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Rodent models for HIV-associated neurocognitive disorders

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Abstract

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. As 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. Here, 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.

Keywords

human immunodeficiency virus type one; rodent model; neuroinflammation; cognitive impairment; HIV-associated neurocognitive disorders

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 enabled 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 triggering a range of adverse clinical outcomes. Up to 27% of HIV-1 infected people show identifiable cognitive dysfunctions, 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 is from limited discernable abnormalities to a multinucleated giant cell encephalitis [5]. Research activities serve to improve diagnosis, understand disease processes, and improve drug delivery and therapeutics. These can be best achieved through experimental systems that reflect chronic viral infection and neuronal function [6].

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HIV targets brain mononuclear phagocytes (MPs; monocyte-derived macrophages and microglia) and immune suppression speeds viral 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 (for example, 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 would provide virus susceptible target cells, including CD4+ T lymphocytes, dendritic cells, monocytes and macrophages that display receptors and co-receptors 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 viral 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 to mimic human disease. In particular, models that reflected viral neuropathologies and pneumonitis were sought, since lentivirus infections commonly caused 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. In 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. Like HIV, all lentiviruses are species-specific [19]. The common features of lentiviruses include a long incubation period, MP tropism, immune abnormalities and deficiencies, nervous and pulmonary system inflammation, and a range of co-morbid 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 what is 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 along with hypoactivity and limb weakness. Other pro-viral transgenic mouse models (full-length or *gag-pol* deleted mutant HIV-1pNL4-3 and full length HIV-1_{JR-CSF}) were developed [24, 25]. Transgenic mice expressing CD4 targeted expression of hu-cycT1 and HIV-1_{JR-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 5 months age, were observed in this model as well as neuroinflammation [28, 29]. This rat model has also been used successfully to reflect co-morbid 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, were found to be neurotoxic [31]. Transgenic expression of gp120/gp160 under a 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 micro- gliosis 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 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] and 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 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, limited virus may elicit detrimental effects on the brain through increased production of pro-inflammatory cytokines and chemokines, and as such, lead to neuronal dysfunction that could not be reflected in these models. Certainly, the amount 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 need 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 of HIV-1 entry and replication in rodents [46] were partially overcome by the insertion of human HIV-specific receptors (CD4) and co-receptors (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 establishing infection. HIV replication was defective because host factors that are needed in the later stage of viral replication were lacking in murine and rat cells [52, 53]. For these reasons, concomitant neuropathology has not been realized in such systems.

Chimeric Viruses

The development of the viruses or transgenic rodents to bypass species-mediated restriction of HIV-1 entry is an attractive modeling approach. In contrast to what has been described in CD4/CCR5 rodents with respect to restrictions in viral replication, several studies with rodent cells indicated that primary mouse cells are permissive to late stages of the virus life cycle [49]. Bypassing the restrictions in virus entry, through DNA transfection or through pseudotyped virus, permits efficient HIV-1 expression and the production of infectious progeny virus in rodent cells [54]. Continuous virus infection and cell-to-cell spread has not been achieved in such models. This was overcome, in part, by molecular viral approaches. Indeed, more recently, the host species range of HIV-1 was expanded from primate to rodent by replacing the coding region of its surface envelope glycoprotein, gp120, with the envelope-coding region from ecotropic murine leukemia virus that facilitates the viral entry into rodent cells [55]. Two chimeric viruses, EcoHIV on a backbone of clade B NL4-3 and EcoNDK on a backbone of clade D, were generated. EcoHIV and EcoNDK established systemic infection in mice after one inoculation. This experimental infection reproduced characteristics of HIV-1 infection in its human host, including viral targeting of lymphocytes and macrophages, induction of antiviral immune responses, neuroinvasiveness, and expression of inflammatory and antiviral factors in the brain. These models showed that the viral structural and regulatory proteins can be produced at some level during EcoHIV replication, and the mice are responsive to HIV-1 antigens. The EcoHIV strain, which had the highest viral burden in the spleen, showed significant increases in the expression of C3, interleukin-1 beta (IL-1 β), monocyte chemoattractant protein-1 (MCP-1), and signal transducers and activators of transcription-1 (STAT-1) in the brain. These models are used for the screening of antiviral drugs and vaccines [56, 57], but limitations abound in relation to reflecting target cell tropism and spread of infection at the levels seen in humans.

Murine lymphocytes, macrophages and astrocytes produce viral RNA, protein, and infectious progeny following infection with HIV-1 pseudotyped by vesicular stomatitis virus envelope glycoprotein G (HIV/VSV) [54, 58]. HIV/VSV can be used to infect murine bone marrow derived macrophages (BMMs), followed by subsequent injection into the brains of wild type mice [59]. Mouse BMMs may more adequately reflect cell trafficking and allow for the study of both innate and adaptive immune responses that follow viral infection. Immune modulation of T cells by Copolymer 1 was found to result in decreased microglial activation and neuroprotection around the injection site of HIV-1 infected BMMs [60]. Adoptive transfer of T regulatory lymphocytes (Treg) also induces neuroprotection in these mice. Treg-mediated the down-regulation of pro-inflammatory cytokines, oxidative stress, and viral replication. In parallel, Treg effects the up-regulation of neurotrophins and leads to

neuroprotection [61]. Tregs readily migrate across the BBB, are retained within virus-induced neuroinflammatory sites and modulate microglial responses [62].

Human reconstitution models

The interest in humanized rodent models of neuroAIDS (ie. models that include a functional human immune system) has spanned decades of research and inquiry [63–65]. The creation of rodent model systems that faithfully reflect human disease must take into account the interplay between viral infection in the periphery and the nervous system. Moreover, the abilities to address questions regarding the viral and host factor interactions as well as the role of lymphoid infection and immune activation on neuropathology is of paramount importance.

The principal pathogenic features of viral infection in the brain include the induction of microgliosis, astrogliosis and neuronal apoptosis. Simply stated, any successful model needs to recapitulate inflammatory responses in the brain, as well as in other tissues where active viral replication occurs. There must also be the development of specific antiviral immunity. Viral infection is well known to induce both humoral and cellular antiretroviral activities. Measures of viral load in relationship to CD4+ T cells and end-organ disease, such as the CNS, need be addressed. To achieve all, a model should support chronic HIV infection. A major question is whether it is possible to model sustained HIV-1 replication and induce murine neuropathology? Immunodeficient mice that accept human cell grafts susceptible to HIV infection have been attractive platforms. Nonetheless, the pathobiological disease events seen in these mice have distinct features from human disease.

HIV-1-infected monocyte-derived macrophages (MDMs) injected into mouse brains

Early attempts to reflect HIV disease of the CNS were re-evaluated following the introduction of cART. Historically, encephalitis fueled by active viral replication in MPs reflected the histopathological correlate of disease in the pre-cART era [66]. Indeed, persistence of HIV-1 nearly exclusively was seen in macrophages [67]; hence, the first attempt to induce pathogenesis of HIV encephalitis (HIVE) was done by injecting virus-infected human peripheral blood mononuclear cells into the brain of immunodeficient mice [63]. Certain aspects of human pathogenesis were reproduced, however, the model proved insufficient to adequately reflect gliosis, giant cell formation, and pro-inflammatory cytokine expression.

After years of investigation, injections of HIV-1 infected human MDMs into the basal ganglia of immunodeficient mice proved remarkably similar to human HIVE. This gave rise to the name HIV encephalitic (HIVE) mice [68]. Indeed, histopathological changes observed in murine brains paralleled those in human HIVE [Figure 1(i)âââââ]. This included HIV-1 infected human macrophages, the formation of multinucleated giant cells, astrogliosis, microglial activation and neuronal cell death. Subsequently, the model was used to examine neuronal function in HIVE. This was done by using a range of behavioral [69–71] and electrophysiological tests [72]. Mice injected with MDMs developed cognitive impairments and associated deficits in synaptic long-term potentiation. HIV-1 viral strain-specific differences in inducing neuropathogenesis were also assessed [73]. Importantly, this mouse model has been used extensively for evaluating new drugs and delivery systems for HIVE. Indeed, among the spectrum of mouse models used to study HIV-1 neuropathogenesis (see Table 1), HIVE mice have been the most widely used for the development of therapeutics to date. Antiretrovirals were assessed to reduce HIV-1 replication within the brain in HIVE mice [74–79]. Different antiretroviral drug delivery systems to maximize the drug brain entry and eradicate the viral reservoirs were tested [79, 80]. As macrophage-induced inflammation affects hippocampal plasticity and neuronal development in HIVE mice [81]

adjunctive medicines that can reduce microglial activation resulting in neuroprotection were developed using these mice [60, 82, 83]. Interestingly, during these investigations it was found that dexamethasone, while profoundly reducing brain inflammatory responses, was directly neurotoxic [84]. Despite the many advantages of reconstituted human cell systems for studying neuroAIDS, these models nevertheless show limitations as they reflect only the static nature of brain disease and are unable to mimic progressive infection.

Lymphoid reconstitution

Neuropathogenesis associated with HAND has a strong correlation with the efficacy of antiviral adaptive immune responses, the levels of activation of the peripheral lymphoid system and CD4⁺ cell decline [85], activation of brain microglial cells and/or immigrated macrophages [6]. CD8⁺ and CD4⁺ T lymphocytes are present in cerebrospinal fluid (CSF) [86], perivascular cuffs of HIV brains [87], and often found as scattering cells in white matter tracks [88]. The neurological dysfunctions have also been found to correlate with increased numbers of CD8⁺ T cells in SIV infected monkeys [88].

Studies have examined the peripheral immunity in HIV mice in order to better understand the adaptive immune response in HIV neuropathogenesis. Non-obese diabetic (NOD) mice on a Severe Combined Immunodeficiency (*scid*) background were used for improved reconstitution with human (hu) peripheral blood lymphocytes (huPBLs). HIV-1-infected human macrophages were injected into the brains of the mice reconstituted with syngeneic huPBL [65, 89]. This huPBL-HIVE model showed that HIV-1-infected cells in the brain could spread the virus to the human lymphocytes infiltrating the brain [Figure 1(ii)]. The infection is disseminated throughout the blood of mice, and HIV-1-specific cellular immune responses were detected in the periphery of these mice [65]. The presence of lymphocytes facilitates elimination of infected cells from the brain and the restoration of tissue integrity. The huPBL-HIVE model allowed investigations of inhibitors of indoleamine 2,3-dioxygenase (IDO), PPAR gamma agonists and cannabinoid 2 (CB2) receptor agonists [90, 91]. IDO activity is linked to immunosuppression by its ability to inhibit lymphocyte proliferation and to neurotoxicity through the generation of quinolinic acid and other toxins [90]. Using the huPBL-HIVE model, the ability of the IDO inhibitor 1-methyl-d-tryptophan to stimulate the generation of cytotoxic lymphocytes (CTLs) and clearance of virus-infected macrophages from the brain was shown [90]. *In vivo* findings obtained in this model underscored the ability of PPAR gamma agonists to reduce HIV-1 replication in lymphocytes and brain macrophages, thus offering a new therapeutic intervention for both systemic and nervous system infections [92]. CB2 receptor activation has been proposed to be beneficial through its anti-inflammatory and immunosuppressive activities in mouse models of HAND [91].

Unfortunately, one of the limitations of the huPBL-HIVE model is that the engraftment of the human PBLs often induces graft vs. host disease (GVHD), which is a result of the human immune cells recognizing the host (mouse) cells as foreign and subsequently initiates an immune-response against the host. This leads to the death of the animals within 4–5 weeks of engraftment, thus limiting the usage of this model for long-term studies [65].

Human CD34⁺ cells and mouse lymphoid tissue re-population

HIVE and huPBL/HIVE mouse models allow only short-term studies since they are acute models of HIV. The goal is to have a chronic infection model for long-term studies to investigate the ongoing relationship of peripheral infection to brain immune cell ingress, neuroinflammation and resulting neurodegeneration. Partial deletion of common cytokine receptor gamma chain ($\gamma_c^{-/-}$, or interleukin-2 receptor γ_c) on a NOD/ShiJic-*scid* (NOG) mouse background [93] and a complete deletion of γ_c^{null} on NOD/LtSz-*scid* (NSG) [94]

generated a new mouse model [95] (Figure 2). These animals have longer life spans, do not develop lymphomas, and do not reject human cells due to the absence of lymphocytes (including natural killer cells) and weak xeno-reactive innate immune responses.

The long-term immune reconstitution of NOG/NSG mice by human hematopoietic stem cells (HSCs) provides a new approach to study HAND. The major characteristics of chimeric animals are the higher rate of HSC acceptance with low doses of irradiation [96]; stable population of human cells in mouse bone marrow [97]; occupancy of murine thymus with human thymocytes and their maturation into T cell subsets [62, 98, 99]; complete reconstitution of residual murine lymph nodes with human T, B and dendritic cells [59, 100]; formation of splenic white pulp [94]; and the distribution of human cells of macrophage lineage throughout brain, the meninges and perivascular spaces [100]. The degree of reconstitution of mouse bone marrow by human cells determines the amount of human cells of macrophage lineage accumulation with age in CD34⁺ cell transplanted NSG animals. These “humanized” mice support chronic HIV-1 replication and recapitulate the course of viral replication with loss of CD4⁺ T cells, development of HIV-1-specific CTL, and humoral immune responses [62, 96, 101]. Productive peripheral infection accelerated the entry of human cells - including activated HLA-DR⁺ lymphocytes and macrophages - into the brain. Viruses were found to disseminate to the brain by the infiltration of peripheral infected cells. In particular, HIV-1 p24-positive cells with macrophage and lymphocyte morphology were observed in the meninges and perivascular spaces of infected animals [100]. Astro- and micro- gliosis and neuronal protein losses were commonly seen (Figure 3), and neurodegeneration was found to correlate with peripheral viral load and CD4⁺ cell depletion [102]. Similar to what is observed in SIV infected monkeys, CD8⁺ T cell depletion initiated by cM-T807 antibodies after infection accelerated the disease course, increased *HIV-1gag* RNA and increased inducible brain nitric oxide synthase expression [103]. The development of meningitis, and more rarely meningoencephalitis, was observed. These findings, taken together, demonstrate natural HIV-1 progression within the CNS of “humanized” rodents [100]. These data are supported by longitudinal non-invasive proton spectroscopic and diffusion tensor imaging of a small cohort of HIV-1 infected humanized mice co-registered with CD4⁺ T lymphocyte number, peripheral viral load, and brain histopathology, demonstrating concordant immune and neuronal abnormalities [102].

Recently, humanized mice were used for testing the effectiveness of nanoformulated antiretroviral therapies (called nanoART). The goal of the work was to increase dosing intervals from one day to one week, and as such, positively affect adherence to complex treatment regimens and reduce cumulative drug toxicities. To this end, the performance of crystalline atazanavir and ritonavir nanoART were tested using macrophages as indicator cells to determine uptake, release and anti-retroviral responses [104, 105]. Physical characteristics evaluated included particle size, surfactant coating, and shape for their abilities to affect drug cell uptake and anti-retroviral efficacy in cells [104, 105]. Based on the efficacy data in cells, the best performing nanoART were tested in humanized mice; these were used as an *in vivo* platform for further drug development [106]. The evaluation endpoints included viral gene expression and CD4⁺ T lymphocyte counts. Newborn NSG animals transplanted with human CD34⁺ cells were infected over months with HIV-1 prior to weekly drug treatments. Limited toxicity and sustained levels of the drug in both the tissues and plasma were observed [106]. Interestingly, HIV-1-infected mice that were treated with nanoART-retained numbers of human CD4⁺, CD8⁺ T cells and CD14⁺ monocytes and showed reduced viral loads (Figure 4). Such findings strongly suggest that the NSG “humanized” mouse model is likely to be a valuable tool for evaluating the efficacy of long-acting antiretroviral drugs.

It is important to mention that this mouse model is not without limitations. With humanization of the mouse immune system, infection of natural viral target cells is achieved. However, variability in the numbers of grafted human cells can significantly impact the pathobiological outcomes elicited by viral infection. Moreover, peripheral immunopathology, as seen in human lymphoid tissues following HIV-1 infection, is only partially replicated in humanized mice because mice lack human stromal elements. Divergent mouse-human and human-mouse receptor-ligand interactions, mixed populations of human and mouse macrophages, altered levels of persistent infection dependent on human cell engraftments, and the lack of significant numbers of microglia, are additional reasons that underlie the variability of infection observed in these mouse models.

Conclusions

The overarching goal in establishing a suitable animal model for human disease is to reflect the disease in all facets. This is a daunting task for chronic lentiviral infections as the virus is species-specific. A number of directives have been suggested to address such limitations. This includes employing related viruses, bypassing viral receptors and improving human-mouse reconstitutions. SIV or SHIV infected monkey models have proven to be a relevant model to mimic HIV-1 pathogenesis. Rodent models have also gained in relevance in recent years for understanding HIV-1 pathobiology. Ease in handling and cost-effectiveness have made mice and rats more accessible. However, while transgenic models are most suitable for understanding specifics of virus-induced neuropathology, mouse models of HIVE have proven necessary for testing antiretrovirals and adjunctive therapies. A substantive step forward in recent years has been the realization of a new mouse model with an engrafted human immune system. This system is better-suited for examining relationships between peripheral infection and neuropathobiology as compared to previous rodent models. Importantly, it is highly specific, as brain disease develops only as a consequence of HIV-1 infection and immune suppression. Such a model will also allow studies of the molecular basis of brain abnormalities seen at defined stages of HIV-1 infection. Furthermore, it will be valuable for the study and development of therapeutics for neuroAIDS, including long-term studies of cART, since the grafts will survive for the life of the mouse.

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Box 1**Outstanding questions**

- What neuronal abnormalities parallel neurocognitive deficits in HAND?
- How does persistent low-level HIV replication affect neuroinflammatory responses and related CNS injury?
- What is the extent of interactions between human immune activation and murine CNS disease?
- What are the relationships between CNS disease and systemic immune activation or immune suppression?
- Why do neurological injuries and cognitive deficits prevail when cART nearly attenuates viral replication?
- How well do human cell-derived inflammatory products (ie. cytokines and chemokines) interact with mouse neural receptors?
- What is the role of cART in the cause of neurological damage during chronic HIV infection?
- Does neuroinflammation persist in cART-treated patients?
- Can the CNS viral reservoir be eradicated by cART when the BBB is penetrated?
- Is there an optimal regiment of cART for blood brain barrier penetrance and for minimizing neurological deficits?
- Can adjunctive CNS therapies complement and extend the actions of cART? For instance, can neuroprotective drugs be given in addition to cART to lessen the occurrence of HAND?
- What are the changing molecular mechanisms of HAND that influence CNS disease onset and progression?
- Will common neurodegenerative disorders such as Alzheimer's and Parkinson's diseases influence the neuropathogenesis of HIV infection?
- What are the best means to improve rodent models to reflect the changing patterns of HIV disease of the CNS?

Figure 1. Schematic overview of HIVE mouse models for neuroAIDS

(i) Human monocytes differentiated into macrophages (MDM) in presence of macrophage colony stimulating factor (M-CSF) are infected with HIV-1_{ADA}, followed by the stereotactic injection of these cells into the basal ganglia of immune deficient mice. Histopathological features of human HIVE were induced that consisted of multinucleated giant cells, astrogliosis and microglial activation and neuronal loss around the injection site (bottom panel) [107]. Standard HIVE models have been widely used to test anti-retroviral, immune modulatory and adjunctive therapies for neuroAIDS [74–79]. Nonetheless, these models show limitations in reflecting aspects of HIV-1 neuropathogenesis. Although perivascular macrophages and microglia are the major drivers of brain pathology during HIV-1 disease, correlations have been noted between HIV-associated neurocognitive disorders and immune suppression [88]. To this end, an alternative mouse model (called huPBL/HIVE) (ii) was developed [89] that includes an adaptive immune cell component to assess its role beyond the innate response (ie. virus-infected macrophage-associated pathologies). Herein, replicate human lymphocytes were isolated from the same donor leukopak, then injected intraperitoneally (i.p.) to reconstitute the peripheral immune system of *NOD/scid* mice. A week after reconstitution, HIV-1 infected MDMs were injected into the basal ganglia of these mice. In brains of *huPBL-HIVE* mice, lymphocytes (arrows, bottom panel) are in close contact with the infected MDMs (arrow heads). Viruses spread to lymphocytes, and the infection disseminates throughout the blood and peripheral immune organs. HIV-1 specific cytotoxic T cell responses were also detected in these mice [89]. Hence, the clearance of viral-infected MDMs was observed. *huPBL-HIVE* mice permits the investigation of immune modulators for disease. However, HuPBL engraftment induces graft versus host disease due to the over-activation of human lymphocytes towards the host cells. To avoid this result, a third model was developed. (iii) HIV-1/VSV infected bone marrow-derived macrophages were injected into the brains of immunocompetent C57/B16 mice. HIV-1/VSV pseudotyped chimeric virus is used to bypass the restriction of infection of murine cells. Similar to the *huPBL/HIVE* model, infiltration of lymphocytes (arrows) into the injected area was seen with a faster clearance of infected macrophages [59], due to the immune response from the donor mouse lymphocytes. Due to the lack of viral spread to mouse cells, there is no viral dissemination into lymphocytes and the periphery, and multinucleated giant cells are also not formed. Abbreviations: i.c., intracranial; MNGC, multinucleated giant cell; NOD/SCID, non-obese diabetic severe combined immunodeficiency; PBL, peripheral blood lymphocytes. Immunohistochemical stainings adapted, with permission, from [60] (i), [89] (ii) and [59] (iii).

Figure 2. “Humanized” NOD/NSG mouse models for chronic HIV-1 infection

A human CD34⁺ stem cell reconstituted mouse model was developed to permit long-term studies assessing the relationship between peripheral infection and brain pathology. Human CD34⁺ stem cells (HSC) isolated from umbilical cord blood were injected intrahepatically into 1 day-old irradiated pups. A complete human immune system is developed in NOD/LtSz-*scid* g_c^{null} NSG mice [91, 100, 102, 108]. The injected HSC reach mouse lymphoid organs including bone marrow, spleen, lymph nodes, gut and develop into a broad range of immune cell lineages. The presence of human CD34⁺ stem cells, myeloblasts, B cell precursors, erythroblasts, promyelocytes, granulocytes and monocytes in mouse bone marrow were detected. Human T cell development occurred in the mouse thymus, as evidenced by the presence of CD4/CD8 T cells. In lymphoid tissue, distinct follicles filled with human T, B lymphocytes and macrophages were observed. Lymph nodes were also reconstituted with human lymphocytes, macrophages and dendritic cells. A mature human immune system develops in the mouse by 20–22 weeks [108].

Figure 3. HIV-1 neuropathology in “humanized” NSG mice

Chronic HIV-1 infection for 8 weeks leads to reductions in CD4⁺ T lymphocytes in lymphoid tissue and accelerated entry of human cells into the brain. Increased ingress of HIV-1 infected blood borne macrophages into the meninges and perivascular spaces of the brain were observed. These pathobiological events induce micro- and astro- glial inflammation and neuronal dysfunction, as evidenced by protein abnormalities. **A**, Microglial activation and nodule formation in white matter tracts within the brain stem as evidenced by immunocytochemical staining (indicated in brown color) for the ionized calcium binding adaptor molecule-1 (anti-Iba-1, arrowheads). **B**, Perivascular accumulation of mouse macrophages and microglia (Iba-1 staining) in the cerebellum (arrowhead). **C**, Astrocyte activation in white matter tracts within the cerebellum around blood vessels (and other brain regions, not shown) is evident by the immunopositive staining for glial fibrillary acidic protein (arrows). **D**, Pattern of distribution of immune competent human cells visualized by staining for human HLA-DR (a MHC class II cell surface receptor, brown color). Human cells are along the cerebellar fissures, in granular cell layers and in perivascular space (arrows). Insert to **D**, is an adjusted section of the vessel shown with a cell stained for HIV-1p24. **E**, HLA-DR staining shows human leukocytes in the meninges with an adjusted section stained for HIV-1p24 shows a high proportion of infected cells (insert to **E**). **F**, Microglia-like cells stained for HIV-1p24 were rarely observed in parenchyma. The blue hematoxylin counterstaining was used for nuclei [Images adapted from [100] (A–C) and [102] (D–F)]. (G–H) Immunofluorescence labeling of dendrites (red staining for Microtubule-Associated Protein 2, MAP2) and synapses (green staining for synaptophysin, SYN) in the cortex of control uninfected (**G**) and HIV-1 infected (**H**) mice (15 weeks of age)

show dendritic and synaptic protein integrity losses in the infected case. Cell nuclei indicated by 4',6-diamidino-2-phenylindole (DAPI) staining (blue) [102]. At the figure bottom is a schematic representation of the fields of view **A** – **F** taken from the sagittal sections (blue squares – bright field images), and **G** and **H** taken from coronal section (red – immunofluorescent) are shown. Images reproduced, with permission, from [100] and [102].

Figure 4.

Schematic model illustrating the use of **HIV-1 infected “humanized” NOD/NSG mice** for testing nanoformulated antiretroviral therapies (nanoART). This schematic reflects the potential of long-acting antiretrovirals to reduce viral load and protect both CD4⁺ T cells and the CNS against HIV-1 associated injuries [106]. The colors reflect normal immune tissue homeostasis (green and yellow) and tissues damaged by chronic viral replication (red). CD4⁺ T lymphocyte decline is observed following HIV-1 infection [108, 109]. In addition, infected and immune activated lymphocytes and macrophages have been observed in lymphoid organs and peripheral blood [108, 109]. Infected animals have been treated with nanoART [106], which can gain entry into monocyte-macrophages (center of picture) and serve as a long-term drug depot leading to the suppression of viral replication and protection of CD4⁺ T cell numbers (green and yellow). The nanoformulation may also facilitate ART delivery to the nervous system, although this has yet to be experimentally demonstrated.

Table 1

Rodent models of neuroAIDS.

Name	Method	Neuropathology	Immuno- and gross pathology	Utility
<i>Transgenic models</i>				
<i>HIV-1</i> Tg mice	Gag-pol- depleted HIV-1 pNL4-3 genome [24]	No description	growth failure	HIV-1-associated renal disease (reviewed in [110])
<i>HIV-1_{JR-CSF}</i> Tg mice	Full length infectious proviral clone of HIV-1 _{JR-CSF} [49]	No description	None	<i>In vivo</i> regulation of HIV-1 production by factors that activate the immune system [111]
<i>HIV-1_{JR-CSF}-hu- cycT1</i> Tg mice	CD4 targeted expression of hu-cycT1 and HIV-1 _{JR-CSF} [112]	Glial activation following LPS, GM- CSF increased	CD4 ⁺ cell depletion	Pathogenesis [26]
NF-L-HIV Tg mice	HIV genome under the control of the NF-L promoter [23]	~50% of 7–12 months old animals had peripheral axonal degeneration	None	Pathogenesis
NFLgp160-Xba Tg mice NFHgp160-Xba Tg mice	HIV-1gp160-Xba under NF-L and NF-H promoters [113, 114]	Neuronal damage/loss, gliosis and microglial reactivity; immunocyte entry into the brain; and chronic perivascular inflammation in leptomeninges	splenic reactive hyperplasia	Pathogenesis
<i>gfap</i> -HIVgp120 Tg mice	HIV-1 gp120 under the control of the GFAP promoter [32]	Astrogliosis, microglial activation, dendritic vacuolization and neuronal loss in neocortex	None	Pathogenesis [115] and therapeutics [116]
<i>gfap-Tat</i> Tg mice	Dox-regulated Tat expression under the control of the GFAP promoter [33]	Disruption of cerebellum and cortex, death at 5–7 days after Dox treatment	growth failure	Pathogenesis [117] and therapeutics [118]
<i>mbp</i> -HIV Tg mice <i>mbp</i> -Nef Tg mice	HIV or Nef under the MBP promoter in [119, 120]oligodendrocytes	Vacuolar changes in spinal cord but infrequent in brain. Late onset hind limb paralysis.	None	Pathobiology
<i>c-fms</i> -Vpr Tg mice	Vpr expression linked to the macrophage-specific murine exon 2 <i>c-fms</i> (M- CSF receptor)[36, 121]	Activation of caspase-6 leading to astrocyte apoptosis, diminished expression of astrocyte-specific markers, hyperexcitable neurobehavioral phenotype	No description	Pathobiology
<i>HIV-1</i> Tg rats	<i>gag-pol</i> -deleted HIV-1 provirus regulated by the viral promoter [27]	Behavioral and motor abnormalities: circling behavior and hind-limb paralysis.	cataracts, weight loss, lung, skin, kidney and heart damage, loss of lymphocytes	Therapeutics (vitamin A and morphine) [122]
<i>Human reconstitution models</i>				
SCID-HIVE mice	Intracranial injection of human HIV-1 infected MDM SCID-HIVE [63, 68]	Focal encephalitis	Limited	Pathogenesis [69, 73, 81, 123, 124]; and therapeutics
hu-PBL SCID- HIVE mice	Intracranial injection of human HIV-1- MDM with autologous PBL reconstitution hu-PBL SCID- HIVE [89]	Accelerated focal encephalitis	Adaptive immune response, Graft vs host disease	Pathogenesis [65, 125] and therapeutics

Name	Method	Neuropathology	Immuno- and gross pathology	Utility
HIV-1 infected huNSG mice	HSC transplantation leads to complete human immune system development, also called humanized NSG	Glial activation, meningitis and neurodegeneration. At 15 weeks after viral infection drop in NAA concentration in cortex and loss of neuronal structural proteins	Viral replication, immune activation, loss of CD4 ⁺ T lymphocytes.	Pathogenesis [100, 102, 108, 109], diagnostics and therapeutics. Ongoing studies on cART [106] and adjunctive therapeutics

Abbreviations: cycT1, cyclin T1; Dox, doxycycline; GFAP, glial fibrillary acidic protein; GMCSF, granulocyte macrophage colony stimulating factor; HIV-1JR-CSF, human immunodeficiency virus type one strain JR-CSF; HIVE, HIV-1 encephalitis; hu-PBL, human peripheral blood lymphocytes; LPS, lipopolysaccharide; MBP, myelin basic protein; MCSF, macrophage colony stimulating factor; MDM, monocyte-derived macrophages; NAA, N-acetylaspartate; NF-L, NF-H; neurofilaments (light and heavy chains, respectively); NOD, nonobese diabetic; SCID, severe combined immunodeficiency; Tat, trans-activator of transcription; Tg, transgenic.