mg/kg, SQ for 8 weeks	Reduced viremia
Igarashi, 2001[84]	SHIVDH12R	5 or 21 days post	Tenofovir, 30 mg/kg, SQ for 4 weeks	Reduced viremia only in early treatment
Spring, 2001[128]	SIVmac239	1 week post	Tenofovir, 30 mg/kg SQ for 28 days	Reduced viremia
Van Rompay, 2002[88]	SIVmac251	3 weeks post	Lamivudine or emtricitabine, 8 mg/kg SQ chronic	Little effect on viremia but selection for M184V mutants
Shen, 2003[96]	SIV/17E-Fr	50 days post	Tenofovir (20 mg/kg) SQ + emtricitabine (50 mg/kg) SQ chronic	Reduced viremia

SQ = subcutaneously; d = days; ZDV = zidovudine

long-term suppression of viral replication, enhanced HIV-specific CD4+ T-cell proliferative responses, and delayed disease progression.[80–83]

When macaques were treated with short-term drug regimens during the acute viremia stage, the duration of the benefits after treatment was withdrawn depended on the virus that was used. With pathogenic SHIV isolates, short-term drug-induced suppression of acute viremia was usually effective to induce strong antiviral immune responses that controlled virus replication and delayed disease for an extended time in the absence of drug treatment.[13,76,77,84] In contrast, with highly virulent SIV isolates such as SIVmac251, it has been more difficult to achieve sustained control of viremia and disease when short-term drug treatment is given (see review[85,86]).

A few macaque studies have investigated the effects of antiviral therapy on established, chronic SIV infection (i.e., after the acute viremia stage). Initial studies with AZT were not very successful in reducing viremia once SIV infection was established,[31,87] but this was probably due to the relative weakness of AZT monotherapy to inhibit virus replication of the highly virulent SIVmac251 isolate. Lamivudine (3TC) and emtricitabine ((−)-FTC) treatment of SIVmac251-infected infant macaques also had little effect on viremia and disease course; yet, there was rapid selection for the development of drug-resistant mutants with the M184V mutation in RT, suggesting that drug levels were sufficient.[88] While initial studies with adefovir (PMEA) showed antiviral efficacy against SIV, subsequent studies demonstrated tenofovir to be the most effective compound so far in this animal model to reduce infectious PBMC-associated and plasma virus levels, reduce plasma RNA levels, and delay disease progression.[37,89–92] Surprisingly, for tenofovir therapy, the emergence of viral mutants with reduced susceptibility does not always lead to a rebound in viremia (see below).

Studies with tenofovir have also demonstrated that the efficacy of antiviral drug therapy is strongly dependent on the immune system. While tenofovir treatment initiated during early stages of SIV infection is usually very effective, strong and persistent reduction of chronic viremia is more difficult to achieve in a host who has high viremia with a highly virulent virus and is immunosuppressed at the start of treatment.[93] In addition, temporary CD8+ cell depletion of macaques during acute viremia (through injection of anti-CD8 monoclonal antibody) transiently reduced the efficacy of simultaneous tenofovir treatment in reducing viremia of wild-type virus, demonstrating the important role of CD8+ cell mediated immune responses during antiviral drug therapy.[94]

Tenofovir's antiviral efficacy in SIV-infected macaques has sparked many other drug studies in this animal model. Tenofovir-containing regimens have been used to gain a better understanding of disease pathogenesis and drug therapy and to test additional intervention strategies. Combination treatment of SIV-infected macaques was found to improve

immune responses against other organisms such as mycobacterium.[95] The macaque model has also been used to investigate the viral reservoirs during drug treatment: SIV-infected pigtailed macaques treated with tenofovir and emtricitabine were found to have viral reservoirs in resting CD4+ T lymphocytes.[96]

A number of studies have combined antiviral drug treatment with other strategies aimed at boosting antiviral immune responses, so that when drug treatment was stopped, viremia was controlled better. These immunotherapeutic strategies include structured treatment interruption,[97] active immunization (with vaccines such as NYVAC and ALVAC, with or without IL-2 administration[98–101]), and immune reconstitution via administration of autologous CD4+ T-cells collected prior to SIV infection.[102] These studies demonstrate the usefulness of the SIV macaque model to design and test immunotherapeutic strategies, aimed at invoking the immune system to assist in reducing virus replication.

Antiviral Drug Resistance

The Value of Animal Models in Studying Drug Resistance

Although HAART has led to significant advances in the clinical management of HIV-infected patients, many individuals, however, do not show the desired strong and persistent suppression of viral replication, and eventually show disease progression. Although other factors, such as compliance and toxicity, also contribute to reduced efficacy and tolerability of antiviral drug therapy, a major limiting factor is the emergence of viral mutants with reduced in vitro susceptibility to antiviral drugs. Due to the high mutation rate of the virus, incomplete suppression of virus replication leads to the selection of viral variants with mutations that allow better replication in the presence of drugs.

While the correlation between specific mutations in the viral genome and in vitro reduced susceptibility has been well documented for many antiviral drugs, many questions remain regarding the exact clinical implications of these drug-resistant variants in vivo and how to use this information to make treatment decisions. If drug resistance means that the drug is no longer effective, then it can as well be withdrawn; but if there is still a partial response, then it will be counterproductive to discontinue drug administration unless better alternatives can be offered.[103–105] Some data suggest that drugs can still have therapeutic effects even in the presence of drug-resistant virus. For example, interruption of RT inhibitors in many patients with drug-resistant virus results in increased viremia, suggesting that these drugs still partially suppressed virus replication.[106] In addition, in patients receiving protease-inhibitor containing regimens, there is often immunological and clinical benefit despite virologic failure

(i.e., so-called discordant results), and it has been hypothesized that the altered pathogenesis of drug-resistant variants may play a role in this phenomenon.[107–109]

A major question about drug resistance concerns the replicative fitness and virulence of drug-resistant mutants relative to wild-type virus. Because the mutations that cause drug resistance are either undetectable or are present at very low frequency in the absence of the selection pressure exerted by drug treatment, drug resistance mutations are expected to reduce the ability of the virus to replicate. But primary drug resistance mutations are often followed by the emergence of compensatory mutations to improve replicative fitness. But to what degree are drug-resistant mutants attenuated in virulence (i.e., their ability to cause disease)? Are drug-resistant mutants significantly attenuated compared to wild-type virus, so that the purpose of continuing drug treatment is to prevent reversion to the more virulent wild-type form?

While many studies have focused on determining the in vitro growth kinetics of drug-resistant HIV mutants, they can never completely predict their in vivo virulence, which is also determined by complex factors (including many tissue- and cell-specific factors) that are difficult to mimic in vitro, such as drug pharmacokinetics, compensatory mutations, and the dual role of the immune system, which can assist in controlling virus replication, but when activated, can also promote virus replication (Figure 3). Studies in the SIV-macaque model have demonstrated repeatedly that the correlation between in vitro viral growth properties/cell tropism and in vivo replica-

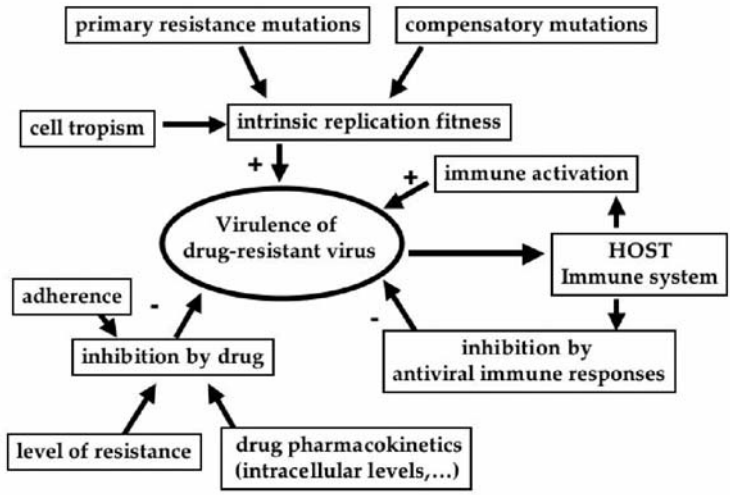

FIGURE 3. Complex interactions of viral and host factors and drug features determine the virus's ability to weaken the immune system.

tive ability/virulence is often weak, as virus isolates that are able to replicate well in vitro can be severely attenuated in replicative fitness and virulence following inoculation in macaques.[110,111] Thus, the extrapolation of results from in vitro growth kinetic studies to decisions affecting clinical management of HIV-infected patients should be performed with caution.

Some information regarding the replicative potential and stability of drug-resistant viral mutants in vivo can be gathered from case reports, such as those documenting primary infection with drug-resistant HIV-1, as well as those monitoring the reversion of drug-resistant virus to wild-type following discontinuation of drug treatment. An animal model, however, allows approaches that are impossible and unethical to use in humans, but which are the most direct ways to study the virulence and clinical implications of drug-resistant virus: animals can be inoculated with drug-resistant viral mutants or their wild-type counterparts, and their replication fitness and virulence can be compared in drug-treated versus untreated animals. Although the SIV macaque model has been underutilized in this research area, several macaque studies have addressed the emergence or virulence of drug-resistant SIV (see below).

Drug Resistance Studies in the SIV and SHIV: Macaque Model

Several methods have been used to generate drug-resistant SIV variants, such as in vitro and in vivo drug selection procedures, as well as site-directed mutagenesis of molecular clones. Treatment of RT-SHIV infected macaques with nevirapine gave rise to the emergence of the Y181C and K103N mutations in RT, similar to observations in nevirapine-treated HIV-1 treated patients.[14]

In vitro selection of SIV for resistance to lamivudine (3TC) gave rise to the expected mutations M184I and M184V in RT; the role of these mutations in conferring high-level resistance of SIV to lamivudine was confirmed by site-directed mutagenesis.[112,113] Treatment of SIV-infected infant macaques with lamivudine or emtricitabine ((−)-FTC) also gave rise to the emergence of M184V mutants within 5 weeks of treatment.[88] The clinical implications of the M184V mutation were subsequently studied by inoculating juvenile macaques with SIVmac239 clones with either wild-type sequence or the M184V mutation in RT (SIVmac239-184V). In comparison to wild-type virus, SIVmac239-184V was replication impaired, based on virus levels 1 week after infection, and on the reversion of SIVmac239-184V to wild-type sequence in untreated animals within 8 weeks after inoculation. However, this reduced replication fitness was not sufficient to affect viral virulence, as animals inoculated with SIVmac239-184V and treated with (−)-FTC (to prevent reversion) developed similar viremia from 2 weeks after infection onward, and had a similar disease course and survival as animals infected with wild-type SIVmac239.[88] In a different study, the

M184V mutation did not revert in macaques inoculated with SIVmac239 containing both the M184V and E89G mutations; however, the M184V mutation in that study was engineered with two base changes in codon 184 (instead of the single base change that is normally seen during in vitro or in vivo selections).[114]

Using site-directed mutagenesis, the mutations that are most commonly found in AZT-resistant HIV-1 (at codons 67, 70, 215, and 219) have been inserted into the molecular clone SIVmac239. The resulting SIVmac239 mutant was AZT-resistant in vitro; inoculation of macaques with this AZT-resistant SIVmac239 and short-term treatment with AZT during the primary viremia resulted in reduced efficacy of AZT treatment to delay seroconversion.[115] Remarkably, however, these mutations in SIV RT were not selected for in vivo, as AZT-treated SIVmac251-infected macaques developed a glutamine-to-methionine substitution at codon 151 of RT (Q151M),[30,116] associated with high-level (>100-fold) in vitro resistance to AZT. Inoculation of the Q151M SIVmac isolate into naive newborn macaques demonstrated that this mutation did not significantly reduce viral replication and viral virulence; the Q151M mutation (which is the result of two base changes) was also very stable in the absence of AZT treatment.[116] This Q151M mutation has not been found in HIV-1-infected patients receiving AZT monotherapy, but has been found at low frequency in HIV-1-infected patients receiving sequential or combination therapy with dideoxynucleoside analogues.[117–119] However, the Q151M mutation is found frequently in HIV-2-infected patients receiving NRTI therapy.[120,121] This latter observation indicates that due to much sequence homology, HIV-2 and SIV use similar pathways, distinct from those of HIV-1, to develop resistance to these antiviral drugs.

Treatment of SIVmac251-infected infant macaques with tenofovir resulted in the emergence of virus with fivefold reduced in vitro susceptibility to tenofovir and a lysine-to-arginine substitution at codon 65 (K65R) of RT. The emergence of K65R was followed by additional RT mutations, which were likely to be compensatory mutations.[37] Tenofovir also selects for the K65R mutation in HIV-1 in vitro,[122] and is found at low frequency (~2%) in HIV-1-infected patients after long-term treatment with tenofovir-containing combination regimens.[123,124] The SIV macaque model has provided important information regarding the clinical implications of K65R viral mutants during tenofovir treatment. Although some SIVmac251-infected animals show an increase in viremia following the emergence of K65R viral mutants, many animals continue to suppress viremia to low or undetectable levels for years (from 1 to >7 years).[37,125] This success in suppressing replication of the highly virulent SIVmac251 isolate with tenofovir monotherapy is unprecedented in this animal model. To investigate whether these findings were caused by an attenuating effect of the K65R mutation on viral virulence, K65R SIV isolates were inoculated into new

animals and in the absence of tenofovir treatment, were found to be as virulent as wild-type SIVmac based on their ability to induce high viremia and rapid disease.[125] In subsequent studies, cell depletion experiments (using injection of anti-CD8 monoclonal antibody) revealed that the suppressed viremia of K65R SIV mutants during tenofovir treatment is due to the development of strong CD8+ cell-mediated antiviral immune responses that are promoted during tenofovir treatment.[94] Continued tenofovir treatment was required to maintain suppression of K65R SIV replication,[94] because tenofovir withdrawal led to an increase in viremia. Thus, both tenofovir and CD8+ cells were required to achieve optimal suppression of virus replication. These observations of reduced viremia of K65R SIV mutants associated with improved antiviral immune responses in tenofovir-treated macaques are consistent with clinical observations in HIV-1 infected human patients: although K65R mutants of HIV-1 are infrequently (~2%) detected after long-term tenofovir treatment, their emergence is generally not associated with a rebound in viremia.[124] Another surprising finding for tenofovir was that, even for tenofovir-treated animals where K65R viral mutants were able to cause high viremia, continued tenofovir treatment was still beneficial, as it prolonged disease-free survival more than predicted based on viral RNA levels and CD4+ T-cell counts.[93,125] Because prolonged survival in the presence of high viremia has not been observed with other antiviral drugs in the SIV-macaque model, these observations suggest that tenofovir treatment may have rather unusual interactions with the immune system that need to be elucidated by further studies.

The SIV animal model has also been studied for interactions between drug resistance mutations. Similar to findings for HIV-1, the lamivudine-selected M184V mutation in RT renders SIV hypersusceptible to tenofovir in vitro. In vitro and in vivo experiments with SIV demonstrated that in the presence of lamivudine and tenofovir, there is strong selection pressure against the M184V mutation but for the emergence of the K65R mutation.[113]

6. CONCLUSIONS

At the start of the HIV pandemic, the role of nonhuman primate models of HIV infection and AIDS in the drug development process was very limited. In recent years, however, a better understanding of disease pathogenesis and the development of better technological methods to monitor infection have made nonhuman primate models more suitable for antiviral drug testing. The encouraging data from drug studies in primates that have subsequently been confirmed in human studies are leading to a growing recognition of the relevance of this animal model. Accordingly, nonhuman primate models of HIV infection will continue to grow as

excellent and adaptable tools to rapidly gather data that can provide a solid scientific foundation to guide human clinical trials.

REFERENCES

1. De Clercq, E., New anti-HIV agents and targets, *Medicinal Res. Rev.* 22: 531–565 (2002).
2. Centers for Disease Control and Prevention, Update: Trends in AIDS incidence-United States, 1996, *MMRW* 46: 862–867 (1997).
3. Finzi, D., J. Blankson, J. D. Siciliano, J. B. Margolick, K. Chadwick, T. Pierson, K. Smith, J. Lisziewicz, F. Lori, C. Flexner, T. C. Quinn, R. E. Chaisson, E. Rosenberg, B. Walker, S. Gange, J. Gallant, and R. F. Siciliano, Latent infection of CD4+ T-cells provides a mechanism for lifelong persistence of HIV-1, even in patients on effective combination therapy, *Nature Med.* 5: 512–517 (1999).
4. Letvin, N. L., and N. W. King, Immunologic and pathologic manifestations of the infection of rhesus monkeys with simian immunodeficiency virus of macaques, *J. Acquir. Immune Defic. Syndr.* 3: 1023–1040 (1990).
5. Desrosiers, R. C., The simian immunodeficiency viruses, *Ann. Rev. Immunol.* 8: 557–578 (1990).
6. Bohm, R. P., L. N. Martin, B. Davison-Fairburn, G. B. Baskin, and M. Murphey-Corb, Neonatal disease induced by SIV infection of the rhesus monkey (Macaca mulatta), *AIDS Res. Hum. Retroviruses* 9: 1131–1137 (1993).
7. Marthas, M. L., K. K. A. Van Rompay, M. Otsyula, C. J. Miller, D. Canfield, N. C. Pedersen, and M. B. McChesney, Viral factors determine progression to AIDS in simian immunodeficiency virus-infected newborn rhesus macaques, *J. Virol.* 69: 4198–4205 (1995).
8. Black, P. L., M. B. Downs, M. G. Lewis, M. A. Ussery, G. B. Dreyer, S. R. J. Petteway, and D. M. Lambert, Antiretroviral activities of protease inhibitors against murine leukemia virus and simian immunodeficiency virus in culture, *Antimicrob. Agents Chemother.* 37: 71–77 (1993).
9. Sager, P. R., J. C. Cradock, C. L. Litterst, L. N. Martin, K. F. Soike, M. Murphey-Corb, P. A. Marx, C. C. Tsai, A. Fridland, A. Bodner, L. Resnick, and R. F. Schinazi, *In vitro* testing of therapeutics against SIV and HIV, *Ann. N.Y. Acad. Sci.* 616: 599–605 (1990).
10. De Clercq, E., HIV inhibitors targeted at the reverse transcriptase, *AIDS Res. Hum. Retroviruses* 8: 119–134 (1992).
11. Überla, K., C. Stahl-Hennig, D. Böttiger, K. Mätz-Rensing, F. J. Kaup, J. Li, W. A. Haseltine, B. Fleckenstein, G. Hunsmann, B. Öberg, and J. Sodroski, Animal model for the therapy of acquired immunodefiency syndrome with reverse transcriptase inhibitors, *Proc. Natl. Acad. Sci. USA* 92: 8210–8214 (1995).
12. Balzarini, J., E. De Clercq, and K. Überla, SIV/HIV-1 hybrid virus expressing the reverse transcriptase gene of HIV-1 remains sensitive to HIV-1-specific reverse transcriptase inhibitors after passage in rhesus macaques, *J. Acquir. Immune Defic. Syndr. Human Retrovirol.* 15: 1–4 (1997).
13. Mori, K., Y. Yasumoti, S. Sawada, F. Villinger, K. Sugama, B. Rosenwirth, J. L. Heeney, K. Überla, S. Yamazaki, A. A. Ansari, and H. Rübsammen-Waigmann, Suppression of acute viremia by short-term postexposure prophylaxis of simian/human immunodeficiency virus SHIV-RT-infected monkeys with a novel reverse transcriptase inhibitor (GW420867) allows for development of potent antiviral immune responses resulting in efficient containment of infection, *J. Virol.* 74: 5747–5753 (2000).

14. Zuber, B., D. Böttiger, R. Benthin, P. Ten Haaft, J. Heeney, B. Wahren, and B. Öberg, An in vivo model for HIV resistance development, *AIDS Res. Hum. Retroviruses* 17: 631–635 (2001).
15. Ha, J. C., C. Nosbisch, S. H. Conrad, G. C. Ruppenthal, G. P. Sackett, J. Abkowitz, and J. D. Unadkat, Fetal toxicity of zidovudine (azidothymidine) in macaca nemestrina: preliminary observations, *J. Acquired Immune Defic. Syndr.* 7: 154–157 (1994).
16. Lopez-Anaya, A., J. D. Unadkat, L. A. Schumann, and A. L. Smith, Pharmacokinetics of zidovudine (azidothymidine). II. Development of metabolic and renal clearance pathways in the neonate, *J. Acquir. Immune Defic. Syndr.* 3: 1052–1058 (1990).
17. Lopez-Anaya, A., J. D. Unadkat, L. A. Schumann, and A. L. Smith, Pharmacokinetics of zidovudine (azidothymidine). I. Transplacental transfer, *J. Acquir. Immune Defic. Syndr.* 3: 959–964 (1990).
18. Lopez-Anaya, A., J. D. Unadkat, L. A. Schumann, and A. L. Smith, Pharmacokinetics of zidovudine (Azidothymidine). III. Effect of pregnancy, *J. Acquir. Immune Defic. Syndr.* 4: 64–68 (1991).
19. Odineces, A., C. Pereira, C. Nosbisch, and J. D. Unadkat, Prenatal and postpartum pharmacokinetics of stavudine (2′,3′-didehydro-3′-deoxythymidine) and didanosine (dideoxyinosine) in pigtailed macaques (*Macaca nemestrina*), *Antimicrob. Agents Chemother.* 40: 2423–2425 (1996).
20. Odinecs, A., C. Nosbisch, and J. D. Unadkat, Zidovudine does not affect transplacental transfer of systemic clearance of stavudine (2′,3′-didehydro-deoxythymidine) in the pigtailed macaque (*Macaca nemestrina*), *Antimicrob. Agents Chemother.* 40: 1569–1571 (1996).
21. Odinecs, A., C. Nosbisch, R. D. Keller, W. L. Baughman, and J. D. Unadkat, In vivo maternal-fetal pharmacokinetics of stavudine (2′,3′-didehydro-3′-deoxythymidine) in pigtailed macaques (*Macaca nemestrina*), *Antimicrob. Agents Chemother.* 40: 196–202 (1996).
22. Pereira, C. M., C. Nosbisch, H. R. Winter, W. L. Baughman, and J. D. Unadkat, Transplacental pharmacokinetics of dideoxyinosine in pigtailed macaques, *Antimicrob. Agents Chemother.* 38: 781–786 (1994).
23. Pereira, C. M., C. Nosbisch, and J. D. Unadkat, Pharmacokinetics of dideoxyinosine in neonatal pigtailed macaques, *Antimicrob. Agents Chemother.* 38: 787–789 (1994).
24. Pereira, C. M., C. Nosbisch, W. L. Baughman, and J. D. Unadkat, Effect of zidovudine on transplacental pharmacokinetics of ddI in the pigtailed macaque (*Macaca nemestrina*), *Antimicrob. Agents Chemother.* 39: 343–345 (1995).
25. Qian, M., T. Bui, R. J. Y. Ho, and J. D. Unadkat, Metabolism of 3′-azido-3′-deoxythymidine (AZT) in human placental trophoblasts and Hofbauer cells, *Biochem. Pharmacol.* 48: 383–389 (1994).
26. Qian, M., G. Chandrasena, R. J. H. Ho, and J. D. Unadkat, Comparison of rates of intracellular metabolism of zidovudine in human and primate peripheral blood mononuclear cells, *Antimicrob. Agents Chemother.* 38: 2398–2403 (1994).
27. Ravasco, R. J., J. D. Unadkat, C.-C. Tsai, and C. Nosbisch, Pharmacokinetics of dideoxyinosine in pigtailed macaques (*Macaca nemestrina*) after intravenous and subcutaneous administration., *J. Acquired Immune Defic. Syndr.* 5: 1016–1018 (1992).
28. Tuntland, T., C. Nosbisch, W. Baughman, J. Massarella, and J. Unadkat, Mechanism and rate of placental transfer of zalcitabine (2′,3′-dideoxycytidine) in *Macaca nemestrina*, *Am. J. Obstet. Gynecol.* 174: 856–863 (1996).
29. Tarantal, A. F., R. J. Spanggord, and A. G. Hendrickx, Pre- and postnatal treatment of the rhesus macaque (*Macaca mulatta*) with azidothymidine: I. Fetal studies, *Pediatr. AIDS HIV Infect.* 5: 10–19 (1994).
30. Van Rompay, K. K. A., M. G. Otsyula, M. L. Marthas, C. J. Miller, M. B. McChesney, and N. C. Pedersen, Immediate zidovudine treatment protects simian immunodeficiency virus-infected newborn macaques against rapid onset of AIDS, *Antimicrob. Agents Chemother.* 39: 125–131 (1995).

31. Böttiger, D., L. Ståhle, S.-L. Li, and B. Öberg, Long-term tolerance and efficacy of 3′-azido-thymidine and 3′-fluorothymidine treatment of asymptomatic monkeys infected with simian immunodeficiency virus, *Antimicrob. Agents Chemother.* 36: 1770–1772 (1992).
32. Wingfield, C., J. Booth, P. Sheridan, J. Detmer, and J. Turczyn, SIV 4.0, performance characteristics of an exceptionally sensitive, quantitative assay for SIV RNA using branched DNA technology, in: *20th Annual Symposium on Nonhuman Primate Models for AIDS*, Monterey, CA (2002).
33. Lifson, J. D., M. A. Nowak, S. Goldstein, J. L. Rossio, A. Kinter, G. Vasquez, T. Wiltrout, C. Brown, D. Schneider, L. Wahl, A. L. Lloyd, J. Williams, W. R. Elkins, A. S. Fauci, and V. M. Hirsch, The extent of early viral replication is a critical determinant of the natural history of simian immunodeficiency virus infection, *J. Virol.* 71: 9508–9514 (1997).
34. Leutenegger, C. M., J. Higgins, T. B. Matthews, A. F. Tarantal, P. A. Luciw, N. C. Pedersen, and T. W. North, Real-time Taqman PCR as a specific and more sensitive alternative to the branched-chain DNA assay for quantitation of simian immunodeficiency virus RNA, *AIDS Res. Hum. Retroviruses* 17: 243–251 (2001).
35. Van Rompay, K. K. A., M. Otsyula, M. L. Marthas, and N. C. Pedersen, Simian immunodeficiency virus infection of newborn and infant rhesus macaques: an animal model for testing antiretroviral drugs, *Inter. Antiviral News* 2: 5–6 (1994).
36. Van Rompay, K. K. A., and M. L. Marthas, Non-human primate models for testing anti-HIV drugs, in: *Antivirals against AIDS*, edited by J. Kreuter, R. Unger, and Rüebsamen-Waigmann (Marcel Dekker, New York, 2000), pp. 295–320.
37. Van Rompay, K. K. A., J. M. Cherrington, M. L. Marthas, C. J. Berardi, A. S. Mulato, A. Spinner, R. P. Tarara, D. R. Canfield, S. Telm, N. Bischofberger, and N. C. Pedersen, 9-[2-(Phosphonomethoxy)propyl] adenine therapy of established simian immunodeficiency virus infection in infant rhesus macaques, *Antimicrob. Agents Chemother.* 40: 2586–2591 (1996).
38. Grob, P. M., Y. Cao, E. Muchmore, D. D. Ho, S. Norris, J. W. Pav, C.-K. Shih, and J. Adams, Prophylaxis against HIV-1 infection in chimpanzees by nevirapine, a nonnucleoside inhibitor of reverse transcriptase, *Nature Med.* 3: 665–670 (1997).
39. Van Rompay, K. K. A., M. B. McChesney, N. L. Aguirre, K. A. Schmidt, N. Bischofberger, and M. L. Marthas, Two low doses of tenofovir protect newborn macaques against oral simian immunodeficiency virus infection, *J. Infect. Dis.* 184: 429–438 (2001).
40. Wyand, M. S., The use of SIV-infected rhesus monkeys for the preclinical evaluation of AIDS drugs and vaccines, *AIDS Res. Hum. Retrovir.* 8: 349–356 (1992).
41. Fazely, F., W. A. Haseltine, R. F. Rodger, and R. M. Ruprecht, Postexposure chemoprophylaxis with ZDV or ZDV combined with interferon-a: failure after inoculating rhesus monkeys with a high dose of SIV, *J. Acquir. Immune Defic. Syndr.* 4: 1093–1097 (1991).
42. Lundgren, B., D. Bottiger, E. Ljungdahl-Ståhle, E. Norrby, L. Ståhle, B. Wahren, and B. Öberg, Antiviral effects of 3′-fluorothymidine and 3′-azidothymidine in cynomolgus monkeys infected with simian immunodeficiency virus, *J. Acquir. Immune Defic. Syndr.* 4: 489–498 (1991).
43. McClure, H. M., D. C. Anderson, A. A. Ansari, P. N. Fultz, S. A. Klumpp, and R. F. Schinazi, Nonhuman primate models for evaluation of AIDS therapy, *Ann. N.Y. Acad. Sci.* 616: 287–298 (1990).
44. Van Rompay, K. K. A., M. L. Marthas, J. D. Lifson, C. J. Berardi, G. M. Vasquez, E. Agatep, Z. A. Dehqanzada, K. C. Cundy, N. Bischofberger, and N. C. Pedersen, Administration of 9-[2-(phosphonomethoxy)propyl] adenine (PMPA) for prevention of perinatal simian immunodeficiency virus infection in rhesus macaques, *AIDS Res. Hum. Retrovir.* 14: 761–773 (1998).
45. Van Rompay, K. K. A., C. J. Berardi, N. L. Aguirre, N. Bischofberger, P. S. Lietman, N. C. Pedersen, and M. L. Marthas, Two doses of PMPA protect newborn macaques against oral simian immunodeficiency virus infection, *AIDS* 12: F79–F83 (1998).

46. Böttiger, D., P. Putkonen, and B. Öberg, Prevention of HIV-2 and SIV infections in cynomolgus macaques by prophylactic treatment with 3′-fluorothymidine, *AIDS Res. Hum. Retrovir.* 8: 1235–1238 (1992).
47. Böttiger, D., L. Vrang, and B. Öberg, Influence of the infectious dose of simian immunodeficiency virus on the acute infection in cynomolgus monkeys and on the effect of treatment with 3′-fluorothymidine, *Antivir. Chem. Chemother.* 3: 267–271 (1992).
48. Tsai, C.-C., K. E. Follis, A. Sabo, R. F. Grant, C. Bartz, R. E. Nolte, R. E. Benveniste, and N. Bischofberger, Preexposure prophylaxis with 9-(-2-phosphonylmethoxyethyl) adenine against simian immunodeficiency virus infection in macaques, *J. Infect. Dis.* 169: 260–266 (1994).
49. Tsai, C.-C., K. E. Follis, T. W. Beck, A. Sabo, R. F. Grant, N. Bischofberger, and R. E. Benveniste, Prevention of simian immunodeficiency virus infection in macaques by 9-(2-phosphonylmethoxypropyl) adenine (PMPA), *Science* 270: 1197–1199 (1995).
50. Böttiger, D., N. G. Johansson, B. Samuelsson, H. Zhang, P. Putkonen, L. Vrang, and B. Öberg, Prevention of simian immunodeficiency virus, SIVsm, or HIV-2 infection in cynomolgus monkeys by pre- and postexposure administration of BEA-005, *AIDS* 11: 157–162 (1997).
51. Otten, R. A., D. K. Smith, D. R. Adams, J. K. Pullium, E. Jackson, C. N. Kim, H. Jaffe, R. Janssen, S. Butera, and T. M. Folks, Efficacy of postexposure prophylaxis after intravaginal exposure of pig-tailed macaques to a human-derived retrovirus (human immunodeficiency virus type 2). *J. Virol.* 74: 9771–9775 (2000).
52. Van Rompay, K. K. A., M. D. Miller, M. L. Marthas, N. A. Margot, P. J. Dailey, R. P. Tarara, D. R. Canfield, J. M. Cherrington, N. L. Aguirre, N. Bischofberger, and N. C. Pedersen, Prophylactic and therapeutic benefits of short-term 9-[2-(phosphonomethoxy)propyl] adenine (PMPA) administration to newborn macaques following oral inoculation with simian immunodeficiency virus with reduced susceptibility to PMPA, *J. Virol.* 74: 1767–1774 (2000).
53. Van Rompay, K. K. A., K. A. Schmidt, J. R. Lawson, R. Singh, N. Bischofberger, and M. L. Marthas, Topical administration of low-dose tenofovir disoproxyl fumarate to protect infant macaques against multiple oral exposures of low doses of simian immunodeficiency virus, *J. Infect. Dis.* 186: 1508–1513 (2002).
54. Miller, C., Z. Rosenberg, and N. Bischofberger, Use of topical PMPA to prevent vaginal transmission of SIV, in: *Ninth International Conference on Antiviral Research*, Fukushima, May 19–24 (1996).
55. Manson, K. H., M. S. Wyand, C. Miller, and A. R. Neurath, Effect of a cellulose acetate phtalate topical cream on vaginal transmission of simian immunodeficiency virus in rhesus monkeys, *Antimicrob. Agents Chemother.* 44: 3199–3202 (2000).
56. Wyand, M. S., K. H. Manson, C. J. Miller, and A. R. Neurath, Effect of 3-hydroxyphthaloyl-b-lactoglobulin on vaginal transmission of simian immunodeficiency virus in rhesus monkeys, *Antimicrob. Agents Chemother.* 43: 978–980 (1999).
57. Weber, J., A. Nunn, T. O'Connor, D. Jeffries, V. Kitchen, S. McCormack, J. Stott, N. Almond, A. Stone, and J. Darbyshire, "Chemical condoms" for the prevention of HIV infection: evaluation of novel agents against SHIV98.6PD in vitro and in vivo, *AIDS* 15: 1563–1568 (2001).
58. Centers for Disease Control and Prevention, Update: provisional Public Health Service recommendations for chemoprophylaxis after occupational exposure to HIV, *MMRW* 45: 468–472 (1996).
59. Centers for Disease Control and Prevention, Management of possible sexual, injecting-drug-use, or other nonoccupational exposure to HIV, including considerations related to antiretroviral therapy. Public Health Service Statement, *MMRW* 47: 1–14 (1998).
60. Mofenson, L. M., and M. G. Fowler, Interruption of materno-fetal transmission, *AIDS* 13: S205–S14 (1999).

61. Connor, E. M., R. S. Sperling, R. Gelber, P. Kiselev, G. Scott, M. J. O'Sullivan, R. VanDyke, M. Bey, W. Shearer, R. L. Jacobson, E. Jiminez, E. O'Neill, B. Bazin, J.-F. Delfraissy, M. Culnane, R. Coombs, M. Elkins, J. Moye, P. Stratton, J. Balsley, for the Pediatric AIDS Clinical Trials Group Protocol 076 Study Group, Reduction of maternal-infant transmission of human immunodeficiency virus type 1 with zidovudine treatment, *N. Engl. J. Med.* 331: 1173–1180 (1994).
62. Centers for Disease Control and Prevention, Administration of zidovudine during late pregnancy and delivery to prevent perinatal HIV transmission-Thailand, 1996–1998, *MMWR* 47: 151–154 (1998).
63. Wade, N. A., G. S. Birkhead, B. L. Warren, T. T. Charbonneau, P. T. French, L. Wang, J. B. Baum, J. M. Tesoriero, and R. Savicki, Abbreviated regimens of zidovudine prophylaxis and perinatal transmission of the human immunodeficiency virus, *N. Engl. J. Med.* 339: 1409–1414 (1998).
64. The Petra Study Team, Efficacy of three short-course regimens of zidovudine and lamivudine in preventing early and late transmission of HIV-1 from mother to child in Tanzania, South Africa and Uganda (Petra Study): a randomized, double-blind, placebo-controlled trial, *Lancet* 359: 1178–1186 (2002).
65. Moodley, D., J. Moodley, H. Coovadia, G. Gray, J. McIntyre, J. Hofmyer, C. Nikodem, D. Hall, F. Gigliotti, P. Robinson, L. Boshoff, J. L. Sullivan, for the South African Intrapartum Nevirapine Trial (SAINT) Investigators, A multicenter randomized controlled trial of nevirapine versus a combination of zidovudine and lamivudine to reduce intrapartum and early postpartum mother-to-child transmission of human immunodeficiency virus type 1, *J. Infect. Dis.* 187: 725–735 (2003).
66. Guay, L. A., P. Musoke, T. Fleming, D. Bagenda, M. Allen, C. Nakabiito, J. Sherman, P. Bakaki, C. Ducar, M. Deseyve, L. Emel, M. Mirochnick, M. G. Fowler, L. Mofenson, P. Miotti, K. Dransfield, D. Bray, F. Mmiro, and J. B. Jackson, Intrapartum and neonatal single-dose nevirapine compared with zidovudine for prevention of mother-to-child transmission of HIV-1 in Kampala, Uganda: HIVNET 012 randomized trial, *Lancet* 354: 795–802 (1999).
67. Eshleman, S. H., M. Mracna, L. A. Guay, M. Deseyve, S. Cunningham, M. Mirochnick, P. Musoke, T. Fleming, M. G. Fowler, L. M. Mofenson, F. Mmiro, and J. B. Jackson, Selection and fading of resistance mutations in women and infants receiving nevirapine to prevent HIV-1 vertical transmission (HIVNET012), *AIDS* 15: 1951–1957 (2001).
68. Martin, L. N., M. Murphey-Corb, K. F. Soike, B. Davison-Fairburn, and G. B. Baskin, Effects of initiation of 3′-azido-3′-deoxythymidine treatment at different times after infection of rhesus monkeys with simian immunodeficiency virus, *J. Infect. Dis.* 168: 825–835 (1993).
69. Tsai, C.-C., K. E. Follis, R. F. Grant, R. E. Nolte, C. R. Bartz, R. E. Benveniste, and P. R. Sager, Effect of dosing frequency on ZDV prophylaxis in macaques infected with simian immunodeficiency virus, *J. Acquired Immune Defic. Syndr.* 6: 1086–1092 (1993).
70. Joag, S. V., Z. Li, L. Foresman, D. M. Pinson, E. B. Stephens, R. Raghavan, J.-F. Navé, P. Casara, and O. Narayan, Early treatment with 9-(2-phosphonylmethoxyethyl) adenine reduces virus burdens for a prolonged period in SIV-infected rhesus macaques, *AIDS Res. Hum. Retrovir.* 13: 241–246 (1997).
71. Le Grand, R., P. Clayette, O. Noack, B. Vaslin, F. Theodoro, G. Michel, P. Roques, and D. Dormont, An animal model for antilentiviral therapy: effect of zidovudine on viral load during acute infection after exposure of macaques to simian immunodeficiency virus, *AIDS Res. Hum. Retrovir.* 10: 1279–1287 (1994).
72. Le Grand, R., B. Vaslin, J. Larghero, O. Neidez, H. Thiebot, P. Sellier, P. Clayette, N. Dereuddre-Bosquet, and D. Dormont, Post-exposure prophylaxis with highly active antiretroviral therapy could not protect macaques from infection with SIV/HIV chimera, *AIDS* 14: 1864–1866 (2000).

73. Van Rompay, K. K. A., P. J. Dailey, R. P. Tarara, D. R. Canfield, N. L. Aguirre, J. M. Cherrington, P. D. Lamy, N. Bischofberger, N. C. Pedersen, and M. L. Marthas, Early short-term 9-[2-(phosphonomethoxy)propyl] adenine (PMPA) treatment favorably alters subsequent disease course in simian immunodeficiency virus-infected newborn rhesus macaques, *J. Virol.* 73: 2947–2955 (1999).
74. Watson, A., J. McClure, J. Ranchalis, M. Scheibel, A. Schmidt, B. Kennedy, W. R. Morton, N. L. Haigwood, and S.-L. Hu, Early postinfection antiviral treatment reduces viral load and prevents CD4+ cell decline in HIV type 2-infected macaques, *AIDS Res. Hum. Retrovir.* 13: 1375–1381 (1997).
75. Rausch, D. M., M. P. Heyes, E. A. Murray, and L. E. Eiden, Zidovudine treatment prolongs survival and decreases virus load in the central nervous system of rhesus macaques infected perinatally with simian immunodeficiency virus, *J. Infect. Dis.* 172: 59–69 (1995).
76. Rosenwirth, B., P. ten Haaft, W. M. J. M. Bogers, I. G. Nieuwenhuis, H. Niphuis, E.-M. Kuhn, N. Bischofberger, J. L. Heeney, and K. Überla, Antiretroviral therapy during primary immunodeficiency virus infection can induce persistent suppression of virus load and protection from heterologous challenge in rhesus macaques, *J. Virol.* 74: 1704–1711 (2000).
77. Smith, M. S., L. Foresman, G. J. Lopez, J. Tsay, D. Wodarz, J. D. Lifson, A. Page, C. Wang, Z. Li, I. Adany, S. Buch, N. Bischofberger, and O. Narayan, Lasting effects of transient postinoculation tenofovir [9-R-(2-phosphonomethoxypropyl) adenine] treatment of SHIVKU2 infection of rhesus macaques, *Virology* 277: 306–315 (2000).
78. Hodge, S., J. De Rosayro, A. Glenn, I. C. Ojukwu, S. Dewhurst, H. M. McClure, N. Bischofberger, D. C. Anderson, S. A. Klumpp, and F. J. Novembre, Postinoculation PMPA treatment, but not preinoculation immunomodulatory therapy, protects against development of acute disease induced by the unique simian immunodeficiency virus SIVsmmPBj, *J. Virol.* 73: 8630–8639 (1999).
79. Lifson, J. D., J. L. Rossio, R. Arnaout, L. Li, T. L. Parks, D. K. Schneider, R. F. Kiser, V. J. Coalter, G. Walsh, R. J. Imming, B. Fisher, B. M. Flynn, N. Bischofberger, M. J. Piatak, V. M. Hirsch, M. A. Nowak, and D. Wodarz, Containment of simian immunodeficiency virus infection: cellular immune responses and protection from rechallenge following transient postinoculation antiretroviral treatment, *J. Virol.* 74: 2584–2593 (2000).
80. Berrey, M. M., T. Schacker, A. C. Collier, T. Shea, S. J. Brodie, D. Mayers, R. Coombs, J. Krieger, T.-W. Chun, A. Fauci, S. G. Self, and L. Corey, Treatment of primary human immunodeficiency virus type 1 infection with potent antiretroviral therapy reduces frequency of rapid progression to AIDS, *J. Infect. Dis.* 183: 1466–1475 (2001).
81. Kinloch-de Loës, S., B. J. Hirschel, B. Hoen, D. A. Cooper, B. Tindall, A. Carr, J.-H. Saurat, N. Clumeck, A. Lazzarin, L. Mathiesen, F. Raffi, F. Antunes, J. Von Overbeck, R. Lüthy, M. Glauser, D. Hawkins, C. Baumberger, S. Yerly, T. V. Perneger, and L. Perrin, A controlled trial of zidovudine in primary human immunodeficiency virus infection, *N. Engl. J. Med.* 333: 408–413 (1995).
82. Lafeuillade, A., C. Poggi, C. Tamalet, N. Profizi, C. Tourres, and O. Costes, Effects of a combination of zidovudine, didanosine, and lamivudine on primary human immunodeficiency virus type 1 infection, *J. Infect. Dis.* 175: 1051–1055 (1997).
83. Rosenberg, E. S., M. Altfeld, S. H. Poon, M. N. Phillips, B. M. Wilkes, R. L. Eldridge, G. K. Robbins, R. T. D'Aquila, P. J. R. Goulder, and B. D. Walker, Immune control of HIV-1 after early treatment of acute infection, *Nature* 407: 523–526 (2000).
84. Igarashi, T., C. R. Brown, Y. Endo, A. Buckler-White, R. Plishka, N. Bischofberger, V. M. Hirsch, and M. A. Martin, Macrophage are the principal reservoir and sustain high virus loads in rhesus macaques after the depletion of CD4+ T-cells by a highly pathogenic simian immunodeficiency virus/HIV type 1 chimera (SHIV): implications for HIV-1 infections of humans, *Proc. Natl. Acad. Sci. USA* 98: 658–663 (2001).

85. Lifson, J. D., and M. A. Martin, One step forwards, one step back, *Nature* 415: 272–273 (2002).
86. Feinberg, M. B., and J. P. Moore, AIDS vaccine models: challenging challenge viruses, *Nature Med.* 8: 207–210 (2002).
87. Van Rompay, K. K. A., M. L. Marthas, R. A. Ramos, C. P. Mandell, E. K. McGowan, S. M. Joye, and N. C. Pedersen, Simian immunodeficiency virus (SIV) infection of infant rhesus macaques as a model to test antiretroviral drug prophylaxis and therapy: oral 3′-azido-3′-deoxythymidine prevents SIV infection, *Antimicrob. Agents Chemother.* 36: 2381–2386 (1992).
88. Van Rompay, K. K. A., T. B. Matthews, J. Higgins, D. R. Canfield, R. P. Tarara, M. A. Wainberg, R. F. Schinazi, N. C. Pedersen, and T. W. North, Virulence and reduced fitness of simian immunodeficiency virus with the M184V mutation in reverse transcriptase, *J. Virol.* 76: 6083–6092 (2002).
89. Tsai, C.-C., K. E. Follis, T. W. Beck, A. Sabo, N. Bischofberger, and P. J. Dailey, Effects of (R)-9-(2-phosphonylmethoxypropyl) adenine monotherapy on chronic SIV infection, *AIDS Res. Hum. Retrovir.* 13: 707–712 (1997).
90. Tsai, C.-C., K. E. Follis, A. Sabo, R. Grant, and N. Bischofberger, Efficacy of 9-(2-phosphonylmethoxyethyl) adenine treatment against chronic simian immunodeficiency virus infection in macaques, *J. Infect. Dis.* 171: 1338–1343 (1995).
91. Nowak, M. A., A. L. Lloyd, G. M. Vasquez, T. A. Wiltrout, L. M. Wahl, N. Bischofberger, J. Williams, A. Kinter, A. S. Fauci, V. M. Hirsch, and J. D. Lifson, Viral dynamics of primary viremia and antiretroviral therapy in simian immunodeficiency virus infection, *J. Virol.* 71: 7518–7525 (1997).
92. Silvera, P., P. Racz, K. Racz, N. Bischofberger, C. Crabbs, J. Yalley-Ogunro, J. Greenhouse, J. Bo Jiang, and M. G. Lewis, Effect of PMPA and PMEA on the kinetics of viral load in simian immunodeficiency virus-infected macaques, *AIDS Res. Hum. Retrovir.* 16: 791–800 (2000).
93. Van Rompay, K. K. A., R. Singh, L. Brignolo, J. R. Lawson, K. A. Schmidt, B. Pahar, C. Wingfield, D. R. Canfield, R. P. Tarara, N. Bischofberger, and M. Marthas, The clinical benefits of tenofovir for simian immunodeficiency virus-infected macaques are larger than predicted by its effects on standard viral and immunological parameters, *J. Acquir. Immune Defic. Syndr.* 36: 900–914 (2004).
94. Van Rompay, K. K. A., R. P. Singh, B. Pahar, D. L. Sodora, C. Wingfield, J. R. Lawson, K. A. Reimann, M. L. Marthas, and N. Bischofberger, CD8+ cell-mediated suppression of virulent simian immunodeficiency virus during tenofovir treatment, *J. Virol.* 78: 5324–5337 (2004).
95. Shen, Y., L. Shen, P. Sehgal, D. Zhou, M. Simon, M. Miller, E. A. Emini, B. Henckler, L. Chalifoux, N. Sehgal, M. Gastron, N. L. Letvin, and Z. W. Chen, Antiretroviral agents restore mycobacterium-specific T-cell immune responses and facilitate controlling a fatal tuberculosis-like disease in macaques coinfected with simian immunodeficiency virus and Mycobacterium bovis BCG, *J. Virol.* 75: 8690–8696 (2001).
96. Shen, A., M. C. Zink, J. L. Mankowski, K. Chadwick, J. B. Margolick, L. M. Carruth, M. Li, J. E. Clements, and R. F. Siciliano, Resting CD4+ T lymphocytes but not thymocytes provide a latent viral reservoir in a simian immunodeficiency virus-*Macaca nemestrina* model of human immunodeficiency virus type 1 infected patients on highly active antiretroviral therapy, *J. Virol.* 77: 4938–4949 (2003).
97. Lori, F., M. G. Lewis, J. Xu, G. Varga, D. E. J. Zinn, C. Crabbs, W. Wagner, J. Greenhouse, P. Silvera, J. Yalley-Ogunro, C. Tinelli, and J. Lisziewicz, Control of SIV rebound through structured treatment interruptions during early infection, *Science* 290: 1591–1593 (2000).
98. Hel, Z., J. Nacsa, B. Kelsall, W.-P. Tsai, N. Letvin, R. Washington Parks, E. Tryniszewska, L. Picker, M. G. Lewis, Y. Edghill-Smith, M. Moniuszko, R. Pal, L. Stevceva, J. D. Altman, T. M. Allen, D. Watkins, J. V. Torres, J. A. Berzofsky, I. M. Belyakov, W. Strober, and G.

Franchini, Impairment of gag-specific CD8+ T-cell function in mucosal and systemic compartments of simian immunodeficiency virus mac251-and simian-human immunodeficiency virus KU2-infected macaques, *J. Virol.* 75: 11483–11495 (2001).

99. Tryniszewska, E., J. Nacsa, M. G. Lewis, P. Silvera, D. Montefiori, D. Venzon, Z. Hel, R. W. Parks, M. Moniuszko, J. Tartaglia, K. A. Smith, and G. Franchini, Vaccination of macaques with long-standing SIVmac251 infection lowers the viral set point after cessation of antiretroviral therapy, *J. Immunol.* 169: 5347–5357 (2002).
100. Hel, Z., J. Nacsa, W.-P. Tsai, A. Thornton, L. Giuliani, J. Tartaglia, and G. Franchini, Equivalent immunogenicity of the highly attenuated poxvirus-based ALVAC-SIV and NYVAC-SIV vaccine candidates in SIVmac251-infected macaques, *Virology* 304: 125–134 (2002).
101. Nacsa, J., J. Stanton, K. J. Kunstman, W.-P. Tsai, D. I. Watkins, S. M. Wolinsky, and G. Franchini, Emergence of cytotoxic T lymphocyte escape mutants following antiretroviral treatment suspension in rhesus macaques infected with SIVmac251, *Virology* 305: 210–218 (2003).
102. Villinger, F., G. T. Brice, A. E. Mayne, P. Bostik, K. Mori, C. H. June, and A. A. Ansari, Adoptive transfer of simian immunodeficiency virus (SIV) naive autologous CD4+ T-cells to macaques chronically infected with SIV is sufficient to induce long-term nonprogressor status, *Blood* 99: 590–599 (2002).
103. Darby, G., and B. A. Larder, The clinical significance of antiviral drug resistance, *Res. Virol.* 143: 116–120 (1992).
104. Richman, D. D., The clinical significance of drug-resistant mutants of human immunodeficiency virus, *Res. Virol.* 143: 130–131 (1992).
105. Richman, D. D., Resistance, drug failure, and disease progression, *AIDS Res. Hum. Retrovir.* 10: 901–905 (1994).
106. Deeks, S. G., T. Wrin, T. Liegler, R. Hoh, M. Hayden, J. D. Barbour, N. S. Hellmann, C. J. Petropoulos, J. M. McCune, M. K. Hellerstein, and R. M. Grant, Virologic and immunologic consequences of discontinuing combination antiretroviral-drug therapy in HIV-infected patients with detectable viremia, *N. Engl. J. Med.* 344: 472–480 (2001).
107. Deeks, S. G., R. Hoh, R. M. Grant, T. Wrin, J. D. Barbour, A. Narvaez, D. Cesar, K. Abe, M. B. Hanley, N. S. Hellmann, C. J. Petropoulos, J. M. McCune, and M. K. Hellerstein, CD4+ T-cell kinetics and activation in human immunodeficiency virus-infected patients who remain viremic despite long-term treatment with protease inhibitor-based therapy, *J. Infect. Dis.* 185: 315–323 (2002).
108. Deeks, S. G., J. D. Barbour, J. N. Martin, M. S. Swanson, and R. M. Grant, Sustained CD4+ T-cell response after virologic failure of protease inhibitor-based regimens in patients with human immunodeficiency virus infection, *J. Infect. Dis.* 181: 946–953 (2000).
109. Barbour, J. D., T. Wrin, R. M. Grant, J. N. Martin, M. R. Segal, C. J. Petropoulos, and S. G. Deeks, Evolution of phenotypic drug susceptibility and viral replication capacity during long-term virologic failure of protease inhibitor therapy in human immunodeficiency virus-infected adults, *J. Virol.* 76: 11104–11112 (2002).
110. Kestler, H. W., III, D. J. Ringler, K. Mori, D. L. Panicalli, P. K. Sehgal, M. D. Daniel, and R. C. Desrosiers, Importance of the *nef* gene for maintenance of high virus loads and for development of AIDS, *Cell* 65: 651–662 (1991).
111. Lohman, B. L., M. B. McChesney, C. J. Miller, E. McGowan, S. M. Joye, K. K. A. Van Rompay, E. Reay, L. Antipa, N. C. Pedersen, and M. L. Marthas, A partially attenuated simian immunodeficiency virus induces host immunity that correlates with resistance to pathogenic virus challenge, *J. Virol.* 68: 7021–7029 (1994).
112. Cherry, E., M. Slater, H. Salomon, E. Rud, and M. A. Wainberg, Mutations at codon 184 in simian immunodeficiency virus reverse transcriptase confer resistance to the (−) enantiomer of 2′,3′-dideoxy-thiacytidine, *Antimicrob. Agents Chemother.* 41: 2763–2765 (1997).

113. Murry, J. P., J. Higgins, T. B. Matthews, V. Y. Huang, K. K. A. Van Rompay, N. C. Pedersen, and T. W. North, Reversion of the M184V mutation in simian immunodeficiency virus reverse transcriptase is selected by tenofovir, even in the presence of lamivudine, *J. Virol.* 77: 1120–1130 (2003).
114. Newstein, M. C., and R. C. Desrosiers, Effects of reverse-transcriptase mutations M184V and E89G on simian immunodeficiency virus in rhesus monkeys, *J. Infect. Dis.* 184: 1262–1267 (2001).
115. Böttiger, D., S. D. Kemp, B. A. Larder, H. Zhang, L. Vrang, and B. Öberg, AZT resistant SIVmac is refractile to AZT therapy in monkeys, in: *14th Annual Symposium on Nonhuman Primate Models for AIDS, October 23–26*, Portland (OR) (1996).
116. Van Rompay, K. K. A., J. L. Greenier, M. L. Marthas, M. G. Otsyula, R. P. Tarara, C. J. Miller, and N. C. Pedersen, A zidovudine-resistant simian immunodeficiency virus mutant with a Q151M mutation in reverse transcriptase causes AIDS in newborn macaques., *Antimicrob. Agents Chemother.* 41: 278–283 (1997).
117. Schmit, J.-C., J. Cogniaux, P. Hermans, C. Van Vaeck, S. Sprecher, B. Van Remoortel, M. Witvrouw, J. Balzarini, J. Desmyter, E. De Clercq, and A.-M. Vandamme, Multiple drug resistance to nucleoside analogues and non-nucleoside reverse transcriptase inhibitors in an efficienty replicating HIV-1 patient strain, *J. Infect. Dis.* 174: 962–968 (1996).
118. Shafer, R. W., M. A. Winters, A. K. N. Iversen, and T. C. Merigan, Genotypic and phenotypic changes during culture of a multinucleoside-resistant human immunodeficiency virus type 1 strain in the presence and absence of additional reverse transcriptase inhibitors, *Antimicrob. Agents Chemother.* 40: 2887–2890 (1996).
119. Schmit, J. C., B. Clotet, L. Ruiz, P. Hermans, S. Sprecher, V. Arendt, M. Leal, E. Lissen, T. Harrer, E. De Clercq, M. Witvrouw, and A.-M. Vandamme, Multiple dideoxynucleoside analogue-resistant HIV-1 in Europe, in: *International Workshop on HIV Drug Resistance, Treatment Strategies and Eradication*, St. Petersburg (Florida), 25–28 June (1997).
120. Colson, P., M. Henry, N. Tivoli, H. Gallais, J. A. Gastaut, J. Moreau, and C. Tamalet, Polymorphism and drug-selected mutations in the reverse transcriptase gene of HIV-2 from patients living in southern France, *Antiviral Ther.* 8: S161 (2003).
121. Descamps, D., F. Damond, S. Matheron, G. Collin, P. Campa, P. Foiny, S. Delarue, S. Pueyo, G. Chêne, F. Brun-Vézinet, and the French ANRS HIV-2 Cohort Study Group, High frequency of selection of the Q151M mutation in HIV-2-infected patients receiving nucleoside reverse transcriptase inhibitor-containing regimen, *Antiviral Ther.* 8: S162 (2003).
122. Wainberg, M. A., M. D. Miller, Y. Quan, H. Salomon, A. S. Mulato, P. D. Lamy, N. A. Margot, K. E. Anton, and J. M. Cherrington, In vitro selection and characterization of HIV-1 with reduced susceptibility to PMPA, *Antiviral Ther.* 4: 87–94 (1999).
123. Margot, N. A., E. Isaacson, I. McGowan, A. K. Cheng, R. T. Schooley, and M. D. Miller, Genotypic and phenotypic analyses of HIV-1 in antiretroviral-experienced patients treated with tenofovir DF, *AIDS* 16: 1227–1235 (2002).
124. Margot, N. A., E. Isaacson, I. McGowan, A. Cheng, and M. D. Miller, Extended treatment with tenofovir disoproxil fumarate in treatment-experienced HIV-1-infected patients: genotypic, phenotypic, and rebound analyses, *J. Acquired Immune Defic. Syndr.* 33: 15–21 (2003).
125. Van Rompay, K. K. A., J. M. Cherrington, M. L. Marthas, P. D. Lamy, P. J. Dailey, D. R. Canfield, R. P. Tarara, N. Bischofberger, and N. C. Pedersen, 9-[2-(Phosphonomethoxy) propyl] adenine (PMPA) therapy prolongs survival of infant macaques inoculated with simian immunodeficiency virus with reduced susceptibility to PMPA, *Antimicrob. Agents Chemother.* 43: 802–812 (1999).
126. Balzarini, J., L. Naesens, J. Slachmuylders, H. Niphuis, I. Rosenberg, A. Holy, H. Schellekens, and E. De Clercq, 9-(2-phosphonylmethoxyethyl) adenine (PMEA) effec-

tively inhibits retrovirus replication in vitro and simian immunodeficiency virus infection in rhesus monkeys, *AIDS* 5: 21–28 (1991).
127. Lori, F., R. C. Gallo, A. Malykh, A. Cara, J. Romano, P. Markham, and G. Franchini, Didanosine but not high doses of hydroxyurea rescue pigtail macaque from a lethal dose of SIVsmmpbj14, *AIDS Res. Hum. Retrovir.* 13: 1083–1088 (1997).
128. Spring, M., C. Stahl-Hennig, N. Stolte, N. Bischofberger, J. Heeney, P. ten Haaft, K. Tenner-Ràcz, P. Ràcz, D. Lorenzen, G. Hunsmann, and U. Dittmer, Enhanced cellular immune responses and reduced CD8+ lymphocytes apoptosis in acutely SIV-infected rhesus macaques after short-term antiretroviral treatment, *Virology* 279: 221–231 (2001).

7

FIV as a Model for HIV: An Overview

ELLEN E. SPARGER

1. INTRODUCTION

Animal models for human immunodeficiency virus (HIV) infection play a key role in understanding the pathogenesis of AIDS and the development of therapeutic agents and vaccines. As the only lentivirus that causes an immunodeficiency resembling that of HIV infection, in its natural host, feline immunodeficiency virus (FIV) has been a unique and powerful model for AIDS research. FIV was first described in 1987 by Niels Pedersen and co-workers as the causative agent for a fatal immunodeficiency syndrome observed in cats housed in a cattery in Petaluma, California.[1,2] Since this landmark observation, multiple studies have shown that natural and experimental infection of cats with biological isolates of FIV produces an AIDS syndrome very similar in pathogenesis to that observed for human AIDS. FIV infection induces an acute viremia associated with T-cell alterations including depressed CD4:CD8 T-cell ratios and CD4 T-cell depletion, peripheral lymphadenopathy, and neutropenia.[3–13] In later stages of FIV infection, the host suffers from chronic persistent infections that are typically self-limiting in an immunocompetent host, as well as opportunistic infections, chronic diarrhea and wasting, blood dyscracias, significant CD4 T-cell depletion, neurologic disorders, and B-cell lymphomas.[2,6,9,12–14] Importantly, chronic FIV infection induces a progressive lymphoid and CD4 T-cell depletion in the infected cat. The primary mode of natural FIV transmission appears to be blood-borne facilitated by fighting and biting.[13,15] However, experimental infection through transmucosal routes (rectal and vaginal mucosa and perinatal) have been well documented for specific FIV isolates.[16–23] Accordingly, FIV disease pathogenesis exhibits striking similarities to that described for HIV-1 infection.[24] Recent observations regarding functions of FIV accessory and structural genes, FIV

ELLEN E. SPARGER • School of Veterinary Medicine, University of California Davis, CA 95616

tropism, and immunopathogenesis have further corroborated similarities shared by FIV and HIV-1. This chapter will serve as an overview of the FIV animal model for HIV AIDS and as such, will focus on FIV molecular biology and virology and address recent developments in FIV viral vector development as well as nondomestic FIV biology. FIV pathogenesis, vaccine development, and antiviral therapies are critical topics for discussion regarding the value of the FIV animal model and will also be described briefly in this overview, but will be examined in more significant detail in subsequent chapters.

2. FIV GENOME, STRUCTURE, AND GENE FUNCTION

Virion Structure

The morphology of the FIV virus particle is similar to that of other lentiviruses.[1,14,25] The mature extracellular virion is spherical to ellipsoid, 100 to 125 nm in diameter, and bordered by an outer envelope with poorly defined short projections or knobs. An elongated conical shell surrounds an eccentrically positioned electron-dense viral nucleoid. A polygonal electron-lucent halo is often visible between the core and a granular layer located just inside the envelope. Similar to other lentiviruses, the density of FIV was shown to be 1.15–1.17 g/cm^3 by continuous sucrose gradient centrifugation.[26]

Typical of other retroviruses, the FIV genome consists of an identical pair of single-stranded RNA molecules that are approximately 9,200 bases in length and densely packed within the virion by their association with nucleocapsid protein (NC, p7).[27,28] A $t\text{-}RNA^{lys}$ is hydrogen-bonded to each RNA molecule at the primer binding site (PBS) located within the 5′ terminal 180 bases of the genome and serves as the primer for negative strand reverse transcription. The ribonucleoprotein complex is contained within a protein core largely composed of the viral capsid protein (CA, p24) that is associated with and surrounded by a roughly spherical shell consisting of myristylated matrix protein (MA, p14). Also contained in the viral capsid are viral enzymes involved in particle maturation and replication of the viral RNA genome including protease (PR), reverse transcriptase (RT), integrase (IN), and dUTPase (DU).[29–31] NC, CA, and MA are expressed from the *gag* gene, while PR, RT, DU, and IN are products of the *pol* gene. Outside the matrix coat is a lipid bilayer of the virion envelope, which confers the characteristic icosahedral morphology to the enveloped retrovirus. Embedded within the lipid bilayer are viral envelope glycoproteins, with the transmembrane subunit (TM, gp40) present as a single-pass transmembrane protein anchor, and the surface unit (SU, gp95) as an entirely extravirion protein bound to TM.[27] Both TM and SU are the products of

the *env* gene. *Env* gene products mediate binding of the virus to cell surface receptors and fusion with the target cell membrane[32–36] and are critical targets for host humoral and cellular immune responses.

Genome Organization and Expression

Sequence organization of the FIV genome is similar to that of HIV-1 and other lentiviruses.[37,38] Flanked by two long terminal repeats (LTR) at both ends, the FIV proviral DNA genome contains three large open reading frames (ORFs), *gag*, *pol*, and *env*, encoding internal structural proteins, RT and other viral enzymes, and envelope proteins, respectively, as well as various small ORFs encoding regulatory and accessory proteins (Figure 1). The FIV genome also contains nonencoding regulatory sequences important for virus replication. These sequences include transcriptional elements within the LTRs, a posttranscriptional regulatory sequence located in 3′ half of the genome,[39–43] and encapsidation determinants within the U5 domain and the first 90–300 nucleotides of *gag*.[44,45] Other critical noncoding sequences include the central polypurine tract (cPPT) involved in priming plus-strand DNA synthesis and the central termination sequence (CTS) important for formation of a central DNA flap during reverse transcription.[46]

The approximately 355 bp long FIV LTR accommodates multiple regulatory sequences and is composed of three domains designated U3, R, and U5. Located at each terminus of the proviral DNA genome, the LTRs are bordered by two bp-inverted repeats. Critical transcriptional regulatory sequences located in the FIV U3 domain consist of enhancer elements including AP-1, ATF (also known as the cAMP response element or CRE), and cEBP sites. These *cis*-acting elements have been shown to be important for FIV LTR promoter activity in vitro and for virus replication in vitro

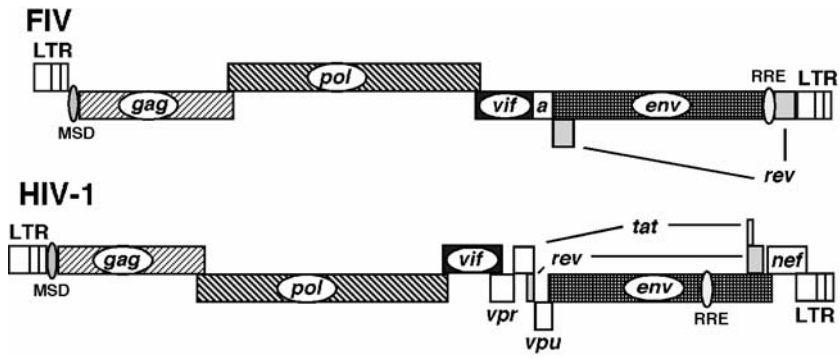

FIGURE 1. Comparison of FIV and HIV-1 genomic organization.

and in vivo.[39,40,43,47–49] Previous studies also demonstrated binding of these specific LTR sequences by cellular proteins using DNase I footprinting and gel shift assays.[39,50] Although *cis*-acting transcriptional elements within the FIV U3 domain differ from those described as critical for the HIV LTR (NFκβ and SP1),[51,52] FIV-encoded AP-1 and ATF sequences are positive regulatory elements that respond to host cell activation states,[40] a property also shared by the NFκβ site within the HIV-1 LTR. Other retroviruses encoding LTRs regulated by ATF/CREB family of transcription factors include human T-lymphotropic virus type (HTLV-1) and bovine leukemia virus (BLV).[53–56] Similarly, an AP-1 site encoded by the visna virus LTR was found to be critical for basal activity and for transactivation of the viral LTR resulting from interactions of the visna virus putative transcriptional transactivator (Tat) protein with cellular transcription factors Fos and Jun.[57–59] Interestingly the structure of Orf-A, an FIV accessory gene product previously regarded to be a Tat protein, is very similar to that of visna virus Tat.[60] Collectively, these observations suggest that FIV LTR promoter activity may be regulated by multiple cell activation pathways involving possible interactions between a viral accessory protein (Orf-A) with cellular proteins that bind either AP-1, ATF, or cEBP elements. However, a definitive characterization of these potential interactions has not yet been reported. Knowledge of potential complex interactions between the FIV LTR and viral and cellular proteins that are most likely involved in regulation of FIV expression is still rudimentary at best.

The FIV LTR is also distinguished from the HIV-1 LTR by its strong basal promoter activity that does not require activation by a viral transactivator.[39,40,43,61] Similarly, other animal lentiviruses including caprine arthritis encephalitis virus (CAEV) and visna virus (VV) also encode LTR promoters capable of high basal levels of transcription in the absence of a viral transactivator.[62,63] In contrast, LTRs encoded by primate lentiviruses including HIV and simian immunodeficiency virus (SIV), include a transcriptional element designated TAR (Tat responsive element) that possesses a stem-loop structure for binding of the virus encoded transactivator Tat and other cellular proteins.[64,65] Tat transactivation of the HIV LTR is required for elongation of initiated RNA transcripts and for efficient transcription of viral genes. The FIV LTR does not contain a stem-loop structure of similar complexity nor does FIV encode a transcriptional transactivator with structure or activity similar to that described for HIV Tat.[39,40,43,61] Previous reports have proposed that the gene product of FIV accessory gene *orf-A* may encode a viral transactivator or FIV Tat. However, these studies have generated conflicting data regarding the ability of FIV-encoded Orf-A to transactivate the FIV LTR and revealed either no effect, a small effect, or a moderate effect imposed by Orf-A on FIV LTR-directed gene expression in transient expression assays.[39,40,43,61,66,67] Taken together, these data suggest that the FIV LTR may be regulated in part by accessory protein Orf-

A although by mechanisms unlike those described for the HIV LTR and HIV Tat.

Examination of FIV mRNA species from infected cells revealed the presence of at least five short multiply spliced transcripts in addition to unspliced genomic RNA and *env*-containing singly spliced transcripts.[42,66,68] Nuclear export of unspliced and singly spliced FIV mRNA transcripts involves binding of a posttranscriptional regulatory sequence, designated the *rev* response element (RRE), by the FIV regulatory protein Rev. The FIV RRE is a 243 nucleotide sequence that forms a stem-loop structure within viral mRNA species and serves as a binding site for FIV regulatory protein Rev and is structurally and functionally similar to the HIV-1 RRE.[41] However, the FIV RRE is located at the 3′ terminus of the *env* gene and partially overlaps the 3′ LTR, whereas the HIV-1 RRE is positioned between the junction of the SU and TM open reading frames within *env*. Binding of the FIV RRE by the viral protein Rev is critical for cytoplasmic accumulation of unspliced and singly spliced FIV mRNA transcripts and for FIV structural (Gag and Env) and enzymatic (Pol) protein expression.[41,69]

FIV Structural and Enzymatic Proteins

FIV Gag proteins are necessary and sufficient for the formation of the noninfectious virus-like particles. Similar to other lentivirus systems, expression of FIV Gag polyprotein precursor (p50) from the *gag* gene within the unspliced genome RNA is dependent on viral Rev. Nevertheless, the FIV Gag polyprotein, when expressed in the absence of other viral structural proteins such as *env* gene products, is capable of self-assembly into virus particles that are released from the plasma membrane of Gag-expressing cells.[70] Formation of mature virus particles, however, requires cleavage of the FIV Gag polyprotein by virus-encoded PR during or shortly after budding from the cell to generate three mature Gag proteins: MA, CA, and NC (Figure 2).[28,71] Examination of the proteolytic sites within the FIV Gag polyprotein revealed processing of Gag proteins similar to that for HIV-1 Gag proteins, and in particular to HIV-1 CA protein.[71] Within the mature virion, MA is attached to the viral lipid membrane while CA forms the virus core, and NC is present in the virus core in a ribonucleoprotein complex with the viral RNA genome.[72]

Reports describing studies focused on FIV Gag protein processing and characterization of functional domains are still limited.[27,28,70,71] Similar to HIV MA, myristylation of FIV MA is required for targeting of MA to the plasma membrane during late events of viral assembly.[70,71] In contrast to observations for HIV-1 MA, targeting of FIV MA to the cellular plasma membrane is not dependent on a N-terminal polybasic domain that is present in both FIV and primate lentivirus MA proteins. Instead, this conserved polybasic domain (lysine-rich) in FIV MA appears necessary for

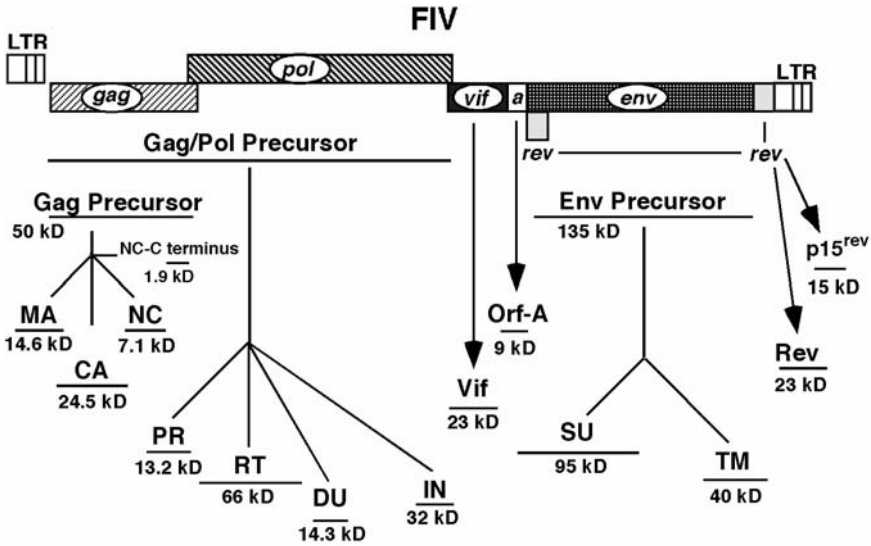

FIGURE 2. Proteins encoded by FIV. Sizes of primary gene products after processing are shown.

either particle assembly or release.[70] The role of FIV MA in other steps of virus replication, where HIV-1 MA is thought to be important, such as early postentry events,[73] has not yet been determined.

The principal function of FIV NC involves encapsidation of full-length, unspliced viral genomic RNA into virions. Similar to other retroviruses, FIV NC protein contains two copies of a zinc finger motif, which has been characterized as a zinc-binding moiety for HIV-1 RNA.[74] Although studies describing FIV NC interactions with viral RNA are few, recent observations suggest that FIV NC binds to viral RNA at, or upstream of, the PBS and may thereby initiate RNA dimerization and promote initiation of minus strand DNA.[75] However, the role of NC in viral RNA encapsidation or determinants responsible for FIV NC binding of RNA have not yet been reported. Interestingly, examination of proteolytic cleavage of NC revealed a secondary cleavage site within the C-terminus of NC that produces a mature NC 7.1kD protein and a C-terminus 1.9kD protein.[71] Although further characterization of this C-terminus 1.9kD protein has not yet been described, amino acid sequence of this cleavage product contains a PSAP motif similar to the PTAP motif also characterized as a "late" or L domain encoded by the HIV-1 p6 Gag protein.[76] This motif within HIV-1 p6 functions in virus particle budding and release[77,78] and is a binding motif for proteins encoding WW domains.[79] Interactions of the L domain of HIV-1 p6 with cellular protein TSG101 are reported to be critical for HIV-1

budding[80–82] and are under investigation as potential targets for antiviral therapeutics. Examination of the significance and potential function of this C-terminal cleavage product of FIV NC as a L domain for FIV Gag may be warranted and provide another lentiviral model for characterizing cellular proteins important for lentivirus assembly and release.

In addition to MA, CA, and NC proteins encoded by FIV *gag*, the FIV Gag polyprotein contains a CA-NC spacer region shown to regulate temporal Gag processing for several retroviruses including HIV-1, bovine immunodeficiency virus (BIV), and Rous sarcoma virus (RSV).[83] The FIV CA-NC spacer region consists of nine residues and contains a LAEAL motif also found in the HIV-1 CA-NC spacer region and reported to be indispensable for HIV-1 Gag assembly.[84,85] Although a recent study showed that the FIV CA-NC spacer region was capable of functionally replacing the BIV CA-NC spacer region for BIV Gag assembly, studies examining the FIV CA-NC spacer region in FIV Gag assembly have not been reported. This observation, however, suggests that FIV Gag may provide another model for examination of this Gag motif in HIV-1 assembly.

The FIV *pol* gene positioned downstream of *gag*, encodes four enzymes: protease (PR), reverse transcriptase (RT), dUTPase (DU), and integrase (IN) (Figure 2). FIV *pol* overlaps the *gag* gene by 109 nucleotides and is in a −1 reading frame with respect to that of *gag*. Similar to other retroviruses, *pol* is translated as a Gag-Pol fusion polyprotein produced by ribosomal frameshifting[86] facilitated by a consensus frameshift signal sequence of GGGAAAC within the *gag-pol* overlap region, together with a sequence displaying potential for a pseudoknot tertiary structure immediately downstream of the signal sequence.[87] The Pol polypeptide of the Gag-Pol fusion precursor protein is cleaved by viral PR into functional enzymes during virus assembly.

FIV PR is a 14.3 kD protein that facilitates processing of Gag and Gag-Pol polyproteins into individual structural and enzymatic proteins during assembly and maturation of the virus particle.[88] Focus on antiviral therapies targeted to HIV-1 PR have promoted interest in FIV PR as a model for design of protease inhibitors as well as structural studies characterizing FIV PR. Based on three-dimensional crystal structure analysis, FIV PR is a homodimeric aspartyl proteinase with quarternary structures very similar to those of HIV-1 PR despite a conservation of only 27 amino acids between the two enzymes.[89,90] However, each monomer of FIV protease is composed of 116 amino acids compared to 99 amino acids for HIV-1 PR. Regardless of similarities observed between FIV PR and HIV-1 PR, FIV PR exhibits a substrate specificity that is restricted to FIV Gag cleavage sites and excludes sites within HIV-1 Gag.[90,91] Multiple residue substitutions are required within FIV PR to modify this specificity to include HIV-1 Gag cleavage sites.[92–94] Furthermore, residues peripheral to the active site of PR, as well as those within the active site, influence binding of substrate by stabilizing

crucial residues within the active site that directly contact substrate and may account for differences in substrate specificities observed between FIV and HIV-1 PR activities.[95] Regardless of disparate substrate specificity displayed by FIV PR and HIV-1 PR, similarities in their structure have been utilized in the development of broad-based inhibitors that will bind both HIV PR and FIV PR.[94,96,97] These comparative analyses should significantly increase the understanding of the molecular basis for lentivirus PR substrate specificity and may possibly facilitate the development of PR inhibitors less susceptible to resistance development.

Reverse transcriptases are encoded by all retroviruses and are RNA-dependent DNA polymerases that reverse transcribe viral genomic RNA into a double-stranded proviral DNA copy that is subsequently integrated into the host cellular genomic DNA.[98] FIV RT is comparable to HIV-1 RT in amino acid sequence, structure and physical properties, catalytic activities, and susceptibility to multiple nucleoside analogs.[99–101] Amino acid sequence analysis reveals a 48% identity and 67% similarity between HIV-1 RT and FIV RT.[101] Like HIV-1 RT, FIV RT exists as a heterodimer consisting of a 66 kD subunit (p66) and a 51 kD subunit (p51), each of which contains a common N-terminus and are present in equimolar concentrations.[100] The p51 subunit is generated by cleavage of the RNase H domain from C-terminus of p66. The RNase H domains in FIV RT and HIV-1 RT function in reverse transcription to degrade RNA from the DNA-RNA hybrid.[102] Importantly, FIV RT and HIV-1 RT exhibit a similar susceptibility to multiple nucleoside analogs.[100,103–105] However, FIV RT resistance to specific nucleoside analogs such as 3′-azido-3′-deoxythymidine (AZT), 2′,3′-dideoxyinosine (ddI), 2′,3′-didehydro-3′-deoxythymidine (d4T), and 2′,3′-dideoxycytidine (ddC) does not map to homologous residues within similarly drug-resistant HIV-1 RT mutants. In contrast, FIV RT and HIV-1 RT susceptibilities to nucleoside analog (−)-+-L-2′,3′-dideoxy-3′-thiacytidine (3TC) map to corresponding codon, M184 in the YMDD active site of the RT palm subdomain.[106,107] Other studies also revealed that a unique 3TC-resistant FIV mutant encoded a novel proline to serine change at position 156, analogous to proline residue 157 residing within the template grip of HIV-1 RT.[108] This observation suggested that mutations within a region (template grip) close to, but distinct from the RT active site could influence substrate recognition, a conclusion further substantiated by examination of homologous HIV-1 RT mutant P157S.[103] Furthermore, functional studies assessing chimeric RT molecules composed of FIV and HIV-1 p51 and p66 subunits demonstrated the importance of p51 in maintaining optimal structural integrity of RT and shed some light on significance of the p51 subunit.[109,110] These studies also produced observations showing the lack of FIV RT sensitivity to non-nucleoside RT inhibitors (NNRTI) found to block HIV-1 replication, although amino acids lining the NNRTI-specific pocket of HIV-1 RT exhibit a higher similarity to the correspond-

ing FIV RT residues than to HIV-2 RT.[109,110] Catalytic activity of HIV/FIV chimeric RTs was also found to be significantly decreased compared to wild type HIV and FIV RTs, despite similarities observed between the two molecules.[109] In summary, similarities and differences observed between HIV-1 and FIV RT have generated somewhat limited support for use of FIV RT as a model for HIV-1 RT-targeted drug design and studies of drug resistance both in cell culture and in vivo.[111]

A DU gene product is expressed from the *pol* gene in genomes from nonprimate lentiviruses including FIV and the type-D retroviruses, but not from primate lentiviruses.[30] FIV DU resides immediately downstream of RT in the Pol polyprotein and is packaged in active form in FIV virions. DU catalyzes the hydrolysis of dUTP to dUMP and inorganic pyrophosphate (PPi), and is believed to minimize misincorporation of dUTP into DNA, which can be mutagenic.[112] For those viruses encoding a DU, enzymatic activity is required for productive viral replication in cells such as primary macrophages that express low dUTPase activity.[113–115] Infection of cats with a DU mutant of FIV resulted in fivefold increase in the number of mutations observed in the viral genome.[114,115] Although HIV-1 does not encode a dUTPase activity, recruitment of a cellular DNA repair enzyme, uracil DNA glycosylase (UNG), into HIV-1 virions by accessory protein Vpr, also acts to modulate viral mutation rate.[116–119] Accordingly, HIV Vpr activity imparts a similar effect to that of FIV DU on virus replication but through a different mechanism.

Integration of double stranded proviral DNA into the host genome is a function of all retroviral integrase proteins and is a distinguishing feature of retrovirus replication.[120] FIV IN is a 32 kD protein that is approximately 37% identical to HIV-1 IN by amino acid sequence.[121] Similar to other retroviral IN proteins, FIV IN contains three domains including an N-terminal domain, a central catalytic core domain, and a C-terminal domain.[122] Studies testing activity of a recombinant FIV IN expressed in *E. coli* revealed that FIV IN exhibits a relaxed sequence requirement for site-specific cleavage and integration of viral DNA termini and is active on FIV, HIV, and Moloney murine leukemia virus (MoMLV) DNA termini.[121] A difference noted between FIV IN and HIV-1 IN was their choice of nucleophiles in vitro with FIV IN preferentially using the 3′ OH viral DNA ends and HIV-1 IN using H_2O and glycerol. In vitro analyses of recombinant FIV IN also demonstrated that the central catalytic core domain determined target site selection and the importance of a central aspartic acid (D118) in 3′ terminus processing and joining activities.[123,124] Virus replication studies testing FIV IN mutants in the context of FIV vectors showed that mutation of either D66 and or both D66 and D118 within the catalytic core domain blocked transduction of dividing fibroblast and integrations, as would be predicted for type I IN mutants.[125] These observations for mutants involving FIV IN residues D66 and D118 that correspond to D64 and D116 in

the catalytic triad of HIV-1 IN, characterized properties of catalytic core IN mutants for a non-HIV-1 lentivirus, and verified similarities between FIV and HIV-1 IN.

In addition to a direct role in integration of proviral DNA into host cell genomic DNA, HIV-1 IN is a component of the PIC that also contains newly synthesized proviral DNA, viral MA and Vpr, and the viral central DNA flap. Each of these viral components are thought to contribute to nuclear import of the PIC[126] and IN may play the primary role.[127] Similar to HIV-1 IN, FIV IN exhibits karyophilic properties. Determinants encoded by FIV IN for nuclear import map to a N-terminal zinc-binding domain and to a region rich with basic residues near the C-terminal domain, rather than to a canonical nuclear localization signal (NLS).[128] The NLS for HIV-1 IN is also thought to involve a bipartite signal that instead includes a 13 residue peptide within the central core domain of IN, but does not include the N-terminal zinc-binding domain, suggesting that mechanisms for nuclear import of HIV-1 and FIV integrase molecules are different.[128]

The *env* gene of FIV and other lentiviruses is the most diverse viral gene in size and sequence.[73] Lentivirus envelope proteins play a major role in the virus life cycle by encoding determinants that interact with cell surface receptor and mediate fusion between the lipid bilayer of the viral envelope and host cell plasma membrane. Accordingly, variation in viral envelope proteins, particularly the surface glycoprotein, affects virus host cell tropism and fusogenicity, as well as virus replication. In addition, the Env glycoproteins contain epitopes that elicit immune responses important for both diagnosis and protective immunity.

FIV *env* expression from a singly spliced mRNA is Rev-dependent, similar to other structural proteins. In contrast to primate lentivirus Env proteins, FIV Env and other nonprimate lentiviruses Env proteins encode a lengthy N-terminal presequence upstream of the hydrophobic region of the Env signal peptide (Figure 3).[37,38,129,130] This N-terminal presequence of FIV Env, containing 149 amino acids, represents a 20 kD polypeptide and includes the L region of *env* that encodes the N-terminal exon of FIV Rev.[41,69] The early gene product of FIV *env* is a full-length uncleaved precursor 145–150 kD glycoprotein that is subsequently processed to a 130 kD precursor (gp130) by cleavage of the N-terminal 20 kD polypeptide and hydrophobic signal sequence.[129,130] Precursor gp130 is then transported to the Golgi and proteolytically cleaved to produce mature FIV surface glycoprotein (SU) gp95 and characteristic hydrophobic membrane-spanning glycoprotein (TM) gp40. SU forms a noncovalent association with TM, which anchors the envelope complex to the lipid bilayer. Studies using glycosylation inhibitors have confirmed extensive glycosylation of FIV envelope proteins similar to that observed for HIV Env and demonstrated cell type-specific glycosylation of Env.[129,131] These studies also verified the role of envelope protein glycosylation in virus infectivity.[129] Sig-

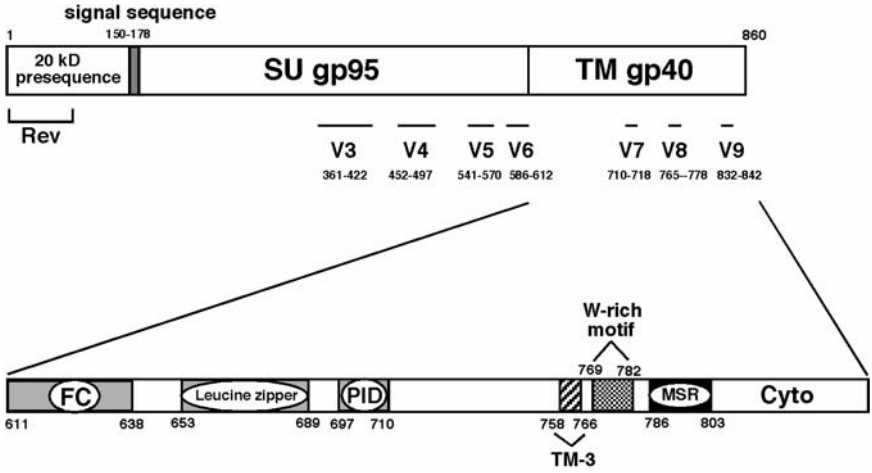

FIGURE 3. Schematic representation of variable regions, functional domains, and various epitopes encoded by FIV *env* gene products SU and TM. Amino acid positions indicated for each domain or epitope are based on deduced amino acid sequence of FIV molecular clone 34TF10.[38] Variable regions (V3 through V9) are shown as previously described.[134,142] Functional domains and epitopes within TM as previously reported,[133,137,138,454] include: FC, fusion peptide; leucine zipper region; principal immunodominant domain, PID; a TM3 epitope analogous to neutralizing HIV-1 2F5 epitope; a tryptophan (W)-rich motif important for infectivity; the membrane-spanning region, MSR, and the cytoplasmic domain, Cyto.

nificance, structure, or function of this 20 kD N-terminal polypeptide cleavage product, apart from proper Env processing, is not well understood, although one study revealed that partial deletion of this peptide produced a virus unable to infect primary feline astrocytes while still infectious for feline lymphocytes and macrophages.[132]

Structural models based on x-ray crystallography or NMR spectroscopy indicate that HIV-1 envelope proteins form trimers on the viral surface. Furthermore, SU binding of the primary receptor, CD4, results in conformational changes facilitating formation of a ternary complex composed of CD4, SU, and coreceptor molecules (β-chemokine receptors CCR-5 and CXCR-4). This ternary complex triggers additional conformation changes in TM that mediate fusion of envelope proteins with the cellular plasma membrane.[73] Models for spatial folding of FIV Env SU and TM proteins based on predictive algorithms using Env amino acid sequence from multiple isolates, reveal structural similarities to HIV-1 envelope proteins, including conserved and hypervariable domains for both FIV SU and TM.[133–135] A principal immunodominant domain (PID) within the extracellular region of TM that is conserved among lentivirus transmembrane glycoproteins, including HIV-1 TM, has also been described for FIV TM (Figure 3).[133,136] Despite conservation of the PID, mutation of this TM

domain was not found to alter FIV infectivity.[136] In contrast, disruption of a tryptophan-rich domain also conserved among lentivirus TM proteins and located in the extracellular region immediately upstream of the membrane-spanning domain abrogated virus entry.[34] Findings from a second study demonstrated a role for the FIV TM tryptophan-rich domain in Env-mediated fusion between viral and cellular membranes, thus providing a probable mechanism by which this domain affects virus infectivity.[137] Finally, synthetic peptides modeled on this tryptophan-rich domain were found to inhibit FIV replication.[138] These findings parallel other data demonstrating the importance of a similar tryptophan-rich domain encoded by HIV TM for HIV-1 entry and HIV-1 fusiogenic effects[139,140] and suggest that FIV and HIV-1 TM may share one mechanism necessary for fusion of viral and cellular membranes. However, a more detailed structural analysis of FIV envelope proteins and their interactions with cell surface receptors based on x-ray crystallography will be needed for a thorough comparison of FIV and HIV-1 envelope proteins.

As stated earlier in this chapter, envelope proteins exhibit the greatest diversity in amino acids among all proteins encoded by lentiviruses. By comparison of different HIV isolates, five interspersed conserved (C1 to C5) and five variable domains (V1–V5) were identified for HIV-1 *env* gene.[73] Similarly, analysis of amino acid sequences derived from the *env* gene of different isolates of FIV resulted in identification of nine variable regions (V1–V9), separated by regions that are more conserved (Figure 3).[134,141–143] Furthermore, examination of V3, V4, and V5 domains within SU-coding sequence revealed up to 26% genetic diversity for different isolates and allowed separation of FIV isolates into five clades, or subtypes, designated A to E[134,142,144–150] and possibly a sixth subtype arising from a subtype B cluster found in Texas.[151] Diversity between subtypes varies from 17 to 26% whereas variation within a subtype ranges from 2.5 to 15%, findings that are similar to those for HIV-1 isolates.[142] Although geographic separation is most likely a major factor for emerging genetic diversity of FIV, individual subtypes have been identified in geographically isolated regions of the world (Table I). FIV subtype A and B isolates have been detected in United States, Europe, Japan, and Australia, whereas subtype C variants have been found in North America, Europe, and Taiwan. Subtype A isolates have also been detected in Central and South America[149] and all three FIV subtypes A, B, and C have been isolated from a single location (Munich, Germany).[150] Subtype D and E isolates, characterized so far, are few and restricted to Japan[147] and Argentina[145] respectively. Multiple genetic analyses indicate that the vast majority of FIV isolates are either subtype A or B. Interestingly, findings from one report revealed twice as many synonymous site changes within FIV subtype B envelope V3 to V4 domains compared to those detected for subtype A envelope, although both subtype A and B envelopes encoded similar numbers of nonsynonymous site changes.[150]

TABLE I
Geographic Distribution of FIV Subtypes

Subtype	Location
A	United States, Europe, Japan, Australia, Central and South America
B	United States, Europe, Japan, and Australia
C	North America, Europe, and Taiwan
D	Japan
E	Argentina

The greater diversity observed for subtype B suggests that this FIV subtype may represent an older virus. Significant diversity of FIV subtype B variants was also shown for viruses isolated from cats in Italy where this subtype is highly prevalent.[144] Findings from this study were generated by phylogenetic analysis focused on sequences from a Gag fragment from 32 isolates and on SU sequences from four isolates. Finally, higher diversity for this subtype was further supported by two recent studies. Examination of both Gag and envelope sequences derived from FIV subtype B isolates in Austria, where subtype B predominates, revealed subtype B clusters with sufficient genetic diversity to support their designation as subclades.[146] A second study characterized a cluster of FIV isolates from Texas more closely related to FIV subtype B viruses than to other subtypes, yet clearly distinct from this subtype.[151] Findings from this study suggested that this virus cluster was either a separate subtype that recently emerged from subtype B or represented a subgroup within subtype B. Collectively, these findings strengthen the hypothesis of FIV subtype B as an older virus existing within the domestic cat population for a longer time period than other FIV subtypes. Host adaptation and lower virulence has been suggested to be associated with FIV B subtype isolates but not confirmed by rigorous examination.

Several reports have also presented evidence of both naturally occurring[149,150] and experimentally induced[152] intersubtype recombinant viruses derived from recombination within the *env* gene. Observation of intersubtype recombinants involving a common break-point within *gag* was also recently reported.[153] These findings for FIV agree with reports of recombinant HIV-1 isolates generated by divergent HIV subtypes in geographic regions where multiple HIV subtypes are found.[154–156] Identification of multiple subtypes, significant divergence within the major subtype B viruses, and confirmation of naturally occurring FIV recombinants are relevant issues for FIV vaccine design and suggest that vaccines specific to geographic area and subtype prevalence may be required. Similar issues exist for HIV-1 vaccine design and provide an opportunity for the FIV

vaccine model to address the importance of multisubtype vaccine approaches.

Regulatory and Accessory Genes

Only one regulatory gene (*rev*) and two accessory genes (*vif* and *orf-A*) have been characterized for the FIV genome. Although several research groups have shown that FIV Rev and FIV Vif provide functions in virus replication similar to those described for analogous HIV-1 proteins, exhaustive examination of these FIV gene products has not been reported. FIV accessory genes, that are clearly analogous to primate lentivirus regulatory and accessory genes, including *tat*, *vpr*, *vpu*, *vpx*, or *nef*, have not been identified. Early reports proposed that FIV Orf-A encoded a HIV-1 Tat gene product. However, studies testing Orf-A transactivation of the FIV LTR did not demonstrate Orf-A activity comparable to HIV-1 Tat.[39,40,43,61] Recent findings suggest that FIV Orf-A may express functions more similar to those of HIV-1 Vpr or perhaps Vpu, although data supporting this premise are very limited.[157,158] However, the possibility that viral functions and activities expressed by multiple unique HIV-1 accessory proteins may be encompassed by a single FIV accessory protein along with specific domains within FIV structural proteins warrants further examination.

Genomes of all members of lentivirus subfamily encode for a HIV-1 Rev-like posttranscriptional regulatory protein that is expressed early in the viral life cycle from a multiply spliced mRNA species containing either *orf-A* and the L region of *env* and *orf-H*, or the L region of *env*[42,66] and *orf-H* only.[68] FIV Rev is a 23 kD protein that is expressed from two exons derived from the L region of *env* and from *orf-H* (Figure 2). The first *rev* exon extends through the 5′ terminal L region of the *env* gene and is in the same open reading frame as *env*. The second exon (*orf-H*) is located at the 3′ terminus of the *env* gene and overlaps the 5′ terminus of the 3′ LTR. Similar to HIV-1 Rev, FIV Rev is a nucleolar protein that binds as multimers to the *cis*-acting regulatory RRE sequence contained within unspliced and singly spliced viral mRNAs to promote nuclear export of these mRNA species to the cytoplasm and to also increase mRNA stability and protein translation.[41,68,69,159,160] FIV Rev contains a highly basic domain similar to that described for other lentivirus Rev proteins. This basic domain located in the second exon, most likely serves as an RRE binding domain, although studies confirming the function of this FIV Rev element have not been reported. Another viral mRNA containing only the second Rev exon (*orf-H*) has also been identified and encodes for a truncated Rev protein designated $p15^{rev}$.[66] $P15^{rev}$ contains the highly basic domain and effector domain of Rev but lacks the N terminus derived from the first exon. This truncated Rev protein exhibited Rev activity by in vitro assays but only 20% of the activity observed for wild type full-length FIV Rev. The significance

of $p15^{rev}$ in virus replication has not been characterized. Expression of FIV structural and enzymatic proteins, including Vif, is dependent on Rev expression and activity. Accordingly, the Rev regulatory system is essential for productive virus replication.

Although FIV Rev shares similar activities and functions with HIV-1 Rev, the FIV Rev effector (activation) domain located immediately downstream of the basic domain, contains a nuclear export signal (NES) that differs significantly in amino acid sequence from those described for Rev effector domains of primate and ungulate lentiviruses.[60,161,162] The FIV Rev effector domain/NES lacks organized hydrophobic residues (leucine-rich clusters) and a core tetramer, which are properties that define the HIV-1 Rev NES.[163] Instead, the FIV Rev effector domain/NES is characterized by basic residues and dispersed leucine residues similar to that observed for equine infectious anemia virus (EIAV) Rev. However, both FIV and EIAV Rev effector domains can functionally replace the effector domain of HIV-1 Rev, a finding that suggests FIV and HIV-1 Rev proteins utilize similar cellular pathways for their activity.[162] Similarity between FIV Rev and HIV-1 Rev is further supported by studies demonstrating that nuclear export of FIV and EIAV Rev proteins, as well as Rev function, is inhibited by leptomycin B, a drug previously shown to block HIV-1 Rev NES interactions with CRM1 (exportin 1).[161] This observation suggests that binding of CRM1 for nuclear export may be a property that FIV Rev shares with HIV-1 Rev, regardless of their dissimilar activation domains. Another functional property shared by the FIV and HIV-1 Rev regulatory systems involves an interaction with cellular eukaryotic initiation factor 5A (eIF-5A), which is also associated with Rev's nuclear export of unspliced and singly spliced viral mRNA.[164–166] Biologically active eIF-5A was previously shown to be required for HIV-1 Rev function and to specifically facilitate binding of Rev/RRE complex to CRM1.[167] Activation of eIF-5A requires synthesis of the unique amino acid hypusine, which is sustained by deoxyhypusine synthetase.[168] A recent report revealed that 1,8-diaminooctane, an inhibitor of deoxyhypusine synthetase, significantly inhibited FIV replication and specifically restricted FIV Rev function.[166] Taken together, these observations demonstrate similarities shared between HIV-1 and FIV Rev proteins and validate FIV Rev as a model for potential antiviral therapies that target HIV-1 Rev.

An accessory gene designated as *vif* is conserved among all the lentiviruses with the exception of EIAV[73] and has been determined to encode a factor necessary for virion cell-free infectivity, although not required for virion production.[169,170] FIV *vif* resides at the 3′ terminus of the *pol* gene in the viral genome and is translated from a singly spliced mRNA[171] to express a 23-kD basic protein (Figure 2). The role of FIV Vif in viral infectivity for primary feline lymphocytes and macrophages has been clearly established.[172–174] However, biochemical characterization of FIV Vif has been limited and restricted to one report describing nuclear

localization for this FIV accessory protein.[175] Studies regarding mechanisms of Vif function have so far focused instead on primate lentivirus Vif proteins. Early studies demonstrated that the replication phenotype of *vif*-deleted HIV mutants was cell type-dependent and specifically dependent of the cell producing the virion.[176] Cell types, including primary lymphocytes and macrophages as well as specific T-cell lines, required the presence of HIV-1 Vif expression for production of infectious HIV and were designated as "nonpermissive" for replication of *vif*-deleted mutants of HIV-1. However, specific human T-cell lines designated as "permissive cells," including SupT-1 cells and Jurkat cells, supported productive virus replication for HIV-1 *vif*-deleted variants. Cell fusion experiments showed that heterokaryons formed between permissive and nonpermissive cells displayed the nonpermissive phenotype, suggesting that nonpermissive cells naturally express an antiviral activity that inhibits the replication of *vif*-deficient virus.[177] Virions produced in nonpermissive cells in the absence of the *vif* gene are impaired for reverse transcription of genomic RNA and therefore fail to establish full-length proviruses after entry into a target cell. The small number of reports focused on FIV Vif to date have not revealed a feline cell line "permissive" for *vif* deletion mutants of FIV. This observation may be due in part to the very few established feline T-cell lines that are available and permissive for primary wild type isolates of FIV.

Recent reports have revealed major breakthroughs in the understanding of HIV-1 Vif functions and interactions with host cell proteins. Sheehy and colleagues first identified a cellular protein designated CEM15 as the "nonpermissive" cell factor responsible for production of noninfectious virus particles in the absence of HIV-1 Vif.[178] CEM-15 was later identified as APOBEC3G, a cell protein closely related to APOBEC1 and a member of the cytidine deaminase family of nucleic acid-editing enzymes. Numerous studies have now established APOBEC3G as a cellular factor that exerts an antiviral effect by deamination of cytosines to uracils in single-stranded minus-strand DNA during reverse transcription, resulting in either degradation of newly synthesized minus-strand DNA or guanidine to adenine hypermutations in the final double-stranded proviral DNA product.[179–181] In the absence of HIV-1 Vif, APOBEC3G is packaged into virions, allowing this cellular protein to exert its antiviral effect during reverse transcription after virion entry into a target cell. However, if present during virion assembly, HIV-1 Vif forms a complex with human APOBEC3G that targets the cellular factor for proteosomal degradation and thereby prevents virion encapsidation of APOBEC3G to facilitate particle infectivity.[182–184] These advances in the elucidation of HIV-1 Vif function have generated new enthusiasm for designing antiviral therapeutics targeting Vif and Vif-APOBEC3G interactions. Recent studies have also confirmed that other primate lentivirus (SIV) Vif proteins also target APOBEC3G in simian cells, although these interactions appear to be species-specific for some of

the simian species. Whether nonprimate lentivirus Vif proteins, including FIV Vif, function similarly by interacting with a species-specific APOBEC3G analog has yet to be determined. Evidence that supports this possibility is derived from reports demonstrating that APOBEC3G degradation is mediated by the HIV-1 Vif SLQ(Y/F)LA domain, an amino acid motif that is conserved among all lentivirus Vif proteins including FIV Vif.[173,182,183]

FIV gene *orf-A* (also referred to as *orf-2*) encodes a 77 amino acid accessory protein previously implicated to encode a Tat-like protein and is critical for efficient viral replication in peripheral blood mononuclear cells (PBMC) in vitro and in vivo.[61,157,185–188] Although the *orf-A* gene product contains a cysteine-rich domain within its 3′ terminus, similar to Tat proteins encoded by ungulate lentiviruses, this gene product does not include core and basic domains comparable to those found in Tat proteins encoded by primate lentiviruses, EIAV, or BIV. Amino acid sequence alignments of FIV Orf-A with visna virus and CAEV Tat proteins reveal a similar organization of conserved putative domains, including N-terminal hydrophobic, central leucine-rich, and C-terminal cysteine-rich regions.[60] In addition, FIV Orf-A encodes previously unrecognized conserved tryptophans at positions 43 and 66 positioned similarly to conserved tryptophan residues 63 and 85 of visna virus Tat. Two recent studies demonstrated a moderate upregulation of the FIV LTR promoter activity by coexpression of Orf-A in transient reporter gene expression assays.[66,67] In contrast, earlier reports revealed either a small effect or no effect imposed by Orf-A on FIV LTR-directed gene expression.[39,40,43,61] These findings suggest that Orf-A very likely drives FIV-LTR-directed transcription, but by indirect mechanisms involving interactions with cellular transcription factors.

A recent report revealed Orf-A to be important in the late steps of the FIV life cycle involved in virion formation and in early steps involved in virus infectivity and mapped critical Orf-A domains needed for these steps in replication.[157] Central leucine-rich and C-terminal cysteine-rich regions, along with a conserved central tryptophan (residue 43) within Orf-A, were shown to be critical determinants for efficient virus replication and infectivity. The leucine-rich domain was important for infectivity, whereas tryptophan 43 and the cysteine-rich domain were important for both infectivity and virion formation. Importantly, deletions and point mutations in *orf-A* imposed a small or no effect on FIV LTR-driven viral gene expression and no effect on viral protein expression. These findings suggested that *orf-A* represents a FIV-encoded analog more similar to accessory genes *vpr*, *vpu*, or *nef* rather than the regulatory *tat* gene encoded by the primate lentiviruses. This concept was further supported by another recent study using mammalian expression plasmids encoding wild type or deletion mutant Orf-A proteins fused to the C′-terminus of green fluorescent protein (GFP) to evaluate Orf-A subcellular localization and effects on cell function.[158] Findings from this study demonstrated nuclear localization

for the GFP-Orf-A fusion protein and allowed mapping of a NLS (residues 44–54) critical for nuclear import of FIV Orf-A. Furthermore, assessment of cell cycle profiles of cells transiently expressing GFP-Orf-A revealed that Orf-A causes an arrest at G2 of the cell cycle. These novel findings suggested that Orf-A is a nuclear protein that expresses some properties similar to those reported for HIV-1-encoded Vpr. Collective data generated from both reports suggest the possibility that Orf-A may encode specific functions attributed to several different HIV-1 encoded accessory protein but not all functions characterized for a single HIV-1 accessory protein. Additional support for this possibility is provided by a recent report showing that CAEV Tat, which shares structural homology with FIV Orf-A, also localizes to the nucleus and arrest cells in G2.[189] Verification of these findings for FIV Orf-A from other research groups and for Orf-A proteins encoded by multiple FIV isolates will be needed to confirm the similarities between FIV Orf-A and HIV-1 Vpr and other HIV-1 accessory proteins.

3. FIV TROPISM

FIV Receptor Usage

Natural and experimental infection of cats with biological and molecularly cloned isolates of FIV consistently induce an acute viremia associated with T-cell alterations including depressed CD4:CD8 T-cell ratios and CD4 T-cell depletion.[4–7,10,11,21,190–194] Early studies revealed that targets for FIV in vitro and in vivo included CD4 T-cells, macrophages, dendritic cells, microglia, and astrocytes similar to those for HIV infection in humans, but also included CD8 T-cells, and B-cells (Table II).[4,18,23,186,195–210] Early reports also demonstrated that continuous passage of particular FIV isolates in cell culture selected for virus variants capable of replication in feline adherent cell lines, including Crandell feline kidney cells (CrFK) and G355-5 cells, as well as established feline interleukin (IL)-2-independent T-cell lines.[2,25,42,211] Importantly, experimental inoculation studies in cats revealed that cell culture-adapted viruses represented a particular subset of viral variants that exhibited reduced replication and virulence in vivo.[111,192,197,212]

FIV infection of feline CD4-negative adherent cell lines provided indirect evidence that FIV differs with HIV-1 and does not utilize CD4 as a primary receptor. In addition, direct evidence refuting FIV usage of CD4 was provided by studies revealing an absence of virus infectivity for non-lymphoid cells expressing feline CD4.[213] Subsequent reports described blocking of FIV infectivity by an antibody specific for feline CD9, a cell surface antigen belonging to the four-transmembrane-spanning domain superfamily (TM4SF).[214,215] However, anti-CD9 antibody was later shown to

TABLE II
Comparison of FIV and HIV-1 Replication Properties In Vitro

Property	FIV	HIV-1
Permissive primary cells	PBMC, CD4 T-cells, CD8 T-cells, macrophages, various other cell types including microglia and astrocytes	PBMC, CD4 T-cells, macrophages various other cell types including microglia and astrocytes
Permissive cell lines	IL-2-dependent T-cell lines IL-2-independent T-cell lines fibroblastic adherent cell lines (CrFK and G355-5)	Various CD4 T-cell lines macrophage cell lines
Cytopathic effects	Syncytium, giant cell formation cell lysis, apoptosis	Same as FIV
Receptors		
Primary isolates	CD134 and CXCR4	CD4 and CCR5 or dual-tropic (R5X4)
Adapted isolates	CD134 and CXCR4 or CXCR4 only	CD4 and CXCR4 or other "orphan" chemokine receptors or CXCR4 only
Host range	Feline cells only (?)	Human, some nonhuman primate cells
Viral tropism determinants	Env: V3 region and TM Orf-A, U3 domain of LTR	Env: V3, V1/V2 Vpr

block virus infection by inhibition of virus release and not by interference with virus-receptor binding.[216]

A major breakthrough in characterization of FIV cell surface receptors resulted from observations showing that cell culture-adapted FIV isolates primarily utilize the β-chemokine receptor CXCR4 for infection in a similar fashion to T-cell line-adapted isolates of HIV-1 (Table II).[33,217] Induced cell surface expression of CXCR4 was shown to mediate a susceptibility to FIV infection[33,218] that could be abrogated by treatment with natural ligands for CXCR4 such as stromal cell-derived factor (SDF-1) and with CXCR4 antagonists including AMD3100.[219–221] Furthermore, critical determinants for FIV infection were mapped to the extracellular loop of CXCR4.[218,222] Multiple reports have now confirmed that cell culture-adapted FIV isolates are capable of using CXCR4 exclusively for virus entry and infection.[33,36,218–221,223,224]

Results of subsequent reports suggesting FIV usage of chemokine receptors CCR5 and CCR3[223,225] have conflicted with observations of other investigators and may have resulted from enhanced expression of CXCR4 associated with ectopic expression of CCR5.[224] Use of other chemokine receptors by particular FIV isolates is not clear at this time and is under investigation. Similar to HIV-1 envelope proteins, binding of FIV Env to

other cell surface makers, including heparan sulfate proteoglycans (HSPGs) and DC-SIGN, has been shown and is specific for particular isolates.[35,36] Binding of DC-SIGN was observed with recombinant Env proteins derived from primary and cell culture-passaged FIV isolates, whereas binding of HSPGs was observed only with a cell culture-passaged FIV strain. Although virus entry was shown to be mediated exclusively by CXCR4, virus infectivity was enhanced by factors including temperature or HSPGs, which either increase concentration of or binding for CXCR4, respectively.[226] The roles of binding of DC-SIGN and HSPGs by FIV Env in FIV infection and pathogenesis in vivo are currently not well understood and warrant further examination.

Previous studies confirming CXCR4 as a receptor also revealed that CXCR4 expression was not sufficient for infectivity of primary FIV isolates and suggested the likehood that primary isolates required a second receptor for binding and entry. One report describing coimmunoprecipitation of a recombinant FIV envelope protein with a 40 kD cellular protein provided further evidence of a non-CXCR4 receptor for FIV.[36] A second major advance in FIV receptor biology was the recent identification of this cellular protein as CD134, a 43 kD cell surface marker, and the receptor utilized by primary FIV isolates in conjunction with CXCR4.[32,227] CD134 is a member of the tumor necrosis factor-receptor superfamily and is expressed primarily on activated CD4 T-cells (mouse and human) after T-cell receptor (TCR) engagement.[228] A recent report confirmed a similar phenotype for feline CD134 by showing up-regulation of CD134 expression on mitogen-activated feline CD4 T-cells.[226] Binding of cell surface CD134 with cellular CD134 ligands provides a costimulatory signal that results in proinflammatory effects, as well as proliferation, migration, and cytokine production by memory T-cells. Low level CD134 expression has also been reported for activated CD8 T-cells, activated B-cells, and macrophages in mice and humans[229–231] but has not been confirmed for the same feline cell lineages.

So far, preliminary reports indicate that infection by primary isolates requires binding of both CD134 and CXCR4.[32,227] Assessment of receptor usage by larger panels of primary FIV isolates will be needed to determine if additional chemokine receptors may also be used for FIV infection, as previously observed for HIV-1, and may be associated with specific FIV replication/pathogenic phenotypes. Whether infection of CD8 T-cells, B-cells, and macrophages previously observed in later stages of FIV infection is due to CD134-independent usage of CXCR4 expressed on these cell populations, or results from usage of both CD134 and CXCR4, has not yet been clarified. Clearly, usage of CD134 as a receptor by primary FIV isolates explains the specific targeting of the CD4 T-cell population for depletion observed with FIV infection and the similarities in the acquired immunodeficiencies induced by both HIV-1 and FIV. Furthermore, these obser-

vations elucidate a mechanism by which FIV and HIV-1 utilize unique receptors (CD4 and CD134) to target a similar subset of T-cells (i.e., activated and resting memory CD4 T-cells) to impart disease pathogenesis.[232–237] Accordingly, these findings further validate the importance of FIV infection as a model for examination of HIV-1-induced AIDS. Examination of pathogenic effects and cellular dysfunction imposed by virus-binding of CD134 expressed on CD4 T-cells in vitro will also be important for defining potential viral mechanisms for induction of feline acquired immunodeficiency in vivo.

Viral Determinants for Cell Tropism

Replication phenotypes in vitro and in vivo clearly distinguish cell culture-adapted FIV isolates from primary isolates. Most primary FIV isolates examined to date require both CD134 and CXCR4 for infection and exhibit a cell tropism restricted to primary feline PBMC, selected IL-2-dependent T-cell lines, and possibly primary macrophages and astrocytes,[2,42,61,196–199,238] although testing for tropism to the latter two cell types has been infrequently reported (Table II). In contrast, FIV variants passaged in vitro utilize CXCR4 solely for efficient infection and replication in feline adherent cell lines including CrFK cells,[33,36,218–221,223,224] as well as feline PBMC, IL-2-dependent and independent feline T-cell lines and macrophages.[2,42,61,187,190,197,211,239–242] These highly passaged isolates, however, exhibit severely restricted virus replication and pathogenicity in vivo as discussed above.[111,192,197,212] Multiple studies using FIV molecular clones have mapped Env as a major determinant that expands cell tropism to include feline adherent cell lines including CrFK cells and G355–5 cells (feline glial cell line), as well as feline astrocytes (Table II).[33,212,223,243–248] Similar to the V3 domain of HIV-1 Env, the V3 domain of the FIV SU encodes specific tropism determinants[212,223,243,244,249–251] in addition to important neutralizing antibody epitopes (Figure 3).[252–254] Critical amino acid residues mapped within the FIV Env V3 domain appear to determine adherent cell tropism by modifying the overall charge of the V3 loop.[212,243,244] A recent report describing the evolution of a nonpathogenic FIV isolate to a more pathogenic virus in vivo over time suggested that mutation E409K within the V3 loop not only imposed CrFK cell tropism but also contributed to virus attenuation.[212] This hypothesis is supported by the consistent observation of severely restricted replication in vivo observed for viruses exhibiting a tropism for CrFK cells in vitro.[111,192,197,212] Other Env determinants affecting FIV cell tropism for adherent cells have been mapped to the second constant domain within SU, the extracellular domain of TM,[247] and the cytoplasmic domain of TM.[245] Although V3 and V4 domains of FIV SU are also reported to influence FIV macrophage and brain microglia tropism,[209,255] viral determinants critical for macrophage tropism as well as the frequency

of macrophage tropism across many primary isolates have not been well examined for FIV. Mechanisms by which these important Env residues or domains contribute to virus infectivity (for all cell types) either by effects on Env conformation or binding affinity to either CXCR4 or CD134 are also not well understood at this time and warrant investigation to further characterize the FIV animal model.

Multiple reports confirmed that the gene product of accessory gene *orf-A* is a critical determinant for infection of feline PBMC[61,157,187,197] but is not required for infection of adherent cell lines including CrFK cells. However, the role of Orf-A in FIV tropism for feline macrophages has received only limited study and is unclear based on conflicting data reported from different studies.[61,187,197] Mechanisms by which Orf-A impacts virus infectivity of PBMC are also undetermined at this time.

Interestingly, early studies suggested that restriction of productive FIV infection in human cells resulted primarily from a block in FIV transcription rather than a block in virus entry.[40,256–259] The capability of FIV isolates to establish latent infections in human cells[258] was later explained by demonstration of cell culture adapted-FIV usage of human CXCR4 for fusion and infection of human cells.[33] Subsequent studies focused on FIV vector development revealed that the FIV U3 domain was the determinant responsible for restricted virus replication in human cells and showed that replacement of U3 with the cytomegalovirus immediate early promoter produced FIV vectors capable of viral gene expression in human cells.[225,260] Studies reported by one research group showing that FIV is capable of productive infection of primate cells both in vitro and in vivo[261,262] have not been confirmed by other independent researchers but suggest FIV infection of nonfeline cells may warrant further examination.

4. EPIDEMIOLOGY AND TRANSMISSION

Similar to other lentiviruses, the presence of FIV-specific antibodies signifies an established virus infection that will persist throughout the remaining lifetime of the host. Antibody detection ELISAs and other immunochromatographic methods (excluding western blot) have served as the primary screening diagnostic assays for FIV infection in clinical veterinary practice and epidemiologic studies.[263–266] Although most FIV-infected cats produce antibodies to both Gag and Env structural proteins, a small proportion of cats will test positive for antibodies specific to only one of these two structural proteins.[267] Therefore, currently available commercial FIV antibody assays include both FIV Gag and Env recombinant antigens for optimal sensitivity as well as specificity.[264] Current and earlier serologic studies have shown that FIV is enzootic worldwide.[13,15,264,266,268–273] Evaluation of mutations within virus subtypes suggests that FIV has been

present in domestic cats for a significant period of time, especially when compared to the relative short evolution of HIV.[142,150] FIV prevalence varies greatly depending on geographic location and other variables of the tested cat populations. Among clinically healthy cats, the prevalence of FIV may be as low as 1% or less as observed in central European countries and the United States, or as high as 30% as reported in Japan and Australia.[14,273] The seroprevalence rates in sick cats appear to be several times higher than those in their healthy counterparts and reflect the disease-inducing potential of FIV.[13,15] Age and gender also markedly affect FIV prevalence. Most infections are acquired after one year of age, and prevalence increases up to approximately 10 years of age and then remains stable or tends to decline as the mean lifespan of a domestic cat is about 15 years.[274] Viruses similar to FIV have been documented in several nondomestic felids such as lions, panthers, and bobcats.[269,275–282] The large genetic diversity observed for lentiviruses among different nondomestic felids and between nondomestic feline lentiviruses and FIV, however, do not support the likelihood that nondomestic feline lentiviruses contribute significantly to the circulation of FIV in domestic cats. There is little evidence that FIV is transmissible to any other species including humans[13] with the exception of a recent single report describing experimental FIV infection of nonhuman primates (cynomolgus macaques).[262]

Precise modes of natural FIV transmission among domestic cats are not yet clear. Nevertheless, strong epidemiologic evidence implies that biting and fighting may be the predominant route of transmission (Table III). The importance of this route of transmission is corroborated by observations of the highest prevalence of infection in rural feral cat populations and urban areas containing a high density of freely roaming cats, as in Japan, and a higher prevalence of infection in adult tomcats.[13,15,270,273,283–287,288] In fact, one epidemiologic study revealed that a pattern of increased FIV infection in orange cats compared to nonorange cats correlated with the pattern of more aggressive behavior also exhibited by orange cats.[289] In addition, evidence of virus in saliva harvested from infected cats[290] and an infection experiment involving virus inoculation by simulating biting further substantiate that transmission by this route is highly effective.[13]

Early epidemiologic and experimental infection studies refuted the possibility of naturally occurring vertical transmission of FIV.[2,13,291] However, later experimental inoculation studies confirmed virus transmission to newborn kittens from queens either acutely or chronically infected with different FIV isolates.[19,20,292–294] Such transmission appeared to occur via in utero[294] and postnatal routes,[19,20,293,295] although intrapartum transmission has also been implicated.[19] Interestingly, recent studies provided evidence of vertical transmission resulting in occult infection of kittens characterized by the presence of viral DNA in tissues in the absence of replicating

TABLE III
Comparison of FIV and HIV-1 Infection Properties In Vivo

Property	FIV	HIV-1
Transmission (natural)	Blood (bite wounds), vertical (?)	Blood, vertical, sexual
Transmission (experimental)	Blood (bite wounds), vertical, vaginal and rectal mucosa	Not applicable
Cell tropism	CD4 T-cells, CD8 T-cells, B cells, macrophages, dendritic cells, microglia follicular dendritic cells CD4+CD25+ T-cells, thymocytes, megakaryocytes	CD4 T-cells, macrophages dendritic cells, microglia follicular dendritic cells thymocytes, CD8 T-cells and others (?), CD4+CD25+ T-cells (?)
Tissue tropism	Blood, lymphoid tissues, gastrointestinal tract, CNS genital tract, liver, kidney	Blood, lymphoid tissues, gastrointestinal tract, CNS genital tract
Host range	Restricted to felids	Restricted to humans and some nonhuman primates
Immunopathology	Lymphoid follicular hyperplasia (early) and CD8α+βlow T-cell subset expansion, followed by lymphoid depletion, CD4 T-cell depletion, cytokine dysregulation, and AIDS	Same as FIV

virus in peripheral blood, as well as absence of antiviral antibody apart from maternal antibody.[206,296,297] Although these occult infections resulting from vertical transmission were considered regressive or transient as reported by one study,[296] the duration of persistence of this type of infection has not been thoroughly examined. These covert or occult infections resemble previously described restricted FIV infections that were detectable only by PCR amplification of viral DNA from blood or tissues and that resulted either from inoculation with extremely attenuated viruses[192] or from persistent but nontraumatic contact between naive and infected cats.[298] Furthermore, similar sequestered virus infections in the absence of antibody have been reported for SIV infection[299,300] and implicated in particular human populations at high risk for HIV infection.[301,302] Collectively, these findings suggest the possibility that the incidence of natural FIV infection by vertical transmission may be underestimated, since diagnostic assays focus on antibody or virus in peripheral blood only.

Detection of virus in semen of infected cats has been observed[303,304] and experimental infection by artificial insemination has also been reported.[305,306] Numerous experimental studies have shown the feasibility of infection by the vaginal route[16–18,21–23,307–311] and demonstrated the utility

of this feline animal model for examination of mucosal transmission and viral pathogenesis. Although these observations and experimental studies suggest the possibility of sexual transmission of FIV, definitive observations of natural transmission by this route so far have not been reported.

5. VIRUS INFECTION AND HOST RESPONSES

Virus Infection in Cell Culture

Primary feline PBMC activated by concanavalin A (ConA) and specific feline IL-2-dependent T-cell lines, including FET-1 cells,[242] MYA-1 cells,[312] FCD4E cells,[201] 104C-1 cells,[227] and MCH5–4 cells,[211] have proven to be highly permissive for propagation of biological and specific molecularly cloned isolates of FIV. As described in the section discussing FIV receptor usage, productive virus replication in these cell types results from expression of feline CXCR4 and CD134 cell surface molecules that has been confirmed, at least for activated feline PBMC and 104C-1 cells (Table II).[32,227] Established feline adherent cell lines, including CrFK cells and G355-5 cells, as well as feline IL-2-independent lymphoid cell lines such as 3201 cells, MCH5-4DL, and 104-C1DL, have been used for propagation of CD134-independent isolates.[13,25,42,211] Feline IL-2-independent lymphoid cell lines (FL-4 cells) chronically infected with FIV isolate FIV-Petaluma were also generated for production of virus for use in whole killed virus vaccines and diagnostics.[242]

Assays used to confirm virus replication and production in vitro have included FIV p24Gag antigen capture ELISAs, RT assays, indirect immunofluorescence or immunocytochemical assays for viral structural proteins, and PCR assays for viral nucleic acids. Quantitative real-time PCR assays for both FIV RNA and DNA have been developed for quantitation of viral nucleic acids of different isolates in either cell culture supernatants, plasma, or cells.[11,157,190,212,309,313–316] The appearance of virus-induced cytopathic effects (CPE) consisting of syncytium, giant cell formation, and cell lysis (Table II) in feline PBMC and T-cell lines may be PBMC donor and FIV isolate-dependent and has proven less dependable for use as a marker of virus infection in these cell types.[1,212,317,318] In contrast, infection of adherent cell lines CrFK and G355-5 and IL-2-independent lymphoid cells lines (3201, MCH5-4DL, 104-C1DL) with CD134-independent FIV isolates may result in prolific CPE that is most consistently observed in CrFK cells and which may serve as a indicator of infection for these isolates.[13,32,211,238,241,242,248] CD134-independent FIV strains have been selected by extensive cell culture passage of biological isolates and mimic CD4-independent HIV-1 isolates that are also extremely cytopathic and efficient for replication in established T-cell lines in vitro.[73]

Virus Localization in the Host

Most knowledge regarding acute FIV infection and virus localization and dissemination has been derived from experimental inoculation studies using both biological and molecularly cloned FIV isolates. Factors that may affect virus localization and distribution during the acute phase of infection are virus pathogenicity, virus tropism, titer of virus inocula, presence of virus-infected cells in the inocula, route used for virus infection, and age of host. Experimental studies testing highly pathogenic and attenuated FIV isolates, viruses inoculated by parenteral or mucosal routes, and cell-free or cell-associated virus inocula have been reported. However, careful examination of effects imposed by each of these variables on viral distribution and ultimate disease, as well as mechanisms for these effects, has yet to be described. Regardless, reports so far describe a virus distribution pattern for pathogenic FIV isolates in vivo that is similar to that reported for HIV-1 (Table III).[319–321]

Early published experimental infection studies designed to examine FIV localization in vivo used either intraperitoneal, intravenous, intramuscular,[4,7,186,200,201,322–324] intrathecal, or bone marrow inoculation[198] of specific pathogen-free cats with various FIV isolates. Blood and tissues harvested from infected cats were assayed for infection by virus isolation from PBMC, viral nucleic acid detection by PCR or in situ hybridization, or viral antigen detection by immunocytochemical analyses. Later reports described experimental studies testing virus localization after virus mucosal delivery by either vaginal, rectal, or oral/nasal routes,[16,18,21,23,307,309,325] in addition to either intraperitoneal or intravenous routes[193,197,202,207,326–328] or intracranial injection.[329,330] Virus localization in systemic lymphoid and central nervous system (CNS) tissues after FIV proviral DNA inoculation has also been examined.[331,332] Collectively, these studies revealed localization of virus in PBMC and plasma, peripheral, and systemic lymphoid tissues, small and large intestinal tracts, and CNS tissues within 10 to 21 days after inoculation of the host, regardless of route of infection tested. In fact, one report described virus detection in gastrointestinal mucosa and associated lymphoid tissue by 1 to 3 days after oral/nasal infection and rapid dissemination of virus to systemic lymphoid tissues, bone marrow, and PBMC within 7 to 10 days after infection.[23] Similarly, virus was detected in vaginal mucosa and spleen within 3 days after vaginal delivery of virus.[23] However, virus infection of vaginal mucosa has been examined in very few studies and was observed only in those testing vaginal delivery of specific virus isolates (FIV-B-2542 and FIV-PPR)[16,23,309] and was not observed after vaginal infection with other FIV isolates[18,23] or after intravenous virus infection.[207] Similar to observations for the SIV animal model,[299,333] experimental FIV infection by mucosal routes generally required higher titered virus inocula than

required by parenteral injection,[16,309] and one study reported a higher efficiency for infection with cell-free virus inocula compared to cell-associated virus.[334] Virus has also been detected in other nonlymphoid organs including liver and kidney.[4,200,207,332] Importantly, FIV infection of the CNS has been well documented[4,198,200,202,255,294,324,335,336] and utilized as an animal model for HIV-1-induced neurologic deficits.[210,324,329,330,332,337–344]

Kinetics of virus emergence in blood and individual tissues has varied depending on the route of inoculation, virus strain, and infectious titer of virus inocula. Virus load in peripheral blood based on either virus isolation from PBMC or plasma viral RNA quantitation, may peak anywhere from 14 to 56 days after experimental infection.[4,11,21,191,200] Significant virus loads during the acute stage of infection may also be observed in both peripheral and systemic lymph nodes, gastrointestinal tissues (predominantly submucosa and lamina propria), spleen, thymus, and bone marrow[4,23,185,193,200,207] and will precede the appearance of peak viremia in peripheral blood. These findings generally mimic virus distribution during acute infection for both HIV-1 and pathogenic SIV isolates.[319,321,345] It is important to note the similar robust virus replication in gastrointestinal mucosal lymphoid tissue (GALT) observed during early time points of FIV,[4,200,207] SIV,[346,347] and HIV-1[348–350] infection. Although virus has been detected in the CNS during both acute and chronic phases of infection, quantitative data describing virus load in specific CNS tissues has been scarce. Virus loads in the PBMC and plasma generally decrease to lower set points during chronic asymptomatic infection, although this finding may be variable and dependent on virus strain pathogenicity[17,212] and has not been as well defined for experimental FIV infection as reported for experimental SIV infection of rhesus macaques. FIV loads in individual tissues at sequential time points spanning acute, chronic asymptomatic, and terminal AIDS stages of disease also have not been well examined.

Similar to findings for HIV-1 and SIV infection,[319–321,345,351] cellular subsets targeted during the acute stage of FIV infection have included CD4 T-cells, monocytes, macrophages, mucosal dendritic cells, mature and immature thymocytes, brain microglia and lymph node follicular dendritic cells (FDC) (Table III).[4,193,194,197,199,201–204,207,208,307,327,338,352,353] As discussed in a previous section of this chapter, FIV differs from HIV-1 by exhibiting a broader tropism in vivo that also includes megakaryocytes,[323] CD8 T-cells and B-cells,[186,193,197,201,307] although a few reports have described either HIV-1 or SIV infection of CD8 T-cells.[354,355] The variation in observations from different reports regarding the frequency and stage of infection for which virus or viral nucleic acid are detected in macrophages, CD8 T-cells, and B-cells may relate to differences in virus isolates, routes used for virus infection, age of the host, and virus detection assays. However, several studies report that either T-cells, or specifically CD4 T-cells, in both blood and

tissues, are the predominant cell type harboring virus during the acute stage of infection. Virus load in CD4 T-cell populations decrease over time as the host progresses into chronic infection,[4,194,201,207,208,307,352] while virus loads in macrophages and B-cells appear to increase. The relationship of stage of infection to virus load in other specific cell populations, including CD8 T-cells, mucosal dendritic cells, lymph node follicular dendritic cells, thymocytes, cells within the CNS, and other nonlymphoid tissues, has received little examination so far, although one report revealed a decrease in FIV-infected CD8 T-cells in lymphoid tissues by 10 weeks after infection.[307] A recent report described FIV infection in vivo of CD4+CD25+ T-cells, a cell population reminiscent of immunosuppressive CD4 T regulatory (Treg) cells.[356] This CD4+CD25+ Treg cell population isolated from FIV-infected cats was shown in another recent report to coexpress costimulatory molecules B7.1, B7.2, and CTLA4 and to be anergic and resistant to clonal deletion.[357] These findings warrant further investigation and suggest that CD4+CD25+ Treg cells may serve as an important long-lived reservoir for latent FIV in lymphoid tissues and currently are under examination as potential reservoirs for HIV-1 infection in vivo.

Clinical Disease

FIV infection results in progressive impairment of the immune system, including loss of CD4 T-cells, inverted CD4:CD8 ratios, heightened susceptibility to infectious agents, disruption of immune cell function, and deterioration of major lymphoid tissues and organs of the hosts.[14,17,358] Observations from studies involving either experimental or natural infections,[2,7–9,13,359–363] show that FIV disease course is very similar to that induced by HIV-1 infection and can be similarly divided into four to five stages based on type and severity of the clinical signs of infection. These stages of infection have been described as acute or primary, chronic asymptomatic, persistent generalized lymphadenpathy (PGL), AIDS-related complex (ARC), and feline AIDS (FAIDS). Notably, FIV and HIV-1 infection are usually associated with a prolonged asymptomatic phase that can last 10 years or more for infected humans and cats, and which constitutes most of the lifetime of the infected cat.[360,364] In contrast, pathogenic SIV isolates induce an accelerated progression of immunodeficiency, resulting in death within 2 years of infection of rhesus macaques with a relatively short or absent asymptomatic phase of infection.[365,366]

The acute phase begins 1 to 4 weeks after FIV infection and may span a time period of 2 to 6 months. This stage of infection is characterized by a transient peak in peripheral blood (plasma and PBMC) virus load that is accompanied by a precipitous decline in CD4 T-cell counts and CD4:CD8 T-cell ratios. Depending on pathogenicity of virus isolate and age of the host, clinical and hematological abnormalities may include generalized

lymphadenopathy, mild pyrexia, dullness, depression, anorexia, and neutropenia. Similar to findings for HIV-1 infection, FIV pathogenicity and clinical prognosis correlate to virus replication and load and to severity of clinical signs and hematologic deficits exhibited during the acute phase of infection.[11,21,185,191,192,212,313,367,368] Another property shared by FIV and HIV-1 acute infection[369,370] relates to the effect of age of the host on severity of clinical and hematologic disease at the time of infection. Multiple studies have shown that severity of primary phase FIV-induced disease was increased for neonatal kittens experimentally infected at birth.[190,318,371–374]

Primary FIV infection recedes as the host generates virus-specific immune responses and as virus loads decrease. Concordant with emergence of antiviral immune responses and reduced peripheral blood virus loads is an increase in CD4 T-cell count and CD4 : CD8 T-cell ratio, although peripheral blood CD4 T-cells counts do not usually return to preinfection concentrations. The infected cat enters a relatively asymptomatic phase of FIV infection where control of virus load by host immune responses is presumed and may last for 5 to 6 years or for a significant proportion of the remaining life span of the cat. However, long-term observation of both experimentally and naturally infected cats has shown that peripheral blood CD4 T-cell counts slowly but progressively decrease during the asymptomatic phase and that clinical disease eventually becomes apparent.[8,9]

Careful observation of infected cats over time may reveal the reappearance of PGL as an early manifestation of clinical disease that is associated with vague signs of disease including recurrent fevers and weight loss.[9,13–15] After the appearance of PGL and other vague clinical signs, infected cats generally progress into ARC, a phase characterized by the development of chronic persistent infections with pathogens that are usually self-limiting and involve the oral cavity, upper respiratory tract, ocular tissues, skin, and other body sites. Progression from ARC to FAIDS may be distinguished by infections with opportunistic pathogens, severe wasting, neoplastic disorders including non-T-cell lymphomas, neurologic disease, leukopenia, and anemia. Virus load increases and severe depletion of peripheral blood CD4 T-cells are observed. Survival time is usually less than a year after the onset of FAIDS.

FIV Immunodeficiency and Pathology

A gradual but progressive CD4 T-cell depletion that mimics primary immune deficiency observed in HIV-1 AIDS is the hallmark of immunodeficiency associated with both experimental and natural FIV infection in cats.[5–8,10,11,21,185,190,192,193,200,212,361,375] CD4 T-cell subset depletion has been observed in peripheral blood and lymphoid tissues including thymus,[22,193,374] during both early and chronic stages of infection (Table III). An increase in CD8 T-cell concentration is frequently associated with reduc-

tion in CD4 T-cell counts and either contributes to, or largely accounts for, lower CD4/CD8 T-cell ratios observed during acute and chronic infection with either FIV or HIV-1. This elevation in the CD8 T-cell count is due to an increase in a $CD8\alpha^{+}\beta^{low}$ subset that is concurrent with a reduction in $CD8\alpha^{+}\beta^{high}$ of CD8 T-cells and involves lymphocyte populations from peripheral blood, lymph nodes, and thymus.[212,334,374,376–379] A similar expansion of an $CD8\alpha^{+}\beta^{low}$ T-cell subset has also been observed in HIV-1 infection.[380,381] Furthermore, this cell population has been shown to express markers associated with lymphocyte activation and adhesion and to exhibit antiviral activity[311,376,379] comparable to a noncytolytic CD8 antiviral activity previously described for HIV-1 infection.[382,383]

FIV-induced immunodeficiency is characterized by other defects that are similar to those described for HIV-1 infection and include reduced proliferative T-cell responses to mitogens, dysregulation of cytokine networks, and humoral immune response deficits.[14,17] Several studies have revealed significantly reduced mitogen-induced blastogenic responses from T-cells isolated from cats during acute and chronic FIV infection,[8,309,384–387] a finding that may result from virus-associated defects in T-cell growth and proliferation, as well as defects in cell surface expression of receptors required for transmission of antigen/mitogen signals.[388] Although cytokine responses in different lymphoid tissues harvested from cats acutely infected with FIV have been shown to be heterogenous,[389] findings from a number of reports suggest that dysregulation of cytokines such as IL-10, tumor necrosis factor (TNF)-alpha, IL-6, IL-1, and interferon gamma may play a role in FIV-induced immune deficiency.[307,389–395] Deficits in generation of antibody responses to multiple antigens and specifically to T-cell-dependent immunogens have also been reported for FIV-infected cats.[14,396–398] Bone marrow abnormalities that may result in neutropenia, leukopenias, and pancytopenias,[323,327,363,399] as well as deficits in neutrophil and monocyte/macrophage function, may further impair immunologic function in FIV-infected cats.[203,400–402]

Histologic lesions associated with FIV infection are predominantly localized to lymphoid tissues including GALT for both early and later stages of FIV infection, although severity of acute stage lesions is dependent on virulence and titer of the infecting FIV isolate. Lymph node abnormalities during the acute stage of infection include a mixture of follicular hyperplasia and lymphoid depletion resulting in loss of lymph node architecture and medullary plasmacytosis.[3,7,12,22,190,200,322,353,403] Lymphoid hyperplasia has also been observed in other lymphoid tissues including spleen, bone marrow, and mucosal-associated lymphoid tissue. Several studies have shown the thymus to be a primary target during early FIV infection, with pathologic changes that include thymic atrophy, thymitis, medullary B-cell hyperplasia, and cortical involution.[7,22,185,190,193,200,328,374] Lesions in the gastrointestinal tract have frequently been observed and include severe inflam-

mation, necrosis, and villous atrophy.[3,7,22,200,322] Brain and lung are nonlymphoid tissues that also frequently exhibit pathologic changes. CNS lesions have consistently included gliosis, glial nodules, and perivascular cellular infiltrates and less frequently included inflammatory changes associated with encephalitis and meningitis and neuronal abnormalities such as neuronal stress and neuronal satellitosis.[198,200,324,329,330,332,339,342,344] Giant cell formation is a frequent lesion in SIV and HIV-1-associated neuropathology but has only rarely been observed by histologic examination of CNS tissues from FIV-infected cats.[342,404] Pulmonary lesions associated with FIV infection have consisted of inflammatory infiltrates suggestive of pneumonia.[200,322,332]

Lymphoid depletion and involution within multiple lymphoid tissues have been dominant and consistent findings in tissues harvested from cats during the later stages of FIV infection.[3,12,322] Neoplastic lesions, including B-cell lymphomas and other sarcomas, have also been frequently observed in later stages of both experimental and natural FIV infection.[8,13,405–409] All together, these histologic lesions of lymphoid hyperplasia frequently characterized as B-cell hyperplasia mixed with severe lymphoid depletion, as well as thymic atrophy and the severe inflammatory lesions observed in the gastrointestinal mucosa, mimic pathological changes reported for both HIV-1 and pathogenic SIV[364,410–413] and further support the use of the FIV animal for examination of HIV immunopathogenesis.

Mechanisms for either FIV or HIV-1-induced CD4 T-cell depletion and dysfunction in vivo most likely involve multiple processes such as direct cytolysis from virus infection or from virus-specific immune responses, and indirect strategies including chronic immune activation, cellular dysregulation, and inappropriate killing of uninfected bystander cells.[364,414] In vitro syncytia formation of FIV-infected cells has been well documented and is due to viral envelope fusion with cellular membranes and associated with specific cell types and FIV strains.[224,238,245,248,311,415–417] Programmed cell death or apoptosis has been hypothesized as one of several important mechanisms involved in FIV/HIV-1 immunopathogenesis based on observations of apoptotic cells in virus-infected cell cultures[418–421] and in PBMC[422–425] and lymphoid and thymic tissues[22,425,426] isolated from FIV-infected cats. Recent reports have shown that binding of cell surface chemokine and virus receptor CXCR4 by FIV TM may trigger apoptosis.[137,427] Although some recent reports suggest that the Fas-TNF-α receptor pathway may be important for FIV-induced apoptosis, mechanisms by which FIV uses this pathway for apoptosis are not well understood and are currently under investigation.[419,425,428–431]

A second probable pathway for FIV-induced apoptosis involves the B7-CTLA4 pathway. Data from recent reports indicate that PBMC and lymph node lymphocytes isolated from FIV-infected cell cultures in vitro or freshly isolated from FIV-infected cats are down-regulated for expression of

costimulatory molecule CD28. These cells are instead up-regulated for expression of T-cell costimulatory molecule CTLA4, a cell surface molecule expressed on activated T-cells and reported to induce anergy in activated T-cell subsets.[432–435] Furthermore, these reports presented flow cytometric analyses that verify induction of apoptosis in FIV-infected cultures by T-cells that coexpress CTLA4 and B7 cell surface molecules and suggested that CD4 T-cell depletion and lymph node apoptosis in vivo may partially result from chronic B7-CTLA4-mediated T-cell interactions.[357,432,434] Similarly, reduced CD28 expression and increased CTLA-4 expression have been observed in HIV-1 infection[436–440] and hypothesized to promote immune hyporesponsiveness and apoptosis through binding of CTLA4 with B7 costimulatory molecules. Although mechanisms by which FIV infection induces an increase in B7+CTLA4+ T-cell populations in vivo are not well understood, chronic immune activation in the virus-infected host has been hypothesized as one possible etiology for this effect. These observations further illustrate similarities in FIV and HIV-1-associated disease and potential value of the FIV animal model for in vivo studies focused on mechanisms of immunopathogenesis of lentivirus-induced acquired immunodeficiency.

Immune Responses

Emergence of cellular and humoral host immune responses during primary infection with either FIV or HIV-1 coincides with reduction in peak peripheral blood virus loads.[14,17,24] These responses are thought to be important for controlling virus replication during acute and chronic stages of infection based on CD8 T-cell and B-cell depletion studies involving SIV-infected rhesus macaques.[441–443] Regardless of robust virus-specific immune responses, most infected hosts fail to eliminate the virus, leading to prolonged clinical latency, eventual immunologic exhaustion with subsequent increasing virus loads, and AIDS. Currently, factors responsible for this inability of host responses to effectively clear virus during primary infection are not well understood. Identification of such factors will be necessary for understanding immunopathogenesis of lentiviral infections and design of vaccines capable of inducing sterilizing immunity.

Based on experimental inoculation studies, antibodies to viral proteins SU gp95, CA p24, and MA p14 are the first to appear in serum, usually within 2 to 4 weeks after infection, and are quickly followed by the appearance of antibodies to TM gp40, Gag precursor p50, and reverse transcriptase.[14,28,444,445] Lentiviral Gag proteins are highly expressed immunogenic proteins and FIV-infected cats typically exhibit high titers of antibody specific for viral CA p24. Four B-cell epitopes have been mapped for FIV CA using mouse monoclonal antibodies.[446] However, evidence that CA-specific antibodies function in either FIV or HIV-1 clearance has been scarce. Inter-

estingly, a recent examination of serological responses in HIV-1-infected patients undergoing prolonged antiretroviral therapy with structured treatment interruptions (STIs) showed that kinetics of CA-specific antibody responses revealed clear differences in patients' immune functions. Patients exhibiting rapid and large increases in CA antibody responses also experienced significantly decreased viral set points.[447] Antibodies specific to CA have not been shown to express significant antiviral activity,[448] but may instead reflect enhanced virus-specific CD4 helper activity that is predictive of the capabilities of the host's antiviral immune responses.[447] Similar careful analyses of FIV Gag antibody responses have not been reported but may be useful for characterizing the value of these responses as markers that aid in distinguishing protective from nonprotective vaccine-induced immune responses.

Only antibodies that bind to surface domains of the envelope are thought to exhibit virus neutralizing activity,[449] and accordingly, identification of envelope B-cell epitopes is critical to the characterization of potentially protective host immune responses. Multiple reports have identified the V3 region of SU, carboxy terminal of SU, and a highly conserved PID located within the ectodomain of TM as major immunodominant domains of FIV envelope (Figure 3).[14,133,252,254,450–454] Four or more linear B-cell epitopes have been mapped within the V3 domain of SU,[254,453] and other SU epitopes are localized within V4 and V5 domains.[452,455,456] B-cell epitopes within extracellular TM include the PID previously discussed, a second domain within the extracellular membrane-proximal domain of TM that is downstream of the PID, and a third domain within the intracytoplasmic region of TM.[452,453] In contrast, the tryptophan-rich motif within the extracellular domain of TM that functions in virus fusion and infectivity was not found to be immunogenic.[457]

Although the V3 immunodominant domain is the only FIV Env determinant consistently shown to induce antibodies capable of neutralizing virus in vitro,[252–254] determinants within domains V4 and V5, including residues 481 and 551, were also shown to confer broad neutralization resistance (BNR) in primary isolates passaged in vivo (Figure 3).[455,458–462] The remaining FIV envelope linear epitopes mapped by binding assays are most likely inaccessible for neutralization due to the complex oligomeric structure and extensive glycosylation of native FIV SU, a property shared with HIV-1 SU.[449,454,463] Expression of both neutralizing epitopes and tropism determinants by the FIV hypervariable V3 domain is an important function also described for the V3 domain of HIV-1 surface glycoprotein.[14,17,24,73] It is also significant that FIV SU linear epitopes, capable of inducing neutralizing antibodies, are located within hypervariable regions of envelope that may change in response to selective pressures in vivo.[141]

Importantly, an epitope encoded within the FIV extracellular membrane-proximal domain of TM (designated as the TM3 epitope) is

similarly positioned to that of a HIV-1 epitope (2F5), which is recognized by an extremely potent broadly neutralizing HIV monoclonal antibody (Figure 3).[463,464] Although feline antisera raised against this FIV TM epitope was not shown to exhibit virus neutralizing activity in vitro,[454] a peptide vaccine based on this epitope was shown to be capable of inducing partial immunity to FIV challenge.[465] These observations suggest that this extracellular membrane-proximal TM3 domain may encode a neutralizing epitope conserved across multiple lentiviruses and may support further investigation of this TM determinant in FIV vaccine design.

Neutralizing antibodies emerge in the host as peak peripheral blood virus loads decline during primary infection and are thought to contribute to control of virus load and replication in lentivirus infections including HIV-1, SIV, and FIV.[466] Control of infection by neutralizing antibody is also based on CD8 T-cell and B-cell depletion studies of rhesus macaques infected with either SIV or chimeric SIV/HIV-1 (SHIV) isolates[441,443,467] and on reports describing successful passive immunization of either neonatal kittens or neonatal macaques with hyperimmune serum.[468,469] However, despite apparent successes observed recently with therapeutic administration of exogenous highly potent neutralizing antibodies in nonhuman primate animal models,[470] antibodies induced in the host by virus infection have been consistently ineffective in virus clearance, particularly within lymphoid tissue reservoirs. Furthermore, FIV and HIV-1 assay systems using established cell lines, rather than primary lymphocytes for virus infectivity or highly passaged viruses compared to primary strains directly isolated from infected cats, generate significantly different data regarding neutralization activity for identical serum samples.[454,471–473] Notably, previous studies have shown that both HIV-1 and FIV primary isolates are resistant to neutralization by autologous sera, especially when primary PBMC are used to assay infectivity.[471,474] However, more recent findings regarding HIV-1 neutralizing antibody biology[466] and use of more sophisticated approaches for analysis of antibody activity have triggered renewed interest and support for investigation of humoral immune responses in virus control during acute and chronic HIV-1 infection and for HIV-1 vaccine design. Similar investigations seem warranted in the FIV animal model, especially for examination of antibody kinetics and specificity associated with mucosal versus parenteral routes of virus exposure[475] and for investigation of epitopes conserved across different virus clades as vaccine immunogens.[476,477]

Strong supporting data exist for CD8 T-cell-mediated suppression of virus load in both HIV-1-infected patients and SIV-infected rhesus macaques.[24,383,442,478,479] Investigation of FIV-specific cellular immune responses has previously been restricted by a deficiency of key feline-specific reagents, including antibodies specific for feline cell surface markers and cytokines, as well as a lack of knowledge of FIV T-cell epitopes. However, recent peptide mapping of FIV-specific T-cell epitopes[480,481] and

established assays for FIV-specific CD4, CD8, and cytotoxic T-cell (CTL) activity have now permitted some characterization of cellular immune responses induced by either virus infection or vaccination.[308,481–491] Furthermore, a report showing that adoptive transfer of blood cells isolated from FIV-vaccinated cats induced resistance in MHC-matched recipient cats to FIV challenge infection, provides some evidence of the importance of virus-specific cellular responses in virus clearance.[488]

Longitudinal examinations of cats experimentally infected with FIV reveal virus-specific CTL activity in PBMC within 2 to 7 weeks after infection, time points in primary infection that coincide with rising virus loads and are similar to those reported for CTL emergence in HIV-1 infection.[24,475,482,490,492] Virus-specific CTL activity has also been detected in lymphocytes isolated from lymphoid tissues including systemic and peripheral lymph nodes and spleen during the primary phase of infection.[475,482,492] Assay for CTL activity during chronic stages of infection at 47 and 127 weeks after virus exposure demonstrated persistence of cellular responses, although detection of antiviral CTLs were more consistent from lymphoid tissues compared to peripheral blood.[482,492] Activity specific to FIV Gag rather than Env was more consistently observed in FIV-infected cats described in these reports. However, currently described protocols testing for FIV-specific CTL by chromium release assay are restricted by use of only two immunogens provided by vaccinia recombinant viruses, one of which expresses a Gag protein derived from isolate FIV-GL14 (subtype A) and the other expressing an Env protein encoded by FIV-Petaluma (also subtype A). Therefore, lower Env-specific CTL activity may be partially attributed to deficient assay detection due to variability within recently mapped Env-encoded T-cell epitopes,[481] since cats described in these reports were experimentally infected with FIV isolates distinct from FIV-Petaluma. Measurement of CTL responses to variable proteins such as Env will require virus-specific reagents for optimal assay sensitivity. Characterization of FIV-specific CTL responses will also call for assay of activity against other viral proteins including RT, Rev, and Orf-A. Although limited mapping of Env T-cell epitopes was previously reported,[493] a recent report[481] described peptide mapping of FIV T-cell epitopes across all FIV genes using a feline interferon gamma ELISpot assay.[489] These newly identified epitopes will facilitate use of peptides for other FIV CD8 assays and further characterize FIV CD8 responses to different FIV isolates, as well as examine the occurrence of FIV CTL epitope escape variants, a well-described trend in SIV and HIV infection.[494–496] Another unique test for FIV-specific CTL activity involved assay of perforin expression[488,497] and may also be useful for future examination of FIV-specific CD8 immune responses.

Interestingly, a $CD8\alpha^{+}\ \beta^{low}$ T-cell subset associated with antiviral activity in FIV-infected cats has actually been more carefully examined than the topic of virus-specific CTL activity. This cell population has also been

described for HIV infection[380,381] and was initially distinguished for its expansion in peripheral blood during the primary phase of FIV infection and for a capacity to suppress virus replication in cultured PBMC by release of a soluble factor in vitro.[308,334,377,378,486,498] Significantly, appearance of this cell population correlated with reduction of peak PBMC-associated virus, suggesting possible antiviral activity in vivo as well as in vitro.[308] Other FIV infection studies have confirmed an expansion of this $CD8\alpha^{+}\beta^{low}$ T-cell subset[212,374] and also reveal expression of lymphocyte activation and adhesion markers by this subset.[376,499] However, these studies also show conflicting data on the issue of a restriction of antiviral activity to the $CD8\alpha^{+}\beta^{low}$ subset with some data suggesting that both $CD8\alpha^{+}\beta^{low}$ and $CD8\alpha^{+}\beta^{-}$ subsets are capable of noncytolytic antiviral activity.[311,379,492,499–502] A lack of agreement is also apparent among studies regarding correlations between CD8 T-cell noncytolytic antiviral activity and either CD4 T-cell counts, clinical disease in vivo, distribution patterns of this cell subset in blood and lymphoid tissues, or persistent expansion of the $CD8\alpha^{+}\beta^{low}$ subset throughout the course of virus infection.[212,308,376,379,503] More recent reports indicate that CD8 antiviral activity production could be enhanced or induced in vitro by exposure to either virus-infected cells or to cells expressing an irrelevant antigen, but is not induced by mitogen activation.[334,502,504] These conflicting reports indicate that additional studies using standardized methodologies will be needed for a more precise definition of this CD8 T-cell antiviral activity. However, data generated from almost all studies reported so far suggest that this noncytolytic CD8 antiviral activity is reminiscent of a still undefined secreted CD8 antiviral factor (CAF) previously described for HIV-1 infection.[382,383] Noncytolytic CD8 antiviral factors associated with both HIV-1 and FIV infections, although not clearly defined, have been hypothesized to be associated with the innate immune system. Multiple factors proposed in previous reports to represent human CAF include human β-chemokines RANTES, MIP-1α, MIP-1β, MCP-1, SDF-1, and alpha defensins.[478] These proteins, however, do not meet the criteria of CAF definition,[478] either due to their biochemical nature or because they are not exclusively expressed by CD8 T-cells. Observation of CD8 T-cell antiviral activity in both HIV-1 and FIV infection confirms the importance of this host immune response to lentivirus infection and provides another opportunity for use of the FIV animal model for assessment of HIV-1 immunopathogenesis.

6. FIV VACCINE DEVELOPMENT

A safe efficacious vaccine that prevents the spread of HIV will be essential to arresting the spread of the AIDS epidemic. Studies with nonhuman primates and SIV and chimeric SHIV isolates have demonstrated that

live-attenuated viruses are highly effective;[505–507] however, such vaccines maintain a low level of pathogenicity.[508–510] Other vaccine trials in the rhesus macaque animal model have described noninfectious DNA vaccines that control viremia and suppress clinical disease but do not induce sterilizing immunity against SIV/SHIV infection.[511–513] FIV vaccine research and development have been fairly well supported due to the value of this animal model for HIV vaccine development and to the significance of FIV as a natural pathogen in cats. A wide variety of vaccine approaches have been examined, although efforts have concentrated on particular vaccine methods including whole killed virus (WKV)-based vaccines, DNA vaccines, and viral protein subunit vaccines.[514] A commercial FIV vaccine (Fel-O-Vax FIV, Fort Dodge Animal Health), containing whole killed viruses representing two distinct FIV subtypes, was approved by the USDA in 2002 for use in the domestic pet cat population and is one of only two commercial lentiviral vaccines currently in use, including a live attenuated EIAV vaccine widely used in China.[515,516] However, use of this FIV WKV vaccine is still not widely accepted due to significant issues including interference of vaccine-induced antiviral antibodies with commercial FIV diagnostic assays[517] and the potential of vaccine-induced enhancement of virus infection.[485,518–520]

FIV WKV and Fixed-Cell Vaccines

FIV immunization studies based on conventional WKV or fixed virus-infected cell (FC) vaccines have produced a diverse array of experimental findings. Vaccine efficacy for different and identical FIV WKV-based vaccines has varied greatly, most likely due to modifications of vaccine inactivation procedure, producer cell types used, vaccination schedule, vaccine adjuvants, vaccine doses, routes of challenge, and variability in virulence or subtypes of challenge FIV isolates.[514] WKV-based vaccine studies have reported both complete or partial protection against challenge with homologous and sometimes heterologous FIV isolates and have also described vaccine-induced enhancement of FIV infection. Interestingly, enhancement of challenge virus infection has been more frequently associated with FC vaccines prepared with autologous feline lymphocytes.[485,518,519] In contrast, FC and WKV vaccines based on virus-infected cell lines have proven efficacious, although protection may be considerably reduced against challenge with either heterologous or more virulent FIV isolates.[487,521–526] The current commercial FIV WKV vaccine is composed of two isolates including FIV-Petaluma subtype A and FIV-Shizuoka subtype D, specifically to broaden virus-specific immune responses, and has demonstrated improved protection against multiple subtypes when compared to single subtype WKV vaccines.[487,527] Use of a FIV WKV vaccine as a booster to a priming

immunization with a recombinant canarypoxvirus (ALVAC)-based FIV vaccine also improved protective responses against challenge with isolates distinct from the vaccinating strains.[528] Duration of WKV and FC vaccine-induced protection has been another concern, with some vaccine studies revealing a breakthrough in vaccine-induced protection a year after vaccination despite boostering the primary immunization.[529,530] Another concern relates to differences in WKV or FC vaccine-induced protection observed against similar challenge viruses but delivered by different routes of exposure, including parenteral and mucosal delivery.[526] Collectively these findings suggest that WKV vaccines demonstrate significant potential for development of lentivirus vaccines, but that multiple issues including WKV vaccine-induced enhancement still require attention for achievement of optimal protection.

Conclusions regarding neutralizing antibodies as immune correlates of vaccine protection frequently varied in early reports describing WKV and FC FIV vaccines.[525,531–533] Nonetheless, other early studies, as well as more recent reports, present findings that suggest a correlation between appearance of virus neutralizing antibodies and WKV and FC vaccine-induced protection.[487,522,523,526,534,535] WKV and FC vaccines have also induced potent cellular immune responses that are thought to be important correlates of protection.[484,487,497,535–537] The current commercial FIV WKV vaccine containing inactivated whole viruses of subtypes A and D, elicited strong cellular responses against both vaccine strain viruses and moderate neutralizing antibody activity, particularly when commercial Fort Dodge vaccine adjuvant FD-1 was supplemented with human IL-12. In fact, the commercial dual subtype FIV WKV vaccine administered with human IL-12 provided broad protection against homologous and heterologous virus challenges containing in vivo-derived FIV inoculum.[487,497] However, the actual contribution provided by cellular and humoral immune responses to protection induced by this vaccine approach is still not clear and may ultimately require immune cell depletion studies for determination. Despite reports of FIV WKV and FC vaccine efficacy, acceptance of this vaccine approach in the veterinary community is restricted by concern of vaccine antibody interference with FIV antibody-based diagnostics.[517] Furthermore, exploration of WKV vaccines in the SIV and SHIV animal model has been extremely limited and was discouraged by early studies showing SIV WKV vaccine-induced protection resulted from immune responses to cellular antigens in vaccine preparations, rather than to viral antigens.[538,539] However, renewed enthusiasm for vaccines that induce strong neutralizing antibody responses[466] has lent increased support for multiple modality vaccine approaches that include WKV as a component. Inclusion of a WKV vaccine as a component is expected to enhance and broaden virus-specific humoral immune responses induced by other vaccine components such as

live viral vectors and DNA.[540,541] The sizable body of data already generated for FIV WKV and FC FIV lends strong support for use of the feline animal model for further investigation of WKV and FC vaccine approaches for HIV-1.

FIV Subunit and Peptide Vaccines

Various FIV subunit vaccine approaches have been tested including SU (primarily V3 and C2 epitopes), TM and Gag peptides,[465,480,483,493,524] recombinant SU proteins generated from bacterial expression plasmids administered as single immunogens,[542,543] or in combination with recombinant SU proteins expressed from vaccinia[462,472] or baculovirus vector systems,[544,545] and immunoaffinity-purified SU from FIV-infected cell lysates.[531] Absence of protection against FIV challenge, observed with many of these frequently highly immunogenic subunit approaches, recapitulates findings reported for subunit vaccine approaches in the nonhuman primate model.[546] However, partial protection against challenge infection was revealed in a few reports describing immunization with either immunoaffinity-purified SU[531] or SU proteins conjugated to autologous erythrocytes via biotin-avidin-biotin bridges,[547] although immune correlates of protection were not determined. As described in the section on immune responses, a FIV peptide vaccine based on an extracellular TM3 epitope, similarly positioned to that of the highly neutralizing HIV-1 2F5 epitope, was also shown to be capable of inducing partial immunity to FIV challenge.[465] Vaccine-induced enhancement of FIV challenge virus infection has also been observed, with a subunit approach consisting of coimmunization of a bacterially expressed full-length envelope protein with either SU or SU-TM vaccinia recombinant proteins.[462] However, coinoculation of recombinant SU proteins, with a plasmid expression vector encoding the FIV NC protein, abrogated vaccine-induced enhancement of challenge infection that was observed for vaccination with recombinant SU alone[543] and actually improved vaccine efficacy in another study.[545] Loss of vaccine-induced enhancement and increased vaccine efficacy associated with coinoculation of NC expression plasmid with recombinant SU were hypothesized to result from a plasmid DNA adjuvant effect based on altered cytokine expression profiles and the absence of FIV NC-specific immune responses associated with this approach. All together, these data do not support use of vaccines consisting of single viral protein subunits. Instead, these findings suggest that FIV may provide another vaccine model for testing peptides encoding broadly reactive neutralizing epitopes as components of a multiple modality vaccine that also includes immunogens for induction of cellular responses, a balanced vaccine approach currently of interest in HIV-1 vaccine design.[466,548]

FIV DNA Vaccines

Assessment of other vaccine approaches, including DNA vaccines, attenuated virus vaccines, and live attenuated viral or bacterial vectors for expression of FIV antigens, is still somewhat limited at this time. FIV DNA vaccine approaches, based on deletion mutants of FIV provirus plasmids, have shown considerable efficacy when challenged with less virulent FIV isolates.[174,314,331,549] DNA vaccines, including either defective proviruses or SU and TM expression cassettes along with feline cytokine expression vectors, have shown particular promise in limited studies.[314,549–551] Extremely low or absent humoral immune responses observed with FIV DNA vaccines may partially result from failure to incorporate codon optimization of viral structural genes encoded by these vaccine plasmids, a process found critical for improved immunogenicity of HIV-1 DNA vaccines that do not coexpress the viral Rev protein.[552] DNA vaccine efficacy has been observed despite low antibody responses and may be attributed in some measure to strong virus-specific CTL responses,[553] despite the lack of a clear correlation between measured CTL activity and DNA vaccine-induced protection.[314] Unfortunately, DNA vaccine-induced protection has proven to be reduced or negligible against more pathogenic isolates.[314,523] Moreover, DNA vaccines encoding envelope genes used without cytokine adjuvants have been associated with enhancement of challenge virus infection.[554–556]

FIV Vaccines Using Live Viral and Bacterial Vectors

Mixed results regarding efficacy have been reported for the few FIV vaccines utilizing either live viral or bacterial vectors. Immunization of cats with a replication-defective adenovirus type 5 expressing FIV Env was poorly immunogenic and failed to induce protection against FIV challenge.[557] A vaccine composed of Venezuelan equine encephalitis (VEE) virus replicon particles engineered to express the FIV matrix/capsid region of Gag and full length Env also failed to induce protection against FIV challenge, although the vaccine elicited both humoral and cellular responses.[558] A combination vaccine using priming inoculations with a feline herpes virus (FHV) vector expressing the FIV *gag* gene and a FHV vector expressing FIV *env* and booster inoculations with a FIV FC vaccine also failed to protect immunized cats from FIV challenge infection.[521] In contrast, partial protection against FIV challenge was observed for cats immunized with live attenuated *Salmonella typhimuriaum aroA* strains expressing FIV CA and truncated SU encoding hypervariable regions V3–V5, although correlates of protection were not defined.[559] Partial protection against challenge was also observed by vaccination of cats with a recombinant canarypoxvirus (ALVAC)-based FIV vaccine used alone or in combination with a FIV FC vaccine.[528] Furthermore, a single oral immu-

nization of cats with a live recombinant *Listeria monocytogenes* strain, which both expresses FIV Gag and delivers an FIV truncated Env-expressing plasmid, was found to confer protection by reduction of virus load and virus-induced disease after FIV challenge.[560] The limited number of FIV vaccine studies utilizing live viral vectors is surprising, given the interest and success of multiple modality vaccines consisting of live recombinant viral vectors and DNA expression plasmids recently observed for the SIV/SHIV animal models.[513]

FIV Attenuated Virus Vaccines

Attenuated live virus vaccines have been extensively examined in the SIV/SHIV animal models due to consistent efficacy shown by these viruses for inducing protection against challenge with highly pathogenic SIV and SHIV isolates.[505–507,561] A widely used vaccine based on an attenuated strain of equine lentivirus EIAV has protected 75 million horses and donkeys over the past 30 years in China.[515,516] Studies describing efficacious molecularly cloned attenuated EIAV and CAEV vaccine viruses have also been reported.[562,563] Examination of attenuated FIV vaccines has been limited but shown that this approach is also effective for the feline AIDS animal model. The FIV-Petaluma virus variant used in the commercial FIV WKV vaccine was recently shown to establish a low-level infection of cats without induction of apparent FIV-associated disease.[316] Furthermore, infection with the attenuated FIV-Petaluma variant protected against infection with a different pathogenic FIV isolate (FIV GL8), although of the same subtype. Complete protection was conferred by this attenuated FIV against FIV GL8 delivered by the intraperitoneal route and partial protection was demonstrated against challenge by a mucosal route. Complete protection was also imparted against wild-type FIV challenge by inoculation of cats with infectious molecularly cloned FIV deletion mutants including a LTR mutant encoding a deletion of the AP-1 site[564] and a *vif*-deleted provirus (FIV-Δvif) administered as a DNA vaccine.[174,331] Lastly, superinfection of domestic cats with either nonpathogenic lion or puma nondomestic feline lentiviruses has been shown to confer resistance to infection with pathogenic domestic FIV.[565] Immune correlates were not apparent from any of these attenuated FIV vaccine studies. In fact, the substantial genetic variation observed between domestic and nondomestic feline lentiviruses argues against initial control of challenge virus infection driven by epitope-specific immune responses and instead suggests currently undetermined resistance factors or mechanisms possibly associated the innate immune system. An important limitation of attenuated lentiviral vaccines is the concern for long-term safety as demonstrated by experiments performed by Ruprecht and coworkers, which showed that a SIV *nef* deletion mutant may cause fatal AIDS-like disease in newborn macaques and may become pathogenic after

long-term infection of adult macaques.[508–510] Regardless of whether attenuated lentivirus vaccines will ever be safe enough for general use, examination of these viruses in animal model systems, including FIV, offers an important opportunity to identify and fully characterize immune responses and undefined host resistance factors that confer protection against pathogenic virus infection or disease.

Critical Issues for FIV Vaccine Development

FIV vaccine development faces critical issues that are very similar or identical to those facing HIV-1 vaccines. Characterization of immune correlates of protective immunity for any of the FIV vaccine approaches examined so far has been elusive, a situation that is common to all lentivirus vaccines including those for SIV and SHIV, and must be addressed for successful HIV-1 vaccine design in the future.[566] Vaccine-induced enhancement of pathogenic virus challenge has been consistently observed with FIV and EIAV vaccine approaches,[567] involving either WKV, FC, or protein subunits/peptides and for FIV, was thought to result from enhancing antibodies that might be specific to epitopes within Env hypervariable regions V3–V5 or to the PID epitope.[462,568,569] However, vaccines using recombinant Env proteins with regions V3–V5 deleted were shown to remain capable of inducing enhancement of challenge infection.[569] Occurrence of FIV DNA vaccine-induced enhancement of challenge virus infection despite negligible antibody responses, also suggested mechanisms other than enhancing antibodies, such as lymphoid activation.[554,555] A very recent report described enhancement of challenge virus infection after vaccination of rhesus macaques with an attenuated recombinant varicella-zoster virus vaccine expressing SIV Env.[570] Vaccine-associated enhancement in this study correlated with appearance of robust anamnestic virus-specific CD4 proliferative responses in the absence of strong CD8 responses and again suggested that immune activation may play a role in vaccine-induced enhancement along with cellular responses skewed against CD8 T-cells. These findings suggest major concerns for clinical testing of HIV-1 vaccines for which less than optimal CD8 responses are already predicted and validate use of the FIV animal model for characterization of this potentially devastating complication of HIV vaccine use. A third issue of importance for both future and the current commercial FIV vaccine design as well as future commercial HIV-1 vaccines, concerns interference of vaccine-induced antibodies with FIV diagnostics. All lentivirus diagnostic assay systems utilize seropositivity as a marker for virus infection. PBMC virus isolation and plasma viral RNA detection systems using PCR, although specific, are not sufficiently sensitive to detect all virus infections or currently feasible as routine diagnostic assays. Future HIV-1 vaccine design will even-

tually need to address this issue for which the FIV animal model may prove most useful.

7. FIV AND ANTIVIRAL THERAPIES

Relatively soon after the initial isolation and characterization of FIV, considerable efforts were focused on characterizing this lentivirus as an animal model for antiviral therapies for HIV-1. These studies were encouraged by similarities between FIV and HIV-1 RT in amino acid sequence, structure and physical properties, catalytic activities, and susceptibility to multiple nucleoside analogs, including AZT, 3TC, 9-(2-phosphonomethoxyethyl)adenine (PMEA) and (R)-9-(2-phosphonylmethoxypropyl)-2,6-diaminopurine {(R)-PMPDAP)},[571–575] as discussed earlier in this chapter. Conversely, FIV has proven relatively resistant to therapies including nonnucleoside RT inhibitors and protease inhibitors used in highly active antiretroviral therapy (HAART) protocols for HIV-1-infected patients.[96,109,110,576] Nevertheless, ongoing studies are evaluating protease inhibitors in vivo that show activity against proteases of multiple lentiviruses in vitro, as well as drugs that target lentiviral TM fusion domains conserved across different lentiviruses. Identification of such compounds will be important for design of efficacious HAART protocols for FIV infection and continued development of FIV as a model for HIV-1 antiviral drug therapy.

Examination of FIV susceptibility to AZT in vitro resulted in the first description of emergence of an AZT-resistant lentivirus through virus passage in cell culture[104] and led to a rigorous examination of multiple drug-resistant FIV RT mutants that arise in vitro.[108,577–579] Although FIV resistance to AZT did not map to homologous residues within AZT-resistant HIV-1 RT mutants, both FIV and HIV-1 do share a similar determinant (M184 in the YMDD active site of the RT palm subdomain) for susceptibility to nucleoside analog 3TC.[106,107] Accordingly, a Met-to-Thr mutation in the YMDD motif of RT has been observed in 3TC-resistant mutants for both FIV and HIV-1. Studies assessing drug-resistant FIV mutants in vivo have been very few but have revealed attenuation of an AZT-resistant FIV when inoculated into cats.[111] These findings recommended the potential use of this model for characterizing pathogenesis of other FIV variants resistant to drugs, targeting domains conserved between lentiviral enzymatic proteins.

Examination of FIV susceptibility to nucleoside analogs AZT, PMEA, and (R)-PMPDAP in vitro has been complemented by a variety of in vivo studies that illustrate the value of this animal model for HIV-1 antiviral drug development and assessment of drug efficacy. Studies testing the merit of

AZT as monotherapy for either reducing virus load, improving CD4:CD8 T-cell ratios, or clinical status in both experimental primary infection and chronic natural infection with FIV have produced somewhat mixed results. Altogether, however, investigations have revealed fairly limited efficacy for AZT used as a single therapeutic regimen for FIV infection, as previously found in HIV-1 infection, and frequently reported side effects such as anemia that are also observed in human patients.[580–587] Furthermore, a single report examining combination therapy of AZT and 3TC for cats either acutely or chronically infected with FIV described only slightly improved efficacy compared to AZT treatment alone.[588] In contrast, nucleoside analog PMEA and its derivative (R)-PMPDAP have proven very efficacious as antiviral therapies for FIV infection both in vitro and in vivo by significantly reducing virus load and improving CD4:CD8 T-cell ratios.[572,573,575,581,589,590] Combination therapy consisting of AZT, 3TC, and a third nucleoside analog abacavir also blocked FIV replication in a synergistic manner in vitro.[591] These observations suggest that this drug combination or combinations including PMEA may warrant future assessment as HAART protocols using the FIV animal model.

A shortage of HIV-1 protease inhibitors that effect FIV replication due to differences in substrate specificity displayed by FIV PR and HIV-1 PR[90–95] has hampered use of the FIV animal for analysis of combination drug protocols used in HAART protocols for HIV-1-infected patients. However, attempts to identify a protease inhibitor universally active against multiple lentivirus PRs revealed that a statine-based inhibitor LP-130[96] and a C2-symmetric competitive inhibitor identified as TL-3[93,592] were both capable of inhibiting PR expressed by HIV-1, SIV, and FIV. Interestingly, comparison of crystal structures of FIV PR and HIV-1 PR in complex with TL-3 reveals differences in the position of the flaps in FIV PR and HIV-1 PR, whereas complexes of FIV PR and HIV-1 PR with inhibitor LP-130 are nearly identical in conformation.[593] Recent reports have shown TL-3 to inhibit FIV and HIV-1 replication and to be active against protease inhibitor-resistant HIV-1 mutants in vitro.[93,592] Furthermore, TL-3 treatment of FIV-infected cats reduced virus load and disease, including clinical neurologic dysfunction and severe acute phase immunodeficiency.[343,594] These findings support further testing of TL-3 in combination drug protocols for FIV infection both in cell culture systems and in vivo. Assessment of combination protocols with TL-3 may be particularly warranted for testing a proposed hypothesis that therapy with compounds broadly reactive against proteases of multiple lentiviruses will less likely be associated with emergence of protease inhibitor-resistant virus mutants in vivo.

Recent preliminary studies have also described testing synthetic peptides targeted to determinants within the FIV TM ectodomain, such as the heptad repeat 2 (HR2) domain[595] and the membrane-proximal tryptophan-rich region[138,416] (Figure 3) as antiviral therapeutics. Previous studies

showed that HIV-1 peptides derived from the HR1 and HR2 regions are potent inhibitors of HIV-1 infection and function by blocking virus-mediated cell fusion.[596] Similarly, the membrane-proximal tryptophan-rich region immediately downstream of HR2 within the TM ectodomain has also been shown to be important for virus-induced fusion and infectivity for both HIV-1 and FIV.[34,137,597] Testing of combination FIV therapies that include nucleoside analogs, protease inhibitors such as TL3, and Env peptide inhibitors now proven active against FIV has not yet been reported but looks promising for further development of the FIV model for HAART therapies for HIV-1.

Various other antiviral approaches have shown activity against FIV replication either in vitro or in vivo but have been examined in a limited fashion. Strategies previously shown to successfully target the FIV Rev regulatory system in vitro include a ribozyme directed to the FIV RRE element[598] and small molecule intervention using 1,8-diaminooctane that blocks the formation of hypusine required by eIF-5A, a cellular factor required for HIV-1 Rev function.[166] Cytokines and cellular growth factors including recombinant human interferon-alpha2, human interferon-omega, human interferon-tau, recombinant human GM-CSF, IL-16, and recombinant human insulin growth factor-1, demonstrated limited success as treatment for FIV infection either in cell culture systems or in infected cats.[328,394,599–603] However, cytokines as adjunctive therapy to HAART therapy are currently under evaluation in HIV-1-infected patients[604] and may be worthy of similar examination in the FIV model, where mechanisms may be experimentally characterized. Cytokines may be useful both as immune reconstitution therapeutics and for activating expression of latent reservoir viruses for subsequent elimination by antiviral drugs. In conclusion, animal models such as FIV and SIV provide opportunities for examination of tissues and cell subset reservoirs for virus in hosts undergoing HAART therapy as reported for HIV-1-infected patients[232,605] and, more importantly, characterization of viral mechanisms for persistence within these reservoirs.

8. FIV AS A VIRAL VECTOR

Advances in characterization of FIV molecular virology have facilitated development of FIV vectors as vehicles for gene transfer in both dividing and nondividing cells.[606,607] FIV vector development has equally contributed significantly to the body of knowledge regarding viral determinants of FIV gene expression, virus packaging, and integration. Lentiviruses provide unique vector systems that allow reliable integration of foreign genes into chromosomal DNA of nondividing cells. Although cumulative data generated for HIV-based vectors suggest that safe and effective primate lentivirus

vectors will be possible, concern over the clinical use of gene vectors derived from a pathogenic human retrovirus (HIV-1) may restrict the use of such vectors. FIV has provided the first nonprimate lentivirus vector system capable of gene transfer efficiency comparable to that observed with HIV-based vectors.[608] Development of lentivirus vectors based on FIV has received continued support due to the apparent lack of FIV replication competence in human cells, resulting from restriction of FIV LTR promoter activity. Although cell culture-adapted variants of FIV are capable of utilizing human chemokine receptor CXCR-4 for infection of human cells,[260] FIV LTR-directed gene expression and virus production are abrogated in human cells.[415] Lack of cross-reactivity of FIV proteins with those of HIV is another potential advantage for use of FIV-based vectors. As discussed above, FIV virion structure and genomic organization are typical of other lentiviral genomes except for the absence of specific accessory and regulatory genes such as *vpr*, *vpu*, *nef*, and *tat* and the presence of a *pol*-encoded dUTPase. The less complicated FIV genome provides another advantage for use of this vector system. FIV vectors systems infect human primary cell types from a broad array of tissues, including brain, eye, airway, hematopoietic system, liver, muscle, and pancreas.[607] These and other factors have promoted enthusiasm for FIV vector systems for gene therapy in human disease.

The first FIV vector described was derived from FIV molecular clone 34TF10[38] and documented that FIV-based vectors were capable of transducing nondividing human cells.[260] Subsequent studies reported by other research groups described biology and cell tropism for similar but modified FIV vector systems also based on FIV 34TF10.[225,609,610] All FIV vector systems described to date use three plasmids: a transfer vector for encoding the gene of interest, a packaging vector for expression of structural and enzymatic genes, and a plasmid for expression of the vesicular stomatitis virus (VSV)-G envelope protein for pseudotyping of vector particles.[606] A chimeric FIV LTR, composed of a human cytomegalovirus (hCMV) immediate early gene promoter replacing the FIV U3 element and fused to the R/U5 LTR domains, is found in all FIV transfer vectors and is necessary for FIV vector production from human cells. As stated earlier, studies focused on optimization of FIV vectors system have mapped specific replication domains that include encapsidation determinants,[44,45] a central polypurine tract, and a central termination sequence that generates a central DNA flap in the preintegration complex.[46] Data from these studies revealed a divergence in sequence between the FIV cPTT and the FIV 3′ PTT, and also showed that the FIV cPTT and 3′ PTT are not entirely purine. These findings uncovered differences between FIV and HIV-1 reverse transcription and have been incorporated into FIV vector design. Vector optimization studies have also characterized class I FIV integrase mutants that prevent the integrase reaction but do not perturb other Gag/Pol

functions.[125,607,611] These integrase mutants may be incorporated into preclinical gene therapy studies in animals for providing control vectors that identify tissues with a requirement for vector integration for gene expression. Finally, FIV vectors have been further modified by deleting noncoding and nonstructural FIV sequences as well as specific *gag* sequences for construction of minimal vectors with reduced cytotoxicity, yet efficient gene transfer.[609,612] Continued FIV vector design studies will contribute to further understanding of the FIV life cycle and FIV molecular virology, as well as facilitate development of optimal lentiviral vector systems. Additional studies will also be required to fully characterize and compare relative efficiencies, as well as biosafety of FIV with primate lentivirus vectors.

9. FELINE LENTIVIRUSES OF NONDOMESTIC CATS

Incidence

The initial observation of infection of nondomestic cats with a feline lentivirus[613] was subsequently confirmed by multiple reports revealing either virologic or serologic evidence of infection in both captive and free-ranging populations of several nondomestic feline species.[269,275–282] Species-specific feline lentivirus isolates have since been characterized for lions (FIV-Ple), leopards (FIV-Ppa), pumas/courgars (FIV-Pco), and pallas cats (FIV-Oma).[277,614–617] Nondomestic feline lentivirus infection of free-ranging nondomestic felids is worldwide and includes lion and leopard populations within Africa; cougar populations in southern and western United States, Canada, and South America; cheetahs in Africa; and Pallas cats in central Asia.[269,275,278,281,282,613] Interestingly, the prevalence of feline lentivirus infection in specific nondomestic cat populations, including lions in the Serengeti National Park and Ngorongoro Crater of east Africa and Kruger National Park of South Africa and cougars within the Snowy Mountain Rage in Wyoming, is quite high ranging from 58% (cougars) to 90% (lions).[277,281,282,618] Despite evidence of endemic infection of free-ranging lion and cougar populations, overt disease has not been associated with lentivirus infection in these populations.[619,620] This apparent lack of virulence, along with the significant genetic diversity observed among feline lentiviruses, suggests that FIV-Pco and FIV-Ple are ancient viruses that have adapted to their hosts[282,615,618] and may be comparable to African SIV isolates that are similarly nonpathogenic for their natural hosts.[621,622]

Genomic Diversity

Characterization of a molecularly cloned FIV-Oma proviral genome revealed genomic organization similar to that of domestic cat FIV iso-

lates.[614] However, current knowledge of genomic diversity of nondomestic FIV isolates is based primarily on phylogenetic analyses focused on viral sequences generated from endemic FIV-Ple- and FIV-Pco-infected cat populations. Although lentiviruses infecting nondomestic cats are clearly related to domestic cat FIV, comparison of amino acid and nucleotide sequences derived from the highly conserved *pol* gene of nondomestic (lion, cougar, and pallas cat) and domestic feline FIV isolates reveals considerable variation, ranging from 21 to 30% differences in nucleotides and 19 to 45% in amino acids.[269,277,614,615,618] These data indicate that divergence between domestic and nondomestic cat lentiviral genomes is similar to that observed between HIV-1 and HIV-2. Furthermore, significant genomic diversity reported for cougar FIV-Pco isolates gathered from North and South America defined two principal clades and 15 divergent subclades for this nondomestic FIV.[282,615] Genetic divergence measured for the two FIV-Pco clades was similar to the diversity that distinguishes domestic cat FIV from FIV-Ple isolates. Similarly, analyses of FIV-Ple isolates from lion populations within the Serengeti Reserve and Ngorongoro Crater in southeastern Africa identified three phylogenetic clades exhibiting genetic diversity also similar to that which separates domestic cat FIV from FIV-Pco.[277,618] Sequence diversity within the conserved *pol* genes derived from both FIV-Pco and FIV-Ple isolates is greater than that reported for the few *pol* sequences available for domestic cat FIV. In fact, this *pol* gene variation is similar to diversity measured for the more variable *env* genes used to define unique domestic cat FIV clades. These findings provide additional support to the theory that nondomestic feline lentiviruses are ancient viruses that have existed within cougar and lion species much longer than FIV in domestic cats.

Additional findings from phylogenetic analyses of endemic FIV-Pco- and FIV-Ple-infected cat populations include evidence of vertical transmission (Pco)[282,615] and possible coinfection of different subtypes within a single host (Pco and Ple).[615,618] A recent report examining a large free-ranging lion population that included 13 prides within the Serengeti National Park revealed the presence of all three FIV-Ple subtypes widely dispersed within the population. Infection with the three FIV-Ple subtypes within the same pride and possibly within the same host was also observed.[618] Furthermore, a high incidence (43%) of coinfection with two to three FIV-Ple subtypes within individual animals was reported, although the alternative possibility of infection with subtype-recombinant viruses was not ruled out. Significant genomic divergence between different FIV-Ple subtypes, most notable between subtype C and the two other subtypes (A and B), was also observed for this population of lions. Monophyletic clustering of FIV-Ple sequences was observed for only one of the prides under study.

Findings described above for lion populations within Serengeti National Park contrasted with those generated from a study of a smaller population of wild cougars located in a mountain range in southeast Wyoming.[282] These animals supported an overall FIV-Pco prevalence rate of 58% and a remarkable 100% rate of infection for all adult animals. Although analysis of both *pol* and *env* sequences confirmed the presence of two distinct FIV-Pco lineages within this infected cougar population, sequence diversity between the lineages was low and evidence of coinfection of a single host with viruses of distinct lineages was not found. Importantly, investigation of the evolution of both FIV-Pco *pol* and *env* sequences within this cougar population over time revealed mean rates of 1 to 3% per 10 years, a rate considerably less than rates of 0.3 to 1% per year reported for HIV.[623–626] Collective observations generated so far from phylogenetic analyses of nondomestic FIV isolates suggest that these lentivirus infections provide unique opportunities to evaluate virus dynamics and viral sequence evolution in a natural host for a nonpathogenic lentivirus.

Infection and Replication of Nondomestic Feline Lentiviruses

The extent of genetic divergence described so far between domestic and nondomestic feline FIV isolates would suggest that infection and replication of nondomestic isolates may not be possible in domestic cats or in primary lymphocytes isolated from domestic cats. Reports of in vitro replication properties of nondomestic feline lentiviruses are scarce and have been restricted by the inability of some nondomestic FIV isolates such as cougar FIV-Pco, to replicate in domestic cat primary lymphocytes.[269,617] However, some nondomestic FIV isolates including FIV-Ple and FIV-Oma have been shown to replicate in domestic cat lymphocytes and some feline cell lines including CrFK cells (FIV-Oma) and a feline lymphoid cell line (3201 cells) (FIV-Ple).[275,617] FIV-Pco was also shown to replicate in 3201 cells.[617] Although characterization of receptor usage for nondomestic feline lentiviruses has not yet been reported, these current findings for in vitro growth properties suggest that nondomestic FIV isolates may also be capable of infecting and replicating in vivo in domestic cats. Rare, isolated cases of naturally occurring cross-species transmission of domestic cat FIV to nondomestic cats have been reported, which involved a cougar and a wild-caught Tsushima cat belonging to a subspecies of leopard cats located in Japan.[279,615] Experimental transmission studies have also confirmed the infectivity of both FIV-Pco and FIV-Ple in domestic cats[627,628] and revealed that establishment of persistent FIV-Ple viral infection is possible in the absence of FIV-associated disease. Moreover, prior infection of domestic cats with either FIV-Pco or FIV-Ple appears to impart some resistance to challenge with pathogenic domestic cat FIV based on lower challenge virus

loads and higher CD4 counts measured for FIV-Pco- and FIV-Ple-infected domestic cats compared to naive cats after exposure to domestic cat FIV.[565] Additional studies with rigorous measurement of virus load and careful examination of viral immunopathogenesis over extended time periods will be needed to characterize nondomestic feline lentivirus infection of the domestic cat as a model for HIV infection.

10. OUTLOOK FOR THE FIV ANIMAL MODEL

Significant progress has been made in the development of antiviral drug regimens for HIV-1, although complete elimination of the virus from the host and full restoration of immunocompetency are still not possible. However, a commercial safe and efficacious vaccine for HIV-1 is still not available and is unlikely in the near future, despite exhaustive efforts in HIV-1 vaccine development that began 20 years ago immediately after the initial characterization of HIV-1.[566] Several serious issues that must be resolved for future vaccine success include identification of immune correlates of protection against either lentivirus infection or virus-associated disease. The apparent difficulty of addressing this question was predictable since virus elimination has not been observed during natural infection with immunodeficiency-inducing lentiviruses, even when strong virus-specific cellular and humoral immune responses have been detected in the infected host. Vaccine-induced protection against pathogenic challenge has been difficult to achieve in both the SIV and FIV animal models, and when observed, has been inconsistent. An additional complication is presented by the specter of vaccine-induced immune responses that prove detrimental rather than protective as reported for FIV and more recently in the SIV animal model. The complication of virus strain diversity has barely been addressed by vaccine design in animal models and surely poses a major complication for HIV-1 vaccine success in the future. Successful resolution of these obstacles in vaccine development, and most particularly the lack of understanding of why some vaccines such as attenuated viruses are sometimes protective, absolutely requires continued examination of viral pathogenesis and host immune responses in animal models including both nonhuman primate and nonprimate lentivirus animal models. Elucidation of pathogenic mechanisms used by these immunodeficiency-inducing lentiviruses during the earliest stages of virus exposure and virus-host interactions will be critical for design of vaccines that will have any potential for sterilizing immunity, if such a goal is possible. Similarly, a far more precise understanding of viral mechanisms for immunodeficiency will also be necessary for design of therapies of immune reconstitution in both acutely and chronically HIV-1-infected hosts, which can only be gained through experimental animal model studies.

Significant progress in characterization of FIV infection has been accomplished in the past 10 years, especially regarding molecular characterization of viral proteins, FIV receptor usage, and virus tropism and localization in vivo. Progress in development of FIV vectors for gene therapy has also been accomplished and has contributed considerably to the molecular characterization of FIV. Continued efforts in FIV vaccine and broad-based antiviral therapeutic development has demonstrated the strong potential of the FIV model for investigation of novel vaccine approaches and antiviral drugs. Lastly, similarities observed for host cell targets for virus infection and immunodeficiencies associated with FIV and HIV infection in vivo offer strong support for use of this animal model for crucial studies focused on mechanisms of immunopathogenesis of lentivirus-induced acquired immunodeficiency.

Acknowledgments. We are grateful for our FIV research colleagues and collaborators who have partsicipated in the development of the FIV animal model and who have provided helpful input or references.

REFERENCES

1. N. C. Pedersen, E. W. Ho, M. L. Brown, and J. K. Yamamoto, Isolation of a T-lymphotropic virus from domestic cats with an immunodeficiency-like syndrome, *Science* 235, 790–793 (1987).
2. J. K. Yamamoto, E. Sparger, E. W. Ho, P. R. Andersen, T. P. O'Connor, C. P. Mandell, L. Lowenstine, R. Munn, and N. C. Pedersen, Pathogenesis of experimentally induced feline immunodeficiency virus infection in cats, *Am. J. Vet. Res.* 49, 1246–1258 (1988).
3. P. J. Brown, C. D. Hopper, and D. A. Harbour, Pathological features of lymphoid tissues in cats with natural feline immunodeficiency virus infection, *J. Comp. Path.* 104, 345–355 (1991).
4. A. M. Beebe, N. Dua, T. G. Gluckstern, P. Moore, N. C. Pedersen, and S. Dandekar, The primary stage of feline immunodeficiency virus infection: viral dissemination and cellular targets, *J. Virol.* 68, 3080–3091 (1994).
5. C. D. Ackley, J. K. Yamamoto, N. Levy, N. C. Pedersen, and M. D. Cooper, Immunologic abnormalities in pathogen-free cats experimentally infected with feline immunodeficiency virus, *J. Virol.* 64, 5652–5655 (1990).
6. J. E. Barlough, C. D. Ackley, J. W. George, N. Levy, R. Acevedo, P. F. Moore, B. A. Rideout, M. D. Cooper, and N. C. Pedersen, Acquired immune dysfunction in cats with experimentally induced feline immunodeficiency virus infection: comparison of short-term and long-term infections, *J.A.I. D. S.* 4, 219–227 (1991).
7. L. J. Diehl, C. K. Mathiason-Dubard, L. L. O'Neil, L. A. Obert, and E. A. Hoover, Induction of accelerated feline immunodeficiency virus disease by acute-phase virus passage, *J. Virol.* 69, 6149–6157 (1995).
8. R. V. English, P. Nelson, C. M. Johnson, M. Nasisse, W. A. Tompkins, and M. B. Tompkins, Development of clinical disease in cats experimentally infected with feline immunodeficiency virus, *J. Infect. Dis.* 170, 543–552 (1994).
9. T. Ishida and I. Tomoda, Clinical staging of feline immunodeficiency virus infection, *Nippon Juigaku Zasshi.* 52, 645–648 (1990).

10. C. Novotney, R. V. English, J. Housman, M. G. Davidson, M. P. Nasisse, C. Jeng, W. C. Davis, and M. B. Tompkins, Lymphocyte population changes in cats naturally infected with feline immunodeficiency virus, *AIDS* 4, 1213–1218 (1990).
11. N. C. Pedersen, C. M. Leutenegger, J. Woo, and J. Higgins, Virulence differences between two field isolates of feline immunodeficiency virus (FIV-A Petaluma and FIV-CPGammar) in young adult specific pathogen free cats, *Vet. Immunol. Immunopathol.* 79, 53–67 (2001).
12. B. A. Rideout, L. J. Lowestine, C. A. Hutson, P. F. Moore, and N. C. Pedersen, Characterization of morphological changes and lymphocytes subset distribution in lymph nodes from cats with naturally acquired feline immunodeficiency virus infection, *Vet. Immunol. Immunopathol.* 29, 391–399 (1992).
13. J. K. Yamamoto, H. Hansen, E. W. Ho, T. Y. Morishita, T. Okuda, T. R. Sawa, R. M. Nakamura, and N. C. Pedersen, Epidemiologic and clinical aspects of feline immunodeficiency virus infection in cats from the continental United States and Canada and possible mode of transmission, *J. Am. Vet. Med. Assoc.* 194, 213–220 (1989).
14. M. Bendinelli, M. Pistello, S. Lombari, A. Poli, C. Garzelli, D. Matteucci, L. Ceccherini-Nelli, G. Malvaldi, and F. Tozzini, Feline immunodeficiency virus: an interesting model for AIDS studies and an important cat pathogen, *Clin. Micorbiol. Rev.* 8, 87–112 (1995).
15. T. Ishida, T. Washizu, K. Toriyabe, S. Motoyoshi, I. Tomoda, and N. Pedersen, Feline immunodeficiency virus infection in cats of Japan, *J. Am. Vet. Med. Assoc.* 194, 221–225 (1989).
16. M. J. Burkhard, L. A. Obert, L. L. O'Neil, L. J. Diehl, and E. A. Hoover, Mucosal transmission of cell-associated and cell-free feline immunodeficiency virus, *AIDS Res. Hum. Retroviruses.* 13, 347–355 (1997).
17. M. J. Burkhard and G. A. Dean, Transmission and immunopathogenesis of FIV in cats as a model for HIV, *Curr. HIV Res.* 1, 15–29 (2003).
18. S. A. Bishop, C. R. Stokes, T. J. Gruffydd-Jones, C. V. Whiting, and D. A. Harbour, Vaginal and rectal infection of cats with feline immunodeficiency virus, *Vet. Microbiol.* 51, 217–227 (1996).
19. L. L. O'Neil, M. J. Burkhard, L. J. Diehl, and E. A. Hoover, Vertical transmission of feline immunodeficiency virus, *AIDS Res. Hum. Retroviruses* 11, 171–182 (1995).
20. L. L. O'Neil, M. J. Burkhard, and E. A. Hoover, Frequent perinatal transmission of feline immunodeficiency virus by chronically infected cats, *J. Virol.* 70, 2894–2901 (1996).
21. L. A. Obert and E. A. Hoover, Feline immunodeficiency virus clade C mucosal transmission and disease courses, *AIDS Res. Hum. Retroviruses* 16, 677–688 (2000).
22. L. A. Obert and E. A. Hoover, Relationship of lymphoid lesions to disease course in mucosal feline immunodeficiency virus type C infection, *Vet. Pathol.* 37, 386–401 (2000).
23. L. A. Obert and E. A. Hoover, Early pathogenesis of transmucosal feline immunodeficiency virus infection, *J. Virol.* 76, 6311–6322 (2002).
24. O. J. Cohen and A. S. Fauci, in *Fields Virology*, edited by D. M. Knipe and P. M. Howley (Lippincott Williams & Wilkins, Philadelphia, PA, 2001), 2043–2094.
25. T. S. Tochikura, K. A. Hayes, C. M. Cheney, T. A. Tanabe, J. L. Rojko, L. E. Mathes, and R. G. Olsen, In vitro replication and cytopathogenicity of the feline immunodeficiency virus for feline T4 thymic lymphoma 3201 cells, *Virology* 179, 492–497 (1990).
26. J. Goff, in *Fields Virology*, edited by D. M. Knipe and P. M. Howley (Lippincott Williams & Wilkins, Philadelphia, 2001), 1871–1939.
27. R. Steinman, J. Dombrowski, T. O'Connor, R. C. Montelaro, Q. Tonelli, K. Lawrence, C. Seymour, J. Goodness, N. C. Pedersen, and P. R. Andersen, Biochemical and immunological characterization of the major structural proteins of feline immunodeficiency virus, *J. Gen. Virol.* 71, 701–706 (1990).
28. H. F. Egberink, J. Ederveen, R. C. Montelaro, N. C. Pedersen, M. C. Horzinek, and M. J. Koolen, Intracellular proteins of feline immunodeficiency virus and their antigenic relationship with equine infectious anaemia virus proteins, *J. Gen. Virol.* 71, 739–743 (1990).

29. P. C. Wagaman, C. S. Hasselkus-Light, M. Henson, D. L. Lerner, T. R. Phillips, and J. H. Elder, Molecular cloning and characterization of deoxyuridine triphosphatase from feline immunodeficiency virus (FIV), *Virology* 196, 451–457 (1993).
30. J. H. Elder, D. L. Lerner, C. S. Hasselkus-Light, D. J. Fontenot, E. Hunter, P. A. Luciw, R. C. Montelaro, and T. R. Phillips, Distinct subsets of retroviruses encode dUTPase, *J. Virol.* 66, 1791–1794 (1992).
31. J. Coffin, in *Fundamental Virology*, edited by B. N. Fields and K. M. Knipe (Raven Press, New York, 1991), 645–708.
32. M. Shimojima, T. Miyazawa, Y. Ikeda, E. L. McMonagle, H. Haining, H. Akashi, Y. Takeuchi, M. J. Hosie, and B. J. Willett, Use of CD134 as a primary receptor by the feline immunodeficiency virus, *Science* 303, 1192–1195 (2004).
33. B. J. Willett, L. Picard, M. J. Hosie, J. D. Turner, K. Adema, and P. R. Clapham, Shared usage of the chemokine receptor CXCR4 by the feline and human immunodeficiency viruses, *J. Virol.* 71, 6407–6415 (1997).
34. S. Giannecchini, F. Bonci, M. Pistello, D. Matteucci, O. Sichi, P. Rovero, and M. Bendinelli, The membrane-proximal tryptophan-rich region in the transmembrane glycoprotein ectodomain of feline immunodeficiency virus is important for cell entry, *Virology* 320, 156–166 (2004).
35. A. P. De Parseval, S. V. Su, J. H. Elder, and B. Lee, Specific interaction of feline immunodeficiency virus surface glycoprotein with human DC-SIGN, *J. Virol.* 78, 2597–2600 (2004).
36. A. P. De Parseval and J. H. Elder, Binding of recombinant feline immunodeficiency virus surface glycoprotein to feline cells: role of CXCR4, cell-surface heparans, and an unidentified non-CXCR4 receptor, *J. Virol.* 75, 4528–4539 (2001).
37. R. A. Olmsted, V. M. Hirsch, R. H. Purcell, and P. R. Johnson, Nucleotide sequence analysis of feline immunodeficiency virus: genome organization and relationship to other lentiviruses, *Proc. Natl. Acad. Sci. USA* 86, 8088–8092 (1989).
38. R. L. Talbott, E. E. Sparger, K. M. Lovelace, W. M. Fitch, N. C. Pedersen, P. A. Luciw, and J. H. Elder, Nucleotide sequence and genomic organization of feline immunodeficiency virus, *Proc. Natl. Acad. Sci. USA* 86, 5743–5747 (1989).
39. F. J. Thompson, J. Elder, and J. C. Neil, Cis- and trans-regulation of feline immunodeficiency virus: identification of functional binding sites in the long terminal repeat, *J. Gen. Virol.* 75, 545–554 (1994).
40. E. E. Sparger, B. L. Shacklett, L. Renshaw-Gegg, P. A. Barry, N. C. Pedersen, J. H. Elder, and P. A. Luciw, Regulation of gene expression directed by the long terminal repeat of the feline immunodeficiency virus, *Virology* 187, 165–177 (1992).
41. T. R. Phillips, C. Lamont, D. A. M. Konings, B. L. Schacklett, C. A. Hamson, P. A. Luciw, and J. H. Elder, Identification of the rev transactivation and *rev*-responsive elements of feline immunodeficiency virus, *J. Virol.* 66, 5464–5471 (1992).
42. T. R. Phillips, R. L. Talbott, C. Lamont, S. Muir, K. Lovelace, and J. H. Elder, Comparison of two host cell range variants of feline immunodeficiency virus, *J. Virol.* 64, 4605–4613 (1990).
43. T. Miyazawa, M. Kohmoto, Y. Kawaguchi, K. Tomonaga, T. Toysaki, K. Kiuta, A. Adachi, and T. Mikami, The AP-1 binding site in the feline immunodeficiency virus long terminal repeat is not required for virus replication in feline T lymphocytes, *J. Gen. Virol.* 74, 1573–1580 (1993).
44. M. T. Browning, F. Mustafa, R. D. Schmidt, K. A. Lew, and T. A. Rizvi, Delineation of sequences important for efficient packaging of feline immunodeficiency virus RNA, *J. Gen. Virol.* 84, 621–627 (2003).
45. I. Kemler, R. Barraza, and E. M. Poeschla, Mapping the encapsidation determinants of feline immunodeficiency virus, *J. Virol.* 76, 11889–11903 (2002).
46. T. Whitwam, M. Peretz, and E. Poeschla, Identification of a central DNA flap in feline immunodeficiency virus, *J. Virol.* 75, 9407–9414 (2001).

47. L. Bigornia, K. M. Lockridge, and E. E. Sparger, Construction and in vitro characterization of attenuated FIV LTR mutant viruses, *J. Virol.* 75, 1054–1060 (2001).
48. Y. Inoshima, Y. Miyazawa, and T. Mikami, In vivo functions of the auxiliary genes and regulatory elements of feline immunodeficiency virus, *Vet. Microbiol.* 60, 141–153 (1998).
49. Y. Kawaguchi, K. Tomonaga, K. Maeda, M. Ono, T. Miyazawa, M. Kohmoto, T. Y., and T. Mikami, The C/EBP site in the feline immunodeficiency virus (FIV) long terminal repeart (LTR) is necessary for its efficient replication and is also involved in the inhibition of FIV LTR-directed gene expression by pseudorabies virus ICP4, *Virology* 208, 492–499 (1995).
50. Y. Ikeda, Y. Inoshima, Y. Kawaguchi, K. Maeda, M. Kohmoto, C. Kai, T. Miyazawa, and T. Mikami, Protein-binding properties of the putative AP-1 and ATF sequence in the feline immunodeficiency virus long terminal repeat, *J. Gen. Virol.* 79, 95–99 (1998).
51. B. R. Cullen and W. C. Greene, Regulatory pathways governing HIV-1 replication, *Cell* 58, 423–426 (1989).
52. B. R. Cullen, Human immunodeficiency virus as a protoypic complex retrovirus, *J. Virol.* 65, 1053–1056 (1991).
53. D. Derse, Bovine leukemia virus transcription is controlled by a virus-encoded *trans*-activing factor and by *cis*-acting response elements, *J. Virol.* 61, 2462–2471 (1987).
54. R. P. S. Kwok, M. E. Laurance, J. R. Lundblad, P. S. Goldman, H. M. Shih, L. M. Connor, S. J. Marriott, and R. H. Goodman, Control of cAMP-regulated enhancers by the viral transctivator Tax through CREB and the co-activator CBP, *Nature* 380, 642–646 (1996).
55. L. Willems, R. Kettmann, G. Chen, D. Portetelle, A. Burny, and D. Derse, A cyclic AMP-responsive DNA-binding protein (CREB2) is a cellular transactivator of the bovine leukemia virus long terminal repeat, *J. Virol.* 66, 766–772 (1992).
56. L. J. Zhao and C. Z. Giam, Interaction of the human T-cell lymphotropic virus type 1 (HTLV-1) transcriptional activator Tax with cellular factors that bind specifically to the 21-base-pair repeats in the HTLV-1 enhancer, *Proc. Natl. Acad. Sci. USA* 88, 11445–11449 (1991).
57. B. A. Morse, L. M. Carruth, and J. E. Clements, Targeting of the visna virus tat protein to AP-1 sites: interactions with the bZIP domains of fos and jun in vitro and in vivo, *J. Virol.* 73, 37–45 (1999).
58. J. L. Hess, J. A. Small, and J. E. Clements, Sequences in the visna virus long terminal repeat that control transciptional activity and respond to viral *trans*-activation: involvement of AP-1 sites in basal activity and *trans*-activation, *J. Virol.* 63, 3001–3015 (1989).
59. L. M. Carruth, B. A. Morse, and J. E. Clements, The leucine domain of the visna virus tat protein mediates targeting to an AP-1 site in the viral long terminal repeat, *J. Virol.* 70, 4338–4344 (1996).
60. K. Tomonaga and T. Mikami, Molecular biology of the feline immunodeficiency virus auxiliary genes, *J. Gen. Virol.* 77, 1611–1621 (1996).
61. A. K. Waters, A. P. De Parseval, D. L. Lerner, J. C. Neil, F. J. Thompson, and J. H. Elder, Influence of ORF2 on host cell tropism of feline immunodeficiency virus, *Virology* 215, 10–16 (1996).
62. C. Nueveut, R. Vigne, J. E. Clements, and J. Sire, The visna transcriptional activator TAT: effects on the viral LTR and on cellular genes, *Virology* 197, 236–244 (1993).
63. S. Villet-Hoc, C. Faure, B. Bouzar, G. Verdier, Y. Chelbloune, and C. Legras, Tat proteins of caprine arthritis encephalitis (CAEV) and maedi visna (MV) viruses are not transactivator proteins of MVV and CAEV LTRs, *American Society of Virology Conference (University of Kentucky).* 21st annual meeting, 166 (2002).
64. A. Gatignol, Identification of cellular proteins that bind to the human immunodeficiency virus type 1 trans-activation-responsive TAR element, *Proc. Natl. Acad. Sci. USA* 86, 7828–7832 (1989).

65. R. Marciniak, M. Garcia-Blanco, and P. Sharp, Identification and characterization of a HeLa nuclear protein that specifically binds to the trans-activation-response (TAR) element of human immunodeficiency virus, *Proc. Natl. Acad. Sci. USA* 87, 3624–3628 (1990).
66. A. P. De Parseval and J. H. Elder, Demonstration that orf2 encodes the feline immunodefiency virus tranactivating (Tat) protein and characterization of a unique gene product with partial rev activity, *J. Virol.* 73, 608–617 (1999).
67. U. Chatterji, A. P. De Parseval, and J. H. Elder, Feline immunodeficiency virus Orf-A is distinct from other lentivirus transactivators, *J. Virol.* 76, 9624–9634 (2002).
68. K. Tomonaga, Y. Shin, M. Fukasawa, T. Miyazawa, A. Adachi, and T. Mikami, Feline immunodeficiency virus gene expression: analysis of the RNA splicing pattern and the monocistronic *rev* mRNA, *J. Gen. Virol.* 74, 2409–2417 (1993).
69. T. Kiyomasu, T. Miyazawa, T. Furuya, R. Shibata, H. Sakai, J. Sakuragi, M. Fukasawa, N. Maki, A. Hasegawa, T. Mikami, and A. Adachi, Identification of feline immunodeficiency virus rev gene activity, *J. Virol.* 65, 4539–4542 (1991).
70. M. L. Manrique, C. C. P. Celma, S. A. Gonzalez, and J. L. Affranchino, Mutational analysis of the feline immunodeficiency virus matrix protein, *Virus Res.* 76, 103–113 (2001).
71. J. H. Elder, M. Schnolzer, C. S. Hasselkus-Light, M. Henson, D. A. Lerner, T. R. Phillips, P. C. Wagaman, and S. B. H. Kent, Identification of proteolytic processing sites within the Gag and Pol polyproteins of feline immunodeficiency virus, *J. Virol.* 67, 1869–1876 (1993).
72. R. Swanstrom and J. W. Wills, in *Retroviruses*, edited by J. Coffin, S. H. Hughes and H. E. Varmus (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1997), 263–334.
73. E. O. Freed and M. A. Martin, in *Fields Virology*, edited by D. M. Knipe and P. M. Howley (Lippincott Williams & Wilkins, Philadephia, PA, 2001), 1971–2041.
74. T. L. South, P. R. Blake, R. C. Sowder, L. O. Arthur, L. E. Henderson, and M. F. Summers, The nucleocapsid protein isolated from HIV-1 particles binds zinc and forms retroviral-type zinc fingers, *Biochemistry* 29, 7786–7789 (1990).
75. M. Moscardini, M. Pistello, M. Bendinelli, D. Ficheux, J. T. Miller, C. Gabus, S. F. Le Grice, W. K. Surewicz, and J. L. Darlix, Functional interactions of nucleocapsid protein of feline immunodeficiency virus and cellular prion protein with the viral RNA, *J. Mol. Biol.* 318, 149–159 (2002).
76. E. O. Freed, Viral late domains, *J. Virol.* 76, 4679–4687 (2002).
77. H. G. Gottlinger, T. Dorfman, J. G. Sodroski, and W. A. Haseltine, Effect of mutations affecting the p6 gag protein on human immunodeficiency virus particle release, *Proc. Natl. Acad. Sci. USA* 88, 3195–3199 (1991).
78. M. Huang, J. Orenstein, M. Martin, and E. Freed, p6Gag is required for particle production from full-length human immunodeficiency virus type 1 molecular clones expressing protease, *J. Virol.* 69, 6810–6818 (1995).
79. M. Sudol, Structure and function of the WW domain, *Prog. Biophys. Molec. Biol.* 65, 113–132 (1996).
80. D. G. Demirov, A. Ono, J. M. Orenstein, and E. O. Freed, Overexpression of the N-terminal domain of TSF101 inhibits HIV-1 budding by blocking late domain function, *Proc. Natl. Acad. Sci. USA* 99, 955–960 (2002).
81. J. E. Garrus, U. K. von Schwedler, O. W. Pornillos, S. G. Morham, K. H. Zavitz, H. E. Wang, D. A. Wettstein, K. M. Stray, M. Cote, R. L. Rich, and W. I. Sundquist, Tsg101 and the vacuolar protein sorting pathway are essential for HIV-1 budding, *Cell* 107, 55–65 (2001).
82. J. Martin-Serrano, T. Zang, and P. D. Bieniasz, HIV-1 and *Ebola* virus encode small peptide motifs that recruit Tsg101 to sites of particle assembly to facilitate egress, *Nat. Med.* 7, 1313–1319 (2001).

83. X. Guo, J. Hu, J. B. Whitney, R. S. Russell, and C. Liang, Important role for the CA-NC spacer region in the assembly of bovine immunodeficiency virus Gag protein, *J. Virol.* 78, 551–560 (2004).
84. H. G. Krausslich, M. Facke, A. M. Heuser, J. Konvalinka, and H. Zentgraf, The spacer peptide between human immunodeficiency virus capsid and nucleoscapsid proteins is essential for ordered assembly and viral infectivity, *J. Virol.* 69, 3407–3419 (1995).
85. M. A. Accola, S. Hoglund, and H. G. Gottlinger, A putative a-helical structure which overlaps the capsid-p2 boundary in the human immunodeficiency virus type 1 Gag precursor is crucial for viral particle assembly, *J. Virol.* 72, 2071–2078 (1998).
86. S. Morikawa and D. H. L. Bishop, Identification and analysis of the *gag-pol* ribosomal frameshift site of feline immunodeficiency virus, *Virology* 186, 389–397 (1992).
87. S. Morikawa, T. F. Booth, and D. H. L. Bishop, Analyses of the requirements for the synthesis of virus-like particles by feline immunodeficiency virus Gag using baculovirus vectors, *Virology* 183, 288–297 (1991).
88. K. von der Helm, Retroviral proteases: structure, function and inhibition from a nonanticipated viral enzyme to the target of a most promising HIV therapy, *Biol. Chem.* 377, 765–774 (1996).
89. G. S. Laco, C. Schalk-Hihi, J. Lubkowski, G. M. Morris, A. Zdanov, A. Olson, J. H. Elder, A. Wlodawer, and A. Gustchina, Crystal structures of the inactive D30N mutant of feline immunodeficiency virus protease complexed with a substrate and an inhibitor, *Biochemistry* 36, 10696–10708 (1997).
90. A. Wlodawer, A. Gustchina, L. Reshetnikova, J. Lubkowski, A. Zdanov, K. Y. Hui, E. L. Angleton, W. G. Farmerie, M. M. Goodenow, and D. Bhatt, Structure of an inhibitor complex of the proteinase from feline immunodeficiency virus, *Nat. Struct. Biol.* 2, 480–488 (1995).
91. M. Schnolzer, H. R. Rackwitz, A. Gustchina, G. S. Laco, A. Wlodawer, J. H. Elder, and S. B. Kent, Comparative properties of feline immunodeficiency virus (FIV) and human immunodeficiency virus type 1 (HIV-1) proteinases prepared by total chemical synthesis., *Virology* 224, 268–275 (1996).
92. Y. Lin, Z. Q. Beck, T. Lee, V. Le, G. M. Morris, A. J. Olson, C. Wong, and J. H. Elder, Alteration of substrate and inhibitor specificity of feline immunodeficiency virus protease, *J. Virol.* 74, 4710–4720 (2000).
93. T. Lee, G. S. Laco, B. E. Torbett, H. S. Fox, D. L. Lerner, J. H. Elder, and C. H. Wong, Analysis of the S3 and S3′ subsite specificities of feline immunodeficiency virus (FIV) protease: development of a broad-based protease inhibitor efficacious against FIV, SIV, and HIV in vitro and ex vivo, *Proc. Natl. Acad. Sci. USA* 95, 939–944 (1998).
94. Z. Q. Beck, Y. Lin, and J. H. Elder, Molecular basis for the relative substrate specificity of human immunodeficiency virus type 1 and feline immunodeficiency virus proteases, *J. Virol.* 75, 9458–9469 (2001).
95. Y. C. Lin, Z. Q. Beck, G. M. Morris, A. J. Olson, and J. H. Elder, Structural basis for distinctions between substrate and inhibitor specificities for feline immunodeficiency virus and human immunodeficiency virus proteases, *J. Virol.* 77, 6589–6600 (2003).
96. J. Kervinen, J. Lubkowski, A. Zdanov, D. Bhatt, B. M. Dunn, K. Y. Hui, D. J. Powell, J. Kay, A. Wlodawer, and A. Gustchina, Toward a universal inhibitor of retroviral proteases: comparative analysis of the interactions of LP-130 complexed with proteases from HIV-1, FIV, and EIAV, *Protein Sci.* 7, 2314–2323 (1998).
97. B. Buhler, Y. Lin, G. M. Morris, O. A. J., C. Wong, D. D. RIchman, J. H. Elder, and B. E. Torbett, Viral evolution in response to the broad-based retroviral protease inhibitor TL-3, *J. Virol.* 75, 9502–9508 (2001).
98. M. Hottiger and U. Hubscher, Human immunodeficiency virus type 1 reverse transcriptase, *Biol. Chem. Hopppe Seyler* 377, 97–120 (1996).

99. T. W. North, G. L. North, and N. C. Pedersen, Feline immunodeficiency virus, a model for reverse transcriptase-targeted chemotherapy for acquired immune deficiency syndrome, *Antimicrob. Agents Chemother.* 33, 915–919 (1989).
100. T. W. North, R. C. Cronn, K. M. Remington, R. T. Tandberg, and R. C. Judd, Characterization of reverse transcriptase from feline immunodeficiency virus, *J. Biol. Chem.* 265, 5121–5128 (1990).
101. M. Amacker, M. Hottiger, and U. Hubscher, Feline immunodeficiency virus reverse transcritpase: expression, functional characterization, and reconstitution of the 66- and 51-kilodalton subunits, *J. Virol.* 69, 6273–6279 (1995).
102. R. C. Cronn, J. D. Whitmer, and T. W. North, RNase H activity associated with reverse transcriptase from feline immunodeficiency virus, *J. Virol.* 66, 1215–1218 (1992).
103. G. J. Klarmann, R. A. Smith, R. F. Schinazi, T. W. North, and B. D. Preston, Site-specific incorporation of nucleoside analogs by HIV-1 reverse transcriptase and the template grip mutant P157S, *J. Biol. Chem.* 275, 359–366 (2000).
104. K. M. Remington, B. Chesebro, K. Wehrly, N. C. Pedersen, and T. W. North, Mutants of feline immunodeficiency virus resistant to 3′-azido-3′-deoxythymidine, *J. Virol.* 65, 308–312 (1991).
105. K. M. Remington, Y.-Q. Zhu, T. R. Phillips, and T. W. North, Rapid phenotypic reversion of zidovudine-resistant feline immunodeficiency virus without loss of drug-resistant feline immunodeficiency virus without loss of drug-resistant reverse transcriptase, *J. Virol.* 68, 632–637 (1994).
106. R. A. Smith, K. M. Remington, R. M. Lloyd, R. F. Schinazi, and T. W. North, A novel Met-to-Thr mutation in the YMDD motif of reverse transcriptase from feline immunodeficiency virus confers resistance to oxathiolane nucleosides, *J. Virol.* 71, 2357–2362 (1997).
107. S. M. A. McCrackin and D. G. McBroom, In vitro characterization of FIV-pPPR, a pathogenic molecular clone of feline immunodeficiency virus, and two drug-resistant pol gene mutants, *Am J. Vet. Res.* 62, 588–594 (2001).
108. R. A. Smith, K. M. Remington, B. D. Preston, R. F. Schinazi, and T. W. North, A novel point mutation at position 156 of reverse transcriptase from feline immunodeficiency virus confers resistance to the combination of (-)-beta-2′,3′-dideoxy-3′-thiacytidine and 3′-azido-3′-deoxythymidine, *J. Virol.* 72, 2335–2340 (1998).
109. J. Auwerx, T. W. North, B. D. Preston, G. J. Klarmann, E. de Clercq, and J. Balzarini, Chimeric human immunodeficiency virus type 1 and feline immunodeficiency virus reverse transcriptases: role of the subunits in resistance/sensitivity to non-nucleoside reverse transcriptase inhibitors, *Mol. Pharmacol.* 61, 400–406 (2002).
110. M. Amacker and U. Hubscher, Chimeric HIV-1 and feline immunodeficiency virus reverse transcriptases: critical role of the p51 subunit in the structural integrity of heterodimeric lentiviral DNA polymerases, *J. Mol. Biol.* 278, 757–765 (1998).
111. J. E. Barlough, T. W. North, C. L. Oxford, K. M. Remington, S. Dandekar, M. N. Ellis, and N. C. Pedersen, Feline immunodeficiency virus infection of cats as a model to test the effect of certain *in vitro* selection pressures on the infectivity and virulence of resultant lentivirus variants, *Antiviral Res.* 22, 259–272 (1993).
112. M. H. Gadsen, E. M. McIntosh, J. C. Game, P. J. Wilson, and R. H. Haynes, dUTP pyrophosphatase is an essential enzyme in Saccharomyces cerevisiae, *EMBO J.* 12, 4425–4431 (1993).
113. S. L. Payne and J. H. Elder, The role of retroviral dUTPases in replication and virulence, *Curr. Protein Pept. Sci.* 2, 381–388 (2001).
114. D. L. Lerner, P. C. Wagaman, T. Phillips, O. Prospero-Garcia, S. J. Henriksen, H. S. Fox, F. E. Bloom, and J. H. Elder, Increased mutation frequency of feline immunodeficiency virus lacking functional deoxyuridine-triphosphatase, *Proc. Natl. Acad. Sci, USA* 92, 7480–7484 (1995).

115. W. K. Steagall, M. D. Robek, S. T. Perry, F. J. Fuller, and S. L. Payne, Incorporation of uracil into viral DNA correlates with reduced replication of EIAV in macrophages, *Virology* 210, 302–313 (1995).
116. L. Selig, S. Benichou, M. E. Rogel, L. I. Wu, M. A. Vodicka, J. Sire, R. Benarous, and M. Emerman, Uracil DNA glycosylase specifically interacts with Vpr of both human immunodeficiency virus type 1 and simian immunodeficiency virus of sooty mangabeys, but binding does not correlate with cell cycle arrest, *J. Virol.* 71, 4842–4846 (1997).
117. L. M. Mansky, S. Preveral, L. Selig, R. Benarous, and S. Benichou, The interaction of Vpr with uracil DNA glycosylase modulates the human immunodeficiency virus type 1 in vivo mutation rate, *J. Virol.* 74, 7039–7047 (2000).
118. L. M. Mansky, E. Le Rouzic, S. Benichou, and L. C. Gajary, Influence of reverse transcriptase variants, drugs, and Vpr on human immunodeficiency virus type 1 mutant frequencies, *J. Virol.* 77, 2071–2080 (2003).
119. M. Bouhamdan, S. Benichou, F. Rey, J. Navarro, I. Agostini, B. Spire, J. Camonis, G. Slupphaug, R. Vigne, R. Benarous, and J. Sire, Human immunodeficiency virus type 1 Vpr protein binds to the uracil DNA glycosylase DNA repair enzyme, *J. Virol.* 70, 697–704 (1996).
120. P. O. Brown, in *Retroviruses*, edited by J. M. Coffin, S. H. Hughes, and H. E. Varmus (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1997), 161–204.
121. C. Vink, K. H. Van Der Linden, and R. H. A. Plasterk, Activities of the feline immunodeficiency virus integrase protein produced in *Escherichia coli*, *J. Virol.* 68, 1468–1474 (1994).
122. E. Khan, J. P. Mack, R. A. Katz, J. Kulkosky, and A. M. Skalka, Retroviral integrase domains: DNA binding and the recognition of LTR sequences, *Nucleic Acids Res.* 19, 851–860 (1991).
123. Y. Shibagaki and S. A. Chow, Central core domain of retroviral integrase is responsible for target site selection, *J. Biol. Chem.* 272, 8361–8369 (1997).
124. Y. Shibagaki, M. L. Holmes, R. S. Appa, and S. A. Chow, Characterization of feline immunodeficiency virus integrase and analysis of functional domains, *Virology* 230, 1–10 (1997).
125. D. T. Saenz, N. Loewen, M. Peretz, T. Whitwam, R. Barraza, K. G. Howell, J. M. Holmes, M. Good, and E. M. Poeschla, Unintegrated lentivirus DNA persistence and accessibility to expression in nondividing cells: analysis with class I integrase mutants, *J. Virol.* 78, 2906–2920 (2004).
126. R. A. Fouchier and M. H. Malim, Nuclear import of human immunodeficiency virus type-1 preintegration complexes, *Adv. Virus Res.* 52, 275–299 (1999).
127. B. Bouyac, M., J. D. Dvorin, R. A. Fouchier, Y. Jenkins, B. E. Meyer, L. I. Wu, M. Emerman, and M. H. Malim, HIV-1 infection requires a functional integrase NLS, *Cell* 7, 1025–1035 (2001).
128. C. L. Woodward, Y. Wang, W. J. Dixon, H. Htun, and S. A. Chow, Subcellular localization of feline immunodeficiency virus integrase and mapping of its karyophiclic determinant, *J. Virol.* 77, 4516–4527 (2003).
129. E. B. Stephens, E. Monck, K. Reppas, and E. J. Butfiloski, Processing of the glycoprotein of feline immunodeficiency virus: effect of inhibitors of glycosylation, *J. Virol.* 65, 1114–1123 (1991).
130. E. J. Verschoor, E. G. J. Hulskotte, J. Ederveen, M. J. M. Koolen, M. C. Horzinek, and P. J. M. Rottier, Post-translational processing of the feline immunodeficiency virus envelope precursor protein, *Virology* 193, 433–438 (1993).
131. M. L. Poss, S. W. Dow, and E. A. Hoover, Cell-specific envelope glycosylation distinguishes FIV glycoproteins produced in cytopathically and noncytopathically infected cells, *Virology* 188, 25–32 (1992).

132. T. W. Vahlenkamp, A. De Ronde, P. J. M. Rottier, M. C. Horzinek, H. F. Egberink, and E. J. Verschoor, The feline immunodeficiency virus envelope protein precursor: functional analysis of a leader deletion mutant, *Vet. Microbiol.* 69, 115–116 (1999).
133. G. Pancino, L. Camoin, and P. Sonigo, Structural analysis of the principal immunodominant domain of the feline immunodeficieny virus transmembrane glycoprotein, *J. Virol.* 69, 2110–2118 (1995).
134. G. Pancino, I. Fossati, C. Chapey, S. Castelot, B. Hurtrel, A. Maraillon, D. Katzmann, and P. Sonigo, Structure and variations of feline immunodeficiency virus envelope glycoproteins, *Virology* 192, 659–662 (1993).
135. P. F. Serres, Molecular mimicry betwen the trimeric ectodomain of the transmembrane protein of immunosuppresive lentiviruses (HIV-SIV-FIV) and interleukin 2, *C. R. Acad. Sci. Ser III.* 323, 1019–1029 (2000).
136. G. Pancino and P. Sonigo, Retention of viral infectivity after extensive mutation of the highly conserved immunodominant domain of the feline immunodeficiency virus envelope, *J. Virol.* 71, 4339–4346 (1997).
137. H. Garg, F. J. Fuller, and W. A. F. Tompkins, Mechanism of feline immunodeficiency virus envelope glycoprotein-mediated fusion, *Virology* 321, 274–286 (2004).
138. S. Giannecchini, A. Di Fenza, A. M. D'Ursi, D. Matteucci, P. Rovero, and M. Bendinelli, Antiviral activity and conformational features of an octapeptide derived from the membrane-proximal ectodomain of the feline immunodeficiency virus transmembrane glycoprotein, *J. Virol.* 77, 3724–3733 (2003).
139. K. Salzwedel, J. T. West, and E. Hunter, A conserved tryptophan-rich motif in the membrane-proximal region of the human immunodeficiency virus type 1 gp41 ectodomain is important for Env-mediated fusion and virus infectivity, *J. Virol.* 73, 2469–2480 (1999).
140. I. Munoz-Barroso, K. Salzwedel, E. Hunter, and R. Blumenthal, Role of the membrane-proximal domain in the initial stages of human immunodeficiency virus-1 envelope glycoprotein-mediated membrane fusion, *J. Virol.* 73, 6089–6092 (1999).
141. M. A. Rigby, E. C. Holmes, M. Pistello, A. Mackay, A. J. L. Brown, and J. C. Neil, Evolution of structural proteins of feline immunodeficiency virus: molecular epidemiology and evidence of selection for change, *J. Gen. Virol.* 74, 425–436 (1993).
142. D. L. Sodora, E. G. Shpaer, B. E. Kitchell, S. W. Dow, E. A. Hoover, and J. I. Mullins, Identification of three feline immunodeficiency virus *env* gene subtypes and comparison of evolutionary patterns between FIV and HIV-1, *J. Virol.* 68, 2230–2238 (1994).
143. W. K. Greene, J. Meers, G. del Fierro, P. R. Carnegie, and W. F. Robinson, Extensive sequence variation of feline immunodeficiency virus *env* gene in isolates from naturally infected cats, *Arch. Virol.* 133, 51–62 (1993).
144. M. G. Pistello, E. Cammarota, E. Nicoletti, D. Matteucci, M. Curcio, D. Del Mauro, and M. Bendinelli, Analysis of the genetic diversity and phylogenetic relationship of Italian isolates of feline immunodeficiency virus indicates a high prevalence and heterogeneity of subtype B, *J. Gen. Virol.* 78, 2247–2257 (1997).
145. M. R. Pecoraro, K. Tomonaga, T. Miyazawa, Y. Kawaguchi, S. Sugita, Y. Tohya, C. Kai, M. E. Etcheverrigaray, and T. Mikami, Genetic diversity of Argentine isolates of feline immunodeficiency virus, *J. Gen. Virol.* 77, 2031–2035 (1996).
146. A. Steinrigl and D. Klein, Phylogenetic analysis of feline immunodeficiency virus in Central Europe: a prerequisite for vaccination and molecular diagnostics, *J. Gen. Virol.* 84, 1301–1307 (2003).
147. S. Kakinuma, K. Motokawa, T. Hohdatsu, J. K. Yamamoto, H. Koyama, and H. Hashimoto, Nucleotide sequence of feline immunodeficiency virus: classification of Japanese isolates into two subtypes which are distinct from non-Japanese subtypes, *J. Virol.* 69, 3639–3646 (1995).

148. G. Inada, T. Miyazawa, Y. Inoshima, M. Kohmoto, Y. Ikeda, C. H. Liu, J. A. Lin, T. F. Kuo, and T. Mikami, Phylogenetic analysis of feline immunodeficiency virus isolated from cats in Taiwan, *Arch. Virol.* 142, 1456–1459 (1997).
149. M. A. Carpenter, E. W. Brown, D. W. MacDonald, and S. J. O'Brien, Phylogeographic patterns of feline immunodeficiency virus genetic diversity in the domestic cat, *Virology* 251, 234–243 (1998).
150. M. H. Bachmann, C. K. Mathiason-Dubard, G. H. Learn, A. G. Rodrigo, D. L. Sodora, P. Mazzetti, H. A. Hoover, and J. I. Mullins, Genetic diversity of feline immunodeficiency virus: dual infection, recombination, and distinct evolutionary rates among envelope sequence clades, *J. Virol.* 71, 4241–4253 (1997).
151. E. A. Weaver, E. W. Collisson, M. Slater, and G. Zhu, Phylogenetic analysies of Texas isolates indicate an evolving subtype of the clade B feline immunodeficiency viruses, *J. Virol.* 78, 2158–2163 (2004).
152. M. T. Kyaw-Tanner, W. K. Greene, H. S. Park, and H. S. Robinson, The induction of in vivo superinfection and recombination using feline immunodeficiency virus as the model, *Arch. Virol.* 138, 261–271 (1994).
153. F. Reggeti and D. Bienzle, Feline immunodeficiency virus subtypes A, B and C and intersubtype recombinants in Ontario, Canada, *J. Gen. Virol.* 85, 1843–1852 (2004).
154. E. C. Sabino, E. G. Shpaer, M. G. Morgado, B. T. M. Korber, R. Diaz, V. Bongertz, S. Cavalcante, B. Galvao-Castro, J. I. Mullins, and A. Mayer, Identification of human immunodeficiency virus type 1 envelope genes recombinant between subtypes B and F in two epidemiologically linked individuals in Brazil, *J. Virol.* 68, 6340–6346 (1994).
155. D. L. Robertson, B. H. Hahn, and P. M. Sharp, Recombination in AIDS viruses, *J. Mol. Evol.* 40, 249–259 (1995).
156. D. L. Robertson, P. M. Sharp, F. E. McCutchan, and B. H. Hahn, Recombination in HIV-1, *Nature* 37, 124–126 (1995).
157. M. C. Gemeniano, E. T. Sawai, C. M. Leutenegger, and E. E. Sparger, Feline immunodeficiency virus orf-A is required for virus particle formation and virus infectivity, *J. Virol.* 77, 8819–8830 (2003).
158. M. C. Gemeniano, E. T. Sawai, and E. E. Sparger, Feline immunodeficiency virus Orf-A localizes to the nucleus and induces cell cycle arrest, *Virology* 253, 167–174 (2004).
159. M. H. Malim, L. S. Tiley, D. F. McCarn, J. R. Rusche, J. Hauber, and B. R. Cullen, HIV-1 structural gene expression requires binding of the Rev trans-activator to its RNA target sequence, *Cell* 60, 675–683 (1990).
160. B. K. Felber, M. Hadzopoulou-Cladaras, C. Cladaras, T. Copeland, and G. N. Pavlakis, Rev protein of human immunodeficiency virus type 1 affects the stability and transport of the viral mRNA, *Proc. Natl. Acad. Sci. USA* 86, 1494–1499 (1989).
161. G. C. Otero, M. E. Harris, J. E. Donello, and T. J. hope, Leptomycin B inhibits equine infectious anemia virus Rev and feline immunodeficiency virus Rev function but not the function of the hepatitus B virus posttranscriptional regulatory element, *J. Virol.* 72, 7593–7597 (1998).
162. V. A. Mancuso, T. J. Hope, L. Zhu, D. Derse, T. Phillips, and T. G. Parslow, Posttranscriptional effector domains in the Rev proteins of feline immunodeficiency virus and equine infectious anemia virus, *J. Virol.* 68, 1998–2001 (1994).
163. T. J. Hope, Viral RNA export, *Chem. Biol.* 4, 335–344 (1997).
164. O. Schatz, M. Oft, C. Dascher, M. Schebesta, O. Rosorius, H. Jaksche, M. Dobrovnik, D. Bevec, and J. Hauber, Interaction of the HIV-1 rev cofactor eukaryotic initiation factor 5A with ribosomal protein L5, *Proc. Natl. Acad. Sci. USA* 95, 1607–1612 (1998).
165. D. Bevec, H. Jaksche, M. Oft, T. Wohl, M. Himmellspach, A. Pacher, M. Schebesta, K. Koettnitz, M. Dobrovnik, R. Csonga, F. Lotspeich, and J. Hauber, Inhibition of HIV-1 replication in lymphocytes by mutants of the Rev cofactor eLF-5A, *Science* 271, 10829–10833 (1996).

166. R. A. Hart, J. Billaud, S. J. Choi, and T. R. Phillips, Effects of 1,8-diaminooctane on the FIV Rev regulatory system, *Virology* 304, 97–104 (2002).
167. H. P. Bogerd, A. Echarri, T. M. Ross, and B. R. Cullen, Inhibition of human immunodeficiency virus Rev and human T-cell leukemia virus Rex function, but not Mason-Pfizer monkey virus constitutive transport element activity, by a mutant human nucleoporin targeted to Crm1, *J. Virol.* 72, 8627–8635 (1998).
168. M. H. Park, E. C. Wolff, and J. E. Folk, Hypusine: its post-translational formation in eukaryotic initiation factor 5A and its potential role in cellular regulation, *Biofactors* 4, 95–104 (1993).
169. K. Strebel, D. Daugherty, and K. Clouse, The HIV "A" (sor) gene product is essential for virus infectivity, *Nature* 328, 728–730 (1987).
170. J. Sodroski, W. C. Goh, and C. Rosen, Replicative and cytopathic potential of HTLV-II/LAV with sor gene deletions, *Science* 231, 412–417 (1986).
171. K. Tomonaga, Y. Inoshima, Y. Ikeda, and T. Mikami, Temporal patterns of feline immunodeficiency virus transcripts in peripheral blood cells during the latent stage of infection, *J. Gen. Virol.* 76, 2193–2204 (1995).
172. K. Tomonaga, J. Norimine, Y. Shin, M. Fukasawa, T. Miyazawa, A. Adachi, T. Toyosaki, Y. Kawaguchi, C. Kai, and T. Mikami, Identification of a feline immunodeficiency virus gene which is essential for cell-free virus infectivity, *J. Virol.* 66, 6181–6185 (1992).
173. B. L. Shacklett and P. A. Luciw, Analysis of the *VIF* gene of feline immunodeficiency virus, *Virology* 204, 860–867 (1994).
174. K. Lockridge, S. Himathongkham, E. T. Sawai, M. Chien, and E. E. Sparger, The feline immunodeficiency virus *vif* gene is required for productive infection of feline peripheral blood mononuclear cells and monocyte-derived macrophages, *Virology* 261, 25–30 (1999).
175. U. Chatterji, C. K. Grant, and J. H. Elder, Feline immunodeficiency virus Vif localizes to the nucleus, *J. Virol.* 74, 2533–2540 (2001).
176. D. H. Gabuzda, K. Lawrence, E. Langhoff, E. Terwillinger, T. Dorfman, W. A. Haseltine, and J. Sodroski, Role of *vif* in replication of human immunodeficiency virus type 1 in CD4+ T lymphocytes, *J. Virol.* 66, 6489–6495 (1992).
177. N. Madani and D. Kabat, An endogenous inhibitor of human immunodeficiency virus in human lymphocytes is overcome by the viral vif protein, *J. Virol.* 72, 10251–10255 (1998).
178. A. M. Sheehy, N. C. Gaddis, J. D. Choi, and M. H. Malim, Isolation of a human gene that inhibits HIV-1 infection and is suppressed by the viral Vif protein, *Nature* 418, 646–650 (2002).
179. V. N. Kewal-Ramani and J. M. Coffin, Virology. Weapons of mutational destruction, *Science* 301, 923–925 (2003).
180. Q. Yu, R. Konig, S. Pillai, K. Chiles, M. Kearney, S. Palmer, D. Richman, J. M. Coffin, and N. R. Landau, Single-strand specificity of APOBEC3G accounts for minus-strand deaminatin of the HIV genome, *Nat. Struct. Mol. Biol.* 11, 435–442 (2004).
181. S. P. Goff, Death by deamination: a novel host restriction system for HIV-1, *Cell* 114, 281–283 (2003).
182. R. Mariani, D. Chen, B. Schrofelbauer, F. Navarro, R. Konig, B. Bollman, C. Munk, H. Nymark-McMahon, and N. R. Landau, Species-specific exclusion of APOBEC3G from HIV-1 virions by Vif, *Cell* 114, 21–31 (2003).
183. M. Marin, K. M. Rose, S. L. Kozak, and D. Kabat, HIV-1 Vif protein binds the editing enzyme APOBEC3G and induces its degradation, *Nat. Med.* 9, 1398–1403 (2003).
184. A. M. Sheehy, N. C. Gaddis, and M. H. Malim, The antiretroviral enzyme APOBEC3G is degraded by the protesome in response to HIV-1 Vif, *Nat. Med.* 9, 1404–1407 (2003).

185. R. M. Norway, P. C. Crawford, C. M. Johnson, and A. Mergia, Thymic lesions in cats infected with a pathogenic molecular clone or an ORF-A/2- deficient molecular clone of feline immunodeficiency virus, *J. Virol.* 75, 5833–5841 (2001).
186. G. Dean and E. E. Sparger, In vivo tropism of molecular cloned isolates of FIV, in *Third International Feline Retrovirus Research Symposim.* Fort Collins, CO (1996).
187. M. Pistello, M. Moscardini, P. Mazzetti, F. Bonci, L. Zaccaro, P. Isola, G. Freer, S. Specter, I. D. Matteucc, and M. Bendinelli, Development of feline immunodeficiency virus ORF-A (tat) mutants: in vitro and in vivo characterization, *Virology* 298, 84–95 (2002).
188. K. Tomonaga, T. Miyazawa, J. Sakuragi, T. Mori, A. Adachi, and T. Mikami, The feline immunodeficiency virus ORF-A gene facilitates efficient viral replication in established T-cell lines and peripheral blood lymphocytes, *J. Virol.* 67, 5889–5895 (1993).
189. S. Villet, B. A. Bouzar, T. Morin, G. Verdier, C. Legras, and Y. Chebloune, Maedi-visna virus and caprine arthiritis encphalitis virus genomes encode a Vpr-like but no Tat protein, *J. Virol.* 77, 9632–9638 (2003).
190. S. De Rozieres, C. K. Mathiason, M. R. Rolston, U. Chatterji, E. A. Hoover, and J. H. Elder, Characterization of a highly pathogenic molecular clone of feline immunodeficiency virus clade C, *J. Virol.* 78, 8971–8982 (2004).
191. L. Diehl, C. K. Mathiason-Dubard, L. L. O'Neil, and E. A. Hoover, Plasma viral RNA load predicts disease progression in accelerated feline immunodeficiency virus infection, *J. Virol.* 70, 2503–2507 (1996).
192. E. E. Sparger, A. M. Beebe, N. Dua, J. Elder, S. Himathongkam, M. Torten, and J. Higgins, Infection of cats with molecularly cloned and biological isolates of the feline immunodeficiency virus, *Virology* 205, 546–553 (1994).
193. J. C. Woo, G. A. Dean, N. C. Pedersen, and P. F. Moore, Immunopathologic changes in the thymus during the acute stage of experimentally induced feline immunodeficiency virus infection in juvenile cats, *J. Virol.* 71, 8632–8641 (1997).
194. J. Yang, R. V. English, J. W. Ritchey, M. G. Davidson, T. Wasmoen, J. K. Levy, D. H. Gebhard, M. B. Tompkins, and W. A. F. Tompkins, Molecularly cloned feline immunodeficiency virus NCSU1 YSY3 induces immunodeficiency in specific-pathogen-free cats, *J. Virol.* 70, 3011–3017 (1996).
195. W. C. Brown, L. Bissey, K. S. Logan, N. C. Pedersen, J. H. Elder, and E. W. Collisson, Feline immunodefiency virus infects both CD4+ and CD8+ T lymphocytes, *J. Virol.* 65, 3359–3364 (1991).
196. D. Brunner and N. C. Pedersen, Infection of peritoneal macrophages in vitro and in vivo with feline immunodeficiency virus, *J. Virol.* 63, 5483–5488 (1989).
197. G. A. Dean, S. Himathongkham, and E. E. Sparger, Differential cell tropism of feline immunodeficiency virus molecular clones in vivo, *J. Virol.* 73, 2596–2603 (1999).
198. S. W. Dow, M. L. Poss, and E. A. Hoover, Feline immunodeficiency virus: a neurotropic lentivirus, *J. Acquir. Immune Defic. Syndr.* 3, 658–668 (1990).
199. S. W. Dow, C. K. Mathiason, and E. A. Hoover, In vivo monocyte tropism of pathogenic feline immunodeficiency viruses, *J. Virol.* 73, 6852–6861 (1999).
200. N. Dua, G. Reubel, J. Higgins, and N. C. Pedersen, The primary stage of feline immunodeficiency virus infection, *Vet. Immunol. Immunopathol.* 43, 337–355 (1994).
201. R. V. English, C. M. Johnson, D. H. Gebhard, and M. B. Tompkins, In vivo lympohcyte tropism of feline immunodeficiency virus, *J. Virol.* 67, 5175–5186 (1993).
202. A. Hein, J. P. Martin, F. Koehren, A. Bingen, and R. Dorries, In vivo infection of ramified microglia from adult cat central nervous system by feline immunodeficiency virus, *Virology* 268, 420–429 (2000).
203. A. Kipar, F. S. Boretti, M. M. Meli, K. Failing, M. Reinacher, and H. Lutz, Reduced constitutive cytokine transcription in isolated monocytes of clinically healthy cats, infected with an FIV strain of low pathogenicity, *Vet. Immunol. Immunopathol.* 98, 215–221 (2004).

204. M. Magnani, L. Rossi, A. Fraternale, L. Silvotti, F. Quintavalla, G. Piedimonte, D. Matteucci, F. Baldinotti, and M. Bendinelli, Feline immunodeficiency virus infection of macrophages: in vitro and in vivo inhibition by dideoxycytidine-5′-triphosphate-loaded erythrocytes, *AIDS Res. Hum. Retroviruses* 10, 1179–1186 (1994).
205. K. Nakagaki, K. Nakagaki, K. Takahashi, D. Schols, E. De Clercq, and T. Tabira, CXCR4 is the primary receptor for feline immunodeficiency virus in astrocytes, *J. Neurovirol.* 7, 487–492 (2001).
206. A. Rogers and E. Hoover, Fetal feline immunodeficiency virus is prevalent and occult, *J. Infect. Dis.* 186, 895–904 (2002).
207. A. B. Rogers, C. K. Mathiason, and E. A. Hoover, Immunohistochemical localization of feline immunodeficiency virus using native species antibodies, *Am. J. Pathol.* 161, 1143–1151 (2002).
208. T. Toyosaki, T. Miyazawa, T. Furuya, K. Tomonaga, Y.-S. Shin, M. Okita, Y. Kawaguchi, C. Kai, S. Mori, and T. Mikami, Localizaion of the viral antigen of feline immunodeficiency virus in the lymph nodes of cats at the early stage of infection, *Arch. Virol.* 131, 335–347 (1993).
209. T. W. Vahlenkamp, A. De Ronde, N. N. Schuurman, A. L. Van Vliet, J. Van Drunen, M. C. Horzinek, and H. F. Egberink, Envelope gene sequences encoding variable regions 3 and 4 are involved in macrophage tropism of feline immunodeficiency virus, *J. Gen. Virol.* 80, 2639–2646 (1999).
210. N. Yu, J. N. Billaud, and T. R. Phillips, Effects of feline immunodeficiency virus on astrocyte glutamate uptake: implications for lentivirus-induced central nervous system diseases, *Proc. Natl. Acad. Sci. USA* 95, 2624–2629 (1998).
211. D. L. Lerner, C. K. Grant, A. P. De Parseval, and J. H. Elder, FIV infection of IL-2-dependent and -independent feline lymphocyte lines: host cells range distinctions and specific cytokine upregulation, *Vet. Immunol. Immunopathol.* 65, 277–297 (1998).
212. M. J. Hosie, B. J. Willett, D. Klein, T. H. Dunsford, C. Cannon, M. Shimojima, J. C. Neil, and O. Jarett, Evolution of replication efficiency following infection with a molecularly cloned feline immunodeficiency virus of low virulence, *J. Virol.* 76, 6062–6072 (2002).
213. J. Norimine, T. MIyazawa, Y. Kawaguchi, K. Tomonaga, Y. S. Shin, T. Toyosaki, M. Kohmoto, M. Niikura, Y. Tohya, and T. Mikami, Feline CD4 molecules expressed on feline non-lymphoid cell lines are not enough for productive infection of highlt lymphotropic feline immunodeficiency virus isolates, *Arch. Virol.* 130, 171–178 (1993).
214. B. J. Willet, M. J. Hosie, O. Jarrett, and J. C. Neil, Identification of a putative cellular receptor for feline immunodeficiency virus as the feline homologue of CD9, *Immunology* 81, 228–233 (1994).
215. M. J. Hosie, B. J. Willett, T. H. Dunsford, O. Jarrett, and J. C. Neil, A monoclonal antibody which blocks infection with feline immunodeficiency virus identifies a possible non-CD4 receptor, *J. Virol.* 67, 1667–1671 (1993).
216. A. De Parseval, D. L. Lerner, P. Borrow, B. J. Willett, and J. H. Elder, Blocking of feline immunodeficiency virus infection by a monoclonal antibody to CD9 is via inhibition of virus release rather than interference with receptor binding, *J. Virol.* 71, 5742–5749 (1997).
217. B. J. Willett, M. J. Hosie, J. C. Neil, J. D. Turner, and J. A. Hoxie, Common mechanism of infection by lentiviruses, *Nature* 385, 587 (1997).
218. B. J. Willett, K. Adema, N. Heveker, A. Brelot, L. Picard, M. Alizon, J. D. Turner, J. A. Hoxie, S. Peiper, J. C. Neil, and M. J. Hosie, The extracellular loop of CXCR4 determines its function as a receptor for feline immunodeficiency virus, *J. Virol.* 72, 6475–6481 (1998).
219. M. J. Hosie, N. Broere, J. Hesselgesser, J. D. Turner, J. A. Hoxie, J. C. Neil, and B. J. Willett, Modulation of feline immunodeficiency virus infection by stromal cell-derived factor (SDF-1), *J. Virol.* 72, 2097–2104 (1998).

220. H. F. Egberink, E. De Clercq, A. L. W. Van Vliet, J. Balzarini, G. J. Bridger, G. Henson, M. C. Horzinek, and D. Schols, Bicyclams, selective antagonists of the human chemokine receptor CXCR4, potently inhibit feline immunodeficiency virus replication, *J. Virol.* 73, 6346–6352 (1999).
221. J. Richardson, G. Pancino, R. Merat, T. Leste-Lasserre, A. Maraillon, J. Schneider-Mergener, M. Alizon, P. Sonigo, and N. Heveker, Shared usage of the chemokine receptor CXCR4 by primary and laboratory-adapted strains of feline immunodeficiency virus, *J. Virol.* 73, 3661–3671 (1999).
222. A. Brelot, N. Heveker, K. Adema, M. J. Hosie, B. Willett, and M. Alizon, Effect of mutations in the second extracellular loop of CXCR4 on its utilization by human and feline immunodeficiency viruses, *J. Virol.* 73, 2576–2586 (1999).
223. J. C. Johnston and C. Power, Feline immunodeficiency virus xenoinfection: the role of chemokine receptors and envelope diversity, *J. Virol.* 76, 3626–3636 (2002).
224. B. J. Willett, C. A. Cannon, and M. J. Hosie, Upregulation of surface feline CXCR4 expression following ectopic expression of CCR5: implications for studies of the cell tropism of feline immunodeficiency virus, *J. Virol.* 76, 9242–9252 (2002).
225. J. C. Johnston, M. Gasmi, L. E. Lim, J. H. Elder, J. Yee, D. J. Jolly, K. P. Campbell, B. L. Davison, and S. L. Sauter, Minimum requirements for efficient transduction of dividing and nondividing cells by feline immunodeficiency virus vectors, *J. Virol.* 73, 4991–5000 (1999).
226. A. De Parseval, S. Ngo, P. Sun, and J. H. Elder, Factors that increase the effective concentration of CXCR4 dictate feline immunodeficiency virus tropism and kinetics of replication, *J. Virol.* 78, 9132–9143 (2004).
227. A. De Parseval, U. Chatterji, P. Sun, and J. H. Elder, Feline immunodeficiency virus targets activated CD4+ T-cells by using CD134 as a binding receptor, *Proc. Natl. Acad. Sci. USA* 101, 13044–13049 (2004).
228. A. D. Weinberg, D. E. Evans, C. Thalhofer, T. Shi, and R. A. Prell, The generation of T-cell memory: a review describing the molecular and cellular events following OX40 (CD1334) engagement, *J. Leukocyte Biol.* 75, 1–11 (2004).
229. P. R. Baum, R. B. I. Gayle, F. Ramsdell, S. Srinivasan, R. A. Sorensen, M. L. Watson, M. F. Seldin, E. Baker, G. R. Sutherland, K. N. Clifford, et al., Molecular characterization of murine and human OX40/OX40 ligand systems: identification of a human OX40 ligand as the HTLV-1-regulated protein gp34, *EMBO J.* 13, 3992–4001 (1994).
230. H. Durkop, I. Anagnostopoulos, S. Bulfone-Paus, and H. Stein, Expression of several members of the TNF-ligand and receptor family on tonsillar lymphoid B cells, *Br. J. Haematol.* 98, 863–868 (1997).
231. H. Durkop, U. Latza, P. Himmelreich, and H. Stein, Expression of the human OX40 (hOX40) antigen in normal and neoplastic tissues, *Br. J. Haematol.* 91, 927–931 (1995).
232. J. D. Siliciano, J. Kajdas, D. Finzi, T. C. Quinn, K. Chadwick, J. B. Margolick, C. Kovacs, S. J. Gange, and R. F. Siliciano, Long-term follow-up studies confirm the stability of the latent reservoir for HIV-1 in resting CD4+ T-cells, *Nat. Med.* 9, 727–728 (2003).
233. R. S. Veazey, I. C. Tham, K. G. Mansfield, M. DeMaria, A. E. Forand, D. E. Shvetz, L. V. Chalifoux, P. K. Sehgal, and A. A. Lackner, Identifying the target cell in primary simian immunodeficiency virus (SIV) infection: highly activated memory CD4(+) T-cells are rapidly eliminated in early SIV infection in vivo, *J. Virol.* 74, 57–64 (2000).
234. Z. Q. Zhang, S. W. Wietgrefe, Q. Li, M. D. Shore, L. Duan, C. Reilly, J. D. Lifson, and A. T. Haase, Roles of substrate availability and infection of resting and activated CD4+ T-cells in transmission and acute simian immunodeficiency virus infection, *Proc. Natl. Acad. Sci. USA* 101, 5640–5645 (2004).
235. Z. Zhang, T. Schuler, M. Zupancic, S. Wietgrefe, et al., Sexual transmission and propagation of SIV and HIV in resting and activated CD4(+) T-cells, *Science* 286, 1353–1357 (1999).

236. D. M. Willerford, M. J. Gale, R. E. Benveniste, E. A. Clark, and W. M. Gallatin, Simian immunodeficiency virus is restricted to a subset of blood CD4+ lymphocytes that includes memory cells, *J. Immunol.* 144, 3779–3783 (1990).
237. S. M. Schnittman, H. C. Lane, J. J. Greenhouse, J. S. Justement, M. Baseler, and A. S. Fauci, Preferential infection of CD4+ memory T-cells by human immunodeficiency virus type 1: evidence for a role in the selective T-cell functional defects observed in infected individuals, *Proc. Natl. Acad. Sci. USA* 87, 6058–6062 (1990).
238. T. Hohdatsu, H. Hirabayashi, K. Motokawa, and H. Koyama, Comparative study of the cell tropism of feline immunodeficiency virus isolates of subtypes A, B and D classified on the basis of the env gene V3–V5 sequence, *J. Gen. Virol.* 77, 93–100 (1996).
239. T. Miyazawa, T. Furuya, S. Itagaki, Y. Tohya, K. Nakano, E. Takahashi, and T. Mikami, Preliminary comparisons of the biological properties of two strains of feline immunodeficiency virus (FIV) isolated in Japan with FIV Petaluma strain isolated in the United States, *Arch. Virol.* 108, 59–68 (1989).
240. R. A. Olmsted, A. K. Barnes, J. K. Yamamoto, V. M. Hirsch, R. H. Purcell, and P. R. Johnson, Molecular cloning of feline immunodeficiency virus, *Proc. Natl. Acad. Sci. USA* 86, 2448–2452 (1989).
241. B. J. Willett, M. J. Hosie, T. H. Dunsford, J. C. Neil, and O. Jarrett, Productive infection of T-helper lymphocytes with feline immunodeficiency virus is accompanied by reduced expression of CD4, *AIDS* 5, 1469–1475 (1991).
242. J. K. Yamamoto, C. D. Ackley, H. Zochlinski, H. Louie, E. Pembroke, M. Torten, H. Hansen, R. Munn, and T. Okuda, Development of IL-2-independent feline lymphoid cell lines chronically infected with feline immunodeficiency virus: importance for diagnostic reagents and vaccines, *Intervirology* 32, 361–375 (1991).
243. K. H. Siebelink, J. A. Karlas, G. F. Rimmelzwaan, A. D. Osterhaus, and M. L. Bosch, A determinant of feline immunodeficiency virus involved in Crandell feline kidney cell tropism, *Vet. Immunol. Immunopathol.* 46, 61–69 (1995).
244. E. J. Verschoor, L. A. Boven, H. llaak, A. L. W. Van Vliet, M. C. Horzinek, and A. De Ronde, A single mutation within the V3 envelope neutralization domain of feline immunodeficiency virus determines its tropism for CRFK cells, *J. Virol.* 69, 4752–4757 (1995).
245. D. L. Lerner and J. H. Elder, Expanded host cell tropism and cytopathic properties of feline immunodeficiency virus strain PPR subsequent to passage through interleukin-2-independent T-cells, *J. Virol.* 74, 1854–1863 (2000).
246. M. Kohmoto, T. Miyazawa, K. Tomonaga, Y. Kawaguchi, T. Mori, Y. Tohya, C. Kai, and T. Mikami, Comparison of biological properties of feline immunodeficiency virus isolates using recombinant chimeric viruses, *J. Gen. Virol.* 75, 1935–1942 (1994).
247. T. W. Vahlenkamp, E. J. Verschoor, N. N. Schuurman, A. L. Van Vliet, M. C. Horzinek, H. F. Egberink, and A. De Ronde, A single amino acid substitution in the transmembrane envelope glycoprotein of feline immunodeficiency virus alters cellular tropism, *J. Virol.* 71, 7132–7135 (1997).
248. G. Pancino, S. Castelot, and P. Sonigo, Differences in feline immunodeficiency virus host cell range correlate with envelope fusogenic properties, *Virology* 206, 796–806 (1995).
249. T. Shioda, J. A. Levy, and C. Cheng-Mayer, Small amino acid changes in the V3 hypervariable region of gp120 can affect the T-cell line and macrophage tropism of human immunodeficiency virus type 1, *Proc. Natl. Acad. Sci. USA* 89, 9434–9438 (1992).
250. T. Shioda, J. A. Levy, and C. Cheng-Mayer, Macrophage and T-cell-line tropisms of HIV-1 are determined by specific regions of the envelope gp120 gene, *Nature* 349, 167–169 (1991).
251. J. J. De Jong, A. De Ronde, W. Keulen, M. Tersmette, and J. Goudsmit, Minimal requirements for the human immunodeficiency virus type 1 V3 domain to support the

syncytium-inducing phenotype: analysis by single amino acid substitution, *J. Virol.* 66, 6777–6780 (1992).
252. A. De Ronde, J. G. Stam, P. Boers, H. Langedijk, R. Meloen, W. Hesselink, L. C. Keldermans, A. Van Vliet, E. J. Verschoor, M. C. Horzinek, and H. F. Egberink, Antibody response in cats to the envelope proteins of feline immunodeficiency virus: identification of an immunodominant neutralization domain, *Virology* 198, 257–264 (1994).
253. S. Lombardi, C. Garzelli, M. Pistello, C. Massi, D. Matteucci, F. Baldinotti, G. Cammarota, L. Da Prato, P. Bandecchi, F. Tozzini, and M. Bendinelli, A neutralizing antibody-inducing peptide of the V3 domain of feline immunodeficiency virus envelope glycoprotein does not induce protective immunity, *J. Virol.* 68, 8374–8379 (1994).
254. S. Lombardi, C. Massi, F. Tozzini, L. Zaccaro, P. Bancecchi, C. La Rosa, M. Bendinelli, and C. Garzelli, Epitope mapping of the V3 domain of feline immunodeficiency virus envelope glycoprotein by monoclonal antibodies, *J. Gen. Virol.* 76, 1893–1899 (1995).
255. A. Hein, H. Schuh, S. Thiel, J. P. Martin, and R. Dorries, Ramified feline microglia selects for distinct variants of feline immunodeficiency virus during early central nervous system infection, *J. Neurovirol.* 9, 465–476 (2003).
256. T. Miyazawa, Y. Kawaguchi, M. Kohmoto, K. Tomonaga, and T. Mikami, Comparative functional analysis of the various lentivirus long terminal repeats in human colon carcinoma cell line (SW489 cells) and feline renal cell line (CRFK cells), *J. Vet. Med. Sci.* 56, 895–899 (1994).
257. T. Miyazawa, Y. Kawaguchi, M. Kohmoto, J. Sakuragi, A. Adachi, M. Fukasawa, and T. Mikami, Production of feline immunodeficiency virus in feline and non-feline non-lymphoid cell lines by transfection of an infectious molecular clone, *J. Gen. Virol.* 73, 1543–1546 (1992).
258. Y. Ikeda, K. Tomonaga, Y. Kawaguchi, M. Kohmoto, Y. Inoshima, Y. Tohya, T. Miyazawa, C. Kai, and T. Mikami, Feline immunodeficiency virus can infect a human cell line (MOLT-4) but establishes a state of latency in the cells, *J. Gen. Virol.* 77, 1623–1630 (1996).
259. T. S. Tochikura, A. Tanabe-Tochikura, K. Hayes, A. Lazo, R. T. Bailer, J. R. Blakeeslee, L. J. Lafrado, P. Roy-Burman, R. Pandey, R. G. Olsen, and L. E. Mathes, Fusion activity dissociated from replication ability in feline immunodeficiency virus (FIV) in human cells, *JAIDS* 6, 1297–1300 (1993).
260. E. M. Poeschla, F. Wong-Staal, and D. J. Looney, Efficient transduction of nondividing human cells by feline immunodeficiency virus lentiviral vectors, *Nature Med.* 4, 354–357 (1998).
261. J. Johnston and C. Power, Productive infection of human peripheral blood mononuclear cells by feline immunodeficiency virus: implications for vector development, *J. Virol.* 73, 2491–2498 (1999).
262. J. B. Johnston, M. E. Olson, E. W. Rud, and C. Power, Xenoinfection of nonhuman primates by feline immunodeficiency virus, *Curr. Biol.* 11, 1109–1113 (2001).
263. M. C. Barr, M. B. Pough, R. H. Jacobson, and F. W. Scott, Comparison and interpretation of diagnostic tests for feline immunodeficiency virus infection, *J. Am. Vet. Med. Assoc.* 199, 1377–1381 (1991).
264. R. Groat, G. Maddsen, B. Bartol, P. Skvorak, K. Serlemitsos, and M. Monn. *Upgraded IDEXX diagnostic products for simultaneous detection of antibodies to feline immunodeficiency virus (FIV) gag and env proteins in feline blood samples.* in *25th World Small Animal Veterinary Association / 6th Federation of European Companion Animal Veterinary Association / Voorjaarsdagen Congress.* Amsterdam, The Netherlands (2000).
265. K. Hartmann, R. M. Werner, H. Egberink, and O. Jarrett, Comparison of six in-house tests for the rapid diagnosis of feline immunodeficiency and feline leukemia virus infections, *Vet. Rec.* 149, 317–320 (2001).

266. T. P. O'Connor, Q. J. Tonelli, and J. M. Scarlett, Report of the national FeLV/FIV awareness project, *J. Am. Vet. Med. Assoc.* 199, 1348–1353 (1991).
267. J. D. Fontenot, E. A. Hoover, J. H. Elder, and R. C. Montelaro, Evaluation of feline immunodeficiency virus and feline leukemia virus transmembrane peptides for serological diagnosis, *J. Clin. Microbiol.* 30, 1885–1890 (1992).
268. M. J. Hosie, C. Robertson, and O. Jarrett, Prevalence of feline leukemia virus and antibodies to feline immunodeficiency virus in cats in the United Kingdom, *Vet. Rec.* 125, 293–297 (1989).
269. R. A. Olmsted, R. Langley, M. E. Roelke, R. M. Goeken, D. Adger-Johnson, J. P. Goff, J. P. Alber, C. Packer, M. K. Laurenson, T. M. Caro, L. Scheepers, D. E. Wildt, M. Bush, J. S. Martenson, and S. J. O'Brien, Worldwide prevalence of lentivirus infection in wild felidae species: epidemiologic and phylogenetic aspects, *J. Virol.* 66, 6008–6018 (1992).
270. I. Lee, J. A. Levy, S. Gorman, P. C. Crawford, and M. Slater, Prevalence of feline leukemia virus infection and serum antibodies against feline immunodeficiency virus in unowned free-roaming cats, *J. Am. Vet. Med. Assoc.* 220, 620–622 (2002).
271. M. Uema, Y. Ikeda, and T. Miyazawa, Feline immunodeficiency virus subtype C is prevalent in northern part of Taiwan, *J. Vet. Med. Sci.* 61, 197–199 (1999).
272. I. G. Winkler, M. Lochelt, and R. L. P. Flower, Epidemiology of feline foamy virus and feline immunodeiciency virus infections in domestic and feral cats: a seroepidemiological study, *J. Clin. Microbiol.* 37, 2848–2851 (1999).
273. F. Courchamp and D. Pontier, Feline immunodeficiency virus: an epidemiological review, *C. R. Acad. Sci. III* 317, 1123–1134 (1994).
274. E. Sparger, P. Luciw, J. Elder, J. Yamamoto, L. Lowenstine, and N. Pedersen, Feline immunodeficiency virus is a lentivirus associated with an AIDS-like disease in cats, *AIDS* 3(Suppl 1), S43–49 (1989).
275. M. C. Barr, L. Zou, D. L. Holzschu, L. Pillips, F. W. Scott, J. W. Casey, and R. J. Avery, Isolation of a highly cytopathic lentivirus from a nondomestic cat, *J. Virol.* 69, 7371–7374 (1995).
276. E. W. Brown, S. Miththapala, and S. J. O'Brien, Prevalence of exposure to feline immunodeficiency virus in exotic felid species, *J. Zoo. Wildl. Med.* 24, 357–364 (1993).
277. E. W. Brown, N. Yuhki, C. Packer, and S. J. O'Brien, A lion lentivirus related to feline immunodeficiency virus: epidemiologic and phylogenetic aspects, *J. Virol.* 68, 5953–5968 (1994).
278. H. Lutz, E. Isenbugel, R. Lehmann, R. Sabapara, and C. Wolfensberger, Retrovirus infections in non-domestic felids: serological studies and attempts to isolate a lentivirus, *Vet. Immunol. Immunopathol.* 35, 215–224 (1992).
279. Y. Nishimura, Y. Goto, K. Yoneda, Y. Endo, T. Mizuno, M. Hamachi, H. Maruyama, H. Kinoshita, S. Koga, M. Komori, S. Fushuku, K. Ushinohama, M. Akuzawa, T. Watari, A. Hasegawa, and H. Tsujimoto, Interspecies transmission of feline immunodeficiency virus from the domestic cat to the Tsushima cat, *J. Virol.* 73, 7916–7921 (1999).
280. J. A. Spencer, A. A. Van Dijk, M. C. Horzinek, H. F. Egberink, R. G. Bengis, D. F. Keet, S. Morikawa, and D. H. L. Bishop, Incidence of feline immunodeficiency virus reactive antibodies in free-ranging lions of the Kruger National Park and the Etosha National Park in southern Africa detected by recombinant FIV p24 antigen, *Onderstepoort J. Vet. Res.* 59, 315–322 (1992).
281. R. Hofmann-Lehmann, D. Fehr, M. Grob, M. Elgizoli, C. Packer, J. S. Martenson, S. J. O'Brien, and H. Lutz, Prevalence of antibodies to feline parvovirus, calicivirus, herpesvirus, coronavirus, and immunodeficiency virus and of feline leukemia virus antigen and the interrelationship of these viral infections in free-ranging lions in East Africa, *Clin. Diagn. Lab. Immunol.* 3, 554–562 (1996).

282. R. Biek, A. G. Rodrigo, D. Holley, A. Drummond, C. R. Anderson, H. A. Ross, and M. Poss, Epidemiology, genetic diversity, and evolution of endemic feline immunodeficiency virus in a population of wild cougars, *J. Virol.* 77, 9578–9589 (2003).
283. F. Courchamp, D. Pontier, M. Langlais, and M. Artois, Population dynamics of feline immunodeficiency virus within cat populations, *J. Theor. Biol.* 175, 553–560 (1995).
284. F. Courchamp, N. G. Yoccoz, M. Artois, and D. Pontier, At-risk individuals in feline immunodeficiency virus epidemiology: evidence from a multivariate approach in a natural population of domestic cats (*Felis catus*), *Epidemiol. Infect.* 121, 227–236 (1998).
285. P. Dorny, N. Speybroeck, S. Verstraete, M. Baeke, A. De Becker, D. Berkvens, and J. Vercruysse, Serological survey of Toxoplasma gondii, feline immunodeficiency virus and feline leukemia virus in urban stray cats in Belgium, *Vet. Rec.* 151, 626–629 (2002).
286. E. Holznagel, H. Lutz, D. Steinhauer, and M. Reinacher, Feline immunodeficiency virus (FIV) infection in cats at necropsy: a serological study, *J. Comp. Pathol.* 116, 339–352 (1997).
287. B. J. Luria, J. K. Levy, M. R. Lappin, E. B. Breitschwerdt, A. M. Legendre, J. A. Hernandez, S. P. Gorman, and I. T. Lee, Prevalence of infectious diseases in feral cats in Northern Florida, *J. Feline Med. Surg.* 6, 287–296 (2004).
288. N. C. Pedersen, in *The Retroviridae Vol. 2,* edited by J. A. Levy (Plenum Press, New York, 1993), 181–227
289. D. Pontier, E. Fromont, F. Courchamp, M. Artois, and N. G. Yoccoz, Retroviruses and sexual size dimorphism in domestic cats (*Felis catus L.*), *Proc. R. Soc. Lond. B. Biol. Sci.* 265, 167–173 (1998).
290. D. Matteucci, F. Baldinotti, P. Mazzetti, M. Pistello, P. Bandecchi, R. Ghilarducci, A. Poli, F. Tozzini, and M. Bendinelli, Detection of feline immunodeficiency virus in saliva and plasma by cultivation and polymerase chain reaction, *J. Clin. Microbiol.* 31, 494–501 (1993).
291. K. Ueland and L. Nesse, No evidence of vertical transmission of naturally acquired feline immunodeficiency virus infection, *Vet. Immunol. Immunopathol.* 33, 301–308 (1992).
292. J. J. Callanan, M. J. Hosie, and O. Jarrett, Transmission of feline immunodeficiency virus from mother to kitten, *Vet. Rec.* 128, 332–333 (1991).
293. R. K. Sellon, H. L. Jordan, S. Kennedy-Stoskopf, M. B. Tompkins, and W. A. Tompkins, Feline immunodeficiency virus can be experimentally transmitted via milk during acute maternal infection, *J. Virol.* 68, 3380–3385 (1994).
294. A. B. Rogers and E. A. Hoover, Maternal-fetal feline immunodeficiency virus transmission: timing and tissue tropisms, *J. Infect. Dis.* 178, 960–967 (1998).
295. R. Allison and E. Hoover, Feline immunodeficiency virus is concentrated in milk early in lactation, *AIDS Res. Hum. Retroviruses* 19, 245–253 (2003).
296. L. L. O'Neil, M. J. Burkhard, L. A. Obert, and E. A. Hoover, Regression of feline immunodeficiency virus infection, *AIDS Res. Hum. Retroviruses* 13, 713–718 (1997).
297. R. Allison and E. Hoover, Covert vertical transmission of feline immunodeficiency virus, *AIDS Res. Hum. Retroviruses* 19, 421–434 (2003).
298. S. Dandekar, A. M. Beebe, J. Barlough, T. Phillips, J. Elder, M. Torten, and N. Pedersen, Detection of feline immunodeficience virus (FIV) nucleic acids in FIV-seronegative cats, *J. Virol.* 66, 4040–4049 (1992).
299. C. J. Miller, M. Marthas, J. Torten, N. J. Alexander, J. P. Moore, G. F. Doncel, and A. G. Hendrickx, Intravaginal inoculation of rhesus macaques with cell-free simian immunodeficiency virus results in persistent or transient viremia, *J. Virol.* 68, 6391–6400 (1994).
300. M. B. McChesney, J. R. Collins, D. Lu, X. Lü, J. Torten, R. L. Ashley, M. W. Cloyd, and C. J. Miller, Occult systemic infection and persistent SIV-specific CD4+ T-cell proliferative responses in rhesus macaques that were transiently viremic after intravaginal inoculation of SIV, *J. Virol.* 72, 10029–10035 (1998).

301. P. Palumbo, J. Skurnick, D. Lewis, and M. Eisenberg, PCR analysis of HIV-seronegative, heterosexual partners of HIV-infected individuals, *J. Acquir. Immune Defic. Syndr. Hum. Retrovirol.* 10, 436–440 (1995).
302. G. K. Sahu, J. J. Chen, J. C. Huang, K. M. Ramsey, and M. W. Cloyd, Transient or occult HIV-1 infection in high-risk adults, *AIDS* 15, 1175–1177 (2001).
303. H. L. Jordan, J. Howard, M. C. Barr, S. Kennedy-Stoskopf, J. K. Levy, and W. A. Tompkins, Feline immunodeficiency virus is shed in semen from experimentally and naturally infected cats, *AIDS Res. Hum. Retroviruses* 14, 1087–1092 (1998).
304. H. L. Jordan, Y. Liang, L. C. Hudson, and W. A. Tompkins, Shedding of feline immunodeficiency virus in semen of domestic cats during acute infection, *Am. J. Vet. Res.* 60, 211–215 (1999).
305. H. L. Jordan, J. Howard, R. K. Sellon, D. E. Wildt, W. A. Tompkins, and S. Kennedy-Stoskopf, Transmission of feline immunodeficiency virus in domestic cats via artificial insemination, *J. Virol.* 70, 8224–8228 (1996).
306. H. L. Jordan, J. G. Howard, J. G. Bucci, J. L. Butterworth, R. V. English, S. Kennedy-Stoskopf, M. B. Tompkins, and W. A. Tompkins, Horizontal transmission of feline immunodeficiency virus with semen from seropositive cats, *J. Reprod. Immunol.* 41, 341–357 (1998).
307. R. J. Avery and E. A. Hoover, Gamma interferon/interleukin 10 balance in tissue lymphocytes correlates with down modulation of mucosal feline immunodeficiency virus infection, *J. Virol.* 78, 4011–4019 (2004).
308. J. G. Bucci, R. V. English, H. L. Jordan, T. A. Childers, M. B. Tompkins, and W. A. Tompkins, Mucosally transmitted feline immunodeficiency virus induces a CD8+ antiviral response that correlates with reduction of cell-associated virus, *J. Infect. Dis.* 177, 18–25 (1998).
309. M. J. Burkhard, C. K. Mathiason, K. O'Halloran, and E. A. Hoover, Kinetics of early FIV infection in cats exposed via the vaginal versus intravenous route, *AIDS Res. Hum. Retroviruses* 18, 217–226 (2002).
310. T. R. Moench, K. J. Whaley, T. D. Mandrell, B. D. Bishop, C. J. Witt, and R. A. Cone, The cat/feline immunodeficiency virus animal model for transmucosal transmission of AIDS: nonoxynol-9 contraceptive jelly blocks transmission by an infected cell inoculum, *AIDS* 7, 797–802 (1993).
311. M. Shimojima, Y. Nishimura, T. Miyazawa, Y. Tohya, and H. Akashi, T-cell subpopulations mediating inhibition of feline immunodeficiency virus replication in mucosally infected cats, *Microbes Infect.* 6, 265–271 (2004).
312. T. Miyazawa, T. Furuya, S. Itagaki, Y. Tohya, E. Takahashi, and T. Mikami, Establishment of a feline T-lymphoblastoid cell line highly sensitive for replication of feline immunodeficiency virus, *Arch. Virol.* 108, 131–135 (1989).
313. Y. Goto, Y. Nishimura, K. Baba, T. Mizuno, Y. Endo, K. Masuda, K. Ohno, and H. Tsujimoto, Association of plasma viral RNA load with prognosis in cats naturally infected with feline immunodeficiency virus, *J. Virol.* 76, 10079–10083 (2002).
314. S. T. Dunham, J. N. Flynn, M. A. Rigby, J. MacDonald, J. Bruce, C. Cannon, M. C. Golder, L. Hanlon, D. A. Harbour, N. A. Mackay, N. Spibey, O. Jarrett, and J. C. Neil, Protection against feline immunodeficiency virus using replication defective proviral DNA vaccines with feline interleukin-12 and -18, *Vaccine* 20, 1483–1496 (2002).
315. C. M. Leutenegger, D. Klein, R. Hofmann-Lehmann, C. Mislin, U. Hummel, J. Boni, F. Boretti, W. H. Guenzbug, and H. Lutz, Rapid feline immunodeficiency virus provirus quantitation by polymerase chain reaction using the TaqMan® fluorogenic real-time detection system, *J. Virol. Methods* 78, 105–116 (1999).
316. M. Pistello, D. Matteucci, F. Bonci, P. Isola, P. Mazzetti, L. Zaccaro, A. Merico, D. Del Mauro, N. Flynn, and M. Bendinelli, AIDS vaccination studies using an ex vivo feline immunodeficiency virus model: protection from an intraclade challenge admin-

istered systemically or mucosally by an attenuated vaccine, *J. Virol.* 77, 10740–10750 (2003).
317. K. Tokunaga, Y. Nishino, H. Oikawa, C. Ishihara, T. Mikami, and K. Ikuta, Altered cell tropism and cytopathicity of feline immunodeficiency viruses in two different feline CD4-positive, CD8-negative cell lines, *J. Virol.* 66, 3893–3898 (1992).
318. A. Moraillon, F. Barre-Sinoussi, A. Parodi, R. Moraillon, and C. Dauguet, In vitro properties and experimental pathogenic effect of three strains of feline immunodeficiency viruses (FIV) isolated from cats with terminal disease, *Vet. Microbiol.* 31, 41–54 (1992).
319. A. T. Haase, K. Henry, M. Zupancic, G. Sedgewick, R. A. Faust, H. Melroe, W. Cavert, K. Gebhard, K. A. Staskus, Z. Q. Zhang, P. J. Dailey, H. H. Balfour, A. Erice, and A. S. Perelson, Quantitative image analysis of HIV-1 infection in lymphoid tissue, *Science* 274, 985–989 (1996).
320. G. Pantaleo, C. Graziosi, L. Butini, P. A. Pizzo, S. M. Schnittman, D. P. Kotler, and A. S. Fauci, Lymphoid organs function as major reservoirs for human immunodeficiency virus, *Proc. Natl. Acad. Sci. USA* 88, 9838–9842 (1991).
321. T. A. Reinhart, M. J. Rogan, D. Huddleston, D. M. Rausch, L. E. Eiden, and A. T. Haase, Simian immunodeficiency virus burden in tissues and cellular compartments during clinical latency and AIDS, *J. Infect. Dis.* 176, 1198–1208 (1997).
322. S. Matsumura, T. Ishida, T. Washizu, I. Tomoda, S. Nagata, J. Chiba, and T. Kurata, Pathologic features of acquired immunodeficiency-like syndrome in cats experimentally infected with feline immunodeficiency virus, *J. Vet. Med. Sci.* 55, 387–394 (1993).
323. M. L. Linenberger, A. M. Beebe, N. C. Pedersen, J. L. Abkowitz, and S. Dandekar, Marrow accessory cell infection and alterations in hematopoiesis accompany severe neutropenia during experimental acute infection with feline immunodeficiency virus, *Blood* 85, 941–951 (1995).
324. M. Podell, M. Oglesbee, L. Mathes, S. Krakowka, R. Olmstead, and L. Lafrado, AIDS-associated encephalopathy with experimental feline immunodeficiency virus infection, *J. Acquir. Immune Defic. Syndr.* 6, 758–771 (1993).
325. C. R. Stokes, S. Finerty, T. J. Gruffydd-Jones, C. P. Sturgess, and D. A. Harbour, Mucosal infection and vaccination against feline immunodeficiency virus, *J. Biotechnol.* 73, 213–221 (1999).
326. G. Ryan, D. Klein, E. Knapp, M. J. Hosie, T. Grimes, M. J. Mabruk, O. Jarrett, and J. J. Callanan, Dynamics of viral and proviral loads of feline immunodeficiency virus within the feline central nervous system during the acute phase following intravenous infection, *J. Virol.* 77, 7477–7485 (2003).
327. T. Tanabe and J. K. Yamamoto, Phenotypic and functional characteristics of FIV infection in the bone marrow stroma, *Virology* 282, 113–122 (2001).
328. J. C. Woo, G. A. Dean, A. Lavoy, R. Clark, and P. F. Moore, Investigation of recombinant human insulin-like growth factor type I in thymus regeneration in the acute stage of experimental FIV infection in juvenile cats, *AIDS Res. Hum. Retroviruses* 15, 1377–1388 (1999).
329. J. B. Johnston, C. Silva, and C. Power, Envelope gene-mediated neurovirulence in feline immunodeficiency virus infection: induction of matrix metalloproteinases and neuronal injury, *J. Virol.* 76, 2622–2633 (2002).
330. J. B. Johnston, C. Silva, T. Hiebert, R. Buist, M. R. Dawood, J. Peeling, and C. Power, Neurovirulence depends on virus input titer in brain in feline immunodeficiency virus infection: Evidence for activation of innate immunity and neuronal injury, *J. Neurovirol.* 8, 420–431 (2002).
331. K. Lockridge, M. Chien, P. A. Luciw, and E. E. Sparger, Protective immunity against feline immunodeficiency virus inducedby inoculation with *vif*-deleted proviral DNA, *Virology.* 273, 67–79 (2000).

332. A. J. Phipps, K. A. Hayes, W. R. Buck, M. Podell, and L. E. Mathes, Neurophysiologic and immunologic abnormalities associated with feline immunodeficiency virus molecular clone FIV-PPR DNA inoculation, *J. Acquir. Immune Defic. Syndr.* 23, 8–16 (2000).
333. C. J. Miller, D. W. Kang, M. Marthas, Z. Moldoveanu, H. Kiyono, P. Marx, J. H. Eldridge, J. Mestecky, and J. R. McGhee, Genital secretory immune response to chronic simian immunodeficiency virus (SIV) infection: a comparison between intravenously and genitally inoculated rhesus macaques, *Clin. Exp. Immunol.* 88, 520–526 (1992).
334. J. Bucci, D. Gebhard, T. A. Childers, E. R., M. B. Tompkins, and W. A. Tompkins, The CD8+ phenotype mediating antiviral activity in feline immunodeficiency virus-infected cats is characterized by reduced surface expression of the CD8 β chain, *J. Infect. Dis.* 178, 968–977 (1998).
335. S. W. Dow, M. J. Dreitz, and E. A. Hoove, Feline immunodeficiency virus neurotropism: evidence that astrocytes and microglia are the primary target cells, *Vet. Immunol. Immunopathol.* 35, 23–35 (1992).
336. A. Hein, J. P. Martin, and R. Dorries, In vitro activation of feline immunodeficiency virus in ramified microglial cells from asymptomatically infected cats, *J. Virol.* 75, 8090–8095 (2001).
337. E. Zenger, E. Tiffany-Castiglioni, and E. W. Collisson, Cellular mechanisms of feline immunodeficiency virus (FIV)-induced neuropathogenesis, *Front. Biosci.* 2, 527–537 (1997).
338. M. K. Patrick, J. B. Johnsto, and C. Power, Lentiviral neuropathogenesis: comparative neuroinvasion, neurotropism, neurovirulence, and host neurosusceptibility, *J. Virol.* 76, 7923–7931 (2002).
339. A. Poli, F. Abramo, C. Di Iorio, C. Cantile, M. Carli, C. Pollera, L. Vago, A. Tosoni, and G. Costanzi, Neuropathology in cats experimentally infected with feline immunodeficiency virus: a morphological, immunocytochemical and morphometric study, *J. Neurovirol.* 3, 361–368 (1997).
340. A. Poli, M. Pistello, M. A. Carli, F. Abramo, G. Mancuso, E. Nicoletti, and M. Bendinelli, Tumor necrosis factor-alpha and virus expression in the central nervous system of cats infected with feline immunodeficiency virus, *J. Neurovirol.* 5, 465–473 (1999).
341. T. R. Phillips, O. Prospero-Garcia, D. W. Wheeler, P. C. Wagaman, D. L. Lerner, H. S. Fox, L. R. Whalen, F. E. Bloom, J. H. Elder, and S. J. Henriksen, Neurologic dysfunctions caused by a molecular clone of feline immunodeficiency virus, FIV-PPR, *J. Neurovirol.* 2, 388–396 (1996).
342. A. A. Lackner, S. Dandekar, and M. B. Gardner, Neurobiology of simian and feline immunodeficiency virus infections, *Brain Pathol.* 1, 201–212 (1991).
343. S. Huitron-Resendiz, S. De Rozieres, M. Sanchez-Alavez, B. Buhler, Y. C. Lin, D. L. Lerner, N. W. Henriksen, M. Burudi, H. S. Fox, B. E. Torbett, S. J. Henriksen, and J. H. Elder, Resolution and prevention of feline immunodeficiency virus-induced neurological deficits by treatment with the protease inhibitor TL-3, *J. Virol.* 78, 4525–4532 (2004).
344. M. Hurtrel, J. P. Ganiere, J. F. Guelfi, L. Chakrabarti, M. A. Maire, F. Gray, L. Montagnier, and B. Hurtrel, Comparison of early and late feline immunodeficiency virus encephalopathies, *AIDS* 6, 399–406 (1992).
345. A. T. Haase, Population biology of HIV-1 infection: viral and CD4+ T-cell demographics and dynamics in lymphatic tissues, *Annu. Rev. Immunol.* 17, 635–656 (1999).
346. R. S. Veazey, M. DeMaria, L. V. Chalifoux, D. E. Shvetz, D. R. Pauley, H. L. Knight, M. Rosenzweig, R. P. Johnson, R. C. Desrosiers, and A. A. Lackner, Gastrointestinal tract as a major site of CD4+ T-cell depletion and viral replication in SIV infection, *Science* 280, 427–431 (1998).
347. I. M. Belyakov, Z. Hel, B. Kelsall, V. A. Kuznetsov, J. D. Ahlers, J. Nacsa, D. I. Watkins, T. M. Allen, A. Sette, J. Altman, R. Woodward, P. D. Markham, J. D. Clements, G. Franchini, W. Strober, and J. A. Berzofsky, Mucosal AIDS vaccine reduces disease and

viral load in gut reservoir and blood after mucosal infection of macaques, *Nat. Med.* 7, 1320–1326 (2001).
348. M. Guadalupe, E. Reay, S. Sankaran, T. Prindiville, J. Flamm, A. McNeil, and S. Dandekar, Severe CD4+ T-cell depletion in gut lymphoid tissue during primary human immunodeficiency virus type 1 infection and substantial delay in restoration following highly active antiretroviral therapy, *J. Virol.* 77, 11708–11717 (2003).
349. J. M. Brenchley, T. W. Schacker, L. E. Ruff, D. A. Price, J. H. Taylor, G. J. Beilman, P. L. Nguyen, A. Khoruts, M. Larson, A. T. Haase, and D. C. Douek, CD4+ T-cell depletion during all stages of HIV disease occurs predominantly in the gastrointestinal tract, *J. Exp. Med.* 200, 749–759 (2004).
350. S. Mehandru, M. A. Poles, K. Tenner-Racz, A. Horowitz, A. Hurley, C. Hogan, D. Boden, P. Racz, and M. Markowitz, Primary HIV-1 infection is associated with preferential depletion of CD4+ T lymphocytes from effector sites in the gastrointestinal tract, *J. Exp. Med.* 200, 761–770 (2004).
351. G. Pantaleo, O. J. Cohen, T. Schacker, M. Vaccarezza, C. Graziosi, G. P. Rizzardi, J. Kahn, C. H. Fox, S. M. Schnittman, D. H. Schwartz, L. Corey, and A. S. Fauci, Evolutionary pattern of human immunodeficiency virus (HIV) replication and distribution in lymph nodes following primary infection: implications for antiviral therapy, *Nat. Med.* 4, 341–345 (1998).
352. G. A. Dean, G. H. Reubel, P. F. Moore, and N. C. Pedersen, Proviral burden and infection kinetics of feline immunodeficiency virus in lymphocyte subsets of blood and lymph node, *J. Virol.* 70, 5165–5169 (1996).
353. B. Hurtrel, L. Chakrabarti, M. Hurtrel, J. M. Bach, J. P. Ganiere, and L. Montagnier, Early events in lymph nodes during infection with SIV and FIV, *Res. Virol.* 145, 221–227 (1994).
354. G. A. Dean, G. H. Reubel, and N. C. Pedersen, Simian immunodeficiency virus infection of CD8+ lymphocytes in vivo, *J. Virol.* 70, 5646–5650 (1997).
355. A. De Maria, G. Pantaleo, S. M. Schnittman, J. J. Greenhouse, M. Baseler, J. M. Orenstein, and A. Fauci, Infection of CD8+ T lymphocytes with HIV. Requirement for interaction with infected CD4+ cells and induction of infectious virus from chronically infected CD8+ cells, *J. Immunol.* 146, 2220–2226 (1991).
356. A. Joshi, T. W. Vahlenkamp, H. Garg, W. A. Tompkins, and M. B. Tompkins, Preferential replication of FIV in activated CD4(+)CD25(+) T-cells independent of cellular proliferation, *Virology* 321, 307–322 (2004).
357. T. W. Vahlenkamp, M. B. Tompkins, and W. A. Tompkins, Feline immunodeficiency virus infection phenotypically and functionally activates immunosuppressive CD4+CD25+ T regulatory cells, *J. Immunol.* 172, 4752–4761 (2004).
358. K. Hartmann, Feline immunoderficiency virus infection—an overview, *Vet. J.* 155, 123–137 (1998).
359. A. H. Sparkes, C. D. Hopper, W. G. Millard, T. J. Gruffydd-Jones, and D. A. Harbour, Feline immunodeficiency virus infection. Clinicopathologic findings in 90 naturally occurring cases, *J. Vet. Intern. Med.* 7, 85–90 (1993).
360. T. Ishida, A. Taniguchi, S. Matsumura, T. Washizu, and I. Tomoda, Long-term clinical observations on feline immunodeficiency virus infected asymptomatic carriers, *Vet. Immunol. Immunopathol.* 35, 15–22 (1992).
361. R. Hofmann-Lehmann, E. Holznagel, P. Ossent, and H. Lutz, Parameters of disease progression in long-term experimental feline retrovirus (feline immunodeficiency virus and feline leukemia virus) infections: hematology, clinical chemistry, and lymphocyte subsets, *Clin. Diagn. Lab. Immunol.* 4, 33–42 (1997).
362. E. J. Fleming, D. L. McCaw, J. A. Smith, G. M. Buening, and C. Johnson, Clinical, hematologic, and survival data from cats infected with feline immunodeficiency virus: 42 cases (1983–1988), *J. Am. Vet. Med. Assoc.* 199, 913–916 (1991).

363. G. H. Shelton, M. L. Linenberger, M. T. Persik, and J. L. Abkowitz, Prospective hematologic and clinicopathologic study of asymptomatic cats with naturally acquired feline immunodeficiency virus infection, *J .Vet. Intern. Med.* 9, 133–140 (1995).
364. J. A. Levy, *HIV and the pathogenesis of AIDS* (ASM Press, Washington, DC, 1998).
365. M. D. Daniel, N. L. Letvin, P. K. Sehgal, G. Hunsmann, D. K. Schmidt, N. W. King, and R. C. Desrosiers, Long-term persistent infection of macaque monkeys with the simian immunodeficiency virus, *J. Gen. Virol.* 68, 3183–3189 (1987).
366. M. B. Gardner and P. A. Luciw, Animal models of AIDS, *Faseb J.* 3, 2593–2606 (1989).
367. Y. Inoshima, M. Kohmoto, Y. Ikeda, H. Yamada, Y. Kawaguchi, K. Tomonaga, T. Miyazawa, C. Kai, T. Umemura, and T. Mikami, Roles of the auxiliary genes and AP-1 binding site in the long terminal repeat of feline immunodeficiency virus in the early stage of infection in cats, *J. Virol.* 70, 8518–8526 (1996).
368. M. De Monte, H. Nonnenmacher, N. Brignon, M. Ullmann, and J. P. Martin, A multivariate statistical analysis to follow the course of disease after infection of cats with different strains of the feline immunodeficiency virus (FIV), *J. Virol. Methods* 103, 157–170 (2002).
369. A. P. Kourtis, C. Ibegbu, A. J. Nahmias, F. K. Lee, W. S. Clark, M. K. Sawyer, and S. Nesheim, Early progression of disease in HIV-infected infants with thymus dysfunction, *N. Engl. J. Med.* 335, 1431–1436 (1997).
370. A. J. Nahmias, W. S. Clark, A. P. Kourtis, F. K. Lee, G. Cotsonis, C. Ibegbu, D. Thea, P. Palumbo, P. Vink, R. J. Simonds, and S. R. Nesheim, Thymic dysfunction and time of infection predict mortality in human immunodeficiency virus-infected infants. CDC Perinatal AIDS Collaborative Transmission Study Group, *J. Infect. Dis.* 178, 680–685 (1998).
371. J. Meers, W. F. Robinson, G. M. del Fierro, M. A. Scoones, and M. A. Lawson, Feline immunodeficiency virus: quantification in peripheral blood mononuclear cells and isolation from plasma of infected cats, *Arch. Virol.* 127, 233–243 (1992).
372. K. Tokunaga, K. Shoda, Y. Nishino, S. Mori, Q. Zhong, Y. Zheng, M. Kishi, C. Ishihara, M. Kanda, and K. Ikuta, Maintenance of high virus load even after seroconversion in newborn kittens acutely infected with feline immunodeficiency virus, *Vaccine* 13, 1393–1398 (1995).
373. J. A. George, N. C. Pedersen, and J. Higgins, The effect of age on the course of experimental feline immunodeficiency virus infection, *AIDS Res. Hum. Retroviruses* 9, 897–905 (1993).
374. M. S. Orandle, P. C. Crawford, J. K. Levy, R. Udoji, G. P. Papadi, T. Ciccarone, A. Mergia, and C. M. Johnson, CD8+ thymic lymphocytes express reduced levels of CD8 β and increased interferon γ in cats perinatally infected with JSY3 molecular clone of feline immunodeiciecny virus, *AIDS Res. Hum. Retroviruses* 16, 1559–1571 (2000).
375. G. Hoffmann-Fezer, J. Thum, C. Ackley, M. Herbold, J. Mysliwietz, S. Thefeld, K. Hartmann, and W. Kraft, Decline in CD4+ cell numbers in cats with naturally acquired feline immunodeficiency virus infection, *J. Virol.* 66, 1484–1488 (1992).
376. D. Gebhard, J. Dow, T. Childers, J. Alvelo, M. Tompkins, and W. Tompkins, Progressive expansion of an L-selectin-negative CD8 cell with anti-feline immunodeficiency virus (FIV) suppressor function in the circulation of FIV-infected cats, *J. Infect. Dis.* 180, 1503–1513 (1999).
377. B. J. Willett, M. J. Hosie, J. J. Callanan, J. C. Neil, and O. Jarrett, Infection with feline immunodeficiency virus is followed by the rapid expansion of a CD8+ lymphocyte subset, *Immunology* 78, 1–6 (1993).
378. M. Shimojima, T. Miyazawa, M. Kohmoto, Y. Ikeda, Y. Nishimura, K. Maeda, Y. Tohya, and T. Mikami, Expansion of CD8alpha+beta− cells in cats infected with feline immunodeficiency virus, *J. Gen. Virol.* 79, 91–94 (1998).

379. P. C. Crawford, G. P. Papadi, J. K. Levy, N. A. Benson, A. Mergia, and C. M. Johnson, Tissue dynamics of CD8 lymphocytes that suppress viral replication in cats infected neonatally with feline immunodeficiency virus, *J. Infect. Dis.* 184, 671–681 (2001).
380. A. Oxenius, H. F. Gunthard, B. Hirschel, S. Fidler, J. N. Weber, P. J. Easterbrook, J. I. Bell, R. E. Phillips, and D. A. Price, Direct ex vivo analysis reveals distinct phenotypic patterns of HIV-specific CD8+ T lymphocyte activation in response to therapeutic manipulation of virus load, *Eur. J. Immunol.* 31, 1115–1121 (2001).
381. J. E. Schmitz, M. A. Forman, M. A. Lifton, O. Concepcion, K. A. Reimann, C. S. Crumpacker, J. F. Daley, R. S. Gelman, and N. L. Letvin, Expression of the CD8alpha beta-heterodimer on CD8(+) T lymphocytes in peripheral blood lymphocytes of human immunodeficiency virus– and human immunodeficiency virus+ individuals, *Blood* 92, 198–206 (1998).
382. C. E. Mackewicz, L. C. Yang, J. D. Lifson, and J. A. Levy, Non-cytolytic CD8 T-cell anti-HIV responses in primary HIV-1 infection, *Lancet.* 344, 1671–1673 (1994).
383. J. A. Levy, C. E. Mackewicz, and E. Barker, Controlling HIV pathogenesis: the role of the noncytotoxic anti-HIV response of CD8+ T-cells, *Immunol. Today* 17, 217–224 (1996).
384. M. Torten, M. Franchini, J. E. Barlough, J. W. George, E. Mozes, H. Lutz, and N. C. Pedersen, Progressive immune dysfunction in cats experimentally infected with feline immunodeficiency virus, *J. Virol.* 65, 2225–2230 (1991).
385. D. S. Lin, D. D. Bowman, R. H. Jacobson, M. C. Barr, M. Fevereiro, J. R. Williams, F. M. O. Noronha, F. W. Scott, and R. J. Avery, Suppression of lymphocyte blastogenesis to mitogens in cats experimentally infected with feline immunodeficiency virus, *Vet. Immunol. Immunopathol.* 26, 183–189 (1990).
386. Y. Hara, T. Ishida, H. Ejima, M. Tagawa, S. Motoyoshi, I. Tomoda, M. Shimizu, and K. Shichinohe, Decrease in mitogen-induced lymphocyte proliferative responses in cats infected with feline immunodeficiency virus, *Nippon Juigaku Zasshi* 52, 573–579 (1990).
387. S. A. Bishop, N. A. Williams, T. J. Gruffydd-Jones, D. A. Harbour, and C. R. Stokes, Impaired T-cell priming and proliferation in cats infected with feline immunodeficiency virus, *AIDS* 6, 287–293 (1992).
388. K. Ohno, T. Watari, R. Goitsuka, H. Tsujimoto, and A. Hasegawa, Altered surface antigen expression on peripheral blood mononuclear cells in cats infected with feline immunodeficiency virus, *J. Vet. Med. Sci.* 54, 517–522 (1992).
389. G. A. Dean and N. C. Pedersen, Cytokine response in multiple lymphoid tissues during the primary phase of feline immunodeficiency virus infection, *J. Virol.* 72, 9436–9440 (1998).
390. Y. Liang, L. C. Hudson, J. K. Levy, J. W. Ritchey, W. A. Tompkins, and M. B. Tompkins, T-cells overexpressing interferon-gamma and interleukin-10 are found in both the thymus and secondary lymphoid tissues of feline immunodeficiency virus-infected cats, *J. Infect. Dis.* 181, 564–575 (2000).
391. C. E. Lawrence, J. J. Callanan, B. J. Willett, and O. Jarrett, Cytokine production by cats infected with feline immunodeficiency virus: a longitudinal study, *Immunology* 85, 568–574 (1995).
392. L. A. Kraus, W. G. Bradley, R. W. Engelman, K. M. Brown, R. A. Good, and N. K. Day, Relationship between tumor necrosis factor alpha and feline immunodeficiency virus expressions, *J. Virol.* 70, 566–569 (1996).
393. J. W. Ritchey, J. K. Levy, S. K. Bliss, W. A. Tompkins, and M. B. Tompkins, Constitutive expression of types 1 and 2 cytokines by alveolar macrophages from feline immunodeficiency virus-infected cats, *Vet. Immunol. Immunopathol.* 79, 83–100 (2001).
394. T. Tanabe and J. K. Yamamoto, Feline immunodeficiency virus lacks sensitivity to the antiviral activity of feline IFN-gamma, *J. Interferon Cytokine Res.* 21, 1039–1046 (2001).

395. G. A. Dean, J. A. Bernales, and N. C. Pedersen, Effect of feline immunodeficiency virus on cytokine response to Listeria monocytogenes in vivo, *Vet. Immunol. Immunopathol.* 65, 125–138 (1998).
396. G. H. Reubel, J. W. George, J. E. Barlough, J. Higgins, C. K. Grant, and N. C. Pedersen, Interaction of acute feline herpesvirus-1 and chronic feline immunodeficiency virus infections in experimentally infected specific pathogen free cats, *Vet. Immunol. Immunopathol.* 35, 95–119 (1992).
397. M. Torten, E. E. Sparger, B. A. Rideout, N. C. Pedersen, and P. A. Luciw, in *Vaccines 90: Modern Approaches to New Vaccines Including Prevention of AIDS*, edited by F. Brown, R. M. Chanock, H. S. Ginsberg, and R. A. Lerner (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1990), 375–378.
398. A. Taniguchi, T. Ishida, T. Washizu, and I. Tomoda, Humoral immune response to T-cell dependent and independent antigens in cats infected with feline immunodeficiency virus, *J. Vet. Med. Sci.* 53, 333–335 (1991).
399. G. H. Shelton, M. L. Linenberger, C. K. Grant, and J. L. Abkowitz, Hematologic manifestations of feline immunodeficiency virus infection, *Blood* 76, 1104–1109 (1990).
400. D. S. Lin and D. D. Bowman, Macrophage functions in cats experimentally infected with feline immunodeficiency virus and toxoplasma gondii, *Vet. Immunol. Immunopathol.* 33, 69–78 (1992).
401. P. Kubes, B. Heit, G. van Marle, J. B. Johnston, D. Knight, A. Khan, and C. Power, In vivo impairment of neutrophil recruitment during lentivirus infection, *J. Immunol.* 171, 4801–4808 (2003).
402. M. A. Hanlon, J. M. Marr, K. A. Hayes, L. E. Mathes, P. C. Stromberg, S. Ringler, S. Krakowka, and L. J. Lafrado, Loss of neutrophil and natural killer cell function following feline immunodeficiency virus infection, *Viral Immunol.* 6, 119–124 (1993).
403. A. L. Parodi, F. Femenia, A. Moraillon, F. Crespeau, and J. J. Fontaine, Histopathological changes in lymph nodes of cats experimentally infected with the feline immunodeficiency virus (FIV), *J. Comp. Pathol.* 111, 165–174 (1994).
404. D. A. Gunn-Moore, G. R. Pearson, D. A. Harbour, and C. V. Whiting, Encephalitis associated with giant cells in a cat with naturally occurring feline immunodeficiency virus infection demonstrated by in situ hybridization, *Vet. Pathol.* 33, 699–703 (1996).
405. J. A. Beatty, J. J. Callanan, A. Terry, O. Jarrett, and J. C. Neil, Molecular and immunophenotypical characterization of a feline immunodeficiency virus (FIV)-associated lymphoma: a direct role for FIV in B-lymphocyte transformation?, *J. Virol.* 72, 767–771 (1998).
406. J. A. Beatty, C. E. Lawrence, J. J. Callanan, C. K. Grant, E. A. Gault, J. C. Neil, and O. Jarrett, Feline immunodeficiency virus (FIV)-associated lymphoma: a potential role for immune dysfunction in tumourigenesis, *Vet. Immunol. Immunopathol.* 65, 309–322 (1998).
407. J. A. Beatty, A. Terry, J. MacDonald, E. Gault, S. Cevario, S. J. O'Brien, E. Cameron, and J. C. Neil, Feline immunodeficiency virus integration in B-cell lymphoma identifies a candidate tumor suppressor gene on human chromosome 15q15, *Cancer Res.* 62, 7175–7180 (2002).
408. Y. Endo, K. W. Cho, K. Nishigaki, Y. Momoi, Y. Nishimura, T. Mizuno, Y. Goto, T. Watari, H. Tsujimoto, and A. Hasegawa, Molecular characteristics of malignant lymphomas in cats naturally infected with feline immunodeficiency virus, *Vet. Immunol. Immunopathol.* 57, 153–167 (1997).
409. A. Poli, F. Abramo, F. Baldinotti, M. Pistello, L. Da Prato, and M. Bendinelli, Malignant lymphoma associated with experimentally induced feline immunodeficiency virus infection, *J. Comp. Path.* 10, 319–328 (1994).
410. A. A. Lackner, P. Vogel, R. A. Ramos, J. D. Kluge, and M. Marthas, Early events in tissues during infection with pathogenic (SIVmac239) and nonpathogenic (SIVmac1A11) molecular clones of simian immunodeficiency virus, *Am. J. Pathol.* 145, 428–439 (1994).

411. H. L. Ioachim, C. W. Lerner, and M. L. Tapper, The lymphoid lesions associated with the acquired immunodeficiency syndrome, *J. Am. Surg. Pathol.* 7, 543–553 (1983).
412. P. N. Fultz, H. M. McClure, D. C. Anderson, and W. M. Switzer, Identification and biologic characterization of an acutely lethal variant of simian immunodeficiency virus from sooty mangabeys (SIV/SMM), *AIDS Res. Hum. Retroviruses* 5, 397–409 (1989).
413. M. L. Bonyhadi, L. Rabin, S. Salimi, D. A. Brown, J. Kosek, J. M. McCune, and H. Kaneshima, HIV induces thymus depletion in vivo, *Nature* 363, 728–732 (1993).
414. F. Miedema, Immunological abnormalities in the natural history of HIV infection: mechanisms and clinical relevance, *Immunodefic. Rev.* 3, 173–193 (1992).
415. E. M. Poeschla and D. J. Looney, CXCR4 is required by a nonprimate lentivirus: heterologous expression of feline immunodeficiency virus in human, rodent, and feline cells, *J. Virol.* 72, 6858–6866 (1998).
416. S. Lombardi, C. Massi, E. Indino, C. La Rosa, P. Mazzetti, M. L. Falcone, P. Rovero, A. Fissi, O. Pieroni, P. Bandecchi, F. Esposito, F. Tozzini, M. Bendinelli, and C. Garzelli, Inhibition of feline immunodeficiency virus infection in vitro by envelope glycoprotein synthetic peptides, *Virology* 220, 274–284 (1996).
417. D. C. Bragg, R. B. Meeker, B. A. Duff, R. V. English, and M. B. Tompkins, Neurotoxicity of FIV and FIV envelope protein in feline cortical cultures, *Brain Res.* 816, 431–417 (1999).
418. C. M. Johnson, N. A. Benson, and G. P. Papadi, Apoptosis and CD4+ lymphocyte depletion following feline immunodeficiency virus infection of a T-lymphocyte cell line, *Vet. Pathol.* 33, 195–203 (1996).
419. T. Mizuno, Y. Goto, K. Baba, K. Masuda, K. Ohno, and H. Tsujimoto, TNF-α-induced cell death in feline immunodeficiency virus-infected cells is mediated by the caspase cascade, *Virology* 287, 446–455 (2001).
420. K. Ohno, Y. Okamoto, T. Miyazawa, T. Mikami, T. Watari, R. Goitsuka, H. Tsujimoto, and A. Hasegawa, Induction of apoptosis in a T lymphoblastoid cell line infected with feline immunodeficiency virus, *Arch. Virol.* 135, 153–158 (1994).
421. K. Ohno, T. Nakano, Y. Matsumoto, T. Watari, R. Goitsuka, H. Nakayama, H. Tsujimoto, and A. Hasegawa, Apoptosis induced by tumor necrosis factor in cells chronically infected with feline immunodeficiency virus, *J. Virol.* 67, 2429–2433 (1993).
422. E. Holznagel, R. Hofmann-Lehmann, C. M. Leutenegger, K. Allenspach, S. W. Huettner, U. Forster, E. Niederer, H. Joller, B. J. Willett, U. Hummel, G. L. Rossi, J. Schupbach, and H. Lutz, The role of in vitro-induced lymphocyte apoptosis in feline immunodeficiency virus infection: correlation with different markers of disease progression, *J. Virol.* 72, 9025–9033 (1998).
423. S. A. Bishop, T. J. Gruffydd-Jones, D. A. Harbour, and C. R. Stokes, Programmed cell death (apoptosis) as a mechanism of cell death in peripheral blood mononuclear cells from cats infected with feline immunodeficiency virus (FIV), *Clin. Exp. Immunol.* 93, 65–71 (1993).
424. Y. Momoi, T. Mizumno, Y. Nishimura, Y. Endo, K. Ohno, T. Watari, R. Goitsuka, H. Tsujimoto, and A. Hasegawa, Detection of apoptosis induced in peripheral blood lymphocytes from cats infected with feline immunodeficiency virus, *Arch. Virol.* 141, 1651–1659 (1996).
425. A. Guiot, D. Rigal, and G. Chappuis, Spontanious programmed cell death (PCD) process of lymphocytes of FIV-infected cats: cellular targets and modulation, *Vet. Immunol. Immunopathol.* 58, 93–106 (1997).
426. G. Sarli, S. L. Della, L. Zaccaro, M. Bendinelli, G. Piedimonte, and P. Marcato, Apoptotic fraction in lymphoid tissue of FIV-infected SPF cats, *Vet. Immunol. Immunopathol.* 64, 33–44 (1998).
427. H. Garg, A. Joshi, and W. A. Tompkins, Feline immunodeficiency virus envelope glycoprotein mediates apoptosis in activated PBMC by a mechanism dependent on gp41 function, *Virology* 330, 424–436 (2004).

428. J. Yamazaki, N. Hasebe, S. Nagafuchi, K. Baba, H. Tsujimoto, R. Kano, and A. Hasegawa, Expression of apoptosis-related gene mRNAs in feline T-cells infected with feline immunodeficiency virus (FIV), *Vet. Microbiol.* 101, 1–8 (2004).
429. T. Mizuno, Y. Goto, K. Baba, Y. Momoi, Y. Endo, Y. Nishimura, K. Masuda, K. Ohno, and H. Tsujimoto, Quantitative analysis of Fas and Fas ligand mRNAs in a feline T-lymphoid cell line after infection with feline immunodeficiency virus and primary peripheral blood mononuclear cells obtained from cats infected with the virus., *Vet. Immunol. Immunopathol.* 93, 117–123 (2003).
430. G. Piedimonte, R. Crinelli, L. D. Salda, D. Corsi, M. G. Pennisi, L. Kramer, A. Casabianca, G. Sarli, M. Bendinelli, P. S. Marcato, and M. Magnani, Protein degradation and apoptotic death in lymphocytes during FIV infection: Activation of the ubiquitin-proteasome proteolytic system, *Exper. Cell Res.* 248, 381–390 (1999).
431. E. Mortola, Y. Endo, T. Mizuno, K. Ohno, T. Watari, H. Tsujimoto, and A. Hasegawa, Effect of interleukin-12 and interleukin-10 on the virus replication and apoptosis in T-cells infected with feline immunodeficiency virus, *J. Vet. Med. Sci.* 60, 1181–1185 (1998).
432. M. E. Bull, T. W. Vahlenkamp, J. L. Dow, E. W. Collisson, B. J. Winslow, A. P. Phadke, M. B. Tompkins, and W. A. Tompkins, Spontaneous T-cell apoptosis in feline immunodeficiency virus (FIV)-infected cats is inhibited by IL2 and anti-B7.1 antibodies, *Vet. Immunol. Immunopathol.* 99, 25–37 (2004).
433. I. S. Choi, H. S. Yoo, and E. W. Collisson, Evaluation of expression patterns of feline CD28 and CTLA-4 in feline immunodeficiency virus (FIV)-infected and FIV antigen-induced PBMC, *J. Vet. Sci.* 1, 97–103 (2004).
434. M. B. Tompkins, M. E. Bull, J. L. Dow, J. M. Ball, E. W. Collisson, B. J. Winslow, A. P. Phadke, T. W. Vahlenkamp, and W. A. Tompkins, Feline immunodeficiency virus infection is characterized by B7+CTLA4+ T-cell apoptosis, *J. Infect. Dis.* 185, 1077–1093 (2002).
435. T. W. Vahlenkamp, M. E. Bull, J. L. Dow, J. M. Ball, E. W. Collisson, B. J. Winslow, A. P. Phadke, W. A. Tompkins, and M. B. Tompkins, B7+CTLA4+ T-cells engage in T-T interactions that mediate apoptosis: a model for lentivirus-induced T-cell depletion, *Vet. Immunol. Immunopathol.* 98, 203–214 (2004).
436. Q. Leng, Z. Bentwich, E. Magen, A. Kalinkovich, and G. Borkow, CTLA-4 upregulation during HIV infection: association with anergy and possible target for therapeutic intervention, *AIDS* 16, 519–529 (2002).
437. D. E. Lewis, L. Yang, W. Luo, X. Wang, and J. R. Rodgers, HIV-specific cytotoxic T lymphocyte precursors exist in a CD28-CD8+ T-cell subset and increase with loss of CD4 T-cells, *AIDS* 13, 1029–1033, (1999).
438. E. N. Mugnaini, A. Spurkland, T. Egeland, M. Sannes, and J. E. Brinchmann, Demonstration of identical expanded clones within both CD8+CD28+ and CD8+CD28– T-cell subsets in HIV type 1-infected individuals, *Eur. J. Immunol.* 28, 1738–1742 (1998).
439. O. K. Haffar, M. D. Smithgall, J. G. Wong, J. Bradshaw, and P. S. Linsley, Human immunodeficiency virus type 1 infection of CD4+ T-cells down-regulates the expression of CD28: effect on T-cell activation and cytokine production, *Clin. Immunol. Immunopathol.* 77, 262–270 (1995).
440. J. E. Brinchmann, J. H. Dobloug, B. H. Heger, L. L. Haaheim, M. Sannes, and T. Egeland, Expression of costimulatory molecule CD28 on T-cells in human immunodeficiency virus type 1 infection: functional and clinical correlations, *J. Infect. Dis.* 169, 730–738 (1994).
441. W. E. Johnson, J. D. Lifson, S. M. Lang, R. P. Johnson, and R. C. Desrosiers, Importance of B-cell responses for immunological control of variant strains of simian immunodeficiency virus, *J. Virol.* 77, 375–381 (2003).
442. J. E. Schmitz, M. J. Kuroda, S. Santra, V. G. Sasseville, M. A. Simon, M. A. Lifton, P. Racz, K. Tenner-Racz, M. Dalesandro, B. J. Scallon, J. Ghrayeb, M. A. Forman, M. D. C., E. P. Rieber, N. L. Letvin, and K. A. Reimann, Control of viremia in simian immunodeficiency virus infection by CD8+ lymphocytes, *Science* 283, 857–860 (1999).

443. J. E. Schmitz, M. J. Kuroda, S. Santra, M. A. Simon, M. A. Lifton, W. Lin, R. Khunkhun, M. Piatak, J. Lifson, G. Grosschupff, R. S. Gelman, P. Racz, K. Tenner-Racz, K. A. Mansfield, N. L. Letvin, D. C. Montefiori, and K. A. Reimann, Effect of humoral immune responses on controlling viremia during primary infection of rhesus monkeys with simian immunodeficiency virus, *J. Virol.* 77, 2165–2173 (2003).
444. T. P. O'Connor, S. Tanguay, R. Steinman, R. A. Smith, M. C. Barr, J. K. Yamamoto, N. C. Pedersen, P. R. Andersen, and Q. J. Tonelli, Development and evaluation of immunoassay for detection of antibodies to the feline T-lymphotropic lentivirus (feline immunodeficiency virus). *J. Clin. Microbiol.* 27, 474–479 (1989).
445. M. J. Hosie and O. Jarrett, Serological responses of cats to feline immunodeficiency virus, *AIDS* 4, 215–220 (1990).
446. S. Lombardi, M. Bendinelli, and C. Garzelli, Detection of B epitopes on the p24 gag protein of feline immunodeficiency virus by monoclonal antibodies, *AIDS Res. Hum. Retroviruses* 9, 141–146 (1993).
447. A. Trkola, H. Kuster, C. Leemann, A. Oxenius, C. Fagard, H. Furrer, M. Battegay, P. Vernazza, E. Bernasconi, R. Weber, B. Hirschel, S. Bonhoeffer, H. F. Gunthard, and S. H. C. Study, Humoral immunity to HIV-1: kinetics of antibody responses in chronic infection reflects capacity of immune system to improve viral set point, *Blood* 104, 1784–1792 (2004).
448. P. W. Parren, J. P. Moore, D. R. Burton, and Q. J. Sattentau, The neutralizing antibody response to HIV-1: viral evasion and escape from humoral immunity AIDS, *AIDS* 13, S137–S162 (1999).
449. D. R. Burton, R. A. Williamson, and P. W. Parren, Antibody and virus: binding and neutralization, *Virology* 270, 1–3 (2000).
450. A. Avrameas, A. Strosberg, A. Moraillon, P. Sonigo, and G. Pancino, Serological diagnosis of feline immunodeficiency virus infection based on synthetic peptides from Env glycoproteins, *Res Virol.* 144, 209–218 (1993).
451. S. Lombardi, C. Garzelli, C. La Rosa, L. Zaccaro, S. Specter, G. Malvaldi, F. Tozzini, F. Esposito, and M. Bendinelli, Identification of a linear neutralization site within the third variable region of the feline immunodeficiency virus envelope, *J. Virol.* 67, 4742–4749 (1993).
452. G. Pancino, C. Chappey, W. Saurin, and P. Sonigo, B epitopes and selection pressures in feline immunodeficiency virus envelope glycoproteins, *J. Virol.* 67, 664–672 (1993).
453. C. Massi, S. Lombardi, E. Indino, D. Matteucci, C. La Rosa, F. Esposito, C. Garzelli, and M. Bendinelli, Most potential linear B cell epitopes of Env glycoproteins of feline immunodeficiency virus are immunogenically silent in infected cats, *AIDS Res. Hum. Retroviruses* 13, 1121–1129 (1997).
454. J. Richardson, I. Fossati, A. Moraillon, S. Castelot, P. Sonigo, and G. Pancino, Neutralization sensitivity and accessibility of continuous B cell epitopes of the feline immunodeficiency virus envelope, *J. Gen. Virol.* 77, 759–771 (1996).
455. K. H. Siebelink, G. F. Rimmelzwaan, M. L. Bosch, R. H. Meloen, and A. D. Osterhaus, A single amino acid substitution in hypervariable region 5 of the envelope protein of feline immunodeficiency virus allows escape from virus neutralization, *J. Virol.* 67, 2202–2208 (1993).
456. K. H. Siebelink, W. Huisman, J. A. Karlas, G. F. Rimmelzwaan, M. L. Bosch, and A. D. Osterhaus, Neutralization of feline immunodeficiency virus by polyclonal feline antibody: simultaneous involvement of hypervariable regions 4 and 5 of the surface glycoprotein, *J. Virol.* 69, 5124–5127 (1995).
457. G. Freer, S. Giannecchini, A. Tissot, M. F. Bachmann, P. Rovero, P. F. Serres, and M. Bendinelli, Dissection of seroreactivity against the tryptophan-rich motif of the feline immunodeficiency virus transmembrane glycoprotein, *Virology* 322, 360–369 (2004).

458. M. Bendinelli, M. Pistello, D. Del Mauro, G. Cammarota, F. Maggi, A. Leonildi, S. Giannecchini, C. Bergamini, and D. Matteucci, During readaptation in vivo, a tissue culture-adapted strain of feline immunodeficiency virus reverts to broad neutralization resistance at different times in individual hosts but through changes at the same position of the surface glycoprotein, *J. Virol.* 75, 4584–4593 (2001).
459. G. Cammarota, D. Matteucci, M. Pistello, E. Nicoletti, S. Giannecchini, and M. Bendinelli, Reduced sensitivity to strain-specific neutralization of laboratory-adapted feline immunodeficiency virus after one passage in vivo: association with amino acid substitutions in the V4 region of the surface glycoprotein, *AIDS Res. Hum. Retroviruses* 12, 173–175 (1996).
460. S. Giannecchini, D. Matteucci, A. Ferrari, M. Pistello, and M. Bendinelli, Feline immunodeficiency virus-infected cat sera associated with the development of broad neutralization resistance in vivo drive similar reversions in vitro, *J. Virol.* 75, 8868–8873 (2001).
461. M. Pistello, D. Matteucci, S. Giannecchini, F. Bonci, O. Sichi, S. Presciuttini, and M. Bendinelli, Evolution of two amino acid positions governing broad neutralization resistance in a strain of feline immunodeficiency virus over 7 years of persistence in cats, *Clin. Diagn. Lab. Immunol.* 10, 1109–1116 (2003).
462. K. H. J. Siebelink, E. Tijhaar, R. C. Huisman, W. Huisman, A. De Ronde, I. H. Darby, M. J. Francis, G. F. Rimmelzwaan, and A. D. Osterhaus, Enhancement of feline immunodeficiency virus infection after immunization with envelope glycoprotein subunit vaccines, *J. Virol.* 69, 3704–3711 (1995).
463. D. R. Burton, R. C. Desrosiers, R. W. Doms, W. C. Koff, P. D. Kwong, J. P. Moore, G. J. Nabel, J. Sodroski, I. A. Wilson, and R. T. Wyatt, HIV vaccine design and the neutralizing antibody problem, *Nat. Immunol.* 5, 233–236 (2004).
464. M. B. Zwick, R. Jensen, S. Church, M. Wang, G. Stiegler, R. Kunert, H. Katinger, and D. R. Burton, Anti-human immunodeficiency virus type 1 (HIV-1) antibodies 2F5 and 4E10 require surprisingly few crucial residues in the membrane-proximal external region of glycoprotein gp41 to neutralize HIV-1, *J. Virol.* 79, 1252–1261 (2005).
465. J. Richardson, A. Moraillon, F. Crespeau, S. Baud, P. Sonigo, and G. Pancino, Delayed infection after immunization with a peptide from the transmembrane glycoprotein of the feline immunodeficiency virus, *J. Virol.* 72, 2406–2415 (1998).
466. N. L. Haigwood and L. Stamatatos, Role of neutralizing antibodies in HIV infection, *AIDS* 17 Suppl. 4, S67–71 (2003).
467. R. A. Rasmussen, R. Hofmann-Lehmann, P. L. Li, J. Vlasak, J. E. Schmitz, K. A. Reimann, M. J. Kuroda, N. L. Letvin, D. C. Montefiori, H. M. McClure, and R. M. Ruprecht, Neutralizing antibodies as a potential secondary protective mechanism during chronic SHIV infection in CD8+ T-cell-depleted macaques, *AIDS* 16, 829–838 (2002).
468. R. Pu, S. Okada, E. R. Little, B. Xu, W. V. Stoffs, and J. K. Yamamoto, Protection of neonatal kittens against feline immunodeficiency virus infection with passive maternal antiviral antibodies, *AIDS* 9, 235–242 (1995).
469. K. K. Van Rompay, C. J. Berardi, S. Dillard-Telm, R. P. Tarara, D. R. Canfield, C. R. Valverde, D. C. Montefiori, K. S. Cole, R. C. Montelaro, C. J. Miller, and M. L. Marthas, Passive immunization of newborn rhesus macaques prevents oral simian immunodeficiency virus infection, *J. Infect. Dis.* 177, 1247–1259 (1998).
470. F. Ferrantelli, R. A. Rasmussen, K. A. Buckley, P. L. Li, T. Wang, D. C. Montefiori, H. Katinger, G. Stiegler, D. C. Anderson, H. M. McClure, and R. M. Ruprecht, Complete protection of neonatal rhesus macaques against oral exposure to pathogenic simian-human immunodeficiency virus by human anti-HIV monoclonal antibodies, *J. Infect. Dis.* 189, 2149–2153 (2004).
471. Q. J. Sattentau, Neutralization of HIV-1 by antibody, *Curr. Opin. Immunol.* 8, 540–545 (1996).

472. W. Huisman, J. A. Karlas, K. H. Siebelink, R. C. Huisman, A. de Ronde, M. J. Francis, G. F. Rimmelzwaan, and A. D. Osterhaus, Feline immunodeficiency virus subunit vaccines that induce virus neutralising antibodies but no protection against challenge infection, *Vaccine* 16, 181–187 (1998).
473. F. Baldinotti, D. Matteucci, P. Mazzetti, C. Giannelli, P. Bandecchi, F. Tozzini, and M. Bendinelli, Serum neutralization of feline immunodeficiency virus is markedly dependent on passage history of the virus and host system, *J. Virol.* 68, 4572–4579 (1994).
474. D. Del Mauro, D. Matteucci, S. Giannecchini, F. Maggi, M. Pistello, and M. Bendinelli, Autologous and heterologous neutralization analyses of primary feline immunodeficiency virus isolates, *J. Virol.* 72, 2199–2207 (1998).
475. M. J. Burkhard, C. K. Mathiason, T. Bowdre, and E. A. Hoover, Feline immunodeficiency virus Gag- and Env-specific immune responses after vaginal versus intravenous infection, *AIDS Res. Hum. Retroviruses* 17, 1767–1778 (2001).
476. T. Hohdatsu, S. Fujimori, M. Maeki, N. Suma, K. Motokawa, S. Okada, and H. Koyama, Virus neutralizing antibody titer to feline immunodeficiency virus isolates of subtypes A, B and D in experimentally or naturally infected cats, *J. Vet. Med. Sci.* 59, 377–381 (1997).
477. Y. Inoshima, T. Miyazawa, M. Kohmoto, Y. Ikeda, E. Sato, T. Hohdatsu, C. K. Mathiason-Dubard, E. A. Hoover, and T. Mikami, Cross virus neutralizing antibodies against feline immunodeficiency virus genotypes A, B, C, D and E, *Arch. Virol.* 143, 157–162 (1998).
478. J. A. Levy, The search for the CD8+ cell anti-HIV factor (CAF), *Trends Immunol.* 24, 628–632 (2003).
479. J. D. Lifson, J. L. Rossio, M. J. Piatak, T. Parks, L. Li, R. Kiser, V. Coalter, B. Fisher, B. M. Flynn, S. Czajak, V. M. Hirsch, K. A. Reimann, J. E. Schmitz, J. Ghrayeb, N. Bischofberger, M. A. Nowak, R. C. Desrosiers, and D. Wodarz, Role of CD8(+) lymphocytes in control of simian immunodeficiency virus infection and resistance to rechallenge after transient early antiretroviral treatment, *J. Virol.* 75, 10187–10199 (2001).
480. J. N. Flynn, C. A. Cannon, J. C. Neil, and O. Jarrett, Vaccination with a feline immunodeficiency virus multiepitopic peptide induces cell-mediated and humoral immune responses in cats, but does not confer protection, *J. Virol.* 71, 7586–7592 (1997).
481. G. A. Dean, A. LaVoy, and M. J. Burkhard, Peptide mapping of feline immunodeficiency virus by IFN-gamma ELISPOT, *Vet. Immunol. Immunopathol.* 100, 49–59 (2004).
482. J. A. Beatty, B. J. Willett, E. A. Gault, and O. Jarrett, A longitudinal study of feline immunodeficiency virus-specific cytotoxic T lymphocytes in experimentally infected cats, using antigen-specific induction, *J. Virol.* 70, 6199–6206 (1996).
483. S. Finerty, C. R. Stokes, T. J. Gruffydd-Jones, T. J. Hillman, N. A. Reeves, C. V. Whiting, W. M. Schaaper, K. Dalsgaard, and D. A. Harbour, Mucosal immunization with experimental feline immunodeficiency virus (FIV) vaccines induces both antibody and T-cell responses but does not protect against rectal FIV challenge, *Vaccine* 18, 3254–3265 (2000).
484. J. N. Flynn, J. A. Beatty, C. A. Cannon, E. B. Stephens, M. J. Hosie, J. C. Neil, and O. Jarrett, Involvement of gag- and env-specific cytotoxic T lymphocytes in protective immunity to feline immunodeficiency virus, *AIDS Res. Hum. Retroviruses* 11, 1107–1113 (1995).
485. S. Giannecchini, P. Isola, O. Sichi, D. Matteucci, M. Pistello, L. Zaccaro, D. Del Mauro, and M. Bendinelli, AIDS vaccination studies using an ex vivo feline immunodeficiency virus model: failure to protect and possible enhancement of challenge infection by four cell-based vaccines prepared with autologous lymphoblasts, *J. Virol.* 76, 6882–6892 (2002).
486. C. R. Jeng, R. V. English, T. Childers, M. B. Tompkins, and W. A. Tompkins, Evidence for CD8+ antiviral activity in cats infected with feline immunodeficiency virus, *J. Virol.* 70, 2474–2480 (1996).

487. R. Pu, J. Coleman, M. Omori, M. Arai, T. Hohdatsu, C. Huang, T. Tanabe, and J. K. Yamamoto, Dual-subtype FIV vaccine protects cats against in vivo swarms of both homologous and heterologous subtype FIV isolates, *AIDS* 15, 1225–1237 (2001).
488. R. Pu, M. Omori, S. Okada, S. L. Rine, B. A. Lewis, E. Lipton, and J. K. Yamamoto, MHC-restricted protection of cats against FIV infection by adoptive transfer of immune cells from FIV-vaccinated donors, *Cell. Immunol.* 198, 30–43 (1999).
489. J. Sirriyah, G. A. Dean, A. LaVoy, and M. J. Burkhard, Assessment of CD4+ and CD8+ IFN-gamma producing cells by ELISPOT in naive and FIV-infected cats, *Vet. Immunol. Immunopathol.* 102, 77–84 (2004).
490. W. Song, E. W. Collisson, P. M. Billingsley, and W. C. Brown, Induction of feline immunodeficiency virus-specific cytolytic T-cell responses from experimentally infected cats, *J. Virol.* 66, 5409–5417 (1992).
491. W. Song, E. W. Collisson, J. Li, A. M. Wolf, J. H. Elder, C. K. Grant, and W. C. Brown, Feline immunodeficiency virus (FIV)-specific cytotoxic T lymphocytes from chronically infected cats are induced in vitro by retroviral vector-transduced feline T-cells expressing the FIV capsid protein, *Virology* 209, 390–399 (1995).
492. J. N. Flynn, S. T. Dunham, A. Mueller, C. Cannon, and O. Jarrett, Involvement of cytolytic and non-cytolytic T-cells in the control of feline immunodeficiency virus infection, *Vet. Immunol. Immunopathol.* 85, 159–170 (2002).
493. J. N. Flynn, C. A. Cannon, G. Reid, M. A. Rigby, J. C. Neil, and O. Jarrett, Induction of feline immunodeficiency virus-specific cell-mediated and humoral immune responses following immunization with a multiple antigenic peptide from the envelope V3 domain, *Immunology* 85, 171–175 (1995).
494. D. T. Evans, D. H. O'Connor, P. Jing, J. L. Dzuris, J. Sidney, J. da Silva, T. M. Allen, H. Horton, J. E. Venham, R. A. Rudersdorf, T. Vogel, C. D. Pauza, R. E. Bontrop, R. DeMars, A. Sette, A. L. Hughes, and D. I. Watkins, Virus-specific cytotoxic T-lymphocyte responses select for amino-acid variation in simian immunodeficiency virus Env and Nef, *Nat. Med.* 5, 1270–1276 (1999).
495. P. J. Goulder, R. E. Phillips, R. A. Colbert, S. McAdam, G. Ogg, M. A. Nowak, P. Giangrande, G. Luzzi, B. Morgan, A. Edwards, A. J. McMichael, and S. Rowland-Jones, Late escape from an immunodominant cytotoxic T-lymphocyte response associated with progression to AIDS, *Nat. Med.* 3, 212–217 (1997).
496. P. J. Goulder and B. D. Walker, The great escape—AIDS viruses and immune control, *Nat. Med.* 5, 1233–1235 (1999).
497. M. Omori, R. Pu, T. Tanabe, W. Hou, J. K. Coleman, M. Arai, and J. K. Yamamoto, Cellular immune responses to feline immunodeficiency virus (FIV) induced by dual-subtype FIV vaccine, *Vaccine* 23, 386–398 (2004).
498. T. Hohdatsu, M. Okubo, and H. Koyama, Feline CD8+ T-cell non-cytolytic anti-feline immunodefieicncy virus activity mediated by a soluble factor(s), *J. Gen. Virol.* 79, 2729–2735 (1998).
499. M. Shimojima, Y. Nishimura, T. Miyazawa, Y. Tohya, and H. Akashi, Phenotypic changes in CD8+ peripheral blood lymphocytes in cats infected with feline immunodeficiency virus, *Microbes Infect.* 5, 1171–1176 (2003).
500. S. T. Hohdatsu T., A. Yamazaki, K. Motokawa, H. Kusuhara, T. Kaneshima, and H. Koyama, CD8+ T-cells from feline immunodeficiency virus (FIV) infected cats suppress exogenous FIV replication of their peripheral blood mononuclear cells in vitro, *Arch. Virol.* 147, 1517–1529 (2002).
501. J. N. Flynn, C. A. Cannon, D. Sloan, J. C. Neil, and O. Jarrett, Suppression of feline immunodeficiency virus replication in vitro by a soluble factor secreted by CD8+ T lymphocytes, *Immunology* 96, 220–229 (1999).
502. I. S. Choi, R. Hokanson, and E. W. Collisson, Anti-feline immunodeficiency virus (FIV) soluble factor(s) produced from antigen-stimulated feline CD8(+) T lymphocytes suppresses FIV replication, *J. Virol.* 74, 676–683 (2000).

503. T. Hohdatsu, A. Yamazaki, M. Yamada, H. Kusuhara, T. Kaneshima, and H. Koyama, Ability of CD8(+) T-cell anti-feline immunodeficiency virus activity correlated with peripheral CD4(+) T-cell counts and plasma viremia, *Microbiol. Immunol.* 47, 765–773 (2003).
504. A. P. Phadke, I. S. Choi, Z. Li, E. A. Weaver, and E. W. Collisson, The role of inducer cells in mediating in vitro suppression of feline immunodeficiency virus replication, *Virology* 320, 63–74 (2004).
505. M. S. Wyand, K. H. Manson, M. Garcia-Moll, D. Montefiori, and R. C. Desrosiers, Vaccine protection by a triple deletion mutant of simian immunodeficiency virus, *J. Virol.* 70, 3724–3733 (1996).
506. M. D. Daniel, F. Kirchhoff, S. C. Czajak, P. K. Sehgal, and R. C. Desrosiers, Protective effects of a live attenuated SIV vaccine with a deletion in the nef gene, *Science* 258, 1938–1941 (1992).
507. K. Abel, L. Compton, T. Rourke, D. Montefiori, D. Lu, K. Rothaeusler, L. Fritts, K. Bost, and C. J. Miller, Simian-human immunodeficiency virus SHIV89.6-induced protection against intravaginal challenge with pathogenic SIVmac239 is independent of the route of immunization and is associated with a combination of cytotoxic T-lymphocyte and alpha interferon responses, *J. Virol.* 77, 3099–3118 (2003).
508. J. B. Whitney and R. M. Ruprecht, Live attenuated HIV vaccines: pitfalls and prospects, *Curr. Opin. Infect. Dis.* 17, 17–26 (2004).
509. T. W. Baba, Y. S. Jeong, D. Penninck, R. Bronson, M. F. Greene, and R. M. Ruprecht, Pathogenicity of live, attenuated SIV after mucosal infection of neonatal macaques, *Science* 267, 1820–1825 (1995).
510. T. W. Baba, V. Liska, A. H. Khimani, N. B. Ray, P. J. Dailey, D. Penninck, R. Bronson, M. F. Greene, H. M. McClure, L. N. Martin, and R. M. Ruprecht, Live attenuated, multiply deleted simain immunodeficiency virus causes AIDS in infant and adult macaques, *Nature Med.* 5, 194–203 (1999).
511. R. R. Amara, F. Villinger, J. D. Altman, S. L. Lydy, et al., Control of a mucosal challenge and prevention of AIDS by a multiprotein DNA/MVA vaccine, *Science* 292, 69–74 (2001).
512. D. H. Barouch, S. Santra, J. E. Schmitz, M. J. Kuroda, et al., Control of viremia and prevention of clinical AIDS in rhesus macaques by cytokine-augmented DNA vaccination, *Science* 290, 486–491 (2000).
513. R. R. Amara and H. L. Robinson, A new generation of HIV vaccines, *Trends Mol. Med.* 8, 489–495 (2002).
514. E. W. Uhl, T. G. Heaton-Jones, R. Pu, and J. K. Yamamoto, FIV vaccine development and its importance to veterinary and human medicine: a review, *Vet. Immunol. Immunopathol.* 6714, 1–20 (2002).
515. J. Cohen, HIV/AIDS in China. Vaccine development with a distinctly Chinese flavor, *Science* 304, 1437 (2004).
516. Y. Kono, K. Kobayashi, and Y. Fukunaga, Immunization of horses against equine infectious anemia (EIA) with an attenuated EIA virus, *Natl. Inst. Anim. Health Q. (Tokyo)* 10, 113–122 (1970).
517. J. K. Levy, P. C. Crawford, and M. R. Slater, Effect of vaccination against feline immunodeficiency virus on results of serologic testing in cats, *J. Am. Vet. Med. Assoc.* 225, 1558–1561 (2004).
518. J. A. Karlas, K. H. Siebelink, M. A. Peer, W. Huisman, G. F. Rimmelzwaan, and A. D. Osterhaus, Accelerated viraemia in cats vaccinated with fixed autologous FIV-infected cells, *Vet. Immunol. Immunopathol.* 65, 353–365 (1998).
519. J. A. Karlas, K. H. Siebelink, M. A. Peer, W. Huisman, A. M. Cuisinier, G. F. Rimmelzwaan, and A. D. Osterhaus, Vaccination with experimental feline immunodeficiency virus vaccines, based on autologous infected cells, elicits enhancement of homologous challenge infection, *J. Gen. Virol.* 80, 761–765 (1999).

520. M. J. Hosie, R. Osborne, G. Reid, J. C. Neil, and O. Jarrett, Enhancement after feline immunodeficiency virus vaccination, *Vet. Immunol. Immunopathol.* 35, 191–197 (1992).
521. W. Hesselink, P. J. Sondermeijer, H. Pouwels, E. Verblakt, and C. Dhore, Vaccination of cats against feline immunodeficiency virus (FIV): a matter of challenge, *Vet. Microbiol.* 69, 109–110 (1999).
522. M. J. Hosie, R. Osborne, J. K. Yamamoto, J. C. Neil, and O. Jarrett, Protection against homolgous but not heterologous challenge induced by inactivated feline immunodeficiency virus vaccines, *J. Virol.* 69, 1253–1255 (1995).
523. M. J. Hosie, T. Dunsford, D. Klein, B. J. Willett, C. Cannon, R. Osborne, J. MacDonald, N. Spibey, N. A. Mackay, O. Jarrett, and J. C. Neil, Vaccination with inactivated virus but not viral DNA reduces virus load following challenge with a heterologous and virulent isolate of feline immunodeficiency virus, *J. Virol.* 74, 9403–9411 (2000).
524. S. Finerty, C. R. Stokes, T. J. Gruffydd-Jones, T. J. Hillman, F. J. Barr, and D. A. Harbour, Targeted lymph node immunization can protect cats from a mucosal challenge with feline immunodeficiency virus, *Vaccine* 20, 49–58 (2001).
525. D. Matteucci, M. Pistello, P. Mazzetti, S. Giannecchini, D. Del Mauro, L. Zaccaro, P. Bancecchi, F. Tozzini, and M. Bendinelli, Vaccination protects against in vivo-grown feline immunodeficiency virus even in the absence of detectable neutralizing antibodies, *J. Virol.* 70, 617–622 (1996).
526. D. Matteucci, A. Poli, P. Mazzetti, S. Sozzi, F. Bonci, P. Isola, L. Zaccaro, S. Giannecchini, M. Calandrella, M. Pistello, S. Specter, and M. Bendinelli, Immunogenicity of an anticlade B feline immunodeficiency fixed-cell vrius vaccine in field cats, *J. Virol.* 74, 10911–10919 (2000).
527. T. Hohdatsu, S. Okada, K. Motokawa, C. Aizawa, J. K. Yamamoto, and H. Koyama, Effect of dual-subtype vaccine against feline immunodeficiency virus infection, *Vet. Microbiol.* 58, 155–165 (1997).
528. M. C. Tellier, R. Pu, D. Pollock, A. Vitsky, J. Tartaglia, E. Paoletti, and J. K. Yamamoto, Efficacy evaluation of prime-boost protocol: canarypoxvirus-based feline immunodeficiency virus (FIV) vaccine and inactivated FIV-infected cell vaccine against heterologous FIV challenge in cats, *AIDS* 12, 11–18 (1998).
529. S. A. Bishop, C. R. Stokes, T. J. Gruffydd-Jones, C. V. Whiting, J. E. Humphries, R. Osborne, M. Papnastasopoulou, and D. A. Harbour, Vaccination with fixed feline immunodeficiency virus (FIV) infected cells: protection, breakthrough and specificity of response, *Vaccine* 14, 1243–1250 (1996).
530. D. Matteucci, M. Pistello, P. Mazzetti, S. Giannecchini, D. Del Mauro, I. Lonetti, L. Zaccaro, C. Pollera, S. Specter, and M. Bendinelli, Studies of AIDS vaccination using an ex vivo feline immunodeficiency virus model: protection conferred by a fixed-cell vaccination against cell-free and cell-associated challenge differs in duration and is not easily boosted, *J. Virol.* 71, 8368–8376 (1997).
531. M. J. Hosie, T. H. Dunsford, A. de Ronde, B. J. Willett, C. A. Cannon, J. C. Neil, and O. Jarrett, Suppression of virus burden by immunization with feline immunodeficiency virus Env protein, *Vaccine* 14, 405–411 (1996).
532. P. Mazzetti, S. Giannecchini, D. Del Mauro, D. Matteucci, P. Portincasa, A. Merico, C. Chezzi, and M. Bendinelli, AIDS vaccination studies using an ex vivo feline immunodeficiency virus model: detailed analysis of the humoral immune response to a protective vaccine, *J. Virol.* 73, 1–10 (1999).
533. D. Matteucci, M. Pistello, P. Mazzetti, S. Giannecchini, P. Isola, A. Merico, L. Zaccaro, and A. Rizzuti, AIDS vaccination studies using feline immunodeficiency virus suppresses viraemia levels following intravaginal challenge with infected cells but not following intravenous challenge with cell-free virus, *Vaccine* 18, 119–130 (1999).
534. S. Giannecchini, D. Del Mauro, D. Matteucci, and M. Bendinelli, AIDS vaccination studies using an ex vivo feline immunodeficiency virus model: reevaluation of neutral-

izing antibody levels elicited by a protective and a nonprotective vaccine after removal of antisubstrate cell antibodies, *J. Virol.* 75, 4424–4429 (2001).
535. J. N. Flynn, P. Keating, M. J. Hosie, M. Mackett, E. B. Stephens, J. A. Beatty, J. C. Neil, and O. Jarrett, Env-specific CTL predominate in cats protected from feline immunodeficiency virus infection by vaccination, *J. Immunol.* 157, 3658–3665 (1996).
536. M. C. Tellier, J. Soos, R. Pu, D. Pollock, and J. K. Yamamoto, Development of FIV-specific cytolytic T-lymphocyte responses in cats upon immunisation with FIV vaccines, *Vet. Microbiol.* 57, 1–11 (1997).
537. M. J. Hosie and J. N. Flynn, Feline immunodefiiency virus vaccination: characterization of the immune correlates of protection, *J. Virol.* 70, 7561–7568 (1996).
538. W. L. Chan, A. Rodgers, R. D. Hancock, F. Taffs, P. Kitchin, G. Farrar, and F. Y. Liew, Protection in simian immunodeficiency virus-vaccinated monkeys correlates with anti-HLA class I antibody response, *J. Exp. Med.* 176, 1203–1207 (1992).
539. E. J. Stott, Anti-cell antibody in macaques, *Nature* 353, 393 (1991).
540. N. A. Doria-Rose, C. Ohlen, P. Polacino, C. C. Pierce, M. T. Hensel, L. Kuller, T. Mulvania, D. C. Anderson, P. D. Greenberg, S. L. Hu, and N. Haigwood, Multigene DNA priming-boosting vaccines protect macaques from acute CD4+-T-cell depletion after simian-human immunodeficiency virus SHIV89.6P mucosal challenge, *J. Virol.* 77, 11563–11577 (2003).
541. R. L. Willey, R. Byrum, M. Piatak, Y. B. Kim, M. W. Cho, J. L. Rossio, J. Bess, T. Igarashi, Y. Endo, L. O. Arthur, J. D. Lifson, and M. A. Martin, Control of viremia and prevention of simian-human immunodeficiency virus-induced disease in rhesus macaques immunized with recombinant vaccinia viruses plus inactivated simian immunodeficiency virus and human immunodeficiency virus type 1 particles, *J. Virol.* 77, 1163–1174 (2003).
542. E. J. Verschoor, M. J. Willemse, J. G. Stam, A. L. van Vliet, H. Pouwels, S. K. Chalmers, M. C. Horzinek, P. J. Sondermeijer, W. Hesselink, and A. de Ronde, Evaluation of subunit vaccines against feline immunodeficiency virus infection, *Vaccine* 14, 285–289 (1996).
543. A. Cuisinier, A. Meyer, B. Chatrenet, A. S. Verdier, and A. Aubert, Attempt to modify the immune response developed against FIV gp120 protein by preliminary FIV DNA injection, *Vaccine* 17, 415–425 (1999).
544. H. Lutz, R. Hofmann-Lehmann, K. Bauer-Pham, E. Holznagel, F. Tozzini, M. Bendinelli, G. Reubel, A. Aubert, D. Davis, D. Cox, and E. Young, FIV vaccine studies. I. Immune response to recombinant FIV env gene products and outcome after challenge infection, *Vet. Immunol. Immunopathol.* 46, 103–113 (1995).
545. C. M. Leutenegger, R. Hofmann-Lehmann, E. Holznagel, A. M. Cuisinier, C. Wolfensberger, V. Duquesne, J. Cronier, K. Allenspach, A. Aubert, P. Ossent, and H. Lutz, Partial protection by vaccination with recombinant feline immunodeficiency virus surface glycoproteins, *AIDS Res. Hum. Retroviruses* 14, 275–283 (1998).
546. N. L. Letvin, Progress in the development of an HIV-1 vaccine, *Science* 280, 1875–1880 (1998).
547. L. Chiarantini, D. Matteucci, M. Pistello, U. Mancini, P. Mazzetti, C. Massi, S. Giannecchini, I. Lonetti, M. Magnani, and M. Bendinelli, AIDS vaccination studies using an ex vivo feline immunodeficiency virus model: homologous erythrocytes as a delivery system for preferential immunization with putative protective antigens, *Clin. Diagn. Lab. Immunol.* 5, 235–241 (1998).
548. S. Zolla-Pazner, Identifying epitopes of HIV-1 that induce protective antibodies, *Nat. Rev. Immunol.* 4, 199–210 (2004).
549. M. J. Hosie, J. N. Flynn, M. A. Rigby, C. Cannon, T. Dunsford, N. A. Mackay, D. Argyle, B. J. Willett, T. Miyazawa, D. E. Onions, O. Jarrett, and J. C. Neil, DNA vaccination affords significant protection against feline immunodeficiency virus infection without inducing detectable antiviral antibodies, *J. Virol.* 72, 7310–7319 (1998).

550. F. S. Boretti, C. M. Leutenegger, C. N. Mislin, R. Hofmann-Lehmann, S. Koenig, M. Schroff, C. Junghans, D. Fehr, S. W. Huettner, A. Habel, J. N. Flynn, A. Aubert, N. C. Pedersen, B. Wittig, and H. Lutz, Protection against FIV challenge infection by genetic vaccination using minimalistic DNA constructs for FIV env gene and feline IL-12 expression, *AIDS* 14, 1749–1757 (2000).
551. C. M. Leutenegger, F. S. Boretti, C. N. Mislin, J. N. Flynn, M. Schroff, A. Habel, C. Junghans, S. A. Koenig-Merediz, B. Sigrist, A. Aubert, N. C. Pedersen, B. Wittig, and H. Lutz, Immunization of cats against feline immunodeficiency virus (FIV) infection by using minimalistic immunogenic defined gene expression vector vaccines expressing FIV gp140 alone or with feline interleukin-12 (IL-12), IL-16, or a CpG motif, *J. Virol.* 74, 10447–10457 (2000).
552. L. Deml, A. Bojak, S. Steck, M. Graf, J. Wild, R. Schirmbeck, H. Wolf, and R. Wagner, Multiple effects of codon usage optimization on expression and immunogenicity of DNA candidate vaccines encoding the human immunodeficiency virus type 1 Gag protein, *J. Virol.* 75, 10991–11001 (2001).
553. J. N. Flynn, M. J. Hosie, M. A. Rigby, N. Mackay, C. A. Cannon, T. Dunsford, J. C. Neil, and O. Jarrett, Factors influencing cellular immune responses to feline immunodefiency virus induced by DNA vaccination, *Vaccine* 18, 1118–1132 (2000).
554. J. Richardson, A. Moraillon, S. Baud, A. M. Cuisinier, P. Sonigo, and G. Pancino, Enhancement of feline immunodeficiency virus (FIV) infection after DNA vaccination with the FIV envelope, *J. Virol.* 71, 9640–9649 (1997).
555. J. Richardson, S. Broche, S. Baud, T. Leste-Lasserre, F. Femenia, D. Levy, A. Moraillon, G. Pancino, and P. Sonigo, Lymphoid activation: a confounding factor in AIDS vaccine development?, *J. Gen. Virol.* 83, 2515–2521 (2002).
556. A. M. Cuisinier, V. Mallet, A. Meyer, C. Caldora, and A. Aubert, DNA vaccination using expression vectors carrying FIV structural genes induces immune response against feline immunodeficiency virus, *Vaccine* 15, 1085–1094 (1997).
557. P. Gonin, A. Fournier, W. Oualikene, A. Moraillon, and M. Eloit, Immunization trial of cats with a replication-defective adenovirus type 5 expressing the ENV gene of feline immunodeficiency virus, *Vet. Microbiol.* 45, 393–401 (1995).
558. M. J. Burkhard, L. Valenski, S. Leavell, G. A. Dean, and W. A. Tompkins, Evaluation of FIV protein-expressing VEE-replicon vaccine vectors in cats, *Vaccine* 21, 258–268 (2002).
559. E. J. Tijhaar, W. Huisman, R. C. Huisman, K. H. Siebelink, J. A. Karlas, A. de Ronde, R. van Herwijnen, F. R. Mooi, and A. D. Osterhaus, Salmonella typhimurium aroA recombinants and immune-stimulating complexes as vaccine candidates for feline immunodeficiency virus, *J. Gen. Virol.* 78, 3265–3275 (1997).
560. R. Stevens, K. E. Howard, S. Nordone, M. J. Burkhard, and G. A. Dean, Oral immunization with recombinant listeria monocytogenes controls virus load after vaginal challenge with feline immunodeficiency virus, *J. Virol.* 78, 8210–8218 (2004).
561. A. Kumar, S. Mukherjee, J. Shen, S. Buch, Z. Li, I. Adany, Z. Liu, W. Zhuge, M. Piatak, J. D. Lifson, H. McClure, and O. Narayan, Immunization of macaques with live simian human immunodeficiency virus (SHIV) vaccines conferred protection against AIDS induced by homologous and heterologous SHIVs and simian immunodeficiency virus, *Virology* 301, 189–205 (2002).
562. A. Harmache, C. Vitu, F. Guiguen, P. Russon, G. Bertoni, M. Pepin, R. Vigne, and M. Susan, Priming with tat-deleted caprine arthritis encphalitis virus (CAEV) proviral DNA or live virus protects goats from challenge with pathogenic CAEV, *J. Virol.* 72, 6796–6804 (1998).
563. F. Li, J. K. Craigo, L. Howe, J. D. Steckbeck, S. Cook, C. J. Issel, and R. C. Montelaro, A live attenuated equine infectious anemia virus proviral vaccine with a modified S2 gene provides protection from detectable infection by intravenous virulent virus challenge of experimentally inoculated horses, *J. Virol.* 77, 7244–7253 (2003).

564. M. Kohmoto, T. Miyazawa, E. Sato, K. Uetsuka, Y. Nishimura, Y. Ikeda, G. Inada, K. Doi, and T. Mikami, Cats are protected against feline immunodeficiency virus infection following vaccination with a homologous AP-1 binding site-deleted mutant, *Arch. Virol.* 143, 1839–1845 (1998).
565. S. VandeWoude, C. A. Hageman, S. J. O'Brien, and E. A. Hoover, Nonpathogenic lion and puma lentiviruses impart resistance to superinfection by virulent feline immunofeciency virus, *J.A.I.D.S.* 29, 1–10 (2002).
566. R. C. Desrosiers, Prospects for an AIDS vaccine, *Nat. Med.* 10, 221–223 (2004).
567. M. L. Raabe, C. J. Issel, S. J. Cook, R. F. Cook, B. Woodson, and R. C. Montelaro, Immunization with a recombinant envelope protein (rgp90) of EIAV produces a spectrum of vaccine efficacy ranging from lack of clinical disease to severe enhancement, *Virology* 245, 151–162 (1998).
568. R. Merat, H. Raoul, T. Leste-Lasserre, P. Sonigo, and G. Pancino, Variable constraints on the principal immunodominant domain of the transmembrane glycoprotein of human immunodeficiency virus type 1, *J. Virol.* 73, 5698–5706 (1999).
569. W. Huisman, E. J. Schrauwen, S. D. Pas, J. A. Karlas, G. F. Rimmelzwaan, and A. D. Osterhaus, Antibodies specific for hypervariable regions 3 to 5 of the feline immunodeficiency virus envelope glycoprotein are not solely responsible for vaccine-induced acceleration of challenge infection in cats, *J. Gen. Virol.* 85, 1833–1841 (2004).
570. S. I. Staprans, A. P. Barry, G. Silvestri, J. T. Safrit, N. Kozyr, B. Sumpter, H. Nguyen, H. McClure, D. Montefiori, J. I. Cohen, and M. B. Feinberg, Enhanced SIV replication and accelerated progression to AIDS in macaques primed to mount a CD4 T-cell response to the SIV envelope protein, *Proc. Natl. Acad. Sci. USA* 101, 13026–13031 (2004).
571. R. C. Cronn, K. M. Remington, B. D. Preston, and T. W. North, Inhibition of reverse transcriptase from feline immunodeficiency virus by analogs of 2′-deoxyadenosine-5′-triphosphate, *Biochem. Pharmacol.* 44, 1375–1381 (1992).
572. H. Egberink, M. Borst, H. Niphuis, J. Balzarini, H. Neu, H. Schellekens, E. De Clercq, M. Horzinek, and M. Koolen, Suppression of feline immunodeficiency virus infection in vivo by 9-(2-phosphonomethoxyethyl)adenine, *Proc. Natl. Acad. Sci. USA* 87, 3087–3091 (1990).
573. K. Hartmann, J. Balzarini, J. Higgins, E. De Clercq, and N. C. Pedersen, In vitro activity of acyclic nucleoside phosphonate derivatives against feline immunodeficiency vius in Crandell feline kidney cells and feline peripheral blood lymphocytes, *Antiviral Chem. Chemother.* 6, 13–19 (1994).
574. T. W. North and R. A. LaCasse, Testing HIV-1 drugs in the FIV model, *Nat. Med.* 1, 410–411 (1995).
575. T. W. Vahlenkamp, A. De Ronde, J. Balzarini, L. Naesens, E. De Clercq, M. J. van Eijk, M. C. Horzinek, and H. F. Egberink, (R)-9-(2-phosphonylmethoxypropyl)-2,6-diaminopurine is a potent inhibitor of feline immunodeficiency virus infection, *Antimicrob. Agents Chemother.* 39, 746–749 (1995).
576. L. M. Stolk and J. F. Luers, Increasing number of anti-HIV drugs but no definite cure. Review of anti-HIV drugs, *Pharm. World Sci.* 26, 133–136 (2004).
577. J. M. Gobert, K. M. Remington, Y. Q. Zhu, and T. W. North, Multiple-drug-resistant mutants of feline immunodeficiency virus selected with 2′,3′-dideoxyinosine alone and in combination with 3′-azido-3′-deoxythymidine, *Antimicrob. Agents Chemother.* 38, 861–864 (1994).
578. Y. Q. Zhu, K. M. Remington, and T. W. North, Mutants of feline immunodeficiency virus resistant to 2′,3′-dideoxy-2′,3′-didehydrothymidine, *Antimicrob. Agents Chemother.* 40, 1983–1987 (1996).
579. H. K. Medlin, Y. Q. Zhu, K. M. Remington, T. R. Phillips, and T. W. North, Selection and characterization of a mutant of feline immunodeficiency virus resistant to 2′,3′-dideoxycytidine, *Antimicrob. Agents Chemother.* 40, 953–957 (1996).

580. N. R. Smyth, M. Bennett, R. M. Gaskell, C. McCracken, C. A. Hart, and J. L. Howe, Effect of 3′azido-2′,3′-deoxythymidine (AZT) on experimental feline immunodeficiency virus infection in domestic cats, *Res.Vet. Sci.* 57, 220–224 (1994).
581. K. Hartmann, A. Donath, B. E. Beer, H. F. Egberink, M. C. Horzinek, H. Lutz, G. Hoffmann-Fezer, I. Thum, and S. Thefeld, Use of two virustatica (AZT, PMEA) in the treatment of FIV and of FeLV seropositive cats with clinical symptoms, *Vet. Immunol. Immunopathol.* 35, 167–175 (1992).
582. K. Hartmann, A. Donath, and W. Kraft, AZT in the teatment of feline immunodeficiency virus infection, Part 1, *Feline Pract.* 23, 16–21 (1995).
583. K. Hartmann, A. Donath, and W. Kraft, AZT in the teatment of feline immunodeficiency virus infection, Part 2, *Feline Pract.* 23, 13–20 (1995).
584. K. A. Hayes, L. J. Lafrado, J. G. Erickson, J. M. Marr, and L. E. Mathes, Prophylactic ZDV therapy prevents early viremia and lymphocyte decline but not primary infection in feline immunodeficiency virus-inoculated cats, *J. Acquir. Immune Defic. Syndr.* 6, 127–134 (1993).
585. K. A. Hayes, J. G. Wilkinson, R. Frick, S. Francke, and L. E. Mathes, Early suppression of viremia by ZDV does not alter the spread of feline immunodeficiency virus infection in cats, *J. Acquir. Immune Defic. Syndr.* 9, 114–122 (1995).
586. J. Meers, G. M. del Fierro, R. B. Cope, H. S. Park, W. K. Greene, and W. F. Robinson, Feline immunodeficiency virus infection: plasma, but not peripheral blood mononuclear cell virus titer is influenced by zidovudine and cyclosporine, *Arch. Virol.* 132, 67–81 (1993).
587. K. A. Hayes, A. J. Phipps, S. Francke, and L. E. Mathes, Antiviral therapy reduces viral burden but does not prevent thymic involution in young cats infected with feline immunodeficiency virus, *Antimicrob. Agents Chemother.* 44, 2399–2405 (2000).
588. M. Arai, D. D. Earl, and J. K. Yamamoto, Is AZT/3TC therapy effective against FIV infection or immunopathogenesis?, *Vet. Immunol. Immunopathol.* 85, 189–204 (2002).
589. K. Hartmann, M. Kuffer, J. Balzarini, L. Naesens, M. Goldberg, V. Erfle, F. D. Goebel, E. De Clercq, J. Jindrich, A. Holy, N. Bischofberger, and W. Kraft, Efficacy of the acyclic nucleoside phosphonates (S)-9-(3-fluoro-2-phosphonylmethoxypropyl)adenine (FPMPA) and 9-(2-phosphonylmethoxyethyl)adenine (PMEA) against feline immunodeficiency virus, *J. Acquir. Immune Defic. Syndr. Hum. Retrovirol.* 17, 120–128 (1998).
590. M. S. Philpott, J. P. Ebner, and E. A. Hoover, Evaluation of 9-(2-phosphonylmethoxyethyl) adenine therapy for feline immunodeficiency virus using a quantitative polymerase chain reaction, *Vet. Immunol. Immunopathol.* 35, 155–166 (1992).
591. L. R. Bisset, H. Lutz, J. Boni, R. Hofmann-Lehmann, R. Luthy, and J. Schupbach, Combined effect of zidovudine (ZDV), lamivudine (3TC) and abacavir (ABC) antiretroviral therapy in suppressing in vitro FIV replication, *Antiviral Res.* 53, 35–45 (2002).
592. T. Lee, V. D. Le, D. Lim, Y. C. Lin, G. M. Morris, A. L. Wong, A. J. Olson, J. E. Elder, and C. H. Wong, Development of a new type of protease inhibitors, efficacious against FIV and HIV variants, *J. Am. Chem. Soc.* 121, 1145–1155 (1999).
593. M. Li, G. M. Morris, T. Lee, G. S. Laco, C. H. Wong, A. J. Olson, J. H. Elder, A. Wlodawer, and A. Gustchina, Structural studies of FIV and HIV-1 proteases complexed with an efficient inhibitor of FIV protease, *Proteins* 38, 29–40 (2000).
594. S. De Rozieres, C. H. Swan, D. A. Sheeter, K. J. Clingerman, Y. C. Lin, S. Huitron-Resendiz, S. J. Henriksen, B. E. Torbett, and J. H. Elder, Assessment of FIV-C infection of cats as a function of treatment with the protease inhibitor, TL-3, *Retrovirology* 1, 38 (2004).
595. R. J. Medinas, D. M. Lambert, and W. A. Tompkins, C-Terminal gp40 peptide analogs inhibit feline immunodeficiency virus: cell fusion and virus spread, *J. Virol.* 76, 9079–9086 (2002).

596. C. T. Wild, D. C. Shugars, T. K. Greenwell, C. B. McDanal, and T. J. Matthews, Peptides corresponding to a predictive alpha-helical domain of human immunodeficiency virus type 1 gp41 are potent inhibitors of virus infection, *Proc. Natl. Acad. Sci. USA* 91, 9770–9774 (1994).
597. S. G. Peisajovich and Y. Shai, Viral fusion proteins: multiple regions contribute to membrane fusion, *Biochem. Biophsy. Acta.* 1614, 122–129 (2003).
598. A. Mergia, J. Blackwell, and S. Chari, Inhibition of FIV replication by a ribozyme that targets the Rev response element, *AIDS Res. Hum. Retroviruses* 13, 1333–1339 (1997).
599. K. De Mari, L. Maynard, A. Sanquer, B. Lebreux, and H. M. Eun, Therapeutic effects of recombinant feline interferon-omega on feline leukemia virus (FeLV)-infected and FeLV/feline immunodeficiency virus (FIV)-coinfected symptomatic cats, *J. Vet. Intern. Med.* 18, 477–482 (2004).
600. M. Arai, J. Darman, A. Lewis, and J. K. Yamamoto, The use of human hematopoietic growth factors (rhGM-CSF and rhEPO) as a supportive therapy for FIV-infected cats, *Vet. Immunol. Immunopathol.* 77, 71–92 (2000).
601. C. R. Gregory, J. E. Barlough, R. Clark, S. M. Griffey, J. D. Patz, P. Jardieu, and N. C. Pedersen, Effects of insulin-like growth factor-1 and AZT in cats experimentally infected with FIV, *Feline Pract.* 25, 23–31 (1997).
602. C. H. Pontzer, J. K. Yamamoto, F. W. Bazer, T. L. Ott, and H. M. Johnson, Potent anti-feline immunodeficiency virus and anti-human immunodeficiency virus effect of IFN-tau, *J. Immunol.* 158, 4351–4357 (1997).
603. C. M. Leutenegger, J. B. Huder, C. N. Mislin, F. Lahrtz, R. Hofmann-Lehmann, N. C. Pedersen, and H. Lutz, Molecular characterization of feline interleukin 16: chemotactic activity and effect on feline immunodeficiency virus infection and/or replication, *AIDS Res. Hum. Retroviruses* 16, 569–575 (2000).
604. S. L. Pett and A. D. Kelleher, Cytokine therapies in HIV-1 infection: present and future, *Expert Rev. Anti Infect. Ther.* 1, 83–96 (2003).
605. J. D. Siliciano and R. F. Siliciano, A long-term latent reservoir for HIV-1: discovery and clinical implications, *J. Antimicrob. Chemother.* 54, 6–9 (2004).
606. M. A. Curran and G. P. Nolan, Nonprimate lentiviral vectors, *Curr. Top. Microbiol. Immunol.* 261, 75–105 (2002).
607. D. T. Saenz and E. M. Poeschla, FIV: from lentivirus to lentivector, *J. Gene Med.* 6, S95–S104 (2004).
608. N. Loewen, M. Fautsch, and M. Peretz, Genetic modification of human trabecular meshwork with lentiviral vectors, *Hum. Gene Ther.* 12, 2109–2119 (2001).
609. J. J. Song, B. Lee, J. W. Chang, J. Kim, Y. K. Kwon, and H. Lee, Optimization of vesicular stomatitis virus-G pseudotyped feline immunodeficiency virus vector for minimized cytotoxicity with efficient gene transfer, *Virus Res.* 93, 25–30 (2003).
610. M. A. Curran, S. M. Kaiser, P. L. Achacoso, and G. P. Nolan, Efficient transduction of nondividing cells by optimized feline immunodeficiency virus vectors, *Mol. Ther.* 1, 31–38 (2000).
611. N. Loewen, D. Leske, and Y. Chen, Comparison of wild type and class I integrase mutant-FIV vectors in retina demonstrates sustained expression of integrated transgenes in retinal pigment epithelium, *J. Gene Med.* 5, 1009–1017 (2003).
612. N. Loewen, R. Barraza, T. Whitwam, D. T. Saenz, I. Kemler, and E. M. Poeschla, FIV vectors, *Methods Mol. Biol.* 229, 251–271 (2003).
613. M. C. Barr, P. P. Calle, M. E. Roelke, and F. W. Scott, Feline immunodeficiency virus infection in nondomestic felids, *J. Zoo. Wildl. Med.* 20, 265–272 (1989).
614. M. C. Barr, L. Zou, F. Long, W. A. Hoose, and R. J. Avery, Proviral organization and sequence analysis of feline immunodeficiency virus isolated from a Pallas cat, *Virology* 228, 84–91 (1997).

615. M. Carpenter, E. Brown, and M. Culver, Genetic and phylogenetic divergence of feline immunodeficiency virus in the puma (*Puma concolor*), *J. Virol.* 70, 6682–6693 (1996).
616. R. J. Langley, V. M. Hirsch, S. J. O'Brien, D. Adger-Johnson, R. M. Goeken, and R. Olmsted, Nucleotide sequence analysis of puma lentivirus (PLV-14): genomic organization and relationship to other lentiviruses, *Virology* 202, 853–864 (1994).
617. S. VandeWoude, S. J. O'Brien, K. Langelier, W. D. Hardy, J. P. Slattery, E. E. Zuckerman, and E. A. Hoover, Growth of lion and puma lentiviruses in domestic cat cells and comparisons with FIV, *Virology* 233, 185–192 (1997).
618. J. L. Troyer, J. Pecon-Slattery, M. E. Roelke, L. Black, C. Packer, and S. J. O'Brien, Patterns of feline immunodeficiency virus multiple infection and genome divergence in a free-ranging population of African lions, *J. Virol.* 78, 3777–3791 (2004).
619. M. A. Carpenter and S. J. O'Brien, Coadaptation and immunodeficiency virus: lessons from the Felidae, *Curr. Opin. Genet. Dev.* 5, 739–745 (1995).
620. C. Packer, S. Alitzer, M. Appel, E. Brown, J. S. Martenson, S. J. O'Brien, M. Roelke-Parker, R. Hofmann-Lehmann, and H. Lutz, Viruses of the Serengeti: patterns of infection and mortality in lions, *J. Anim. Ecol.* 68, 1161–1178 (1999).
621. S. Goldstein, I. Ourmanov, C. R. Brown, B. E. Beer, W. R. Elkins, R. Plishka, A. Buckler-White, and V. M. Hirsch, Wide range of viral load in healthy African green monkeys naturally infected with simian immunodeficiency virus, *J. Virol.* 74, 11744–11753 (2000).
622. V. M. Hirsch, G. Dapolito, R. M. Goeken, and B. Campbell, Phylogeny and natural history of the primate lentiviruses, SIV and HIV, *Curr. Opin. Genet. Dev.* 5, 798–806 (1997).
623. R. Shankarappa, J. B. Margolick, S. J. Gange, A. G. Rodrigo, D. Upchurch, H. Farzadegan, P. Gupta, C. R. Rinaldo, G. H. Learn, X. He, X. L. Huang, and J. I. Mullins, Consistent viral evolutionary changes associated with the progression of human immunodeficiency virus type 1 infection, *J. Virol.* 73, 10489–10502 (1999).
624. E. Pelletier, W. Saurin, R. Cheynier, N. L. Letvin, and S. Wain-Hobson, The tempo and mode of SIV quasispecies development in vivo calls for massive viral replication and clearance, *Virology* 20, 644–652 (1995).
625. M. C. Muller-Trutwin, S. Corber, M. D. Tavares, V. M. Herve, E. Nerrienet, M. C. Georges-Courbot, W. Saurin, P. Sonigo, and F. Barre-Sinoussi, The evolutionary rate of nonpathogenic simian immunodeficiency virus (SIVagm) is in agreement with a rapid and continuous replication in vivo, *Virology* 223, 89–102 (1996).
626. T. Leitner and J. Albert, The molecular clock of HIV-1 unveiled through analysis of a known transmission history, *Proc. Natl. Acad. Sci. USA* 96, 10752–10757 (1999).
627. S. VandeWoude, S. J. O'Brien, and E. A. Hoover, Infectivity of lion and puma lentiviruses for domestic cats, *J. Gen. Virol.* 78, 795–800 (1997).
628. S. VandeWoude, C. L. Hageman, and E. A. Hoover, Domestic cats infected with lion or puma lentivirus develop anti-feline immunodeficiency virus immune responses, *J. Acquir. Immune Defic. Syndr.* 34, 20–31 (2003).

8

FIV as a Model for AIDS Pathogenesis Studies

THOMAS W. VAHLENKAMP, MARY B. TOMPKINS, and WAYNE A. F. TOMPKINS

1. INTRODUCTION

The domestic cats (*Catus domesticus*) from which feline immunodeficiency virus (FIV) was first isolated showed clinical signs of immunodeficiency, and the incidence of disease was associated with the prevalence of FIV. The first publication by Niels Pedersen and his collegues[1] at the University of California describes a number of important genomic, structural, and biochemical characteristics of the virus that were found to be remarkably similar to human immunodeficiency virus (HIV). During the past two decades, FIV infection in the domestic cat has become an excellent comparative model for studying several aspects of HIV biology, vaccine development, and particularly immunopathogenesis. Although the lentiviruses genetically most closely related to FIV are those of the small ruminants, maedi-visna virus of sheep, and caprine arthritis encephalitis virus,[2,3] the type of disease produced by FIV is remarkably similar to acquired immunodeficiency syndrome (AIDS) in humans.

HIV infection results in a transient lymphopenia at the time of seroconversion followed by a partial rebound in CD4+ T-cell numbers and the development of a $CD8^+$ lymphocytosis after several weeks. The characteristic decrease in the $CD4^+/CD8^+$ T-cell ratio progresses throughout the asymptomatic stage of infection as $CD4^+$ T-cells continue to decline. By the

THOMAS W. VAHLENKAMP, MARY B. TOMPKINS and WAYNE A. F. TOMPKINS • Immunology Program, College of Veterinary Medicine, North Carolina State University, 4700 Hillsborough St., Raleigh, NC 27606

time HIV+ patients develop AIDS, the $CD4^+$ T-cell numbers are markedly reduced.[4] Parallel with a loss in $CD4^+$ T-cell numbers is a progressive decline in $CD4^+$ T-cell proliferative response to mitogenic and antigenic stimulation that is unrelated to $CD4^+$ cell numbers.[5] The number of $CD8^+$ T-cells also decreases as AIDS develops, but unlike $CD4^+$ T-cell counts, is not a good predictor of disease progression.[6,7] The loss of $CD4^+$ cell numbers and function is highly predictive of the development of AIDS, suggesting that the immunopathogenesis of HIV and the development of AIDS are centered around this selective loss. Despite intensive research in the 20 years after the first isolation of the virus, the mechanisms responsible for the changes in circulating lymphocyte subsets in HIV+ patients are not fully understood.

The development of the FIV animal model with virus-immune system interactions similar to those seen with HIV has greatly facilitated the study of the immunopathogenesis of AIDS, which will be focus of this chapter.

2. COURSE OF THE DISEASE

As in HIV+ patients, progressive immune dysfunctions in naturally or experimentally FIV+ domestic cats usually result in four clinical stages of disease (Figure 1). The acute phase of the infection is characterized

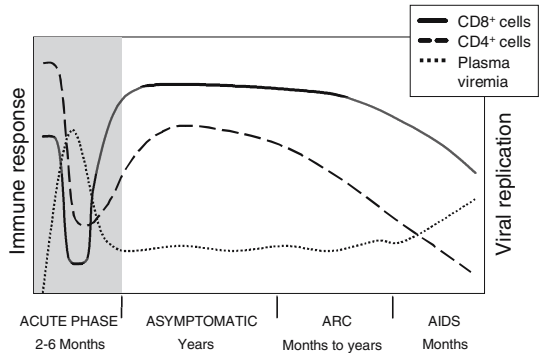

FIGURE 1. Natural history of FIV infection. The acute stage infection is characterized by an initial plasma viremia and a decrease in $CD4^+$ and $CD8^+$ cells in the peripheral blood that is followed by a rebound of $CD4^+$ and $CD8^+$ cells and a marked decline in plasma viremia. The initial phase of the immune response (gray underlayed area) appears to be normal and similar to other viral infections.[198] With transition to the asymptomatic stage of infection a low level plasma viremia persists and $CD4^+$ cell counts in the peripheral blood remain lower than physiologic numbers before the infection. With time of infection $CD4^+$ cells and in later stages also $CD8^+$ cells progressively decrease. Clinical symptoms appear as the infection progresses to the AIDS-related-complex (ARC) and AIDS stages that are characterized by a marked immunodeficiency and an increase in plasma viremia.

by an early peak in plasma viremia generally three to four weeks post-infection. Transient clinical signs may accompany this early period of virus replication, commonly manifested as mild pyrexia, anorexia, depression, and neutropenia. Acute diarrhea, conjunctivitis, and upper respiratory tract symptoms may develop in more severely affected cats.[8] During this period, productive infection is seen within the thymus, mucosal and systemic lymphoid tissues, and bone marrow, and the virus can be detected in plasma, saliva, vaginal secretions, semen, and milk/colostrums.[9–13] Virus also enters the central nervous system early in the course of infection and can be isolated from cerebrospinal fluid.[10] Some cats develop a persistent generalized lymph-adenopathy without obvious secondary or opportunistic infections.[8] Concurrent with the humoral and cellular immune response the level of viral load in plasma decreases and the animals become clinically asymptomatic. Usually the infection remains clinically inapparent for prolonged periods of time, lasting for several years. Despite the lack of clinical disease symptoms, there is progressive immune dysfunction characterized by loss of $CD4^+$ cells, altered cytokine profiles, inability of $CD4^+$ cells to produce interleukin (IL)-2, and loss of T-cell proliferative response to mitogen and major histocompatibility complex (MHC) II restricted recall antigens. Antibody responses in FIV+ cats have been reported to be normal or enhanced,[14] but usually infected cats mount reduced antibody responses to a variety of antigens and microorganisms.[15–17] As immune dysfunctions progress, infected cats eventually move into the AIDS-related complex (ARC) stage of infection. This stage is characterized by recurrent episodes of infection and inflammation, and cats suffer from infections of the oral cavity, weight loss, anemia, leukopenia, upper respiratory tract infections, chronic skin disorders, diarrhea, and lymphoid neoplastic disorders. Neurologic symptoms associated with the ARC stage of FIV infection may include sleep disorders, behavioral changes, and altered visual and auditory evoked potentials.[18–20] As in HIV+ patients,[21] the great majority of lymphoid tumors are of B-cell origin.[8,22,23] Clinical symptoms worsen over a period of months to years, and animals may develop a disease picture comparable to AIDS in man. Cytopenia with $CD4^+$ T-cell numbers less than 200–300/μl of blood is usually seen with this stage of disease that is also characterized by increased plasma viral load, weight loss, pancytopenia, severe emaciation, chronic disease symptoms, and opportunistic infections. In the individual cat the degree of $CD4^+$ cell depletion, however, does not solely predict the clinical outcome, although for the larger population of FIV^+ cats, this correlate can be made.[24,25] Cats usually die within months of developing AIDS-associated clinical disease.[22,23,26,27]

Whatever the mechanisms leading to the loss of $CD4^+$ cell numbers and function, data suggest that FIV and HIV affect the immune system in a similar manner and share a common immunopathogenesis.

3. FELINE IMMUNODEFICIENCY VIRUS (FIV)

A major feature of retrovirus infection is virus persistence in the host. Lentiviruses achieve this through the integration of a reverse transcribed DNA copy of the viral RNA into the host cell chromosomal DNA, which is maintained as provirus for the life of the cell. Lentivirus persistence is also promoted by the establishment of effective mechanisms to evade the host's specific immune responses to viral antigens. The latter can be achieved by constant mutational changes in viral antigens presented to the immune system or by interference with immune regulatory mechanisms essential for the induction of an effective immune response.

Cell Tropism

HIV and FIV are both lymphotropic lentiviruses. The majority of HIV strains utilize the host cell CD4 molecule as the primary receptor. It was originally thought that FIV has a somewhat broader lymphocyte tropism than HIV as it does not utilize the CD4 receptor and has the ability to infect $CD8^+$ cells and B-cells.[28] However, HIV and SIV infection of $CD8^+$ cells and B-cells has been reported, and some strains of HIV and SIV have been demonstrated to enter target cells independent of their CD4 expression (Table I).[29]

In vivo, FIV infects $CD4^+$, $CD8^+$ T-cells, B-cells, cells of the monocyte/macrophage lineage, astrocytes, and megacaryocytes.[1,10,28,30,31] The vast majority of bone marrow cells apparently remain virus-free throughout the course of FIV infection. By in situ hybridization, FIV RNA was detected only in limited numbers of megacaryocytes and mononuclear cells.[32,33] For both FIV and HIV, only low numbers of circulating monocytes have been found

TABLE I
Main Cell Tropism and Chemokine Receptor Usage

Cell Tropism	HIV	SIV	FIV
$CD4^+$ T-cells	+	+	+
$CD8^+$ T-cells	(+)	(+)	+
B-cells	−	−	+
Monocytes/macrophages	+	+	+
Astrocytes/Glia cells	+	+	+
Chemokine receptor[a]			
CCR5	+	+	−
CXCR4	+	−	+

[a] Belong to the seven-transmembrane domain chemokine receptor family

infected.[28,34] Both viruses, however, are prevalent in tissue macrophages and follicular dendritic cells.[33,35]

The principal in vivo target of FIV early after infection is $CD4^+$ T-cells, which carry the highest proviral burden.[28] As the infection progresses to the asymptomatic phase the provirus burden shifts from the $CD4^+$ cells to the $CD21^+$ B-cells and $CD8^+$ cells.[28,32,36] Several reasons may account for the shift in proviral burden within weeks after acute infection. As with HIV infection, FIV can be lytic for $CD4^+$ cells in vitro, raising the possibility that a highly susceptible population of $CD4^+$ cells are infected and rapidly lysed. Immune-mediated destruction of infected $CD4^+$ cells as a means of reducing virus burden may also occur. In support of this the decrease in $CD4^+$ cell proviral burden in FIV^+ cats correlates with the increase in activated $CD8^+$ cells.[37] $CD8^+$ cells may control virus levels by CTL-mediated FIV-infected $CD4^+$ cell lysis or by suppression of virus replication in a noncytotoxic, contact-dependent non-MHC restricted mechanism.[38–41] Whatever the reason, continued viral replication in the $CD4^+$ T-cell population may then depend on a slow renewal of these cells or on a smaller $CD4^+$ T-cell subset supportive of a nonlytic virus infection.

Differential susceptibility of $CD4^+$ cell subsets has been reported for both HIV and SIV.[42,43] Subsets of activated $CD4^+$ cells are preferentially infected by HIV-1 and SIV in vitro and the same subsets of highly differentiated, activated CD45RO expressing $CD4^+$ cells contain the majority of provirus in vivo during the entire course of the infection.[44,45] In vitro studies demonstrated that productive HIV infection is associated with a $CD4^+$ T-cell subset characterized by a partial activation phenotype, as indicated by cell surface expression of the CD25 receptor (IL-2 receptor α chain). Peripheral blood mononuclear cells (PBMC) depleted of $CD25^+$ cells are markedly diminished in their ability to replicate HIV when infected in vitro.[42,43] $CD4^+CD25^+$ cells were also shown to be susceptible to productive HIV infection in vitro, whereas highly purified resting $CD4^+CD25^-$ cells were resistant to infection.[46] Interestingly, a naturally occurring $CD25^+$ subset of $CD4^+$ T-cells (T regulatory cells) with unique immune regulating properties has been described in humans and rodents.[47,48] While the $CD4^+CD25^+$ cells reported to support HIV replication were not identified as T regulatory cells, these observations are of interest, as the partial activated phenotype of $CD4^+CD25^+$ T regulatory cells could make it a particularly favorable target for sustained HIV infection. Studies in our laboratory demonstrated that both $CD4^+CD25^+$ and $CD4^+CD25^-$ cells are susceptible to FIV infection in vitro and in vivo, but only $CD4^+CD25^+$ cells replicate the virus in the absence of mitogenic stimulation. In contrast to $CD4^+CD25^-$ cells, $CD4^+CD25^+$ cells, whether or not infected with FIV, do not proliferate in response to Concanavalin A (Con A) stimulation and are relatively resistant to activation-induced programmed cell death. These observations plus the fact that $CD4^+CD25^+$ cells are partially activated and yet are anergic

and incapable of progressing through the G1 cell cycle suggest that they could represent a long-lived reservoir of productive FIV infection.[49]

Receptor Usage

Despite the similar cell tropism to HIV, there is no evidence that FIV uses the CD4 molecule as a cellular receptor.[50,51] FIV infects a range of $CD4^-$ cell lines, including monocyte/macrophages,[30,52,53] which in cats do not express CD4 and cells of neuronal lineage.[10] CD4 expression on cells also does not correlate with susceptibility to in vitro infection with FIV.[54] It is intriguing that FIV infection induces $CD4^+$ T-cell associated AIDS-like disease in cats and yet does not share a common primary cellular receptor with HIV.

Binding of HIV to the primary receptor induces a conformational change in the envelope glycoprotein that enables interaction with a chemokine coreceptor. Following a second conformational change, the viral envelope glycoprotein fuses with the cellular membrane of the target cell allowing virus entry into cells.[55,56] HIV-1 coreceptors belong to the seven-transmembrane domain chemokine receptor family, with CCR5 and CXCR4 being the main receptors for macrophage and T-cell tropic isolates, respectively (Table I).[57–59] The expression of CCR5 on human peripheral blood T-cells is largely restricted to $CD4^+CD45RO^+$ (memory) cells, while CXCR4 is expressed on B-cells and on both $CD4^+CD45RA^+$ (naïve) and memory T-cells.[60] CCR5-dependent viruses are detected early in HIV infection. In about 50% of infected individuals progression of the disease coincides with the emergence of CXCR4-dependent viruses. The differential expression of CXCR4 and CCR5 on cells of the immune system may provide a basis for the specific targeting of memory T-cells early in infection and the additional cell types targeted with the emergence of CXCR4-dependent strains in the later stages of infection.

FIV strains of clade A and tissue culture adapted strains of clade A and B have been shown to utilize CXCR4 as a receptor for envelope-mediated fusion.[61–63] The second extracellular loop of CXCR4 acts as the primary determinant of usage by the FIV envelope V3 to V5 region,[64,65] and two CXCR4 ligands, stromal cell derived factor 1 alpha (SDF1∀) and the bicyclam AMD3100 are able to inhibit FIV infection.[62,63,66] In addition, the feline CXCR4 displays 94.9% amino acid homology with the human counterpart and FIV is able to utilize both species of CXCR4 for cell infection and syncytia formation. It is unknown whether CXCR4 acts as a primary receptor for some strains of FIV. Certain primate lentivirus strains can utilize CXCR4 independently of the primary receptor CD4.[29] In contrast to the chemokine distribution in the human immune system, the expression of feline CXCR4 in vivo is mainly restricted to monocytes and B-cells and found to be unaltered after FIV infection.[67] CXCR4 expression has also

been reported on freshly isolated thymocytes and on a proportion of T-cells in the lymph nodes. In vitro, expression of CXCR4 was shown to be upregulated on mitogen-activated feline T-cells.[62] If CXCR4 is also upregulated upon T-cell activation in vivo, FIV might selectively target activated $CD4^+$ cells in early stages of the infection, as has been reported.[28,67] In support of this, FIV has been shown to productively infect partially activated CD25 expressing $CD4^+$ T-cells in vitro.[49]

It is, however, likely that FIV also uses alternate cellular receptors, in agreement with the differential receptor usage by HIV strains.[57] Consistent with this, a number of CXCR4+ cell lines are resistant to infection by primary isolates of FIV, and viral infection of T-cells is not consistently inhibited by natural ligands for CXCR4.[68,69] In addition, RANTES, the natural ligand for CCR5, inhibits FIV infection of feline PBMC and antibodies against CCR5 or CCR3 reduce FIV-dependent fusion of human PBMC.[68,70] Feline cell surface molecules can also function as coreceptors for HIV and SIV infection, as HIV-2 and SIV can infect feline cells expressing the human CD4 molecule.[71] These results suggest a shared chemokine receptor usage between primate and feline lentiviruses and a close evolutionary link of entry mechanisms between FIV and HIV. In addition to chemokine receptors, cell surface heparans have been shown to contribute to the envelope glycoprotein binding of cell culture adapted strains of FIV,[69] similar to that described for HIV.[72] FIV envelope binding to primary T-cells that cannot be inhibited by RANTES, SDF-1α, macrophage inflammatory protein 1∃, or heparan also suggests the usage of additional cellular receptor(s) for some FIV strains.[69]

Antibody and complement binding has been shown to enhance susceptibility of B-cells for HIV infection.[73] An indirect antibody and complement-dependent mechanism of viral entry into B-cells might also occur in FIV+ cats. This is supported by the fact that proviral burden in B-cells increases dramatically as the anti-FIV antibody response develops and would explain why B-cells are not susceptible to FIV infection in vitro.[28,74]

Viral Clades

Phylogenetic analysis of the nucleotide sequences encompassing the gag and envelope variable (V) 3 to 5 regions revealed that the degree of divergence among FIV isolates from different places is a function of the geographical distance and segregation.[75–79] Five distinct clades (A-E) of FIV have been identified based on greater than 15 to 20% variability in envelope amino acid sequences. The majority of viruses identified to date belong to clade A or clade B.[75–77,80] While both virus groups are distributed worldwide, clade A viruses have been found to predominate in the western United States, northern Japan, Germany, and South Africa, whereas clade B viruses predominate in eastern Japan, Italy, Portugal, and the central and

eastern United States.[75,77] Clade B viruses are significantly more diverse than clade A and can be distinguished by three evolutionary subgroups.[77] Clade C viruses are common in northern Taiwan, Japan, and Vietnam.[77,81–83] Several clade D viruses have been characterized from Japan and Vietnam. Two Argentine strains represent subtype E viruses.[84]

It is currently not known whether infections with viruses from different clades or superinfections with viruses from different clades are associated with an accelerated disease progression. Experimental studies, however, suggest that FIV isolates from different clades differ in their pathogenicity, tissue cell tropism, and clinical disease.[85–87] In one study, peak plasma viremia and tissue provirus burden were significantly greater in cats infected with clade B virus (FIV-2542) compared to those infected with a clade A virus (FIV-PPR).[13] In another study comparing clade C (FIV-CPGammar) and clade A (FIV-Petaluma) viruses, greater plasma viremia, PBMC proviral burden, tissue viral burden, and histologic lesions were detected in the clade C virus infected cats.[88] Further studies are necessary to determine whether differences in the pathogenicity described for these clade B and clade C viruses can be extrapolated to other members of the clades.

4. IMMUNE RESPONSE TO FIV

The hallmarks of FIV infection, as with HIV infection, is the progressive loss of $CD4^+$ T-cell numbers and immune function. The immune defects in FIV+ cats are characterized by decreased responses to antigen and mitogen stimulation, reduced antibody responses to a variety of antigens, and increased susceptibility to secondary pathogen. As FIV induces a similar disease state as HIV, despite using an alternative cellular receptor and having a broader cell tropism, understanding the immunopathogenesis of FIV is of prime importance to elucidate the mechanism(s) of $CD4^+$ T-cell depletion and the pathogenesis of lentivirus-induced immunodeficiency.

Humoral Immune Response

B-cell numbers do not differ significantly from normal in either naturally or experimentally FIV+ cats.[17,27,89,90] Most naturally and experimentally infected cats develop a polyclonal gammopathy (mainly IgG) directed against nonviral proteins that can be detected as early as 6 weeks post-infection.[89,91,92] The hypergammaglobulinemia correlates histologically with the activation of the B-cell compartment in lymphoid organs.[25]

Antibodies directed against FIV can be detected in serum of experimentally infected animals by ELISA or western blot as early as 2 weeks

postinfection and typically persist throughout the course of the disease.[12,93] Antibody responses against Gag and Env usually develop at approximately the same time and tend to remain high throughout the lifespan of the infected animal.[94,95] Using recombinant, partially overlapping peptides, immunodominant domains have been localized within the surface and transmembrane glycoproteins of FIV. Within the surface glycoprotein, an immunodominant region has been identified within the V3 region, which shares both structural and functional homology with the V3 domain of HIV.[96–99] Other immunodominant domains have been localized to the carboxy terminus of the surface and transmembrane glycoprotein, as well as to at least four different epitopes on the Gag protein.[100–102] In addition, the ectodomain of the FIV transmembrane glycoprotein contains an immunodominant domain, also termed principal immunodominant domain, analogous to that found in other lentiviruses.[98] A pentapeptide comprised between two cysteine residues represents a linear B-cell epitope, which is recognized by 100% of sera from FIV+ cats.[100] Characterization of this domain revealed that chimeric FIV envelope protein containing the principal immunodominant domain of HIV is correctly processed and is capable of inducing syncytia in feline cells, whereas chimeric FIV envelope proteins containing the similar domain of maedi-visna virus (MVV) or equine infectious anaemia virus (EIAV) are nonfunctional. Computer modeling of this domain in FIV, HIV, MVV, and EIAV suggested a conservation of structure between FIV and HIV, whereas there is little similarity in structure between HIV and MVV or EIAV.[98]

Neutralizing Antibodies

The V3 region is an important target for virus-neutralizing antibodies. Synthetic peptides consistent with this region have been found to inhibit FIV-neutralizing activity of pooled immune cat sera, and cat sera raised against these peptides effectively neutralized FIV infectivity for Crandell feline kidney (CrFK) cells.[96] Residual neutralizing activity in the absorbed sera suggests that other nonlinear determinants in the viral envelope are also involved in neutralization. The involvement of the V4 and V5 regions of the surface glycoprotein in virus neutralization has been shown as single amino acid changes in these regions conferred resistance to virus neutralization.[103,104] These data are supported by findings that the V3 to V5 region of the FIV envelope interacts with the second extracellular loop of CXCR4 to mediate fusion.[64,65] It is of interest to note that neutralizing epitopes and also at least in part the immunodominant regions on the viral envelope coincide with genetically highly variable regions.

Although FIV infection induces a strong neutralizing antibody response, the role of these antibodies in controlling virus infection is controversial. The presence of neutralizing antibodies does not correlate with

virus clearance or disease progression in FIV$^+$ cats, as virus neutralizing titers are similar in asymptomatic and symptomatic FIV$^+$ cats.[105,106] Also, sera that exhibited elevated titers of highly efficient, cross-reactive neutralizing activity when assayed on CrFK cells exerted a much lower neutralizing effect when assayed in PBMC, thymocytes or T-cell lines.[103,106,107] In contrast, the activity of neutralizing antibodies in vivo has been shown in adoptive transfer experiments, as sera from vaccinated cats effectively protect cats against subsequent challenge with the homologous strain of FIV.[108,109] Cats without detectable antibody production are also reported to progress more rapidly to terminal immunodeficiency.[110,111]

Cellular Immunity to FIV

CD4$^+$ T-Cells

In healthy domestic cats, CD4$^+$ cells represent approximately 66% of the T-cells in circulation, whereas CD8$^+$ cells represent a much smaller proportion, yielding normal CD4:CD8 ratios between 1.5 and 3 (Figure 2A).[17,27,90] In cats, similar to the situation in humans, CD4$^+$ cells are the main source of IL-2,[112] which explains the "help" that CD8$^+$ cells must receive from CD4$^+$ cells in mounting an immune response. CD4$^+$ T-helper function is manifested by IL-2-promoted expansion and differentiation of natural killer (NK) cells and antigen-specific CD8$^+$ cells into effector cells. As such, antigen-specific CD4$^+$ T-cell responses are essential for controlling viral infection, including HIV and FIV infections.

The rapid expansion of activated CD8$^+$ cells during the acute phase of HIV and FIV infection suggests that in the initial immune response, CD4$^+$ cells are able to provide the necessary T-cell help. Studies suggest, however, that even at the acute stage of infection HIV-1 specific CD4$^+$ immune responses are not sustained as there is a relative absence of CD4$^+$ cell proliferative responses to HIV antigens despite normal CD4$^+$ cell responses to other antigens.[113–115] During the chronic phase of infection, HIV-specific CD4$^+$ T-cell responses in the peripheral blood of HIV$^+$ individuals, in particular those to the envelope, are also often difficult to detect.[116] In patients undergoing continuous highly active antiretroviral therapy (HAART) reconstitution of functional antigen-specific CD4$^+$ T-cell responses to HIV and several other antigens has been observed.[117–119] The restoration of antigen-specific CD4$^+$ T-cell responses in patients undergoing HAART, however, does not always correlate with a significant decrease in plasma viremia.[118]

Activation of CD4$^+$ T-cells has been documented in both FIV and HIV infection as CD4$^+$ cells show an upregulated expression of B7.1 and B7.2 costimulatory molecules.[120–123] Additional evidence of chronic and pro-

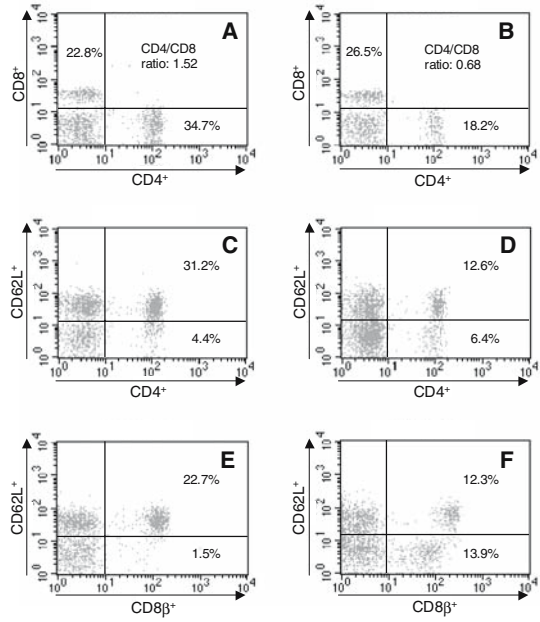

FIGURE 2. FIV infection results in decreased $CD4^+/CD8^+$ cell ratios and activation of T-cell subsets. Flow cytometric analysis of feline $CD4^+$ and $CD8^+$ cells in the peripheral blood derived from control (A, C, E) and FIV^+ cats (B, D, F) are shown. In uninfected animals, the number of $CD4^+$ cells exceeds the number of $CD8^+$ cells (A), whereas in FIV^+ cats the decreased $CD4^+$ cell numbers result in an inverted CD4/CD8 cell ratio (B). Numbers in the upper and lower quadrants represent percentage of positive cells of the total $CD45^+$ lymphocyte population of representative blood samples. Two-color flow cytometric analysis reveals a loss of CD62L expression on $CD4^+$ and $CD8^+$ cells in FIV^+ cats (D, F) as compared to control animals (C, E). Note that the loss of CD62L expression on $CD8^+$ cells is restricted to the $CD8\alpha^+\beta^{low}$ cell population. Numbers in the upper and lower right quadrants represent percentage of positive cells of representative blood samples. The increase in the percentage of $CD4^+CD62L^-$ as well as $CD8^+CD62L^-$ cells progresses throughout the course of FIV infection.

gressive T-cell activation in FIV^+ cats and HIV^+ patients comes from studies investigating the expression of L-selectin (CD62L), a surface marker that is lost on activated T-cells. Analysis of CD62L expression on $CD4^+$ cells in FIV^+ cats demonstrated that with time after infection the percentage of $CD4^+CD62L^-$ cells increase such that the $CD4^+CD62L^-$ cell population represents about 80% of the total $CD4^+$ cells in long-term (>7 years) infected cats (Figure 2C and D).[41] In other words, 80% of the circulating $CD4^+$ as well as $CD8^+$ T-cells (Figure 2E and F) in the blood of chronic, long-term FIV^+ cats have an activation phenotype. A similar phenotype change has been reported for $CD4^+$ cells of HIV^+ patients.[124,125]

$CD8^+$ T-Cells

FIV and HIV infection results in increased total CD8 cell counts in the peripheral blood, which can usually be detected early after infection, remain high throughout the long asymptomatic phase of infection, and decrease only late in the infection prior to the development of AIDS-like disease symptoms.[27,89,126] In both infections, the CD8 lymphocytosis correlates with the emergence of $CD8^+$ cells that provide antiviral immunity through cytotoxic and virus-suppressive mechanisms.[38,40,41,127–129]

Cytotoxic T Lymphocytes (CTL). CTL responses in the peripheral blood of FIV^+ cats can be detected as early as 2 weeks postinfection,[130] which is similar to the very early detection of virus-specific CTL activity in SIV^+ macaques.[131] Early detection of CTL activities corresponds with a reduction in viremia and transition to the asymptomatic phase.[128,130] In chronically FIV^+ cats, CTL responses against Gag, Pol, and Env epitopes are also detectable in the lymph nodes and spleen.[128,129,132] As the lymph node is a known site of virus sequestration and replication following HIV infection, and CTL responses in HIV^+ patients are associated with the maintenance of a low viral burden in the asymptomatic phase of long-term progressors,[133] the demonstration of CTL at these sites in FIV^+ cats may reflect the attempts of the host immune system to control virus replication.[134] In support of this, vaccine-induced protection against FIV challenge has been associated with CTL responses directed against the Env protein.[135]

Noncytolytic Antiviral Activity. In addition to the virus-specific CTL responses, FIV and HIV infection induces a population of nonantigen specific $CD8^+$ effector cells that suppress virus replication in $CD4^+$ cells by a noncytotoxic, contact-dependent, non-MHC restricted mechanism.[38–41] $CD8^+$ effector cells that mediate immunity through cytolytic or noncytolytic suppressor mechanisms can be divided by differential expression of surface markers. Early detection of noncytolytic $CD8^+$ effector cells in FIV^+ cats is associated with reduced expression of the CD8 β chain and increased expression of MHC class II molecules.[14,37,40,41] As reported for HIV^+ patients,[125] FIV-induced $CD8\alpha^+\beta^{lo}$ cells are also characterized by a progressive downregulation of CD62L (Figure 2E, F) resulting in more than 70% CD62L negative cells of the total $CD8\beta^+$ population in the peripheral blood of long-term (>7 years) infected cats.[41] The appearance of these $CD8\alpha^+\beta^{lo}$ cells correlates with the acute stage of infection and the rapid decline in viremia.[38,41,136] In the case of FIV infection, activated $CD8\alpha^+\beta^{lo}CD62L^-$ cells possess strong anti-FIV activity[40,41] and are capable of controlling FIV expression in vivo and in ex vivo cultured PBMC, suggesting that they may play a major role in maintaining the low plasma viremia in cats during the asymptomatic stage of infection.[40,137,138] Further phenotypic characterization of the $CD8^+$ cell population in FIV^+ cats revealed a profound and sustained loss of the naïve $CD8\alpha^+\beta^{hi}CD62L^+CD44^{lo}CD49d^{lo}CD18^{lo}$ phenotype

with a concurrent expansion of an $CD8\alpha^{+}\beta^{lo}CD62L^{-}CD44^{hi}CD49d^{hi}CD18^{hi}$ activation phenotype in the circulation.[41] Similar to FIV infection, in patients with long-term HIV infection, activated $CD8^{+}CD11a^{hi}$ cells, including a major subpopulation of $CD8^{+}CD62L^{-}CD11a^{hi}$ cells, represent approximately 80% of the total $CD8^{+}$ T-cells in the circulation.[124,125] The loss of CD62L and increase in the adhesion molecule (CD44) and integrin (CD49d, CD18) on the surface of $CD8^{+}$ cells is indicative of T-cell activation and therefore suggests that naïve CD8 cells are largely replaced by activated $CD8^{+}$ cells during the course of FIV and HIV infection.

Additional evidence of chronic and progressive $CD8^{+}$ T-cell activation in HIV^{+} patients and FIV^{+} cats comes from the analysis of B7 costimulatory molecules and CTLA4 expression on these cells. Similar to what is seen on $CD4^{+}$ cells, the $CD8^{+}B7^{+}$ phenotype represents approximately 80% of the total $CD8^{+}$ cells in long-term (>7 years) infected cats, and B7 expression has been shown to be largely confined to the $CD8\alpha^{+}\beta^{lo}$ cell population.[122] The role of these activated $CD8^{+}$ T-cells in controlling FIV or HIV infection in vivo is unknown, although Bucci et al.[136] has shown by $CD8^{+}$ cell depletion studies that they have a potent suppressive activity on FIV replication in $CD4^{+}$ cells cultured ex vivo.

5. IMMUNOPATHOGENESIS OF FIV INFECTION

The decline in the number of $CD4^{+}$ T-cells and the inversion of the $CD4^{+}/CD8^{+}$ cell ratio is seen in naturally and experimentally FIV^{+} cats regardless of the route of infection (Figure 2A and B).[12,27,36,110,136] The onset of $CD4^{+}$ T-cell depletion usually coincides with the peak plasma viremia during the acute stage of infection,[139] and after a partial rebound progresses throughout the asymptomatic and symptomatic phases of the infection. Several mechanisms of $CD4^{+}$ cell loss have been proposed. FIV is lytic for $CD4^{+}$ cells in vitro, suggesting that this may occur in vivo as well. Immune-mediated destruction of infected cells may also occur. $CD8^{+}$ CTL and NK cells capable of lysing virus-infected cells have been demonstrated in both FIV and HIV infection.[38,128,129] Due to the high viral load during the acute phase of infection, lytic infections of target cells might at this stage of infection contribute predominantly to the $CD4^{+}$ cell decline. However, as the infection progresses, the percentage of infected cells among the total lymphocyte population is relatively low, indicating that mechanisms other than lysis of infected cells may be involved in the continued $CD4^{+}$ T-cell loss. Whatever the mechanism(s) responsible for $CD4^{+}$ T-cell loss with disease progression, increased secondary infection is a manifestation of this immune dysfunction.

The types of secondary infections seen in HIV and FIV infections are those that are controlled by a functional cell-mediated immune (CMI)

response. While the development of these secondary infections can be attributed to the decline in $CD4^+$ cell numbers and the resulting decrease in cytokines (IL-2, IFN-γ) required for a successful CMI response, numerous studies indicate that the immune dysfunction develops before a significant decrease in $CD4^+$ cell numbers. Clerici et al.[5] described a sequential loss of immune functions in HIV infections beginning with an inability to respond to recall antigens and progressing to lack of response to human leukocyte antigen (HLA) alloantigens and finally to phytohemagglutinin (PHA) stimulation. These defects occurred in many HIV^+ individuals prior to $CD4^+$ cell loss. Similar immune dysfunctions have been described in FIV infection.[17,140] In our laboratory we developed a *Toxoplasma gondii*-FIV coinfection model that allowed us to examine FIV-induced dysfunctions in the CMI response. Normal cats challenged with *T. gondii* develop a transient chorioretinitis that resolves 3 to 4 weeks post-challenge.[141,142] However, as early as 16 weeks after infection with FIV, FIV^+ cats challenged with *T. gondii* develop severe interstitial pneumonia resulting in 50% to 75% mortality.[141,143] This suggests that severe immune dysfunction develops early after lentivirus infection. Several mechanisms responsible for this immune dysfunction have been proposed including cytokine deregulation,[144] defective or altered antigen presentation,[145,146] and inappropriate activation of immune regulatory cells.[147] The remainder of this chapter will address these proposed mechanisms of HIV/FIV immunopathogenesis.

Cytokine Alterations

There have been numerous studies evaluating cytokine levels in FIV infections, suggesting that the perturbations in cytokine production observed in the human immune system following HIV infection are paralleled in FIV^+ cats. Clerici et al.[5] reported that PBMC from HIV^+ individuals had decreased proliferation and IL-2 production in response to recall antigens as compared to seronegative controls. This nonresponsiveness has important consequences, as IL-2 has been shown to determine both the magnitude of a primary cellular immune response, as well as the development of memory cells. In contrast to many other cytokines, IL-2 is not redundant with regards to its role in the generation of a normal immune response and other cytokines or surface ligands cannot substitute for IL-2. In contrast to the lack of IL-2, serum levels of tumor necrosis factor-alpha (TNF-α) are found elevated in all AIDS patients, and approximately 50% of those with ARC, but none in the asymptomatic phase of HIV infection.[148] Lawrence et al.[140] reported similar results (decreased IL-2 and increased TNF-α levels) in FIV^+ cats early after infection. Elevated levels of IL-6 have been reported in the serum of both HIV^+ patients[149,150] and FIV^+ cats,[140] and

both infections have been shown to stimulate production of IL-6 by macrophages.[53,151] In FIV$^+$ cats, peak production of the proinflammatory cytokines IL-6, IL-1, and TNF-α by PBMC was found to coincide with periods of depressed immune responses as evidenced by a concurrent depressed responsiveness of PBMC to mitogen stimulation and the presence of clinical signs.[152] With the division of CD4$^+$ cells into two subsets based on cytokine production, Clerici and Shearer[144] speculated that HIV infection affects these subsets differently, causing a switch from a T-helper-1 (TH1; IL-2 and IFN-γ producers) to a T-helper-2 (TH2; IL-4, IL-5, IL-6, and IL-10 producers) type response to specific antigens. However, further studies did not support a clear TH1 to TH2 switch. Elevated levels of IL-10 and IFN-γ in PBMC and lymph nodes of HIV$^+$ patients have been described by Graziosi et al.[153] and Than et al.[154] The latter also reported increased expression of TNF-α and decreased IL-12 mRNA, whereas IL-2 and IL-4 mRNA were undetectable in both patients and controls. Others have also described decreased IL-12 p40 and p35 synthesis in HIV$^+$ patients.[155] However, decreases in IFN-γ production have also been reported in HIV$^+$ individuals,[156,157] while IL-4 has been reported to increase.[157,158] It is not surprising that the precise changes in cytokine expression leading to HIV-associated immune deficiency, and how such changes may impact immune responses to other antigens, remain controversial. The heterogeneity of the patient populations, including virus burden, disease progression, and exposure to other pathogens, makes meaningful comparisons difficult. Some of these issues have been addressed in FIV$^+$ cats.

FIV infection has provided a powerful animal model to examine lentivirus-induced cytokine dysfunctions, allowing for analysis of constitutive cytokine levels prior to and after FIV infection, as wells as cytokine responses following challenge with secondary pathogens. Using an FIV-*T. gondii* coinfection model, we were able to analyze constitutive cytokine production by lymph node cells from normal and FIV$^+$ cats before and after challenge with *T. gondii*.[159,160] FIV infection caused a marked increase in constitutive IFN-γ, TNF-α, and IL-10 mRNA expression in the lymph nodes during the acute stage infection. Challenge of control cats with *T. gondii* induced an increase in IL-2, IFN-γ, and IL-12 (p40) in lymph nodes, indicative of a normal CMI response, whereas IFN-γ and IL-10, but not IL-2 or IL-12, were increased in lymph nodes of FIV-*T. gondii* coinfected cats. Dean and colleagues[161,162] reported similar findings using an FIV-*Listeria monocytogenes* coinfection model. In both cases, prior to secondary challenge, the levels of IL-10 with respect to IL-12 in the FIV$^+$ cats were greatly elevated compared to negative cats. These data suggest that FIV-induced immunodeficiency may be derived from a failure to generate an IL-12 dependent response, in part due to elevated levels of IL-10, a cytokine known to suppress IL-12 production by dendritic cells.

Altered Antigen Presentation

A number of studies have examined the possibility that the $CD4^+$ T-cell immunodeficiency in HIV^+ patients is the result of inadequate or inappropriate antigen presentation. Defective antigen presentation can lead to T-cell anergy, which is characterized by decreased or no IL-2 production following T-cell receptor (TCR) stimulation. Studies have shown that anergy is more easily induced in IL-2/IFN-γ secreting $CD4^+$ T-cells than in IL-4 secreting $CD4^+$ T-cells.[163,164] The unresponsiveness of $CD4^+$ T-cells in HIV infections resembles anergy, and it has been suggested that it is the result of defective antigen presentation by antigen presenting cells (APC).[145,146] Mechanisms proposed for defective APC function include loss of dendritic cells due to direct virus infection,[165] decreased expression of accessory molecules such as MHC class II or B7 on APC,[145,165,166] and decreased cytokine or cytokine-dependent signaling.[145]

Inadequate APC costimulation is consistent with the observation that T-cells from asymptomatic HIV^+ patients and FIV^+ cats fail to produce IL-2 and proliferate after antigenic stimulation. The lack of IL-2 in the stimulated cells results in anergy and progression to activation-induced programmed cell death or apoptosis,[167,168] leading to the speculation that loss of T-cell function and subsequent immune deficiency in HIV^+ patients and FIV^+ cats is the result of anergy/apoptosis. This speculation is supported by the findings of abnormally high frequencies of apoptotic cells in the lymph nodes of HIV^+ individuals and FIV^+ cats.[169–172]

T-Cell-Mediated Immunopathology

Recent data suggest that the $CD4^+$ T-cell immune hyporesponsiveness in FIV^+ and HIV^+ patients may be unrelated to APC but result from unique immunosuppressive properties of virus activated T-cells. These potentially immunosuppressive T-cells fall into two categories: the activated $B7^+CTLA4^+$ T-cells that are part of the normal T-helper cell immunoregulatory response; and the $CD4^+CD25^+$ T-regulatory cells, a distinct population of $CD4^+$ T-cells with negative immunoregulatory properties.

$B7^+CTLA4^+$ T-Cell-Induced Anergy and Apoptosis

There is recent evidence that HIV and FIV infections may cause immunopathology by disrupting the normal process of activation-induced programmed cell death (apoptosis) that controls the magnitude and duration of the T-cell immune response. Apoptosis regulates the cellular repertoire throughout the life of an organism and is initiated by engagement of specific cell surface receptors with their ligands. The best described examples of receptor interactions that transduce intracellular signals mediating

cell death are between proteins that belong to the TNF family (e.g., FasL with Fas, TNF-α with TNF-α receptor, Apo2L with TRAIL), and interactions between the B7 costimulatory molecules and CTL antigen 4 (CTLA4). Apoptosis induced by proteins of the TNF family induce caspase-mediated cell death in many tissues, whereas the B7-CTLA4 apoptotic pathway induces T-lineage-specific cell death via the suppression of IL-2, resulting in antigen-specific anergy or clonal deletion of previously activated T-cells.[173,174] The inability of $CD4^+$ cells from HIV^+ individuals and FIV^+ cats to produce IL-2 and proliferate in response to recall antigens suggests that the increased apoptosis could be due to B7-CTLA4 interactions.

B7.1 and B7.2 costimulation receptors are normally found on professional APCs, such as dendritic cells, activated monocytes, and B-cells.[175] These molecules interact with CD28 and CTLA4 on T-cells, providing necessary second signals to either stimulate IL-2 synthesis (B7-CD28) thus promoting an immune response or suppression of IL-2 production (B7-CTLA4) and terminating the immune response. CD28 is constitutively expressed on T-cells, whereas CTLA4 is expressed only after T-cells have been activated, 2 to 3 days after peptide-TCR engagement. As the binding avidity of the B7 molecules for CTLA4 is 50–100 times greater than for CD28, negative signaling would dominate over activation on cells expressing both CD28 and CTLA4. While B7.1 and B7.2 are normally found on professional APCs, a number of studies have demonstrated that these molecules are upregulated on activated T-cells in vitro[176,177] and in vivo in situations of chronic antigenic stimulation such as autoimmune disease[178,179] and HIV infection.[121] Our laboratory has recently reported an increase in the percentage of $CD4^+$ and $CD8^+$ T-cells that express B7.1 and B7.2 molecules in FIV^+ cats compared to uninfected cats.[122] The numbers of B7 positive $CD4^+$ and $CD8^+$ T-cells increase with disease progression such that the majority of T-cells (≥75%) in the lymph nodes express B7 molecules in long-term (>7 years) infected cats. Interestingly, the majority of $B7^+CD4^+$ and $B7^+CD8^+$ T-cells in FIV^+ cats also express CTLA4, and we have been able to correlate the expression of these molecules to spontaneous T-cell apoptosis in vitro[180] and in LN and PBMC of FIV^+ cats in vivo.[122] Further, in a more recent study, we have found that T-cell apoptosis in the PBMC of HIV^+ individuals is associated with $CD4^+$ T-cells coexpressing B7 and CTLA4.[123] As ligation of CTLA4 by B7 transduces a signal for downregulation of IL-2 and induction of anergy and apoptosis, we speculated that persistent antigenic stimulation, as seen in HIV and FIV infection, chronically induces $B7.1/2^+CTLA4^+$ T-cells capable of T-T interactions that result in T-cell anergy and apoptosis. Such a mechanism could explain the progressive loss of T-cell immune function and increase in lymph node T-cell apoptosis, which is the hallmark of FIV and HIV infections.

This speculation is supported by the observation that T-cells activated in vitro and shown to express B7 are capable of antigen presentation and

can engage the TCR on other activated T-cells in an antigen specific manner.[181] However, in contrast to professional APC, evidence suggests that T-T cell antigen presentation does not costimulate IL-2 production and proliferation, but primes T-cells for anergy and apoptosis.[182,183] Why T-T cell antigen presentation favors anergy over costimulation is not known. Greenfield et al.[183] demonstrated that while B7.1 expressed on T-cells was capable of binding both CD28 and CTLA4 on costimulated T-cells, B7.2 was capable of binding CTLA4 but not CD28, leading to T-cell anergy. As we have also documented increased expression of CTLA4 on T-cells from FIV$^+$ cats, one can envision activated B7 expressing CD4$^+$ cells being able to present antigen to other T-cells expressing CTLA4, resulting in anergy or apoptosis. The proposed mechanism for T-T antigen presentation leading to anergy and apoptosis is illustrated in Figure 3. As not all anergic cells progress to apoptosis, such a mechanism could explain the decreased

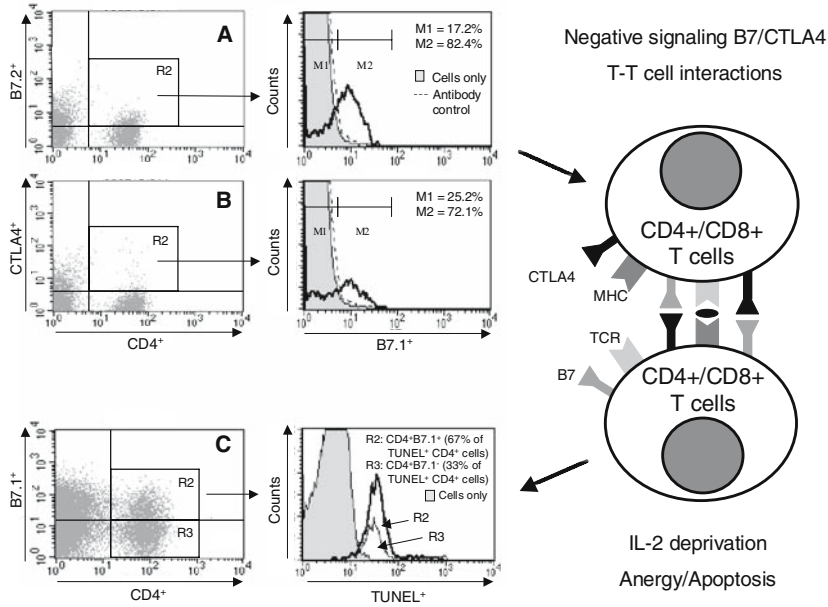

FIGURE 3. Coexpression of B7 and CTLA4 molecules on feline CD4$^+$ and CD8$^+$ cells correlates with the induction of apoptosis. Three-color flow cytometric analysis demonstrating colocalization of B7.1 costimulatory molecules on B7.2 and CTLA4 expressing CD4$^+$ cells. The majority of B7.2$^+$ (A) or CTLA4$^+$ (B) CD4$^+$ cells coexpress B7.1. The phenotype of B7.1$^+$B7.2$^+$CTLA4$^+$ CD4$^+$ cells enables T-T-cell interactions mediated by B7 receptors and CTLA4 capable of bidirectional signaling of anergy or apoptosis. Consistent with this, three-color flow cytometric analysis of apoptotic cells reveals that the majority of TUNEL$^+$ cells are among the B7.1$^+$ phenotype of CD4$^+$ cells (C). Similar up-regulation of costimulatory molecules, coexpression and correlation between B7$^+$CTLA4$^+$ cell phenotypes and apoptosis are also present on CD8$^+$ cells (not shown).

IL-2 production and immune unresponsiveness prior to $CD4^+$ cell loss. Moreover, the T-T cell interactions may not need to be antigen specific in their immunosuppressive function. Bienzle et al.[184] reported that HIV-specific CTL exerted a cytotoxic effect against uninfected, activated $CD4^+$ cells but not against nonactivated $CD4^+$ cells. This effector function was restricted to $CD8^+$ cells from HIV^+ individuals, not antigen specific or MHC-restricted, and killed uninfected $CD4^+$ cells via apoptosis as opposed to a normal CTL-mediated perforin mechanism. The studies of Bienzle et al.[184] are consistent with the reported loss of immunity to primary *T. gondii* infection in the FIV-*T. gondii* coinfection model. The large numbers of FIV-induced $B7^+CD8^+$ or $B7^+CD4^+$ effector cells in the lymph node could interact with activated $CD4^+$ cells responding to *T. gondii* antigens, resulting in anergy and eventual apoptosis and the inability to develop an immune response to *T. gondii*. While there is compelling evidence for a marked expansion of a unique population(s) of phenotypically and functionally activated $CD4^+$ and $CD8^+$ with disease progression in FIV/HIV infection, there is no direct evidence as yet that it plays a role in inhibiting protective T-cell responses to FIV/HIV antigen or to other unrelated pathogens. The most interesting data to date comes from reports by Vahlenkamp et al.[180] and Bull et al.[185] that anti-B7.1 or IL-2 treatment of cultured purified $CD4^+$ cells from FIV^+ cats significantly inhibits spontaneous apoptosis, as would be expected if apoptosis were the result of $B7^+$-$CTLA4^+$ mediated T-T interactions.

$CD4^+$ T Regulatory Cell-Mediated Anergy and Apoptosis

Studies in a number of experimental models have firmly established the existence of a "professional" $CD4^+$ regulatory T-cell population (Treg) that performs an important antiautoimmunity function by inhibiting the activation of autoreactive T-cells, thereby maintaining peripheral self-tolerance.[47,48] Evidence has accumulated that $CD4^+CD25^+$ Treg cells can also suppress immune responses against infectious agents, such as bacteria, fungi, viruses, and intracellular parasites,[186,187] and play a central role in regulating the T-helper-dependent immune response. The defining feature of $CD4^+CD25^+$ Treg cells in both rodents and humans is their ability, when activated through their TCR, to inhibit proliferation and induce apoptosis of other activated $CD4^+$ or $CD8^+$ T-cells in vitro. This in vitro suppression is mediated through a cell contact-dependent mechanism that transduces a signal for transcriptional down-regulation of IL-2, resulting in anergy and apoptosis.[188,189] Although activation of $CD4^+CD25^+$ cells is, with some exceptions (e.g., lipolysaccharide [LPS] and IL-2), antigen-specific, once activated, they suppress $CD4^+$ and $CD8^+$ T-cell responses in an antigen nonspecific manner.[188,190]

Phenotypic analysis of $CD4^+$ Treg cells reveal that, in addition to CD25, Treg cells express CTLA4, and when activated B7.1 and B7.2.[191,192] This

surface phenotype is reminiscent of the activated $CD4^+B7^+CTLA4^+$ cells that we have described in FIV^+ cats and HIV^+ patients that we propose may play a role in $CD4^+$ immune hyporesponsiveness in these lentivirus infections (Figure 3). $CD4^+CD25^+$ T-cells in normal and FIV^+ cats display the salient characteristics of $CD4^+$ Treg cells in humans and rodents,[47,48,187,193,194] as they constitute about 5 to 10% of the peripheral T-cell population, fail to proliferate in response to Con A stimulation (Figure 4B), and are relatively resistant to activation-induced programmed cell death (Figure 4C).[49,195] Phenotypic analysis of $CD4^+CD25^+$ T-cells from FIV^+ cats revealed that these cells also express B7.1, B7.2, and CTLA4,[195] suggesting that they were activated in vivo. Consistent with this interpretation, freshly isolated $CD4^+CD25^+$ T-cells from FIV^+ cats, but not normal cats, are able to suppress the proliferative response of Con A-stimulated autologous $CD4^+CD25^-$ T-cells (Figure 4E). This suppression was contact-dependent and correlates with the downregulation of IL-2 production,[195] providing further support that the $CD4^+CD25^+$ T-cells in FIV^+ cats are activated Treg cells capable of anergy induction.

It is possible that they are FIV antigen specific and activated by chronic antigenemia. It is also possible that they are nonspecifically activated by molecules such as LPS that has been shown to occur in vitro with murine as well as feline $CD4^+CD25^+$ Treg cells. Why Treg cells would be activated in FIV infection is not clear. However, it is easy to imagine their potential role in the immune dysfunctions associated with FIV/HIV infections. One can speculate that chronic FIV antigen presentation, and in particular aberrant antigen presentation by $CD4^+B7^+MCHII^+$ T-cells may activate Treg cells to dampen protective $CD4^+$ T-helper cell-dependent immune responses to FIV antigens. This negative immunoregulatory property of active $CD4^+$ Treg cells is well documented in a number of infectious diseases.[186,187,196,197] The activated $CD4^+CD25^+B7^+$ cells are found predominantly in the lymph node as opposed to the blood of FIV^+ animals, and it is the lymph node where we would expect immune responses to antigen to occur and where we would expect Treg cells to exert their suppressor function. It will be of much interest to further explore the possible role of FIV-activated $CD4^+CD25^+$ Treg cells on the $CD4^+$ T-helper-cell immunodeficiency in infected cats and determine if a similar mechanism contributes to human AIDS.

In addition to their potential role in immunosuppression in HIV and FIV infection, $CD4^+CD25^+$ Treg cells may contribute to the long-term virus persistence in these infections in vivo. In vitro studies have shown that activated $CD4^+$ cells expressing CD25 can be productively infected with HIV in vitro, whereas highly purified resting $CD4^+CD25^-$ cells were resistant to HIV infection.[46] The $CD4^+CD25^+$ cells reported to support HIV replication, however, were not identified as Treg cells. Recent studies in our laboratory using the FIV infection model demonstrated that both $CD4^+CD25^+$ and

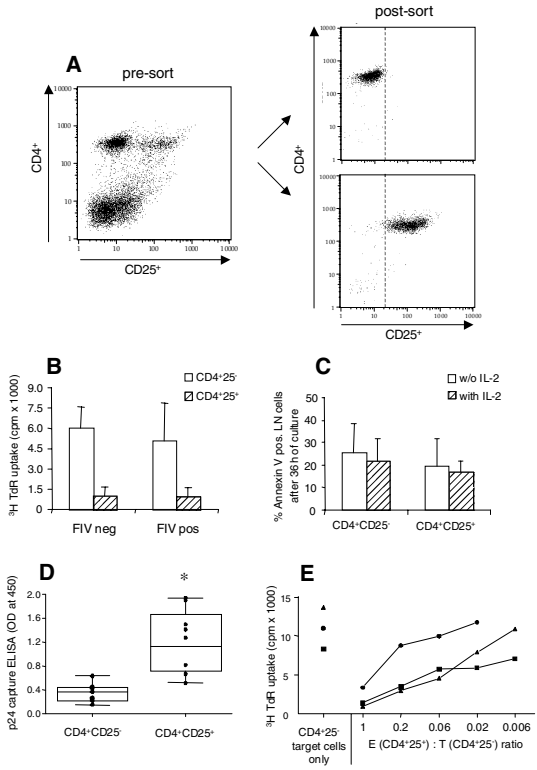

FIGURE 4. Feline $CD4^+CD25^+$ LN cells are anergic and productively infected with FIV. FIV infection results in activation of immunosuppressive function(s) of $CD4^+CD25^+$ T-regulatory cells in vivo. Lymph node (LN) cells of FIV^+ and control cats were sorted (MoFlo® cell sorter, Dako-Cytomation) into $CD4^+CD25^+$ and $CD4^+CD25^-$ cells (>97% purity) (A). Analysis of the purified cell subsets using ^3H TdR incorporation assays reveals that in contrast to $CD4^+CD25^-$ cells, $CD4^+CD25^+$ cells derived from FIV^+ and FIV^- cats are unresponsive to 2-day mitogen stimulation (Con A; 5 μg/ml) (B). As the percentage of Annexin V positive cells among the $CD4^+CD25^-$ and $CD4^+CD25^+$ cells cultured for 36h with or without IL-2 (100IU/ml) does not differ significantly (C), failure of $CD4^+CD25^+$ cells to proliferate upon mitogen stimulation indicates a state of nonresponsiveness/anergy. Coculture of FCD4E cells with in vitro unstimulated $CD4^+CD25^-$ and $CD4^+CD25^+$ cells from FIV^+ cats reveals that FIV can be rescued preferentially ($p < 0.05$) in cocultures with $CD4^+CD25^+$ cells (D), as determined by the presence of FIV-p24 in the culture supernatant. The results (mean ± standard deviation) of at least four independent experiments (B, C, D) are shown. Viral replication and the lack of increased apoptosis in these cells support the hypothesis that the partially activated, anergic $CD4^+CD25^+$ cells might serve as persistent reservoir of FIV replication in vivo. The phenotype of anergic $CD4^+CD25^+$ cells is reminiscent of immunosuppressive $CD4^+$ Treg cells. To test the ability of $CD4^+CD25^+$ cells in FIV^+ cats to suppress the proliferation of stimulated autologous $CD4^+CD25^-$ cells, we performed coculture experiments using Con A (5 μg/ml) stimulated $CD4^+CD25^-$ cells as targets and $CD4^+CD25^+$ cells as effector cells. The ^3H TdR incorporation assays reveal that the proliferation of $CD4^+CD25^-$ cells is markedly inhibited by coculture with unstimulated $CD4^+CD25^+$ cells in a dose-dependent manner as shown for three individual FIV^+ cats (E). As experiments using similar $CD4^+$ cell subpopulations derived from FIV^- cats reveal only a minor inhibition of target cell proliferation, the experiments suggest that FIV infection results in the activation of immunosuppressive $CD4^+CD25^+$ Treg cells in vivo.

$CD4^+CD25^-$ cells are susceptible to FIV infection. In contrast to $CD4^+CD25^-$ cells, which become latently infected, $CD4^+CD25^+$ cells isolated from FIV^+ cats or infected in vitro harbor a productive FIV infection (Figure 4D), yet have functional characteristics of $CD4^+CD25^+$ Treg cells.[49] Thus, $CD4^+CD25^+$ Treg cells could potentially provide a long-term, stable reservoir for FIV, as well as HIV replication. Further experiments will address the role of cellular transcription factors known to regulate FIV replication in the activation state and function of $CD4^+CD25^-$ and anergic $CD4^+CD25^+$ cells. Figure 5 depicts the proposed mechanism of $CD4^+CD25^+$ Treg

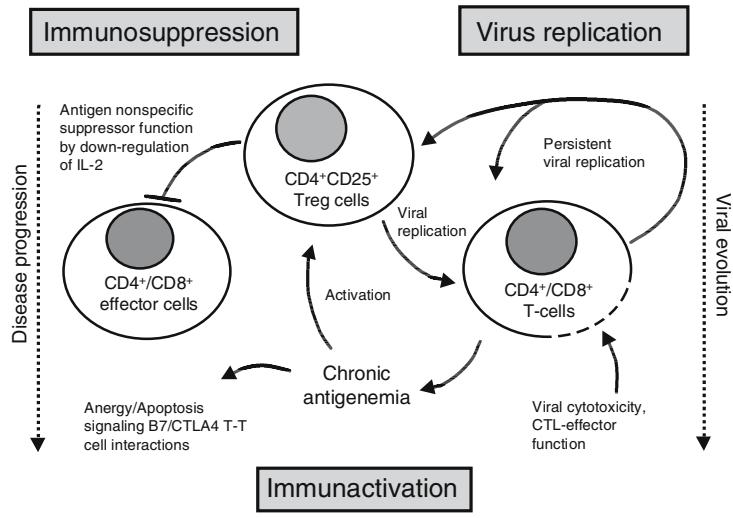

FIGURE 5. Potential role of immunosuppressive $CD4^+CD25^+$ Treg cells in the immunopathogenesis of FIV: A model for immunosuppression and persistent viral replication. FIV infection is characterized by the hallmarks of (i) immunosuppression, (ii) viral persistence and concurrent with disease progression, (iii) immunactivation. Studies in our laboratory show that $CD4^+CD25^+$ Treg cells harbor transcription factors required for lentiviral replication. Despite the fact that these cells are productively infected they are not apoptosis-prone in FIV+ cats, suggesting that these partially activated yet anergic (probably long-lived) regulatory cells might serve as a reservoir for persistent active viral replication in vivo. Infection of activated $CD4^+$ and $CD8^+$ cells supports viral replication throughout the course of the infection. Chronic viremia in infected animals could activate $CD4^+CD25^+$ Treg cells. Activated Treg cells suppress the proliferation of $CD4^+$ and $CD8^+$ effector cells in an antigen nonspecific manner by a transcriptional down-regulation of IL-2. Activated $CD4^+CD25^+$ Treg cells might therefore be responsible for the decreased responsiveness of T-cells to immune stimulation and the lack of IL-2 production detected early during the asymptomatic stage of FIV infection. With disease progression and increasing viral mutational diversity, chronic antigenic stimulation results in a continued activation of $CD4^+$ and $CD8^+$ cells as evidenced by the downregulated expression of adhesion (CD62L) molecules and by the abnormal expression of B7 costimulatory molecules, as well as their ligand CTLA4 leading to anergy/apoptosis, signaling T-T-cell interactions.

cell activation induced immunosuppression of T-cell-mediated immune responses and the possible role of $CD4^+CD25^+$ Treg cells in persistent viral replication.

6. CONCLUSION

FIV is a lentivirus of cats with a pathogenesis and clinical disease pattern that parallels HIV infection.[8,22,25,134] Cats develop an acute infection syndrome, including low-grade fever and transient generalized lymphadenopathy, followed by a long asymptomatic period in which the $CD4^+/CD8^+$ ratio declines due to an early increase in $CD8^+$ and a progressive decrease in $CD4^+$ cells.[28,37] This asymptomatic period is followed by the development of a variety of disorders, many of which mimic AIDS in humans.[8,22] Similar to HIV, the acute stage FIV infection is characterized by a high plasma viremia followed by a marked decline and persistent low-level viremia during the long asymptomatic stage of infection.[24]

The relevance of FIV as a model for HIV immunopathogenesis is underscored by the remarkable parallels in $CD4^+$ and $CD8^+$ phenotypic and functional changes during disease progression. Both infections are characterized by early and long-term changes in cytokine expression (e.g., elevation in IFN-γ and IL-10) and $CD4^+$ T-cell anergy and apoptosis. While the molecular mechanism(s) leading to immunodeficiency and AIDS is not known, recent new studies in the FIV animal model of AIDS suggest that anergy and/or clonal deletion of other antigen activated T-cells may be involved. Whatever, the mechanism(s) leading to $CD4^+$ immune dysfuntion in HIV^+ patients—and there have been many proposed—the FIV infection in cats has been and will continue to be an important animal model to study the immunopathogenesis of HIV infection. Based on data developed principally in the FIV model, one such scenario suggests that progressive deterioration of $CD4^+$ numbers and function characteristics of FIV/HIV infection could result from chronic anergic signaling mediated by virus activated T-cells expressing B7 molecules. More recent data developed in the FIV model support an argument that virus activated $CD4^+CD25^+$ Treg cells may abort protective T-helper-cell responses to antigens.

REFERENCES

1. N. C. Pedersen, E. Ho, M. L. Brown, and J. K. Yamamoto, Isolation of a T-lymphotropic virus from domestic cats with an immunodeficiency-like syndrome, *Science* 235, 790–793 (1987).
2. R. A. Olmsted, A. K. Barnes, J. K. Yamamoto, V. M. Hirsch, R. H. Purcell, and P. R. Johnson, Molecular cloning of feline immunodeficiency virus, *Proc. Natl. Acad. Sci.* 86, 2448–2452 (1989).

3. R. L. Talbott, E. E. Sparger, K. M. Lovelace, W. M. Fitch, N. C. Pedersen, P. A. Luciw, and J. H. Elder, Nucleotide sequence and genomic organization of feline immunodeficiency virus, *Proc. Natl. Acad. Sci.* 86, 5743–5747 (1989).
4. A. Fauci, A. Macher, D. Longo, H. Lane, A. Rook, H. Masur, and E. Gelmann, Acquired immunodeficiency syndrome: Epidemiologic, clinical, immunological, and therapuetic considerations, *Ann. Intern. Med.* 100, 92–106 (1984).
5. M. Clerici, N. Stocks, R. Zajac, R. Boswell, D. Lucey, C. Via, and G. Shearer, Detection of three distinct patterns of T helper cell dysfunction in asymptomatic, human immunodeficiency virus-seropositve patients, *J. Clin. Invest.* 84, 1892–1899 (1989).
6. J. L. Fahey, J. M. Taylor, R. Detels, B. Hofmann, R. Melmed, P. Nishanian, and J. V. Giorgi, The prognostic value of cellular and serologic markers in infection with human immunodeficiency virus type 1, *N. Engl. J. Med.* 322, 166–172 (1990).
7. B. F. Polk, R. Fox, R. Brookmeyer, S. Kanchanaraksa, R. Kaslow, B. Visscher, C. Rinaldo, and J. Phair, Predictors of the acquired immunodeficiency syndrome developing in a cohort of seropositive homosexual men, *N. Engl. J. Med.* 316, 61–66 (1987).
8. T. Ishida and I. Tomoda, Clinical staging of feline immunodeficiency virus infection, *Jpn. J. Vet. Sci.* 52(3), 645–648 (1990).
9. J. K. Yamamoto, H. Hansen, W. E. Ho, T. Y. Morishita, T. Okuda, T. R. Sawa, R. M. Nakamura, W. P. Kau, and N. C. Pedersen, Epidemiologic and clinical aspects of feline immunodeficiency virus infection in cats from the continental United States and Canada and possible mode of transmission, *J. Am. Vet. Med. Assoc.* 194, 213–220 (1989).
10. S. W. Dow, M. L. Poss, and E. A. Hoover, Feline immunodeficiency virus: A neurotropic lentivirus, *J. Acquir. Immune Defic. Syndr.* 3, 658–668 (1990).
11. H. Jordan, J. Howard, W. Tompkins, and S. Kennedy-Stoskopf, Detection of feline immunodeficiency virus in semen from seropositive domestic cats (Felis catus), *J. Virol.* 69(11), 7328–7333 (1995).
12. T. W. Vahlenkamp, H. F. Egberink, M. J. van Eijk, A. M. Slotboom-Kamphorst, E. J. Verschoor, M. C. Horzinek, and A. de Ronde, Competitive reverse transcription-polymerase chain reaction for quantitation of feline immunodeficiency virus, *J. Virol. Meth.* 52(3), 335–346 (1995).
13. M. J. Burkhard, C. K. Mathiason, K. O'Halloran, and E. A. Hoover, Kinetics of early FIV infection in cats exposed via the vaginal versus intravenous route, *AIDS Res. Hum. Retroviruses* 18(3), 217–226 (2002).
14. R. Lehmann, B. von Beust, E. Niederer, M. A. Condrau, W. Fierz, A. Aubert, C. D. Ackley, M. D. Cooper, M. B. Tompkins, and H. Lutz, Immunization-induced decrease of the CD4+:CD8+ ratio in cats experimentally infected with feline immunodeficiency virus, *Vet. Immunol. Immunopathol.* 35(1–2), 199–214 (1992).
15. S. Dawson, N. R. Smyth, M. Bennett, R. M. Gaskell, C. M. McCracken, A. Brown, and C. J. Gaskell, Effect of primary-stage feline immunodeficiency virus infection on subsequent feline calicivirus vaccination and challenge in cats, *AIDS* 5(6), 747–750 (1991).
16. A. Taniguchi, T. Ishida, T. Washizu, and I. Tomoda, Humoral immune response to T-cell dependent and independent antigens in cats infected with feline immunodeficiency virus, *J. Vet. Med. Sci.* 53(2), 333–335 (1991).
17. M. Torten, M. Franchini, J. E. Barlough, J. W. George, E. Mozes, H. Lutz, and N. C. Pedersen, Progressive immune dysfunction in cats experimentally infected with feline immunodeficiency virus, *J. Virol.* 65(5), 2225–2230 (1991).
18. O. Prospero-Garcia, N. Herold, T. R. Phillips, J. H. Elder, F. E. Bloom, and S. J. Henriksen, Sleep patterns are disturbed in cats infected with feline immunodeficiency virus, *Proc. Natl. Acad. Sci. USA* 91(26), 12947–12951 (1994).
19. T. R. Phillips, O. Prospero-Garcia, D. L. Puaoi, D. L. Lerner, H. S. Fox, R. A. Olmsted, F. E. Bloom, S. J. Henriksen, and J. H. Elder, Neurological abnormalities associated with feline immunodeficiency virus infection, *J. Gen. Virol.* 75(Pt 5), 979–987 (1994).

20. T. R. Phillips, O. Prospero-Garcia, D. W. Wheeler, P. C. Wagaman, D. L. Lerner, H. S. Fox, L. R. Whalen, F. E. Bloom, J. H. Elder, and S. J. Henriksen, Neurologic dysfunctions caused by a molecular clone of feline immunodeficiency virus, FIV-PPR, *J. Neurovirol.* 2(6), 388–396 (1996).
21. S. M. Astrin, E. Schattner, J. Laurence, R. I. Lebman, and C. Rodriguez-Alfageme, Does HIV infection of B lymphocytes initiate AIDS lymphoma? Detection by PCR of viral sequences in lymphoma tissue, *Curr. Top. Microbiol. Immunol.* 182, 399–407 (1992).
22. N. C. Pedersen and J. E. Barlough, Clinical overview of feline immunodeficiency virus, *J. Am. Vet. Med. Assoc.* 199(10), 1298–1305 (1991).
23. Y. Goto, Y. Nishimura, K. Baba, T. Mizuno, Y. Endo, K. Masuda, K. Ohno, and H. Tsujimoto, Association of plasma viral RNA load with prognosis in cats naturally infected with feline immunodeficiency virus, *J. Virol.* 76(19), 10079–10083 (2002).
24. N. C. Pedersen, Feline immunodeficiency virus, in: *The retroviruses, vol. 2* edited by J. A. Levy (Plenum Press, New York, 1993).
25. M. Bendinelli, M. Pistello, S. Lombardi, A. Poli, C. Garzelli, D. Matteucci, L. Ceccherini-Nelli, G. Malvaldi, and F. Tozzini, Feline immunodeficiency virus: an interesting model for AIDS studies and an important cat pathogen, *Clin. Microbiol. Rev.* 8(1), 87–112 (1995).
26. T. Ishida, A. Taniguchi, S. Matsumura, T. Washizu, and I. Tomoda, Long-term clinical observations on feline immunodeficiency virus infected asymptomatic carriers, *Vet. Immunol. Immunopathol.* 35(1–2), 15–22 (1992).
27. R. V. English, P. Nelson, C. M. Johnson, M. Nasisse, W. A. Tompkins, and M. B. Tompkins, Development of clinical disease in cats experimentally infected with feline immunodeficiency virus, *J. Infec. Dis.* 170, 543–552 (1994).
28. R. V. English, C. M. Johnson, D. H. Gebhard, and M. B. Tompkins, In vivo lymphocyte tropism of feline immunodeficiency virus, *J. Virol.* 67(9), 5175–5186 (1993).
29. M. J. Endres, P. R. Clapham, M. Marsh, M. Ahuja, J. D. Turner, A. McKnight, J. F. Thomas, B. Stoebenau-Haggarty, S. Choe, P. J. Vance, T. N. Wells, C. A. Power, S. S. Sutterwala, R. W. Doms, N. R. Landau, and J. A. Hoxie, CD4-independent infection by HIV-2 is mediated by fusin/CXCR4, *Cell* 87(4), 745–756 (1996).
30. D. Brunner and N. C. Pedersen, Infection of peritoneal macrophages in vitro and in vivo with feline immunodeficiency virus, *J. Virol.* 63(12), 5483–5488 (1989).
31. W. C. Brown, L. Bissey, D. S. Logan, N. C. Pedersen, J. H. Elder, and E. W. Collisson, Feline immunodeficiency virus infects both CD4+ and CD8+ T-lymphocytes, *J. Virol.* 65, 3359–3364 (1991).
32. A. Beebe, T. Gluckstern, J. George, N. Pedersen, and S. Dandekar, Detection of feline immunodeficiency virus infection in bone marrow of cats, *Vet. Immunol. Immunopathol.* 35, 37–49 (1992).
33. S. Lombardi, A. Poli, C. Massi, F. Abramo, L. Zaccaro, A. Bazzichi, G. Malvaldi, M. Bendinelli, and C. Garzelli, Detection of feline immunodeficiency virus p24 antigen and p24-specific antibodies by monoclonal antibody-based assays, *J. Virol. Methods* 46(3), 287–301 (1994).
34. A. Fauci, S. Schnittman, G. Poli, S. Koenig, and G. Pantaleo, Immunopathogenic mechanisms of human immunodeficiency virus (HIV) infection, *Ann. Intern. Med.* 114, 678–693 (1991).
35. H. Spiegel, H. Herbst, G. Niedobitek, H. D. Foss, and H. Stein, Follicular dendritic cells are a major reservoir for human immunodeficiency virus type 1 in lymphoid tissues facilitating infection of CD4+ T-helper cells, *Am. J. Pathol.* 140(1), 15–22 (1992).
36. G. A. Dean, G. H. Reubel, P. F. Boore, and N. C. Pedersen, Proviral burden and infection kinetics of feline immunodeficiency virus in lymphocyte subsets of blood and lymph node, *J. Virol.* 70, 5165–5169 (1996).

37. B. J. Willett, M. J. Hosie, J. J. Callanan, J. C. Neil, and O. Jarrett, Infection with feline immunodeficiency virus is followed by the rapid expansion of a CD8+ lymphocyte subset, *Immunology* 78(1), 1–6 (1993).
38. C. E. Mackewicz, L. C. Yang, J. D. Lifson, and J. A. Levy, Non-cytolytic CD8 T-cell anti-HIV responses in primary HIV-1 infection, *Lancet* 344(8938), 1671–1673 (1994).
39. J. Levy, C. Mackewicz, and E. Barker, Controlling HIV pathogenesis: the role of the non-cytotoxic anti-HIV response of CD8+ cells, *Immunol. Today* 17, 217–224 (1996).
40. J. Bucci, D. Gebhard, T. Childers, R. English, M. Tompkins, and W. Tompkins, The CD8+ phenotype mediating antiviral activity in FIV-infected cats is characterized by reduced surface expression of the CD8 beta chain, *J. Inf. Dis.* 178, 968–977 (1998).
41. D. Gebhard, J. Dow, T. Childers, J. Alvelo, M. Tompkins, and W. Tompkins, Progressive expansion of an L-selectin-negative CD8 cell with anti-feline immunodeficiency virus (FIV) suppressor function in the circulation of FIV-infected cats, *J. Infect. Dis.* 180, 1503–1513 (1999).
42. O. Ramilo, K. D. Bell, J. W. Uhr, and E. S. Vitetta, Role of CD25+ and CD25-T-cells in acute HIV infection in vitro, *J. Immunol.* 150(11), 5202–5208 (1993).
43. J. Borvak, C. S. Chou, K. Bell, G. Van Dyke, H. Zola, O. Ramilo, and E. S. Vitetta, Expression of CD25 defines peripheral blood mononuclear cells with productive versus latent HIV infection, *J. Immunol.* 155(6), 3196–3204 (1995).
44. S. Schnittman, H. Lane, J. Greenhouse, J. Justement, M. Baseler, and A. Fauci, Preferential infection of CD4+ memory T-cells by human immunodeficiency virus type 1: Evidence for a role in the selective T-cell functional defects observed in infected individuals, *Proc. Natl. Acad. Sci. USA* 87, 6058–6062 (1990).
45. D. Willerford, M. J. Gale, R. Benveniste, E. Clark, and W. Gallatin, Simian immunodeficiency virus is restricted to a subset of blood CD4+ lymphocytes that includes memory cells, *J. Immunol.* 144, 3779–3783 (1990).
46. C. S. Chou, O. Ramilo, and E. S. Vitetta, Highly purified CD25- resting T-cells cannot be infected de novo with HIV-1, *Proc. Natl. Acad. Sci. USA* 94(4), 1361–1365 (1997).
47. K. J. Maloy and F. Powrie, Regulatory T-cells in the control of immune pathology, *Nat. Immunol.* 2(9), 816–822 (2001).
48. E. M. Shevach, CD4+ CD25+ suppressor T-cells: more questions than answers, *Nat. Rev. Immunol.* 2(6), 389–400 (2002).
49. A. Joshi, T. W. Vahlenkamp, H. Garg, W. A. Tompkins, and M. B. Tompkins, Preferential replication of FIV in activated CD4+CD25+ T-cells independent of cellular proliferation, *accepted for publication* (2004).
50. M. J. Hosie, B. J. Willett, T. H. Dunsford, O. Jarrett, and J. C. Neil, A monoclonal antibody which blocks infection with feline immunodeficiency virus identifies a possible non-CD4 receptor, *J. Virol.* 67(3), 1667–1671 (1993).
51. B. J. Willett and M. J. Hosie, The role of the chemokine receptor CXCR4 in infection with feline immunodeficiency virus, *Mol. Membr. Biol.* 16(1), 67–72 (1999).
52. T. W. Vahlenkamp, A. de Ronde, N. N. Schuurman, A. L. van Vliet, J. van Drunen, M. C. Horzinek, and H. F. Egberink, Envelope gene sequences encoding variable regions 3 and 4 are involved in macrophage tropism of feline immunodeficiency virus, *J. Gen. Virol.* 80(Pt 10), 2639–2646 (1999).
53. J. W. Ritchey, J. K. Levy, S. K. Bliss, W. A. Tompkins, and M. B. Tompkins, Constitutive expression of types 1 and 2 cytokines by alveolar macrophages from feline immunodeficiency virus-infected cats, *Vet. Immunol. Immunopathol.* 79(1–2), 83–100 (2001).
54. J. Norimine, T. Miyazawa, Y. Kawaguchi, K. Tomonaga, Y. S. Shin, T. Toyosaki, M. Kohmoto, M. Niikura, Y. Tohya, and T. Mikami, Feline CD4 molecules expressed on feline non-lymphoid cell lines are not enough for productive infection of highly lymphotropic feline immunodeficiency virus isolates, *Arch. Virol.* 130(1–2), 171–178 (1993).
55. S. Wain-Hobson, HIV. One on one meets two, *Nature* 384(6605), 117–118 (1996).

56. P. R. Clapham and A. McKnight, Cell surface receptors, virus entry and tropism of primate lentiviruses, *J. Gen. Virol.* 83(Pt 8), 1809–1829 (2002).
57. H. Deng, R. Liu, W. Ellmeier, S. Choe, D. Unutmaz, M. Burkhart, P. Di Marzio, S. Marmon, R. E. Sutton, C. M. Hill, C. B. Davis, S. C. Peiper, T. J. Schall, D. R. Littman, and N. R. Landau, Identification of a major co-receptor for primary isolates of HIV-1, *Nature* 381(6584), 661–666 (1996).
58. T. Dragic, V. Litwin, G. P. Allaway, S. R. Martin, Y. Huang, K. A. Nagashima, C. Cayanan, P. J. Maddon, R. A. Koup, J. P. Moore, and W. A. Paxton, HIV-1 entry into CD4+ cells is mediated by the chemokine receptor CC-CKR-5, *Nature* 381(6584), 667–673 (1996).
59. Y. Feng, C. C. Broder, P. E. Kennedy, and E. A. Berger, HIV-1 entry cofactor: functional cDNA cloning of a seven-transmembrane, G protein-coupled receptor, *Science* 272(5263), 872–877 (1996).
60. C. C. Bleul, L. Wu, J. A. Hoxie, T. A. Springer, and C. R. Mackay, The HIV coreceptors CXCR4 and CCR5 are differentially expressed and regulated on human T lymphocytes, *Proc. Natl. Acad. Sci. USA* 94(5), 1925–1930 (1997).
61. B. J. Willett, M. J. Hosie, J. C. Neil, J. D. Turner, and J. A. Hoxie, Common mechanism of infection by lentiviruses [letter], *Nature* 385(6617), 587 (1997).
62. J. Richardson, G. Pancino, R. Merat, T. Leste-Lasserre, A. Moraillon, J. Schneider-Mergener, M. Alizon, P. Sonigo, and N. Heveker, Shared usage of the chemokine receptor CXCR4 by primary and laboratory-adapted strains of feline immunodeficiency virus, *J. Virol.* 73(5), 3661–3671 (1999).
63. H. Garg, A. Joshi, and W. A. Tompkins, Membrane expressed feline immunodeficiency virus env glycoprotein mediates apoptosis in activated PBMC by a fusion dependent mechanism, *accepted for publication* (2004).
64. B. J. Willett, K. Adema, N. Heveker, A. Brelot, L. Picard, M. Alizon, J. D. Turner, J. A. Hoxie, S. Peiper, J. C. Neil, and M. J. Hosie, The second extracellular loop of CXCR4 determines its function as a receptor for feline immunodeficiency virus, *J. Virol.* 72(8), 6475–6481 (1998).
65. J. B. Johnston and C. Power, Feline immunodeficiency virus xenoinfection: the role of chemokine receptors and envelope diversity, *J. Virol.* 76(8), 3626–3636 (2002).
66. H. F. Egberink, E. De Clercq, A. L. Van Vliet, J. Balzarini, G. J. Bridger, G. Henson, M. C. Horzinek, and D. Schols, Bicyclams, selective antagonists of the human chemokine receptor CXCR4, potently inhibit feline immunodeficiency virus replication, *J. Virol.* 73(8), 6346–6352 (1999).
67. B. J. Willett, C. A. Cannon, and M. J. Hosie, Expression of CXCR4 on feline peripheral blood mononuclear cells: effect of feline immunodeficiency virus infection, *J. Virol.* 77(1), 709–712 (2003).
68. D. L. Lerner and J. H. Elder, Expanded host cell tropism and cytopathic properties of feline immunodeficiency virus strain PPR subsequent to passage through interleukin-2-independent T-cells, *J. Virol.* 74(4), 1854–1863 (2000).
69. A. de Parseval and J. H. Elder, Binding of recombinant feline immunodeficiency virus surface glycoprotein to feline cells: role of CXCR4, cell-surface heparans, and an unidentified non-CXCR4 receptor, *J. Virol.* 75(10), 4528–4539 (2001).
70. J. Johnston and C. Power, Productive infection of human peripheral blood mononuclear cells by feline immunodeficiency virus: implications for vector development, *J. Virol.* 73(3), 2491–2498 (1999).
71. A. McKnight, P. R. Clapham, and R. A. Weiss, HIV-2 and SIV infection of nonprimate cell lines expressing human CD4: restrictions to replication at distinct stages, *Virology* 201(1), 8–18 (1994).
72. Y. Ohshiro, T. Murakami, K. Matsuda, K. Nishioka, K. Yoshida, and N. Yamamoto, Role of cell surface glycosaminoglycans of human T-cells in human immunodeficiency virus type-1 (HIV-1) infection, *Microbiol. Immunol.* 40(11), 827–835 (1996).

73. G. S. Gras and D. Dormont, Antibody-dependent and antibody-independent complement-mediated enhancement of human immunodeficiency virus type 1 infection in a human, Epstein-Barr virus-transformed B-lymphocytic cell line, *J. Virol.* 65(1), 541–545 (1991).
74. A. M. Beebe, N. Dua, T. G. Faith, P. F. Moore, N. C. Pedersen, and S. Dandekar, Primary stage of feline immunodeficiency virus infection: viral dissemination and cellular targets, *J. Virol.* 68(5), 3080–3091 (1994).
75. D. L. Sodora, E. G. Shpaer, B. E. Kitchell, S. W. Dow, E. A. Hoover, and J. I. Mullins, Identification of three feline immunodeficiency virus (FIV) env gene subtypes and comparison of the FIV and human immunodeficiency virus type 1 evolutionary patterns, *J. Virol.* 68(4), 2230–2238 (1994).
76. S. Kakinuma, K. Motokawa, T. Hohdatsu, J. K. Yamamoto, H. Koyama, and H. Hashimoto, Nucleotide sequence of feline immunodeficiency virus: classification of Japanese isolates into two subtypes which are distinct from non-Japanese subtypes, *J. Virol.* 69(6), 3639–3646 (1995).
77. M. H. Bachmann, C. Mathiason-Dubard, G. H. Learn, A. G. Rodrigo, D. L. Sodora, P. Mazzetti, E. A. Hoover, and J. I. Mullins, Genetic diversity of feline immunodeficiency virus: dual infection, recombination, and distinct evolutionary rates among envelope sequence clades, *J. Virol.* 71(6), 4241–4253 (1997).
78. M. Pistello, G. Cammarota, E. Nicoletti, D. Matteucci, M. Curcio, D. Del Mauro, and M. Bendinelli, Analysis of the genetic diversity and phylogenetic relationship of Italian isolates of feline immunodeficiency virus indicates a high prevalence and heterogeneity of subtype B, *J. Gen. Virol.* 78 (Pt 9), 2247–2257 (1997).
79. T. Hohdatsu, K. Motokawa, M. Usami, M. Amioka, S. Okada, and H. Koyama, Genetic subtyping and epidemiological study of feline immunodeficiency virus by nested polymerase chain reaction-restriction fragment length polymorphism analysis of the gag gene, *J. Virol. Meth.* 70(1), 107–111 (1998).
80. A. Steinrigl and D. Klein, Phylogenetic analysis of feline immunodeficiency virus in Central Europe: a prerequisite for vaccination and molecular diagnostics, *J. Gen. Virol.* 84(Pt 5), 1301–1307 (2003).
81. G. Inada, T. Miyazawa, Y. Inoshima, M. Kohmoto, Y. Ikeda, C. H. Liu, J. A. Lin, T. F. Kuo, and T. Mikami, Phylogenetic analysis of feline immunodeficiency virus isolated from cats in Taiwan, *Arch. Virol.* 142(7), 1459–1467 (1997).
82. M. Uema, Y. Ikeda, T. Miyazawa, J. A. Lin, M. C. Chen, T. F. Kuo, C. Kai, T. Mikami, and E. Takahashi, Feline immunodeficiency virus subtype C is prevalent in northern part of Taiwan, *J. Vet. Med. Sci.* 61(2), 197–199 (1999).
83. K. Nakamura, Y. Suzuki, K. Ikeo, Y. Ikeda, E. Sato, N. T. Nguyen, T. Gojobori, T. Mikami, and T. Miyazawa, Phylogenetic analysis of Vietnamese isolates of feline immunodeficiency virus: genetic diversity of subtype C, *Arch. Virol.* 148(4), 783–791 (2003).
84. M. R. Pecoraro, K. Tomonaga, T. Miyazawa, Y. Kawaguchi, S. Sugita, Y. Tohya, C. Kai, M. E. Etcheverrigaray, and T. Mikami, Genetic diversity of Argentine isolates of feline immunodeficiency virus, *J. Gen. Virol.* 77(Pt 9), 2031–2035 (1996).
85. G. Pancino, S. Castelot, and P. Sonigo, Differences in feline immunodeficiency virus host cell range correlate with envelope fusogenic properties, *Virology* 206(2), 796–806 (1995).
86. T. Hohdatsu, H. Hirabayashi, K. Motokawa, and H. Koyama, Comparative study of the cell tropism of feline immunodeficiency virus isolates of subtypes A, B and D classified on the basis of the env gene V3-V5 sequence, *J. Gen. Virol.* 77(Pt 1), 93–100 (1996).
87. M. de Monte, H. Nonnenmacher, N. Brignon, M. Ullmann, and J. P. Martin, A multivariate statistical analysis to follow the course of disease after infection of cats with different strains of the feline immunodeficiency virus (FIV), *J. Virol. Meth.* 103(2), 157–170 (2002).

88. N. C. Pedersen, C. M. Leutenegger, J. Woo, and J. Higgins, Virulence differences between two field isolates of feline immunodeficiency virus (FIV-APetaluma and FIV-CPGammar) in young adult specific pathogen free cats, *Vet. Immunol. Immunopathol.* 79(1–2), 53–67 (2001).
89. C. D. Ackley, J. K. Yamamoto, N. Levy, N. C. Pedersen, and M. D. Cooper, Immunologic abnormalities in pathogen-free cats experimentally infected with feline immunodeficiency virus, *J. Virol.* 64(11), 5652–5655 (1990).
90. C. Novotney, R. English, J. Housman, M. Davidson, M. Nasisse, C. R. Jeng, W. Davis, and M. Tompkins, Lymphocyte population changes in cats naturally infected with feline immunodeficiency virus, *AIDS* 4, 1213–1218 (1990).
91. A. Poli, C. Giannelli, M. Pistello, L. Zaccaro, D. Pieracci, M. Bendinelli, and G. Malvaldi, Detection of salivary antibodies in cats infected with feline immunodeficiency virus, *J. Clin. Microbiol.* 30(8), 2038–2041 (1992).
92. J. N. Flynn, C. A. Cannon, C. E. Lawrence, and O. Jarrett, Polyclonal B-cell activation in cats infected with feline immunodeficiency virus, *Immunology* 81(4), 626–630 (1994).
93. J. K. Yamamoto, E. Sparger, E. W. Ho, P. R. Andersen, T. P. O'Connor, C. P. Mandell, L. Lowenstine, R. Munn, and N. C. Pedersen, Pathogenesis of experimentally induced feline immunodeficiency virus infection in cats, *Am. J. Vet. Res.* 49(8), 1246–1258 (1988).
94. H. F. Egberink, C. E. Keldermans, M. J. Koolen, and M. C. Horzinek, Humoral immune response to feline immunodeficiency virus in cats with experimentally induced and naturally acquired infections, *Am. J. Vet. Res.* 53(7), 1133–1138 (1992).
95. S. Lombardi, M. Bendinelli, and C. Garzelli, Detection of B epitopes on the p24 gag protein of feline immunodeficiency virus by monoclonal antibodies, *AIDS Res. Hum. Retroviruses* 9(2), 141–146 (1993).
96. S. Lombardi, C. Garzelli, C. La Rosa, L. Zaccaro, S. Specter, G. Malvaldi, F. Tozzini, F. Esposito, and M. Bendinelli, Identification of a linear neutralization site within the third variable region of the feline immunodeficiency virus envelope, *J. Virol.* 67(8), 4742–4749 (1993).
97. G. Pancino, I. Fossati, C. Chappey, S. Castelot, B. Hurtrel, A. Moraillon, D. Klatzmann, and P. Sonigo, Structure and variations of feline immunodeficiency virus envelope glycoproteins, *Virology* 192(2), 659–662 (1993).
98. G. Pancino, L. Camoin, and P. Sonigo, Structural analysis of the principal immunodominant domain of the feline immunodeficiency virus transmembrane glycoprotein, *J. Virol.* 69(4), 2110–2118 (1995).
99. T. W. Vahlenkamp, E. J. Verschoor, N. N. Schuurman, A. L. van Vliet, M. C. Horzinek, H. F. Egberink, and A. de Ronde, A single amino acid substitution in the transmembrane envelope glycoprotein of feline immunodeficiency virus alters cellular tropism, *J. Virol.* 71(9), 7132–7135 (1997).
100. A. Avrameas, A. D. Strosberg, A. Moraillon, P. Sonigo, and G. Pancino, Serological diagnosis of feline immunodeficiency virus infection based on synthetic peptides from Env glycoproteins, *Res. Virol.* 144(3), 209–218 (1993).
101. G. Pancino, C. Chappey, W. Saurin, and P. Sonigo, B epitopes and selection pressures in feline immunodeficiency virus envelope glycoproteins, *J. Virol.* 67(2), 664–672 (1993).
102. A. de Ronde, J. G. Stam, P. Boers, H. Langedijk, R. Meloen, W. Hesselink, L. C. Keldermans, A. van Vliet, E. J. Verschoor, M. C. Horzinek, and et al., Antibody response in cats to the envelope proteins of feline immunodeficiency virus: identification of an immunodominant neutralization domain, *Virology* 198(1), 257–264 (1994).
103. K. H. Siebelink, G. F. Rimmelzwaan, M. L. Bosch, R. H. Meloen, and A. D. Osterhaus, A single amino acid substitution in hypervariable region 5 of the envelope protein of feline immunodeficiency virus allows escape from virus neutralization, *J. Virol.* 67(4), 2202–2208 (1993).

104. K. H. Siebelink, M. L. Bosch, G. F. Rimmelzwaan, R. H. Meloen, and A. D. Osterhaus, Two different mutations in the envelope protein of feline immunodeficiency virus allow the virus to escape from neutralization by feline serum antibodies, *Vet. Immunol. Immunopathol.* 46(1–2), 51–59 (1995).
105. J. K. Yamamoto, T. Okuda, C. D. Ackley, H. Louie, E. Pembroke, H. Zochlinski, R. J. Munn, and M. B. Gardner, Experimental vaccine protection against feline immunodeficiency virus, *AIDS Res. Hum. Retroviruses* 7(11), 911–922 (1991).
106. F. Tozzini, D. Matteucci, P. Bandecchi, F. Baldinotti, K. Siebelink, A. Osterhaus, and M. Bendinelli, Neutralizing antibodies in cats infected with feline immunodeficiency virus, *J. Clin. Microbiol.* 31(6), 1626–1629 (1993).
107. F. Baldinotti, D. Matteucci, P. Mazzetti, C. Giannelli, P. Bandecchi, F. Tozzini, and M. Bendinelli, Serum neutralization of feline immunodeficiency virus is markedly dependent on passage history of the virus and host system, *J. Virol.* 68(7), 4572–4579 (1994).
108. T. Hohdatsu, R. Pu, B. A. Torres, S. Trujillo, M. B. Gardner, and J. K. Yamamoto, Passive antibody protection of cats against feline immunodeficiency virus infection, *J. Virol.* 67(4), 2344–2348 (1993).
109. M. J. Hosie, R. Osborne, J. K. Yamamoto, J. C. Neil, and O. Jarrett, Protection against homologous but not heterologous challenge induced by inactivated feline immunodeficiency virus vaccines, *J. Virol.* 69(2), 1253–1255 (1995).
110. M. Burkhard, L. Obert, N. L. O, L. Diehl, and E. Hoover, Mucosal transmission of cell-associated and cell-free feline immunodeficiency virus, *AIDS Res. Hum. Retroviruses* 13, 347–355 (1997).
111. L. J. Diehl, C. K. Mathiason-Dubard, L. L. O'Neil, and E. A. Hoover, Plasma viral RNA load predicts disease progression in accelerated feline immunodeficiency virus infection, *J. Virol.* 70(4), 2503–2507 (1996).
112. Y. Liang, L. C. Hudson, J. K. Levy, J. W. Ritchey, W. A. F. Tompkins, and M. B. Tompkins, T-cells over expressing IFN-γ and IL10 are found in both the thymus and secondary lymphoid tissues of FIV-infected cats, *J. Infect. Dis.* 181, 564–575 (2000).
113. M. M. Reddy, A. Englard, D. Brown, E. Buimovici-Klien, and M. H. Grieco, Lymphoproliferative responses to human immunodeficiency virus antigen in asymptomatic intravenous drug abusers and in patients with lymphadenopathy or AIDS, *J. Infect. Dis.* 156(2), 374–376 (1987).
114. B. Wahren, L. Morfeldt-Mansson, G. Biberfeld, L. Moberg, A. Sonnerborg, P. Ljungman, A. Werner, R. Kurth, R. Gallo, and D. Bolognesi, Characteristics of the specific cell-mediated immune response in human immunodeficiency virus infection, *J. Virol.* 61(6), 2017–2023 (1987).
115. H. C. Kelker, M. Seidlin, M. Vogler, and F. T. Valentine, Lymphocytes from some long-term seronegative heterosexual partners of HIV-infected individuals proliferate in response to HIV antigens, *AIDS Res. Hum. Retroviruses* 8(8), 1355–1359 (1992).
116. S. Cohen, M. Tuen, and C. E. Hioe, Propagation of CD4+ T-cells specific for HIV type 1 envelope gp120 from chronically HIV type 1-infected subjects, *AIDS Res. Hum. Retroviruses* 19(9), 793–806 (2003).
117. T. Wendland, H. Furrer, P. L. Vernazza, K. Frutig, A. Christen, L. Matter, R. Malinverni, and W. J. Pichler, HAART in HIV-infected patients: restoration of antigen-specific CD4 T-cell responses in vitro is correlated with CD4 memory T-cell reconstitution, whereas improvement in delayed type hypersensitivity is related to a decrease in viraemia, *AIDS* 13(14), 1857–1862 (1999).
118. M. Peruzzi, C. Azzari, L. Galli, A. Vierucci, and M. De Martino, Highly active antiretroviral therapy restores in vitro mitogen and antigen-specific T-lymphocyte responses in HIV-1 perinatally infected children despite virological failure, *Clin. Exp. Immunol.* 128(2), 365–371 (2002).

119. G. A. Hardy, N. Imami, A. K. Sullivan, A. Pires, C. T. Burton, M. R. Nelson, B. G. Gazzard, and F. M. Gotch, Reconstitution of CD4+ T-cell responses in HIV-1 infected individuals initiating highly active antiretroviral therapy (HAART) is associated with renewed interleukin-2 production and responsiveness, *Clin. Exp. Immunol.* 134(1), 98–106 (2003).
120. K. Wolthers, S. Otto, S. Lens, D. Kolbach, R. van Lier, F. Miedema, and L. Meyaard, Increased expression of CD80, CD86 and CD70 on T-cells from HIV-infected individuals upon activation in vitro: regulation by CD4+ T-cells, *Eur. J. Immunol.* 26, 1700–1706 (1996).
121. C. Kochli, T. Wendland, K. Frutig, R. Grunow, S. Merlin, and W. Pichler, CD80 and CD86 costimulatory molecules on circulating T-cells of HIV infected individuals, *Immunol. Lett.* 65, 197–201 (1999).
122. M. B. Tompkins, M. E. Bull, J. L. Dow, J. M. Ball, E. W. Collisson, B. J. Winslow, A. P. Phadke, T. W. Vahlenkamp, and W. A. Tompkins, Feline immunodeficiency virus infection is characterized by B7+CTLA4+ T-cell apoptosis, *J. Infect. Dis.* 185(8), 1077–1093 (2002).
123. M. E. Bull, W. A. Tompkins, P. Menezes, J. J. Eron, C. D. Pilcher, J. L. Schmitz, J. A. Frelinger, and M. B. Tompkins, HIV infection is characterized by CD4+B7+CTLA4+MHCII+ T-cell apoptosis: A model for CD4+ T-cell loss and immunodeficiency, *Ph.D. thesis, North Carolina State University* (2003).
124. R. L. Rabin, M. Roederer, Y. Maldonado, A. Petru, L. A. Herzenberg, and L. A. Herzenberg, Altered representation of naive and memory CD8 T-cell subsets in HIV-infected children, *J. Clin. Invest.* 95(5), 2054–2060 (1995).
125. M. Roederer, J. G. Dubs, M. T. Anderson, P. A. Raju, L. A. Herzenberg, and L. A. Herzenberg, CD8 naive T-cell counts decrease progressively in HIV-infected adults, *J. Clin. Invest.* 95(5), 2061–2066 (1995).
126. H. E. Prince and E. R. Jensen, Three-color cytofluorometric analysis of CD8 cell subsets in HIV-1 infection, *J. Acquir. Immune Defic. Syndr.* 4(12), 1227–1232 (1991).
127. P. Borrow, H. Lewicki, B. H. Hahn, G. M. Shaw, and M. B. Oldstone, Virus-specific CD8+ cytotoxic T-lymphocyte activity associated with control of viremia in primary human immunodeficiency virus type 1 infection, *J. Virol.* 68(9), 6103–6110 (1994).
128. J. Flynn, J. Beatty, C. Cannon, E. Stephens, M. Hosie, J. Neil, and O. Jarrett, Involvement of gag- and env-specific cytotoxic T lymphocytes in protective immunity to feline immunodeficiency virus., *AIDS Res. Hum. Retroviruses* 11, 1107–1113 (1995).
129. J. N. Flynn, P. Keating, M. J. Hosie, M. Mackett, E. B. Stephens, J. A. Beatty, J. C. Neil, and O. Jarrett, Env-specific CTL predominate in cats protected from feline immunodeficiency virus infection by vaccination, *J. Immunol.* 157(8), 3658–3665 (1996).
130. J. Beatty, B. Willett, E. Gault, and O. Jarrett, A longitudinal study of feline immunodeficiency virus-specific cytotoxic T lymphocytes in experimentally infected cats, using antigen-specific induction, *J. Virol.* 70(9), 6199–6206 (1996).
131. Y. Yasutomi, K. A. Reimann, C. I. Lord, M. D. Miller, and N. L. Letvin, Simian immunodeficiency virus-specific CD8+ lymphocyte response in acutely infected rhesus monkeys, *J. Virol.* 67, 1707–1711 (1993).
132. W. Song, E. Collisson, P. Billingsley, and W. Brown, Induction of feline immunodeficiency virus-specific cytolytic T-cell responses from experimentally infected cats, *J. Virol.* 66(9), 5409–5417 (1992).
133. C. R. Rinaldo, Jr., L. A. Beltz, X. L. Huang, P. Gupta, Z. Fan, and D. J. Torpey, III, Anti-HIV type 1 cytotoxic T lymphocyte effector activity and disease progression in the first 8 years of HIV type 1 infection of homosexual men, *AIDS Res. Hum. Retroviruses* 11(4), 481–489 (1995).
134. B. Willett, N. Flynn, and M. Hosie, FIV infection of the domestic cat: an animal model for AIDS, *Immunol. Today* 18, 182 (1997).

135. M. J. Hosie and J. N. Flynn, Feline immunodeficiency virus vaccination: characterization of the immune correlates of protection, *J. Virol.* 70(11), 7561–7568 (1996).
136. J. Bucci, R. English, H. Jordan, T. Childers, M. Tompkins, and W. Tompkins, Mucosally transmitted feline immunodeficiency virus induces a CD8+ antiviral response that correlates with reduction of cell-associated virus, *J. Infect. Dis.* 177, 18–25 (1998).
137. P. C. Crawford, G. P. Papadi, J. K. Levy, N. A. Benson, A. Mergia, and C. M. Johnson, Tissue dynamics of CD8 lymphocytes that suppress viral replication in cats infected neonatally with feline immunodeficiency virus, *J. Infect. Dis.* 184(6), 671–681 (2001).
138. J. N. Flynn, S. Dunham, A. Mueller, C. Cannon, and O. Jarrett, Involvement of cytolytic and non-cytolytic T-cells in the control of feline immunodeficiency virus infection, *Vet. Immunol. Immunopathol.* 85(3–4), 159–170 (2002).
139. J. W. George, N. C. Pedersen, and J. Higgins, The effect of age on the course of experimental feline immunodeficiency virus infection in cats, *AIDS Res. Hum. Retroviruses* 9, 897–905 (1993).
140. C. Lawrence, J. Callanan, and O. Jarrett, Decreased mitogen responsiveness and elevated tumor necrosis factor production in cats shortly after feline immunodeficiency virus infection, *Vet. Immunol. Immunopathol.* 35, 51–59 (1992).
141. M. G. Davidson, J. Rottman, R. V. English, M. R. Lappin, and M. B. Tompkins, Feline immunodeficiency virus predisposes cats to acute generalized toxoplasmosis, *Am. J. Pathol.* 143, 1486–1497 (1993).
142. M. G. Davidson, M. R. Lappin, R. V. English, and M. B. Tompkins, A feline model of ocular toxoplasmosis, *Invest. Ophthalmol. Visual Sci.* 34(13), 3653–3660 (1993).
143. J. Yang, R. English, J. Ritchey, M. Davidson, T. Wasmoen, J. Levy, D. Gebhard, M. Tompkins, and W. Tompkins, Molecularly cloned feline immunodeficiency virus NCSU1 JSY3 induces immunodeficiency in specific-pathogen-free cats, *J. Virol.* 70(5), 3011–3017 (1996).
144. M. Clerici and G. M. Shearer, A T_H1 to T_H2 switch is a critical step in the etiology of HIV infection, *Immunol. Today* 14(3), 107–111 (1993).
145. L. Meyaard, H. Schuitemaker, and F. Miedema, T-cell dysfunction in HIV infection: anergy due to defective antigen-presenting cell function?, *Immunol. Today* 14(4), 161–164 (1993).
146. M. R. Helbert, J. L'Age-Stehr, and N. A. Mitchison, Antigen presentation, loss of immunological memory and AIDS, *Immunol. Today* 14(7), 340–344 (1993).
147. M. Ascher and H. Sheppard, AIDS as immune system activation. II. The panergic imnesia hypothesis, *J. Acquir. Immune Defic. Syndr.* 3, 177–191 (1990).
148. J. Lahdevirta, C. P. Maury, A. M. Teppo, and H. Repo, Elevated levels of circulating cachectin/tumor necrosis factor in patients with acquired immunodeficiency syndrome, *Am. J. Med.* 85(3), 289–291 (1988).
149. P. Gallo, K. Frei, C. Rordorf, J. Lazdins, B. Tavolato, and A. Fontana, Human immunodeficiency virus type 1 (HIV-1) infection of the central nervous system: an evaluation of cytokines in cerebrospinal fluid, *J. Neuroimmunol.* 23(2), 109–116 (1989).
150. E. C. Breen, A. R. Rezai, K. Nakajima, G. N. Beall, R. T. Mitsuyasu, T. Hirano, T. Kishimoto, and M. O. Martinez, Infection with HIV is associated with elevated IL-6 levels and production, *J. Immunol.* 144(2), 480–484 (1990).
151. L. Trentin, S. Garbisa, R. Zambello, C. Agostini, C. Caenazzo, C. Di Francesco, A. Cipriani, E. Francavilla, and G. Semenzato, Spontaneous production of interleukin-6 by alveolar macrophages from human immunodeficiency virus type 1-infected patients, *J. Infect. Dis.* 166(4), 731–737 (1992).
152. C. E. Lawrence, J. J. Callanan, B. J. Willett, and O. Jarrett, Cytokine production by cats infected with feline immunodeficiency virus: a longitudinal study, *Immunology* 85(4), 568–574 (1995).

153. C. Graziosi, G. Pantaleo, K. R. Gantt, J. P. Fortin, J. F. Demarest, O. J. Cohen, R. P. Sekaly, and A. S. Fauci, Lack of evidence for the dichotomy of TH1 and TH2 predominance in HIV-infected individuals, *Science* 265(5169), 248–252 (1994).
154. S. Than, R. Hu, N. Oyaizu, J. Romano, X. Wang, S. Sheikh, and S. Pahwa, Cytokine pattern in relation to disease progression in human immunodeficiency virus-infected children, *J. Infect. Dis.* 175(1), 47–56 (1997).
155. J. Chehimi, S. E. Starr, I. Frank, A. D'Andrea, X. Ma, R. R. MacGregor, J. Sennelier, and G. Trinchieri, Impaired interleukin 12 production in human immunodeficiency virus-infected patients, *J. Exp. Med.* 179(4), 1361–1366 (1994).
156. E. Maggi, D. Macchia, P. Parronchi, M. Mazzetti, A. Ravina, D. Milo, and S. Romagnani, Reduced production of interleukin 2 and interferon-gamma and enhanced helper activity for IgG synthesis by cloned CD4+ T-cells from patients with AIDS, *Eur. J. Immunol.* 17(12), 1685–1690 (1987).
157. L. Meroni, D. Trabattoni, C. Balotta, C. Riva, M. Clerici, and M. Galli, Evidence for type 2 cytokine production and lymphocyte activiation in early phases of HIV-1 infection, *AIDS* 10, 23–30 (1996).
158. M. Clerici, F. T. Hakim, D. J. Venzon, S. Blatt, C. W. Hendrix, T. A. Wynn, and G. M. Shearer, Changes in interleukin-2 and interleukin-4 production in asymptomatic, human immunodeficiency virus-seropositive individuals, *J. Clin. Invest.* 91(3), 759–765 (1993).
159. J. Levy, J. Ritchey, J. Rottman, M. Davidson, Y. Liang, H. Jordan, W. Tompkins, and M. Tompkins, Elevated interleukin-10-to-interleukin-12 ratio in feline immunodeficiency virus-infected cats predicts loss of type 1 immunity to Toxoplasma gondii, *J. Infect. Dis.* 178, 503–511 (1998).
160. J. K. Levy, Y. H. Liang, J. W. Ritchey, M. G. Davidson, W. A. Tompkins, and M. B. Tompkins, Failure of FIV-infected cats to control *T. gondii* challenge correlates with reduced IL2, IL6, and IL12 and elevated IL10 expression by lymph node T-cells, *Vet. Immunol. Immunopathol.* in press (2004).
161. G. A. Dean, J. A. Bernales, and N. C. Pedersen, Effect of feline immunodeficiency virus on cytokine response to Listeria monocytogenes in vivo, *Vet. Immunol. Immunopathol.* 65(2–4), 125–138 (1998).
162. N. C. Pedersen, G. A. Dean, J. Bernales, A. Sukura, and J. Higgins, Listeria monocytogenes and Serratia marcescens infections as models for Th1/Th2 immunity in laboratory cats, *Vet. Immunol. Immunopathol.* 63(1–2), 83–103 (1998).
163. T. M. Williams, K. R. Fox, and J. A. Kant, Interleukin-2: basic biology and therapeutic use, *Hematol. Pathol.* 5(2), 45–55 (1991).
164. D. De Wit, M. Van Mechelen, M. Ryelandt, A. C. Figueiredo, D. Abramowicz, M. Goldman, H. Bazin, J. Urbain, and O. Leo, The injection of deaggregated gamma globulins in adult mice induces antigen-specific unresponsiveness of T-helper type 1 but not type 2 lymphocytes, *J. Exp. Med.* 175(1), 9–14 (1992).
165. S. E. Macatonia, S. Patterson, and S. C. Knight, Primary proliferative and cytotoxic T-cell responses to HIV induced in vitro by human dendritic cells, *Immunology* 74(3), 399–406 (1991).
166. L. J. Eales, O. Moshtael, and A. J. Pinching, Microbicidal activity of monocyte derived macrophages in AIDS and related disorders, *Clin. Exp. Immunol.* 67(2), 227–235 (1987).
167. L. Meyaard, S. Otto, R. Jonker, M. Mijnster, R. Keet, and F. Miedema, Programmed cell death of T-cells in HIV-1 infection, *Science* 257, 217–219 (1992).
168. F. Miedema, Immunological abnormalities in the natural history of HIV infection: mechanisms and clinical relevance, *Immunodefic. Rev.* 3, 173–193 (1992).
169. T. Finkel, G. Tudor-Williams, N. Banda, M. Cotton, T. Curiel, C. Monks, T. Baba, R. Ruprecht, and A. Kupfer, Apoptosis occurs predominantly in bystander cells and not in productively infected cells of HIV- and SIV-infected lymph nodes, *Nat. Med.* 1, 129–134 (1995).

170. C. Muro-Cacho, G. Pantaleo, and A. Fauci, Analysis of apoptosis in lymph nodes of HIV-infected persons. Intensity of apoptosis correlates with the general state of activation of the lymphoid tissue and not with stage of disease or viral burden, *J. Immunol.* 154, 5555–5566 (1995).
171. A. Guiot, D. Rigal, and G. Chappuis, Spontaneous programmed cell death (PCD) process of lymphocytes of FIV-infected cats: cellular targets and modulation, *Vet. Immunol. Immunopathol.* 58, 93–106 (1997).
172. G. Sarli, L. Della Salda, L. Zaccaro, M. Bendinelli, G. Piedimonte, and P. Marcato, Apoptotic fraction in lymphoid tissue of FIV-infected SPF cats, *Vet. Immunol. Immunopathol.* 64, 33–44 (1998).
173. L. Boise, P. Noel, and C. Thompson, CD28 and apoptosis, *Curr. Opin. Immunol.* 7, 620–625 (1995).
174. E. Greenfield, K. Nguyen, and V. Kuchroo, CD28/B7 costimulation: a review, *Crit. Rev. Immunol.* 18, 389–418 (1998).
175. D. J. Lenschow, T. L. Walunas, and J. A. Bluestone, CD28/B7 system of T-cell costimulation, *Annu. Rev. Immunol.* 14, 233–258 (1996).
176. M. Azuma, H. Yssel, J. Phillips, H. Spits, and L. Lanier, Functional expression of B7/BB1 on activated T lymphocytes, *J. Exp. Med.* 177, 845–850 (1993).
177. T. Wyss-Coray, D. Mauri-Hellweg, K. Baumann, F. Bettens, R. Grunow, and W. Pichler, The B7 adhesion molecule is expressed on activated human T-cells: functional involvement in T-T-cell interactions, *Eur. J. Immunol.* 23, 2175–2180 (1993).
178. E. Ranheim and T. Kipps, Elevated expression of CD80 (B7/BB1) and other accessory molecules on synovial fluid mononuclear cell subsets in rheumatoid arthritis, *Arthritis Rheum.* 37, 1637–1646 (1994).
179. D. Folzenlogen, M. Hofer, D. Leung, J. Freed, and M. Newell, Analysis of CD80 and CD86 expression on peripheral blood B lymphocytes reveals increased expression of CD86 in lupus patients, *Clin. Immunol. Immunopathol.* 83, 199–204 (1997).
180. T. W. Vahlenkamp, M. E. Bull, J. L. Dow, E. W. Collisson, B. J. Winslow, A. P. Phadke, W. A. Tompkins, and M. B. Tompkins, B7+CTLA4+ T-cells engage in T-T-cell interactions that mediate apoptosis: A model for lentivirus-induced T-cell depletion, *Vet. Immunol. Immunopathol.* in press (2004).
181. V. Barnaba, C. Watts, B. M. de, P. Lane, and A. Lanzavecchia, Professional presentation of antigen by activated human T-cells, *Eur. J. Immunol.* 24, 71–75 (1994).
182. G. Lombardi, S. Sidhu, T. Dodi, R. Batchelor, and R. Lechler, Failure of correlation between B7 expression and activation of interleukin-2-secreting T-cells, *Eur. J. Immunol.* 24, 523–530 (1994).
183. E. Greenfield, E. Howard, T. Paradis, K. Nguyen, F. Benazzo, P. McLean, P. Hollsberg, G. Davis, D. Hafler, A. Sharpe, G. Freeman, and V. Kuchroo, B7.2 expressed by T-cells does not induce CD28-mediated costimulatory activity but retains CTLA4 binding: implications for induction of antitumor immunity to T-cell tumors, *J. Immunol.* 158, 2025–2034 (1997).
184. D. Bienzle, F. Smaill, and K. Rosenthal, Cytotoxic T-lymphocytes from HIV-infected individuals recognize an activation-dependent, non-polymorphic molecule on uninfected CD4+ lymphocytes, *AIDS* 10, 247–254 (1996).
185. M. E. Bull, T. W. Vahlenkamp, J. L. Dow, E. W. Collisson, B. J. Winslow, A. P. Phadke, M. B. Tompkins, and W. A. Tompkins, Spontaneous T-cell apoptosis in feline immunodeficiency virus (FIV)-infected cats is inhibited by IL-2 and anti-B7.1 antibodies, *accepted for publication* (2004).
186. Y. Belkaid, C. A. Piccirillo, S. Mendez, E. M. Shevach, and D. L. Sacks, CD4+CD25+ regulatory T-cells control Leishmania major persistence and immunity, *Nature* 420(6915), 502–507 (2002).
187. S. Sakaguchi, Regulatory T-cells: mediating compromises between host and parasite, *Nat. Immunol.* 4(1), 10–11 (2003).

188. T. Takahashi, Y. Kuniyasu, M. Toda, N. Sakaguchi, M. Itoh, M. Iwata, J. Shimizu, and S. Sakaguchi, Immunologic self-tolerance maintained by CD25+CD4+ naturally anergic and suppressive T-cells: induction of autoimmune disease by breaking their anergic/suppressive state, *Int. Immunol.* 10(12), 1969–1980 (1998).
189. A. M. Thornton and E. M. Shevach, CD4+CD25+ immunoregulatory T-cells suppress polyclonal T-cell activation in vitro by inhibiting interleukin 2 production, *J. Exp. Med.* 188(2), 287–296 (1998).
190. A. M. Thornton and E. M. Shevach, Suppressor effector function of CD4+CD25+ immunoregulatory T-cells is antigen nonspecific, *J. Immunol.* 164(1), 183–190 (2000).
191. K. Nakamura, A. Kitani, and W. Strober, Cell contact-dependent immunosuppression by CD4(+)CD25(+) regulatory T-cells is mediated by cell surface-bound transforming growth factor beta, *J. Exp. Med.* 194(5), 629–644 (2001).
192. I. Caramalho, T. Lopes-Carvalho, D. Ostler, S. Zelenay, M. Haury, and J. Demengeot, Regulatory T-cells selectively express toll-like receptors and are activated by lipopolysaccharide, *J. Exp. Med.* 197(4), 403–411 (2003).
193. B. Salomon, D. J. Lenschow, L. Rhee, N. Ashourian, B. Singh, A. Sharpe, and J. A. Bluestone, B7/CD28 costimulation is essential for the homeostasis of the CD4+CD25+ immunoregulatory T-cells that control autoimmune diabetes, *Immunity* 12(4), 431–440 (2000).
194. H. Jonuleit, E. Schmitt, M. Stassen, A. Tuettenberg, J. Knop, and A. H. Enk, Identification and functional characterization of human CD4(+)CD25(+) T-cells with regulatory properties isolated from peripheral blood, *J. Exp. Med.* 193(11), 1285–1294 (2001).
195. T. W. Vahlenkamp, M. B. Tompkins, and W. A. Tompkins, Feline immunodeficiency virus (FIV) infection phenotypically and functionally activates immunosuppressive CD4+CD25+ T-regulatory (Treg) cells, *accepted for publication* (2004).
196. B. Singh, S. Read, C. Asseman, V. Malmstrom, C. Mottet, L. A. Stephens, R. Stepankova, H. Tlaskalova, and F. Powrie, Control of intestinal inflammation by regulatory T-cells, *Immunol. Rev.* 182, 190–200 (2001).
197. S. Hori, T. L. Carvalho, and J. Demengeot, CD25+CD4+ regulatory T-cells suppress CD4+ T-cell-mediated pulmonary hyperinflammation driven by Pneumocystis carinii in immunodeficient mice, *Eur. J. Immunol.* 32(5), 1282–1291 (2002).
198. R. A. Seder and R. Ahmed, Similarities and differences in CD4+ and CD8+ effector and memory T-cell generation, *Nat. Immunol.* 4(9), 835–842 (2003).

9

Drugs of Abuse, AIDS, and the FIV Model

MARGARET C. BARR, MARIA PIA TESTA, and TOM R. PHILLIPS

1. INTRODUCTION

Drug abuse is an important route of exposure to human immunodeficiency virus-1 (HIV-1). However, the role that drugs of abuse play in lentivirus disease progression remains unclear. There is a pressing need for appropriate animal models to examine the role of drugs of abuse on lentivirus disease progression. The feline immunodeficiency virus (FIV)/cat model is a legitimate system for the study of lentivirus-induced disease. Not only does FIV produce a disease that is very similar to human acquired immune deficiency syndrome (AIDS), but it is also similar to HIV-1 at both the molecular and biochemical levels. In this chapter, we will concentrate on the interactions of opiates and methamphetamine with lentivirus-induced disease, emphasizing the use of the FIV model.

The 2004 United Nations World Drug Report estimates that 3% of the world's population are users of illicit drugs (United Nations, 2004). Worldwide use of heroin and other opiates is estimated at 15 million users, while the global estimate of amphetamine and methamphetamine users is about 30 million (United Nations, 2004). A recent National Institute on Drug Abuse report (NIDA, 2000) estimates that 2.4 million people in the United Sates have used heroin; this number has remained stable for several years. In contrast, methamphetamine use has been increasing rapidly. In 1994, the National Household Study on Drug Abuse estimated that, in the United States, there were approximately 4 million people who have used methamphetamine. A recent estimate of methamphetamine use more than doubles that figure to 8.8 million (NIDA Research Reports Series, 2002).

MARGARET C. BARR, MARIA PIA TESTA, and TOM R. PHILLIPS • Western University of Health Sciences, College of Veterinary Medicine, 309 East Second Street, Pomona, CA 91766-1854

The precise interaction of drugs of abuse with HIV-1, the etiologic agent of AIDS, is not known. It is clear, however, that through the sharing of contaminated needles and syringes, intravenous drug use has become a common route of HIV-1 exposure. Increasingly, a larger proportion of HIV-infected individuals acquire the virus through drug use or are exposed to drugs of abuse during their disease course. Increased exposure to HIV-1 occurs from the rather obvious practice of sharing contaminated needles and syringes, but sexual transmission of HIV is also increased in the drug-abusing population. Illicit drug use impairs judgment in sexual practices, encourages sex-for-money or sex-for-drug exchanges, and is frequently employed for the purported aphrodisiac-like qualities of some agents (United Nations, 2004; Ross and Williams, 2001). The prevalence of HIV infection easily approaches 50% in certain regional populations of intravenous drug abusers, increasing to 90% in some cases (United Nations, 2004). Thus, in certain populations, there is a convergence of drugs of abuse and HIV-1; yet our understanding of the potential effects that simultaneous exposure to drugs of abuse and HIV-1 might have on disease progression is very limited.

Properly controlled epidemiological studies in the drug abuser population are extremely difficult to conduct. Surreptitious use of drugs, coinfection with other pathogens, time of infection, route of infection, viral strain, genetic factors, malnutrition, stress, environmental factors, dosage of drug used, frequency of drug use, and the lack of compliance with investigator's instructions are all confounding variables. Thus, to truly understand the complex interaction between drugs of abuse and lentivirus infections, both epidemiological studies and animal studies are needed.

2. THE FIV/CAT MODEL FOR AIDS PATHOGENESIS AND DRUG ABUSE

There are seven main reasons that the FIV/cat model is well suited for examining the effects of drugs of abuse on lentivirus neuropathogenesis: (1) both FIV and HIV-1 are lentiviruses and share many common structural and biochemical properties; (2) the clinical syndromes for both FIV and HIV-1 are remarkably similar; (3) the FIV/cat system has been developed into a reproducible disease model, with the establishment of reliable measures (detectable within 2 months postinfection) of both general and neurological disease progression; (4) the cost of cats is relatively low, thus appropriate numbers of animals can be employed in properly controlled studies, increasing the likelihood that meaningful results will be obtained; (5) invasive CNS studies are possible during early stages of the disease;

(6) specific pathogen-free (SPF) cats are available, eliminating confounding interactions with other feline pathogens; and (7) the nervous system of the cat has been well characterized, and the pharmacology of numerous compounds (including morphine and methamphetamine) has been thoroughly investigated in the feline species.

FIV Transmission and Pathogenesis

FIV was first identified as a lentivirus in cats by Niels Pedersen and colleagues (1987) at the University of California at Davis. FIV infection is associated with an immunodeficiency syndrome similar to human AIDS. Animals experimentally infected with FIV develop a lymphadenopathy similar to that found in human AIDS (Pedersen et al., 1987). FIV-induced feline AIDS (FIV-FAIDS) is primarily a disease of free-roaming cats. Bite wounds usually inflicted during cat fights are the primary mode of natural FIV transmission; however, vertical transmission does occur, and FIV has been transmitted experimentally using infected semen (Burkhard and Dean, 2003). Similar to HIV-1, FIV has a long latency, with an average age incidence of approximately 6 years (Ishida and Tomada, 1990). Chronic clinical conditions associated with FIV include: upper and lower respiratory infections, diarrhea, upper and lower urinary tract infections, thrush, skin conditions, gingivitis, lymphadenopathy, weight loss, anemia, ocular conditions, and various malignancies (reviewed in Burkhard and Dean, 2003; Uhl et al., 2002; Willis, 2000). FIV-infected SPF cats have reduced immune functions, including decreased lymphocyte proliferative responses to mitogens and T-dependent antigens (Barlough et al., 1991; Bishop et al., 1992; Lin et al., 1990). Particularly striking is the marked depletion of CD4+ cells, a hallmark of the progression of human AIDS, that is evident in both experimentally and field-infected cats (Burkhard and Dean, 2003). Ishida and Tomada (1990) have studied the natural pathogenesis of FIV and have found that after an acute phase, lasting weeks to months, an asymptomatic phase lasting up to 5 years can be observed prior to the onset of AIDS-related complex and AIDS. Animals typically die of a wasting condition, often exacerbated by opportunistic infections.

FIV Neuropathogenesis

Similar to HIV infection, neurological involvement is also noted early in FIV infection (Fox and Phillips, 2002; Podell et al., 2000). It is clear that infectious virus can be recovered from the brain and cerebrospinal fluid when inoculated by either the intracerebral or peripheral route (Dow et al., 1990; Phillips et al., 1994). In the absence of opportunistic infections,

pathologic findings include perivascular infiltrates of mononuclear cells, diffuse gliosis, glial nodules, and white matter pallor. These FIV-induced lesions are primarily located in the caudate nucleus, midbrain, and rostral brain stem, regions where methamphetamine also causes toxic effects (Hanson et al., 2004). Central nervous system (CNS) lesions are seen as early as 1 month after infection when the virus is given intracerebrally, and as early as 2 months after infection when the virus is inoculated intravenously. Thus, CNS entry for FIV is an early event, even when inoculated peripherally. FIV-infected cats can have delayed pupillary response, delayed righting reflex, anisocoria, delayed auditory and visual evoked potentials, decreased sensory and spinal conduction velocities, and disturbed sleep patterns (Henriksen et al., 1995; Phillips et al., 1994; Podell et al., 1993).

FIV Tropism

One of the major differences between FIV and HIV-1 is receptor usage. It appears that FIV, unlike HIV-1, does not use CD4 as a receptor (Brown et al., 1991; Dow et al., 1990; Hosie et al., 1993). Recently, a primary receptor for FIV has been identified as CD134 (OX40), a T-cell activation antigen and costimulatory molecule (Shimojima et al., 2004). Despite the evolutionary differences between the feline and human lentiviruses, both viruses use receptors that target the infection to a subset of T-cells that are critical for the production of an active immune response. FIV does use the same coreceptors as HIV-1. Like HIV-1, FIV uses the alpha chemokine receptor, CXCR4, as a coreceptor for cell entry (Richardson et al., 1999; Willet et al., 1997). Certain strains of FIV have also been demonstrated to use beta chemokine receptors to mediate FIV infection. Johnston and Power (2002) demonstrated that the V1CSF strain of FIV required the presence of either CCR5 or CCR3 as entry cofactors.

In spite of the partial differences in receptor usage, HIV-1 and FIV display similar patterns of cell tropism and disease pathogenesis within their respective hosts. The primary cellular targets of FIV in vivo include CD4+ and CD8+ T-lymphocytes as well as cells derived from the monocyte/macrophage lineage (Burkhard and Dean, 2003). This host cell range resembles the pattern of cell tropism displayed by HIV-1 (Levy, 1993). Like human AIDS, disease progression in FIV-infected cats is characterized by an inversion of the CD4/CD8 ratio that evolves due to a progressive decline in circulating CD4+ T-cells accompanied by a sustained increase in activated CD8+ T-cells (Burkhard and Dean, 2003).

Primary cultures of the microglia and astrocytes are productively infected with FIV in vitro. HIV also productively infects, in vitro, both astrocyte (Dewhurst et al., 1987; Erfle et al., 1991) and microglia (Sharpless et al., 1992) cultures. FIV does not infect neurons, but it is associated with

neuronal death, especially in the striatum (Zenger et al., 1997). The mechanism of neuronal damage is unclear, possibly resulting from the effects of FIV on the neuron supportive functions of astrocytes, toxic products released from infected microglia, or cytokines produced in response to the viral infection. Astrocytes are by far the most common cell type of the brain and are important in maintaining the neuronal microenvironment in the CNS. One of the most important functions of astrocytes is to regulate the level of extracellular glutamate, a major excitatory neurotransmitter that accumulates as a consequence of neuronal activity. Excessive levels of extracellular glutamate often result in neuronal toxicity and death. In vitro infection of feline astrocytes with FIV can significantly inhibit their glutamate scavenging ability (Billaud et al., 2000; Gruol et al., 1998; Yu et al., 1998). As is predicted by this finding, brain glutamate levels are increased in FIV-infected cats (Power et al., 1997). HIV-1 infected microglia cultures produce the neurotoxic compounds TNF-α, IL-1β, quinolinate, eicosanoids, and Ntox (Gendelman et al., 1994; Genis et al., 1992; Giulian et al., 1996). Microglia infected with FIV probably produce similar neurotoxins, although the relevance of these products in FIV-induced CNS pathogenesis requires further elucidation.

Genetic Characteristics of FIV

Marked similarities between HIV and FIV are also present at structural, molecular, and biochemical levels of the virus (reviewed in Miyazawa et al., 1994). Because FIV, like HIV, is a lentivirus, the two viruses share a number of properties, including a tRNA-lys primer-binding site, translation of the *pol* gene through ribosomal frameshifting, and a magnesium-dependent reverse transcriptase. In addition to the *gag*, *pol*, and *env* genes common to all retroviruses, both FIV and HIV have the coding capacity for small regulatory genes. A region is present between *pol* and *env* that, by analogy with other lentiviruses, encodes a potential transactivating domain (Sparger et al., 1992). Also present is an open reading frame following *pol* that coincides precisely, in size and location, to the HIV viral infectivity factor gene, *vif*. Both FIV Rev and a Rev responsive element (RRE) have been identified (Phillips et al., 1992). These FIV elements regulate viral mRNA expression in a manner that is similar to the HIV system. The reverse transcriptase enzymes of FIV and HIV resemble one another in their physical properties, catalytic activities, and sensitivities to several inhibitors (North et al., 1990a, 1990b).

Thus, the signs, lesions, nature, course, pathogenesis, and etiology of the syndrome in cats is markedly similar to human AIDS, demonstrating the relevance of the FIV/cat system as a model for the human disease. In recent years, our laboratory has concentrated on the use of the FIV/cat model to determine the effects of opiates and amphetamine derivatives on

lentivirus disease progression. Some of our findings, in context with the research of others, are presented below.

3. OPIATES AND THE FIV/CAT MODEL

Opiates have well-known physiological and psychological effects, including analgesia, euphoria, and diminished emotional distress, although these effects can be altered in chronic abusers (White, 2004). They are also considered to be immunosuppressive. The immunomodulatory properties of opiates and endogenous opioids are mediated directly through immune system cells or indirectly through the CNS. Opiates decrease lymphocyte proliferative responses to mitogen, decrease the CD4/CD8 ratio, and suppress natural killer (NK) cell activity. Morphine exposure reduces respiratory burst activity of monocytes. Through these activities, opiates depress primary and anamnestic antibody responses, phagocytic function, and delayed-type hypersensitivity reactions (Peterson et al., 1998).

The Feline Response to Morphine

Morphine is the primary metabolite of heroin, the most commonly abused opiate; thus, morphine is used to study the effects of opiates in vitro and in vivo. The feline pharmacological response to morphine has been thoroughly investigated and is nearly identical to the response seen in humans (de Andres et al., 1984; Harris et al., 1984; Villablanca et al., 1984). From the early studies investigating pharmacology of morphine in cats, a misconception arose that morphine does not induce a typical reaction in the feline species. These studies described an atypical response called feline mania. A dose response analysis was not performed, with morphine being given to cats at dosages that are commonly used in other species (>5–10 mg/kg). It was later shown that when the morphine dosage is reduced to 0.5–3 mg/kg given intraperitoneally, a typical morphine reaction is demonstrated in cats (Fertziger et al., 1974). At this reduced morphine dosage, the feline opiate response is characterized by three stages: (1) an autonomic stage (vomiting, swallowing, vocalization, and salivation); (2) a quiet stage (fixed gaze, sitting, mydriasis); and (3) a head movement stage (aroused, sitting, complex head movements typical of visual tracking, and pouncing/avoidance paw movements). The cats' sleep, urination, and defecation functions are all depressed, and an opiate dependence syndrome develops (de Andres et al., 1984; Harris et al., 1984; Villablanca et al., 1984). It was concluded from these later studies that when given the appropriate morphine dosage, the cat is an excellent model for studying the effects of opiates.

Feline Mu Opiate Receptor

To further develop and validate the feline model for examining the effects of drugs of abuse and their interactions with lentiviruses, an examination of the feline mu opiate receptor (MOR) was conducted. A partial sequencing of the feline MOR revealed a homology of 92 and 93% to the published human MOR sequences. MOR transcripts were present in the feline brain and tonsil, but not in the spleen. Using microphysiometry, the specific receptor-ligand interactions of the human and feline opiate receptors were shown to be similar (Billet et al., 2001).

Effects of Opiates on Lentivirus Infections

Intuitively, illicit drug use would seem likely to cause a deleterious effect on lentivirus disease progression. Because both opiates and lentiviruses are immunosuppressive, it seems logical that the combining of these two agents would lead to increased severity or more rapid progression of disease. However, the literature is filled with many conflicting data. In vitro, opiates have an enhancing effect on HIV-1 replication in several cell systems (Schweitzer et al., 1991, Peterson et al., 1990, 1994), but the in vivo effects of opiates on HIV-1 disease progression are murkier. Some epidemiological studies have demonstrated an increased frequency and severity of HIV-associated illnesses in intravenous drug users (Mientjes et al., 1991; Friedman et al., 1996), while others show no such association (Lyles et al., 1997), or even suggest a delayed progression to AIDS (Spijkerman et al., 1996). Animal studies have been similarly confusing. Morphine increased the severity of pneumonia in herpesvirus- and *Pasteurella*-infected swine, but decreased herpesvirus associated neurologic disease in the same animals (Risdahl et al., 1993). Chronic morphine exposure had no deleterious effects on the progression of Friend virus disease in mice (Starec et al., 1991) and had a protective effect in one study (Veyries et al., 1995). Likewise, contradictory effects were seen in studies on chronic morphine administration to simian immunodeficiency virus-infected monkeys (Donahoe and Vlahov, 1998).

It has been hypothesized that many of the animal studies did not correctly model the pattern of drug use in the abusing population, as many of the animal studies administered morphine in a chronic continuous manner. This pattern more accurately models methadone treatment than a pattern of street use (Kreek, 1990). The scheduling of morphine exposure in a modeling system has the potential to greatly affect the course of the disease. The patterns of chronic continuous exposure, multiple acute exposure, and the pattern of escalating drug use interspersed with periods of withdrawal might very well have different physiological effects, resulting

in different effects on lentivirus disease progression. To address these concerns, two separate FIV studies were conducted.

FIV/Cat Model for Occasional Opiate Abuse

In the first study (Barr et al., 2000), the FIV/cat system was used to model the weekend opiate abuser: the nondependent, nonaddicted, and nontolerant person. Sixteen cats were placed into four groups: cats infected with FIV (FIV-PPR strain) only, cats treated with morphine only, morphine-treated/FIV-infected cats, and untreated/uninfected control cats. Multiple acute morphine exposures, administered over a period of 26 weeks, did not increase the severity of early lentivirus infection. On the contrary, morphine exposure delayed or moderated the FIV-induced disease progression (Table I). Although the animals were exposed to only one injection of morphine (2.0 mg/kg) per day for 2 consecutive days each week, the morphine-treated/FIV-infected animals had a delayed onset of FIV-induced lymphadenopathy, did not develop or had a significant delay in the FIV-induced effects on brain stem auditory evoked potentials, and demon-

TABLE I
Effects of Opiate Exposure in FIV-Infected Cats

Study Paradigm	"Weekend User" Acute, intermittent exposure	"Dependent User" Escalating exposure and withdrawals
Morphine exposure	2.0 mg/kg/day, IM 2 days/week	1.0 mg/kg/day, increasing to 2.0 mg/kg/day, SQ 2-day and 2-week withdrawals
Physical examination	Delayed onset of lymphadenopathy in morphine-treated, FIV+ cats versus untreated, FIV+ cats	No significant differences between treated and untreated, FIV+ cats
T lymphocyte subsets	No significant differences between treated and untreated, FIV+ cats	No significant differences between treated and untreated, FIV+ cats
Plasma viremia	Trend toward lower levels in morphine-treated FIV+ cats—Not significant	No significant differences between treated and untreated, FIV+ cats
Cell-associated viremia	Trend toward lower levels in morphine-treated FIV+ cats—Not significant	No significant differences between treated and untreated, FIV+ cats
Plasma cortisol	No significant differences between treated and untreated, FIV+ cats	Significant elevation in cortisol levels during withdrawal periods in morphine-treated cats
Brainstem auditory evoked potentials	Protection against FIV-induced delays in auditory evoked potentials	Protection against FIV-induced delays in auditory evoked potentials

strated a trend toward decreased virus load. In this study, the pattern of drug exposures did not lead to morphine dependency and signs of withdrawal were not observed.

FIV Model for Opiate Addiction and Withdrawal

In the second study (Barr et al., 2003), morphine treatment of FIV-PPR-infected cats modeled a typical pattern of escalating drug use interspersed with withdrawals. Twenty-four cats were divided into four groups, as described for the first study. The cats were exposed to increasing doses of morphine (from 1.0 mg/kg/day to 1.5 mg/kg/day) or a placebo over 4 weeks, followed by a 2-day mini-withdrawal. Morphine administration was resumed, increasing from 1.5 mg/kg/day to 2.0 mg/kg/day over the next 3 weeks, then discontinued for 2 weeks. This 9-week cycle was repeated once. To demonstrate that stress was associated with morphine withdrawal, plasma cortisol levels were determined during the trial. In the morphine-treated cats, cortisol levels peaked at time points corresponding to each morphine withdrawal. These levels returned to baseline during morphine treatment and were normal when measured several weeks after the final withdrawal. Morphine-treated cats displayed clear behavioral and physical signs of opiate exposure and signs of withdrawal when the drug was stopped. Despite the evidence of stress associated with withdrawals, morphine exposure did not enhance the severity of FIV-related disease (Table I). Similar to the first study, morphine provided a significant protective effect on FIV-associated changes in brainstem auditory evoked potentials. Plasma viremia tended to be decreased in morphine-treated cats, although the effect was not statistically significant.

These studies collectively suggest that opiate exposure is unlikely to adversely affect the progression of acute lentivirus infection and might even be beneficial in controlling lentivirus-associated neurological disease. The pattern of opiate exposure does not seem to affect the outcome of morphine/FIV interactions. The mechanisms of the neuroprotective effect require further investigation.

4. METHAMPHETAMINE AND THE FIV/CAT MODEL

D-methamphetamine hydrochloride is a strong CNS stimulant. The physiological effects of methamphetamine include dilated pupils, increased heart and respiratory rates, elevated blood pressure, hyperthermia, decreased appetite, and increased wakefulness. The psychological effects of methamphetamine intake are associated with a feeling of euphoria, increased alertness and vigor, and increased social interactions (NIDA Research Report, 2004; Yu et al., 2003). High doses of methamphetamine

can cause stereotypical or repetitive behaviors. Methamphetamine can be ingested, smoked, inhaled, or injected, with the intravenous route of exposure to methamphetamine gaining in popularity. Intravenous drug use is often associated with the sharing of contaminated needles and syringes, a common method of HIV-1 transmission. Additionally, methamphetamine reduces inhibitions and increases the likelihood of unsafe sexual practices among its abusers, increasing exposure to HIV-1 (Ross and Williams, 2003).

Feline Response to Methamphetamine

Cats have been frequently used to study the effects of methamphetamine, with doses ranging from 0.04 mg/kg/injection to as high as 50 mg/kg/day (reviewed in Phillips et al., 2000). Additionally, cats, like other species, can be conditioned to self-administer methamphetamine (Balster et al., 1976). What is not apparent from the early literature is that higher methamphetamine doses can only be safely achieved by gradually increasing the level of drug that is given to the animals, and that a number of cats may succumb to toxic methamphetamine reactions. In fact, doses as low as 1.0 mg/kg/day produce readily demonstrable behavioral effects as well as a typical methamphetamine-induced temperature elevation (Phillips et al., 2000; Huitron-Resendiz et al., submitted for publication). The elevation in temperature varies between individual cats (Figure 1) and can be lethal in some cats if it is not controlled. These data show that the feline is very sensitive to low doses of methamphetamine.

Effects of Methamphetamine on Lentivirus Infections

Interactions between methamphetamine and HIV-1 can occur through several means. One area of clear convergence is the effect of these two agents on the CNS. Both lentiviruses and methamphetamine produce toxic changes in the deep gray nuclei, in particular the mesostriatal and corticostriatal neural elements. The neurotoxic properties of methamphetamine have been the subject of two recent reviews (Hanson et al., 2004; Kita et al., 2003). Methamphetamine acts directly on neurons by mimicking the action of the neurotransmitter, dopamine. The neurotoxic effects of methamphetamine on the brain monoamine systems are well characterized and include eventual loss of dopamine and serotonin terminals, the production of reactive oxygen radicals leading to oxidative stress, disruption of mitochondrial function, and neuronal necrosis or apoptosis. On the other hand, the effect of HIV-1 on neurons is believed to be indirect, primarily through the action of toxic products from the virus, such as gp 120 and Tat, and from substances released from astrocytes and microglial cells, such as TNF-α, IL1-β, and superoxides (Gendelman et al., 1994; Genis et al., 1992; Giulian et al., 1996). Neurons are also damaged

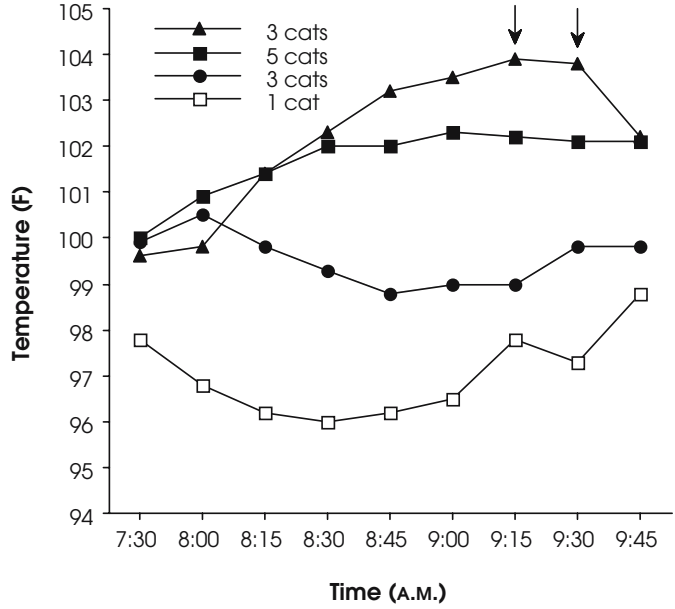

FIGURE 1. Response of cats to methamphetamine (METH). Twelve specific pathogen-free cats were administered 1.0 mg/kg of METH orally at 7:30 a.m., and their body temperature was recorded every 15 minutes over the next 2 hours. Three types of temperature responses were observed in the cats: (1) Four cats (□, ◆) had body temperatures that remained relatively stable, or decreased, following METH exposure; (2) five cats (■) experienced a self-limiting rise in temperature; and (3) three cats (▲) had body temperature increases of more than 4°F, requiring cooling intervention (arrows).

when the virus disrupts the normal support functions of astrocytes, including glutamate uptake and recycling.

A second area of potential interactions between methamphetamine and lentiviruses is in the immune system. Information on the effects of methamphetamine on immune function is limited. Methamphetamine does have some immunomodulatory properties, including the suppression of IL-2 and IFN-γ and the enhancement of TNF-α production in mice (Yu et al., 2003). High doses of methamphetamine also induce apoptosis of thymic and splenic lymphocytes in rats (Isawa et al., 1996).

The FIV model has been used to investigate the effects of simultaneous exposure to methamphetamine and lentivirus infection. Initial investigations into the interaction between methamphetamine and FIV replication were performed in vitro. In a study in our laboratory, a concentration dependent effect of methamphetamine on FIV replication was demonstrated, with the highest concentration of methamphetamine causing a threefold increase in FIV replication (Phillips et al., 2000).

Another research group found that methamphetamine significantly increased FIV replication in a feline astrocyte line, although the effect was lost for astrocyte-adapted virus (Gavrilin et al., 2002).

FIV Model for Binge Use of Methamphetamine

In our most recent study (Huitron-Resendiz et al., submitted for publication), we modeled the bingeing methamphetamine user with sequential, repeated periods of abstinence. Twenty-four cats (six cats per group) were given methamphetamine (1.0 mg/kg, *per os*) or a placebo daily for 5 consecutive days, followed by 2 drug-free weeks. This pattern of methamphetamine exposure was repeated over a 17-week period. Two groups of cats (FIV-infected and methamphetamine-treated/FIV-infected) were infected with FIV-PPR on the fifth day of the first methamphetamine exposure. Some cats experienced severe hyperthermic responses at the 1.0 mg/kg dose (Figure 1); subsequent methamphetamine doses were decreased for these cats. In this model, methamphetamine exposure significantly accelerated and enhanced the FIV-induced CNS functional pathology, as measured by delays in brainstem auditory evoked potentials, an indication of early lentivirus-induced CNS disease. Methamphetamine treatment also accelerated the onset of FIV-induced lymphadenopathy, a clinical sign of FIV infection. Reciprocally, FIV enhanced the methamphetamine-induced effects on brain. In the caudate nucleus, both dopamine (DA) and 3,4-dihydroxyphenylacetic acid (DOPAC) levels were significantly lower and the 5-hydroxyindol-3-acetic acid (5-HIAA)/serotonin (5-HT) ratio was significantly higher in the FIV- and methamphetamine-treated cats relative to the values found in either the methamphetamine-treated or FIV-infected alone groups. Similarly, simultaneous FIV and methamphetamine exposure resulted in lower dopamine transporter (DAT) levels than did methamphetamine exposure alone.

Brain extracts of frontal gray matter, frontal white matter, and caudate from these same cats were examined by proton magnetic resonance spectroscopy (Cloak et al., 2004). Similar to HIV's effect on brain metabolites, the FIV-infected cats exhibited decreases in choline, creatine, and glutamate in the frontal white matter. The decrease in glutamate was an unexpected finding and conflicted with earlier studies (Power et al., 1997); perhaps the early stage of infection or the region of brain selected for evaluation was responsible for the difference in results. The FIV-induced decrease in glutamate normalized in the methamphetamine-treated/FIV-infected cats. Clearly, additional research must be done to determine the effects of methamphetamine and FIV infection on regional brain metabolites.

The results of this study indicate that in concurrent FIV infection and methamphetamine exposure, a dual potentiation occurred. That is,

methamphetamine enhanced certain aspects of FIV-induced CNS disease, and FIV enhanced some of the toxic effects of methamphetamine. The interactions are complex and may vary depending upon the area of the brain that is being examined. Contrary to our predictions, based on earlier in vitro findings, methamphetamine did not increase peripheral virus load. This finding emphasizes the fact that neurologic disease progression does not necessarily mirror progression of systemic FIV infection. Further work must be done to elucidate the mechanisms by which FIV and methamphetamine interact to achieve their neurotoxic synergism, and to determine patterns of FIV expression in the CNS.

5. CONCLUSION

With the two drugs of abuse discussed above, we see very different effects on FIV disease progression. With morphine, enhanced lentivirus disease progression was not detected, and in fact, certain measures of FIV disease progression did not develop or had a significant delay in their onset. However, we see a very different pattern when cats are simultaneously exposed to both methamphetamine and FIV. Not only was FIV disease progression enhanced by methamphetamine, but also FIV significantly enhanced the methamphetamine-induced neurotoxicity. Frequently, in the drug-abusing populations, it is common for several different illicit drugs to be used at the same time. The impact on lentivirus disease progression in any given individual will likely depend upon which drugs are being used, in what combination, and at what dose and frequency of use. The FIV model should be helpful in understanding the underlying mechanisms as well as the complex interactions that are occurring between drugs of abuse and lentivirus disease progression.

REFERENCES

Balster, R. L., Kilbey, M. M., and Ellinwood, E. H., Jr. (1976). Methamphetamine self-administration in the cat. *Psychopharmacologia* 46:229–233.

Barlough, J. E., Ackley, C. D., George, J. W., Levy, N., Acevedo, R., Moore, P. F., Rideout, B. A., Cooper, M. D., and Pedersen, N. C. (1991). Acquired immune dysfunction in cats with experimentally induced feline immunodeficiency virus infection: comparison of short-term and long-term infections. *J. Acquir. Immune Defic. Syndr.* 4:219–227.

Barr, M. C., Billaud, J. N., Selway, D. R., Huitron-Resendiz, S., Osborn, K. G., Henriksen, S. J., and Phillips, T. R. (2000). Effects of multiple acute morphine exposures on feline immunodeficiency virus disease progression. *J. Infect. Dis.* 182:725–732.

Barr, M. C., Huitron-Resendiz, S., Sanchez-Alavez, M., Henriksen, S. J., and Phillips, T. R. (2003). Escalating morphine exposures followed by withdrawal in feline immunodeficiency virus-infected cats: a model for HIV infection in chronic opiate abusers. *Drug Alcohol Depend.* 72:141–149.

Billaud, J. N., Selway, D., Yu, N., and Phillips, T. R. (2000). Replication rate of feline immunodeficiency virus in astrocytes is envelope dependent: implications for glutamate uptake. *Virology* 266:180–188.

Billet, O., Billaud, J. N., and Phillips, T. R. (2001). Partial characterization and tissue distribution of the feline mu opiate receptor. *Drug Alcohol Depend.* 62:125–129.

Bishop, S. A., Williams, N. A., Gruffydd, J. T., Harbour, D. A., and Stokes, C. R. (1992). Impaired T-cell priming and proliferation in cats infected with feline immunodeficiency virus. *AIDS* 6:287–293.

Brown, W. C., Bissey, L., Logan, K. S., Pedersen, N. C., Elder, J. H., and Collisson, E. W. (1991). Feline immunodeficiency virus infects both CD4+ and CD8+ T lymphocytes. *J. Virol.* 65:3359–3364.

Burkhard, M. J. and Dean, G. A. (2003). Transmission and immunopathogenesis of FIV in cats as a model for HIV. *Curr. HIV Res.* 1:15–29.

Cloak, C. C., Chang, L., Ernst, T., Barr, M. C., Huitron-Resendiz, S., Sanchez-Alavez, M., Phillips, T. R., and Henriksen, S. (2004). Methamphetamine and AIDS: ^{1}HMRS studies in a feline model of human disease. *J. Neuroimmunol.* 147:16–20.

de Andres, I., Villablanca, J. R., and Burgess, J. W. (1984). Reassessing morphine effects in cats: II. Protracted effects on sleep-wakefulness and the EEG. *Pharmacol. Biochem. Behav.* 21:923–928.

Dewhurst, S., Sakai, K., Bresser, J., Stevenson, M., Evinger-Hodges, M. J., and Volsky, D. J. (1987). Persistent productive infection of human glial cells by human immunodeficiency virus (HIV) and by infectious molecular clones of HIV. *J. Virol.* 61:3774–3782.

Donahoe, R. M. and Vlahov, D. (1998). Opiates as potential cofactors in progression of HIV-1 infections to AIDS. *J. Neuroimmunol.* 83:77–87.

Dow, S. W., Poss, M. L., and Hoover, E. A. (1990). Feline immunodeficiency virus: a neurotropic lentivirus. *J Acquir. Immune Defic. Syndr.* 3:658–668.

Erfle, V., Stoeckbauer, P., Kleinschmidt, A., Kohleisen, B., Mellert, W., Stavrou, D., and Brack-Werner, R. (1991). Target cells for HIV in the central nervous system: macrophages or glial cells? *Res. Virol.* 142:139–144.

Fox, H. S. and Phillips, T. R. (2002). FIV and neuroAIDS. *J. Neurovirol.* 8:155–157.

Friedman, L. N., Williams, M. T., Singh, T. P., and Frieden, T. R. (1996). Tuberculosis, AIDS, and death among substance abusers on welfare in New York City. *N. Engl. J. Med.* 334:828–833.

Gavrilin, M. A., Mathes, L. E., and Podell, M. (2002). Methamphetamine enhances cell-associated feline immunodeficiency virus replication in astrocytes. *J. Neurovirol.* 8:240–249.

Gendelman, H. E., Lipton, S. A., Tardieu, M., Bukrinsky, M. I. and Nottet, H. S. L. M. (1994). The neuropathogenesis of HIV-1 infection. *J. Leukoc. Biol.* 56:389–398.

Genis, P., Jett, M., Bernton, E. W., Boyle, T., Gelhard, H. A., Dzenko, K., Keane, R. W., Resnick, L., Mizrachi, Y., Volski, D. J., Epstein, L. G., and Gendelman, H. E. (1992). Cytokines and arachidonic metabolites produced during human immunodeficiency virus (HIV)-infected macrophage-astroglia interactions: implications for the neuropathogenesis of HIV Disease. *J. Exp. Med.* 176:1703–1718.

Giulian, D., Yu, J., Tom, D., Li, J., Wendt, E., Lin, S. N., Schwarcz, R., and Noonan, C. (1996). Study of receptor-mediated neurotoxins released by HIV-1-infected mononuclear phagocytes found in human brain. *J. Neurosci.* 16:3139–3153.

Gruol, D. L., Yu, N., Parsons, K. L., Billaud, J. N., Elder, J. H., and Phillips, T. R. (1998). Neurotoxic effects of feline immunodeficiency virus, FIV-PPR. *J. Neurovirol.* 4:415–425.

Hanson, G. R., Rau, K. S., and Fleckenstein, A. E. (2004). The methamphetamine experience: a NIDA partnership. *Neuropharmacology* 47 (Suppl. 1):92–100.

Harris, C. M., Villablanca, J. R., Burgess, J. W., and de Andres, I. (1984). Reassessing morphine effects in cats: III. Responses of intact, caudate nuclei-lesioned and hemispherec-

tomized animals following chronic administration and precipitated withdrawal. *Pharmacol. Biochem. Behav.* 21:929–936.

Henriksen, S. J., Prospero-Garcia, O., Phillips, T. R., Fox, H. S., Bloom, F. E., and Elder, J. H. (1995). Feline immunodeficiency virus as a model for study of lentivirus infection of the central nervous system. In Oldstone, M. and Vitkovic, L. (eds.), *Current topics in Microbiology and Immunology: HIV and Dementia* (vol. 202, pp. 167–186). New York: Springer-Verlag.

Huitron-Resendiz, S., Barr, M. C., Choi, S. J., Crawford, E., Leutenegger, C. M., Parsons, L. H., Henriksen, S. J., and Phillips, T. R. Methamphetamine and lentivirus interactions: reciprocal enhancement of CNS disease. *Submitted for publication.*

Hosie, M. J., Willett, B. J., Dunsford, T. H., Jarrett, O., and Neil, J. C. (1993). A monoclonal antibody which blocks infection with feline immunodeficiency virus identifies a possible non-CD4 receptor. *J. Virol.* 67:1667–1671.

Isawa, M., Maeno, Y., Inoue, H., Koyama, H., and Matoba, R. (1996). Induction of apoptotic cell death in rat thymus and spleen after a bolus injection of methamphetamine. *Int. J. Legal Med.* 109:23–28.

Ishida, T. and Tomoda, I. (1990). Clinical staging of feline immunodeficiency virus infection. *Nippon Juigaku Zasshi* 52:645–648.

Johnston, J. B. and Power, C. (2002). Feline immunodeficiency virus xenoinfection: the role of chemokine receptors and envelope diversity. *J. Virol.* 76:3626–3636.

Kita, T., Wagner, G. C., and Nakashima, T. (2003). Current research on methamphetamine-induced neurotoxicity: animal models of monoamine disruption. *J. Pharmacol. Sci.* 92:178–195.

Kreek, M. J. (1990). Immune function in heroin addicts and former heroin addicts in treatment: pre- and post-AIDS epidemic. *NIDA Res. Monogr.* 96:192–219.

Levy, J. A. 1993. Pathogenesis of human immunodeficiency virus infection. *Microbiol. Rev.* 57:183–289.

Lin, D. S., Bowman, D. D., Jacobson, R. H., Barr, M. C., Fevereiro, M., Williams, J. R., Noronha, F. M., Scott, F. W., and Avery, R. J. (1990). Suppression of lymphocyte blastogenesis to mitogens in cats experimentally infected with feline immunodeficiency virus. *Vet. Immunol. Immunopathol.* 26:183–189.

Lyles, C. M., Margolick, J. B., Astemborski, J., Graham, N. M., Anthony, J. C., Hoover, D. R., and Vlahov, D. (1997). The influence of drug use patterns on the rate of CD4+ lymphocyte decline among HIV-1-infected injecting drug users. *AIDS* 11:1255–1262.

Mientjes, G. H., Miedema, F., van Ameijden, E. J., van den Hoek, A. A., Schellekens, P. T., Roos, M. T., and Coutinho, R. A. (1991). Frequent injecting impairs lymphocyte reactivity in HIV-positive and HIV-negative drug users. *AIDS* 5:35–41.

Miyazawa, T., Tomonaga, K., Kawaguchi, Y., and Mikami, T. (1994). The genome of feline immunodeficiency virus. *Arch. Virol.* 134:221–234.

NIDA. Research Report Series. Heroin: abuse and addiction (1997; reprinted 2000). Bethesda. Updated December 7, 2004. Retrieved from January 6, 2005 http://www.drugabuse.gov/ResearchReports/Heroin/heroin2.html.

NIDA. Research Report Series. Methamphetamine: abuse and addiction (1998; reprinted 2002). Bethesda. Updated December 7, 2004. Retrieved from http://www.nida.nih.gov/ResearchReports/methamph/methamph.html.

North, T. W., Cronn, R. C., Remington, K. M., and Tandberg, R. T. (1990a). Direct comparisons of inhibitor sensitivities of reverse transcriptases from feline and human immunodeficiency viruses. *Br. Vet. J.* 146:468–475.

North, T. W., Cronn, R. C., Remington, K. M., Tandberg, R. T., and Judd, R. C. (1990b). Characterization of reverse transcriptase from feline immunodeficiency virus. *J. Biol. Chem.* 265:5121–5128.

Peterson, P. K., Gekker, G., Hu, S., Anderson, W. R., Kravitz, F., Portoghese, P. S., Balfour, H. H., Jr., and Chao, C. C. (1994). Morphine amplifies HIV-1 expression in chronically infected promonocytes cocultured with human brain cells. *J. Neuroimmunol.* 50:167–175.

Pedersen, N. C., Ho, E. W., Brown, M. L., and Yamamoto, J. K. (1987). Isolation of a T-lymphotropic virus from domestic cats with an immunodeficiency-like syndrome. *Science* 235:790–793.

Peterson, P. K., Sharp, B. M., Gekker, G., Portoghese, P. S., Sannerud, K., and Balfour, H. H., Jr. (1990). Morphine promotes the growth of HIV-1 in human peripheral blood mononuclear cell cocultures. *AIDS* 4:869–873.

Peterson, P. K., Molitor, T. W., and Chao, C. C. (1998). The opioid-cytokine connection. *J. Neuroimmunol.* 83:63–69.

Phillips, T. R., Billaud, J. N., and Henriksen, S. J. (2000). Methamphetamine and HIV-1: potential interactions and the use of the FIV/cat model. *J. Psychopharmacol.* 14:244–250.

Phillips, T. R., Lamont, C., Konings, D. A. M., Shacklett, B. L., Hamson, C. A., Luciw, P. A., and Elder, J. H. (1992). Identification of the rev transactivation and rev-responsive elements of feline immunodeficiency virus. *J. Virol.* 66:5464–5471.

Phillips, T. R., Prospero-Garcia, O., Puaoi, D. L., Lerner, D. L., Fox, H. S., Olmsted, R. A., Bloom, F. E., Henriksen, S. J., and Elder, J. H. (1994). Neurological abnormalities associated with feline immunodeficiency virus infection. *J. Gen. Virol.* 75:979–987.

Podell, M., March, P. A., Buck, W. R., and Mathes, L. E. (2000). The feline model of neuroAIDS: understanding the progression towards AIDS dementia. *J. Psychopharmacol.* 14:205–213.

Podell, M., Oglesbee, M., Mathes, L., Krakowka, S., Olmsted, R., and Lafrado, L. (1993). AIDS-associated encephalopathy with experimental feline immunodeficiency virus infection. *J. Acquir. Immune Defic. Syndr.* 6:758–771.

Power, C., Moench, T., Peeling, J., Kong, P. A., and Langelier, T. (1997). Feline immunodeficiency virus causes increased glutamate levels and neuronal loss in brain. *Neuroscience* 77:1175–1185.

Richardson, J., Pancino, G., Merat, R., Leste-Lasserre, T., Moraillon, A., Schneider-Mergener, J., Alizon, M., Sonigo, P., and Heveker, N. (1999). Shared usage of the chemokine receptor CXCR4 by primary and laboratory-adapted strains of feline immunodeficiency virus. *J. Virol.* 73:3661–3671.

Risdahl, J. M., Peterson, P. K., Chao, C. C., Pijoan, C., and Molitor, T. W. (1993). Effects of morphine dependence on the pathogenesis of swine herpesvirus infection. *J. Infect. Dis.* 167:1281–1287.

Ross, M. W. and Williams, M. L. (2001). Sexual behavior and illicit drug use. *Annu. Rev. Sex Res.* 12:290–310.

Schweitzer, C., Keller, F., Schmitt, M. P., Jaeck, D., Adloff, M., Schmitt, C., Royer, C., Kirn, A., and Aubertin, A. M. (1991). Morphine stimulates HIV replication in primary cultures of human Kupffer cells. *Res. Virol.* 142:189–195.

Sharpless, N. E., O'Brien, W. A., Verdin, Kufta, C. V., Chen, I. S., and Dubois, D. M. (1992). Human immunodeficiency virus type 1 tropism for brain microglial cells is determined by a region of the env glycoprotein that also controls macrophage tropism. *J. Virol.* 66:2588–2593.

Shimojima, M., Miyazawa, T., Ikeda, Y., McMonagle, E. L., Haining, H., Akashi, H., Takeuchi, Y., Hosie, M. J., and Willett, B. J. (2004). Use of CD134 as a primary receptor by the feline immunodeficiency virus. *Science* 303:1192–1195.

Sparger, E. E., Shacklett, B. L., Renshaw, G. L., Barry, P. A., Pedersen, N. C., Elder, J. H., and Luciw, P. A. (1992). Regulation of gene expression directed by the long terminal repeat of the feline immunodeficiency virus. *Virology* 187:165–177.

Spijkerman, I. J., Langendam, M. W., Veugelers, P. J., van Ameijden, E. J., Keet, I. P., Geskus, R. B., van den Hoek, A., and Coutinho, R. A. (1996). Differences in progression to AIDS

between injection drug users and homosexual men with documented dates of seroconversion. *Epidemiology* 7:571–577.

Starec, M., Rouveix, B., Sinet, M., Chau, F., Desforges, B., Pocidalo, J. J., and Lechat, P. (1991). Immune status and survival of opiate- and cocaine-treated mice infected with Friend virus. *J. Pharmacol. Exp. Ther.* 259:745–750.

Uhl, E. W., Heaton-Jones, T. G., Pu, R., and Yamamoto, J. K. (2002). FIV vaccine development and its importance to veterinary and human medicine: a review. FIV vaccine 2002 update and review. *Vet Immunol. Immunopathol.* 90:113–132.

United Nations, Office on Drugs and Crime. World Drug Report (2004). Vienna. Retrieved from January 3, 2005 http://unodc.org/world_drug_report.html.

Veyries, M. L., Sinet, M., Desforges, B., and Rouveix, B. (1995). Effects of morphine on the pathogenesis of murine Friend retrovirus infection. *J. Pharmacol. Exp. Ther.* 272:498–504.

Villablanca, J. R., Harris, C. M., Burgess, J. W., and de Andres, I. (1984). Reassessing morphine effects in cats: I. Specific behavioral responses in intact and unilaterally brain-lesioned animals. *Pharmacol. Biochem. Behav.* 21:913–921.

White, J. M. (2004). Pleasure into pain: the consequences of long-term opioid use. *Addict. Behav.* 29:1311–1324.

Willett, B. J., Picard, L., Hosie, M. J., Turner, J. D., Adema, K., and Clapham, P. R. (1997). Shared usage of the chemokine receptor CXCR4 by the feline and human immunodeficiency viruses. *J. Virol.* 71:6407–6415.

Willis, A. M. (2000). Feline leukemia virus and feline immunodeficiency virus. *Vet. Clin. North Am. Small Anim. Pract.* 30:971–986.

Yu, N., Billaud, J. N., and Phillips, T. R. (1998). Effects of feline immunodeficiency virus on astrocyte glutamate uptake: implications for lentivirus-induced central nervous system diseases. *Proc. Natl. Acad. Sci. USA* 95:2624–2629.

Yu, Q., Larson, D. F., and Watson, R. R. (2003). Heart disease, methamphetamine, and AIDS. *Life Sciences* 73:129–140.

Zenger, E., Tiffany-Castiglioni, E., and Collisson, E. W. (1997). Cellular mechanisms of feline immunodeficiency virus (FIV)-induced neuropathogenesis. *Front. Biosci.* 2:d527–537.

10

FIV as a Model for AIDS Vaccine Studies

STEPHEN DUNHAM and OSWALD JARRETT

1. INTRODUCTION

Many experimental strategies have been adopted in experiments to protect cats from FIV infection by vaccination, and some have been successful. The interest in developing a vaccine arose both because FIV is a common cause of morbidity and mortality in pet cats and because the feline virus provides a model for its counterpart in man, human immunodeficiency virus (HIV), for which an effective vaccine is urgently required to halt the current tragic pandemic of acquired immunodeficiency syndrome (AIDS). Shortly after the discovery of FIV and its characterization as a lentivirus,[54] attempts were made to produce a vaccine and success was soon achieved with relatively simple inactivated virus or inactivated virus-infected cell vaccines.[82] Further development of this approach led to the introduction in 2002 of the first commercial vaccine against FIV.[59] With an estimated prevalence of the infection of up to 25% in populations of pet cats, an effective FIV vaccine could have a significant influence on animal welfare. In addition, this success poses the question of whether a similar strategy might produce an effective vaccine against HIV.

Many candidate HIV vaccines have been constructed and a number are currently undergoing clinical trials in human populations. The results from the first phase III trial, which was designed to test the ability of a vaccine based on the envelope surface glycoprotein (SU) to protect against exposure to HIV in a high-risk population, were not encouraging. Much of the slow progress in producing HIV vaccines to take to phase III trials is because there is no ideal surrogate host in which to carry out experiments that would confidently predict whether a candidate vaccine was

STEPHEN DUNHAM AND OSWALD JARRETT • Retrovirus Research Laboratory, Institute of Comparative Medicine, University of Glasgow, Faculty of Veterinary Medicine, Bearsden Road, Glasgow G61 1QH, U.K.

indeed protective or would identify the correlates of protection. Even in the chimpanzee, the putative source of the simian immunodeficiency virus (SIV) from which HIV was originally derived, the dynamics of experimental HIV infection are sufficiently different from those in man to render the model problematic. SIV itself is a plausible model system in which to test vaccine strategies and has been used extensively for this purpose as described in Chapters 5 and 6. Although these simian viruses do not appear to cause disease in their natural hosts—various primate species in Africa—they do cause a fatal immunodeficiency upon inoculation of Asian macaques that is very similar to human AIDS. This outcome may very well mimic the situation that has developed in man, who has yet to coevolve sufficiently with HIV to render that virus less pathogenic. Many SIV vaccines have been tested in macaques, but still there is no consensus about what constitutes a vaccine that could serve as an example for HIV. The apparent effectiveness of the recently introduced commercial FIV vaccine raises the questions of how good FIV is as a comparative model system for HIV in vaccination studies, and what weight should be put on the results from FIV vaccine trials in making decisions about the type of HIV vaccine to be tested in clinical trials in man.

In this review we examine the similarities between FIV and HIV that make the feline virus an excellent model with which to pursue vaccine strategies for human AIDS. We then discuss the crucial issues that must be considered and the choices that have to be made in producing FIV vaccines. Finally, we discuss the results of experimental trials of a selection of FIV vaccines that might influence the development of vaccines for HIV.

2. RELEVANCE OF FIV TO HIV VACCINATION

How similar are FIV and HIV? Certainly there are differences between the two viruses in biology and natural history. Thus, FIV has a different genome content and organization from HIV, as illustrated in Figure 1. In nature FIV is transmitted mainly by biting, rather than through a mucosal surface like HIV. Following infection, a proportion of infected cats develop AIDS, although many individuals do not develop terminal disease.[1] To enter cells, primary isolates of FIV use CD134 as a receptor[66] and CXCR4 as a coreceptor.[78] In contrast HIV-1 uses CD4 as a primary receptor and CCR5 and other chemokine receptors, including CXCR4, as a coreceptor. However, despite these differences, both viruses critically target $CD4^+$ T lymphocytes and the outcome of infection is remarkably similar in cats and man. In the present context, the dynamics of infection and the resulting immune response are so much alike following infection with either virus that FIV should be able to provide valuable information to aid the development of HIV vaccines. It is the common features of FIV and HIV rather

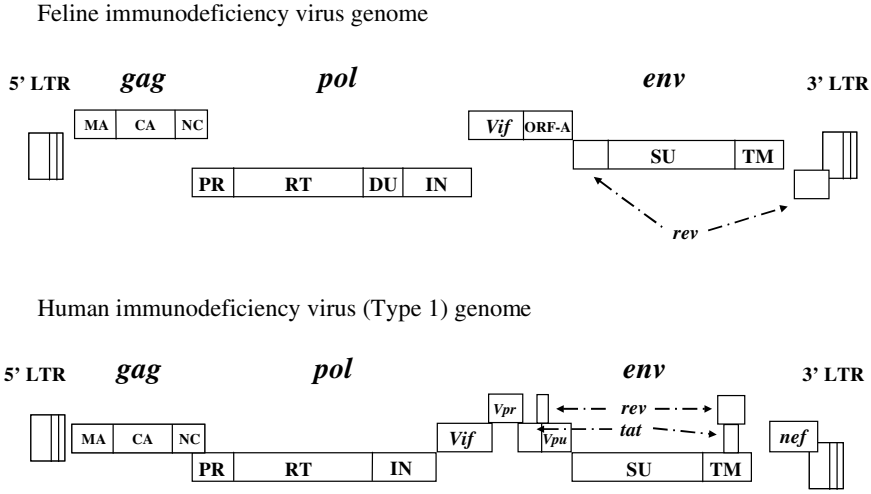

FIGURE 1. Comparison of FIV and HIV-1 genomes.

than their differences that should guide us to the essential requirements for successful protection by vaccination against each infection.

The FIV Genome

FIV, like HIV, is a complex retrovirus, with a genome approximately 10 kb in size, containing accessory genes in addition to *gag*, *pol*, and *env* (Figure 1). A comparison of the nucleotide sequences of representative FIV and HIV sequences (FIV Petaluma M25381 and HIV NC_001802) reveals an overall homology of approximately 63% in *gag-pol* and 76% in *env*. The corresponding proteins exhibit lower homology of 43% and 35% respectively (comparisons made using GCG Bestfit, University of Wisconsin). The feline *pol* gene, like HIV, encodes protease, integrase, and reverse transcriptase proteins, which have analogous functions in each of the viruses. FIV *pol* also encodes an additional enzyme, deoxyuridine pyrophosphatase (DU), not found in primate lentiviruses, which reduces cellular concentrations of dUTP, preventing viral mutations due to its misincorporation into viral cDNA. The activity of viral DU is particularly important in nondividing cells such as macrophages, which have low endogenous levels of the host enzyme. FIV mutants lacking DU are consequently unable to replicate productively in macrophages.[40] Although DU is absent in HIV, its role is assumed by HIV Vpr, which directs incorporation of uracil DNA glycosylase (UNG2) into HIV virions, thereby reducing misincorporation of uracil and facilitating replication in macrophages.[9]

The export of unspliced and singly spliced viral RNAs from the nucleus requires the presence of Rev, which is encoded by both HIV and FIV genomes. Rev mediates nuclear export by binding to the Rev response element (RRE). For FIV the RRE is located 3′ to the transmembrane (TM) protein of Env, whereas in HIV the RRE is 5′ to TM. The *vif* (viral infectivity factor) gene is also common to both FIV and HIV and functions in a similar way for both viruses. Vif expression is critical for viral dissemination in vivo and infection of nonpermissive cells including lymphocytes and macrophages. Recent evidence has shown that HIV Vif acts by abrogating the effect of the cellular antiretroviral factor APOBEC3G,[65] and it is likely that FIV Vif acts in an analogous manner. FIV lacks a *tat* gene and its corresponding transactivation response (TAR) element. Additionally, the FIV genome does not encode Nef, Vpr, or Vpu. It does, however, encode an additional accessory protein designated ORF-A or ORF-2. ORF-A was initially considered as a *tat*-like gene because of its ability to transactivate viral transcription at low levels. However, it shares several properties with HIV Vpr, including nuclear localization and induction of G2 cell cycle arrest.[22]

Knowledge of the genetic structure of FIV allows the use of virus mutants as experimental vaccines. For example, the deletion of accessory genes such as ORF-A or *vif* can result in the production of attenuated viruses that can be utilized as vaccines.[43] DNA vaccines have also been produced using full-length molecular clones of provirus, which are rendered replication defective by in-frame deletion of either the reverse transcriptase or integrase genes.[16,29]

FIV Infection in Cats

Most natural FIV infections are acquired by biting, presumably through the inoculation of virus, or virus-infected cells, from the saliva of persistently infected cats. Cats are readily infected by parenteral inoculation of virus, for example, by the intravenous, intraperitoneal, or intramuscular route. Transmission from mother to her kittens may occur but only a proportion of the offspring become persistently infected. Although neither oronasal or venereal spread has been documented in nature, cats can be infected by experimental inoculation of virus into the nose, mouth, vagina, and rectum[51] and the virus can be recovered from semen following natural or experimental infection.[37] In each case, inoculation results in the infection of lymphoid and myelomonocytic cells, which become reservoirs that maintain a persistent infection.

FIV closely resembles HIV in the dynamics of its growth in vivo. Thus, in the first few days following experimental inoculation, the virus grows in

dendritic cells, macrophages, and $CD4^+$ T lymphocytes and is found in the plasma within 2 weeks. The level of virus in the plasma and proviral DNA in the blood mononuclear cells rises over a period, reaching a peak at about 8 to 12 weeks. During this period, mild to moderate clinical signs, for example anorexia, depression, and pyrexia, may be observed that are associated with the initial uninhibited growth of the virus. These conditions generally subside rapidly, although signs such as generalized lymphadenopathy, due to increased numbers and size of active germinal centers in the cortex of the lymph nodes, may continue for several weeks or months. The fall in plasma viral load heralds the beginning of the so-called asymptomatic phase that can last for many years, and in many cases for life, during which the cat is quite healthy. It is assumed that virus replication is brought under control by the developing immune response to the virus. $CD8^+$ FIV-specific cytotoxic T-cells (CTL) can be detected in the blood within one week of infection.[3] Rather later, at around the same time as the peak in virus load, anti-FIV antibodies, including virus neutralizing antibodies, appear in the plasma.[17]

Another hallmark of early FIV infection by certain isolates is the appearance of a population of $CD8^+$ T-cells termed $CD8^{low}$,[77] which has the phenotype α^+/β^{low}. These cells serve as a marker of immune activation by more virulent strains of FIV and functionally may contribute to the non-cytolytic activity for FIV mediated by $CD8^+$ T-cells.

The final outcome of FIV infection, following experimental or natural challenge, is variable. During the asymptomatic phase the plasma virus load is stable, but a progressive decline in $CD4^+$ T lymphocyte numbers occurs. In an as yet unknown proportion of naturally infected cats, this decline results in a functional immunodeficiency that leads to clinical disease and death. In one experiment, in which cats were infected as kittens with the FIV-GL8 strain and were maintained for 10 years, approximately half of the cats developed serious and ultimately fatal conditions that could be ascribed to the infection (John Callanan, personal communication). By contrast, in a completely closed household of 26 pet cats observed over a 10-year period, natural FIV spread slowly among the cats but did not appear to cause any significant disease.[1] Clearly, in vaccine trials with FIV or HIV, the efficacy of vaccination must be measured by the capacity of the vaccine to prevent infection rather than disease.

As discussed below in more detail, strains of FIV differ in virulence. Viruses of high virulence are defined here as those that establish high virus loads, cause a reduction in $CD4^+$ T lymphocyte numbers, and activate $CD8^{low}$ T-cells. By contrast, viruses of low virulence establish infections so that plasma viral RNA and proviral DNA or infectious virus may be difficult to detect. These viruses do not cause a decline in $CD4^+$ T-cell numbers or induce a rise in $CD8^{low}$ T-cells.

Immune Responses to FIV Infection

Although the immune response to natural or experimental FIV infection does not eliminate the virus from its host, as in HIV infection, it is sufficiently powerful to contain the infection for many years. Consequently, it is not unreasonable to believe that the quality of this response might be one to emulate by vaccination and that if the immune system is appropriately primed before exposure, that more effective control of the virus may be possible. As noted above, the responses that accompany FIV infection and are presumed to play a major role in inhibiting viral infection are the induction of CTL and virus neutralizing antibodies. Although our ability to define precisely the details of feline immune responses is fairly rudimentary because of a relative lack of reagents, compared to those available for man and macaques, there is in fact very little difference between FIV, SIV, and HIV in our understanding of the appropriate immune responses that block viral infection. Early studies on HIV vaccination concentrated on raising virus neutralizing antibodies and hence on generating vaccines that incorporated SU that contains the targets for these antibodies. It was considered desirable to include antigens that could induce antibodies that would neutralize viruses of as many clades of virus as possible. Later research concluded that CTL were crucial in protective immunity and several vaccine approaches have been developed, which include peptide epitopes recognized by appropriate haplotypes of populations in which the vaccine might be applied. This same cycle of opinion has occurred in FIV vaccine research.

3. CRUCIAL FACTORS IN FIV VACCINE DEVELOPMENT STRATEGIES

Viral Immunogens

An unresolved issue for both FIV and HIV is which virus strain or isolate should be used as a source of immunogen in a vaccine. There are a number of FIV strains that have been used as antigens or challenge viruses, including viruses with differences in antigenicity, clade, and virulence.

Antigenic Variation

A major consideration in the development of any vaccine is whether there is antigenic variation between virus isolates to which the target population might be exposed. With their high rates of mutation and recombination, together with the selection pressure applied by the antiviral

immune responses of the host, lentiviruses might be expected to be highly antigenically variable; and so it has proved.

The classical method of antigenic typing of viruses is by virus neutralization. Unfortunately, as for HIV, the measurement of virus neutralizing antibodies against FIV has been, and remains, difficult. In early studies, neutralization by sera, from infected or vaccinated cats of FIV adapted for growth in the feline fibroblast cell line CRFK, was reported.[17] However, this type of CRFK-tropic, and probably CD134-independent, FIV does not represent the majority of viruses that are isolated from naturally infected cats and are probably transmitted in the field (Hayley Haining and Margaret Hosie, personal communication). It is likely that neutralization of these viruses in this assay is mediated through blocking of the interaction between the V3 region of SU and CXCR4 by anti-V3 antibodies, as virus neutralizing antibodies can be completely absorbed by a peptide that represents the V3 loop (Robert Osborne, personal communication). Cats vaccinated with an inactivated virus vaccine that had high levels of virus neutralizing antibodies measured in this way were protected from challenge with the CRFK-tropic FIV-Pet but were only partially protected against FIV-GL8.[32] These antibodies may have contributed to protection from FIV-Pet, although this is not known for certain. However, it is unlikely that anti-V3 antibodies alone were responsible, because in other experiments cats vaccinated with V3 peptides that include an immunodominant B-cell epitope were not protected against FIV-Pet.[44] These findings suggest that other immune mechanisms are required for protection against most naturally occurring viruses.

Neutralization assays employing feline blood mononuclear cells or T-cell lines to measure residual infectivity, such as MBM or Mya-1, are generally considered to be more appropriate indicators of antibody activity because they may be relevant to immunity in vivo. However, serum virus neutralizing antibody titers in these systems have generally been low, making interpretation of results difficult. Some of the problems of measuring antibody in this way in cats vaccinated with inactivated virus or virus-infected cells were resolved by preabsorbing the sera with the cells in which the virus was prepared.[50] This procedure appears to remove anticell activity in the sera and thereby enhances virus neutralizing antibodies titers. Using this technique, virus neutralizing antibodies have been demonstrated in vaccinated cats, but again it is not clear whether this activity operates in vivo or contributes to protection.

Clades

An unresolved and very important issue about HIV vaccination is whether clades are relevant to vaccination. Do clades represent different

antigenic types and if so, should vaccines be designed to include antigens of the prevalent clade(s) in a particular geographic area? FIV appears to be an excellent model in which to answer these questions. FIV exists in at least 5 clades or subtypes (A–E), and in some geographical areas these clades are distinct. Thus, in analyses of FIV isolates made in Italy and the UK, it was found that all of the Italian isolates belonged to clade B,[55] while all of the UK isolates were of clade A (Alexandra Müller and Mauro Pistello, personal communication). In Austria and southern Germany there appears to be a mixture of the two clades, including recombinants.[69] This curious distribution may be due to the patterns of migration of cats and the evolution of FIV in these cat populations. Additional clades and recombinants have been identified throughout the world.[62]

Unfortunately it has not yet been possible to resolve the issue of whether FIV clades represent serotypes because of the difficulties in antigenic typing using virus neutralization, as described above. An indication that there may be clade-specific differences comes from a comparison of the neutralization of viruses representing five different clades,[36] although insufficient isolates were examined to provide a comprehensive view of antigenic relationships. A potential further complication is the finding of type-specific neutralization within viruses of clade A using the CRFK-based assay.[52] There have been few reports of antigenic relationships between primary FIV isolates, predictably because of the technical difficulties encountered. One study of 15 Italian isolates of clade B confirmed their resistance to neutralization and failed to reveal any correlation between behavior in neutralization assays and genetic relatedness or epidemiological distribution.[13] Experience in the UK with clade A viruses has been similar (Alexandra Müller, personal communication). This resistance appears to be highly conserved in FIV, as infection of cats with neutralization-sensitive virus results in the evolution of a neutralization-resistant population with time.[4] Clearly this is a very important field of study that urgently requires further effort to clarify.

A pragmatic approach to the issue, which is feasible in the cat but not in man or macaques, has been to determine whether a vaccine that contains antigen(s) derived from one clade can protect against challenge with a virus of another clade. So far this has not been done in a systematic way, although Pu et al.[59] showed that cats vaccinated with an inactivated cell vaccine containing a mixture of clades A and D were protected more effectively against challenge with viruses of clades A, B, or C than cats vaccinated with a clade A virus alone. The commercial vaccine derived from that experimental vaccine protected completely against a further clade B virus.[58] This information is of practical significance as FIV of clade B is widely distributed in the United States and parts of Europe. The generality of these results needs to be confirmed by the use of further challenge viruses rep-

resenting all FIV clades. This cross-clade protection implies, however, that there are immunogens in common among clades.

Virulence

While the virulence of a virus used for the challenge of vaccinated cats has to be taken into account in assessing vaccine efficacy, as discussed below, it is not so clear if virulence is an important consideration in the choice of virus to be used in a vaccine. Do virulent viruses have other phenotypic characteristics that make them more attractive as immunogens?

Cell tropism is one characteristic that is almost certainly an important factor in virulence. Like HIV, FIV has a tropism for $CD4^+$ T lymphocytes. As described previously, the primary receptor for FIV is CD134[66] and the coreceptor is CXCR4.[78] It is probable that the viruses transmitted in nature, accordingly most relevant to vaccine development, require both of these molecules for virus entry into cells. Viruses in a minority of primary isolates of FIV, as well as certain "laboratory-adapted" strains, are able to infect cells directly through an interaction between the V3 region of the SU protein and CXCR4.[78,79] Although only a few of these viruses have been tested for their capacity to replicate in vivo, those that have been inoculated into cats establish lower virus loads, and consequently are considered to be less virulent, than more "primary" isolates. By contrast, it is considered that the CXCR4-tropic HIVs that appear during the terminal phases of infection are somehow associated with the decline in immune responsiveness of the host, and therefore are more virulent than the primary CCR5-tropic viruses that are initially present after infection. Experience with FIV suggests that in fact the viruses that enter cells using only the coreceptor may be a *product* of the immunodeficiency rather than the cause. It is relevant to this review that in the cat there appears to be pressure on these CXCR4-tropic FIVs to evolve into viruses with a phenotype more like that of the primary isolates, which use both receptors.[33] Direct inoculation of a CXCR4 tropic FIV that established a very low virus load was followed, almost 3 years later, by the emergence of a virus population that appeared to have a change in receptor usage. When inoculated into kittens, these variant viruses established a higher virus load than the parental virus, similar to that following infection with a "primary" isolate. Consequently, there appeared to be selection pressure on the virus to evolve a phenotype that was more suited for long-term survival in its host.

The association between receptor usage, cell tropism, and virulence has yet to be fully determined. It is important that this association be unraveled because a great deal depends on the answer, particularly the choice of viruses that should be included in a vaccine for HIV or FIV and

which should be used as challenge viruses in FIV vaccine trials to measure efficacy.

Immune Responses to Vaccination and Correlates of Protection

It is often asserted that to be effective a vaccine should induce an immune response that is equivalent to that which occurs in individuals who have been challenged with the cognate infectious agent and have subsequently recovered. The problem with defining the type of response to HIV or FIV that should be induced is that these viruses establish persistent infections from which the infected individual never recovers, even though they possess strong antiviral immune responses. Accordingly, it can also be argued that since the responses that are observed in the course of the infection do not eliminate the virus, they may not necessarily be indicators of the type of immunity that would be desirable in a protective vaccine.

The protection that is afforded by inactivated virus vaccines against FIV has been exploited to determine the immune correlates of vaccine-induced protection. An experiment with an inactivated virus vaccine that was manipulated to protect only a proportion of vaccinated cats allowed a comparison of the immune responses following vaccination, which resulted in either protection or the establishment of a persistent infection.[21,28] While virus neutralizing antibodies appeared to be correlated with protection in the period immediately following virus challenge, CTL induced early after vaccination correlated with protection from further challenge 8 months later. These results suggested that perhaps a more universal response, in which both virus neutralizing antibodies and CTL were induced, is beneficial in vaccination. That each component is important in protection is suggested by the results of experiments in which protection could be transferred from successfully vaccinated cats by either serum[25] or blood mononuclear cells.[61] Whether the conclusions from these experiments with inactivated virus vaccines are valid also for other types of vaccines is not known. Since vaccines other than inactivated vaccines have not been entirely successful, it is not yet possible to determine directly the correlates of whatever protection they have provided.

Challenge Systems

Although the measurement of immune responses elicited by vaccination may give a valuable indication of the immunogenicity of a vaccine, it is still not entirely clear what immune responses may be protective. A broad range of cellular and humoral responses may be required or specific antibody responses to defined epitopes may be essential. Furthermore, the

contribution to protection made by nonspecific innate immune responses (e.g., NK cells) is uncertain. In the absence of such correlates of protection, the assessment of vaccine efficacy is only possible using a challenge model. Further, the choice of an appropriate challenge is clearly important. There are four factors that should be considered: choice of virus strain, source of virus, challenge route, and dose of inoculum.

Choice of Challenge Virus Strains

There are a number of viral strains that have been used in efficacy studies, as illustrated in Tables I, II, and III. These strains vary significantly in their virulence, as assessed by viral loads attained and by reduction in $CD4^+$ T-lymphocyte numbers following challenge. A further consideration is whether a homologous or heterologous challenge should be used. Choice of challenge strain may be influenced by geography; for example, in northern Europe clade A viruses predominate, while in central and southern Europe, clade B viruses are more common. Studies on field cases of FIV have shown that the majority of infected cats have relatively high viral loads and have depressed $CD4^+$ T-lymphocyte counts. This supports the use of more pathogenic virus as a challenge strain for vaccine studies. In addition, this provides a realistic model for HIV, where high viral loads and suppression of CD4 counts are typically seen in infected patients. The feline model offers good opportunities to study the effect of viral variation on the ability of vaccines to protect against challenge by viruses of different clades and of different pathogenicity.

Source and Type of Virus Inoculum

It is believed that FIV is transmitted mainly by biting and, therefore, through the traumatic transfer of virus or virus-infected cells in the saliva. The dilemma in choosing the most appropriate source of virus is to balance simulation of natural infection with the practicalities of obtaining a suitable and reproducible inoculum. Infected saliva has not been used because of the difficulties in obtaining sufficient quantities of acceptable quality. The materials that have been most widely employed are free virus or infected cells grown in cultures of feline T-cells, either primary blood mononuclear cells or cell lines. The use of virus derived from molecular clones may be preferable, as it ensures consistency between experiments and avoids viral attenuation that can occur with long-term culture in vitro. Increasingly, virus-containing plasma from cats infected with an appropriate FIV strain is used. Although virus grown in vitro is more readily controlled, virus obtained ex vivo may represent more closely the state of the virus that is transmitted naturally; for example, in terms of its resistance or susceptibility to neutralizing antibodies.

TABLE I
DNA and Recombinant Viral Vectored Vaccines

Vaccine Formulation (a)	Immunization Route (b)	Vaccine Virus (c), Immunogen	Adjuvant/ Formulation	Challenge Virus (c), Dose, Route (number of challenge where sequential challenges given)	Number of Cats Protected (Effect on viral loads)	Reference
VEE-FIV	SC	FIV $NCSU_1$—construct expressed Gag and Env		FIV $NCSU_1$, 5×10^5 cells, vaginal	0/4	8
Control (VEE-GFP)	SC			FIV $NCSU_1$, 5×10^5 cells, vaginal	0/3	
Proviral DNA ΔIN	IM	FIV GL8—proviral DNA with integrase deletion		FIV Pet, 25 CID_{50}, IP	1/6	16
Proviral DNA ΔIN	IM	FIV GL8—proviral DNA with integrase deletion	IL-18 DNA	FIV Pet, 25 CID_{50}, IP	2/6	
Proviral DNA ΔIN	IM	FIV GL8—proviral DNA with integrase deletion	IL-12 + IL-18 DNA	FIV Pet, 25 CID_{50}, IP	2/6	
Proviral DNA ΔRT	IM	FIV GL8—proviral DNA with reverse transcriptase deletion	IL-18 DNA	FIV Pet, 25 CID_{50}, IP	2/6	
Proviral DNA ΔRT	IM	FIV GL8—proviral DNA with reverse transcriptase deletion	IL-12 + IL-18 DNA	FIV Pet, 25 CID_{50}, IP	0/6	
Control (pBR328)	IM			FIV Pet, 25 CID_{50}, IP	0/6	
Proviral DNA ΔIN	IM	FIV GL8—proviral DNA with integrase deletion		FIV Pet, 25 CID_{50}, IP (2)	1/1	16
Proviral DNA ΔIN	IM	FIV GL8—proviral DNA with integrase deletion	IL-18 DNA	FIV Pet, 25 CID_{50}, IP (2)	2/2	
Proviral DNA ΔIN	IM	FIV GL8—proviral DNA with integrase deletion	IL-12 + IL-18 DNA	FIV Pet, 25 CID_{50}, IP (2)	2/2	

Proviral DNA ΔRT	IM	FIV GL8—proviral DNA with reverse transcriptase deletion	IL-18 DNA	FIV Pet, 25 CID_{50}, IP (2)	0/1	
Control (pBR328)	IM			FIV Pet, 25 CID_{50}, IP	0/4	
Proviral DNA ΔIN	IM	FIV GL8—proviral DNA with integrase deletion		FIV GL8, 10 CID_{50}, IP (3)	0/1 (↓)	16
Proviral DNA ΔIN	IM	FIV GL8—proviral DNA with integrase deletion	IL-18 DNA	FIV GL8, 10 CID_{50}, IP (3)	0/2 (↓)	
Proviral DNA ΔIN	IM	FIV GL8—proviral DNA with integrase deletion	IL-12 + IL-18 DNA	FIV GL8, 10 CID_{50}, IP (3)	0/2 (↓)	
Control (pBR328)	IM			FIV GL8, 10 CID_{50}, IP	0/4	
pCMV-Env	ID + IM	FIV 34TF10—Env DNA		FIV Pet, 10 CID_{50}, IP	1/4	63
pCMV-Env	TLN	FIV 34TF10—Env DNA		FIV Pet, 10 CID_{50}, IP	0/4 (↑, early)	
Control (pUC18)	ID + IM			FIV Pet, 10 CID_{50}, IP	1/4	
Control (pCMV-S)	ID + IM			FIV Pet, 10 CID_{50}, IP	0/4	
MIDGE DNA	ID – gene gun	FIV Z2—Env gp140 DNA		FIV Z2, 25 $TCID_{50}$, IP	0/4	6, 41
MIDGE DNA	ID – gene gun	FIV Z2—Env gp140 DNA	IL-12 DNA	FIV Z2, 25 $TCID_{50}$, IP	3/4 (↓)	
MIDGE DNA	ID – gene gun	FIV Z2—Env gp140 DNA	IL-16 DNA	FIV Z2, 25 $TCID_{50}$, IP	0/4 (↓)	
MIDGE DNA	ID – gene gun	FIV Z2—Env gp140 DNA	CpG	FIV Z2, 25 $TCID_{50}$, IP	0/4 (↓)	
Control (gold particles)	ID – gene gun			FIV Z2, 25 $TCID_{50}$, IP	0/4	
Proviral DNA ΔRT	IM	FIV Pet—proviral DNA with reverse transcriptase deletion	IFN-γ DNA	FIV Pet, 10 CID_{50}, IP	1/6	26
Proviral DNA ΔRT	IM	FIV GL8—proviral DNA with reverse transcriptase deletion	IFN-γ DNA	FIV Pet, 10 CID_{50}, IP	3/6	
Control (IFN-γ DNA alone)	IM		IFN-γ DNA	FIV Pet, 10 CID_{50}, IP	0/6	
Proviral DNA ΔRT	IM	FIV Pet—proviral DNA with reverse transcriptase deletion	IFN-γ DNA	FIV GL8, 10 CID_{50}, IP	0/6	

TABLE I
Continued

Vaccine Formulation (a)	Immunization Route (b)	Vaccine Virus (c), Immunogen	Adjuvant/ Formulation	Challenge Virus (c), Dose, Route (number of challenge where sequential challenges given)	Number of Cats Protected (Effect on viral loads)	Reference
Proviral DNA ΔRT	IM	FIV GL8—proviral DNA with reverse transcriptase deletion	IFN-γ DNA	FIV GL8, 10 CID_{50}, IP	0/6	
Control (IFN-γ DNA alone)	IM		IFN-γ DNA	FIV GL8, 10 CID_{50}, IP	0/6	
Proviral DNA ΔVif	IM	FIV PPR—proviral DNA with *vif* deletion		FIV PPR, 50 $TCID_{50}$, IP	3/3	43
Control (medium)	IM				0/2	
Proviral DNA ΔRT	IM	FIV Pet—proviral DNA with reverse transcriptase deletion		FIV Pet, 25 CID_{50}, IP	1/5	29
Proviral DNA ΔRT	IM	FIV Pet—proviral DNA with reverse transcriptase deletion	IFN-γ DNA	FIV Pet, 25 CID_{50}, IP	3/5	
Control (PBS or IFN-γ DNA alone)	IM		IFN-γ DNA	FIV Pet, 25 CID_{50}, IP	0/10	
Proviral DNA ΔRT	IM	FIV Pet—proviral DNA with reverse transcriptase deletion	IFN-γ DNA	FIV Pet, 25 CID_{50}, IP	2/5	29
Control (IFN-γ DNA alone)	IM		IFN-γ DNA	FIV Pet, 25 CID_{50}, IP	0/5	
ALVAC-FIV	IM	FIV VFr—construct expresses Env, Gag and Pro		FIV Pet, 50 CID_{50}, IP	2/3	71

ALVAC-FIV + ICV	IM + SC	FIV VFr—Env, Gag and Pro + FIV Pet – infected cell vaccine	SAF-MDP	FIV Pet, 50 CID_{50}, IP	3/3	
Control (ALVAC + single dose ICV)	IM + SC	FIV Pet—infected cell vaccine	SAF-MDP	FIV Pet, 50 CID_{50}, IP	0/3	
ALVAC-FIV + ICV	IM + SC	FIV VFr—Env, Gag and Pro + FIV Pet – infected cell vaccine	SAF-MDP	FIV Bang, 75 CID_{50}, IP (2)	1/3 (↓)[1]	71
Control (none)				FIV Bang, 75 CID_{50}, IP	0/3	
pTΔ20ds DNA	IM	FIV 34TF10—Env DNA	Post 25% sucrose	FIV Pet, 10 CID_{50}, IP	0/7 (↑, early)	64
pn14 DNA	IM	FIV 34TF10—Env DNA[2]	Post 25% sucrose	FIV Pet, 10 CID_{50}, IP	0/7 (↑, early)	
pn92 DNA	IM	FIV 34TF10—Env DNA[2]	Post 25% sucrose	FIV Pet, 10 CID_{50}, IP	0/7 (↑, early)	
Control (pUC18)	IM		Post 25% sucrose	FIV Pet, 10 CID_{50}, IP	0/7	
Env (gp120) DNA	IM	FIV Gasser—NC (p10) and Env (gp120) DNA		FIV Gasser, 100 $TCID_{50}$, IP	0/4 (↓ in 2 cats)	11
Env (gp120) DNA + NC (p10) DNA	IM	FIV Gasser—NC (p10) and Env (gp120) DNA		FIV Gasser, 100 $TCID_{50}$, IP	0/4	
Control (pCMV DNA)	IM			FIV Gasser, 100 $TCID_{50}$, IP	0/4	
Ad-Env		FIV Wo—construct expresses Env	Montanide ISA 708 or 206	FIV Wo, 20 CID_{50}, IP	0/4	24
Control (Ad-pseudorabies gp50)			Montanide ISA 708 or 206	FIV Wo, 20 CID_{50}, IP	0/4	

(a) VEE: Venezuela equine encephalitis virus replicon particles expressing FIV Gag and Env or green fluorescent protein (GFP); MIDGE: minimalistic immunogenic defined gene expression; pCMV-S: DNA expressing S region of hepatitis B virus; ALVAC-FIV: canarypox vector expressing Env, Gag, and Pro proteins of FIV; ICV: inactivated infected cell vaccine prepared from FL-4 cell line; Ad-Env: replication defective human adenovirus serotype 5 expressing FIV Env.
(b) TLN: targeted lymph node; ID: intradermal.
(c) FIV $NCSU_1$: FIV North Carolina State University 1; FIV GL8: FIV Glasgow 8; FIV VFr: FIV Ville Franche; FIV 34TF10: FIV molecular clone derived from FIV Pet; FIV-Z2: FIV Zurich 2; Pet: FIV Petaluma; PPR: FIV San Diego PPR; FIV Wo: French isolate of FIV.
[1] Single cat remained FIV negative by both virus isolation and PCR, two cats were transiently infected as determined by virus isolation and/or PCR.
[2] Contain mutations in the principal immunodominant domain.

TABLE II
Inactivated Whole Virus and Cell-Associated Virus Vaccines

Vaccine Formulation (a)	Immunization Route (b)	Vaccine Virus (c), Immunogen	Adjuvant/ Formulation (d)	Challenge Virus (e), Dose, Route (number of challenge where sequential challenges given)	Number of Cats Protected (Effect on viral loads)	Reference
Fixed FIV infected autologous PBMC	SC	FIV M2—inactivated infected cell vaccine	Incomplete Freund's	FIV M2, 5 CID_{50}, IP	3/5[1]	23
Auto. PBMC + AT2-FIV	SC	FIV M2—inactivated infected cell vaccine	Incomplete Freund's	FIV M2, 5 CID_{50}, IP	2/5[1]	
Auto. PBMC + AT2-FIV + Peptide SU_5	SC	FIV M2—inactivated infected cell vaccine	Incomplete Freund's	FIV M2, 5 CID_{50}, IP	2/5[1]	
Auto. PBMC + AT2-FIV + Peptide TM_{59}	SC	FIV M2—inactivated infected cell vaccine	Incomplete Freund's	FIV M2, 5 CID_{50}, IP	1/5[1]	
Control (Auto. PBMC)	SC		Incomplete Freund's	FIV M2, 5 CID_{50}, IP	4/4[1]	
Control (Auto PBMC + SU_5 + TM_{59})	SC		Incomplete Freund's	FIV M2, 5 CID_{50}, IP	4/5[1]	
Fixed FL-4 cells	TLN	FIV Pet—inactivated infected cell vaccine	Quil A	FIV Pet, 100 CID_{50}, rectal	0/4	18
Control (fixed FeT-J cells)	TLN			FIV Pet, 100 CID_{50}, rectal	3/4	
Inactivated FIV	SC	FIV Pet + FIV Shi – whole virus, dual subtype vaccine	FD-1	FIV Bang, 10 CID_{50}, IV	4/5	59
Control (FeT-J cell lysate/PBS)	SC		FD-1	FIV Bang, 10 CID_{50}, IV	0/4	

Inactivated FIV	SC	FIV Pet + FIV Shi – whole virus, dual subtype vaccine	FD-1	FIV Bang, 100 CID_{50}, IV	2/5	59
Inactivated FIV	SC	FIV Pet—whole virus	FD-1	FIV Bang, 100 CID_{50}, IV	0/4	
Inactivated FIV	SC	FIV Shi—whole virus	FD-1	FIV Bang, 100 CID_{50}, IV	1/4	
Control (PBS)	SC		FD-1	FIV Bang, 100 CID_{50}, IV	0/5	
Inactivated FIV	SC	FIV Pet + FIV Shi – whole virus dual subtype vaccine	FD-1	FIV Pet, 50 CID_{50}, IV	4/4	59
Inactivated FIV	SC	FIV Pet—whole virus	FD-1	FIV Pet, 50 CID_{50}, IV	1/5	
Control (PBS)	SC		FD-1	FIV Pet, 50 CID_{50}, IV	0/5	
Inactivated FIV	SC	FIV Shi—whole virus	FD-1	FIV Shi, 50 CID_{50}, IV	1/3	59
Control (PBS)	SC		FD-1	FIV Pet, 50 CID_{50}, IV	0/3	
Inactivated FIV	SC	FIV Pet + FIV Shi – whole virus dual subtype vaccine	FD-1	FIV Pet, 20 or 25 CID_{50}, IV	6/6	59
Control (PBS)	SC		FD-1	FIV Pet, 20 or 25 CID_{50}, IV	0/9	
Inactivated FIV	rectal +/– IP or IN	FIV GL8—whole virus	Cholera toxin	FIV GL8, 10 CID_{50}, rectal	0/12	19
Control (water)	rectal			FIV GL8, 10 CID_{50}, rectal	0/4	
Inactivated FIV	SC	FIV Pet—whole virus derived from FL-4 cell line	MF59	FIV Pet, 10 CID_{50}, IP	5/5	26

TABLE II
Continued

Vaccine Formulation (a)	Immunization Route (b)	Vaccine Virus (c), Immunogen	Adjuvant/ Formulation (d)	Challenge Virus (e), Dose, Route (number of challenge where sequential challenges given)	Number of Cats Protected (Effect on viral loads)	Reference
Control (adjuvant only)	SC		MF59	FIV Pet, 10 CID_{50}, IP	1/5	
Inactivated FIV	SC	FIV Pet—whole virus derived from FL-4 cell line	MF59	FIV GL8, 10 CID_{50}, IP	2/5	
Control (adjuvant only)	SC		MF59	FIV GL8, 10 CID_{50}, IP	0/5	
Inactivated FIV	SC	FIV Pet—whole virus derived from FL-4 cell line	MF59	FIV AM6, 10 CID_{50}, IP	3/5	
Control (adjuvant only)	SC		MF59	FIV AM6, 10 CID_{50}, IP	1/5	
Fixed FIV infected cells Control (none)	SC	FIV M2—inactivated infected cell vaccine	Incomplete Freund's	NA – natural challenge under field conditions.	0/12 5/14	49
Homologous RBC coated with FIV	IP	FIV M2—whole virus		FIV M2, 10 CID_{50}, IV	2/4[2]	10
Control (RBC coated with BSA)	IP			FIV M2, 10 CID_{50}, IV	0/4[3]	
Homologous RBC coated with FIV	IP	FIV M2—whole virus		FIV M2, 10 CID_{50}, IV (2)	1/4[4]	10

Control (RBC coated with BSA)	IP			FIV M2, 10 CID_{50}, IV (2)	0/2	
Fixed FIV infected autologous PBMC	IV + SC	FIV 19k1—whole virus		FIV 19k1, 10 CID_{50}, IM	0/3 (↑, early)	38
Control (autologous PBMC)	IV + SC			FIV 19k1, 10 CID_{50}, IM	0/2	
Inactivated FIV	SC	FIV Pet—whole virus derived from FL-4 cell line	tMDP/SAF-M	FIV-Pet, 50 CID_{50}, IP	4/5	27
FIV envelope	SC	FIV Pet—gp120 purified envelope	tMDP/SAF-M	FIV-Pet, 50 CID_{50}, IP	1/5 (↓)	
Control (adjuvant only)	SC		tMDP/SAF-M	FIV-Pet, 50 CID_{50}, IP	0/5	

(a) AT2-FIV: virus inactivated with 2,2′-dithiodipyridine (aldrithiol-2 [AT-2]) prior to incubation with autologous PBMC for 2 hours followed by PFA fixation; Peptide SU_5 and Peptide TM_{59}: peptides designed to enhance virus-receptor interaction dependent epitopes were added to autologous PBMC—AT-2 inactivated virus after 1 hour and incubated for further hour; Auto PBMC: autologous peripheral blood mononuclear cells.
(b) TLN: targeted lymph node; IN: intranasal.
(c) FIV GL8: FIV Glasgow 8; FIV M2—FIV Milan 2; Pet: FIV Petaluma; FIV Shi: FIV Shizuoka; FIV 19k1: molecular clone of FIV Amsterdam 19.
(d) Quil A: semipurified saponin adjuvant derived from *Quillaia saponaria* bark; FD-1: Fort Dodge commercial adjuvant; MF 59: Chiron oil/water adjuvant; tMDP: threonyl muramyl dipeptide; SAF-M: syntax adjuvant formulation.
(e) As c. above and: FIV AM6: FIV Amsterdam 6; FIV-Bang: FIV Bangston; NA—not applicable.
[1] Cats in control groups remained FIV negative due to low dose of challenge virus. All negative cats were boosted and rechallenged with 10 CID_{50} FIV M2 (IP); following this all animals became infected.
[2] Virus isolation negative for all cats but two cats transiently positive by PCR for PBMC proviral DNA.
[3] Virus isolation negative for 3/4 cats; 2/4 cats became seropositive; 4/4 cats positive by PCR for PBMC proviral DNA.
[4] One cat remained virus isolation negative but had previously been positive by PCR for PBMC proviral DNA.

TABLE III
Recombinant Protein, Peptide, and Nonviral Vectored Vaccines

Vaccine Formulation (a)	Immunization Route (b)	Vaccine virus (c), Immunogen	Adjuvant/ formulation (d)	Challenge Virus (e), Dose, Route (number of challenge where sequential challenges given)	Number of Cats Protected (Effect on viral loads)	Reference
LMgag/env	Oral	FIV $NCSU_1$ – Gag, Env		FIV $NCSU_1$, 75000 $TCID_{50}$ and 75000 FIV infected PBMC, vaginal	0/5 (↓)	70
Control (Wt-LM)	Oral			FIV $NCSU_1$, 75000 $TCID_{50}$ and 75000 FIV infected PBMC, vaginal	0/5	
Control (PBS)	Oral			FIV $NCSU_1$, 75000 $TCID_{50}$ and 75000 FIV infected PBMC, vaginal	0/5	
Env C2 peptide	TLN or rectal	conserved Env peptide	Quil A	FIV GL8, 10 CID_{50}, rectal	0/8	18
Control (water)	rectal			FIV GL8, 10 CID_{50}, rectal	0/4	
Env V3 MAP	rectal +/− IP	conserved Env peptide	Cholera toxin	FIV GL8, 10 CID_{50}, rectal	0/8	19
Env V3 MAP	rectal +/− IP	conserved Env peptide	Quil A	FIV GL8, 10 CID_{50}, rectal	0/8	
Env V3 MAP	rectal	conserved Env peptide		FIV GL8, 10 CID_{50}, rectal	0/4	
Control (water)	rectal			FIV GL8, 10 CID_{50}, rectal	0/4	
Palmitoyl thiester Env V3 peptide	SC	conserved Env peptide	IFA	FIV GL8, 10 CID_{50}, rectal	0/4	19
Palmitoyl thiester Env V3 peptide	SC	conserved Env peptide	ISCOM	FIV GL8, 10 CID_{50}, rectal	0/4	
Env V3 peptide	SC, IN or rectal	conserved Env peptide	Cholera toxin	FIV GL8, 10 CID_{50}, rectal	0/12	
Control (water)	SC			FIV GL8, 10 CID_{50}, rectal	0/4	
FIV NC DNA + Env SU rec. protein	IN (DNA)/SC (rEnv)	FIV Gasser—NC DNA; FIV Bang rec. Env SU		FIV Gasser, 100 $TCID_{50}$, IP	1/4	12

FIV NC DNA + Env SU rec. protein	IM (DNA)/SC (rEnv)	FIV Gasser—NC DNA; FIV Bang rec. Env SU		FIV Gasser, 100 $TCID_{50}$, IP	0/4	
pCI-neo DNA + Env SU rec. protein	IM (DNA)/SC (rEnv)	FIV Bang—recombinant Env SU		FIV Gasser, 100 $TCID_{50}$, IP	0/4 (↑)	
Control (pCI-neo DNA + PBS)	IM (DNA)/SC (PBS)			FIV Gasser, 100 $TCID_{50}$, IP	0/4	
ISCOM-FIV	SC	FIV Am6c—purified virus	ISCOM	FIV AM19, 20 CID_{50}, IM	0/6	34
ISCOM-FIV + ISCOM-vGR657 × 15	SC	FIV Am19—Env with SU/TM deletion	ISCOM	FIV AM19, 20 CID_{50}, IM	0/6 (↑, early)	
ISCOM-vGR657 × 15 + FIV-Gag-ISCOM	SC	FIV Am19 Env + FIV 19k1 Gag	ISCOM	FIV AM19, 20 CID_{50}, IM	0/5	
Control (CrFK-ISCOM)	SC		ISCOM	FIV AM19, 20 CID_{50}, IM	0/6 (↑, early)	
Control (SIV Env ISCOM)	SC		ISCOM	FIV AM19, 20 CID_{50}, IM	0/6	
Control (PBS)	SC			FIV AM19, 20 CID_{50}, IM	0/6	
Env SU recombinant protein	SC	FIV Z2—Env SU protein, *E. coli* expressed	AlOH + QS21	FIV Z2, 20 $TCID_{50}$, IP	0/5 (↓)	42
Env SU recombinant protein	SC	FIV Z2—Env SU protein, baculovirus expressed	AlOH + QS21	FIV Z2, 20 $TCID_{50}$, IP	0/5 (↓)	
Env SU recombinant protein	SC	FIV Z2—Env SU protein, baculovirus expressed	Freund's + rabies NC	FIV Z2, 20 $TCID_{50}$, IP	0/5 (↓)	
Control (PBS)	SC			FIV Z2, 20 $TCID_{50}$, IP	0/7	
Multiepitope peptide	SC	FIV Pet + GL8—Env V3, TM and p24 multiepitope peptide	Quil A	FIV Pet, 25 CID_{50}, IP	0/7	20
Control (adjuvant only)	SC		Quil A	FIV Pet, 25 CID_{50}, IP	0/3	

TABLE III
Continued

Vaccine Formulation (a)	Immunization Route (b)	Vaccine virus (c), Immunogen	Adjuvant/ formulation (d)	Challenge Virus (e), Dose, Route (number of challenge where sequential challenges given)	Number of Cats Protected (Effect on viral loads)	Reference
SL3261-FIV	Oral + IP	FIV 19k1 – Gag, Env		FIV 19k1, 30 CID_{50}, IM	0/3 (↓)	72
SL3261-FIV + ISCOM-FIV	SC/Oral + IP	FIV 19k1 – Gag, Env	ISCOM	FIV 19k1, 30 CID_{50}, IM	0/3	
SL3261-CtxB + ISCOM-FIV	SC/Oral + IP	FIV 19k1 – Gag, Env	Cholera toxin/ ISCOM	FIV 19k1, 30 CID_{50}, IM	0/3	
Control (SL3261-CtxB + ISCOM-SIV)	SC/Oral + IP		Cholera toxin/ ISCOM	FIV 19k1, 30 CID_{50}, IM	0/3	
ISCOM-vGR657	SC	FIV Am19 – native Env	ISCOM	FIV AM19, 20 CID_{50}, IM	0/6 (↑, early)	67
ISCOM-vGR657 × 15	SC	FIV Am19 – Env with SU/TM deletion	ISCOM	FIV AM19, 20 CID_{50}, IM	0/6 (↑, early)	
vGR657 × 15	SC	FIV Am19 – Env with SU/TM deletion	Quil A	FIV AM19, 20 CID_{50}, IM	0/6 (↑, early)	
β-gal-FIV Env fusion protein	SC	FIV Am19 – native Env	Quil A	FIV AM19, 20 CID_{50}, IM	0/6	
Control (SIV Env ISCOM)	SC		ISCOM	FIV AM19, 20 CID_{50}, IM	0/6s	
Control (PBS)	SC			FIV AM19, 20 CID_{50}, IM	0/6	

(a) LMgag/env: *Listeria monocytogenes* expressing FIV Gag and delivering plasmid pND14-Lc-*env* DNA expressing FIV envelope; Wt-LM: Wild type *Listeria monocytogenes*; Env C2 peptide: 29 aa peptide of second conserved region of FIV envelope; Env V3 MAP: multiple antigenic peptide of V3 (variable loop 3) region of envelope; rec.: recombinant; ISCOM-FIV: immunostimulating complexes incorporating purified FIV virus; ISCOM-vGR657×15: immunostimulating complexes incorporating FIV envelope expressed from recombinant vaccinia virus, containing deletion between Env SU and TM domains which facilitates incorporation into ISCOMs; CrFK-ISCOM: immunostimulating complexes incorporating soluble CrFK cell proteins; SIV Env ISCOM: immunostimulating complexes incorporating simian immunodefieciency virus envelope; PBS: phosphate buffered saline; SL3261-FIV and SL3261-CtxB:*Salmonella typhimurium aroA* mutant expressing either FIV Gag and Env (SL3261-FIV) or cholera toxin B (SL3261-CtxB); ISCOM-SIV: immunostimulating complexes incorporating simian immunodefieciency virus Gag and Env; β-gal FIV Env fusion protein: E. coli expressed FIV envelope-β-galactosidase fusion protein.
(b) TLN: targeted lymph node; IN: intranasal.
(c) FIV $NCSU_1$: FIV North Carolina State University 1; FIV-Bang: FIV Bangston; FIV Am6c/Am 19: FIV Amsterdam 6c/19. FIV-Z2: FIV Zurich 2; Pet: FIV Petaluma; FIV GL8: FIV Glasgow 8; FIV 19k1: molecular clone of FIV Amsterdam 19.
(d) Quil A: semipurified saponin adjuvant derived from *Quillaia saponaria* bark; AlOH: aluminium hydroxide; QS21: saponin adjuvant derived from *Quillaia saponaria* bark.
(e) As c. above.

Route of Challenge

In assessing the efficacy of FIV vaccines in the development of products for use in the target species, the challenge should be delivered in a manner that is relevant to the route of infection by which natural transmission occurs. This being the case, establishing the outcome of housing vaccinated and nonvaccinated cats together with healthy persistently infected cats should provide the most natural and realistic measure of vaccine efficacy. However, for practical use, direct parenteral inoculation of virus has to be employed. In attempts to provide a model of sexual transmission of HIV, mucosal challenge by FIV has also been used.

Natural Challenge. A clear advantage of the FIV model over the SIV model is that ultimately the testing of a vaccine in its natural host under field conditions is possible. This provides the most realistic assessment of how well the vaccine performs in its intended environment. The prevalence of FIV is quite high in many populations of cats, with estimates ranging from 1–12% in the healthy pet cat population and up to 100% in small populations. This high prevalence might suggest that it would be easy to test FIV vaccines in a natural setting with relatively small numbers of cats, either under experimental conditions or in the field. Unfortunately, this is not the case. In situations of natural transmission among pet cats in the field it would be difficult, if not impossible, to obtain permission from regulatory authorities to carry out the experiment; keeping pet or shelter cats in a situation in which unvaccinated individuals might be infected is not appealing.

In the experimental situation, which simulates natural transmission, the problem is achieving a sufficiently high incidence of transmission of the infection, particularly among cats that are well socialized and nonaggressive toward each other. Group sizes need to be large, to ensure statistically meaningful results, because a portion of vaccinates is likely to remain uninfected following challenge. The experience of many is that cats housed with FIV-infected animals may remain uninfected over periods of years. However, in some cases regular, if slow, spread of infection has been recorded.[1]

These potential problems ensure that testing of the majority of candidate vaccines must still be performed in experimental animals, with challenge virus being administered artificially. How can we make these experiments more realistic? Should we consider using a dose of virus that represents a more natural challenge or use multiple lower doses of virus? The hope is that vaccines that are able to prevent infection in an experimental setting with a "robust" challenge may be more likely to provide significant protection in pet cats in the field.

Inoculation of Challenge Virus. As discussed above, there is a difference in the route of natural transmission of FIV and HIV: HIV is transmitted across mucosal surfaces, while FIV is spread mainly by biting. Consequently,

FIV can serve as a model for HIV vaccination in two ways. In the first, more obvious model, which we have been describing until now, the principles established in eliciting protection from a natural challenge may be able to be applied to HIV. The second model involves manipulation of the FIV system by attempting to protect cats against virus administered by the vaginal or rectal routes, which are not the natural means of transmission of FIV but may be more relevant to HIV.

Cats may be infected vaginally, rectally, or orally,[51] although these mucosal routes require higher doses of virus than intramuscular, intraperitoneal, or intravenous inoculation to produce persistent infection. Infection may be more readily established by rectal or vaginal routes with cell-associated virus than cell-free virus.[5] This is in agreement with the infectious doses required to infect macaques with SIV by these routes.[2,68] Vaginal inoculation is more efficient than rectal inoculation, while oronasal administration is the least effective method of infection.[7]

Clearly the route of viral challenge may influence the ability of vaccines to protect against infection or disease. While infection by the intramuscular and intraperitoneal routes requires relatively low amounts of virus for infection, mucosal infection by either the vaginal, rectal, or intranasal/oral routes requires large amounts: up to four logs higher than that required to establish infection by the intravenous or intraperitoneal routes. The implication of this difference is that HIV may similarly be more readily transmitted by intravenous drug use than by sexual transmission. What remains to be determined, although seems very likely, is whether vaccination may more readily prevent infection by mucosal compared to parenteral inoculation. The desired immune responses required to prevent infection are almost certainly different depending on the route of exposure to virus. Examples of the results of vaccination trials using mucosal challenge are discussed below.

Dose of Challenge Virus. The dose of viral inoculum used for challenge is typically chosen to be sufficiently high to ensure that all nonvaccinated controls become infected; usually 10–50 median cat infectious doses of virus are used. However, this dose is likely to be far greater than would be encountered in a natural challenge. It is possible, therefore, that vaccination trials carried out using these challenge doses may underestimate the potential efficacy of vaccines in a field setting. This contention is supported by the vaccine trial by Matteucci et al.,[49] which was performed in a population of feral cats in a private shelter where FIV was endemic, with a prevalence of 29 to 58%. Animals were vaccinated with six doses of a FIV clade B paraformaldehyde-inactivated infected cell vaccine and observed for a 28-month period after the first immunisation. Although 5 of 14 control animals became infected, all 12 vaccinates remained free of infection. This result contrasts with the relatively modest success obtained in laboratory cats using similar vaccines.

Measurement of the Outcomes of Vaccination

In assessing the success of vaccine experiments, four potential outcomes may be envisaged. The vaccine may provide robust protection, inducing sterilizing immunity, where it is not possible to isolate infectious virus or detect viral RNA or proviral DNA by the polymerase chain reaction (PCR) following challenge. Although they may not provide sterilizing immunity, vaccines may provide significant protection, leading to a decrease in viral loads in the blood or lymphoid tissues following challenge. Alternatively, vaccines may fail to provide any degree of protection, or worse, may actually enhance infection following challenge. Examples of each of these outcomes may be seen in Tables I–III. Many vaccines have failed to induce significant protection. This has been the outcome even when vaccination has induced significant anti-viral immune responses. By contrast, some degree of protection has been achieved with vaccines that failed to activate one or another arm of a specific immune response. These findings further emphasize the need for viral challenge when assessing vaccine efficacy.

With experience of lentiviral vaccine development, the realization that the quality of the immune response induced by vaccination might be insufficient to provide sterilizing immunity against infection has given rise to the hope that it might control subsequent infection sufficiently to reduce the transmissibility of the virus from the infected individual and to prevent the progressive decline to disease and death. Confirmation that the risk of transmission can be reduced by vaccination should be possible with FIV, as it is should be perfectly feasible to quantify virus or proviral DNA in saliva.[46] Surprisingly, very little work appears to have been done on this important subject.

The most worrying result for a candidate vaccine is the finding that infection is enhanced following challenge. This outcome has been observed with a number of different types of FIV vaccine, including inactivated whole cell vaccines, some envelope subunit vaccines, and DNA vaccines encoding viral envelope. A number of potential mechanisms for such enhancement have been proposed and are discussed below.

4. EXAMPLES OF FIV VACCINES

Inactivated Virus and Virus-Infected Cell Vaccines

In spite of the number of variables associated with the challenge system, if a vaccine can be shown to be effective in cats against a virulent challenge with FIV, given the biological similarities between the two lentiviruses, it must be considered to be a serious candidate for development as an HIV vaccine. With this in mind, what lessons have we learned

from the cat? The results of recent FIV vaccine trials shown in Tables I–III illustrate the number of candidate vaccines that are in development. While the earliest FIV vaccines to be studied were those based on virus or infected cells inactivated with paraformaldehyde, since then many approaches have been tried. These include peptide and recombinant proteins, DNA vaccines, and viral vectors. However, despite the application of new technologies to FIV vaccine development, and while DNA vaccines have shown early promise, inactivated virus and infected cell vaccines remain the most successful.

Inactivated Virus Vaccines

The whole inactivated virus vaccine pioneered by Yamamoto and her colleagues provided complete protection against challenge with homologous virus (FIV-Pet)[82] and another strain of clade A.[81] Hosie et al.[32] confirmed these results and showed subsequently that this vaccine, although not providing full protection against challenge with a heterologous virus (FIV-GL8), significantly reduced the virus load.[26] The reasons for the difference in the degree of protection achieved against challenge by the two viruses were not entirely clear but, as discussed above, a strong possibility is that the greater virulence of the FIV-GL8 strain was responsible. A virus that establishes a high virus load might be more difficult to protect against by vaccination, either because it grows more rapidly following infection or establishes infection in cells that are not accessible to the immune response induced by the vaccine. Another possible explanation is that the two viruses differ in antigenicity. In the CRFK-based neutralization assay, it was shown that although FIV-Pet and FIV-GL8, both of which are of clade A, were cross-neutralized by sera derived from cats infected with either virus, there was a degree of antigenic strain specificity.[52] A third possibility, mentioned previously, is that the FIV-Pet virus was more readily neutralized than FIV-GL8 by the particular antibody induced by vaccination, such that the effective dose of virus was rapidly reduced immediately following challenge.

If the virulence of a virus indicates a phenotype that should be incorporated into a vaccine in order to provide effective protection, then viruses such as FIV-GL8 should be prime candidates. However, early attempts to produce an effective inactivated virus vaccine based on FIV-GL8 were unsuccessful.[31] This lack of efficacy was ascribed to the poor preservation of SU on the vaccine virus, compared to the FIV-Pet from FL4 cells. In an attempt to produce a vaccine containing FIV-GL8 particles with high levels of SU, a molecular clone was modified to produce a virus in which the endocytosis signal in the cytoplasmic tail of Env was mutated, thereby promoting an accumulation of virus at the infected cell surface and incorporation of SU into viral particles.[30] A vaccine derived from these particles

inactivated with paraformaldehyde provided partial protection from virulent FIV-GL8 challenge. Although the formulation of the two FIV-GL8 vaccines was quite different, it may be that the significant improvement of efficacy of the second vaccine was due to better preservation of SU on the vaccine virus particles. However, the degree of protection achieved by the second vaccine against FIV-GL8 challenge was not markedly greater than that afforded by a previous vaccine based on FIV-Pet.[26] This outcome suggests that factors other than the quality of SU are necessary for protection against challenge by viruses of high virulence.

Inactivated Virus-Infected Cell Vaccines

Vaccines based on inactivated FIV-infected cells have also provided good protection. This material is attractive as a commercial proposition because it does not require expensive concentration or purification, and it contains the full range of viral proteins. In their initial experiments, Yamamoto et al.[82] obtained essentially equivalent protection with vaccines containing either paraformaldehyde-inactivated whole virus or inactivated infected cell vaccines. In these experiments the vaccines were derived from the interleukin-2-independent FL4 cell line that is chronically infected with FIV-Pet, and the challenge was the homologous virus administered by the intraperitoneal route. In further developments of this approach, the vaccine was found to protect also against another virus of the same clade.[81] The inclusion of cells infected with a second strain of FIV, FIV-Shi, belonging to clade D, extended the efficacy of the vaccine, which now protected against challenge by three different FIV strains: FIV-Pet (clade A), FIV-Ban (clade B), and FIV-Shi (clade D). The commercial derivative of this vaccine was shown subsequently to protect against FIV-FC1, a "primary" isolate of clade B, a common clade in the United States.[60] This virus was considered to be of high virulence as it caused a reduction in $CD4^+$ T-cell numbers in unvaccinated cats.

In an attempt to set a benchmark against which other vaccines could be judged, we tested the ability of this commercial vaccine to protect against challenge with the virulent UK isolate, FIV-GL8. The vaccine failed to provide sterile protection in any cats regardless of whether they were challenged by the intramuscular, intraperitoneal, or oronasal route. This raises the question of whether FIV-GL8 represents an excessive challenge. Studies of UK field isolates have shown that they typically produce high viral loads and depress $CD4^+$ T-cell numbers, such that FIV-GL8 appears to be representative. However, it is not clear if there are other factors that may compromise the ability of vaccines to protect against this isolate. Ideally, vaccines would be tested against a panel of well-characterized field isolates, but unfortunately this is impractical at present.

Confirmation of the efficacy of vaccines containing FIV-infected cells was provided by Matteucci et al.[47] A vaccine based on paraformaldehyde-inactivated cells of the MBM feline lymphoblast line infected with FIV-M2 protected cats when challenged with ex vivo, cell-free virus 4 months after vaccination. No significant protection was recorded when the cats were challenged 12 or 28 months after vaccination, indicating that the duration of immunity was short. Nevertheless, this vaccine protected cats in a natural setting over a period of 24 months, as described above.[49]

In contrast to these successes, other types of inactivated cell vaccines have not proven to be as protective. The substrate in which the vaccine virus is grown may have an effect on efficacy. Thus, a vaccine based on paraformaldehyde-inactivated CRFK cells or feline thymocytes infected with FIV-UT113 failed to protect cats on challenge with homologous virus.[75] An even more alarming outcome was found in later trials of vaccines containing inactivated FIV-infected autologous T-cells.[38] The rationale for these experiments was that FIV antigens would be presented in a MHC-matched manner. However, the challenge with homologous virus was found to enhance the challenge, indicated by a shorter period between challenge and the appearance of viraemia in the vaccinated cats versus the controls. Similar results were obtained by Giannecchini et al.[23] in testing four vaccines based on inactivated infected autologous lymphoblasts.

Inactivated HIV Vaccines

There has been comparatively little interest in developing inactivated virus vaccines for HIV, despite the efficacy of some FIV vaccines and the extremely successful use of many killed vaccines for other human diseases. The main reason for the lack of interest is the danger arising from incomplete inactivation of infectious virus and the high cost of production for a virus that is required for large-scale use in poor countries.

Recently, inactivated HIV has been used as the boost in a prime-boost vaccination regime in an attempt to protect macaques from infection with a SIV-HIV recombinant virus (SHIV).[80] The rationale for using inactivated virus particles was that they might induce high levels of virus neutralizing antibodies to complement the cell-mediated immunity likely to be elicited by the priming component, a vaccinia virus recombinant expressing HIV and SIV structural proteins. The vaccine failed to prevent infection with the challenge, although virus loads were very much reduced compared to those in unvaccinated control animals, and the vaccinated macaques remained healthy over a period of 21 months while the controls developed AIDS within this period.

Attenuated Virus and DNA Vaccines

Attenuated virus vaccines offer several advantages over inactivated vaccines. They can be relatively inexpensive to produce, often do not require an adjuvant for efficacy, and produce a robust immune response. Of relevance to the development of lentivirus vaccines is the capacity of attenuated vaccines to induce both antibody and cell-mediated immune responses. Viruses have traditionally been attenuated by prolonged passage in cell culture. However, the introduction of defined changes using molecular techniques offers a more refined and perhaps safer approach. The deletion of accessory lentiviral genes provides a method to reduce virulence without completely disabling virus replication and dissemination in vivo and has been extensively pursued in the SIV model. Attempts to produce a *nef* deleted SIV vaccine were based on the discovery that a number of individuals infected with an HIV variant deleted in *nef* showed slow disease progression. Although this approach showed initial promise, the eventual development of disease, both in vaccinated macaques and HIV-infected individuals, led to an obvious reluctance to pursue such vaccines for HIV.[76]

A further opportunity provided by the FIV model is the availability of viruses of low virulence that can be tested for their ability to protect against subsequent challenge with a more virulent virus. The possibility that FIV-Pet, attenuated by prolonged growth in vitro, could protect against a subsequent virulent challenge has been studied. Modest protection was seen against FIV-M2 administered intravenously.[57] Excellent protection was achieved against intraperitoneal challenge with cell-free FIV-GL8 but not cell-associated vaginal challenge.[56] Although such vaccines are unlikely to be considered safe enough for clinical development, they may be able to provide a useful insight into both viral determinants of pathogenesis and immune correlates of protection.

A difficulty encountered with the use of attenuated virus vaccines is that of growing viral stocks to sufficient titers in vitro to produce the quantities required for vaccination. DNA vaccines offer an alternative method for exploring the immunogenicity of proviruses that can be rendered defective by selective deletions. The first successful example of this approach used a deletion in the reverse transcriptase gene to produce a DNA vaccine (ΔRT) that provided significant protection against challenge with the homologous virus, FIV-Pet.[29] This encouraged the hope that vaccine protection might extend to more virulent isolates. However, subsequent studies have failed to demonstrate such significant levels of protection against FIV-GL8, using proviral DNA vaccines with either the ΔRT vaccine or a similar vaccine with a deletion in integrase.[16,26] Attempts to improve levels of protection by using a variety of cytokine adjuvants and a prime-boost approach have also been unsuccessful. One of the most

promising results shown by DNA vaccination, against FIV-PPR, used a proviral DNA vaccine attenuated by a deletion in *vif*.[43] Unfortunately, the safety of such a vaccine is uncertain, with the possibility that the attenuated virus may revert to a virulent phenotype.

If DNA vaccines were to be developed for widespread use, then one concern that would need to be addressed is the use of vectors that do not contain antibiotic resistance genes. A system to accomplish this has been tested in the FIV model. Minimalistic, immunogenic defined gene expression (MIDGE) constructs encoding FIV Env and feline IL-12 and lacking unnecessary vector sequences have shown significant protection against challenge with FIV-Z2.[6,41]

The experience with DNA vaccines for FIV mirrors that of DNA vaccines for SIV and other viral pathogens. Although they have shown early promise, they have yet to produce levels of protection, when used alone, that suggest they may be applied to HIV. This has led to several approaches to try to improve their efficacy, including the use of cytokine adjuvants, inoculation of DNA vaccines in combination with viral vectors or proteins in a prime-boost approach, use of improved delivery systems, and alteration of codon usage to improve expression levels. Some of these approaches are currently also being tried for FIV. Should improved efficacy be attained then this would be of great value to the development of other lentiviral vaccines.

Protein and Peptide Vaccines

Early efforts to develop both HIV and FIV vaccines attempted to induce protective neutralizing antibodies. Although the majority of successful experiments in the FIV model have used whole inactivated or infected cell preparations, as outlined above, experiments have also been performed using subunit and peptide vaccines (Table III). These have largely been unsuccessful and indeed this result reflects the great challenge that is faced in developing antigens that will induce potent, broadly reactive antibodies that can mediate protection from HIV. It is likely that to be successful such vaccines will have to comprise protective epitopes that are very similar to those in the virus. Since the majority of these are conformationally dependent, it is expected that peptide immunogens are less likely to achieve this. The use of envelope gp120 as a vaccine has met with poor success, most likely due to the lack of authentic conformation of the monomer. This outcome has been seen in the FIV system where the relatively poor efficacy of a purified gp120 vaccine was in contrast to the robust protection provided by the whole inactivated virus from which the purified preparation was derived.[27] The lack of efficacy of gp120 in animal models, however, does not appear to be a lesson that was heeded in the clinical development of an HIV vaccine, where gp120 was pursued at great cost as a prototypic HIV vaccine, as discussed above.

Viral Vectored Vaccines

Like DNA vaccines, vaccination with viral vectors is effective at generating cell-mediated immune responses. Although relatively few studies have been performed with viral vectors for vaccination against FIV, one of the most effective vaccines for the other pathogenic feline retrovirus, FeLV, is a canarypox vector, which expresses FeLV Env and Gag proteins. Cats immunized with a "prime" of canarypox virus encoding FIV *env* and *gag-pol* followed by a "boost" of inactivated FIV-infected cell vaccine were solidly protected against homologous challenge with clade A FIV-Pet. When subsequently challenged with clade B FIV-Bang, cats showed full to partial protection.[71] The idea of combining a vaccine that can generate potent cell-mediated immunity with one that can generate potent antibody responses is one which has being adopted in an effort to develop an effective human AIDS vaccine.[45] However, early data from a phase I and II clinical trial in humans of the prime-boost combination of canarypox and gp120 or gp160 are not encouraging. In this trial, individuals who became infected subsequent to vaccination showed no delay in the progression of infection compared to those receiving placebo.[39]

Enhancement of Challenge by Vaccination

The disturbing phenomenon of enhancement of the FIV challenge following vaccination, evidenced by an acceleration of the appearance of viraemia, a higher virus load, or an increase in the proportion of cats becoming infected compared to control cats, has been observed with several different types of vaccines. This effect has not been recognized in macaques vaccinated with SIV.* Therefore, it is not known whether enhancement is a danger to be avoided in HIV vaccination, although it is a concern, as sera from infected individuals may amplify HIV infection in vitro and the enhancing activity in serum has been suggested as a component in the progression to AIDS.

Two plausible mechanisms have been advanced to account for enhancement of FIV infection by vaccination. The first implicates antibody-mediated infection of target cells, for example, by Fc or complement receptors. In experiments to evaluate several Env products as immunogens, enhancement produced in cats vaccinated with Env expressed by a vaccinia

* Since going to press enhancement has been recognized in the SIV model Ref: Staprans, S. I., Barry, A. P., Silvestri, G., Safrit, J. T., Kozyr, N., Sumpter, B., Nguyen, H., McClure, H., Montefiori, D., Cohen, J. I., Feinberg, M. B. (2004). Enhanced SIV replication and accelerated progression to AIDS in macaques primed to mount a CD4 T cell response to the SIV envelope protein. Proc. Natl. Acad. Sci. U.S.A 101, 13026–13031.

recombinant was transferable to naïve cats by plasma from the vaccinated animals.[67] Definition of the regions of Env that might be responsible for this effect proved to be elusive.[35] Pancino and Sonigo[53] showed that the highly conserved structure of the principal immunogenic determinant of TM, maintained through two delineating cysteines, was essential for viral infectivity and suggested that the induction of antibodies to this region, which represent a large proportion of serum anti-FIV antibodies in infected cats, might be a mechanism that has evolved to enhance viral entry and growth.

However, that the induction of antibodies specific for Env is not the only possible mechanism involved is evident from experiments in which enhancement was produced in cats immunized with vaccines that did not include Env and consequently could not elicit anti-Env antibodies; for example, a recombinant p24 (Gag) vaccine adjuvanted with immune stimulating complexes.[31] In addition, enhancement might be due to the generation of effectors other than antibodies. Thus, Richardson et al.[63] showed that a likely mechanism for increased viral growth in cats after challenge was an expansion of activated T lymphocytes, induced by vaccination with a DNA Env vaccine that did not induce anti-FIV antibodies. In a previous trial of a similar vaccine, which had resulted in enhancement, the vaccinated cats had been challenged shortly after vaccination at a time when lymphocyte activation might have been at a peak. In the second trial, in an attempt to minimize this effect, the vaccinated cats were not challenged until 10 weeks after the final vaccination. The cats immunized by the intradermal/intramuscular route did not exhibit enhanced infection after challenge. However, the appearance of virus in the blood of some cats, in which vaccine was targeted to mesenteric lymph nodes, was very much accelerated. These cats had FIV-specific helper T-cell proliferative responses and their cells were more susceptible than normal cells to infection with FIV ex vivo. Although the number of subjects in these experiments was small, the results suggest that activated FIV-specific T-cells might be more susceptible to infection than nonspecific cells, as has been demonstrated for HIV.[14] It is also possible that vaccination results in a change in the population of cells that are susceptible to infection. For example, the quantity and distribution of cells expressing receptors for FIV may be increased.

Of great concern for the welfare of cats is the finding that enhancement has been seen in trials of vaccines based on inactivated infected FL4 cells, which resulted in the production of the commercial FIV vaccine,[15] and with other inactivated cell vaccines.[23,38] In cats challenged 4 weeks after receiving their third dose of commercial vaccine, plasma viral loads were elevated at 2 weeks postchallenge compared to unvaccinated controls. Viral loads fell below those of controls by 8 weeks postchallenge, but the early rise suggests that wider viral dissemination and establishment of viral reservoirs may have occurred.[15]

Clearly, the potential for enhancement is of great importance, not just for the use of the FIV vaccine, but also for the development of HIV vaccines. It has been suggested that increasing the length of time following vaccination prior to challenge may reduce the risk of enhancement. However, prevention of exposure to the virus while the immune response "matures" and the possibly increased susceptibility to infection declines seems an unlikely proposition for a commercial vaccine.

Vaccination Against Mucosal Challenge

The development of mucosal vaccines against HIV is undoubtedly a rapidly growing area, given increased impetus with the finding that the gastrointestinal mucosa represents a major viral reservoir.[74] As discussed above, much effort has been applied to developing the FIV model to accommodate challenge by a mucosal route that is relevant to HIV. Some degree of protection from vaginal challenge has been achieved by vaccination. Although parenteral vaccination with an inactivated virus vaccine did not protect against challenge with homologous virus, the vaccinated cats had significantly lower virus loads than control cats.[48] In another approach, a recombinant vaccine consisting of an alphavirus replicon that delivered FIV *gag* and *env* given subcutaneously induced serum and mucosal anti-FIV antibodies but did not protect from vaginal challenge with FIV-NCSU-1, a virulent strain.[8]

The use of bacteria as vectors for mucosal vaccine delivery is also an area of growing interest for lentivirus vaccination. Early attempts to develop *Salmonella* as a vector for FIV vaccines met with only modest success.[72,73] In contrast, in a more recent trial of a recombinant *Listeria monocytogenes* vector expressing FIV Gag and Env, a single oral dose of vaccine provided significant protection.[70] Both vaccinated and control cats became infected following challenge with the virulent NCSU-1 strain of FIV, but the vaccinated cats had extremely low virus loads compared to the controls and did not suffer from the depletion of lymphocytes in the intestine or mesenteric lymph nodes as was found in the control cats.

Vaccine trials using rectal challenge have also been carried out. Mucosal immunization, with either a SU V3 peptide or inactivated infected cells, adjuvanted with quil A and cholera B toxin, although inducing FIV-specific antibody and T-cell proliferation, did not protect cats from rectal challenge with the homologous virus, FIV-GL8.[19] More successful results were obtained with an inactivated FL4 cell vaccine when immunization was targeted to the iliac lymph nodes and rectal challenge was with homologous cell-free FIV-Pet.[18] It is not known whether this difference in outcome in the second trial was due to a better preserved immunogen, more effective immunization, or because of the lower virulence of the FIV-Pet challenge. Challenge with cell-associated virus proved to be un-

expectedly inefficient, even in control cats vaccinated with inactivated uninfected cells, which suggested that an anticell immune response had been induced that might have protected from challenge in a non-FIV-specific fashion.

As for parenteral challenge systems, the success or failure of vaccination against mucosal challenge could be due to a variety of factors, including choice of antigen delivery system and strain of challenge virus.

5. CONCLUSIONS

In this short review we have discussed some of the ways in which the development of FIV vaccines has been approached. The driving force behind many of these attempts has been to produce a vaccine that could be used in the field to prevent the spread of FIV among domestic cats. In addition it is recognized that if this end were to be achieved, the methodology might very well be applicable to the development of a vaccine against HIV in humans. So far, success has been tantalizingly close: a commercial vaccine has become available and an experimental vaccine has been shown to protect against challenge in a natural setting. Other vaccines have provided partial protection. Paradoxically, a valuable outcome of some of the trials of FIV vaccines that have failed to provide complete protection is that several confounding factors have been identified, which, by their resolution, may provide the basis for more effective vaccines in the future. For example, the commercial vaccine has been found to protect against some virus strains but not others. This result may be due to differences in virulence between the viruses used for challenge. Sufficient well-characterized FIV strains are now available to test if this is the reason. Another possibility is that there are antigenic differences between the viruses. Again, the availability of a very large number of primary FIV isolates of different clades from around the world means that the antigenic relationships between individual viruses and clades might be defined, and the practical significance of any difference might be tested directly by experimental challenge. The question of whether partial protection reduces the transmissibility of FIV can be determined by the quantification of virus in the saliva of vaccinated and challenged cats. Enhancement of infection following vaccination is a concern for the application of HIV vaccines in the field. Further definition of the factors responsible for enhancement and those that are necessary to overcome enhancement by certain FIV vaccines should be made.

Despite the opportunities provided by FIV to resolve these issues, which undoubtedly also apply to HIV vaccine development, the feline model has been relatively neglected by the AIDS research community. The crucial problems that have arisen in the course of FIV vaccine development will only be resolved by careful, systematic analysis of the viral and host factors

responsible. We have no doubt that given more generous resources, many of these problems could be overcome, and the central tenets for the production of effective vaccines against both FIV and HIV could be established.

Acknowledgements. We are grateful to Dr. Margaret Hosie for her helpful comments on this manuscript.

REFERENCES

1. Addie D. D., Dennis J. M., Toth S., Callanan J. J., Reid S., and Jarrett O. Long-term impact on a closed household of pet cats of natural infection with feline coronavirus, feline leukaemia virus and feline immunodeficiency virus. *Vet. Rec.* 2000;146:419–424.
2. Baba T. W., Trichel A. M., An L., Liska V., Martin L. N., Murphey-Corb M., and Ruprecht R. M. Infection and AIDS in adult macaques after nontraumatic oral exposure to cell-free SIV. *Science* 1996;272:1486–1489.
3. Beatty J. A., Willett B. J., Gault E. A., and Jarrett O. A longitudinal study of feline immunodeficiency virus-specific cytotoxic T lymphocytes in experimentally infected cats, using antigen-specific induction. *J. Virol.* 1996;70:6199–6206.
4. Bendinelli M., Pistello M., Del Mauro D., Cammarota G., Maggi F., Leonildi A., Giannecchini S., Bergamini C., and Matteucci D. During readaptation in vivo, a tissue culture-adapted strain of feline immunodeficiency virus reverts to broad neutralization resistance at different times in individual hosts but through changes at the same position of the surface glycoprotein. *J. Virol.* 2001;75:4584–4593.
5. Bishop S. A., Stokes C. R., Gruffydd-Jones T. J., Whiting C. V., and Harbour D. A. Vaginal and rectal infection of cats with feline immunodeficiency virus. *Vet. Microbiol.* 1996;51:217–227.
6. Boretti F. S., Leutenegger C. M., Mislin C., Hofmann-Lehmann R., Konig S., Schroff M., Junghans C., Fehr D., Huettner S. W., Habel A., Flynn J. N., Aubert A., Pedersen N. C., Wittig B., and Lutz H. Protection against FIV challenge infection by genetic vaccination using minimalistic DNA constructs for FIV env gene and feline IL-12 expression. *AIDS* 2000;14:1749–1757.
7. Bucci J. G., English R. V., Jordan H. L., Childers T. A., Tompkins M. B., and Tompkins W. A. Mucosally transmitted feline immunodeficiency virus induces a CD8+ antiviral response that correlates with reduction of cell- associated virus. *J. Infect. Dis.* 1998;177:18–25.
8. Burkhard M. J., Valenski L., Leavell S., Dean G. A., and Tompkins W. A. Evaluation of FIV protein-expressing VEE-replicon vaccine vectors in cats. *Vaccine* 2002;21:258–268.
9. Chen R., Le Rouzic E., Kearney J. A., Mansky L. M., and Benichou S. Vpr-mediated incorporation of UNG2 into HIV-1 particles is required to modulate the virus mutation rate and for replication in macrophages. *J. Biol. Chem.* 2004;279:28419–28425.
10. Chiarantini L., Matteucci D., Pistello M., Mancini U., Mazzetti P., Massi C., Giannecchini S., Lonetti I., Magnani M., and Bendinelli M. AIDS vaccination studies using an ex vivo feline immunodeficiency virus model: homologous erythrocytes as a delivery system for preferential immunization with putative protective antigens. *Clin. Diagn. Lab. Immunol.* 1998;5:235–241.
11. Cuisinier A. M., Mallet V., Meyer A., Caldora C., and Aubert A. DNA vaccination using expression vectors carrying FIV structural genes induces immune response against feline immunodeficiency virus. *Vaccine* 1997;15:1085–1094.

12. Cuisinier A. M., Meyer A., Chatrenet B., Verdier A. S., and Aubert A. Attempt to modify the immune response developed against FIV gp120 protein by preliminary FIV DNA injection. *Vaccine* 1999;17:415–425.
13. Del Mauro D., Matteucci D., Giannecchini S., Maggi F., Pistello M., and Bendinelli M. Autologous and heterologous neutralization analyses of primary feline immunodeficiency virus isolates. *J. Virol.* 1998;72:2199–2207.
14. Douek D. C., Brenchley J. M., Betts M. R., Ambrozak D. R., Hill B. J., Okamoto Y., Casazza J. P., Kuruppu J., Kunstman K., Wolinsky S., Grossman Z., Dybul M., Oxenius A., Price D. A., Connors M., and Koup R. A. HIV preferentially infects HIV-specific CD4+ T-cells. *Nature* 2002;417:95–98.
15. Dunham S. P., Bruce J., MacKay S., Golder M. C., Jarrett O., and Neil J. Efficacy of feline immunodeficiency virus vaccines Fel-O-Vax and FIVGL8dIN DNA vaccine: Effect of route of challenge on outcome. Seventh International Feline Retrovirus Research Symposium, Pisa, Italy. 2004.
16. Dunham S. P., Flynn J. N., Rigby M. A., Macdonald J., Bruce J., Cannon C., Golder M. C., Hanlon L., Harbour D. A., Mackay N. A., Spibey N., Jarrett O., and Neil JC. Protection against feline immunodeficiency virus using replication defective proviral DNA vaccines with feline interleukin-12 and -18. *Vaccine* 2002;20:1483–1496.
17. Fevereiro M., Roneker C., Laufs A., Tavares L., and de Noronha F. Characterization of two monoclonal antibodies against feline immunodeficiency virus gag gene products and their application in an assay to evaluate neutralizing antibody activity. *J. Gen. Virol.* 1991;72(Pt 3):617–622.
18. Finerty S., Stokes C. R., Gruffydd-Jones T. J., Hillman T. J., Barr F. J., and Harbour D. A. Targeted lymph node immunization can protect cats from a mucosal challenge with feline immunodeficiency virus. *Vaccine* 2001;20:49–58.
19. Finerty S., Stokes C. R., Gruffydd-Jones T. J., Hillman T. J., Reeves N. A., Whiting C. V., Schaaper W. M., Dalsgaard K., and Harbour D. A. Mucosal immunization with experimental feline immunodeficiency virus (FIV) vaccines induces both antibody and T-cell responses but does not protect against rectal FIV challenge. *Vaccine* 2000;18:3254–3265.
20. Flynn J. N., Cannon C. A., Neil J. C., and Jarrett O. Vaccination with a feline immunodeficiency virus multiepitopic peptide induces cell-mediated and humoral immune responses in cats, but does not confer protection. *J. Virol.* 1997;71:7586–7592.
21. Flynn J. N., Keating P., Hosie M. J., Mackett M., Stephens E. B., Beatty J. A., Neil J. C., and Jarrett O. Env-specific CTL predominate in cats protected from feline immunodeficiency virus infection by vaccination. *J. Immunol.* 1996;157:3658–3665.
22. Gemeniano M. C., Sawai E. T., and Sparger E. E. Feline immunodeficiency virus Orf-A localizes to the nucleus and induces cell cycle arrest. *Virology* 2004;325:167–174.
23. Giannecchini S., Isola P., Sichi O., Matteucci D., Pistello M., Zaccaro L., Del Mauro D., and Bendinelli M. AIDS vaccination studies using an ex vivo feline immunodeficiency virus model: failure to protect and possible enhancement of challenge infection by four cell-based vaccines prepared with autologous lymphoblasts. *J. Virol.* 2002;76:6882–6892.
24. Gonin P., Fournier A., Oualikene W., Moraillon A., and Eloit M. Immunization trial of cats with a replication-defective adenovirus type 5 expressing the ENV gene of feline immunodeficiency virus. *Vet. Microbiol.* 1995;45:393–401.
25. Hohdatsu T., Pu R., Torres B. A., Trujillo S., Gardner M. B., and Yamamoto J. K. Passive antibody protection of cats against feline immunodeficiency virus infection. *J. Virol.* 1993;67:2344–2348.
26. Hosie M. J., Dunsford T., Klein D., Willett B. J., Cannon C., Osborne R., Macdonald J., Spibey N., Mackay N., Jarrett O., and Neil J. C. Vaccination with inactivated virus but not viral DNA reduces virus load following challenge with a heterologous and virulent isolate of feline immunodeficiency virus. *J. Virol.* 2000;74:9403–9411.

27. Hosie M. J., Dunsford T. H., de Ronde A., Willett B. J., Cannon C. A., Neil J. C., and Jarrett O. Suppression of virus burden by immunization with feline immunodeficiency virus Env protein. *Vaccine* 1996;14:405–411.
28. Hosie M. J., and Flynn J. N. Feline immunodeficiency virus vaccination: characterization of the immune correlates of protection. *J. Virol.* 1996;70:7561–7568.
29. Hosie M. J., Flynn J. N., Rigby M. A., Cannon C., Dunsford T., Mackay N. A., Argyle D., Willett B. J., Miyazawa T., Onions D. E., Jarrett O., and Neil J. C. DNA vaccination affords significant protection against feline immunodeficiency virus infection without inducing detectable antiviral antibodies. *J. Virol.* 1998;72:7310–7319.
30. Hosie, M. J., Klein, D., Binley, J. M., Dunsford, T. H., Jarrett, O., Neil, J. C., Knapp, E., Giannecchini, S., Matteucci, D., Bendinelli, M., Hoxie, J. A., and Willett, B. J. Vaccination with an inactivated virulent feline immunodeficiency virus engineered to express high levels of Env. *J. Virol.* 2005;79:1954–1957.
31. Hosie M. J., Osborne R., Reid G., Neil J. C., and Jarrett O. Enhancement after feline immunodeficiency virus vaccination. *Vet. Immunol. Immunopathol.* 1992;35:191–197.
32. Hosie M. J., Osborne R., Yamamoto J. K., Neil J. C., and Jarrett O. Protection against homologous but not heterologous challenge induced by inactivated feline immunodeficiency virus vaccines. *J. Virol.* 1995;69:1253–1255.
33. Hosie M. J., Willett B. J., Klein D., Dunsford T. H., Cannon C., Shimojima M., Neil J. C., and Jarrett O. Evolution of replication efficiency following infection with a molecularly cloned feline immunodeficiency virus of low virulence. *J. Virol.* 2002;76:6062–6072.
34. Huisman W., Karlas J. A., Siebelink K. H., Huisman R. C., de Ronde A., Francis M. J., Rimmelzwaan G. F., and Osterhaus A. D. Feline immunodeficiency virus subunit vaccines that induce virus neutralising antibodies but no protection against challenge infection. *Vaccine* 1998;16:181–187.
35. Huisman W., Schrauwen E. J., Pas S. D., Karlas J. A., Rimmelzwaan G. F., and Osterhaus A. D. Antibodies specific for hypervariable regions 3 to 5 of the feline immunodeficiency virus envelope glycoprotein are not solely responsible for vaccine-induced acceleration of challenge infection in cats. *J. Gen. Virol.* 2004;85:1833–1841.
36. Inoshima Y., Miyazawa T., Kohmoto M., Ikeda Y., Sato E., Hohdatsu T., Mathiason-Dubard C., Hoover E. A., and Mikami T. Cross virus neutralizing antibodies against feline immunodeficiency virus genotypes A, B, C, D and E. *Arch. Virol.* 1998;143:157–162.
37. Jordan H. L., Howard J., Barr M. C., Kennedy-Stoskopf S., Levy J. K., and Tompkins W. A. Feline immunodeficiency virus is shed in semen from experimentally and naturally infected cats. *AIDS Res. Hum. Retroviruses* 1998;14:1087–1092.
38. Karlas J. A., Siebelink K. H., Peer M. A., Huisman W., Cuisinier A. M., Rimmelzwaan G. F., and Osterhaus A. D. Vaccination with experimental feline immunodeficiency virus vaccines, based on autologous infected cells, elicits enhancement of homologous challenge infection. *J Gen. Virol.* 1999;80:761–765.
39. Lee D., Graham B. S., Chiu Y. L., Gilbert P. B., McElrath M. J., Belshe R. B., Buchbinder S. P., Sheppard H. W., Koblin B. A., Mayer K. H., Keefer M. C., Mulligan M. J., and Celum C. L. Breakthrough infections during phase 1 and 2 prime-boost HIV-1 vaccine trials with canarypox vectors (ALVAC) and booster dose of recombinant gp120 or gp160. *J. Infect. Dis.* 2004;190:903–907.
40. Lerner D. L., Wagaman P. C., Phillips T. R., Prospero-Garcia O., Henriksen S. J., Fox H. S., Bloom F. E., and Elder J. H. Increased mutation frequency of feline immunodeficiency virus lacking functional deoxyuridine-triphosphatase. *Proc. Natl. Acad. Sci. USA.* 1995;92:7480–7484.
41. Leutenegger C. M., Boretti F. S., Mislin C. N., Flynn J. N., Schroff M., Habel A., Junghans C., Koenig-Merediz S. A., Sigrist B., Aubert A., Pedersen N. C., Wittig B., and Lutz H. Immunization of cats against feline immunodeficiency virus (FIV) infection by using minimalistic immunogenic defined gene expression vector vaccines expressing FIV gp140

alone or with feline interleukin-12 (IL-12), IL-16, or a CpG Motif. *J. Virol.* 2000;74:10447–10457.
42. Leutenegger C. M., Hofmann-Lehmann R., Holznagel E., Cuisinier A. M., Wolfensberger C., Duquesne V., Cronier J., Allenspach K., Aubert A., Ossent P., and Lutz H. Partial protection by vaccination with recombinant feline immunodeficiency virus surface glycoproteins. *AIDS Res. Hum. Retroviruses* 1998;14:275–283.
43. Lockridge K. M., Chien M., Dean G. A., Stefano C. K., Montelaro R. C., Luciw P. A., and Sparger E. E. Protective immunity against feline immunodeficiency virus induced by inoculation with vif-deleted proviral DNA. *Virology* 2000;273:67–79.
44. Lombardi S., Garzelli C., Pistello M., Massi C., Matteucci D., Baldinotti F., Cammarota G., Da Prato L., Bandecchi P., and Tozzini F. A neutralizing antibody-inducing peptide of the V3 domain of feline immunodeficiency virus envelope glycoprotein does not induce protective immunity. *J. Virol.* 1994;68:8374–8379.
45. Malkevitch N. V., and Robert-Guroff M. A call for replicating vector prime-protein boost strategies in HIV vaccine design. *Expert. Rev. Vaccines* 2004;3:S105–S117.
46. Matteucci D., Baldinotti F., Mazzetti P., Pistello M., Bandecchi P., Ghilarducci R., Poli A., Tozzini F., and Bendinelli M. Detection of feline immunodeficiency virus in saliva and plasma by cultivation and polymerase chain reaction. *J. Clin. Microbiol.* 1993;31:494–501.
47. Matteucci D., Pistello M., Mazzetti P., Giannecchini S., Del Mauro D., Lonetti I., Zaccaro L., Pollera C., Specter S., and Bendinelli M. Studies of AIDS vaccination using an ex vivo feline immunodeficiency virus model: protection conferred by a fixed-cell vaccine against cell-free and cell-associated challenge differs in duration and is not easily boosted. *J. Virol.* 1997;71:8368–8376.
48. Matteucci D., Pistello M., Mazzetti P., Giannecchini S., Isola P., Merico A., Zaccaro L., Rizzuti A., and Bendinelli M. AIDS vaccination studies using feline immunodeficiency virus as a model: immunization with inactivated whole virus suppresses viraemia levels following intravaginal challenge with infected cells but not following intravenous challenge with cell-free virus. *Vaccine* 1999;18:119–130.
49. Matteucci D., Poli A., Mazzetti P., Sozzi S., Bonci F., Isola P., Zaccaro L., Giannecchini S., Calandrella M., Pistello M., Specter S., and Bendinelli M. Immunogenicity of an anti-clade B feline immunodeficiency fixed-cell virus vaccine in field cats. *J. Virol.* 2000;74:10911–10919.
50. Mazzetti P., Giannecchini S., Del Mauro D., Matteucci D., Portincasa P., Merico A., Chezzi C., and Bendinelli M. AIDS vaccination studies using an ex vivo feline immunodeficiency virus model: detailed analysis of the humoral immune response to a protective vaccine. *J. Virol.* 1999;73:1–10.
51. Moench T. R., Whaley K. J., Mandrell T. D., Bishop B. D., Witt C. J., and Cone R. A. The cat/feline immunodeficiency virus model for transmucosal transmission of AIDS: nonoxynol-9 contraceptive jelly blocks transmission by an infected cell inoculum. *AIDS* 1993;7:797–802.
52. Osborne R., Rigby M., Siebelink K., Neil J. C., and Jarrett O. Virus neutralization reveals antigenic variation among feline immunodeficiency virus isolates. *J. Gen. Virol.* 1994;75(Pt 12):3641–3645.
53. Pancino G., and Sonigo P. Retention of viral infectivity after extensive mutation of the highly conserved immunodominant domain of the feline immunodeficiency virus envelope. *J. Virol.* 1997;71:4339–4346.
54. Pedersen N. C., Ho E. W., Brown M. L., and Yamamoto J. K. Isolation of a T-lymphotropic virus from domestic cats with an immunodeficiency-like syndrome. *Science* 1987; 235:790–793.
55. Pistello M., Cammarota G., Nicoletti E., Matteucci D., Curcio M., del Mauro D., and Bendinelli M. Analysis of the genetic diversity and phylogenetic relationship of Italian

isolates of feline immunodeficiency virus indicates a high prevalence and heterogeneity of subtype B. *J. Gen. Virol.* 1997;78(Pt 9):2247–2257.
56. Pistello M., Matteucci D., Bonci F., Isola P., Mazzetti P., Zaccaro L., Merico A., del Mauro D., Flynn N., and Bendinelli M. AIDS vaccination studies using an ex vivo feline immunodeficiency virus model: protection from an intraclade challenge administered systemically or mucosally by an attenuated vaccine. *J. Virol.* 2003;77:10740–10750.
57. Pistello M., Matteucci D., Cammarota G., Mazzetti P., Giannecchini S., del Mauro D., Macchi S., Zaccaro L., and Bendinelli M. Kinetics of replication of a partially attenuated virus and of the challenge virus during a three-year intersubtype feline immunodeficiency virus superinfection experiment in cats. *J. Virol.* 1999;73:1518–1527.
58. Pu R., Coleman J., Coisman J., Sato E., Tanabe T., Arai M., and Yamamoto J. K. Dual-subtype FIV (Fel-O-Vax FIV) protection against heterologous subtype B FIV isolate. *J. Feline Med. Surg.* 7:65–70.
59. Pu R., Coleman J., Omori M., Arai M., Hohdatsu T., Huang C., Tanabe T., and Yamamoto J. K. Dual-subtype FIV vaccine protects cats against in vivo swarms of both homologous and heterologous subtype FIV isolates. *AIDS* 2001;15:1225–1237.
60. Pu R., Coleman J., and Yamamoto J. K. Dual-subtype FIV vaccine protection against virulent heterologous subtype B virus. Seventh International Feline Retrovirus Research Symposium, Pisa, Italy. 2004.
61. Pu R., Omori M., Okada S., Rine S. L., Lewis B. A., Lipton E., and Yamamoto J. K. MHC-restricted protection of cats against FIV infection by adoptive transfer of immune cells from FIV-vaccinated donors. *Cell Immunol.* 1999;198:30–43.
62. Reggeti F., and Bienzle D. Feline immunodeficiency virus subtypes A, B and C and intersubtype recombinants in Ontario, Canada. *J. Gen. Virol.* 2004;85:1843–1852.
63. Richardson J., Broche S., Baud S., Leste-Lasserre T., Femenia F., Levy D., Moraillon A., Pancino G., and Sonigo P. Lymphoid activation: a confounding factor in AIDS vaccine development? *J. Gen. Virol.* 2002;83:2515–2521.
64. Richardson J., Moraillon A., Baud S., Cuisinier A. M., Sonigo P., and Pancino G. Enhancement of feline immunodeficiency virus (FIV) infection after DNA vaccination with the FIV envelope. *J. Virol.* 1997;71:9640–9649.
65. Sheehy A. M., Gaddis N. C., and Malim M. H. The antiretroviral enzyme APOBEC3G is degraded by the proteasome in response to HIV-1 Vif. *Nat. Med.* 2003;9:1404–1407.
66. Shimojima M., Miyazawa T., Ikeda Y., McMonagle E. L., Haining H., Akashi H., Takeuchi Y., Hosie M. J., and Willett B. J. Use of CD134 as a primary receptor by the feline immunodeficiency virus. *Science* 2004;303:1192–1195.
67. Siebelink K. H., Tijhaar E., Huisman R. C., Huisman W., de Ronde A., Darby I. H., Francis M. J., Rimmelzwaan G. F., and Osterhaus A. D. Enhancement of feline immunodeficiency virus infection after immunization with envelope glycoprotein subunit vaccines. *J. Virol.* 1995;69:3704–3711.
68. Sodora D. L., Gettie A., Miller C. J., and Marx P. A. Vaginal transmission of SIV: assessing infectivity and hormonal influences in macaques inoculated with cell-free and cell-associated viral stocks. *AIDS Res. Hum. Retroviruses* 1998;14(Suppl 1):S119–S123.
69. Steinrigl A., and Klein D. Phylogenetic analysis of feline immunodeficiency virus in Central Europe: a prerequisite for vaccination and molecular diagnostics. *J. Gen. Virol.* 2003;84:1301–1307.
70. Stevens R., Howard K. E., Nordone S., Burkhard M., and Dean G. A. Oral immunization with recombinant *Listeria monocytogenes* controls virus load after vaginal challenge with feline immunodeficiency virus. *J. Virol.* 2004;78:8210–8218.
71. Tellier M. C., Pu R., Pollock D., Vitsky A., Tartaglia J., Paoletti E., and Yamamoto J. K. Efficacy evaluation of prime-boost protocol: canarypoxvirus-based feline immunodeficiency virus (FIV) vaccine and inactivated FIV- infected cell vaccine against heterologous FIV challenge in cats. *AIDS* 1998;12:11–18.

72. Tijhaar E. J., Huisman W., Huisman R. C., Siebelink K. H., Karlas J. A., de Ronde A., van Herwijnen R., Mooi F. R., and Osterhaus A. D. *Salmonella typhimurium* aroA recombinants and immune-stimulating complexes as vaccine candidates for feline immunodeficiency virus. *J. Gen. Virol.* 1997;78(Pt 12):3265–3275.
73. Tijhaar E. J., Siebelink K. H., Karlas J. A., Burger M. C., Mooi F. R., and Osterhaus A. D. Induction of feline immunodeficiency virus specific antibodies in cats with an attenuated *Salmonella* strain expressing the Gag protein. *Vaccine* 1997;15:587–596.
74. Veazey R., and Lackner A. The mucosal immune system and HIV-1 infection. *AIDS Rev.* 2003;5:245–252.
75. Verschoor E. J., van Vliet A. L., Egberink H. F., Hesselink W., van Alphen W. E., Joosten I., Boog C. J., Horzinek M. C., and de Ronde A. Vaccination against feline immunodeficiency virus using fixed infected cells. *Vet. Immunol. Immunopathol.* 1995;46:139–149.
76. Whitney J. B., and Ruprecht R. M. Live attenuated HIV vaccines: pitfalls and prospects. *Curr. Opin. Infect. Dis.* 2004;17:17–26.
77. Willett B. J., Hosie M. J., Callanan J. J., Neil J. C., and Jarrett O. Infection with feline immunodeficiency virus is followed by the rapid expansion of a CD8+ lymphocyte subset. *Immunology* 1993;78:1–6.
78. Willett B. J., Hosie M. J., Neil J. C., Turner J. D., and Hoxie J. A. Common mechanism of infection by lentiviruses [letter]. *Nature* 1997;385:587.
79. Willett B. J., Picard L., Hosie M. J., Turner J. D., Adema K., and Clapham P. R. Shared usage of the chemokine receptor CXCR4 by the feline and human immunodeficiency viruses. *J. Virol.* 1997;71:6407–6415.
80. Willey R. L., Byrum R., Piatak M., Kim Y. B., Cho M. W., Rossio J. J., Jr., Bess J. J., Jr., Igarashi T., Endo Y., Arthur L. O., Lifson J. D., and Martin M. A. Control of viremia and prevention of simian-human immunodeficiency virus-induced disease in rhesus macaques immunized with recombinant vaccinia viruses plus inactivated simian immunodeficiency virus and human immunodeficiency virus type 1 particles. *J. Virol.* 2003;77:1163–1174.
81. Yamamoto J. K., Hohdatsu T., Olmsted R. A., Pu R., Louie H., Zochlinski H. A., Acevedo V., Johnson H. M., Soulds G. A., and Gardner M. B. Experimental vaccine protection against homologous and heterologous strains of feline immunodeficiency virus. *J. Virol.* 1993;67:601–605.
82. Yamamoto J. K., Okuda T., Ackley C. D., Louie H., Pembroke E., Zochlinski H., Munn R. J., and Gardner M. B. Experimental vaccine protection against feline immunodeficiency virus. *AIDS Res. Hum. Retroviruses* 1991;7:911–922.

11

FIV as a Model for HIV Treatment

KATRIN HARTMANN and CHRISTIANE STENGEL

1. INTRODUCTION

Human immunodeficiency virus (HIV) infection shares close similarities with feline immunodeficiency virus (FIV) infection in respect to molecular structure, replication properties, and pathogenesis of disease. FIV causes an immunodeficiency syndrome in domestic cats comparable to acquired immunodeficiency syndrome (AIDS) in humans.

As anti-HIV drugs and combination therapies are only partially effective in controlling viral replication and rapid emergence of resistance limits their efficacy, development of new drugs is crucial. Furthermore, the question of whether therapy should be started early in infection or when disease is more advanced has become a major point of discussion. Identification of suitable animal models that mimic HIV infection and the evolving clinical syndrome seen in humans is essential for development and testing of antiviral agents in vivo. The FIV model can be used to investigate pharmacodynamic and pharmacokinetic properties of anti-HIV compounds and can help to determine their antiviral potency. Studies in experimentally infected cats allow evaluation of drug efficacy in preexposure models, in recent infection as well as in chronic infection with defined infection status. In addition, a major advantage of the FIV/cat model for evaluation of antiviral drugs is the fact that cats are naturally infected with the virus, and treatment studies can be performed without experimental infection in naturally exposed animals. FIV infection is an important problem in the cat population as 2 to 30% of pet cats worldwide are naturally infected by FIV. Treatment studies in naturally infected cats are not only helpful in the development of new drugs against HIV but also of major importance for veterinary medicine as they provide a potential treatment for pet cats, thus

KATRIN HARTMANN and CHRISTIANE STENGEL • Medizinische Kleintierklinik, Ludwig-Maximilians-Universität München, Germany

improving clinical signs, increasing quality of life, and prolonging survival time in these animal patients.

2. DEVELOPMENT OF THE FIV/CAT MODEL

For using the FIV/cat system as an animal model for treatment of HIV infection, several in vitro and in vivo parameters have been developed.

In Vitro Parameter

First, potential compounds have to be screened in FIV-infected cell cultures for their anti-FIV efficacy and cytotoxicity in feline cells. A number of different feline cells have been used including Crandell feline kidney (CrFK) cells or primary lymphocyte cultures that can be infected with FIV. For investigation of the antiviral efficacy in vitro, different assays can be used, for example, measurement of reverse transcriptase (RT) activity or FIV p24 antigen concentration in cell culture supernatants, immunohistochemical staining, counting of infected cells as well as polymerase chain reaction (PCR) to determine provirus or virus load in culture cells or supernatants, respectively. Cytotoxicity can be determined in vitro using trypan blue staining of viable cells or tests to evaluate enzymatic cell function (e.g., MTT or XTT assays).

In Vivo Parameters

Usefulness of a variety of parameters to determine efficacy, compare activity, and detect side effects of antiviral compounds in FIV-infected cats has been evaluated. Clinical parameters, like quality of life and improvement of clinical signs, need to be objectively quantified to allow statistical analysis. This can only be achieved by performing placebo-controlled double-blind studies.

Karnofsky's Score

To evaluate quality of life and well-being of cats, the Karnofsky's score was modified for cats. The Karnofsky's score, evaluating quality of life in human patients and commonly used for HIV-infected individuals (Fischl et al., 1990), was originally developed by David A. Karnofsky in 1948 for patients suffering from lung cancer (Karnofsky et al., 1948). This score determines the ability of a patient to carry on normal activities in life by using a scale from 0 to 100%.

This index has been modified for cats (Hartmann and Kuffer, 1998) taking playing, grooming, sleeping, eating, sexual, and comfort behavior

of the feline patients into consideration. The modified Karnofsky's score enables judgment of quality of life and well-being in cats which otherwise would be very difficult to interpret. Objectivity is given by a classification oriented in details concerning general condition as well as changes in behavior. Establishing an objective quantifiable scale is especially important for animal studies because the patient itself is not able to give information about well-being and life quality, which should be one main criteria for treatment success.

Clinical Signs

Under natural conditions, FIV-related clinical signs develop after a long latent time period over years, as in HIV. Symptoms are characterized by a progressive immunosuppression and appearance of opportunistic infections like viral (e.g., feline calicivirus, feline herpesvirus, and poxvirus infection, feline infectious peritonitis (FIP)), protozoal (e.g., toxoplasmosis, cryptosporidiosis, fungal (e.g., candidiasis, cryptococcosis), and bacterial (e.g., mycobacteriosis) infections. In both diseases, cofactors like secondary infections play an important role for manifestation of the immunodeficiency (Torten et al., 1990; Reubel et al., 1994). Progression of both types of infection takes a parallel course, and a similar classification in different stages of disease is possible. Neurological signs are described in FIV and HIV infection, which show similar histopathological pictures (Dow et al., 1990). In addition, hematological changes during FIV infection are comparable to those of HIV-infected patients (Scadden et al., 1989).

FIV infection progresses through several stages, much like HIV infection in humans. Recognized clinical stages in cats include an acute phase, a clinically asymptomatic phase of variable duration, and a terminal phase of infection. Attempts have been made to define the clinical course in different stages analogous to those of HIV infection; there is, however, often no sharp distinction between the phases in cats and not all stages will be apparent in some cats.

The primary phase of infection is characterized by clinical signs of varying severity such as fever, diarrhea, stomatitis, conjunctivitis, uveitis, and generalized lymphadenopathy and by laboratory changes such as lymphopenia and neutropenia. Signs persist from a few days to several weeks before disappearing. Mortality during the initial stage is low, and in natural infection clinical signs are usually not noticed by the owners. Following the first stage, cats enter a long period of clinically normal appearance (latent stage) before finally progressing to the terminal stage. Although no clinical signs are present, a progressive decrease in $CD4^+$ cells and CD4/CD8 ratio and a hypergammaglobulinemia can usually be demonstrated during the latent stage. Cats in the terminal stage with AIDS-like disease suffer

from opportunistic infections, myelosuppression, tumors, or neurologic signs. The span between the primary and the terminal stages has not been precisely determined, but it can last up to 5 years or more. Some cats may carry FIV for life with minimal disease problems. Cofactors such as exposure to secondary infections determined by environmental conditions play an important role in disease progression, as it has been shown that indoor only cats in single cat households have a significant longer symptom-free period and life expectancy than cats that have contact with other cats and infectious agents.

Many FIV-infected cats are clinically completely healthy; others present with a history of recurrent illnesses with periods of relative health between episodes. In a follow-up study in naturally FIV-infected cats, the rate of progression was variable, with death occurring in about 18% of infected cats within the first 2 years of observation (about 5 years after the estimated time of infection). An additional 18% developed increasingly severe disease, but more than 50% of the infected cats remained clinically asymptomatic during the 2 years (Barr and Phillips, 2000). In a survey study of 826 naturally FIV-infected cats examined at North American Veterinary Teaching Hospitals, the most common disease syndromes diagnosed in the FIV-positive patients were stomatitis, neoplasia (especially malignant lymphoma and cutaneous squamous cell carcinoma), ocular inflammation (uveitis and chorioretinitis), anemia and leukopenia, opportunistic infections, renal insufficiency, lower urinary tract disease, and endocrinopathies such as hyperthyroidism and diabetes mellitus (Shaw et al., 1990; Levy, 2000), some of which are most likely associated with the older age in which these cats were presented (e.g., endocrinopathies, renal insufficiency) than with their FIV infection.

Stomatitis. Chronic ulceroproliferative stomatitis is the most common disease syndrome found in cats naturally infected with FIV; depending on the investigated population, up to 50% of FIV-infected cats may suffer from oral lesions. The proliferative stomatitis characteristically originates in the fauces and gradually spreads rostrally, especially along the maxillary teeth. The lesions are often painful and tooth loss is common. Severe stomatitis can lead to anorexia and emaciation.

Histologically, the mucosa is invaded by plasma cells and lymphocytes, accompanied by variable degrees of neutrophilic and eosinophilic inflammation. The cause of the syndrome is uncertain, although the histologic findings suggest an immune response to chronic antigenic stimulation or immune dysregulation. In addition, circulating lymphocytes of cats with stomatitis have higher than normal expression of inflammatory cytokines (Levy, 2000), further implicating immune activation in the pathogenesis of this condition. Concurrent calicivirus infection is often identified in the oral cavity of cats with FIV-associated stomatitis and may be one of the infectious cofactors capable of inducing stomatitis in combination with FIV.

Other infectious agents may also play a role. Trichomoniasis in the oral cavity is found significantly more often in FIV-infected cats than in FIV-free animals. Oral *Candida* infection has been described in cats with FIV infection, similar to the opportunistic infection in HIV-infected humans. Recently, *Bartonella* infections have been discussed as being a potential cofactor in chronic proliferative stomatitis.

Neurologic Disease. Both central and peripheral neurologic disease complicates the course of HIV infection of humans, and the same is true for FIV. The dementia of human AIDS is often characterized by a slight decline in cognitive ability or behavior, changes that may be too subtle to recognize in cats. About 5% of clinically diseased FIV-infected cats have neurological abnormalities as a predominant clinical feature of their disease. Although the majority of FIV-infected cats do not manifest clinically observable neurologic dysfunction, a much higher proportion of naturally and experimentally infected cats exhibit microscopic lesions in their central nervous system (CNS) (Dow et al., 1990; Barr and Phillips, 2000). However, as in HIV infection, histopathologic lesions only poorly correlate with detected clinical abnormalities. Experimental studies suggest that brain lesions may occur in the absence of massive infections, and abnormal neurologic function has been documented in FIV-infected cats with only mild to moderate histologic evidence of inflammation.

Uncommonly, neurologic signs are caused by opportunistic infections such as toxoplasmosis, cryptococcosis, or FIP; mostly, abnormal neurologic function is the result from a direct effect of the virus on CNS cells. Experimentally, neurologic expression of FIV infection is highly strain-dependant. The virus infects the brain early, with virus-induced CNS lesions sometimes developing within 2 months after experimental infection (Barr and Phillips, 2000). However, these early CNS signs are frequently transient in nature and may resolve after a few weeks or months. The reversal nature of these early neurologic signs may reflect resolution of transient lesions or the adaptive ability of the brain.

Neurological aberrations seen in naturally infected cats tend to be more behavioral than motor. Twitching movements of the face and tongue, psychotic behavior, dementia, loss of bladder and rectal control, compulsive roaming, and disturbed sleep pattern have been recognized. Other signs described are nystagmus, ataxia, convulsions, and intention tremor. In addition, sensitive electrodiagnostic tests such as nerve conduction velocity and brain stem auditory evoked potentials may detect abnormalities in clinically normal FIV-infected cats (Phillips et al., 1994; Barr and Phillips, 2000).

FIV has been isolated from cerebrospinal fluid (CSF), cerebral cortex, nucleus caudatus, midbrain, cerebellum, and rostral and caudal brain stem. Pathologic findings include the presence of perivascular infiltrates of mononuclear cells, diffuse gliosis, glial nodules, and white matter pallor.

These lesions are usually located in the caudate nucleus, midbrain, and rostral brain stem. Brain cells that have been shown to be infected by FIV include microglia and astrocytes. The virus does not infect neurons; however, neuronal death has been associated with FIV infection (Barr and Phillips, 2000). Especially forebrain signs are often a result of direct neuronal injury from the virus. The mechanism of neuronal damage is unclear. Neuronal apoptosis has been discussed. Neuronal damage may also result from the effects of FIV on the neuron supportive functions of astroytes, toxic products released from infected microglia, or cytokines produced in response to viral infection. In vitro studies support the hypothesis that FIV infection of CNS cells, in particular astrocytes, may impair normal metabolism (Zenger et al., 1995; Barr and Phillips, 2000). Documented abnormalities of astrocyte function include altered intercellular communication, abnormal glutathione reductase activity that could render cells more susceptible to oxidative injury, and alterations in mitochondrial membrane potential that disrupt energy-producing capacities of the cell (Zenger et al., 1995; Sellon, 1998). Astrocytes are by far the most common cell type of the brain and are important in maintaining CNS neuronal microenvironment. One of the most important functions of astrocytes is to regulate the level of extracellular glutamate, a major excitatory neurotransmitter that accumulates as a consequence of neuronal activity. Excessive extracellular glutamate often results in neuronal toxicity and death. It has been shown that infection of astrocytes with FIV can significantly inhibit the glutamate-scavenging ability of feline astrocytes, potentially resulting in neuronal damage (Yu et al., 1998).

Tumors. FIV-infected cats have a higher incidence of certain types of tumors, as is the case in HIV-infected humans. Statistically, FIV-infected cats are five times more likely to develop malignant lymphoma or leukemia (mainly B cell) than noninfected cats, compared to 62 times for feline leukemia virus (FeLV) infected cats and 77 times for cats with dual FIV/FeLV infection (Callanan et al., 1996; Sellon, 1998). Neoplastic diseases that have been reported besides malignant lymphomas include squamous cell carcinomas, fibrosarcomas, mast cell tumors, and myeloid tumors. In the survey study at North American Veterinary Teaching Hospitals including 826 naturally FIV-infected cats, the second most common neoplasia was cutaneous squamous cell carcinoma (Levy, 2000). This may not only be the result of FIV infection being associated with tumor development but also be influenced by the fact that FIV-infected cats are usually free-roaming by the time the infection is detected and therefore might have increased exposure to sun when compared to indoor only animals.

It is not exactly known how FIV is associated with these cancers. Lentiviruses are not considered to be directly oncogenic. Due to the fact that older cats are more likely to both be infected with FIV and develop tumors, it is difficult to determine the exact role of FIV in the neoplastic

process. It is known that infection with immunosuppressive lentiviruses is associated with increased cancer risk, but most studies have suggested indirect mechanisms. Examination of five tumors of cats naturally or experimentally infected with FIV with molecular probes to screen for integrated viral sequences was not able to detect integrated FIV genome, suggesting that the role of FIV in lymphomagenesis is generally indirect (Terry et al., 1995). There are several theories about the association of tumors with FIV infection. FIV might increase cancer incidence by decreasing tumor immunosurveillance mechanisms; it might promote tumor development through immunostimulatory effects by replicating in lymphocytes; it might impair immunological control of oncogenic FeLV infection as well as control of overgrowth of transformed lymphoid cells; or it might allow other cancer-causing agents to be activated (Hartmann, 1998). An exception was recently found in a B-cell lymphoma in which the tumor cells contained a single integrated FIV genome. Genome analysis indicated that FIV integration in this case resulted in promoter insertion and truncation of a conserved gene on feline chromosome B3, whereas the unaffected allele of the gene appeared to be transcriptionally down-regulated. This gene is located within a region of frequent tumor-specific deletions. These new observations demonstrate that FIV may indeed also have a direct mutagenic potential (Beatty et al., 2002).

Immunological Changes

Immunological changes are similar in FIV and HIV infections. In both infections, there is a selective decrease of the $CD4^+$ cell count and the CD4/CD8 ratio. Furthermore, a progressive reduction of the stimulability of the T-lymphocytes can be seen, in combination with an unspecific B-cell stimulation (Hara et al., 1990; Taniguchi et al., 1990). Changes in cytokine pattern (Lehmann et al., 1992; Ohashi et al., 1992; Lawrence et al., 1995) and a reduced antibody response against T-cell dependent synthetic polypeptides (Torten et al., 1991) develop during the course of infection, which resemble immune dysfunction in humans with AIDS.

The immune response of the cat to the retroviral infection follows the same pattern as in HIV-infected patients. Antibodies against *env*-proteins are regularly and early produced, whereas antibodies against core proteins may appear later (Rimmelzwaan et al., 1994).

FIV replicates in $CD4^+$ and $CD8^+$ lymphocytes, in B lymphocytes, in macrophages, as well as in astrocytes and microglia cells. As in HIV, some FIV strains replicate preferentially in lymphocytes and only minimally in macrophages, while other strains are able to replicate equally well in both cell types. Replication in different cell types is thought to be associated with use of different cellular receptors and to be responsible for different clinical manifestations. Virus replication in cells of the monocytes/

macrophage lineage can result in disease manifestation in the CNS. While authors agree that during the early phase of infection with FIV, the major reservoirs of infected cells in the peripheral blood are $CD4^+$ lymphocytes, the shift in viral tropism and receptor usage during chronic infection is still a matter of discussion (Willett et al., 2002). FIV and HIV target cells by binding their external envelope glycoprotein to a cellular receptor, and for both viruses interaction with a chemokine receptor (CXCR4 and CCR5) on the cellular surface (of lymphocytes and macrophages, respectively) is essential to mediate fusion between the viral and host cell membrane. Like HIV, FIV infects $CD4^+$ cells in vitro and in vivo, but does not use the feline CD4 molecule as a cellular receptor. The nature of the primary receptor is still in discussion. Initially, the CD9 molecule was considered because antibody blockage of the CD9 receptor inhibits FIV infection. Meanwhile, it was demonstrated that CD9 blockage prevents the release of the virus rather than the binding (DeParseval et al., 1997). It has been hypothesized that the chemokine receptor CXCR4 acts as primary receptor for FIV and that CCR5 receptors may influence FIV infection by modulating the expression of CXCR4 (Willett et al., 2002). Recently it was shown that FIV targets activated $CD4^+$ T-cells by using CD134 as a binding receptor (DeParseval et al., 2004).

FIV can be isolated from lymphocytes at the earliest between days 10 and 14 after infection. Viremia rapidly increases until day 21, peaks between weeks 7 and 8, and then decreases gradually until virus load increases again in the terminal stage. Conversely, when the virus peaks, $CD4^+$ cells decrease (Diehl et al., 1995). Within the first few weeks of infection, both $CD4^+$ and $CD8^+$ cells decline. The initial lymphopenia is followed by a strong immune response, which is characterized by the production of anti-FIV antibodies and a rebound in $CD8^+$ cells above preinfection levels. This results in a persistent inversion of the CD4/CD8 ratio. Over time, both $CD4^+$ and $CD8^+$ cells gradually decline. Decrease of $CD4^+$ cells depends on several mechanisms and is not only caused by a reduced live span of infected lymphocytes but also characterized by programmed active cell death, apoptosis, of uninfected cells. In HIV, distinctive clinical stages can be defined based on absolute $CD4^+$ cell count. A $CD4^+$ cell count of 200/μl or less is an AIDS-defining condition in HIV infection. It is more difficult to assign clinical stages of disease to cats based on $CD4^+$ cell counts, mainly because FIV appears to be less pathogenic than HIV. Although chronic inflammatory conditions and opportunistic infections are more common in cats with low $CD4^+$ cells, other cats with low counts appear to remain healthy.

Apart from the quantitative decrease of $CD4^+$ cells, FIV-infected cats show a dysfunction of immune cells (e.g., loss of ability of lymphocytes to proliferate in response to stimulation). In addition, a significant perturbation of cytokine production can be detected that contributes to the immunodeficiency. Cell-mediated immunity is more profoundly affected

than humoral immunity. Despite being target cells of viral infection, there are no changes in number or proportions of B-cells after FIV infection. Chronic inflammatory conditions, neoplasia, and infections with intracellular organisms are more common than infections controlled by antibodies. FIV-infected cats appear to respond adequately to vaccination and frequently develop a polyclonal hypergammaglobulinemia characteristic of nonspecific stimulation of humoral immunity.

Provirus and Virus Load

Development of quantitative PCR and RT-PCR to quantify provirus and virus load, respectively, has created very important tools for treatment studies in experimentally and naturally infected cats. Virus expression levels have been shown to correlate with disease stages in HIV and FIV infections, and it has been shown that not only HIV RNA levels but also FIV RNA levels play an important role in predicting the rapidity of disease progression (Diehl et al., 1996). These findings enhance the utility of the FIV model in assessing and evaluating anti-HIV therapy strategies aimed at reducing replicative virus load. However, while PCR is a very sensitive and specific method if used in research cats and experimental conditions, it can pose a problem if naturally infected cats are used. Various PCR assays have been shown to be incapable of detecting some FIV strains in the field due to the marked variability of the viral genome. PCR reagents, including primer and probe sequences, are often selected based on genetic sequences of a few well-characterized FIV strains. How well these reagents detect the wide variety of genetically divergent strains present in the environment is unknown, but failure rates of up to 50% have been reported (Klein et al., 1999). Additionally, virus load is often too low to be detected be RT-PCR, and it has been shown that some laboratory cats with documented FIV infection have even insufficient circulating provirus copies for detection by conventional PCR. Therefore, cats entering treatments studies, either in experimental or natural conditions, should be pretested for provirus or preferentially virus load and only cats with detectable levels should be included in theses studies.

3. TREATMENT STUDIES IN FIV-INFECTED CATS

Many compounds have been shown to be active against FIV, in cell culture and in infected cats. Most of the FIV treatment studies are aimed to screen new compounds in the FIV/cat model to document a potential benefit for HIV-infected patients. Many of these substances are in an experimental state and will never appear on the market or be accessible to veterinarians even if some have very good efficacy against FIV. In this

chapter, an overview of all published treatment studies performed with compounds that are commercially available at the moment will be given.

Inhibitors of the Viral Replication Cycle

"True antivirals" are compounds that interfere with one step (or several steps) in the viral replication cycle. Closer scrutiny of the relationship of the virus to the cell reveals several points at which the viral cycle can be interrupted, including adsorption to and penetration of the cell, uncoating of the viral nucleic acid, the various stages of nucleic acid replication, assembly of new viral particles, and release of infectious virions (if the cell is not destroyed).

The most common antiretroviral drugs are inhibitors of the reverse transcription inhibiting the retroviral enzyme RT (e.g., nucleoside analogues). Drugs with broader spectrums inhibit other viral enzymes like DNA or RNA polymerases and thus interfere with virus genome replication (e.g., forscarnet) or by inhibiting proteases (e.g., protease inhibitors) that are important for the splitting of precursor proteins during virus assembly. Other drugs target the virus entry by binding to specific receptors that the virus uses for adsorption (e.g., bicyclams, a new class that inhibits the CXCR4 receptor important for HIV and FIV entry), by acting as fusion inhibitors preventing the conformational changes of the virus necessary for the fusion process (e.g., T20), or by interfering with uncoating (e.g., amantadine). Antivirals used for HIV treatment at the moment belong to one of four classes of compounds: nucleoside analogues (nucleoside reverse transcriptase inhibitors, NRTI), nonnucleoside reverse transcriptase inhibitors (NNRTI), protease inhibitors, and entry inhibitors (newest group). In addition, some immunomodulatory substances are being investigated.

While many nucleoside analogues have been used against FIV, most NNRTI are highly specific for HIV-1 and not active against FIV. Only foscarnet, a relatively old compound belonging to the NNRTI, has proven activity against FIV. Most protease inhibitors active against HIV also are not effective against FIV and therefore only have been investigated in in vitro studies. Efficacy against FIV of the new fusion inhibitors (e.g., T20) that recently came on the market is mainly undetermined yet, although there are experimental fusion inhibitors with proven activity against FIV (Garg et al., 2004). Other compounds (e.g., bicyclams) interfering with virus entry by binding to cellular receptors (e.g., CXCR4) are active against FIV (Hartmann et al., 2002; Stengel et al., 2003).

Nucleoside Analogues

In the event of viral replication, it is the process of nucleic acid replication (which is extremely rapid relative to most mammalian cells) that has

proved to be the most vulnerable point of attack. The most clinically useful antiviral agents are nucleoside analogues interacting with this specific step. These nucleoside analogues are derivatives of nucleosides, so-called antimetabolites. A nucleoside consists of a nitrogenous base covalently attached to a sugar (ribose in RNA, 2-deoxyribose in DNA); a nucleotide consists of a nitrogenous base, a sugar, and a phosphate group. A nucleic acid (RNA and DNA) contains a chain of nucleotides covalently linked to form a sugar-phosphate backbone with protruding nitrogenous bases. For integration of these nucleotides (or monophosphates) into the nucleic acid, three high energy phosphate groups have to be bound to the nucleoside first (triphosphates), 2 of which are removed, releasing their energy during prolongation of the nucleic acid chain.

Nucleoside analogues are very similar molecules to the "true" nucleoside. They equally have to be phosphorilated intracellularly to become active compounds. Due to their structural similarities, they can bind to the active center of enzymes (e.g., RT and other polymerases) and block enzyme activity. Many of them also can be integrated in developing DNA or RNA strands, but due to differences in the molecular structure, the next nucleotide cannot be attached. This leads to chain termination or nonfunctional nucleic acids.

Nucleoside analogues can be divided into antimetabolites with "wrong" base (e.g., ribavirin), antimetabolites with "wrong" sugar (e.g., zidovudine), and antimetabolites with "wrong" base and "wrong" sugar (e.g., didanosine). Nucleoside analogues are not only accepted as false substrates by viral enzymes but also by cellular enzymes, and this is the major cause of their toxicity. Selectivity, however, results from differences in DNA synthesis of infected and noninfected cells; in noninfected cells, DNA synthesis is intermittent, while it is continuous and fast in infected cells.

Zidovudine. Zidovudine, 3′-azido-2′,3′-dideoxythymidine, AZT, was the first drug to be approved for treatment of HIV infection. Originally, AZT was developed as a potential anticancer drug in 1964 but appeared to be not very active. In 1985, its anti-HIV activity was demonstrated in vitro, and approval from the U.S. Food and Drug Administration (FDA) was granted in 1987 for HIV-infected patients. AZT inhibits replication of retroviruses and has a mild inhibitory effect on the replication of herpes viruses. It inhibits new infection of cells but not replication of viruses already present in infected cells. The inhibitory effect of AZT is 100 times higher against HIV-RT than mammalian cell DNA polymerases (Greene and Watson, 1998a). It has been used in experimental and in clinical trials in FIV-infected cats.

AZT is a thymidine derivative with a chemical structure very similar to thymidine but a missing OH-group at the 3′-position of the sugar. There are 2 major modes of action. AZT is converted to its triphosphate intracellularly. The first phosphorilation to AZT monophosphate is performed very fast and effectively by cellular kinases. Phosphorylations to the

di- and triphosphate, however, are slow and limiting steps. AZT monophosphate competitively inhibits the enzyme monophosphate kinase responsible for the second and third steps in nucleoside phosphorylation. This leads to a depletion of the nucleoside triphosphate pool necessary for DNA production. This mechanism explains its anti-herpes virus activity and also its side effects. The more important mode of action occurs after conversion to AZT triphosphate. AZT triphosphate blocks the retroviral RT, the enzymes that converts viral RNA into DNA. Due to its similarity to thymidine triphosphate it can enter the active center of RT and is integrated into the developing DNA strand. The missing OH-group at the sugar, however, leads to DNA chain termination. AZT monophosphate, incorporated in the DNA, cannot be cleaved from the active center, and thus, the enzyme is blocked.

It has been shown that AZT inhibits FIV replication in vitro and in vivo (Greene and Watson, 1998a); it reduces plasma virus load, improves the immunological and clinical status of naturally FIV-infected cats, increases quality of life, and prolongs life expectancy. In a placebo-controlled trial, AZT improved stomatitis and increased the CD4/CD8 ratio in naturally FIV-infected cats (Hartmann et al., 1995a, 1995b, 1998). Neurologic abnormalities also tend to respond favorably to treatment with AZT. In some cats with FIV-associated neurologic signs, there is marked improvement within the first days of therapy. As is the case in HIV, there is evidence that FIV can become resistant to nucleoside analogues. AZT-resistant mutants of FIV can arise already after 6 months' use. A single point mutation in the FIV gene was identified that can create resistance to AZT (Smith et al., 1998). In humans, resistance to AZT frequently develops, but the addition of 3TC to a therapeutic protocol can cause AZT-resistant strains to revert to AZT-sensitive strains. A combination of these two drugs might be a promising approach in FIV-infected cats to prevent resistance development.

AZT should be used at a dosage of 5 to 10 mg/kg every 12 hours orally or by subcutaneous injection. The higher dose should be used carefully as side effects develop easily. For subcutaneous injection, the lyophilized product (marketed for IV injection in humans) should be diluted in isotonic NaCl solution (5 ml) to prevent local irritation. For oral application, syrup (raspberry flavor) or gelatin capsules (compounded individually for each cat) can be given. AZT is rapidly and completely absorbed from the gastrointestinal tract, and absorption is unaffected by the presence of food. AZT is metabolized in the liver and is rapidly excreted through the urine (Greene and Watson, 1998b). It is widely distributed throughout the body, penetrates into CNS and CSF, and crosses placenta. In human medicine, it is successfully used to treat HIV-infected pregnant women to prevent infection of the child. Pregnancy of FIV-infected queens is a potential indication for AZT treatment if the owner wants the kittens to be delivered,

although in contrast to HIV-infected women in utero transmission occurs infrequently in natural FIV infection.

Side effects of AZT in cats are similar to the side effects reported in humans. Thus, regular blood cell counts are necessary because nonregenerative anemia is a common side effect, especially if higher dosages are used. Complete blood counts should be performed weekly for the first month. If values are stable after the first 4 weeks, a monthly control is recommended. Cats with bone marrow suppression should not be treated because of the risk of life-threatening anemia. In cats with concurrent chronic renal failure dosage should be reduced to avoid toxicity. Studies in which cats were treated with AZT for 2 years showed that AZT is well tolerated in most FIV-infected cats. Hematocrit declines within 3 weeks of initiating treatment to about 60% of baseline but comes back up afterward in most cases even without discontinuation of treatment. If hematocrit drops below 20%, discontinuation is recommended and anemia usually resolves within a few days (Hartmann et al., 1995b). Neutropenia is less frequent than anemia. Other side effects in cats, including vomiting or anorexia, rarely develop. One side effect sometimes positively noted by owners is the development of a fuller and shiny hair coat. AZT should not be combined with drugs that are myelosuppressive. Concurrent use of nonsteroidal antiphlogistics has to be monitored carefully as it can delay AZT metabolism, result in kidney toxicity, or lead to neutropenia.

Stavudine. Stavudine, 2′,3′-didehydro-2′,3′-dideoxythymidine, d4T, is a thymidine-based nucleoside analogue and was the fourth drug to receive FDA approval for use against HIV infection. D4T is closely related in mode of action to AZT, since both are thymidine analogues.

D4T is active against FIV in vitro (Balzarini et al., 1996; Zhu et al., 1996). Mutants of FIV resistant to d4T and cross-resistant to several other antivirals including AZT, didanosine, and foscarnet have been found. A single point mutation in the RT-encoding region of the *pol* gene responsible for the resistance was identified (Zhu et al., 1996). No in vivo data in FIV-infected cats are published so far.

Didanosine. Didanosine, 2′,3′-dideoxyinosine, ddI, is also used to treat HIV infection in humans (second anti-HIV drug approved by FDA). DdI is an inosine analogue, an antimetabolite containing a "wrong" base and a "wrong" sugar molecule. It is intracellularily converted to the active substance ddI triphosphate that competitively inhibits RT. It is less bone marrow suppressive in humans but is less active against HIV than AZT.

DdI is active against FIV (Gobert et al., 1994) in vitro. Controlled in vivo studies confirming the efficacy in cats with FIV infection are not available so far.

Zalcitabine. Like AZT, zalcitabine, 2′,3′-dideoxycytidine, ddC, was originally developed as an antitumor agent, and 20 years later its antiretroviral activity was detected. It is currently used to treat HIV infection

in humans (third drug approved). DdC is an analogue of the nucleoside 2′-desoxicytidine. The active compound is the intracellularily produced ddC triphosphate that acts as RT inhibitor.

In vitro, antiviral efficacy has been demonstrated against FIV (Medlin et al., 1996) but no in vivo data exist demonstrating its efficacy in FIV-infected cats. A mutant of FIV resistant to ddC was selected in cell culture that showed cross resistance to other antiviral compounds (e.g., ddI, foscarnet) (Medlin et al., 1996).

Lamivudine. Lamivudine, (2*R*,cis)-4-amino-1-(2-hydroxymethyl-1,3-oxathiolan-5-yl)-(1H)-pyrimidin-2-one, 3TC, the youngest of the approved nucleoside analogues against HIV, is the (-)enantiomer of a dideoxy analogue of cytidine with activity against HIV and hepatitis B virus (HBV). Intracellularly, 3TC is phosphorylated to its active triphosphate metabolite, 3TC triphosphate. The principal mode of its antiretroviral action is the inhibition of the RT via DNA chain termination after incorporation into the viral DNA. 3TC triphosphate is also a weak inhibitor of mammalian DNA polymerases α and β, and mitochondrial DNA polymerase, and this explains its anti-HBV activity. 3TC is often combined with AZT in HIV-infected patients as both drugs show a synergistic effect. However, HIV mutants exist that are resistant to both 3TC and AZT.

3TC is active against FIV in vitro (Arai et al., 2002; Bisset et al., 2002). Combination of AZT and 3TC had synergistic anti-FIV activities in primary peripheral blood mononuclear cell cultures (Arai et al., 2002). FIV mutants resistant to 3TC, containing a point mutation in the RT gene, were selected in vitro and showed cross resistance to AZT (Smith et al., 1998). One in vivo study was performed in experimentally FIV-infected cats that were treated with a high dose AZT/3TC combination (each drug 100 or 150 mg/kg/day). Combination protected some cats when the treatment was started before experimental infection. However, AZT/3TC treatment had no anti-FIV activity in chronically infected cats. Severe side effects including fever, anorexia, and marked hematological changes were observed in some of the cats with such high dosage dual drug treatment (Arai et al., 2002).

The pharmacokinetic of 3TC in cats shows considerable similarity to pharmacokinetics of AZT in cats and to that of 3TC in humans (Jordan et al., 2001). Thus, in naturally infected cats 3TC dosages similar to AZT are probably recommended.

Ribavirin. Ribavirin, 1-β-D-ribofuranosyl-1H-1,2,4-triazole-3-carboxamide, RTCA, is a broad-spectrum triazole nucleoside that has marked in vitro antiviral activity against a variety of DNA and RNA viruses. The strongest antiviral activity is against RNA respiratory viruses and herpes viruses. RTCA has been effective in HIV infection, Lassa fever (a human arenavirus infection), and in hantavirus infections. It is also used in humans with hepatitis C and is part of the standard protocol in these patients. Systemic use,

however, is limited due to the development of dose-dependant hemolytic anemia in humans. Therefore, as single drug it is currently mainly used to treat people with respiratory syncytial viral infection by aerosol route. If used as an aerosol, only low concentrations appear in the systemic circulation, and side effects are tolerable (Greene and Watson, 1998a).

RTCA is also a nucleoside analogue but in contrast to other anti-HIV compounds that act primarily to inhibit RT activity by causing premature chain termination during the transcription of DNA from the single-stranded RNA template, RTCA allows DNA synthesis to occur, but prevents the formation of viral proteins, probably by interfering with capping of viral mRNA. In vitro, RTCA antagonizes the action of AZT, probably by feedback inhibition of thymidine kinase so that the AZT is not phosphorylated.

RTCA is active against a number of viruses in vitro including FIV (Smyth et al., 1994), however, there are no controlled in vivo studies. As in humans, the most common side effect in cats found in several studies (already in low dosages of 11 mg/kg) is hemolysis that develops as a result of sequestration of the drug in red blood cells. In addition, a dose-related toxic effect on bone marrow, primarily on megakaryocytes (resulting in thrombocytopenia and hemorrhage) and erythroid precursors and later or in higher dosages suppressing also numbers of neutrophils was seen. Liver toxicity also occurs (Povey, 1978; Weiss et al., 1993a). Weiss and coworkers (1993b) tried to decrease the toxicity of RTCA by incorporating it into lecithin-containing liposomes and giving it at a lower dosage (5 mg/kg) intravenously to cats. They were, however, not able to reach therapeutic concentration with this treatment regimen. Overall, side effects have limited the systemic use of RTCA in feline medicine.

Nonnucleoside Reverse Transcriptase Inhibitors (NNRTI)

Most NNRTI are highly specific for HIV-1 and not active against FIV (Auwrex et al., 2004). Only foscarnet is active against other retroviruses besides HIV.

Foscarnet. Foscarnet, trisodium phosphonoformate hexahydrat, PFA, is a pyrophosphate analogue that inhibits virus-specific DNA and RNA polymerase and RT. It has a wide spectrum of activity against DNA and RNA viruses including retroviruses. It has been administered to treat HIV infection especially if coinfection with cytomegaly virus is present, but significant nephrotoxicity has limited its use. Renal dysfunction is evident in most humans after 2 weeks of therapy (Greene and Watson, 1998a). PFA is only virustatic, and after treatment is stopped, virus replication is activated.

PFA inhibits DNA polymerase activity by preventing pyrophosphate exchange. The action is selective in that the corresponding mammalian polymerase is much less susceptible to inhibition. In addition, activity of PFA against retroviruses seems to be due to a different mechanism. Like

the nucleoside analogues, PFA inhibits RT activity, but it binds to the enzyme at a site distinct from that of the nucleoside triphosphates. The effect is noncompetitive and reversible. PFA does not need to be activated by phosphorylation.

In vitro, PFA has been shown to be active against FIV. As for HIV, PFA-resistant FIV strains can develop (Gobert et al., 1994). No reliable data exist on its efficacy in cats against FIV in vivo, and its use in veterinary medicine is limited due to toxicity.

PFA has been used in experimental FeLV infection, and in these studies it was mostly administered intravenously by continuous infusion because of its short half-life, although oral application is possible. It should not be given as a fast bolus (maximum intravenous rate 1 mg/kg/min). It penetrates the blood-brain barrier. Most of the drug is excreted in urine mainly unmetabolized. PFA accumulates in bone matrix. Renal clearance (Swenson et al., 1990) and bone deposition (Swenson et al., 1991) in cats are age-dependant (faster clearance in younger animals, but more bone deposition). As in humans, it causes nephrotoxicity and myelosupression in cats. PFA also chelates divalent cations such as calcium, so that hypocalcemia, hypomagnesemia, and hypokalemia may develop (Ryrfeld et al., 1992). PFA is toxic to epithelium cells and mucous membranes. Gastrointestinal side effects are common. Ulceration of genital epithelium may occur as the drug is excreted in the urine unmetabolized. Oral administration can cause irritation of mucous membranes and oral bleeding. Recommended dose for cats is 13.3 mg/kg every 8 hours orally or intravenously (as infusion of <1 mg/kg/min) (Greene and Watson, 1998b).

Entry Inhibitors

Drugs recently developed for inhibition of the virus entry can be classified in fusion inhibitors and drugs interfering with the entry process by binding to the cellular receptors (e.g., CXCR4). In the group of fusion inhibitors only one drug has been licensed for HIV infection so far (T20). Compounds binding to the cellular receptors have not been licensed for treatment of HIV infection but are commercially available as stem cell activators for patients receiving bone marrow transplants.

The HIV and FIV fusion and entry process begins with interaction between the trimeric envelope complex (a cluster of proteins on the outer coat of the lentivirus referred to as gp160) and both the CD4 and a chemokine receptor (either CCR5 or CXCR4) on the cell surface. This complex is made up of 3 transmembrane glycoproteins (gp41), which anchor the cluster to the virus and 3 extracellular glycoproteins (gp120), which contain the binding domains for both CD4 and the chemokine receptors. The first step in fusion involves the high affinity attachment of the CD4 binding domains of gp120 to the N-terminal membranes distal

domains of CD4. Once gp120 is bound with the CD4 protein, the envelope complex undergoes a structural change, bringing the chemokine binding domains of gp120 into proximity with the chemokine receptor, allowing for a more stable two-pronged attachment. Antagonists of CXCR4 and CCR5 act here. If the virus latches onto both CD4 and the chemokine receptor, additional conformational changes allow for the N-terminal fusion peptide of gp41 to enter the CD4 cell membrane. There are 2 heptad repeat sequences, HR1 and HR2, of gp41 that interact resulting in collapse of the extracellular portion of p41 to form a hair pin structure. In the absence of an inhibitor, the hair pin structure brings virus and cell membrane close together, allowing fusion of the membrane and subsequent entry of viral RNA. The fusion inhibitor T20 acts here by mimicking HR2, resulting in a botched formation of the hair pin (Eron and Hogan, 2002).

T20. T20 is a peptide fusion inhibitor of HIV. It is a conserved 36 amino acid synthetic C-peptide that corresponds to a region of the C-helix structure of gp41. The C-peptide goes to work after gp120 has successfully docked with the CD4 receptor. T20 mimics the activity of the C-helix structure. This effectively blocks the conformational changes that are required to secure virus cellular membrane fusion. Clinical trials in HIV-infected patients revealed that T20 has good antiviral efficacy and is well tolerated (Kilby et al., 2002).

T20 has been tested against a variety of viruses, including HIV-2, SIV, and FIV, and was found to show only minimal antiviral effect (Medinas et al., 2002). However, peptides created specifically to inhibit FIV fusion are highly active against FIV but not HIV (Giannecchini et al., 2003; Garg et al., 2004). If the primary amino acid sequence of HIV and FIV HR regions would exhibit greater sequence homology, peptides from these regions would most probably display greater antiviral cross-reactivity (Medinas et al., 2002). Therefore, the recent commercially available peptide fusion inhibitors are not useful for treatment of FIV infection, but new compounds of this class developed in the future might be more promising.

AMD3100. AMD3100, 1,1′-[1,4-phenylenebis(methylene)]bis-1,4,8,11-tetraazacyclotetradecane octahydrochloride, JM3100, SID791, belongs to the class of bicyclams. AMD3100 is licensed as a stem cell activator for patients that undergo bone marrow transplantation. It is active against HIV in vitro and also in clinical trials in HIV-infected patients but has not been licensed for HIV because it caused cardial toxicity in some human patients. Likely, related compounds that are less toxic will come on the market soon.

AMD3100 is the prototype of low-molecular-weight compounds that have been shown to act as potent and selective antagonists of the chemokine receptor CXCR4. Blocking the CXCR4 receptor triggers the movement of stem cells out of the bone marrow and into circulating blood. Once in the circulating blood, these stem cells can be collected and used

to restore the immune system of cancer patients who have had treatments that destroyed their immune cells. Alternatively, blocking the CXCR4 receptor also leads to inhibition of the entry of a number of lentiviruses. CXCR4 is the main coreceptor for T-cell-line-adapted (T-tropic) HIV strains (Berson et al., 1996; Feng et al., 1996; Hori et al., 1998; Zhang et al., 1998). A close correlation between anti-HIV-1 activity and interaction with CXCR4 has been found for a series of bicyclams (Donzella et al., 1998; DeClercq et al., 2001). Recently, it was shown that FIV strains also use CXCR4 for cell fusion and viral entry and that a high degree of homology exists between the human and feline CXCR4 (Willett et al., 1997). CXCR4 plays an important role for FIV entry in the cell but it is currently unknown if receptors other than CXCR4 are necessary for infection with FIV (Richardson et al., 1999; Frey et al., 2001).

Since FIV binds to both human and feline CXCR4 and given the amino acid sequence homology between the chemokine receptors of both species, bicyclams were investigated as potential inhibitors of FIV infection. A number of bicyclams, including AMD3100, are highly active against FIV in vitro (Egberink et al., 1999). In a placebo-controlled double-blind study in which 40 naturally FIV-infected cats were treated for 6 weeks, it was shown that treatment with AMD3100 caused a statistically significant improvement in the proviral load in FIV-infected cats. Patients receiving AMD3100 did not show side effects during treatment. Cardiac function was monitored carefully and was not compromised in the cats. Interestingly, a statistically significant reduction of the serum magnesium level without producing clinical signs was observed due to the administration of AMD3100 (Hartmann et al., 2002; Stengel et al., 2003).

In this study a potential resistance development was also investigated; FIV strains of all cats treated with the bicyclam were collected and cultured in the presence of AMD3100. There was, however, no development of resistance to AMD3100 (Hartmann et al., 2002; Stengel et al., 2003).

AMD3100 is administered at 0.5 mg/kg every 12 hours subcutaneously. Side effects in cats are not described if cats are treated over a period of 6 weeks.

Immunomodulators

Immunomodulators or immunostimulatory agents or biological response modifiers are widely used against FIV-infected cats. It has been suggested that these agents benefit infected animals by restoring compromised immune function, thereby allowing the patient to control viral burden and recover from associated clinical syndromes. These substances modify the responses of immunocompetent cells through cytokines or other mechanisms. Some of them have not only an effect on the immune system but also a true antiviral activity (e.g., interferons (IFN)).

Interferons

IFN are polypeptide molecules produced by vertebrate cells in response to viral infections or certain inert substances, such as double-stranded RNA, and other microbial agents. In humans, there are at least 3 characterized types of IFN: IFN-α (formerly leukocyte IFN), IFN-β (formerly fibroblast IFN), and IFN-γ. IFN-α and IFN-β are structurally similar, being produced in response to viral infection or polyribonucleotide administration. IFN-γ is structurally distinct and is produced by T-lymphocytes in response to a specific antigenic stimulus. Human IFN have been manufactured by recombinant DNA technology and are available on the market. IFN are not strictly species-specific in their effects, although their biologic activity and toleration are greater in cells of genetically related species. IFN-α and IFN-β have antiviral activity. IFN-α has been licensed for the treatment of people with myelogenous leukemia, papillomatosis, and HIV infection. IFN-β is licensed for adjunctive treatment of various lymphatic and disseminated neoplasms and for treatment of symptomatic AIDS patients. IFN-γ is licensed for the treatment of people with chronic granulomatous diseases and is considered to be an immunostimulant in animals rather than an antiviral. Recently, recombinant feline IFN-ω, closely related to human IFN-α in its structure and effects, came on the market in Japan and some European countries and is licensed for the treatment of several virus infections in cats and dogs.

Human Interferon-α. Interferon-α, IFN-α, has immunomodulatory and antiviral activity. IFN-α is active against many DNA and RNA viruses, although sensitivity varies (e.g., myxoviruses are susceptible, whereas adenoviruses are not). In people, high-dose parenteral IFN-α administration has shown some efficacy against influenzaviruses, rhinoviruses, herpes viruses, and papillomaviruses. IFN-α has also been shown to inhibit oncogenic transformation induced by retroviruses. IFN-α is not only applied systemically but also topically, intranasally, ocularly, and intralesionally (e.g., in papillomavirus infections).

IFN-α acts as a cytokine, and therefore has an immunomodulatory effect, but also has a direct antiviral effect by inducing a general "antiviral state" of IFN-α-exposed cells that protects against virus replication. It is not virucidal but merely inhibits viral nucleic acid and protein synthesis. It binds to specific cell receptors that activate enzymes inhibiting synthesis, assembly, and release of viruses.

Human IFN-α is marketed as a recombinant product (rHuIFN-α) produced by a cloned human IFN-α gene expressed in *Escherichia coli.* There are two common treatment regimens for use of human IFN-α in cats: subcutaneous injection of high dose (10^4 to 10^6 IU/kg every 24 hours) IFN-α or oral application of low dose (1 to 50 IU/kg every 24 hours) IFN-α. When given parenterally in high doses, application leads to detectable serum

levels. However, given parenterally to cats it becomes ineffective after 3 to 7 weeks because of the development of neutralizing antibodies against the human IFN-α that limits its activity. In a study by Zeidner and coworkers (1990) in which cats were treated with human IFN-α subcutaneously, cats became refractory to therapy after 3 or 7 weeks, respectively, depending upon whether a high (1.6×10^6 IU/kg) or lower (1.6×10^4 IU/kg) dose was used (Zeidner et al., 1990).

Orally it can be given for a longer period as no antibodies develop. Given orally (as is done by many veterinarians to treat FIV infection), however, IFN-α is inactivated by gastric acid and, like other proteins, destroyed by trypsin and other proteolytic enzymes in the duodenum; therefore, it is not absorbed and cannot be detected in the blood after oral administration (Cantell and Pyhala, 1973). Thus, direct antiviral effects are unlikely after oral application; instead, it seems to have immunomodulatory activity. IFN-α may bind to mucosal receptors in the oral cavity stimulating the local lymphoid tissue leading to cytokine release by lymphatic cells in the oral or pharyngeal area, triggering a cascade of immunologic responses that finally act systemically (Koech and Obel, 1990). Tompkins (1999) showed that orally administered IFN-α induces cytokine responses in buccal mucosal lymphoid structures, including upregulation of IFN-α expression and downregulation of interleukin-4. In mice studies, it was shown that subcutaneous administration of murine IFN-α had an antiviral effect, while oral administration only caused an immunomodulatory effect; infection of mice with encephalomyocarditisvirus resulted in death in 100% of mice if not treated, in 40% survival if mice were treated with 2×10^5 IU/mouse murine IFN-α orally, and in 90% survival if it was given at the same dose intraperitoneally (Schelleken et al., 2001). The rational behind the use of low doses (*versus* high doses) orally is to mimic natural defense processes. In studies comparing low oral doses IFN-α with higher oral doses, increasing the dose did not improve the effect (Cummins et al., 1999).

Human IFN-α is active against FIV in vitro (Tanabe and Yamamoto, 2001). It is frequently used in the field for treatment of FIV-infected cats. There are, however, no placebo-controlled studies that evaluate the effect of either low dose oral or high dose parenteral use of human IFN-α in FIV-infected cats. However, use of oral human IFN-α should be considered very critically due to the expected immunomodulatory activities but lack of antiviral activity after oral use. An unspecific stimulation of the immune system is contraindicated in FIV infection as immunostimulation can lead to an increased virus replication in HIV infection caused by activation of latently infected lymphocytes and macrophages, and therefore can effect a progression of disease. In cell culture, it was demonstrated that stimulation of FIV-infected cells is consistently associated with enhanced production of FIV (Sellon, 1998).

Feline Interferon-ω. Recently, the corresponding feline IFN, feline IFN-ω, was licensed for use in veterinary medicine in some European countries and Japan. IFN are species-specific and the feline IFN clearly differs from the human one not only concerning its antigenicity (therefore causing antibody development in animals) but also concerning its antiviral efficacy in feline cells. Therefore, even if feline IFN-ω is used long term, cats will not develop antibodies. In addition, the fact that it is the homologous species IFN in cats, it is expected to be more effective that human IFN-α.

Feline IFN-ω is a recombinant product which is produced by baculoviruses containing the feline sequence for this IFN that replicate in silkworms; subsequently, IFN-ω is purified out of homogenized silkworm preparation (Ueda et al., 1993a). Besides antiviral activity, antitumor effects of feline IFN-ω against canine neoplastic cells, antiproliferation, and anticolony-forming activities were demonstrated in vitro; thus, feline IFN-ω might be useful for treatment of some feline neoplastic conditions (Priosoeryanto et al., 1995; Tateyama et al., 1995), but in vivo studies are missing so far.

Feline IFN-ω is active against FIV in vitro. So far, only one study has been performed to investigate efficacy against FIV infection in vivo; 62 naturally FIV-infected cats were treated with IFN-ω at 10^6IU/kg subcutaneously every 24 hours on 5 consecutive days in 3 series (day 0, 14, 60) in a placebo-controlled multicenter trial in France. This study did not show significant changes in survival rate in treated cats when compared to the placebo group, although some clinical improvement was noted (Maynard et al., 2000).

Feline IFN-ω has similar pharmacokinetic properties to human IFN-α and is distributed primarily in the liver and kidneys, is catabolized rapidly mainly in the kidneys, and is excreted in the urine without residual accumulation in the body (Ueda et al., 1993b). Recommendation for treatment in cats with acute virus infections is 2.5×10^6IU/kg intravenously or subcutaneously every 24 hours for 3 consecutive days. In chronic virus infections, a treatment protocol of IFN-ω at 10^6IU/kg subcutaneously every 24 hours on 5 consecutive days in 3 series (day 0, 14, 60) is suggested. No side effects have been reported.

Growth Factors

Besides IFN, other cytokines have been used to treat FIV-infected cats. They do not have a direct antiviral effect but some regulate bone marrow function and thus are thought to have a beneficial effect in virus infections that interfere with hematopoiesis. Hematopoietic growth factors are glycoproteins that affect the growth and differentiation of blood cells, including erythrocytes, platelets, monocytes, granulocytes, and lymphocytes. Several of the feline hematopoietic growth factors have been cloned but

have only been used in research trials and are not on the market. Thus, human factors have to be used in veterinary medicine instead, but cats develop antibodies against the human products limiting the length of treatment.

Filgastrim. Filgastrim, granulocyte colony-stimulation factor, G-CSF, is on the market as recombinant human product (rHuG-CSF) for treatment of neutropenia in humans with various conditions (e.g., HIV infection, antitumor and antiviral treatment, transplantations). The human product also increases blood neutrophil counts in various animals, either in healthy individuals or in animals with certain neutropenic conditions. Neutrophilia usually occurs by day 7 after application and reaches maximal levels from 10 to 14 days of treatment. In cats, short-term increases in neutrophil counts are followed by neutropenia with continued use owing to development of neutralizing antibodies to this heterologous product (dose-dependent after 10 days to 7 weeks), and treatment should be used for not more than 3 weeks (Arai et al., 2001). Potential use in virus infections are neutropenias associated with FIV, FeLV, or parvovirus infections or concurrent with antiviral chemotherapy to avoid neutropenic side effects.

G-CSF has been used in FIV-infected cats. In one study, a small number of naturally FIV-infected cats was treated but no significant changes were seen when compared to untreated cats (Kraft and Kuffer, 1995). In a recent study, G-CSF was used in chronically experimentally FIV-infected cats using 5 μg/kg every 12 hours for 14 days. All treated cats developed increases in neutrophils; all treated FIV-infected cats but no placebo-treated infected cats, however, developed a significant increase in virus load of FIV in peripheral blood mononuclear cells during the treatment. It was hypothesized that this increase in virus replication may be due to either enhanced infection of lymphocytes or enhanced expression of FIV by infected lymphocytes (Arai et al., 2001). Therefore, use of G-CSF in FIV-infected cats should be considered carefully.

G-CSF is used in cats at 5 μg/kg subcutaneously every 24 hours for 3 to 5 consecutive days in acute virus infections and potentially up to 21 days in chronic virus infections. Potential side effects include bone discomfort, splenomegaly, allergic reactions, and fever (Greene, 1998; Arai et al., 2001). There is a risk of development of persistent antibodies against endogenous feline granulocyte colony-stimulation factor after 10 days (if used in a higher dose) to 7 weeks, resulting in rebound neutropenia.

Erythropoietin. Erythropoietin, EPO, is on the market as recombinant human product (rHuEPO) and is effectively used in cats and dogs with nonregenerative anemia due to endogenous erythropoietin deficiency in chronic renal failure. EPO treatment does not only increase red blood cell counts but also megacaryocyte and platelet numbers in animals and humans with clinical disease (Ogilvie, 1995). In a recent study, human EPO also increased white blood cell counts in cats (Arai et al., 2001).

In one study, FIV-infected cats were treated with human EPO (100 IU/kg subcutaneously 3 times a week for 2 weeks). All treated cats had a gradual increase in red blood cells, hemoglobin concentration, and packed cell volume, but also increased white blood cell counts consisting of either increased neutrophils, lymphocytes, or a combination. In contrast to treatment with filgrastim, no increased virus loads were observed, and thus, human EPO can be used safely in FIV-infected cats (Arai et al., 2001). EPO therefore may be indicated in FIV-infected cats with cytopenias.

Recommended dosage is 100 IU/kg subcutaneously every 48 hours until desired packed cell volume is reached, then as needed to maintain the packed cell volume. Anti-EPO antibodies may develop in 25 to 30% of treated animals after 6 months to 1 year. Binding of these antibodies not only to the human EPO but also the bodies own EPO nullifies their physiologic actions on erythroid progenitor cells, causing bone marrow failure and refractory anemia. Anti-EPO antibodies, however, dissipate after discontinuation of treatment and anemia resolves. Side effects include systemic hypertension, seizures, and iron depletion and develop from pathophysiologic adaptation to increased red blood cell volume. Thus, packed cell volume has to be monitored regularly in animals with long-term treatment.

Insulin-Like Growth Factor-1. Insulin-like growth factor-1, IGF-1, is on the market as recombinant human product (rHuIGF-1). Insulin-like growth factors resemble insulin in structure, receptor composition, and action; 2 insulin-like growths factors (type 1 and 2) have been characterized and both have broad anabolic actions that include stimulated differentiation of a wide variety of cell types, amino acid transport, protein synthesis in muscle, glucose transport and lipid formulation in fat, and fatty acid synthesis in the liver. In addition, IGF-1 has the ability to induce thymic growth and to stimulate T-cell function. Thymus involvement and the development of thymic lesions in HIV infection are hypothesized to alter thymus function and to limit T-cell maturation and replenishment of the peripheral lymphoid pool. Therapeutic modulation to protect or enhance thymus function may therefore ameliorate peripheral lymphopenia and retard disease progression in lentivirus infections.

In two experimental studies, effect of human IGF-1 in FIV-infected cats, its impact on the thymus and development of side effects were investigated (Gregory et al., 1997; Woo et al., 1999). Treatment with human IGF-1 resulted in a significant increase in thymus size and weight and evidence of thymic cortical regeneration in FIV-infected cats. Inflammation in the thymus was reduced as evidenced by a reduced numbers of B-cells. No side effects were described in these two studies, although it had been observed earlier that subcutaneous infusion of 2800 μg/kg IGF-1 over 24 hours can result in marked hypoglycemia and shock in fasted cats (Gregory et al., 1997). Although of particular concern that IGF-1 could induce T-cell stim-

ulation that might increase FIV replication, virus load was not increased during treatment in either of the 2 studies. These studies suggest that treatment with human IGF-1 may be effective in enhancing thymus function and replenishing the peripheral T-cell pool especially in young FIV-infected cats (Gregory et al., 1997; Woo et al., 1999). There are no field studies so far to show usefulness in naturally FIV-infected cats.

Human IGF-1 is applied through continuous subcutaneous infusion in a dose of 1000 µg/kg/24 hours for 30 days using surgically implanted minipumps. Cats should be fed *ad libidum* to avoid hypoglycemia, and blood glucose levels should be monitored regularly.

Other Drugs With Immunomodulatory Activity

A number of drugs (e.g., levamisole) used for different reasons in humans and animals also have immunomodulatory activity. IFN inducers are immunomodulatory compounds, that are widely used in virus-infected cats and dogs. These compounds induce synthesis of IFN and other cytokines. Several macromolecules and certain microorganisms are known to produce antiviral and other antimicrobial activities in the host. Most of the substances known to have this effect have a structure similar to double-stranded DNA that can be of microbial origin or new synthetic nucleic acid polymers. Therapy and prophylaxis with IFN inducers have become more attractive in veterinary medicine in the past years. Effects of these compounds on unspecific defense functions are difficult to test, and the results (positive and negative) in special in vitro test systems cannot simply be transferred to in vivo situations and vice versa. Most of the reports that do appear in the literature about these compounds are difficult to interpret due to unclear diagnostic criteria, lack of clinical staging or follow-up, the natural variability of the course of disease, the lack of placebo control groups, small numbers of animals used, and additional supportive treatments given. Although reports of uncontrolled studies sometimes suggest dramatic clinical improvement, these effects are usually not observed when followed by controlled studies.

There is no conclusive evidence from controlled studies that these drugs have any beneficial effect on health or survival of asymptomatic animals with virus infections. An unspecific stimulation of the immune system might even be contraindicated in cats with FIV infection, because it has been shown in HIV infection that these drugs can lead to an increased virus replication caused by activation of latently infected lymphocytes and macrophages, and therefore can effect a progression of disease. In cell culture it was demonstrated that stimulation of FIV-infected cells is consistently associated with enhanced production of FIV (Sellon, 1998). Therefore, unspecific immune stimulation should be avoided in FIV-infected cats.

Acemannan. Acemannan is a water-soluble long-chained complex carbohydrate (mannan) polymer derived from aloe vera plant and licenced for veterinary medicine. Acemannan is thought to be taken up by macrophages, stimulating them to release cytokines, which stimulate cell-mediated immune responses including cytotoxicity. In murine tissue culture, acemannan induces macrophages to produce tumor necrosis factor-α and interleukin-1. Acemannan is also thought to have a true antiviral effect as it interferes with viral glycosylation and has been shown to inhibit growth of HIV, Newcastle disease virus, and influenza virus. Acemannan has been used as an adjuvant in vaccines or by itself to enhance regression of tumors, for example, in the management of postvaccinal injection-site sarcomas in cats (Harris et al., 1991; King et al., 1995). It also may accelerate wound healing.

Acemannan has been used in FIV-infected cats in a study including 49 FIV-infected cats with clinical signs receiving acemannan either by intravenous or subcutaneous injection once weekly for 12 weeks or by daily oral administration for 12 weeks. The reported beneficial effects observed in this study are not clear because cats were concurrently treated with antibiotics and other symptomatic or supportive therapies and the study did not contain a control group (Yates et al., 1992). Acemannan treatment must be considered with caution in FIV-infected cats because of its immunostimulatory effects.

Acemannan is used systemically at 2 mg/kg subcutaneously or intraperitoneally every 7 days for 6 weeks. Subcutaneous injection rarely can cause hypersensitivity reactions to the drug. Necrosis can occur at the injection site (Fogleman et al., 1992). Very high intraperitoneal doses can cause abdominal pain, vomiting, and diarrhea; an atypical microgranulomatous peritonitis with mononuclear/macrophage infiltrates in the liver, spleen, and peritoneal surface has been described (Greene and Watson, 1998b).

Levamisole. Levamisole is a broad-spectrum anthelmintic, used, for example, as heartworm preventive in cats and dogs (Dillon, 1984; Reinemeyer et al., 1995) that nonspecifically stimulates cell-mediated immunity in a variety of species. Levamisole was detected as immunomodulatory substance when treatment applied against nematode infestation did not only kill the parasites but also improved clinical signs of other infections. Levamisole influences phosphodiesterase activity leading to increase of cyclic guanosin-3′,5′-monophosphate and decrease of cyclic adenosine monophosphate. Increase in cyclic guanosin-3′,5′-monophosphate in lymphocytes stimulates proliferative and secretory responses. It also potentiates mononuclear cells in phagocytosis, chemotaxis, and intracellular destruction of bacteria. Levamisole has been used in cats with mammary tumors, but had no significant effect (MacEwen et al., 1984).

Levamisole has been given to FIV-infected cats (Cotter et al., 1991), but its effect has never been substantiated by controlled studies. It remains investigational.

Toxicity of levemisole is relatively high; hypersalivation, vomiting, diarrhea, and CNS signs have been observed similar to signs observed in nicotine poisoning (Hsu, 1980). Morphologic lesions, characterized by perivascular, nonsuppurative, or granulomatous meningo-encephalitis, were described in the CNS of treated dogs. Potential facilitation of the cell-mediated immune system may produce the lesions in the CNS by causing the body to react against latent agents (Greene, 1998).

Lactoferrin. Lactoferrin is a protein of bovine origin that binds iron, reducing its availability for bacteria. It has been used for its local immunomodulatory effect in the oral cavity, and it has been shown to increase phagocytic activity of neutrophils (Sato et al., 1996). A protective effect of lactoferrin during lethal bacteremia has been reported in mice. In a study in healthy humans, orally administered lactoferrin increased the phagocytic activity of peripheral mononuclear cells in some but not all individuals (Yamauchi et al., 1998).

Lactoferrin has been used in cats with FIV infection and stomatitis. It was applied topically (40 mg/kg every 24 hours for 14 days) to the oral mucosa of the cats with intractable stomatitis (FIV-infected cats and FIV-negative control cats) and improved clinical signs of disease (pain-related response, salivation, appetite, and oral inflammation) in all cats independent of their FIV status (Sato et al., 1996).

Lactoferrin is administered at 40 mg/kg every 24 hours for 14 days. The powder must be purchased from chemical suppliers and has to be mixed in solution. The solution should be applied locally with a syringe; it should not be mixed with food. Side effects are not described.

REFERENCES

Arai, M., Darman, J., Lewis, A., Yamamoto, J. K., and Darmen, J., 2000, The use of human hematopoietic growth factors (rhGM-CSF and rhEPO) as a supportive therapy for FIV-infected cats, *Vet Immunol Immunopathol* 77:71–92.

Arai, M., Earl, D. D., and Yamamoto, J. K., 2002, Is AZT/3TC therapy effective against FIV infection or immunopathogenesis? *Vet Immunol Immunopathol* 85:189–204.

Auwerx, J., Esnouf, R., DeClercq, E., and Balzarini, J., 2004, Susceptibility of feline immunodeficiency virus/human immunodeficiency virus type 1 reverse transcriptase chimeras to non-nucleoside RT inhibitors, *Mol Pharmacol* 65:244–251.

Balzarini, J., Egberink, H., Hartmann, K., Cahard, D., Vahlenkamp, T., Thormar, H., DeClercq, E., and McGuigan, C., 1996, Antiretrovirus specificity and intracellular metabolism of 2′,3′-didehydro-2′,3′-dideoxythymidine (stavudine) and its 5′-monophosphate triester prodrug So324, *Mol Pharmacol* 50:1207–1213.

Barr, A. C., Phillips, T. R., 2000, FIV and FIV-related diseases, in: *Textbook of Veterinary Internal Medicine*, 5th ed., S. J. Ettinger, E. C. Feldman, eds., WB Saunders, Philadelphia, pp. 433–438.

Beatty, J., Terry, A., MacDonald, J., Gault, E., Cevario, S., O'Brien, S. J., Cameron, E., and Neil, J. C., 2002, Feline immunodeficiency virus integration in B-cell lymphoma identifies a candidate tumor suppressor gene on human chromosome 15q15, *Cancer Res* 62:7175–7180.

Berson, J. F., Long, D., Doranz, B. J., Rucker, J., Jirik, F. R., and Doms, R. W., 1996, A seven-transmembrane domain receptor involved in fusion and entry of T-cell-tropic human immunodeficiency virus type 1 strains, *J Virol* 70:6288–6295.

Bisset, L. R., Lutz, H., Boni, J., Hofmann-Lehmann, R., Luthy, R., and Schupbach, J., 2002, Combined effect of zidovudine (ZDV), lamivudine (3TC) and abacavir (ABC) antiretroviral therapy in suppressing in vitro FIV replication, *Antiviral Res* 53:35–45.

Callanan, J. J., Jones, B. A., Irvine, J., Willett, B. J., McCandlish, I. A., and Jarrett, O., 1996, Histologic classification and immunophenotype of lymphosarcomas in cats with naturally and experimentally acquired feline immunodeficiency virus infections, *Vet Pathol* 33:264–272.

Cantell, K., and Pyhala, L., 1973, Circulating interferon in rabbits after administration of human interferon by different routes, *J Gen Virol* 20:97–104.

Cotter, S. M., 1991, Management of healthy feline leukemia virus-positive cats, *J Am Vet Med Assoc* 199:1470–1473.

Cummins, J. M., Beilharz, M. W., and Krakowka, S., 1999, Oral use of interferon. *J Interferon Cytokine Res* 19:853–857.

DeClercq, E., Schols, D., Bridger, G., and Henson, G., 2001, CXCR4 receptor antagonist, AMD3100, is a potent inhibitor of HIV infection, *Abstracts of Papers American Chemical Society* 221:Medi24.

DeParseval, A., Lerner, D. L., Borrow, P., Willett, B. J., and Elder, J. H., 1997, Blocking of feline immunodeficiency virus infection by a monoclonal antibody to CD9 is via inhibition of virus release rather than interference with receptor binding, *J Virol* 71:5742–5749.

DeParseval, A., Chatterji, U., Sun, P., and Elder, J. H., 2004, Feline immunodeficiency virus targets activated CD4+ T-cells by using CD134 as a binding receptor, *Proc Natl Acad Sci USA* 101:13044–13049.

Diehl, L. J., Mathiason-Dubard, C. K., O'Neil, L. L., and Hoover, E. A., 1995, Longitudinal assessment of feline immunodeficiency virus kinetics in plasma by use of a quantitative competitive reverse transcriptase PCR, *J Virol* 69:2328–2332.

Diehl, L. J., Mathiason-Dubard, C. K., O'Neil, L. L., and Hoover, E. A., 1996, Plasma viral RNA load predicts disease progression in accelerated feline immunodeficiency virus infection, *J Virol* 70:2503–2507.

Dillon, R., 1984, Feline dirofilariasis, *Vet Clin North Am Small Anim Pract* 14:1185–1199.

Donzella, G. A., Schols, D., Lin, S. W., Este, J. A., Nagashima, K. A., Maddon, P. J., Allaway, G. P., Sakmar, T. P., Henson, G., De Clercq, E., and Moore, J. P., 1998, AMD3100, a small molecule inhibitor of HIV-1 entry via the CXCR4 co-receptor, *Nat Med* 4:72–77.

Dow, S. W., Poss, M. L., and Hoover, E. A., 1990, Feline immunodeficiency virus: a neurotropic lentivirus, *J Acquir Immune Defic Syndr* 3:658–668.

Egberink, H. F., DeClercq, E., VanVliet, A. L., Balzarini, J., Bridger, G. J., Henson, G., Horzinek, M. C., and Schols, D., 1999, Bicyclams, selective antagonists of the human chemokine receptor CXCR4, potently inhibit feline immunodeficiency virus replication, *J Virol* 73:6346–6352.

Eron, J. J., and Hogan, C. M., 2002, Entry and fusion inhibitors: an update, *PRN Notebook* 7:16–22.

Feng, Y., Broder, C. C., Kennedy, P. E., and Berger, E. A., 1996, HIV-1 entry cofactor: functional cDNA cloning of a seven-transmembrane, G protein-coupled receptor, *Science* 272:872–877.

Fischl, M. A., Richman, D. D., Hansen. N., Collier, A. C., Carey, J. T., Para, M. F., Hardy, W. D., Dolin, R., Powderly, W. G., Allan, J. D., Wong, B., Merigan, T. C., McAuliffe, V. J., Hyslop, N. E., Rhame, F. S., Balfour, H. H., Spector, S. A., Volberding, P., Pettinelli, C., and Anderson, J., 1990, The safety and efficacy of zidovudine (AZT) in the treatment of subjects with mildly symptomatic human immunodeficiency virus type 1 (HIV) infection, *Ann Intern Med* 112:727–737.

Fogleman, R. W., Chapdelaine, J. M., Carpenter, R. H., McAnalley, B. H., 1992, Toxicologic evaluation of injectable acemannan in the mouse, rat and dog. *Vet Hum Toxicol* 34:201–205.

Frey, S. C., Hoover, E. A., and Mullins, J. I., 2001, Feline immunodeficiency virus cell entry, *J Virol* 75:5433–5440.

Garg, H., Fuller, F. J., and Tompkins, W. A., 2004, Mechanism of feline immunodeficiency virus envelope glycoprotein-mediated fusion. *Virology* 321:274–286.

Giannecchini, S., DiFenza, A., D'Ursi, A. M., Matteucci, D., Rovero, P., and Bendinelli, M., 2003, Antiviral activity and conformational features of an octapeptide derived from the membrane-proximal ectodomain of the feline immunodeficiency virus transmembrane glycoprotein, *J Virol* 77:3724–3733.

Gobert, J. M., Remington, K. M., Zhu, Y. Q., and North, T. W., 1994, Multiple-drug-resistant mutants of feline immunodeficiency virus selected with 2′,3′-dideoxyinosine alone and in combination with 3′-azido-3′-deoxythymidine, *Antimicrob Agents Chemother* 38:861–864.

Greene, C. E., 1998, Immunoprophylaxis and immunotherapy, in: *Infectious Diseases of the Dog and Cat*, 2nd ed., C. E. Greene, ed., WB Saunders, Philadelphia, pp. 717–750.

Greene, C. E., and Watson, A. D. J., 1998a, Antiviral drugs, in: *Infectious Diseases of the Dog and Cat*, 2nd ed., C. E. Greene, ed., WB Saunders, Philadelphia, pp. 6–9.

Greene, C. E., and Watson, A. D. J., 1998b, Antimicrobial drug formulary, in: *Infectious Diseases of the Dog and Cat*, 2nd ed., C. E. Greene, ed., WB Saunders, Philadelphia, pp. 790–919.

Gregory, C. R., Barlough, J. E., Clark, R., Griffey, S. M., Patz, J. D., Jardieu, P., and Pedersen, N. C., 1997, Effects of insulin-like growth factor-1 and AZT in cats experimentally infected with FIV, *Feline Pract* 25:23–31.

Hara, Y., Ishida, T., Ejima, H., Tagawa, M., Motoyoshi, S., Tomoda, I., Shimizu, M., and Shichinohe, K., 1990, Decrease in mitogen-induced lymphocyte proliferative responses in cats infected with feline immunodeficiency virus, *Jpn J Vet Sci* 52:573–591.

Harris, C., Pierce, K., King, G., Yates, K. M., Hall, J., and Tizard, I., 1991, Efficacy of acemannan in treatment of canine and feline spontaneous neoplasms, *Mol Biother* 3:207–213.

Hartmann, K., 1995a, AZT in the treatment of feline immunodeficiency virus infection. Part 1, *Feline Pract* 23:16–21.

Hartmann, K., 1995b, AZT in the treatment of feline immunodeficiency virus infection. Part 2, *Feline Pract* 23:13–20.

Hartmann, K., 1998, Feline immunodeficiency virus infection, *Brit Vet J* 155:123–137.

Hartmann, K., and Kuffer, M., 1998, Karnofsky's score modified for cats, *Eur J Med Res* 3: 95–98.

Hartmann, K., Stengel, S., Klein, D., Egberink, H., and Balzarini, J., 2002, Efficacy of the chemokine receptor inhibitor 1,1′-bis-1,4,8,11-tetraazacyclotetradekan against feline immunodeficiency virus infection, in: *Abstracts 6th International Feline Retrovirus Research Symposium*, Amelia Island, USA.

Hori, T., Sakaida, H., Sato, A., Nakajima, T., Shida, H., Yoshie, O., and Uchiyama, T., 1998, Detection and delineation of CXCR-4 (fusin) as an entry and fusion cofactor for T-tropic

(correction of T-cell-tropic) HIV-1 by three different monoclonal antibodies, *J Immunol* 160:180–188.

Hsu, W. H., 1980, Toxicity and drug interactions of levamisole, *J Am Vet Med Assoc* 176:1166–1169.

Jordan, H. L., Pereira, A. S., Cohen, M. S., and Kashuba, A. D., 2001, Domestic cat model for predicting human nucleoside analogue pharmacokinetics in blood and seminal plasma, *Antimicrob Agents Chemother* 45:2173–2176.

Karnofsky, D. A., Abelmann, W. H., Craver, L. F., and Burchenal, J. H., 1948, The use of the nitrogen mustards in the palliative treatment of carcinoma, *Cancer* 11:634–656.

Kilby, J. M., Lalezari, J. P., Eron, J. J., Carlson, M., Cohen, C., Arduino, R. C., Goodgame, J. C., Gallant, J. E., Volberding, P., Murphy, R. L., Valentine, F., Saag, M. S., Nelson, E. L., Sista, P. R., and Dusek, A., 2002, The safety, plasma pharmacokinetics, and antiviral activity of subcutaneous enfuvirtide (T-20), a peptide inhibitor of gp41-mediated virus fusion, in HIV-infected adults, *AIDS Res Hum Retroviruses* 18:685–693.

King, G. K., Yates, K. M., Greenlee, P. G., Pierce, K. R., Ford, C. R., McAnalley, B. H., and Tizard, I. R., 1995, The effect of acemannan immunostimulant in combination with surgery and radiation therapy on spontaneous canine and feline fibrosarcomas, *J Am Anim Hosp Assoc* 31:439–447.

Klein, D., Janda, P., Steinborn, R., Mueller, M., Salmons, B., and Guenzburg, W. H., 1999, Proviral load determination of different feline immunodeficiency virus isolates using real-time polymerase chain reaction: influence of mismatches on quantification, *Electrophoresis* 20:291–299.

Koech, D. K., and Obel, A. O., 1990, Efficacy of Kemron (low dose oral natural human interferon alpha) in the management of HIV-1 infection and acquired immune deficiency syndrome (AIDS), *East Afr Med J* 67:64–70.

Kraft, W., and Kuffer, M., 1995, Treatment of severe neutropenias in dogs and cats with filgrastim, *Tierärztl Prax* 23:609–613.

Lawrence, C. E., Callanan, J. J., Willett, B. J., and Jarrett, O., 1995, Cytokine production by cats infected with feline immunodeficiency virus: a longitudinal study, *Immunology* 85:568–574.

Lehmann, R., Joller, H., Haagmans, B. L., and Lutz, H., 1992, Tumornecrosis faktor α levels in cats experimentally infected with feline immunodeficiency virus: effects on immunization and feline leukemia virus infection, *Vet Immunol Immunopathol* 35:61–69.

Levy, J. K., 2000, CVT update: feline immunodeficiency virus, in: *Kirk's Current Veterinary Therapy XIII. Small Animal Practice*, J. D. Bonagura, ed., WB Saunders, Philadelphia, pp. 284–288.

MacEwen, E. G., Hayes, A. A., Mooney, S., Patnaik, A. K., Harvey, H. J., Passe, S., and Hardy, W. D. Jr., 1984, Evaluation of effect of levamisole on feline mammary cancer, *J Biol Response Mod* 3:541–546.

Maynard, L., DeMari, K., and Lebreux, B., 2000, Efficacy of a recombinant feline omega interferon in the treatment of symptomatic FeLV- or FeLV- and FIV-positive cats, in: *Abstracts 10th Annual Congress of the European Society of Veterinary Internal Medicine (ESVIM)*, Neuchâtel, Switzerland.

Medinas, R. J., Lambert, D. M., and Tompkins, W. A., 2002, C-Terminal gp40 peptide analogs inhibit feline immunodeficiency virus: cell fusion *and* virus spread, *J Virol* 76:9079–9086.

Medlin, H. K., Zhu, Y. Q., Remington, K. M., Phillips, T. R., and North, T. W., 1996, Selection and characterization of a mutant of feline immunodeficiency virus resistant to 2′,3′-dideoxycytidine, *Antimicrob Agents Chemother* 40:953–957.

Ogilvie, G. K., 1995, Hematopoietic growth factors: frontiers for cure, *Vet Clin North Am Small Anim Pract* 25:1441–1456.

Ohashi, T., Goitsuka, R., Watari, T., Tsujimoto, H., and Hasegawa, A., 1992, Elevation of feline interleukin 6-like activity in feline immunodeficiency virus infection, *Clin Immunol Immunopathol* 65:207–211.

Phillips, T. R., Prospero Garcia, O., Puaoi, D. L., Lerner, D. L., Fox, H. S., Olmsted, R. A., Bloom, F. E., Henriksen, S. J., and Elder, J. H., 1994, Neurological abnormalities associated with feline immunodeficiency virus infection, *J Gen Virol* 75:979–987.

Povey, R. C., 1978, Effect of orally administered ribavirin on experimental feline calicivirus infection in cats, *Am J Vet Res* 39:1337–1341.

Priosoeryanto, B. P., Tateyama, S., Yamaguchi, R., and Uchida, K., 1995, Antiproliferation and colony-forming inhibition activities of recombinant feline interferon (rFeIFN) on various cells in vitro, *Can J Vet Res* 59:67–69.

Reinemeyer, C. R., Faulkner, C. T., Assadi-Rad, A. M., Burr, J. H., and Patton, S., 1995, Comparison of the efficacies of three heartworm preventives against experimentally induced infections with Ancylostoma caninum and Toxocara canis in pups, *J Am Vet Med Assoc* 206:1710–1715.

Reubel, G. H., Gregg, A. D., George, J. W., Barlough, J. E., and Pedersen, N. C., 1994, Effects of incidental infections and immune activation on disease progression in experimentally feline immunodeficiency virus-infected cats, *J Acquir Immune Defic Syndr* 7:1013–1015.

Richardson, J., Pancino, G., Merat, R., Leste-Lasserre, T., Moraillon, A., Schneider-Mergener, J., Alizon, M., Sonigo, P., and Heveker, N., 1999, Shared usage of the chemokine receptor CXCR4 by primary and laboratory-adapted strains of feline immunodeficiency virus, *J Virol* 73:3661–3671.

Rimmelzwaan, G. F., Siebelink, K. H., Broos, H., Drost, G. A., Weijer, K., Vvan Herwijnen, R., and Osterhaus, A. D., 1994, Gag- and env-specific serum antibodies in cats after natural and experimental infection with feline immunodeficiency virus, *Vet Microbio* 39:153–165.

Ryrfeldt, A., Nordgren, T., and Lundstrom, J., 1992, Hypocalcemia induced by foscarnet (Foscavir) infusion in dogs, *Fundam Appl Toxicol* 18:126–130.

Sato, R., Inanami, O., Tanaka, Y., Takase, M., and Naito, Y., 1996, Oral administration of bovine lactoferrin for treatment of intractable stomatitis in feline immunodeficiency virus (FIV)-positive and FIV-negative cats, *Am J Vet Res* 57:1443–1446.

Scadden, D. T., Zon, L. I., and Groopman, J. E., 1989, Pathophysiology and management of HIV-associated hematologic disorders, *Blood* 74:1455–1463.

Schellekens, H., Geelen, G., Meritet, J.-F., Maury, C., and Tovey, M. G., 2001, Oromucosal interferon therapy: Relationship between antiviral activity and viral load, *J Interferon Cytokine Res* 21:575–581.

Sellon, R. K., 1998, Feline immunodeficiency virus infection, in: *Infectious Diseases of the Dog and Cat*, 2nd ed., C. E. Greene, ed., WB Saunders, Philadelphia, pp. 84–96.

Shaw, S. E., Robertson, I. D., Robinson, W. F., Alexander, R., and Sutherland, R. J., 1990, Feline immunodeficiency virus: disease associations, *Australian Vet Pract* 20:194–198.

Smith, R. A., Remington, K. M., Preston, B. D., Schinazi, R. F., and North, T. W., 1998, A novel point mutation at position 156 of reverse transcriptase from feline immunodeficiency virus confers resistance to the combination of (-)-beta-2′,3′-dideoxy-3′-thiacytidine and 3′-azido-3′-deoxythymidine, *J Virol* 72:2335–2340.

Smyth, N. R., McCracken, C., Gaskell, R. M., Cameron, J. M., Coates, J. A., Gaskell, C. J., Hart, C. A., and Bennett, M., 1994, Susceptibility in cell culture of feline immunodeficiency virus to eighteen antiviral agents, *J Antimicrob Chemother* 34:589–594.

Stengel, C., Klein, D., Egberink, H., Balzarini, J., and Hartmann, K., 2003, Placebo-controlled double-blind treatment study in naturally feline immunodeficiency virus-infected cats using the chemokine receptor inhibitor 1,1′-bis-1,4,8,11-tetraazacyclo-tetradekan (AMD3100), in: *Research Abstracts of the 21st Annual American College of Veterinary Internal Medicine (ACVIM) Forum*, Charlotte, NC.

Swenson, C. L., Sams, R. A., Polas, P. J., Michael, D. F., and Mathes, L. E., 1990, Age-related differences in pharmacokinetics of phosphonoformate in cats, *Antimicrob Agents Chemother* 34:871–874.

Swenson, C. L., Weisbrode, S. E., Nagode, L. A., Hayes, K. A., Steinmeyer, C. L., and Mathes, L. E., 1991, Age-related differences in phosphonoformate-induced bone toxicity in cats, *Calcif Tissue Int* 48:353–361.

Tanabe, T., and Yamamoto, J. K., 2001, Feline immunodeficiency virus lacks sensitivity to the antiviral activity of feline IFN-gamma, *J Interferon Cytokine Res* 21:1039–1046.

Taniguchi, A., Ishida, T., Konno, A., Washizu, T., and Tomoda, I., 1990, Altered mitogen response of peripheral blood lymphocytes in different stages of feline immunodeficiency virus infection, *Jpn J Vet Sci* 52:513–518.

Tateyama, S., Priosoeryanto, B. P., Yamaguchi, R., Uchida, K., Ogiwara, K., and Suchiya, A. T., 1995, In vitro growth inhibition activities of recombinant feline interferon on all lines derived from canine tumours, *Res Vet Sci* 59:275–277.

Terry, A., Callanan, J. J., Fulton, R., Jarrett, O., and Neil, J., 1995, Molecular analysis of tumours from feline immunodeficiency virus (FIV)-infected cats: an indirect role for FIV? *Int J Cancer* 61:227–232.

Tompkins, W. A., 1999, Immunomodulation and therapeutic effects of the oral use of interferon-α: Mechanism of action, *J Interferon Cytokine Res* 19:817–828.

Torten, M., Franchini, M., Barlough, J. E., George, J. W., Mozes, E., Lutz, H., and Pedersen, N. C., 1991, Progressive immune dysfunction in cats experimentally infected with feline immunodeficiency virus, *J Virol* 65:2225–2230.

Torten, M., Rideout, B. A., Luciw, P. A., Sparger, E. E., and Pedersen, N. C., 1990, Co-infection of cats with feline immunodeficiency virus (FIV) and feline leukemia virus (FeLV) enhances the severity of FIV infection and affects the distribution of FIV DNA in various tissues, in: *Animal Models in AIDS*, H. Schellekens, M. C. Horzinek, eds., Elsevier Science Publishers, Amsterdam, pp. 209–219.

Ueda, Y., Sakurai, T., and Yanai, A., 1993a, Homogeneous production of feline interferon in silkworm by replacing single amino acid code in signal peptide region in recombinant baculovirus and characterization of the product, *J Vet Med Sci* 55:251–258.

Ueda, Y., Sakurai, T., Kasama, K., Satoh, Y., Atsumi, K., Hanawa, S., Uchino, T., and Yanai, A., 1993b, Pharmacokinetic properties of recombinant feline interferon and its stimulatory effect on 2′,5′-oligoadenylate synthetase activity in the cat, *J Vet Med Sci* 55:1–6.

Weiss, R. C., Cox, N. R., and Boudreaux, M. K., 1993a, Toxicologic effects of ribavirin in cats, *J Vet Pharmacol Ther* 16:301–316.

Weiss, R. C., Cox, N. R., and Martinez, M. L., 1993b, Evaluation of free or liposome-encapsulated ribavirin for antiviral therapy of experimentally induced feline infectious peritonitis, *Res Vet Sci* 55:162–172.

Willett, B. J., Cannon, C. A., and Hosie, M. J., 2002, Upregulation of surface feline CXCR4 expression following ectopic expression of CCR5: implications for studies of the cell tropism of feline immunodeficiency virus, *J Virol* 76:9242–9252.

Willett, B. J., Picard, L., Hosie, M. J., Turner, J. D., Adema, K., and Clapham, P. R., 1997, Shared usage of the chemokine receptor CXCR4 by the feline and human immunodeficiency viruses, *J Virol* 71:6407–6415.

Woo, J. C., Dean, G. A., Lavoy, A., Clark, R., and Moore, P. F., 1999, Investigation of recombinant human insulin-like growth factor type I in thymus regeneration in the acute stage of experimental FIV infection in juvenile cats, *AIDS Res Hum Retroviruses* 15:1377–1388.

Yamauchi, K., Wakabayashi, H., Hashimoto, S., Teraguchi, S., Hayasawa, H., and Tomita, M., 1998, Effects of orally administered bovine lactoferrin on the immune system of healthy volunteers, *Adv Exp Med Biol* 443:261–165.

Yates, K. M., Rosenberg, L. J., Harris, C. K., Bronstad, D. C., King, G. K., Biehle, G. A., Walker, B., Ford, C. R., Hall, J. E., and Tizard, I. R., 1992, Pilot study of the effect of acemannan

in cats infected with feline immunodeficiency virus, *Vet Immunol Immunopathol* 35:177–189.

Yu, N., Billaud, J. N., and Phillips, T. R., 1998, Effects of feline immunodeficiency virus on astrocyte glutamate uptake: implications for lentivirus-induced central nervous system diseases, *Proc Natl Acad Sci USA* 95:2624–2629.

Zeidner, N. S., Myles, M. H., Mathiason-DuBard, C. K., Dreitz, M. J., Mullins, J. I., and Hoover, E. A., 1990, Alpha interferon (2b) in combination with zidovudine for the treatment of presymptomatic feline leukemia virus-induced immunodeficiency syndrome, *Antimicrob Agents Chemother* 34:1749–1756.

Zenger, E., Collisson, E. W., Barhoumi, R., Burghardt, R. C., Danave, I. R., and Tiffany Castiglioni, T. E., 1995, Laser cytometric analysis of FIV-induced injury in astroglia, *Glia* 13:92–100.

Zhang, L., He, T., Huang, Y., Chen, Z., Guo, Y., Wu, S., Kunstman, K. J., Brown, R. C., Phair, J. P., Neumann, A. U., Ho, D. D., and Wolinsky, S. M., 1998, Chemokine coreceptor usage by diverse primary isolates of human immunodeficiency virus type 1, *J Virol* 72:9307–9312.

Zhu, Y. Q., Remington, K. M., and North, T. W., 1996, Mutants of feline immunodeficiency virus resistant to 2′,3′-dideoxy-2′,3′-didehydrothymidine, *Antimicrob Agents Chemother* 40:1983–1987.

12

Equine Infectious Anemia Virus as a Model for Lentiviral Pathogenesis

SUSAN L. PAYNE, WAH-SENG LIM,
FREDERICK J. FULLER, and JUDITH M. BALL

1. INTRODUCTION

Equine infectious anemia virus (EIAV), the etiologic agent of equine infectious anemia (EIA) or swamp fever, is a unique macrophage-tropic lentivirus. EIA, described in the veterinary literature in the mid-19th century, was one of the first diseases determined to be caused by a "filterable agent."[1] However, studies of the virus languished as methods for its culture in horse leukocytes were not described until the 1960s, and even then difficulty working with equine cultures forestalled its detailed characterization. EIAV was adapted to growth in a persistently infected cell line in 1973, and evidence that EIAV was a retrovirus was presented in 1976.[1] A detailed characterization of viral structural proteins was finally available in the early 1980s.[1] Although of considerable veterinary importance, studies of EIAV remained relatively obscure in comparison to the oncogenic retroviruses, especially those affecting more amenable animal models such as chickens and mice. EIAV and the other so-called "slow viruses" of ungulates, maedi-visna virus (MVV) of sheep and caprine-arthritis encephalitis virus (CAEV) of goats, garnered some attention due to reports that described their ability to form viral swarms during persistent infection. Descriptions of the EIAV genome appeared shortly after reports that HIV shared sequence homology with the animal lentiviruses, a finding that placed a new urgency on understanding the molecular biology and patho-

SUSAN PAYNE, WAH-SENG LIM, and JUDITH BALL • Department of Pathobiology, College of Veterinary Medicine, Texas A&M University, College Station TX 77845
FREDERICK J. FULLER • Department of Microbiology, Pathology and Parasitology, College of Veterinary Medicine, North Carolina State University, Raleigh, NC 27650

genesis of these viruses, and reinvigorated attempts at vaccine development.[1] Overall, the pace of EIAV research has remained slow in comparison to primate lentiviruses, primarily due to the lack of reagents and other difficulties of working with this large animal model. However, as described below, the use of EIAV as a comparative system has contributed to our understanding of HIV replication and disease.

EIAV Genome Organization

A schematic of the EIAV genome is shown in Figure 1; at 8.2 kb it is considerably smaller than the genomes of the primate lentiviruses. The long terminal repeats (LTRs) range from 305 to 320 bp and the enhancer region, found in U3, varies extensively between cell-culture adapted and wild-type virus strains.[2] The overall organization of EIAV *gag* and *pol* is similar to other lentiviruses; EIAV *gag* and *pol* overlap and protease (PR) is in-frame with reverse transcriptase (RT). The EIAV capsid protein (CA or p26) shares 30% of its amino acids (aa) with HIV and the overall base composition of the EIAV genome, at 60 to 62 mole percent A + T, is typical for lentiviruses.[1] A major difference between EIAV and the primate lentiviruses is found in the organization of *pol*, which in the case of EIAV also encodes the enzyme deoxyuridine triphosphatase (dUTPase or DU).[3]

EIAV *env* encodes an approximately 860 residue precursor polypeptide that is cleaved and processed to generate a surface unit glycoprotein (SU or gp90) of approximately 440 aa and a transmembrane protein (TM or gp45) of approximately 400 aa.[1] SU is highly glycosylated and the

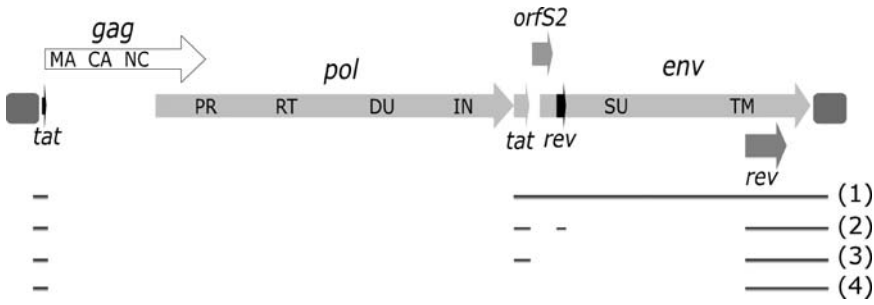

FIGURE 1. Schematic diagram of the EIAV genome and the major spliced mRNAs. Open reading frames are indicated by arrows. Orfs encoding *pol*, *tat*, *env*, and *rev* exon 1 are in the same reading frame. The major spliced viral mRNAs are: (1) A singly spliced product that serves as a tricistronic mRNA encoding Tat, S2, and Env. (2) A 4-exon mRNA that encodes Tat and Rev. (3) A three exon mRNA that encodes Tat, but lacks the first coding exon of Rev. (4) A singly spliced mRNA that encodes TTM, a protein comprised of Tat and TM sequences. As described in the text, Rev-minus mutants fail to produce the 3-exon mRNA.

numbers and positions of potential N-linked glycosylation sites vary among isolates, particularly during chronic infection.[1] EIAV *env* contains variable and conserved domains that are generally similar to those mapped for HIV SU and TM.[1] EIAV TM has two hydrophobic domains with the first presumed to be the surface exposed fusion domain and the second the membrane-spanning domain.[1,4] TM is glycosylated on sites located on the N-terminal surface exposed region of the protein and has a long cytoplasmic domain (about 200 aa) typical of other lentiviruses.[1] EIAV TM may occur in two forms in mature virions. Both uncleaved (45kDa) and cleaved (32–35kDa glycosylated and 20kDa unglycosylated) TM products have been isolated from purified virions.[1,4] The cleaved TM products have been confirmed by protein sequencing, and the cleavage site has been placed at a His-Leu bond located 240 aa from the amino terminus. This cleavage product is not present in cell lysates, suggesting that cleavage occurs exclusively in free virus.[1,4]

Another difference between EIAV and the primate lentiviruses is the relative simplicity of the EIAV genome with only three small open reading frames (orfs) in addition to *gag*, *pol*, and *env*. These small orfs are used to produce four auxiliary proteins (Figure 1). A transactivator protein, Tat, is required for efficient transcription from the EIAV LTR and shares properties with HIV-1 Tat (HTat).[2] EIAV Tat (ETat) is 83 aa with a basic domain and a hydrophobic core sequence (Figure 2A) and lacks the cysteine rich domain found in HTat. The functional domains of ETat have been mapped using EIAV/HIV-1 Tat chimeras to activate chimeric promoters. It has been demonstrated that the activation domains of ETat and HTat are interchangeable despite the fact that they share no apparent aa sequence homology.[2]

The entire 26 aa carboxyl terminal region, including the basic domain of ETat, is required for interaction with EIAV TAR (ETAR), located at positions +1 to 25 of the nascent transcript.[5] However, unlike the case for HIV-1, no strong interaction between ETat and ETAR can be demonstrated in vitro.[5] ETAR binding and transcriptional activation of the EIAV LTR require binding of ETat to equine cyclin T1. ETat also forms a complex with human cyclin T1, but this complex fails to bind ETAR and does not transactivate the EIAV LTR.[5] The amino terminal region of cyclin T1 interacts with ETat; a comparison of equine and human cyclin T1 proteins reveals a leucine at position 29 of the equine protein, in place of a valine in the human protein.[5] Binding of an ETat-cyclin T1 complex to ETAR requires L29 such that ETat will productively interact with human cyclin T1 carrying a V29L substitution.[5] The ability to create EIAV/HIV chimeric proteins and to use ETat to compete with HTat for access to cellular cofactors, such as cyclin T1, has provided a powerful tool to clarify mechanisms of HTat function and underscores the utility of using EIAV as a model system.

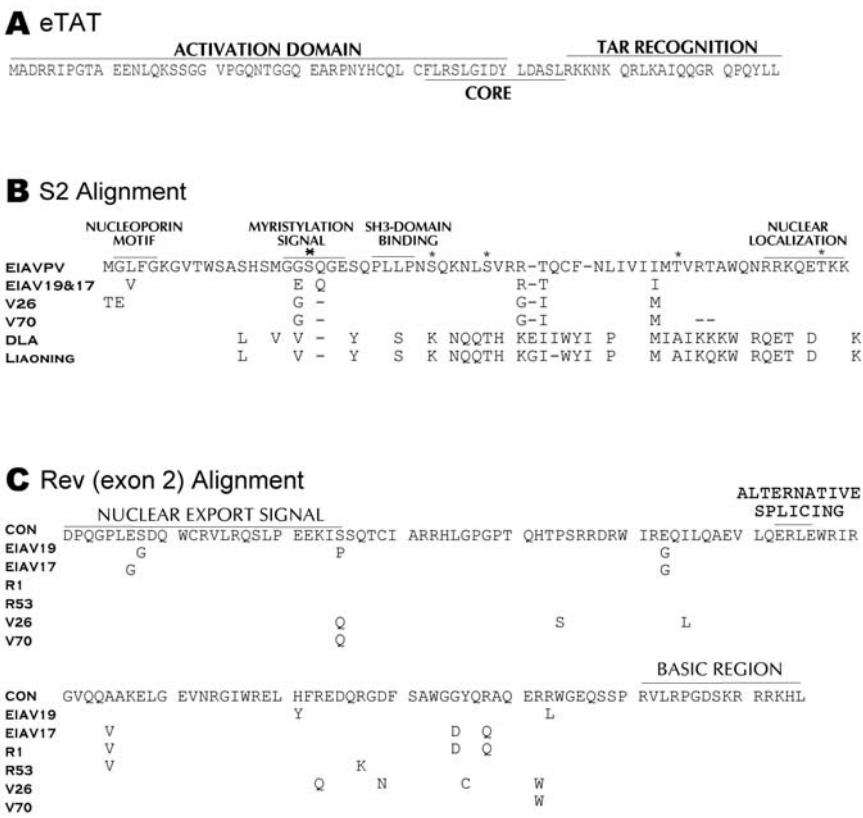

FIGURE 2. EIAV auxilliary proteins. (A) Complete aa sequence of Tat with the major functional regions indicated. Tat sequences are highly conserved among EIAV strains. (B) A comparison of S2 sequences with proposed functional motifs as indicated. Potential phosphorylation sites are indicated (* casein kinase II; * protein kinase C). $EIAV_{PV}$ is a biologically cloned, virulent stock derived by horse passage of Malmquist EIAV. $EIAV_{19}$ and $EIAV_{17}$ are molecular clones with identical S2 sequences. $EIAV_{19}$ is derived from FEA cells infected with avirulent Malmquist virus. V26 and V70 are Japanese strains of EIAV. V70 is the virulent parent of the highly attenuated V26 vaccine strain that was derived by passage in equine leukocytes. Liaoning is a highly virulent Chinese strain. DLA (donkey leukocyte adapted) is an attenuated vaccine strain that was derived from Liaoning by passage in donkey leukocytes. (C) A comparison of Rev exon 2 sequences with function domains indicated. The origins of R1 and R53 are described in the text.

The second auxiliary protein of EIAV, Rev, influences viral mRNA splicing and transport and has obvious similarities to HIV Rev.[2] The third auxiliary protein of EIAV is the product of the S2 orf. S2 is a 7kDa protein of unknown function, but S2-mutants of EIAV replicate very poorly in vivo.[6] EIAV Rev and S2 proteins are discussed in more detail below. A fourth auxiliary protein, containing regions of both the Tat and TM proteins (TTM),

also has been demonstrated, but its function remains undetermined.[2] TTM is generated from a transcript that fuses exon 1 to exon 4. The 27kDa protein contains 29 aa from Tat fused to 218 aa from TM and was identified using peptide antisera against the amino terminus of Tat and the extreme C-terminus of TM.[2]

Examination of EIAV splicing patterns reveals some interesting features of EIAV gene expression.[2] Exon 1 of all spliced RNAs is the first coding exon of *tat* and at least three abundant viral mRNAs encode complete Tat proteins (Figure 1). Tat translation initiates at a CUG codon, thus adequate Tat expression appears to derive from suboptimal expression of several different RNAs. The predominant singly spliced viral RNA is functionally tricistronic, expressing Tat, S2, and Env.

Cell Tropism

EIAV is primarily macrophage-tropic. The absence of T-cell tropism is a significant difference between EIAV and the primate lentiviruses and certainly has a major impact on the clinical manifestations and outcome of infection. It has been clearly demonstrated that during the acute disease episode, the bulk of virus production takes place in mature tissue macrophages.[7] The virus burden measured in the blood on the day of sacrifice correlates to the extent of virus replication in macrophages, and quantitative studies indicate that most of the viral burden during acute disease is accounted for by virus replication in tissues rich in macrophages, such as spleen and liver.[7] Endothelial cells also harbor EIAV during acute infection, but the overall contribution of this cell type to total virus burden is unknown.[8] Sensitive *in situ* hybridization and PCR techniques have been used to compare the tissue sites and levels of EIAV replication during clinical and subclinical infections, and to determine if cells other than macrophages might be an important reservoir for viral persistence.[9] A non-macrophage reservoir (such as endothelial or dendritic cells) has not been detected during the carrier stage. Virus production continues in tissue macrophages, but at levels that are significantly reduced relative to periods of clinical disease.[9]

In contrast to tissue macrophages, peripheral blood mononuclear cells (PBMC) contain proviral DNA but generally express little virus, generating less than 1% of the total viral burden.[7] The percentage of circulating monocytes harboring proviral DNA is estimated to be less than 10%.[10] In vitro studies of EIAV expression suggest complex regulation of EIAV expression in monocytes versus macrophages, with expression increasing upon differentiation.[10,11] Upon in vitro infection of nonadherent monocytes, virus expression is delayed by 2 to 3 days as compared to infection of adherent cells.[11] Similarly, when PBMC are recovered from EIAV infected horses, virus expression is initially absent, but increases with differentiation of the

cultured monocytes, at approximately 6 to 8 days postplating.[10] In addition, treatment of equine monocyte-derived macrophages (eMDM), with either lipopolysaccharides (LPS) or phorbol myristate acetate (PMA), decreases the production of infectious virus.[12] Treatment of cells with increasing amounts of LPS increases the level of virus inhibition and although the cause for the inhibition is not fully understood, early events, up to and including transcription of multiply spliced mRNA, are not inhibited in LPS-treated macrophages.[12]

Field strains of EIAV can be propagated in both eMDM and equine bone marrow–derived macrophages (eBMDM); infection of these macrophage cultures is highly cytopathic. Unfortunately, it can be difficult to work with primary equine macrophages as their viability and suitability for EIAV culture can vary widely between donor animals. Also, neither eMDM nor eBMDM can be maintained in culture for long periods of time. Due to these limitations, strains of EIAV that replicate in nonmyeloid cells have been isolated. The Malmquist strain of EIAV has been recovered by blind passage of the Wyoming field strain in equine dermis (ED) cells,[13] and many derivatives of the Malmquist strain are commonly used today. The Malmquist strain can also be cultured in cell lines of canine (Cf2Th and D17) and feline (FEA) origin; however, these cell lines do not support replication of field strains of EIAV. Recently cultured equine endothelial cells have been shown to support replication of some EIAV strains.[14] As with many viruses, strains of EIAV that are extensively passaged in culture (in either myeloid or nonmyeloid cells) generally become attenuated as a result. It should be noted that in vivo attenuation does not correlate with the ability of EIAV to produce cytopathic infection in eMDM. Some attenuated EIAVs replicate to high titer in eMDM, and some virulent stocks can be propagated in nonmyeloid cells.[15]

2. DISEASE INDUCTION BY EIAV

A unique feature of the EIAV life cycle is transmission by deer and horse flies.[16] Virus transmission is purely mechanical and can occur if a fly is interrupted during a blood meal and moves from an infected to an uninfected animal. Transmission decreases with distance between horses, as biting flies seldom travel more than 100 yards to complete a blood meal. Virus transmission also has been reported to occur through semen or from a mare to a foal, although these transmissions occur infrequently. Virus also can be readily transmitted by blood-contaminated veterinary instruments.[16]

While EIAV infects all members of the family Equidae, the disease is best described in *E. callabus* (domestic horses and ponies); due to their small size, Shetland ponies are common experimental animals. An acute disease episode generally occurs 1 week to a month following exposure to

the virus, lasting no more than several days.[17] Acute disease is characterized by fever and thrombocytopenia with many animals also displaying diarrhea, CNS depression, anorexia, and petechiae on mucus membranes.[16] High-titer plasma viremia is coincident with the acute febrile episode,[17] and there is a correlation between severity of disease and level of virus replication.[18] The acute disease episode is fatal in a small percentage of animals.[17]

The chronic or persistent phase of EIA is typified by discrete, periodic episodes of clinical illness. These episodes usually occur within 1 year of the initial infection, but may also appear years later, particularly in response to stress or upon administration of immunosuppressive drugs.[16] Fever and thrombocytopenia are indicators of discrete disease episodes, and recurring cycles of illness lead to the classic symptoms of edema, weight loss, and anemia.[16] As during the acute disease episode, high-titer viremia accompanies febrile episodes, but virus replication per se does not correlate with disease, as titers of up to 10^4 copies of viral RNA have been measured in plasma in the absence of clinical disease.[18] It has been shown at both the protein and nucleotide sequence levels that viruses recovered during chronic EIA vary in their *env* (and overlapping *rev*) sequences.[1,19,20] Following a period of chronic disease many infected horses and ponies become inapparent carriers. While the level of circulating virus in carriers is low, transfer of whole blood efficiently transmits infection to naive animals.[16]

EIAV-Induced Anemia

The development of anemia is a result of both intravascular and extravascular hemolysis and impaired bone marrow responses.[17] EIAV SU hemaglutinates erythrocytes and complement-coated erythrocytes are present in infected horses, suggesting that circulating virus-antibody complexes attach to erythrocytes via Fc or complement receptors, leading to complement-mediated lysis. Erythrophagocytosis is also evident in splenic and bone marrow macrophages from acutely infected horses. Impaired bone marrow responses also contribute to EIAV-induced anemia, as evidenced by the development of anemia in severe combined immunodeficency (SCID) foals.[17] SCID foals do not produce antibody and their erythrocytes lack antibody and complement on their surface. As discussed below, suppression of erythropoiesis by proinflammatory cytokines likely contributes to the anemia in SCID foals and during acute febrile episodes in immunocompetent horses.

EIAV-Induced Thrombocytopenia

The cause of EIAV-induced thrombocytopenia has been investigated extensively as it is a consistent clinical finding.[17] Various mechanisms, both

immune and nonimmune mediated, have been postulated, but a review of relevant studies suggests the thrombocytopenia associated with febrile episodes is likely due to platelet destruction with removal, as well as reduced production by bone marrow megakaryocytes (MK cells). Strong support for nonimmune-mediated mechanisms of thrombocytopenia is derived from the SCID foal model. SCID foals lack functional T- and B-cells, but have normal macrophages and are readily infected by EIAV.[21] Infected SCID foals develop severe thrombocytopenia during acute infection, ruling out a role for T- or B-cell involvement in the process. Examination of MK cells in the bone marrow during EIAV-induced thrombocytopenia has failed to demonstrate significant alterations in MK cell numbers and there is no evidence that MK cells are infected by EIAV.[21–23] However, evidence does exist for a primary production deficit of platelets.[21] In support of this hypothesis, Tornquist and Crawford[23] show inhibition of MK maturation and growth in vitro upon addition of plasma from prethrombocytopenic animals. These sera contain elevated levels of TNF-α and TGF-β, proinflammatory cytokines that have been demonstrated to have suppressive effects on MK colony growth in other species.[24] The addition of antibodies to TNF-α and TGF-β to inhibitory prethrombocytopenic plasma partially decreases the suppressive effects of the sera, indicating a role for these cytokines in either reducing proliferation of early MK progenitor cells or by inhibiting MK maturation.[23] In addition, injection of purified TNF-α induces severe thrombocytopenia in mice and humans possibly by stimulating secretion of platelet agonists.[25,26] Other studies reveal IL-1 and IL-6 are capable of directly activating platelets in vitro[27,28] and platelet activation in vivo has been observed in thrombocytopenic ponies acutely infected with EIAV.[22] These studies support a model of platelet loss due to the accelerated removal of in vivo activated platelets from the circulation. Indeed, platelets from acutely EIAV-infected horses have increased surface-bound fibrinogen, ultrastructural changes, and hypofunctional aggregation responses to agonists in vitro consistent with in vivo platelet activation.[22] Activation of platelets could cause degranulation or formation of aggregates resulting in their removal from circulation. Both a primary production deficit of platelets and platelet loss due to in vivo platelet activation could be functioning to produce the severe thrombocytopenia associated with acute EIA, and both mechanisms for platelet loss could be the result of altered cytokine production by infected macrophages.

Role of Virally Induced Cytokines in Disease

Mounting evidence suggests that virally induced cytokines cause many of the clinical abnormalities seen during EIA febrile episodes.[22,29,30] Using cell-based assays of cytokine activity, significant increases in the proinflam-

matory cytokines IL-6, TNF-α, and TGF-β have been demonstrated during viremic phases.[22,29,30] Increased TNF-α has been correlated to the level of viremia, severity of clinical signs and vaccine-induced disease enhancement in experimental infections, with higher levels of TNF-α present in sera of ponies infected with virulent $EIAV_{PV}$ than in that of avirulent $EIAV_{PR}$-infected ponies.[29] TNF-α levels also are elevated in Arabian foals infected with the highly virulent $EIAV_{WYO}$ field strain and in SCID foals infected with the virulent, culture-adapted $EIAV_{WSU5}$.[30] Likewise, increased IL-6 levels are found in sera of ponies experimentally infected with $EIAV_{WYO}$,[22] and in media following in vitro infection of eBMDM with EIAV.[31] These enhanced levels of IL-6 and TNF-α activity may be responsible for fever, thrombocytopenia, anemia, anorexia, hypergammaglobulinaneamia, and polyclonal B-cell activation frequently observed in EIAV-infected equids.

Proinflammatory cytokines are predominantly produced by macrophages[32] and are well known for their pyrogenic activities.[33] The inflammatory effects of these cytokines can cause anorexia and wasting during viremic episodes in EIAV-infected horses[1] as seen in HIV AIDS.[34] Elevated TNF-α and IL-1 activities promote migration of leukocytes, activation of endothelial cells, and stimulation of the hypothalamic pituitary axis.[35] TNF-α and IL-1 may exacerbate EIA anemia by activating macrophages and neutrophils, enhancing phagocytosis of complement-coated erythrocytes, and suppressing erythropoiesis by down regulating erythropoietin production and dysregulating iron metabolism.[36] IL-1 induces expression of prostanglandins,[37] which can result in fever, anorexia, and lethargy, common symptoms of EIA. IL-6 participates in regulating various steps of hematopoiesis in vivo and is a potent cofactor of hematopoietic progenitor cells in vitro.[38] Together with IL-10, IL-6 increases total serum globulin and IgM concentrations, which are commonly seen in EIAV-infected horses.[39]

We have reported the use of an equine-specific ribonuclease protection assay (RPA) to quantitate mRNA levels of 10 cytokines[40] and have utilized this newly developed assay to show significant differences in cytokine induction in eMDM exposed to virulent and avirulent EIAV clones.[41] Virulent $EIAV_{17}$ induces significant increases in proinflammatory cytokines, whereas avirulent $EIAV_{19}$ fails to induce any of the tested cytokines above that of control levels. These data are consistent with the hypothesis that cytokines directly contribute to EIA pathogenesis. Notably, the proinflammatory cytokines are elevated during febrile episodes when circulating virus is readily detectable, suggesting these cytokines may affect EIA pathogenesis by modulating viral replication in vivo. There are several ways the proinflammatory cytokines could contribute to enhanced replication of EIAV in vivo, including (a) recruitment of uninfected monocytes to sites of viral replication via monocyte-chemotactic factors; (b) promotion of monocyte migration into tissue by the stimulation of adhesion molecules;[42]

and (c) induction of nonmonocytic cells to produce EIAV-activating molecules.

Immune System Alterations

EIAV infection does not result in severe immunodeficiency, but alterations in lymphocyte subsets have been noted, particularly during periods of robust virus replication. For example, the mitogen-induced proliferative responses of peripheral blood lymphocytes (PBL) isolated from ponies during febrile episodes or in PBL exposed to acute sera are significantly suppressed.[43,44] A direct virus effect for this process is likely, as the addition of high concentrations of infectious or heat-inactivated EIAV to eMDM is able to directly suppress mitogen-induced proliferative responses.[44] The addition of virus after addition of mitogen does not alter proliferative responses, suggesting that the initial activation of mononuclear cells is altered. Antigen-specific lymphocyte proliferation is also suppressed during febrile episodes.[44]

EIAV-induced abnormalities of lymphocyte counts have been demonstrated in both acute and carrier animals. Inapparent carriers have significantly decreased T-lymphocyte numbers and modest increases in B-cells.[39] During acute febrile episodes, or during recrudescent disease, more profound differences are seen. T-cell subsets ($CD4^+$, $CD5^+$, and $CD8^+$) are significantly decreased in febrile animals, while there is an increase in circulating B-cells.[39,45] Thus febrile animals have significantly decreased T- to B-cell ratios in comparison to seronegative horses. Increased B-cell numbers may contribute to the elevated concentrations of gamma globulin that are a common finding in EIAV-infected horses. Alternatively (or in addition) B-cells may be activated due to secretion of inflammatory cytokines as described above.

3. IMMUNE RESPONSES THAT CONTROL EIAV REPLICATION

Immune responses are required to control EIAV infection and usually are effective, although elimination of the virus is never achieved.[1] The importance of immune system control during long-term infection is demonstrated by disease recrudescence upon experimental immune suppression and the development of disease upon transfer of whole blood from long-term inapparent carriers to naive recipients.[16] Antibodies to EIAV CA as well as to group-specific determinants of SU can be detected early after infection (within weeks). Neutralizing antibodies directed against SU and TM are not detected until at least a month after infection and are seldom detected until well after the resolution of the initial febrile episode.[1] The poor initial neutralizing antibody responses to EIAV may result from the fact that the viral SU and TM proteins are heavily glycosy-

lated. Detailed studies of antibody avidity and epitope specificity indicate that the development of a fully mature humoral immune response takes from 6 to 8 months and is characterized by moderate to high avidity antibodies that are capable of recognizing conformational epitopes.[46,47] The poorly neutralizing antibodies present at earlier times postinfection (pi) are of low avidity and are directed primarily at linear epitopes.[46] Delay in development of an effective neutralizing humoral immune response may explain why the chronic phase of EIAV, with recurrent febrile periods, generally occurs during the first year pi. A recent study shows that serum neutralization titers to an infecting virus stock are lower in ponies that develop chronic EIA than in those that do not progress to the chronic stage of disease.[47] Once the humoral immune response matures, it remains relatively constant for up to 3 years.[46] Despite the slow development of effectively neutralizing antibody, its role in the control of virus replication is underscored by the fact that viruses that emerge to replicate to high titer during chronic infection are unique populations that have temporarily escaped host immune control.[1]

The first suggestion that EIAV strains are antigenically heterogeneous came from virus neutralization assays of viruses recovered during chronic EIA, and in 1973 it prompted the hypothesis that "the persistence of virus in the blood of infected horses might be maintained by a consecutive development of antigenically distinct virus populations not susceptible to antibodies previously produced, and that the increase in such variants might be responsible for febrile relapse."[48] Since that time, EIAV antigenic variation has been examined extensively. Immunologic studies, as well as molecular analyses of viral variants isolated during discrete febrile episodes, illustrate that chronic EIA in particular is a period of dynamic interaction between host and virus, during which variant viruses emerge by escaping from immune surveillance.[1] As regards immune escape and genetic variation, *env* sequences have been analyzed most extensively.[1,20,47] The development of an *env* quasispecies during infection is best characterized in experimental infections where the genetic makeup of the infecting stock is known. In studies using the biologically cloned stock $EIAV_{PV}$ (whose *env* sequence divergence is about 0.2%), *env* divergence ranges from 0.09 to 2.6% upon pair-wise clone comparisons.[49] Analysis of SU sequences reveals that discrete populations of SU sequences are recovered from febrile episodes and suggests that the virus population associated with any given disease episode is replaced during subsequent disease episodes.[49] Eight discrete variable regions of SU have been identified with mutations consisting of blocks of nucleotide substitutions as well as insertions and deletions, particularly within the principal neutralizing domain (PND), a region of the SU protein against which the neutralizing antibody is directed.[1,47] In studies of a virulent EIAV stock originating in Japan,[19] SU variation recovered from 22 distinct febrile episodes is reminiscent of that reported for $EIAV_{PV}$.

Direct evidence that SU variation is responsible for neutralization escape has been obtained by recovery and analysis of virus-neutralizing mouse monoclonal antibodies recognizing EIAV SU.[1] These strongly neutralizing antibodies map to epitopes in the V3 domain of SU, a region predicted to form a surface loop; this region of SU is similar to the V3 loop of HIV-1.[1] A panel of *env* variants recovered from sequential febrile episodes in an experimentally infected horse has been used to further characterize the effects of *env* variation on neutralization phenotype and to specifically define neutralizing determinants in *env*.[47] This study reveals that *env* sequences found during later febrile episodes are increasingly neutralization-resistant and that the V3 and V4 regions of EIAV SU contribute individually to serum neutralization resistance.[47]

Characterization of cell-based immune responses to EIAV has been challenging due to the scarcity of equine specific reagents. However, the role of cytotoxic T-lymphocyte (CTL) activity in the control of EIAV replication has been firmly established.[50–53] Cellular immune responses play a major role in controlling virus replication early after infection due to the delay in the development of neutralizing antibody. EIAV-specific $CD8^+$ and $CD4^+$ CTL are present within 7 to 10 days pi, a time of appearance that correlates extremely well with control of initial viremic episodes.[50,53] CTL activity is MHC restricted and a majority of CTL are $CD8^+$[53]. Memory CTL (CTLm) has also been demonstrated after in vitro stimulation of PBL from carrier horses.[50,51] CTL recognize epitopes on MA, CA, SU, TM, and S2, and a majority of animals have CTL directed to MA and CA.[52,54] However, one difficulty of measuring CTL responses to EIAV is that vector-transduced target cells present a single set of epitopes that are likely not recognized by CTL directed against viral variants that arise in infected horses.

Immune reconstitution of SCID foals is being investigated as a means of studying protective immune responses to EIAV.[55] If successful, these studies will provide a method for determining the critical components of a protective immune response to EIAV. In a recent study, a SCID foal treated with adoptively transferred virus-stimulated lymphocytes derived from a persistently infected half sibling donor became lymphocyte engrafted and developed EIAV-specific CTL and neutralizing antibody which controlled virus replication.[55] These data indicate the importance of the equine model in studying lentivirus immune status and how it relates to disease.

4. VACCINES

The emergence of the AIDS epidemic in the 1980s renewed interest in the development of an EIAV vaccine, an effort that had been largely abandoned after a reliable serodiagnostic test for the virus became avail-

able in the 1970s.[1,16] Montelaro et al. and Issel have extensively tested inactivated whole virus and subunit vaccines.[1] An inactivated whole virus vaccine, prepared from avirulent, culture-adapted $EIAV_{PR}$, protects over 90% of horses against homologous challenge. Although it fails to protect against infection by a heterologous, virulent strain ($EIAV_{PV}$), the vaccinated animals experience a milder disease than unvaccinated controls.[1] A subunit vaccine of lectin-purified viral glycoproteins also protects vaccinees against challenge by homologous virus. However, results of heterologous challenge of vaccinees is troubling, as half of the challenged animals display more severe symptoms than unvaccinated controls, suggestive of immune enhancement.[1]

Additional vaccine trials of subunit vaccines have been performed to investigate immune enhancement, and a subunit vaccine consisting of baculovirus-expressed EIAV SU (rgp90 vaccine) has been extensively tested in three trials. Of 17 vaccinated ponies challenged with $EIAV_{PV}$ the following results have been reported: Seven animals developed more severe disease than unvaccinated control animals indicative of immune enhancement, seven animals experienced moderate disease, and three were infected but remained asymptomatic.[18] The different vaccine protocols used in the study generate different serologic responses such that there is no apparent correlation between antibody levels and the frequency of disease enhancement. A particulate vaccine consisting of $EIAV_{PV}$ conjugated to glutaraldehyde-activated iron-oxide beads also has been tested. Recipients of this vaccine are not protected against infection with 300 50% horse infectious doses (HID_{50}) of homologous virus, but on a positive note, they have delayed progression to disease and lower viral RNA loads as compared to nonimmunized controls.[56]

While use of an inactivated vaccine and protection against high-titer virus challenge are laudable goals, they are not likely to be achieved for lentivirus vaccines. Thus, live attenuated vaccines and use of more natural challenge doses of virus have been tested in the EIAV system. Li et al.[57] have constructed an EIAV provirus ($EIAV_{UK\Delta S2}$) lacking a functional S2 gene. While the precise function of EIAV S2 is unknown, it is known that the protein product is required for robust virus replication in vivo but is dispensable in vitro.[6] $EIAV_{UK\Delta S2}$ replicates to low levels in horses and ponies, without disease production.[57] $EIAV_{UK\Delta S2}$-infected animals develop "mature" humoral immune responses[50] by 6 months pi that are characterized by moderate to high avidity antibody capable of recognizing conformational epitopes. $EIAV_{UK\Delta S2}$ vaccinated horses are protected from challenge with multiple low doses (10 HID_{50}) of $EIAV_{PV}$. Not only is protection from disease achieved, but RT-PCR of plasma and tissue samples has revealed no evidence of the challenge virus.[57] Similar results have also been obtained after challenge with a higher virus dose (300 HID_{50}) of $EIAV_{PV}$. In contrast to the S2-deletion vaccine, another method of attenuation, removal of DU,

does not protect against challenge.[58] EIAV clones lacking DU replicate at very low levels in cultured eMDM,[3] thus it is not surprising that $EIAV_{\Delta DU}$ replicates very poorly in Shetland ponies.[58] $EIAV_{\Delta DU}$ does not protect against infection or disease following a challenge (at 15 months pi) with 300 HID_{50} of $EIAV_{PV}$,[59] suggesting that there is a threshold level of virus replication that must be achieved in order to generate protective immune responses.

To determine predictive correlates of protection, humoral immune responses of ponies to vaccines developed by Hammond et al. have been comprehensively analyzed by quantitative, qualitative, and functional assays.[59] While no single measured parameter provides a statistically significant correlate of protection, multiple parameters can be used as correlates of protection. For example, the combination of high end point titer of IgG along with recognition of conformational epitopes correlates with protection. In contrast to the ability to correlate specific humoral responses to protection, no clear correlates to disease enhancement have been determined.[59]

An attenutated EIAV vaccine was tested in Japan in the 1970s and the attenuated and parental virulent strains recently have been sequenced.[60] The attenuated strain, V26 has been compared to V70, the virulent parental strain, revealing the following differences: S2 sequences diverge by 6% and S2 of the attenuated virus lacks the S2 initiation codon. The LTRs are 10.7% divergent with a large insertion in the U3 region of V26. *Gag* sequences are 1.22% divergent, *pol* is 1.05% divergent, *env* is 1.65% divergent, and there are no differences in either *tat* or the *env* PND.[60] Finally, an attenuated EIAV vaccine has been developed in China and has been widely used since the 1980s.[1,16] The vaccine, generated by passage in donkey leukocyte cultures, protects about 85% of vaccinees from challenge with virulent EIAV.

5. MOLECULAR CHARACTERIZATION OF VIRULENCE DETERMINANTS

To examine roles of viral gene products in disease production, sequence comparisons have been made of viruses recovered from different stages of disease and between attenuated and virulent strains. Infectious molecular clones also provide a powerful tool to examine and map the virulence determinants of EIAV. Two different types of virulence determinants can be considered. The first affects the ability of the virus to replicate to high titer in the relevant host cells, eMDM, without impacting virus replication in other cell types. EIAV DU is an example of this type of virulence determinant. A second, and perhaps more interesting, group of virulence determinants are those that do not manifest themselves in vitro, but

are critical in vivo. These virulence determinants involve more subtle interactions between virus and host. In this group, S2, *env*, and the viral LTRs can be powerful determinants of virulence, though their respective roles in the disease process remain to be determined.[6,61]

S2 Protein

Strong evidence for expression of EIAV S2 derives from the presence of high titer antibodies in experimentally and naturally infected equids and the presence of the S2 orf in all EIAV field strains.[1] The EIAV S2 protein has been shown to play an important role in viral pathogenesis as its disruption on the background of virulent clones is highly attenuating and results in substantially lower levels of plasma viral RNA relative to parental viruses[6] (Fuller unpublished). S2 is not necessary for viral replication in vitro,[62] and the protein has proven to be difficult to express to high levels in cultured cells.[63] As shown in Figure 1, the open reading frame that encodes S2 is located between *pol* and *env*, immediately following the second exon of *tat* and overlaps the amino terminus of *env*.[1] S2 translation likely occurs as a consequence of leaky ribosome scanning of a singly spliced tricistronic mRNA encoding Tat, S2, and Env.[1]

The requirement for S2 in EIAV disease expression prompts speculation as to its function and a comparison of S2 aa sequences from various strains reveals some interesting features (Figure 2B). S2 has a predicted size of 7kDa. The amino-terminal half of the protein is conserved, while the carboxyl terminal half of the protein is divergent. The S2 sequences of two Chinese strains differ by approximately 50% from Wyoming-derived and Japanese strains. In contrast, the vaccine (DLA) and virulent (Liaoning) Chinese strains differ from each other at only four positions (Figure 2B). The virulent Japanese strain (V70) differs from Wyoming S2 at seven positions, while the Japanese vaccine strain (V26) lacks an initiator methionine, a feature that could certainly be the basis for attenuation.[64] In contrast, $EIAV_{17}$ and $EIAV_{19}$ (molecular clones producing virulent and avirulent virus stocks respectively) have identical S2 sequences, indicating that while S2 is necessary, it is not sufficient to confer virulence.

A scan for predicted motifs in S2 reveals highly conserved domains. A completely conserved myristiylation signal is predicted to begin at residue 7, which if utilized would remove the first six aa from the protein. If myristiylation does occur, a predicted nucleoporin motif (GLFG) would be removed that is present in several EIAV isolates. It is possible that two populations of S2 could be present in infected cells, one population that is cleaved, myristiylated, and directed to the plasma membrane, and a second that is uncleaved and traffics to the nucleus. The GLFG motif is apparently not absolutely required for virulence, as it is not present in the acutely virulent $EIAV_{17}$. Other motifs that have been identified in EIAV S2 include a

Src-homology region 3 (SH3) binding motif (PxxP), found in all S2 sequences, a casein kinase II substrate domain, and two to three protein kinase C (PKC) substrate sites. All of the S2 sequences also have basic regions at their C-termini that could serve as nuclear localization domains.

As regards genomic location and expression, S2 resembles the HIV-1 auxiliary protein Vpu,[65] however, it has little overall aa sequence homology to this or other HIV auxiliary proteins. HIV-1 Vpu is an 81 aa protein that promotes degradation of CD4, interacts with proteosome members to inhibit IκB degradation, promotes HIV particle release, and may have ion channel activity.[65] HIV-1 Vpu is an integral membrane protein that contains a high proportion of charged residues in its cytoplasmic domain and has a pair of serine residues that are constitutively phosphorylated by casein kinase II.[65] The integral membrane domain of HIV Vpu is important in particle release and channel activity, while the cytoplasmic domain appears to be most important for its other activities. Like HIV-1 Vpu, EIAV S2 has a pair of serine residues that could be substrates for casein kinase II. SIV lacks a Vpu protein, but the particle release enhancing activity of HIV Vpu is found in the TM proteins of some SIV isolates.[65]

EIAV S2 also has some features in common with the Nef proteins of the primate lentiviruses. These include its effect on virulence and the presence of both myristiylation signals and an SH3-binding PxxP motif that is similar to the one found in SIV Nef.[66] A combination of membrane localization (via myristiylation) and a PxxP motif suggests that S2 may function to interact in cell-signaling pathways as has been firmly established for HIV-1 Nef.[66] It is interesting to speculate that the activities of EIAV S2 may include some of those identified for Nef (cell activation and signaling) and Vpu (protein degradation). Our lab has recently investigated the ability of bacterially expressed S2 to interact with SH3 family members using a commercially available protein interaction screen and the results indicate that S2 does interact with some SH3 domains (Payne and Fuller unpublished).

Rev Variation and Activity Correlate with Stage of Disease

EIAV Rev (eRev) is a 165 aa protein whose first 29 aa are derived from the SU region of *env*, with the remainder encoded by the S3 orf that overlaps TM.[2] ERev is required for virus replication and influences both viral mRNA splicing and transport.[67,68] ERev-minus mutants export reduced levels of intron-containing (*gag*) RNA from the nucleus, as compared to the wild-type virus; in this regard ERev is similar to HIV Rev (HRev). The ERev nuclear export signal (NES) is located toward the N-terminus of orf S3, as indicated in Figure 2C.[69] This domain, rich in polar residues, is functionally interchangeable with the leucine rich effector domain of HRev.[2] ERev export function is inhibited by leptomycin B, a drug that

inhibits HRev export via host cell CRM1.[70] Therefore, ERev export activity is either CRM1-dependent or uses another leptomycin B-sensitive export protein.[70]

ERev-minus mutants display alterations in the relative abundance of spliced mRNAs as compared to wild type virus, with the loss of a 3 exon multiply spliced transcript and an increase in a 4 exon transcript.[2,68] ERev regulates splicing by binding to a purine-rich exon splicing enhancer (ESE) located proximal to the 5′ splice site of exon 3, inhibiting exon 3 splicing, probably via interactions with the host cell protein ASF/SF2, a member of the serine- and arginine-rich family of splicing factors.[67,71] Mutations in the central region of ERev (Figure 2C) eliminate production of the 3 exon alternatively spliced transcript and the mutant ERev fails to bind the ESE.[67] Mutations in the C-terminal basic region, the nuclear localization signal, of ERev also reduce the levels of the 3 exon transcript, however, mutations in the NES do not affect alternative splicing.[68]

Sequence variation occurs in the overlapping TM/rev region of the EIAV genome. This region encodes the carboxyl terminus of TM, predicted to be internal in the virus particle and thus not expected to play a major role in virus neutralization. Therefore, it has been speculated that *rev* variation may be important in EIAV infection, with differences in ERev activity conferring distinct selective advantages at different times during infection.[69]

Longitudinal examination of ERev shows that subpopulations coexist in infected animals.[64,72] Many of the observed ERev variants differ in a central region of the protein (designated the "variable region" in Figure 2C) that has not yet been assigned a function based on site-directed mutagenesis. However, chimeric proviral clones containing these naturally occurring *rev* genotypes differ in their rate and overall level of virus replication in ED cells, and these ERev variants display significantly different levels of export activity of mRNA in transient expression assays.[64,72] An ERev group (clade B) that predominates in the Wyoming virus inoculum also predominates during the acute disease episode in Wyoming-infected horses.[64,72] The clade B group is represented by the R1 sequence shown in Figure 2C. R1 is very similar, differing by only two residues from $EIAV_{17}$ Rev, indicating that the virulent $EIAV_{17}$ clone contains a Rev that is representative of the Wyoming field strain.[61] Clade B Revs also predominate during long periods of inapparant disease in Wyoming virus-infected horses.[64,72]

A second group of ERevs (clade A) has been identified as the majority population during periods of chronic disease in Wyoming-infected horses.[64,72] The R53 ERev sequence shown in Figure 2C represents clade A and appears closely related to the ERev sequence of the avirulent, fibroblast-adapted $EIAV_{19}$ clone. Assays of ERev nuclear export activity show significantly greater nuclear export for R53 than for R1. Virus replication

assays in ED cells indicate that virus with R53 ERev on the background of $EIAV_{19}$ replicates to similar levels as $EIAV_{19}$, while R1 on the same genetic background yields decreased amounts of virus.[64] Thus higher nuclear export activity of ERev correlates with increased virus production in fibroblasts. If the ERev phenotypes measured in fibroblasts are similar in vivo, increased virus expression may well result in the recurrent febrile episodes that constitute the chronic phase of EIA. In contrast, as the immune system of the infected animal matures, decreased ERev activity (thus decreased particle production) could promote viral persistence by reducing CTL killing of infected cells.[64] It is not entirely clear why the ERev variants that predominate during the acute febrile episode (and present on an acutely virulent clone) have lower ERev activity than those variants present during the chronic disease phase.

LTR Variation

Another variable region in the EIAV genome is the transcriptional enhancer found in U3 of the LTR. Retroviral LTRs play a critical role in virus expression, and LTR variation has long been recognized to play a key role in the biologic phenotypes of retroviruses. Enhancer motifs commonly found in EIAV LTRs include PU.1 binding motifs that interact with the myeloid/B cell specific transcription factor, PU.1 as well as MDBP, PEA-2, Oct, and CRE sites.[2] Variation in EIAV U3 includes deletions, duplications, and point mutations that change the nature of these transcription factor-binding motifs. LTR sequences are divergent between culture-adapted and field strains of EIAV and these divergent LTRs influence in vitro growth characteristics.[15,73] LTRs representing virulent, unpassaged EIAVs have very similar sequences that contain 3 PU.1 sites and a CRE site.[2] In contrast, fibroblast adapted EIAV LTRs, as well as those from healthy seropositive carrier animals, show extensive variation.[2] As illustrated in Figure 3, these LTRs generally have lost the 5′-most PU.1 site, but have gained other transcription factor-binding motifs such as PEA-2 and Oct.[2] Assays of the promoter activity of the Wyoming field strain LTR reveal that it does not support high levels of transcription in nonmyeloid cells.[15,73] In Cf2Th, FEA, and D17 cells, the Wyoming LTR is transactivated by ETat, but overall levels of reporter gene expression are significantly lower than those produced by the $EIAV_{19}$ LTR.[15] However, having a mutation of the Wyoming LTR generate a PEA-2 site is sufficient to increase gene expression in fibroblasts.[15,73] The inability of the Wyoming LTR to drive virus expression in fibroblasts has also been demonstrated using molecular clones isogenic in all but LTR sequences.[15] Thus there is a strong selective advantage, upon passage in cultured cells, for alterations in the Wyoming enhancer.

Selective advantages for variant LTRs in cultured macrophages and in vivo are less clear. Fibroblast-adapted EIAV strains contain PU.1 sites, and

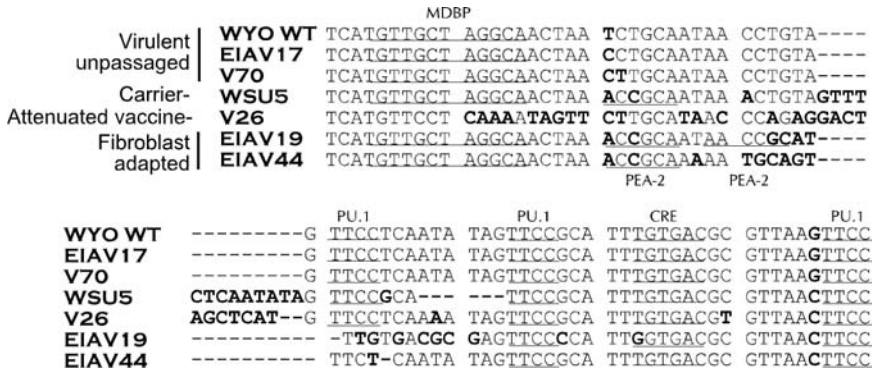

FIGURE 3. A comparison of the enhancer region of selected EIAV isolates showing variation in transcription-factor binding sites. The sites indicated have been demonstrated to interact with proteins from either nuclear or fibroblast nuclear extracts.[2] The origin of each LTR is indicated.

viruses with these LTRs can replicate to high titer in eMDM.[41] However, the ability of a specific LTR to support replication in cultured eMDM does not correlate with a role in acute disease. The influence of the EIAV LTR on the development of acute disease is best demonstrated by comparing a pair of molecular clones that differ only in their LTR sequences.[61] Replacing the LTRs of $EIAV_{17}$ (acutely virulent) with the LTRs from $EIAV_{19}$ (culture-adapted, avirulent) results in loss of virulence (compare Figure 4A to 4C), even at a high-infecting virus dose.[41] The LTR swap does not change the ability of clone-derived virus to replicate in eMDM, as $EIAV_{17E/19L}$ is cytopathic and replicates to similar levels as $EIAV_{17}$.[41] Finally, as mentioned above, LTR sequences recovered from healthy EIAV carriers are quite variable[2] (Payne and Ball unpublished), with enhancers that seem to resemble those from culture-adapted viruses (Figure 3). Clearly additional studies are needed, particularly as regards the regulation of virus expression in vivo in monocytes versus macrophages, an area of study yet to be addressed in any detail, in order to understand the role of EIAV enhancer sequences in disease production and long-term viral persistence.

Env Variation

We have begun to analyze *env* variation as it impacts the acute virulence phenotype of EIAV using a series of related molecular clones.[41,61] The viruses $EIAV_{19}$ and $EIAV_{17}$ share the same *gag*, *pol*, *tat*, and S2 genes, differing in *env*, *rev*, and LTRs (Figure 4). $EIAV_{19}$ is highly attenuated in that we have been unable to passage this virus to virulence. In contrast, $EIAV_{17}$ causes severe disease in our Shetland pony model;[74] an example of such an

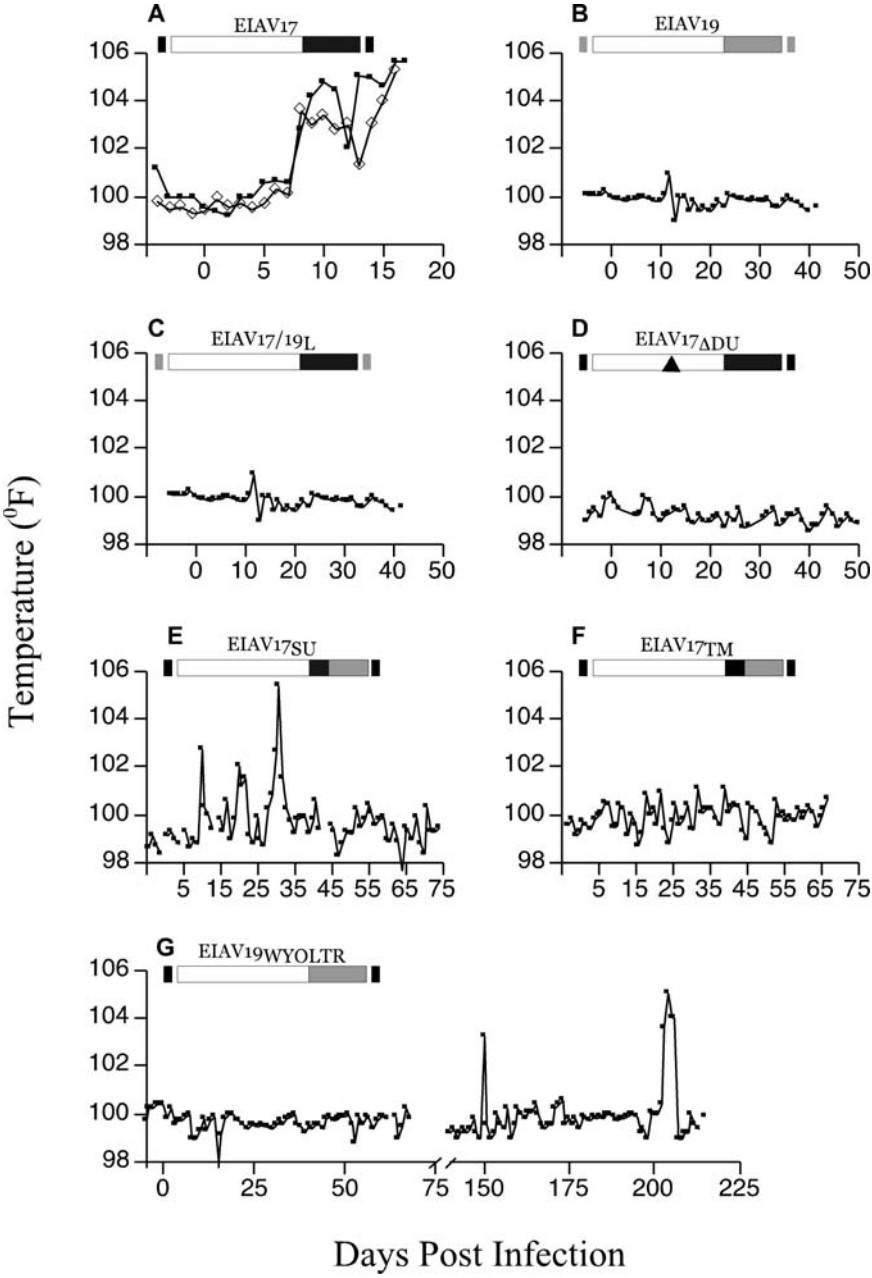

FIGURE 4. Temperature profiles of Shetland ponies infected with virus stocks derived from the indicated clones. The parental clones are: $EIAV_{19}$, that produces virus that is highly attenuated (panel B) and replicates in both eMDM and nonmyeloid cell lines,[75] and $EIAV_{17}$ that was derived by replacing the *env* and LTRs of $EIAV_{19}$ with sequences derived from the Wyoming field strain.[74] $EIAV_{17}$ produces virus that is acutely virulent in a Shetland pony model (panel A). Panels D through G show representative clinical outcomes of ponies infected with virus stocks derived from the indicated clones.[61] The open boxes in each genome schematic represent the *gag*, *pol*, and S2 regions that are identical between $EIAV_{19}$ and $EIAV_{17}$. The light gray boxes indicate the *env/rev* and LTR sequences of $EIAV_{19}$. The black boxes indicate the *env/rev* and LTR sequences of the $EIAV_{17}$ parent. The infecting virus in panel D was derived from DU-deleted version of $EIAV_{17}$ (Payne and Fuller unpublished).

infection is shown in Figure 4A. A series of chimeric clones have been constructed and used to test the contribution of *env* and LTR sequences to the acute virulence phenotype of $EIAV_{17}$. Results of pony inoculations reveal that LTR and *env/rev* sequences contribute to virulence, as replacement of either region with sequences from $EIAV_{19}$ abrogates the virulence phenotype. *Env* contributions to virulence phenotype also have been examined with the $EIAV_{17SU}$ and $EIAV_{17TM}$ (SU or TM region and LTR of $EIAV_{17}$) on the backbone of $EIAV_{19}$. Shetland pony infections show that SU contributes more strongly to the virulence phenotype of the parental clone as $EIAV_{17SU}$ causes febrile episodes in experimentally infected ponies while a similar dose of $EIAV_{17TM}$ does not (compare Figure 4E and 4F). The disease-producing dose of $EIAV_{17SU}$ is approximately 30 times greater (as determined by 50% infectious dose determinations in eMDM) than the lethal dose of $EIAV_{17}$, suggesting that both the $EIAV_{17}$ TM and SU proteins contribute to disease expression.

DU

Several nonprimate retroviruses, including EIAV, CAEV, MVV, and FIV, encode DU.[3] In host cells the enzyme plays a central role in dNTP biosynthesis: hydrolyzing dUTP (produced from dCTP) to produce dUMP that is required for synthesis of dTTP. Maintaining low dUTP concentration is important in the cell as dUTP can be readily incorporated into DNA where it becomes a target for the DNA-repair enzyme uracil-N-glycosylase (UNG). Knock-out mutants in dUTPase have a phenotype of chromosomal fragmentation that results from this repair process. The requirement for dUTPase among the retroviruses is less well understood, but studies suggest a role for the enzyme in facilitating replication in nondividing cells[3] where one might expect to find suboptimal dNTP pools.

EIAV DU is expressed as part of the Pol polyprotein and is packaged into virions where it is available to function during the early phases of the retroviral life cycle.[3] The requirement for EIAV DU in nondividing eMDM is quite stringent, but the enzyme is not required in dividing cell lines.[3] Transfection of $EIAV_{19\Delta DU}$ into permissive cell lines produces a virus that replicates at or near parental levels; however, when these viruses are passed on to eMDM replication is impaired. RT activity is significantly reduced compared to the parent virus and no cytopathic effects are evident.[3] Examination of early steps in replication of $EIAV_{19\Delta DU}$ in eMDM demonstrates that viral DNA synthesis is not impaired; in fact, PCR analysis suggests an increased rate of proviral DNA synthesis for the DU-deleted virus.[3] A likely explanation for this result is that dNTP concentrations are limited in differentiated eMDM and $EIAV_{19\Delta DU}$ is able to utilize dUTP, facilitating DNA synthesis. In support of this explanation, the presence of uracil in proviral DNA from $EIAV_{19\Delta DU}$-infected eMDM can be demonstrated by treating DNA

from infected eMDM with the enzyme UNG to cleave uracil-containing DNA prior to amplification with viral-specific PCR primers.[3] This treatment reduces the synthesis of viral-specific PCR product only in $EIAV_{19\Delta DU}$ infected eMDM, strongly suggesting that this viral DNA contains some level of uracil. Further examination of the replication cycle of $EIAV_{19\Delta DU}$ in eMDM shows an approximately 100-fold decrease in viral transcripts. Thus the uracil-containing provirus is not a competent template for transcription.[3] The molecular basis for the reduction in viral transcripts has not been determined but several possibilities exist. Uracil-containing DNA might become fragmented during efforts at DNA repair in the nucleus, could be defective in integration, or may be a poor template for host-cell transcription machinery.

As EIAV replicates primarily in macrophages in vivo, it is not surprising that DU is required for efficient replication in the horse and for development of disease. Shetland ponies infected with DU-deleted viruses ($EIAV_{\Delta DU}$) have peak virus levels that are at least 10- to 100-fold lower than in ponies infected with the parental virus.[58] As shown in Figure 4A, $EIAV_{17}$ causes severe disease when administered to Shetland ponies,[74] but $EIAV_{17\Delta DU}$ (Figure 4D) causes no clinical disease, even when administered at high doses. Animals infected with $EIAV_{17\Delta DU}$ are also slower to seroconvert, and viral sequences are not recovered (Payne and Fuller unpublished). Since EIAV is primarily transmitted via mechanical transfer, and occurs most efficiently during episodes of viremia, it seems very unlikely that a DU-deleted EIAV could be naturally maintained in an equine population.

Taken together, molecular analyses indicate that the virulence phenotype of EIAV is complex and is associated with several genes. S2 is required for robust replication and disease production in vivo but is not sufficient to confer virulence. Rev and LTR variants that are found during different phases of infection (chronic versus inapparant for example) have counterparts in virulent and avirulent molecular clones. SU or TM variants also contribute to virulence phenotype, perhaps by induction of cytokines that impact virus replication in vivo.

REFERENCES

1. Montelaro, R. C., Ball, J. M. and Rushlow, K. E., 1993, *The Retroviridae,* Plenum Press, pp. 257–360.
2. Maury, W., 1998, Regulation of equine infectious anemia virus expression, *J. Biomed. l Sci.* 5:11–23.
3. Payne, S. L. and Elder, J. H., 2001, The role of retroviral dutpases in replication and virulence, *Curr. Prot. & Pep. Sci.* 2:381–388.
4. Rice, N. R., Henderson, L. E., Sowder, R. C., Copeland, T. D., Oroszlan, S. and Edwards, J. F., 1990, Synthesis and processing of the transmembrane envelope protein of equine infectious anemia virus, *J. Virol.* 64:3770–3778.

5. Taube, R., Fujinaga, K., Irwin, D., Wimmer, J., Geyer, M. and Peterlin, B. M., 2000, Interactions between equine cyclin T1, Tat, and TAR are disrupted by a leucine-to-valine substitution found in human cyclin T1, *J. Virol.* 74:892–898.
6. Li, F., Leroux, C., Craigo, J. K., Cook, S. J., Issel, C. J. and Montelaro, R. C., 2000, The S2 gene of equine infectious anemia virus is a highly conserved determinant of viral replication and virulence properties in experimentally infected ponies, *J. Virol.* 74:573–579.
7. Harrold, S. M., Cook, S. J., Cook, R. F., Rushlow, K. E., Issel, C. J. and Montelaro, R. C., 2000, Tissue sites of persistent infection and active replication of equine infectious anemia virus during acute disease and asymptomatic infection in experimentally infected equids, *J. Virol.* 74:3112–3121.
8. Oaks, J. L., Ulibarri, C. and Crawford, T. B., 1999, Endothelial cell infection in vivo by equine infectious anaemia virus, *J. Gen. Virol.* 80:2393–2397.
9. Oaks, J. L., McGuire, T. C., Ulibarri, C. and Crawford, T. B., 1998, Equine infectious anemia virus is found in tissue macrophages during subclinical infection, *J. Virol.* 72:7263–7269.
10. Sellon, D. C., Walker, K. M., Russell, K. E., Perry, S. T., Covington, P. and Fuller, F. J., 1996, Equine infectious anemia virus replication is upregulated during differentiation of blood monocytes from acutely infected horses, *J. Virol.* 70:590–594.
11. Maury, W., 1994, Monocyte maturation controls expression of equine infectious anemia virus, *J. Virol.* 68:6270–6279.
12. Smith, T. A., Davis, E. and Carpenter, S., 1998, Endotoxin treatment of equine infectious anaemia virus-infected horse macrophage cultures decreases production of infectious virus, *J. Gen. Virol.* 79:747–755.
13. Malmquist, W. A., Barnett, D. and Becvar, C. S., 1973, Production of equine infectious anemia antigen in a persistently infected cell line, *Arch. Gesamte Virusforsch.* 42:361–370.
14. Maury, W., Oaks, J. L. and Bradley, S., 1998, Equine endothelial cells support productive infection of equine infectious anemia virus, *J. Virol.* 72:9291–9297.
15. Payne, S. L., La Celle, K., Pei, X. F., Qi, X. M., Shao, H., Steagall, W. K., Perry, S. and Fuller, F., 1999, Long terminal repeat sequences of equine infectious anaemia virus are a major determinant of cell tropism, *J. Gen. Virol.* 80:755–759.
16. Cook, R. F., Issel, C. J. and Montelaro, R. C., 1996, *Virus Infections of Equines,* Elsevier Sciences, pp.297–323.
17. Sellon, D. C., Fuller, F. J. and McGuire, T. C., 1994, The immunopathogenesis of equine infectious anemia virus, *Virus Res.* 32:111–138.
18. Raabe, M. L., Issel, C. J., Cook, S. J., Cook, R. F., Woodson, B. and Montelaro, R. C., 1998, Immunization with a recombinant envelope protein (rgp90) of EIAV produces a spectrum of vaccine efficacy ranging from lack of clinical disease to severe enhancement, *Virology* 245:151–162.
19. Zheng, Y. H., Sentsui, H., Nakaya, T., Kono, Y. and Ikuta, K., 1997, In vivo dynamics of equine infectious anemia viruses emerging during febrile episodes: Insertions/duplications at the principal neutralizing domain, *J. Virol.* 71:5031–5039.
20. Leroux, C., Craigo, J. K., Issel, C. J. and Montelaro, R. C., 2001, Equine infectious anemia virus genomic evolution in progressor and nonprogressor ponies, *J. Virol.* 75:4570–4583.
21. Crawford, T. B., Wardrop, K. J., Tornquist, S. J., Reilich, E., Meyers, K. M. and McGuire, T. C., 1996, A primary production deficit in the thrombocytopenia of equine infectious anemia, *J. Virol.* 70:7842–7850.
22. Russell, K. E., Perkins, P. C., Hoffman, M. R., Miller, R. T., Walker, K. M., Fuller, F. J. and Sellon, D. C., 1999, Platelets from thrombocytopenic ponies acutely infected with equine infectious anemia virus are activated in vivo and hypofunctional, *Virology* 259:7–19.
23. Tornquist, S. J. and Crawford, T. B., 1997, Suppression of megakaryocyte colony growth by plasma from foals infected with equine infectious anemia virus, *Blood.* 90:2357–2363.

24. Maciejewski, J. P., Weichold, F. F., and Young, N. S., 1994, HIV-1 suppression of hematopoiesis in vitro mediated by envelope glycoprotein and tnf-alpha, *J. Immunol.* 153:4303–4310.
25. Michelmann, I., Bèockmann, D., Nèurnberger, W., Eckhof-Donovan, S., Burdach, S. and Gèobel, U., 1997, Thrombocytopenia and complement activation under recombinant TNF alpha/IFN gamma therapy in man, *Ann. Hematol.* 74:179–184.
26. Tacchini-Cottier, F., Vesin, C., Redard, M., Buurman, W. and Piguet, P. F., 1998, Role of TNFR1 and TNFR2 in TNF-induced platelet consumption in mice, *J. Immunol.* 160:6182–6186.
27. Bar, J., Zosmer, A., Hod, M., Elder, M. G. and Sullivan, M. H., 1997, The regulation of platelet aggregation in vitro by interleukin-1beta and tumor necrosis factor-alpha: Changes in pregnancy and in pre-eclampsia, *Thromb. Haemost.* 78:1255–1261.
28. Oleksowicz, L., Mrowiec, Z., Zuckerman, D., Isaacs, R., Dutcher, J. and Puszkin, E., 1994, Platelet activation induced by interleukin-6: Evidence for a mechanism involving arachidonic acid metabolism, *Thromb. Haemost.* 72:302–308.
29. Costa, L. R., Santos, I. K., Issel, C. J. and Montelaro, R. C., 1997, Tumor necrosis factor-alpha production and disease severity after immunization with enriched major core protein (p26) and/or infection with equine infectious anemia virus, *Vet. Immunol. Immunopathol.* 57:33–47.
30. Tornquist, S. J., Oaks, J. L. and Crawford, T. B., 1997, Elevation of cytokines associated with the thrombocytopenia of equine infectious anaemia, *J. Gen. Virol.* 78:2541–2548.
31. Swardson, C. J., Lichtenstein, D. L., Wang, S., Montelaro, R. C. and Kociba, G. J., 1997, Infection of bone marrow macrophages by equine infectious anemia virus, *Am. J. Vet. Res.* 58:1402–1407.
32. Murtaugh, M. P., Baarsch, M. J., Zhou, Y., Scamurra, R. W. and Lin, G., 1996, Inflammatory cytokines in animal health and disease, *Vet. Immunol. Immunopathol.* 54:45–55.
33. Dinarello, C. A., 1999, Cytokines as endogenous pyrogens, *J. Infect. Dis.* 179 Suppl 2:S294–304.
34. Chang, H. R., Dulloo, A. G. and Bistrian, B. R., 1998, Role of cytokines in aids wasting, *Nutrition* 14:853–863.
35. Tracey, K. J. and Cerami, A., 1994, Tumor necrosis factor: A pleiotropic cytokine and therapeutic target, *Annu. Rev. Med.* 45.
36. Means, R. T., Jr., 2000, The anaemia of infection, *Baillieres Best Pract. Res. Clin. Haematol.* 13:151–162.
37. Dinarello, C. A., 1996, Biologic basis for interleukin-1 in disease, *Blood.* 87:2095–2147.
38. Heike, T. and Nakahata, T., 2002, Ex vivo expansion of hematopoietic stem cells by cytokines, *Biochim. Biophys. Acta.* 1592:313–321.
39. Russell, K. E., Walker, K. M., Miller, R. T. and Sellon, D. C., 1998, Hyperglobulinemia and lymphocyte subset changes in naturally infected, inapparent carriers of equine infectious anemia virus, *Am. J. Vet. Res.* 59:1009–1015.
40. Lim, W. -S., Edwards, J. F., Boyd, N. K., Payne, S. L. and Ball, J. M., 2003, Simultaneous quantitation of equine cytokine mrnas using a multi-probe ribonuclease protection assay, *Vet. Immunol. Immunopathol.* 91:45–51.
41. Lim, W. -S., Payne, S. L., Edwards, J. F., Kim, I. and Ball, J. M., 2004, Differential effects of virulent and avirulent equine infectious anemia virus molecular clones on primary equine macrophage cytokine expression, *Virology* 332:295–306.
42. Sampson, M. J., Davies, I. R., Brown, J. C., Ivory, K. and Hughes, D. A., 2002, Monocyte and neutrophil adhesion molecule expression during acute hyperglycemia and after antioxidant treatment in type 2 diabetes and control patients, *Arterioscler Thromb Vasc Biol* 22:1187–1193.

43. Gerencer, M., Valpotic, I., Jukic, B., Tomaskovic, M. and Basic, I., 1989, Qualitative analyses of cellular immune functions in equine infectious anemia show homology with aids, *Arch. Virol.* 104:249–257.
44. Newman, M. J., Issel, C. J., Truax, R. E., Powell, M. D., Horohov, D. W. and Montelaro, R. C., 1991, Transient suppression of equine immune responses by equine infectious anemia virus (EIAV), *Virology* 184:55–66.
45. Murakami, K., Sentsui, H., Shibahara, T. and Yokoyama, T., 1999, Reduction of CD4+ and CD8+ T lymphocytes during febrile periods in horses experimentally infected with equine infectious anemia virus, *Vet. Immunol. Immunopathol.* 67:131–140.
46. Hammond, S. A., Li, F., McKeon, B. M., Sr., Cook, S. J., Issel, C. J. and Montelaro, R. C., 2000, Immune responses and viral replication in long-term inapparent carrier ponies inoculated with equine infectious anemia virus, *J. Virol.* 74:5968–5981.
47. Howe, L., Leroux, C., Issel, C. and Montelaro, R. C., 2002, Equine infectious anemia virus envelope evolution in vivo during persistent infection progressively increases resistance to in vitro serum antibody neutralization as a dominant phenotype, *J. Virol.* 76:10588–10597.
48. Kono, Y., Kobayashi, K. and Fukunaga, Y., 1973, Antigenic drift of equine infectious anemia virus in chronically infected horses, *Arch. Gesamte Virusforsch.* 41:1–10.
49. Leroux, C., Issel, C. J. and Montelaro, R. C., 1997, Novel and dynamic evolution of equine infectious anemia virus genomic quasispecies associated with sequential disease cycles in an experimentally infected pony, *J. Virol.* 71:9627–9639.
50. Hammond, S. A., Cook, S. J., Lichtenstein, D. L., Issel, C. J. and Montelaro, R. C., 1997, Maturation of the cellular and humoral immune responses to persistent infection in horses by equine infectious anemia virus is a complex and lengthy process, *J. Virol.* 71:3840–3852.
51. McGuire, T. C., Zhang, W., Hines, M. T., Henney, P. J. and Byrne, K. M., 1997, Frequency of memory cytotoxic T lymphocytes to equine infectious anemia virus proteins in blood from carrier horses, *Virology* 238:85–93.
52. Zhang, W., Lonning, S. M. and McGuire, T. C., 1998, Gag protein epitopes recognized by ELA-A-restricted cytotoxic t lymphocytes from horses with long-term equine infectious anemia virus infection, *J. Virol.* 72:9612–9620.
53. McGuire, T. C., Tumas, D. B., Byrne, K. M., Hines, M. T., Leib, S. R., Brassfield, A. L., O"Rourke, K. I. and Perryman, L. E., 1994, Major histocompatibility complex-restricted CD8+ cytotoxic T lymphocytes from horses with equine infectious anemia virus recognize env and gag/pr proteins, *J. Virol.* 68:1459–1467.
54. McGuire, T. C., Leib, S. R., Lonning, S. M., Zhang, W., Byrne, K. M. and Mealey, R. H., 2000, Equine infectious anaemia virus proteins with epitopes most frequently recognized by cytotoxic T lymphocytes from infected horses, *J. Gen. Virol.* 81:2735–2739.
55. Mealey, R. H., Fraser, D. G., Oaks, J. L., Cantor, G. H. and McGuire, T. C., 2001, Immune reconstitution prevents continuous equine infectious anemia virus replication in an arabian foal with severe combined immunodeficiency: Lessons for control of lentiviruses, *Clin. Immunol.* 101:237–247.
56. Hammond, S. A., Cook, S. J., Falo, L. D., Jr., Issel, C. J. and Montelaro, R. C., 1999, A particulate viral protein vaccine reduces viral load and delays progression to disease in immunized ponies challenged with equine infectious anemia virus, *Virology* 254:37–49.
57. Li, F., Craigo, J. K., Howe, L., Steckbeck, J. D., Cook, S., Issel, C. and Montelaro, R. C., 2003, A live attenuated equine infectious anemia virus proviral vaccine with a modified S2 gene provides protection from detectable infection by intravenous virulent virus challenge of experimentally inoculated horses, *J. Virol.* 77:7244–7253.
58. Lichtenstein, D. L., Rushlow, K. E., Cook, R. F., Raabe, M. L., Swardson, C. J., Kociba, G. J., Issel, C. J. and Montelaro, R. C., 1995, Replication in vitro and in vivo of an equine infectious anemia virus mutant deficient in dutpase activity, *J. Virol.* 69:2881–2888.

59. Hammond, S. A., Raabe, M. L., Issel, C. J. and Montelaro, R. C., 1999, Evaluation of antibody parameters as potential correlates of protection or enhancement by experimental vaccines to equine infectious anemia virus, *Virology* 262:416–430.
60. Zheng, Y. H., Sentsui, H., Kono, Y. and Ikuta, K., 2000, Mutations occurring during serial passage of Japanese equine infectious anemia virus in primary horse macrophages, *Virus Res.* 68:93–98.
61. Payne, S. L., Pei, X. F., Jia, B., Fagerness, A. and Fuller, F. J., 2004, Influence of long terminal repeat and env on the virulence phenotype of equine infectious anemia virus, *J. Virol.* 78:2478–2485.
62. Li, F., Puffer, B. A. and Montelaro, R. C., 1998, The S2 gene of equine infectious anemia virus is dispensable for viral replication in vitro, *J. Virol.* 72:8344–8348.
63. Yoon, S., Kingsman, S. M., Kingsman, A. J., Wilson, S. A. and Mitrophanous, K. A., 2000, Characterization of the equine infectious anaemia virus S2 protein, *J. Gen. Virol.* 81:2189–2194.
64. Baccam, P., Thompson, R. J., Li, Y., Sparks, W. O., Belshan, M., Dorman, K. S., Wannemuehler, Y., Oaks, J. L., Cornette, J. L. and Carpenter, S., 2003, Subpopulations of equine infectious anemia virus rev coexist in vivo and differ in phenotype, *J. Virol.* 77:12122–12131.
65. Bour, S. and Strebel, K., 2003, The HIV-1 Vpu protein: A multifunctional enhancer of viral particle release, *Microbes. Infect.* 5:1029–1039.
66. Fackler, O. T. and Baur, A. S., 2002, Live and let die: Nef functions beyond HIV replication, *Immunity* 16:493–497.
67. Chung, H. and Derse, D., 2001, Binding sites for rev and ASF/SF2 map to a 55-nucleotide purine-rich exonic element in equine infectious anemia virus RNA, *J. Biol. Chem.* 276:18960–18967.
68. Harris, M. E., Gontarek, R. R., Derse, D. and Hope, T. J., 1998, Differential requirements for alternative splicing and nuclear export functions of equine infectious anemia virus rev protein, *Mol. Cell. Biol.* 18:3889–3899.
69. Belshan, M., Harris, M. E., Shoemaker, A. E., Hope, T. J. and Carpenter, S., 1998, Biological characterization of rev variation in equine infectious anemia virus, *J. Virol.* 72:4421–4426.
70. Otero, G. C., Harris, M. E., Donello, J. E. and Hope, T. J., 1998, Leptomycin B inhibits equine infectious anemia virus rev and feline immunodeficiency virus rev function but not the function of the hepatitis B virus posttranscriptional regulatory element, *J. Virol.* 72:7593–7597.
71. Belshan, M., Park, G. S., Bilodeau, P., Stoltzfus, C. M. and Carpenter, S., 2000, Binding of equine infectious anemia virus rev to an exon splicing enhancer mediates alternative splicing and nuclear export of viral mRNAs, *Mol. Cell. Biol.* 20:3550–3557.
72. Belshan, M., Baccam, P., Oaks, J. L., Sponseller, B. A., Murphy, S. C., Cornette, J. and Carpenter, S., 2001, Genetic and biological variation in equine infectious anemia virus rev correlates with variable stages of clinical disease in an experimentally infected pony, *Virology* 279:185–200.
73. Maury, W., Bradley, S., Wright, B. and Hines, R., 2000, Cell specificity of the transcription-factor repertoire used by a lentivirus: Motifs important for expression of equine infectious anemia virus in nonmonocytic cells, *Virology* 267:267–278.
74. Payne, S. L., Qi, X. M., Shao, H., Dwyer, A. and Fuller, F. J., 1998, Disease induction by virus derived from molecular clones of equine infectious anemia virus, *J. Virol.* 72:483–487.
75. Payne, S. L., Rausch, J., Rushlow, K., Montelaro, R. C., Issel, C., Flaherty, M., Perry, S., Sellon, D. and Fuller, F., 1994, Characterization of infectious molecular clones of equine infectious anaemia virus, *J. Gen. Virol.* 75:425–429.

13

Studies of the Structure of Caprine Arthritis-Encephalitis Virus Surface Envelope Glycoprotein

ISIDRO HÖTZEL and WILLIAM P. CHEEVERS

1. INTRODUCTION

Caprine arthritis-encephalitis virus (CAEV) is a monocyte/macrophage tropic lentivirus which causes an immune-mediated disease complex in domestic goats characterized mainly by progressive arthritis of carpal joints.[1,2] Joint lesions caused by CAEV consist of synovial membrane hyperplasia and villous hypertrophy with extensive angiogenesis and perivascular infiltration of mononuclear cells accompanied by significant cortical hyperplasia of regional lymph nodes.[3] The mononuclear cell phenotypes, comprising perivascular infiltrates, include MHC class II activated macrophages, $CD4^+$ and $CD8^+$ $\alpha\beta$ T lymphocytes, and, predominantly, B lymphocytes and plasma cells.[3,4] CAEV-induced arthritis follows a chronic, progressive course with clinical signs of periarticular swelling, accumulation of synovial fluid containing inflammatory cells and radiographic changes of soft tissue mineralization, and erosion of articular surfaces.[5–7] Lesions contain CAEV infected macrophages[8] and virus are readily recovered from synovial fluid, synovial fluid cells, and explants of synovial tissue.[5,6] Previous studies have established that immune responses to CAEV envelope glycoproteins contribute to the development of arthritis.

CAEV-induced arthritis is specifically associated with type 2 immune responses to viral antigens, particularly surface (SU) and transmembrane (TM) glycoproteins encoded by the envelope (*env*) gene. Long-term non-

ISIDRO HÖTZEL • Department of Veterinary Microbiology and Pathology, Washington State University, Pullman, WA 99164-7040 WILLIAM P. CHEEVERS • Department of Veterinary Microbiology and Pathology, Washington State University, Pullman, WA 99164-7040

progressor (LTNP) goats that remain clinically asymptomatic have a predominant population of SU-responsive Th1 lymphocytes[9] and IgG2 biased antibody response to SU (J. D. Trujillo and W. P. Cheevers, unpublished results), whereas goats with arthritis have SU-responsive Th2 lymphocytes[9] with high serum and synovial fluid titers of polyclonal and CAEV-reactive IgG1.[10–14] Increased IgG1 response to CAEV SU is initiated prior to development of clinical arthritis,[14] and selective induction of IgG2 responses by vaccination with plasmid DNA expressing the CAEV *env* gene suppresses development of arthritis following challenge infection.[15]

In summary, previous studies have established that immune responses to CAEV envelope glycoproteins contribute to the development of arthritis. This review emphasizes recent structural studies of the SU of CAEV, with particular reference to structural similarities between small ruminant lentiviruses and HIV-1.

2. PRIMARY STRUCTURE OF CAEV SU

The envelope glycoprotein of CAEV is expressed as a precursor glycoprotein which is processed by cellular enzymes into a surface envelope glycoprotein with an apparent molecular mass of 135 kDa (gp135) and a transmembrane glycoprotein with an apparent molecular mass of 38 kDa (gp38).[16,17] After processing, gp135 and gp38 remain associated through noncovalent interactions to form a heterodimer anchored to the cell and viral envelope membranes by the hydrophobic membrane spanning region of gp38. TM moieties of different heterodimers are presumed to interact to form the oligomeric envelope glycoprotein complexes inducing cell-to-cell and virus-to-cell fusion.

Sequence Variability of CAEV SU

The mature SU of CAEV is 546 to 550 amino acids long, corresponding to a polypeptide of a predicted molecular mass of 63 kDa.[18–20] Comparison of SU sequences from different CAEV strains has defined four conserved (C1 to C4) and five variable regions (V1 to V5) (Figure 1).[5] Analysis of neutralization variants and blood and tissue proviruses from goats persistently infected with the CAEV-63 strain has identified two short hypervariable SU regions within the quasispecies (Figure 1).[21] Hypervariable region 1 (HV1) is located within the C3 region and occurs in a sequence strictly conserved in all small ruminant lentiviruses except CAEV-63 and 1244. Sequence variation in the HV1 region appears to occur only in some persistently infected goats with progressive arthritis. The four-amino acid HV2 region is located at the carboxy end of the V4 region and coincides with part of the visna virus principal neutralization domain.[21,22]

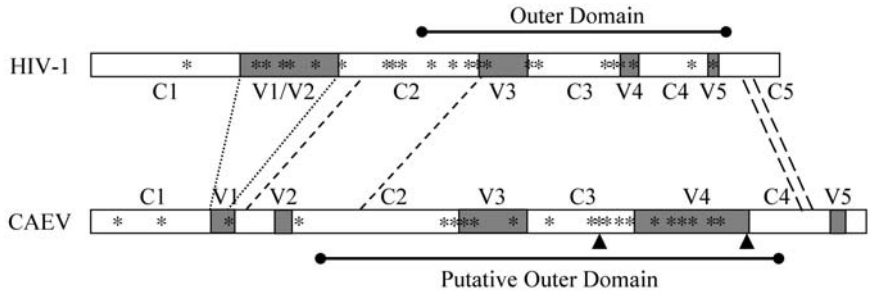

FIGURE 1. Schematic representation of CAEV and HIV-1 SU. Gray boxes represent variable (V1 to V5) regions and white boxes represent the conserved (C1 to C5) regions of HIV-1 and CAEV SU. Asterisks indicate N-linked glycosylation sites in HIV-1 HXBc2 SU and consensus potential N-linked glycosylation sites of CAEV SU. Dashed lines indicate the corresponding regions of CAEV and HIV-1 SU according to the alignment shown in Figure 2. Dotted lines indicate the putative structural analogue of the HIV-1 V1/V2 loop structure in CAEV SU as shown in Figure 4. Arrowheads indicate the positions of hypervariable regions of CAEV-63 SU (HV1 and HV2, left and right, respectively). Sequences comprising the outer domain of HIV-1 SU and the putative outer domain of CAEV SU are indicated. Variable and conserved regions and consensus N-linked glycosylation sites of CAEV SU are according to reference 20.

Sequence changes in the HV2 region occur in most proviruses or viruses isolated from infected goats regardless of disease progression and may be extensive within the quasispecies of an infected goat. Curiously however, HV2 sequences of independent CAEV strains are very similar, conforming to the consensus sequence Arg-Asn-Gly-Lys.[20] Whether this HV2 consensus sequence is an artifact of tissue culture or reflects a common sequence in circulating strains is not known.

Glycosylation of CAEV SU

CAEV SU has about 21 N-linked glycosylation sites, 17 of which are conserved in all strains.[20] Given that the apparent molecular mass of SU is 135 kDa, most if not all of these potential N-linked glycosylation sites are probably glycosylated. O-linked oligosacharides have been reported as a major component of CAEV SU glycans.[23] However, complete digestion of CAEV SU with PNGaseF results in a polypeptide of about 60 kDa,[24] indicating that O-linked oligosacharides constitute only a minor fraction of CAEV SU glycans. Potential N-linked glycosylation sites are asymmetrically distributed throughout SU with most of the conserved sites clustered in its carboxy terminal half, particularly between amino acids 355 and 454 in the C3 and V4 regions (Figure 1).[19,20] Desialylation of CAEV SU increases the susceptibility of CAEV to proteases, neutralization by immune sera, and binding of monoclonal and polyclonal antibodies to SU.[23,24] Sialic acids on

CAEV SU may thus constitute an important mechanism of immune evasion. Visna virus SU has a similar number and distribution of potential N-linked glycosylation sites as CAEV SU and is also sialylated. However, this virus is more susceptible to neutralization and proteolysis than CAEV,[23] indicating that other structural features of SU also contribute to these biological properties.

3. COMPARATIVE STRUCTURAL MODEL OF CAEV SU

Little amino acid sequence similarity has been recognized between the SU of divergent lentiviruses. This apparent lack of sequence similarity is presumed to be due to the rapid rate of sequence variation of lentiviral SU to evade the evolving immune responses mounted by their hosts coupled to the adaptation of different lentiviruses to different host environments and receptors. In contrast, a clear sequence similarity is observed between the TM of different lentiviruses as well as between the TM of lentiviruses and betaretroviruses.[25–28] The different conservation of SU and TM is not surprising since TM is less accessible to humoral immune responses than SU and interacts with less variable elements (the unexposed surface of SU, viral matrix proteins, envelope and target cell lipid bilayers and itself). In fact, regions of HIV-1 SU not exposed on the oligomer surface are less variable than exposed regions, indicating that the host is the major selective factor in SU sequence variation.[29]

Three studies have proposed possible conserved structural elements of retroviral SU. The first study proposed a conserved "knob and socket" structure for the interaction between TM and SU in all retroviruses, the knob being formed by the cysteine loop of TM and the socket by the carboxy-terminal region of SU.[30] However, the predicted conserved socket structure does not reflect any sequence similarity in SU. A second study proposed a common tertiary structure based on similar distributions of conserved and variable domains in the SU of lentiviruses, but did not identify any sequence conservation.[31] Another study identified three putative domains in the SU of retroviruses defined by predicted secondary structure motifs.[32] However, some of these predicted secondary structures did not agree with the subsequent crystal structure of HIV-1 gp120,[33] and the general validity of this proposed structural model remains uncertain.

A Partial CAEV SU Structural Model Based on Sequence Similarity With HIV-1 gp120

We used the HIV-1 gp120 crystal structure as a framework for interpretation of sequence similarities between primate and small ruminant lentiviruses.[33,34] Using this approach, we found a partial but statistically and

structurally significant sequence similarity between the second conserved (C2) region of gp120 of primate lentiviruses and the gp135 of CAEV and the closely related maedi-visna virus of sheep (Figure 2).[34] Interestingly, amino acid similarity is clustered in short motifs corresponding to β-strands in the HIV-1 gp120 while loop regions are usually not conserved. The clusters of gp120:gp135 sequence similarity generally mirrors the clusters of sequence similarity within the small ruminant and primate lentiviruses, respectively, suggesting a common structural constraint on sequence variation in gp135 and gp120. Five cysteines are conserved between gp120 and gp135. Four of these cysteines form two disulfide bonds within the proximal region of the gp120 inner domain while the fifth represents the first cysteine of the gp120 V3 loop (Figure 2). Although sequence variation encompasses the whole gp120 C2 region (Figures 1 and 2), the most conserved regions are those corresponding to gp120 β-strands 4 to 8 in the virion-proximal region of the inner domain (Figure 3). Another important structural element in this region is β-strand 25 of gp120 formed by a sequence in the carboxy terminal C5 region about 20 amino acids before the cleavage site between SU and TM (Figure 3). Consistent with the con-

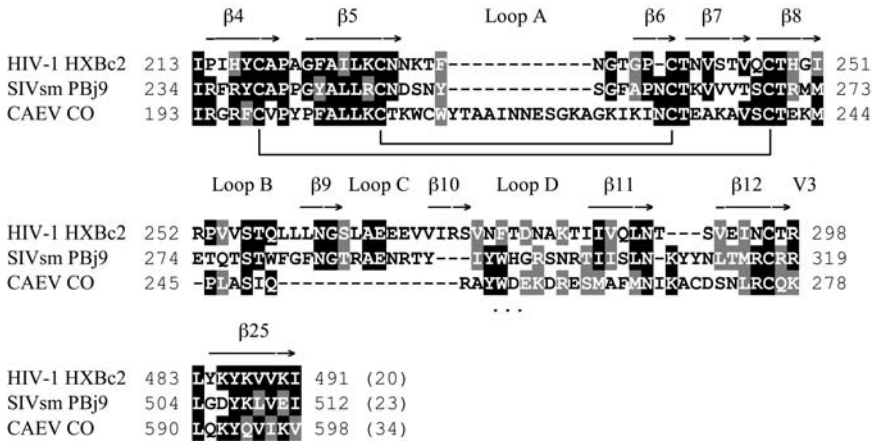

FIGURE 2. Alignment of the HIV-1 gp120 C2 (amino acids 213 to 298) and C5 (amino acids 483 to 491) regions with the SIV gp120 and CAEV gp135. Black and gray backgrounds indicate amino acid identity or similarity, respectively. Dashes represent gaps introduced for optimal alignment. Lines connecting cysteine residues indicate disulfide bonds of HIV-1 gp120. Arrows indicate positions of β-strands 4 to 12 and 25 of HIV-1 gp120. Positions of loops A through D and V3 of HIV-1 are indicated above the alignment. Dots indicate a nonaligned region of 185, 185, 312 amino acids in HIV-1, SIVsm, and CAEV SU, respectively. Numbers indicate amino acid positions from the *env* gene initiation codon and numbers in parentheses indicate amino acids from the last residue of the alignment to the SU/TM cleavage site. Figure adapted from references 34 and 35.

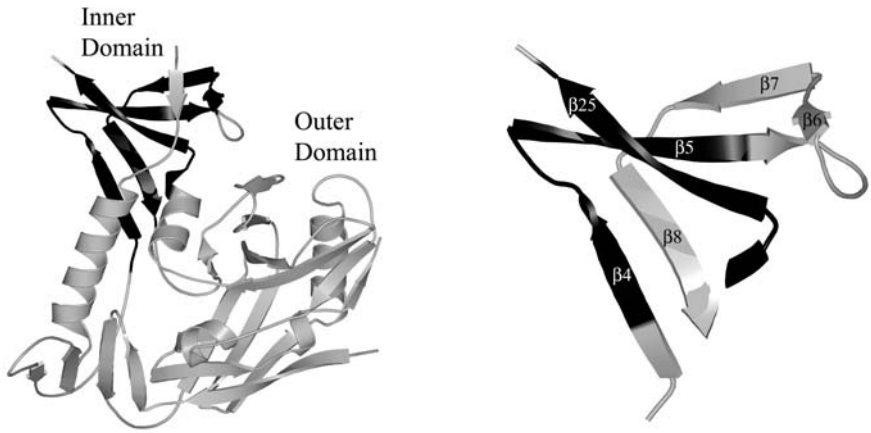

FIGURE 3. Location of regions of sequence similarity between HIV-1 gp120 and CAEV gp135. Left panel: Ribbon diagram of the CD4-bound gp120 core. The target cell and envelope membranes are located toward the bottom and the top of the figure, respectively. The gp120 inner and outer domains are indicated. The region most conserved between HIV-1 gp120 and CAEV gp135 (β-strands 4 to 8 and 25 in the inner-proximal region) is indicated in black. Right panel: Detail of the inner-proximal region of HIV-1 gp120 indicating the position of β-strands 4 to 8 and 25 and loop A. The β4/β5 and β25 regions highlighted in black are conserved in most lentiviruses as shown in Figure 4. Figure adapted from reference 41.

servation of β-strands 4 to 8 of gp120 in gp135, a sequence similar to that of gp120 β-strand 25 is also present in gp135 in a similar position relative to the cleavage site between SU and TM (Figure 2).[35] Therefore, the inner-proximal domain of HIV-1 gp120 shares sequence and structural similarities with the gp135 of small ruminant lentiviruses.

General Conservation of the β4/β5 and β25 Region in the SU of Lentiviruses and Betaretroviruses

Two elements of the sequences conserved between gp135 and gp120, a 12-amino acid motif overlapping HIV-1 β-strands 4 and 5 and the 9-amino acid conserved sequence overlapping β25, are also conserved in the SU of other lentiviruses except the feline immunodeficiency virus (Figures 3 and 4).[35] The β4/β5 motif is also present in the SU of all endogenous and exogenous betaretroviruses.[35] This is not surprising given the closer sequence similarity between the TM of lentiviruses and betaretroviruses relative to other retroviral groups combined with the fact that β-strands 4 and 5 are located in a region of gp120 proximal to TM and the envelope oligomer axis.[36] Therefore, these two sequence motifs appear to form a highly conserved structure in the SU of lentiviruses (Figure 3), and perhaps in the SU of betaretroviruses as well, participating in SU-TM interactions.

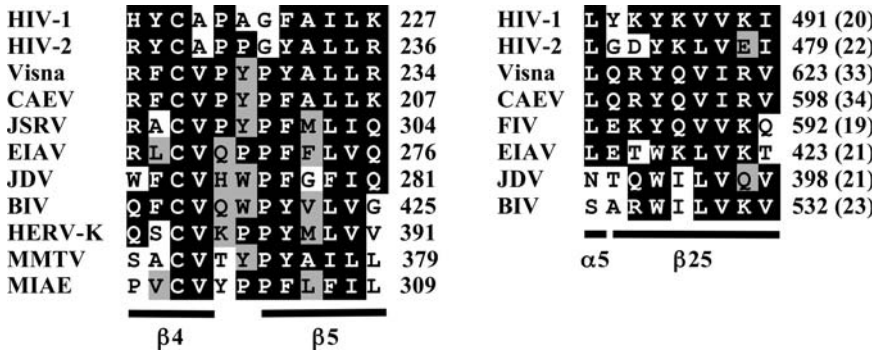

FIGURE 4. Alignment of the β-strand 4, 5, and 25 regions of HIV-1 gp120 with the SU of lentiviruses and betaretroviruses. Numbers at the right of the alignment indicate amino acid position in Env. Black backgrounds represent identical amino acids or conservative variations between the lentiviruses and betaretroviruses. Gray backgrounds indicate identical amino acids or conservative substitutions between the lentiviruses and betaretroviruses which are not conserved with the residues with black backgrounds. Numbers in parentheses indicate the distance between the last amino acid of the alignment and the carboxy terminus of SU. Lines indicate regions in β-strands 4, 5, and 25 and α-helix 5. JSRV, jaagsiekte sheep retrovirus; JDV, jembrana disease virus; BIV, bovine immunodeficiency virus; FIV, feline immunodeficiency virus; HERV-K, human endogenous retrovirus K; MMTV, mouse mammary tumor virus; MIAE, mouse intracisternal A-type element. Figure adapted from reference 35.

Potential Loop Regions of CAEV SU

The V1/V2 and V3 loop of HIV-1 gp120 are important structures involved in immune evasion by masking the receptor binding regions from the immune system. The gp120 V3 loop is also important for coreceptor binding and tropism determination. The regions of sequence conservation between CAEV gp135 and HIV-1 gp120 include most of the gp120 C2 region which is located between the V2 and V3 loop regions. Therefore, the regions flanking the gp135 sequences conserved with the gp120 C2 region may define structural analogues of the gp120 V1/V2 and V3 loops in gp135. Using this rationale, a potential V3 loop analogue has been proposed for gp135.[34] This sequence is flanked by two cysteine residues that may form a short disulfide loop with the consensus sequence "CQKRPGGC." This sequence is included in the gp135 C2 region and is relatively conserved between CAEV strains. Interestingly, this sequence includes an immunodominant linear epitope recognized by sera from naturally infected goats.[20]

Although there are no cysteines in the region immediately upstream from the gp135 sequence conserved with gp120 C2, the highly variable V1 region of gp135 is located in a similar location relative to the conserved β4 cysteine as the gp120 V1/V2 loop structure (Figures 1 and 4). The V1

CAEV gp135	(V1)N X_{18} C	198
EIAV gp90	C X_6 C X_{39} C X_5 C X_4 C X_{32} C X_{29} C N X_8 C X_{13} C	267
HIV-1 gp120	$CX_{19}CX_{44}CX_6CX_4CX_{25}CX_{38}CNX_7CX_{12}C$	218
	β1 α1 β2 V1 V2 β3 β4	

FIGURE 5. Alignment of the amino-terminal region of CAEV, EIAV, and HIV-1 SU. Amino acid spacing is shown by "X" followed by a number. Cysteines residues are shown as "C." The underlined cysteine residues are located in the β-strand 4 motif conserved in most lentiviruses and betaretroviruses. The β-strand, α-helix V1 and V2 regions of HIV-1 gp120 are shown below the alignment. The position of the V1 region of CAEV gp135 is shown. "N" indicates the putative conserved N-linked glycosylation site of lentiviral SU. The position of the last amino acid residue in the alignment is shown on the right. Figure modified from reference 37.

region is about 18-amino-acids long but deletions within this region are observed in some CAEV strains. In addition, 7 to 10 of the 18 amino acids of V1 have charged side chains in different strains despite the sequence variability in this region. The hydrophilic properties and apparent lack of secondary structure of V1 sequences indicate that at least part of this region forms a surface-exposed loop of gp135. Therefore, gp135 V1 may be structurally analogous to the HIV-1 gp120 V1/V2 loop.

A structural analogue of the V1/V2 loop structure has also been proposed for the equine infectious anemia lentivirus (EIAV) gp90.[37] A conserved potential N-linked glycosylation site in EIAV gp90 is located in the same position relative to the putative V2 loop analogue and the conserved β-strand 4 cysteine as the asparagine 197 glycan of gp120 modulating neutralization sensitivity in HIV-1 (Figure 4).[37] Interestingly, CAEV gp135 also has a strictly conserved potential N-linked glycosylation site within V1[20] in a similar location relative to the conserved β-strand 4 cysteine as EIAV gp90 and HIV-1 gp120 (Figure 4). This conserved glycan structure may represent a common structure involved in neutralization resistance in different lentiviruses.

Domain Model of CAEV SU

The position of the colinear β4/β5 and β25 motifs can be used to define putative domains of SU conserved in different lentiviruses. The sequence comprising the outer domain of HIV-1 gp120 is located entirely between the sequences of β-strands 8 and 25 (Figure 1).[33] This would suggest a similar outer domain between the conserved β4/β5 and β25 motifs of the SU of other lentiviruses.[35] The distance between the β-strands 8 and 25 in CAEV and HIV-1 SU (Figure 3) would suggest a putative outer domain for CAEV SU about 100 amino acids longer than the HIV-1 gp120 outer domain (Figure 1).[35]

Similar to HIV-1 gp120, the putative outer domain, but not the inner domain, of CAEV SU is highly glycosylated (Figure 1).[19,20] Glycosylation of the outer domain of gp120 is an important mechanism of immune evasion as epitopes in this carbohydrate-rich surface that are exposed on the oligomer surface may appear as "self" to the immune system, resulting in the poor induction of antibody responses against this outer domain surface.[38] The heavy glycosylation of the putative CAEV SU outer domain may have a similar role in immune evasion.

A possible two-domain structure of CAEV SU may also have implications in receptor binding. Binding of HIV-1 gp120 to CD4 is mediated by residues in the interface between the inner and outer domains,[33] and a similar receptor binding structure may therefore occur in CAEV and other small ruminant lentiviruses. In this regard, it is noteworthy that part of the loop B sequence of HIV-1 appears to be conserved in CAEV and other small ruminant lentiviruses (Figure 2). Included within this conserved loop B sequence is the Thr-257 residue, which is one of the gp120 residues lining the hydrophobic "Phe-43 cavity" critical for CD4 binding.[33] The loop B analogue of CAEV gp135 may therefore form an equivalent structure indirectly involved in receptor binding and receptor-induced conformational changes of gp135, similar to the presumed role of gp120 loop B during CD4 binding.[33] In addition, structural heterogeneity due to interdomain flexibility of gp120 may have an important role in immune evasion by HIV-1, through inhibition of the induction or binding of antibodies to the receptor binding site.[39] Assuming a two-domain structure for gp135, a similar mechanism may be involved in the low induction or high resistance of CAEV and certain MVV strains to neutralizing antibodies.[40]

Experimental Evidence of the Structural Model

Interference assays have shown that CAEV and the Icelandic MVV strain K1514 use different receptors to infect and fuse susceptible goat cells.[41] To test whether the putative V3 loop analogue of gp135 has any effect on receptor usage, the CAEV SU sequence was replaced with that of the corresponding sequence from MVV K1514. CAEV envelopes with the heterologous V3 loop analogue sequence retained functionality and receptor usage identical to that of the wild-type CAEV envelope in fusion-based receptor interference assays (I. Hötzel, unpublished results). Furthermore, a CAEV envelope with the whole V3 loop analogue replaced by the sequence Cys-Gly-Ala-Gly-Cys was capable of inducing syncytia (but not infectivity) in goat cells (I. Hötzel, unpublished results), indicating that this region is not absolutely required for functional activity. Therefore, it seems unlikely that the putative gp135 V3 loop analogue has any role in receptor binding and usage. Whether this region has any role in immune evasion,

similar to the masking of receptor binding sites by the HIV-1 gp120 V3 loop,[42] remains to be determined.

The HIV-1 gp120 β4/β5 turn is located in a domain facing gp41 and close to β25 residue I491 which interacts with gp41.[43] Therefore, the gp120 β4/β5 turn may interact with gp41, although this has not has not been tested. The partial CAEV gp135 structural model thus predicts a role for the Pro-Tyr-Pro motif in the putative β4/β5 turn of gp135 in interactions with gp38. We have recently tested this prediction and found that mutations in the β4/β5 Pro-Tyr-Pro motif of CAEV gp135 increase the dissociation of gp135 from gp38.[44] Furthermore, similar to HIV-1 gp120, mutations in both the amino and carboxy-terminal domains of gp135 also increase gp135 shedding, indicating that gp120 and gp135 share other structural elements interacting with TM. In addition, immunological evidence indicates a similar folding of the proximal region of the HIV-1 gp120 inner domain formed by β-strands 5 and 25 in CAEV gp135. A monoclonal antibody recognizing a conformation-dependent epitope of CAEV gp135 failed to react specifically with gp135 with mutations in the β5 proline residue or a residue just downstream from putative β-strand 25.[44] This indicates that, similar to the corresponding HIV-1 gp120 regions, these two regions are located close to each other in the folded gp135 despite their distance in the primary structure. These findings support the general validity of the gp135 inner domain structural model based on the sequence similarity shown in Figure 2 and indicate that the SU of distantly related lentiviruses, and perhaps betaretroviruses, share common structures for interactions with TM.

The role of the gp120 inner-proximal domain in HIV-1 envelope glycoprotein intersubunit interaction, infectivity, and syncytium formation has been recently tested.[45] Consistent with the role of inner-proximal residues in the interaction of gp135 with gp38 in CAEV, mutation of some residues in this conserved gp120 inner-proximal domain affects intersubunit interaction. Interestingly, some mutations in the inner-proximal region affected the efficiency of fusion mediated by the envelope glycoprotein independently of stability of intersubunit interactions, suggesting that this region has a role in the modulation of steps after receptor binding leading to membrane fusion.

4. SUMMARY

The surface envelope glycoprotein of CAEV is the ligand for receptor interaction and an important target of host immune responses that control virus replication and disease status. A complete understanding of host-virus interactions, particularly with regard to SU structure and function, will be required to determine the mechanisms of disease pathogenesis and to

develop effective vaccine strategies. This review summarizes recent studies on the structural properties of CAEV gp135 SU with emphasis on higher intramolecular order predicted by the crystal structure of HIV-1 gp120. This review is intended to serve as baseline information for further studies on the structure, antigenic properties, and function of the SU of CAEV, MVV, and other lentiviruses.

REFERENCES

1. O. Narayan, M. Zink, M. Gorrell, S. Crane, D. Huso, P. Jolly, M. Saltarelli, R. Adams, and J. Clements, The lentiviruses of sheep and goats, in *The Retroviridae*, edited by J. Levy (New York: Plenum Press, 1993), vol. 2, pp. 229–256.
2. R. Garry, A. Krieg, W. Cheevers, R. Montelaro, H. Golding, C. Fermin, and W. Gallaher, Retroviruses and their roles in chronic inflammatory diseases and autoimmunity, in *The Retroviridae*, edited by J. Levy (New York: Plenum Press, 1995), vol. 4, pp. 491–603.
3. M. J. Wilkerson, W. C. Davis, T. V. Baszler, and W. P. Cheevers, Immunopathology of chronic lentivirus-induced arthritis. *Am. J. Pathol.* 146, 1433–1443 (1995).
4. U. von Bodungen, F. Lechner, H. Pfister, H. R. Vogt, W. P. Cheevers, G. Bertoni, T. W. Jungi, and E. Peterhans, Immunohistology of the early course of lentivirus-induced arthritis. *Clin. Exp. Immunol.* 111, 384–390 (1998).
5. W. P. Cheevers, D. P. Knowles, T. C. McGuire, D. R. Cunningham, D. S. Adams, and J. R. Gorham, Chronic disease in goats orally infected with two isolates of caprine arthritis-encephalitis lentivirus. *Lab. Invest.* 58, 510–517 (1988).
6. W. P. Cheevers, D. P. Knowles, and L. K. Norton, Neutralization resistant antigenic variants of caprine arthritis-encephalitis lentivirus associated with progressive arthritis. *J. Infect. Dis.* 164, 679–685 (1991).
7. M. J. Wilkerson, W. C. Davis, and W. P. Cheevers, Peripheral blood and synovial fluid mononuclear cells phenotypes in lentivirus-induced arthritis, *J. Rheumatol.* 22, 8–15 (1995).
8. F. Lechner, H. Vogt, H. F. Seow, G. Bertoni, W. P. Cheevers, U. von Bodungen, A. Zurbriggen, and E. Peterhans, Expression cytokine mRNA in lentivirus-induced arthritis. *Am. J. Pathol.* 151, 1053–1065 (1997).
9. W. P. Cheevers, J. C. Beyer, and D. P. Knowles, Type 1 and type 2 cytokine gene expression by viral gp135 surface protein-activated T lymphocytes in caprine arthritis-encephalitis lentivirus infection. *J. Virol.* 71, 6259–6263 (1997).
10. G. C. Johnson, D. S. Adams, and T. C. McGuire, Pronounced production of polyclonal immunoglobulin G1 in the synovial fluid of goats with caprine arthritis-encephalitis virus infection. *Infect. Immun.* 41, 805–815 (1983).
11. D. P. Knowles, W. P. Cheevers, T. C. McGuire, T. A. Stem, and J. R. Gorham, Severity of arthritis is predicted by antibody response to gp135 in chronic infection with caprine arthritis-encephalitis virus. *J. Virol.* 64, 2396–2398 (1990).
12. T. C. McGuire, D. P. Knowles, W. C. Davis, A. L. Brassfield, T. A. Stem, and W. P. Cheevers, Transmembrane protein oligomers of caprine arthritis-encephalitis lentivirus are immunodominant in goats with progressive arthritis. *J. Virol.* 66, 3247–3250 (1992).
13. G. Bertoni, M. Zahno, R. Zanoni, H. Vogt, E. Peterhans, G. Ruff, W. Cheevers, P. Sonigo, and G. Pancino, Antibody reactivity to the immunodominant epitopes of the caprine arthritis-encephalitis virus gp38 transmembrane protein associates with the development of arthritis. *J. Virol.* 68, 7139–7147 (1994).

14. L. L. Perry, M. J. Wilkerson, G. A. Hullinger, and W. P. Cheevers, Depressed CD4+ T lymphocyte proliferative response and enhanced antibody response to viral antigen in chronic lentivirus-induced arthritis. *J. Infect. Dis.* 171, 328–334 (1995).
15. W. P. Cheevers, K. R. Snekvik, J. D. Trujillo, N. M. Kumpula-McWhirter, K. J. Pretty On Top, and D. P. Knowles, Prime-boost vaccination with plasmid DNA encoding caprine arthritis-encephalitis lentivirus Env and viral SU suppresses challenge virus and development of arthritis. *Virology* 306, 116–125 (2003).
16. W. P. Cheevers, T. A. Stem, D. P. Knowles, and T. C. McGuire, Precursor polypeptides of caprine arthritis-encephalitis lentivirus structural proteins, *J. Gen. Virol.* 69, 675–681 (1988).
17. T. C. McGuire, D. P. Knowles, W. C. Davis, A. L. Brassfield, T. A. Stem, and W. P. Cheevers, Transmembrane protein oligomers of caprine arthritis-encephalitis lentivirus are immunodominant in goats with progressive arthritis, *J. Virol.* 66, 3247–3250 (1992).
18. M. Saltarelli, G. Quérat, D. Konings, R. Vigne, and J. E. Clements, Nucleotide sequence and transcriptional analysis of molecular clones of CAEV which generate infectious virus, *Virology* 179, 347–364 (1990).
19. D. P. Knowles, W. P. Cheevers, T. C. McGuire, A. L. Brassfield, W. G. Harwood, and T. A. Stem, Structure and genetic variability of envelope glycoproteins of two antigenic variants of caprine arthritis-encephalitis lentivirus, *J. Virol.* 65, 5744–5750 (1991).
20. S. Valas, C. Benoit, C. Baudry, G. Perrin, and R. Z. Mamoun, Variability and immunogenicity of caprine arthritis-encephalitis virus surface glycoprotein, *J. Virol.* 74, 6178–6185 (2000).
21. I. Hötzel, N. Kumpula-McWhirter, and W. P. Cheevers, Rapid evolution of two discrete regions of the caprine arthritis-encephalitis virus envelope surface glycoprotein during persistent infection, *Virus Res.* 84, 17–25 (2002).
22. R. Skraban, S. Matthíasdóttir, S. Torsteinsdóttir, G. Agnarsdóttir, B. Gudmundsson, G. Georgsson, R. H. Meloen, O. S. Andrésson, K. A. Staskus, H. Thormar, and V. Andrésdottir, Naturally occurring mutations within 39 amino acids in the envelope glycoprotein of maedi-visna virus alter the neutralization phenotype, *J. Virol.* 73, 8064–8072 (1999).
23. D. L. Huso, O. Narayan, and G. W. Hart, Sialic acids on the surface of caprine arthritis-encephalitis virus define the biological properties of the virus, *J. Virol.* 62, 1974–1980 (1988).
24. F. Özyörük, W. P. Cheevers, G. A. Hullinger, T. C. McGuire, M. Hutton, and D. P. Knowles, Monoclonal antibodies to conformational epitopes of the surface glycoprotein of caprine arthritis-encephalitis virus: potential application to competitive-inhibition enzyme-linked immunosorbent assay for detecting antibodies in goat sera, *Clin. Diag. Lab. Immunol.* 8, 44–51 (2001).
25. M. J. Braun, J. E. Clements, and M. A. Gonda, The visna virus genome: evidence for a hypervariable site in the env gene and sequence homology among lentivirus envelope proteins. *J. Virol.* 61, 4046–4054 (1987).
26. H. Toh, and T. Miyata, Is the AIDS virus recombinant? *Nature* 316, 21–22 (1985).
27. M. A. McClure, M. S. Johnson, D. F. Feng, and R. F. Doolittle, Sequence comparisons of retroviral proteins: relative rates of change and general phylogeny, *Proc. Natl. Acad. Sci. USA* 85, 2469–2473 (1988).
28. D. F. York, R. Vigne, D. W. Verwoerd, and G. Quérat, Nucleotide sequence of the jaagsiekte retrovirus, an exogenous and endogenous Type D and B retrovirus of sheep and goats, *J. Virol.* 66, 4930–4939 (1992).
29. R. Wyatt, P. D. Kwong, E. Desjardins, R. W. Sweet, J. Robinson, W. A. Hendrickson, and J. Sodroski, The antigenic structure of the HIV gp120 envelope glycoprotein, *Nature* 393, 705–711 (1998).

30. T. F. Schulz, B. A. Jameson, L. Lopalco, A. G. Siccardi, R. A. Weiss, and J. P. Moore, Conserved structural features in the interaction between retroviral surface and transmembrane glycoproteins? *AIDS Res. Hum. Retroviruses* 8, 1571–1580 (1992).
31. G. Pancino, H. Ellerbrok, M. Sitbon, and P. Sonigo, Conserved framework of envelope glycoproteins among lentiviruses. *Curr. Top. Microbiol. Immunol.* 188, 77–105 (1994).
32. W. R. Gallaher, J. M. Ball, R. F. Garry, A. M. Martin-Amedee, and R. C. Montelaro, A general model for the surface glycoproteins of HIV and other retroviruses, *AIDS Res Hum Retroviruses* 11, 191–202 (1995).
33. P. D. Kwong, R. Wyatt, J. Robinson, R. W. Sweet, J. Sodroski, and W. A. Hendrickson, Structure of an HIV gp120 envelope glycoprotein in complex with the CD4 receptor and a neutralizing human antibody, *Nature* 393, 648–659 (1998).
34. I. Hötzel, and W. P. Cheevers, Sequence similarity between the envelope surface unit (SU) glycoproteins of primate and small ruminant lentiviruses, *Virus Res.* 69, 47–54 (2000).
35. I. Hötzel, and W. P. Cheevers, Conservation of human immunodeficiency virus type 1 gp120 inner-domain sequences in lentivirus and type A and B retrovirus envelope surface glycoproteins, *J. Virol.* 75, 2014–2018 (2001).
36. P. D. Kwong, R. Wyatt, Q. J. Sattentau, J. Sodroski, and W. A. Hendrickson, Oligomeric modeling and electrostatic analysis of the gp120 envelope glycoprotein of human immunodeficiency virus, *J. Virol.* 74, 1961–1972 (2000).
37. I. Hötzel, Conservation of the human immunodeficiency virus type 1 gp120 V1/V2 stem/loop structure in the equine infectious anemia virus (EIAV) gp90, *AIDS Res. Hum. Retroviruses*, 19, 923–924 (2003).
38. R. Wyatt, and J. Sodroski, The HIV-1 envelope glycoproteins: fusogens, antigens, and immunogens, *Science* 280, 1884–1888 (1998).
39. P. D. Kwong, M. L. Doyle, D. J. Casper, C. Cicala, S. A. Leavitt, S. Majeed, T. D. Steenbeke, M. Venturi, I. Chaiken, M. Fung, H. Katinger, P. W. I. H. Parren, J. Robinson, D. Van Ryk, L. Wang, D. R. Burton, E. Freire, R. Wyatt, J. Sodroski, W. A. Hendrickson, and J. Arthos, HIV-1 evades antibody-mediated neutralization through conformational masking of receptor-binding sites, *Nature* 420, 678–682 (2002).
40. O. Narayan, D. Sheffer, D. E. Griffin, J. Clements, and J Hess, Lack of neutralizing antibodies to caprine arthritis-encephalitis lentivirus in persistently infected goats can be overcome by immunization with inactivated *Mycobacterium tuberculosis*, *J. Virol.* 49, 349–355 (1984).
41. I. Hötzel, and W. P. Cheevers, Differential receptor usage of small ruminant lentiviruses in ovine and caprine cells: host range but not cytopathic phenotype is determined by receptor usage, *Virology* 301, 21–31 (2002).
42. R. Wyatt, N. Sullivan, M. Thali, H. Repke, D. Ho, J. Robinson, M. Poshner, and J. Sodroski, Functional and immunologic characterization of human immunodeficiency virus type 1 envelope glycoproteins containing deletions of the major variable regions, *J. Virol.* 67, 4557–4565 (1993).
43. E. Helseth, U. Olshevsky, C. Furman, and J. Sodroski, Human immunodeficiency virus type 1 gp120 envelope glycoprotein regions important for association with the gp41 transmembrane glycoprotein, *J. Virol.* 65, 2119–2123 (1991).
44. I. Hötzel, and W. P. Cheevers, Caprine arthtitis-encephalitis virus envelope surface glycoprotein regions interacting with the transmembrane glycoprotein: structural and functional parallels with the human immunodeficiency virus type 1 gp120, *J. Virol.*, in press (2003).
45. X. Yang, E. Mahony, G. H. Holm, A. Kassa, and J. Sodroski, Role of the gp120 inner domain β-sandwich in the interaction between the human immunodeficiency virus envelope glycoprotein subunits, *Virology*, 313, 717–125 (2003).

14

Ethical Issues in the Use of Animal Models of Infection and Some Practical Refinements

DAVID B. MORTON

1. INTRODUCTION

Other chapters in this book have drawn the reader's attention to the devastation caused by HIV infection, not only to the individuals concerned but also to their families and dependants. Moreover, there are direct effects on others in the community such as doctors, nurses, health workers and care givers and; as well as running the risk of themselves becoming infected, they are exposed daily to the harrowing tragedies of those afflicted by this disease. AIDS not only drastically reduces life expectancy of the individual, but also the quality of that life. In addition to these costs are the indirect costs that further burden a stretched national economy, particularly in regard to health care services, as spending on AIDS reduces unit resources for all those that are in the system. A choice between caring for someone with AIDS in their last few weeks or days of life, or saving the life of a newborn, or repairing a fractured bone, should never have to be faced, but it is in many countries on a daily basis. Set against this background how can some people value the lives of nonhuman animals to the point where they might want to restrict or even stop the use of animals to gain insights and cures for these HIV diseases? The notion that animals have a right to a life and a right not to suffer would mean just that. So is the issue of animal rights, which would stop all the current uses of animals in research, simply a privileged armchair view from the developed Western world put forward

DAVID B. MORTON • Centre for Biomedical Ethics, Department of General Practice and Primary Care, Primary Care Clinical Sciences Building, University of Birmingham, Edgbaston, Birmingham B15 2TT, UK

by those who are relatively healthy and have just had a good meal? Or, is animal rights a notion that reflects the thoughts of those wishing to improve the lot of vulnerable beings through actions that do not involve the use of animals in research? Perhaps there is a philosophically intermediate position that might permit the continued use of animals while not losing sight of the scientific and humanitarian objectives that are so badly needed? In this chapter I wish to examine the various moral positions that can be held and to translate these into practical ethical issues that can be addressed by those seeking to use animals in their research as well as those that oppose this use of animals. Neither camp will be satisfied, but as it was once stated "Better a dissatisfied Socrates than a satisfied pig."

2. SHOULD WE USE ANIMALS IN RESEARCH ON HIV?

The main objections to the use of animals in research are that during their use they *will* inevitably experience pain and distress of various types and, nearly always, they will die. Those who believe that animals, like humans, have a right to a life and a right not to be caused to suffer, state that it is wrong to cause animals to suffer and to take their lives notwithstanding any benefit that may come from those uses (e.g., food, cures for disease, and so on). Any such benefits are seen as ill-gotten gains on par with torture and the use of humans in research without their consent. Those who hold an animal rights view might argue that such "natural rights" are necessary to protect the vulnerable, such as human babies and the young, and they see animals in the same group as these types of humans. Exploiting one vulnerable group of animals (chimps, mice) to save another group (humans with AIDS) is unacceptable unless one can find a morally relevant difference between the two groups. Both nonhuman animals and human beings are living animals who are able to suffer and have an interest in continuing to live a life that is valuable to them. But in addition, humans, because they are able to make moral choices unlike animals, have a duty not to exploit other animals (including other humans) in any way that would cause them to suffer or die. To do so is no different in principle to tribalism, racism, sexism, and ageism and has been termed "speciesism" (Ryder, 1975). So if we assign such strong rights to animals (not to suffer in any way and to live) then it follows they cannot be used in research on HIV, FIV, SIV, and so on.

Another view might be to argue that while the lives of nonhuman animals that are able to experience pain, distress, suffering, and lasting harm (as well as happiness, i.e., they are sentient beings) are valuable, that value can be overridden in some circumstances. Such a caveat demands that the benefits of any use outweigh the harms that are inflicted. This leads to some form of analysis of the burdens and benefits, sometimes referred

to as a harm benefit or cost benefit analysis. Thus there has been a shift in the philosophical position from one of absolute rights to one where certain prima facie rules form a baseline and, providing only the minimum of suffering is caused, the use of animals might be permitted for some greater good. This change in emphasis is closely correlated with duties on those who use animals to be very careful how they act. This approach would apply to the use of human subjects in research as well. So rather than having an absolute ban on the use of animals in HIV research, this utilitarian approach requires that the (research) objective is so worthwhile that it outweighs any suffering inflicted on the animals concerned, and that level of suffering is the minimum needed to obtain the scientific objective. Unlike the animal rights position it also means that killing animals is not wrong in a good cause. Note that this utilitarian approach, of necessity, involves predictions of both the harms to the animals as well as the hoped-for outcome. In many ways the harms are easier to predict than the outcomes, and it is the very nature of research that there are long- and short-term goals. Basic research on the genetic makeup of the virus, its mode of infection, and trying to understand how disease is caused are long-term goals that inform applied research. The development of treatments to help those who are suffering from the disease, as well as developing preventive strategies such as vaccines, are often based on that fundamental knowledge.

There is no easy way in which to weigh harms done to the animals against the benefits that may accrue from the research; it is not a mathematical formula, rather a value judgment that takes opinions from those concerned (e.g., members of an ethics committee). There may be some notion that severe pain and severe distress should never be caused to animals (as in UK law) or that substantial pain and distress should not be caused when the research benefit is a gain in knowledge rather than a potential substantive contribution to a serious human or animal disease. This should not be taken to mean that it is simply a matter of opinion, rather that any view has to be justified by explanation (see Smith & Boyd, 1992). Ethics is not about holding an opinion; it is the justification of that view that is key to taking a particular decision and the consistent application of principles on which that opinion is based.

3. THE THREE RS: AN ETHICAL FRAMEWORK TO DEVELOP RESEARCH STRATEGIES AND PROJECT EVALUATION

One ethical framework to decide whether a proposed use of animals in a research project is acceptable was developed by Russell and Burch (1959) during the late 1950s. It still forms the cornerstone of all legislation controlling animal research worldwide. Its principles are that one should

Replace animals whenever possible by using nonsentient means such as in vitro techniques, computer modeling and so forth; *Refine* the experimental design so as to minimize any suffering; and *Reduce* the number of animals to the minimum needed to achieve an acceptable statistical standard. The use of the word "alternative" can be applied to all three Rs, but it is commonly accepted that the term applies when no animals are needed at all (i.e., a true replacement, such as the use of permanent cell culture lines or computer modeling). Killing an animal for tissue would not be a replacement as an animal could still "suffer" as a result of its husbandry or its manner of death (it could experience fear on being removed from its cage even though it was immediately humanely killed) and would be classified as a refinement; similarly with procuring tissues under terminal anesthesia. In many ways the three Rs overlap, particularly refinement and reduction as the total suffering involved in a research project is a product of the two (i.e., the number of animals multiplied by the duration and intensity of suffering).

One way of starting to apply a practical ethical evaluation in animal research is to ask questions: Is the work worth doing? and Is it possible to carry out this scientific procedure and cause less suffering to the animals concerned? The first question is often avoided by stating that it has been peer reviewed and it therefore must be worth doing. But consider for a minute the quality of research into cancer some decades ago when President Richard Nixon provided extra funds: did it work? To be ethical, science has to be of the highest quality and well designed.

The level of suffering should always be kept to the minimum as it would be unethical to cause extra *avoidable suffering* than was necessary to achieve the scientific objective (see Figure 1). This means that only methods that can be classified as *good laboratory-animal practice* should be used. Animal researchers should refine their care and use of experimental animals for moral and legal reasons, as well as for economic reasons: if an animal dies before the experiment is completed or it suffers to an extent that it did not yield reliable scientific data, then that life has been wasted at best, and at worst it has provided misleading information. Poor animal well-being caused by poor animal health, poor care or poor husbandry, or clumsy experimental technique will lead to animal suffering, which may well confound an animal's response to the scientific variable being investigated, thus adding to the biological variation. It may just skew the research data with no significance, or it may totally alter the scientific interpretation of that data. The results obtained are then not only misleading but in the long run may lead to more animals having to be used. Paying attention to animal well-being, therefore, makes sense for reliable, humane, and economic science.

In the present context of infectious disease it is unlikely that replacement alternatives can be used to totally replace whole living animals.

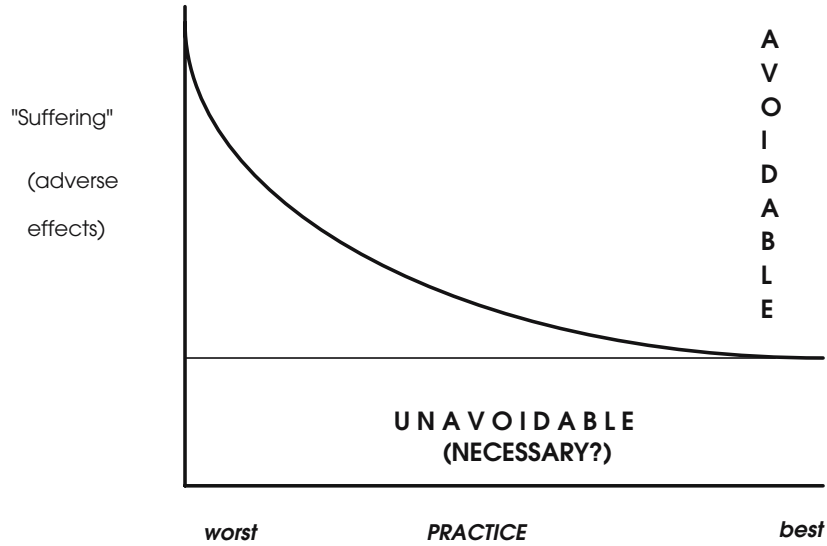

FIGURE 1. How animal suffering is increased by not using best practice. There will always be some suffering that cannot be avoided or relieved (i.e., the suffering that is inevitable or necessary to achieve the scientific objective).

However, it is also likely that a considerable amount of in vitro work can be carried out to good effect before animal research is started. In vitro techniques are cheaper and often needed to answer questions about cell-cell interactions as well as the changes in cell function as a result of infection. Replacement alternatives can be seen as adjuncts to the in vivo research and should precede work in vivo whenever possible so that animals can be used to answer more specific questions only obtainable in living animals.

Refinement can be defined as: Those methods which avoid or alleviate or minimize the potential pain, distress or other adverse effects suffered by the animals involved, or which enhance animal well-being. Approximately 20 years ago, animal welfare was considered mainly in the light of animal health—an absence of disease—and scientists would have concentrated on the potential effects of infectious microorganisms on their research (Pakes et al., 1984). While disease is still an important issue today, it is less so than before thanks to the high health standards maintained by the breeders of laboratory animals. Moreover, other areas are emerging as important to animals, particularly psychological well-being.

I now wish to illustrate some practical ways in which animals can suffer, how these adverse states can be recognized, and how "best practice" or, a preferably phrase for me is "good practices," can be identified and put into effect.

Animal Suffering

Animal suffering is a generic term used rather loosely to refer to an animal's adverse reactions when exposed to some environmental change or internal physiological or psychological perturbation. It should be possible to use physiological and psychological indices to recognize when animals are suffering, and this is a prerequisite to be able to assess, measure, and even separate these emotional conditions. Several general references are worth reading (Duncan & Maloney, 1986; AVMA, 1987; Dawkins, 1990, 1992, 1993; Smith & Boyd, 1991; ILAR/NRC, 1992; Townsend, 1993; Morton, 1997, Paul-Murphy et al., 2004). While physiological outcomes of the stress response can be measured, such as hormonal levels of catecholamines, corticosteroids, endorphins, or target organ responses such as heart rate and blood pressure, these adverse states are often not clearly separated from each other. For example, animals in pain or which are disabled may also be anxious and frightened (see Figure 2). Animals are also able to

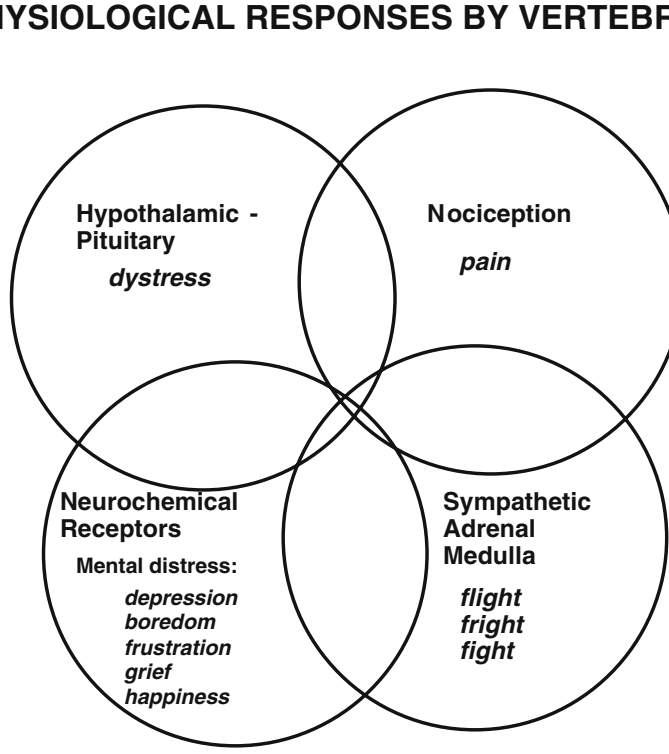

FIGURE 2. Indicates the theoretical measurable adverse physiological and psychological states, showing that they can occur independently as well as at the same time. It is likely that on many occasions they will occur at the same time and the circles should therefore overlap more closely.

suffer psychologically and this is seen as abnormal behavior, for example, some mammals in captivity show signs of boredom and frustration (mental distress) (Poole, 1992). Such signs can often be mitigated or reversed through "enriching" the environment (or rather making it less barren!). Refinement includes enrichment (Reese, 1991; Dean, 1999, Bayne et al., 2002) and because we are under a moral obligation to reduce all avoidable suffering to the minimum necessary through utilizing good practices, then attention must be paid to the husbandry of animals as well as to the experimental procedures that are carried out on them.

How Much Is an Animal Suffering?

First, it is important to *recognize* when an animal is suffering as this is the fundamental starting point for its assessment, avoidance, and alleviation. To assess whether an animal is suffering or not it is important to observe the animal carefully for signs of abnormality, and to use the degree to which it has deviated from normality as an indicator of the magnitude of that suffering (Morton & Griffiths, 1985; Morton, 2000; Morton & Hau, 2002). Detecting early signs of such deviation in an individual animal through a thorough clinical examination can be extremely valuable and provide extra information with which to interpret the scientific data and base further research (see "Score Sheets" below). Such measures of adverse states can be easily quantified such as bodyweight (or the amount eaten and drunk), temperature, behavior, heart rate, respiration rate; or detected qualitatively on reliable indicators of health and well-being such as posture, fecal consistency, pattern of respiration (e.g., labored), position of eyelids (e.g., closed or open), ambulation (e.g., lameness of varying degrees). All these signs can be objectively and reliably scored, and whether they are scored quantitatively or qualitatively, it is in their *interpretation* where the subjective element comes in.

Animals, Species, Speciesism, and Extrapolation

A range of animal species is used in research depending on the particular disease and the questions being asked. In some countries (e.g., the UK) special consideration has to be given to companion animals such as dogs, cats, and equidae (e.g., horses, ponies) and in other countries the use of primates has been banned especially the great apes (e.g., chimpanzees in the UK, the Netherlands). An ethical issue here is that the closer the species is to man, the greater is the ethical concern to use that species in ways we would not use human beings. Primate species other than great apes are used in research, but there is still concern due to their advanced neurophysiological development compared with lower species, their requirement for appropriate facilities (i.e., not just a box as is traditional for rabbits and rodents), for their care and husbandry, as well as their

potential to suffer more like humans. This latter point is contentious as it could be that more mammalian species suffer more than we currently think, but the data are weak. However, there is an argument that studies on pain and other neurological diseases that we can model in animals and that disable humans psychologically as well as physiologically, may result in similar feelings in animals as they do in humans. Until it can be proven otherwise, animals should be given the benefit of the doubt.

What is known is that humans and nonhuman mammals share many genes (> 90%) and so it is not surprising that results obtained in species such as rats and mice can be fruitfully used in the study of diseases of humans, as well as other animals. This obviously may not be 100% but, when carrying out research on the same species that the disease naturally affects (e.g., as is often the case in farm animals), the results become more reliable, and any variation will be due to extraneous factors such as their husbandry and housing or particular strain or breed differences.

An important point about animals is that, like humans, they are individuals and while group /herd/ flock averages are helpful in establishing benchmarks for normal parameters, other factors can make one animal very different from another. Such differences can be attributable to that animal's earlier life experiences (e.g., a lack of socialization in puppies to other dogs and humans, or an adverse experience in relation to a human) (Davis & Balfour, 1992; ILAR, 2002). So the attending clinician and the scientists always have to bear that in mind as it may result in some animals requiring more careful handling and coaxing, or more analgesia postoperatively than others, and individual responses may affect the data collected.

Score Sheets

An approach to the recognition and assessment of animal suffering is to use score sheets to examine in detail the impact on the animals of the scientific procedures involved, and to score those in some way. Wallace et al. (1990) tried to do this prospectively, but this approach assumes that all researchers will be equally competent on all occasions, and that all animals will react the same. An alternative approach is to look at it from the point of view of what the animal is telling us, no matter what the procedure or species of animal, or who is carrying out the procedure. Many laboratories are approaching this difficult topic of the recognition and assessment of suffering in animals (and nonverbal humans, babies) using score sheets, that is, a list of cardinal clinical signs encountered in a particular scientific procedure (see CCAC, 1998; Morton, 2000). These are developed through experience and, by and large, are unique to the system of husbandry, to the specific experiment, as well as to the species, and even the breed or strain of animal being used. It is not possible to make a general score sheet

for all experiments and for all species. One only has to consider the different potential adverse effects of a rejection of a skin transplant compared with a kidney or heart transplant rejection to appreciate that different clinical signs will be seen.

Practically, it is important to develop a disciplined approach and strategy to the recognition of adverse effects in animals (see Morton & Griffiths, 1985). At the beginning of an assessment, the animal should be viewed from a distance, and its natural undisturbed behavior and appearance noted. Next, as the observer approaches the cage or pen, the animal will inevitably start to take notice and interact with the observer and that interaction can be used to determine whether it is responding normally (an animal may be inquisitive or show signs of fear). Finally, a detailed clinical examination can be carried out by restraining the animal in some way and observing its appearance carefully and then making clinical measurements (e.g., of body weight, body condition, temperature) in addition to its behavior as it may have become more aggressive, fearful, or lethargic.

A list of clinical signs is developed by observing the first few animals undergoing a novel scientific procedure very closely. The list can then be modified with experience until a set of cardinal signs that most animals will show during that experiment and that are relevant to the assessment of suffering is determined. These key clinical signs are set out against time in a score sheet (see Figure 3). On the left-hand side are listed clinical and behavioral signs, and along the top the days and the time of the observation. The method of scoring is that clinical signs can only be recorded as being present or absent indicated by a plus or a minus sign (or sometimes a +/− if an observer is unsure). The convention is that negative signs indicate normality or within the normal range, and plus signs indicate compromised animal well-being. In this way, it is possible to visually scan a score sheet to gain an impression of an animal's well-being: the more plus signs, the more that animal has deviated from normality with the inference that it is suffering more than it was earlier. Clinical treatments and other observations are also recorded. It is important to note that animals can be scored at any time and it should certainly be more than once daily during critical periods when an animal's condition could predictably give rise to concern (e.g., in the immediate postoperative period; or in a study on infection at the time of bacteraemia or septicaemia).

At the bottom of the sheet there are guidance notes for the animal caretakers about what they should provide in terms of husbandry and care. There are also guidelines on how to score qualitative clinical signs such as diarrhea and respiration, as well as criteria by which to judge humane endpoints. If an animal has to be put down, there is guidance about what other actions should be taken such as tissues to be retrieved and kept in formal saline; this helps ensure that the maximum information is always obtained from any animal in the study.

RAT No.		**ANIMAL ISSUE No.**					
DATE OF STARVING:		**PRESTARVED WEIGHT:**					
DATE							
DAY							
TIME							
FROM A DISTANCE							
Inactive							
Isolated							
Walking on tiptoe							
Hunched posture							
Starey coat							
Type of breathing*							
Grooming							
ON HANDLING							
Not inquisitive and alert							
Not eating							
Not drinking/average amount drunk (ml)							
Bodyweight (g)							
% change from start							
Body temperature (C)							
Pale or sunken eyes							
Dehydration							
Diarrhea 0 to 3 (+m or +b)**							
Distended abdomen/swollen							
Vocalization on gentle palpation							
Nothing abnormal detected (NAD)							
Given 5ml saline s/c or p.o.							
Other signs noted: Loss of body condition							
SIGNATURE:							

Special husbandry requirements:
Animals should be kept on grid cage with tray and cleaned twice daily, and mouse box for enrichment
Two bottles should be provided for each cage and filled twice daily
Deprivation of water overnight may be sufficient to cause death by dehydration.
Autoclaved diet must be provided.
Scoring details
* Breathing: R = rapid; S = shallow; L = labored; N= normal
**0 = normal; 1 = loose feces on floor; 2 = pools of feces on floor; 3 = running out on handling;
+ 'm' = feces contain mucus; + 'b' = feces contain blood
Humane endpoints and actions
1. Any animals showing signs of coma within the first 24–48 hr will be humanely killed
2. Any animals weighing less than the starting weight after 7 days will be humanely killed
3. Any animal showing tiptoe or slow ponderous gait will be humanely killed
Inform scientist, named veterinary surgeon, and animal technician in day-to-day care if any of 1 to 3 above are seen
Scientific Measures
Animals that have to be put down should have their kidneys placed in formal saline and the pots clearly labeled

FIGURE 3. Animal score sheet (blank) for Streptozotocin diabetes model.

While these sheets take time to fill in, it is not difficult for an experienced person, such as an animal caretaker, to see if an animal is unwell so the time taken can be reduced by simply scoring that the animal is normal by ticking the NAD box (nothing abnormal detected). However, if an animal is not normal, it does take time to check it and to make judgments over what actions to take, but that is the price for practicing humane science.

In order to promote good care and good continuity of care we allocate an animal technician to be responsible for liaising with the scientists and other technical staff, and also to maintain and update the score sheets. The roles of the technician in charge are:

- to check that the appropriate licenses are in order and match them with what the scientist actually intends to do that day to the animal(s);
- to check the score sheet is appropriate before the experiment begins;
- to know the purpose of the experiment and the scientific objectives, and to become familiar with the scientific procedures to be carried out on the animals and the clinical signs that may occur;
- to ensure all personnel (technicians, scientists) know how to use score sheets and can recognize the clinical signs and interpret the signs clearly into humane endpoints;
- to check that technicians not familiar with that experiment, say doing a weekend or holiday rotation, are informed about animals;
- to liaise with licensees over the experiment timing, numbers of animals, equipment, endpoints;
- to update the score sheets based on new signs or combinations of signs observed; and
- to report to the responsible persons any concerns over the animals or personnel involved.

This scheme of scoring clinical signs for the recognition and assessment of adverse effects on animals during scientific procedures has been shown to have several advantages, which include the following.

- Closer observation of animals is now carried out by all staff at critical times in the experiment as the sheets have indicated those times that are critical for the animal, and when the animals find their circumstances most aversive.
- Subjective assessments of suffering by staff and scientists are avoided, thereby promoting more fruitful dialogue, as evidence-based opinion becomes possible based on the clinical signs. In a sense they empower the animal technicians and help them demonstrate to less experienced persons why an animal is “not right.”
- Consistency of scoring is increased as the guidance is clear and the scoring options are limited.

- Single signs or combination of signs can be used to indicate overall severity of the procedure, as well as alleviative therapies or scientific procedures at set points in the experiment (e.g., blood sampling).
- The sheets help to determine the effectiveness of any therapy intended to relieve adverse effects.
- The sheets help to determine which experimental models cause the least pain and distress by comparing alternative animal models, thus helping to refine scientific procedures.
- The sheets help to train those inexperienced in the assessment of adverse effects.

As mentioned above the sheets are constantly being updated with further experience; it is surprising how the process never seems to stop as new staff pick up new signs, or new signs develop as the experimental model is slightly modified, or staff start to perceive patterns of adverse effects that, when taken as a whole, indicate early death or early deterioration sufficient to warrant the animal being killed on scientific grounds alone. Such information has led to better animal care as well as providing useful scientific information such as the recognition of neurological deficits, times of epileptic fits or weight loss, as well as unexpected findings such as urinary retention in a model of renal failure. Furthermore, by picking up signs of poor animal well-being early, humane endpoints can be implemented sooner rather than later, which avoids animals being inadvertently lost from an experiment. In the UK, where severity limits are imposed on each scientific procedure, the sheet can be used to indicate when such limits have been reached, or are about to be reached, or may have to be reviewed, by a precise observation of clinical signs. The score sheet system provides a visual aid, opens up discussion between interested parties, and helps focus attention on to the animal's condition throughout the procedures.

Alleviation of Adverse States

It is fairly evident that pain can be alleviated in animals through a variety of strategies. Perhaps the most important is preemptive analgesia (Flecknell & Waterman-Pearson, 2000) where pain is anticipated and appropriate drugs given at an early stage before an animal experiences pain and develops "wind-up." Wind-up is sensitization of the central nervous system that produces hyperalgesia and allodynia. (Hyperalgesia is an increased sensitivity to painful stimuli and allodynia is a painful response to a nonpainful stimulus.) Opiates are good for acute pain and if nonsteroidal antiinflammatory drugs are given at the same time, then the pain due to tissue repair and the release of prostaglandin derivatives from the action of cyclo-oxygenases can be mitigated. This is true also for chronic

pain, such as in arthritis, and visceral pain. The general rule is that if the equivalent condition is painful in humans, then it should be assumed that it is painful in animals until proven otherwise, and relief should be given. Any potential confounding effect of pain relief treatment on a potential therapy can always be studied in its own right as a subsequent experiment (refinement is considered to trump reduction). First, the potential of a new treatment should be demonstrated humanely, and then any confounding or synergistic effect should be studied.

Other states such as distress, or just "feeling unwell" as humans might have with a viral infection are more difficult to deal with, as the presenting clinical signs (or symptoms in humans) are often less well defined, such as malaise, headache, reduced appetite, and reduced (social) interactions with other animals and humans. This is the advantage of keeping animals in groups or pairs and having enrichment in their cages as it provides additional scoring measures for such interactions. Humans feel lethargic, whereas animals show this by reduced activity, and so are less responsive when disturbed; comparing affected animals with normal naïve animals can quickly reveal significant differences. The experience of the animal caretakers can often be very helpful in these situations. The degree of change from normal is more difficult to assess as behavioral studies are time consuming and some species, such as rodents, may only reveal such differences at night—their normal activity times as they sleep during the daytime.

Avoidance of Adverse States

As mentioned earlier in this chapter, it may be possible to minimize any pain and suffering in two key areas.

Technical Skills

The first is that the research staff should be *trained* and *competent* in carrying out the scientific techniques, including scoring adverse states in animals. In some countries competency has to be demonstrable in some way to an animal care and use committee or government inspector and may include attending an accredited training course, as well as some assessment of competence, analogous to a driving test. Training to learn about driving is very different from being a good driver.

Experimental Design

If, after due consideration by an authority, such as an ethics committee or national controlling body, that it is acceptable to use animals in the

proposed research because it will provide useful and important new information, and that using animals is the only way to achieve the scientific objective, the next step is to decide how best to carry out the work. This step focuses on research strategies and the use of appropriate statistical methods is prerequisite. It is important to appreciate that using too few animals in an experiment is just as unethical as using too many, as the research may have to be repeated. Researchers should seek professional advice on the number of animals required to provide statistically significant results before embarking on an experiment. There are now a range of statistical approaches that enable better, more meaningful use of small numbers of animals. (Festing et al., 2002) The key to good study design, from the point of view of animal number reduction, is to define clearly in advance exactly what the purpose of the test or experiment is, and what actions or decisions will be taken on the basis of particular results. Then researchers should opt for the lowest probability level that will meet their essential information needs. There is no point in aiming for a very small margin of error (by using greater numbers of animals) if that degree of precision is not going to influence what is done with the results.

One way in which researchers can determine in advance the appropriate number of animals to use in an experiment is by carrying out pilot studies on small numbers of animals. This practice, which is now becoming commonplace as it reduces animal numbers, may also provide valuable information on variations between animals (in terms of test outcomes), any likely adverse effects that may be encountered, and any practical problems that are likely to be encountered during the main study. For example, differences in diet, bedding, staff, cleaning materials, husbandry, and environment have all been shown to affect animals' responses to chemicals in various ways (van den Heuvil et al., 1990). Similarly, different species may respond differently to a test substance. Pilot studies need not involve any "waste" of animals, since the data can often be incorporated into the main study results. Historical data are also useful, particularly for determining the most useful means of study control, which may be a positive control (for example, a standard challenge of a known infectious organism) or a negative control without the active principle (i.e., vehicle only). Using such background data can influence the number of animals needed for a given study, and may even do away with the need for a control group altogether if the control outcome is so well known and accepted that it does not need to be demonstrated once again. For example, researchers drawing on historical data will know that total pancreatectomy leads to diabetes mellitus, with a consequent rise in blood sugar and death in less than 10 days. These data can serve as a de facto control. The reason for running a control group should be carefully considered as well as the number of animals needed in that group.

However, there are also issues not directly related to statistics that also have to be considered in order to refine experiments, for example, to stage the experimental work incrementally and to carefully review it after each experiment (Morton, 1998, 2002). Another strategy is to cause a minimum harm before a greater one in order to provide proof of concept. For example, one does not need to test the effectiveness of a novel analgesic by administering a severe stimulus, as if the potential agent will not work on a minor stimulus then it will be unlikely to work on a greater one. In terms of infections, it would be worth trying to see if a therapy worked at an early stage rather than when a disease has progressed more, as a failure to eliminate an infection early on does not bode well for success at a later stage when a disease has become more widespread.

Humane Endpoints

A general definition of a humane endpoint is "the earliest stage at which an experiment can be terminated"; the OECD (2001) defined it as "The earliest indicator in an animal experiment of severe pain, severe distress, suffering or impending death." It may be the time when the scientific objective has been achieved, after all what is the point of going on? Or it may be that an animal is so physiologically abnormal or so psychologically disturbed that the scientific outcome measures will be skewed and no longer of value (see ILAR, 2000). In the past, it was not uncommon to use the death of a test animal as an endpoint, meaning the measurable outcome of the experiment. This is changing as awareness grows that death is rarely related to the experimental variable under study, but rather to indirect effects such as dehydration and starvation. At the 1996 World Congress on Alternatives to animal testing, workshop participants arrived at the following statement on humane endpoints: "In principle, death should not be an endpoint in any experiment or test, but if death is proposed as an endpoint, it must be justified with increasingly strong arguments as the anticipated overall pre-death suffering increases" (Mellor & Morton, 1997, p. 297). In studies on infection the scientific objective may have been achieved when testing a vaccine and a vaccinated animal is showing signs of infection that would be unacceptable in a human subject, or that it is showing signs of disease that will inevitably proceed to death (in some studies death has been the classical endpoint).

Even aside from the ethical considerations, which are self-evident, there are good scientific reasons for avoiding death as a necessary outcome of animal experiments. For example, histological analysis and culture counts are severely compromised when samples are collected postmortem. In infection studies, the counting of bacteria or plaque forming units at set points before death may be more valuable than using death as an endpoint.

Little work seems to have been done in this area and there is scope for research aimed at determining, and refining, humane endpoints (Morton, 1999). One approach is to note the clinical signs preceding death closely, find out which signs are irrevocably linked with death, and then use such signs as prelethal endpoints. This idea of using early clinical signs to predict later ones requires validation studies showing that animals would have normally progressed in that way and that such surrogate endpoints can be relied upon. Grant-awarding bodies have a moral obligation to support this sort of research aimed at developing and validating humane endpoints. Pioneers of such research is Klaus Cussler of the Paul Ehrlich Institute in Germany and Coenraad Hendriksen in the Netherlands whose teams have been investigating surrogate endpoints for use in a vaccine potency tests (see Hendricksen & Morton, 1999). Data recently presented by the group showed that mice vaccinated against rabies, if unprotected or inadequately protected and subsequently given a challenge doses of rabies virus, went through a predictable series of clinical signs. Animals showing slow circular movements invariably progressed to death—the traditional endpoint for the test—so this behavior could reliably be used as an alternative endpoint (Cussler et al., 1999). In other studies in mice, researchers have used a body temperature of less that 35°C as a prelethal endpoint for some types of infection models (Soothill et al., 1992). This approach can be used in a variety of tests, including acute toxicity testing for novel compounds; assessment of the virulence of microorganisms or parasites; and pharmaceutical efficacy research, such as the vaccine potency test or the Rodent Protection Test for novel antibiotics (Acred et al., 1994).

4. GUIDANCE FOR ETHICS COMMITTEES AND PUBLICATION OF DATA

I have summarized some pointers for IACUCs, or the equivalent depending on the country, in Tables I and II. Table I draws attention to the proven value of the model and provides some of the details that may make it essential for that model to work. It is based on examples where a change in some of those parameters can change the outcome. It also brings into question the fidelity of the model: How good a model is it? Has it been used to develop new therapies successfully? and so on. There is little point in refining experimental methods but not passing on that information to others. So many details, tricks of the trade, remain buried in-house, but there is a moral obligation to pass that knowledge on, including the recognition, assessment, avoidance, and alleviation of animal suffering (see Morton, 1992). Table II raises the issues about the housing and husbandry of the animals as well as methods for refining the experimental design.

TABLE I
Animal Model Data

1. Model of:

Purpose of the research (benefit)

2. Purpose that model can be used for (e.g., human disease, drug evaluation)
3. Advantages and disadvantages (scientifically and in its relevance and application to human or animal disease or treatment)
4. Clinical trials or medicine (drug) evaluation indicating relevance or predictability of the model to its intended purpose (2 above)

Animal details

5. Species of animal and experimental details (e.g., strain, inbred/outbred, sex, age, weight, health status, acclimation period, other)
6. Husbandry special or critical requirements (e.g., caging or pen type, animal kept singly or in groups, diet, bedding, isolator or filtration boxes, breeding details, other)
7. Methodological details (e.g., equipment required, manual skills required, dosing and timings, timings of measurements, where advice can be obtained)
8. Refinement aspects (e.g., humane endpoints, score sheet giving cardinal signs, useful tests, and any other information)
9. Scoring of signs and severity grading (severity grading: give average and maximum expected)

Alternative models and staging strategies

10. Replacement (any alternative methods for all or part of the model available, such as prescreening)
11. What information is necessary or would be useful before in vivo work begins?
12. Are pilot studies necessary for dose sighting? Are the doses chosen the minimum necessary to obtain an indication of effectiveness before proceeding to more detailed trials (e.g., dose response studies)?
13. Success rate of the model (give mean and range and methods used to determine success)
14. Statistics (How are the data to be handled? What are the best means of analysis, data transformation, etc.?)
15. If lethality is an endpoint, please justify. If a LD 50% dose is being obtained justify the scientific necessity for a precise estimation in relation to the subsequent extrapolation to human or animal therapies.
16. Ethical commentary
17. References:
18. Keywords for literature searches

5. CONCLUSION

This chapter deals with some of the underlying philosophical issues concerning the use of animals in research. Taking the view that it is unethical to carry out research that is badly designed, or that causes more animal pain, distress, and suffering than is necessary to achieve the scientific objective, I have concentrated on practical ways in which that may be

TABLE II
Refinement Considerations

1. CONSIDER HOW ENVIRONMENTAL ASPECTS MIGHT AFFECT THE ANIMALS AND THE EXPERIMENT

Husbandry (e.g., enrichment):

- Complexity of environment
- Interaction with humans and other animals (avoiding single caging or single penning of animals, stable groups, in sight of others
- Novelty (e.g., diet, enrichment objects)

2. CONSIDER HOW SCIENTIFIC PROCEDURES CAN BE IMPROVED

Controls (necessity or use of historical data)
Staging of experiments (critical experiments, relationship in vitro to in vivo experiments)
Surgery and anesthesia (asepsis, modern anesthetics, analgesics)
Euthanasia (humane, skilled persons)
Statistics and experimental design (too many/too few?; right design; obtain professional advice before starting work)
Endpoints (humane and scientific, avoid painful endpoints where possible)
Monitoring adverse effects (score sheets)
Education, training, and competence
Question tradition and obtain data to support scientific and humane suppositions
Database for animal models

identified and remedied. The guidance I have provided in the tables, I hope, will act as an *aide memoire* for animal care and use/ethics committees.

REFERENCES

Acred, P., Hennessey, T. D., MacArthur-Clarke, J. A., Merrikin, D. J., Ryan, D. M., Smulders, H. C., Troke, P. F., Wilson, R. G., & Straughan, D. W. (1994) Guidelines for the welfare of animals in rodent protection tests. A report from the Rodent Protection Test Working Party. *Laboratory Animals* 28, 1–8.

AVMA (1987) Colloquium on recognition and alleviation of animal pain and distress. *J. Amer. Vet. Med. Assn.*, 191, 1184–1294.

Bayne, K. A. L., Mench, J. A., Beaver, B. V., & Morton, D. B. (2002) Laboratory animal behavior. In: *Laboratory animal science* ACLAM series (2nd ed., pp. 1240–1264). New York: Elsevier.

CCAC (1998) *Guidelines on: choosing an appropriate endpoint in experiments using animals for research and testing*. Available from Canadian Council on Animal Care, Constitution Square, Tower II, 315–350 Albert Street, Ottawa, Ontario, Canada K1R 1B1. ISBN 0-919087-33-7. pp. 30.

Cussler, K., Morton, D. B., & Hendriksen, C. F. M. (1999) Humane endpoints in vaccine research and quality control. In: *Humane endpoints in animal experiments for biomedical research*. C. F. M. Hendriksen & D. B. Morton (Eds.) (pp. 95–101). London: Royal Society of Medicine.

Davis, H., & Balfour, D. (1992) *The inevitable bond: examining scientist-animal interactions*. Cambridge: Cambridge University Press.

Dawkins, M. S. (1990) From an animal's point of view: motivation, fitness and animal welfare. *Behavioural Brain Science* 13: 1–61.

Dawkins, M. S. (1992) *Animal suffering: the science of animal welfare*. 2nd ed. *London: Chapman & Hall*.

Dawkins, M. S. (1993) *Through our eyes only. The search for animal consciousness*. New York: W. H. Freeman Spektrum.

Dean, S. W. (1999) Environmental enrichment of laboratory animals used in regulatory toxicology studies. *Laboratory Animals* 33: 309–327.

Duncan, I. J. H., & Molony, V. (1986) *Assessing pain in farm animals*. Commission of the European Communities Brussels.

Ellery, A. W. et al. (1985) Working Committee for the Biological Characterization of laboratory animals/GV-SOLAS. Guidelines for specification of animals and husbandry methods when reporting the results of experiments. Laboratory Animals 19(2): 106–108.

Festing M. F. W., Overend P., Gaines Das R., Cortina Borja M., & Berdoy M. (2002) The Design of Animal Experiments: Reducing the Number of Animals in Research through Better Experimental Design. Laboratory Animal Handbooks Series, 14. London: Royal Society of Medicine Press.

Flecknell, P. A., & Waterman-Pearson, A. (Eds.) (2000) *Pain management in animals*. London: WB Saunders: pp. 147–60.

Hendriksen, C. F. M., & Morton. D. B. (Eds.) (1999) Humane endpoints in animal experiments for biomedical research. London: *Royal Society of Medicine*.

ILAR Institute of Laboratory Animal Resources (National Research Council) (2000) Humane endpoints for animals used in biomedical research and testing. *ILAR* 41(2).

ILAR Institute of Laboratory Animal Resources (National Research Council) (2002) Implications of human-animal interactions and bonds in the laboratory. *ILAR* 43(1).

ILAR/NRC Institute of Laboratory Animal Resources and National Research Council. (1992) *Committee on Pain and Distress in Laboratory Animals: Recognition and alleviation of pain and distress in laboratory animals*. Washington, DC: National Academy Press.

Mellor, D. J., & Morton, D. B. (1997) Humane endpoints in research and testing. Synopsis of the workshop. In: *Animal alternatives, welfare and ethics* (pp. 297–299). L.F.M. van Zutphen & M. Balls. (Eds.) Amsterdam, The Netherlands. *Elsevier Science BV*.

Morton, D. B. (1992, April 11) A fair press for animals. *New Scientist* 1816: 28–30.

Morton, D. B. (1997) Ethical and refinement aspects of animal experimentation. In: *Veterinary vaccinology* (pp. 763–785). P.-P. Pastoret, J. Blancou, P. Vannier & C. Verscheuren. Amsterdam: Elsevier Science BV.

Morton, D. B. (1998, June) The importance of non-statistical design in refining animal experimentation. ANZCCART Facts Sheet. *ANZCCART News* 11(2) Insert. ANZCCART, PO Box 19, Glen Osmond, SA5064, Australia.

Morton, D. B. (1999) Ethical aspects of the use of animal models of infection. In: *Handbook of animal models of infection* (pp. 29–48). Otto Zak (Ed.). San Diego: Academic Press.

Morton, D. B. (2000) A systematic approach for establishing humane endpoints. *ILAR Journal* 2000, 41(2): 80–86.

Morton, D. B. (2002) The importance of non-statistical experimental design in refining animal experiments for scientists, IACUCs, and other ethical review panels. *In*: Applied ethics in animal research. philosophy, regulation, and laboratory applications (pp. 49–178). John P. Gluck, Tony DiPasquale, F. Barbara Orlans (Eds.). West Layfayette, IN: Purdue University Press.

Morton, D. B., & Griffiths, P. H. M. (1985). Guidelines on the recognition of pain, distress and discomfort in experimental animals and hypothesis for assessment. *Veterinary Record* 116:431.

Morton D.B., & Hau J. (2002) Welfare assessment and humane endpoints. In: *CRC handbook of laboratory animal science Essential Principles and Practices* (vol. 1., 2nd ed., pp. 457–486). Jann Hau and Gerald LVan Hoosier, Jr. (Eds.). Boca Raton, FL: CRC Press.

OECD (2001) Environmental health and safety publications series on testing and assessment no. 19: Guidance document on the recognition, assessment, and use of clinical signs as humane endpoints for experimental animals used in safety evaluation environment directorate. Retrieved from http://www.oecd.org/ehs/.

Pakes, S. P., Yue-Shoung, L. & Meunier, P. C. (1984) Factors that complicate animal research. In: *Laboratory animal medicine* (pp. 649–665). J. G. Fox, B. J. Cohen & F. M. Loew (Eds.). San Diego: Academic Press.

Paul-Murphy, J., Ludders, J. W., Robertson, S., Gaynor, J. S., Hellyer, P. W. & Wong, P. (2004) The need for a cross-species approach to the study of pain in animals. *J. Amer. Vet. Med. Assoc.* 224: 692–697.

Poole, T. B. (1992). The nature and evolution of behavioural needs in mammals. *Animal Welfare* 1: 203–220.

Reese, E. P. (1991) The role of husbandry in promoting the welfare of animals. In: *Animals in biomedical Research* (pp. 155–192). CFM Hendriksen & HBWM Köeter (Eds.). *Amsterdam: Elsevier.*

Russell W. M. S. and Burch R. L. (1959) *The principles of humane experimental technique.* London: Methuen.

Ryder, R. D. (1975) *Victims of science—The use of animals in research. National Anti-vivisection Society.* Davis-Pointer Ltd. GB.

Smith, J. A., & Boyd, K. (Eds.) (1991) Lives in the balance. Oxford: Oxford University Press

Soothill, J. S., Morton, D. B., & Ahmad, A. (1992). The HID50 (hypothermia-inducing dose 50): an alternative to the LD50 for measurement of bacterial virulence. *Internatl. J. Exper. Pathol.* 73: 95–98.

Townsend, P. (1993). Control of pain and distress in small laboratory animals. *Animal Technology* 44: 215–226.

van den Heuvel, M. J., Clark, D. G., Fielder, R. J., Koundakjian, P. P., Oliver, G. J. A., Pelling, D., Tomlinson, N. J., & Walker, A. P. (1990). The international validation of a fixed dose procedure as an alternative to the classical LD50 test. *Food Chemical Toxicol.* 28: 469–482

Wallace, J., Sanford, J., Smith, M. W., & Spencer, K. V. (1990) The assessment and control of the severity of scientific procedures on laboratory animals. Report of the Laboratory Animal Science Association Working Party. *Laboratory Animals* 24: 97–130.

15

Future Perspectives

MAURO PISTELLO

Animal research has served a major role in the development of modern medicine and as clearly inferred from Dr. Gardner's "Historical Perspective" and chapters focused on specific lentiviruses, much of today's vast knowledge concerning the biology and pathogenesis of viral infections has been achieved by in vivo animal studies. Although a never-ending issue of political controversy, there is little doubt that progress in medicine and science are intimately connected to animal research.

An understanding in depth of mechanisms of pathogenesis and, perhaps more importantly, development and assessment of vaccines against lentiviruses require systematic and well-controlled in vivo experiments. Fortunately, continuous advances in basic and applied research have permitted improvement of conventional in vitro techniques and development of alternative methods replacing many in vivo experimentation and have allowed adherence to the "Three Rs", the enunciation of ethic principles of animal research proposed in 1959: refinement, reduction, and replacement. Scientists pledge to refine their techniques so as to induce the minimum suffering, reduce the number of animals used, and replace animals with other techniques whenever possible. While it is obvious that every conscientious scientist designs and sets up experiments in such a way as to minimize animal use, it is not obvious, especially to the nonscientific community, why animal research is still necessary (mandatory for microbiology and many other medical disciplines) in today's world.

The recent advent of molecular techniques and improvement of new immunological assays permits dissecting and understanding important key aspects of the natural history of virus infection and virus-host relationships. In human immunodeficiency virus (HIV) and other lentiviral infections, it has been clearly documented that the extent of virus replication plays an important role in accelerating the course of disease and, on the opposite side, both neutralizing antibodies and cell-mediated immune responses provide effective protection from infection and disease progression. It is not known, however, which neutralizing antibodies successfully block virus

MAURO PISTELLO • University of Pisa, Pisa, Italy

infectivity in vivo and their effective titer or the type of CD4+ and CD8+ T-cells that coordinate the antiviral immune response and kill productively and latently infected cells. Finally, cells and tissue compartments where lentiviruses hide and persist are not clearly defined. The lack of understanding of these issues and the absence of definitive experimental evidence that certain types of immune responses are indeed immune correlates of protection support the view that more basic research (i.e., experiments with animal models) is needed for cure of disease and development of the efficacious vaccines.

In this context, computer models of infection and disease and the technologies developed in the past few years have helped clarify some of these issues. The endless increase of computing capacity has allowed development of defining models of virus replication kinetics, persistence and clearance from the host's body, and efficacy of an immune response. Although a computer model is only as successful as the person who develops the model, and is therefore limited by what is already known about a disease, such a model is undoubtedly useful for predicting the course of infection and disease and calculating the efficiency of antiviral treatments. Practical implications of mathematical models either applied to humans or animals are countless. Such models, however, need to be tested and validated in vivo. Ad hoc animal model experiments in which virus and host factors are monitored and compared to computing model outputs are essential steps before entering into human research and ultimately clinical use.

Likewise, thanks to the amazingly rapid increase of computing power and development of new compounds and labeling techniques, in vivo imaging has reached sensitivity and resolution levels unthinkable only a few years ago. Positron emission tomography (PET), for instance, is one of the most widely used imaging techniques and is extensively used in clinical settings for analyzing tissue metabolism and detecting tumor and inflammatory processes. Today PET imaging can detect in a human body femptomols of radio-labeled substrate with a spatial resolution below $2\,mm^3$. Such performances and noninvasiveness have propelled PET scan analysis into a remarkably powerful tool for early and precise diagnosis of a wide variety of human diseases. Thanks to versatility and recent cost reduction, as well as scaling down of instrumentation, PET analysis is currently widely used in animal research. When appropriately delivered, PET reporter genes encoding receptors that bind positron-emitting ligand probes or enzymes that modify positron-emitting substrate probes readily permit visualization of cells expressing or retaining the PET reporter probe. Recently, an adenovirus expressing herpes virus type 1 thymidine kinase injected into mice was readily traced following injection of ganciclovir radio-labeled with ^{18}F. Application of this technique for monitoring the journey of lentivirus in the body might give useful information about the primary sites of virus

replication and, even more importantly, the sites where the virus hides and persists during the chronic phase of infection or antiviral treatment. Finally, PET imaging could also be useful in vaccine experiments for studying the countermeasures adopted by the virus itself to avoid the adoptive immune response.

Further emerging fields in which animal research is manifestly mandatory are developments of virus vectors and new strategies for drug and vaccine delivery. Virus vectors are a novel procedure to devise useful components to an otherwise dangerous substance. In nature, a virus can carry a genome and its harmful cargo into specific cells. Within these cells, a virus can activate its machinery, replicate, egress, and eventually cause the cell's death. It has been hypothesized that, if appropriately engineered, a virus may deliver heterologous genetic material into specific cells. This property can be exploited for treating genetic diseases and for immunization purposes. Transduction, as this phenomenon has been called following its discovery in bacteriophages, has been successful with a variety of engineered viruses. When integrated into proteins interacting with a cell receptor(s), the vector particles also evince a different cell tropism compared to their unmodified counterpart and are therefore capable of penetrating cells otherwise resistant to wild-type particles.

Lentivirus-derived vectors (lentivectors) have been found to be very good as a vehicle for prolonged duration of expression of a heterologous gene (transgene) and also have unmatched capability of delivering a transgene into quiescent cells, which constitute the overwhelming majority of cells in a living organism. However, a few lentivectors have been shown to transduce terminally differentiated cells such as neurons, lymphocytes, dendritic cells, muscular fibers, and so forth. Needless to say, virus vectors must be tested for safety, efficiency as a carrier of a transgene, and therapeutic benefit in vivo. Whereas virus vectors have been first employed for correcting genetic defects, in subsequent experiments vectors have been used for delivering immunogens for possible vaccination against several neoplasms and infectious diseases. In this context, other delivery systems such as biopolymers, conjugated antigens, electric and biological devices, alone or in different combinations, are being tested in vivo, once again underlining the importance and necessity of animal research for developing safe and efficacious vaccines and antiviral compounds.

Last but not least and, albeit controversial, it is of value to mention genetically modified animals and animal cloning. These techniques can indeed be useful in basic research. Genetically modified animals, although not a natural model per se, might permit successful study and dissection of host factors important for containing infection and disease. On the other hand, injection of intact virus, deleted mutants or different virus amounts into cloned animals may help us to understand the genetic determinants of virulence.

Previous chapters and this brief and certainly incomplete review emphasize the crucial role of animal research for studying lentiviral infection and devising new preventative and therapeutic intervention strategies. Although progress in biomedical research has permitted some alternatives and surrogates for animal use, there is little doubt that future medical science advances remain heavily dependent upon animal research. There are many good reasons to be proud of past achievements using animals and to expect that similar breakthroughs will follow in the future.

Index