Journal of NeuroVirology, 10(suppl. 1): 113–117, 2004

ISSN: 1355-0284 print / 1538-2443 online
DOI: 10.1080/13550280490270815

Taylor & Francis
Taylor & Francis Group

Comparative neurovirulence in lentiviral infections: The roles of viral molecular diversity and select proteases

Christopher Power, Kunyan Zhang, and Guido van Marle

Departments of Clinical Neurosciences and Microbiology and Infectious Diseases, University of Calgary, Calgary, Alberta, Canada

All lentiviruses infect the brain, causing chronic neurological disease in their respective hosts. To examine the relationship(s) between lentivirus molecular diversity and the development of neurological disease, we examined *in vitro* and *in vivo* models of lentivirus neurovirulence using different recombinant viruses derived from human (HIV-1) and feline (FIV) immunodeficiency viruses. Both *in vitro* and *in vivo* studies of FIV neurovirulence showed that the FIV envelope derived from a neurovirulent strain was a principal determinant of neuropathogenesis, although systemic immunosuppression was also an integral feature of FIV neurovirulence. Studies of HIV-1 envelope sequences derived from brain or blood indicate that molecular diversity is greater in viruses from patients with HIV-associated dementia (HAD), compared to nondemented individuals. Moreover, the hypervariable V3 domain of HIVgp120, regardless of the HIV-1 clade from which it was derived, was an important region for mediating neurotoxicity *in vitro* but the level of viral replication did not influence neurotoxicity. For both the HIV-1 and FIV envelopes and HIV-1 Tat, induction of matrix metalloproteinase (MMP)-2 in macrophages was a consistent finding. Neurotoxicity caused by supernatants from HIV-infected or transfected macrophages, containing MMP-2, was greater than direct neurotoxicity levels caused by direct exposure of neurons to virus in assays of total neuronal death, but not in assays of neuronal apoptosis. Proteinase-activated receptor (PAR)-1 and its ligand thrombin were also induced during HIV infection, chiefly on astrocytes. PAR-1 activation resulted in gliosis and neurobehavioral changes in an animal model and resulted in *N*-methyl-D-aspartate (NMDA) receptor-mediated neuronal death. These findings suggest that the lentivirus envelope, which is a domain of extensive molecular diversity in brain-derived lentivirus isolates, directly influences neuropathogenesis through the activation of select proteases, underscoring the importance of concentrating on individual viral genes and proteases in the development of neuroprotective agents for HIV-related neurological disease. *Journal of NeuroVirology* (2004) **10(suppl. 1),** 113–117.

Keywords: dementia; FIV; HIV-1; matrix metalloproteinases; neurotoxicity; proteinase-activated receptors

Address correspondence to Dr. Christopher Power, Neurovirology Laboratory, Neuroscience Research Group, Departments of Clinical Neuroscience and Microbiology and Infectious Diseases, 3330 Hospital Drive NW, Calgary, Alberta, Canada T2N 4N1. E-mail: power@ucalgary.ca

The authors thank Belinda Ibrahim for assistance with manuscript preparation. These studies were supported by the Canadian Institutes of Health Research, Alberta Heritage Foundation for Medical Research, and the Canadian Foundation for AIDS Research.

Received 15 February 2003; accepted 15 April 2003.

Like many retroviruses, human immunodeficiency virus type 1 (HIV-1) infects the nervous system (neurotropism) and causes neurological disease (neurovirulence) in a subset of infected persons (Johnson, 1998). Together with other lentiviruses, including simian (SIV) and feline (FIV) immunodeficiency viruses, HIV-1 infection is associated with central nervous system damage resulting in cognitive manifestations, including HIV-associated dementia (HAD) and minor cognitive and motor

disorder (MCMD) (Power and Johnson, 2001). Similarly, HIV-1 infection has also been associated with the development of distal sensory polyneuropathy (DSP) and other types of neuropathy in the peripheral nervous system (Keswani *et al*, 2002). There is abundant evidence from other neurotropic retrovirus models that the envelope gene sequence and expression is pivotal in the development of neurovirulence (Poulsen *et al*, 1998). Similarly, among the lentiviruses, including HIV, FIV, and SIV, there are compelling data to suggest that envelope-encoded gene products contribute to neurovirulence (Power and Johnson, 2001). However, among lentiviruses and other retroviruses, other genes seem to participate in the development of neurovirulence, but the role of the envelope remains predominant amongst each of these neurotropic viruses. Herein, we describe studies implicating the role of the envelope gene from both FIV and HIV in the development of neurovirulence using *in vivo* and *in vitro* models. We also examine host gene responses in relation to the development of neurovirulence, including analysis of the several proteases expressed in the brain. The present studies highlight the concept that neurological disease occurrence in the context of an infection requires unique properties of the infectious agent together with a pathogenic host immune response.

FIV molecular diversity and neurovirulence

Earlier studies indicate that FIV causes immune suppression, is neurotropic, and utilized CXCR4 for infection (Bendinelli *et al*, 1995), but more recent studies from our laboratory and others suggest that the FIV utilized both CXCR4 and CCR5 for infection of lymphocytes and macrophages, depending on the viral strain (Johnston and Power, 2002). We have also reported that the neurovirulent FIV strain, V1CSF, induced neurological disease in a neonatal feline model (Johnston *et al*, 2002a; Power *et al*, 1998a). To examine the role of the envelope in FIV neuropathogenesis, we constructed recombinant viruses in which we cloned the entire FIV envelope into a molecular clone derived from the non-neurovirulent strain Petaluma. The resulting chimeric virus, FIV-Ch, readily infected peripheral blood mononuclear cells and macrophages from cats (Johnston *et al*, 2000). *In vivo* studies using the same viruses indicated that the recombinant virus, FIV-Ch, induced comparable levels of neurobehavioral impairment to those observed in animals infected with VICSF, but, in contrast, both mock-infected animals and animals infected with Petaluma exhibited minimal neurobehavioral impairment over a 12-week period following infection (Johnston *et al*, 2002b). These neurobehavioral findings were associated by a significant reduction in CD4 lymphocyte count in both the V1CSF- and FIV-Ch–infected animals, relative to the other groups. Neuropathological analysis revealed that *c-fos* expression in neurons was increased in the brains of those animals infected with V1CSF and FIV-Ch, which was accompanied by dysmorphic neurons and apparent neuronal loss compared to mock- and Petaluma-infected animals. Similarly, the extent of inflammation reflected by astrogliosis and microgliosis was greater amongst animals infected by V1CSF and its daughter chimeric virus, FIV-Ch, compared to mock- and Petaluma-infected animals. These studies suggest that the envelope gene is pivotal in the determination of neurovirulence in this lentivirus animal model and, furthermore, this was reflected by pathological changes including inflammation and neuronal injury.

HIV-1 molecular diversity and neurovirulence

There are numerous studies implicating the role of HIV envelope gene, including its gene products, gp120 and gp41, in the neuropathogenesis of HIV infection (Nath, 1999). Earlier studies from our laboratory as well as others suggest that viral diversity in brain-derived HIV-1 sequences was associated with the development of HAD. Notably, mutations in the V1 (Power *et al*, 1998b) and V3 (Power *et al*, 1994) hypervariable loops of the envelope surface unit (gp120), together with sequence differences in the *tat* gene (Bratanich *et al*, 1998), were associated with neurocognitive impairment. In addition, analysis of recombinant viruses containing a brain-derived V3 region revealed that all viruses uniformly used CCR5 as a coreceptor for infection (Chan *et al*, 1999) and were exclusively macrophage-tropic. More recently, we investigated HIV envelope diversity in blood and brain from patients with and without HAD. In both blood- and brain-derived HIV V3 sequences, we found that that the extent of sequence diversity as well as the number of nonsynonymous mutations was greater in patients (Figure 1A) who exhibited HIV-associated dementia (van Marle *et al*, 2002). These latter findings were complemented by studies suggesting that sera from patients with HAD exhibited a relative inability to neutralize CCR5 (R5)-dependent viruses compared to CXCR4 (X4)-dependent viruses and sera from acquired immunodeficiency syndrome (AIDS) patients without dementia (Figure 1B). Hence, an inability to neutralize and eliminate R5 viruses may permit this select population of viruses to enter the nervous system and ultimately cause disease.

The extent of viral diversity within HIV is immense, although most studies of HIV-1 molecular heterogeneity are based on plasma or peripheral blood-derived viruses from B clade viruses isolated in North America and Western Europe. Studies from our laboratory suggest that viral diversity in brain-derived sequences from patients infected with viruses from different HIV-1 clades indicated that like viral sequences derived from blood and plasma, those

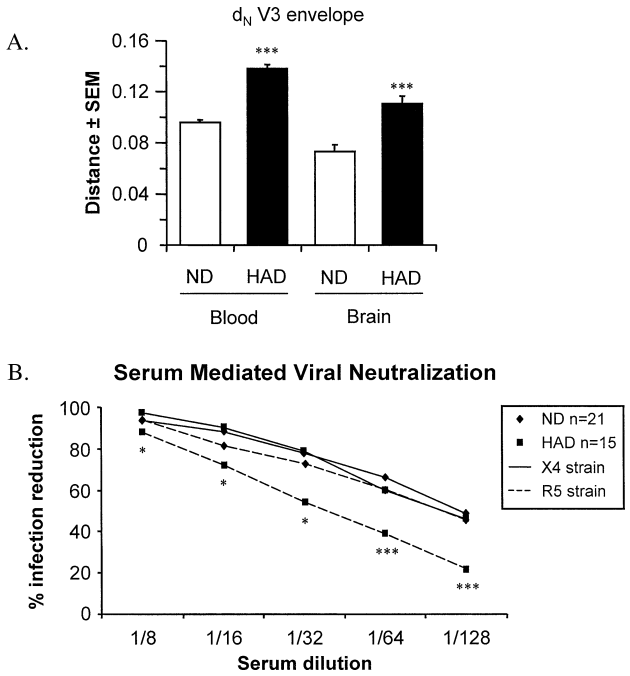

Figure 1 HIV-1 molecular diversity in blood and brain and differential neutralization of R5-dependent virus. (**A**) Comparison of blood- and brain-derived HIV-1 gp120 V3 sequences revealed that molecular diversity was greater in patients with HAD compared to nondemented AIDS patients (ND), as measured by nonsynonymous (amino acid changing) mutation rates. (**B**) Sera from HAD patients exhibited lower neutralization efficiencies of a R5 virus compared to ND patients, but the neutralization efficiencies of an X4 virus was similar for both groups.

viruses derived from brains exhibited marked sequence diversity among patients (Zhang *et al*, 2001). Moreover, in a small subset of viral sequences, we found evidence of viral recombination, which would also contribute to the extent of viral diversity. To explore the functional consequences of viral diversity amongst non-B clade viruses, we constructed recombinant viruses in which we inserted the C2V3 envelope region of the non-B clade virus into a molecular clone of HIV-1 and subsequently examined the biological features of the recombinant viruses. Although many of the recombinant viruses expressed viral envelope protein, they failed to replicate in peripheral blood mononuclear cells or macrophages (Zhang *et al*, 2003). In a subset of viruses that did replicate, all were found to be R5-dependent viruses. Surprisingly, all of the recombinant viruses, regardless of whether they replicated or not, were found to induce neurotoxicity *in vitro* mediated by both necrosis and apoptosis, although apoptosis predominated. These studies are similar to earlier studies from our laboratory in which we showed that recombinant viruses containing the C2V3 of patients with and without HAD were R5-dependent viruses. However, recombinant viruses constructed from patients with HAD were significantly more neurotoxic in *in vitro* assays in which supernatants from infected macrophages were applied to human neuronal cell lines (Power *et al*, 1998b), again emphasizing the importance of the envelope gene in neurovirulence.

Lentivirus-induced protease expression in brain

Given earlier studies implicating the role of matrix metalloproteinases (MMPs) in the development of HIV-associated neurological disease (Conant *et al*, 1999), we examined macrophages infected by recombinant viruses derived from patients with HAD and found increased expression of signal transduction and activation of transcription (STAT-1) and Janus kinase (JAK-1) relative to macrophages infected by AIDS patients without dementia (Johnston *et al*, 2000). Additionally, increased MMP-2 and, to a lesser extent, MMP-9 release from macrophages was also observed, which accompanied the STAT/JAK pathway activation and release of neurotoxins in supernatants from infected macrophages, providing a direct link between the expression of MMPs and neuronal injury. Studies from our laboratory also suggest that HIV-1 Tat regulates the expression of MMP-2 and -7 and that the sequence diversity within HIV Tat influences the relative expression of MMP-2, which caused neurotoxicity *in vitro* (Johnston *et al*, 2001). Similarly, FIV V1CSF and FIV-Ch induced STAT-1/JAK-1 signaling in feline macrophages that was associated with increased MMP-2 release (Johnston *et al*, 2000). We examined the *in vivo* expression of MMP-2 and -9 together with tumor necrosis factor (TNF)-α by measuring mRNA and protein levels in brain following FIV infection (Johnston *et al*, 2002b). MMP-2 protein and mRNA was increased in the brains of those animals infected with the neurovirulent FIV strains, V1CSF and FIV-Ch, whereas MMP-9 was increased amongst all of the FIV-infected groups but not in the mock-infected animals. Likewise TNF-α was increased at the mRNA level in the animals infected by V1CSF and FIV-Ch, but it was not increased in mock- or Petaluma-infected animals. Of interest, MMP-2 cleaves the potentially neurotoxic molecule stromal cell-derived factor (SDF-1) *in vitro* (McQuibban *et al*, 2001). Furthermore, cleaved SDF-1 fails to block HIV-1 infection *in vitro*, unlike the uncleaved SDF-1, although previous studies indicate that MMP-cleaved chemokines may abrogate inflammation (McQuibban *et al*, 2000). Studies of cleaved chemokines' effects in the nervous system are currently in progress. Very recent studies from our laboratory indicate that cleaved SDF-1 is highly cytotoxic to neurons both in vitro and in vivo through activation of a G protein coupled receptor (Zhang et al, 2003).

The relative role of different proteases in the central nervous system and their relationship to pathogenesis has recently attracted increased attention. Although MMPs are clearly important for both neural ontogeny and pathogenesis (Yong *et al*,

2001), other proteases have been implicated in pathogenesis, including the serine protease, thrombin. The proteinase-activated receptors (PARs), which are activated by thrombin or tryptase, are also expressed within the central and peripheral nervous systems (Hollenberg, 2002). PAR-1 is expressed on astrocytes and neurons and, moreover, PAR-1 overexpression is associated with astrogliosis and death of motor neurons in transgenic mice (Festoff *et al*, 2000). The PARs are known to mediate a complex cascade of signaling pathways, which can result in the release of proinflammatory molecules as well as cell death through apoptosis, depending on the cell type on which the individual PAR is expressed. To explore the role of PAR-1 expression in the brain during HIV infection, we compared mRNA levels of PAR-1 and prothrombin in HIV-infected and control patients. These studies suggested that PAR-1 and prothrombin mRNA levels were increased in the brains of patients with HIV infection compared to HIV-1 seronegative controls (Boven *et al*, 2003). Immunocytochemistry showed that PAR-1 was chiefly localized on activated astrocytes in patients with HIV encephalitis. Prothrombin expression was detected in both neurons and astrocytes in brains of patients with HIV encephalitis, although it was minimally expressed, like PAR-1, in control patients. PAR-1 was also found to regulate the expression of interleukin (IL)-1β and inducible nitric oxide synthase (iNOS) in astrocytes *in vitro* using thrombin and the activating PAR-1 peptide ligand, TF. Moreover, supernatants from human fetal astrocytes applied to human fetal neurons showed that supernatants from thrombin- as well as TF-treated astrocytes caused significantly greater neuronal death than control supernatants. Neurotoxicity was blocked by the *N*-methyl-D-aspartate (NMDA) receptor antagonist, MK801, suggesting that the ligand for the NMDA receptor might be glutamate, which was released by thrombin- and TF-treated astrocytes. Using a rodent model of Parkinson's disease (Ungerstedt and Arbuthnott, 1970), we stereotactically implanted the TF peptide in the striatum of CD1 mice and showed that there was markedly increased astrogliosis and microgliosis together, with increased iNOS expression, in those animals that received the implant of TF peptide relative to animals implanted with an inactive control peptide. These studies were complemented by behavioral studies suggesting that the TF peptide induced significantly greater increase in neurobehavioral abnormalities measured by ipsiversive rotary movements in animals that received the TF peptide relative to controls. These studies underline the importance of the proteinase-activated receptors in the pathogenesis of neurologic disease. The precise mechanisms by which PARs are upregulated remain unknown, but recent studies indicate that expression of the HIV gp120 in astrocytes resulted in increased PAR-1 expression (Noorbaksh and Power, unpublished). Moreover, multiple parallel protease-mediated pathways driven by viral expression and sequence diversity contribute to neuronal injury and death causing dementia. Future studies will concentrate on the precise biochemical mechanisms by which viral sequence diversity induces these pathogenic signaling pathways in the brain.

References

Bendinelli M, Pistello M, Lombardi S, Poli A, Garzelli C, Matteucci D, Ceccherini-Nelli L, Malvaldi G, Tozzini F (1995). Feline immunodeficiency virus: an interesting model for AIDS studies and an important cat pathogen. *Clin Microbiol Rev* **8:** 87–112.

Boven LA, Henry SD, Vergnolle N, Hollenberg M, Power C (2003). Upregulation of proteinase-activated receptor 1 expression in astrocytes during HIV encephalitis. *J Immunol* **170**: 2638–2646.

Bratanich AC, Liu C, McArthur JC, Fudyk T, Glass JD, Mittoo S, Klassen GA, Power C (1998). Brain-derived HIV-1 tat sequences from AIDS patients with dementia show increased molecular heterogeneity. *J NeuroVirol* **4:** 387–393.

Chan SY, Speck RF, Power C, Gaffen SL, Chesebro B, Goldsmith MA (1999). V3 recombinants indicate a central role for CCR5 as a coreceptor in tissue infection by human immunodeficiency virus type 1. *J Virol* **73:** 2350–2358.

Conant K, McArthur JC, Griffin DE, Sjulson L, Wahl LM, Irani DN (1999). Cerebrospinal fluid levels of MMP-2, 7, and 9 are elevated in association with human immunodeficiency virus dementia. *Ann Neurol* **46:** 391–398.

Festoff BW, D'Andrea MR, Citron BA, Salcedo RM, Smirnova IV, Andrade-Gordon P (2000). Motor neuron cell death in wobbler mutant mice follows overexpression of the G-protein-coupled, protease-activated receptor for thrombin. *Mol Med* **6:** 410–429.

Hollenberg MD (2002). PARs in the stars: proteinase-activated receptors and astrocyte function. *Am J Physiol Cell Physiol* **283:** C1347–C1350.

Johnson RT (1998). *Viral infections of the nervous system*, 2nd ed. Philadelphia: Lippincott-Raven.

Johnston JB, Jiang Y, van Marle G, Mayne MB, Ni W, Holden J, McArthur JC, Power C (2000). Lentiviral infection in the brain induce matrix metalloproteinase expression: the role of envelope diversity. *J Virol* **74:** 7211–7220.

Johnston JB, Power C (2002). Feline immunodeficiency virus xenoinfection: The role of chemokine receptors and envelope diversity. *J Virol* **76:** 3626–3636.

Johnston JB, Silva C, Hiebert T, Buist R, Peeling J, Dawood MR, Power C (2002a). Neurovirulence depends on virus input titer in brain in feline immunodeficiency virus infection: evidence for activation of innate immunity and neuronal injury. *J NeuroVirol* **8:** 420–431.

Johnston JB, Silva C, Power C (2002b). Envelope gene-mediated neurovirulence in feline immunodeficiency virus infection: induction of matrix metalloproteinases and neuronal injury. *J Virol* **76:** 2622–2633.

Johnston JB, Zhang K, Silva C, Shalinsky D, Conant K, Corbett D, Yong VW, Power C (2001). HIV-1 Tat neurotoxicity is prevented by matrix metalloproteinase inhibitors. *Ann Neurol* **49:** 230–241.

Keswani SC, Pardo CA, Cherry CL, Hoke A, McArthur JC (2002). HIV-associated sensory neuropathies. *AIDS* **16:** 2105–2117.

McQuibban GA, Butler GS, Gong JH, Bendall L, Power C, Clarke-Lewis I, Overall CM (2001). Matrix metalloproteinase cleavage of the chemokine stromal cell derived factor-1 results in loss of chemokine activity. *J Biol Chem* **276:** 43503–43508.

McQuibban GA, Gong JH, Tam EM, McCulloch CA, Clark-Lewis I, Overall CM (2000). Inflammation dampened by gelatinase A cleavage of monocyte chemoattractant protein-3. *Science* **289:** 1202–1206.

Nath A (1999). Pathobiology of human immunodeficiency virus dementia. *Semin Neurol* **19:** 113–127.

Patrick M, Johnston JB, Power C (2002). Lentivirus neuropathogenesis: comparative neuroinvasion, neurotropism, neurovirulence, and host susceptibility. *J Virol* **76:** 7923–7931.

Poulsen DJ, Robertson SJ, Favara CA, Portis JL, Chesebro BW (1998). Mapping of a neurovirulence determinant within the envelope protein of a polytropic murine retrovirus: induction of central nervous system disease by low levels of virus. *Virology* **248:** 199–207.

Power C, Buist R, Johnston JB, Del Bigio MR, Ni W, Dawood MR, Peeling J (1998a). Neurovirulence in feline immunodeficiency virus-infected neonatal cats is viral strain specific and dependent on systemic immune suppression. *J Virol* **72:** 9109–9115.

Power C, Johnson RT (2001). Neurovirological and neuroimmunological aspects of HIV infection. *Adv Virus Res* **56:** 579–624.

Power C, McArthur JC, Johnson RT, Griffin DE, Glass JD, Perryman S, Chesebro B (1994). Demented and nondemented patients with AIDS differ in brain-derived human immunodeficiency virus type 1 envelope sequences. *J Virol* **68:** 4643–4649.

Power C, McArthur JC, Nath A, Wehrly K, Mayne M, Nishio J, Langelier T, Johnson RT, Chesebro B (1998b). Neuronal death induced by brain-derived human immunodeficiency virus type 1 envelope genes differs between demented and nondemented AIDS patients. *J Virol* **72:** 9045–9053.

Ungerstedt U, Arbuthnott GW (1970). Quantitative recording of rotational behaviour in rats after 6-hydroxydopamine lesions of nigrostriatal dopamine system. *Brain Res* **24:** 485–493.

van Marle G, Rourke SB, Zhang K, Silva C, Ethier J, Gill MJ, Power C (2002). HIV dementia patients exhibit reduced viral neutralization and increased envelope sequence diversity in blood and brain. *AIDS* **16:** 1905–1914.

Yong VW, Power C, Forsyth P, Edwards D (2001). Metalloproteinases in the biology and pathology of the nervous system. *Nat Rev Neurosci* **2:** 502–511.

Zhang K, Hawken M, Rana F, Silva C, Gartner S, Power C (2001). Human immunodeficiency Virus type 1 clade A and neurotropism: molecular evolution, recombination and co-receptor utilization. *Virology* **283:** 19–30.

Zhang K, Rana F, Silva C, Ethier J, Wehrly K, Chesebro B, Power C (2003). Human immunodeficiency virus type 1 envelope-mediated neuronal death: Uncoupling of viral replication and neurotoxicity. *J Virol* **77:** 6899–6912.

Zhang K, McQuibban GA, Silva C, Butter GS, Johnston JB, Clark-Lewis I, Overall CM, Power C (2003). HIV-induced metalloproteinase processing of the chemokines stromal cell-derived factor-1 causes neurodegeneration. *Nat Neurosci*.