Journal of NeuroVirology, 13: 168–172, 2007

ISSN: 1355-0284 print / 1538-2443 online
DOI: 10.1080/13550280601178218

informa
healthcare

Short Communication

Ceftriaxone protects against the neurotoxicity of human immunodeficiency virus proteins

Jeffrey A Rumbaugh, Guanhan Li, Jeffrey Rothstein, and Avindra Nath

Departments of Neurology and Neuroscience, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA

Human immunodeficiency virus (HIV) proteins Tat and gp120 have been implicated in the pathogenesis of HIV dementia by various mechanisms, including down-regulation of excitatory amino acid transporter-2 (EAAT2), which is responsible for inactivation of synaptic glutamate. Recent work indicates that beta-lactam antibiotics are potent stimulators of EAAT2 expression. The authors treated mixed human fetal neuronal cultures with recombinant gp120 or Tat, in the presence or absence of ceftriaxone, and determined neurotoxicity by measuring mitochondrial membrane potential and neuronal cell death. Ceftriaxone produced dose-dependent attenuation of the neurotoxicity and neuronal cell death caused by both viral proteins. This study demonstrates that this class of drugs may have therapeutic efficacy in HIV dementia. *Journal of NeuroVirology* (2007) **13,** 168–172.

Keywords: beta lactam; excitotoxicity; glutamate; neuroimmunology; neurotoxicity; viral

Human immunodeficiency virus (HIV)-associated dementia (HIVD) affects cognitive, motor, and behavioral domains. Highly active antiretroviral therapy (HAART) has minimized the incidence of severe forms of HIVD, but has made HIV infection a chronic disease, thus actually increasing the prevalence of HIVD (Dore *et al*, 2003; McArthur *et al*, 2003; Neuenburg *et al*, 2002; Sacktor, 2002; Sacktor *et al*, 2002). Furthermore, HAART only targets select viral proteins, such as reverse transcriptase, protease, and integrase. Untargeted proteins, like Tat and gp120, likely have a much greater role in the neuropathogenesis of HIVD. In the brain, only macrophages and microglia are productively infected, whereas astrocytes have a restricted infection in which viral proteins are expressed, but replication of the viral genome does not occur (Saito *et al*, 1994). Such astrocytes can produce potentially toxic viral proteins, such as Tat (Ranki *et al*, 1995; Tornatore *et al*, 1994), which in turn induce glial cells and macrophages to produce other neurotoxic substances (Chen *et al*, 1997), such as tumor necrosis factor-α (Lafortune *et al*, 1996). After viral integration into the human genome, production of Tat and gp120 is unimpeded by HAART. It is thus apparent that HAART is not sufficient to control HIVD, and other neuroprotective strategies must be developed.

Astrocytes efficiently absorb glutamate, thus preventing excitotoxicity that might be caused by glutamate accumulation (Drejer *et al*, 1983). Excitatory amino acid transporter-2 (EAAT2) is the glutamate transporter predominantly expressed in astrocytes and responsible for most glutamate uptake in the brain (Anderson and Swanson, 2000; Robinson, 1998). This ability of astrocytes to absorb glutamate is attenuated by gp120, a protein that forms the surface spikes of the HIV virion and has numerous effects on glial cells. Importantly, Gp120 down-regulates the EAAT2 glutamate transporter mRNA and protein expression, resulting in extensive and lasting disruption in glutamate uptake, in primary human fetal astrocyte cultures (Wang *et al*, 2003, 2004). Gp120 not only inhibits glutamate absorption, but also stimulates calcium-dependent glutamate release from astrocytes (Lipton, 1994; Wahl *et al*, 1989) via a

Address correspondence to Jeffrey A. Rumbaugh, 600 North Wolfe Street, Pathology 509, Baltimore, MD 21287, USA. E-mail: jrumbaug@jhmi.edu

The authors gratefully acknowledge Phillip Ray for preparation of recombinant Tat protein. This work was supported by NIH grants to J. Rumbaugh and A. Nath.

Received 2 August 2006; revised 1 November 2006; accepted 13 December 2006.

cyclooxygenase-2 dependent mechanism (Corasaniti *et al*, 2006). The increased levels of extracellular glutamate stimulate the neuronal glutamate *N*-methyl-D-aspartic acid (NMDA) receptor, resulting in excitotoxic cascades.

Tat is the primary transactivator protein for HIV (Rosen *et al*, 1988) and has been implicated in the neurotoxicity of HIVD by multiple mechanisms. Tat directly stimulates the NMDA receptor (Prendergast *et al*, 2002), and also promotes the NMDA receptor's phosphorylation, leading to its further stimulation (Haughey *et al*, 2001). Tonic stimulation of this receptor can lead to neuronal death by the same mechanisms as for gp120-mediated glutamate excitotoxicity. Tat sensitizes neurons so that normally physiological levels of glutamate cause significant excitotoxicity and massive derangement in intracellular calcium (Nath *et al*, 2000).

The neurotoxic effects of Tat and gp120 are synergistic (Bansal *et al*, 2000; Nath *et al*, 2000). By decreasing stimulation of glutamate receptors, absorption of glutamate by astrocytes could attenuate the excitotoxic effects of Tat, which, as just described, are largely mediated through the glutamate receptors. Therefore, gp120's ability to disrupt astrocytic absorption of glutamate perhaps accounts, at least in part, for the synergism between these two virotoxins. Recent data using organotypic slice cultures from temporal lobectomy specimens have suggested that Tat, like gp120, can down-regulate expression of the EAAT2 glutamate transporter (C. Pardo, personal communication). Furthermore, Western blot analysis has demonstrated a decrease in EAAT2 levels, at least in some brain regions, in autopsy specimens of patients with HIV dementia compared to patients with HIV but no dementia and compared to healthy controls (C. Pardo, personal communication). Thus, both Tat and gp120 may attenuate astrocytic absorption of glutamate, and a therapy which could counter this effect, by up-regulating EAAT2, might be very beneficial in preventing or ameliorating HIVD.

Ceftriaxone was protective in murine models of ischemic injury and motor neuron disease (Rothstein *et al*, 2005), via a mechanism presumably directly applicable to the toxic effects of Tat and gp120 in HIVD. Particularly, ceftriaxone up-regulates the EAAT2 glutamate transporter expression and function (Rothstein *et al*, 2005), which might counter the down-regulation of this same gene by gp120 and Tat. Astrocytes would then have a greater ability to absorb glutamate, decreasing excitotoxicity mediated by glutamate receptors. We thus investigated if ceftriaxone could provide neuroprotection against Tat and gp120 in an *in vitro* model of HIV dementia.

Mixed neuronal-glial cultures were prepared, as previously described (Magnuson *et al*, 1995), from human fetal brain specimens of 12 to 15 weeks' gestational age, with consent from women undergoing elective termination of pregnancy, as approved by the Johns Hopkins University Institutional Review Board. Briefly, meninges and blood vessels were removed and the specimens washed in Opti-MEM (GIBCO). The tissue was dissociated with a 20-gauge needle and syringe, then pelleted at 270 $\times g$ for 10 min. Cells were resuspended in OptiMEM with 5% heat inactivated fetal bovine serum and 0.2% N-2 supplement to encourage neuronal growth and prevent glial proliferation. Antimicrobial solution (1000 units penicillin G per ml, 10 μg streptomycin per ml, and 25 μg amphotericin B per ml in 0.9% NaCl) was added at a final concentration of 1%. Cells were maintained in tissue culture flasks for at least 4 weeks, then plated in 96-well plates for 3 to 7 days before use in neurotoxicity assays. These cells contained approximately 70% neurons as determined by immunostaining for microtubule associated protein-2, 30% astrocytes by immunostaining for glial fibrillary acidic protein, and 1% microglia by immunostaining for CD68.

Ceftriaxone was obtained from Sigma-Aldrich, St. Louis, MO. Recombinant gp120IIIB was obtained from the AIDS Repository of the National Institutes of Health.

The human fetal mixed neuronal-glial cell cultures were preincubated in ceftriaxone at concentrations detailed in the figure legends. At the time of performing the experiment, culture medium was replaced by Locke's buffer (154 mM NaCl, 5.6 mM KCl, 2.3 mM $CaCl_2$, 1 mM $MgCl_2$, 3.6 mM $NaHCO_3$, 5 mM glucose, and 5 mM *N*-2-hydroxyethylpiperazine-*N*-2-ethanesulfonic acid, pH 7.2), with or without 300 pM gp120. Controls included untreated cultures and cultures treated with 3-nitropropionic acid (Aldrich Chemical Company, Milwaukee, WI), a highly neurotoxic compound (Olsen *et al*, 1999). Mitochondrial potential was monitored in the cultures by using 5,5',6,6-tetrachloro-1,1',3,3'-tetraethylbenzimidazole carbocyanide iodide (JC-1) dye (Molecular Probes), as we have previously described (Turchan *et al*, 2001). We performed this assay at 6 h after exposure to gp120, a time period we have previously shown to have optimal kinetics for consistent measurements (Nath *et al*, 2000). In the absence of mitochondrial damage, membrane potential remains high and JC-1 forms aggregates with a red fluorescence. With mitochondrial damage, membrane potential decreases and JC-1 remains in monomers, giving a green fluorescence. Measurements were therefore made using a fluorescent plate reader (Molecular Dynamics) with excitation at 485 nm and emission at 538 nm and 590 nm. A ratio of fluorescence at 590 nm to that at 538 nm provides an indication of mitochondrial health. Results were normalized so that control mean values were equal to 100. The means $\pm$ standard error of the means were calculated and the data analyzed by one-way analysis of variance (ANOVA) and the Bonferroni post-test.

At toxic doses of gp120 (>300 pM), ceftriaxone produced dose-dependent attenuation of neurotoxicity. Ceftriaxone at doses of <1 μM provided minimal neuroprotection, whereas at doses of 10 to

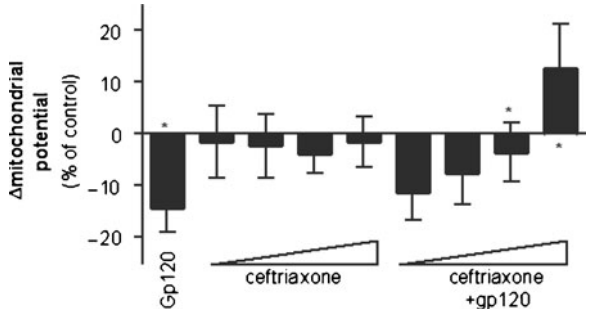

Figure 1 Ceftriaxone protects against gp120-induced neurotoxicity. Mixed human neuronal cultures were exposed to gp120 (300 pM) and ceftriaxone (0.1, 1, 10, and 100 μM, respectively), either alone or in combination. Mitochondrial membrane potential was measured 6 h later. Gp120 alone caused significant toxicity, whereas ceftriaxone alone produced no significant change compared to untreated controls. However, when incubated together, ceftriaxone, in a dose dependent manner, significantly attenuated the toxicity caused by gp120 alone. ($^*P < .05$) Toxicity was measured as a loss of mitochondrial potential compared to untreated control cultures. Data represent mean ± SEM.

100 μM, ceftriaxone provided increasingly significant ($P < .05$) neuroprotection, returning the mitochondrial membrane potential to the same level as untreated controls (Figure 1).

We next tested whether ceftriaxone could also attenuate the neurotoxicity of the Tat protein. Recombinant Tat1–72 was produced in our laboratory. Details of Tat production and purification have been published previously (Ma and Nath, 1997; Turchan *et al*, 2001). Briefly, because Tat is a nonglycosolated protein, we inserted the genes into an *Escherichia coli* vector, PinPoint Xa-2 (Promega), which allowed expression of Tat as a fusion protein naturally biotinylated at the N-terminus. The fusion protein was cleaved such that there were no overhanging or deleted amino acids. The protein was then desalted and endotoxin removed. It was then lyophilized and stored in anaerobic conditions. It was greater than 99% pure as analyzed by high-performance liquid chromatography (HPLC) and silver-stained gel electrophoresis. Each batch is monitored for purity by Western blot analysis and for endotoxin contamination by Litmus amebocyte lysate assay (Associates of Cape Cod).

We performed the neurotoxicity experiments with Tat as detailed for gp120 experiments, except using 200 nM Tat in place of 300 pM gp120. We found that ceftriaxone, at doses >0.1 μM, protects against Tat-induced neurotoxicity as well (Figure 2).

We recognized that mitochondrial membrane potential is an intermediate measurement of neuronal viability, and therefore tested ceftriaxone's ability to protect against Tat- and gp120-induced cell death. The human fetal mixed neuronal-glial cell cultures were used for these assays as described above, but instead of using JC-1 dye, we stained the cells with 0.4% trypan blue at 16 h after exposure to gp120 or Tat, a time point chosen based on previous work (Nath *et al*, 2000). We used trypan blue exclusion as

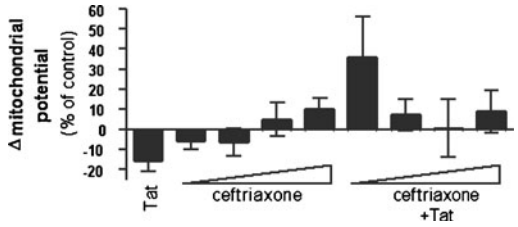

Figure 2 Ceftriaxone protects against Tat-induced neurotoxicity. Mixed human neuronal cultures were exposed to Tat (200 nM) and ceftriaxone (0.1, 1, 10, and 100 μM, respectively), either alone or in combination. Mitochondrial membrane potential was measured 6 h later. Tat alone caused significant toxicity, whereas ceftriaxone alone produced no significant change compared to untreated controls. However, when incubated together, ceftriaxone, at all doses, attenuated the toxicity caused by Tat alone. Toxicity was measured as a loss of mitochondrial potential compared to untreated control cultures. Data represent mean ± SEM.

a measure of neuronal cell death because it measures both apoptotic and necrotic cell death and we have extensive experience in using this assay for characterizing Tat-induced neurotoxicity. The dead cells were quantitated as described previously (Magnuson *et al*, 1995; Nath *et al*, 1996; Turchan *et al*, 2001). Briefly, cultures were assayed by neuronal cell counts determined from 10 fields at predetermined coordinates. Each field was coded and counted without knowledge of its experimental identity, and each experiment was done in triplicate. The means ± standard error of the means were calculated and the data analyzed by one way ANOVA and the Bonferroni posttest. We found that ceftriaxone was protective against Tat- and gp120-induced cell death in a dose dependent manner, as measured by trypan blue exclusion (Figure 3).

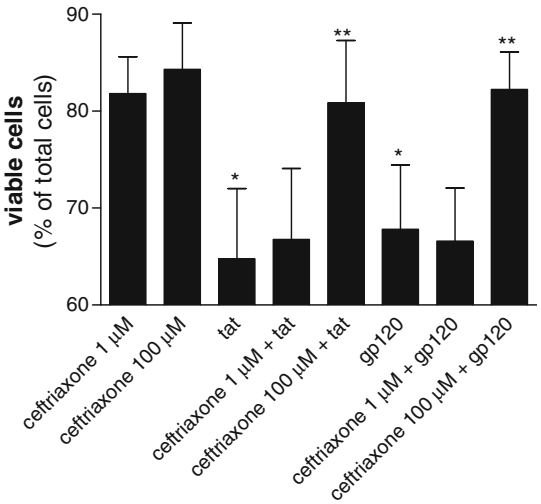

Figure 3 Ceftriaxone protects against Tat- and gp120-induced neuronal cell death. Mixed human neuronal cultures were exposed to Tat (200 nM), gp120 (300 pM), and ceftriaxone (1 or 100 μM), either alone or in combination. Cell death was measured by uptake of trypan blue 16 h later. Tat and gp120 alone each caused significant neuronal death ($^*P < .05$). However, when incubated together, ceftriaxone at 100 μM returned levels of cell death to that seen with ceftriaxone alone ($^{**}P < .05$). Data represent mean ± SEM.

To determine ceftriaxone's effect on Tat's primary function in the viral life cycle, we also used a long terminal repeat–(LTR-chloramphicol acetyltransferase (CAT)) assay (Ma and Nath, 1997) to test whether ceftriaxone might influence Tat-mediated LTR transactivation or directly activate LTR. However, we found no effect. At doses between 0.1 and 1000 μM, ceftriaxone alone did not transactivate LTR, nor did its presence affect Tat's ability to transactivate LTR (data not shown).

These studies thus demonstrate that ceftriaxone, at easily attainable pharmacologic levels (serum 30 to 300 μM; cerebrospinal fluid [CSF] 15 to 30 μM) (Scheld *et al*, 1984), can protect human neurons against toxicity produced by the HIV proteins, gp120 and Tat. One likely mechanism for this protective effect is stimulation of the EAAT2 glutamate transporter gene, counteracting the down-regulation induced by viral proteins, and thus preventing excitotoxicity. As with HIV and human astrocytes, disruption of astrocytic glutamate uptake has been demonstrated in feline astrocytes infected with feline immunodeficiency virus (Billaud *et al*, 2000; Yu *et al*, 1998), and in human astrocytes infected with human T-cell leukemia virus (HTLV)-1 (Szymocha *et al*, 2000), suggesting a common neurotoxic mechanism among various retroviruses (Wang *et al*, 2003). This class of drugs may have therapeutic efficacy in HIV dementia. In fact, several beta-lactam antibiotics have shown neuroprotective potential (Rothstein *et al*, 2005). We have focused our studies on ceftriaxone because of its known ability to penetrate the CSF.

Ceftriaxone is already Food and Drug Administration (FDA) approved and widely used for the treatment of bacterial meningitis. There are several challenges to using ceftriaxone in this patient population, or, indeed, for any neuroprotective indication. We do not know whether ceftriaxone will be effective as treatment for HIV dementia, reversing some of the neurological dysfunction and cognitive impairments produced by viral proteins, or only as prophylaxis against development of HIVD in those at risk. In any case, it is likely that an agent used for these purposes would have to be taken chronically. Chronic use of ceftriaxone would be problematic because it is only available as an intravenous formulation. Chronic use in relatively large populations would probably also promote antibiotic resistance to ceftriaxone and other beta-lactam agents.

Clearly, significant expansion of experimental data is required before a ceftriaxone-based agent will ever be clinically useful. Nevertheless, a small pilot trial of ceftriaxone in patients with HIVD may be reasonable, looking for clinical evidence to support further development of such agents. Ultimately, it will be necessary to develop oral agents that maintain ceftriaxone's neuroprotective and CSF-penetrating abilities, preferably without the antibiotic properties. Though the goal of having such agents available for clinical use in patients with HIVD, amyotrophic lateral sclerosis, and other neurological conditions, is likely still years away, the promise of such agents remains very exciting.

References

Anderson CM, Swanson RA (2000). Astrocyte glutamate transport: review of properties, regulation, and physiological functions. *Glia* **32:** 1–14.

Bansal AK, Mactutus CF, Nath A, Maragos W, Hauser KF, Booze RM (2000). Neurotoxicity of HIV-1 proteins gp120 and Tat in the rat striatum. *Brain Res* **879:** 42–49.

Billaud JN, Selway D, Yu N, Phillips TR (2000). Replication rate of feline immunodeficiency virus in astrocytes is envelope dependent: implications for glutamate uptake. *Virology* **266:** 180–188.

Chen P, Mayne M, Power C, Nath A (1997). The Tat protein of HIV-1 induces tumor necrosis factor-α production: Implications for HIV associated neurological diseases. *J Biol Chem* **272:** 22385–22388.

Corasaniti MT, Bellizzi C, Russo R, Colica C, Amantea D, Di Renzo G (2003). Caspase-1 inhibitors abolish deleterious enhancement of COX-2 expression induced by HIV-1 gp120 in human neuroblastoma cells. *Toxicol Lett* **139:** 213–219.

Dore GJ, McDonald A, Li Y, Kaldor JM, Brew BJ (2003). Marked improvement in survival following AIDS dementia complex in the era of highly active antiretroviral therapy. *AIDS* **17:** 1539–1545.

Drejer J, Meier E, Schousboe A (1983). Novel neuron-related regulatory mechanisms for astrocytic glutamate and GABA high affinity uptake. *Neurosci Lett* **37:** 301–306.

Haughey NJ, Nath A, Mattson MP, Slevin JT, Geiger JD (2001). HIV-1 Tat through phosphorylation of NMDA receptors potentiates glutamate excitotoxicity. *J Neurochem* **78:** 457–467.

Lafortune L, Nalbantoglu J, Antel JP (1996). Expression of tumor necrosis factor alpha (TNF alpha) and interleukin 6 (IL-6) mRNA in adult human astrocytes: comparison with adult microglia and fetal astrocytes. *J Neuropathol Exp Neurol* **55:** 515–521.

Lipton SA (1994). AIDS related dementia and calcium homeostasis. *Ann N Y Acad Sci* **594:** 189–196.

Ma M, Nath A (1997). Molecular determinants for cellular uptake of Tat protein of human immunodeficiency virus type 1 in brain cells. *J Virol* **71:** 2495–2499.

Magnuson DS, Knudsen BE, Geiger JD, Brownstone RM, Nath A (1995). Human immunodeficiency virus type 1 tat activates non-*N*-methyl-D-aspartate excitatory amino acid receptors and causes neurotoxicity. *Ann Neurol* **37:** 373–380.

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, Haughey NJ, Jones M, Anderson C, Bell JE, Geiger JD (2000). Synergistic neurotoxicity by human immunodeficiency virus proteins Tat and gp120: protection by memantine. *Ann Neurol* **47:** 186–194.

Nath A, Psooy K, Martin C, Knudsen B, Magnuson DS, Haughey N, Geiger JD (1996). Identification of a human immunodeficiency virus type 1 Tat epitope that is neuroexcitatory and neurotoxic. *J Virol* **70:** 1475–1480.

Neuenburg JK, Brodt HR, Herndier BG, Bickel M, Bacchetti P, Price RW, Grant RM, Schlote W (2002). HIV-related neuropathology, 1985 to 1999: rising prevalence of HIV encephalopathy in the era of highly active antiretroviral therapy. *J Acquir Immune Defic Syndr* **31:** 171–177.

Olsen C, Rustad A, Fonnum F, Paulsen RE, Hassel B (1999). 3-Nitropropionic acid: an astrocyte-sparing neurotoxin in vitro. *Brain Res* **850:** 144–149.

Prendergast MA, Rogers DT, Mulholland PJ, Littleton JM, Wilkins LH, Jr., Self RL, Nath A (2002). Neurotoxic effects of the human immunodeficiency virus type-1 transcription factor Tat require function of a polyamine sensitive-site on the *N*-methyl-D-aspartate receptor. *Brain Res* **954:** 300–307.

Ranki A, Nyberg M, Ovod V, Haltia M, Elovaara I, Raininko R, Haapasalo H, Krohn K (1995). Abundant expression of HIV Nef and Rev proteins in brain astrocytes in vivo is associated with dementia. *AIDS* **9:** 1001–1008.

Robinson MB (1998). The family of sodium-dependent glutamate transporters: a focus on the GLT-1/EAAT2 subtype. *Neurochem Int* **33:** 479–491.

Rosen CA, Terwilliger E, Dayton A, Sodroski JG, Haseltine WA (1988). Intragenic cis-acting art gene-responsive sequences of the human immunodeficiency virus. *Proc Natl Acad Sci USA* **85:** 2071–2075.

Rothstein JD, Patel S, Regan MR, Haenggeli C, Huang YH, Bergles DE, Jin L, Dykes Hoberg M, Vidensky S, Chung DS, Toan SV, Bruijn LI, Su ZZ, Gupta P, Fisher PB (2005). Beta-lactam antibiotics offer neuroprotection by increasing glutamate transporter expression. *Nature* **433:** 73–77.

Sacktor N (2002). The epidemiology of human immunodeficiency virus-associated neurological disease in the era of highly active antiretroviral therapy. *J NeuroVirol* **8(Suppl 2):** 115–121.

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.

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.

Scheld WM, Rocha H, Sande MA, Bryan JP (1984). Rationale for clinical trials evaluating ceftriaxone in the therapy of bacterial meningitis. *Am J Med* **77:** 42–49.

Szymocha R, Akaoka H, Dutuit M, Malcus C, Didier-Bazes M, Belin MF, Giraudon P (2000). Human T-cell lymphotropic virus type 1-infected T lymphocytes impair catabolism and uptake of glutamate by astrocytes via Tax-1 and tumor necrosis factor alpha. *J Virol* **74:** 6433–6441.

Tornatore C, Chandra R, Berger JR, Major EO (1994). HIV-1 infection of subcortical astrocytes in the pediatric central nervous system. *Neurology* **44:** 481–487.

Turchan J, Anderson C, Hauser KF, Sun Q, Zhang J, Liu Y, Wise PM, Kruman I, Maragos W, Mattson MP, Booze R, Nath A (2001). Estrogen protects against the synergistic toxicity by HIV proteins, methamphetamine and cocaine. *BMC Neurosci* **2:** 3.

Wahl LM, Corcoran ML, Pyle SW, Arthur LO, Harel-Bellan A, Farrar WL (1989). Human immunodeficiency virus glycoprotein (gp120) induction of monocyte arachidonic acid metabolites and interleukin 1. *Proc Natl Acad Sci USA* **86:** 621–625.

Wang Z, Pekarskaya O, Bencheikh M, Chao W, Gelbard HA, 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.

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). Effects of human immunodeficiency virus type 1 on astrocyte gene expression and function: potential role in neuropathogenesis. *J NeuroVirol* **10(Suppl 1):** 25–32.

Yu N, Billaud JN, Phillips TR (1998). Effects of feline immunodeficiency virus on astrocyte glutamate uptake: implications for lentivirus-induced central nervous system diseases. *Proc Natl Acad Sci USA* **95:** 2624–2629.