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Human immunodeficiency virus type 1 transport across the in vitro mouse brain endothelial cell monolayer

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Abstract

Human immunodeficiency virus type 1 (HIV-1) is associated with a neuroinflammatory dementia. Cognitive impairment remains a common complication of late-stage HIV-1 infection. Previous studies have shown that entry of HIV-1 into the central nervous system (CNS) occurs soon after infection. For these reasons, it is important to understand how HIV-1 crosses the BBB. We used primary mouse brain microvessel endothelial cell (MBEC) monolayer models to study interactions between brain endothelial cells and radioactively labeled HIV-1 CL4 (^{131}I-HIV-1), which had been rendered noninfectious with aldithiol, and compared to radioactively labeled bovine serum albumin (^{131}I-BSA or ^{125}I-BSA) and detected HIV-1 on MBEC monolayer with electron microscopic analysis. The permeability of the monolayers to HIV-1 was measured by determining the percent material transported (PMT). Luminal to abluminal PMT of ^{131}I-HIV-1 was 4.65 times greater than that of the much smaller ^{131}I-BSA, showing that the MBEC monolayer is more permeable to HIV-1 than to BSA. Electron microscopy showed that HIV-1 was transported through a trans-cellular pathway from luminal side to basolateral space with some virus associated with the nucleus. Unlabeled HIV-1 did not affect the transport of ^{131}I-HIV-1 or break down the MBEC monolayer. Wheatgerm agglutinin (WGA) increased ^{131}I-HIV-1 penetration across the MBEC monolayer, consistent with absorptive endocytosis as the mechanism for HIV-1 penetration. The enhanced transport of HIV-1 was unidirectional, as the abluminal to luminal PMT of ^{131}I-HIV-1 was not different from that of BSA nor enhanced by WGA. Characterization of the radioactivity transported from the luminal to abluminal chamber on Sepharose 4B-200 columns showed the transported radioactivity represented intact virus. MBEC monolayers preloaded from the luminal surface with ^{131}I-HIV-1 showed most of the virus was retained by the endothelial cells, while the remainder was effluxed mainly to the luminal surface. MBEC monolayers preloaded from the abluminal surface with ^{131}I-HIV-1 retained little virus and most of the virus was effluxed mainly to the abluminal surface. In conclusion, cell-free, intact ^{131}I-HIV-1 crossed brain endothelial cell monolayers unidirectionally in the luminal to abluminal direction through an adsorptive endocytotic pathway. HIV-1 taken up from luminal side by monolayers of brain endothelial cells was mainly released to the luminal side. HIV-1 efflux mechanisms are different from influx mechanisms.

Keywords: Human immunodeficiency virus type 1 (HIV-1); AIDS; Blood–brain barrier (BBB); Brain endothelial cell monolayer; In vitro model; Electron microscopy; Adsorptive endocytosis; Transport mechanism; gp120

Introduction

AIDS dementia complex is a major problem in human immunodeficiency virus type 1 (HIV-1)-infected individuals. HIV-1 is associated with a neuroinflammatory dementia. Cognitive impairment remains a common

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complication of a late stage of HIV-1 infection (Pessin et al., 2003). Up to half of HIV-1-positive individuals demonstrate neuropathological findings at autopsy. The blood–brain barrier (BBB) can retard the passage of compounds and pathogens from the blood into the central nervous system (CNS). Brain endothelial cells express many barrier functions and are an essential component of the BBB. T cell surface antigen CD4 is an essential component of the receptor of HIV-1 (Dalgleish et al., 1984; Klatzmann et al., 1984), and CCR5 and CXCR4, which are the seven-transmembrane G-protein-coupled chemokine receptors, are the major co-receptors for HIV-1 (Dragic et al., 1996; Feng et al., 1996). HIV-1 entry into a target cell requires gp120 and the CD4 receptor as well as the co-receptor CCR5/CXCR4 (McMichael and Hanke, 2003). However, adult brain endothelial cells are weakly positive for CD4 and negative for CXCR4 and CCR5 (Willey et al., 2003). Therefore, brain endothelial cells express other unknown molecular targets used by HIV-1 for invading the brain.

Previous studies have shown that HIV-1 enters the CNS soon after infection. Although the brain endothelial cells do not express the major receptor molecules for HIV-1, the virus can cross the BBB. For cell-free virus, gp120, the viral coat glycoprotein, plays an essential role in that transport across the BBB (Banks et al., 2001). However, the molecular mechanisms for HIV-1 entry into brain endothelial cells may be in other ways fundamentally different from that of viral entry into T cells (Argyris et al., 2003). Adsorptive endocytosis (AE) is a main pathway by which HIV-1 enters brain endothelial cells (Banks and Kastin, 1998; Banks et al., 1997, 1998, 2001). HIV-1 is known to be taken up and released by brain endothelial cells (Banks et al., 2001; Liu et al., 2002). However, fundamental questions remain about such uptake and release. For example, it is unclear the degree to which HIV-1 is degraded within the brain endothelial cell, whether uptake and transport disrupt the BBB, and whether transcytosis of virus is related to the adsorptive endocytosis, which seems to control initial internalization. It is also unclear whether virus taken up by the brain is subsequently released from the luminal or abluminal surface of brain endothelial cells (Banks et al., 2001). The former would simply be recycling virus back into the circulation, whereas the latter would constitute transcytosis across the BBB.

It is also not known whether HIV-1 can cross brain endothelial cells in the brain to blood direction. Since it is assumed that the brain acts as a reservoir, protecting virus circulating anti-virals, it is important to know what route HIV-1 can take to reenter the circulation. Understanding the cellular pathways HIV-1 uses is important to an understanding of the pathology of neuro-AIDS and could provide new targets for clinical therapy. Here, we used in vitro mouse brain endothelial cell (MBEC) monolayers to investigate these pathways.

Materials and methods

Mouse brain endothelial cell monolayer and HIV-1 uptake

HIV-1 CL4 was rendered noninfective by covalently binding the nucleocapsid zinc finger motifs with 2,2′-dithiodipyridine (aldrithiol-2; AT-2). AT-2 inactivated HIV-1 retains conformational and functional integrity of envelope proteins (Arthur et al., 1998; Rossio et al., 1998). HIV-1 CL4 was radioactively labeled with ^{131}I by the chloramine-T method, a method that preserves viral coat glycoprotein activity (Frost, 1977; Montelaro and Rueckert, 1975). One mCi of ^{131}I (New England Nuclear, Boston, MA), 10 μg of chloramine-T, and 5 μg of the virus was incubated together for 60 s (Banks et al., 2001). The iodinated virus was purified by filtration on Sepharose 4B-200 (Sigma-Aldrich Co., St. Louis, MO) columns. BSA (5 μg) was labeled by the chloramine-T method. One mCi of ^{131}I or ^{125}I, 10 μg of chloramine-T, and 5 μg of BSA were incubated together for 60 s. The iodinated BSA was purified by filtration on 4B-200 columns (Banks et al., 2001).

The protocol for isolating mouse brain endothelial cells (MBEC) (Banks et al., 2004) was modified from that for rat brain endothelial cells (Abbott et al., 1992; Deli and Joo, 1996; Kis et al., 2002). Brains from anesthetized CD-1 mice were cleaned of meninges and homogenized with a hand-held scalpel. The homogenate was digested in a collagenase solution (1 mg/ml collagenase type 2 in 288 U/ml of DNase I; Sigma Chemical Company) at 37°C for 60 min. Neurons, astrocytes, and Schwann cells were removed by centrifuging in DMEM with 20% BSA. The partially purified mixture was digested again (1 mg/ml collagenase/dispase with 288 U/ml DNase I at 37°C for 30 min). Finally, the endothelial cells were purified on a 33% Percoll gradient (Amersham Biosciences) centrifuged at 1000 × *g* for 10 min.

The MBEC were placed in culture dishes (Falcon) coated with 0.1 mg/ml fibronectin (Sigma) and incubated at 37°C with 5% CO_2 (Banks et al., 2004; Kis et al., 2002) in endothelial cell culture medium [20% plasma derived serum (PDS, Quad Five MT) containing 1 ng/ml basic fibroblast growth factor (Sigma) and DMEM] (Banks et al., 2004). Cell culture medium was changed every 2–3 days. MBEC were typically 70–80% confluent by day 7.

MBEC (4.0×10^4 cell/insert) cultured to 70–80% confluence were added to Transwell™ culture inserts (Coster, 24-well format, 3470) and cultured for 3 more days. Transwells had a culture plate (abluminal side) volume of 0.6 ml, an insert volume of 0.1 ml, and polyester membrane pore size of 0.4 μm. ^{131}I-HIV (1×10^6 cpm/ml), ^{131}I-BSA (1×10^6 cpm/ml) or ^{125}I-BSA (1×10^6 cpm/ml) was added to the luminal or abluminal chamber. The side opposite to that to which the radioactive material was added was the collecting chamber. The collecting chamber was sampled 2, 5, 10, 15, 20, and 30