INFECTIONS OF FELINE LEUKEMIA VIRUS AND FELINE IMMUNODEFICIENCY VIRUS

Takayuki Miyazawa

Research Center for Emerging Infectious Diseases, Research Institute for Microbial Diseases, 3-1 Yamadaoka, Suita-city, Osaka 565-0871, Japan

TABLE OF CONTENTS

1. Abstract
2. Introduction
3. Classification of feline retroviruses
   3.1. RD-114 virus
   3.2. Endogenous FeLV-related sequences
   3.3. Mac-1
   3.4. FeLV
   3.5. FIV
   3.6. FeFV
4. General feature of FeLV infection
   4.1. The genomic structure of FeLV
   4.2. Immune responses to FeLV
5. Subgroups of FeLV
6. Natural resistance to FeLV-B
7. Receptors of FeLV-A, B and C
8. Cofactor for infection of FeLV-T
   8.1. FeLIX
   8.2. Paradox of FeLIX
9. Discordant clinical samples
10. General feature of FIV infection
    10.1. The genomic structure of FIV
    10.2. Immune responses to FIV
11. Receptors of FIV
12. Concluding remarks
13. Acknowledgements
14. References

1. ABSTRACT

Feline retrovirus infections have been extensively studied for more than 30 years as an animal model for the persistent infections and pathogenesis caused by retroviruses in general. Two retroviruses, feline leukemia virus (FeLV) and feline immunodeficiency virus (FIV), have been recognized as causative agents of a variety of diseases including proliferative and degenerative diseases. Recent studies revealed the receptors of FeLV, its variants and FIV. FeLVs utilize at least three distinct receptors, two of which have been successfully cloned and characterized. Furthermore, an FeLV variant which induces severe immunodeficiency, utilizes a truncated envelope of the endogenous FeLV as coreceptor or cofactor for viral entry. FIV utilizes as receptor one of the chemokine receptors, CXCR4 which also is a coreceptor for the T-lymphotropic human immunodeficiency virus. This review provides an overview to the infections of FeLV and FIV, specifically focuses on the viral genomic structures, FeLV variants, the immune responses and recent findings on the receptors for FeLV and FIV. Better understanding of retroviral persistence and pathogenesis will aid the development of prophylactic vaccines and therapeutic medicine to interfere with retrovirus infections.

2. INTRODUCTION

Feline retroviruses can be divided into two groups. Endogenous retroviruses are those that are transmitted vertically from parents to offspring via the germ lines. Their proviral DNA sequences can be found naturally integrated in the chromosomes of all cells of the hosts. There are at least three feline endogenous retroviruses: the RD-114 virus, endogenous feline leukemia virus (enFeLV) and the Mac-1. Exogenous retroviruses are transmitted horizontally. No footprint of the virus can be found in uninfected cells of the hosts. There are three feline exogenous retroviruses: the FeLV, feline immunodeficiency virus (FIV) and the feline foamy virus (FeFV).

Among the retroviruses, both FeLV and FIV are common and important pathogens of the domestic cats. FeLV was known to cause leukemia and was first isolated from a cat that had developed lymphosarcoma. However, FeLV can induce a variety of diseases, both proliferative and degenerative, but neoplastic disease is a relatively rare outcome of the virus-host interaction. The most frequent clinical manifestation of FeLV infection is severe immunodeficiency that gave secondary opportunistic
Infections of FeLV and FIV

Infections. Recent studies have revealed that the degenerative diseases and immunodeficiency are induced by variant forms of FeLV. On the other hand, FIV causes persistent infection. It induces acquired immunodeficiency syndrome (AIDS) in cats. FIV infection in cats is a useful small animal model and mirrors the pathogenesis of the human immunodeficiency virus (HIV) infection in humans. Studies of feline retrovirus infections in cats help us to better understand persistent infections and pathogenesis of retroviruses in general. The information also provides insight to assist with the development of prophylactic vaccines and therapeutic medicine against retrovirus infections.

3. CLASSIFICATION OF FELINE RETROVIRUSES

3.1. RD-114 virus

The RD-114 virus is an endogenous retrovirus of domestic cats (1, 2). In earlier studies, RD-114 virus is regarded as xenotropic, i.e., it does not productively infect feline cells, but only non-feline cells including human cells. However, my laboratory has recently shown that RD-114 virus can productively infect feline fibroblasts, such as AH927 and QN10S cells (Miyazawa et al., unpublished observation). Moreover, pseudotype assays by use of recombinant murine leukemia virus (MuLV) with an RD-114 envelope to infect feline cells revealed the presence of the receptors for RD-114, indicating that the RD-114 virus might be polytropic (Miyazawa et al., unpublished observation). The RD-114 viruses are expressed in cats, but only a single copy is active (3). Cats do not have antibodies to RD-114 virus, regardless of their health status, indicating that cats might be immunologically tolerant to RD-114 proteins (4). There is no evidence that RD-114 virus causes any disease in cats, or that RD-114 sequences recombine with exogenous feline retroviruses.

The envelope (env) gene of RD-114 has high homology to that of the baboon endogenous retrovirus (BaEV). Hence, a cross-species transmission in ancient times is suspected. However, recent demonstration by Van der Kuyl et al. (ref. 5) that the RD-114 virus is actually a recombinant between a newly recognized feline endogenous retrovirus sequence, FeEV (Felis catus endogenous retrovirus), and the env gene of BaEV indicates that RD-114 is not an equivalent of BaEV in cats (5). The FeEV is closely related to the primate Papio cynocephalus endogenous virus (5).

3.2. Endogenous FeLV-related sequences

The domestic cats and a group of wild Felidae harbor endogenous sequences related to FeLV (6-8). A number of the enFeLV sequences have been molecularly cloned (9, 10). Most of the enFeLV sequences are full-length copies, although a significant minority is truncated (10). Expression of the enFeLV proviral DNA is limited to subgenomic transcripts, and no infectious virus is produced (11-14). However, enFeLV sequences can recombine with exogenous FeLV to produce recombinant viruses and are involved in FeLV pathogenesis as described in Sections 5, 6 and 8 of this review.

3.3. Mac-1

The Mac-1 is an endogenous primate retrovirus originally isolated from a macaque cell line (15). The Mac-1 sequences are found in a number of carnivores, indicating that they have been present for several million years. The Mac-1 sequences are not related to the RD-114 or enFeLV sequences. In the cats, the Mac-1 sequences do not produce infectious viruses. There is no evidence that Mac-1 sequences recombine with exogenous feline retroviruses.

3.4. FeLV

The FeLV was first isolated from a cat that had developed lymphosarcoma in Scotland in 1964 (16). The FeLV is a member of the genus Gammaretroviruses. Seroepidemiological surveys conducted in the United Kingdom showed that the prevalence of FeLV was 4.5% and 17.5% in healthy and sick cats, respectively (17). Infection rates in Europe, the United States, Canada, Taiwan, and Japan ranged from 1.3% to 11.5% (18). Replication-competent FeLV isolates can be classified into three subgroups, A, B, and C (19, 20). The subgroup phenotype is determined by the viral envelope that utilizes three distinct host cell receptors. Interference to superinfection by a virus of the same subgroup arises from the blockade of receptors by the envelope glycoproteins expressed from the integrated viruses.

3.5. FIV

The FIV was first isolated in 1986 from a cat with an AIDS-like disease in the United States (21). The FIV is a member of the genus Lentiviruses. Infection by FIV in cats has been associated with AIDS-related diseases including stomatitis, gingivitis, anemia, neurological dysfunctions and others (22, 23). Seroepidemiological surveys conducted in several countries have revealed that the prevalence of FIV infection in cats varies from 1% to 15% in healthy cats and from 3% to 44% in diseased cats (24). Clinical epidemiological studies revealed that a wide range of clinical signs is seen in cats infected with FIV (17, 23, 25, 26). Generally, nonspecific signs, hematological changes and secondary infections such as chronic gingivitis/stomatitis, upper respiratory tract infection and diarrhea frequently occur (17, 23, 25, 26). Like HIV-1 infection in humans, FIV infection in cats can be staged into five phases (26): the acute phase, the asymptomatic carrier phase, the persistent generalized lymphadenopathy phase, the AIDS-related complex phase, and the AIDS stage. The terminal AIDS stage is usually characterized by a number of chronic infections of a secondary or opportunistic nature and/or neurological disorders.

3.6. FeFV

The FeFV has been isolated from normal and neoplastic tissues of cats (27-29). The FeFV is a member of the genus Spumaviruses. No clinical disease is known to be associated with FeFV infection. The virus is generally considered to be apathogenic. Isolation of FeFV has been reported in many countries (29-31). Seroepidemiological surveys conducted in Japan and Taiwan revealed that the infection rates of FeFV in domestic cats varied from 13.7% to 28% (32-34).
Infections of FeLV and FIV

4. GENERAL FEATURE OF FELV INFECTION

4.1. The genomic structure of FeLV

The FeLV possesses the essential structural and enzymatic genes \( \text{gag} \), \( \text{pol} \) and \( \text{env} \) which are flanked by long terminal repeats (LTR’s; ref. 35 and figure 1). The 5’-LTR regulates viral transcription. At least ten proteins are encoded by the FeLV genome. The \( \text{gag} \) gene encodes the viral structural proteins p15 (matrix protein, MA), p12 (unknown function), p27 (capsid protein, CA) and p10 (nucleocapsid protein, NC). The \( \text{pol} \) gene encodes the enzymatic proteins p14 (protease, PR), p80 (reverse transcriptase, RT), and p46 (integrase, IN). The \( \text{env} \) gene encodes the envelope proteins (Env), i.e., the gp70 surface unit (SU) and the p15E transmembrane (TM) proteins.

The glycocylated Gag protein (gp40\text{gag}) is synthesized and processed independently of the precursor Gag polyprotein (pr65\text{gag}) of the internal virion proteins. Translation of glycosylated Gag is initiated upstream of, and in frame with, that for pr65\text{gag}, yielding a protein pr80\text{gag} with a unique N-terminus of 15kDa but also carrying all of the components of pr65\text{gag} (35). Unlike pr65\text{gag}, pr80\text{gag} is glycosylated, cleaved by a cellular protease and released from FeLV-infected cells (36). The cleaved product of 40 kDa glycoprotein has epitopes of CA, p12, and NC, but not MA (36). This 40 kDa protein is not thought to be incorporated into the virion, and may be a type II integral membrane protein with a signal/anchor sequence. The function of this 40 kDa glycosylated Gag protein is unknown at present. However, in the studies of the glycocyslated Gag protein of MuLV, interruption of the expression of the glycocyslated Gag has demonstrated that the protein facilitates virus spread (37-39).

4.2. Immune responses to FeLV

Immunological responses to FeLV develop after viral exposure leading to resistance of the host to persistent infection. Most of the cats exposed to FeLV develop a temporary infection, with or without a transient viremia, and either completely recover or establish a latent infection (40). In one study, after exposure to FeLV, 28% of the cats become persistently infected and 42% become immune to the virus, whereas the remaining 30% become neither immune nor infected. Of the 28% of cats that become persistently infected, 83% will die of a FeLV-induced disease within 3.5 years in the follow-up (41, 42). Only about 10% of these cats develop lymphosarcoma, whereas more than 50% die of the immunosuppressive effects of the virus (42-44).

Both humoral and cellular immune responses are important for controlling FeLV infection. Virus neutralizing (VN) antibodies are thought to play a role in the recovery from infection, because passive transfer of immune serum provides protection (45). Exposed cats with protective titers of VN antibodies repel the virus and are resistant to subsequent viral infection (43, 46).

The FeLV can be broadly divided into subgroups A, B and C (19, 20). No persistently FeLV-infected cats have protective VN antibodies to FeLV-A and -B. However, in cats persistently infected with FeLV-A and -B, 45% of the cats have substantial level of VN antibodies to
Infections of FeLV and FIV

FeLV-C. The reason for the discordant immune response to FeLV-C is unknown. In persistently infected cats, antibody responses against the FeLV gag antigens MA, p12, CA, and NC are not beneficial (42).

The cellular immune response is also necessary for the control of viral replication and clearance from the host. Vaccination of domestic cats by use of FeLV cDNAs affords protection against viremia and the development of latency without inducing antiviral antibodies (47). Recently Flynn et al. (ref. 48) compared the FeLV-specific cytotoxic T-lymphocyte (CTL) responses in vaccine-protected, transiently viremic, and persistently viremic cats. In vaccine protected cats, immunity was associated with the detection of higher level of virus-specific CTL effectors to Gag/PR and Env as compared to that observed in unvaccinated control, persistently viremic cats. Likewise, higher levels of virus-specific CTLs were also observed in transiently viremic cats which recovered following exposure to FeLV. In cats that controlled their infection, CTL recognition of Gag/PR antigens was significantly higher than the recognition of Env antigens. The results indicate that virus-specific CTL plays a very significant role in determining the outcome of FeLV infection in either vaccinated cats or cats naturally recovering from FeLV exposure.

5. SUBGROUPS OF FeLV

FeLV was originally classified into three receptor interference subgroups: A, B and C (19, 20). FeLV-A is the ecotropic, transmissible form of FeLV. FeLV-B and -C have acquired an altered receptor specificity, compared to FeLV-A, and an expanded host range that includes human cells. FeLV-B arises in vivo through recombination between FeLV-A and enFeLV (49, 50). Acquisition of enFeLV sequences encoding portions of the SU Env gene in FeLV, including receptor binding domain, leads to changes in cell tropism because of the change in receptor specificity (49, 50). The FeLV-C is derived from FeLV-A infected cats following mutation. The crucial determinants of the FeLV-C subgroup are located in the variable region 1 (Vr 1) of the env gene (51, 52). Sequence analysis of FeLV-C isolates shows that each differs at Vr 1, but the alterations vary with each other (52). Nonetheless, they share the property of recognizing the same subgroup C host cell receptor.

Isolation of FeLV from infected cats reveals an unusual pattern of subgroup distribution. The FeLV-A has been found in all infected cats either alone (50%), in combination with FeLV-B (49%), or together with FeLV-B and FeLV-C (1%) (43, 53). The common FeLV-A isolates are generally of low to moderate pathogenicity and the ultimate source of all the pathogenic variants. FeLV-B viruses are more common in leukemic cats than in FeLV-A infected healthy cats. FeLV-B may contribute to the leukemogenic process or, alternatively, leukemic cats have longer standing infections, allowing more opportunity for FeLV-B recombinants to arise (54). FeLV-C viruses are rare, and their isolation occurs invariably in anemic cats (53).

Besides the three FeLV subgroups, there are variants associated with feline AIDS (FAIDS). These FeLV variants were initially isolated as complex mixtures of FeLVs from domestic cats infected with a cloned transmissible FeLV-A. Cloning from one of these isolated mixtures gave a replication-defective virus 61C, and a replication-competent avirulent clone, 61E (55, 56). Coinfection of 61C and 61E in cat induces FAIDS (55, 56). A replication competent chimera, EEEC, that induces FAIDS in cats was produced by recombination between the 5' LTR-gag-pol region of 61E and the env-3'LTR of 61C. The EEEC virus has a tropism for T cells with cytopathicity, and was designated as FeLV-T by Andersen et al. (ref. 57). The phenomenon was reproduced wherein FeLV-T variants evolved from FeLV-A during the course of an in vivo infection (58, 59). The sequence of the FeLV-T envelope is most closely related to FeLV-A. The envelope gene of an FeLV-T molecular clone (EVEC) encodes an N-terminal 6-amino-acid deletion, a C-terminal 6-amino-acid insertion, and scattered changes in 11 residues compared to the envelope gene of the parental avirulent FeLV-A clone 61E. As a consequence of these changes, this Env protein is incapable of establishing superinfection resistance (60, 61), allowing the accumulation of large amount of unintegrated proviral DNA in infected cells, and causes cell death (55, 62).

6. NATURAL RESISTANCE TO FeLV-B

Feline fibroblasts are highly permissive for infection with FeLV-B, however, cats are highly resistant to infection with FeLV-B in the absence of FeLV-A as a helper virus (54). Natural resistance to FeLV-B can be overcome by infection with phenotypic mixtures of FeLV-A and FeLV-B, but replication of FeLV-B is restricted and delayed when compared with that of the associated FeLV-A (54, 63).

McDaugall et al. (ref. 14) proposed that this restriction is caused by enFeLV. A subset of defective enFeLV proviruses is highly expressed in a variety of primary tissues, including lymphoid tissues of cats. At least two mRNA species, a 4.5 kb mRNA containing gag and env genes and a 2kb mRNA containing env gene, are expressed in feline lymphoid cell lines. Cloning of enFeLV cDNA from two FeLV-free lymphoma cell lines, 3201 and MCC, revealed a long open reading frame (ORF) encoding a truncated env gene product corresponding to the N-terminal portion of SU Env. The enFeLV env ORF expressed an intracellular product of 35 kDa which was also shed from cells in stable form. Expression of the 35 kDa protein correlated with the enFeLV mRNA levels in cells but was inversely correlated with the susceptibility to infection by FeLV-B. Cell culture supernatant containing the 35 kDa protein specifically blocked the infection of permissive fibroblasts by FeLV-B isolates. Likely, the truncated enFeLV Env (35kDa) mediated resistance by receptor blockade. An extension of this interpretation is that this form of enFeLV expression mediates the natural resistance of cats to infection with FeLV-B in the absence of FeLV-A (14). This natural resistance phenomenon is comparable to that observed for the murine retrovirus resistance genes Fv-4 (64) and Rmce (65, 66).
Infections of FeLV and FIV

7. RECEPTORS OF FELV SUBGROUPS A, B AND C

FeLV subgroups A, B and C utilize distinct receptors. Receptor interferences between FeLV-B, gibbon ape leukemia virus (GaLV) and wooly monkey sarcoma virus have been demonstrated using syncytial assays and pseudotype assays based on recombinant murine leukemia virus (67, 68). O’Hara et al. (ref. 69) reported the cloning of the GaLV receptor, as Pit1, the sodium-dependent inorganic phosphate symporter that has ten hydrophobic potential membrane-spanning sequences and five extracellular loops (70, 71). The Pit1 conferred sensitivity to infection by FeLV-B, indicating that it is the FeLV-B receptor (68). Interestingly, the amphotropic MuLV receptor cloned in 1994 (72, 73) showed significant identity to the Pit1 gene. It also functions as a sodium-dependent inorganic phosphate transporter and was named Pit2. Boomer et al. (ref. 74) demonstrated that certain FeLV-B isolates can also use human Pit2 and hamster Pit2 for entry. These observations are in parallel to that described for the amphotropic MuLV variant 10A1 that can use both Pit1 and Pit2 to infect susceptible cells (75).

Recently, the receptor for FeLV-C was also cloned (76, 77). The receptor (FLVCR) carries 12 hydrophobic potential membrane-spanning sequences and appears to belong to the major-facilitator superfamily (MFS) of transporters. The FLVCR is most closely related to the organic phosphate antiporter and anion/cation symporter subfamilies, both of which transport organic anions. The FLVCR mRNA as a 2.0kb transcript is expressed in all hematopoietic tissues including peripheral blood lymphocytes, and is most abundant in the fetal liver. This is consistent with evidence that FeLV-C preferentially infects different lineages of hematopoietic cells in vivo (78).

A putative FeLV-A receptor on cat cells has been identified by immunoprecipitation as a 70kDa molecule (79). It is not known whether the FeLV-A receptor is related to the FeLV-C receptor, although the molecular mass of the putative FeLV-A receptor is consistent with the size of MFS transporters. Cloning of the FeLV-A receptor gene is needed to resolve this issue.

8. COFACTOR FOR INFECTION OF FELV-T

8.1. FeLIX

Despite the similarity in \textit{env} gene sequence between FeLV-A and FeLV-T, interference assays suggest that FeLV-A, exemplified by clone 61E and FeLV-T, exemplified by clone 61C use distinct receptors (80). Recently Anderssen et al., (ref. 57) reported the identification of a cellular cofactor that is necessary for infection by FeLV-T. This protein, designated FeLIX for feline leukemia virus infection "x-cessory" factor is expressed from the enFeLV genome, and corresponds to a truncated version of the FeLV SU. This molecule is identical to the 35kDa protein of the truncated Env of enFeLV reported previously by McDaugall et al. (ref. 14). The FeLIX is nearly identical in sequence to the receptor binding domain of the FeLV-B envelope. FeLIX is necessary but not sufficient for FeLV-T infection. It acts in concert with Pit1 to permit infection by FeLV-T (81). Besides FeLIX, the untruncated full-length form of the FeLV-B SU Env also is able to permit infection by FeLV-T (81; Miyazawa \textit{et al.}, unpublished observations). Of the FeLV, MuLV and GaLV Env’s, only those with receptor-binding domains derived from enFeLV can render cells permissive for FeLV-T. Although FeLV-B SU’s can bind to Pit1 and Pit2, they efficiently mediate FeLV-T infection of cells expressing the Pit1 receptor, but only inefficiently of cells expressing the Pit2 receptor (81). Expression analysis of feline Pit1 and FeLIX suggest that FeLIX is likely the primary determinant of FeLV-T tropism.

8.2. Paradox of FeLIX

As mentioned above in Section 8.1, FeLIX is identical to the 35 kDa truncated Env of enFeLV (14). The FeLIX/35kDa protein harboring receptor binding domain of FeLV should bind to Pit1, the receptor of FeLV-B, interfere with FeLV-B binding and leads to the inhibition of infection by the virus. However, Anderssen \textit{et al.} (ref. 57) reported that FeLIX did not inhibit the infection of a feline fibroblast cell line (AH927 cells) by FeLV-B. In contrast, McDaugall \textit{et al.} (ref. 14) showed that addition of a culture supernatant from the 3201 feline thymic lymphoma cells that produce high amounts of the FeLIX/35kDa protein inhibited FeLV-B infection of feline fibroblasts (14). In my laboratory, FeLV-B infection of the AH927 cells was marginally inhibited by addition of a culture supernatant of COS-7 cells transfected with the expression plasmid of the FeLIX/35kDa protein as compared to that of mock-transfected COS-7 cells. Likewise, the AH927 cells stably expressing the FeLIX/35kDa protein by transfection with the expression plasmid was still permissive to FeLV-B (unpublished observations). Thus, the inhibitory effect of the FeLIX/35kDa protein on FeLV-B infection is only partial \textit{in vitro}, but might be sufficient to confer resistance to FeLV-B in cats.

As discussed, FeLIX/35kDa acting in concert with Pit1 permits FeLV-T infection of cells (81). The 3201 cells that secrete high amount of FeLIX/35kDa protein and express Pit1 can readily be infected by FeLV-T. However, there is no direct evidence that FeLV-T associates with Pit1. My laboratory also showed that certain cells expressing Pit1 and susceptible to FeLV-B, are not permissive to FeLV-T even in the presence of FeLIX/35kDa protein (unpublished observations). Hence, it is likely that FeLV-T may not utilize Pit1 but an unidentified molecule as receptor.

9. DISCORDANT CLINICAL SAMPLES

Several methods to detect FeLV infection in cats are available. The most widely used sample for diagnostic testing is blood. In most cats with a persistent infection, both infectious virus and free viral CA (p27) protein are present in the plasma and viral antigen is demonstrable in the neutrophils. Infectious virus may be detected by virus isolation (VI), CA protein by enzyme-linked
Infections of FeLV and FIV

Figure 2. Genomic organization of FIV. The gag gene encodes the MA, CA and NC. The pol gene encodes PR, RT, dUTPase (DU) and IN. The env gene encodes SU and TM envelope glycoproteins. In addition to the genes, FIV has three auxiliary genes, vif, ORF-A and rev. The solid black lines represent the encoded proteins.

Immunosorbent assay (ELISA) and antigen in the cytoplasm of neutrophils by immunofluorescence (IF) on blood smears. Accumulated evidence suggests that results obtained from the VI and IF tests of the samples are in concordance (82). However, in a study testing 2000 blood samples, approximately 10% of the samples tested positive for the CA protein by ELISA were tested negative by VI, while all samples tested negative by ELISA also were tested negative by VI (83). The cats that were ELISA-test positive but VI-negative were designated as ‘discordant’ cats. The discordant state was not limited to one particular ELISA test. Five other commercial tests gave comparable results, and were in good agreement (83). Some of the discordant cats have proviral FeLV DNA in their peripheral blood leukocytes (PBL). Nested PCR detected the FeLV genome in the PBL of 33% of the discordant samples (84) indicating a proportion of the discordant cats harbor FeLV. In addition, 18% of the discordant cats were tested positive by a single-step PCR assay (84). It was estimated that the frequency of FeLV-positive cells was one in 1.5 x 10^7 PBL. One expects that this high frequency of FeLV-positive cells should give positive results in VI. My laboratory recently showed that the FeLIX/35kDa protein is present abundantly in the sera of cats (manuscript in preparation). Based on the observation that the FeLIX/35kDa protein acts in concert with Pit1 to permit infection by FeLV-T (81), we hypothesized that the current laboratory protocol for VI might have contributed to the observed discordance in our diagnostic results. The current protocol requires extensive washing of the indicator cells, such as the QN10S cells, after exposure to and adsorption of the serum samples. If only the FeLV-T variants were in the serum samples, the abundant presence of the FeLIX/35kDa protein would function as a cofactor to permit the initial infection of the indicator cells. Subsequent washing would have removed the FeLIX/35kDa protein limiting the spread of FeLV-T in culture, and registered the VI as negative. To test this hypothesis, my laboratory has recently established new indicator cells constitutively expressing the FeLIX/35kDa protein to allow the infection of FeLV-T variants (manuscript in preparation). This method should open the way to assess the prevalence of the FeLV-T variants in the field samples.

10. GENERAL FEATURE OF FIV INFECTION

10.1. Genome structure of FIV

The gag gene encodes a polypeptide that is processed into the three major structural proteins (figure 2): the p15 MA, p25 CA and the p7 NC (85-87). The pol gene encodes the p13 PR, p61 RT, p13 IN and the p14 protease-like proteins (85-87). It overlaps with gag, as are in the cases with primate lentiviruses, and is translated via ribosomal frame-shift (88). The FIV protease-like protein has a dUTPase activity which might reduce the mutation rate of FIV by protection against nucleotide misincorporation and by DNA repair (89). The dUTPase is lacking in the primate lentiviruses, but is present in the equine infectious anemia virus, visna virus and the caprine arthritis virus (90). The env gene encodes 95 kDa SU and the 40 kDa TM Env glycoproteins (85, 86). Both Env proteins are heavily glycosylated. The pattern of glycosylation varies depending on the cell types in which the virus has been maintained (91). The primary translation product of env is a precursor glycoprotein of 145 to 150 kDa (gp145) that is rapidly reduced in size to 130 kDa.
Infections of FeLV and FIV

(gp130). This gp130 is subsequently processed into the mature Env glycoproteins i.e., the gp95 SU and the gp40 TM by proteolytic cleavage (92). The FIV has extensive sequence variation in the env gene (93-95). Based on the differences found in the variable V3 to V5 region of env, FIV can be divided into five distinct subtypes, from A to E, similar to those that have been described for the HIV (95-97).

Genome complexity is characteristic to all lentiviruses. In addition to the structural genes, i.e., gag, pol and env, the FIV genome contains several short ORF’s located between pol and env, within env and at the 3’ end of the viral genome, which encode a number of auxiliary proteins.

The vif gene of FIV is similar in size and in genomic location to the vif genes of primate lentiviruses. Although the vif mutant of FIV produces virion-associated reverse transcriptase at a normal level upon transfection, cell-free virus prepared from the transfected cells do not infect feline CD4 positive T cells (98). The vif-defective mutant virus also does not grow efficiently in cats (99, 100).

The ORF-A of FIV is located between the vif and env genes, and is comparable to the location of the first exon of tat in the genomes of primate and non-primate lentiviruses. In primate lentiviruses, mutation of tat severely impairs viral replication, because Tat has transcriptional activation activity in trans, and is essential for viral replication (101). In contrast, the FIV ORF-A gene product appears to have only a low, if any, trans-activation activity (102-106). Although ORF-A-defective mutant retains the ability to replicate in cells, it has reduced replication rate in cultured feline cells, such as T cell lines, and especially in primary peripheral blood mononuclear cells (PBMC’s; ref. 104). In addition, ORF-A-defective mutant replicates in vivo at relatively low levels in cats (99, 100). Similarly, the cloned FIV, 34TF10, which has stop codons in the ORF-A gene, did not grow efficiently either in vitro or in vivo (107, 108).

Lentiviruses encode a trans-acting regulatory protein Rev that functions in the stabilization and transport of the unspliced and incompletely spliced viral mRNAs from the nucleus to cytoplasm. The Rev binds to the REV responsive element (RRE), a specific cis-acting target sequences, which is found in unspliced and singly spliced viral mRNAs. Like other lentiviruses, the FIV rev consists of two exons. The first exon utilizes nucleotides coding for the first 81 codons of the Env precursor (109). The second exon is located immediately downstream of env and overlaps with the 3’-LTR. The FIV RRE has been mapped to the 3’ end of env (110, 111), while the RRE of visna and the primate lentiviruses are located within the env gene. The presence of the Rev protein is a common feature of lentiviruses and is critical for the replication of the progeny virus.

10.2. Immune responses to FIV

Cats naturally infected with FIV produce antibodies to the viral proteins SU, TM, the p55 Gag precursor, CA and MA. After experimental infection, cats produce antibody to CA first at about 4 weeks after infection, and subsequently antibodies to MA, p55 and SU (112). A dominant epitope of CA recognized by murine B cells is highly conserved among FIV strains (113). However, some sera from infected cats cannot react with CA, but react with SU, suggesting that some cats do not develop antibody to the CA (114). Previously we found that anti-CA antibodies disappeared during the asymptomatic period in a cat infected with FIV and died with AIDS (115). It might be possible that the level of anti-CA antibody defines the prognosis. Several epitopes in the SU and TM are recognized by feline B cells, and two linear epitopes in Env are known to undergo positive selection for change to escape host immunity (93).

Antiviral CTL activity has been extensively studied as a possible mode of vaccine immunity against FIV (119-122). In an adoptive transfer study, serum-free blood cells from vaccinated cats clearly protect against homologous virus challenge, while serum-free blood cells from unvaccinated controls do not (123). Flynn et al. (ref. 119) also demonstrated that cats immunized with a whole-FIV vaccine elicited strong CTL responses to Gag and Env and were protected against low-dose challenge with homologous virus (119).

11. RECEPTORS OF FIV

Primary isolates of FIV have a cellular tropism restricted to PBMC’s, thymocytes, macrophages, brain cells (astrocytes), and interleukin-2-dependent T-cell lines (124-128). Certain primary isolates of FIV classified as subtype A can be readily adapted to replicate in an epithelial cell line, the Crandell feline kidney (CRFK) cells (129, 130). However, FIV isolates classified as subtypes B and D could not be adapted to replicate in these cells (86, 130, 131). The adapted viruses retain their parental cellular tropism, but also acquire the ability to infect CRFK cells as well as other non-lymphoid cells (132, 133). Chimeric and sequencing analyses of the env gene from the CRFK-tropic viruses suggest that the principal determinant of the CRFK tropism resides in the V3 loop of the SU Env (133, 134).

The FIV was thought to be highly species-specific (23). However, transfection of infectious FIV molecular clones have shown that the virus can replicate in non-feline non-lymphoid cell lines (135). Furthermore, CRFK-tropic FIV can readily infect human PBMC’s (136) and Molt-4 cells, a human T lymphoma cell line (137).
Infections of FeLV and FIV

Surprisingly, the CRFK-tropic FIV also can infect cynomolgus macaques (Macaca fascicularis) and induces clinical signs, including the depletion of CD4-positive cells (138). Species-specificity of FIV might be less stringent than previously considered.

The primary receptor for HIV is CD4. However, the feline CD4 homologue is not sufficient for the productive infection of a highly T-lymphotropic FIV (139). Hosie et al. (ref. 140) generated a monoclonal antibody which blocks FIV infection. This monoclonal antibody also recognized feline CD9, a molecule that spans the membrane four times (141). It was proposed that CD9 might be a receptor for FIV. However, subsequent studies showed that the inhibitory effects of the antibody impacted FIV infection at a stage after viral entry (142, 143).

Recently, it was shown that a CRFK-adapted FIV uses the chemokine receptor CXCR4 for cell fusion and viral entry (144). The CXCR4 is the coreceptor for HIV’s that are T-lymphotropic. The CRFK-tropic FIV can use both the feline and human homologues of CXCR4 as receptor (144, 145), and infection of these primary cells can be efficiently inhibited by the stromal cell-derived factor 1 (SDF-1) alpha and the bicyclam AMD3100. Both SDF-1 alpha and AMD3100 are CXCR4 ligands (146-148). The envelope of CRFK-tropic FIV also has been shown to interact directly with CXCR4 (146). It is still unknown whether CXCR4 acts as a primary receptor for FIV in like manner to certain isolates of the primate lentiviruses that utilize CXCR4 independently of the primary receptor CD4 (149). It is possible that the CRFK-tropic FIV isolates may have gained independence from a CD4-like factor which is required by primary isolates (150). The CXCR4 might not be a sole (co)receptor for FIV. The SDF-1 alpha did not inhibit infection of the MYA-1 cell line, an interleukin-2 dependent feline T cell line, which is highly sensitive to FIV, indicating the possible existence of a CXCR4-independent pathway of infection (146). Furthermore, antibodies against CCR3 and CCR5 chemokine receptors also inhibited infection of human PBMC’s by FIV (137).

12. CONCLUDING REMARKS

The FeLV and FIV belong to different genera, Gammaaretoviruses and Lentiviruses, respectively. Both viruses commonly induce immunodeficiency in cats. Hence, infection of cats by these viruses serves as useful experimental model to study the pathological mechanisms of immunodeficiency induced by retroviruses in general.

The FeLV-FAIDS variant, FeLV-T, infects cells by using FeLIX/35 kDa protein as a coreceptor or cofactor. Although this protein confers on cats the resistance to FeLV-B, FeLV-T can infect feline cells in the presence of the protein. Therefore, the FeLIX/35 kDa protein is not beneficial to the host in this aspect. In addition, the protein may influence the immune responses against FeLV, since cats may be tolerant to most of the epitopes of the protein sharing many epitopes with exogenous FeLVs, especially with those of the FeLV-B Env. Humoral immune responses against Env to neutralize FeLV are responsible for the recovery of cats from FeLV infection, but most cats do not elicit sufficient levels of Env-targeting VN antibodies. In contrast, the CTL responses against the Gag proteins may be important to the clearance of FeLV in FeLV-infected cats. Cells infected with FeLV produce the glycosylated Gag protein of 40kDa that has epitopes of CA protein. The functional significance and the disease relationship of this protein in cats are unclear. However, by analogy with MuLV infection in mice, this FeLV glycosylated Gag protein might play a significant role in viral spread. Further studies are needed to elucidate the influence of the FeLX/35kDa protein and the glycosylated Gag in cats against FeLV infection.

FIV contains several auxiliary genes characteristic of the genus Lentiviruses. In primate lentiviruses, several auxiliary genes are responsible for pathogenesis, with the nef gene being most important. In the case with FIV, there is no homologue of the nef gene, although there are at least three auxiliary proteins, i.e., the dUTPase, ORF-A and VIF; they are not essential to virus replication in vitro. The importance of these proteins in FIV pathogenesis is unclear, and infectious molecular clones of FIV that can induce AIDS in a relatively short period to study viral pathogenesis are sadly lacking at this time.

Recent studies revealed that FIV utilizes the CXCR4 as a primary or secondary receptor. However, this evidence does not explain well the expanded cell tropism of the CRFK-adapted FIV. In FeLV infection, the change of the cell tropism in the FeLV variants is associated with disease progression. In HIV infection, macrophage and T-lymphotropic isolates utilize CCR5 and CXCR4, respectively. Macrophage-tropic isolates are predominant early in infection, while T-lymphotropic isolates appear later and the presence of the T-lymphotropic isolates is generally associated with disease progression. There is no evidence that CRFK-adapted FIV can induce diseases faster than lymphotropic FIV. Further studies are needed to clarify the determinant of the broadened cell tropism and its implication on the variants in the pathogenesis of FIV infection.

13. ACKNOWLEDGEMENTS

This work was supported in part by grants from the Ministry of Education, Science, Sports and Culture of Japan.

14. REFERENCES


Infections of FeLV and FIV


Infections of FeLV and FIV


Infections of FeLV and FIV


Infections of FeLV and FIV


103. Miyazawa T., M. Kohmoto, Y. Kawaguchi, K. Tomonaga, T. Toyosaki, K. Ikuta, A. Adachi & T. Mikami:
Infections of FeLV and FIV

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)


Infections of FeLV and FIV


**Infections of FeLV and FIV**


**Key words:** FeLV, FIV, Infection, Receptor, Immunity, Retrovirus, Cats, Review

**Send correspondence to:** Dr. T. Miyazawa: Research Center for Emerging Infectious Disease, Research Institute for Microbial Diseases (BIKEN), Osaka University, 3-1 Yamadaoka, Suita, Osaka 565-0871, Japan, Tel: +81-(6)-6879-8343, Fax:+81-(6)-6879-8269, E-mail: takavet@mc.kcom.ne.jp