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Plenary Session 6

Neuronal signalling, dysfunction and apoptosis/Viral latency (1) and reactivation

Chairpersons: D. Gilden (Denver, USA)
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70

Neuronal signaling, dysfunction and apoptosis

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A significant proportion of HIV-1 infected individuals develop impairment of the central nervous system (CNS) that can culminate in dementia, paralysis, and death. HIV-1 enters the brain at the early stage of infection and resides primarily in a limited number of macrophages/microglia and astrocytes. Infection of these cells, however, may not explain the massive neuronal pathology which is seen in AIDS associated dementia suggesting a role for factors released from HIV-1 infected cells that trigger a cascade of events leading to neurodegeneration. One of the viral proteins with potent regulatory activity is Tat, which is secreted by infected cells and can affect neighboring uninfected cells by transcellular means. Results from our recent studies show that Tat can influence multiple biological events that lead to neuronal injury. For example, treatment of neuronal cells with Tat affects MAPK/ERK1/2 activity, the downstream central component of the nerve growth factor (NGF) signaling pathway. This can affect Egr-1, a transcription factor that is regulated by MAPK/ERK1/2 and is responsible for the stimulation of p35 protein. p35 is a neuron-specific activator of cdk5, a cyclin dependent kinase that phosphorylates several neuronal proteins including neurofilament and plays an important role in neuronal differentiation and survival. Accordingly, our data indicate that treatment of cells with Tat severely decreases p35 expression. In parallel, Tat can bind to the cellular protein, Pur(alpha), which associates with cdk5. Further, results from Pur(alpha) knockout animals revealed a decrease in p35 activity, pointing to the importance of Pur(alpha) association with cdk5 in the activity of cdk5: p35 complex. The cooperativity between HIV-1 Tat and the cellular protein, Pur(alpha), which results in de-regulation of the NGF signal transduction pathway in neuronal cells will be discussed.

71

HIV-1 induced neuronal apoptosis: mechanisms, pathways and protection

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Neuronal apoptosis within the CNS is a characteristic feature of AIDS dementia complex (ADC), and may represent a common pathway of cell death induced by neurotoxins released by HIV-infected macrophages. In primary neuronal cell systems macrophage-released cellular products and HIV proteins have been demonstrated to over-stimulate the N-methyl-D-aspartate (NMDA) subtype of glutamate receptor, leading to apoptosis. However, while potential neurotoxins in ADC have been extensively studied, the mechanisms and pathways underlying neuronal apoptosis in HIV-1 infection are not fully understood. In brain injury models, neuronal apoptosis may result from activation of the intrinsic (bcl-2-regulated mitochondrial) or extrinisic (Fas, TNF-a regulated death receptor) pathways, although which pathway predominates in CNS HIV infection is unknown. The Bcl-2 family proteins, Bcl-2 and Bcl-xL, block apoptosis initiated by the intrinsic pathway but often not the extrinsic pathway, and whether they protect against HIV is also unknown. Activation of AKT/protein kinase B may block both pathways, and some evidence suggests AKT activation may protect neurons against HIV envelope neurotoxicity.

To better understand the mechanisms of HIV-induced neuronal apoptosis and to test neuroprotective strategies, we developed a unique in vitro model, utilizing the NT2 human neuronal cell line, primary astrocytes and macrophages, and primary CNS HIV-1 isolates. We found that NT2.N neurons are protected against HIV-infected macrophages by NMDA receptor antagonists, similar to primary neurons. We established stable NT2.N neuronal lines that over-express Bcl-2 or Bcl-xL (NT2.N/bcl-2 and NT2.N/bcl-xL) and found that such neurons were resistant to apoptosis induced by R5, X4, or R5/X4 primary HIV-1isolates. This inhibition was overcome by a Bcl-2 antagonist. Thus, the Bcl-2 family of proteins protects neurons against the spectrum of primary HIV-1 isolates and the intrinsic apoptosis pathway contributes significantly to HIV/macrophage induced neuronal apoptosis. Modulation of the bcl-2 pathway as well activation of AKT/protein kinase B may offer adjunctive neuroprotection against ADC.

72

Herpes simplex virus type I (HSV-1) latency: the many functions of the HSV-1 latency associated gene

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HSV-1 establishes latent infection in human peripheral sensory ganglia and can reactivate to produce recurrent disease. Latent HSV-1 infection is a continuum that requires viral ability to establish latent infection in neuronal tissue, mainly if not exclusively in neuronal cells, maintenence of the latent infection for the entire life of the host and reactivation, namely resumption of viral replication in peripheral muco-cutaneous tissues to infect new hosts. During latent HSV-1 infection, restricted gene expression takes place. The latency associated gene gives rise to two latency-associated transcripts (LATs), 2.0 and 1.5 kb in size and is located within the repeat regions of the viral genome. Using viral mutants, unable to express this gene and neuronal cell lines and a transgenic mouse model that express the LATs we provide evidence that HSV-1 latency-associated gene may participate in all functions required for latency.

73

Analysis of alpha-herpesvirus genes expressed during latency

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Primary infection by herpes simplex virus type 1 (HSV-1) can cause clinical symptoms in the peripheral and central nervous system, upper respiratory tract, gastro-intestinal tract, and is the leading cause of corneal blindness due to an infectious agent. Recurrent ocular shedding leads to corneal scarring that can progress to vision loss. Latency is established in sensory neurons of trigeminal ganglia (TG) but can periodically reactivate and spread. Viral gene expression and replication occur when HSV-1 infects a neuron, but many neurons survive infection. During latency, the only abundant viral RNA expressed is the latency-associated transcript (LAT). LAT is anti-sense to ICP0; a viral gene that is crucial for efficient infection, suggesting LAT interferes with ICP0 expression and thus promotes latency. Although the majority of LAT is located in the nucleus of latently infected neurons, alternative splicing occurs in TG suggesting LAT isoforms have unique biological properties. Our previous studies have demonstrated that LAT inhibits apoptosis (programmed cell death) in TG of infected rabbits and transiently transfected cells. These studies have also linked the anti-apoptotic functions of LAT to reactivation from latency. In productively infected neuro-2A cells (murine neuroblastoma cells), LAT inhibits cleavage and activation of caspase 9, but not caspase 3. LAT also had subtle effects of caspase 8 cleavage in productively infected neuro-2A cells. In contrast, LAT had not little effect on caspase cleavage in productively infected NIH3T3 cells (mouse fibroblasts). Caspase 9 plays a crucial role in neuronal apoptosis suggesting that the ability of LAT to inhibit caspase 9 cleavage is important for the latency-reactivation cycle. Current studies are focusing on understanding the mechanism by which LAT interferes with caspase 9 cleavage in neuronal cells.