HIV-1 Tat-Mediated Effects on Focal Adhesion Assembly and Permeability in Brain Microvascular Endothelial Cells

Hava Karsenty Avraham, Shuxian Jiang, Tae-Hee Lee, Om Prakash and Shalom Avraham

*J Immunol* 2004; 173:6228-6233; doi: 10.4049/jimmunol.173.10.6228
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The blood-brain barrier (BBB) is a network formed mainly by brain microvascular endothelial cells (BMECs). The integrity of the BBB is critical for brain function. Breakdown of the BBB is commonly seen in AIDS patients with HIV-1-associated dementia despite the lack of productive HIV infection of the brain endothelium. The processes by which HIV causes these pathological conditions are not well understood. In this study we characterized the molecular mechanisms by which Tat mediates its pathogenic effects in vitro on primary human BMECs (HBMECs). Tat treatment of HBMECs stimulated cytoskeletal organization and increased focal adhesion sites compared with control cells or cells treated with heat-inactivated Tat. Pretreatment with Tat Abs or with the specific inhibitor SU-1498, which interferes with vascular endothelial growth factor receptor type 2 (Flk-1/KDR) phosphorylation, blocked the ability of Tat to stimulate focal adhesion assembly and the migration of HBMECs. Focal adhesion kinase (FAK) was tyrosine-phosphorylated by Tat and was found to be an important component of focal adhesion sites. Inhibition of FAK by the dominant interfering mutant form, FAK-related nonkinase, significantly blocked HBMEC migration and disrupted focal adhesions upon Tat activation. Furthermore, HIV-Tat induced permeability changes in HBMECs in a time-dependent manner. Tat also impaired BBB permeability, as observed in HIV-1 Tat transgenic mice. These studies define a mechanism for HIV-1 Tat in focal adhesion complex assembly in HBMECs via activation of FAK, leading to cytoskeletal reorganization and permeability changes. The Journal of Immunology, 2004, 173: 6228–6233.

Some 20% of adult and 50% of pediatric AIDS patients develop neurological disorders. HIV enters the CNS early after the primary infection (1–3). Despite extensive investigation of HIV-1 neuroinvasion, the mechanisms of viral entry into the CNS and HIV pathology in the brain are not clearly understood.

HIV-1 Tat has been implicated in HIV encephalitis (2, 4). Several findings suggest that extracellular Tat plays an important role in the pathogenesis of HIV-1-associated dementia (HAD): 1) Tat is actively secreted by the infected cells of HAD patients (5, 6); 2) anti-Tat Abs are frequently detected in the serum of HAD patients (7, 8); 3) Tat mRNA is elevated in patients with HAD (9, 10); 4) a single intraventricular injection of Tat leads to macrophage infiltration, progressive glial activation, and neuronal death, pathologies that are also observed in HAD brains (11); 5) Tat has been detected in the serum of HIV-infected patients (12); 6) Tat is highly neurotoxic, causing neurotoxicity in the picomolar range (13); and 7) Tat can be detected in the brains of patients with HIV encephalitis (3, 14).

Tat is a transcriptional activator of viral gene expression produced early after infection and is essential for virus replication. The protein is composed of 86–104 aa encoded by two exons. In the portion encoded by the first exon (72 aa), four distinct regions can be recognized (N-terminal, cysteine-rich, core, and basic). The second exon encodes the C-terminal region containing the RGD sequence (15). During acute infection of T cells by HIV-1, Tat is released from cells in an active form and via a leaderless secretory pathway that is specific and resembles that of IL-1 and fibroblast growth factor (FGF) (16). Tat easily enters different cell types, contributing to transactivation of the HIV-1 long terminal repeat promoter in latently infected cells (for review, see Ref. 4). Alternatively, it acts as a soluble mediator affecting the physiologic functions of cells, including T and B cells, monocytes, chondrocytes, and neurons (16–20). However, one of the most relevant targets of Tat is the vascular system, where it activates a proinflammatory and angiogenic program. Tat can up-regulate the expression of endothelial cell (EC) adhesion molecules as well as induce ECs to proliferate and release proteolytic enzymes. In addition, Tat induces EC adhesion to the extracellular matrix (ECM) and stimulates ECs to undergo in vitro morphogenesis (21) and in vivo angiogenic activity. HIV-1 Tat was shown to modulate gene expression, growth, and angiogenic activity in ECs by interacting with the vascular endothelial growth factor (VEGF) receptor type 2 (VEGFR-2 [Flk-1/KDR]) through an RGD amino acid motif. However, because most of these studies were performed in HUVEC, the specific effects of Tat on the permeability and function of brain microvascular ECs remain to be established.

Attachment of cells to the ECM (e.g., laminin, fibronectin, and collagen) is mediated by structures called focal adhesions, which connect the ECM with plasma membranes and the underlying actin.
cytoskeletal network. Attachment of cells to the ECM results in the clustering of integrin receptors and initiates the recruitment of numerous cytoplasmic proteins to the focal adhesion complex. This complex includes both structural and catalytically active signaling proteins. Signaling through focal adhesions regulates a variety of cellular processes, including cell growth, migration, and apoptosis (22). Focal adhesions are dynamic structures, and therefore, their formation and breakdown are regulated by many different extracellular stimuli.

Activation of tyrosine kinases is a prerequisite for focal adhesion assembly, because inhibitors of tyrosine kinase activity block cell adhesion and focal adhesion formation (23). Clustering of integrins appears sufficient to recruit focal adhesion kinase (FAK) and tensin, leading to the autophosphorylation and activation of FAK (24). FAK is recruited to clustered integrins by a direct interaction of the N-terminal domain of FAK and the cytoplasmic domain of the β integrin subunits. Subsequent tyrosine phosphorylation of FAK at Tyr^377 (proximal to the kinase domain) creates a binding site for Src, resulting in the formation of a complex consisting of two tyrosine kinases. The formation of the FAK/Src complex leads to the activation of Src and the subsequent activation of downstream signals (23, 24). In some cells the C-terminal domain of FAK is expressed as a separate protein, called FRNK (FAK-related non-kinase), whose overexpression results in inhibition of the rate of cell spreading and cell migration (25, 26). In this study we explored the effects of HIV-1 Tat on focal adhesion assembly, migration, and permeability in human brain microvascular ECs (HBMECs). In addition, we examined the effects of Tat on brain endothelium in Tat transgenic mice.

Materials and Methods

HIV-1 Tat protein

Recombinant HIV-1 Tat protein was obtained from ImmunoDiagnostics (Woburn, MA; product 1002). The full-length (two exons and 86 aa) recombinant HIV-1 Tat was produced in the Escherichia coli expression system and is 99% pure. The purified recombinant HIV-1 Tat was found to be endotoxin-free using the Limulus amoebocyte lysate assay (Sigma-Aldrich, St. Louis, MO; <0.01 endotoxin Units/ml). This protein was biologically active and assessed by its rescue of Tat-defective provirus replication in HLM-1 cells. As a control for all experiments, we used heat-inactivated Tat protein. Tat protein was heat-inactivated by incubation at 85°C for 30 min. The concentration of Tat in all experiments was 4 ng/ml (285 pM) or 10 ng/ml (712.5 pM), as indicated specifically.

Culture of HBMECs

HBMECs were obtained from Cell Systems (Kirkland, WA). Cells were cultured in complete serum-free cell culture medium (CSC) containing 10% FCS, which was supplemented with EC growth factor, Endogro (100 ng/ml), as described in the manufacturer’s manual. HBMEC cultures were stained with anti-factor VIII, a marker for ECs, and expressed γ-glutamyl transpeptidase, an enzyme specific for CNS ECs (27). Serum- and growth factor-free medium was used beginning 4 h before the start of the experiment. HBMECs were used up until passages 5–6. For the proposed signal transduction studies, postconfluent HBMECs at 80–90% confluence were incubated in serum-deprived medium with 0.5% FBS for 4 h. Cells were then stimulated with 10 nM heparin with or without HIV-1 Tat protein in medium containing 0.5% FBS for the indicated time periods.

Western blot analysis

For the Western blot analysis, cells were lysed, and the lysates were measured for protein concentration using a bicinechonic acid protein assay reagent (Pierce, Rockford, IL). Approximately 30 μg of protein was separated by SDS-PAGE. After electrophoresis to polyvinylidene difluoride membranes, the membranes were blocked in 5% nonfat milk in PBS-0.05% Tween 20 (PBST) for 2 h at room temperature, then incubated overnight at 4°C with primary Abs. Membranes were washed four times with PBST for >40 min and incubated with secondary HRP-conjugated Abs. After three washes with PBST, detection was conducted using the ECL chemiluminescence reagent (Amersham Biosciences, Piscataway, NJ).

Permeability assay

HBMECs (10^5 cells) were seeded onto fibronectin-coated polycarbonate membranes with a pore size of 0.4 μm in a two-chamber system (Collaborative Biomedical Products, Bedford, MA) and maintained confluent for 7 days. The cells on the upper chamber were starved in 1% FCS medium at 37°C for 24 h and then the test sample was added. The lower chamber was loaded with 0.8 ml of fresh medium, and the upper chamber was filled with 0.5 ml of prewarmed medium containing Lucifer Yellow (at 1.0 mg/ml) and test sample. After incubation for 2 h, 50-µl aliquots of medium were withdrawn from the lower chamber. Samples were diluted with 0.1 ml of distilled H2O and 100 μl of 0.15% deoxycholate, and the protein was precipitated by the addition of 150 μl of 50% TCA. The tubes were placed on ice for at least 20 min. The supernatants were then centrifuged, and the lower chamber diluted with 1 ml of Hanks’ buffer to a final volume of 2.0 ml. Measurements were performed in a luminescence spectrometer (LS-5B; PerkinElmer, Wellesley, MA). The paired Student’s t test was used to determine statistical difference.

Adenovirus constructs and infections

The replication-deficient recombinant adenovirus mediating overexpression has been previously described (27). A replication-defective adenovirus encoding GFP-tagged FRNK (Adv-GFP-FRNK) was constructed as previously reported (27). An adenovirus expressing GFP alone (Adv-GFP) was used to control for the nonspecific effects of adenoviral infection. HBMECs were infected at a multiplicity of infection of 10 for 2 h at 25°C under gentle agitation with replication-defective adenoviruses diluted in medium. The medium was then replaced with virus-free medium, and the cells were cultured for an additional 48 h and assayed for cell migration and focal adhesion assembly.

Cell migration assay

The migration assay used a modified Boyden chamber and was performed essentially as previously described (28). Cells were grown to 50% confluence and then either untreated or infected with adenoviral constructs as described below for 2 h. Cells were lifted from the dish using nondenzytric cell dissociation solution (Sigma-Aldrich), centrifuged for 2 min at 1000 rpm, and resuspended in medium 199 containing 0.5% BSA. The cells were then plated on fibronectin precoated Transwells (Costar, Cambridge, MA) at a density of 10^5 cells/well. Medium 199 with Tat (10 ng/ml) was used as a chemotaxant in the lower wells. The cell migration assay was performed for 8 h at 37°C in a CO2 incubator. Cells that had migrated through the Transwell were counted under a fluorescent microscope. Four different fields were counted for each experiment, and all samples were performed in triplicate.

Focal adhesion assay

HBMECs were seeded onto the indicated substrate-coated coverslips: fibronectin (20 μg/ml) and gelatin (0.2%). After a 4-h incubation in serum-free CSC medium, the cells were stimulated with Tat (10 ng/ml) for the indicated times. The cells were then fixed in 4% paraformaldehyde for 10 min, permeabilized with 0.1% Triton X-100 in PBS for 5 min at room temperature, and treated with blocking buffer (0.1% goat serum and 0.1% BSA in PBS). Polyclonal anti-FAK (1/500) Abs generated in our laboratory, as detailed previously (28), were used for this study. mAb for vinculin (1/250) or control Ab was added, followed by incubation with goat anti-rabbit IgG conjugated to fluorescein (1/200). For inhibition of focal adhesion assembly, murine anti-Tat mAb was used (ImmunoDiagnostics; product 1102). This Ab has binding specificity for the N terminus of Tat. For the labeling of F-actin, Texas Red-phalloidin (Molecular Probes, Eugene, OR; 1/1000) was used. The cells were then washed again and mounted in anti-fading compound (phenylendiamine; 1 mg/ml 50% glycerol with PBS). The fluorescent signals were detected by optical sectioning using a TCS-NT confocal laser-scanning microscope (Leica, Deerfield, IL). A minimum of 200 cells/condition were evaluated for the presence of focal adhesions. Cells that are positive usually have ~15–30 plaques/cell. Cells with fewer than five to seven plaques per cell were scored as negative.

Analyses of brain microvascular ECs (BMECs) isolated from HIV-1 Tat transgenic mice

BMECs were isolated from cerebral cortex capillaries by enzymatic digestion at 37°C, separated by 25% BSA at 1000 × g for 15 min, and then separated from RBC with 5% horse serum in a 50% Percoll gradient as previously described (29). BMECs were purified by anti-Thy 1.1 and rabbit complement, and were maintained in F-10 medium supplemented with 4% FBS, 5% penicillin/streptomycin, 1% glutamine, 1% heparin, 0.7% endothelial mitogen, and 15% horse serum. Primary BMECs were grown at
37°C with 5% CO₂. For the permeability assays, murine brain endothelial cultures were incubated with biotin-labeled albumin (4 ng/ml) for 1.5 h at 37°C. After several washings with PBST, peroxidase-conjugated streptavidin (1/10,000 in PBS) was added for 45 min at room temperature. The monolayers were washed, and 0.1% O-phenylenediamine in a pH 4.5 citrate buffer-0.015% H₂O₂ was added for 10 min at room temperature, followed by blocking with H₂SO₄. The color that developed was analyzed by spectrophotometry at 428 nM.

Data analysis

Results are expressed as the mean ± SD. Differences among means were considered significant at p < 0.05. Data were analyzed using the SigmaStat statistical software package (version 1.0; Jandel Scientific, San Rafael, CA).

Results

Tat induced the formation of focal adhesions

Cell adhesion to the ECM is a highly dynamic process involving structures heterogeneous with respect to size, composition, and orientation to actin filaments. The largest and tightest structures are usually referred to as focal adhesions, which link the actin cytoskeleton to the ECM by integrin receptor complexes. Actin-binding proteins that colocalize with integrins in focal adhesions include actin, talin, vinculin, and tensin. Focal contacts were detected by staining the fixed cells with either mAb to vinculin, a cytoskeletal protein that localizes at focal adhesion contacts, or FAK mAb (23). To determine whether Tat induced the formation of focal adhesions, HBMECs were treated with Tat for 1 or 4 h and then analyzed by a focal adhesion assay. As shown, Tat mediated focal adhesion assembly in a time-dependent manner, which was blocked by treatment with either specific Abs for Tat or the specific inhibitor for VEGFR-2, SU-1498 (Fig. 1).

Effects of integrins and Tat signaling on focal adhesion formation

Next we examined the ability of HBMECs to assemble focal adhesions when plated on integrin-dependent (e.g., fibronectin) or integrin-independent (e.g., gelatin) substrates in the presence of Tat. HBMECs plated on laminin or fibronectin stimulated cytoskeleton organization and increased focal adhesion sites in the presence of Tat compared with the vehicle-treated cells (Table I). In contrast, these changes were significantly reduced in cells plated on gelatin (Table I) or cells plated on poly-L-lysine (Table I).

Tyrosine phosphorylation of FAK in HBMECs

Because we observed an effect of Tat on focal adhesion complex formation, we analyzed the potential activation of FAK, a critical component of focal adhesion assembly. FAK was tyrosine-phosphorylated after Tat stimulation for 5–15 min, and by 30 min its phosphorylation was reduced to basal levels (Fig. 2). These results indicate that Tat can induce the phosphorylation of a key component of focal adhesions.

Effects of GFP-FRNK on Tat-induced focal adhesion assembly and migration

To specifically determine the effects of FAK on focal adhesion assembly and to target FAK-dependent signaling in HBMECs upon Tat treatment, we used a replication-defective adenovirus encoding FRNK that specifically inhibits FAK function (30). As shown in Fig. 3, overexpression of FRNK significantly inhibited HBMEC migration and disrupted focal adhesions induced by Tat, whereas control adenovirus had no effect on HBMEC migration and focal adhesion assembly.

HIV-Tat impaired BBB permeability in HBMECs

Because Tat induced the migration of HBMECs through modulation of FAK, Tat may also affect the integrity and permeability of the lining of the endothelium. Therefore, we examined the effects of Tat on HBMEC permeability. HBMECs were cultured to confluence on porous polycarbonate membranes coated with human fibronectin in a two-chamber system. At the initiation of the experiments, the medium in both chambers was changed to CSC medium containing 1% PBS, then Tat was added to the upper chamber. A significant increase in transcytosis (as indicated by the rise in absorbance (OD)) was observed 6 h after HIV-1 Tat treatment (Fig. 4B), indicating that HIV-1 Tat resulted in increased HBMEC permeability. These results were comparable with the observed increase in transcytosis upon TNF-α stimulation of HBMECs (Fig. 4A), which was used as a positive control. The

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**Figure 1.** Effects of Tat on focal adhesion assembly. HBMECs were grown and preincubated with Tat mAb or control Ab (1/500 dilution) or with the inhibitor SU-1498 (10 ng/ml) for 30 min at 37°C. Cells were next washed and incubated with Tat or DMEM (control), then fixed and examined for the presence of focal adhesions. Results are expressed as the mean percentage of cells positive for focal adhesions ± SD (n = 3). * Tat significantly induced focal adhesion assembly at 4 h compared with the control (p < 0.05).

**Figure 2.** FAK kinase is tyrosine-phosphorylated upon Tat stimulation of HBMECs. Five hundred micrograms of total cell lysates obtained from Tat-treated HBMECs for the indicated times were immunoprecipitated (IP) with FAK Abs. Blots were probed with anti-Py20, then stripped and reprobed with anti-FAK Abs for the Western blot (WB) analysis.

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**Table 1.** Tat-induced focal adhesions required ECM protein

| Treatment          | Cells Positive for Focal Adhesions (%) |
|--------------------|---------------------------------------|
| **HBMECs**         |                                       |
| Tat (10 ng/ml)     | 53 ± 7                                |
| Heat-inactivated Tat (10 ng/ml) | 15 ± 3 |
| Control            | 13 ± 4                                |
| Laminin            | 68 ± 6                                |
| FN                 | 16 ± 4                                |
| Gelatin            | 13 ± 3                                |
| Poly-L-lysine      | 15 ± 4                                |

*HBMECs plated on 10 μg/ml each of laminin, fibronectin, gelatin, or poly-L-lysine were treated with vehicle (control), Tat (10 ng/ml), or heat-inactivated Tat (10 ng/ml), as indicated. Results are expressed as the mean percentage of cells positive for focal adhesions ± SD (n = 3).
Tat-induced permeability was inhibited by Tat-specific Abs (Fig. 4C), indicating the specific effects of Tat on HBMEC permeability. No effects on HBMEC permeability were observed with heat-inactivated Tat (±1.2 ± 0.2 at 20 h). Thus, HIV-1 Tat injures the integrity of the HBMEC lining and thereby may enhance spreading of the disease to the peripheral tissues.

Several Tat-transgenic mouse models were generated to study the effects of Tat in HIV-1 neuropathogenesis (31, 32). Tat expression in the brain caused failure to thrive, a hunched gesture, tremors, ataxia, slow cognitive and motor movements, seizures, and premature death (32). The neuropathology of these mice was characterized by damage of the cerebellum and cortex, brain edema, astrocytosis, degeneration of neuronal dendrites, neuronal apoptosis, and increased infection of activated monocytes and T lymphocytes. However, the effects of Tat on brain endothelium were not addressed directly in these models. To test whether alteration in HBMEC permeability upon exposure to HIV-1 Tat is intrinsic to the BBB changes in vivo, we isolated brain ECs from Tat-transgenic mice and control mice and measured cell permeability changes in brain endothelium found in vivo upon Tat exposure are due to the action of Tat protein.

**Tat effects on focal adhesion assembly were not mediated through VEGF**

Our previous studies have shown that VEGF regulates focal adhesion assembly in HBMECs through activation of FAK and related adhesion focal tyrosine kinase (28). Because Tat is known to induce the expression of some cytokines and growth factors in ECs, we examined whether the effects of Tat on focal adhesion assembly in HBMECs are mediated via VEGF induction. Conditioned medium was obtained from HBMECs treated with either HIV-1 Tat or heat-inactivated Tat over various Tat concentrations (0, 4, 10, 50, and 100 ng/ml) or for various times (0, 6, 24, and 48 h), and then assessed for the secretion of basic FGF and VEGF using specific ELISA cytokine assays (R&D Systems, Minneapolis, MN). No secretion of VEGF or basic FGF was observed after HIV-1 Tat treatment of brain ECs (data not shown). These results suggest that the effects of Tat on HBMECs are not mediated by VEGF.

**Discussion**

The BBB plays a critical role in regulating cell trafficking through the CNS due to several unique anatomical features, including the presence of interendothelial tight junctions that form highly selective impermeable seals between the cells. Previous studies have demonstrated BBB perturbations during HIV encephalitis (1, 2, 4). However, the basis of these permeability changes and their relationship to the infiltration of HIV-1-infected monocytes, a critical event in the pathogenesis of the disease, are not clear.

HAD is a syndrome of progressive motor, cognitive, and behavioral impairment that occurs in a significant number of patients with AIDS. HIV-1 induces dementia with alarming frequency worldwide. Although highly active antiretroviral therapy has resulted in a decrease in the incidence of HIV-1 infection, it does not seem to provide complete protection from or reversal of HAD (33, 34). The prevalence of HAD may increase as people live longer with AIDS.

The HIV transregulatory protein Tat plays an important intracellular as well as extracellular role in the molecular pathway involved in HIV-1-induced CNS pathology. We hypothesize that Tat interacts with brain endothelium and causes endothelial activation and cell injury, thereby altering BBB permeability and integrity. These changes result in the breakdown of the BBB and facilitate entry of HIV into the CNS, thus contributing to the pathology in brain. In this study we characterized the molecular mechanisms by which Tat mediates its pathogenic effects on human brain endothelium, resulting in breakdown of the BBB. Specifically, we have elucidated the functional aspects of Tat-mediated effects on focal adhesion assembly, migration, and permeability in brain endothelium. We have shown that Tat increased the focal adhesion assembly of brain endothelium as well as the migration and permeability of brain ECs. These changes may lead to the breakdown of the BBB, as observed in the brains of HIV patients.

The ability of cells to form cell contacts, adhere to the ECM, change morphology, and migrate is essential for wound healing, metastasis, cell survival, and the immune response. These events depend on the binding of integrins to the ECM and on the assembly of focal adhesions, which are complexes comprising scaffolding and signaling proteins organized by adhesion to the ECM. In this study we found that Tat regulated the assembly of focal adhesions in HBMECs in an integrin-dependent manner. Tat induced the formation of focal adhesions in HBMECs when seeded on fibronectin, and pretreatment with Tat-specific Abs or with the specific inhibitor SU-1498, which inhibits VEGFR-2 receptor.
HBMECs were untreated or pretreated with Tat Ab (1/50) and then incubated with HIV-1 Tat for the indicated times. Microvascular ECs were cultured to confluence on Transwell polycarbonate membranes and then incubated with HIV-1 Tat for the indicated times. The amount of LY in the lower chamber was measured at an OD of 428 nm.

**FIGURE 4.** Effects of HIV-1 Tat on the fluid phase permeability of HBMECs. A, Microvascular ECs were cultured to confluence on Transwell polycarbonate membranes and then incubated with TNF-α at a concentration of 10 ng/ml for 6 and 24 h. After 4 h of exposure to Lucifer Yellow (LY), the amount of LY in the lower chamber was measured at an OD of 428 nm. * Significant increase in permeability compared with time zero (p < 0.05). B, Microvascular ECs were cultured to confluence on Transwell polycarbonate membranes and then incubated with HIV-1 Tat at a concentration of 10 ng/ml for 6 and 24 h. After 4 h of exposure to LY, the amount of LY in the lower chamber was measured at an OD of 428 nm. * Significant increase in permeability compared with time zero (p < 0.05). C, Microvascular ECs were cultured to confluence on Transwell polycarbonate membranes and then incubated with HIV-1 Tat for the indicated times. HBMECs were untreated or pretreated with Tat Ab (1 μg) or control Ab phosphorylation, blocked the ability of Tat to stimulate focal adhesion assembly. These effects of Tat on focal adhesion assembly were very similar to those observed with VEGF (28). This suggests that Tat mimics VEGF-mediated effects in brain endothelium via its binding to VEGFR-2, leading to VEGFR-2 activation and VEGFR-2-mediated signaling in BMEC. These Tat effects were not mediated by VEGF, because Tat did not induce VEGF secretion in HBMECs.

In addition, we demonstrated that exposure of HBMECs to HIV-1 Tat induced the tyrosine phosphorylation of FAK and focal adhesion assembly in HBMECs. Adhesive interactions with ECM components play a critical role in regulating a variety of intracellular signaling pathways that control cell growth, survival, and differentiation. The integrin family of transmembrane cell surface receptors mediates cell contact with the ECM and is responsible for initiating the formation of focal adhesion structures, which tether the integrin cytoplasmic tail with the actin cytoskeleton. The identification and characterization of FAK as a component of Tat-mediated signaling provide additional mechanisms for Tat signaling in brain endothelium. Abrogation of FAK expression and/or activation by FRNK, which inhibits FAK phosphorylation, significantly inhibited the focal adhesion assembly and migration of HBMECs. Therefore, FAK is an important component of focal adhesions in HBMECs and acts as a mediator of Tat function in brain endothelium.

There are several Tat-transgenic mouse models available (35–38) with phenotypic abnormalities observed in the brain. Transgenic mice expressing HIV-1 Tat protein under regulation of the astrocyte-specific glial fibrillary acidic protein promoter and doxycycline were also recently generated (32). Tat expression in the brains of these mice caused failure to thrive, hunched gesture, tremor, ataxia, slow cognitive and motor movement, seizures, and premature death (32). Neuropathologies of these mice were characterized by breakdown of the BBB, breakdown of the cerebellum and cortex, brain edema, astrocytosis, and degeneration of neuronal dendrites as well as neuronal apoptosis. These observations are similar to those noted in the brains of AIDS patients and provide evidence of a critical role of Tat protein in HIV-1 neuropathogenesis (32). Furthermore, HIV-1 transgenic rats that develop HIV-related pathology and immunologic dysfunction were also generated (39). These transgenic rats contain a gag-pol-detected HIV-1 provirus regulated by the viral promoter. Unlike mice with the same transgene, efficient viral gene expression occurred in the lymph nodes, spleen, thymus, and blood, suggesting functional Tat. A recent report by Lin et al. (40) described Tat-transgenic mice that secreted a chimera between Tat lacking the positive elongation factor b and GFP from β cells of the pancreas. No deleterious side effects of Tat were found in this mouse model (40), because Tat lacking the positive elongation factor b is deleted from
the HIV long-terminal repeat of Tat in these mice (41). Regardless, most Tat-transgenic mice showed phenotypic abnormalities in their brains similar to those observed in the brains of AIDS patients. Therefore, in this study we analyzed the permeability of brain endothelium in Tat-transgenic mice. BMEC isolated from Tat-transgenic mice showed increased permeability to albumin, which was blocked by anti-Tat-specific Ab, but not by isotype-matched control Ab (Fig. 4E). Thus, changes in the permeability of brain endothelium found in vivo upon Tat exposure could be due to the action of Tat protein.

Taken together, this study demonstrates that Tat regulates BMEC cytoskeletal reorganization. In this regard, Tat induced changes in focal adhesion assembly leading to changes in the migration of BMECs and in BMEC permeability, which may result in the breakdown of the BBB in HIV patients.

Acknowledgments

We thank Dr. Jerome E. Groopman for his help, advice, and support. We also thank Heather Kil for typsetting assistance, Janet Delhanty for editing of the manuscript, and Tae-Aug Kim and Philip Chen for technical help in performing some of these experiments.

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