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Plenary Session 3

Cell activation and differentiation/Animal models (2)/Virus-host interactions

Chairpersons: A. Nath (Lexington, USA)
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HIV infection of astrocytes—an uneasy truce

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Astrocytes are the most abundant cells of the central nervous system. As a cell population, they appear to be quite heterogenous and fulfilll numerous crucial functions in the CNS. These include essential neuronal support functions, and maintenance of barriers controlling influx of compounds into the brain. In addition astrocytes are capable of communicating with other cells in the CNS.

Astrocytes can contribute to diseases of the central nervous system both by direct mechanisms, such as loss of essential functions and secretion of neurotoxic factors and indirectly by stimulating production of neurotoxic factors by other cells of the CNS. On the other hand astrocytes have various means of protecting neurons from toxic effects of for example excitatory amino acids, ammonia and possibly also of cytotoxic pathogens. Because they are essential for functionality of the CNS, the role of astrocytes is important to consider in the context of infectious diseases of the CNS.

HIV components have been detected in astrocytes of infected individuals, confirming that astrocytes can act as target cells for HIV *in vivo*. *In vitro* studies indicate that virus-cell interactions are different in astrocytes from other HIV target cells. In addition these studies show that astrocytes tolerate persistent infection with HIV and stable production of HIV regulatory factors, indicating that they are capable of curbing toxic effects of HIV. Here I will review cellular models used to study interaction of HIV with astrocytes, compare various aspects of the HIV life cycle in astrocytes with other cells and discuss potential beneficial and harmful effects of astrocytes in HIV-associated pathogenesis.

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Microglia activation in the pathogenesis of HIV-dementia: insights provided by the SIV/macaque model

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HIV-infection leads to neurological disease in a significant proportion of AIDS patients. Since not neurons but cells of monocytic/microglial origin are the main target of infection within the brain, indirect mechanisms have been postulated as cause of HIV-dementia. These include neurotoxic effects of viral proteins or cellular products secreted due to an aberrant activation of these cells. In order to determine the role of these mechanisms for the pathogenesis HIV-dementia we have used the infection of macaques with simian immunodeficiency virus (SIV) as an animal model.

We have isolated microglial cells from uninfected and infected monkeys and have analyzed their phenotype by three color flow cytometry. In addition, viral load, cytokines and excitotoxic substances were measured in the cerebrospinal fluid (CSF), brain parenchyma and supernatants of isolated microglia of SIV infected macaques.

Microglia isolated from infected animals displayed an increased expression of activation markers such as MHCII and CD14 and of the costimulatory molecules CD80 and CD86. The level of expression of these antigens correlated with disease progression and the occurence of clinically overt neurological signs. Similarly, the production of viral antigen, TNF alpha and glutamate, but not IL-1 beta and IL-6 was increased in animals with neurological disease. Longitudinal assessment of CSF revealed that autochthonous viral replication must take place for a prolonged period before neurological signs are manifested. Treatment of isolated microglia with different drugs (Pentoxyphyllin, Iloprost, Rolipram) inhibited TNF alpha production.

Our results show that viral load and TNF alpha are important factors in the pathogenesis of HIV-dementia. High replication of SIV within microglia leads to increased activation of these cells. As TNF alpha also stimulates viral replication, a vicious circle is induced. Therefore, substances inhibiting TNF alpha production may provide a valuable tool to decrease both viral load and the detrimental effects of microglia activation.

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Genome-wide transcriptional profiling reveals distinct classes of genes upregulated in SIV neuroAIDS

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Objective: CNS functional and pathological abnormalities are devastating sequelae of HIV infection. The basis of the cogntive and motor disorders induced by HIV infection remain unknown. We instituted a genome-wide screen for

RNAs upregulated in SIV-infected animals with CNS functional and pathological abnormalities.

Method: Six rhesus macaques were infected with SIVmac182 and treated with a CD8-depleting antibody to induce a rapid progression to simian AIDS. Six uninfected animals served as controls. Frontal lobe RNA samples were analyzed using Affymetrix Human GeneChips, with parametric statistical testing performed with a 0.05 p-value cutoff followed by multiple testing correction.

Results: Monkeys progressed to simian AIDS with high viral loads, delays in neurophysiological evoked potentials, and defects in behavioral testing, most consistently on motor tasks. CNS pathology showed abundant macrophages in the brain. Statistical analysis of the GeneChip studies revealed that 116 of the 12,927 genes examined were significantly elevated, and greater than 2-fold increased, in the RNA from SIV-infected animals relative to controls. These genes can be grouped into functional categories, including those inducible by interferon and viruses, cell cycle regulators, critical components in antigen processing, cellular structural proteins, and a subset of products of activated macrophages. Real-time PCR, in situ, and immunohistochemical analysis has confirmed the upregulation of many of these mRNA and proteins in neurons, astrocytes, endothelial cells, and microglia/macrophages. Many of the upregulated genes, including osteopontin, CD163, 5-LO activating protein, tissue transglutaminase, HCgp39, Mac2BP, Glut5, and IDO are products of activated macrophages, whereas a small number, such as TIMP1, are found in neurons.

Conclusion: In these animals with CNS functional abnormalities, macrophage infiltration was consistently found in the brain. Candidate neuropathogenic, as well as potentially neuroprotective, molecules have been identified. The cellular molecular signatures reveal the interplay between destructive and protective mechanisms in the infected brain.

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Brain endothelial cells in HIV neuroinvasion and reservoir formation

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Human immunodeficiency virus (HIV) infection of the central nervous system (CNS) is one of the more challenging aspects of HIV disease, despite the success of highly active antiretroviral therapy (HAART) in reducing peripheral viral load. The mechanisms of viral entry into the brain are incompletely understood, as are the sequelae following virus entry. Brain cells infected with HIV are predominantly macrophages and microglia, with fewer but significant numbers of microvascular endothelial cells and astrocytes also demonstrating immunohistochemical evidence of infection. The importance of CNS macrophage infection is underscored by the fact that neurotoxic cellular or viral components are released from these cells. The importance of endothelial cell infection is less well understood and is a matter of continuing debate. Following exposure to cellular or viral factors, endothelial cells may exhibit compromised blood brain barrier (BBB) function and promote increased leukocyte recruitment. Direct infection of the BBB with HIV may also be a key event in neuroinvasion and neuropathogenesis.

Low levels of replication-competent virus have been demonstrated in patients on HAART, leading to the concept of viral reservoirs. Reservoir studies have focused primarily on CD4+ resting T cells, but additional tissue compartments including the brain have been proposed. Complete HIV eradication will not be possible until all sources of reservoir virus are discovered and eliminated. Evaluation of the CNS with identification of the cell types that function as reservoirs is thus a critical issue. The ability of brain endothelial cells to maintain a persistent infection with HIV or SIV identifies these cells as potential reservoirs.

The simian immunodeficiency virus (SIV)/macaque model is an appropriate and pertinent model with which to complement HIV pathogenesis studies. Our studies have focused on how HIV and SIV interact with and cross the BBB, as well as the potential role of the CNS as a viral reservoir. We present here our *in vitro* results with HIV and SIV infection of brain endothelial cells, as well as the results of an *in vivo* study of neurotropic SIV in the macaque model of neuropathogenesis and reservoir formation.

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The art of survival during viral persistence

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Resolution of acute CNS infection by the neurotropic JHM strain of mouse hepatitis virus results in a chronic infection characterized by viral antigen and RNA, the absence of detectable infectious virus and focal ongoing demyelination. CD8+ T cells, the major effectors of viral clearance during acute infection, utilize differential effector mechanisms to inhibit replication in a cell type specific manner. While perforin mediated cytolysis controls virus in microglia/macrophages and astrocytes, IFN-g regulates replication in oligodendroglia. Analysis of JHM pathogenesis in mice unable to secrete anti-viral antibody confirmed a primary role of cell mediated immunity and a redundant role for humoral immunity in resolution of acute disease. However, infectious virus reactivated within the CNS of antibody deficient mice following initial viral clearance. This finding indicated that T cells remaining in the CNS of immune competent persistently infected mice are insufficient in controlling persistent infection. Indeed, reactivation in antibody deficient mice was not associated with increased CNS T cells, but can be prevented via transfer of neutralizing antibody. A vital role of humoral immunity during persistence in wild type mice is supported by the accumulation of virus specific antibody secreting cells following clearance of infectious virus, in contrast to the progressive decline, but continued maintenance of T cells. Thus, although a potent cell mediated immune response controls acute infection, humoral immunity maintains viral persistence within the CNS. This experimental model demonstrates that the CNS provides an environment for the retention of both long lived T cells and plasma cells that maintain virus in a persistent, but non infectious form. The low turnover of virus, T cells and B cells may thus constitute a unifying feature for the art of survival at the site of infection and ultimately the host. Therefore, JHM infection illustrates the dichotomy between immune effectors effective in regulating acute and persistent CNS infections to minimize immune pathology.