Journal of NeuroVirology (1997) 3, 168–173
© 1997 Journal of NeuroVirology, Inc.
http://www.jneurovirol.com

Short Communication

Human immunodeficiency virus type 1 Tat protein induces death by apoptosis in primary human neuron cultures

Deborah R New[1,2], Meihui Ma[3], Leon G Epstein[5,6,7], Avindra Nath[3,4], and Harris A Gelbard[5,6,8]

Departments of [1]Neurobiology and Anatomy, [2]Dental Research, [5]Neurology, [6]Pediatrics, [7]Microbiology and Immunology, [8]Pharmacology and Physiology, University of Rochester Medical Center, 601 Elmwood Ave., Rochester, New York, 14642, USA; Departments of [3]Medical Microbiology, and [4]Internal Medicine (Section of Neurology), University of Manitoba, 523-730 William Ave., Winnipeg, Manitoba, R3E OW3, Canada

Neuronal loss in HIV encephalopathy remains a mystery since HIV-1 productively infects macrophage and microglia and only rarely infects neurons in the central nervous system. Apoptosis is a mechanism which may account for the loss of neurons in HIV-1 infected brain. Putative toxic factors that result in neuronal cell death in HIV-1 infection include the regulatory protein Tat, since this protein is known to be released from HIV-1 infected cells. Here we show that Tat induces cell death by apoptosis in cultured human fetal neurons producing characteristic morphological and biochemical features associated with apoptosis. These findings suggest that Tat may play an important role as a secreted, soluble neurotoxin in HIV-1 associated dementia.

Keywords: apoptosis; brain; central nervous system; dementia; HIV-1; pathogenesis; Tat; human cortical neuron cultures

Introduction

Neurodegeneration associated with infection of the central nervous system (CNS) by HIV-1 results in a CNS dysfunction termed HIV-1 associated dementia (Navia *et al*, 1986). The pathogenesis of HIV-1 associated dementia is not clear, but it is unlikely that productive infection of neurons with HIV-1 is responsible. In fact, HIV-1 infected neurons have rarely been demonstrated in post-mortem tissue, while macrophages and microglia are primarily found to be productively infected with the virus (Takahashi *et al*, 1996; Bernton *et al*, 1992; Wiley *et al*, 1986, 1991; Watkins *et al*, 1990). HIV-1 infection in the brain results in widespread myelin pallor, reactive astrogliosis, alterations of neocortical dendritic processes and neuronal loss without necrosis (Epstein and Gendelman, 1993; Sharer, 1992; Wiley *et al*, 1991). Several studies have demonstrated that HIV-1 infection of macrophages and microglia results in the release of soluble toxic factors that mediate neuronal death in *in vitro* models of HIV-1 neurotoxicity (Gelbard *et al*, 1994; Genis *et al*, 1992; Merrill and Chen, 1991; Giulian *et al*, 1990).

Recent evidence in post-mortem AIDS brain tissue indicates that neuronal apoptosis was present in cases with productive HIV-1 infection (Adle-Biassette *et al*, 1995; Gelbard *et al*, 1995; Petito and Roberts, 1995), but the cause of this pathological finding remains to be determined. Several authors have cited indirect mechanisms of neurotoxicity via macrophage-astroglial interactions mediated by a number of soluble factors including the cytokine tumor necrosis factor-alpha, and the phospholipid mediator platelet-activating factor (Gelbard *et al*, 1994; Genis *et al*, 1992; Merrill and Chen, 1991). Also *in vitro* studies indicate that the HIV-1 regulatory protein known as Tat, is released from infected cells (Ensoli *et al*, 1992). Several studies have shown Tat_{1-86} and Tat peptides which contain the basic region within the first exon amino acid sequence are neurotoxic (Nath *et al*, 1996; Magnuson *et al*, 1995; Philippon *et al*, 1994; Hayman *et al*, 1993). Tat is a viral transactivator, and is expressed early in the viral life cycle (Sodroski *et al*, 1985). It activates transcription directed by the HIV-1 long terminal repeat (LTR), which in turn leads to increased expression of all viral genes, (Arya *et al*, 1985; Sodroski *et al*, 1985) and some cellular genes (Ensoli *et al*, 1992; Buonaguro *et al*, 1992; Helland *et al*, 1991; Vogel *et*

Correspondence: HA Gelbard
Received 18 September 1996; revised 5 December 1996; accepted 5 December 1996

al, 1988). This protein has been shown to induce neurotoxicity in human fetal neuron cultures which is mediated via excitatory amino acid receptors (Nath *et al*, 1996; Magnuson *et al*, 1995). The Tat protein has also been linked to apoptotic cell death in cultured peripheral blood mononuclear cells as well as a CD4-positive ($CD4^+$) T cell line (Li *et al*, 1995; Purvis *et al*, 1995; Westendorp *et al*, 1995a, b).

In this study, we demonstrate that recombinant-Tat_{1-86} and Tat_{1-72} containing the basic region induces apoptosis in cultured human fetal cortical neurons in a dose-dependent fashion.

In situ *detection of Tat induced apoptosis in human fetal neurons*

Second trimester human cortical neuronal cultures, 28 days post-explantation, were treated with recombinant Tat_{1-86} protein at various concentrations for 18 h then TUNEL stained for the free 3′-OH ends of cleaved DNA. Tat-induced apoptosis was found to be dose-dependent and was detectable at doses as low as 0.125 μM (Figure 1). The dose response curve generated from our data indicates an LD_{50} for neuronal apoptosis of 0.5 μM. Doses of 1 μM or greater resulted in detached cells (data not shown). Positively stained neurons were observed with chromatin aggregation, nuclear condensation, and apoptotic bodies, features consistent with apoptosis (Figure 2B). Similar apoptosis results were achieved with Tat_{1-72}. Neuron cultures were also treated with a vehicle control, where only rare TUNEL staining was observed (Figure 2A).

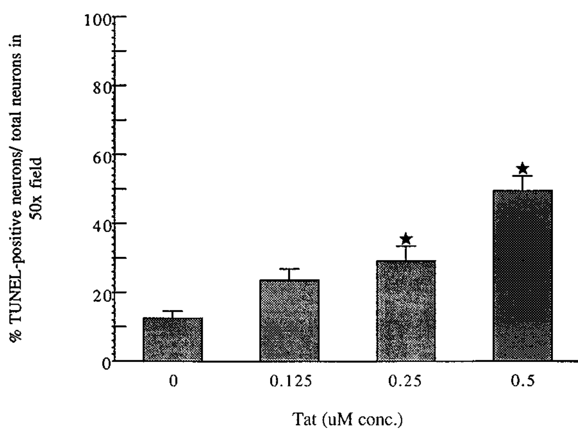

Figure 1 Primary human fetal neuron cultures were treated with Tat at the following doses; 0, 0.125, 0.25 and 0.5 μM for 18 h. Cultures were fixed and TUNEL stained, the positive apoptosis immunostained neurons were analyzed in 16 random fields of each treatment by computerized morphometry. The histogram bars represent the average percent of TUNEL-positive apoptotic cells per total neurons per 50× field, SEM is shown by vertical lines (★=$p<0.001$ *vs* control). This initial dose response curve at 18 h indicates an LD_{50} at 0.5 μM, higher doses at 1 μM and greater were also analyzed, but resulted in detached cells.

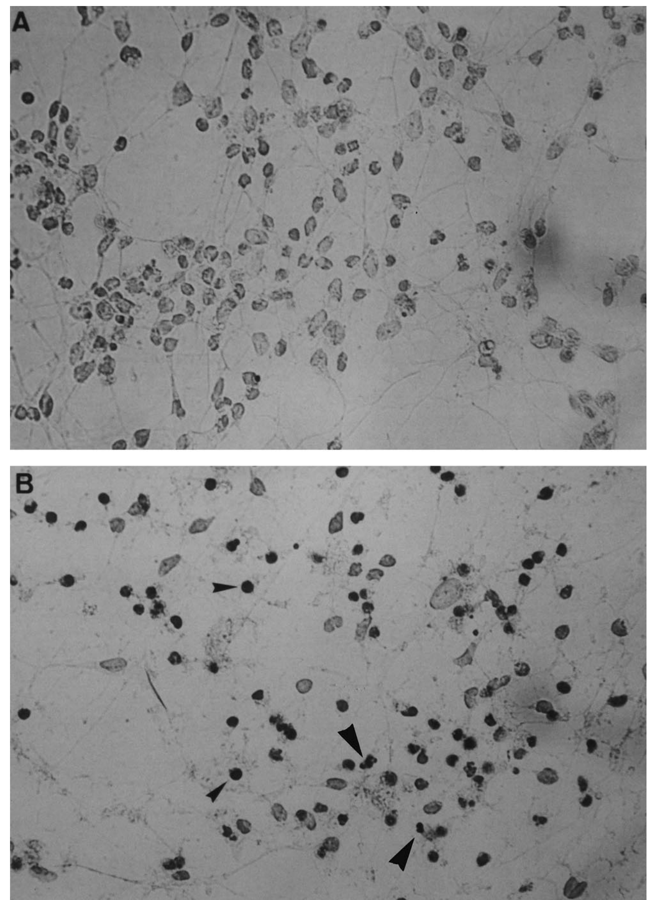

Figure 2 Primary human fetal neurons cultured for 28 days were treated with vehicle control (A) or 0.5 μM of Tat (B) for 18 h. Neurons were subsequently fixed and stained *in situ* for new 3′-OH DNA ends generated by DNA fragmentation that results in chromatin condensation, a morphologic feature of apoptosis. The TUNEL assay or active labeling of cells by end labeling utilizing the 'Apoptag kit' (Oncor, Gaithersburg, MD) is indicated by the black precipitate seen as chromatin condensation (small arrows), and apoptotic bodies (large arrows).

Electron microscopy analysis of Tat treated neurons

Morphological indicators of apoptosis in the human fetal neurons treated with recombinant Tat_{1-72} were anlayzed by transmission electron microscopy (Figure 3). A number of changes consistent with apoptosis were observed in a subpopulation of neurons. Most commonly, condensation of the nuclear chromatin with relative preservation of the cytoplasmic structures, and the nuclear and cell membranes was noted (Figure 3A, B, D). Blebbing of the cell membrane was seen in some cells (Figure 3C). These cells showed only minimal changes in the nucleus and hence cell membrane blebbing was recognized as an early morphological change. Other cells showed partitioning and condensation of cytoplasm and nuclear material into membrane bound apoptotic bodies demonstrating end stage apoptotic changes. No phagocy-

tic cells were seen in these sections. The above morphological changes were absent in the untreated cultures or in cultures treated with solutions from which Tat had been immunoabsorbed (data not shown).

Cellular DNA analysis for Tat induced apoptosis
DNA was extracted from recombinant Tat_{1-72} treated neurons and analyzed by agarose gel electrophoresis. A characteristic DNA cleavage ladder pattern specific for apoptosis was observed (Figure 4, Lane 2). No laddering was observed in the untreated cultures (Figure 4, Lane 3).

The phenomenon of apoptosis is usually associated with development, homeostasis, and aging processes. In HIV-1 infection, apoptosis occurs in the $CD4^+$ subpopulation of lymphocytes in the peripheral blood as a consequence of the infection (Meyaard *et al*, 1992). The protein Tat also induces apoptosis in $CD4^+$ lymphocytes (Ehret *et al*, 1996; Westendorp *et al*, 1995a,b). In the CNS, it has been suggested that pathological apoptosis occurs resulting in the loss of neurons (Adle-Biassette *et al*, 1995; Gelbard *et al*, 1995; Petito and Robert, 1995). This study focused on whether the HIV-1 regulatory protein Tat may play a role in mediating neuronal apoptosis.

Neuronal loss in HIV-1 infection is not directly due to the virus but may be due to toxic viral products that are secreted from infected cells. The HIV-1 regulatory protein Tat is one of many viral and cellular products secreted from HIV-1 infected cells. It has been suggested that Tat may be transported transcellularly from either infected or transfected cells via direct cell to cell contact in amounts sufficient to transactivate Tat-responsive promoter elements (Frankel and Pabo, 1988; Helland *et al*, 1991; Marcuzzi *et al*, 1992a,b). This suggests that exogenous Tat may affect uninfected cells during the course of HIV-1 infection.

Here we show convincing evidence that the HIV-1 protein Tat induced the morphological and biochemical features of apoptosis in cultured human neurons. Tat-induced apoptosis was demon-

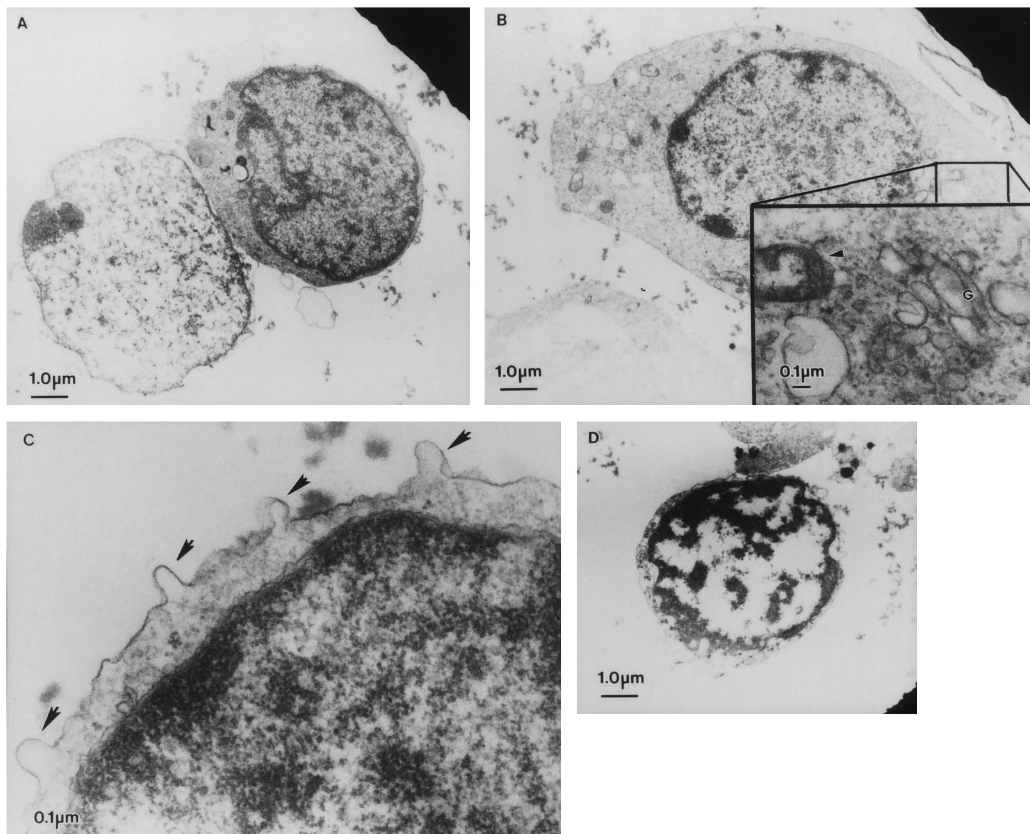

Figure 3 Morphological characteristics of Tat-induced apoptosis. (A) Two neurons are seen in close proximity. The one on the right shows normal morphological features while the other neuron shows degradation and condensation of the nuclear chromatin with preservation of the nuclear and cell membrane. (B) Condensation of nuclear chromatin with relative preservation of the cytoplasmic structures. Insert shows normal mitochondria and golgi apparatus. (C) Arrows show typical blebbing of the cytoplasmic cell membrane. (D) Another cell with clumping of the chromatin, degradation of cytoplasmic structures, but relative preservation of the nuclear and cell membranes.

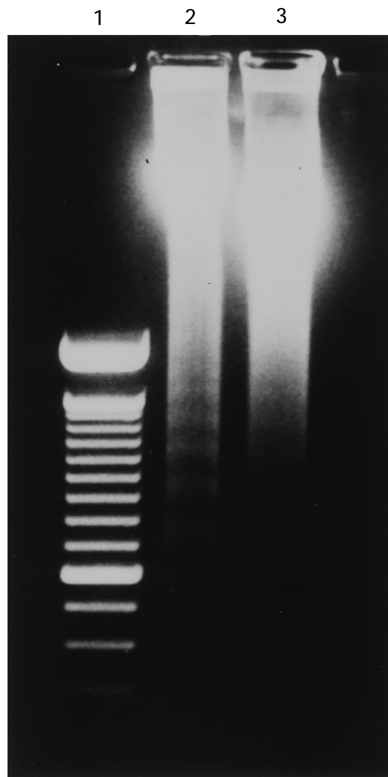

Figure 4 Tat induced DNA laddering. Lane 1. Molecular weight markers (1 kb) Lane 2. DNA extracts from Tat treated human fetal neurons analyzed by agarose gel electrophoresis show DNA laddering. Lane 3. Untreated neurons show absence of DNA laddering.

strated by several assays. First, we detected the presence of apoptotic neurons by demonstrating chromatin condensation and the formation of apoptotic bodies by *in situ* nick end labeling. We also demonstrated chromatin condensation and aggregation that abuts the inner surface of the nuclear membrane, and blebbing of the cytoplasmic cell membrane along with complete preservation of the integrity of the cytoplasmic organelles in the electron micrographs. Finally we demonstrated the typical DNA fragmentation ladder characteristic of apoptosis in the neuron cultures treated with Tat.

Recently, Tat was shown to activate non-NMDA excitatory amino acid (EAA) receptors and cause neurotoxicity in cultured neurons leading to increases in intracellular calcium and cell death (Nath *et al*, 1996; Magnuson *et al*, 1995). The neurotoxic domain resides within a conformationally dependent epitope within the first exon of Tat between residues 31 to 61 (Nath *et al*, 1996). These increases in intracellular calcium may induce apoptosis by activation of cellular enzymes (Conant *et al*, 1996).

Thus, recombinant Tat containing the basic region induces death in a subpopulation of cultured human fetal cortical neurons with the characteristic morphological and biochemical features of apoptosis. Tat-induced necrosis of neurons was not observed at the light or electron microscope level. Our studies indicate that the first exon of Tat_{1-72} is sufficient to cause cell death. Tat-induced apoptosis was only observed in cortical neurons and not in astrocytes (New, Angel, unpublished observations). We speculate that Tat-induced apoptosis can occur by more than one pathway, including glutamate receptor activation, oxidative stress and signaling via the pro-inflammatory cytokine TNF-α. The finding that Tat is a potent HIV-1-induced neurotoxin with an LD_{50} of 0.5 μM suggests that it may play a highly significant role in mediating neuronal apoptosis.

Methods

Human fetal brain tissue between gestational ages of 13 to 16 weeks were obtained and processed for culture as previously described (Gelbard *et al*, 1994). Cultured neurons were treated with full length recombinant Tat_{1-86} protein. Once it was determined that only the basic region of the protein was required to elicit apoptosis, recombinant Tat_{1-72} was used to complete these experiments. Since the pathological levels of Tat in the HIV-infected brain are unknown we initially ran dose response experiments with concentrations of Tat used for these experiments ranging from 0.125 μM to 2.4 μM.

Recombinant HIV-1 Tat_{1-86} was expressed and purified as described before (Herrmann and Rice, 1993). Briefly a glutathione S-transferase Tat_{1-86} fusion protein (AIDS research and reference reagent program, NIAID, NIH; Herrmann and Rice, 1993) was expressed and purified by adsorption to glutathione-sepharose beads (Pharmacia). Recombinant Tat_{1-72} was also prepared as described before (Magnuson *et al*, 1995). Tat_{1-72} was expressed as a fusion protein with a naturally biotinylated protein at the N-terminus in *E. coli* DH5aF'IQ (Gibco BRL). The biotin portion of the fusion protein was adsorpted to Softlink™ soft-release avidin resin (Promega), then cleaved from the resin with factor Xa, a serine endopeptidase (Boehringer Mannheim). Dithiothreitol (DTT) was added in each step of the purification of Tat_{1-86} and Tat_{1-72}. Tat protein was suspended in a buffer containing 50 mM Tris (pH 8.0), 100 mM NaCl, 1 mM $CaCl_2$ and 0.5 mM DTT. The Tat protein was 95% pure by gel electrophoresis. The purified product was further analyzed by Western immunoblot analysis with a polyclonal antibody (AIDS Research and Reference Reagent Program, NIAID, NIH; Hauber *et al*, 1987). Its biological activity was measured by β-galactosidase (β-gal) gene activation in an HIV-1 long terminal repeat (LTR)-β-gal plasmid which had been transfected into HeLa cells (AIDS Repository, NIH) (Conant *et*

al, 1996). An *E. coli* strain containing the GST expression plasmid without the Tat sequence was expressed and purified as a sham Tat and used as a vehicle control.

Human fetal neuron cultures were treated with recombinant Tat for 18 h then stained to detect free 3′-OH ends of newly cleaved DNA in situ as described previously (Talley *et al*, 1995). Tat-induced apoptotic cells were stained with the *in situ* terminal deoxynucleotidyl transferase-mediated digoxigenin-dUTP nick end labeling (TUNEL) assay (Oncor, Gaithersburg, MD). Digitized images of TUNEL stained neurons in 16 or greater microscopic fields were analyzed for numbers of positively stained neuronal nuclei divided by total numbers of neurons per 50× field using computerized morphometry (Imaging Research Inc., Onatrio, Canada). Data were expressed as means ± SEM, with significance determined by one-way ANOVA.

Electron microscopy analysis of neurons was performed following 18 h treatment with 1 μM Tat. The cells were scraped and fixed in 2% glutaraldehyde, washed in a 0.1 M sodium cacodylate solution, suspended in agarose. The cells in agarose were cut into 1 mm^3 blocks and fixed in 1% osmium tetroxide. The cells were dehydrated in successively increasing concentrations of acetone followed by propylene oxide and then embedded in Epon 812/araldite 502 (Marivac, Halifax, NS). Ultra-microtome sections of cells were stained with Reynolds lead citrate and viewed on a transmission electron microscope (Hayat 1981). Control cultures were similarly processed for electron microscopy.

DNA extraction and electrophoresis analysis was performed on human fetal neuronal cultures (4×10^6 cells) treated with 2.4 μM Tat_{1-72} for 18 h. The neurons were separated by vigorous shaking and harvested by centrifugation at 400 × g for 5 min. The cell pellet was lysed in 0.5 ml lysis buffer (20 mM Tris-HCl pH 7.5, 4 mM EDTA, 3% SDS, 0.5 mg proteinase K per ml) at 50°C for 1 h. RNase H (20 g/ml, Boehringer Mannheim) was then added and the incubation continued for another 1 h. The lysate was centrifuged at 12 000 × g for 10 min and the supernatant was extracted with phenol followed by chloroform-isoamyl alcohol (24:1) and precipitated with ethanol at −70°C. The DNA pellet was dried and resuspended in TE buffer (10 mM Tris-HCl pH 8.0, 1 mM EDTA) and analyzed by 1.8% agarose gel electrophoresis.

Acknowledgements

This work was supported by the NIH grant K16 DE00159 for DR New. The Medical Research Council of Canada and the National Health and Research Development Program provided funding for M Ma and A Nath. LG Epstein and HA Gelbard were funded in part by a generous grant from the Charles A Dana Foundation and by the NIH grant PO1 NS31492-04. We thank Mark Bernier for technical assistance.

References

Adle-Biassette H, Levy Y, Colombel M, Poron F, Natchev S, Keohane C, Gray F (1995). Neuronal apoptosis in HIV infection in adults. *Neuropath Appl Neurobio* **21:** 218–227.

Arya SK, Guo C, Josephs SF, Wong-Staal F (1985). Transactivator gene of human T-lymphotrophic virus type III (HTLV-III). *Science* **229:** 69–73.

Bernton E, Bryant H, Decoster M, Orenstein JM, Ribas J, Meltzer MS, Gendelman HE (1992). No direct neuronotoxicity by HIV-1 virions or culture fluids from HIV-1 infected T cells or monocytes. *AIDS Res Hum Retroviruses* **8:** 495–503.

Buonaguro L, Barillari G, Chang HK, Bohan CA, Kao V, Morgan R, Gallo RC, Ensoli B (1992). Effects of the human immunodeficiency virus type 1 Tat protein on the expression of inflammatory cytokines. *J Virol* **66:** 7159–7167.

Conant K, Ma M, Nath A, Major EO (1996). Extracellular human immunodeficiency virus type 1 Tat protein is associated with an increase in both NF-kB binding and protein kinase C activity in primary human astrocytes. *J Virol* **70:** 1384–1389.

Ehret A, Westendorp MO, Herr I, Debatin K-M, Heeney JL, Frank R, Krammer PH (1996). Resistance of chimpanzee T cells to human immunodeficiency virus type 1 Tat-enhanced oxidative stress and apoptosis. *J Virol* **70:** 6502–6507.

Ensoli, B, Buonaguro L, Barillari G, Fiorelli V, Gendelman R, Morgan R, Wingfield P, Gallo R (1992). Release, uptake, and effects of extracellular human immunodeficiency virus type 1 Tat protein on cell growth and viral transactivation. *J Virol* **67:** 277–287.

Epstein LG, Gendelman HE (1993). Human immunodeficiency virus type 1 infection of the nervous system: Pathogenetic mechanisms. *Ann Neurol* **33:** 429–436.

Frankel AD, Pabo CO (1988). Cellular uptake of the Tat protein from human immunodeficiency virus. *Cell* **55:** 1189–1193.

Gelbard HA, James HJ, Sharer LR, Perry SW, Saito Y, Kazee A, Blumberg BM, Epstein LG (1995). Apoptotic neurons in brains from paediatric patients with HIV-1 encephalitis and progressive encephalopathy. *Neuropath Appl Neurobio* **21:** 208–217.

Gelbard HA, Nottet HSLM, Swindells S, Jett M, Dzenko K, Genis P, White R, Wang L, Choi Y, Zhang D, Lipton SA, Tourtellotte WW, Epstein LG, Gendelman HE (1994). Platelet-activating factor: a candidate human immunodeficiency virus type 1-induced neurotoxin. *J Virol* **68:** 4628–4635.

Genis P, Jett M, Bernton EW, Gelbard HA, Dzenko K. Keane R, Resnick L, Volsky DJ, Epstein LG, Gendelman HE (1992). Cytokines and arachidonic acid metabolites produced during HIV-infected macrophage-astroglial interactions: Implications for the neuropathogenesis of HIV disease. *J Exp Med* **176:** 1703–1718.

Giulian D, Vaca K, Noonan CA (1990). Secretion of neurotoxins by mononuclear phagocytes infected with HIV-1. *Science* **250:** 1593–1596.

Hauber J, Perkins A, Heimer E, Cullen B (1987). Trans-activation of human immunodeficiency virus gene expression is mediated by nuclear events. *Proc Natl Acad Sci USA* **84:** 6364–6368.

Hayat MA (1981). Fixation for electron microscopy. Academic Press. New York, New York.

Hayman M, Arbuthnott G, Harkiss G, Brace H, Filippi P, Philippon V, Thomson D, Vigne R, Wright A (1993). Neurotoxicity of peptide analogues of the transactivating protein tat from maedi-visna virus and human immunodeficiency virus. *Neuroscience* **53:** 1–6.

Helland D, Welles J, Caputo A, Haseltine W (1991). Transcellular transactivation by the human immunodeficiency virus type 1 Tat protein. *J Virol* **65:** 4547–4549.

Herrmann CH, Rice AP (1993). Specific interaction of the human immunodeficiency virus Tat proteins with a cellular protein kinase. *Virology* **197:** 601–608.

Li CJ, Friedman DJ, Wang C, Metelev V, Pardee AB (1995). Induction of apoptosis in uninfected lymphocytes by HIV-1 Tat protein. *Science* **268:** 429–431.

Magnuson DSK, 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.

Marcuzzi A, Weinberger J, Weinberger OK (1992a). Transcellular activation of the human immunodeficiency virus type 1 long terminal repeat in co-cultured lymphocytes. *J Virol* **66:** 4228–4232.

Marcuzzi A, Weinberger J, Weinberger OK (1992b). Transcellular activation of the human immunodeficiency virus type 1 long terminal repeat in T lymphocytes requires CD4-gp120 binding. *J Virol* **66:** 4536–4539.

Merrill JE, Chen ISY (1991). HIV-1, macrophages, glial cells, and cytokines in AIDS nervous system disease. *FASEB J* **5:** 2391–2397.

Meyaard L, Otto SA, Jonker RR, Mijnster MK, Keet RPM, Miedema F (1992). Progammed death of T cells in HIV-1 infection. *Science* **257:** 217–219.

Nath A, Psooy K, Martin C, Knudsen B, Magnuson DSK, 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.

Navia BA, Jordan BD, Price RW (1986). The AIDS dementia complex: I. Clinical features. *Ann Neurol* **19:** 517–524.

Petito CK, Roberts B (1995). Evidence of apoptotic cell death in HIV encephalitis. *Am J Pathol* **146:** 1121–1130.

Philippon V, Vellutini C, Gambarelli D, Harkiss G, Arbuthnott G, Metzger D, Roubin R, Filippi P (1994). The basic domain of the lentiviral Tat protein is responsible for damages in mouse brain: Involvement of cytokines. *Virology* **205:** 519–529.

Purvis SF, Jacobberger JW, Sramkoski RM, Patki AH, Lederman MM (1995). HIV type 1 Tat protein induces apoptosis and death in jurkat cells. *AIDS Res Hum Retro* **11:** 443–450.

Sharer LR (1992). Pathology of HIV-1 infection of the central nervous system (Review). *J. Neuropath.* and Exp. *Neurol* **51:** 3–11.

Sodroski J, Rosen C, Wong-Staal F, Salahuddin SZ, Popovic M, Arya S, Gallo RC, Haseltine WA (1985). Trans-acting transcriptional regulation of human T-cell leukemia virus type III long terminal repeat. *Science* **227:** 171–173.

Takahashi K, Wesselingh SL, Griffin DE, McArthur JC, Johnson RT, Glass JD (1966). Localization of HIV-1 in human brain using polymerase chain reaction/in situ hybridization and immunocytochemistry. *Ann Neurol* **39:** 705–711.

Talley A, Dewhurst S, Perry S, Dollard S, Gummuluru S, Fine S, New D, Epstein L, Gendelman H, Gelbard H (1995). Tumor Necrosis Factor Alpha-Induced Apoptosis in Human Neuronal Cells: Protection by the Antioxidant N-Acetylcysteine and the Genes bcl-2 and crm A. *Mol Cell Biol* **15:** 2359–2366.

Vogel J, Hinrichs SH, Reynolds RK, Luciw PA, Jay G (1988). The HIV *tat* gene induces dermal lesions resembling Kaposi's sarcoma in transgenic mice. *Nature* **335:** 606–611.

Walkins BA, Dorn HH, Kelly WB, Armstrong RC, Potts BJ, Michael F, Kufta CV, Dubois-Dalcq M (1990). Specific tropism of HIV-1 for microglial cells in primary human brain cultures. *Science* **249:** 549–553.

Westendorp MO, Frank R, Ochsenbauer C, Stricker K. Dhein J, Walczak H, Debatin K-M, Krammer PH (1995a). Sentitization of T cells to CD95-mediated apoptosis by HIV-1 Tat and gp120. *Nature* **375:** 497–500.

Westendorp MO, Shatrov VA, Schulze-Osthoff K, Frank R, Kraft M, Los M, Krammer PH, Droge W, Lehmann V (1995b). HIV-1 Tat potentiates TNF-induced NF-kB activation and cytotoxicity by altering the cellular redox state. *EMBO J* **14:** 546–554.

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 USA* **83:** 7089–7093.

Wiley CA, Masliah E, Morey M, Lemere C, DeTeresa R, Grafe M, Hansen L, Terry R (1991). Neocortical damage during HIV infection. *Ann Neurol* **29:** 651–657.