Journal of NeuroVirology, 8(suppl. 1): 43–44, 2002
 ISSN 1355–0284/02 $12.00+.00
DOI: 10.1080/13550280290049985

Basic Science Workshop 5

Viral proteins and blood brain barrier

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The HIV protein Tat-induced induction of inflammatory responses in the brain tissue

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Impaired function of the brain vasculature and the breakdown of the blood-brain barrier might be critical factors in the mechanisms of HIV-1 virus trafficking in the central nervous system (CNS). Evidence indicates that the viral gene product, protein Tat, is partially responsible for these effects. In the present study, we tested the hypothesis that Tat can upregulate adhesion molecules and inflammatory cytokines and, thereby facilitating entry of infected monocytes to the brain. C57BL/6 mice were injected with 25 mg Tat into the right hippocampus, and expression of inflammatory mediators such as adhesion molecules (ICAM-1 and VCAM-1), chemokines (MCP-1) and inflammatory cytokines (TNF-a) were determined by RT-PCR and immunocytochemistry. The most marked induction of VCAM-1, MCP-1 and TNF-a protein expression was observed at the site of Tat injection. However, induction of the ICAM-1, MCP-1 and TNF-a genes also was evident in discrete regions of the brain, such as hippocampus, frontal cortex, and corpus striatum. A series of double immunostaining studies was performed to determine the localization of inflammatory mediator expression in the brain tissue. These experiments revealed strong inflammatory responses from macrophages/microglia and astrocytes. Vascular brain endothelium also overexpressed inflammatory mediators; however, this expression was less intense as compared to perivascular macrophages or glial cells. Our studies also revealed a strong infiltration of vascular monocytes into the brain tissue in response to Tat injections. These data demonstrate that intracerebral administration of Tat can induce profound proinflammatory effects, associated with monocyte infiltration, in the brain tissue. These results are relevant to the mechanisms of the blood-brain barrier breakdown and HIV entry into the CNS. (Supported by grants from NINDS and NIMH).

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The blood-brain barrier in the neuropathogenesis of HIV-1 infection

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Objectives: The presence of perivascular monocytic infiltration is a major hallmark of HIV-associated dementia (HAD) and therefore the function and properties of the blood-brain barrier (BBB) during HAD were investigated.

Methods: Immunohistochemical staining of brain tissue obtained from AIDS patients with and without HAD was performed. Cocultures of HIV-infected macrophages and primary human brain microvascular endothelial cells (HBMECs) were also performed in order to mimick pathophysiological processes that are relevant for the neuropathogenesis of HIV-1 infection.

Results: Immunohistochemical analysis for zonula occludens-1 (ZO-1), a tight junction protein, and CD68, a macrophage marker, on brain tissue obtained from AIDS patients revealed that loss of tight junction immunoreactivity was highly correlated with monocyte infiltration in HAD. Coculturing of HIV-infected macrophages and primary HBMECs resulted in immune activation as indicated by increased expression of interleukin-1b (IL-1b). It was also found that the endothelial adhesion molecule E-selectin was induced in cocultures of HIV-infected macrophages and HBMECs when compared to uninfected cocultures. In addition, cocultures of HIV-infected macrophages and brain endothelium showed an up-regulation of cyclooxygenase-2 (COX-2) expression by both cell types. This up-regulation occurs via an IL-1b-dependent mechanism in macrophages and via an IL-1b-independent mechanism in endothelial cells. Furthermore, interactions between HIV-infected macrophages and HBMECs resulted in an up-regulation of monocyte chemotactic protein-1 (MCP-1), macrophage inflammatory protein (MIP)-1a, MIP-1b and RANTES during cell-cell contact as well as in a trans-well system. Analogously to the COX-2 expression data, macrophage, but not endothelial, chemokine expression was altered by a neutralizing anti-IL-1b antibody.

Conclusion: These data suggest that the presence of perivascular macrophages results in immune activation and loss of the BBB thereby facilitating infiltration of more monocytic cells into the brain hence enhancing disease progression.

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Caspase inhibition activates HIV in latently infected cells

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Stimulation of TNF-R1 triggers both caspase-dependent and caspase-independent signaling activities. The caspase-dependent signaling pathway induces apoptotic cell death in susceptible cells, whereas the caspase-independent signaling cascade leads to activation of NF-kB and induces anti-apoptotic signaling activities. Stimulation of NF-kB via TNF-R1 is known to activate HIV replication in infected cells. Here we show that the broad range caspase inhibitor ZVAD activates HIV replication in the chronically infected T cell line ACH-2. Virus activation was caused by a sensitization of TNF-R1 towards endogenously produced TNF-a. Neutralizing anti-TNF-a antibodies completely abolished the virus inducing activity of ZVAD. Treatment of cells with TNF-a in the presence of ZVAD caused increased expression of TNF-a and induced enhanced virus replication. Activation of CD95, another member of the TNF-receptor family, similarly triggered HIV replication, which was further enhanced in the presence of ZVAD. Our data show that caspase inhibitors sensitize both CD95 and TNF-R1 to mediate activation of HIV in latently infected cells. Activation of HIV replication in latent virus reservoirs is currently discussed as a therapeutic strategy to achieve eradication of HIV in patients treated with antiretroviral therapy. Our results point to a novel role for caspase inhibitors as activators of virus replication *in vivo*.

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IL-1beta-activated astrocytes express Fas Ligand: novel pathways to neuronal injury in HIV-1-associated dementia

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Astrocytes function as immune effector cells of the brain and participate in both brain homeostasis and disease pathogenesis. Indeed, astrogliosis is a prominent feature of all types of neural injury including HIV-1-associated dementia (HAD). Based on these observations, we hypothesized that activation of astrocytes with pro-inflammatory stimuli such as IL-1 beta may convert these cells into neurotoxic immune effectors. We utilized primary human astrocytes and neurons to study whether secretory factors produced by IL-1 beta-activated astrocytes cause neuronal injury. Neurotoxicity was analyzed by DNA fragmentation ELISA and lactate dehydrogenase (LDH) release assay. Caspase activation in human neurons was studied. Fas ligand (FasL) levels in culture supernatants and biological fluids from patients were analyzed by ELISA. Gene microarray analysis, quantikine mRNA ELISA, electrophoretic mobility shift assay and reporter constructs harboring luciferase gene under the control of FasL promoter were utilized for molecular analysis of FasL regulation in human astrocytes. Culture supernatants from activated astrocytes and human FasL led to elevated levels of LDH release and DNA fragmentation in neurons. Concomitant caspase activation was observed in neuronal cultures. Gene microarray analysis of IL-1 beta-activated astrocytes showed an upregulation in FasL. Astrocytes stimulated with IL-1 beta showed activation of NFkB and produced soluble and cell-associated FasL. Primary human astrocytes transfected with FasL promoter-luciferase reporter constructs showed significant upregulation in luciferase activity with IL-1 beta activation. In addition, elevated levels of FasL were observed in cerebrospinal fluid of HAD patients ($n = 12$) when compared to HIV-seropositive individuals without cognitive impairment ($n = 11$) and seronegative controls ($n = 6$). We propose that expression of FasL by activated astrocytes reveals a unique pathway of neuronal injury in HAD. Our data suggest that FasL expression by astrocytes could lead to neuronal demise and is relevant for the pathogenesis of HAD and perhaps other neurodegenerative disorders.