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Mechanisms of leukocyte trafficking into the CNS

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HIV-1 encephalitis occurs in up to one-third of HIV-1-infected individuals. The mechanisms through which this pathology develops are thought to involve viral passage across the blood-brain barrier (BBB), as well as entry of HIV-infected and/or uninfected inflammatory cells into the central nervous system (CNS). Viral proteins and cytokines may also contribute to the pathogenesis of encephalitis. We show that the chemokines SDF-1 and MCP-1 induce transmigration of uninfected human lymphocytes and monocytes across our model of the BBB, a co-culture of human fetal astrocytes and endothelial cells. We also demonstrate that the HIV-1 protein Tat induces adhesion molecule expression and chemokine production by human fetal astrocytes and microglia, which could further contribute to leukocyte entry into the CNS. Finally, our data indicate that inflammatory cytokines modulate the expression of CXCR4, a co-receptor for HIV-1, on human fetal astrocytes, suggesting that these cytokines may potentially modulate the infectability of astrocytes by HIV-1. These findings support the hypothesis that there may be several different mechanisms that contribute to the development and progression of HIV-1 encephalitis. *Journal of NeuroVirology* (2000) **6,** S82–S85.

Keywords: blood-brain barrier; leukocyte transmigration; tat; adhesion molecules; chemokines; CXCR4

Introduction

A major complication of HIV-1 infection, particularly in children, is encephalitis, with approximately one-third of those infected with HIV-1 developing HIV-1 encephalitis (Lipton and Gendelman, 1995). The mechanisms that mediate HIV-1 infection of the CNS as well as the entry of inflammatory cells into the CNS are still not well understood, although crossing of the blood-brain barrier (BBB) is most likely involved. It is also unclear whether cellular components of the BBB are productively infected with HIV-1. To address these issues we developed a tissue culture model of the BBB using human astrocytes and endothelial cells (EC) cocultured on opposite sides of a porous membrane (Hurwitz *et al*, 1993). We demonstrated that certain chemokines mediate the transmigration of specific sets of leukocytes across this coculture system, thereby establishing a model for inflammatory cell infiltration of the CNS (Weiss *et al*, 1998). In addition, we showed that the HIV-1-derived protein Tat facilitates leukocyte transmigration (Weiss *et al*, 1999). Our data further demonstrate that astrocytes, a key cell in the structure and integrity of the BBB, as well as microglia, respond to Tat by elaborating chemokines. Astrocytes also express surface adhesion molecules in response to Tat that may further direct leukocytes within the CNS parenchyma (Woodman *et al*, 1999). In addition, we demonstrated that cytokines found within the CNS of HIV-1 infected individuals modulate the expression of the HIV-1 co-receptor, CXCR4, on astrocytes, and therefore may facilitate not only leukocyte transmigration but also infection of astrocytes.

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SDF-1 and MCP-1 mediate leukocyte transmigration across a coculture model of the BBB

We developed a coculture model of the BBB in which human fetal astrocytes and human umbilical vein endothelial cells are grown on opposite sides of a tissue culture membrane with 3 μm pores, which allows the astrocytes to be in physical proximity to the endothelial cells. When astrocyte foot processes are in contact with the EC, the EC express BBB-specific properties, such as Glut-1 and γ-G1. In addition, the coculture exhibits barrier properties, as demonstrated by studies with [^{3}H]-Inulin (Hayashi *et al*, 1997) or with Evans Blue Dye coupled to BSA (Kevil *et al*, 1998). EC or astrocytes cultured alone on inserts, or together on a membrane with pores too small for astrocyte foot processes to traverse the membrane, do not demonstrate BBB properties.

Using this model, different chemokines are added to the bottom chamber of the coculture, while 3×10^5 unactivated PBMC obtained from healthy adult volunteers are added to the top chamber. The number of PBMC that transmigrate to the lower chamber in a given time is then analyzed by flow cytometry. We demonstrated that MCP-1 (100 ng/ml) mediates predominantly monocyte transmigration across the coculture after 2.5 h) and that the adhesion protein ICAM-1 is essential for this process (Weiss *et al*, 1998).

SDF-1 acts as a chemoattractant for both resting (unactivated, naive), and activated T cells, as well as for monocytes (Bleul *et al*, 1996). SDF-1 acts on a repertoire of unactivated and activated cells, and therefore it may participate in the early recruitment of mononuclear cells into the CNS, and it may also act to amplify ongoing cell recruitment. Lymphocytes exhibited an exuberant response to SDF-1 (50 ng/ml) after 4 h (2081 ± 1129 lymphocytes transmigrated in the presence of SDF-1 *vs* 199 ± 116 lymphocytes transmigrated when no SDF-1 was present) (Figure 1), with minimal monocyte transmigration (857 ± 236 monocytes transmigrated in the presence of SDF-1 *vs* 506 ± 35 monocytes transmigrated when no SDF-1 was present). It is unclear whether SDF-1 directly mediates monocyte transmigration or whether SDF-1-induces additional chemoattractants to facilitate their transmigration.

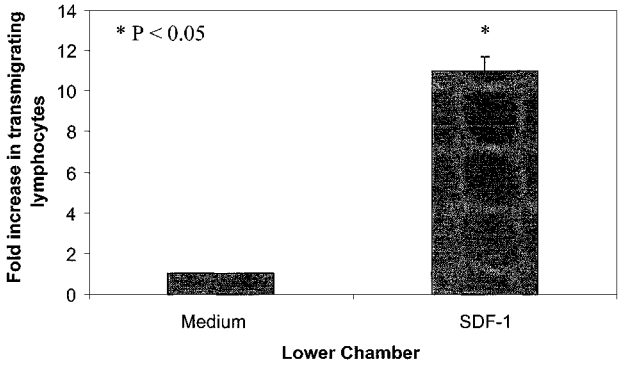

Figure 1 SDF-1 is a potent chemoattractant for lymphocyte across the BBB. SDF-1 (50 ng/ml) was placed in the lower chamber of a BBB co-culture, which consists of human fetal astrocytes and an EC monolayer grown on opposite sides of a 3 μm porous membrane on a transwell insert. The astrocytes and EC were obtained from different donors. PBMC obtained from healthy adult volunteers were placed in the upper chamber and allowed to transmigrate in response to the chemokine for 4 h. Transmigrated PBMC were distinguished by flow cytometry: monocytes (CD45+/CD14+) and lymphocytes (CD45+/CD14−). *P*-value was calculated using Student's two-tailed, paired *t*-test (n=3).

Tat-mediated transmigration, chemokine and adhesion molecule production

The HIV-1 transactivator protein, Tat, is known to be present in the serum of HIV-1-infected indivi-

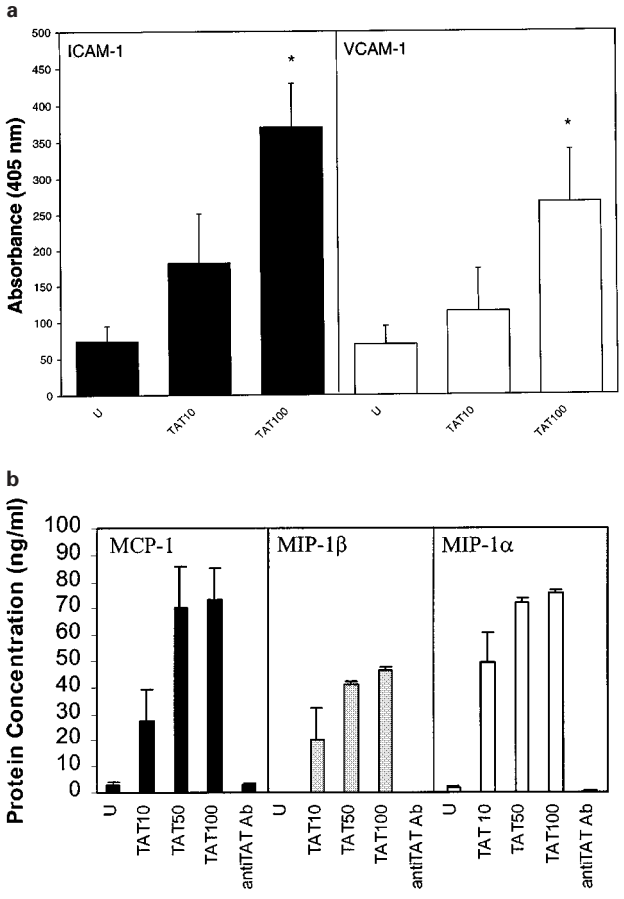

Figure 2 (**a**) HIV-1 Tat induced ICAM-1 and VCAM-1 expression by astrocytes. Astrocytes were treated with 10 ng/ml (TAT10) or 100 ng/ml (TAT100) of Tat for 24 h. ICAM-1 and VCAM-1 induction was demonstrated by cell-based ELISA (n=7, $P \leq 0.05$) using Wilcoxon Sign Rank Test. (**b**) HIV-1 Tat induced MCP-1, MIP-1α, MIP-1β expression by microglia. Microglia were treated with 10 ng/ml (TAT10), 50 ng/ml (TAT50), or 100 ng/ml (TAT100) of Tat for 24 h. MCP-1 (black), MIP-1α (grey), MIP-1β (white) induction was demonstrated by protein ELISA. Treatment of microglia with 100 ng/ml of Tat that was immunoeluted with purified rabbit anti-Tat antibody abrogated the expression of each of these chemokines (representative experiment shown).

duals (Wesselingh *et al*, 1993; Wiley *et al*, 1996) and its transcript is elevated in brains of patients with HIV-1 dementia (Westendorp *et al*, 1995; Piller *et al*, 1998). Tat added to the bottom well of the coculture model induced astrocytes to produce MCP-1, resulting in monocyte transmigration across the BBB model. This Tat-induced transmigration can be blocked by antibodies to MCP-1 (Weiss *et al*, 1999). In addition, we and others demonstrated that Tat induces adhesion molecule expression by EC (Dhawan *et al*, 1997) and astrocytes (Woodman *et al*, 1999) (Figure 2a), and that these adhesion proteins facilitate leukocyte transmigration *in vitro*. These *in vitro* data are supported by the finding of increased density of ICAM-1 and VCAM-1 positive EC and astrocytes in ADC patients (Seilhean *et al*, 1997). MCP-1, MIP-1α and MIP-1β have been detected in the CNS and cerebral spinal fluid of HIV-infected individuals (Bernasconi *et al*, 1996; Nuovo and Alfieri, 1996; Conant *et al*, 1998). We demonstrated that Tat induces MCP-1, MIP-1α and MIP-1β chemokine expression by cultured astrocytes and microglia, which would enhance transmigration and perhaps modulate the infectability of CNS microglia and leukocytes (McManus *et al*, submitted) (Figure 2b).

Cytokines modulate CXCR4 expression on astrocytes

It is still controversial as to whether cellular components of the BBB can be productively infected with HIV-1. Astrocytes express the chemokine receptor CXCR4, which is also a coreceptor for HIV-1 (Feng *et al*, 1996; Gabuzda *et al*, 1998) We demonstrated that IL-1β and γ-IFN, cytokines found within the CNS of HIV-infected humans and/or SIV-infected monkeys (Tyor *et al*, 1992; Lane *et al*, 1996), modulate the surface expression of this receptor, and may therefore contribute to the infectability of astrocytes. IL-1 upregulates CXCR4 surface expression, while γ-IFN downmodulates both constitutive and IL-1-induced expression (Figure 3). We are currently examining the functional consequences of this regulation.

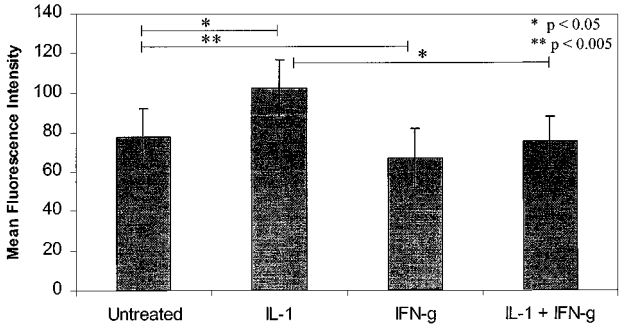

Figure 3 IL-1 increases cell surface expression of CXCR4 by fetal astrocytes at 16 h. IFN-γ not only decreases the basal level of CXCR4 expression, but also negates the inductive effect of IL-1 at 16 h. Human fetal astrocytes were treated with IL-1 (2 U/ml) and/or IFN-γ (200 U/ml) for 16 h, then analyzed for CXCR4 expression by flow cytometry. Primary antibodies were CXCR4 (12G5) and mouse myeloma IgG2a (isotype-matched control antibody). *P*-values were calculated using Student's two-tailed, paired *t*-test (n=4).

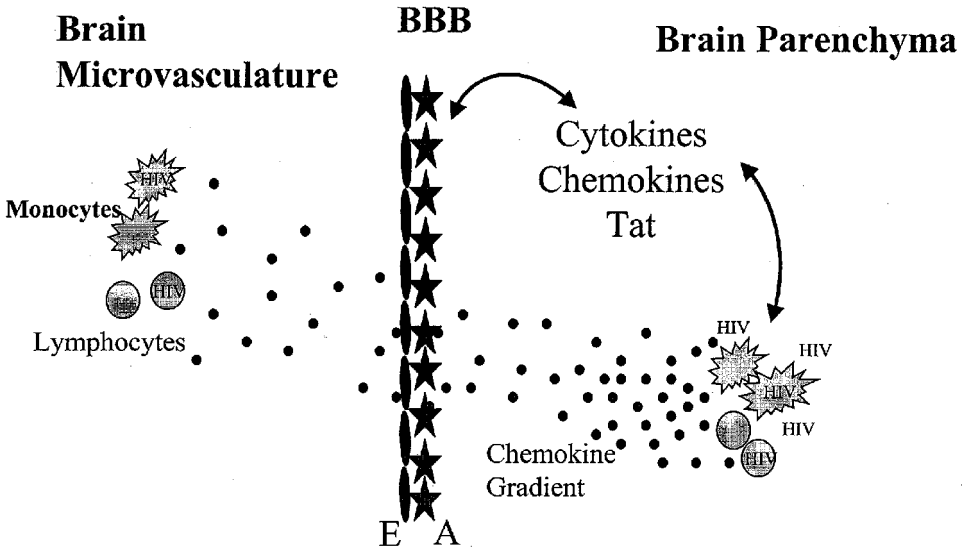

Figure 4 Mechanisms for entry of HIV-infected leukocytes into the central nervous system. There is normal trafficking of leukocytes into the CNS, potentially in response to constitutive SDF-1 production. As part of this process, HIV-infected leukocytes may also enter the brain. The interaction between infected cells, normal leukocytes, and the surrounding glia may induce production/upregulation of chemoattractants, cytokines, and adhesion molecules, which could contribute further to CNS entry of leukocytes and HIV. These chemoattractants include chemokines such as MCP-1 and SDF-1, and HIV products such as Tat protein. In addition, the cytokines IL-1 and IFN-γ may modulate the expression of chemokine recepotrs and adhesion molecules on astrocytes and endothelial cells, augmenting the influx of leukocytes. Since both astrocytes and endothelial cells express the chemokine receptor CXCR4, a co-receptor for HIV-1, cytokine-mediated modulation of CXCR4 may result in altered susceptibility to HIV infection. E=Endothalial cells, A=Astrocytes.

Summary
NeuroAIDS is a major complication of HIV-1 infection. The mechanisms by which the CNS becomes infected are still unclear, but the subsequent inflammation associated with CNS infection contributes significantly to the disease process. Leukocyte trafficking into the CNS, either of infected or uninfected cells, is facilitated by the expression of chemokines within the CNS parenchyma, as well as adhesion proteins on the surface of both endothelial cells and astrocytes. We demonstrate that the HIV-1-derived protein Tat can induce the expression of these mediators by cells of the CNS, and therefore may contribute to the ongoing recruitment of cells into the brain, augmenting inflammation. In addition, proinflammatory factors within the CNS can modulate the expression of chemokine receptors on the surface of BBB components as well as on infiltrating cells, further facilitating their susceptibility to HIV infection. Figure 4 is a schematic representation of this ongoing process.

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