Journal of NeuroVirology, 10(suppl. 1): 1–6, 2004

ISSN: 1355-0284 print / 1538-2443 online
DOI: 10.1080/13550280490427532

Taylor & Francis
Taylor & Francis Group

Guest Editorial

Viral and host genetic factors regulating HIV/CNS disease

Jeymohan Joseph[1] and Toby Behar[2]

[1]*Center for Mental Health Research on AIDS, National Institute of Mental Health, Bethesda, Maryland, USA;*
[2]*Neural Environment Cluster, National Institute of Neurological Disorders and Stroke, Bethesda, Maryland, USA*

Jeymohan Joseph

Toby Behar

The National Institute of Mental Health and the National Institute of Neurological Disorders and Stroke convened a joint symposium entitled "Viral and Host Genetic Factors Regulating HIV/CNS Disease" on November 20–22, 2002 in Washington DC. The purpose of this meeting was to review studies of viral genetics that examined molecular diversity of HIV-1 and how it reflects viral trafficking into the CNS, mechanisms of pathogenesis, and compartmentalized evolution of drug resistance in the context of currently available highly active anti-retroviral therapy (HAART). Research on compartmentalized viral evolution is germane to the ongoing debate concerning viral reservoirs in the central nervous system (CNS) and the potential for reseeding drug resistant strains of HIV-1 to the periphery. The role of HIV-1 diversity in regulating interactions with host receptors on CNS-derived cells and the functional impact on neuropathogenesis were also examined. In addition a session was devoted to highlighting emerging findings that emphasized the impact of host genetic factors on susceptibility and progression of HIV-associated CNS disease. This special issue is devoted to papers elaborating the topics presented at the symposium. These papers not only summarize important recent progress in understanding how host and viral genetics factors influence HIV neuropathogenesis, but they also highlight new emerging questions that will guide future research efforts.

Compartmentalization of HIV-1 infection in the CNS/CSF

A variety of factors may determine discordance or concordance of viral sequences and resistance patterns in the CNS vs. peripheral compartments. Trafficking of immune cells between compartments, compartment-specific evolution, and the capacity of antiretrovirals to penetrate the blood-brain barrier may influence the observed sequence in different compartments.

Several reports focused on how HIV-1 evolves in the CNS compartment in the context of HAART. The tissue environment in the CNS may impact the evolution of HIV such that CNS virus exhibits genotypic and phenotypic differences from virus found in the periphery. Viral genes may be subject to selective pressures that result in discordance at multiple loci. Clinically, cognitive impairment associated with HIV-induced dementia may be a result of viral genetic discordance and independent replication in the CNS compartment (Ellis *et al*, 2000; Bestetti *et al*, 2004).

Another report examined HIV gp160 and protease-reverse transcriptase (pro-RT) sequences from lymph node, bone marrow and multiple regions of brain parenchyma. Co-mingling, as well as close homology, was observed among gp160 sequences recovered from different regions of the same brain, and between brain and marrow sequences, whereas lymph node sequences formed independent clusters. Overall, pro-RT sequences from lymph node exhibited predominantly antiretroviral-resistant genotype, whereas brain sequences were essentially wild type; the latter may be a result of either viral latency or limited drug penetrance into brain. Both patterns were represented in bone marrow. The phylogenetic relationships observed for both *env* and *pol* further suggest that during late-stage HIV-1 infection, marrow-derived monocytes transport HIV-1 into the brain (Liu *et al*, 2000).

The role of immune cell trafficking in the spread of virus to various compartments was further confirmed

in studies that used partitioning methodologies to investigate the association between infection in CNS and in tissues where macrophages may be resident (lung, GI tract and bone marrow). In a cohort of HIV-infected patients, non lymphoid tissues contained a virus population genetically distinct from those recovered from lymph node and generally resembled those found in the CNS, implying a shared infected cell type and route of dissemination (Wang *et al*, 2001). These findings were also confirmed in the SIV model (Williams *et al*, 2002) where the infected macrophages in the CNS and similar populations of cells in the lung, gut and bone marrow were found to express common immune phenotypes (CD14, CD45, PCNA positive).

Novel approaches have been used to study the evolution of virus and its compartmentalization. For example, an ultraintensive CSF sampling approach was developed whereby intrathecal lumbar catheters were inserted and maintained in the spinal canal for prolonged periods of time, allowing continuous sampling of CSF. Results from these ultraintensive sampling studies confirmed that there is discordance between HIV-1 nucleotide sequences in CSF and plasma, including mutations associated with drug resistance (Haas 2004). Another novel approach used strategic treatment interruption (STI), a method that provides an avenue to study dynamics of infection and host responses in the CSF relative to blood. The STI studies underscored the relationship between CSF viral load and CSF lymphocytosis. The data from the STI studies were conceptualized in a model that defines three types of CSF infection: (1) initiated from without (push); (2) initiated from within (pull) and (3) amplified by positive feedback within the CNS (Price and Deeks 2004).

A well-recognized problem affecting the validity of studies investigating HIV compartmentalization in CNS/CSF is the accurate sampling of small viral populations. The heteroduplex-tracking assay (HTA) has recently been adapted for use as an alternative to cloning and sequencing. HTA is a gel-based assay that can be used to compare viral genotypes in small populations. The conformation of the heteroduplex is very sensitive to sequence differences, thereby generating varying rates of migration for different genotypes, which are displayed in the gel as discrete bands. The compartmentalization of HIV-1 in blood plasma versus CSF was studied during the primary and chronic stage infection taking into account the varying degrees of neurologic dysfunction. Limited compartmentalization was observed during primary infection (1/5 patients) while compartmentalization during chronic infection was more extensive (9/11 patients). These findings suggest that there is an independent source of replication for the virus in the CNS and there may be different selective pressures in the two compartments. No correlation was noted, however, between the extent of compartmentalization and CNS disease in this sample of subjects (Swanstrom 2002, personal communication).

In summary, the various reports at the symposium devoted to HIV compartmentization provide evidence that viral discordance can been found between CNS and peripheral compartments. In contrast, several investigators presented evidence that common HIV-1 sequences have been found in CNS, bone marrow, and GI tract-derived monocyte/macrophages, a population that serves as a carrier of virus to different compartments.

The basis for viral diversity and discordant resistance patterns is a widely debated issue. One explanation for this discordance is that the CNS and periphery represent different compartments of viral replication and that virions generated in each compartment have differential access to CSF and plasma. Alternatively, the state of activation and rates of turnover of HIV-infected cell populations may affect the observed viral sequences. Sequence analysis of cell-free virus provides a snapshot of viral sequences in the cells that produced the virus, while kinetics of viral decay in response to therapy largely reflect turnover rates of infected cells. Differences in turnover rates of different cells types or subpopulations may result in apparent sequence discordance in cross-sectional analysis. Productively infected lymphocytes turn over much more rapidly than monocytes/macrophages, and virus population in the plasma is largely derived from lymphocytes.

The extent to which the vigorous anti-HIV immune response influences viral replication and sequence diversity in the CNS is uncertain. Anti-retroviral agents also differ in their abilities to achieve effective concentration in the CNS, and such differences may be magnified as accumulation of resistance mutations incrementally reduces drug susceptibility. It is therefore important to consider not only viral susceptibility, but also local tissue drug concentrations and immune response in studies of viral discordance in the CNS compartments. Discordant antiretroviral susceptibility patterns in the CNS may also imply a need for CNS-specific targeting of anti-retroviral therapy, since viral evolution is in part a result of varying microenvironments.

Regional and cell-type-specific compartmentalization of HIV-1

Another important area of focus of the meeting was to define viral genetic factors regulating cell-type specific and regional compartmentalization of HIV-1 in the CNS. Several cell populations within the CNS compartment, including multinucleate giant cells (MNGC's), neurons, and astrocytes were examined for their capacity to be infected and support replication of HIV-1.

In one study using the SIV model, single cell RNA analysis was used to examine the relationship

between variants obtained from the entire brain and those from individual mutinucleate giant cells. Brain-specific variants were dominant in all the clones obtained from individual MNGC's indicating that they are most likely responsible for giant-cell formation. In addition, two less-frequent sequences were noted. These included sequences closely related to the spleen; they most likely represent recent neuroinvasion as well as sequences that were closely related or identical to the initial molecularly cloned inoculum, suggesting long-term residence within the brain. Finally, mosaic sequences were found in some giant cells, indicating that MNGC's may be a site of viral recombination (Rhyzova *et al*, 2002).

Intriguing data were presented at the meeting regarding the potential for HIV-1 to infect neurons. Neurons in tissue culture are able to sustain transient productive infection, as documented by their production of small amounts of HIV-1 p24 for brief periods. In vivo studies were performed by amplifying HIV-1 gene sequences from groups of pyramidal neurons from CA1, CA3 and CA4 regions of the hippocampus isolated from human brain sections by laser capture microdissection. Amplified *Nef* sequences were more frequent than *gag* sequences and were more common in the CA3 and CA4 neurons than in the CA1 neurons. In addition, differential vulnerability to injury and death in hippocampal CA3 and CA4 neurons were demonstrated which correlated with chemokine receptor diversity (Petito, 2004).

Another cell population in the brain that is being intensely studied is the astrocyte. Using laser capture microdissection on brain tissue sampled from HIV-1 infected individuals, it was demonstrated that HIV-1 DNA could be detected in cortical and basal ganglia derived astrocytes. Furthermore using gene microarray analysis it was determined that HIV-1 alters the program of gene expression in astrocytes, including changes in transcripts encoding cytokines, G-coupled protein receptors, transcription factors and other proteins. Exposure of astrocytes to HIV-1 or gp120 in vitro impairs the cell's ability to transport L-glutamate due to transcriptional inhibition of the EAAT2 glutamate transporter gene. Inhibition of the capacity of astrocytes to clear glutamate is likely to affect neuronal activity or survival and contribute to HIV neuropathogenesis (Wang *et al*, 2004).

Human CNS-derived progenitor cells have been used to model HIV-1 infection of astrocytes in vitro. HIV-1 causes a restricted or persistent infection in CNS- derived cells that are on a lineage pathway of differentiation into astrocytes. Treatment with specific cytokines such as TNF-α results in reactivation of virus in these cells. These studies suggest that persistently infected astrocytes may serve as a viral reservoir in the brain (Lawrence and Major, 2002).

Astrocytes may also play a role in controlling viral replication in CNS by producing IFN-β, which inhibits active HIV/SIV replication in macrophages. The inhibition of viral replication by IFN-β is likely to be mediated by the induction of a dominant-negative isoform of the transcription factor C/EBP-β (Barber *et al*, 2004).

In addition to cell type-specific compartmentalization, HIV-1 molecular diversity was observed in different regions of the CNS. Previous studies have shown that two CCAAT/enhancer binding protein (C/EBP) binding sites (site I and II) are critically important for efficient HIV-1 replication within cells of the monocyte lineage, a primary cell type infected with HIV-1. Sequence variation in C/EBP sites I and II has been shown to alter the affinity of C/EBP factors to these sites. Distinct LTR populations with specific C/EBP site II configurations were found in different neuroanatomical regions of the brain. This finding may result from differences in the molecular architecture of the LTR, viral entry pathways and or/brain microenvironments (Burdo *et al*, 2004). Various models could be developed to account for regional diversity of HIV in the CNS. Burdo *et al* (2004), suggest that a preferred model is one in which viral infection in the CNS involves multiple brain entry pathways leading to the delivery of viral genomes containing LTR sequences unique to specific brain regions. This would be more likely to occur during the later stages of infection, when there is increased infiltration of macrophages across the blood-brain barrier. This model would predict that the evolution of HIV-1 LTR would be driven by selective pressures in the peripheral immune system rather than in the brain of HIV-1 infected patients and the sequences would be distributed in the brain late in the course of infection.

Host genetic and molecular markers associated with HIV dementia

There is increasing interest in identifying host genetic markers associated with susceptibility to HIV-associated dementia as well as disease progression. Not only would such linkages serve as predictors of an individual's susceptibility to disease, but they would also provide important clues regarding mechanisms of pathogenesis.

Two studies presented at the symposium are highlighted below. In one, the influence of genetic variation of MCP-1 on HIV-1 neuropathogenesis was examined in a large cohort of HIV-1 infected adults and children. In adults, homozygosity for the MCP-1-2578G allele was associated with a 50% reduction in the risk of acquiring HIV-1. However once HIV-1 infection was established, this same MCP-1 genotype was associated with accelerated disease progression and a 4.5-fold increased risk of HAD. The mutant MCP-1 allele conferred greater transcriptional activity via differential DNA-protein interactions, enhanced protein production in vitro, increased serum MCP-1 levels and MP infiltration into tissues (Gonzalez *et al*, 2002).

Another study examined allelic association with common polymorphisms in candidate genes that are postulated to play a role in the pathogenesis of HIV-related neurologic complications. DNA was extracted from frozen brain samples and determination of APOE4, TNF-2, IL-1B*2 and IL-1RN*2 polymorphisms was performed by PCR and RFLP mapping. No consistent association between these candidate polymorphic alleles and the pathologic finding of HIV encephalitis or vacuolar myelopathy was detected. This preliminary study was not sufficiently powered to exclude a modest but clinically significant effect. Future studies will require much larger sample sizes and technical advances to allow screening of a large number of candidate loci (Diaz-Arrastia *et al*, 2004).

In addition to host genetic markers, there is a critical need to identify molecular markers linked with HIV-associated dementia. Such markers could be beneficial for diagnosis and tracking individual response to therapy. Two studies presented at the symposium suggested that there is a possibility of developing HAD-specific biomarkers. One study alluded to the existence of a unique subset of circulating peripheral monocytes for HAD. The emergence of this monocyte population in blood is associated with a unique proteomic signature that is abrogated after treatment. These findings may provide a basis for identifying an effective marker for the diagnosis of dementia in HIV-infected individuals, and might also be used for measuring therapeutic efficacy (Wojna *et al*, 2004).

Another HIV-dementia associated molecular marker has recently been identified and termed OTK18. OTK18 protein appears to be specifically expressed in mononuclear phagocytes sampled from HIVE brain tissue, but not in brain tissue of HIV-infected patients without neurological disease. OTK18 is also up regulated in the plasma of HIV infected patients, including those with HIVD and may correlate with disease prognosis (Limoges 2002, personal communication).

HIV viral genetics and neuropathogenesis-functional studies

Diversity in chemokine receptors and their utilization by various strains of HIV may have major implications for infection of CNS-derived cells as well as inflammatory events post infection. Macrophage-tropic CCR5 utilizing strains have been predominantly associated with HIV neuropathogenesis. However, recent research has shown that HIV envelopes can interact with CXCR4 on a number of neuronal and astroglial cell types in the CNS. A CNS variant (tybe) has recently been identified that replicates in primary macrophages, and also possesses a syncytia-inducing (SI) phenotype. Fusion assays indicate that the tybe envelope utilizes CXCR4 exclusively for macrophage infection, underscoring the need to address the role of CXCR4 dependent HIV-1 in the development of HIV dementia. Both R5 and X4 gp120 activate intracellular signals in macrophages through CCR5 and CXCR4. These findings suggest that both X4 and R5 HIV-1 contribute to HIVE pathogenesis, and that gp120/chemokine receptor interactions in monocyte/macrophages (M/M) trigger signal transduction pathways that affect M/M function and provide a mechanism for CNS injury (Yi *et al*, 2004).

Additional evidence for the utilization of CXCR4 in CNS neuropathogenesis has been provided in studies using animal models. Macrophage-tropic strains of SHIV that use CXCR4 have been identified. The neuropathogenic potential of X4 SHIVs was contingent upon concurrent co-infection in the brain with opportunistic pathogens. IL-4 is produced as a result of opportunistic infections and has a major role in promoting neuropathogenesis of macrophage-tropic X4 viruses (Buch *et al*, 2004). CXCR4 is also involved in pathways leading to neuronal apoptosis and differentiation. Recent studies have demonstrated that activation of the chemokine receptor CXCR4 by SDF-1α or gp120 modulates the activity of two important regulators of cell survival and apoptosis-the tumor suppressor protein Rb and the transcription factor E2F1-in dividing cells as well as differentiated neurons (Brandimarti *et al*, 2004).

Viral sequence diversity may also affect synthesis of different proteases in the central nervous system, and their relationship to pathogenesis has recently attracted increased attention. While matrix metalloproteinases are clearly important for both neural ontogeny and pathogenesis, 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 system. PAR-1 is expressed on astrocytes and neurons and its over expression is associated with astrogliosis and death of motor neurons in transgenic mice. The PARs are known to mediate a complex cascade of signaling pathways that can result in the release of pro-inflammatory molecules as well as cell death through apoptosis depending on the cell type on which the individual PAR is expressed. Multiple parallel protease-mediated pathways driven by viral expression and sequence diversity contribute to neuronal injury and death, causing dementia (Power *et al*, 2004). Additional research is needed on precise biochemical mechanisms by which viral sequence diversity induces these pathogenic signaling pathways in the brain.

Small-animal models for viral genetic studies

Non-human primates have served as valuable models to study neuropathogenesis, viral compartmentalization, anti-viral immune responses and to address

difficult questions relating to latent viral reservoirs in the CNS. This is evident by the many studies using non-human primate models reported at this symposium. However, the enormous costs as well as relative scarcity of animals has generated a critical need for developing small animal models for research in HIV neuropathogenesis. One such model is a non-transgenic severe combined immune deficiency mouse implanted with infected human monocyte derived macrophages. This model exhibits the hallmarks of HIV encephalitis (astrogliosis, activation of macrophages and mouse microglia, and degeneration of neurons). In addition, neuroinflammatory responses, neuronal dysfunction and behavioral dysfunction seen in HAD were reproduced in this animal model. This model has also been used to define the relative roles of neurotropic strains and brain replication in CNS disease onset and progression (Nukuna *et al*, 2004).

Another advance presented at the symposium was the development of a rat transgenic for human CD4 (hCD4) and the human chemokine receptor (hCCR5) that express the transgenes in CD4+ T lymphocytes, macrophages, and microglia. In ex vivo cultures, CD4+ T lymphocytes, macrophages and microglia from hCD4/hCCR5-transgenic rats were susceptible to infection by HIV-1 R5 viruses leading to expression of abundant levels of HIV-1 gene products. Primary rat macrophages and microglia, but not lymphocytes from double-transgenic rats, could be productively infected by various recombinant and primary R5 strains of HIV-1. Preliminary results indicate that HIV can transit the blood-brain barrier and has the capacity for some degree of persistence in the CNS (Keppler *et al*, 2002). Further refinement of this transgenic rat model may make it possible to address key questions in HIV viral genetics and neuropathogenesis that have thus far been elusive using humans or non-human primate models.

Future research priorities

The papers presented in this supplement of the *Journal of NeuroVirology* reflect many of the research presentations at the symposium and our most current understanding of the role of viral and host genetic factors in regulating HIV neuropathogenesis. These studies are critical for further research on mechanisms of HIV compartmentalization, establishment of latent reservoirs and evolution of drug resistance in the CNS. Several gaps in our research knowledge identified at the meeting represent important areas of research emphasis for the future. These include (1) establishing whether HIV DNA in the brain can give rise to transcriptionally active virus; (2) identifying mechanisms of evolution of discordance in sequences as well as decay of HIV in the brain vs. periphery; (3) establishing mechanisms by which latency can occur in astrocytes, how reactivation is effected and how this process relates to HIV encephalopathy; (4) assessing the impact of viral diversity on neuropathogenesis, particularly studying the role of CXCR4 viruses in the human brain; (5) studying the impact of immune response in the brain and periphery on regulating viral diversity: (6) using animal models such as SIV to determine if the brain is truly a reservoir of latent viral infection; (7) developing a panel of host genetic risk factors that are associated with the development of HIV-related neuropathology and response to therapy; and (8) implementing proteomics and protein profiling to characterize and understand HIV-related neuropathology and response to HAART. It is also critical to expand viral and host genetic research globally because the epidemic is emerging as a major threat in developing countries. In the process of conducting AIDS research world-wide, we have the opportunity to perform comparative studies of the biology of this infection, and its clades, with the added bonus of furthering our knowledge of viral genetics and evolution in the presence and absence of HAART.

References

Barber SA, Herbst DS, Bullock BT, Gama L, Clements JE (2004). Innate immune responses and control of acute SIV replication in the CNS. *J Neurovirol* **10**(Suppl 1): S15–S20.

Bestetti A, Presi S, Pierotti C, Bossolasco S, Sala S, Racca S, Carrera P, Lazzarin A, Cinque P (2004). Long-term virological effect of HAART in cerebrospinal fluid and relationship with genotypic resistance. *J Neurovirol* **10**(Suppl 1): S52–S57.

Brandimarti B, Khan MZ, Fatatis A, Meucci O (2004). Regulation of cell cycle proteins by chemokine receptors: a novel pathway in HIV neuropathogenesis. *J Neurovirol* **10**(Suppl 1): S108–S112.

Buch S, Sui Y, Potula R, Pinson D, Adany I, Li Z, Huang M, Li S, Dhillon N, Major E, Narayan O (2004). Role of interleukin-4 and monocyte chemoattractant protein-1 in the neuropathogenesis of X4-SHIV infection in macaques. *J Neurovirol* **10**(Suppl 1): S118–S124.

Burdo TH, Gartner S, Mauger D, McArthur J, Wigdahl B (2004). Region-specific distribution of HIV-1 LTRs containing specific configurations of C/EBP site II in brains derived from demented and non-demented patients. *J Neurovirol* **10**(Suppl 1): S7–S14.

Diaz-Arrastia R, Gong Y, Kelly CJ, Gelman BB (2004). Host genetic polymorphisms in HIV related neurologic disease. *J Neurovirol* **10**(Suppl 1): S67–S73.

Ellis RJ, Gamst AC, Capparelli E, Spector SA, Hsia K, Wolfson T, Abramson I, Grant I, McCutchan JA (2000). Cerebrospinal fluid HIV RNA originates from both local CNS and systemic sources. *Neurology* **54:** 927–936.

Gonzalez E, Rovin BH, Sen L, Cooke G, Dhanda R, Mummidi S, Kulkarni H, Bamshad MJ, Telles V, Anderson SA, Walter EA, Stephan KT, Deucher D, Mangano A, Bologna R, Ahuja SS, Dolan MJ, Ahuja SK (2002). HIV-1 infection and AIDS dementia are influenced by a mutant MCP-1 allele linked to increased monocyte infiltration

of tissues and MCP-1 levels. *Proc Natl Acad Sci USA* **99**: 13795–13800.

Haas DW (2004). Sequence heterogeneity and viral dynamics in cerebrospinal fluid and plasma during antiretroviral therapy. *J Neurovirol* **10**(Suppl 1): S33–S37.

Keppler OT, Welte FJ, Ngo TA, Chin PS, Patton KS, Tsou CL, Abbey NW, Sharkey ME, Grant RM, You Y, Scarborough JD, Ellmeier W, Littman DR, Stevenson M, Charo IF, Herndier BG, Speck RF, Goldsmith MA (2002). Progress toward a human CD4/CCR5 transgenic rat model for de novo infection by human immunodeficiency virus type 1. *J Exp Med* **195**: 719–736.

Lawrence DM and Major EO (2002). HIV-1 and the brain: connections between HIV-1-associated dementia, neuropathology and neuroimmunology. *Microb Infect* **4**: 301–308.

Liu Y, Tang XP, McArthur JC, Scott J, Gartner S (2000). Analysis of human immunodeficiency virus type 1 gp160 sequences from a patient with HIV dementia: evidence for monocyte trafficking into brain. *J Neurovirol* **6**(Suppl 1): S70–S81.

Nukuna A, Gendelman HE, Limoges J, Rasmussen J, Poluektova L, Ghorpade A, Persidsky Y (2004). Level of HIV-1 infection in macrophages determines the severity of HIV-1 encephalitis in SCID mice. *J Neurovirol* **10**(Suppl 1): S82–S90.

Petito CK (2004). HIV-1 compartmentalization in the central nervous system. *J Neurovirol* **10**(Suppl 1): S21–S24.

Power C, Zhang K, van Marle G (2004). Comparative neurovirulence in lentiviral infections: the roles of viral molecular diversity and select proteases. *J Neurovirol* **10**(Suppl 1): S113–S117.

Price RW, Deeks SJ (2004). Antiretroviral drug treatment interruption in HIV infected adults: clinical and pathogenic implications for the central nervous system. *J Neurovirol* **10**(Suppl 1): S44–S51.

Ryzhova EV, Crino P, Shawver L, Westmoreland SV, Lackner AA, Gonzalez-Scarano F (2002). Simian immunodeficiency virus encephalitis: analysis of envelope sequences from individual brain multinucleate giant cells and tissue samples. *Virol* **297**: 57–67.

Wang TH, Donaldson YK, Brettle RP, Bell JE, Simmonds P (2001). Identification of shared populations of human immunodeficiency virus type 1 infecting microglia and tissue macrophages outside the central nervous system. *J Virol* **75**: 11686–11699.

Wang Z, Trillo-Pazos G, Kim SY, Canki M, Morgello S, Sharer LR, Gelbard HA, Su ZZ, Kang DC, Brooks AI, Fisher PB, Volsky DJ (2004). Effect of HIV-1 on astrocyte gene expression and function: potential role in neuropathogenesis. *J Neurovirol* **10**(Suppl 1): S25–S32.

Williams K, Schwartz, Corey S, Orandle M, Kennedy W. Thompson I, Alvarez X, Brown C, Gartner S, Lackner A (2002). Proliferating cellular nuclear antigen expression as a marker of perivascular macrophages in simian immunodeficiency virus encephalitis. *Am J Pathol* **161**: 575–585.

Wojna V, Carlson KA, Luo X, Mayo R, Melendez LM, Kraiselburd E, Gendelman HE (2004). Proteomic fingerprinting of HIV-1 associated dementia from patient monocyte-derived macrophages: A case study. *J Neurovirol* **10**(Suppl 1): S74–S81.

Yi Y, Lee C, Liu QH, Freedman BD, Collman RG (2004). Chemokine receptor utilization and macrophage signaling by HIV-1 gp120: Implications for neuropathogenesis. *J Neurovirol* **10**(Suppl 1): S91–S96.