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Murine models for human immunodeficiency virus type 1–associated dementia: The development of new treatment testing paradigms

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The neuroimmune events leading to human immunodeficiency virus (HIV)-1–associated dementia (HAD) are linked to macrophage secretory neurotoxins (cellular and viral toxins). To study such events, we developed a murine model of HIV-1 encephalitis (HIVE), the pathological equivalent of HAD. Severe combined immunodeficient (SCID) mice injected with HIV-1–infected monocyte-derived macrophages (MDMs) into basal ganglia exhibited many of the pathological features of human HIVE. Moreover, behavioral and cognitive abnormalities in the HIVE mice were associated with neuronal dysfunction and decreased synaptic density. Thus, the rationale for testing novel therapeutic approaches (anti-inflammatory, antiretroviral, or neuroprotective) in the HIVE SCID mice is clear. Animals treated with anti-inflammatory compounds (platelet-activating factor [PAF] antagonist and tumor necrosis factor [TNF]-α release inhibitor) showed a marked reduction in brain inflammation and a reduction in neuronal injury. Comparative analyses of highly active antiretroviral therapy (HAART) regimens provided direction for which one might be most efficient for reduction of viral load in infected brain tissue. Moreover, modifications of the HIVE model might serve as a vehicle for testing vaccine approaches. Reconstitution of immunodeficient animals with syngeneic T lymphocytes followed by injection of HIV-1–infected MDMs in the brain resulted in cytotoxic antivirus T lymphocyte (CTL) response. CD8-positive T cells migrated to the sites of human MDMs, leading to the cell-mediated destruction of HIV-1 infected MDMs. These results, taken together, strongly support the use of HIVE SCID mouse model as a novel system for studies of the neuropathogenesis of HIV-1 infection, as well as for testing novel therapeutic and vaccine interventions for human disease. *Journal of NeuroVirology* (2002) **8(suppl. 2),** 49–52.

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Introduction

Central nervous system (CNS) disease occurs at the late stage human immunodeficiency virus type 1 (HIV-1) infection. Before introduction of highly active antiretroviral therapy (HAART), up to half of virus-infected individuals demonstrated neuropathological changes at autopsy, and a quarter had a triad of clinical cognitive, behavioral, and motor abnormalities, ranging from mild motor/cognitive deficits to overt dementia (HIV-1–associated dementia [HAD]) (McArthur *et al*, 1993; Gendelman *et al*, 1997). Despite the diminished incidence of HAD to 11% in patients on HAART (Maschke *et al*, 2000), the greater life expectancy of infected individuals suggests that the prevalence of such complications of viral infection may increase as a result of viral mutation and/or failure or intolerance of HAART (Sacktor *et al*, 2001). A proportional increase in HAD compared with other acquired immunodeficiency syndrome (AIDS)-defining illnesses and a marked

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increase in the median $CD4^+$ T cell count at HAD diagnosis have been reported following HAART (Dore *et al*, 1999). The host-viral interactions leading to HAD remain elusive.

HIV-1 infection of CNS

Virus-infected mononuclear phagocytes (MPs; microglia and perivascular macrophages) serve as reservoirs for productive HIV-1 infection within the CNS (Koenig *et al*, 1986; Wiley *et al*, 1986). A substantial amount of data suggest that neuroimmune events leading to HAD are associated with MP secretory products (cellular and viral toxins) produced during HIV-1 encephalitis (HIVE) following MP activation (Gendelman *et al*, 1997; Kaul *et al*, 2001). Pathological analyses performed in postmortem brain tissue demonstrated that microglial activation is the best correlate for HAD (Persidsky *et al*, 1999). Diffuse microglial activation in HAD may explain the apparent paradox of how relatively small numbers of infected perivascular macrophages can produce widespread neurological dysfunction. Our previous works showed that such MP activation and, to a lesser extent, HIV-1 infection and reactive astrogliosis resulted in prominent chemokine production, macrophage brain infiltration, and neurological decline (Persidsky *et al*, 1999). Although HIV-1 enters the brain early following viral infection, productive viral replication and brain MP invasion occurs years later and only in some infected people (Gendelman *et al*, 1997). The onset of neurological disease coincides with immunodeficiency and progression to AIDS, suggesting that virus-specific immune responses (including cytotoxic $CD8^+$ T cells) play a protective role in the CNS (Sopper *et al*, 1998). Defective chemotactic responses in brain could result in a breakdown in such protective mechanisms. Because α-chemokines mediate attraction of T lymphocytes into CNS, we hypothesized that α-chemokine deficit could alter T-cell responses in HIVE. To our surprise, *in vitro* experiments proved that lymphocyte chemotactic responses remained operative, and $CD8^+$ lymphocytes were found in increased numbers in HIVE brains as compared to brain tissue from seropositive patients without encephalitis (Poluektova *et al*, 2001). One plausible explanation is that circulating HIV-specific $CD8^+$ T cells may be partially anergic and unable to eliminate HIV-1–infected cells *in vivo*, in the setting of impaired $CD4^+$ T cells (Pitcher *et al*, 1999; Trimble *et al*, 2000). Activated $CD8^+$ cells (secreting high levels of interferon-γ) can act as instigators of microglia and astrocyte activation. The activated glia affect MP influx into the brain. Such events are regulated by β-chemokines. MP brain infiltration also results in significant structural (tight junctions) and functional abnormalities (down-regulation of specialized transport systems protecting the brain from toxic insults) of microvasculature associated with the intensity of HIVE (Persidsky *et al*, 2000). Findings in human brain tissue affected by HAD directed our works during the development of animal models for the disease.

Animal model systems for HAD

Animal models serve as a major tool for studies of pathogenesis. To these ends, we have developed a nontransgenic murine animal model system for HAD where HIV-1–infected monocyte derived macrophages (MDMs) were stereotactically inoculated into the basal ganglia/cortex of severe combined immunodeficient (SCID) mice (Persidsky *et al*, 1996). The hallmarks of HIVE (astrogliosis, activation of macrophages and mouse microglia, and degeneration of neurons) were demonstrated in this model, and neuroinflammatory responses seen in HAD were reproduced in these animals (Persidsky *et al*, 1997). Our recent works demonstrated a relationship between cognitive impairment, synaptic physiology, and neuronal morphology in HIVE animals. This study integrating behavioral testing, *ex vivo* electrophysiologic assessment (neuronal function), and neuropathologic analyses (Zink *et al*, 2002) allows comprehensive testing of new treatment paradigms *in vivo* using this animal system. Cognition was examined using HIVE SCID mice in the Morris Water Maze (MWM; model for memory acquisition and storage [King *et al*, 1999]) at 4, 8, and 15 days after injection with HIV-1–infected and uninfected MDM vehicle (sham), and unmanipulated controls. By day 8, mice in the HIV-1 MDM and MDM groups developed cognitive impairment, as evidenced by failure to acquire spatial information. MWM testing at day 15 showed no evidence of cognitive impairment in any tested groups.

Compromised MWM performance was then correlated with altered synaptic physiology. Animals were sacrificed at the same three postinjection time points. The hippocampal formation ipsilateral to the injection was dissected and sectioned; excitatory postsynaptic potentials were recorded within the CA1 region. At 6 to 8 and 14 to 16 days after injection, HIV-1 MDM and MDM mice consistently displayed reduced synaptic enhancement compared to sham-animals. Notably, reduction in long-term potentiation (electrophysiologic correlate for learning and memory) was most pronounced in the HIV-1 MDM mice. Transient changes in mouse cognition and progressive changes in synaptic physiology were compared with morphological changes in the hippocampus. Digital image analysis of neuronal synaptic density was performed, using antibodies against synaptophysin (SP), and neurofilaments (NF; a cytoskeletal protein localized primarily in neuronal axons) and neuronal dendritic arbor were detected by immunostaining for microtubule-associated protein-2 (MAP-2). Significant reduction in SP staining was found in HIV-1 MDM–inoculated (58% to 61%) and MDM-inoculated groups (44% to 54%) as compared to control animals at days 4, 8, and 15 post inoculation.

Similar results were obtained by using two other neuronal markers, NF and MAP-2.

The ability to quantitatively evaluate cognitive impairment, neuronal dysfunction, and neuropathology in this model system makes it useful for the studies of pathogenesis and for therapeutic testing. Indeed, HIVE SCID mice treated with a platelet-activating factor (PAF) antagonist and a matrix metalloproteinase/TNF-α release inhibitor showed a marked reduction in brain inflammation, astrogliosis, and microglia activation (Persidsky *et al*, 2001). These findings demonstrate that diminution of neuroinflammatory responses, independent of viral replication level, can ameliorate HIVE pathology. Currently, new adjunctive therapies for HAD are under investigation at our Center for Neurovirology and Neurodegenerative Disorders.

Further refinement of the animal model allowed us to demonstrate spread of viral infection among the inoculated human macrophages in the mouse brains. These studies permitted comparative assessment of different antiretroviral drugs by measurements of viral load and numbers of infected human macrophages. Our experiments demonstrate that two nucleoside reverse transcriptase inhibitors (abacavir and lamivudine) or their combination significantly reduce viral load in SCID mice with HIVE (Limoges *et al*, 2000, 2001). Testing of novel drug delivery systems (including block copolymers) is currently under investigation.

Our most recent *in vivo* experiments were designed to determine whether primary cellular immune responses could be generated and lead to the elimination of productively infected MPs. Peripheral blood lymphocytes (PBLs, inoculated intraperitoneally) were used to reconstitute the peripheral immune system of non-obese-diabetic (NOD)–SCID mice. One week later, HIVE was established in NOD–SCID animals after stereotactic injection of syngeneic human HIV-1–infected MDMs into the basal ganglia, generating human PBL–NOD–SCID HIVE mice (Poluektova *et al*, 2002). Flow cytometric tests demonstrated successful lymphocyte engraftment and showed development of HIV-1 *gag*- and *pol*-specific cytotoxic lymphocytes in mouse spleens, which were supported by immunohistochemical tests. The number of productively infected MDMs was markedly reduced during a 2-week observation period. These changes were accompanied by profound glial activation and evidence of blood brain barrier compromise in microvessels. This was associated with a prominent lymphocyte migration. Cell to cell contact did not appear to contain tight junctions (TJs). Loss of TJs was demonstrated by immunostaining for occludin (TJ protein) in microvessels featuring lymphocyte and monocyte infiltration. Future works in the human PBL–NOD–SCID HIVE model is designed for vaccine testing and to explore the role played by T cells in the pathogenesis of HIV-1 infection of the brain.

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