Optimizing animal models for HIV-associated CNS dysfunction and CNS reservoir research

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Introduction

Understanding the mechanisms of HIV-associated CNS dysfunction and establishment of CNS viral reservoirs are priority research areas for the NeuroHIV field. Over the past decade, both the clinical and pathophysiological characteristics of HIV-induced central nervous system (CNS) dysfunction have changed. Early in the pandemic, dementia and encephalitis dominated the clinical and pathological picture; and macrophage-derived mediators and HIV-1 proteins were the key pathogenic mechanisms implicated in causing CNS dysfunction. However, with the advent of combination antiretroviral therapy (cART), the spectrum of CNS disorders has transformed. While, HIV-associated CNS dysfunction still affects a considerable proportion of people living with HIV (PLHIV), the signs and symptoms are generally mild, and do not correlate with the classical findings of HIV-related neuropathology.

Animal models have been central to the study of HIV-associated CNS dysfunction. However, the relationship of many of the animal models to the current clinical picture of the CNS disorders in PLHIV is unclear. No model is perfect, and it would be useful to uncover the models that have the greatest utility to address important questions. Given the change in the clinical presentation and pathology associated with HAND (HIV-associated neurocognitive disorders), it is critical to re-evaluate prior models and assess newer models as well as elucidate what may be needed to fill the critical gaps. In addition, these models can have great utility in HIV-1 eradication and cure efforts. While the CNS is one of the potential reservoirs, these *in vivo* models can lead to significant discoveries in other organ systems as well.

In October 2016, the Division of AIDS Research, NIMH (National Institute of Mental Health) hosted a meeting entitled “Optimizing Animal Models for Evolving HAND Phenotypes and CNS Reservoirs Research” in conjunction with the 14th International Symposium of NeuroVirology, Toronto, Canada. Many different animal models were presented, and their utility for understanding HAND pathogenesis, its prevention, and treatment, as well as the applicability to eradication/cure studies, were highlighted. This was followed by a moderated open discussion to stimulate communication and presentation of ideas from scientists in the field.

This special issue of the Journal of Neurovirology is a collection of articles from the many of speakers who presented their respective animal models at the NIMH sponsored meeting. In addition, a few additional authors who are working in this research area also contributed to this special issue. Our goal for publishing this issue is to have a valuable resource for the field to identify appropriate animal models based on their research questions relating to HIV-associated CNS dysfunction and CNS reservoirs.

Setting the stage

The first two papers in this issue examine the changing clinical phenotype of HIV-associated CNS dysfunction and associated neuropathologic findings. This sets the stage for examining if the animal models currently in use reflect the evolving pathological and clinical presentations of HIV-associated CNS dysfunction in the current ART era.

Sacktor’s (Sacktor 2017) review of the field indicates in cART-treated patients that the prevalence of HIV-associated dementia has declined substantially, and milder forms of HAND, predominate. PLHIV with mild neurocognitive disorder (MND) can still have significant functional impairment in particular activities of daily living. Also, the mean survival for an individual diagnosed with HIV dementia has increased dramatically. Further, in HIV+ individuals on cART with a

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suppressed systemic viral load, the majority of individuals with HAND remain stable, with only a small proportion showing deterioration. Extrapyramidal signs are now less common in patients with HAND on cART. In the cART era, HIV-associated CNS dysfunction may have a mixed pattern of both cortical and subcortical features with greater deficits in executive functioning and working memory. Despite the milder clinical phenotype, in the cART era, patients with HIV-associated CNS dysfunction still have persistent laboratory and neuroimaging abnormalities in the CNS even with systemic viral suppression. As the HIV+ patient population ages, cerebrovascular disease risk factors such as hypertension, diabetes, and hypercholesterolemia are increasingly recognized as causative influences responsible for cognitive impairment in HIV+ patients on cART.

Gelman et al. (Gelman et al. 2017) focus on how to develop experimental models of HAND and CNS HIV-1 latency that best imitate the CNS pathophysiology in diseased humans in the era of viral suppression. The paper emphasizes that models of HIV encephalitis (HIVE) with active CNS viral replication that were developed in the early years of the AIDS pandemic have minimal clinical relevancy to the current forms of HAND observed in virally suppressed patients. They suggest that improved models of HAND should incorporate the neurochemical, neuroimmunological, and neuropathological features of virally suppressed patients. Common anomalies in these patients as established in autopsy brain specimens include brain endothelial cell activation and neurochemical imbalances of synaptic transmission, and classical forms neurodegeneration may not be as crucial. With regard to latent HIV with viral suppression, human brain specimens have a pool of latent proviral HIV-1 DNA in the CNS, that is small relative to the total body pool and it does not change substantially over the years. The CNS pool of latent virus probably differs from lymphoid tissues, because the mononuclear phagocyte system sustains productive infection (versus lymphocytes). These and yet-to be discovered aspects of the human CNS of virally suppressed patients need to be better defined and addressed in experimental models. This paper reiterates that in order to maintain clinical relevancy, models of HAND and viral latency should faithfully emulate the current clinical findings in virally suppressed individuals.

Mouse models expressing single HIV viral protein

Thaney et al. (Thaney et al. 2017) describe a transgenic (tg) mouse model expressing the envelope protein gp120 of HIV-1 in the brain astrocytes under the control of a glial fibrillary acidic protein promoter. These GFAP-gp120tg mice manifest several key neuropathological features observed in AIDS brains, such as decreased synaptic and dendritic density,

increased numbers of activated microglia, and pronounced astrogliosis. Recent studies show that brains of GFAP-gp120tg mice mimic the findings observed in neurocognitively impaired HIV patients such as differentially regulated genes; activation of innate immunity and cellular signaling pathways; disturbed neurogenesis; and learning deficits. Based on these findings, the authors suggest that GFAP-gp120tg mouse model can be used to investigate neurodegenerative mechanisms and develop therapeutic strategies targeting gp120 to mitigate the consequences associated with HIV-1 infection of the CNS.

Langford et al. (Langford et al. 2017) provide a review of the creation of a doxycycline-inducible astrocyte-specific HIV-1 Tat transgenic mice (iTat) to understand the contributions of Tat protein to HIV/neuroAIDS and the underlying molecular mechanisms of HIV-1 Tat neurotoxicity in the context of a whole organism and independently of HIV-1 infection. Tat expression levels in the brains of iTat mice were in the physiologically relevant range of 1–5 ng/ml and led to astrogliosis, loss of neuronal dendrites, and neuroinflammation. This paper indicates that iTat mice have enabled studies investigating the direct effects of Tat on astrocytes, astrocyte-mediated Tat neurotoxicity, understanding of Tat impaired neurogenesis, to decipher Tat-induced loss of neuronal integrity, and comprehend exosome associated Tat release and uptake.

Severe combined immunodeficient mouse HIV encephalitis model

Tyor and Bimonte-Nelson (Tyor and Bimonte-Nelson 2017) outline a mouse model of HAND which demonstrates mild behavioral deficits and has been used to investigate cART and novel treatments for HAND. This model also shows correlations between impaired cognitive functioning due to HIV-1 in the brain and pathological parameters such as gliosis and neuronal abnormalities. A recent advancement utilizes the object recognition test to monitor mouse behavior before and after therapy, a paradigm that models how humans would be identified with cognitive dysfunction prior to the institution of a novel cognitive treatment. The authors postulate that this model is well suited for preclinical testing of novel therapies and provides correlations of mild cognitive impairment with pathological markers that can give further insights into the pathophysiology of HAND.

Humanized mouse models for HIV CNS reservoir research

Honeycutt and Garcia (Honeycutt and Garcia 2017) summarize several humanized mouse models for evaluating

NeuroHIV and cure strategies. Of particular interest is the recently described myeloid only mouse model (MoM). MoM is generated by injecting human hematopoietic stem cells into pre-conditioned NOD/SCID mice. These animals are reconstituted with human myeloid and B cells enabling systemic analysis of HIV-1 infection in tissue macrophages. This model is able to address a major critique pertaining to the presence of virus in macrophages, being attributed to phagocytosis of infected T cells. While most studies of HIV-1 persistence have focused on T cells, this paper emphasizes that the MoM model offers a unique avenue to study persistence exclusively in tissue macrophages. This is important when developing strategies for targeting HIV infection in the CNS, as the predominant targets for infection of the brain are myeloid-derived cells such as macrophages and microglia.

Llewellyn et al. (Llewellyn et al. 2017) describe a strategy to repopulate the brains of immune-deficient NOD SCID gamma (NSG) mice with human microglial cells. When radiation or busulfan conditioned immune-deficient NSG mice are transplanted with human hematopoietic stem cells, these cells enter the brain and differentiate into microglia. Upon infection of these mice with replication competent HIV, virus was readily detected in these bone marrow-derived human microglia. Their previous studies have identified members of the CoREST repression complex as key regulators of HIV-1 latency in microglia in both rat and human microglial cell lines. Further, they demonstrate, a monoamine oxidase (MAO) and potential CoREST CNS penetrant inhibitor, phenelzine, is able to stimulate HIV-1 production in human microglial cell lines. Human glia recovered from the brains of HIV-infected humanized mice also responded to phenelzine. The authors indicate that these studies provide strong evidence that HIV-1 can establish latency in primary microglia. Further, they emphasize that the humanized mice they have developed show great promise as a model system for the development of strategies aimed at reducing the CNS reservoir.

SIV models

Beck et al. (Beck et al. 2017) outline studies with SIV infection of pigtailed macaques to generate a highly representative and well-characterized animal model for HIV neuropathogenesis that provides an excellent opportunity to study and develop prognostic markers of HAND. This model is generated by inoculating pigtailed macaques (*Macaca nemestrina*) intravenously with both neurovirulent SIV/17E-Fr strain and the immunosuppressive CD4+ T cell-depleting SIV swarm SIV/DeltaB670 strain which lead to consistent development of AIDS and prototypic SIV encephalitis within a 3-month period. Similar to observations in HIV-infected patients receiving antiretroviral therapy (ART), ongoing neurodegeneration, and inflammation are present in SIV-infected

pigtailed macaques treated with suppressive ART. By developing quantitative viral outgrowth assays that measures both CD4+ T cells and macrophages harboring replication competent SIV as well as a highly sensitive mouse-based viral outgrowth assay, the authors indicate that they have positioned the SIV/pigtailed macaque model to advance our understanding of latent cellular reservoirs, including potential CNS reservoirs, to promote HIV cure research. The authors also state that, in addition to contributing to our understanding of the pathogenesis of HAND, the SIV/pigtailed macaque model also provides an excellent opportunity to test innovative approaches to eliminate the latent HIV reservoir in the brain.

Mallard and Williams (Mallard and Williams 2018) describe a CD8 depletion model with SIVmac251 infection of *Rhesus macaque* with and without ART. Virus enters the CNS early, and macrophage activation correlates with CNS disease, as well as inflammation outside of the CNS. Antiretroviral therapy in HIV+ humans and SIV+ Rhesus macaques results in non-detectable plasma virus, decreased, or non-detectable viral RNA or protein in the CNS. But, viral DNA rebounds following therapy interruption, demonstrating the presence of replication competent virus in the CNS within myeloid cells. In this brief review, they discuss their findings using a Rhesus macaque model of SIV-associated CNS infection and pathology, focusing on monocyte/macrophage activation and the link between CNS and cardiac disease. They also describe recent studies using adjunctive therapy targeting monocytes/macrophages with ART to prevent or diminish CNS pathology that may be associated with HAND.

Feline immunodeficiency virus model

Power (Power 2017) describes a feline immunodeficiency virus (FIV) model that causes immunosuppression through virus-mediated CD4+ T cell depletion in feline species. FIV infection mimics HIV-1 infection of the CNS in humans, as evidenced by a virus-induced disease in the nervous system. FIV enters the brain soon after primary infection and is detected as FIV-encoded RNA, DNA, and proteins in microglia, macrophages, and astrocytes. FIV infection activates neuroinflammatory pathways including cytokines, chemokines, proteases, and ROS with accompanying neuronal injury and loss. Neurobehavioral deficits during FIV infection are manifested as impaired motor and cognitive functions. Several treatment strategies have been tried in this model, including the therapeutic benefits of antiretroviral therapies, other protease inhibitors, anti-inflammatory, and neurotrophic compounds. Recent studies have documented insulin's anti-viral, anti-inflammatory, and neuroprotective effects in this model. Treatment with insulin suppressed FIV replication of lymphocytes and diminished cytokine/chemokine activation in infected microglia. Intranasal (IN) insulin delivery for

6 weeks suppressed FIV expression in the brain, reduced neuroinflammation, and protected neurons in hippocampus striatum and neocortex in FIV-infected animals. Intranasal insulin also improved neurobehavioral outcomes in the FIV model. This paper emphasizes that FIV infection of the nervous system model provides an in vivo model for discovering and evaluating disease mechanisms as well as developing therapeutic strategies for NeuroHIV in humans.

HIV-1 transgenic rat models

Two papers describe the use of HIV-1 transgenic rats for behavioral studies. HIV-1 transgenic rats contain a gag-pol-deleted HIV-1 provirus located on chromosome 9 in F344/N strain. McLaurin et al. (McLaurin et al. 2017) describe utility of the transgenic rats in longitudinal experimental designs to determine the progression of HAND. They begin by validating the integrity of auditory and visual sensory system function in transgenic rats, using cross-modal prepulse inhibition and locomotor activity assessments. Next, they document the progression of neurocognitive impairments, including temporal processing and long-term episodic memory, in the HIV-1 Tg rat. The authors state that the transgenic rat model can serve as an advantageous model for assessing neurocognitive deficits caused by HIV-1 and can possibly help in the development of neurorestorative and preventive treatments.

Royal et al. (Royal et al. 2018) have utilized the transgenic rat model to study interactions between smoking and HIV infection. The HIV-1 transgenic rats were tested using behavioral tests such as the rotarod test (assessing motor coordination, balance, motor learning), the novel object recognition test (assess recognition memory), and the open field test (examines general locomotor activity). These studies, demonstrated that F344 and HIV1Tg rats show differential behavioral and immune effects from environmental exposures. In this publication, the authors highlight the utility of the HIV-1 transgenic rat model for studying behavioral changes as a result of exposure to environmental toxins and HIV.

In summary, this special issue summarizes our current knowledge about a variety of animal models used to study HIV-associated CNS dysfunction and CNS viral reservoirs. The discussions at the NIMH meeting as well as the papers presented in this special issue did not make any specific recommendation on the utility of using one model over the other. It was widely felt that the choice of the model will be dictated by the questions being pursued. It is critical that the investigator be aware of the limitations of the model and comprehend whether the findings match the phenotype observed in virally suppressed patients.

We hope that this will serve as an important resource for the field and help stimulate further research in the area. We would also like to take this opportunity to thank all the authors for

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