Review

Rodent models for HIV-associated neurocognitive disorders

Santhi Gorantla, Larisa Poluektova and Howard E. Gendelman

Center for Neurodegenerative Disorders and Department of Pharmacology and Experimental Neuroscience, University of Nebraska Medical Center, Omaha, NE, USA

Human immunodeficiency virus (HIV)-associated neurocognitive disorders (HAND) reflect the spectrum of neural impairments seen during chronic viral infection. Current research efforts focus on improving antiretroviral and adjunctive therapies, defining disease onset and progression, facilitating drug delivery, and halting neurodegeneration and viral resistance. Because HIV is species-specific, generating disease in small-animal models has proved challenging. After two decades of research, rodent HAND models now include those containing a human immune system. Antiviral responses, neuroinflammation and immunocyte blood–brain barrier (BBB) trafficking follow HIV infection in these rodent models. We review these and other rodent models of HAND and discuss their unmet potential in reflecting human pathobiology and in facilitating disease monitoring and therapeutic discoveries.

Introduction

The Holy Grail for HIV research activities is the elimination of virus from an infected human host. Notwithstanding prodigious research efforts, vaccination has so far failed, and combination antiretroviral therapy (cART) has permitted low-level persistent viral replication. The latter is observed in defined reservoirs with notable disease morbidities. One such morbidity is central nervous system (CNS) disease, which affects cognitive, behavioral and motor functions that trigger a range of adverse clinical outcomes. Up to 27% of HIV-1-infected people show identifiable cognitive dysfunction, and 84% show definable deficits in cognition, physiology and behavior [1]. The clinical manifestations of HIV-associated neurocognitive disorders (HAND), interestingly, range from very limited deficits in information processing to profound dementia [2–4]. Brain pathology ranges from limited discernable abnormalities to multinucleated giant cell encephalitis [5]. Research activities serve to improve diagnosis, understand disease processes, and improve drug delivery and therapeutics. These can best be achieved through experimental systems that reflect chronic viral infection and neuronal function [6].

HIV targets brain mononuclear phagocytes (MPs; monocyte-derived macrophages and microglia) and immune suppression speeds virus growth and related neuronal injuries. Viral and cellular MP products elicit local metabolic dysfunctions and disrupt neuronal networks. Paracrine regulations of immune secreted bioactive products (e.g. chemokines, cytokines, arachidonic metabolites, quinolinic acid, Fas and Fas ligand among others) induce inflammation and affect disease tempo [7]. How systemic infection, immune activation and nervous system infection drive neuronal damage can be addressed through relevant animal models [8,9]. The following are important requirements for reflecting HAND in animals. First, an animal model should provide virus-susceptible target cells, including $CD4^+$ T lymphocytes, dendritic cells, monocytes and macrophages, that display receptors and coreceptors for viral infection and possess the host cell machinery to complete the viral life-cycle. Second, animals need to be infected through known portals of virus entry (i.e. the genitourinary system, rectum and blood). Third, infection and immune activation need be continuous for prolonged time-periods to model the chronic nature of disease. Fourth, BBB function should be altered as a consequence of viral infection to permit leukocyte transmigration. Fifth, virus target cells in the nervous system and viral reservoirs need be maintained. Here, we review recent progress in the development of rodent models that fulfill many of these requirements and, as such, provide an important means to study the pathophysiology of HAND. The noted limitations of the models together with the pathways towards improvements are discussed.

Animal models of lentiviral infections

HIV is a lentivirus and any review of animal models of HAND must take into consideration the unique molecular and biological properties of lentiviruses. Since the first appearance of the acquired immunodeficiency syndrome (AIDS) and the discovery of its causative agent, HIV, laboratory and animal models were quickly generated which mimic human disease. In particular, models that reflected viral neuropathologies and pneumonitis were sought because lentivirus infections commonly cause such tissue injuries as a result of persistent replication in MPs [10,11]. Notably, maedi–visna virus [12], ovine progressive pneumonia virus [13], equine infection anemia [14] and caprine arthritis–encephalitis viruses [15] infect MPs and induce severe tissue damage in the face of modest immune abnormalities. By contrast, simian immunodeficiency virus (SIV), simian-HIV (SHIV) [16], bovine immunodeficiency virus (BIV) [17], and feline immunodeficiency virus (FIV) [18] all induce profound immunodeficiency with tropism for both $CD4^+$ T cells and MP. All lentiviruses,

Corresponding author: Gendelman, H.E. (hegendel@unmc.edu)
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including HIV, are species-specific [19]. The common features of lentiviruses include an extended incubation period, MP tropism, immune abnormalities and deficiencies, nervous and pulmonary system inflammation, and a range of comorbid diseases [20]. However, working with larger animals is both cumbersome and costly, the viruses have unique biological and molecular properties in their hosts, and the end-organ damage observed with these viruses is often divergent from that observed in humans with HIV.

Rodent models of HIV disease

Rodent models can reflect HIV-1 pathogenesis and be used for therapeutic testing and vaccines [21]. Although HIV-1 does not infect rodent cells, mice and rat models can be used to reflect viral infection either by creating transgenic rodents expressing viral genes or by transplanting HIV-1 infected cells into immunodeficient rodent tissues. The main advantage of developing rodent models is that, in addition to the ease in handling and housing the animals, there are well-established methodologies for manipulating the rodent genome [22]. However, limitations abound in reflecting end-organ disease. In the case of transgenic models, there is no spread of infection and inferring causal biological ties to natural infection from the expression of specific viral proteins is not always possible. In the case of human cell reconstitution models, human–mouse cytokine–receptor interactions and limitations in graft survival for any prolonged time-period make these models of more limited value. These are especially notable for CNS disease.

HIV-1 provirus and its constituents in transgenic rodents

Virus and viral proteins affect neuroinflammatory and neurodegenerative activities. As a first attempt, full-length HIV-1 proviral DNA was inserted into the mouse genome and the virus was expressed in neurons under the transcriptional control of the neurofilament promoter [23]. The transgene was expressed in the anterior thalamic and spinal motor neurons, and animals developed axonal degeneration together with hypoactivity and limb weakness. Other proviral transgenic mouse models (full-length or *gag–pol*-deleted mutant HIV-1pNL4-3 and full length HIV-$1_{JR\text{-}CSF}$) were developed [24,25]. Transgenic mice expressing CD4 targeted expression of hu-cycT1 and HIV-$1_{JR\text{-}CSF}$ were capable of producing glial inflammatory responses [26]. A HIV-1 NL4-3 provirus devoid of Gag and Pol expressed in a transgenic rat (HIV-Tg) demonstrated immune dysfunction as well as behavioral and motor abnormalities [27]. Deficits in learning beginning at five months of age were observed in this model as well as neuroinflammation [28,29]. This rat model has also been used successfully to reflect comorbid effects of drugs of abuse on the CNS [30].

Individual viral subgenomic fragments have been used to create transgenic rodents capable of eliciting systemic pathologies as well as neuropathologies (Table 1). Expression of the HIV proteins, the regulatory transactivator of transcription protein (Tat) and the envelope glycoprotein gp120, was found to be neurotoxic [31]. Transgenic expression of gp120/gp160 under the regulatory control of modified mouse glial fibrillary acidic protein (GFAP) promoter or a neuron-specific promoter induced neurotoxicity, but less than that observed with Tat [32,33]. Regarding the former, HIV-1gp120 mice elicit a reactive astro- and microgliosis with readily demonstrable neuronal losses in the neocortex with dendritic vacuolation [32]. Behavioral studies show age-dependent memory impairments, and the model successfully delineated cellular pathways for gp120 neurotoxicity [34,35]. With respect to the latter, the transgenic mouse expressing HIV-1 Tat under the control of a doxycycline-dependent astrocyte-specific GFAP promoter showed Tat-dependent neural abnormalities and premature death [33]. Astrogliosis, degeneration of neuronal dendrites, neuronal apoptosis, and infiltration of activated monocytes and T cells reflected a role for Tat in viral neuropathogenesis. Transgenic mice expressing viral protein R (Vpr) under the control of the c-Fms promoter, to express the protein in myeloid cells, demonstrated neuronal and glial apoptosis and behavioral abnormalities ([36], reviewed in [37]). Transgenic expression of another HIV accessory protein, Nef, under the control of the CD4 promoter, resulted in systemic immune abnormalities [38]. In addition to HIV-1 proteins, transgenic expression of the HIV-1 long terminal repeat (LTR) revealed that it is most active in brain tissue when it is derived from a neurotropic strain [39]. As in any model, there are notable limitations for the transgenic systems. Indeed, in the cART era, neurocognitive dysfunctions persist even with limited CNS virus. In this setting, low levels of virus may elicit detrimental effects on the brain through increased production of proinflammatory cytokines and chemokines, and as such, lead to neuronal dysfunction that could not be reflected in these models. Certainly, the amounts of virus and specific viral proteins in the brain do not directly link to HAND severity [40]. Hence, transgenic models expressing viral proteins may not mimic events involved in the natural onset and progress of HIV neuropathogenesis. The transgenic models also fail in their abilities to reproduce disease complexities. Indeed, the interplay between peripheral viral replication and brain pathology needs to be addressed in any model system. Although HIV-1 and human host protein interactive networks are established [41,42], the complexities of virus-associated effects on host immunity and related neurotoxic activities require dynamic and relevant model systems that accurately reflect human disease.

Human C-X-C chemokine receptor type 4 (CXCR4)/C-C chemokine receptor type 5 (CCR5)

Significant attempts were made in generating a viable system for chronic viral infections in rodents [43,44]. One attractive approach has been to engineer immunocompetent transgenic rodents that are susceptible to HIV-1 infection. This includes engineering both T lymphocytes and macrophages, the target cells for HIV-1 infection in human hosts, in animal models [45]. Towards this end, the multiple blocks to HIV-1 entry and replication in rodents [46] were partially overcome by the insertion of human HIV-specific receptors (CD4) and coreceptors (CCR5 or CXCR4). Development of human CD4/CCR5 or CXCR4 transgenic rats [47,48], mice [49,50] and rabbits [51] was achieved. However, multiple defects in completing the HIV-1 life-cycle in rodent cells and overall viral infectivity of rodent-cell-generated viruses remained obstacles in