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Strain-Specific Viral Distribution and Neuropathology of Feline Immunodeficiency Virus

Craig Miller¹, Helle Bielefeldt-Ohmann², Martha MacMillan¹, Salvador Huitron-Resendiz³, Steven Henriksen⁴, John Elder⁵, and Susan VandeWoude¹

¹ Department of Microbiology, Immunology, and Pathology, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, CO, USA

² School of Veterinary Science, University of Queensland, Gatton Campus, Gatton, Qld, Australia

³ Department of Neuropharmacology, The Scripps Research Institute, La Jolla, CA, USA

⁴ Graduate College of Biomedical Sciences, Western University of Health Sciences, Pomona CA, USA

⁵ Department of Molecular Biology, The Scripps Research Institute, La Jolla, CA, USA

Abstract

Feline immunodeficiency virus (FIV) is a naturally-occurring lentivirus of domestic cats, and is the causative agent of feline AIDS. Similar to human immunodeficiency virus (HIV), the pathogenesis of FIV involves infection of lymphocytes and macrophages, and results in chronic progressive immune system collapse and death. Neuropathologic correlates of FIV infection have not yet been elucidated, and may be relevant to understanding HIV-associated neurologic disease (neuroAIDS). As in HIV, FIV strains have been shown to express differential tendencies towards development of clinical neuroAIDS. To interrogate viral genetic determinants that might contribute to neuropathogenicity, cats were exposed to two well-characterized FIV strains with divergent clinical phenotypes and a chimeric strain as follows: FIV_{PPR} (PPR, relatively apathogenic but associated with neurologic manifestations), FIV_{C36} (C36, immunopathogenic but without associated neurologic disease), and Pcenv (a chimeric virus consisting of a PPR backbone with substituted C36 env region). A sham inoculum control group was also included. Peripheral nerve conduction velocity, CNS imaging studies, viral loads and hematologic analysis were performed over a 12 month period. At termination of the study (350 days post-inoculation), brain sections were obtained from four anatomic locations known to be involved in human and primate lentiviral neuroAIDS. Histological and immunohistochemical evaluation with seven markers of inflammation revealed that Pcenv infection resulted in mild inflammation of the CNS, microglial activation, neuronal degeneration and apoptosis, while C36 and PPR strains induced minimal neuropathologic changes. Conduction velocity aberrations were noted peripherally in all three groups at 63 weeks post-infection. Pcenv viral load in this study was intermediate to the parental strains (C36 demonstrating the highest viral load and PPR the lowest). These results collectively

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Corresponding Author: Craig Miller, 1619 Campus Delivery, Colorado State University, Fort Collins, CO 80523-1619, 970-491-7696, craig.miller@colostate.edu.

Conflict of interest statement

All authors declare that there is no conflict of interest.

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suggest that (i) 3' C36 genomic elements contribute to viral replication characteristics, and (ii) 5' PPR genomic elements contribute to CNS manifestations. This study illustrates the potential for FIV to provide valuable information about neuroAIDS pathogenesis related to genotype and viral kinetics, as well as to identify strains useful to evaluation of therapeutic intervention.

Keywords

Lentiviral neuropathology; neuroAIDS; feline immunodeficiency virus

Introduction

Feline immunodeficiency virus (FIV) is a naturally-occurring lentivirus of domestic cats, and is widely considered to be a valuable model to study human immunodeficiency virus (HIV)-associated neurologic disease (neuroAIDS) development (Fletcher et al., 2008; Podell et al., 2000; Power et al., 1998). Similarities between FIV and HIV infections in the central nervous system (CNS) have been demonstrated through brain-entry mechanisms via the blood-brain and blood-choroid plexus barriers (Dallasta et al., 1999; Gonzalez-Scarano and Martin-Garcia, 2005; Kramer-Hammerle et al., 2005; Luabeya et al., 2000; Ryan et al., 2005), presence of astrogliosis and microgliosis in FIV and HIV-infected individuals (Everall et al., 1995; Lenhardt et al., 1988; Meeker et al., 1997; Gray et al., 1996), perivascular lympho-plasmacytic and mononuclear cell infiltration of the meninges and choroid plexus (Gray et al., 1996; Ryan et al., 2005), and overall immunodeficiency and neurological impairment (Podell et al., 1993). In addition, the structural and functional diversity that may result in the varying pathogenicity of different HIV isolates are paralleled in FIV (de Rozieres et al., 2008; Sodora et al., 1994). The viral genomic determinants responsible for these varying degrees of pathogenicity are not fully understood and further phenotypical characterization of these elements may lead to the development of more effective treatment modalities (de Rozieres et al., 2008).

Five clades (A–E) have been established for FIV and most FIV subtypes are classified within clades A and B. FIV isolates in general have been shown to exhibit varying degrees of pathogenicity with one previously-characterized clade C isolate (FIV_{CPG}) causing severe disease and high mortality (de Rozieres et al., 2004; Diehl et al., 1995; Obert et al., 2000; Sodora et al. 1994). This clade C isolate, while markedly immunopathogenic in young cats, has not been previously associated with neurologic disease, despite high peripheral viral loads (de Rozieres et al., 2004). In contrast, other FIV isolates, such as those belonging to clade A, have been demonstrated to exhibit a predilection for neurologic disease development (Phillips et al., 1996; Phipps et al., 2000; Prospero-Garcia et al., 1994). The reason for the varied neurotropism in individual FIV isolates remains unclear and investigation of the molecular basis of various FIV strains may help to better understand neuroAIDS progression *in vivo*.

To interrogate viral genetic determinants that might contribute to neuropathogenicity, cats were exposed to two well-characterized FIV strains with divergent clinical phenotypes and a chimeric strain as follows: FIV_{PPR} (PPR, relatively apathogenic but associated with neurologic manifestations), FIV_{C36} (C36, immunopathogenic but without associated neurologic disease), and Pcenv (a chimeric virus consisting of a PPR backbone with substituted C36 env region) (de Rozieres et al., 2004, 2008; Phillips et al., 1990, 1996). Examination of neuropathogenicity associated with *in vivo* infection from these strains was performed by a variety of methods. Brain sections from four anatomic locations known to be involved in human and primate lentiviral neuroAIDS were examined for pathogenic change by histological and immunohistochemical methods (Zink et al., 2006; Fletcher et al., 2008).

In addition, cerebral spinal fluid (CSF) obtained from infected cats, as well as the aforementioned brain sections, were used to determine CNS viral load by RNA extraction and RT-PCR. Brainstem auditory evoked potential changes (BAEPs) were also assessed in all cats in this study at specified intervals. The findings of this study are presented in this report and reflect that the Pcenv chimera appears to be more neuropathogenic than both parental strains and that CNS viral load is not predictive of neuropathogenicity during FIV infection.

Methods

In vivo infections, cloning and analysis of Pcenv sequences, and FIV proviral DNA and plasma RNA quantitation were all performed as previously described (de Rozieres et al., 2008). The Pcenv chimeric virus was constructed with PPR genetic elements *ltr*, *rev2*, *gag* or *pol* and with C36 genetic elements *vif*, *orfA*, *env*, and the first exon of *rev* (Reviewed in Figure 1 as presented in de Rozieres et al., 2008). Twenty, 14–16 week-old, SPF cats (Cedar River Laboratories, Ames, IA, and an SPF colony at Colorado State University [CSU], Fort Collins, CO) (de Rozieres et al., 2008) were maintained in barrier rooms within AAALAC-international accredited animal facilities in accordance with CSU IACUC-approved protocols. Three groups of cats (each n=5) were inoculated intravenously with 1 ml normalized plasma obtained from cats previously infected with either PPR, Pcenv, or C36 strains as previously described (de Rozieres et al., 2008). An additional group (n=5) was administered naïve plasma and used as non-infected controls. Blood samples were taken on days -7, 3, 7, 10, 14, 17, 21, 28, 35, 46, 55, 67, 81, 95, 111, 138, 173, 216, and 259 for detection of circulating virus and PBMC provirus using a previously described technique (de Rozieres et al., 2008). Animals were monitored daily for clinical signs of illness. One cat from the PPR group and one cat from the C36 group were euthanized prior to day 173 post-inoculation due to allergic dermatitis unrelated to FIV infection.

At 350 days post-inoculation, necropsy was performed on all 18 remaining cats following cerebral spinal fluid (CSF) collection, which was carried out ante-mortem. CSF was collected, while the animal was under general anesthesia, from the cerebellomedullary cistern using a 20-gauge, 1.5-inch spinal needle and a sterile collection tube. The CSF was then frozen at -80°C for RNA purification. The brain was removed from the skull of each cat and the left hemisphere was fixed in 10% neutral-buffered formaldehyde for histology sectioning; the right hemisphere was used for fresh tissue sampling. Tissue samples (200–300mg) were taken at the time of necropsy from the following neuroanatomical locations: frontal lobe, caudate nucleus, basal ganglia, and cerebellum. Each sample was frozen at -80°C for RNA purification. RNA purification was performed on frozen tissue samples using a FastRNA kit and homogenizer (MP Biomedicals). RNA was also purified from 140 μl of frozen CSF using an RNeasy Mini Kit (Qiagen). SuperscriptII (Invitrogen), random primers (Invitrogen), and RNase Out (Invitrogen) were used to prepare cDNA from the sample of viral RNA by reverse transcription. Real-time PCR was performed on an iCycler thermocycler (Bio-Rad) to quantify viral RNA by $\Delta\Delta\text{CT}$ and GAPDH-formulated standard curve as previously described (Livak and Schmittgen, 2001). Statistical analysis of viral RNA was achieved by Kruskal-Wallis test to examine if viral load differed among FIV strains for each tissue individually. Viral loads were then compared among tissues for each strain individually, also using a Kruskal-Wallis test. For all significant results, pair-wise comparisons were made by post-hoc analysis.

The left hemisphere of brain tissue was fixed in 10% formalin for 24 hours prior to tissue collection for histological examination. Tissue samples were taken from each hemisphere at the following locations: frontal lobe, caudal nucleus, basal ganglia, cerebellum, which are areas known to be associated with neuroAIDS in primates (Zink et al., 2006; Fletcher et al.,

2008). The tissues from the left hemisphere were processed, embedded in paraffin and sections collected on charged slides (Superfrost; Colorado Histo-Prep, Fort Collins, CO) with one slide of each tissue stained with hematoxyline and eosin for light microscope examination. Immunohistochemistry (IHC) was performed on the remaining consecutive sections using previously described protocols (Bielefeldt-Ohmann et al., 2008; Tolnay et al., 2010), employing the following primary antibodies specific for: vascular endothelial growth factor (VEGF) Ab-1 (Santa Cruz Biotechnology), the oligodendrocyte-specific transcription factor OLIG-2 (Anti-Olig-2) (Sigma-Aldrich), the astrocyte-specific glial fibrillary acidic protein (GFAP) (Millipore), the microglia/activated macrophage-specific anti-ionized calcium binding adapter molecule 1 (Iba-1) antibody (Wako Pure Chemical Industries, Ltd.), antibody specific for phospho-p38 MAPK (P38-MAPK) (Cell Signal Technology), the neuron-specific 200 kD neurofilament heavy (NFH) (Abcam), and apoptosis marker activated caspase-3 (Abcam). All tissues were scored using the following criteria: 0 = no apparent pathology/change, 0.5 = minimal change (such as minimal vasocentric lymphocyte infiltration in meninges), 1 = mild change (such as mild perivascular lymphocyte infiltration in meninges and brain), 2 = moderate change (such as mild neuronal degeneration with satellitosis and mild to moderate perivascular lymphocyte infiltration in meninges and/or brain), 3 = marked changes (as for 2, but more extensive and with apparent gliosis), 4 = severe change (such as neuronal necrosis/drop-out accompanied by gliosis and perivascular and neuropil infiltration of lymphocytes and monocytes).

Brainstem auditory evoked potential changes (BAEPs) were evaluated in all cats in this study at 5, 16, and 63 weeks post-inoculation using a previously described method (Gong et al., 2000; Huitron-Resendiz et al., 2004). Six waves were identified in the short BAEP complex by visual inspection, P1 to P6. Interpeak interval latencies were measured to assess the transmission of stimuli along the brainstem portion of the primary auditory pathway.

A complete anatomic brain magnetic resonance imaging (MRI) scan with diffusion weighted imaging (DW-MRI) was performed on all cats at 5, 16, and 63 weeks post-inoculation with a General Electric Signa LX 1.5 Tesla MR HiSpeed Plus System (Milwaukee, USA). Images were obtained with cats under general anesthesia maintained with isoflurane gas via a non-rebreathing circuit and positive pressure ventilation. MRI and DW-MRI were performed as previously described (Bucy et al., 2010).

Results

Histological assessment by light microscopy

Tissues from the neuroanatomical locations outlined above were examined for lesions by light microscopy. All brain sections from all non-infected animals were unremarkable histologically except for one animal, which had a focally limited lesion of unknown etiology located in a sulcus of the cerebral cortex. There was limited lympho-plasmacytic infiltration in the meninges of three of the PPR-infected animals. All other PPR sections were histologically normal. All C36-infected brain tissues had minimal to mild lympho-plasmacytic meningitis and minimal neuronal degeneration in the neocortex. Two C36-infected animals also exhibited mild neuronal degeneration in regions of the basal ganglia. All Pcnv-infected animals had mild lympho-plasmacytic meningitis. In three of the animals, the meningitis extended into the arachnoid of penetrating vessels appearing as mild vascular cuffing of scattered vessels in the cortex, cerebellum and basal ganglia. In the same three animals there was also scattered neuronal degeneration, satellitosis and infiltration of lymphocytes in the neuropil (Figure 2).

Immunohistochemical assessment of CNS tissues

Immunohistochemistry for CNS-specific cells and inflammation markers was carried out to assess possible effects of the infection not discernable by conventional histological examination. Vascular endothelial growth factor (VEGF) is an inflammation-associated glycoprotein induced in response to hypoxia and inflammatory mediators and stimulates angiogenesis, while phospho-p38-MAPK expression indicates that the TNF-alpha signaling pathway has been stimulated (Ferrara et al., 2003; Herlaar and Brown, 1999). Apoptosis or effects potentially leading to apoptosis was assessed by immunelabeling for activated caspase 3 (Porter and Jänicke, 1999). In addition, cell specific markers for oligodendrocytes, astrocytes, microglia and neurons (anti-Olig-2, -GFAP, -Iba-1 and -NFH antibodies, respectively) were used to detect and assess the status of these cells in various brain regions.

All tissues were scored as described in Methods and the data are presented in Table 1. IHC for the non-infected animals were unremarkable for all markers tested, with the following exceptions. In the animal with the aforementioned histological lesion, there was upregulation of nuclear and, rarely, cytoplasmic expression of activated caspase-3, suggestive of a neurologic insult of unknown etiology. One other non-infected animal in this group exhibited microglial activation based upon increased Iba-1 expression of unknown etiology as the brain was histologically normal. There were no discernable differences between the PPR-infected group and the non-infected animals with regard to the markers examined by IHC. Two C36-infected individuals showed mild to no microglial activation and the other two individuals displayed marked microglial activation based upon increased Iba-1 expression (Table 1). Other immunohistochemical changes, including mild nuclear and cytoplasmic expression of activated caspase 3 and mild upregulation of VEGF, were consistent with mild inflammation. Pcnv-infected animals exhibited microglial activation; Iba-1 expression was more intense and the microglia were hypertrophic with increased branching, compared to the uninfected and PPR animals (Figure 3). Widespread nuclear and cytoplasmic expression of activated caspase 3 in one animal, mild upregulation of VEGF, and in another animal, of phospho-p38-MAPKa expression were consistent with a mild inflammatory response. While statistical significance was not attained in any group versus another, the trend for more significant activation in cats infected with chimeric FIV-PCnv is apparent. Pcnv-infected animals exhibited changes similar to those noted for C36, but in general tissues from these animals demonstrated the greatest degree of IHC staining, both in terms of intensity and of number of positive cells (Figures 2, 3, 4 and Table 1).

Assessment of CNS RNA *gag* viral load

RT-PCR was performed to quantify the CNS RNA *gag* viral load in each of the animals. RNA was purified from CSF collected ante-mortem and from frozen tissues collected at necropsy from the neuroanatomical locations corresponding to those examined histologically, including the frontal lobe, caudate nucleus, basal ganglia, and cerebellum. The CNS RNA viral loads were compared to peripheral viral loads that were obtained using a previously described protocol (de Rozieres et al., 2008). Analysis of peripheral viremia demonstrated that circulating virus during *in vivo* infection is highest for C36, followed by Pcnv, then PPR (de Rozieres et al., 2008). Mean levels of viral RNA present in the CSF are shown in Figure 5 and Table 2. C36 infected cats had 10–50 times greater viral load than the other two groups. Measurements of RNA *gag* viral load in CNS tissues revealed that like in the CSF, the highest amount of viral RNA was present in cats infected with C36, followed by cats infected with Pcnv, and cats infected with PPR having the least amount of viral RNA in the CNS (Figure 5 and Table 2). Further analysis revealed that viral load differed significantly between viral strains for all tissues (Table 2). Viral loads did not differ significantly among tissues for each strain individually (C36 $X^2_{4,18} = 5.541$, $P = 0.236$; PPR $X^2_{4,18} = 2.788$, $P = 0.594$; Pcnv $X^2_{4,18} = 2.300$, $P = 0.681$) and viral RNA levels were

similar across all four anatomic locations for individual groups. The most interesting observation noted was that, while Pcnv exhibited the highest degree of pathological changes based upon histological and immunohistochemical evaluation, its viral load was intermediate to the parental strains (C36 demonstrating the highest viral load and PPR the lowest). Therefore, the degree of cellular activation and neuropathology detected was not proportional to replicating virus in the CNS.

Brainstem auditory evoked potential changes (BAEPs) and magnetic resonance imaging (MRI)

Six waves were identified in the short BAEP complex by visual inspection, as previously described (Gong et al., 2000; Huitron-Resendiz et al., 2004). BAEP waves from all FIV-infected cats showed characteristic delays in the latency of the P3 ($p < 0.01$, Figure 6), P4 ($p < 0.01$), P5 ($p < 0.001$) and P6 ($p < 0.01$) waves, compared to the control group, suggesting disruptions in the auditory stimuli conduction at the level of the brainstem. P3 – P1 interpeak interval latencies exhibited significant delays ($p < 0.05$), compared to control, whereas P6 – P4 interpeak latencies did not show significant abnormalities. Therefore, delays in the P3 wave were responsible for the delays noted in P4, P5 and P6 delays. Cortical AEPs did not show significant differences in the latency of their waves compared to those of the control group. Neuroimaging results from MRI did not produce any significant differences in regard to brain diffusion. This is contrary to findings from a previous study that revealed increased brain diffusion in both PPR and C36-infected cats (Bucy et al., 2010).

Discussion

During the course of infection, peripheral viral load of Pcnv is intermediate to its parental strains; with PPR viral load being lower and C36 viral load being higher than Pcnv (de Rozieres et al., 2008; Thompson, 2009). As demonstrated in Figure 5, this trend is mirrored in the CNS by viral RNA load in several regions and CSF. PPR-infected cats in this study had little to no detectable virus in CNS tissues, consistent with findings in peripheral blood mononuclear cells (de Rozieres et al., 2008; Thompson, 2009). While Pcnv RNA was detectable in CNS, C36 was detected at 10-fold greater levels (Figure 5). Despite lower viral RNA levels, Pcnv exhibited the greatest degree of histological and immunohistochemical evidence of inflammation.

This finding raises the question as to which genetic components of the Pcnv chimeric virus contribute to its neurovirulence and replication characteristics. FIV is classified into subtypes based upon *env* diversity (Sodora et al., 1994), and residue changes in this gene may contribute directly to clade-associated pathogenicity as these changes alter cell tropism (Vahlenkamp et al., 1997; Verschoor et al., 1995). There is considerable evidence of neuropathologic development in cats experimentally infected with both FIV A and B subtypes, such as PPR (Phillips et al., 1996; Phipps et al., 2000), FIV_{MD} (Phillips et al., 1994; Podell et al., 1993, 1997, 1999; Prospero-Garcia et al., 1994), FIV_{NCSU1} (Meeker et al., 1997), and FIV_{V1CSF} (Power et al., 1998). PPR has been shown to be a neurotropic virus clone capable of inducing pathogenic changes in the CNS (Phillips et al., 1996; Phipps et al., 2000) to a greater extent than C36 (de Rozieres et al., 2004). The finding that Pcnv expressed a more neurovirulent component, based on IHC results, therefore suggests that viral determinants underlying this phenotype lie within the 5' PPR long terminal repeat (LT), *rev2*, *gag* or *pol* genetic elements, and is less dependent upon *env* determinants.

C36 has been shown repeatedly to replicate to a high degree compared to other FIV strains (de Rozieres et al., 2008), and as demonstrated here and previously, Pcnv attains replication characteristics intermediate to C36 and PPR. The chimeric virus incorporated C36 *env* and

accessory regulatory genes *vif*, *rev*, and *orfA*. These 3' genomic elements of C36 putatively contribute to enhanced virulence of Pccnv through enhanced replicative capabilities gained during the course of *in vivo* infection (de Rozieres et al., 2008). Env is known to play a role in host cell tropism and receptor attachment (Sielbelink et al., 1995; Vahlenkamp et al., 1997; Verschoor et al., 1995; Willet et al., 1997) and C36 Env was shown to differ significantly from clade A and B Env proteins in regions V3 to V5 (de Rozieres et al., 2004), a region known to contain macrophage-tropic determinants in which genetic alterations may result in a T-cell-tropic phenotype (Vahlenkamp et al., 1999). It is hypothesized that by increasing the target-cell range, pathogenicity may be increased through expanding the spectrum of cells that are susceptible to infection, resulting in enhanced replication rate (de Rozieres et al., 2004). It is also possible that 3' C36 open reading frame-encoded elements play a role in viral replication that results in a higher viral load set point.

Contrary to previously published observations (de Rozieres et al., 2004), neuropathology associated with PPR infection was in most cases below detectable levels, and, of the two parental virus strains, the higher degree of neuropathogenic change, albeit mild, was noted in C36 infected cats. This uncharacteristic finding is likely due to the nature of the inoculum used in this study, which was untitered pooled plasma from cats infected with PPR, C36, or Pccnv. Circulating PPR levels were consistently low throughout this study, with two of the five PPR infected cats being aviremic for nearly six months between days 81 and 173 PI, whereas viral loads in C36 and Pccnv infected cats were consistently detected (Thompson, 2009). Given that BAEP latency, considered a highly sensitive measure of FIV-induced neurodysfunction (Huitron-Resendiz et al., 2004), was altered in PPR infected cats despite demonstrable pathology and very low viral loads, it is possible that neuroAIDS progression would have occurred in PPR cats at later time points.

The recording of the auditory evoked potentials is a method for studying the function and integrity of the auditory pathway during the early stages of neurological disorders, including HIV (Samuelsson et al., 2006). The BAEP is the most commonly studied of all auditory potentials and it reflects the transmission time of stimuli along the brainstem portion of the primary auditory pathway. It has been accepted that the acoustic nerves generate the I and II peaks, the ipsilateral cochlear nucleus generates peak III, the superior olivary complex generates peak IV, and both the lateral lemniscus and the contralateral inferior colliculus generate peak V. Inasmuch as patients infected with HIV show significant increased peak latencies and interpeak latencies in BAEPs compared to normal subjects, in recent years the BAEP has been used as a tool to study the effects of HIV on the CNS (Matas et al., 2010). These alterations are observed even in asymptomatic HIV-seropositive patients, suggesting that BAEP can be a useful tool for detecting early neurological alteration caused by the HIV infection even in asymptomatic stages (Ollo et al., 1991). Because similar alterations in the BAEPs have been reported in cats infected with FIV, cats have been used as an animal model to study the effects of lentivirus in the CNS. BAEP in cats include five to eight peaks positive to vertex, and their neuronal generators in the cat have been described previously (Melcher et al., 1996a, b, c). The present study showed significant delays in the generation of P3, P4, P5 and P6 following the auditory stimulation, in all FIV-infected cats compared to control animals. These delays in the latency of the last components of the BAEPs are in concordance with Phillips et al (1994, 1996) and de Rozières et al (2004) who reported delays in the BAEPs peaks of FIV-infected animals, suggesting impairments in the brainstem transmission of infected cats.

A previous MRI neuroimaging study (Bucy et al., 2010) detected increased brain diffusion at 17.5 – 18 wk post-inoculation in neurologically asymptomatic cats with FIV infection from both PPR and C36; a finding consistent with the proposed mechanism of neuronal

injury during HIV and FIV infection due to indirect effects from cytokines, chemokines and glutamate released by activated leukocytes and microglial cells (Bell, 1998; Eggert et al., 2009; Gray et al., 1996; Meeker, 2007; Poli et al., 1999). Both viral strains resulted in similarly increased brain diffusion despite differences in peripheral viremia and proviral load, implicating indirect threshold-associated effects from secondary cytokines (Bucy et al., 2010). In this study, however, MRI results did not show any variances in cats infected with PPR, C36, or Pcenv. We propose that this is a result of lower viral loads, as viral loads in this study were 2–4 order of magnitudes lower when compared to that of the aforementioned study (Bucy et al., 2010).

Future *in vivo* studies should assess the virulence of additional chimeras constructed with individual substitutions of C36 genes *env*, *vif*, *orfA*, and *rev*, situated on the PPR backbone, as well as explore markers of neuropathogenicity that might be associated with 5' elements of PPR. By assessing individual gene products from each parental isolate, the virulence and viral replication mechanisms of each specific genetic element during infection may be further elucidated.

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Figure 1.

Pcenv is a chimeric virus containing the 3' regulatory elements *vif*, *orfA*, *rev1*, and *env* from a highly virulent clade C strain (C36) and the 5' backbone of a moderately virulent clade A strain (PPR) (from de Rozieres et al., 2008).

Figure 2.

PcenV-infected cats display mild neuropathology. (A) From the parietal lobe neocortex of a C36-infected cat (*C36-3*). This is also representative of the sham-infected control cats. (B) The corresponding area in the brain of a cat infected with PcenV (cat *PcenV-5*), displaying neuronal degeneration, seen as chromatin condensation and cytoplasmic shrinkage, accompanied by satellitosis, i.e., localization of microglial cells adjacent to affected neurons (examples indicated with arrows). (C) Microphotograph of neuropil in the brainstem of a sham-infected cat (*NI-2*), and (D) the corresponding area in a PcenV-infected cat (*PcenV-5*) with multifocal infiltration of lymphocytes (arrow). Scale bar = 150µm

Figure 3.

Microphotographs demonstrating activation of microglial cells (detected by IHC for Iba1) in brains of Pcenv infected cats (B; cat *Pcenv-3*) compared to control and PPR-cats (A; cat *NI-4*). Most cats infected with C36 had mild (C; cat *C36-4*) to marked microglial activation, the latter similar to Pcenv infected cats. Activated microglial cells displayed hypertrophied perikarya and increased numbers and length of dendrites (compare inserts in panel A, B, and C). Scale bars = 100 μ m. Inserts are from 50 $\times$ magnifications of originals.

Figure 4.

Expression of inflammation markers (phospho-p38-MAPK [A-C] and VEGF [D-F]) in brains from FIV-infected and sham-infected cats. (A) Sham-infected cat NI-4, (B) Pcenv-infected cat *Pcenv-2*, (C) C36-infected cat *C36-2*, (D) PPR-infected cat *PPR-1*, (E) Pcenv-infected cat *Pcenv-1*, (F) C36-infected cat *C36-1*. Brains of Pcenv-infected cats showed more intense immunolabeling for these markers compared to all other groups. All microphotos are from the parietal lobe. Scale bars = 100 μ m.

Figure 5.

RNA *gag* viral load is highest for C36, followed by Pcenv, then PPR. Pcenv viral load is intermediate to PPR and C36 in all brain tissues sampled, CSF, and plasma. Pcenv viral load was on average 10-fold greater than PPR and 10-fold less than C36. Viral load did not vary significantly in different neuroanatomical locations for each viral strain. PPR viral load was undetectable in the frontal lobe of all cats in that group. Data is presented as an average of all animals/group/tissue-site.

Figure 6.

Percentage of change in the latency of P3 compared to control cats (dotted line). BAEP waves from all FIV-infected cats showed characteristic delays in the latency of the P3 wave compared to the control group. FIV-PCenv infected cats showed an increase in the latency of P3 both 16 and 63 weeks post infection; however, this increase at 16 weeks did not reach significant values ($p < 0.07$).

IHC Average Group Scores

Table 1

Immunohistochemical scores presented as an average of all animals group tissue site on a scale from 0–4 (described in text). PCnv and C36 demonstrate higher mean scores for Iba-1a, and only PCnv demonstrates slight activation compared to controls as measured by Activated Caspase 3 staining. Iba-1a= microglia/activated macrophage-specific anti-ionized calcium binding adapter molecule 1; VEGF vascular endothelial growth OLIG-2; actCasp3=astrocyte-specific astrocyte-specific glial fibrillary acidic protein; p38-MAPK=phospho-p38 MAPK (P38-MAPK).

Group	Tissue	Iba-1a	VEGF	actCasp3	p38-MAPK
Non-infected	Parietal lobe	0	0.9	0.3	0
	Caudate nucleus	0.8	0.6	0.1	0
	Basal ganglia	0.8	1	0.3	0
	Cerebellum	0.5	0.9	0.3	0
	Average Score	0.5	0.8	0.2	0
PPR	Parietal lobe	0.6	1	0.4	0
	Caudate nucleus	0.6	1.3	0	0
	Basal ganglia	0.5	0.4	0.1	0
	Cerebellum	0	0.5	0	0.3
	Average Score	0.4	0.8	0.1	0.1
PCnv	Parietal lobe	1	1	0.1	0.4
	Caudate nucleus	0.7	0.8	0.3	0.1
	Basal ganglia	1.3	1.1	0.4	0
	Cerebellum	1.2	1.1	0.6	0
	Average Score	1.1	1.0	0.4	0.1
C36	Parietal lobe	1.5	0.8	0	0
	Caudate nucleus	1.5	0.5	0	0
	Basal ganglia	1.3	0.5	0.1	0
	Cerebellum	1.5	0.9	0.1	0
	Average Score	1.4	0.7	0.1	0

Kruskal-Wallis Test of mean RNA gag viral load

Table 2

Viral RNA load (Mean ± SE) differs among viral strains within tissues. Significant pair-wise comparisons among tissues represented by **a**, **b** and **c**. Mean viral RNA load is presented as RNA copies/million cells or per ml CSF

	Frontal Lobe	Caudate Nucleus	Basal Ganglia	Cerebellum	CSF
Pcenv	Mean	118.20 _a	323.00 _{ab}	320.20 _b	144.40 _a
	SE	87.06	261.99	144.93	42.59
PPR	Mean	0.00 _b	44.25 _a	7.50 _a	4.25 _a
	SE	0.00	35.25	7.50	4.25
C36	Mean	1695.25 _c	729.00 _b	1411.50 _b	2121.25 _b
	SE	458.11	340.26	946.06	956.05
$\chi^2_{2,11}$	10.172	6.565	8.203	7.933	8.414
<i>P</i>	0.006	0.038	0.017	0.019	0.015