Journal of NeuroVirology (2000) 6, 221–228

www.jneurovirol.com

Short Communication

Detection of HIV-1 Tat and JCV capsid protein, VP1, in AIDS brain with progressive multifocal leukoencephalopathy

Luis Del Valle[1], Sidney Croul[1,2], Susan Morgello[3], Shohreh Amini[1], Jay Rappaport[1] and Kamel Khalili*[,1]

[1]Center for Neurovirology and Cancer Biology, College of Science and Technology, Temple University, Philadelphia, PA 19122, USA; [2]Department of Pathology and Laboratory Medicine, MCP Hahnemann University School of Medicine, Philadelphia, PA 19102, USA and [3]Department of Pathology, Mount Sinai Medical Center, New York, NY 10029, USA

HIV-1 infection can lead to severe central nervous system (CNS) clinical syndromes in more than 50% of HIV-1 positive individuals. Progressive multifocal leukoencephalopathy (PML) is the frequent opportunistic infection of the CNS which is seen in as high as 5% of AIDS patients. Results from previous cell culture studies showed that the HIV-1 regulatory protein, Tat can potentiate transcription of the human neurotropic virus, JCV, the causative agent for PML in cells derived from the human CNS. In this communication we examine the presence of the HIV-1 regulatory protein, Tat, as well as the HIV-1 and JCV structural proteins, p24 and VP1, respectively in AIDS/PML clinical samples. We demonstrate high level expression of the JCV capsid protein, VP1, in oligodendrocytes and to some degree in astrocytes of AIDS with PML. In HIV-1+ samples expression of HIV-1 core protein, p24 was detected in perivascular monocytic cells and to a lesser extent in astrocytes and endothelial cells. A lack of p24 expression in oligodendrocytes suggested no infection of these cells with HIV-1. Interestingly, HIV-1 Tat was detected in various infected cells as well as in uninfected oligodendrocytes from HIV-1+ tissue, supporting the earlier *in vitro* findings that secreted Tat from the infected cells can be localized in the neighboring uninfected cells. The presence of Tat in oligodendrocytes was particularly interesting as this protein can up-modulate JCV gene transcription and several key cell cycle regulatory proteins including cyclin E, Cdk2, and pRb. The data presented here provide *in vivo* evidence for a role of HIV-1 Tat in the pathogenesis of AIDS/PML by acting as a positive regulatory protein that affects the expression of JCV and other cell regulatory proteins in the CNS. *Journal of NeuroVirology* (2000) **6,** 221–228

Keywords: PML; AIDS; JC virus; central nervous system; immunohistochemistry

HIV-1 infection of the central nervous system (CNS) induces a variety of clinical manifestations (Price *et al*, 1991). Progressive Multifocal Leukoencephalopathy (PML) is among the most common HIV-1 associated CNS complications (Berger and Concha 1995). PML is a subacute CNS demyelinating disease that results from cytolytic infection of oligodendrocytes with the human neurotropic polyomavirus, JCV (Walker, 1985). Although PML is recognized to occur in patients with a variety of immunodeficient conditions due to altered T-cell surveillance, the most frequent immunodeficiency which results in reactivation of JCV is the Acquired Immunodeficiency Syndrome (AIDS). PML is found in 4–8% of AIDS patients and is now considered an AIDS defining illness (Berger and Concha 1995; Berger *et al*, 1987). The relatively high rate of PML in AIDS patients in comparison with patients suffering from other severe forms of T-cell suppressive illnesses suggests that HIV-1 infection of the CNS may contribute to the increased frequency of PML. Previously, a direct role for HIV-1 in JCV reactivation was investigated by *in vitro* cell culture system and the results showed that the HIV-1

*Correspondence: K Khalili, Center for Neurovirology and Cancer Biology, College of Science and Technology, Temple University, 1900 North 12th Street, 015-96, Philadelphia, PA 19122, USA
Accepted 24 March 2000

Table 1 Clinical data and histopathological findings

Case	*Age and gender*	*Diagnosis*	*Associated diseases*	*Demyelination*	*Astrocytosis*	*Inclusions*	*Inflammation*
1	44 years, Male	PML (HIV+)	Tuberculosis, lung adenocarcinoma	++++	++++	++++	+++
2	6 years, Male	PML (HIV+)	HIV+, pneumonia	+	++	++	++
3	48 years, Male	PML (HIV+)	HIV+, pneumonia	++++	++++	++++	+++
4	33 years, Male	PML (HIV+)	Common variable immunodeficiency pseudomonas, pneumonia	++++	++++	+++	++
5	44 years, Female	No pathology	HIV+, B cell lymphoma, cirrhosis	N/F	N/F	N/F	N/F
6	41 years, Male	Cerebral infarctions	None	N/F	++++	N/F	++
7	41 years, Female	Creutzfeld Jacob Disease	None	N/F	++++	N/F	N/F
8	56 years, Male	No pathology	Cardiac transplant, renal failure	N/F	N/F	N/F	N/F
9	47 years, Male	No pathology	Hodgkin's disease, endocarditis	N/F	N/F	N/F	N/F

N/F, not found.

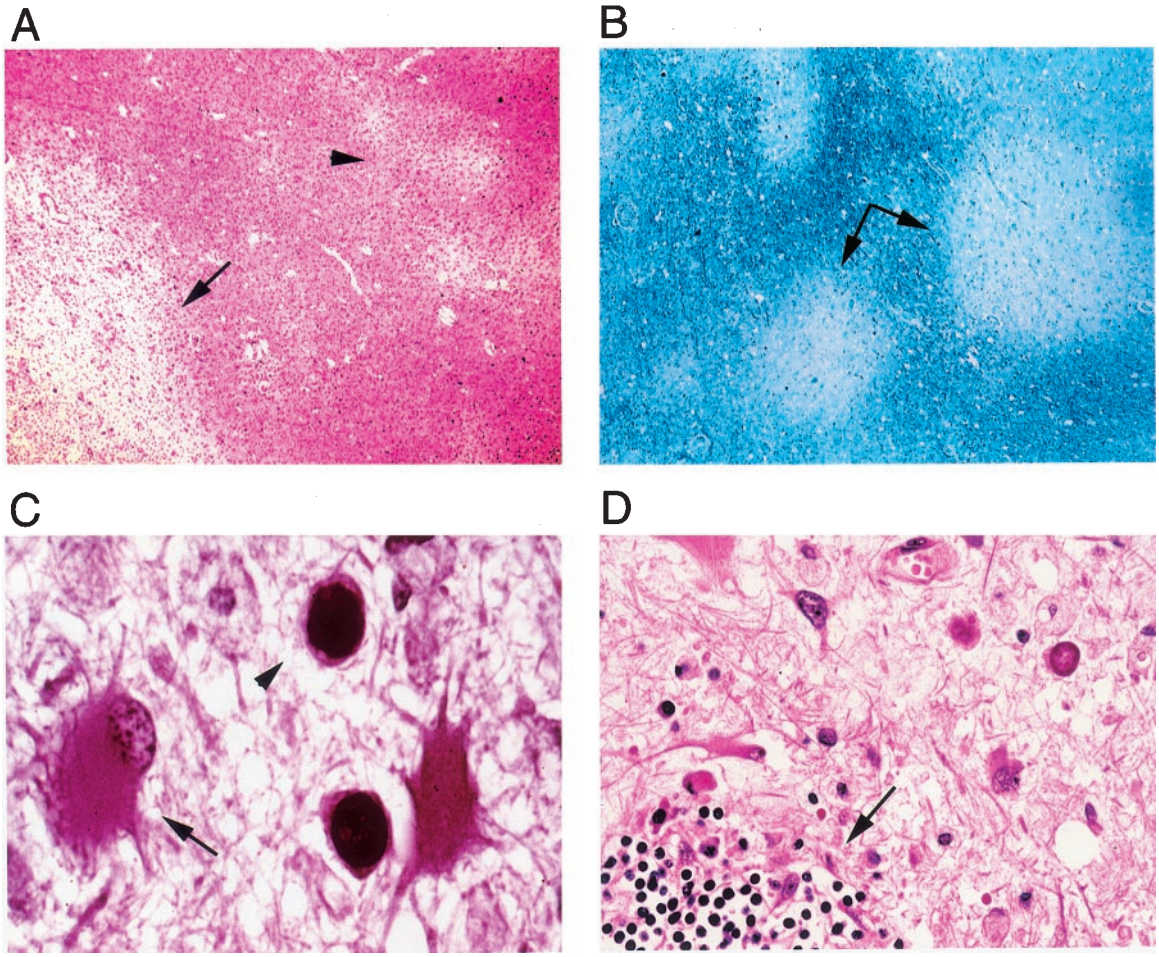

Figure 1 Histological evaluation of AIDS/PML. (**A**,**B**) display demyelinated lesions from AIDS/PML samples as examined by H & E (**A**) and Luxol Fast Blue (**B**). The areas of demyelination are shown by arrows and an arrowhead. (**C**) Detection of oligodendrocytes with glassy intranuclear inclusion (arrowhead) and atypical astrocytes with eccentric eosinophilic cytoplasm and speckled, notched nuclei (arrow). (**D**) Perivascular inflammatory cells (arrow). Original magnifications, **A** and **B** 20×, **C** 100×, and **D** 40×.

regulatory protein, Tat, stimulates transcription of the JCV promoter in glial cells (Chowdhury *et al*, 1990; 1992; 1993; Tada *et al*, 1990).

Tat is a 14 kDa protein which is produced at the early stage of HIV-1 infection and potentiates transcription and replication of the HIV-1 genome (Liu *et al*, 1999). To exert its activity, Tat localizes itself in close proximity to the transcription start site by binding to the TAR RNA sequence positioned in the leader of HIV-1 transcripts and/or interacting with various cellular regulatory proteins (Hottiger and Nabel, 1998; Xiao *et al*, 1998). Interestingly, a region similar to HIV-1 TAR has been detected in the JCV genome which confers Tat responsiveness to the transcription of JCV in glial cells (Chowdhury *et al*, 1992). Furthermore, expression of Tat in glial cells, in the absence of HIV-1, stimulated transcription of the JCV late genome which is responsible for the viral capsid proteins including VP1 (Tada *et al*, 1990). These observations have provided a molecular model for communication of HIV-1 and JCV via the Tat transcription factor. However, the caveat of this model stemmed from studies ruling out HIV-1 infection of oligodendrocytes, the cell type which is infected by JCV. On the other hand, other studies, showed that HIV-1 Tat can be secreted from the infected cells and be taken up by the neighboring uninfected cells (Frankel and Pabo, 1988) supporting a model in which secretion of Tat from the HIV-1 infected brain cells such as microglia and astrocytes can be localized in non-infected cells such as oligodendrocytes. Functionally, results from several cell culture studies showed that soluble Tat is active after uptake and can induce transcription of Tat responsive genes in the cells (Nath *et al*, 1999; Sawaya *et al*, 1998). In this study, we have examined the presence of Tat and JCV late gene product in brain tissue from AIDS patients with PML and demonstrated accumulation of Tat in oligodendrocytes infected with JCV. Further, we demonstrated expression of several Tat-responsive cell cycle regulatory proteins in various CNS cells.

The most common histological features of PML include extensive demyelinated plaques, the presence of atypical astrocytes within these plaques and eosinophilic intranuclear inclusions in oligodendrocytes. Other features such as microglial nodules and perivascular lymphocytic cuffing are less frequently observed in PML. Table 1 summarizes the clinical data and presents the pathologic features of the tissues which were used in this

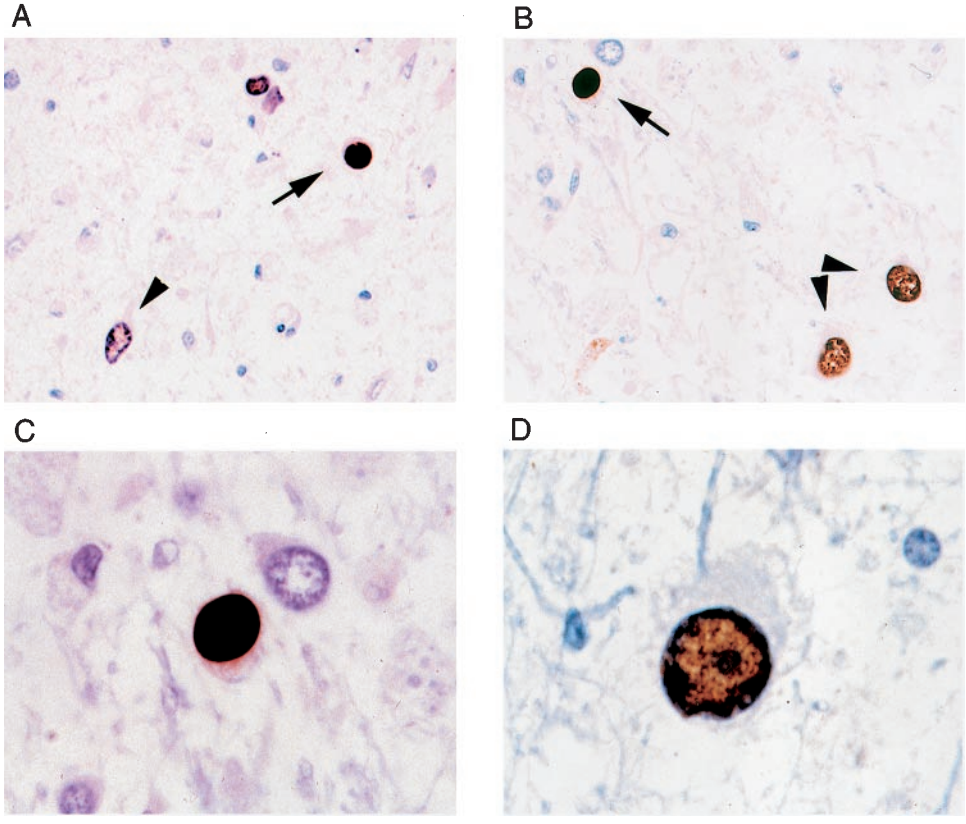

Figure 2 Immunohistochemical evaluation of AIDS/PML with anti-JCV VP1 antibody. (**A**, **B**, **C**) Detection of VP1 positive stained oligodendrocytes within and adjacent to demyelinated lesions (arrows). (**A**, **B**, **D**) Low, but detectable, levels of staining was observed in smaller numbers of astrocytes (arrowheads). Original magnifications, **A** and **B** 40×, **C** and **D** 100×.

study. In general, all four cases displayed plaques of demyelination with atypical reactive astrocytes typified by eccentric eosinophilic cytoplasm. Figure 1 illustrates representative data depicting demyelinated plaques (Figure 1A,B), reactive astrocytes and oligodendrocytes with viral inclusions within nuclei (Figure 1C). Also, in accord with previous observations (Aksamit *et al*, 1990), perivascular infiltration of inflammatory cells was observed in HIV-1 positive PML (Figure 1D).

The presence of JCV in the lesions was determined by immunohistochemistry using an antibody which recognized the JCV late protein, VP1. In all cases with PML, anti-VP1 antibody strongly stained oligodendrocytes, while a weak staining was also detected in astrocytes. Figure 2 illustrates nuclear staining of several oligodendrocytes and astrocytes from AIDS/PML and non-AIDS/PML samples. For detection of HIV-1 infection, tissues were reacted with anti-p24 antibody which recognizes the 24 kDa HIV-1 core protein. As shown in Figure 3A, HIV-1 core protein was detected in the cytoplasm of perivascular monocytes indicative of HIV-1 replication in these cells. In addition, p24 was also detected, albeit to a lesser degree, in the cytoplasm of reactive astrocytes (Figure 3B), endothelial cells (Figure 3C), but not in oligodendrocytes (Figure 3D). These observations corroborate with the previous findings pointing to the productive replication of HIV-1 in monocytic cells, weak replication in astrocytes and endothelial cells and virtually no infection in oligodendrocytes.

In a different set of experiments, we evaluated the presence of the HIV-1 regulatory protein, Tat. Surprisingly, we observed immunolocalization of Tat in the nuclei of several oligodendrocytes with JC virus inclusion (Figure 4A,B). This is an important observation as the oligodendrocytes showed no signals corresponding to HIV-1 core protein, p24 suggesting that oligodendrocytes may have taken up the Tat protein which is released by the infected cells in brains of AIDS patients.

Previous cell culture studies reported de-regulation of several cellular proteins including those which are important for cell cycle events by HIV-1 in glial cells (Kundu *et al*, 1997). As such, we sought to examine the level of expression of cyclin E, cyclin-dependent kinase 2 (Cdk2) and the tumor suppressor protein, pRb, in the clinical samples. As shown in Figure 5, the oligodendrocytes from HIV-1/PML expressed cyclin E (Figure 5A), Cdk2 (Figure 5B), and pRb (Figure 5C). The cells from non-

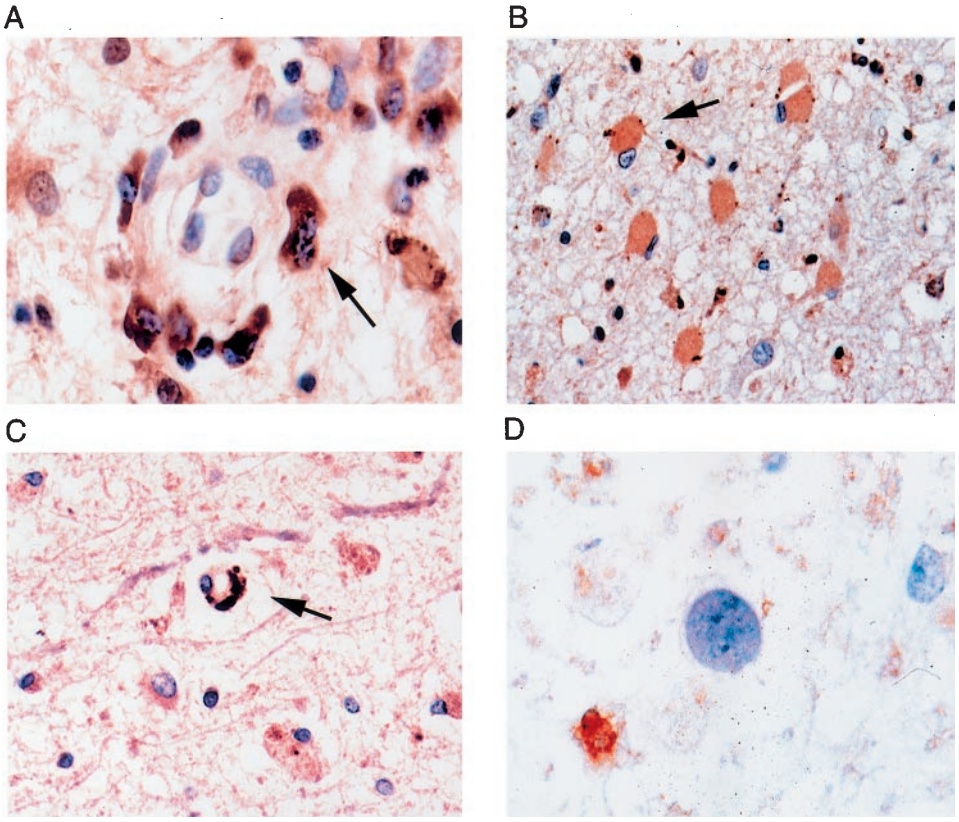

Figure 3 Immunohistochemical staining of AIDS/PML samples with anti-HIV-1 p24 antibody. (**A**) Detection of p24 in perivascular monocytes (arrow). (**B**) Cytoplasmic staining of atypical reactive astrocytes (arrow). (**C**) Occasional detection of p24 positive endothelial cells (arrow). (**D**) Negative staining of oligodendrocytes with p24 antibody. Original magnifications, **A** and **D** 100×, **B** and **C** 40×.

infected tissue showed no sign for expression of these proteins. Also, astrocytes from the AIDS/PML showed strong positive signals for those regulatory proteins (Figure 5D–F). These observations altogether demonstrate the presence of HIV-1 Tat in glial cells of HIV-1 infected brain and de-regulated expression of the cellular proteins which are involved in the control of glial cell function. Table 2 summarizes the results from the immunohistochemical evaluations of the clinical samples used in this study.

In summary, in this study we present data which, along with the previous *in vitro* findings, support a model in which HIV-1 Tat, in the absence of infection of oligodendrocytes may participate in the reactivation of JCV and development of PML. Our results show that in PML lesions, HIV-1 core protein, p24, is present predominantly in perivascular monocytes and to a lesser degree in astrocytes. On the other hand, the JCV capsid protein, VP1, was detected mainly in oligodendrocytes and secondarily in astrocytes. Tat immunostaining of oligodendrocytes is of particular interest suggesting the proposition that Tat is taken up by these cells. Thus, based on our previous *in vitro* observations and the data presented here, it is likely that the presence of Tat in oligodendrocytes results in increased transcription of the JCV late gene which is responsible for VP1 production in these cells. It is important to note that the brain tissue from case 5 (shown in Table 1), an HIV-1 positive patient with no neurologic disease showed no immunoreactivity for HIV-1 and JCV related proteins indicating that their expression is closely related to the pathologic process. Two immunosuppressed cases, 8 and 9, showed no staining for JCV related proteins further supporting the notion that their expression is disease related.

Tat can also affect expression of several cellular genes such as the regulators of cell growth and differentiation. The previous data from cell culture studies revealed that Tat may alter astrocytic cell growth *in vitro* by altering expression of cyclin E and its partner Cdk2, and their downstream target, pRb. Our data show expression of cyclin E, Cdk2, and pRb in oligodendrocytes and astrocytes of all PML cases, i.e. HIV-1 positive and HIV-1 negative. This result is not surprising since the JCV regulatory protein, T-antigen, by itself can alter the expression and activity of these proteins (Krynska *et al*, 1997). Interestingly, we observed an increase in the expression of cyclin E, Cdk2, and pRb in monocytes

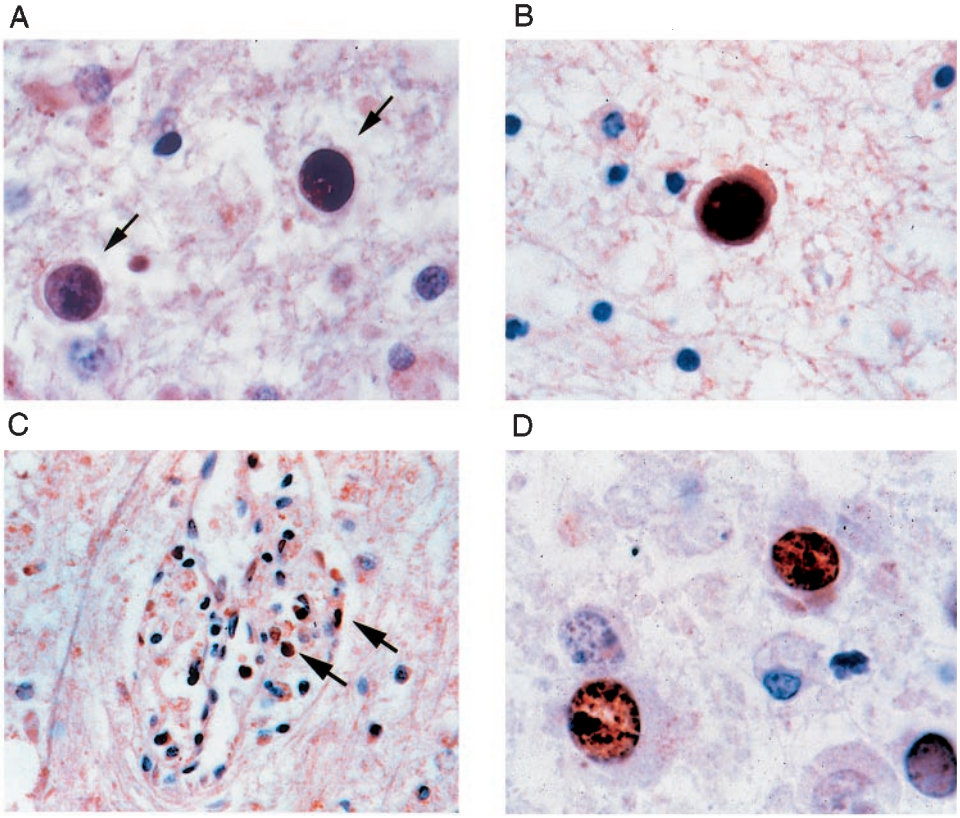

Figure 4 Detection of Tat in AIDS/PML samples. (**A**, **B**) Immunoreactivity of oligodendrocyte nuclei with anti-Tat antibody. (**C**) Detection of Tat in perivascular monocytic cells. (**D**) Weak staining of astrocytes with Tat antibody. Original magnifications, **A**, **B** and **D** 100×, **C** 40×.

and microglia of HIV-1 positive PML cases (data not shown) suggesting that these alterations are HIV-1 related and may be induced by Tat protein.

Taken together, these observations, which are consistent with previous findings (Bonwetsch *et al*, 1999), provide *in vivo* evidence for the presence of HIV-1 Tat in various CNS cells including those which are HIV-1 negative and provide supporting *in vivo* evidence for the earlier cell culture studies pointing to the role of Tat in the pathogenesis of PML in AIDS patients.

Materials and methods

Tissue samples

Three autopsy samples of HIV-1 positive PML patients and one autopsy of HIV-1 negative PML patient were obtained from the Manhattan HIV Brain Bank (R24 MH59724) at Mount Sinai Medical Center (New York, NY, USA) and utilized in these studies. All samples were formalin-fixed and paraffin-embedded. Formalin-fixed and paraffin-embedded control brain tissue was obtained from the Hahnemann University Hospital archives which includes autopsy frontal cortex and white matter from a patient with HIV-1 with no CNS pathology, two samples of normal cortex and white matter from patients without history or pathologic evidence of HIV-1 and two samples of cortex and white matter from patients with CNS lesions in the absence of HIV-1. Table 1 illustrates clinical data for the patient samples.

Histologic and immunohistochemical analysis

Four micron thick sections were stained with hematoxylin and eosin to confirm tissue diagnoses. Immunohistochemistry was performed by deparaffinizing four micron sections in xylene, followed by rehydration and nonenzymatic antigen retrieval in 0.01 M sodium citrate buffer (pH 6.0) at 95°C for 40 min. After cooling to room temperature (approximately 20 min), slides were rinsed and endogenous peroxide was quenched by incubation in MeOH/3% H_2O_2 for 20 min. This was followed by a wash in phosphate buffer saline (PBS) and blocking in PBS plus 1% BSA and 5% normal horse serum for 2 h at room temperature. Slides were then incubated overnight with primary antibodies. To detect JC virus VP1 protein, a mouse monoclonal antibody to recombinant JCV VP1 (a generous gift from Dr Eugene O Major, NINDS/NIH, Bethesda, MD, USA) was used at 1 : 1000 dilution. HIV-1 p24 core protein was detected with a rabbit polyclonal antibody against baculovirus derived with IIIB p24 at a 1 : 1000 dilution (Intracel Corp., Rockville, MD, USA). Tat was detected with rabbit polyclonal antiserum HIV-1_{BH10} generated against recombinant HIV-1 Tat at 1 : 2000 dilution (a generous gift from Dr Avindra Nath, Depart-

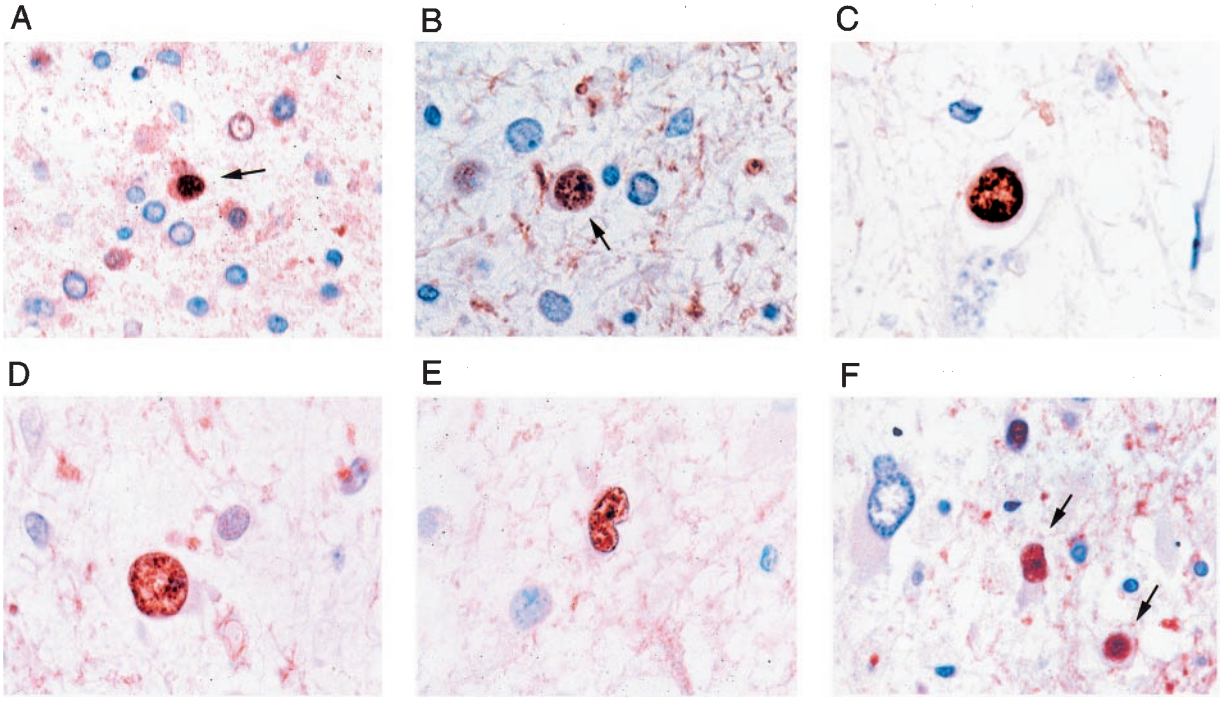

Figure 5 Detection of cell cycle regulators in glial cells of AIDS/PML samples. (**A**, **B**, **C**) The nuclei of oligodendrocytes in the demyelinated lesions showed positive staining to cyclin E (**A**), Cdk2 (**B**), and pRb (**C**). (**D**, **E**, **F**) Depict positive nuclear staining of astrocytes with cyclin E, cdk2, and pRb (**D**–**F**, respectively). Original magnifications, all panels 100×.

Table 2 Immunohistochemical findings

Case	*VP-1*	*HIV p24*	*Tat*	*Cyclin E*	*Cdk2*	*pRb*
1	++++ Oligo ++Astro	+ Astro +++ Mono ++ Endo	+++ Oligo + Astro ++ Mono	++ Oligo + Astro	++ Oligo	++ Oligo ++ Astro + Mono
2	++++ Oligo ++ Astro	++ Astro ++ Mono	+++ Oligo +++ Micro	+ Oligo + Micro	++ Oligo + Astro	+++ Oligo + Astro + Micro
3	++++ Oligo ++ Astro	++ Astro +++ Mono +++ Endo	++++ Oligo ++ Astro + Micro	+++ Oligo ++ Astro	++ Oligo + Mono	+++ Oligo
4	++++ OLigo ++ Astro	None	None	+ Oligo	++ Oligo + Astro	+++ Oligo +++ Astro
5	None	+ Endo	None	None	None	None
6	None	+ Endo	None	None	None	None
7	None	+ Endo	None	None	None	None
8	None	+ Endo	None	None	None	None
9	None	+ Endo	None	None	None	None

Abbreviations: Oligo, oligodendrocyte; Astro, astrocyte; Mono, monocyte; Micro, microglia; Endo, endothelium; N/D, not determined.

ment of Neurology, University of Kentucky College of Medicine, Lexington, KY, USA). Cyclin E was detected with a rabbit polyclonal antibody, C-19 at 1 : 1000 dilution, Cdk2 was detected with a rabbit polyclonal antibody, SC-163 1 : 500 dilution, and pRb was detected with a mouse monoclonal antibody to the human protein, 14001A 1 : 100 dilution (Pharmigen). Positive controls for Cyclin E, Cdk2, and pRb included human medulloblastoma specimens. Negative controls consisted of tissues incubated in buffer for monoclonal antibodies or normal rabbit serum for polyclonal antibodies without primary antibody. Primary antibodies were detected with biotinylated anti-mouse or anti-rabbit secondary antibodies, avidin biotin peroxidase complex and diaminobenzidine chromagen according to manufacturer's instructions (Vectastain Elite ABC Peroxidase Kit, Vector Laboratories, Burlingame, CA, USA). Following a light counterstain with hematoxylin, sections were dehydrated and coverslipped with Permount.

Evaluation of stained slides

Each slide was examined independently by two microscopists for the characteristics of the lesions found with H & E staining. In addition, they estimated the presence of immunohistochemical stain product with the various antibodies and the percentage of positive cells. Percentages were converted into a four level scale where +=1–25%, ++=26–50%, +++=51–75%, and ++++=76–100% positive nuclei immunohistochemically. For the antibodies used in this study only nuclear localization of staining was accepted as positive. All differences between the two examiners were resolved by examining the tissues together to achieve consensus.

Acknowledgments

We wish to thank Dr Eugene O Major and Avindra Nath for their generous gifts, and past and present members of the Center for Neurovirology and Cancer Biology for their insightful discussion and sharing of ideas. The HIV-1 and PML tissues were obtained from the Manhattan HIV Brain Bank (R24 MH59724) at Mount Sinai Medical Center, New York, NY, USA. We would also like to thank Cynthia Schriver for editorial assistance and preparation of the manuscript. This work was made possible by grants awarded by the NIH to J Rappaport, S Amini and K Khalili.

References

Aksamit AJ, Gendelman HE, Orenstein JM, Pezeshkpour GH (1990). AIDS-associated progressive multifocal leukoencephalopathy (PML): comparison to non-AIDS PML with in situ hybridization and immunohistochemistry. *Neurology* **40:** 1073–1078.

Berger JR, Concha M (1995). Progressive multifocal leukoencephalopathy: the evolution of a disease once considered rare. *J NeuroVirology* **1:** 5–18.

Berger JR, Kaszovitz D, Post JD, Dickinson G (1987). Progressive multifocal leukoencephalopathy associated with human immunodeficiency virus infection: a review of the literature with a report of sixteen cases. *Ann Intern Med* **107:** 87.

Bonwetsch R, Croul S, Richardson MW, Lorenzana C, Del Valle L, Sverstiuk AE, Amini S, Morgello S, Khalili K, Rappaport J (1999). Role of HIV-1 Tat and CC chemokine MIP-1α in the pathogenesis of HIV associated central nervous system disorders. *J Neurovirology* **5:** 685–694.

Chowdhury M, Taylor JP, Tada H, Rappaport J, Wong-Staal F, Amini S, Khalili K (1990). Regulation of the human neurotropic virus promoter by JCV T-antigen and HIV-1 Tat protein. *Oncogene* **5:** 1737–1742.

Chowdhury M, Taylor JP, Chang C-F, Rappaport J, Khalili K (1992). Evidence that a sequence similar to TAR is important for induction of the JC virus late promoter by human immunodeficiency virus type 1 Tat. *J Virol* **66:** 7355–7361.

Chowdhury M, Kundu M, Khalili K (1993). GC/GA rich sequence confers Tat-responsiveness to human neurotropic virus promoter, JCV_L, in cells derived from CNS. *Oncogene* **8:** 887–892.

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

Hottiger MO, Nabel GJ (1998). Interaction of human immunodeficiency virus type 1 Tat with the transcriptional coactivators p300 and CREB binding protein. *J Virol* **72:** 8252–8256.

Krynska B, Gordon J, Otte J, Franks R, Knobler R, Giordano A, DeLuca A, Khalili K (1997). Role of cell cycle regulators in tumor formation in transgenic mice expressing the human neurotropic virus, JCV, early protein. *J Cell Biochem* **67:** 223–230.

Kundu M, Guermah M, Roeder RG, Amini S, Khalili K (1997). Interaction between cell cycle regulator, E2F-1, and NF-kappa B mediates repression of HIV-1 gene transcription. *J Biol Chem* **272:** 29468–29474.

Liu Y, Sune C, Garcia-Blanco MA (1999). Human immunodeficiency virus type 1 Tat-dependent activation of an arrested RNA polymerase II elongation complex. *Virology* **255:** 337–346.

Nash A, Conant K, Chen P, Scott C, Major EO (1999). Transient exposure to HIV-1 Tat protein results in cytokine production in macrophages and astrocytes. A hit and run phenomenon. *J Biol Chem* **274:** 17098–17102.

Price RW, Sidtis JJ, Brew BJ (1991). AIDS dementia complex and HIV-1 infection: a view from the clinic. *Brain Pathol* **1:** 155–162.

Sawaya BE, Thatikunta P, Denisova L, Brady J, Khalili K, Amini S (1998). Regulation of TNFα and TGFβ-1 gene transcription by HIV-1 Tat in CNS cells. *J Neuroimmunol* **87:** 33–42.

Tada H, Rappaport J, Amini S, Lashgari M, Wong-Stahl F, Khalili K (1990). Cell type transactivation of human neurotropic virus JCV promoter by HIV-TAT protein. *Proc Natl Acad Sci USA* **87:** 3479–3483.

Walker DL (1985). Progressive multifocal leukoencephalopathy. In: *Handbook of Clinical Neurology*, **vol. 47,** PJ Vinken *et al*, (eds). Elsevier Science Publishing, Inc., New York, pp 503–524.

Xiao H, Tao Y, Greenblatt J, Roeder RG (1998). A cofactor, TIP30, specifically enhances HIV-1 Tat-activated transcription. *Proc Natl Acad Sci USA* **95:** 2146–2151.