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Mechanisms of the Blood–Brain Barrier Disruption in HIV-1 Infection

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SUMMARY

1. Alterations of brain microvasculature and the disruption of the blood–brain barrier (BBB) integrity are commonly associated with human immunodeficiency virus type 1 (HIV-1) infection. These changes are most frequently found in human immunodeficiency virus-related encephalitis (HIVE) and in human immunodeficiency virus-associated dementia (HAD).

2. It has been hypothesized that the disruption of the BBB occurs early in the course of HIV-1 infection and can be responsible for HIV-1 entry into the CNS.

3. The current review discusses the mechanisms of injury to brain endothelial cells and alterations of the BBB integrity in HIV-infection with focus on the vascular effects of HIV Tat protein. In addition, this review describes the mechanisms of the BBB disruption due to HIV-1 or Tat protein interaction with selected risk factors for HIV infection, such as substance abuse and aging.

KEY WORDS: HIV; AIDS; blood–brain barrier; brain endothelial cells; inflammatory responses; tight junction; Tat.

INTRODUCTION

Central nervous system (CNS) complications, such as human immunodeficiency virus-related encephalitis (HIVE) and HIV-associated dementia (HAD), are frequently associated with infection by human immunodeficiency virus type 1 (HIV-1). Most pathological and epidemiological studies on the frequency of neurological disease in HIV infection were generated before the introduction of the highly active antiretroviral therapy (HAART). Before the era of HAART, the development of CNS neuropathology occurred in approximately 40–90% of patients with HIV infection (Budka, 1991). HIVE developed in approximately one-third of HIV-infected

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patients and HAD affected 15–20% of the patients in the late stages of AIDS and approximately 50% of infected children (Lipton and Gendelman, 1995). In addition, HAD was considered the most common cause of dementia worldwide among people aged 40 or less and a significant independent risk factor for death due to AIDS (Ellis *et al.,* 1997).

The widespread use of HAART appeared effective in reversing some of the manifestations of HAD (Filippi *et al.,* 1998; Skolnick, 1998). These observations are consistent with earlier findings after the adoption of zidovudine therapy (Portegies, 1995). The effectiveness of HAART in decreasing the rate of HAD indicates that the virus and/or viral-related products are responsible for mediating the functional impairment in the brain. Although antiretroviral therapy represents a significant advancement in the treatment of HIV and the management of HIV dementia, it is believed that pathological changes in the CNS will continue to be serious complications in patients with HIV infection (Cohen and McArthur, 2001; Dougherty *et al*., 2002). At least two processes might be responsible for the persistence of the CNS complications in the course of HIV infection: (a) the development of resistance to HAART and/or and (b) biological and/or therapeutic inadequate control of the virus in cellular reservoirs within the brain.

Brain microvascular endothelial cells (BMEC) are the major structural and functional element of the blood–brain barrier (BBB). Under physiological conditions, this vascular barrier selectively regulates the intracellular and paracellular exchange of macromolecules and cells between the circulation and CNS. Alterations of brain microvasculature and the disruption of the BBB are commonly found in AIDS patients (Buttner*et al*., 1996; Petito and Cash, 1992). Moreover, such changes are more frequent in AIDS patients with dementia, as compared to nondemented AIDS patients or seronegative controls (Power *et al*., 1993). For example, accumulation of serum proteins in white matter glia was observed in the brains of 12 out of 12 patients with AIDS dementia, as compared to 6 out of 12 AIDS patients without dementia. Moreover, serum protein-immunopositive cortical neurons were found in the frontal cortex of 11 out of 12 demented AIDS patients as compared to only 3 out of 12 AIDS patients without dementia. Because no differences in demyelination were noted between demented and nondemented AIDS patients, it was concluded that alterations in BBB are mainly responsible for the development of dementia in the course of HIV infection (Power *et al*., 1993). It also was suggested that a breakdown in the BBB can predict the development of HAD. Moreover, dysfunction of BMEC may play a major role in the breakdown of the BBB and HIV-1 entry into the brain. Thus, this review will focus on the changes in brain vasculature observed in HIV-infected patients as well as on alterations of BMEC metabolism induced in cultured endothelial cells by HIV Tat protein.

EVIDENCE OF THE DISRUPTION OF THE BBB IN HIV-INFECTED PATIENTS

A growing line of evidence indicates structural and functional perturbations of the BBB during HIV infection (Giovannoni *et al*., 1998; McArthur *et al*., 1992;

Petito and Cash, 1992). Such changes are observed in pathological studies (Power *et al*., 1993; Rhodes and Ward, 1991), CSF studies (Singer *et al*., 1994) and by dynamic magnetic resonance imaging (Avison*et al*., 2002; Tofts and Kermode, 1991). Several distinctive processes can be responsible for the disruption of the BBB in HIV-infected patients. For example, morphological changes, such as an increase in diameter of cortical vessels, surface area density, and volume fractions, are observed during HIV infections (Weis *et al*., 1996). These alterations can be associated with thinning of the basal lamina, a decreased immunoreactivity of collagen IV, and loss of glycoproteins in the membrane of endothelial cells (Buttner*et al*., 1996; Weis *et al*., 1996).

Another process which can contribute to the disturbances of the BBB is related to apoptosis of endothelial cells. Morphological studies indicated that brain endothelial cells can undergo apoptosis during HIV infection. For example, apoptosis of endothelial cells was detected in the brain sections in 10 of 11 AIDS patients. Endothelial cell apoptosis was more frequent in small and medium-size vessels throughout the gray and white matter (Shi *et al*., 1996). Apoptotic death of endothelial cells also was observed in the brains of macaques injected with neurovirulent simian immunodeficiency virus (Adamson *et al*., 1996). It was suggested that apoptosis of endothelial cells may contribute to the disruption of the BBB in AIDS patients (Shi *et al*., 1996).

Disruption of the BBB in the course of HIV infections also can result from the disruption of tight junctions. It is generally known that the presence of high resistance interendothelial tight junctions, which consist of continuous strands between the outer leaflets of adjacent cerebral endothelial cell membranes, is the most distinct feature of the BBB. Tight junctions are created by several integral membrane proteins, such as occludins, claudins, or junction adhesion molecules, associated cytoplasmic proteins (Dejana *et al*., 2001; Gonzalez-Mariscal *et al*., 2000; Tsukita *et al*., 2001). Clinical studies indicated that HIV infection can lead to alterations in structure and functions of junctional proteins. Specifically, fragmentation and loss of immunoreactivity for occludin and zona occludens-1 (ZO-1) were observed in patients who died due to HIVE (Dallasta *et al*., 1999). In addition, immunohistochemical analyses in HAD patients revealed that loss of ZO-1 immunoreactivity was highly correlated with monocyte infiltration and the degree of dementia (Boven *et al*., 2000). Finally, disruptions of the BBB associated with accumulation of perivascular macrophages were demonstrated in simian immunodeficiency virus encephalitis (Luabeya *et al*., 2000).

HIV-1 ENTRY INTO THE CNS: THE ROLE OF BRAIN ENDOTHELIAL CELLS AND INFLAMMATORY RESPONSES

It is believed that HIV-1 can enter the CNS early in the course of infection (An *et al*., 1999). After entering the CNS, the virus may reside in microglia and macrophages of the perivascular space (Gartner, 2000) and induce the development of HIVE or HAD at the later stages of HIV infection. However, the mechanisms of

this process and the establishment of viral reservoirs in the CNS are still obscure. It has been shown that dysfunction of BMEC can be directly involved in the process of HIV-1 entry into the CNS (Wu *et al*., 2000). According to the leading hypothesis, HIV-1 trafficking into the brain might be mediated through a "Trojan horse" mechanism, in which HIV-infected CD4+ T-lymphocyte and/or circulating monocytes enter the CNS through breaches of the BBB (Liu *et al*., 2000).

To support this hypothesis, a growing line of evidence indicates that induction of inflammatory reactions may play a critical role in HIV-1 entry into the brain. Indeed, infiltration of leukocytes into the brain parenchyma during inflammatory diseases of the CNS, such as HIVE or HAD, requires leukocyte recruitment and adhesion, followed by monocyte migration across the cerebral endothelium (Couraud, 1998; Feuerstein *et al*., 1994). These processes are mediated by adhesion molecules located on both BMEC and leukocytes. Adhesion molecules involved in leukocyte adhesion and migration belong either to the selectin family such as P-selectin and E-selectin or to the immunoglobulin superfamily, such as intercellular adhesion molecule-1 (ICAM-1) or vascular cell adhesion molecule-1 (VCAM-1). These molecules are either present on BMEC in a resting state or can be expressed or further upregulated following stimulation by different proinflammatory factors (Brayton *et al*., 1998; Defazio *et al*., 1998; Dufour *et al*., 1998; Hess *et al*., 1994). Elevated levels of soluble ICAM-1 in cerebrospinal fluid of the patients with HIV-associated neurological diseases confirm the significance of upregulation of adhesion molecules in the pathogenesis of this disease (Heidenreich *et al*., 1994). Upregulation of adhesion molecules is directly involved in the migration of blood monocytes into the CNS. For example, a relationship between upregulation of adhesion molecules and neurological complications of HIV infections was observed in the brain tissue of AIDS patients with encephalitis (Nottet *et al*., 1996).

In addition to expression of adhesion molecules, BMEC can produce a spectrum of inflammatory cytokines, including interleukin (IL)-1 β (Frigerio *et al*., 1998), IL-6 (Zidovetzki *et al*., 1998), and tumor necrosis factor- α (TNF- α) (Botchkina *et al*., 1997) as well as chemokines, such as monocyte chemoattractant protein-1 (MCP-1) (Zhang *et al.,* 1999) and IL-8/CXCL8 (Hofman *et al*., 1998).

It is well known that redox-responsive transcription factors, and primarily nuclear factor- κB (NF- κB), play a critical role in the development of inflammatory responses. NF- κB regulates production of inflammatory cytokines and adhesion molecules (Berliner *et al*., 1995). Indeed, NF- κB binding sites were identified in the promoter regions of genes encoding for adhesion molecules (ICAM-1, VCAM-1, and E-selectin) and inflammatory cytokines (IL-1 β, IL-6, and TNF- α), growth factors, chemokines, and manganese superoxide dismutase. Although other transcription factors are also required for expression of these genes, NF- κB constitutes an important component of transcriptional regulation of these genes (Siebenlist *et al*., 1994; Stade *et al*., 1990). It is interesting that expression of inflammatory cytokines is dependent on activated NF- κB and, in turn, these cytokines can stimulate activation of this transcription factor. Thus, it appears that inflammatory cytokines use NF- κB to amplify their own signals (Berliner *et al*., 1995). The role of inflammatory mediators in the pathogenesis of neurological complications associated with HIV infection was studied in animal models in which HIV-infected monocytes were

inoculated into the basal ganglia and cortex of mice with severe combined immunodeficiency disease. Using this experimental design, it was shown that HIV-infected monocytes/macrophages stimulate upregulation of TNF- α, IL-6, VCAM-1, and E-selectin expression (Persidsky *et al*., 1997). The role of NF- κB in the stimulation of inflammatory response in the brains of HIV-infected patients was supported by the observation that activation of this transcription factor was found in the brains of children with HIV-1 encephalitis. The increased immunoreactivity for p50 and p65 subunits of NF- κB was associated primarily with microglia and macrophages (Dollard *et al*., 1995).

In addition to the "Trojan horse" hypothesis, it has been proposed that HIV-1 can invade the brain through simple entry of circulating free virus, or via binding of the virus to endothelial cells with subsequent passage across the BBB. This hypothesis is supported by the observation that BMEC can be permissively infected by HIV-1 in vitro (Moses and Nelson, 1994) as well as by a neuroinvasive variant of the simian immunodeficiency virus (Edinger *et al*., 1997; Strelow *et al*., 1998). The mechanisms of this process are not fully understood because BMEC do not possess the CD4 receptors or galactosylceramide binding sites which are used by gp120 (the glycoprotein of the HIV-1 viral coat) to enter other cell types (Banks *et al*., 1998). It has been hypothesized that HIV infection of BMEC may lead to the dysfunction of the BBB and thus facilitate HIV-1 entry into the CNS as well as the flow of cytokines or other blood-borne factors into the brain (Moses and Nelson, 1994). However, it should be noted that using a PCR/in situ hybridization technique, no HIV-infected endothelial cells were found in the brain of adult patients with AIDS, including patients with HAD (Takahashi *et al*., 1996). Therefore, it has been hypothesized that the effects of HIV infection can be mediated by soluble factors released from the infected cells. One of such viral factors is the Tat protein which plays a critical role in viral gene expression and replication (Nath and Geiger, 1998). Indeed, the present review is focused on the effects of Tat on the BBB functions. However, it should be noted that other viral proteins also can induce dysfunction of the BMEC and compromise integrity of the BBB. For example, it was demonstrated that exposure of BMEC to gp120, the glycoprotein viral coat of HIV-1, can increase endothelial permeability in a dose-dependent manner via a signaling pathway which involves substance P (Annunziata *et al.,* 1998). In addition, it was shown that gp120 can induce the passage of HIV-1 across the BBB by induction of adsorptive endocytosis (Banks *et al.,* 1998, 1999). HIV Nef protein also was demonstrated to induce disruption of the BBB. Specifically, intracisternal administration of Nef resulted in the breaching of the BBB as measured by Evans blue extravasation and increased matrix metalloproteinsase-9 activity. Pretreatment with MMP inhibitor attenuated Nef-mediated disruption of the BBB (Sporer *et al.,* 2000).

ROLE OF HIV-1 TAT PROTEIN IN DISRUPTION OF THE BBB

Among different HIV-1 proteins, Tat may play the most critical role in induction of HIV-related neuropathology. Due to its highly positively charged cell attachment domain (Weeks *et al*., 1995), Tat is able to cross cell membranes and induce a variety

of cellular effects. To support the role of Tat in the development of neuropathological changes associated with HIV infection, elevated Tat protein and mRNA levels have been detected in the brains of AIDS patients (Hudson *et al*., 2000; Kruman *et al*., 1999; Valle *et al*., 2000) and in macaques injected with simian immunodeficiency virus (Kruman *et al*., 1999). Elevated concentrations of Tat can disrupt the barrier function of the BBB. For example, it has been demonstrated that exposure to Tat can increase endothelial permeability in vitro and in vivo (Arese *et al*., 2001; Kim *et al*., 2003; Oshima *et al*., 2000; Pu *et al*., 2003). In addition to its effect upon viral gene transcription, Tat is capable of modulating the expression of several host genes, including genes which regulate inflammatory responses.

It also should be noted that a transient in vitro or in vivo exposure to Tat is sufficient to produce cellular alterations, such as neuronal excitation, an increase in intracellular calcium or activation of glial cells (Haughey *et al*., 2001; Jones *et al*., 1998; Nath *et al*., 1999). It was demonstrated that a single injection of Tat into the brain can cause progressive neuropathological changes for several days, even though the Tat protein itself cannot be detected within 6 h of injection (Jones *et al*., 1998). This Tat-induced pattern of cellular alterations was called the "hit and run" phenomenon (Nath *et al*., 1999). Results of these experiments further support observations that HIV-induced biological responses, including neuropathological effects, are not directly correlated with viral load and/or viral protein levels. Potential mechanisms of Tat-induced injury to BMEC and the disruption of the BBB are illustrated in Fig. 1.

Role of Tat Protein in Induction of Inflammatory Processes in Brain Endothelial Cells

As stipulated in the "Trojan horse" theory of HIV entry into the CNS via infected lymphocytes and/or monocytes, inflammatory responses within the brain vasculature have critical significance for the development of neuropathology associated with HIV infection (Fig. 1). Evidence indicates that Tat may be an important mediator of HIV-mediated inflammatory responses (Arese *et al*., 2001; Bruce-Keller *et al*., 2001; Kruman *et al*., 1998, 1999; Rappaport *et al*., 1999). We and others have reported that brain injections of Tat can stimulate infiltration of mononuclear cells and inflammatory responses both through the blood vessels and through the choroid plexus (Fig. 2) (Jones *et al*., 1998; Pu *et al*., 2003). In addition, several in vitro studies based on a variety of non-CNS cell types have demonstrated stimulatory effects of Tat on expression of a variety of inflammatory genes, such as MCP-1 (Conant *et al*., 1996), TNF-α transforming, growth factor-β (Sawaya *et al*., 1998) and adhesion molecules (Pu *et al.,* 2003). Other cytokines and chemokines which can be induced by Tat include TNF- β, IL-2, IL-6, CXCR4, chemokine receptor 5, IL-2 receptors, and IL-4 receptors (Buonaguro *et al*., 1992; Ehret *et al*., 2001; Gibellini *et al*., 1994; Husain *et al*., 1996; Sastry *et al*., 1990; Scala *et al*., 1994; Secchiero *et al*., 1999; Weiss *et al*., 1999).

MCP-1 appears to be one of the most important chemokines in the pathogenesis of HIV infection and its levels are markedly and consistently elevated in the brains and CSF of patients with HAD (Cinque *et al*., 1998; Kelder *et al*., 1998). MCP-1 stimulates chemotaxis and transmigration of monocytes, lymphocytes, and

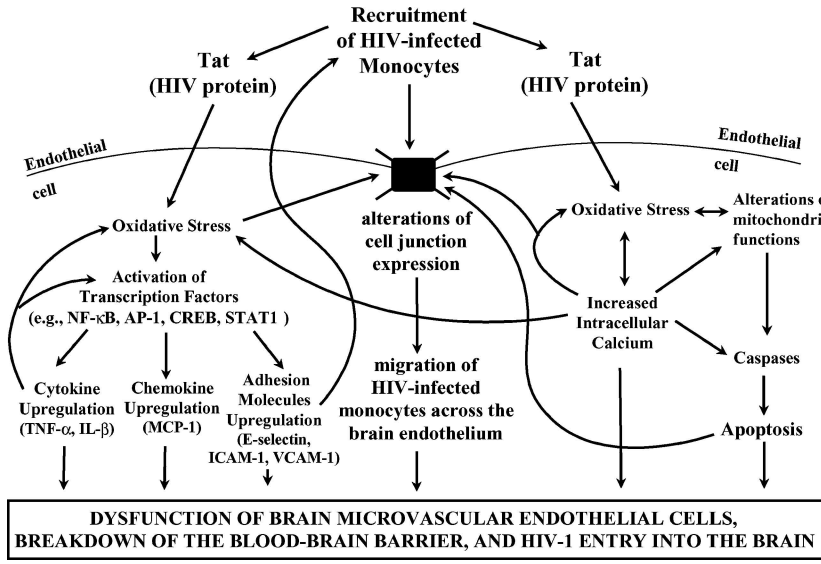

Fig. 1. Schematic diagram of the described mechanisms of Tat-induced endothelial cell dysfunction and the breakdown of the BBB. Tat can induce oxidative stress in brain endothelial cells and stimulate activation of oxidative stress-related responses (e.g., activation of transcription factors and expression of inflammatory genes) and levels of intracellular calcium. Upregulation of inflammatory cytokines can further contribute to cellular oxidative stress and activation of transcription factors, leading to potentiation of inflammatory responses and recruitment of HIV-infected monocytes, which produce Tat protein. In addition, Tat can alter expression of cell junction proteins and thus disrupt integrity of the BBB. Activation of caspases and apoptosis of brain endothelial cells can further exacerbate loss of BBB integrity. Tat-induced breakdown of BBB may allow the entry of HIV-infected monocytes into the CNS. Abbreviations: AP-1, activator protein-1; CREB, cAMP responsive element binding protein; ICAM-1, intercellular adhesion molecule-1; IL-1 β, interleukin-1 β; MCP-1, monocyte chemoattractant protein-1; NF- κB, nuclear factor- κB; STAT1, signal transducers and activators of transcription; TNF-α, tumor necrosis factor-α; VCAM-1, vascular cell adhesion molecule-1.

granulocytes (Mukaida *et al.*, 1998) and may be involved in upregulation of adhesion molecules and cytokines (Jiang *et al.*, 1992). MCP-1 expression also is known to be regulated by cellular redox status. The MCP-1 promoter contains binding sequences for both NF- κB and activator protein-1 (AP-1) (Shyy *et al.*, 1990) and both of these transcription factors were shown to regulate induction of the MCP-1 genes (Martin *et al.*, 1997). Due to chemotactic properties of MCP-1, it can be assumed that Tat-induced strong overexpression of this chemokine can lead directly to monocyte transmigration across the brain endothelium. To support the role of MCP-1 in monocyte trafficking, recent in vitro studies provided evidence that Tat-induced MCP-1 expression can induce monocyte transmigration across a model of the BBB (Weiss *et al.*, 1999).

Research on Tat has been concentrated primarily on its injury induction to astrocytes and neurons and only limited information is available on the influence of this protein on endothelial cell metabolism. It was demonstrated that cellular

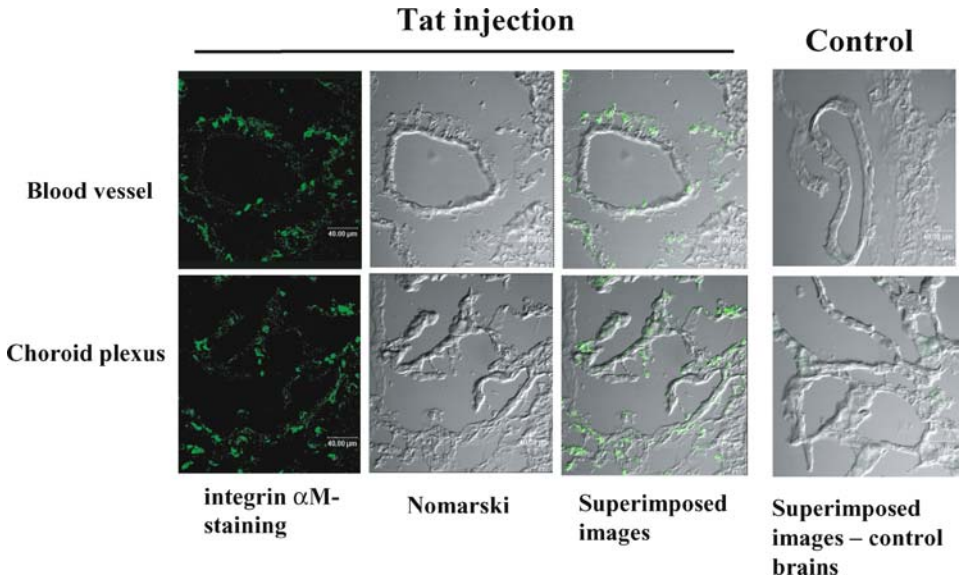

Fig. 2. Tat stimulates infiltration of monocytes into the brain. Tat (25 μg/μL) was injected into the mouse hippocampus and monocyte migration was studied 24 h after the injection. The brain vessels and the choroid plexus were visualized using the Nomarski technique and/or stained for the presence of the integrin α M-immunoreactive cells (marker of monocytes and macrophages). Superimposed images of the corresponding optical sections showed increased migration of the integrin αM-immunoreactive cells (stained in green). Only single monocytes/macrophages were detected in the corresponding sections in the control animals. Modified from Pu *et al.* (2003).

exposure to Tat can activate BMEC and induce expression of E-selectin as well as production of IL-6 and plasminogen activator inhibitor-1 (PAI-1) (Hofman *et al.*, 1994; Zidovetzki *et al.*, 1998). In addition, it was shown that Tat-induced IL-6 mRNA expression in BMEC is regulated by a signal transduction mechanism which can involve protein kinase C and cAMP-dependent protein kinase (PKA) (Zidovetzki *et al.*, 1998). We demonstrated that exposure of BMEC to Tat results in a dose-dependent increase in cellular oxidative stress, a decrease in intracellular glutathione and activation of NF-κB (Toborek *et al.*, 2003). Tat-induced activation of NF-κB also was confirmed in other cell types (Bruce-Keller *et al.*, 2001; Conant *et al.*, 1998; Nath *et al.*, 1999). As discussed previously, NF-κB is a transcription factor which regulates expression of several adhesion molecules, inflammatory cytokines, and chemokines.

Our research also demonstrated that Tat can activate DNA binding and trans-activation of AP-1 in cultured BMEC (Toborek *et al.*, 2003). AP-1 also is a classical transcription factor in which activity is regulated by cellular redox status and which is involved in the regulation of cellular inflammatory responses. Tat-mediated activation of AP-1 in endothelial cells is in agreement with literature reports (Kumar *et al.*, 1998). For example, it was shown that Tat can induce expression of *c-fos* mRNA and protein as well as AP-1 binding activity in Jurkat cells (Gibellini *et al.*, 2001; Manna and Aggarwal, 2000). Specific mechanisms of Tat-induced AP-1 activation are not known. However, it was shown that Tat can induce the mitogen-activated protein

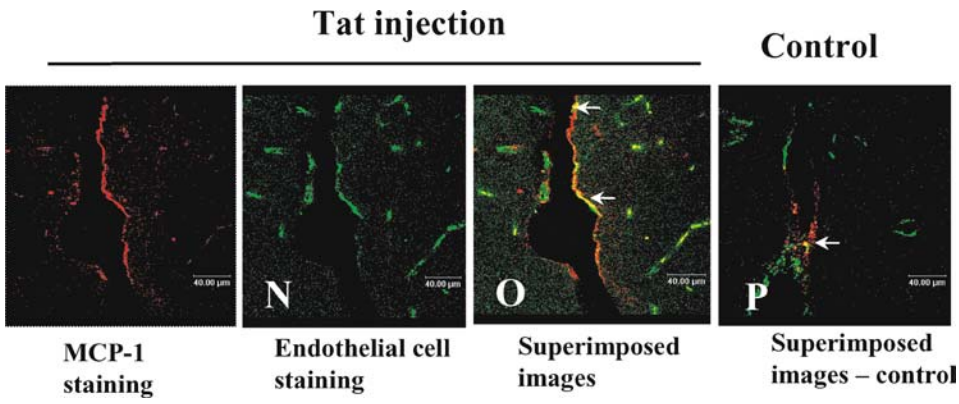

Fig. 3. Tat induces expression of MCP-1 on the brain endothelium. Tat (25 μg/ μL) was injected into the mouse hippocampus and MCP-1 expression was studied 24 h after the injection. The brain sections were stained for MCP-1 or von Willebrand factor (marker of endothelial cells). Regions of the colocalization, which reflect the additive effect of superimposed red and green pixels, are depicted in yellow (arrows). Increased MCP-1 immunoreactivity was observed in Tat-injected animals. Only slight constitutive expression of MCP-1 was detected on endothelial cells of control animals. Modified from Pu *et al.* (2003).

kinase (MAPK) cascade (Manna and Aggarwal, 2000) and stimulation of MAPK can lead to AP-1 activation.

We also have evidence that Tat can target the vascular system and induce inflammatory responses in vivo. To address this phenomenon, Tat was injected into the right hippocampus and induction of several inflammatory mediators was determined in brain tissue. In addition, we employed double-immunostaining techniques to determine cellular sources of the upregulated inflammatory proteins. It appeared that Tat can markedly upregulate expression of MCP-1 (Fig. 3) and VCAM-1 on brain endothelial cells. In contrast, Tat-induced expression of TNF- α and ICAM-1 was not associated with brain vasculature (Pu *et al.*, 2003).

Effects of Tat Protein on Endothelial Cell Apoptosis and Viability

Using different peripheral endothelial cell lines, conflicting results of Tat-mediated effects on cell proliferation were reported. For example, in a model system of immortalized peripheral endothelial cells, profound effects of Tat on stimulation of growth, migration, and morphogenesis of endothelial cells were observed. These effects resulted in enhanced angiogenesis in Tat-treated cultures (Albini *et al.*, 1996; Iurlaro *et al.*, 1998). In contrast, using a different endothelial cell line, exposure to Tat inhibited cell growth in the presence of fibronectin in the culture system. Without added fibronectin as a culture substrate, no effect of Tat on endothelial cell growth was observed. Similar results were obtained in cells transfected with the tat gene which endogenously produced Tat (Cavallaro *et al.*, 1997).

Recently, compelling evidence was presented that exposure to Tat can induce apoptosis of BMEC (Kim *et al.*, 2003). Several mechanisms, such as induction of caspases and dysregulation of nitric oxide production and activation of phosphoinositol

3-kinase (PI3 kinase) could be responsible for the proapoptotic effects of Tat in cultured endothelial cells. Indeed, inhibition of nitric oxide synthase by *N*-nitro-L-arginine methyl ester or inhibition of PI3 kinase by wortmannin attenuated Tat-induced apoptosis (Kim *et al*., 2003). In addition, using nonendothelial cells it was demonstrated that Tat can disrupt calcium homeostasis (Haughey and Mattson, 2002) and bind to tubulin/microtubules, leading to the alteration of microtubule network and activation of a mitochondria-dependent apoptotic pathway (Chen *et al*., 2002). However, comparing the proapoptotic effects of several different proteins encoded by HIV-1, it was shown that Tat can induce BMEC apoptosis to a lesser extent than gp120, Vpr, and Nef (Acheampong *et al*., 2002). Tat-mediated apoptosis could be further potentiated by coexposure to ethanol (Acheampong *et al*., 2002).

Role of Tat in the Disruption of Tight Junctions

There is only very limited information available on the effects of Tat on tight junction proteins. Due to the role of Tat protein in the pathogenesis of HIV-1 infection and since intact tight junctions are essential for maintaining a functional BBB, we studied the effects of Tat on expression and distribution of tight junction proteins in BMEC. Exposure to Tat altered distribution and/or protein levels of claudin-1, claudin-5, and ZO-2 in BMEC (Fig. 4). Among tight junction proteins studied, expression of claudin-5 appeared to be most affected in Tat-treated cultures (András *et al*., 2003). Disturbances expression of tight junction proteins may severely compromise the BBB integrity. For example, among a large family of claudins, claudin-1 and claudin-5 are believed to be most important with regards to structure and function of the tight junctions of the BBB (Liebner *et al*., 2000). Claudin-5 localizes primarily on the external leaflet of the endothelial cell membranes, claudin-1 is associated with the internal membrane leaflet (Furuse *et al*., 1998; Morita *et al*., 1999) and the tightness of the BBB may depend on the ratio of claudin-1 to claudin-5 (Liebner *et al*., 2000). Therefore, a Tat-induced decrease in both claudin-1 and claudin-5 can affect endothelial permeability and be responsible for infiltration of the CNS by inflammatory cells through the breaches in claudin continuity.

DISRUPTION OF THE BBB AS THE RESULT OF THE INTERACTION OF HIV-1 WITH SELECTED RISK FACTORS FOR HIV INFECTION

Selective populations appear to be especially prone to HIV infection. For example, drug abuse and alcoholism are common in HIV-infected patients and recent evidence demonstrates that aging may also be associated with increased risk of HIV infection. Complex factors, such as high-risk sexual behavior or compromised immune defense may be in part responsible for this phenomenon. On the other hand, abused drugs, alcohol, or aging-related factors can interact with HIV proteins at the molecular and cellular levels, compromising the integrity of the BBB, and contributing to HIV-1 neuropathology.

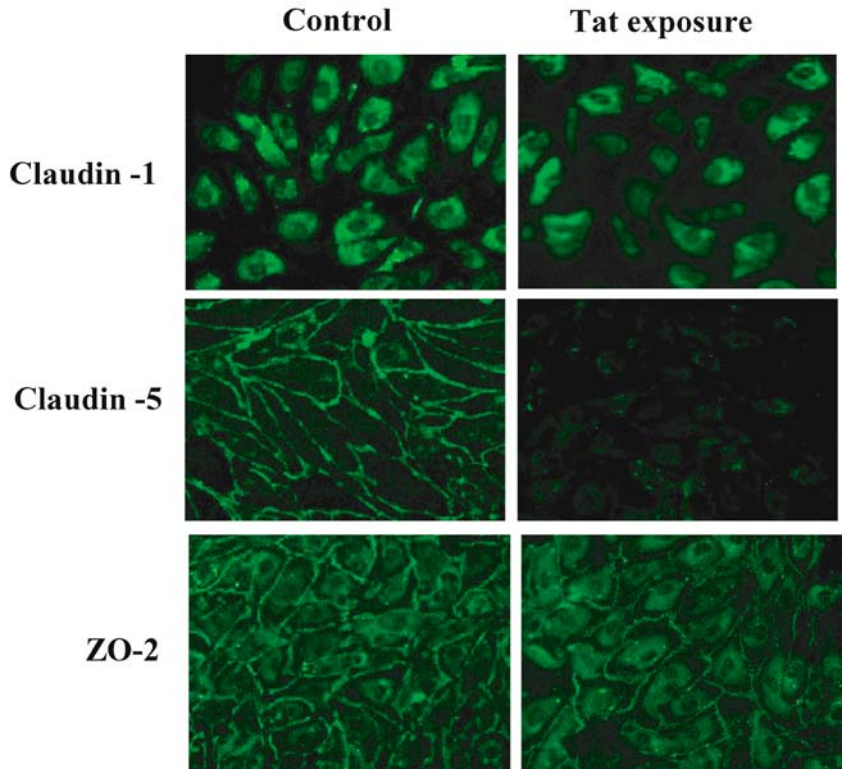

Fig. 4. Tat decreases expression and distribution of claudin-1, claudin-5, and zona occludens-2 (ZO-2) in cultured brain endothelial cells. Confluent primary cultures of BMEC were treated with 100 nM Tat for 24 h and expression of tight junction proteins was determined by immunofluorescence microscopy. Modified from Andras *et al.* (2003).

Interaction Between Tat and Abused Drugs

Drug abuse is a common risk factor for HIV infection. Apart from practices of drug users which can directly lead to HIV infection (e.g., needle-sharing and unsafe sex practices with HIV-positive partners), abused drugs can influence specific cellular and molecular processes which can further contribute to the development of AIDS. Thus, potential interactive effects of Tat and abused drugs become an emerging problem in HIV pathogenesis.

Our research primarily focused on the vascular effects of methamphetamine (METH), because it is currently the third most popular psychostimulant drug used in USA (Pulse Check, 2000), and the repeated use of METH causes neurological deficits that may lead to serious psychiatric and neurological symptoms (Lan *et al.*, 1998). It has been shown that Tat and METH exert similar toxicological profiles. For example, METH toxicity, at least in part, is mediated via generation of cellular oxidative stress (Jayanthi *et al.*, 1998). In addition, it can activate redox-responsive transcription factors such as NF- κB, AP-1, and cAMP responsive element binding

protein (CREB) in BMEC, leading to the activation of genes related to cell death and inflammatory responses (Lee *et al*., 2001). These facts raised the possibility that Tat and METH may interact in an additive or synergistic fashion to cross-potentiate their cytotoxic effects. Indeed, DNA binding activities of NF-κB, AP-1, and CREB in the frontal cortex and hippocampus were more pronounced in mice injected with Tat plus METH as compared to the effects of Tat or METH alone. In addition, in selected brain regions, but primarily in corpus striatum, exposure to Tat plus METH resulted in potentiation of TNF-α, IL-β, and ICAM-1 gene expression (Flora *et al*., 2003). These results indicate that Tat and METH can cross-amplify their cellular effects, leading to alterations of redox-regulated inflammatory pathways in the brain (Flora *et al*., 2003; Maragos *et al*., 2002). Such synergistic proinflammatory stimulation may have significant implications in HIV-infected patients who abuse drugs (Nath *et al*., 2002).

Interaction Between HIV and Alcohol Abuse

Ethanol abuse has been suggested to be correlated with enhanced development of HIV. For example, ethanol abuse is frequently associated with high-risk sexual behaviors and abuse of intravenous drugs (Biglan *et al*., 1990). Chronic alcohol abuse can induce profound pathological changes in the CNS, such as dilatation of ventricles, widening of sulci, patchy loss of neurons, disturbances of cerebral blood flow (Tyor and Middaugh, 1999) and the disruption of the BBB (Banks, 1999). Indeed, ethanol-induced vascular changes closely resemble those observed in HAD. It also has been shown that ethanol-mediated disturbance of immune function may further exacerbate HAD (Balla *et al*., 1994; Tyor and Middaugh, 1999).

Several studies demonstrated that exposure to ethanol may both increase susceptibility of cells to HIV infection and stimulate HIV replication in infected cells (Bagasra *et al*., 1989, 1996). Ethanol also can contribute to the impaired oxidative metabolism in the brains of AIDS patients (Meyerhoff *et al*., 1995) and the development of HIV/AIDS-associated encephalitis or dementia (Biglan *et al*., 1990; Fein *et al*., 1995). In support of the hypothesis of the relationship between ethanol and HIV-associated CNS pathology, clinical studies revealed that ethanol abuse can exacerbate the decline in frontal lobe physiology in HIV-positive patients (Fein *et al*., 1995). It also was recently shown that ethanol can potentiate apoptosis of BMEC by several HIV proteins, such as Nef, Vpr, Tat, and gp120 (Acheampong *et al*., 2002). Ethanol and Tat can both induce production of inflammatory cytokines through the NF-κB-mediated pathway (Jokelainen *et al*., 2001).

Interaction Between HIV and Alzheimer's Disease

Epidemiological studies provide compelling evidence that HIV/AIDS is an emerging problem in an aging population (Justice *et al*., 2001). Recently published data indicate that approximately 10% of Americans diagnosed with HIV and 14% of Americans living with AIDS are of age 50 or older (CDC; The HIV/AIDS Surveillance Report). Because older people are not tested for HIV on a regular basis, this rate could even be higher. In addition, the numbers of new cases of older people with

HIV/AIDS are rapidly growing. It is estimated that the number of people of age 65 or older with newly diagnosed AIDS increased 10-fold in the last 10 years (Justice *et al*., 2001). Due to the variety of sociological, biological and medical implications (Adler *et al*., 1997; Goodkin *et al*., 2001; Justice *et al*., 2001), this trend may even increase in the near future. These facts are of significant concern because elderly patients develop AIDS more rapidly and have higher morbidity and mortality rates (Adler *et al*., 1997). In addition, older patients are more likely to develop AIDS-associated dementia (Ferro and Salit, 1992).

HAD is characterized by cognitive and motor dysfunctions, which clinically strongly resemble symptoms of Alzheimer's disease (AD). In addition, several recent reports provided evidence that the development of AD may involve similar cellular processes as those involved in HAD. Well-known pathogenic processes associated with AD are related to the deposition of amyloid β-peptide (A β) aggregates in brain tissue, stimulation of oxidative stress, dysregulation of calcium homeostasis, inflammatory responses, apoptotic cell death, and neurodegeneration (Mattson, 2002). It is striking to notice that such cellular events are also linked to HAD. Indeed, recent evidence indicated that AD may stimulate the development of dementia in HIV-infected patients. It was demonstrated that A β plaques are prevalent in HIV-infected patients (Esiri *et al*., 1998), deposits of amyloid β precursor protein (APP) are associated with HIV replication in the brain (Nebuloni *et al*., 2001), and HIV-positive patients who possess ApoE4 allele (a known risk factor for AD and formation of A β aggregates) are more prone to the development of dementia than patients who do not express this allele (Corder *et al*., 1998). Recent reports also indicated that A β can stimulate HIV infection of CD4 positive cells (Wojtowicz *et al*., 2002), the presence of APP-rich lesions are correlated with HAD (Nebuloni *et al*., 2001), and the levels of Par-4, a protein which plays an important role in AD neurodegeneration, have also been linked to the development of HIV encephalitis (Kruman *et al*., 1999). Finally, profound vascular changes and the disruption of the BBB are hallmarks of both AD and HIV dementia.

CONCLUSION

Vascular changes and the disruption of the BBB contribute to HIV-1 neuropathology. Compromised BBB integrity may allow HIV-1 entry into the CNS with the subsequent formation of virus reservoir within the brain and potential development of encephalitis or dementia. It appears that HIV Tat protein can be in part responsible for these effects. Indeed, evidence indicates that Tat can induce oxidative stress, compromise viability and disrupt tight junctions in brain endothelial cells. In addition, Tat can interact with abused drugs or aging-related factors and thus contribute to the development of HIV neuropathology in selective populations prone to HIV infection.

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