Biological Nature of Feline Immunodeficiency Virus

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ABSTRACT. Feline immunodeficiency virus (FIV) was first isolated in 1986 from a cat with an acquired immunodeficiency syndrome (AIDS)-like disease. This virus has many characteristics in common with human immunodeficiency virus which is an etiological agent of AIDS in humans and is classified as a member of the lentivirus genus of the retrovirus family. Since the discovery of FIV, many researchers have studied the virus extensively from clinical, biological, and genetic aspects. In this review, the biological nature of FIV is summarized in four sections, i.e., morphological and biochemical properties of FIV, biological properties of FIV, immunological aspects of FIV infection, and clinical aspects of FIV infection. This review includes some recent, unpublished data from our and other groups.—KEY WORDS: biology, feline, FIV, immunodeficiency, immunology.


Feline immunodeficiency virus (FIV), previously called feline T lymphotropic lentivirus was first isolated in 1986 from specific pathogen-free kittens inoculated with either peripheral blood or plasma from a cat with an acquired immunodeficiency syndrome (AIDS)-like disease in the United States [62]. This virus possesses many of the characteristic properties of the lentiviruses, including the morphology of the virion, cell tropism for T lymphocytes and monocytes/macrophages, and Mg²⁺-dependent reverse transcriptase (RT) activity, a genome organization, and the persistent, lifelong infection in the host. Therefore, FIV has been classified as a member of the lentivirus genus of the retrovirus family.

Seroepidemiological surveys conducted in several countries have revealed that FIV is prevalent worldwide and the incidence of FIV infection in cats varied from 1% to 15% in healthy cats and from 3% to 44% in diseased cats in various countries [4, 9, 15–17, 24, 28, 37, 70–72]. In retrospective seroepidemiological surveys conducted in Japan and the United States, sera positive for FIV were found in the samples collected in 1968 [15, 72], suggesting that FIV did not arise in recent years but existed in these countries as early as in 1968.

The purpose of this review is to summarize briefly the biological nature of FIV in vitro and in vivo.

MORPHOLOGICAL AND BIOCHEMICAL PROPERTIES OF FIV

Morphology. Morphologically FIV is quite similar to other lentiviruses including primate lentiviruses and the visna virus [93]. The virus buds from the plasma membrane of infected cells in the same manner as other retroviruses. Individual FIV particles are variable from spherical to ellipsoid and 105 to 125 nm in diameter. The core is composed of a conical shell that surrounds an eccentric electron-dense nucleoid.

Biochemical property. Like other lentiviruses, the RT of FIV requires Mg²⁺ for its activity. The optimal molar of the ion for the maximal RT activity is identical to that of primate lentiviruses [93]. Molecular clonings of several FIV isolates from geographically distant areas have been reported [38, 41, 50, 58, 59, 66, 73, 78]. Figure 1 shows the genomic organization of FIV. The gag gene encodes the matrix (p15), capsid (p24) and nucleocapsid (p10) proteins. The pol gene encodes the protease (p14), RT (p62), integrase (p31) and dUTPase (p15) proteins. The env gene

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Fig. 1. (A) Graphic representation of the open reading frames of the FIV TM2 proviral DNA [38]. In addition to the coding regions of gag, pol and env, the virus contains short open reading frames vif, A and rev. The gag gene encodes matrix (MA), capsid (CA), and nucleocapsid (NC) proteins. The pol gene encodes protease (PR), reverse transcriptase (RT), dUTPase (DU) and integrase (IN). The env gene encodes surface envelope (SU) and transmembrane (TM) glycoproteins. (B) Schematic view of the putative binding sites for the enhancer/promoter proteins in the long terminal repeat of the TM2 strain of FIV.
encodes the surface envelope glycoprotein (gp120) and transmembrane glycoprotein (gp42). In addition to the gag, pol, and env genes, several auxiliary genes have been found in the FIV genome. The vir gene product controls the cell-free virus infectivity in susceptible cells [84]. The rev gene product functions in the stabilization and transport of incompletely spliced viral RNA from the nucleus to cytoplasm [33, 65]. Between the vir and env genes, a small open reading frame (ORF) called A is present. The ORF A gene has a cysteine-rich motif for tat protein found in other lentiviruses. Although the primate lentiviral tat gene product increases the level of transcription of viral mRNA, trans-activational activity of FIV has not been detected and found that the ORF A gene is dispensable for viral replication in two feline T lymphoblastoid cell lines (Tomonaga et al., submitted).

The long terminal repeat (LTR) of FIV contains cis-acting regulatory elements including the TATA box for the initiation of the transcription. In the U3 region of the LTR, putative binding sites for several enhancer/promoter proteins such as AP-1, AP-4, NFkB, C/EBP, and ATF are present [38, 41, 66]. Among them, AP-1 binding site has been shown to be important for basal promoter activity [32, 46, 76]. AP-1 consists of two oncogene products, c-Jun and c-Fos. The expression level of c-Fos increased in association with the activation of macrophages [19]. Therefore, it is likely that FIV replication increases when macrophages are activated by immunological modulators such as interleukin-1 (IL-1) and tumor necrosis factor α (TNFα). More detailed information on the molecular biology of FIV will be described separately.

BIological properties of FIV

Cell tropism: FIV is highly species-specific and can infect primary feline T lymphocytes, monocytes/macrophages, and brain cells in vivo [7, 11, 30, 42, 43, 93]. Independent researchers reported that FIV did not infect Con A-stimulated primary human and canine lymphocytes, several human lymphoblastoid cell lines (HuT-78, H9, CEM and Raji cells), several primate non-lymphoid cells (HeLa, SW480, Vero, and COS cells) and murine primary spleen cultures, HT-2c cells (murine T lymphoblastoid cell line) [45, 93]. The only exception is human astrocytoma cell line U-373 cells in which FIV can infect productively [11], FIV causes cytopathic effects characterized by cell death, syncytium formation, and/or cell lysis in feline T lymphoblastoid cell lines such as 3201 [82], MYA-1 [43] and FeL-039 cells [83]. However, these cytopathic effects differ among strains of FIV and cell lines used as described later [42, 43, 83]. Apoptosis (programmed cell death) occurs in MYA-1 cells infected with FIV (Ohno et al., submitted). In contrast, Crandell feline kidney (CRFK) cells, and feline lymphoblastoid (FL-74) cells which are chronically infected with feline leukemia virus, can support the replication of some strains of FIV without showing any apparent cytopathic effects [30, 93]. Recently Ohno et al. [56] reported that the CRFK cells infected with FIV were killed by TNFα in the presence of nontoxic concentration of TNFα to uninfected normal CRFK cells.

Different cell tropism was observed among FIV strains [30]. An original U.S. isolate (Petaluma strain) and a Japanese isolate, KYO-1 strain, can infect CRFK cells which are feline non-lymphoid lined cells, and brain cell cultures [30]. On the other hand, the second U.S. isolate (PPR strain), two Japanese isolates (TM1 and TM2 strains) and a Dutch isolate (Amsterdam-19 strain) cannot infect CRFK cells (41, 45, 66, 73). In addition, cell tropism of FIV differs among strains even in T cell lines (MYA-1 and FeL-039 cells) of similar cell surface phenotypes [83].

Characteristics of lymphoblastoid cell lines sensitive to FIV: FIV can replicate in several feline lymphoblastoid cell lines, such as FL74 [93], 3201 [82], MYA-1 [43], and FeL-039 [83] cells as described. While FL74 and 3201 cells can grow without IL-2 in the medium, the growth of the MYA-1 and FeL-039 cells are dependent on the presence of IL-2 in the medium. There are differences in susceptibility to FIV among these cell lines. FL74 cells are not susceptible to TM1 and TM2 strains of FIV isolated in Japan [41]. Although 3201 cells had been reported to be unsusceptible to Petaluma strain of FIV in an earlier study [93]. Tochikura et al. [82] reported that the cells can be infected with the strain. Subsequently, Willett et al. [89] reported that Glasgow-8 strain of FIV isolated in the United Kingdom can infect the cells only after prolonged cocultivation with FIV-infected cells. Recently, Tokunaga et al. [83] examined the kinetics of FIV replication in MYA-1 and FeL-039 cells. Although all FIV strains examined were highly cytopathic to MYA-1 cells, the infectivity in FeL-039 cells was highly dependent on the strains, i.e., some were noninfectious, and others were highly cytopathic or less cytopathic. These results indicate that MYA-1 cells are more sensitive to FIV than FeL-039 cells. From these observations, MYA-1 cells appear to be most sensitive to FIV infection and would be useful for virus isolation, titration, and virus neutralization tests.

Flow cytometric analysis revealed that 80%, 46%, 83% and 100% of the MYA-1 cells were positive for pan-T, feline CD4, IL-2 receptor α-subunit, and major histocompatibility complex class II antigens, respectively [47]. Similarly, 3201 and FeL-039 cells have been reported to be positive for feline CD4 and negative for feline CD8 [82, 83]. The percentage of feline CD4 on MYA-1, FeL-039 and 3201 cells varies depending on the culture condition [47, 82, 83, and unpublished observation].

Although persistent infection of some strains of FIV in MYA-1, FeL-039 and 3201 cells were reported [44, 82, 83], it seems difficult to establish a permanent FIV producer cell line using the MYA-1 and FeL-039 cells [44, 83]. However, it is of interest that Yamamoto et al. [90] established two IL-2-independent FIV producer T lymphoblastoid cell lines (FL-4 and FL-6) after selecting cells from an IL-2 dependent cells (FeT1) cocultured with normal peripheral blood lymphocytes infected with FIV.
Paraformaldehyde-inactivated FL-4 cells induced a strong antibody response to the gag and env antigens in immunized cats [90] and are used for vaccine development [92].

Relationship of FIV with feline CD4 molecule: In the lymph nodes of the cats infected with FIV, we detected doubly positive lymphocytes with FIV-antigens and feline CD4 molecules, but could not detect those cells with FIV-antigens and feline CD8 molecules [87]. Tokunaga et al. [83] reported that feline CD4 positive lined cells lost their reactivity to anti-feline CD4 monoclonal antibody (mAb) after persistent infection with FIV. These observations suggest that FIV may down-modulate feline CD4 expression and/or has a selective tropism for the feline CD4 subpopulation of feline T cells. On the other hand, Brown et al. [5] reported that FIV could infect both CD4 and CD8 T cells in vitro. Furthermore, Hosie et al. [25] isolated a mAb which blocked infection of CRFK cells by a strain of FIV which could infect the cells. The antibody designated vpg15 recognizes cellular protein of 24 kDa which is apparently different from the feline CD4 molecule. Recently, we cloned a cDNA encoding the feline CD4 glycoprotein [51] and examined whether the CRFK cells expressing feline CD4 stably are susceptible to the highly lymphotropic FIV (TM1 strain) infection [52]. However, we could not find any evidence for the FIV infection in the CD4-expressing CRFK cells. Until now, there has been no report in respect of whether feline CD4 glycoprotein can bind to FIV envelope or a mAb against feline CD4 can block FIV infection. To investigate the role of feline CD4 in FIV infection, more detailed studies are needed.

Titration method: Titration of FIV can be made by inoculation of the serially diluted viruses into an FIV sensitive T lymphoblastoid cell line (MYA-1 cells) [31]. The value of 50% tissue culture infective dose (TCID50) in this system has linear correlation with the value expressed as the counts by the Mg2+-dependent RT activity assay [31]. The RT activity is considered to reflect the activity of both infectious and uninfected viruses, and therefore the same counts of RT activity does not mean the same TCID50 value if the strains were different. In fact, the value of TCID50 per RT activity counts of the TM1 strain isolated in Japan was much lower than that of the Petaluma strain isolated in the United States [31]. Enzyme-linked immunoabsorbent assay (ELISA) for detection of p24 (capsid) antigen has also been developed using anti-p24 mAb [81]. Quantification of FIV-infected cells in the infected cats was established by Meers et al. [40] using MYA-1 cells.

IMMUNOLOGICAL ASPECTS OF FIV INFECTION

Humoral and cellular immune responses: After natural infection of cats with FIV, they produce antibodies to gp120, gp42, p55 (Gag precursor), p24, and p15 (see section Biochemical Property). After experimental infection, antibody to p24 developed first (about 4 weeks after infection) and subsequently those to p15, p55 and gp120 followed [23]. Sera from cats infected with Japanese isolates and the U.S. isolate had cross-reactive antibodies to p24 antigen of both FIV strains [42]. Recently, a predominant epitope of p24 protein recognized by murine B cells was determined [39] and the epitope was highly conservative among FIV strains. However, some sera could not react with the p24 antigen but react with gp120, suggesting that some cats infected with FIV did not develop the antibody to the p24 [14]. In human immunodeficiency virus (HIV) infection, it has been reported that more than half of the AIDS patients with antibodies to HIV envelope protein (gp130) did not have antibodies against HIV major core protein (p24), although most serum samples from AIDS-related complex patients reacted with the both proteins [3]. However, the relationship between disease progress and the absence of the anti-p24 antibodies in cats infected with FIV is unknown at present. Pancino et al. [60] reported that several epitopes recognized by feline B cells are present in surface (SU1 to SU5) and transmembrane envelope proteins (TM1 to TM4), and two linear epitopes (SU3 and TM4) are submitted to positive selection for change to escape from the host immunity.

Two viral neutralization (VN) assays for FIV Petaluma strain which can infect CRFK cells were developed. One method was developed by use of anti-FIV gag mAbs and normal CRFK cells [13], and another method was developed using CRFK cells which are adapted to a medium containing 0.5% fetal calf serum and produce syncytia after FIV infection [88]. Neutralizing antibodies in cats experimentally infected with FIV Petaluma strain were detected 3 to 4 weeks post infection (p.i.) [13]. Antibody titers progressively increased during the first year of infection and reached high titers which were maintained for 2.5 years p.i. [13]. Recently, the VN assay applicable to other strains of FIV which cannot infect CRFK cells was established [74]. Sequence analysis of an escape mutant from virus neutralization revealed that an epitope for virus neutralization is present in the 5th hypervariable region of the surface envelope protein [74]. In addition, within envelope protein there is a region which contains a putative major neutralizing epitope comparable to V3 region of HIV [61]. Song et al. [75] reported that FIV-specific cytolytic T cells could be detected as early as 7 to 9 weeks p.i., and proved that the cytotoxicity was mediated by T cells which were positive for feline CD8 and restricted with major histocompatibility complex class I. The target molecule or epitope is unknown at present. There is no report on antibody-dependent cell cytotoxicity by killer cells, complement-dependent cytolysis nor natural killer cell activity in cats infected with FIV.

Immune disorders: The depressed proliferative responses (hypoblastogenesis) of the peripheral blood mononuclear cells to mitogens have been reported in cats infected with FIV [18, 36, 79]. The number of circulating IL-2 receptor positive cells in cats infected with FIV is higher than those in uninfected cats, whereas induction of
IL-2 receptor by Con A-stimulation is depressed in cats infected with FIV [57]. Primary humoral immune response to a T cell dependent antigen (sheep red blood cells) was reported to be suppressed even in asymptomatic carrier cats infected with FIV [80]. Similarly, antibody response to the synthetic T cell dependent immunogen diminished 25 to 44 months after infection, while that to the synthetic T cell independent antigen was not affected [86].

Elevation of total plasma protein and hypergammaglobulinemia are observed [2]. Recently, Ohashi et al. [55] reported that the plasma IL-6 level was significantly higher in FIV-infected cats than in uninfected healthy controls. Furthermore, they suggested that overproduction of IL-6 might contribute to the polyclonal B cell activation observed in FIV-infected cats. Immune mediated disorders such as Coomb's positive anemia, thrombocytopenia and arthritis were also reported [22].

The mAbs against CD4 and CD8 homologues in cats have been established [1, 34]. A significant decrease of the CD4/CD8 T cell ratio has been shown in cats infected with FIV [2, 21, 53, 85]. In addition, the decrease of CD4/CD8 T cell ratio was accelerated when cats were dually infected with FIV and feline leukemia virus [63].

It was reported that peritoneal macrophages from cats infected with FIV had a decreased ability of IL-1 secretion and an increased anti-microbial activity [35].

**CLINICAL ASPECTS OF FIV INFECTION**

**Transmission and clinical signs:** FIV infection is found mainly in older, free roaming, male cats. FIV can be recovered from blood, serum, plasma, cerebrospinal fluid and saliva of infected cats [11, 91, 93]. Bites seem to be the most efficient and important mode of transmission, explaining the higher incidence of FIV infection in male, free roaming cats [91]. Several studies revealed that the infection rate in outdoor cats was 5 to 20 times higher than that in the population kept strictly indoors [22, 29, 91].

**Table 1. Clinical abnormalities associated with FIV infection**

<table>
<thead>
<tr>
<th>General signs</th>
<th>Lethargy, Inappetence, Weight loss, Lymphadenopathy, Fever of unknown origin, Vomiting</th>
</tr>
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<tbody>
<tr>
<td>Other signs</td>
<td>Renal disease, Neoplasia, Neurological dysfunctions</td>
</tr>
<tr>
<td>Secondary infections</td>
<td>Chronic gingivitis, Periodontitis, Chronic stomatitis, Ear infections, Chronic upper respiratory infections, Opportunistic infections, Chronic skin infections, Bacterial urinary tract infections, Chronic abscesses, Chronic keratitis, Anemia, Leucopenia, Neutropenia, Lymphopenia, Myeloproliferative disorders, Hyper gammaglobulinemia, Increase of IL-6 level, Decrease of CD4/CD8 ratio</td>
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Clinical epidemiological study revealed that a wide range of clinical signs is seen in cats infected with FIV [24, 27, 28, 37, 64, 91]. Clinical signs commonly observed in FIV infection are shown in Table 1. Generally, nonspecific signs, hematological changes and secondary infections such as chronic gingivitis/stomatitis, upper respiratory tract infection and diarrhea frequently occur [24, 27, 28, 37, 64, 91]. The stage of FIV-infection in cats was proposed to be divided into five different phases as observed in HIV-infection in humans [27]; 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.

**Histopathology:** There are a few reports on the histopathological features of FIV infection. Follicular hyperplasia of lymph node is commonly observed in the infection [6, 68, 93]. In addition, follicular involution or mixed hyperplasia is also observed [6, 68], indicating that the pathological features of lymph nodes of the cats infected with FIV are very similar to those observed in HIV and simian immunodeficiency virus (SIV) infections [8, 12, 49]. In the central nervous system, encephalopathy similar to that of HIV and SIV infections was also observed in the cats infected with FIV [26]. Moderate gliosis and glial nodules were found commonly in both experimentally and naturally infected cats with FIV, suggesting the neuropathogenesis of the virus. Immunohistochemical stainings for FIV gag protein in lymph nodes revealed that FIV antigens were localized in follicular dendritic cells and sparsely in small lymphocytes of paracortical area [87].

**Diagnosis:** Several methods for diagnosis of FIV infection are available. Usually, detection of anti-FIV antibody in serum is the most important for diagnosis, because FIV can be isolated from all the cats with antibody to FIV. However, it has been reported that some FIV-infected cats do not have antibody to the virus, while the genome of FIV can be detected by polymerase chain reaction (PCR) [10]. For detection of anti-FIV antibody in serum or plasma, the immunofluorescence assay [15, 43, 62, 91], Western blotting (immunoblotting) assay [28, 54], radioimmunoprecipitation method [54], and ELISA using either the whole virus [54] or FIV gag-antigen expressed by E. coli [14, 67] had been developed. For detection of viral genome, PCR using specific primers for FIV is used [20, 48, 69]. The definitive test for FIV infection in cats is either virus isolation or detection of viral genome by PCR, since infection with FIV is lifelong. However, both methods are laborious and involve intensive procedures, and therefore it is not appropriate for screening.

**CONCLUSION**

Recent discovery of FIV made it possible to investigate the role of the virus in the pathogenesis of AIDS-like
disease in cats and establishment of the disease as an useful animal model for human AIDS. However experimental induction of the disease is rather difficult to achieve by FIV inoculation of cats. Production of a highly virulent FIV infectious molecular clone by genetic engineering and establishment of highly susceptible cat population by genetic selection or immunosuppression are urgently required to solve the problem. Further intensive research on the pathogenesis of FIV is needed.

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