Journal of NeuroVirology, 10(suppl. 1): 21–24, 2004

ISSN: 1355-0284 print / 1538-2443 online
DOI: 10.1080/13550280490268241

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Taylor & Francis Group

Human immunodeficiency virus type 1 compartmentalization in the central nervous system

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HIV-1 replicates in monocyte-derived cells in multiple organ systems; in the central nervous system, productive infection is confined to monocyte lineage cells in the brain. In addition, productive viral infection takes place in the choroid plexus, where the incidence of infection is actually higher than in brain and is present prior to the onset of AIDS and immunosuppression. Restricted or latent infection occurs in astrocytes and neurons. The presence of perineuronal CD4+ lymphocytes, as well as activated microglia, support the potential for a trans-receptor mechanism of viral entry whereas intrinsic gene profiles do not appear to participate in conferring selective neuronal vulnerability or resistance to infection. *Journal of NeuroVirology* (2004) **10(suppl. 1),** 21–24.

Keywords: chemokine receptors; choroid plexus; neurons, microarrays; T lymphocytes

Viral compartmentalization: Organ and regional specificity

Multiple factors influence the localization of human immunodeficiency virus type 1 (HIV-1) in the central nervous system (CNS). The virus enters the central nervous system early in the course of systemic HIV-1 infection (Resnick *et al*, 1988). The potential for HIV-1 to elicit an actual HIV encephalitis (HIVE) with productive brain infection in the CNS requires immunosuppression, although the ages of viral quasispecies in acquired immunodeficiency syndrome (AIDS) brains suggests that the onset of brain infection may precede the onset of AIDS by several years (Hughes *et al*, 1997). HIV-1 gene sequences in the V3 loop of HIV-1 may confer neurotropism as well as neurovirulence (Korber *et al*, 1994; Power *et al*, 1994; Reddy *et al*, 1996; DiStefano *et al*, 1996; Chen *et al*, 2000). Brain-derived isolates are macrophage-tropic and their *env* sequences tend to exhibit negative or neutral charges when compared to systemic viral isolates.

The normal characteristics of the CNS blood-brain barrier (BBB) may influence viral compartmentalization. Brain capillaries have tight junctions between adjacent endothelial cells and a paucity of pinocytosis; this unique characteristic of the brain vasculature requires the presence encircling astrocytic foot processes around the capillaries. The BBB limits entry of infected immune cells into brain parenchyma, whereas the open junctions of the capillaries in the choroid plexus (CPx) stroma may facilitate viral entry into this structure. We hypothesized that the greater vascular permeability of the choroid plexus also might render it more vulnerable to HIV-1 infection and facilitate hematogeneous dissemination of the virus to the brain when our early studies noted a high incidence of immune complexes and *Toxoplasma gondii* infection in choroid plexus of AIDS patients (Falangola and Petito, 1993; Falangola *et al*, 1994). We found that the HIV-1 infection was more frequent in the CPx (52%) than in the brain (20%) (Falangola *et al*, 1995) and that infection was confined to CPx stromal macrophages and dendritic cells (Falangola *et al*, 1995; Hanly and Petito, 1998), a finding replicated in experimental models of HIV as well (Bragg *et al*, 2002). Follow-up studies showed that CPx viral sequences are admixtures of brain and blood sequences (Chen *et al*, 2000) and are common in HIV-1–infected asymptomatic patients (Petito *et al*,

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The author gratefully acknowledges the major contributions of multiple colleagues, including Drs. Fatima Falangola, Charles Wood, Micheline McCarthy, Becky Adkins, and Jorge Torres-Muñoz. This review is supported by the NIH (R01-NS39177).

Received 3 February 2003; revised 20 February 2003; accepted 20 February 2003.

1999). These findings support the hypothesis that hematogeneous dissemination to CPx may precede brain infection and suggest that the CPx be a sanctuary or reservoir of productive CNS virus.

Viral compartmentalization also shows striking regional variability within the brain itself. The initial pathology studies found a higher incidence in, and greater frequency of, HIVE lesions in the basal ganglia and cerebral white matter when compared with cerebral cortex (see Sharer, 1992, for review). This regional compartmentalization was confirmed subsequently with semiquantitative immunohistochemical studies (Achim *et al*, 1994; Brew *et al*, 1995). Virus and HIVE also localize to temporal lobe, as initially described by de la Monte *et al* (1987) and, more recently, by regional quantification of HIV DNA (Fujimura *et al*, 1997) and HIV RNA (Wiley *et al*, 1998). Although the mechanism for viral localization in brain is not clear, we raised the hypothesis that the proximity between the cerebrospinal fluid (CSF) and areas of HIVE concentration may result from dissemination of virus from infected CPx to brain via CSF pathways (Falangola *et al*, 1995).

Viral compartmentalization: Cellular specificity in the brain

HIV localization displays a dramatic cellular compartmentalization that is related directly to the presence or absence of the requisite HIV-1 cell-surface receptors needed for viral entry. Monocytes, microglia, and multinucleated giant cells sustain productive viral infection *in vivo* and *in vitro* and their vulnerability to infection correlates with the macrophagetropism exhibited *in vitro* by isolates from brain homogenates and CSF. All express CD4 as well as chemokine coreceptors, both of which are needed for viral entry. In contrast, primary neuroectodermal cells, specifically astrocytes and neurons, are incapable of sustaining productive infection *in vitro*, although may transiently produce low levels of HIV-1 p24, presumptive evidence of productive infection. Both cell types express HIV-1 chemokine coreceptors but neither express CD4 receptors. There is good evidence to suggest that astrocytes *in vivo* can harbor restricted, nonproductive HIV-1 infection because they can contain regulatory proteins such as Nef or Rev, but not structural proteins such as Gag or Env (Kohleisen *et al*, 1992; Tornatore *et al*, 1994; Saito *et al*, 1994; Guillemin *et al*, 2000).

The potential for HIV-1- to infect neurons *in vivo* is under active investigation. In tissue culture, neurons are able to sustain transient productive infection, as documented by their production of small amounts of HIV-1 p24 for brief periods (Li *et al*, 1990; Ensoli *et al*, 1997). Amplified neuronal gene sequences in autopsy brains have been found by *in situ* hybridization in two initial studies but not in three subsequent ones (see Torres-Muñoz *et al*, 2001, for references).

We explored the potential for HIV-1 to infect neurons by amplifying HIV-1 gene sequences from groups of 20 or 100 pyramidal neurons from the CA1, CA3, and CA4 subregions of the hippocampus; neurons were isolated from human brain sections by laser capture microdissection (PixCell II laser capture microscope [LCM], Arcturus) (Torres-Muñoz *et al*, 2001). Amplified *Nef* sequences were more frequent than *gag* sequences and were more common in the CA3 and CA4 neurons than in the CA1 neurons. Our observation that microdissected neurons in AIDS brains contain amplified HIV-1 gene sequences has confirmed by Trillo-Pazos, Volsky, and colleagues (Trillo-Pazos *et al*, 2002, and in press).

We hypothesized that the distribution of neuronal chemokine receptors in AIDS patients contributed to a selective vulnerability to HIV-1–induced neuronal injury and death. We selected the hippocampus as a model system for selective vulnerability because its subregions are well-defined and because its subregions display selective neuronal injury or death in diseases such as hypoxia-ischemia, epilepsy, and Alzheimer's disease. We used immunohistochemistry to evaluate gliosis and chemokine coreceptors in postmortem brains of normal controls and of AIDS patients with and without HIVE ($n = 10$–11 per group) (Petito *et al*, 2001). We found that the CA3–4 subregions of the hippocampus were more damaged than the CA1 subregion. They displayed high levels of reactive gliosis, neuronal loss, and frequent inflammatory infiltrates of HIVE. Semiquantification of neuronal HIV-1 chemokine coreceptors expression documented not only the highest levels of CXCR4 and CCR5 in the CA3–4 neurons, but also an AIDS-associated increase in neuronal CXCR4 and decrease in neuronal CCR5. These results supported the hypothesis that neuronal damage may be related to neuronal levels of chemokine receptors.

Differential gene expression in hippocampal neurons

We next tested the hypothesis that differential gene expression profiles might underlie the vulnerability of the CA3–4 hippocampal neurons to AIDS-related brain injury (Torres-Muñoz *et al*, 2002). Our initial studies have used control normal brains to determine if constitutive gene differences in hippocampal subregions, particularly of neuronal HIV-1 chemokine coreceptors, might be related to neuronal injury.

We selected HIV-1–negative autopsy cases with normal brains, a postmortem intervals <24 h, and archival fresh-frozen hippocampus (some material from the University of Miami NICHD Brain and Tissue Bank for Developmental Disorders, HD83284). We extracted total RNA (PicoPure RNA Isolation Kit, Arcturus) from 600 pyramidal neurons from the CA1 subregion of the hippocampus and 600 pyramidal neurons from the CA3 subregion of the hippocampus removed by microdissection with the LCM.

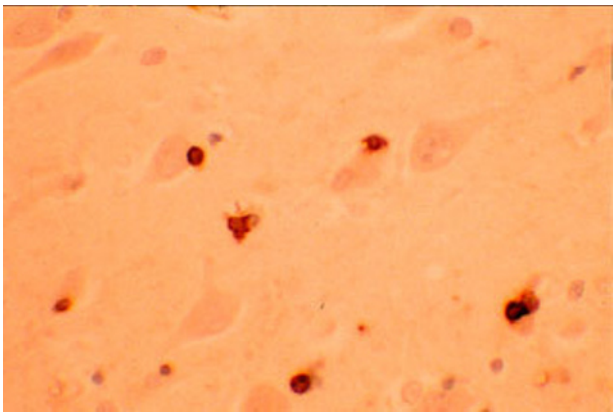

Figure 1 Hippocampus of AIDS patients with HIVE displays cell-to-cell contact between pyramidal neurons and perineuronal CD45RO+ T lymphocytes.

Five of the nine controls had sufficient quantities of high quality aRNA and adequate hybridization for both neuronal groups for analysis of the cDNA microarrays.

Less than 1% of the 8000 mRNAs were differentially expressed in CA1 *versus* CA3 pyramidal neurons (43 enriched genes in CA1 neurons and 17 enriched genes in CA3 neurons). Enriched genes in CA1 neurons included two associated with cell injury (activating transcription factor 3 and clusterin). With the sole exception of enriched prosataglanin E synthase in CA3 neurons, none of the genes known to be enriched in 'warm' ischemia (Dash *et al*, 2002) or during a posthypoxic interval (Gozal *et al*, 2002) were detected in our samples. None of 14 coded glutamate receptor genes displayed a differential expression. Only two of eight chemokine receptor genes included in the hybridization were enriched and these were CCR1 and CCR5 in CA1 neurons. This result was unexpected because CCR1 has not previously been detected in brain and because CCR5 immunoreactivity is strongest in the CA3 neurons. Possible explanations for the apparent discrepancy include unequal postmortem mRNA degradation or rapid transport or breakdown of CCR5 within the neuronal cytoplasm.

These preliminary microarray findings confirm the validity of using microdissected human brain cells for gene expression analysis. They suggest that selective vulnerability of hippocampal subregions in disease states may be more related to extrinsic differences in the extracellular environment or cellular composition rather than to intrinsic differences in neuronal gene expression.

HIV-1 compartmentalization in the CNS: Contribution of T lymphocytes

The mechanism of astrocyte and neuronal infection by HIV-1 is unclear because both cell types lack the requisite CD4 receptor utilized for viral entry in T cells and monocyte lineage cells. We examined the possibility that a trans-receptor mechanism might participate in neuronal infection as Speck *et al* (1999) showed for astrocytes *in vitro*. In this scenario, the gp120 of HIV-1 first interacts with the CD4+ receptor of a perineuronal CD4+ T lymphocyte or microglia and undergo its requisite spatial configuration to allow the subsequent interaction between gp120 and the neuronal or astrocytic chemokine coreceptor.

We used immunohistochemical markers to identify T-cell subsets in formalin-fixed paraffin blocks of hippocampus from AIDS brains with and without hippocampal HIVE and uninfected controls with normal brains (Petito *et al*, 2003). The number of activated/memory CD45RO+ T cells in brain parenchyma significantly increased in hippocampal regions with local HIVE (1.14 ± cells/high power field (hpf)), whereas hippocampal regions without HIVE in AIDS patients had the same (low) number of CD45RO+ T cells as HIV-1–uninfected controls (0.003 ± 0.09 T cells/hpf, and 0.03 ± 0.09 T cells/hpf, respectively). Perivascular infiltrates contained more CD4+ than CD8+ cells, whereas parenchymal T lymphocytes contained more CD8+ than CD4+ cells. Rarely, colocalization of T lymphocytes and HIV-1 p24 antigen were observed on serial sections.

Cell-to-cell contact between neurons and both T lymphocytes and activated microglia occurred in five of the six AIDS cases with hippocampal HIVE and included activated/memory T cells (Figure 1), CD4+ and CD8+ T cells, and CD4+ microglia. Parenchymal T cells were rare or absent in AIDS hippocampal regions in the absence of HIVE, and cell-to-cell contact between neurons and either T lymphocytes or microglia were not observed in controls, the HIVE AIDS cases, and the hippocampal subregions without local HIVE.

Several hypotheses may be derived from the above studies. First, T-tropic virus may be present in AIDS brains, a finding of importance in view of the fact that both neurons and astrocytes are more vulnerable to infection and injury with T-tropic than with M-tropic viral strains. Second, *trans*-receptor–mediated viral entry in neurons may occur in AIDS brains with HIVE. Lastly, T lymphocyte–mediated neuronal injury may be important in brain injury and neuronal loss occurring in AIDS patients.

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