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# Humanized mouse models for HIV-1 infection of the CNS

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## Abstract

Since the onset of the HIV epidemic, there has been a shift from a deadly diagnosis to the management of a chronic disease. This shift is the result of the development of highly effective drugs that are able to suppress viral replication for years. The availability of these regimens has also shifted the neurocognitive pathology associated with infection from potentially devastating to a much milder phenotype. As the disease outcome has changed significantly with the availability of antiretroviral therapy, there is an opportunity to re-evaluate the currently available models to address the neurocognitive pathology seen in suppressed patients. In the following, we seek to summarize the current literature on humanized mouse models and their utility in understanding how HIV infection leads to changes in the central nervous systems (CNS). Also, we identify some of the unanswered questions regarding HIV infection of the CNS as well as the opportunities and limitations of currently existing models to address those questions. Finally, our conclusions indicate that the earlier humanized models used to study HIV infection in the CNS provided an excellent foundation for the type of work currently being performed using novel humanized mouse models. We also indicate the potential of some humanized mouse models that have not been used as of this time for the analysis of HIV infection in the brain.

## Introduction

Human immunodeficiency virus (HIV) is the causative agent of acquired immune deficiency syndrome (AIDS) and represents a significant economical and health burden worldwide.

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Conflict of Interest

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HIV-associated dementia is, when untreated, a progressive clinical syndrome that occurs as a primary manifestation of HIV infection and is part of the overarching group of HIV-associated neurocognitive disorders (HAND). Historically, HIV infection led to cognitive impairment in a significant fraction of infected people. This cognitive impairment was thought to be the result of HIV encephalitis (HIVE) characterized by astrogliosis, activated microglia and microglial nodules, multinucleated giant cells, and neuronal loss (Gras and Kaul 2010). The significant progress made in recent years in HIV treatment has dramatically reduced morbidity and mortality; however, HIV cognitive impairment has remained a significant problem for many infected individuals with approximately half of all treated patients suffering from some level of cognitive impairment (Clifford and Ances 2013). In the pre-antiretroviral therapy (ART) era, HIV infection was associated with high levels of virus in the basal ganglia and hippocampus as well as robust microglial activation, particularly in patients with HIVE (Wiley et al. 1998; Anthony and Bell 2008). The introduction of ART, by controlling systemic viral replication and maintaining a more intact immune system, has reduced the incidence of various opportunistic infections such as CMV that would otherwise infect the central nervous system (CNS), and seems to limit lymphocyte infiltration into the CNS (Anthony and Bell 2008; Kranick and Nath 2012). Activation of macrophage/microglia in the CNS has been demonstrated in ART-treated patients, and it has been suggested that this ongoing inflammation, which may be caused by viral blipping and/or microbial translocation beginning early in the gastrointestinal tract, contributes to cognitive dysfunction (Albright et al. 2003; Chen et al. 2014). However, whether direct or indirect the link between inflammation in the CNS and manifestations of cognitive impairment is yet to be determined. The increase in the incidence of milder forms of HAND may be exacerbated due to the effects of aging in the infected population, although this remains controversial (McPhail and Robertson 2011). Additionally, it has been proposed that the brain may act as a tissue sanctuary for HIV due to limited drug penetrance into the CNS allowing for low levels of replication (McPhail and Robertson 2011). However, the CNS penetrance of many antiretroviral drugs has since been evaluated, and drugs with improved CNS penetration yield better neurocognitive outcomes, suggesting this problem could be overcome with the proper ART regimen (Letendre et al. 2004; Patel et al. 2009; Smurzynski et al. 2011).

Another remaining question for HIV CNS infection is whether or not T cells, microglia, or CNS macrophages are reservoirs for HIV in the brain, and if these cells are capable of re-establishing systemic infection after therapy interruption. In two post-mortem studies of human samples, microglia were determined to be latently infected, defined by the authors as presence of viral DNA with an absence of viral RNA or protein (p24) production (Thompson et al. 2011; Desplats et al. 2013). However, this definition falls short of the more stringent definition that is widely accepted in the HIV eradication field, whereby latency is defined as a reversible non-productive state of viral infection (Siliciano and Greene 2011). Without evidence to show that microglia with integrated viral DNA can become activated, produce virus, and lead to the subsequent infection of new cells, it is still necessary to determine whether or not microglia can truly be latently infected and act as a viral reservoir in the CNS.

Human studies of HIV infection in the CNS are limited to non-invasive imaging, cerebral spinal fluid (CSF) sampling, and post-mortem analyses (Langford et al. 2006; Masters and Ances 2014). The limited ability to directly probe brain tissue for HIV-infected cells significantly hinders efforts to track temporal and spatial relationships during acute or chronic infection. This in turn hinders our ability to elucidate the molecular basis of neuropathology in HIV infection. In this regard, animal models have become increasingly important because cellular, biochemical, molecular, and behavioral data can be compared to histological studies. Furthermore, preclinical evaluations of therapeutic drug treatment protocols can be monitored for multiple parameters to assess efficacy in the CNS. It is expected that these animal models that include both non-human primates and humanized mice will serve to gain novel insight into the molecular and genetic basis of HIV neuropathology and to answer long-standing questions: 1) how does HIV enter the CNS? 2) are T cells, microglia or macrophages reservoirs for HIV in the CNS? 3) does HIV cause direct neuronal damage leading to cognitive impairments, or is the CNS immune response to HIV what mediates the cognitive effects? and 4) what is the role of antiretroviral therapy in cognitive impairment? In the following as a prelude to the characterization of the humanized mouse models we first briefly describe progress made using non-human primate models of CNS infection. We then describe the different humanized mouse models of CNS infection and indicate their individual pros and cons. Finally, we summarize research areas where opportunities still exist to further utilize these models and where significant improvements remain to be made that could make these systems better models to study HIV infection of the CNS.

## Non-Human Primate Models of HIV infection of the CNS

HIV only infects humans and chimpanzees thus limiting experimentation. An alternative model is the rhesus macaque. These animals have been utilized extensively to study simian immunodeficiency virus (SIV)-induced CNS disease and from these studies significant information has been obtained regarding the kinetics of CNS SIV infection, virus compartmentalization, and the tropism of the several SIV variants most often present in the CNS (Veazey et al. 2008; Matsuda et al. 2013; Milush et al. 2013). In addition, these models have been extremely useful in investigating the efficacy of antiretroviral drugs to control HIV infection in the CNS (Annamalai et al. 2010). Analyses of the different populations of immune cells present in the CSF and the CNS of infected animals have shown the presence of SIV-specific $CD8^+$ cytotoxic T lymphocytes (CTLs) (von Herrath et al. 1995). The number of highly activated SIV-specific CTLs is increased in the brain during early SIV infection, and these cells are believed to contribute to the control of the infection (Marcondes et al. 2001). Macaques infected with a chimeric virus of SIV and HIV (SHIVSF162P3N) have been shown to develop giant cell SIV encephalitis, whereby the membranes of infected CNS macrophages fuse, a prominent feature of pre-ART era HIVE (7/43 infected animals, 16.3%) (Harbison et al. 2014). In the pigtail macaque model, non-CNS penetrating highly active antiretroviral therapy (HAART) has been shown to control virus RNA levels in the periphery and brain, with lower levels of CNS inflammation compared to untreated SIV-infected controls (Graham et al. 2011) Despite their significant contributions and direct relevance to human disease, non-human primate models of HIV infection in the CNS have been underutilized due to their limited availability, requirement

for specialized housing facilities, and high cost to maintain. Additionally, these models often utilize strains of SIV that have been continually passaged in order to cause CNS disease, limiting their ability to mimic a more natural infection as seen in humans (Matsuda, Brown et al. 2013).

## Humanized mice for HIV research

Mice are naturally refractory to HIV infection. However, immunodeficient mice are capable of receiving transplanted human donor cells and tissues that can support the study of HIV infection. Mice repopulated with human cells and/or implanted with human tissue have been generically called “humanized mice.” Over the last 20 years, humanized mice have been extensively used to study HIV replication, pathogenesis, transmission, immune responses, and novel antiretroviral therapies (Denton et al. 2010; Gorantla et al. 2010; Denton et al. 2014). For HIV infection of the CNS, two major humanization strategies have been employed: direct injection models where human cells are directly injected into the brain of mice or systemic transplant models in which transplanted human cells migrate into the brain of mice. In the following paragraphs and in Table 1, we summarize different types of humanized mice used to study HIV infection in the CNS.

## Direct injection mouse models of HIV infection of the CNS

In order to investigate the contribution of HIV-infected cells to HIV encephalitis (HIVE), several groups have injected HIV-infected human peripheral blood monocyte-derived macrophages (MDMs) or infected human microglia cells directly into the brains of severe combined immunodeficiency deficient (SCID) mice that lack T cells and B cells (SCID-HIVE mice) (Tyor et al. 1993; Persidsky et al. 1996). In this model, approximately half of the injected macrophages are infected ($p24^+$). Injection of HIV-infected human cells into the brains of mice resulted in neuropathology similar to that seen in HIV-infected humans with HIVE, including encephalitis, astrogliosis, multinucleated giant cell formation, infiltration of mononuclear phagocytes, and decreased microtubule-associated protein-2 (MAP-2) expression (Persidsky, Limoges et al. 1996; Persidsky et al. 1997). MAP-2 acts as a scaffolding protein that stabilizes the growth of microtubules, and MAP-2 loss has been associated with HIV infection in the brains of human patients (Lim and Halpain 2000; Desplats, Dumaop et al. 2013). The pathological changes seen in infected animals mimicking HIVE were also seen in uninfected controls, although the severity of pathology was diminished, suggesting that the presence of virus was necessary for the most severe pathology (Persidsky, Buttini et al. 1997). The direct injection model results in pathological changes in approximately seven days. Injection of infected macrophages or microglia (with uninfected MDM injections for control animals) into the basal ganglia (a region of the brain shown to be affected in humans) has been done in 3-4 week old mice (Persidsky, Buttini et al. 1997). Seven days post injection, HIV-infected cells could be found near the site of injection, in the basal ganglia as well as the cortex, putamen, and in close association with cerebral microvessels (Persidsky et al. 1999). Interestingly, a pronounced accumulation of mouse monocytes and microglia was noted near locations where human HIV-infected microglial cells were present, perhaps in part due to a local inflammatory response from the host at the site of injection. In summary, injection of HIV-infected human macrophages or microglia into the brain of immunodeficient mice results in astrogliosis, monocyte trans-

endothelial migration, and chemokine expression, reflecting similar abnormalities observed in the brains of HIV-infected patients with HIVE. Even though this model of HIVE has been extensively used, it is not ideal because of the trauma at the injection site, leading to severe inflammation from xenoreactivity, and because the human myeloid cells are placed in a microenvironment of foreign mouse cells. Another limitation of this model is that the studies are very short-lived (typically four weeks or less), and this makes it difficult to conduct long-term HIV persistence studies. While the SCID-HIVE model has been extensively used as a model for the more severe pathology associated with untreated or poorly managed HIV infection, there is limited evidence these animals recapitulate the less severe pathology seen in ART-suppressed patients.

To reflect the dynamic interaction between infected myeloid cells and the lymphoid effectors of the adaptive immune system, Poluektova et al. developed a model in which mice previously reconstituted systemically with human peripheral blood leukocytes (PBLs) were inoculated with HIV-infected autologous macrophages in the brain (Poluektova et al. 2002; Poluektova et al. 2004). The authors reported HIV-specific immune responses as determined by granzyme-positive $CD8^+$ T cells, by tetramer staining, and by IFN-gamma ELISPOT assays (Poluektova, Munn et al. 2002). In huPBL/SCID-HIVE mice, PBLs were usually found in the meninges, choroid plexus, and the ventricles which are regions patrolled by circulating immune cells (Ousman and Kubes 2012). In infected animals, CNS entry of lymphocytes (including $CD8^+$ T cells) was highest at seven days post macrophage injection, and their numbers decreased over the next 14 days. Additionally, giant multinucleated cells were present in the brains of these animals and were found to be in close proximity to human $CD8^+$ T cells. In huPBL/SCID-HIVE mice, elevated levels of IL-1beta and IL-6 were seen for three weeks, followed by a reduction in the numbers of HIV-infected macrophages in the CNS compared with SCID-HIVE mice suggesting that the additional immune cells were able to eliminate some of the infected cells (Poluektova, Gorantla et al. 2004). Additionally, in both the SCID-HIVE and huPBL/SCID-HIVE mouse models, multinucleated giant cells, astrogliosis, activation of the resident microglial cells as well as neuronal loss were reported (Gorantla et al. 2012). In summary, the huPBL/SCID-HIVE model of brain infection, despite its inherent limitations with limited time for experiments and the traumatic nature of injection, has served to delineate some of the interplay between infected macrophages in the brain and peripheral lymphocytes infiltrating the CNS.

## Systemic human reconstituted mouse models of HIV infection of the CNS

To model HIV infection systemically, many transplantation strategies have been utilized in various immunodeficient mouse strains. Mosier et al. described a mouse model where SCID mice were transplanted with human peripheral blood leukocytes (Mosier et al. 1991). One of the major advances in the development of robust and long-lived humanized mouse models lay in the mouse strains available. The same humanization approach for hu-PBL-SCID mice was performed in NOD/SCID mice yielding improved levels of human reconstitution (huPBMC-NOD/SCID) (Koyanagi et al. 1997). Human peripheral blood mononuclear cells obtained from normal individuals were administered to NOD/SCID mice via intraperitoneal injection; once reconstituted, these mice could then be infected parenterally with HIV-1 (Miura et al. 2003). In huPBMC-NOD/SCID mice, human $CD3^+$ T cells have been

consistently found in the meninges in close association with microvessels of both infected and uninfected animals (Miura, Misawa et al. 2003). However, under these conditions no human macrophages were detected in the brains of these mice, and there was no overt neuropathology except for sparse astrogliosis in the brains of infected mice. To induce human macrophage infiltration into the CNS, animals were administered bacterial endotoxin-derived lipopolysaccharide (LPS) (Miura, Misawa et al. 2003). Leukocyte infiltration was most evident in the perivascular regions of the cortex, the basal ganglia, the hippocampus, and the cerebellum (Miura, Misawa et al. 2003). Human macrophages and T cells infiltrated the CNS of both uninfected and HIV-infected mice, and microglial nodule formation was observed in both groups. LPS treatment also up-regulated tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) expression on the infiltrating macrophages and was postulated to contribute to both bystander killing of uninfected $CD4^+$ T cells and neuronal apoptosis in this model (Miura, Misawa et al. 2003).

The next advancement in humanization strategies was the introduction of hematopoietic stem cells (HSC) in place of peripheral blood-derived cells. These animals are created by injecting human $CD34^+$ HSC isolated from human umbilical cord blood, bone marrow or human fetal liver into the liver of sub-lethally irradiated newborn immunodeficient mice (the hu-NSG and hu-NOG models) (Gorantla et al. 2010; Dash et al. 2011). Alternatively, adult NOD/SCID mice can be transplanted intravenously with HSC (Asheuer et al. 2004). In all these mice there is robust and systemic reconstitution with human hematopoietic cells. Hu-NSG and hu-NOG mice support long-term systemic HIV infection, complete with $CD4^+$ T cell depletion and virus-specific responses from $CD8^+$ T cells (Denton and Garcia 2011). In the hematopoietic stem cell reconstituted models, human HLA-DR- expressing cells are present in the cortex, meninges, and brain stem of uninfected mice, and the numbers of these cells increase during HIV infection. Additionally, infected cells are found in the perivascular spaces and the meninges of the brain with a concomitant increase in $CD8^+$ T cells (Gorantla, Makarov et al. 2010).

Using the hu-NSG mouse model, $^1$H-magnetic resonance spectroscopy (MRS) was performed which demonstrated a significant decrease in N-acetyl aspartate (NAA) concentration in the cerebral cortex. These animals were infected five months post-humanization and were followed for up to 15 weeks post-HIV infection. Uninfected and nonhumanized NSG mice were used as controls. NAA is often used as an indicator of viability within neurons, thus suggesting that the murine neurons are undergoing apoptosis or that there are other neuronal abnormalities formed following infection (Dash, Gorantla et al. 2011). In another study, hu-NSG mice were depleted of $CD8^+$ T cells mimicking accelerated HIV infection, as previously done in rhesus macaques (Sopper et al. 2002; Kim et al. 2008; Veazey, Acierno et al. 2008; Gorantla, Makarov et al. 2010). $CD8^+$ T cell depletion resulted in increased HIV Gag expression in the cerebellum and in increased inducible nitrous oxide synthase (iNOS) expression in both the cerebellum and cortex (Gorantla, Makarov et al. 2010; Gorantla, Makarov et al. 2010). This is notable as oxidative stress induced by iNOS can cause neuronal damage and has been implicated in cognitive impairment. Overall, these systemically reconstituted humanized mice mimic a natural influx of HIV-infected cells into the brain and have the important benefit that they can be studied over longer periods of time.

## Therapeutic interventions

Studies performed in the SCID-HIVE model of infection demonstrated that while highly active ART (HAART), consisting of zidovudine/lamivudine/indinavir, was able to significantly reverse many of the pathological changes seen in HIV infection (astrogliosis, microgliosis, and reduction in TNF-alpha mRNA as well as HIV-RNA levels), cognitive manifestations were still present compared to uninfected animals (Kapadia et al. 2005; Cook-Easterwood et al. 2007). Recently, HAART, consisting of atazanavir, tenofovir, and emtricitabine, administered systemically over 10 days in SCID-HIVE mice, has been shown to significantly reduce the neuropathology usually seen in these animals (Koneru et al. 2014). Specifically, these mice had fewer murine macrophages/microglia and decreased levels of astrogliosis in the CNS compared to HIV-infected animals. Additionally, Koneru et al. determined the concentration of each component of the HAART regimen within the brain. They found that the drugs were able to penetrate into the brain within one hour, but only atazanavir was maintained at a steady concentration for four hours (last time analyzed). Both tenofovir and emtricitabine were undetectable in the brain at four hours post-administration. Additionally, the authors noted a significant decrease in HIV p24-positive cells in the brain after HAART, but viremia persisted in the treated animals. A limitation of this study is the extremely short period of therapy, only 10 days. In another HAART study of SCID-HIVE mice, nano-encapsulated 5'-triphosphates of NRTIs (nano-NRTIs) were evaluated. The study found that nano-NRTIs were associated with lower levels of apoptosis and reactive oxygen species formation in the brain and were also able to suppress brain viral loads up to 10-fold with treatment every other day for a period of two weeks (Gerson et al. 2014). This model suggests that, if appropriately delivered, current antiretroviral regimens can mitigate HIV replication in the brain. It should be noted that in the absence of HIV infection, ART drugs themselves, have been shown to cause changes in cognition, including increased anxiety and memory impairment in mice (Pistell et al. 2010; Romao et al. 2011).

## HIV-associated manifestations of neuropathology in humanized mice

As CNS manifestations in HIV-infected patients are the primary driver behind these models, it would be advantageous to have a model that could also recapitulate the neurocognitive effects seen in humans. In ART-treated patients, these manifestations include impairments in memory and learning, specifically with regard to prospective memory (ability to "remember to remember") (Clifford and Ances 2013). In the SCID-HIVE mouse model, cognitive traits were characterized for learning and memory using a Morris water maze (Cook-Easterwood, Middaugh et al. 2007). The authors found that all HIV-infected animals, regardless of treatment status, exhibited cognitive deficits compared to uninfected, vehicle-treated, or HAART-treated animals. These cognitive deficits were not correlated with TNF-alpha levels in the brain. Another study in the SCID-HIVE mouse model demonstrated isolate-specific differences in neuropathology (Rao et al. 2008). Mice infected with HIV-$1_{ADA}$ made significantly more errors in a water escape task, which probes working memory, compared to uninfected control mice. However, mice infected with HIV-$1_{indie\text{-}C1}$ made fewer errors compared to animals infected with the HIV-$1_{ADA}$ and did not show significant impairment compared to uninfected control mice. HIV-$1_{indie\text{-}C1}$-infected mice also had decreased astrogliosis compared with HIV-$1_{ADA}$-infected animals despite both groups of mice having similar viral loads in the blood (Rao, Sas et al. 2008). The authors interpreted these results as

clade-specific differences in neurocognitive ability; however, evaluations of additional isolates from each clade would be useful to further delineate these differences. To date, HSC-transplanted models, such as the hu-NSG and hu-NOG models, are lacking in any assessment of behavioral or cognitive testing.

### Limitations of current mouse models

In terms of reproducing HIV neuropathogenesis in the current models of humanized mice, the limitations are numerous and include the lack of HIV-infected microglia in the murine CNS and the lack of HIV receptors on murine neuron and glial cells which precludes testing the direct effects of the HIV envelope protein. It is hypothesized that infected microglial cells are a major source of HIV in the CNS (Takahashi et al. 1996; Wiley et al. 1996; Gonzalez-Scarano and Martin-Garcia 2005). Human hematopoietic stem cells have not been demonstrated to replace the mouse microglial cells in the brain. As microglia cells are derived from precursor present in the yolk sac during embryonic development it is not likely that adult hematopoietic stem cells transplanted to adult or even neonate animals can give rise to human microglia cells in the brain of transplanted cells. Newer models where microglia repopulate the brain of transplanted mice without the trauma of an intracranial injection will have to be developed (Ginhoux et al. 2010; Schulz et al. 2012).

Productive HIV replication does not seem to occur in human neurons, oligodendrocytes, or astrocytes; however, HIV products could contribute to neuronal dysfunction. HIV proteins that may act as neurotoxins such as gp120 are known to bind to receptors on human neurons and glial cells, and this may interfere with their normal function and result in neuronal death (Maung et al. 2012). HIV gp120 does not properly bind to murine CCR5 (Atchison et al. 1996) or murine CXCR4 (Parolin et al. 1998) or result in neuropathology in the hippocampus or subventricular regions, which are the areas where neurogenesis and migration of chemokine receptor-expressing progenitor cells may occur in adult mice. Yet, administration or expression of gp120 does result in neuronal perturbations that may be partly mediated by activated microglia/macrophages (Medders et al. 2010). Therefore, any resulting neuropathology associated with HIV infection in humanized mice may be a result of bystander effects such as secretion of cytokines, oxidative intermediates, proteases, etc., by immune cells, particularly macrophages, that may perturb supporting astrocytes, oligodendrocytes, or neurons themselves. Although transgenic expression of gp120 can result in neuropathology (Toggas et al. 1994), it is not clear if the mechanism includes direct binding to murine CCR5 or murine CXCR-4 on neurons or glia. Additional genetic or molecular modifications will be needed in the current humanized mouse models to more closely mimic the human CNS microenvironment.

## Conclusions

Humanized mice for HIV research represent a relatively new tool in the field that has been shown to be able to provide critical new insight into the biology and pathogenesis of HIV/AIDS in vivo (Gorantla, Makarov et al. 2010; Dash et al. 2012; Palmer et al. 2013; Watkins et al. 2013; Denton, Long et al. 2014; Salgado et al. 2014). The majority of HIV infection studies of the brain have centered on the severe pathological manifestations of HIVE,

although in the post-ART era the appropriateness of these models to address the milder pathology now seen in treated patients must be readdressed. The more recently developed systemically reconstituted humanized mouse models may offer a unique tool for evaluating a natural influx of HIV into the brain with limited trauma. Also, there is a lack of behavioral studies in these human hematopoietic stem cell transplanted animals. This does not negate the fact that there is still a benefit offered by these models to elucidate relevant issues regarding the pathology of HIV infection of the CNS without behavioral studies. Namely, the longer treatment studies afforded by the stem cell transplant mouse models of infection could more clearly address the effects on long-term ART on the brain and its effect on reversing some of the sequela of HIV infection.

Although there has been some characterization of the brains of NOD/SCID mice reconstituted with human $CD34^+$ stem cells, there has been no evaluation of HIV-1 infection in the brains of these animals (Asheuer, Pflumio et al. 2004) and this model does not permit the study of immune responses to HIV. Despite some obvious limitations such as the fact that the human component present is limited to immune cells and that these cells exist in a chimeric environment with endogenous mouse cells the use of humanized mouse models to study HIV infection in the CNS holds significant promise for potentially serve to better understand the contribution of adaptive and innate immune cells. While several different humanized mouse models have been employed to date (Gorantla et al. 2012), there is a need to continue to develop and evaluate new models that might recapitulate additional pathologies seen in infected human brains and that could serve to shed light into several long-standing questions in the field. For example, the humanized bone marrow/liver/thymus (BLT) mouse model has been extensively used to investigate important aspects of HIV-associated pathology, transmission, prevention and more recently HIV persistence (Denton et al. 2012; Denton, Long et al. 2014). Unfortunately, BLT mice have not been used for studies of HIV infection of the CNS. BLT mice hold significant promise for these types of studies since they have been shown to recapitulate key aspects of HIV infection and its response to antivirals (Denton, Krisko et al. 2010; Wahl et al. 2012). This could open the door for future experiments aimed at investigating HIV persistence in the CNS and novel approaches to eradicate it. Humanized mouse models could serve to address a long standing questions in the field such as how HIV enters into the brain: does HIV enter the CNS as a free virus or as a cell-associated virus within T cells or macrophages? Using some of the systemically reconstituted mouse models, experiments could address the requirements for HIV to enter into the brain via T cells using strictly T cell-tropic strains of HIV and newer humanized mouse models that contain a full complement of human T cells while essentially devoid of any human myeloid cells (Honeycutt et al. 2013). It is also unknown whether HIV causes direct neuronal damage leading to cognitive impairments or the CNS immune response to HIV mediates the cognitive effects. If so, what are the molecular basis and viral determinants of these effects? The previous literature indicating that HIV proteins alone are capable of inducing CNS pathology strongly suggest that the use of novel models where HIV proteins are produced in the context of human myeloid and T cells will provide highly relevant information in this regard. Also, the ability of models where different drugs individually and/or in combination can be evaluated for penetrance and effectiveness in the CNS could inform clinical practice and novel approaches to eradicate HIV from the CNS.

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## Table 1

Summary of the current humanized mouse models for HIV-1 CNS pathology studies

| Model | Method of humanization | Human cell reconstitution of CNS | Characterization of CNS pathology | Characterization of behavior/learning | Evaluation of ART in the CNS |
|---|---|---|---|---|---|
| **Direct brain injection mouse models** | | | | | |
| **SCID-HIVE** | Injection of HIV-1 infected MDMs into the brain of SCID mice | Human macrophages present throughout brain | Encephalitis, astrogliosis, multinucleated giant cells, infiltration of murine mononuclear phagocytes, decreased MAP-2 expression | Impaired learning and memory | **Zidovudine/ Lamivudine/ Indinavir:** decreased levels of MAP-2, TNF-alpha mRNA, viral load, and astrogliosis **Atazanavir/ Tenfovir/ Emtricitabine:** decreased inflammatory response |
| **huPBL/HIVE** | Injection of HIV-1 infected MDMs into basal ganglia of NOD/SCID mice previously injected with human peripheral blood lymphocytes | Human macrophages present throughout brain | Multinucleated giant cells and T cell infiltration into the brain | Not evaluated to date | Not evaluated to date |
| **Systemic reconstitution mouse models** | | | | | |
| **huPBMC** | Injection of human PBMCs into adult NOD/ SCID mice | Human T cells present in meninges (but no macrophages), after LPS injection human macrophages migrate to the brain | After LPS administration astrocytosis, microglial nodules (nodules present with/ without HIV), and upregulation of TRAIL are observed | Not evaluated to date | Not evaluated to date |
| **huNSG/huNOG** | Injection of human HSC into the liver of newborn NSG/NOG mice | Macrophage repopulation of the meninges/ perivascular spaces | HIV infection led to an increase in the number of human cells in the brain; loss of neuronal integrity | Not evaluated to date | Not evaluated to date |
| **huNOD/SCID** | Tail-vein injection of human HSC into adult NOD/SCID mice | Human Alu sequences present in cerebral cortex, cerebellum, colliculus and olfactory bulbs suggesting the presence of human cells | not evaluated to date | | |
| **BLT** | Implantation of human thymus and liver under the kidney capsule followed by autologous HSC transplant into NSG mice | Not evaluated to date | | | |