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Basic Science Workshop 2

HIV—dementia

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HIV-1 Tat inhibits the amyloid beta degrading enzyme, neprilysin

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Since the introduction of highly active antiretroviral drugs (HAART), the incidence of HIV-1-associated dementia complex (ADC) has declined and acuity of the disease appears to have decreased. However, as the life expectancy for patients with HIV increases, we hypothesize that there will be a resurgence in ADC in an aging AIDS population with the added complication of age-related Alzheimer's disease (AD). It was our objective to determine ways in which HIV might alter or increase amyloid beta (A beta), similar to the accumulation of A beta seen in AD. A membrane bound metalloendopeptidase, neprilysin (NEP), also known as CD10, is the primary A beta-degrading enzyme in the brain. We found abundant membrane-associated NEP in human brain cell aggregates using Western blot analyses. Using human brain cell aggregates and a quantitative enzymatic assay, we determined that A beta is degraded by NEP. Using a fluorescent competition assay, we found that nanomolar concentrations of Tat inhibited 85% of NEP activity compared to thiorphan, a specific NEP inhibitor. These results have important consequences for an aging AIDS population. This is especially significant when it is known that A beta increases in the normal aging brain. We speculate that in the brain, HIV-infected cells release Tat, which inhibits NEP and interferes with normal A beta catabolism. Over time, this imbalance could result in a neuropathologic accumulation of A beta. In addition to HAART, limiting A beta-induced neurodegeneration by enhancing NEP activity could be a valuable adjunct therapeutic target.

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Potential links between tumor necrosis factor apoptosis inducing ligand (TRAIL) and HIV-1 associated dementia

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Dysfunction in mononuclear phagocyte (MP; brain macrophage and microglia) immune function plays a prominent role in the pathogenesis of HIV-1-associated dementia (HAD). In particular, pro-inflammatory factors produced by MP, as a consequence of immune activation and viral infection, can induce neuronal injury. Tumor necrosis factor related apoptosis inducing ligand (TRAIL) is a type II integral membrane protein which interacts with at least four receptors expressed on multiple cell types including neurons. As this ligand is upregulated by immune stimuli, we examined whether HIV-1 infected and immune activated MP can mediate neurotoxic activities through TRAIL. The expression of TRAIL and its receptors in human brain, as well as, on human monocytes, monocyte-derived macrophages (MDM) and neurons was demonstrated by Western Blot, immunohistochemical assays, RT-PCR and florescence activated flow cytometry (FACS). TRAIL mRNA and cell surface protein levels were increased in MDM after HIV-1 infection and/or lipopolysaccharide, tumor necrosis factor alpha and interferon gamma mediated activation. Macrophages expressing TRAIL were found in HIV encephalitic human brains. A dose dependent effect of recombinant human TRAIL (rhTRAIL, 0.5 to 50 ng/ml) on neuronal apoptosis was observed by TUNEL assay and DNA fragmentation ELISA. Neuronal apoptosis was enhanced by HIV-1 protein gp120 (1 nM, $p < 0.01$). Further, rhTRAIL-mediated neuronal apoptosis was blocked by TRAIL neutralizing antibody as well as inhibitors of caspases 3, 8 and 9. These results support a role for TRAIL in MP-mediated neurotoxicity during HAD.

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Involvement of Vpr-mediated trans-activation in HIV-1-associated dementia

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Numerous host and viral factors participate in the genesis of HIV-1-associated dementia (HIVD). Previous studies have suggested that viral gene expression in CNS cells of the monocyte/macrophage lineage plays a significant role in the production of neurotoxic viral proteins and/or infectious virus leading to the dysfunction of perivascular macrophages, brain microglial cells, astrocytes and neurons. HIV-1 replication is regulated, in part, by interactions between cellular transcription factors and cis-acting elements within the LTR and the viral transactivators, Tat and Vpr. Vpr is a multi-faceted protein involved in cell cycle arrest, nuclear import and viral transcription. The hypothesis guiding these studies is that C/EBP factors and the molecular evolution of the transactivator proteins Tat and Vpr and specific cis-acting elements in the LTR play an important role in viral-induced neurologic dysfunction. Studies are in progress to assess the functional impact of sequence variation in C/EBP

sites I and II with respect to Vpr- and Tat-mediated transactivation. A correlation between high affinity Vpr interaction with LTR C/EBP site I and HIVD has also been established. To this end, the interaction of Vpr with C/EBP site I from 45 LTRs from three non-demented patients and 90 LTRs from seven demented patients was examined. Competition EMS analysis was utilized to examine Vpr binding to probes containing variant C/EBP sites. Approximately 89% of LTRs derived from clinically demented patients displayed a relatively high affinity Vpr binding phenotype, while 11% exhibited a relatively low affinity binding phenotype. In contrast, examination of LTRs derived from patients without evident dementia revealed that 51% displayed relatively high affinity Vpr binding, while 49% exhibited a low affinity binding phenotype. Additionally, 95% of peripheral blood-derived LTRs displayed a relatively high affinity Vpr binding phenotype, while only 5% displayed a relatively low affinity Vpr binding phenotype. LTRs derived from brains of demented patients, but not from non-demented patients, exhibit C/EBP and Vpr binding phenotypes similar to LTRs derived from peripheral blood.

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Chemokine receptors and HIV-induced neurodegeneration: are cell cycle proteins involved?

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This study aims to evaluate the role of cell cycle proteins in the neuronal death associated with HIV infection. The hypothesis to be tested is that chemokines regulate the activity of cell cycle proteins in differentiated neurons, thus contributing to the maintenance of neurons in a quiescent state, and that the inappropriate induction of cell cycle proteins by the HIV-1 envelope protein gp120 results in the delivery of an apoptotic signal. To test this hypothesis, we are studying the effect of the chemokine SDF-1 alpha, the natural CXCR4 ligand, and CXCR4-using variants of gp120 on the activity and expression of proteins involved in cell cycle progression and apoptosis. We focused our attention on the CDK/Rb/E2F-1 pathway as alterations of Rb and E2F1 have been recently shown in the brain of monkeys with SIV encephalitis and patients with HIVE. Thus, we have been analyzing the changes in expression, localization and phosphorylation/activation of Rb and E2F-1 induced by SDF-1 alpha and gp120 in primary neuronal and glial cultures from rat brain as well as in human cell lines expressing recombinant CXCR4. The overall purpose of these initial experiments has been to establish a correlation between the activation of CXCR4 and the CDK/Rb/E2F-1 pathway in different cellular models, while studying the changes of Rb and E2F-1 under experimental conditions that promote neuronal death. Our initial data indicate that changes in the nuclear and cytosolic levels of Rb are associated with apoptosis in differentiated neurons as well as in proliferating cells, and that SDF-1 alpha is able to affect the expression and, possibly, the phosphorylation of Rb. In fact, we found that the chemokine affects the cellular compartmentalization of both Rb and E2F-1 by increasing their cytosolic content. This could prevent the expression of pro-apoptotic genes whose transcription is regulated by E2F-1. Alternatively, Rb and/or E2F-1 may be implicated in additional interactions with cytosolic proteins involved in the regulation of cell survival. (Supported by amfAR 02-816-30RG and NIH/NIDA 15014-01 grants to O.M.)