Dual lentivirus infection potentiates neuroinflammation and neurodegeneration: viral copassage enhances neurovirulence

Amir Afkhami-Goli, 1, 2 Shu-Hong Liu, 3 Yu Zhu, 1 Joseph M Antony, 1 Hosseinali Arab, 2 and Christopher Power 1, 3

1 Departments of Medicine and Medical Microbiology and Immunology, University of Alberta, Edmonton, Alberta, Canada; 2 Department of Pharmacology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran; and 3 Department of Clinical Neurosciences, University of Calgary, Calgary, Alberta, Canada

Infection by multiple lentiviral strains is recognized as a major driving force in the human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS) epidemic, but the neuropathogenic consequences of multivirus infections remain uncertain. Herein, we investigated the neurovirulence and underlying mechanisms of dual lentivirus infections with distinct viral strains. Experimental feline immunodeficiency virus (FIV) infections were performed using cultured cells and an in vivo model of AIDS neuropathogenesis. Dual infections were comprised of two FIV strains (FIV-Ch and FIV-PPR) as copassaged or superinfected viruses, with subsequent outcome analyses of host immune responses, viral load, neuropathological features, and neurobehavioral performance. Dual infections of feline macrophages resulted in greater IL-1β (interleukin-1β), TNF-α (tumor necrosis factor α), and IDO (indoleamine 2,3-dioxygenase) expression and associated neurotoxic properties. FIV co-infection and sequential superinfection in vivo also induced greater IL-1β, TNF-α, and IDO expression in the basal ganglia (BG) and cortex (CTX), compared to the monovirus- and mock-infected groups, although viral loads were similar in single virus/C1 and dual virus/C1 infected animals. Immunoblot analyses disclosed lower synaptophysin immunoreactivity in the CTX resulting from FIV super- and co-infections. Cholinergic and GABAergic neuronal injury was evident in the CTX of animals with dual FIV infections. With increased glial activation and neuronal loss in dual FIV-infected brains, immunohistochemical analysis also revealed elevated detection of cleaved caspase-3 in dysmorphic neurons, which was associated with worsened neurobehavioral abnormalities among animals infected with the copassaged viruses. Dual lentivirus infections caused an escalation in neuroinflammation and ensuing neurodegeneration, underscoring the contribution of infection by multiple viruses to neuropathogenesis. Journal of NeuroVirology (2009) 15, 139–152.

Keywords: FIV; nervous system; dual infection; neuroinflammation; neuron; glia; apoptosis

Address correspondence to Dr. Christopher Power, Department of Medicine (Neurology), 611 HMRC, University of Alberta, Edmonton, AB, T6G 2H3 Canada. E-mail: chris.power@ualberta.ca

Amir Afkhami-Goli and Shu-Hong Liu contributed equally to this study.

These studies were supported by the Canadian Institutes of Health Research (CIHR). A.A.-G. was supported by a scholarship from the Iranian Ministry of Science. J.M.A. was supported by a Studentship from the Multiple Sclerosis Society of Canada. Y.Z. holds a Fellowship from the CIHR. C.P. holds a Canada Research Chair (Tier 1) in Neurological Infection and Immunity and is an Alberta Heritage Medical Research Senior Scholar. The authors thank Martine Ooms and Neda Shariat for technical assistance and Stephanie Skinner and Leah DeBlock for manuscript preparation.

Received 26 June 2008; revised 18 August 2008; accepted 7 October 2008
**Introduction**

Infection by multiple strains of human immunodeficiency virus type 1 (HIV-1) represents an important source of viral diversity in the current HIV/AIDS (acquired immunodeficiency syndrome) epidemic (Taylor *et al.*, 2008). Indeed, several of the principal HIV-1 strains identified globally are derived from recombination between different primary strains, which present major challenges in terms of developing vaccines (Klausner *et al.*, 2003) and implementing antiretroviral therapies (Kantor and Katzenstein, 2004). A hallmark of all pathogenic lentivirus infections is extensive viral genetic diversity as a consequence of the error-prone nature of the viral reverse transcriptase (RTase) and a propensity for viral recombination, coupled with high rates of virion production that are modulated by immune selection (Patrick *et al.*, 2002). The pivotal step in retroviral recombination is the simultaneous infection by two or more viral strains of the same cell during a single transmission event (coinfection) or through sequential viral infection during multiple transmission events (superinfection). To date, much of the knowledge about dual retroviral infections is derived from *ex vivo* studies (Kim *et al.*, 1993, 1996), clinical case reports documenting dual infection with HIV-1 and HIV-2 (Andersson *et al.*, 1999; Sarr *et al.*, 2000) or by examining viruses belonging to different HIV-1 subtypes as superinfections (Fang *et al.*, 2004; Takehisa *et al.*, 1997) or coinfections (Iversen *et al.*, 1999; Ramos *et al.*, 1999; Becker-Pergola *et al.*, 2000; Long *et al.*, 2000; Thomson *et al.*, 2001). Indeed, there is compelling evidence that both drug-sensitive and -resistant HIV-1 strains exist concurrently in infected individuals and each strain predominates depending on the antiretroviral regimen efficacy (Brumme and Harrigan, 2006). While bearing viral interference in mind and the fact that, at least in the case of superinfections, the primary viral infection may result in subsequent resistance to the latter infection(s) (reviewed in Nethe *et al.*, 2005), there is no widespread consensus regarding the real prevalence of dual HIV strain infection, although the widespread detection of circulating recombinant form (CRFs) is compelling evidence that dual infection occurs (Takek *et al.*, 2004).

Feline immunodeficiency virus (FIV) is a member of the lentivirus subfamily; it causes persistent infection in domestic cats and shares many of the immunological and neurological properties with HIV (Bendinelli *et al.*, 1995). FIV causes neurological disorders in 20% to 40% of naturally infected cats by entering the nervous system and infecting parenchymal microglia, perivascular macrophages, and astrocytes (Power, 2001). Neuropathogenic effects mediated by FIV infection are viral strain specific (Power *et al.*, 1998; Johnston *et al.*, 2002b) and range from impaired motor activity, seizures, as well as behavioral abnormalities such as psychomotor slowing, aggressiveness, disrupted sleep and arousal patterns (Phillips *et al.*, 1994; Prospero-Garcia *et al.*, 1999). Neuroimmune activation during FIV infection is accompanied by neuronal injury in the basal ganglia and cortical regions. Indeed, microglial and astroglial activation are cardinal features of FIV infection, although multinucleated giant cells are rarely observed in FIV infection (Power *et al.*, 1997; Noorbakhsh *et al.*, 2006).

Although dual lentiviral infections occur *in vivo* (Blackard *et al.*, 2002), the neurologic consequences of dual infection in terms of viral properties such as neurotropism, replication kinetics, neuroimmune responses, and ensuing disease progression have not been fully investigated. Previous studies of HIV-1 have focused on a blood-derived evidence of dual lentivirus infection, reflecting largely T-cell infection. However, the extent of dual infection of monocytoïd cells, which are the principal target cells in the brain, is unknown. Dual HIV-1 infection of brain is apparent from several reports (Smit *et al.*, 2004), with evidence of viral recombination (Zhang *et al.*, 2001) and an association with HIV-associated dementia (Salemi *et al.*, 2005). We hypothesized that dual lentivirus infection with two distinct neurovirulent lentivirus strains would worsen neurologic disease by amplifying the underlying pathogenic mechanisms. The present studies indicated that infection by two FIV strains, known to cause neurologic disease, resulted in enhanced neuroinflammation and neurodegeneration, which was dependent on the specific infection paradigm.

**Results**

**Dual infection with FIV-Ch and FIV-PPR**

The sensitivity of the FIV *pol* primers was assessed by amplifying 10-fold serial dilutions of FIV-Ch and FIV-PPR plasmid added to equal amounts of healthy (uninfected) feline peripheral blood mononuclear cell (PBMC)-derived cDNA (Figure 1A). To determine if both FIV-Ch and FIV-PPR were detected in dual-infected primary feline monocyte-derived macrophage (MDM) cultures, we digested the polymerase chain reaction (PCR) amplicons for FIV-Ch and FIV-PPR *pol* with Dra I, revealing different digestion patterns with a 214-bp undigested band for FIV-Ch but 120- and 94-bp digested bands for FIV-PPR. Indeed, different restriction digestion patterns of *pol* amplicons obtained from FIV-infected MDMs showed that each viral strain was detectable. In MDMs superinfected simultaneously with both viruses (FIV-SI) or infected with copassaged viruses (FIV-CP), at day 3 (data not shown) and day 6 post infection (p.i.) (Figure 1B) amplicons corresponding to both FIV strains were evident. These studies indicated that both viruses were present in MDMs...