Proc. Natl. Acad. Sci. USA
Vol. 93, pp. 6073–6078, June 1996
Medical Sciences

Human immunodeficiency virus *tat* gene transfer to the murine central nervous system using a replication-defective herpes simplex virus vector stimulates transforming growth factor beta 1 gene expression

(AIDS/central nervous system disease/cytokines/Tat)

SIYAMAK RASTY*, PRAKASH THATIKUNTA†, JENNIFER GORDON†, KAMEL KHALILI†, SHOHREH AMINI†‡, AND JOSEPH C. GLORIOSO*§

*Department of Molecular Genetics and Biochemistry, University of Pittsburgh School of Medicine, Pittsburgh, PA 15261; and †Molecular Neurovirology Section of Jefferson Institute of Molecular Medicine, Department of Biochemistry and Molecular Biology, Thomas Jefferson University, Philadelphia, PA 19107

Communicated by Darwin J. Prockop, Jefferson Medical College, Philadelphia, PA, January 18, 1996 (received for review October 24, 1995)

ABSTRACT **The high incidence of neurological disorders in patients afflicted with acquired immunodeficiency syndrome (AIDS) may result from human immunodeficiency virus type 1 (HIV-1) induction of chemotactic signals and cytokines within the brain by virus-encoded gene products. Transforming growth factor β1 (TGF-β1) is an immunomodulator and potent chemotactic molecule present at elevated levels in HIV-1-infected patients, and its expression may thus be induced by viral trans-activating proteins such as Tat. In this report, a replication-defective herpes simplex virus (HSV)-1 *tat* gene transfer vector, dSTat, was used to transiently express HIV-1 Tat in glial cells in culture and following intracerebral inoculation in mouse brain in order to directly determine whether Tat can increase TGF-β1 mRNA expression. dSTat infection of Vero cells transiently transfected by a panel of HIV-1 long terminal repeat deletion mutants linked to the bacterial chloramphenicol acetyltransferase reporter gene demonstrated that vector-expressed Tat activated the long terminal repeat in a trans-activation response element-dependent fashion independent of the HSV-mediated induction of the HIV-1 enhancer, or NF-κB domain. Northern blot analysis of human astrocytic glial U87-MG cells transfected by dSTat vector DNA resulted in a substantial increase in steady-state levels of TGF-β1 mRNA. Furthermore, intracerebral inoculation of dSTat followed by Northern blot analysis of whole mouse brain RNA revealed an increase in levels of TGF-β1 mRNA similar to that observed in cultured glial cells transfected by dSTat DNA. These results provided direct *in vivo* evidence for the involvement of HIV-1 Tat in activation of TGF-β1 gene expression in brain. Tat-mediated stimulation of TGF-β1 expression suggests a novel pathway by which HIV-1 may alter the expression of cytokines in the central nervous system, potentially contributing to the development of AIDS-associated neurological disease.**

Acquired immunodeficiency syndrome (AIDS)-related neurodegenerative disorders occur in approximately one-half of children and one-third of adults afflicted with the disease (1). Deterioration of brain tissue, eventually leading to dysfunction of cognition, dementia, or even paralysis occurs in the absence of infection of neuronal tissue by the human immunodeficiency virus type 1 (HIV-1), the etiologic agent of AIDS, and in the absence of other malignancies or opportunistic infections (1, 2). Such discrepency between the severity of neurodegeneration and the lack of detectable HIV-1 within neurons suggests that virus- or host-derived diffusable factors may induce a pathologic process by altering the normal pattern of expression of cellular genes.

The HIV-1 trans-activating protein Tat, a potent activator of the viral long terminal repeat (LTR; refs. 3–5), influences the expression of a number of cellular genes. Tat also stimulates the growth of cell lines derived from Kaposi sarcoma lesions *in vitro* (6), and transgenic mice expressing Tat develop dermal lesions similar to Kaposi sarcoma (7). Tat released from HIV-1-infected cells can enter uninfected neighboring cells in which it can activate expression of cellular genes (8–11). In this manner, Tat may play a role in stimulating expression of tumor necrosis factor β (12), and through cytokine induction, it may inhibit antigen-induced proliferation of lymphocytes (13). In glioblastoma cells, Tat can also activate the expression of a number of extracellular matrix proteins (14) and stimulate the expression of heterologous genes linked to the transforming growth factor β1 (TGF-β1) promoter (11).

In this study, a replication-defective herpes simplex virus (HSV)-1 Tat expression vector, dSTat, was engineered for *tat* gene transfer to astroglial cells in culture and to mouse brain by intracranial inoculation and evaluated for Tat-mediated activation of TGF-β1 gene expression both *in vitro* and *in vivo*. Tat expression by dSTat was demonstrated by immunofluorescence assays of cultured human astrocytic glial U87-MG cells infected by the vector. Using a series of LTR-chloramphenicol acetyltransferase (CAT) deletion constructs in transfection–infection assays, it was demonstrated that vector-expressed Tat trans-activated the HIV-1 LTR in a trans-activation response element (TAR)-dependent manner distinct from the HSV-mediated stimulation of the HIV-1 LTR through the NF-κB enhancer domain. Following transfection of dSTat viral DNA into astrocytic glial U87-MG cells, TGF-β1 mRNA production was shown to be substantially elevated relative to transfection of control HSV-1 vector DNA lacking the *tat* expression cassette. This induction of TGF-β1 gene expression was also evident *in vivo* upon microinjection of dSTat into mouse brain. Tat-mediated activation of TGF-β1 expression in the central

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: CNS, central nervous system; HSV, herpes simplex virus; TGF-β1, transforming growth factor β1; LTR, long terminal repeat; CAT, chloramphenicol acetyltransferase; SV40, simian virus 40; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; IL, interleukin; TAR, trans-activation response element.

‡To whom reprint requests should be addressed at: Molecular Neurovirology Section of Jefferson Institute of Molecular Medicine, Department of Biochemistry and Molecular Biology, Thomas Jefferson University, 233 South 10th Street, Room 419, Philadelphia, PA 19107. e-mail: amini1@jeflin.tju.edu.

§To whom reprint requests should be addressed at: Department of Molecular Genetics and Biochemistry, University of Pittsburgh School of Medicine, E1240 Biomedical Science Tower, Pittsburgh, PA 15261. e-mail: michele@hoffman.mgen.pitt.edu.

nervous system (CNS), and possibly that of other cytokines and immunomodulatory molecules, may contribute to neuronal damage in AIDS patients.

MATERIALS AND METHODS

Plasmids. pUCX1 is a pUC19-based plasmid containing the 3.5-kb HSV-1 *Bam*HI P fragment that contains the thymidine kinase (*tk*) gene. pJG100 (15) is a derivative of pUCX1, which contains the 34-bp *lox*P recombination site of bacteriophage P1 inserted into the *tk* coding sequence of pUCX1. The *lox*P site in pJG100 is present on a 364-bp *Nae*I–*Sna*BI DNA fragment from pBS64 (16), which was cloned into the *Sna*BI site within the *tk* coding sequence of pUCX1, thus inactivating the *tk* gene. pSVTat contains the simian virus 40 (SV40) early promoter–enhancer cloned upstream of the HIV-1 SF_2 *tat* cDNA (7), followed by the SV40 early poly(A) site. pSVTatlox (see Fig. 1) contains the SV40 promoter-*tat*-poly(A) cassette of pSVTat subcloned into the *Bam*HI site of pJG116 (17), a *lox*P vector carrying the *E. coli lacZ* gene driven by the human cytomegalovirus immediate-early promoter–enhancer. pCD23, pCD23ΔTAR, pCD52, and pCD52ΔTAR have been described previously (18). pAct-Tat contains the *tat* gene of HIV-1 under the control of human β-actin promoter (11, 18). pNL4–3 is a previously described HIV-1 proviral DNA construct (19).

Cells. Vero cells were obtained from American Type Culture Collection (ATCC). E5 is a Vero-derived cell line stably transfected with the HSV-1 *ICP4* gene driven by its own promoter (20) and was obtained from Neal DeLuca, University of Pittsburgh. Vero and E5 cells were maintained in minimal essential medium (MEM; GIBCO) supplemented with 10% (vol/vol) fetal bovine serum. U87-MG, a cell line derived from human glioblastoma, was obtained from ATCC, and was maintained in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum. The cell line 5-10 is a U87-MG-derived cell line stably transfected by the HIV-1 *tat* gene under the control of the SV40 late promoter and has been previously described (14). Primary human fetal glial cell cultures were prepared by careful removal of brain meninges from fresh brain tissue of 10- to 21-week-old abortive human fetuses, followed by preparation of tissue as described (21). Briefly, brain tissue was mechanically dissociated, and the resulting cell suspension was plated at a density of 1×10^6 cells per 60-mm dish and incubated for 4–5 days. The cells were then washed to remove nonadherent cells, and supplied with DMEM/10% fetal bovine serum. After 1 week, the culture medium was removed and replaced with serum-free medium, and cultures were placed on a shaker platform at 200 rpm at 37°C overnight to obtain purified cultures of astrocytes. Cells detached from the culture were removed and discarded, and the adherent astrocytic cells were washed and refed with fresh DMEM/10% fetal bovine serum. To assess the purity of the primary culture, cells were grown on glass chamber slides, fixed with ice-cold methanol, and incubated with anti-GFAP antibody. The samples were then incubated with a fluorescein-conjugated mouse anti-IgG secondary antibody and evaluated for fluorescence. The results indicated that type 1 astrocytes constituted the majority of the primary cell population. For transfection assays, the cells were seeded at 5×10^5 cells per 60-mm dish.

Viruses. d120 is an *ICP4* deletion mutant of HSV-1 strain KOS (22) and was obtained from Neal DeLuca. dTKlox was derived from d120 by marker transfer of a *Bam*HI digest of plasmid pJG100 into d120 viral DNA on E5 cells, followed by selection of tk^- viral progeny in the presence of 100 μg of araT per ml (17). dSTat was constructed by Cre-*lox* recombination of plasmid pSVTatlox into dTKlox viral DNA (17), followed by transfection of E5 cells.

Immunofluorescence. U87-MG or 5-10 cells were seeded on glass coverslips in 12-well tissue culture plates at 1×10^5 cells per well. Sixteen to 24 h later, U87-MG cultures were infected at a multiplicity of infection of 10.0 with either dSTat or dTKlox, or they were mock-infected with growth medium alone. Twelve to 15 h after infection of U87-MG cells, both infected U87-MG cultures and uninfected 5-10 monolayers were rinsed three times with PBS, fixed for 2 min in cold (−20°C) acetone, air-dried, and blocked with 10% goat serum in PBS for 1 h. Coverslips were incubated overnight with a 1:200 dilution of a polyclonal anti-Tat antibody (no. 705, AIDS Research and Reference Reagent Program, Division of AIDS, National Institute of Allergy and Infectious Diseases, National Institutes of Health) at room temperature, followed by 1-h incubations with a 1:1000 dilution of biotin-conjugated anti-rabbit IgG secondary antibody (Amersham) and a 1:1000 dilution of Texas Red-conjugated streptavidin (GIBCO/BRL). Immunoreactivities were visualized by fluorescence microscopy.

Transfections and CAT Assays. Plasmid and viral DNA transfections were carried out by the calcium phosphate precipitation method as described (23). For CAT assays, at 24 h after transfection of plasmids into subconfluent Vero monolayers in 60-mm dishes, the cells were either mock-infected or infected with dTKlox or dSTat at multiplicity of infection of 10.0. Cell lysates were prepared 24 h after infection and were processed for CAT activity as described (24) using equal concentrations of protein from each lysate as determined by a Bio-Rad protein assay kit. Percent conversion was calculated by scanning the thin-layer chromatography plate using a Radioimaging Analytic System (AMBIS).

RNA Extraction and Northern Analysis. Following transfection, total cellular RNA from two 100-mm dishes was prepared by the hot acid-phenol procedure (25). Poly(A)$^+$ RNA was prepared using a poly(A)$^+$ RNA purification kit (Qiagen). For primary fetal glial cell cultures, 48 h following transfection of 5×10^5 cells, total RNA was prepared and analyzed by Northern blot. For detection of expression of TGF-β1 in mouse brain following injection with viral vectors, 3- to 4-month-old male Swiss Webster mice were intracerebrally injected with a 23-gauge needle while under anesthesia with avertin. Animals received 10^6 plaque forming units in 25 μl of PBS alone, PBS with dSTat, or PBS with dTKlox, and animals were killed 48 h after inoculation. Brain tissue was then harvested for preparation of poly(A)$^+$ RNA. Fifteen micrograms of total RNA or 2.0 μg of poly(A)$^+$ RNA was subjected to electrophoresis in denaturing 1.2% formaldehyde/agarose gels, transferred to nylon membranes (Amersham), and hybridized to ^{32}P-dCTP labeled TGF-β1 or glyceraldehyde 3-phosphate dehydrogenase (GAPDH) cDNA probes prepared by random-primed labeling (26) using a Boehringer Mannheim kit. Following autoradiography, RNA levels were quantitated by densitometric scanning of Northern blots, and levels of TGF-β1 RNA were calculated after normalization to GAPDH RNA levels.

RESULTS

Construction of a Replication-Defective HSV-1 Vector Expressing HIV-1 Tat. Using a cell-free Cre-*lox* recombination system (15, 17), we recombined pSVTatlox, an HIV-1 SF_2 *tat* expression plasmid containing a bacteriophage P1 *lox*P recombination sequence, into the genome of dTKlox, a replication-defective HSV-1 vector derived from the *ICP4* deletion mutant d120 (ref. 22; Fig. 1). dTKlox contained a *lox*P site inserted into the thymidine kinase (*tk*) gene of d120, thus making it mutant in both *ICP4* and *tk*, and a potential recipient of a variety of *lox*P-containing shuttle plasmids (17). In addition to the SV40 early promoter–enhancer-driven *tat* expression cassette, pSVTatlox contained a *lacZ* reporter gene under transcriptional control of the human cytomegalovirus immediate-early promoter–enhancer (Fig. 1). Following Cre-mediated recombination of pSVTatlox into dTKlox DNA and transfection of

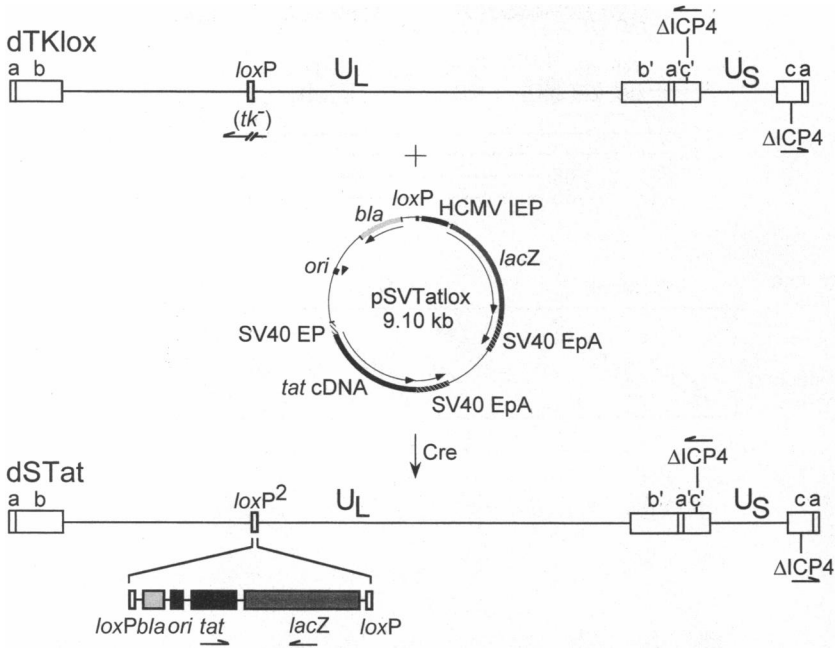

FIG. 1. Schematic diagram of the viral vectors used in this study (not drawn to scale). dTKlox is a tk^- derivative of the ICP4$^-$ HSV-1 mutant, d120 (22), generated by marker transfer of a 364-bp plasmid fragment containing the 34-bp *lox*P recombination site of bacteriophage P1 (15, 17) into the *tk* coding sequence of d120. dSTat was constructed by Cre-*lox*P recombination of plasmid pSVTatlox into dSTat viral DNA, followed by transfection of ICP4-complementing E5 cells (20). U_L and U_S, unique long and short sequences of the HSV-1 genome, respectively, flanked by inverted repeats; *bla*, β-lactamase; *ori*, plasmid origin of replication; IEP, immediate-early promoter; EP, early promoter; and EpA, early poly(A) signal.

ICP4-complementing E5 cells, recombinant tk^- viral progeny, designated dSTat, were identified and plaque-purified based on their blue plaque phenotype following overlay of the E5 cell monolayer with Bluo-gal. The genomic structure of dSTat was confirmed by Southern blot analysis of viral DNA (data not shown). To examine the possibility that a wild-type, ICP4$^+$ recombinant virus may have been generated by passaging stocks of dSTat on E5 cells, or that Tat expressed by the dSTat may allow the viral lytic cycle to proceed under nonpermissive conditions, dSTat infection of noncomplementing Vero cells was examined and compared to infection by dTKlox and the parental d120 virus. No progeny virions were detected by standard HSV plaque assays following infection of Vero cells by any of the three viruses following high multiplicity of infections (data not shown). These results confirmed that the dSTat is totally compromised for replication in noncomplementing cells.

Efficacy of Tat expression by dSTat *in Vitro*. The ability of dSTat to express Tat in cultured U87-MG cells was examined by indirect immunofluorescence using an anti-Tat polyclonal antibody (Fig. 2). Substantial expression of Tat protein was observed in dSTat-infected U87-MG cells (Fig. 2*A*) and in Tat-producing U87-MG derived 5-10 cells (ref. 14; Fig. 2*D*), but not in dTKlox- or mock-infected U87-MG cells (Fig. 2 *B* and *C*, respectively). These results indicated that dSTat is capable of expressing Tat in cultured human astrocytic glial cells at a level comparable to that detected in the same cell line engineered to constitutively express Tat (14).

Expression of Functional Tat by dSTat *in Vitro*. To determine whether Tat expressed from dSTat was functional, a transfection–infection assay was employed using a panel of HIV-1 LTR deletions linked to the bacterial CAT reporter gene (Fig. 3). Twenty-four hours after transient transfection of Vero cells by the LTR-CAT plasmid constructs, monolayers were either mock-infected or infected by the control vector dTKlox or by dSTat, and levels of vector-mediated transactivation of the HIV-1 LTR were measured by CAT activity. Because one or more immediate-early HSV-1 gene products are capable of trans-activating the HIV-1 LTR through interactions with the NF-κB enhancer domain of the LTR (27–34), CAT assays were performed using an LTR deletion construct containing the Tat-responsive TAR sequence but lacking the HSV-stimulated NF-κB domain to determine whether vector-expressed Tat, not an HSV-1 gene product, was responsible for

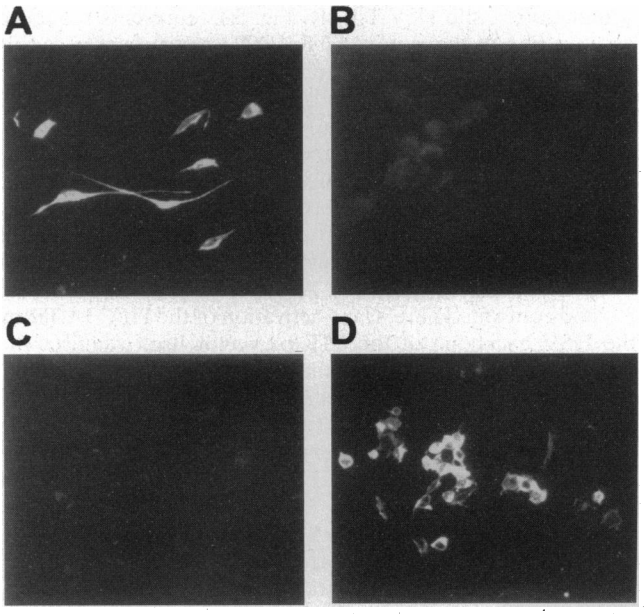

FIG. 2. Indirect immunofluorescence analysis of Tat expression in dSTat-infected glial cells. U87-MG (*A–C*) or Tat$^+$ 5-10 (*D*) cells were plated at low density on glass coverslips, and 16–24 h later, U87-MG monolayers were either mock-infected (*C*), or infected at multiplicity of infection of 10.0 with (*A*) dSTat or (*B*) dTKlox. Twelve to 15 h after infection, cells were prepared for indirect immunofluorescence using an anti-Tat polyclonal antibody, a biotin-conjugated anti-rabbit IgG secondary antibody, and Texas Red-conjugated streptavidin.

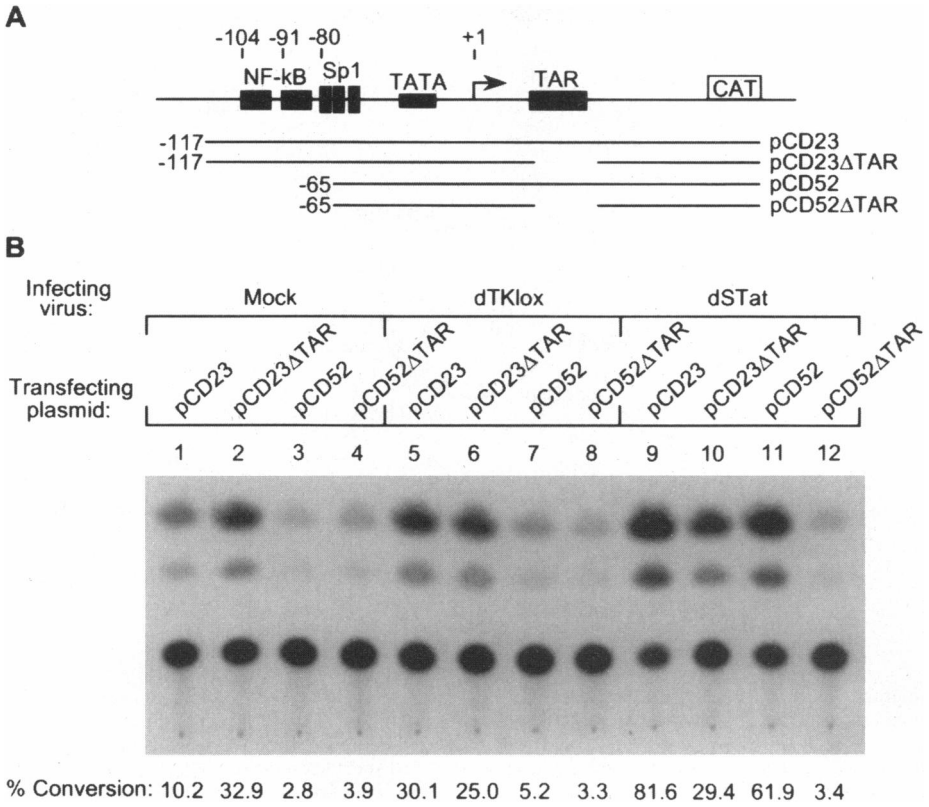

FIG. 3. TAR-dependent trans-activation of the HIV-1 LTR by dSTat. (*A*) Diagram of LTR-CAT deletion constructs showing relevant cis-elements (not drawn to scale). 5′-LTR sequences in pCD23 span from nucleotide −117 to around nucleotide +80 relative to the HIV-1 transcription start site, and contain the NF-κB and TAR elements. pCD23ΔTAR is a TAR-deleted mutant of pCD23 lacking nucleotides +24 to +80; pCD52 is an NF-κB-deleted derivative of pCD23 lacking nucleotides −117 to −65; and pCD52ΔTAR is missing both TAR and NF-κB domains. NF-κB, twice-repeated 11-bp binding site for transcription factor NF-κB; Sp1, GC-rich binding site for transcription factor Sp1; TATA, putative TATA box; and TAR, trans-activation response element. Numbering is relative to the transcription start site, which is designated +1. (*B*) CAT assay analysis of the deletion constructs transiently transfected into Vero cells and infected at multiplicity of infection of 10.0 with the designated viruses at 24 h after transfection. A representative experiment is shown.

stimulation of the HIV-1 LTR. Fig. 3*A* represents sequence organization of the various LTR-CAT constructs used in this experiment. dSTat-mediated trans-activation of the full-length LTR in pCD23-transfected cells resulted in much higher CAT activity than that produced by the control dTKlox infection (Fig. 3*B*, compare lanes 5 and 9). Furthermore, following transfection with either pCD23ΔTAR or pCD52, it became evident that this enhanced level of CAT activity was dependent on the presence of the TAR element and not the NF-κB enhancer in the LTR-CAT construct (Fig. 3*B*, compare lanes 6 and 10, and lanes 7 and 11). As reported previously (28, 30, 33) and confirmed here, trans-activation of the HIV-1 LTR by the HSV backbone of the dTKlox vector itself requires the presence of the NF-κB domain and not the TAR sequence. The presence of the TAR element led to an enhanced trans-activation of the LTR by dSTat and not by dTKlox. These results confirmed that dSTat expresses functional Tat capable of trans-activating the HIV-1 LTR in a TAR-dependent fashion.

Induction of TGF-β1 mRNA Expression in Glial Cells by Tat. HIV-1 Tat, expressed from transient transfection of Tat-producing plasmids or intact dSTat viral DNA, was examined for its effect on the expression of TGF-β1 mRNA in primary human fetal glial cultures or in an established human glioblastoma cell line. Primary human fetal glial cells were transfected with 15 μg of pNL4-3, an HIV-1 proviral DNA construct (Fig. 4*A*, lane 2) or with 15 μg of control salmon sperm DNA (Fig. 4*A*, lane 1). Total RNA prepared from transfected cells was subjected to Northern blot analysis using a 1.0-kb fragment of TGF-β1 cDNA as probe. The same blot was rehybridized to a probe generated from a 1.3-kb fragment of GAPDH cDNA to confirm that each well contained similar amounts of RNA. A greater than 3-fold increase in TGF-β1 mRNA level was detected upon introduction of HIV-1 proviral DNA into these cells. U87-MG human astrocytic glial cells were transfected with 0, 4, 10, and 20 μg of a Tat producer plasmid, pAct-Tat. Forty-eight hours after transfection, total RNA was subjected to Northern blot analysis as described above using TGF-β1 and GAPDH probes (Fig. 4*B*). A dose-dependent pattern of TGF-β1 mRNA expression was observed with increasing levels of Tat expression, showing the effect of Tat expression on the transcription of TGF-β1. Transfection of 25.0 μg of dSTat viral DNA into U87-MG cells also resulted in an ≈4-fold increase in the level of TGF-β1 mRNA compared to that obtained following transfection of control dTKlox viral DNA (Fig. 4*C*). The upregulation of TGF-β1 mRNA by Tat expressed from dSTat thus paralleled that observed using the Tat expression plasmid pAct-Tat and confirmed our previous findings that link Tat expression to enhanced CAT expression from TGF-β1 promoter-CAT constructs transfected into U87-MG cells (11).

Activation of TGF-β1 mRNA synthesis by dSTat *in Vivo*. To determine the effect of Tat on levels of TGF-β1 mRNA expression *in vivo*, dSTat or the control dTKlox vector were injected into the mouse brain. As can be seen in Fig. 5, intracerebral injection of dSTat (lane 3) induced TGF-β1 mRNA levels in the mouse brain by ≈3-fold, compared with injection of the control dTKlox vector (lane 2) or buffer alone (lane 1). Furthermore, it appeared that this increase in steady-state levels of TGF-β1 mRNA was indeed due to expression of

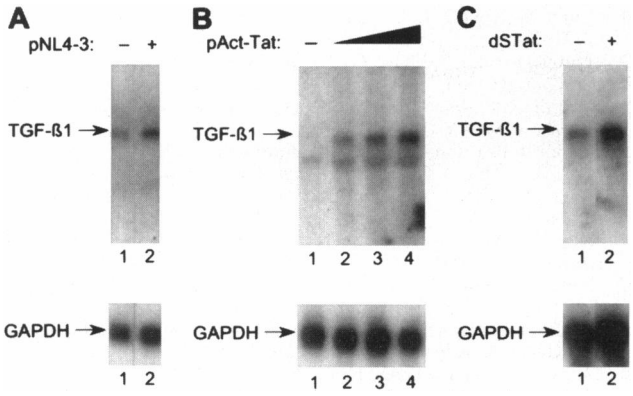

FIG. 4. Effect of Tat on TGF-β1 mRNA expression in glial cells. (*A*) Total RNA from primary human fetal glial cells transfected with control salmon sperm DNA (lane 1) or the HIV-1 proviral DNA construct, pNL4–3 (lane 2). (*B*) Total RNA from U87-MG glial cells transfected with 0, 4, 10, and 20 μg of the Tat expression plasmid, pAct-Tat (lanes 1–4, respectively). (*C*) Poly(A)$^+$ RNA from U87-MG cells transfected with 25 μg of the control dTKlox vector (lane 1) or dSTat (lane 2). Fifteen micrograms of total RNA or 2.0 μg of poly(A)$^+$ RNA from each sample was fractionated by electrophoresis in 1.2% agarose–formaldehyde gels, transferred to nylon membranes, and hybridized to ^{32}P-labeled cDNA probes. *Upper*, Northern blot probed with a 1.0-kb fragment of a TGF-β1 cDNA. *Lower*, same blot reprobed with a 1.3-kb fragment of a GAPDH cDNA.

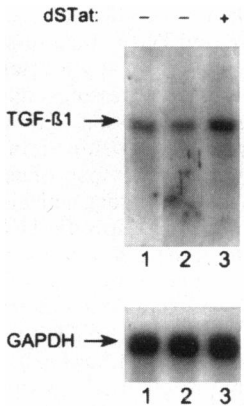

FIG. 5. Effect of intracerebral dSTat injection on TGF-β1 mRNA expression *in vivo*. Swiss Webster mice at 3–4 months of age were injected intracerebrally with 25 μl of PBS alone (lane 1) or PBS containing 10^6 plaque-forming units of control dTKlox (lane 2) or dSTat (lane 3). Two micrograms of poly(A)$^+$ RNA, isolated from whole brain tissue, was subjected to Northern blot analysis using TGF-β1 (*Upper*) or GAPDH (*Lower*) cDNA probes as described in Fig. 4.

Tat from dSTat and not from any HSV-1 immediate-early gene products expressed from the replication-defective vector's backbone *in vivo*, as these same gene products are expected to be expressed from the control dTKlox vector *in vivo* (Fig. 5, lane 2). Of note, during the course of these studies (i.e., 48 h), the injected animals showed no detectable signs of neurological impairment. Furthermore, at autopsy no gross anatomical abnormalities and no signs of inflammatory response were observed.

DISCUSSION

Two HIV-1 proteins have been shown to exhibit neurotoxic properties: the envelope glycoprotein gp120 (35–37), and the nonstructural regulatory protein Tat (38–40). gp120 has been reported to exert its neurotoxic properties by dramatically raising intracellular neuronal Ca^{2+} concentrations and stimulating NMDA excitatory amino acid receptors (35, 36). Chemically synthesized Tat peptides have been shown to bind to rat neuronal cell membranes, to depolarize cockroach giant interneurons, and to be potent and lethal neurotoxic agents upon interventricular injections in mice (38). Tat peptide-mediated neurotoxicity has also been demonstrated by intrastriatal injections of rats with synthetic Tat peptides from visna virus, an HIV-related lentivirus (39). In that study, neurotoxic effects, including loss of striatonigral neurons with microglial cell infiltration and astrocytosis, were shown to be blocked by NMDA receptor antagonists (39). More recently, micromolar concentrations of recombinant Tat protein have been shown to induce depolarization of young rat and adult human neurons and to be toxic to cultured human fetal neurons through activation of non-NMDA excitatory amino acid receptors (40).

In HIV-1 infection of the CNS, gp120, and Tat may also exert their neurotoxic effects through induction of immunoregulatory mediators and inflammatory cytokines with potentially harmful activities such as interleukin (IL)-1 (41, 42), tumor necrosis factor α (41, 43, 44), and TGF-β (11). Expression of IL-1 and tumor necrosis factor α may in turn activate the synthesis of IL-6, prostaglandins, and TGF-β (45), resulting in a cytokine cascade and an autocrine feedback loop that could perpetuate the neuronal damage and also activate HIV-1 replication. Our earlier findings have shown the ability of HIV-1 Tat to activate the expression of a number of cellular genes including those encoding extracellular matrix proteins in Tat-producing glioblastoma cells (14). In addition, Tat-mediated transcriptional activation from the TGF-β1 promoter has been demonstrated in cultured glial cells (11). These data corroborate earlier observations that TGF-β1 gene expression is enhanced among HIV-1-positive individuals (46, 47). Although the importance of these findings in the pathogenesis of AIDS CNS disease remains unclear, it has been speculated that TGF-β1, a potent chemoattractant, may act to recruit infected monocytes into the brain, thereby perpetuating and enhancing CNS dysfunction (48). Elevated levels of TGF-β1 could deregulate expression of other cellular genes, including neurotoxic cytokines, and directly or indirectly contribute to the clinical complications observed in AIDS neuropathology.

In the present study we examined the effect of Tat introduced into the murine CNS by intracerebral inoculation of a recombinant, replication-defective HSV-1 Tat expression vector, dSTat, on expression of TGF-β1, a candidate gene involved in AIDS-related neurodegenerative disease. dSTat expressed functional Tat protein in cells in that it (*i*) transactivated the HIV-1 LTR in a TAR-dependent manner in cultured cells transfected with an HIV-1 LTR-CAT reporter gene construct and (*ii*) upregulated transcription of the TGF-β1 gene both in cultured glial cells and in the mouse brain. The SV40 early promoter–enhancer was used to drive the expression of *tat* in the context of a replication-defective HSV-1 vector. Although the SV40 promoter–enhancer could not support long-term *tat* expression from dSTat in the mouse brain beyond 2 days as determined by *in situ* hybridization analysis of *tat* mRNA expressed in the injected hippocampus (data not shown), the observed effect on TGF-β1 upregulation was easily detectable at 2 days after intracerebral injection of dSTat. This observation suggested that very minute quantities of Tat are required to exert a physiological effect *in vivo*. Recently we have stereotactically injected dSTat into the hippocampus of rats and mice. Results of 5-bromo-4-chloro-3-indolyl β-D-galactoside (X-gal) staining, immunohistochemistry using an anti-HSV polyclonal antibody, and *in situ* hybridization to *tat* mRNA using an antisense *tat* riboprobe all indicate that granule and pyramidal neurons of the dentate gyrus of the hippocampus are infected by the vector. It would be expected that Tat expressed in these neurons is either upregulating TGF-β1 gene expression in the vector-infected

neuronal cells itself, or secretion of Tat from the infected neuron induces increased TGF-β1 expression in a neighboring neuron or glial cell. We are currently attempting to assess the identity of the cell type expressing high levels of TGF-β1 message using *in situ* hybridization. The mechanism by which Tat induces expression of TGF-β1 in brain tissue remains to be investigated. Results from previous transient transfection of glial cells have demonstrated that activation of TGF-β1 promoter activity by *tat* requires specific DNA sequences which are located upstream of the transcription start site (11). Thus, it appears that, at least in cell culture, *tat* exerts its stimulatory action on TGF-β1 through a TAR-independent pathway (18, 49, 50). The inability of Tat to directly bind to the DNA responsive elements of TGF-β1 has led us to speculate that an intermediary cellular protein, perhaps with a binding activity, is involved in this regulatory event. More recently we have identified a GC/GA binding protein which exhibits affinity for HIV-1 Tat protein (C. P. Krachmarov, L. L. Chepenik, S. M. Barr, J. N. Brady, K.K., and E. M. Johnson, unpublished data). Of particular interest is the notion that the Tat-responsive element of TGF-β1 contains the GC/GA motif. Development of the *in vivo* model for studying TGF-β1 expression will allow us to launch a molecular approach to unravel the mechanism involved in activation of TGF-β1 promoter in brain cells. Future studies are intended to determine whether the normal patterns of expression of other cytokine genes, including those encoding tumor necrosis factor α, IL-1, IL-6, and granulocyte–macrophage colony-stimulating factor are also altered as a result of dSTat-mediated delivery of Tat into the mouse brain. The results presented in this report point to the utility of recombinant, replication-defective HSV-1 vectors for analysis of the effects of short-term expression of diffusable HIV-1 gene products, such as Tat, on normal patterns of CNS gene expression upon direct gene transfer to the mouse brain. Generation of similar HSV-1 vectors capable of expressing other HIV-1 gene products may provide experimental tools useful in further exploring mechanisms by which expression of HIV-1 gene products in the brain may alter normal CNS physiology and lead to AIDS-associated neurological disease.

The following reagent was obtained through the AIDS Research and Reference Reagent Program, Division of AIDS, National Institute of Allergy and Infectious Disease, National Institutes of Health: antiserum to HIV-1 Tat from Dr. Bryan Cullen. We thank Dr. Paul A. Luciw for the gift of pSVTat plasmid and Drs. James Cavalcoli and Johnny Huard for invaluable technical assistance in immunofluorescence assays. We are also grateful to Dr. Michael Zeira, former member of our laboratory, for contributing to the data on TGF-β1 activation in primary glial cell cultures. We thank Cynthia Schriver for her editorial assistance in preparation of this manuscript. This work was made possible by grants awarded by National Institutes of Health to J.G., K.K., and S.A.

1. Price, R. W., Brew, B., Sidtis, J., Rosenblum, M., Scheck, A. C. & Cleary, P. (1988) *Science* **239,** 586–592.
2. de la Monte, S. M., Ho, D. D., Schooley, R. T., Hirsch, M. S. & Richardson, E. P., Jr. (1987) *Neurology* **37,** 562–569.
3. Arya, S. K., Guo, C., Josephs, S. F. & Wong-Staal, F. (1985) *Science* **229,** 69–73.
4. Sodroski, J., Rosen, C., Wong-Staal, F., Salahuddin, S. Z., Popovic, M., Arya, S., Gallo, R. C. & Haseltine, W. A. (1985) *Science* **227,** 171–173.
5. Southgate, C. D. & Green, M. R. (1991) *Genes Dev.* **5,** 2496–2507.
6. Ensoli, B., Barillari, G., Salahuddin, S. Z., Gallo, R. C. & Wong-Staal, F. (1990) *Nature (London)* **345,** 84–86.
7. Vogel, J., Hinrichs, S. H., Reynolds, R. K., Luciw, P. A. & Jay, G. (1988) *Nature (London)* **335,** 606–611.
8. Frankel, A. D. & Pabo, C. O. (1988) *Cell* **55,** 1189–1193.
9. Helland, D. E., Welles, J. L., Caputo, A. & Haseltine, W. A. (1991) *J. Virol.* **65,** 4547–4549.
10. Marcuzzi, A., Weinberger, J. & Weinberger, O. K. (1992) *J. Virol.* **66,** 4228–4232.
11. Cupp, C., Taylor, J. P., Khalili, K. & Amini, S. (1993) *Oncogene* **8,** 2231–2236.
12. Buonaguro, L., Barillari, G., Chang, H. K., Bohan, C. A., Kao, V., Morgan, R., Gallo, R. C. & Ensoli, B. (1992) *J. Virol.* **66,** 7159–7167.
13. Viscidi, R. P., Mayur, K., Lederman, H. M. & Frankel, A. D. (1989) *Science* **246,** 1606–1608.
14. Taylor, J. P., Cupp, C., Diaz, A., Chowdhury, M., Khalili, K., Jimenez, S. A. & Amini, S. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 9617–9621.
15. Gage, P. J., Sauer, B., Levine, M. & Glorioso, J. C. (1992) *J. Virol.* **66,** 5509–5515.
16. Sauer, B., Whealy, M., Robbins, A. & Enquist, L. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 9108–9112.
17. Rasty, S., Goins, W. F. & Glorioso, J. C. (1995) *Methods Mol. Genet.* **7,** 114–130.
18. Taylor, J. P., Pomerantz, R., Bagasra, O., Chowdhury, M., Rappaport, J., Khalili, K. & Amini, S. (1992) *EMBO J.* **11,** 3395–3403.
19. Adachi, A., Gendelman, H. E., Koenig, S., Folks, T., Willey, R., Rabson, A. & Martin, M. A. (1986) *J. Virol.* **59,** 284–291.
20. DeLuca, N. A. & Schaffer, P. A. (1987) *Nucleic Acids Res.* **15,** 4491–4511.
21. Cole, R. & deVellis, J. (1989) *Dissection and Tissue Culture Manual of the Nervous System*, eds. Shohar, de Vellis, Vernadakis & Haber (Liss, New York), pp. 121–133.
22. DeLuca, N. A., McCarthy, A. M. & Schaffer, P. A. (1985) *J. Virol.* **56,** 558–570.
23. Graham, F. L. & Van der Eb, A. J. (1973) *Virology* **52,** 456–467.
24. Gorman, C. M., Moffat, L. F. & Howard, B. H. (1982) *Mol. Cell. Biol.* **2,** 1044–1051.
25. Queen, C. & Baltimore, D. (1983) *Cell* **33,** 741–748.
26. Feinberg, A. P. & Vogelstein, B. (1983) *Anal. Biochem.* **132,** 6–13.
27. Golden, M. P., Kim, S., Hammer, S. M., Ladd, E. A., Schaffer, P. A., DeLuca, N. & Albrecht, M. A. (1992) *J. Infect. Dis.* **166,** 494–499.
28. Vlach, J. & Pitha, P. M. (1992) *J. Virol.* **66,** 3616–3623.
29. Vlach, J. & Pitha, P. M. (1993) *J. Virol.* **67,** 4427–4431.
30. Gimble, J. M., Duh, E., Ostrove, J. M., Gendelman, H. E., Max, E. E. & Rabson, A. B. (1988) *J. Virol.* **62,** 4104–4112.
31. Gendelman, H. E., Phelps, W., Feigenbaum, L., Ostrove, J. M., Adachi, A., Howley, P. M., Khoury, G., Ginsberg, H. S. & Martin, M. A. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 9759–9763.
32. Ostrove, J. M., Leonard, J., Weck, K. E., Rabson, A. B. & Gendelman, H. E. (1987) *J. Virol.* **61,** 3726–3732.
33. Mosca, J. D., Bednarik, D. P., Raj, N. B., Rosen, C. A., Sodroski, J. G., Haseltine, W. A., Hayward, G. S. & Pitha, P. M. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 7408–7412.
34. Nabel, G. J., Rice, S. A., Knipe, D. M. & Baltimore, D. (1988) *Science* **239,** 1299–1302.
35. Lipton, S. A., Sucher, N. J., Kaiser, P. K. & Dreyer, E. B. (1991) *Neuron* **7,** 111–118.
36. Lipton, S. A. (1993) *Ann. Neurol.* **33,** 227–228.
37. Toggas, S. M., Masliah, E., Rockenstein, E. M., Rall, G. F., Abraham, C. R. & Mucke, L. (1994) *Nature (London)* **367,** 188–193.
38. Sabatier, J. M., Vives, E., Mabrouk, K., Benjouad, A., Rochat, H., Duval, A., Hue, B. & Bahraoui, E. (1991) *J. Virol.* **65,** 961–967.
39. Hayman, M., Arbuthnott, G., Harkiss, G., Brace, H., Filippi, P., Philippon, V., Thomson, D., Vigne, R. & Wright, A. (1993) *Neuroscience* **53,** 1–6.
40. Magnuson, D. S., Knudsen, B. E., Geiger, J. D., Brownstone, R. M. & Nath, A. (1995) *Ann. Neurol.* **37,** 373–380.
41. Wahl, L. M., Corcoran, M. L., Pyle, S. W., Arthur, L. O., Harel-Bellan, A. & Farrar, W. L. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 621–625.
42. Clouse, K. A., Cosentino, L. M., Weih, K. A., Pyle, S. W., Robbins, P. B., Hochstein, H. D., Natarajan, V. & Farrar, W. L. (1991) *J. Immunol.* **147,** 2892–2901.
43. Merrill, J. E., Koyanagi, Y., Zack, J., Thomas, L., Martin, F. & Chen, I. S. (1992) *J. Virol.* **66,** 2217–2225.
44. Merrill, J. E., Koyanagi, Y. & Chen, I. S. (1989) *J. Virol.* **63,** 4404–4408.
45. Morganti-Kossmann, M. C., Kossmann, T. & Wahl, S. M. (1992) *Trends Pharmacol. Sci.* **13,** 286–291.
46. Kekow, J., Wachsman, W., McCutchan, J. A., Cronin, M., Carson, D. A. & Lotz, M. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 8321–8325.
47. Kekow, J., Wachsman, W., McCutchan, J. A., Gross, W. L., Zachariah, M., Carson, D. A. & Lotz, M. (1991) *J. Clin. Invest.* **87,** 1010–1016.
48. Wahl, S. M., Allen, J. B., McCartney-Francis, N., Morganti-Kossmann, M. C., Kossmann, T., Ellingsworth, L., Mai, U. E., Mergen-hagen, S. E. & Orenstein, J. M. (1991) *J. Exp. Med.* **173,** 981–991.
49. Bagasra, O., Khalili, K., Seshamma, T., Taylor, J. P. & Pomerantz, R. J. (1992) *J. Virol.* **66,** 7522–7528.
50. Taylor, J. P., Kundu, M. & Khalili, K. (1992) *Virology* **195,** 780–785.