FELINE IMMUNODEFICIENCY VIRUS: A CONCISE REVIEW

Luis Isamu Barros Kanzaki 1, and David J. Looney 2,3

1 Federal University of Amapa, Departament of Microbiology, Rodovia Juscelino Kubitscheck de Oliveira, Km 02, Macapa, Amapa, Brazil. CEP 68902-280, Brasil, 2 San Diego VA Healthcare System, Infectious Disease 9-111F, 3350 La Jolla Village Drive, San Diego, CA 92161, 3 University of California, San Diego. Department of Medicine, 0678, La Jolla, CA 92037-0678

TABLE OF CONTENTS

1. Abstract
2. Introduction
3. Structural and Molecular Characterization
4. Epidemiological Features
5. Clinical Features
6. Features of Virus Replication
7. FIV Vector Systems
8. Pathogenesis and Immunity
9. FIV Vaccine Development
10. Perspective
11. Concluding Remarks
12. Acknowledgment
13. References

1. ABSTRACT

Among non-primate vertebrates, feline immunodeficiency virus (FIV) infection in the cat may be the closest model of human immunodeficiency virus (HIV) infection and acquired immunodeficiency syndrome (AIDS). Clinical evolution and immunological and virological relationships between human HIV/AIDS and disease produced by FIV infection in cats are very close. These similarities should facilitate progress in the understanding of mechanisms of viral infection and immunopathology, and make this model potentially very valuable in evaluation of experimental therapeutic approaches to AIDS in man. Development of feline immunodeficiency virus vectors bearing therapeutic genes targeting different human diseases is a promising strategy for gene therapy, despite some recent studies which suggest that despite lack of evidence of infection of man by FIV, additional epidemiological surveillance may be indicated to determine if transmission can occur from this close companion to humans in some circumstances.

2. INTRODUCTION

Feline Immunodeficiency Virus (FIV) was first isolated by Pedersen et al (1) in 1986, from a domestic cat (Felis catus) with chronic opportunistic infection and neurological disease, presenting a clinical picture reminiscent of human acquired immunodeficiency syndrome. Retrospective serological analysis performed by Shelton et al (2) in the U.S. and Furuya et al (3) in Japan showed widespread evidence of FIV infection among cats as early as 1968, indicating that dissemination of FIV is not likely to be recent. In contrast, there is no evidence of human infection, despite opportunity for frequent exposure (4).

Like human immunodeficiency virus (HIV) and simian immunodeficiency virus (SIV), FIV is a member of the retroviridae family, lentivirinae subfamily, genus lentivirus (1,5), though FIV is more closely related to ungulate lentiviruses (equine infectious anemia virus or EIAV, visna virus, caprine arthritis encephalitis virus or CAEV) than to primate lentiviruses genetically. Like these other lentiretroviruses, and unlike murine retroviruses, FIV possesses the capability of infecting non-dividing cells as well as dividing cells (6).

The biology of FIV has attracted special attention due to clinical and pathologic similarities to human HIV infection (1) as a potential small animal model of AIDS (7), similarities in genomic organization and structural and non-
structural genes between FIV and HIV-1 which provide opportunities for comparative virology and elucidation of gene function, and similarities in replication and cell tropism, including the use of the CXCR4 coreceptor for viral entry. Another important reason for interest in FIV is its potential for use in vector systems for gene therapy of both infectious diseases, including AIDS, inherited diseases as cystic fibrosis and many other diseases (6-10), where the use of FIV instead of primate lentivirus may reduce concerns about recombination, mobilization, or exposure (and potentially seroconversion) to HIV proteins.

3. STRUCTURAL AND MOLECULAR CHARACTERIZATION

Mature FIV virions measure 105-125 nm in diameter, exhibiting spherical to ellipsoid morphology, with sparse and short spikes on its envelope (1,12). The FIV virion genome is 9,200 nucleotides in length, and FIV virions are diploid, containing two copies of positive strand RNA. In the general manner of all exogenous retroviruses, the FIV genome contains from 5' to 3' gag, pol, and env flanked at both sides by long terminal repeat (LTR) sequences. Each LTR is comprised of untranslated 3' (U3, promoter/enhancer elements), repeat (R, from the RNA start site, containing the polyadenylation signal), and untranslated 5' (U5) regions. The FIV proviral genome generates 6 viral mRNA classes of 9.8 kb, 5.0 kb, 4.4 kb, 2.0/1.9 kb and 1.2 kb which can be grouped in 3 classes: the unspliced full-length mRNA (9.8 kb), the singly spliced medium sized mRNAs (5.0 and 4.4 kb) and the small commonly multiply spliced mRNA (2.0/1.9 kb doublet and 1.2 kb), which represent the gag/pol, vif/env, env, orf2/rev and rev transcripts respectively. The gag gene encodes the 15kDa matrix (MA), 25 kDa capsid (CA) and 7/10 kDa nucleocapsid (NC) proteins and the pol gene encodes the 13 kDa protease, 61 kDa reverse transcriptase, 14 kDa dUTPase and 13 kDa integrase, while the env gene encodes 120/100 kDa surface (SU) and 44 kDa transmembrane (TM) envelope glycoproteins. The gag gene encodes a 23-29 kDa protein and the ORF2/ tat like gene encodes a 9 kDa protein. The rev gene overlaps the 5' env gene and the 3'LTR encoding a 23 kDa protein. Additional 2 mRNAs derived from the env region were identified coding for Rev-related proteins of 11 and 15 kDa (5,12,13,14,15). Also, an antisense open reading frame is transcribed antisense to the rev responsive element at the 3' terminus of the env sequence, producing a theoretical product of molecular weight 11.4 kDa, designated ASP (16).

Among lentiviruses, only FIV, equine infectious anemia virus (EIAV) and Visna/Maedi virus encode a deoxouridine pyrophosphatase (dUTPase), which is responsible for deoxouridine triphosphate (dUTP) degradation, which may aid viral replication by reducing or avoiding misincorporation of dUTP in the viral genome and providing substrate (dUMP) for deoxothymidine triphosphate (dTTP) synthesis (17). As in other lentiviruses, the envelope gene product is proteolyzed into surface (SU) and transmembrane (TM) proteins. Molecular diversity in FIV isolates occurs mainly in variable segments of the envelope gene, but glycosylation sites and cysteine residues are largely or completely conserved. Proper folding and modification of the envelope is necessary for receptor-mediated tropism of the virus, fusion activity, and recognition by host cell immune responses to infection (7,12). Comparisons among FIV, HIV and other lentiviral structural models of the spatial folding of TM and SU glycoproteins exhibited considerable similarities, presumably indicating the existence of core conserved env sequences with certain levels of variation, mainly determined by pressure of the host immune response and other exogenous factors (7,18).

As in other lentiviruses, FIV gag gene generates an MA, CA and NC proteins during the budding process by a viral protease cleavage of a precursor polyprotein (p55). FIV morphogenesis mechanism directed by the MA protein is similar in HIV and SIV even though there is few amino acid sequence similarity in the MA protein among these viruses (18). A conserved cysteine residue 190 (Cys 190) of FIV, HIV and all immunodeficiency retroviral gag protein plays a vital role in the capsid protein assembly, and is indispensable for virus infectivity (20).

FIV and HIV protease are structurally similar, but amino acid sequence and mutational analyses have shown differences in enzymatic activity; FIV protease is more efficient at cleaving at sites with hydrophobic residues such as valine in the P2 position, compared with either asparagine (basic) or glutamine or glutamic acid (acidic) residues in this position (21). Conversely, FIV protease seems to prefer hydrophilic residues such as serine at the P1 position, rather than hydrophobic residues such as valine. This pattern of preferences are also typical of HIV proteases found in strains resistance to current protease inhibitor drugs, and FIV protease contains residues identical to those associated with resistance in HIV protease at six different homologous locations.

The viral infectivity factor (vif) has been detected in all lentiviruses except in EIAV and appears to be essential for production of infectious viral particles in some producer cells, including primary peripheral blood mononuclear cells and monocyte derived macrophages (22). Although primate and non primate lentiviruses' vif genes have little amino acid sequence similarity, a single conserved motif (cysteine region) is critical for viral infectivity (23). As for HIV, FIV vif is present in both the cytoplasm and nucleus, and appears to colocalize with gag in the cytoplasm (24,25). Whether the same or different cellular proteins, such as HP68 (26) or CEM15/Apobec3G (27), are involved in interactions with HIV and FIV vif is currently unknown.

Just as for all other lentiviruses, FIV rev is vital for viral replication and packaging, acting by transporting unspliced and singly spliced viral mRNA from nuclear localization to the cytoplasm by interaction with the cis-acting Rev-responsive element (RRE) and a cellular factor, the chromosomal region maintenance protein (Crm 1) (12,14,22). FIV and EIAV rev both possess atypical nuclear export signals (NES) which bind to CRM1 (or exportin), and, distinct from HIV and other lentiviruses, do
not present organized hydrophobic residues and core tetramer motifs (12). The FIV RRE is 243 nucleotides in length, and is located at the 3’ end of the env region, rather than more centrally as in the env gene of HIV (12) FIV does not appear to have an equivalent of the nef gene of primate lentiviruses.

4. EPIDEMIOLOGICAL FEATURES

FIV is a cosmopolitan infectious agent with a broad range of the Felidae family hosts (28) including domestic cats, lions (Panthera leo), leopards (Panthera pardus), tigers (Panthera tigris), pumas (Felis concolor), snow leopards (Panthera uncia), jaguars (Panthera onca), Pallas cat (Felis manul), flat-headed cats (Ictailurus planiceps), Cheetahs (Acinonix jubatus) and bobcats (Lynx rufus).

Seroepidemiological surveys have shown an overall seroprevalence of 11.04 % (9,750 out of 85,529) worldwide, among both healthy and ill felines screened in North America, Asia, Europe and Oceania. It is estimated that approximately 44 million cats are FIV infected as the total cat population in the world is around 400 million. This is likely to be an underestimate since it does not take into account the fact that that 10 to 15 % of FIV infected cats are seronegative (28). The highest FIV prevalence is found among ill cats, ranging from 47% in England (29) 30% in Japan (30) 21 % in Australia (26) to 19% in Canada (27).

FIV infection occurs mainly through biting among cats fighting for territorial demarcation and during mating. The virus has been isolated from saliva, blood, serum, plasma, and genital secretions (28,31,32). Horizontal transmission appears to be predominantly mediated by biting, but the presence of FIV has also been demonstrated in semen (34) and vaginal washings in experimental transmission; therefore, male and female reciprocal FIV transmission in the wild may possibly proceed by sexual routes as well (33,34). Vertical transmission of virus during acute maternal experimental infection occurs, either through neonatal exposure to virus present in milk, or infection of the fetus in utero (31,35,33).

FIV viral isolates have been grouped in 4 subtypes, A, B, C and D, based on molecular analysis using a heteroduplex mobility assay employing the V3-V4 regions of envelope. Eighty percent of FIV viral isolates worldwide belong to the A or B subtypes, but viral recombinants of A/B, B/D and A/C subtypes are also observed. Evidence suggests FIV subtype B is the oldest, and is more host adapted than the others (36).

5. CLINICAL FEATURES

Infection by FIV can be classified in four stages: acute, asymptomatic, non specific, and terminal AIDS-like disease. The acute or initial phase is primarily characterized by clinical signs that could persist for several weeks, which include hyperthermia, diarrhea, inflammatory process of the conjunctivae, gingiva, and uvea, generalized lymphadenopathy, jaundice, secondary bacterial sepsis, and neutropenia, usually related to opportunistic infection (31). The latent or asymptomatic phase can last up to 5 years, during which infected animals seem apparently healthy, although the virus can usually be isolated.

The clinical literature indicates that about half of FIV infected cats develop non specific signs of the third phase disease, including generalized lymphadenopathy, recurrent fever, apathy, leukopenia, anemia, anorexia, weight loss, chronic stomatitis and behavioral abnormalities. These signs can last from several months to one year. Animals that progress to the terminal AIDS-like phase develop AIDS related complex symptoms, opportunistic infections (including cryptococcosis, toxoplasmosis, and a variety of other viral infections), neoplasia, and neurological abnormalities which may eventually lead to the death of the host (7,31).

Diagnosis is easily accomplished either using a variety of inexpensive commercial ELISA kits, or alternatively using PCR based techniques (37). Most animal shelters screen for both feline leukemia and feline immunodeficiency virus routinely. As noted above, the use of DNA based screening may detect up to 15% additional animals which are infected, yet nonetheless seronegative.

Treatment at present is largely supportive, although the combination of lamivudine (3TC) and zidovudine (AZT) has been shown to be synergistically inhibitory in vitro (38) and use of AZT has been explored (39). The use of AZT alone in juvenile infected cats reduces viral burden, but does not seem to prevent thymic atrophy or immunodeficiency (40).

6. FEATURES OF VIRUS REPLICATION

FIV infects a variety of cell types, exhibiting tropism for both CD4+ and CD8+ T-cells. In addition, FIV readily infects non-dividing cells, including macrophages and microglia targets (35-37). Initially, studies suggested that CD9 might represent a primary receptor for FIV. CD9 resembles human CD9 closely, except that it is not glycosylated in the first extracellular loop (41). Later studies showed that while CD9 monoclonal antibody blocked FIV infection, this effect was mediated at a postentry stage of the viral life cycle (42). Like HIV-I, FIV also utilizes CXCR4, the CXC chemokine receptor for SDF-1, as a coreceptor or perhaps as a sole receptor for cell entry (37,43). It has been shown that a 40 kDa protein, preliminary detected in the surface of primary T cells could possibly be utilized as a coreceptor for FIV besides CXCR4 (45). Also, surface heparan sulfate proteoglycans (HSPGs) may function as coreceptors for FIV cell entry, as occurs with HIV-I (45,46). Utilization of common receptors or coreceptors by distantly related lentiviruses suggests a common link to induction of disease (43,44,45,47, 49).
viral nucleocapsid in the cell cytoplasm (43,44,45,49). The viral RNA is reverse transcribed by the viral reverse transcriptase yielding an incompletely paired circularized DNA. A DNA flap, overlying the central polypurine tract (cPPT) and ending in central termination sequences (CTS), plays a key role in the transport of preintegration complexes into the nucleus (46). There are three forms of retroviral RNA reverse transcribed products: the early (LTR), intermediate (LTR-Gag) and late (circular) viral DNA forms. The viral integrase catalyses the viral DNA integration in the host cell genome. While an integrated provirus is the final product of reverse transcription, two circularized forms of unintegrated viral DNA may coexist in the nucleus during productively FIV infection (50,51).

7. CROSS PACKAGING AND PRIMATE INFECTION

FIV is capable of packaging HIV-1, HIV-2, and SIV RNAs, but not type C or D retrovirus RNA (unpublished, 52). While some investigators have found FIV RNA to be packaged by SIV or HIV-1, our studies show that FIV RNA is very poorly packaged by primate lentiviruses (relative efficiency <0.1%). This asymmetric cross packaging suggests that the requirements for encapsidation by FIV are less stringent than those for primate lentivirus.

One report has indicated infection of human PBMC by FIV (54), and a recent report indicates that *Cynomolgus macaques* show evidence of limited virus replication (virus was detectable from 4 to 9 weeks after infection using nested PCR) after injection of autologous cells infected *in vitro* at a high multiplicity of infection with a virus preparation of uncloned FIV grown in feline cells. The two animals showed decline of CD4 lymphocyte count of ~60% at up to 12 weeks. Note, however, the potential role of other endogenous feline viruses, such as RD114 which might pseudotype FIV, was not addressed by the experimental methodologies used. These results must be considered very preliminary, and should not be extrapolated to suggest that human infection can occur, given the demonstrated lack of human seroreactivity despite exposure to pets and in veterinary practice.

8. FIV VECTOR SYSTEMS

Several groups have developed vector systems based on FIV (46,55) and have explored use in a variety of systems. The use of FIV avoids concerns about inadvertent seroconversion to HIV proteins with laboratory or clinical use of vector preparations that occur when using HIV-1 based lentiviral vectors, and may provide additional layer of insurance against mobilization of vector in vivo by HIV infection, though this is also obviated by the use of SIN vectors.

FIV vector systems have been used in experiments investigating transduction of retina, brain, adrenal cells, middle ear epithelium, primary lymphocytes and T-lymphoblastic cell lines, human terminally differentiated neurons, and other tissues *in vitro* and in animal model systems (8-11). The range and tropism of vector pseudotyped with different envelopes is still being defined, as is the optimal structure and elements to be included in both vector and packaging constructs. Similarly, the suitability of FIV systems for transduction of hematopoetic stem cells, and embryonic stem cell is under investigation (56,57), though some results suggest that there may be blocks to efficient transgene expression from FIV vectors in some cell types. (58). These may possibly be avoided by introduction of additional 3’ deletions.

9. PATHOGENESIS AND IMMUNITY

The mechanism by which FIV produces selective CD4 depletion and immunodeficiency, despite infection of both CD4 and CD8 lymphocytes as well as other cell types, is currently unknown. The use of CXCR4 as a receptor by some FIV strains is intriguing, given that destruction of CD4 cells in HIV disease seems to be accelerated in individuals with strains utilizing CXCR4 (syncytium inducing or SI strains). However, immunodeficiency also occurs without use of this coreceptor in both FIV and HIV induced disease. Like HIV, FIV produces thymic atrophy, and can effect production of new immune precursor cells in the bone marrow (59-61).

Replication in cells of monocyte/macrophage cell lineage is thought to be required for neurovirulence, but it is not sufficient, as both neurovirulent and non-neurovirulent strains can infect macrophages and mixed glial cell cultures with equal efficiency (62). Some have hypothesized that central nervous system (CNS) surveillance by T lymphocytes may cause release of factors inducing FIV replication in microglial cells or macrophages in the brain and/or which are directly toxic to neurons (such as matrix metalloproteinases) (49,62,63,64). FIV envelope may also directly produce neurotoxicity, through alterations in glutamate uptake and calcium signaling. Glutamate uptake by FIV infected astrocytes is decreased, while glutamate levels are high in the brains of cats with neurological disease. In the presence of low concentrations of glutamate, FIV envelope protein disturbing cellular calcium flux when infused into the CNS (49,65).

10. FIV VACCINE DEVELOPMENT

Partial protection (protection against the development of FIV disease) has been found among cats naturally infected by non pathogenic lentiviruses, including lions and pumas. Domestic cats infected with FIV strains from these animals appear to be relatively protected from FIV disease, if not infection (66). The mechanisms involved in this protection are not known, but viral interference, production of antiviral factors by CD8+ lymphocytes, CD8+ cytotoxic lymphocyte activity, and other innate immune responses have all been hypothesized to be involved (67).

FIV infection, particularly mucosal models of infection and maternal transmission, may provide an excellent model for development of an HIV vaccine. Already many lessons have been learned. Vaccines
incorporating multiple subtypes of FIV strains confer broader range protection against wild type strains of FIV than vaccines incorporating a single subtype (68). A vaccine utilizing an FIV vif-deleted mutant appears to confer broad and durable protection against homologous wild type challenge (69) somewhat analogous to nef-deleted SIV vaccine in non-human primates. FIV LTR mutants with deletion of the ATF-1 binding site, or other cis-acting transcriptional elements producing attenuation of virus replication, have been envisioned (68-70).

11. PERSPECTIVE

It is interesting to reflect that at the time when the first cases of human AIDS were described feline immunodeficiency virus infection had been silently occurring in our pets for many years, perhaps into antiquity. While some preliminary results suggest the possibility that human infection with FIV might be possible, domestic cats have been domesticated since the Egyptian Middle Kingdom, and have close physical contact with both adults and children, with no evidence of natural human infection. Perhaps through study of this terrible affliction of our feline friends, we will one day learn how to control HIV, and *Felis catus* will have given one more gift to humanity. Hopefully our study of HIV will help veterinarians learn how to return the favor.

12. ACKNOWLEDGMENTS

We wish to acknowledge the assistance of the CAPES/MEC/Brazil and the UCSD Center for AIDS Research, 2 P30 AI36214, in support of Dr. Kanzaki’s work at UCSD.

13. CONCLUDING REMARKS

The potential use of FIV based systems for gene therapy, and the use of FIV models of infection for the study of neurological and immunodeficiency disease, drug therapy of lentiviral infection, and as a model for development of HIV vaccine have prompted intense interest in the biology of feline immunodeficiency virus. However, while much has been learned about the virology of FIV, and the pathogenesis of disease in the last decade, much more remains to be elucidated.

14. REFERENCES


17. Tye BK, P.O. Nyman, I.R. Lehman, S. Hochmanhauser & B. Weiss: Transient accumulation of Okazaki fragments as a result of uracil incorporation into nascent DNA. *Proc Natl Acad Sci USA* 74, 154-157 (1977)


40. Kathleen AH, A.J. Phipps & S. Francke: Antiviral therapy reduces viral burden but does not prevent thymic involution in young cats infected with Feline
FIV Review


FIV Review


**Key Words:** Feline Immunodeficiency Virus, Human Immunodeficiency Virus, Lentivirus, Animal models, Vaccine, Review

**Send correspondence to:** David J. Looney, Dept. of Medicine 0678, UCSD/SCRB 302, La Jolla, CA 92037. Tel: 858-552-8585 x2626, Fax: 858-642-6476, E-mail: dlooney@ucsd.edu