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Effects of human immunodeficiency virus type 1 on astrocyte gene expression and function: Potential role in neuropathogenesis

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Neurodegeneration and dementia caused by human immunodeficiency virus type 1 (HIV-1) infection of the brain are common complications of acquired immunodeficiency syndrome (AIDS). Introduction of highly active antiretroviral therapy (HAART) reduced the incidence of HIV-1–associated dementia, but so far had no effect on the high frequency of milder neurological disorders caused by HIV-1. This indicates that some neuropathogenic processes persist during limited HIV-1 replication in the central nervous system (CNS). The authors are evaluating the hypothesis that interaction of HIV-1 with astrocytes, which bind HIV-1 but support limited productive HIV-1 infection, may contribute to these processes by disrupting astrocyte functions that are important for neuronal activity or survival. Using laser-capture microdissection on brain tissue samples from HIV-1–infected individuals, we found that HIV-1 DNA can be detected in up to 1% of cortical and basal ganglia astrocytes, thus confirming HIV-1 infection in astrocytes from symptomatic patients. Using rapid subtraction hybridization, the authors cloned and identified 25 messenger RNAs in primary human fetal astrocytes either up-regulated or down-regulated by native HIV-1 infection or exposure to gp120 *in vitro*. Extending this approach to gene microarray analysis using Affymetrix U133A/B gene chips, the authors determined that HIV-1 alters globally and significantly the overall program of gene expression in astrocytes, including changes in transcripts coding for cytokines, G-coupled protein receptors, transcription factors, and others. Focusing on a specific astrocyte function relevant to neuropathogenesis, the authors showed that exposure of astrocytes to HIV-1 or gp120 *in vitro* impairs the ability of the cells to transport L-glutamate and the authors related this defect to transcriptional inhibition of the EAAT2 glutamate transporter gene. These findings define new pathways through which HIV-1 may contribute to neuropathogenesis under conditions of limited virus replication in the brain. *Journal of NeuroVirology* (2004) **10(suppl. 1),** 25–32.

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HIV-1 neurologic diseases in the era of HAART

HIV-1 enters and colonizes the central nervous system (CNS) early in infection, triggering slowly progressing neurological disorders and, in a subset of patients, HIV-1–associated dementia (HAD or HIV-D) (Navia and Price, 1998). The clinical studies mirror virological findings that HIV-1 infection in the brain includes three states: establishment of infection in the brain very soon after infection in the periphery, a quiescent state of limited virus replication, and extensive virus replication at late stages of HIV-1 disease coinciding with immunodeficiency. Experimental infection in the simian immunodeficiency virus (SIV)-macaque model permitted an accurate description of this course of lentiviral infection in the brain (Zink *et al*, 1999). Late-stage HIV-1 infection in the CNS was frequently observed in patients prior to the introduction of highly active antiretroviral therapy (Navia and Price, 1998) and was characterized by abundant infection of brain microglia and macrophages (Gartner *et al*, 1986; Genis *et al*, 1992) and a general correlation between advanced immunodeficiency, CNS inflammation, and development of prominent neurological diseases (Navia and Price, 1998). It has been suggested that cellular and viral products made by the highly infected cells trigger inflammatory and neurotoxic reactions leading to neuronal cell death (Lipton and Gendelman, 1995).

HAART has altered the patterns of systemic HIV-1 infection and the neurological syndromes caused by the virus. In most patients, HAART reduces the virus burden, permits return of some immunological functions, and generally extends patient's life (Vandamme *et al*, 1998). HAART was also found to bring about dramatic reductions in the incidence of HAD and in the severity of the neurological disease in patients with low CD4 counts (McArthur *et al*, 2003; Sacktor *et al*, 2001a) and to reduce viral load and markers of immune activation such as neopterin and β_2-microglobulin in the cerebrospinal fluid (Ellis *et al*, 2002; McArthur *et al*, 2003). The effects of HAART on HIV-1 burden in the brain are not yet known. Nevertheless, recent studies show that a significant proportion of patients on HAART continue to exhibit neurological impairments and progress to HAD despite reduced plasma viremia and improved immunological parameters (Kusdra *et al*, 2002; Sacktor *et al*, 2001b). The proportion of new HAD cases in patients on HAART with improved CD4 counts was shown to be actually increasing in one study (Sacktor *et al*, 2001a) and the prevalence of HAD is also on the rise (McArthur *et al*, 2003). Furthermore, HAART seems to have limited benefit for minor cognitive/motor disorder or sensory neuropathy, both of which currently afflict more than 30% of HIV-1–positive adults (McArthur *et al*, 2003; Sacktor *et al*, 2002). These results indicate that certain neuropathogenic processes persist during limited HIV-1 replication in the absence of overt neuroinflammation, and that they may have been masked by the rapid course of HIV-1 disease in the pre-HAART era. Understanding the nature and causes of these processes will be critical to management of HIV-1 neurological disease as it evolves into a chronic condition.

Potential role of astrocytes in HIV-1 neuropathogenesis: An overview

Several cell types in the brain, including microvascular endothelial cells, neurons, and astrocytes, can support a limited, low-productive HIV-1 infection (Bagasra *et al*, 1996; Takahashi *et al*, 1996; Trillo-Pazos *et al*, 2003). We have been interested in the course of HIV-1 replication in human astrocytes and its effects on astrocyte physiology (Canki *et al*, 2001; Dewhurst *et al*, 1987b; Su *et al*, 2002; Trillo-Pazos *et al*, 2003; Wang *et al*, 2003). Four aspects of astrocyte activity prompt investigation of their role in HIV-1 neuropathogenesis: (a) astrocytes are critical to physiological brain function; (b) they serve as an abundant target for low-productive, noncytopathic infection with HIV-1; (c) interaction of HIV-1 with astrocytes *in vitro* impairs gene expression and cellular function; and (d) astrocyte dysfunction causes deleterious effects in the nervous system. The importance of astrocytes in HIV-1 neuropathogenesis has been convincingly argued previously (Conant and Major, 1998; Brack-Werner, 1999). Below we discuss the four noted elements in light of our research findings concerning the course and consequences of HIV-1 interactions with astrocytes. This discussion is not intended as a comprehensive review.

Critical functions of astrocytes required for neuronal activity and survival

Astrocytes are responsible for essential functions that impact the physiological activity and survival of neurons, the focus of damage in HAD. These include maintaining brain homeostasis, serving as a component of the blood-brain barrier, and responding to pathogens and brain injury (reviewed in Danbolt, 2001). Another central activity of astrocytes is uptake of intrasynaptic glutamate; disruption of this function results in neuronal death due to glutamate excitotoxicity, a pathological condition implicated in amyotrophic lateral sclerosis, Alzheimer's disease, stroke, epilepsy, as well as HAD (reviewed in Danbolt, 2001). More recent data indicate that astrocytes display some of the excitable properties of neurons, such as presence of functional neurotransmitter receptors and Ca^{2+}-dependent neurotransmitter release (Iino *et al*, 2001; Sharma and Vijayaraghavan, 2001), and that they actively participate in neural signal transmission by regulating synapse formation and synaptic transmission, and forming glial-neuronal

synapses (Oliet *et al*, 2001; Parpura, 2000; Ullian *et al*, 2001). Astrocytes have also been shown to induce neurogenesis from adult stem cells (Song *et al*, 2002). It should be clear that disruption of any of these astrocyte functions by HIV-1 would contribute to the progression of HIV-1–mediated neuropathogenesis.

Astrocytes can be infected by HIV-1 and serve as an abundant viral target in the brain

In contrast to T cells and macrophages, HIV-1 infection of astrocytes *in vitro* is inefficient, both in terms of the overall virus production and the proportion of cells expressing viral antigens (Dewhurst *et al*, 1987a; Nath *et al*, 1995; Tornatore *et al*, 1991). Following a small burst of virus production, from about 0.5% of cells in our hands, the virus persists in infected cells for prolonged periods in a low productive, noncytolytic state. Increased virus production can be induced by treatment of infected cells with physiologic stimuli such as tumor necrosis factor-α (TNF-α) (Tornatore *et al*, 1991). Several factors may contribute to this atypical pattern of HIV-1 infection. Some reports indicated that astrocytes may restrict HIV-1 replication at the level of viral RNA processing or translation, leading to preferred expression of viral regulatory rather than structural gene products (Neumann *et al*, 1995; Tornatore *et al*, 1994). The paucity of viral transcripts coding for structural proteins was attributed to inefficient Rev function (Ludwig *et al*, 1999). Alternatively, because astrocytes do not express surface CD4 (Ma *et al*, 1994; Willey *et al*, 2003), a primary block to HIV-1 infection could be inefficient entry. Indeed, stable expression of CD4 in human glioma cells H4 permitted efficient infection of these cells with HIV-1 (Shahabuddin *et al*, 1996; Volsky *et al*, 1992), as did expression of CD4 and CXCR4 on SVG-A astrocytic cells (Schweighardt and Atwood, 2001; Schweighardt *et al*, 2001). Inefficient HIV-1 entry also appears to be a major reason responsible for the restricted infection of human primary astrocytes (Canki *et al*, 2001; Willey *et al*, 2003). When this restriction was bypassed by infection of human fetal astrocytes with HIV-1 pseudotyped with MuLV envelope or VSV-G protein, the cells permitted robust viral expression (Canki *et al*, 2001). Viral antigens were detected in up to 70% of astrocytes at the peak of infection, the cells expressed the three major viral transcripts and fully processed core and envelope proteins, and they produced infectious progeny virus at levels similar to infected T cells (Canki *et al*, 2001). The requirement for CD4 for efficient infection of primary astrocytes with native HIV-1 was demonstrated directly by conferring expression of the receptor with an adenovirus vector (Willey *et al*, 2003). Such CD4-expressing astrocytes were susceptible to productive infection by some but not all viral strains tested, de-

pending on the coreceptor they recognized (Willey *et al*, 2003). These results also indicate that HIV-1 strains differ in their capacity to utilize native receptors on astrocytes *in vitro* and raises the possibility that a similar diversity in tropism to astrocytes may exist among HIV-1 in the brain (Sabri *et al*, 1999). The identity of the HIV-1 receptor(s) on astrocytes is unknown but previous work indicates that the cells efficiently bind gp120 and virus particles, possibly through a high affinity receptor (Hao and Lyman, 1999; Ma *et al*, 1994). Overall, although infection of normally CD4-negative astrocytes with native HIV-1 is inefficient, the cells have the capacity to bind and replicate HIV-1 and they can serve as a source of infectious virus in the CNS. Further studies are required to elucidate HIV-1 interactions with astrocyte surface receptors and the identity of these receptors, as they apparently are the primary determinants of HIV-1 infection in astrocytes and may mediate global, functional effects of the virus on the cells discussed below. Increased attention should also be directed to the potential heterogeneity among astrocytes as target cells for HIV-1, both with respect to their ability to support HIV-1 infection and regarding virus-induced cellular changes.

Because most of HIV-1 replication in the brain occurs in macrophages and microglia (Sharer *et al*, 1985; Wiley *et al*, 1986), the significance of HIV-1 infection of astrocytes for neurological disease has been debated (Wiley *et al*, 1999). Early attempts to detect productively infected astrocytes in brain tissues were generally unsuccessful (Conant *et al*, 1998; Wiley, 1996; Wiley *et al*, 1986). However, because astrocytes support only a low-productive, restricted mode of virus infection, technical difficulties to detect low levels of viral products in autopsy brain tissues can be expected. Using more sensitive methods of virus detection, HIV-1 infection of astrocytes *in vivo* has been well documented (Nuovo *et al*, 1994; Saito *et al*, 1994; Takahashi *et al*, 1996; Tornatore *et al*, 1994; Trillo-Pazos *et al*, 2003). Some of these studies indicated that the frequency of HIV-1–positive astrocytes could reach 1% in specific regions of the brain (Nuovo *et al*, 1994; Takahashi *et al*, 1996). We confirmed these estimates by using laser capture microdissection (LCM) to examine the presence of HIV-1 DNA in different brain cell types dissected from archival postmortem brain tissues with HIV-1 encephalitis (Trillo-Pazos *et al*, 2003). Cell lineage and virus antigen expression were determined by immunocytochemistry prior to LCM. HIV-1 *gag* DNA was consistently detected by this procedure in 1 to 20 p24-positive cells and in pools of 50 to 100 individually dissected CD68-positive microglia/macrophages and 100 to 200 astrocytes from HIV-1–positive patients but not in HIV-1–negative controls (Trillo-Pazos *et al*, 2003). Considering that the adult brain contains 10^{11} to 10^{12} astrocytes (Schubert, 1984), even a small frequency of infection will affect a significant number of cells. Thus astrocytes may provide

a sizable sanctuary to HIV-1 in the CNS, which could be further influenced by a virus strain-specific recognition of surface receptors on astrocytes (Willey *et al*, 2003). Antiviral treatment arrests virus transmission but does not affect proviral DNA in cellular sanctuaries in astrocytes or other cells (Finzi, 1997). Induction of extant virus by physiological stimuli (Chun *et al*, 1997; Tornatore *et al*, 1991) provides a mechanism for secretion of infectious progeny virus into the CNS compartment and in the periphery.

HIV-1 down-modulates glutamate transporter EAAT2 in human astrocytes and impairs L-glutamate transport by the cells *in vitro*

L-Glutamate is the major excitatory neurotransmitter in the brain. Astrocytes maintain low levels of synaptic glutamate by high-affinity uptake and defects in this function may lead to neuronal cell death by excitotoxicity (Danbolt, 2001). Glutamate excitotoxicity is also considered a central end-point defect in HAD (Lipton and Gendelman, 1995). It has been suggested that free gp120 released from HIV-1–infected macrophages and microglial cells can indirectly contribute to excitotoxicity through interaction with astrocytes (Benos *et al*, 1994; Holden *et al*, 1999). This interaction was shown to stimulate glial NA^+/H^+ exchange and increase secretion of excitatory amino acids in rat astrocytes (Benos *et al*, 1994), whereas similar gp120-induced events in human astrocytes subsequently lead to *N*-methyl-D-aspartate (NMDA) receptor–dependent increase in intracellular Ca^{2+} in neurons, which may result in neuronal cell death (Holden *et al*, 1999). We investigated the effects of HIV-1, as well as gp120, upon glutamate uptake and expression of glial glutamate transporters EAAT1 and EAAT2 in fetal human astrocytes *in vitro* (Wang *et al*, 2003). Exposure of astrocytes to HIV-1 or gp120 inhibited glutamate uptake up to 90% between 6 h to 3 days of observation, with a 59% reduction in V_{max} for glutamate transport and commensurate 40% to 70% decline in steady-state levels of EAAT2 RNA and protein. Treatment of astrocytes with TNF-α decreased the expression of both EAAT1 and EAAT2, but neither HIV-1 nor gp120 were found to induce TNF-α production by astrocytes. Thus HIV-1 and gp120 induce transcriptional downmodulation of the EAAT2 transporter gene in human astrocytes and coordinately attenuate glutamate transport by the cells *in vitro*. Preliminary survey of frozen brain tissues by immunoblotting indicated that tissues from acquired immunodeficiency syndrome (AIDS) patients contained lower levels of EAAT2 protein than did tissues from HIV-1–negative controls (Trillo-Pazos *et al*, submitted). Although this approach cannot relate the decline in EAAT2 expression to infection of astrocytes *in vivo,* these studies suggest that reduction of the ability of HIV-1–infected astrocytes to take up glutamate may con-

tribute to the development of neurological disease. To facilitate investigation of transcriptional regulation of glutamate transporter genes, we cloned the human EAAT2 promoter and demonstrated that TNF-α induces decreased EAAT2 transcriptional activity and consequently decreased mRNA and protein levels in primary human fetal astrocytes (Su *et al*, 2003b). Preliminary studies indicate that HIV-1 down-modulates EAAT2 promoter activity in astrocytes, providing a clue for molecular action of HIV-1 on glutamate transport at this level. We also cloned and characterized the unusually long (9684 base pairs) 3′-untranslated RNA region (3′-UTR) of EAAT2 to investigate its involvement in HIV-1 modulation of EAAT2 at the level of post-transcriptional regulation of RNA expression (Kim *et al*, 2003a). Finally, we cloned and partially characterized the promoter for EAAT1 (Kim *et al*, 2003b) to study relative effects of HIV-1 and gp120 on the EAAT1 and EAAT2 gene expression. These studies provide tools for detailed molecular analysis of the deleterious effects of HIV-1, TNF-α, and other neuropathogenic entities upon glutamate transporter gene expression in human astrocytes.

HIV-1 interaction with astrocytes reprograms cellular gene expression

The lasting and extensive inhibition of glutamate transport in astrocytes by HIV-1 or gp120 (Wang *et al*, 2003) indicated that HIV-1 exerts global effects upon astrocyte physiology extending beyond the small proportion of cells that can replicate virus. To determine the extent of these changes, we initiated studies to establish the profile of cellular gene expression in astrocytes exposed to HIV-1 or gp120. We employed rapid subtraction hybridization (RaSH) to identify human genes whose expression in astrocytes is altered at discrete time points after HIV-1/NL4-3 infection and also determined their expression after exposure to gp120$_{MN}$ (Kang *et al*, 2002). We described that 15 transcripts, including 13 known and 2 novel genes, were induced in human fetal astrocytes by either HIV-1 infection or gp120 exposure (Su *et al*, 2002). Some of these astrocyte elevated genes (AEGs) were induced early, 6 h or 24 h after infection, and others were up-regulated later (Su *et al*, 2002). Several of the astrocyte gene products detected in our differential gene expression screens in infected astrocytes in culture could affect neuronal functions through a signaling network with astrocytes (Bezzi and Volterra, 2001). For example, G-proteins, such as AEG2 (Su *et al*, 2002) are receptor-associated signal-transduction molecules that can profoundly affect cellular signaling leading to pathophysiology in the brain (Lee *et al*, 2001). Using the same approach, 10 genes were identified that display reduced expression in human fetal astrocytes following HIV-1 infection, 2 of which are novel (Su *et al*, 2003a). Like the set of AEGs, differential gene expression was

Table 1 Families of transcripts regulated by HIV-1 in astrocytes as determined by Affymetrix global arrays

Transcript	Up-regulated	Down-regulated
Actin binding	3	1
Alternative splicing	4	1
Apoptosis	4	1
Calcium binding	0	4
Cytokine	18	0
DNA binding	5	0
Endoplasmic reticulum	0	5
Glycoprotein	8	0
G-coupled protein receptor	7	0
Homeo box DNA binding	3	2
Hydrolase	21	13
Ion channel	8	3
Lyase	2	3
Neurotransmitter transporter	4	0
Nuclear protein	10	15
Oxidoreductase	10	1
Proteoglycan	0	3
Transcription factor	21	9
Transferase	15	11
Transmembrane protein	15	2
Transport protein	7	7
Zink finger	5	2
Miscellaneous	108	104
ESTs	371	356
Total	649	543

observed at different times after virus exposure. Most of the transcripts were suppressed late in infection and they include several genes whose products are important to normal function of the CNS, such as neuronatin and neuroendocrine differentiation factor. These studies provided compelling evidence of the profound and global effects of HIV-1 on gene expression in human primary astrocytes.

A recent study employed a different technology, tissue-specific cDNA microarrays to identify transcripts either up- or down-regulated by HIV-1 or gp120 exposure of human fetal astrocytes (Galey *et al*, 2003). Using immuno- or neuromicroarrays of 1100 oligonucleotides each, many genes were identified of significance to astrocyte function in the CNS. HIV-1 and gp120 exposure resulted in opposite effects in a number of cases, possibly due to the fact that the gp120 was derived from a different virus than that used for infection. HIV-1 altered expression of many more genes surveyed in this study than did gp120, implicating products of HIV-1 infection in the changes observed (Galey *et al*, 2003). This result contrasts with our findings of similar changes in astrocyte gene expression in cells exposed to HIV-1 and gp120 (Su *et al*, 2002, 2003a; Wang *et al*, 2003). One possible reason for the discrepancy could be methodological differences between our systems and those of Galey *et al*. Another potential variable is the heterogeneity of astrocyte populations isolated from fetal brain tissue and the inevitable genetic diversity among the human tissues used. For example, we observed extensive variability among batches of astrocytes with respect to their ability to take up glutamate and respond to HIV-1 or gp120 (Wang *et al*, 2003).

We have also begun to perform large-scale screens for changes in gene expression in HIV-1–infected astrocytes through cDNA microarray analysis using Affymetrix U133A/B chips. We have tested multiple batches of astrocytes by this approach, screening over 44,000 human transcripts. Data were analyzed using Data Mining Tool, GeneSpring, and SAM (Statistical Analysis of Microarrays) to determine statistical significance. Table 1 shows a current summary of the protein families of the differentially expressed transcripts obtained in this extensive data set. Together, different genetic approaches promise to define an objective, unbiased spectrum of changes in astrocyte function through alterations in gene expression.

Conclusion: Relevance of HIV-1–induced functional changes in astrocytes to neuropathogenesis

We believe that it is important to consider two separable interactions of HIV-1 with astrocytes, both of which may have consequences upon cell function. A small minority of astrocytes in a population can be productively infected by the virus in culture or carry and potentially produce virus in the brain. These cells are clear candidates for dysfunction caused directly by HIV-1 replication and viral products such as Tat and Nef. In addition, astrocytes respond globally to binding of HIV-1 or gp120 by reduced expression of glutamate transporters (Wang *et al*, 2003) as well as by widespread dysregulation of expression of many other cellular genes (Su *et al*, 2002, 2003; Galey *et al*, 2003). It should be emphasized that these effects of HIV-1/gp120 are felt throughout the cell population, indicating a scale of response of real significance in neuropathogenesis. In our hands, the mechanism of alteration in gene expression involves ligation of surface receptors by gp120, because the same pattern and extent of change was observed in astrocytes infected by intact HIV-1 or exposed to purified gp120 (Su *et al*, 2002, 2003a). This aspect of HIV-1/gp120 regulation of cellular gene expression in astrocytes involves the common function of HIV-1 to bind to the cells, rather than the restricted function of HIV-1 to productively infect them. We suggest that the surface receptors on astrocytes shown to bind HIV-1/gp120 (Hao and Lyman, 1999; Ma *et al*, 1994) can transduce signals that alter cellular gene expression and thus cellular functions; however, this receptor less frequently forms a complex competent to mediate HIV-1 fusion and entry into cells. We look forward to new investigations that will clarify these and other interactions of HIV-1 with astrocytes that contribute to neuropathogenesis and ultimately the findings that will provide the means to control this arm of HAD.

References

Bagasra O, Lavi E, Bobroski L, Khalili K, Pestaner JP, Tawadros R, Pomerantz RJ (1996). Cellular reservoirs of HIV-1 in the central nervous system of infected individuals: identification by the combination of in situ polymerase chain reaction and immunohistochemistry. *AIDS* **10**: 573–585.

Benos DJ, Hahn BH, Bubien JK, Ghosh SK, Mashburn NA, Chaikin MA, Shaw GM, Benveniste EN (1994). Envelope glycoprotein gp120 of human immunodeficiency virus type 1 alters ion transport in astrocytes: implications for AIDS dementia complex. *Proc Natl Acad Sci U S A* **91**: 494–498.

Bezzi P, Volterra A (2001). A neuron-glia signalling network in the active brain. *Curr Opin Neurobiol* **11**: 387–394.

Brack-Werner R (1999). Astrocytes: HIV cellular reservoirs and important participants in neuropathogenesis. *AIDS* **13**: 1–220.

Canki M, Thai JNF, Chao W, Ghorpade A, Potash MJ, Volsky DJ (2001). Highly productive infection with pseudotyped human immunodeficiency virus type 1 (HIV-1) indicates no intracellular restrictions to HIV-1 replication in primary human astrocytes. *J Virol* **75**: 7925–7933.

Chun T-W, Stuyver L, Mizell SB, Ehler LA, Mican JAM, Baseler M, Lloyd AL, Nowak MA, Fauci AS (1997). Presence of an inducible HIV-1 latent reservoir during highly active antiretroviral therapy. *Proc Natl Acad Sci U S A* **94**: 13193–13197.

Conant K, Garzino-Demo A, Nath A, McArthur JC, Halliday W, Power C, Gallo RC, Major EO (1998). Induction of monocyte chemoattractant protein-1 in HIV-1 Tat-stimulated astrocytes and elevation in AIDS. *Proc Natl Acad Sci U S A* **95**: 3117–3121.

Conant K, Major EO (1998). Astrocytes as mediators of CNS injury in AIDS. In: *The neurology of AIDS*. Gendelman HE, Lipton AS, Epstein L, Swindells S (eds). New York: Chapman & Hall, pp. 147–155.

Danbolt NC (2001). Glutamate uptake. *Prog Neurobiol* **65**: 1–105.

Dewhurst S, Bresser J, Stevenson M, Sakai K, Evinger-Hodges MJ, Volsky DJ (1987a). Susceptibility of human glial cells to infection with human immunodeficiency virus (HIV). *FEBS Lett* **213**: 138–143.

Dewhurst S, Sakai K, Bresser J, Stevenson M, Evinger-Hodges MJ, Volsky DJ (1987b). Persistent productive infection of human glial cells by human immunodeficiency virus (HIV) and by infectious molecular clones of HIV. *J Virol* **61**: 3774–3782.

Ellis RJ, Moore DJ, Childers ME, Letendre S, McCutchan JA, Wolfson T, Spector SA, Hsia K, Heaton RK, Grant I (2002). Progression to neuropsychological impairment in human immunodeficiency virus infection predicted by elevated cerebrospinal fluid levels of human immunodeficiency virus RNA. *Arch Neurol* **59**: 923–928.

Finzi D, Hermankova M, Pierson T, Carruth LM, Buck C, Chaisson RE, Quinn TC, Chadwick K, Margolick J, Brookmeyer R, Gallant J, Markowitz M, Ho DD, Richman DD, Siliciano RF (1997). Identification of a reservoir for HIV-1 in patients on highly active antiretroviral therapy. *Science* **278**: 1295–1300.

Galey D, Becker K, Haughey N, Kalehua A, Taub D, Woodward J, Mattson M, Nath A (2003). Differential transcriptional regulation by HIV-1 and gp120 in human astrocytes. *J NeuroVirol* **9**: 358–371.

Gartner S, Markovits P, Markovitz DM, Kaplan MH, Gallo RC, Popovic M (1986). The role of mononuclear phagocytes in HTLV-III/LAV infection. *Science* **233**: 215–219.

Genis P, Jett M, Bernton EW, Boyle T, Gelbard HA, Dzenko K, Keane RW, Resnick L, Mizrachi Y, Volsky DJ, *et al* (1992). Cytokines and arachidonic metabolites produced during human immunodeficiency virus (HIV)-infected macrophage-astroglia interactions: implications for the neuropathogenesis of HIV disease. *J Exp Med* **176**: 1703–1718.

Hao H-N, Lyman WD (1999). HIV infection of fetal human astrocytes: the potential role of a receptor-mediated endocytic pathway. *Brain Res* **823**: 24–32.

Holden CP, Haughey NJ, Nath A, Geiger JD (1999). Role of Na^+/H^+ exchangers, excitatory amino acid receptors and voltage-operated Ca^{2+} channels in human immunodeficiency virus type 1 gp120-mediated increases in intracellular Ca^{2+} in human neurons and astrocytes. *Neuroscience* **91**: 1369–1378.

Iino M, Goto K, Kakegawa W, Okado H, Sudo M, Ishiuchi S, Miwa A, Takayasu Y, Saito I, Tsuzuki K, Ozawa S (2001). Glia-synapse interaction through Ca^{2+}-permeable AMPA receptors in Bergmann glia. *Science* **292**: 926–929.

Kang D-c, Jiang H, Su Z-z, Volsky DJ, Fisher PB (2002). RaSH-Rapid subtraction hybridization. In: *Analysing gene expression*. Lorkowski S, Cullen P (eds). Germany: Wiley-VCH Verlag GmbH, pp 206–214.

Kim S-Y, Chao W, Volsky DJ (2003a). Cloning and characterization of the 3′-untranslated region of the human excitatory amino acid transporter 2 transcript. *J Neurochem* **86**: 1458–1467.

Kim S-Y, Choi SY, Chao W, Volsky DJ (2003b). Transcriptional regulation of human excitatory amino acid transporter 1 (EAAT1): cloning of the EAAT1 promoter and characterization of its basal and inducible activity in human astrocytes. *J Neurochem* **87**: 1485–1498.

Kusdra L, McGuire D, Pulliam L (2002). Changes in monocyte/macrophage neurotoxicity in the era of HAART: implications for HIV-associated dementia. *AIDS* **16**: 31–38.

Lee DK, George SR, Cheng R, Nguyen T, Liu Y, Brown M, Lynch KR, O'Dowd BF (2001). Identification of four novel human G protein-coupled receptors expressed in the brain. *Brain Res Mol Brain Res* **86**: 13–22.

Lipton S, Gendelman HE (1995). Dementia associated with the acquired immunodeficiency syndrome. *New Engl J Med* **233**: 934–940.

Ludwig E, Ceccherini-Silberstein F, van Empel J, Erfle V, Neumann M, Brack-Werner R (1999). Diminished Rev-mediated stimulation of human immunodeficiency virus type 1 is a hallmark of human astrocytes. *J Virol* **73**: 8279–8289.

Ma M, Geiger JD, Nath A (1994). Characterization of a novel binding site for the human immunodeficiency virus type 1 envelope protein gp120 on human fetal astrocytes. *J Virol* **68**: 6824–6828.

McArthur JC, Haughey N, Gartner S, Conant K, Pardo C, Nath A, Sacktor N (2003). Human immunodeficiency virus-associated dementia: an evolving disease. *J NeuroVirol* **9**: 205–221.

Nath A, Hartloper V, Furer M, Fowke KR (1995). Infection of human fetal astrocytes with HIV-1: viral tropism

and the role of cell to cell contact in viral transmission. *J Neuropath Exp Neurol* **54:** 320–330.

Navia AD, Price WR (1998). Clinical and biologic features of the AIDS dementia complex. In: *The neurology of AIDS*. Gendelman HE, Lipton AS, Epstein L, Swindells S (eds). New York: Chapman & Hall, pp 229–240.

Neumann M, Felber BK, Kleinschmidt A, Froese B, Erfle V, Pavlakis GN, Brack-Werner R (1995). Restriction of human immunodeficiency virus type 1 production in a human astrocytoma cell line is associated with a cellular block in Rev function. *J Virol* **69:** 2159–2167.

Nuovo GJ, Gallery F, MacConnell P, Braun A (1994). In situ detection of polymerase chain reaction-amplified HIV-1 nucleic acid and tumor necrosis-alpha RNA in the central nervous system. *Am J Pathol* **144:** 659–666.

Oliet SHR, Piet R, Poulain DA (2001). Control of glutamate clearance and synaptic efficacy by glial coverage of neurons. *Science* **292:** 923–926.

Parpura VH PG (2000). Physiological astrocytic calcium levels stimulate glutamate release to modulate adjacent neurons. *Proc Natl Acad Sci U S A* **97:** 8629–8634.

Sabri F, Tresoldi E, Di Stefano M, Polo S, Monaco MC, Verani A, Fiore RJ, Lusso P, Major E, Chiodi F, Scarlatti G (1999). Nonproductive human immunodeficiency virus type 1 infection of human fetal astrocytes: independence from CD4 and major chemokine receptors. *Virol* **264:** 370–384.

Sacktor N, Lyles RH, Skolasky R, Kleeberger C, Selnes OA, Miller EN, Becker JT, Cohen B, McArthur JC (2001a). HIV-associated neurologic disease incidence changes: Multicenter AIDS Cohort Study, 1990–1998. *Neurology* **56:** 257–260.

Sacktor N, McDermott MP, Marder K, Schifitto G, Selnes OA, McArthur JC, Stern Y, Albert S, Palumbo D, Kieburtz K, De Marcaida JA, Cohen B, Epstein L (2002). HIV-associated cognitive impairment before and after the advent of combination therapy. *J NeuroVirol* **8:** 136–142.

Sacktor N, Tarwater PM, Skolasky RL, McArthur JC, Selnes OA, Becker J, Cohen B, Miller EN (2001b). CSF antiretroviral drug penetrance and the treatment of HIV-associated psychomotor slowing. *Neurology* **57:** 542–544.

Saito Y, Sharer LR, Epstein LG, Michaels J, Mintz M, Louder M, Golding K, Cvetkovich TA, Blumberg BM (1994). Overexpression of Nef as a marker for restricted HIV-1 infection of astrocytes in postmortem pediatric central nervous tissues. *Neurology* **44:** 474–481.

Schubert D (1984). *Developmental biology of cultured nerve, muscle, and glia*. New York: John Wiley and Sons.

Schweighardt B, Atwood WJ (2001). HIV type 1 infection of human astrocytes is restricted by inefficient viral entry. *AIDS Res Hum Retroviruses* **17:** 1133–1142.

Schweighardt B, Shieh JTC, Atwood WJ (2001). CD4/CXCR4-independent infection of human astrocytes by a T-tropic strain of HIV-1. *J NeuroVirol* **7:** 155–162.

Shahabuddin M, Bentsman G, Volsky B, Rodriguez I, Volsky DJ (1996). A mechanism of restricted human immunodeficiency virus type 1 expression in human glial cells. *J Virol* **70:** 7992–8002.

Sharer LR, Cho ES, Epstein LG (1985). Multinucleated giant cells and HTLV-III in AIDS encephalopathy. *Hum Pathol* **16:** 760.

Sharma G, Vijayaraghavan S (2001). Nicotinic cholinergic signaling in hippocampal astrocytes involves calcium-induced calcium release from intracellular stores. *Proc Natl Acad Sci U S A* **98:** 4148–4153.

Song H, Stevens CF, Gage FH (2002). Astroglia induce neurogenesis from adult neural stem cells. *Nature* **417:** 39–44.

Su Z-z, Kang D-c, Chen Y, Pekarskaya O, Chao W, Volsky DJ, Fisher PB (2002). Identification and cloning of human astrocyte genes displaying elevated expression after infection with HIV-1 or exposure to HIV-1 envelope glycoprotein by rapid subtraction hybridization, RaSH. *Oncogene* **21:** 3592–3602.

Su Z-z, Kang D-c, Chen Y, Pekarskaya O, Chao W, Volsky DJ, Fisher PB (2003a). Identification of gene products suppressed by human immunodeficiency virus type 1 infection or gp120 exposure of primary human astrocytes by rapid subtraction hybridization. *J NeuroVirol* **9:** 372–389.

Su Z-z, Leszczyniecka M, Kang D-c, Sarkar D, Chao W, Volsky DJ, Fisher PB (2003b). Insights into glutamate transport regulation in human astrocytes: cloning of the promoter for excitatory amino acid transporter 2 (EAAT2). *Proc Natl Acad Sci U S A* **100:** 1955–1960.

Takahashi K, Wesselingh SL, Griffin DE, McArthur JC, Johnson RT, Glass JD (1996). Localization of HIV-1 in human brain using polymerase chain reaction/in situ hybridization and immunocytochemistry. *Ann Neurol* **39:** 705–711.

Tornatore C, Meyers K, Atwood W, Conant K, Major EO (1994). Temporal patterns of human immunodeficiency virus type 1 transcripts in human fetal astrocytes. *J Virol* **68:** 93–102.

Tornatore C, Nath A, Amemiya K, Major EO (1991). Persistent human immunodeficiency virus type 1 infection in human fetal glial cells reactivated by T-cell factor(s) or by the cytokines tumor necrosis factor alpha and interleukin-1 beta. *J Virol* **65:** 6094–6100.

Trillo-Pazos G, Diamanturos A, Rislove L, Menza T, Chao W, Belem PSS, Morgello S, Sharer L, Volsky DJ (2003). Detection of HIV-1 DNA in microglia/macrophages, astrocytes and neurons isolated from brain tissue with HIV-1 encephalitis by laser capture microdissection. *Brain Pathol* **13:** 144–154.

Ullian EM, Sapperstein SK, Christopherson KS, Barres BA (2001). Control of synapse number by glia. *Science* **291:** 657–660.

Vandamme AM, Vaerenbergh K, De Clerq E (1998). Anti-human immunodeficiency virus drug combination strategies. *Antivir Chem Chemother* **9:** 187–203.

Volsky B, Sakai K, Reddy MM, Volsky DJ (1992). A system for the high efficiency replication of HIV-1 in neural cells and its application to anti-viral evaluation. *Virol* **186:** 303–308.

Wang Z, Pekarskaya O, Bencheikh M, Chao W, Gelbard H, Ghorpade A, Rothstein JD, Volsky DJ (2003). Reduced expression of glutamate transporter EAAT2 and impaired glutamate transport in human primary astrocytes exposed to HIV-1 or gp120. *Virology* **312:** 60–73.

Wiley CA (1996). Polymerase chain reaction in situ hybridization—opening pandora's box. *Ann Neurol* **39:** 691–692.

Wiley CA, Achim CL, Christopherson C, Kidane Y, Kwok S, Masliah E, Mellors J, Radhakrishnan L, Wang G, Soontornniyomkij V (1999). HIV mediates a productive infection of the brain. *AIDS* **13:** 2055–2059.

Wiley CA, Schrier RD, Nelson JA, Lampert PW, Oldstone MBA (1986). Cellular localization of human immunodeficiency virus infection within the brains of acquired immune deficiency syndrome patients. *Proc Natl Acad Sci U S A* **83:** 7089–7093.

Willey SJ, Reeves JD, Hudson R, Miyake K, Dejucq N, Schols D, De Clercq E, Bell J, McKnight A, Clapham PR (2003). Identification of a subset of human immunodeficiency virus type 1 (HIV-1), HIV-2, and simian immunodefi-

ciency virus strains able to exploit an alternative coreceptor on untransformed human brain and lymphoid cells. *J Virol* **77:** 6138–6152.

Zink MC, Suryanarayana K, Mankowski JL, Shen A, Piatak M Jr, Spelman JP, Carter DL, Adams RJ, Lifson JD, Clements JE (1999). High viral load in the cerebrospinal fluid and brain correlates with severity of simian immunodeficiency virus encephalitis. *J Virol* **73:** 10480–10488.