Central and peripheral reservoirs of feline immunodeficiency virus in cats: a review

Chrissy D. Eckstrand,¹,* Ellen E. Sparger² and Brian G. Murphy³

Abstract

Infection with feline immunodeficiency virus (FIV), a lentivirus similar to human immunodeficiency virus (HIV), results in lifelong viral persistence and progressive immunopathology in the cat. FIV has the ability to infect and produce infectious virus in a number of different cell types. FIV provirus can also be maintained in a replication-competent but transcriptionally quiescent state, facilitating viral persistence over time. Immediately after the initial infection, FIV infection quickly disseminates to many anatomical compartments within the host including lymphoid organs, gastrointestinal tract and brain. Collectively, the anatomic and cellular compartments that harbour FIV provirus constitute the viral reservoir and contain foci of both ongoing viral replication and transcriptionally restricted virus that may persist over time. The relative importance of the different phenotypes observed for infected cells, anatomic compartment, replication status and size of the reservoir represent crucial areas of investigation for developing effective viral suppression and eradication therapies. In this review, we discuss what is currently known about FIV reservoirs, and emphasize the utility of the FIV-infected cat as a model for the HIV-infected human.

INTRODUCTION

Feline immunodeficiency virus (FIV) is a lentivirus that shares similarities to human immunodeficiency virus (HIV) and is present in both domestic and feral cat (Felis catus) populations worldwide. FIV prevalence varies by geographic region with the incidence in North America reported at 2.5 % (2006), 5.4 % in Bangkok, Thailand (2014), 9.5 % in Ankara, Turkey (2013), 23.2 % in Japan (2008) and 31.1 % in peninsular Malaysia (2012), equating to millions of FIV-infected animals worldwide [1–5].

Infection with FIV results in lifelong viral persistence and progressive immune dysfunction; however the clinical outcomes of FIV-infected cats are variable, with many FIV-infected cats living approximately normal lifespans [6]. Although FIV has been associated with unfavourable outcomes such as opportunistic infections, neoplasia, wasting and death, the precise determinants of morbidity remain unclear. Contributory factors including viral/host genetics, age of host, viral load during acute virus infection, housing environment, stress, cohabitation with other cats and concurrent disease status have been proposed [7–12]. Efforts to control lentiviral infections primarily focus on two broad strategies: preventing new infections and suppressing viral replication in already infected individuals, both of which are particularly relevant for HIV-infected humans. The complete elimination of virus-infected cells from the host is a laudable goal. However, practical and effective viral eradication strategies do not yet exist for animals or humans infected with lentiviruses in spite of highly effective viral suppression therapies and several decades of research focused on the pathogenesis of lentiviral agents [13].

The identification of permissive cell types and anatomical compartments harbouring virus have been important goals in research related to immunodeficiency-inducing lentiviruses. Such information is critical to the development of effective eradication therapies which depend upon the tissue penetration of various pharmaceutical agents. The relative importance of the specific cell types and anatomical compartments that constitute the lentiviral reservoir in an infected host remain controversial [13, 14].
Multiple factors play roles in defining a cell’s permissiveness to lentiviral infection and productive replication and include the appropriate membrane receptors for virus attachment, fusion and entry, a temporally appropriate milieu of nuclear transcription factors, activation state, target cell subset, tissue micro-environment, and presence of specific host cellular restriction factors like tetherin, APOBEC3 family and others [11, 13, 15, 16]. These same factors also determine the identity of cells that serve as reservoirs for persistent latent virus [13]. This review discusses what is currently known about the cellular and anatomical reservoirs in the FIV-infected cat and point out similarities shared by the FIV- and HIV-infected host. For the purpose of this review, reservoirs relevant to viral pathogenesis will be defined as cells and tissues that persistently harbour replication-competent provirus. The FIV-infected cat offers the opportunity to investigate the virologic and cellular features of central viral reservoirs that are typically inaccessible in living HIV-infected humans, and may also serve as a model for assessing the effectiveness of viral suppression and eradication therapies.

**FIV BACKGROUND**

Feline immunodeficiency virus was initially isolated in the mid-1980s from a cohort of group-housed domestic cats in northern California exhibiting a variety of non-specific illnesses such as gingivitis, enteritis, dermatitis, weight loss and death [17]. Virus isolation, electron microscopy, reverse transcriptase enzyme assays and Western blot analyses identified a T-lymphotropic retrovirus in the cats with clinical disease [17, 18]. Subsequent to this discovery, investigations into naturally and experimentally infected domestic cats have revealed a plethora of information about the virus and disease pathogenesis; as a result, the FIV-infected cat has become a well-recognized translational model for investigation of HIV pathogenesis, as well as development of antiviral therapeutics and vaccines. The FIV-infected cat is the only naturally occurring HIV animal model for a clinical immunodeficiency that is characterized by a progressive CD4+ T cell depletion, opportunistic infections and various entities including wasting and neurological disorders [19, 20]. As such, FIV is distinguished from other experimental animal models such as simian immunodeficiency virus (SIV)-infection of non-natural hosts including the Asian macaque species and the HIV-infected humanized mouse [21]. Similar to HIV-infected humans, FIV infection follows a relatively predictable course of disease through three phases: the acute, chronic asymptomatic and terminal stages [22–24]. Numerous reports have described the virologic and immunopathologic aspects of acute and early asymptomatic phases of FIV infection through experimental inoculation studies. However, investigation of the late asymptomatic phase and transition into FIV disease syndrome (F AIDS) has been primarily restricted to clinical cohorts of cats naturally infected with FIV [7, 23, 25]. This is perhaps due to the cost of maintaining and housing experimentally infected cats in specific pathogen free (SPF) facilities for prolonged time frames.

**FIV RECEPTOR USAGE**

Early reports revealed the presence of FIV proviral DNA in specific cell subsets including CD4+ and CD8+ T cells, macrophages and dendritic cells by sorting of these cell populations from experimentally infected cats or analysis of tissues by immunohistochemistry (IHC) [26–31]. Subsequent studies revealed that FIV utilizes two cell surface receptors identified as the costimulatory molecule CD134 (OX40) and the chemokine receptor CXCR4 for cellular attachment and entry into these specific cellular targets [32–34]. Primary binding of the cell by the virion is mediated between the FIV Env-SU protein and the cell surface receptor CD134, a costimulatory marker expressed by activated CD4+ T cells. Binding instigates a conformational change in Env, which facilitates subsequent binding between the cell surface expressed chemokine receptor CXCR4 and the V3 region of Env-SU [35, 36]. Binding of a primary cell surface receptor to facilitate Env interactions with a surface chemokine receptor is a mode of cell entry that is also well characterized for other immunodeficiency-inducing lentiviruses such as HIV-1 and SIV [37, 38].

CD134 is a transmembrane glycoprotein within the tumour necrosis factor superfamily, and serves as the initial binding receptor equivalent to the CD4 cell surface receptor in HIV binding [35]. Human and mouse CD134 are predominantly expressed on activated CD4+ T cells including regulatory T cells and T follicular helper (Tfh) cells, and more transiently expressed on activated CD8+ T cells. CD134 expression by natural killer (NK) cells, NKT cells and neutrophils has also been reported [39–41]. Currently, expression of feline CD134 has been demonstrated on the surface of CD4+ T and CD8+ T cells, and B220 (CD45R)+ B cells, but not on peripheral blood-derived monocytes [42, 43]. However, feline monocyte-derived macrophages, tissue-derived CD14 + macrophages and activated CD8+ T cells were shown to express CD134 surface expression that was further enhanced with lipopolysaccharide activation [33, 42]. In a separate study, cellular CD134 mRNA was detected in feline T and B lymphocytes, dendritic cells and macrophages; the highest expression, which increased with activation, was detected in activated T lymphocytes [43]. Overall, CD134 expression correlated with immune cell populations shown to be susceptible to FIV infection in vitro or to be positive for viral DNA in the infected cat.

Early reports showed that different CD134 binding-domain patterns may be viral strain-dependent [35, 44, 45]. Furthermore, CD134 domains required for FIV Env binding and infection with more pathogenic FIV strains were mapped to two cysteine-rich domains (CRD1 and CRD2), whereas less pathogenic strains only required binding to CRD1 based on in vitro analyses. These findings agreed with evidence for FIV Env-CD134 binding patterns changing over time in individually infected animals, which may ultimately influence viral cell surface affinity due to nucleotide base changes in env and reflect isolates sensitive to circulating anti-CD134 autoantibodies [44–46]. Recent studies further...
characterized FIV Env changes associated with CD134 binding of distinctive FIV isolates that emerge in naturally infected cats during disease progression [47]. Variants characterized as Env recombinant viruses have also been described [48]. Notably, specific changes in the env gene sequence mapped over longitudinal time points of infection resulted in FIV variants that lost dependence for binding to the CRD2 domain of CD134 for virus entry. These CRD2-independent isolates, which are characteristic of the later stages of FIV infection, were also shown to be less efficient for transmission and replication during acute infection when compared to CRD2-dependent isolates in experimental infection studies. These findings generated a hypothesis that CRD2-dependent FIV isolates, which are efficiently transmitted in vivo and able to achieve higher virus loads during early infection, may be analogous to CCR5-dependent HIV-1 isolates that also transmit much more efficiently during primary HIV-1 infection of humans when compared to later-emerging CXCR4-tropic variants in HIV-1 infection [49, 50]. Specific CD4+ T cell subsets that may be specific targets for these CRD2-dependent FIV isolates and their role in viral pathogenesis remain to be determined.

Flow cytometric analysis revealed that chemokine receptor CXCR4 is a co-receptor shared by both FIV and HIV-1 and is expressed on feline peripheral blood-derived monocytes, B-cells and activated T cells [51, 52]. These findings for feline CXCR4+ cell subsets overall coincide with cells that express FIV primary receptor feline CD134, and also account for most cellular subsets shown to infected with FIV in vivo, as described below. FIV Env domains responsible for binding of CXCR4 and CD134 have also been mapped and found to be independent of each other, as expected [36, 53]. Additionally, CXCR4 expression has been detected in lymph node, thymic and bone marrow-derived CD4+, CD8+ T lymphocytes, CD21+ B lymphocytes and unfractionated CD4+/CD8-/CD21- cells [54]. Results from this report also revealed FIV infection in both CXCR4+ and CXCR4- cell populations, as determined by quantitative polymerase chain reaction (qPCR) assay of isolated cell populations for FIV proviral DNA. These results suggested the possibility that FIV may be capable of infecting cells in a CXCR4-independent manner, although this finding has not been tested further or confirmed by subsequent studies. Interestingly, laboratory-adapted FIV isolates have been shown to be capable of infecting cells independent of CD134 by use of heparin sulphate proteoglycans (HSPGs) and DC-SIGN as binding receptors to promote infection and facilitate binding of CXCR4 in the absence of CD134 [53]. FIV Env binding of HSPG and DC-SIGN are properties shared with HIV-1 [55, 56] wherein HIV-1 Env binding of these receptors on dendritic cells may facilitate transmission of HIV-1 virions to T cell targets.

However, usage of these alternative receptors appears to be restricted to laboratory-adapted FIV isolates and the role of these receptors in FIV pathogenesis is currently unknown. Accordingly, HIV-1 and FIV utilize similar strategies for entry of target cells by Env binding of a primary cell surface receptor, CD4 and CD134, respectively, that facilitates Env interactions with either the CCR5 (HIV-1 only) or CXCR4 (HIV-1 and FIV) chemokine coreceptors [37, 49]. Furthermore, targeting CD4+ T cells through either CD4 or CD134 surface proteins results in a similar immunopathogenesis, characterized by a progressive CD4+ T cell depletion, lymphoid depletion and immune exhaustion during the chronic phases of HIV-1 and FIV infections, respectively.

**CELL RESERVOIRS OF FIV**

Specific cell reservoirs of FIV are discussed with the understanding that a cellular reservoir contains integrated replication-competent provirus and persists over time. The biology of FIV cellular reservoirs is complex and dynamic. Critical factors include different cell types that are permissive to FIV infection, infecting strain of FIV, changes in absolute CD4+ and CD8+ T cell numbers, cell surface receptor expression modulation over time, stage of infection, evolution of the viral genotype over time in the infected host, host-adaptive immune responses and mechanisms of viral latency. Similar to HIV-1, FIV infects and rapidly expands during the acute stage of the disease, and then persists in peripheral blood and tissue CD4+ T cells [26, 27, 52, 57]. However, CD4+ T cells are not the only relevant reservoirs as the virus also infects and disseminates to a wide variety of other leukocytes during the acute stage of infection, including CD8+ T cells, B-cells, monocytes/macrophages and other long-lived cells such as dendritic cells and megakaryocytes [26, 27, 29, 31, 58, 59]. Evidence supporting that these leukocytes are susceptible and permissive to FIV include PCR for proviral DNA and viral RNA, in situ hybridization for intracellular FIV genomic RNA and/or DNA, immunofluorescence (IF) or IHC assessment of tissues for detection of viral antigen, and either reverse-transcriptase (RT) activity assays or enzyme-linked immunosorbant assay (ELISA) for detection of extracellular viral protein. Table 1 summarizes key studies that have identified FIV cell reservoirs and the modalities used to detect infection or susceptibility to infection. It is important to note that the leukocyte subsets that have proven most consistently infected in vivo and susceptible to infection in vitro are CD4+ T cells and monocyte/macrophages, which are also target cell reservoirs for HIV [13, 49, 54, 60–62]. Evidence thus far is conflicting regarding productive infection of dendritic cells in vivo [29, 30, 54, 63], although multiple studies support productive FIV infection of cultivated dendritic cells [43, 63, 64]. Likewise, reports may demonstrate productive HIV-1 infection of dendritic cells in vitro [65], but in vivo analyses suggest that extracellular virions bound to the cell surface of dendritic cells are the primary mode by which these cells transmit virus infection to CD4 T cells [13, 66]. Evidence for productive replication of other subsets such as CD8+ T cells and B cells in vivo or in vitro has not been reported. These findings point once more to the similarities in cell tropism shared by FIV and HIV-1.
<table>
<thead>
<tr>
<th>Author and Year</th>
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<th>Method(s) of evaluation</th>
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FIV is capable of infecting and replicating in cell types other than traditional leukocytes. Feline central nervous system (CNS)-derived glial cells, including astrocytes and microglia, have been shown to support FIV replication in vivo and in vitro [67–71]. Infection of the feline CNS by FIV is proposed to result from trafficking of infected B and T cells, as well as macrophages, through the blood–brain barrier and into the choroid plexus based on experimental cat inoculation studies [72] and ex vivo choroid plexus epithelial cultures [73]. It is perhaps not surprising that microglia can support FIV infection, as these cells arise from bone marrow myeloid progenitor cells similar to other tissue-based macrophages. However, it is puzzling that astrocytes seem to support FIV replication, as these glial cells are derived from neuroectoderm. Billaud et al. demonstrated that astrocytes not only support FIV replication in vitro, but that viral replication is dependent upon the env sequence and that the replication rate in astrocytes differs between FIV strains [70]. However, other studies failed to show productive FIV infection of astrocytes [74]. Similarly, findings for HIV-1 infection of astrocytes have been conflicting. Astrocytes were shown to support HIV infection in both in vivo and in vitro studies, with the human mannose receptor reported to be essential for CD4-independent infection of this cell type [75, 76]. However, more recent studies reported inconsistent findings regarding HIV infection of astrocytes and suggest that infection of astrocytes is highly restricted, may involve CD81 vesicles and may internalize virus particles without productive virus replication [77, 78]. Regardless, the overall findings indicate that astrocyte infection or internalization lead to cellular dysfunction and facilitate transfer and dissemination of HIV-1 within the CNS. The role and nature of FIV interactions with astrocytes in virus-induced disease of the CNS, and similarities shared with HIV, remain to be determined.

There are relatively few reports describing longitudinal studies designed to investigate changes within infected leukocyte subsets according to the stage of FIV infection. Evidence that proviral DNA loads may shift between cellular subsets as disease progresses, especially as CD4+ T cells are depleted, has been presented in a limited number of reports [26, 27, 79]. The hallmark of FIV infection in the acute and early asymptomatic phases of infection is an inverted peripheral blood CD4:CD8 T cell ratio initially due to a decrease in CD4+ T cells and in some cases a concurrent expansion of CD8+ T cells [60, 80–82]. The abrupt decline in peripheral CD4+ T cell count typically re bounds, although a return to pre-infection values may not be observed. This decline in peripheral CD4+ T cells counts during acute infection has been reported to coincide with even more severe declines in CD4+ T cell frequencies in lymphoid tissues with the severity dependent on the FIV isolate used for inoculation and route of infection [60]. Importantly, relatively few studies have monitored the frequency of CD4+ and CD8+ T cell subsets into the late stages of infection. However, findings from these few studies, including experimental and natural infections, show that CD4+ T cell counts continue to progressively decline to very low levels in blood and lymphoid tissues and may be associated with declining peripheral CD8+ T cell frequencies and clinical signs of immunodeficiencies [7, 19, 83]. Interestingly, significant declines in CD4+ T cells counts are not always associated with clinical signs of acquired immunodeficiencies and other factors such as environmental stresses may determine disease onset [7, 24, 79].

Early studies investigating FIV-infected target cell populations during experimental infection relied on IHC analysis with a limited array of cell surface markers, in situ hybridization for viral nucleic markers and PCR analysis frequently dependent on nested-PCR protocols. Based on these reports [26, 27, 31, 81], highest viral loads were detected in CD4+ T cells. However, findings regarding shifts in proviral DNA loads to other subsets varied between these early studies to reflect greater loads in B cells, CD8+ T cells or macrophages depending on the virus isolate tested. Later reports and summaries [29, 54, 60, 79, 84, 85] also show variable findings. Yet, overall, these later studies reported that CD4+ T cells followed by CD21+ B cells and monocyte/macrophages have the highest proviral DNA loads in blood and lymphoid tissue, including the mucosa of the gastrointestinal tract. Dendritic cells have also been reported to be positive for proviral DNA in the few studies that analysed this subset, although the relevance of FIV infection of dendritic cells in vivo is still poorly understood [29, 30, 54]. Targeting of CD4+ T cells for a progressive depletion in the periphery and in lymphoid tissues along with targeting of other immune cell subsets including monocyte/macrophages, and dendritic cells parallels findings reported for HIV-1 infection in humans and correlates with the onset of a clinical immunodeficiency in the FIV-infected cat.

**FIV TISSUE RESERVOIRS**

From a broader perspective, anatomical sites that harbour FIV-infected cells have also been an active area of investigation in research focused on lentiviral pathogenesis. The topic of anatomical reservoirs of lentiviral persistence became particularly important with the advent of antiretroviral drug therapy (ART) for HIV-infected humans. While ART is highly effective for suppressing HIV viral replication, there is evidence that incomplete anatomically localized suppression of viral replication occurs due to inadequate tissue drug concentrations in particular anatomical sites such as the brain [86–90]. As is true for cellular reservoirs, technical challenges impede the identification of tissue reservoirs infected with replication-competent provirus. Multiple methodologies have been utilized to identify FIV-infected cells in tissues, including PCR to identify provirus in tissue homogenates, tissue localization of virus by IHC and in situ hybridization, electron microscopy, and virus isolation/outgrowth assays. Optimally, biologically relevant tissue reservoirs should be identified using a combination of these techniques.
Anatomical reservoirs frequently discussed in the HIV field are the brain, gut-associated lymphoid tissue (GALT), lymph nodes and the reproductive tract [13, 14, 88, 91]. Many of these tissues have been investigated as tissue reservoirs in the FIV-infected cat as well, particularly during the acute and early asymptomatic phases of infection [60, 62, 79, 92]. A thorough understanding of tissue reservoir distribution and biology requires an understanding of the early pathogenesis and dissemination of virus.

FIV inoculation in cats is believed to occur primarily through blood transmission during fighting (biting), however many other routes of experimental inoculation have proven to be effective for viral transmission, including oral, rectal/vaginal mucosal transfer, intravenous, intramuscular, intraperitoneal, subcutaneous injection and transplacental transfer [17, 19, 93–97]. Infection can be achieved by inoculation of either cell-associated or cell-free virus, with cell-free virus being apparently more efficient (at least with FIV-C) [30, 60, 93]. Viral inoculation is followed by robust cell-associated viral replication in the peripheral blood, resulting in transient plasma viraemia and systemic viral dissemination [60, 81, 98]. Obert and Hoover demonstrated that within 2 days of oro-nasal or vaginal mucosal exposure, FIV traffics from the mucosa to the regional lymph nodes via dendritic cells or CD3+ T cells transfer with subsequent systemic spread to secondary and tertiary lymphoid organs [30]. Other important target tissues in early FIV dissemination include additional lymphoid organs such as the intestinal tract mucosa, thymus, spleen, tonsil and bone marrow [27, 29, 31, 81, 94, 99–103]. Fig. 1 summarizes important tissue and cellular reservoirs known to harbour virus in FIV-infected cats.

Additionally FIV-infected cells can be detected in the CNS, liver, kidney, lung and salivary gland during the acute phase [74, 81, 94, 97]. Table 1 lists the studies of tissue reservoirs of FIV. Rogers et al. described a modified IHC technique for the detection of FIV protein (undefined FIV antigen) in formalin-fixed, paraffin-embedded tissue by using feline serum from an experimentally FIV-infected cat [29]. The study demonstrated that, within 3 weeks of inoculation, FIV disseminates to many tissue types including lymph nodes, spleen, intestinal lamina propria, intestinal Peyer’s patches (lymphoid follicles), thymus, bone marrow and resident tissue histiocytic cells such as Kupffer cells of the liver [29]. Interestingly, brain was not identified as a tissue for FIV infection. However, other reports have demonstrated that different FIV strains vary in their tropism for the brain and for different compartments of the brain [70, 104]. These findings point to some similarities in viral pathogenesis for the CNS shared by FIV and HIV-1 ([74, 105] – review with references therein). Indeed, based on multiple reports, the brain has proven to be an important target during early FIV infection and, similar to HIV, has been associated with various pathologic features such as encephalitis, microglial activation and, in some cases, degenerative changes like myelin pallor ([74, 105] and references therein; [104, 106]).

Multiple reports have shown virus localized to various compartments of the brain by qPCR or by in situ hybridization for FIV nucleic acid. However, relatively few in vivo studies have tracked specific cell populations within the CNS that are infected with FIV, with a few reports confirming infection of resident microglia in experimentally infected cats [69, 107]. FIV infection of the CNS warrants further scrutiny to determine the value of this animal model for testing therapeutic approaches to purge the HIV-1 reservoirs within the CNS.

There are fewer studies that have examined the long-term persistence of FIV in tissue sites. However, several studies indicate that tissues targeted by FIV in the acute phase also harbour replication-competent provirus in the later stages of infection. Dean et al. demonstrated the presence of replication-competent provirus in the peripheral blood and lymph node out to 70 months (5.8 years) after infection with FIV [27]. Pistello et al. demonstrated the presence of abundant provirus in lymphoid tissues, and lesser amounts in the brain, lung and kidney 3 years post super-infection (inoculation followed by second exposure) with FIV-Petaluma [108]. Infection of bone marrow cells in naturally infected asymptomatic cats has also been reported, although the identity of infected subsets was not determined [25]. Two recent investigations into tissue reservoirs by our laboratory demonstrated the presence of proviral DNA within the popliteal lymph node, mesenteric lymph node, spleen and mucosa of the small intestines during the late asymptomatic phase of infection (5–6 years post inoculation), in the face of a relatively quiescent or inactive viral harbour replication-competent provirus in spite of relatively quiescent or inactive viral replication in the peripheral blood [79, 92, 109]. Interestingly, our studies revealed that, firstly, lymph node germinal centres contained an increased frequency of CD3+ T cells compared to uninfected cat tissues. These observations suggested a proliferation of resident Tfh cells or infiltration of CD8+ T cells into the B cell follicles, although CD8+ T cells are generally restricted from entering B cell follicles. Secondly, in our most recent report, a novel in situ hybridization technique (RNAscope) for detection of FIV RNA identified germinal centres in the mesenteric lymph node, spleen and intestinal Peyer’s patches as microanatomical reservoirs of viral transcription in the chronically FIV-infected cat [92].

**FIV LATENCY**

Multiple investigative modalities have been used to identify cellular reservoirs of FIV, including the detection and quantification of proviral DNA, viral RNA, viral protein, reverse transcriptase activity and other techniques described in detail in a recent review by Ammersbach and Bienzle [110]. While the detection and quantification of these various viral components can provide supportive evidence of viral replication, they do not ultimately prove a cell’s ability to produce infectious virus. The ‘gold standard assay’ for the identification of replication-competent and infectious lentiviral reservoir cells is the ex vivo viral outgrowth assay.
(VOA) which has been used extensively for investigation of HIV-1 latency in humans [111] and in our laboratory for detection of cells containing transcriptionally silent infectious provirus in cats chronically infected with FIV [84, 92]. For assessment of virus infection by VOA, an enriched (purified) cell type such as CD4+ T cells are obtained from an infected individual and are cultured \textit{ex vivo}. To reverse potential viral latency, cultured cells are stimulated for varying amounts of time with mitogenic compounds such as phytohemagglutinin or concanalin-A and/or allogeneic peripheral blood mononuclear cells (PBMCs) to instigate a mixed lymphocyte reaction [112]. In the VOA, \textit{ex vivo} stimulation is an attempt to ‘reverse latency’ by activating viral transcription, viral protein production, virion assembly and release from cells containing replication-competent provirus. For detection of FIV infection, cell-free (clarified) culture supernatant from the activated cells is then passaged onto uninfected SPF feline PBMCs or another susceptible cell type. These cells are then cultured, and subsequently tested for the presence of proviral DNA by standard or quantitative PCR. Detection of proviral DNA in the secondary SPF cell culture confirms that supernatant from the primary cell culture contained replication-competent and infectious virus. The VOA can also be used to quantify the size of the reservoir by initially preparing serial dilutions of the reservoir cell of interest [113]. Although this assay is most sensitive for virus detection, major limitations of the VOA are that \textit{ex vivo} stimulation may fail to reverse latency (induce viral transcription) of all the replication-competent proviruses present within the tested reservoir population, is labour intensive and may yield variable results [13]. As a result, apparently ‘non-inducible’ cells harbouring replication-competent provirus will fail to be identified [114].

Lentiviral latency refers to cells with integrated replication-competent provirus that are transcriptionally inactive. Latently infected cells can be identified by detecting proviral DNA with a concurrent absence of detectable viral RNA and viral proteins. Lentiviral latency allows the integrated and replication-competent provirus to remain essentially invisible to both the host immune system and antiretroviral therapies and thus allows the virus to persist over time within the infected cell and host. Latently infected cells are thus of key importance when considering viral reservoirs, particularly within a host on ART. McDonnel \textit{et al.} reviewed FIV latency and the strengths of the FIV-infected cat as a model of the HIV-infected human [61]. Different cohorts of naturally and experimentally infected cats have demonstrated either undetectable or consistently detectable plasma viraemia during the asymptomatic phase, which may be due to differences in viral strains, inoculating dose, age of the animal or other factors [7, 84, 98, 104, 115–118].

The cellular mechanisms involved in FIV latency have been identified in both \textit{in vitro} and \textit{in vivo} infection systems [52, 84, 119–122]. McDonnel \textit{et al.} reported that peripheral blood-derived CD4+ T cells latently infected with replication-competent, infectious virus were infrequent (estimated at approximately 1 cell in 10^5 peripheral CD4+ T cells). This report also revealed that latent infection was characterized by the association of the FIV 5’ long terminal repeat (LTR) promoter with host histone proteins that were hypermethylated and deacetylated, consistent with a condensed chromatin state [123]. Subsequent reports demonstrated that latently FIV-infected cells could be reactivated \textit{ex vivo} through the administration of histone deacetylase inhibitors suberoylanilide hydroxamic acid, valproic acid, trichostatin A, and sodium butyrate (NaB) and a histone
methyltransferase inhibitor 3-deazanoplanocin A [124, 125]. Similar findings were reported in two studies by Tada-fumi and Chan who used histone deacetylase NaB and protein kinase-C inhibitors, respectively, to reverse FIV latency [120, 126]. Lastly, only short, abortive transcripts (R region of the LTR) along with an association of RNA polymerase II with the LTR by chromosomal immunoprecipitation were detected in cells latently infected with FIV [123]. These findings provided indirect and direct evidence that RNA polymerase II activity is paused on the FIV promoter, a finding also reported for cells latently infected with HIV [123, 127, 128]. These findings collectively reveal that circulating CD4+ T cells serve as a reservoir for latent FIV infections with latency characterized by at least one similar regulatory mechanism, repressive chromatin, that has also been described for HIV-1 latency in patients on ART [129]. Assogba et al. suggested that low dose viral inocula (10^2–10^3 copies), delivered by the mucosal route, and CD8+ T cells may also play important roles in inducing latency in CD4+ cells and continued viral suppression [121]. The same study described an infection state proposed as latency, in which proviral DNA was undetectable in blood- and tissue-derived cells harvested from experimentally infected cats [121]. However, activation of cultivated tissue lysates depleted of CD8+ T cells ex vivo resulted in virus production. The identity of cellular subsets serving as reservoirs for latent virus within these infected tissues was not determined. A different report showed that FIV preferentially replicated in activated CD4+CD25+ T cells and that CD4+CD25+ T cells served as a productive reservoir resistant to apoptotic cell death [52]. In contrast, CD4+CD25- cells were shown to be reservoirs for latent FIV with the potential to reactivate. This observation suggests that less-activated CD4+ T cells, as defined by the absence of CD25 expression, may preferentially harbour latent FIV infection – a finding that agrees with the observation of resting CD4+ T cells being a major reservoir for latent HIV-1 infection in patients on ART [13]. CD4+ T cells are the most frequently investigated cell type with regards to FIV latency. However, testing with additional markers for feline T cell activation and memory will be necessary to fully characterize the CD4+ T cell subsets that are the predominant reservoirs for latent FIV infection and to determine similarities between latency FIV and HIV-1 for CD4 + T cell infections. Finally, a previous report revealed that monocytes isolated 12–16 weeks after experimental FIV inoculation contained detectable proviral DNA in the absence of detectable viral protein. Moreover, virus production from these monocytes could be subsequently induced when stimulated ex vivo [58]. Monocytes and macrophages have also been identified as reservoirs for latent HIV-1 infection in patients on ART [13]. Taken together, observations to date suggest that FIV may share similarities in cellular reservoirs and molecular mechanisms for latency with HIV-1, although FIV latency remains less well characterized compared to HIV-1 latency, particularly regarding mechanisms of latency. These few reports described for FIV suggest that epigenetic factors, early virus infection events such as inoculating virus concentrations, immune restriction and cell activation, may play a role in FIV latency, factors shared by HIV-1 latency. One report has described FIV proviral DNA integration sites, which were revealed to be similar to those for HIV-1 [130]. However, any relationship between FIV integration sites and virus latency is currently undetermined. Furthermore, in contrast to HIV-1 infection, FIV latency may be apparent in the absence of ART and a common characteristic in cats naturally infected with FIV, particularly in environments free of stress and exposure to other pathogens, such as a single-cat household [7]. However, this dissimilarity suggests that FIV infection may provide a unique opportunity to examine mechanisms and interventions of lentiviral latency in the absence of ART.

CONCLUSIONS

Since the initial identification of FIV, there has been an extensive history of investigations into cellular and tissue viral reservoirs of FIV that have greatly contributed to understanding the pathogenesis of disease in the FIV-infected cat. Additionally, there is no shortage of research and review papers highlighting the strengths and advantages of using the FIV-infected cat as an animal model for the HIV-infected human. Many tissue reservoirs of FIV are very similar to HIV and include (but are not limited to) lymphoid tissues, including the gastrointestinal tract, and the CNS. FIV infects similar leukocyte subsets as HIV and most likely utilizes the same cells, such as dendritic cells and astrocytes, to facilitate infection of T cell targets. Importantly, FIV infection offers the opportunity to investigate the relevance of cellular and tissue reservoirs that may not be readily accessible in HIV-infected people (i.e. CNS). Furthermore, examination of the differences between FIV and HIV-1 infections, including receptor usage and clinical outcomes, may also provide unique insights to the viral mechanisms of lentiviral pathogenesis. As the lentivirus field evolves to investigate new antiretroviral and eradication strategies such as the gene-editing tool, CRISPR, one should consider the advantages of utilizing the affordable, outbred, tractable, naturally occurring FIV cat disease model for testing the safety and efficacy of such therapies.

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