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Basic Science Workshop 3

Neuropathology

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Identification and characterization of a nuclear export-signal in HTLV-I Tax and intracellular distribution of the viral transactivator in human astrocytes

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The human T cell leukemia virus type I (HTLV-I) transactivator protein Tax has been shown to transactivate the viral LTR and a number of cellular promoters. The role of Tax in the pathogenesis of HTLV-I associated myelopathy/tropical spastic paraparesis (HAM/TSP) has yet to be clearly defined, however, infection and/or activation of astrocytes is thought to play a role in disease progression. In many cell types, Tax has been shown to traffick to the nucleus utilizing the characterized nuclear localization sequence (NLS), although a small amount appears to be present in the cytoplasm. To facilitate studies of intracellular localization, recombinant plasmids were constructed fusing Tax and carboxy terminal truncation mutants of Tax to green fluorescent protein (GFP). In HeLa (cervical carcinoma) and Jurkat (T lymphocyte) cells, Tax localized primarily to the nucleus with a small amount present in the cytoplasm, a result consistent with previously reported observations. However, in astrocytic U-87 MG cells there was also a significant amount of Tax in the cytoplasm, a result consistent with reported observations with HTLV-I-infected astrocytes. Unexpectedly, the Tax-delta-214-GFP truncation mutant, which still contains the Tax NLS, localized equally to the cytoplasm and nucleus in U-87 MG cells while localizing almost exclusively in the cytoplasm in HeLa and Jurkat cells. Examination of the Tax amino acid sequence reveals a putative nuclear export signal between amino acids 188-210 (tNES). When this sequence was fused to GFP (tNES-GFP), the chimeric protein accumulated predominantly within the cytoplasm of both HeLa and astrocyte cells. However, after treatment with leptomycin B (10 M, 4 hr), a specific inhibitor of the exportin/CRM-1 export pathway, tNES-GFP localization was enhanced in the nucleus. These results suggest that the tNES-GFP chimeric protein uses the exportin/CRM-1 pathway for nuclear export. Whether the increased accumulation of Tax in the astrocytic cytoplasm plays a role in HTLV-I neuropathogenesis remains to be determined.

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Mechanisms of regulation of replication of HIV-1 and JC virus in the CNS provide a model for regulation of cellular gene expression by sequence-specific single-stranded DNA- and RNA-binding protein Pur-alpha

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Previous results have indicated that the Tat protein of HIV-1 can interact with cellular protein Pura in U-87MG cells to stimulate transcription of both the HIV-1 genome and the late promoter of JC virus (JCV). Pura binds highly specifically to Tat and to TAR RNA, and Pura overexpression enhances transcription of the HIV-1 genome. Tat and Pura function synergistically to stimulate transcription at the JCV late promoter more than 100-fold. JCV has been shown to infect primarily oligodendrocytes and HIV-1 to infect primarily other glial cells of the brain. We find, however, that oligodendrocytes avidly incorporate Tat from the extracellular medium in functional form, as determined both biochemically and by indirect immunofluorescence microscopy. In the absence of Tat, highly elevated Pura in transfected glial cells inhibits DNA replication initiated at the JCV origin. At lower levels of Pura, however, the replication is enhanced, and presence of extracellular Tat at less than 10-11 M stimulates such replication in KG-1 oligodendrocytes. This stimulatory effect of Tat is dependent upon Pura in an in vitro replication system. The interaction of Pura with TAR RNA is mimicked by the interaction of Pura with BC200, implicated in targeting proteins to neuronal dendrites and influencing translation in the human CNS. In both cases Pura regulates transcription and binds to the resulting transcript. We report that this binding is dependent on a highly specific polynucleotide secondary structure that is present in both RNA molecules as well as in 7SL RNA, another RNA type reported to bind to Pura *in vivo*. The mechanism of Pura regulation of HIV-1 gene expression may provide a model for a novel cellular mechanism in which a given protein regulates not only transcription, but also disposition of the resulting RNA transcript.

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Expression of glutamate transporter EAAT-1 in the brain of HIV infected patients

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Objectives: Recent *in vitro* and SIV-macaque studies by our group showed that activated macrophages/microglia (AMM) express glutamate transporters EAAT suggesting that, besides their classical neurotoxic properties, they may play a neuroprotective role by clearing extracellular glutamate. The present study was undertaken to test this hypothesis in human. Methods Samples from cerebral cortex with underlying white matter, basal ganglia, and brainstem from 12 HIV infected cases at different stages of the disease and 3 controls were immunostained for EAAT-1, p24, GFAP, HLA-DR and CD68. Apoptotic cells were identified by *in situ* end labelling.

Results: EAAT-1 immunostains were clearly positive in all HIV infected cases and scarse or negative in controls. EAAT-1 expression did not correlate with that of GFAP. It correlated with HLA-DR and CD68 expression although differently according to the disease stage. In the 5 cases with acute HIV-encephalitis (HIVE) apoptotic neurons were numerous, EAAT-1 expression by AMM was strong, superposable to that of HLA-DR or CD68, in the white matter, particularly in microglial nodules (MGN) and multinucleated giant cells (MGCs). It was milder in the cortex, involving perivascular microglia and MGN: satellite microglia was exceptionnally stained. In one case with "burnt out" HIVE, apoptotic neurons were frequent; EAAT-1 expression in the white matter was superposable to that of HLA-DR and CD68, involving diffuse scattered microglial cells. In the grey mater, it was milder than HLA-DR and CD68 expression, involving perivascular microglia and, occasionally, satellite cells. In 3 AIDS patients without HIVE as in 3 pre-AIDS cases, apoptotic cells were rare; EAAT-1 expression in the white matter was much weaker than HLA-DR and CD68 expression. There was a better correlation in the grey matter where satellite cells were stained predominantly.

Conclusion: Our findings tend to confirm that AMM, particularly satellite cells, may play a neuroprotective role in the early stages of HIV infection. This may explain the contrast between the early observation of microglial activation, in pre-AIDS cases, and the late occurrence of neuronal loss.

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Dysregulation of the glutamatergic system plays an important role in the pathogenesis of HIV dementia

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HIV-1-associated cognitive/motor complex is one of the major neurological complications in patients with AIDS. Clinical and neuroimaging data suggest that basal ganglia dysfunction plays an important role in the pathogenesis of HIV-associated dementia. Dysregulation of the glutamatergic system resulting in calcium-mediated neuronotoxicity might represent an important component.

Human excitatory amino acid transporters (EAATs) are members of a family of high affinity sodium-dependant transporter molecules that regulate the neurotransmitter concentrations at the excitatory glutamatergic synapses of the central nervous system. EAATs play an essential role in regulation of the re-uptake of glutamate, thereby modulating synaptic signaling at post-synaptic receptors and protecting neurones from glutamate excitotoxicity. By use of commercially available monoclonal antibodies we analyzed the differential expression and distribution of the glial EAAT 1 and 2 on paraffin-sections of selected brain regions. Our postmortem series included cases with HIV encephalitis (HIVE), HIV-positive patients without HIVE, HIV-negative patients with Parkinson's disease and Huntington's disease, as well as HIV-negative controls.

In HIV-positive patients without HIVE the differential expression of EAATs did not differ from normal controls. In HIVE both EAAT 1 and 2 were up-regulated in the cerebral cortex. The basal ganglia revealed striking over-expression of EAAT 1, partially also of EAAT 2 resembling the changes in those cases with Huntington's disease and Parkinson's disease. Our results suggest that glutamatergic dysregulation as well as imbalance of astroglial transport play an important role in the pathogenesis of HIV dementia.