

Modeling HIV-associated neurocognitive disorders in mice: new approaches in the changing face of HIV neuropathogenesis

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It is well established that infection with the human immunodeficiency virus (HIV) leads to immune suppression. Less well known is the fact that long-term, progressive HIV disease is associated with the development of cognitive deficits. Since the introduction of combined antiretroviral therapy (cART), the clinical presentation of HIV infection has evolved into a chronic illness with very low levels of viral replication and chronic immune activation, with compliant affected individuals surviving for decades with a high quality of life. Despite these advances, many HIV-infected individuals develop some degree of neurodegeneration and cognitive impairment. The underlying pathophysiological mechanisms are not well understood, and there are no effective treatments. Thus, there is an unmet need for animal models that enable the study of HIV-associated neurocognitive disorders (HAND) and the testing of new therapeutic approaches to combat them. Here, we review the pros and cons of existing mouse models of HIV infection for addressing these aims and propose a detailed strategy for developing a new mouse model of HIV infection.

A call to action: the changing face of HIV infection

The advent of combined antiretroviral therapy (cART) resulted in a profound decrease in mortality rates in people infected with the human immunodeficiency virus (HIV). cART greatly extends the lifespan of HIV-infected individuals by reducing viral load, preserving immune function and decreasing the risk of lethal opportunistic infections. With the introduction of cART, HIV infection evolved from a certain death sentence into a life-long disease that is manageable with drug therapy. Despite this promising success, a serious comorbidity that remains prevalent in nearly 30% of cART-treated HIV-infected individuals are the HIV-associated neurocognitive disorders (HAND), a spectrum of neurological complications that range from asymptomatic cognitive impairments to severe dementia (Simioni et al., 2010). HAND remains a major concern for HIV-infected individuals because it can result in impaired daily living and a reduced quality of life. In severe instances, HAND can contribute to increased mortality in HIV-infected individuals because their cognitive dysfunction results in cART noncompliance.

Since the emergence of HIV in humans in the 1980s, numerous mouse models have been established in an attempt to mimic the progression of human HIV disease. Given the current changes in the clinical presentation of HIV infection, we review here the contributions of mouse models to the understanding of the

neuropathology that underlies HAND. We also address their limitations, and explore the possibility of developing a new mouse model that would be relevant to the current clinical presentation of HAND in HIV infection.

Mechanisms that contribute to HAND

There are several mechanisms that are thought to contribute to the development of HAND in cART-treated individuals. These include: (1) ongoing viral replication in the brain due to low brain penetration of cART, (2) infiltration of activated cytotoxic CD8⁺ T cells into the brain, (3) indirect neurotoxicity from the extracellular release of the HIV Tat protein (despite control of viral replication by cART) and (4) direct neurotoxicity of the cART drugs themselves. These mechanisms are discussed in detail below.

Many cART regimens exhibit low blood-brain barrier (BBB) uptake, so it can be difficult to establish therapeutic drug levels in the brain. This creates a scenario in which, although viral loads are well controlled in the periphery, viral replication continues to occur in the brain because the antiretroviral drugs are present at subtherapeutic concentrations. Failure of cART to control HIV replication in the brain is an important mechanism that contributes to the development of HAND because it provides a favorable environment for the evolution of drug-resistant HIV. The ongoing replication of HIV in the brain can also play a role in the second mechanism implicated in the persistence of HAND: the presence of activated T cells in the central nervous system (CNS). This phenomenon has been termed immune reconstitution inflammatory syndrome and can manifest as an acute or chronic form (reviewed in Johnson and Nath, 2011). Host mechanisms for the control of HIV replication in the CNS are not completely understood; however, animal models indicate that CD8⁺ T cells enter the brain early in the course of infection and persist in the brain in a highly activated state (Marcondes et al., 2007; Marcondes et al., 2001). These chronically activated T cells could contribute

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to HIV-mediated neuropathology either through direct cytotoxic killing of infected brain cells or through indirect mechanisms such as the release of proinflammatory cytokines.

A third mechanism that can contribute to HAND is the fact that cART cannot prevent the production of early viral proteins once proviral DNA has been formed. Current cART regimens inhibit HIV replication by targeting HIV protease or HIV reverse transcriptase. Because of this, the drugs do not block production of the early viral proteins Tat, Rev and Nef (Nath, 2002). This is especially problematic for Tat because, even in the presence of successful cART-mediated suppression of HIV replication, infected cells still release Tat protein into the extracellular space where it can exert neurotoxic effects by interacting with neighboring cells (Li et al., 2009; Chauhan et al., 2003). The final mechanism that can contribute to HAND is the fact that cART drugs demonstrate neurotoxicity. This has been shown indirectly in HIV-infected individuals through neuropsychological evaluation (Robertson et al., 2010) and through in vitro studies (Robinson et al., 2007). However, the exact molecular processes that mediate this toxicity in the brain remain to be determined.

Mechanisms involved in neuronal injury during brain infection with HIV

To enter the brain, HIV must first cross the BBB. Previous work has suggested that HIV is transported across the BBB either by infected macrophages (Nottet et al., 1996; Persidsky et al., 1997) or as free viral particles (Banks et al., 2001). HIV enters the brain early in the course of infection (Davis et al., 1992), and can be detected in brain macrophages, microglia and astrocytes in pre-symptomatic individuals, suggesting that these cell types are

important reservoirs for the virus (Thompson et al., 2011). HIV cannot directly infect neurons. Instead, HIV-mediated neurotoxicity occurs as a result of indirect mechanisms. One key process that mediates neurotoxicity is the release of soluble neurotoxic viral proteins (Tat and gp120) and proinflammatory molecules (cytokines and free radicals) from productively infected brain macrophages and microglia. As described previously, these HIV-infected cells can maintain a persistent low-level infection because the brain is poorly accessed by most antiretroviral drugs. This promotes accumulation of chronically activated uninfected T cells in the CNS. The release of pro-inflammatory cytokines by these T cells provides another indirect mechanism that contributes to neuronal injury (reviewed in Johnson and Nath, 2011). Fig. 1 shows a summary of how these indirect mechanisms result in neuronal injury associated with HIV infection in the brain.

Brain cells infected by HIV

Brain macrophages and microglia

The key cells that support productive HIV infection in the brain are the macrophages and microglia (Koenig et al., 1986; Wiley et al., 1986). Brain macrophages consist of several distinct cell populations. For the purpose of this review, we focus on the perivascular macrophages. Whereas microglia are a long-lived static population of cells, perivascular macrophages are a dynamic population that is periodically replenished by monocytes from the peripheral blood (Kennedy and Abkowitz, 1997). Perivascular macrophages seem to be a major target for the dissemination of HIV infection to the brain from the periphery. Interestingly, it has been shown that HIV-infected monocytes can cross the BBB more readily than uninfected monocytes (Persidsky et al., 1999). These

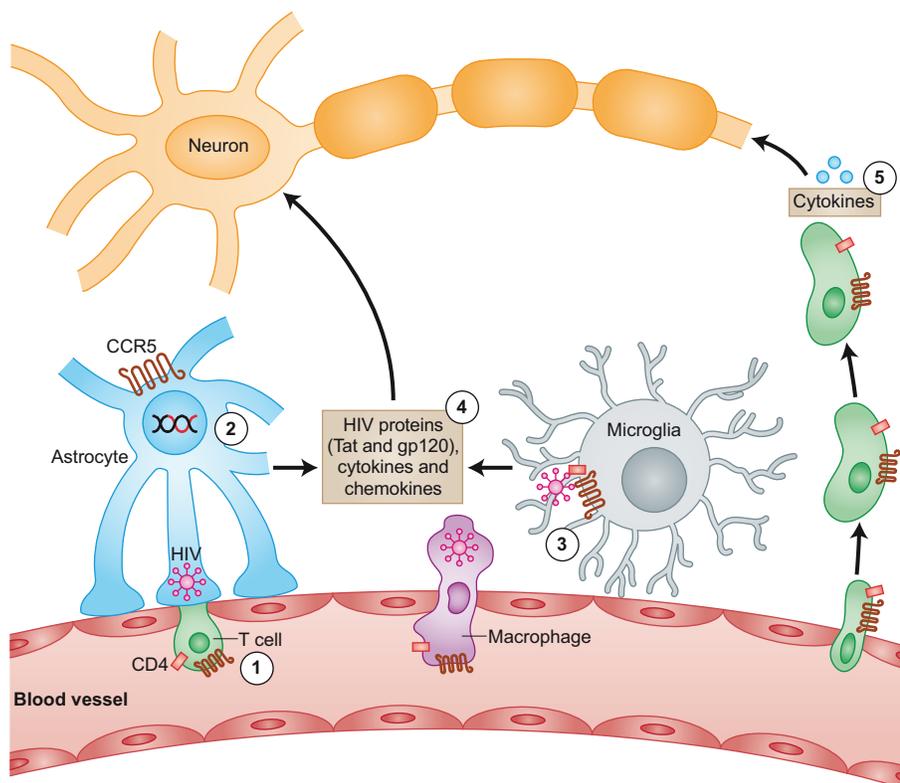


Fig. 1. Cellular mechanisms that contribute to the development of HAND. (1) HIV can infect astrocytes by direct cell-cell contact with infected T cells through the virological synapse. (2) After infecting astrocytes, HIV integrates into the genome where it remains latent. (3) HIV-infected macrophages enter the brain early after initial infection. Next, HIV infection spreads to perivascular macrophages and microglia, a process that occurs when HIV enters the cell after binding to the CD4-receptor-CCR5 co-receptor complex. (4) HIV-infected astrocytes and microglia cause neuronal injury indirectly by releasing soluble toxic viral proteins (Tat and gp120) and proinflammatory molecules (cytokines and chemokines). (5) Ongoing low levels of HIV replication in the CNS despite the use of cART promotes the entry of chronically activated T cells, which cause neuronal injury indirectly by releasing pro-inflammatory cytokines.

findings are important because HIV-infected monocytes that cross the BBB can repopulate the resident macrophages of the perivascular space with HIV-infected cells.

Astrocytes

Perivascular astrocytes are another cell receiving increasing attention as an important reservoir for HIV (Churchill et al., 2009; Eugenin et al., 2011). Although in vivo and cell culture studies indicate that HIV infects astrocytes, the role of these cells in HIV neuropathology is not fully understood (Kramer-Hämmerle et al., 2005). Recent studies show that, in individuals with HAND, as many as 19% of the perivascular astrocytes are infected with HIV (Churchill et al., 2009). Because astrocytes outnumber neurons by 10:1, this represents a large amount of infection in the brain, which was previously under-recognized. The impact of astrocyte infection might be substantial because these cells demonstrate aberrant intracellular signaling and abnormal interactions with the brain endothelial cells of the BBB (Eugenin et al., 2011). Astrocyte-mediated disruption of the BBB could play a role in the development of HAND because it would allow increased numbers of HIV-infected or activated monocytes or lymphocytes to traffic into the perivascular space, thereby exposing the brain to additional HIV-infected cells and proinflammatory insults.

Mouse models of HIV: a brief history and evidence of cognitive dysfunction

Because HIV is not infectious to rodents, creating a small animal model that can accurately mimic the human pathology has proven to be quite challenging. A variety of cutting-edge techniques have been employed in an effort to establish an HIV mouse model. These small animal models have evolved drastically in their level of

complexity over the last 30 years (Table 1) (Poluektova, 2011). Although in vivo HIV models have been established in other species, mouse models confer several advantages that help to maintain their popularity. These include: (1) the ease of handling the animals, (2) the low relative cost associated with their production and maintenance, and (3) the well-established and easy-to-use methods for manipulating the mouse genome. As with all mouse models of human disease, however, a major challenge is the difficulty in predicting how preclinical findings will translate in a clinical setting.

Transgenic mouse models

Transgenic mouse models were among the first tools created for the study of HIV pathology in vivo. HIV demonstrates a limited capacity for replication in mouse cells because numerous key proteins are either missing or are altered in such a way that the virus cannot interact with them. For example, the absence of any of the following host factors can result in restricted infectivity of HIV in the mouse: CD4, CCR5, cyclin T1, APOBEC, Fut-2, TRIM5 α , Lv-1, Ref-1, cyclophilin, host-splicing inhibitor p32 and certain restriction factors. Early attempts to confer susceptibility to HIV infection in transgenic mice relied on the use of genetic techniques to introduce human host factors individually (single transgenic) or in combination (double transgenic). These efforts indicate that, although it is possible to create transgenic mouse models that support viral entry, the primary barrier to HIV infectivity in the mouse is that the cells are unable to support HIV replication. This is because, after successfully entering the mouse T cell, HIV encounters a block that prevents viral integration into the mouse genome, and subsequent viral production cannot occur (Tervo et al., 2008). There are several HIV proteins that are toxic

Table 1. Summary of mouse models currently used for the study of HAND

Model	Disadvantages	Advantages
HIV transgenics		
gp120 expressed under non-inducible <i>GFAP</i> promoter; Tat expressed under non-inducible or inducible <i>GFAP</i> promoter	<ul style="list-style-type: none"> • Viral protein is not expressed in brain macrophages, the primary cell type targeted for HIV infection • Non-inducible models: presence of viral protein throughout the lifespan might promote survival of abnormally robust neuronal populations • Doxycycline-inducible models: doxycycline is neuroprotective, and its use might confound results 	<ul style="list-style-type: none"> • Model is non-infectious, so risk to lab workers is minimal because there is no exposure to actual HIV • Care of mouse colony is less technically challenging because immune suppression is not necessary to create these models • Neurodegeneration and glial cell activation mimics that of HIVE
HIS mice		
Human CD34 ⁺ cells injected into either a NOG, NSG or BRG immunodeficient mouse; reconstituted mice then infected with HIV	<ul style="list-style-type: none"> • Unclear whether the functional capacity of the transplanted human immune system is completely reconstituted and maintained in recipient mice • Lack of consistent results attributed to use of different sources for human donor cells; reproducibility in these models needs to be optimized 	<ul style="list-style-type: none"> • Immune dysfunction mimics that observed in HIV-infected individuals • Provides opportunity to study the effects of antiretroviral drugs and the peripheral immune dysfunction on the brain
HIVE mouse		
HIV-infected macrophages intraparenchymally injected into CB17-SCID mouse	<ul style="list-style-type: none"> • Microglial response is short-lived, so model does not allow long-term observation • Stereotaxic injection can cause traumatic brain injury • Older CB17-SCID mice susceptible to developing graft-versus-host response to human macrophages 	<ul style="list-style-type: none"> • Has the potential to incorporate HIV-infected human macrophages into brain • Well-characterized model with neuronal changes similar to HIVE in humans

to neurons, including gp120, gp140, Tat, Nef, Vpr and Rev (Nath, 2002). The discovery that gp120 and Tat can be released as soluble proteins led to initial studies involving injection of these proteins directly into the mouse brain (Jones et al., 1998). Eventually, transgenic mouse models expressing gp120 or Tat were created in an attempt to better replicate the neuropathology present in HAND.

gp120 transgenic mice

The HIV envelope glycoprotein gp120 is the most prominent viral antigen found in the lysates of HIV-infected cells (Schneider et al., 1985). In the periphery, the overproduction and shedding of gp120 by HIV-infected cells is thought to play a major role in the protective antibody response against HIV (Schneider et al., 1986). In the CNS, however, HIV-infected macrophages and astrocytes can shed gp120 into the extracellular space as a soluble protein, so gp120 has the potential to interact with uninfected neurons. A transgenic gp120 mouse model was generated by inserting the portion of the HIV *env* gene that encodes gp120 into the mouse genome under the control of a glial fibrillary acidic protein (*GFAP*) promoter, resulting in gp120 protein expression by brain astrocytes. Secretion of gp120 in these transgenic mice is associated with neuropathological changes in the neocortex and hippocampus, indicating that soluble gp120 is neurotoxic (Toggas et al., 1994). These animals also have impaired proliferation and differentiation of neural progenitor cells (Lee et al., 2011; Okamoto et al., 2007). Similarly, behavioral testing using the Morris water maze shows that the gp120 transgenic mouse demonstrates an age-dependent impairment in spatial reference memory, indicating that overexpression of gp120 in older mice confers a susceptibility to cognitive decline (D'hooge et al., 1999). Subsequent studies indicate that the underlying mechanism for neuronal injury in these mice involves enhanced glutamate signaling via N-methyl-D-aspartate (NMDA) receptors and abnormal activation of Ca²⁺-dependent signaling pathways (Krucker et al., 1998; Lipton, 1994; Toggas et al., 1996). Via interactions with CXCR4, gp120 can also upregulate the nicotinic acetylcholine receptor, particularly in the striatum, leading to dysregulation of Ca²⁺ signaling in neurons (Ballester et al., 2012). These mice also develop hyperphosphorylation of tau protein, a condition that causes tau to dissociate from the neuronal microtubule cytoskeleton. Following its detachment from microtubules, tau then self-associates inside the cell into tangles of paired helical filaments. The presence of these tau aggregates in the gp120 mouse is notable: these are a histopathological feature that is common in the Alzheimer's disease brain (Alonso et al., 2001). Studies have shown that tau hyperphosphorylation in the gp120 mouse can be prevented by combined treatment with erythropoietin and insulin growth factor-1 (Kang et al., 2010), or with an inhibitor of cyclin dependent kinase-5 (Patrick et al., 2011).

Tat transgenic mice

Tat, a protein that is involved in HIV transcription, is the first protein produced by HIV following infection. After HIV-infected cells secrete Tat, this protein can enter most cell types in the body via its arginine-rich basic domain. Unlike gp120, Tat production is not impacted in HIV-infected individuals on cART. This is important because, even in the absence of viral replication, a large amount of Tat protein is present. One report showed that nearly

33% of HIV-infected individuals have detectable Tat in serum, ranging from 0.1 to 40 ng/ml (Xiao, 2000). To better understand the contribution of Tat protein to the progression of HAND, several Tat transgenic models have been created whereby Tat expression occurs constitutively throughout the lifespan of the mouse in most tissues (Choi et al., 2000; Garza et al., 1996; Vellutini et al., 1995; Vogel et al., 1988). Interestingly, Tat protein could not be detected in the brain and these animals did not develop any obvious neurological abnormalities. Hence, two laboratories created separate lines of a brain-tissue-specific Tat transgenic mouse that expresses Tat protein using a *GFAP* promoter with a doxycycline-inducible component (Fitting et al., 2010; Kim et al., 2003). Like the gp120 transgenic mouse, these Tat transgenic mice demonstrated neuropathological changes. However, in one of the lines, the damage was largely localized to the cerebellum and cortex (Kim et al., 2003). In the second line, the abnormalities were found to be more subtle, with a reduction in spine density, dendritic abnormalities, and widespread disruption of the opioid system in the brain (Fitting et al., 2010). Subsequent studies showed that there are several potential mechanisms that mediate Tat toxicity in the brain, such as toxicity to dopaminergic neuronal populations (Aksenov et al., 2008; Bansal et al., 2000; Wallace et al., 2006) and activation of ryanodine receptors resulting in increased dopamine transporter levels (Norman et al., 2008; Perry et al., 2010). Dysregulated expression of microRNAs has also been found to contribute to neuronal dysfunction in *GFAP-Tat* transgenic mice (Chang et al., 2011).

New application for transgenic mouse models: development of adjunctive therapies

Transgenic mouse models that express HIV proteins are useful because they provide valuable information about the acute toxicity of viral proteins. Although the use of these models has declined recently, they are still relevant for HIV research because they are reflective of the current HIV-infected population. Specifically, since the advent of cART, HIV-infected individuals are now characterized by low viral loads yet high levels of neurotoxic Tat protein. Thus, one promising approach for deterring the development of HAND in HIV-infected individuals would be the development of biologics (e.g. antibodies, antisense reagents or siRNA) to specifically target neurotoxic viral proteins such as Tat. Anti-Tat biologics are an attractive adjunctive therapy for HIV because they are specifically designed to target viral proteins and are therefore less likely to produce off-target side effects.

Caveats of transgenic mouse models

Studies conducted with gp120 and Tat transgenic mice have provided clues about the mechanisms involved in HIV-associated neuropathology. For example, we now know that the presence of toxic viral protein alone is sufficient to cause neuropathology and the development of cognitive deficits. Although the available HIV transgenic mouse models are useful for exploring adjunctive therapies, they have several limitations. For example, both the Tat and gp120 transgenics express their respective transgene in astrocytes through the presence of a *GFAP* promoter. However, the primary cell type that supports productive brain infection of HIV is the macrophage, so these mouse models would be more pertinent if they contained a cell-specific promoter for the expression of HIV

neurotoxic proteins in macrophages instead of astrocytes. A second caveat to the non-inducible Tat and gp120 transgenic mouse models is that the mice express these viral proteins throughout their lifespan. This is problematic because expression of neurotoxic proteins during development can lead to an abnormally robust neuronal population because susceptible neurons are eliminated early in development and replaced by less vulnerable cells. Finally, the use of doxycycline in the Tat-inducible transgenic mouse is problematic because this antibiotic has the potential to act as a neuroprotective compound both in vivo and in vitro (Cho et al., 2009; Jantzie et al., 2006), increasing the risk of false negatives. Furthermore, this inducible promoter is leaky, so small amounts of Tat protein can be expressed in early stages of development. This leakiness might explain why an immune response to Tat does not occur in these animals after Tat induction. However, this can be viewed as an advantage in that the model allows studies of the effects of Tat on the brain in an adult animal without the need for immunosuppressive drugs.

Humanized mouse models

Humanized mouse models are generated by transplanting human cells or tissues into genetically modified immunodeficient mice. Mice with a humanized immune system can sustain long-term chronic HIV replication and are susceptible to infection through the natural routes by which humans are exposed to the virus. Here, we refer to this paradigm as the human immune system (HIS) mouse model. Early HIS mouse models were generated by surgically grafting human fetal lymphoid organs (bone marrow, liver and thymus) in an immunodeficient mouse to generate, and mature, human immune cells. These procedures required great surgical skill along with unrestricted access to a system for procurement of human fetal tissue (Legrand et al., 2009). To establish a more widely accessible method for the generation of HIS mouse models, a technique was established whereby a recipient immunodeficient mouse was simply injected with a suspension of either human fetal hematopoietic progenitor cells isolated from umbilical cord blood (CD34⁺ cells) or adult human peripheral blood mononuclear cells (PBMCs).

Generation of the more recent HIS mouse models has been made possible by the development of immunodeficient mouse strains with severe defects in both innate and adaptive immunity. These include the NOD/Shi-*scid* *IL2r γ* ^{-/-} (NOG) (Ito et al., 2002), the NOD/LtSz-*scid* *IL2r γ* ^{-/-} (NSG) (Shultz et al., 2005) and the BALB/cRag2^{-/-} γ ^{-/-} (BRG) mouse. HIS models have been established in the NOG and NSG mice by intravenous administration of CD34⁺ cells (NOG/CD34⁺ or NSG/CD34⁺, respectively). Newborn BRG pups are typically engrafted with human CD34⁺ cells administered by intrahepatic injection (BRG/CD34⁺) (Traggiai et al., 2004). Mice are then infected with HIV at a later time. In terms of HIV research, the greatest advantage of the HIS mouse models is that NOG/CD34⁺, NSG/CD34⁺ and BRG/CD34⁺ mice can all support both a sustained HIV infection and an antiviral immune response within a single physiological system (Berges et al., 2010; Berges et al., 2006; Sato et al., 2010b; Traggiai et al., 2004). In addition, they are susceptible to infection by natural routes: for example, in the BRG/CD34⁺ mouse, human immune cells populate mucosal tissues such as the gut and vaginal tracts, so the mice are readily infected through intravaginal and

intrarectal exposures (Berges et al., 2008). Because of this, the HIS mouse models can be used to study complex issues that are relevant to the current HIV-infected human population. Comprehensive reviews have already been published on the immune response to HIV in these models (Denton and Garcia, 2011; Sato and Koyanagi, 2011), so we focus below on discussing their potential for use in the study of HAND.

Determining the mechanisms involved in HAND

Of the HIS mouse models, only the NSG/CD34⁺ mouse has been characterized for its neuropathology. Histopathological examination of these mice revealed that they mimic aspects of the human neuroimmunology: for example, human CD163⁺/CD14⁺ macrophages were found to repopulate the meninges and perivascular spaces of these mice (Gorantla et al., 2010). In addition, proton magnetic resonance spectroscopy and morphological analysis of cortical neurons in HIV-infected NSG/CD34⁺ mice showed abnormalities in neuronal structures (Dash et al., 2011). Although other neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease tend to act focally in targeted regions, HIV causes broad neurodegeneration with an emphasis on subcortical and deep gray matter structures. To determine whether the HIS mouse models demonstrate neuropathology comparable to that of an HIV-infected individual, more extensive imaging of these animal models with techniques such as two-photon microscopy and magnetic resonance imaging (MRI) will be necessary to track disease progression and assess how well it correlates with clinical data. These models would be especially useful if they could provide neuroimaging signals or biomarkers that could be used to detect presymptomatic neurological changes caused by HIV infection.

Testing cART for neurotoxicity

The most recent application of HIS mouse models has been extensive efficacy studies of cART therapies. Although it is well accepted that HIV infection can result in neuronal damage and the development of cognitive deficits, less well understood is what role cART plays in this matter. Although cART regimens with good CNS penetration are more effective in suppressing HIV replication in the brain, increasing the CNS penetration of cART does not always result in increased cognitive performance (Marra et al., 2009). A likely cause of this is neurotoxicity of the anti-HIV drugs themselves, although such neurotoxicity has not been proven. As in HIV-infected individuals, initiation of cART in HIV-infected BRG/CD34⁺ mice can suppress viremia (Choudhary et al., 2009; Sango et al., 2010) and recover CD4⁺ T cell counts (Choudhary et al., 2009). This indicates that the HIS mouse models can support a sustained HIV infection that is manageable with cART. Thus, this model could be used to examine which cART treatments are the least neurotoxic when administered over extended periods of time.

HIV evolution in brain reservoirs of cART-treated mice

HIV-infected individuals are often co-infected with more than one strain of HIV. This can result in the exchange of genetic material between the viral strains, a process referred to as viral recombination. Recombination can increase viral diversity, thereby increasing the probability that drug-resistant strains will emerge. In HIV-infected individuals on cART, if viral replication in the brain

is not properly suppressed, viral recombination can occur in this reservoir. Additionally, tissue-specific factors in the brain might put evolutionary pressure on the virus, resulting in neurotropic strains (Holman et al., 2010). The HIS mouse model has great potential for studying HIV recombination and evolution in a physiological system. Studies have already shown that these models can be used to look at the evolution of a single strain of HIV. The NOG/CD34+ mice have been used to track mutations in the proviral DNA from the original HIV-1_{JR-CSF} inoculum (Sato et al., 2010a). Additionally, viral populations isolated from HIV-infected BRG/CD34+ mice indicate that genotypic divergence occurs in these animals at a rate that is similar to that observed in humans over the same amount of time (Ince et al., 2010). However, mouse brain cells are not susceptible to HIV infection, so in their current form the models cannot be used to study viral evolution in the brain.

Caveats of HIS mouse models

HIS mouse models are useful for various applications, but their main drawback is the lack of consistency from mouse to mouse. This is due to the highly variable nature of human immune cells from person to person. These features can result in differences in both the level and timing of HIV infection in HIS mouse models. One approach to dealing with this limitation is to transplant large numbers of HIS mice with cells from the same donor to create experimental replicates. Another shortcoming of the HIS mouse model is that it is not clear whether the transplanted human immune system in a mouse functions in the same way as in humans. For example, once the CD34+ progenitor cells differentiate in the HIS mouse, immature human T cells seem to be 'educated' in the mouse thymus (Ishikawa et al., 2005). Because this is not the typical route of human T cell maturation, it raises questions about how well these engrafted human T cells can 'see' and become activated in response to antigens in the HIS mouse. As discussed earlier, T cells become chronically hyperactivated during chronic HIV infection, a condition that is thought to play a role in the progression of HAND (Johnson and Nath, 2011). If mouse thymic 'education' does not properly train the transplanted T cells, then this important component of chronic HIV infection might be impaired or absent in the HIS mouse model. To increase the translational capacity of the transplanted human immune system in the HIS mouse, a graft of the thymus from the autologous human donor could be carried out (Stoddart et al., 2011).

The HIV encephalitis mouse model

Similar to the HIS mouse model, the HIV encephalitis (HIVE) mouse model involves grafting human immune cells into severe combined immunodeficient (SCID) mice. Unlike the HIS mouse models described above, in the HIVE mouse model cells already infected with HIV are implanted directly into the brain. Specifically, human macrophages are infected with a macrophage-tropic strain of HIV-1 (HIV-1_{ADA}). They are then stereotaxically injected into the basal ganglia (Eggert et al., 2010), putamen or cortex (Persidsky et al., 1996). Following implantation, these mice exhibit virus-infected macrophages that persistently express HIV p24 antigen and are viable in the brain for up to 5 weeks (Persidsky et al., 1996). The mouse brain also shows many of the histopathological features of HIVE, including HIV-infected perivascular and parenchymal macrophages, astrocytosis, microglial activation, neuronal

apoptosis, and dendritic damage (Persidsky and Gendelman, 1997). In recent years, much research effort has been focused on the discovery and design of neuroprotective compounds to be used as adjunctive therapies in cART-treated HIV-infected individuals. The HIVE mouse model has proven to be useful for efficacy studies to screen potential neuroprotective compounds, such as sodium valproate (Dou et al., 2003), for their applicability in the setting of HIV-associated neuroinflammation.

Caveats of the HIVE mouse model

The HIVE mouse model is a macrophage-centric model that can be used to study the contributions of HIV-infected human macrophages to HAND. The advantage of the HIVE model over a transgenic HIV mouse model is that the HIV-infected human macrophages can produce complete viral particles. This model was developed nearly two decades ago but, because of its complexity, it has largely been used by only a single research group. A disadvantage of this model is that it does not allow for long-term observation of HIV infection because the inflammatory response in the brain peaks within the first 2 weeks after macrophage implantation (Persidsky et al., 1996). Another caveat associated with this model is that stereotaxic injection of the human macrophages can result in traumatic brain injury to the mouse. This can confound interpretation of results because it is difficult to differentiate injection-induced injury from infection-induced injury. Finally, studies with this model were conducted in the CB17-SCID mouse strain, which, owing to an age-related 'leakiness' in the SCID-generated immune deficiency, the minimal host immune cells in this strain could potentially mount a xenoresponse to the transplanted human cells. Thus, future studies conducted in this model would be greatly improved if a more completely immune-deficient mouse strain is used for implantation, such as the NOG, NSG or BRG mouse.

Proposed future direction for the field: a new humanized mouse model for HAND

Although the HIV-infected HIS mouse models have been characterized extensively for their ability to recapitulate the human immune response against HIV (Zhang et al., 2009), there are few studies demonstrating the presence of learning and memory impairments in these animals. Similarly, the HIS mouse models cannot be used to study brain reservoirs of HIV infection because they do not contain HIV-infected microglia or astrocytes. Thus, an appropriate model to assess the HIV-associated neuropathology of HAND is not currently available. Here, we propose a strategy for the design of a new mouse model for HIV research, the HIS-CNS mouse. The technical details regarding the creation of this HIS-CNS mouse are summarized in Fig. 2. Essentially, the HIS-CNS mouse will consist of a chimeric mouse that is transplanted postnatally with both a human immune system and HIV-permissive cells from the human brain (microglia and astrocytes). When the HIS-CNS mouse reaches maturity (8 weeks of age), it can be infected with HIV using various routes of exposure (e.g. intravenous, intrarectal, intravaginal). The advantage of the HIS-CNS mouse is that both the transplanted human lymphoid and brain tissue will be permissive to HIV infection, thereby creating an inexpensive easy-to-house mouse model that can be used to study the neuropathological

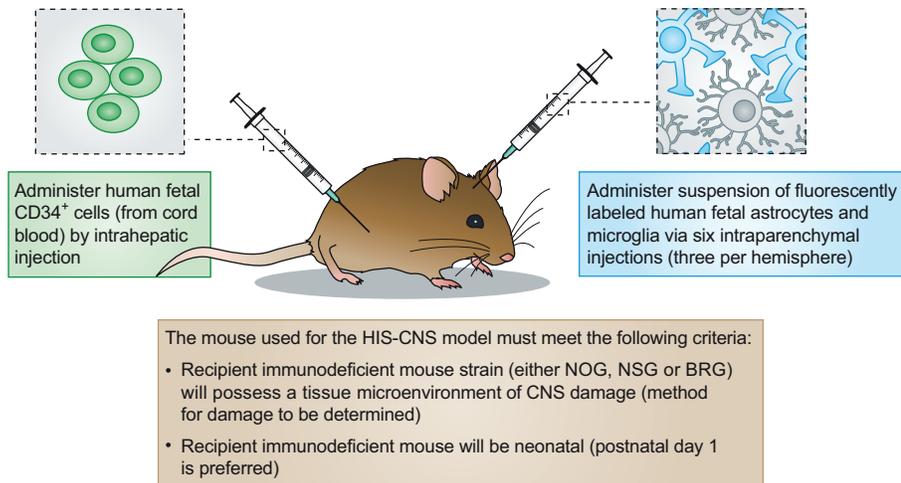


Fig. 2. Proposed strategy for the development of a new mouse model of HIV infection: the HIS-CNS mouse. Here, we propose a strategy in which 1-day-old immunodeficient mice are transplanted with human fetal microglia and astrocytes (intracerebrally), as well as with CD34⁺ human hematopoietic progenitor cells (intrahepatically). Prior to transplantation, human cells would be fluorescently labeled by transducing them with lentiviral vectors, to allow tracking of human cells in recipient mice. After the human donor cells have engrafted in the recipient mouse, the human-mouse chimera would then be susceptible to HIV infection, both in the periphery and in the CNS, and could therefore be used to study the pathophysiology of HAND.

mechanisms that contribute to the development of HAND. In addition, the HIS-CNS mouse will provide a novel model that could be used for evaluating the effectiveness of new strategies to eradicate brain reservoirs of HIV and for evaluating the effects of long-term antiretroviral therapy on the brain. This model, which uses human brain cells and HIV, provides distinct advantages over non-human primate models infected with SIV, because SIV is genetically distinct from HIV and does not respond to antiretroviral drugs in the same manner.

Previous studies have successfully implanted human brain cells into the mouse brain (Roy et al., 2006; Tamaki et al., 2002; Tamaki et al., 2009). These human cell transplantation studies were initiated in response to a growing interest in developing cell replacement strategies for the treatment of neurological diseases (Dihné et al., 2011; Dyson and Baker, 2011; Joers and Emborg, 2009). Unfortunately, the cell types used (neurons, oligodendrocytes and neural stem cells) are not permissive to HIV infection and therefore would not be applicable to the HIS-CNS mouse model. Despite this limitation, the literature regarding xenotransplantation of these human CNS cells into the mouse brain is worth reviewing because some of the problems associated with the technique will probably apply to all cells of the human brain, including our cells of interest, the microglia and astrocytes. Collectively, these studies have shown that there are four factors that play a key role in the success of human brain tissue engraftment in immunodeficient mouse models. In the following paragraphs, we outline these parameters and provide details on how they are incorporated into our proposed strategy for the development of the HIS-CNS mouse model.

The first parameter that we considered when developing our strategy to transplant human astrocytes and microglia into the mouse brain was the permissiveness of the mouse tissue microenvironment. For example, rats subjected to chemical demyelination show significant remyelination when transplanted with human oligodendrocyte progenitor cells. Conversely, when the same cell type is transplanted into rats with an intact, healthy brain, very few human oligodendrocyte progenitors survive (Windrem et al., 2002). This indicates that CNS damage provides a tissue microenvironment that encourages successful engraftment of human brain cells, possibly because factors in the damaged brain

encourage the donor cells to migrate and mature. Thus, we suggest transplanting the human astrocytes and microglia into an injured mouse brain to increase the probability that they will engraft successfully.

The second parameter to be considered when creating the plan for the HIS-CNS mouse is the age of the recipient mouse. Studies conducted in the HIS mouse model indicate that the human immune system is reconstituted more efficiently in neonates compared with adult mice (Ishikawa et al., 2005; Ito et al., 2002). For example, in BRG/CD34⁺ mice, at 8 weeks following transplantation of human CD34⁺ cells, neonates show engraftment of 80%, whereas mice transplanted at 1 week of age show 30% and mice transplanted at 2 weeks of age show only 10% (Gimeno et al., 2004). The cause of this age-dependent decline in the efficiency of engraftment is not clear, but it is possible that trophic factors are optimally expressed in the early neonatal stage (Gimeno et al., 2004; Legrand et al., 2006). Thus, we suggest engraftment of the HIS-CNS mice with both the human lymphoid and brain tissue on postnatal day 1 to take advantage of this perinatal period when the mice demonstrate increased tolerance to engrafted tissue (Ridge et al., 1996).

The third parameter to be considered is the source of the human lymphoid and brain tissue for engraftment into the HIS-CNS mouse. In general, cells isolated from human fetal tissue seem to have an advantage over cells isolated from adult tissue. For example, a side-by-side comparison shows that human fetal oligodendrocyte progenitor cells demonstrate greater mitotic behavior when transplanted into the mouse brain compared with adult human oligodendrocyte progenitor cells (Windrem et al., 2004). Although there are no publications regarding the transplantation of human microglia and/or astrocytes into the mouse brain at present, based on the findings described above for oligodendrocytes, it is likely that the same principles will apply for all human CNS cell types. Thus, we propose injecting the mice at postnatal day 1 with a cell suspension of human fetal microglia and astrocytes. We plan to administer the cells in six separate intraparenchymal injections (three bilateral, all into the cerebral cortex; at least 5 × 10⁴ cells/injection). We have adapted this protocol from a method used previously for the successful engraftment of human oligodendrocytes into the mouse brain (Windrem et al., 2008).

Following administration of the fetal brain tissue, the mice will immediately receive an intrahepatic injection of human fetal hematopoietic CD34⁺ progenitor cells (from cord blood). We have selected this route of administration because it is an established method that has been used to successfully reconstitute the human immune system in the HIS mouse (Traggiai et al., 2004).

The final point we considered for our strategy to develop the HIS-CNS mouse is the challenge of tracking the human astrocytes and microglia post-transplantation, because many antibodies are not species-specific. A potential solution to this problem would be to transduce the human astrocytes and microglia with lentiviral vectors that express fluorescent proteins, prior to transplantation into the mouse brain (Tamaki et al., 2002). This method would almost eliminate the risk of false positives when tracking the cells after engraftment, and would allow not only the success of graft implantation but also the degree of cell engraftment in the mouse brain to be easily determined. Although viral transduction can alter the behavior of cells, it does not seem to change their ability to engraft into and repopulate the mouse CNS. For example, when NOD-SCID mice were transplanted with human neurospheres that had been transduced with a lentiviral vector expressing green fluorescent protein (GFP), more than 98% of 200 transplanted animals had human cell engraftment. Additionally, these cells demonstrated the potential for long-term survival, because they were detectable in the hippocampus more than 16 weeks later (Tamaki et al., 2002). On the basis of these findings, we propose a strategy for the HIS-CNS mouse model whereby mixed cultures of human fetal astrocytes and microglia will be transduced with lentiviral vectors that express green fluorescent protein before transplantation into the mouse brain. Once they are implanted into the mouse CNS, the fluorescent signal will allow us to easily differentiate the implanted human brain tissue from the mouse brain tissue when we assess the HIS-CNS mice for histological abnormalities during post-mortem analysis.

Although an HIV-infected HIS-CNS mouse model offers great promise for preclinical research on the mechanisms that contribute to the development of HAND, two concerns associated with these animals are: (1) the challenge associated with obtaining the large amount of human fetal tissue that will be necessary to create such models and (2) the fact that such a model is prone to variability between animals, limiting reproducibility. Tissue engineering approaches could play a key role in overcoming these challenges. For example, a recent study showed that polymer scaffolds encapsulating primary human hepatocytes can be used to create human ectopic artificial livers when implanted in immunocompetent mice with normal liver function (Chen et al., 2011). Post-transplantation, these hepatocytes successfully engrafted and expanded after only 2 weeks (Chen et al., 2011). This is a great advantage over traditional techniques because scaffold-free human hepatocytes cannot successfully engraft into a mouse unless the mouse has been subjected to some degree of liver injury and is immunocompromised. In addition, human hepatocytes engrafted without a scaffold typically take months to properly engraft and expand (de Jong et al., 2010). The results of these studies suggest that this strategy of using polymer scaffolds would be ideal for implantation of human CNS tissue in the HIS-CNS mouse, owing to two main advantages. First, it would increase the consistency of human CNS tissue transplants from mouse to

mouse, because polymer scaffolds allow for higher implantation efficiency and thereby reduce the number of human donor cells needed to establish a tissue graft. Because fewer cells are required, larger numbers of mice could be implanted from the same human tissue donor, thus increasing reproducibility in the HIS-CNS model. Second, previous studies indicate that the polymer scaffolds eliminate the requirement of tissue injury for successful engraftment (Chen et al., 2011; de Jong et al., 2010). As mentioned previously, in order to successfully engraft human microglia and astrocytes in the HIS-CNS mouse, it will probably be necessary to injure the mouse CNS prior to cell transplantation. Using polymer scaffolds to implant human microglia and astrocytes in the mouse brain might eliminate the need for an injured mouse CNS, thereby eliminating one of the technical challenges associated with developing the HIS-CNS model.

Other considerations regarding implantation of human CNS cells into mice include species mismatch between glial-neuronal interactions, long-term survival of human cells in the brain and unresolved ethical issues related to humanization of the mouse brain. It needs to be determined which regions of the brain and how much of the brain can and should be safely humanized.

Conclusion

Animal models of disease allow us to study questions about pathophysiology and treatment, but we cannot expect them to exactly mimic human disease. As detailed in this review, a single small animal model to study all aspects of HAND is not currently available. Instead, the multiple mouse models of HIV that are available are used to address different questions related to HAND, depending on which aspect of the disease they recapitulate.

Owing to the introduction of cART, the presentation of disease in a typical HIV-infected individual is now much different than when the disease was first described. Thus, the criteria that were used to create mouse models of HIV should now be revisited. In the previous section, we proposed the development of a human-mouse chimeric model that would improve on currently available models in that it would recapitulate most features of HAND in a single physiological system containing both a human immune system and human CNS cells (Fig. 2). This proposed HIS-CNS model offers an advantage over the transgenic HIV, HIS and HIVE mouse models because it would contain both human brain macrophages and astrocytes capable of being infected with HIV. In addition to enabling studies of the pathological mechanisms underlying HAND, the effects on the brain of long-term cART exposure could be addressed. It is hoped that this approach will contribute to the development of new antiviral and neuroprotective treatments for HAND.

COMPETING INTERESTS

The authors declare that they do not have any competing or financial interests.

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REFERENCES

- Aksenov, M. Y., Aksenova, M. V., Silvers, J. M., Mactutus, C. F. and Booze, R. M. (2008). Different effects of selective dopamine uptake inhibitors, GBR 12909 and WIN 35428, on HIV-1 Tat toxicity in rat fetal midbrain neurons. *Neurotoxicology* **29**, 971-977.
- Alonso, A., Zaidi, T., Novak, M., Grundke-Iqbal, I. and Iqbal, K. (2001). Hyperphosphorylation induces self-assembly of tau into tangles of paired helical filaments/straight filaments. *Proc. Natl. Acad. Sci. USA* **98**, 6923-6928.

- Ballester, L. Y., Capo-Velez, C. M., Garcia-Beltran, W. F., Ramos, F. M., Vazquez-Rosa, E., Rios, R., Mercado, J. R., Melendez, R. I. and Lasalde-Dominicci, J. A. (2012). Up-regulation of the neuronal nicotinic receptor $\alpha 7$ by HIV-GP120: potential implications for HIV associated neurocognitive disorder. *J. Biol. Chem.* **287**, 3079-3086.
- Banks, W. A., Freed, E. O., Wolf, K. M., Robinson, S. M., Franko, M. and Kumar, V. B. (2001). Transport of human immunodeficiency virus type 1 pseudoviruses across the blood-brain barrier: role of envelope proteins and adsorptive endocytosis. *J. Virol.* **75**, 4681-4691.
- Bansal, A. K., Mactutus, C. F., Nath, A., Maragos, W., Hauser, K. F. and Booze, R. M. (2000). Neurotoxicity of HIV-1 proteins gp120 and Tat in the rat striatum. *Brain Res.* **879**, 42-49.
- Berges, B. K., Wheat, W. H., Palmer, B. E., Connick, E. and Akkina, R. (2006). HIV-1 infection and CD4 T cell depletion in the humanized Rag2- γ mouse model. *Retrovirology* **3**, 76.
- Berges, B. K., Akkina, S. R., Folkvord, J. M., Connick, E. and Akkina, R. (2008). Mucosal transmission of R5 and X4 tropic HIV-1 via vaginal and rectal routes in humanized Rag2- γ mouse. *Virology* **373**, 342-351.
- Berges, B. K., Akkina, S. R., Remling, L. and Akkina, R. (2010). Humanized Rag2- γ mouse can sustain long-term chronic HIV-1 infection lasting more than a year. *Virology* **397**, 100-103.
- Chang, J. R., Mukerjee, R., Bagashev, A., Del Valle, L., Chabrashvili, T., Hawkins, B. J., He, J. J. and Sawaya, B. E. (2011). HIV-1 Tat protein promotes neuronal dysfunction through disruption of microRNAs. *J. Biol. Chem.* **286**, 41125-41134.
- Chauhan, A., Turchan, J., Pocernich, C., Bruce-Keller, A., Roth, S., Butterfield, D. A., Major, E. O. and Nath, A. (2003). Intracellular human immunodeficiency virus Tat expression in astrocytes promotes astrocyte survival but induces potent neurotoxicity at distant sites via axonal transport. *J. Biol. Chem.* **278**, 13512-13519.
- Chen, A. A., Thomas, D. K., Ong, L. L., Schwartz, R. E., Golub, T. R. and Bhatia, S. N. (2011). Humanized mice with ectopic artificial liver tissues. *Proc. Natl. Acad. Sci. USA* **108**, 11842-11847.
- Cho, Y., Son, H. J., Kim, E. M., Choi, J. H., Kim, S. T., Ji, I. J., Choi, D. H., Joh, T. H., Kim, Y. S. and Hwang, O. (2009). Doxycycline is neuroprotective against nigral dopaminergic degeneration by a dual mechanism involving MMP-3. *Neurotox. Res.* **16**, 361-371.
- Choi, J., Liu, R. M., Kundu, R. K., Sangiorgi, F., Wu, W., Maxson, R. and Forman, H. J. (2000). Molecular mechanism of decreased glutathione content in human immunodeficiency virus type 1 Tat-transgenic mice. *J. Biol. Chem.* **275**, 3693-3698.
- Choudhary, S. K., Rezk, N. L., Ince, W. L., Cheema, M., Zhang, L., Su, L., Swanstrom, R., Kashuba, A. D. and Margolis, D. M. (2009). Suppression of human immunodeficiency virus type 1 (HIV-1) viremia with reverse transcriptase and integrase inhibitors, CD4+ T-cell recovery, and viral rebound upon interruption of therapy in a new model for HIV treatment in the humanized Rag2- γ mouse. *J. Virol.* **83**, 8254-8258.
- Churchill, M. J., Wesselingh, S. L., Cowley, D., Pardo, C. A., McArthur, J. C., Brew, B. J. and Gorry, P. R. (2009). Extensive astrocyte infection is prominent in human immunodeficiency virus-associated dementia. *Ann. Neurol.* **66**, 253-258.
- D'hooge, R., Franck, F., Mucke, L. and De Deyn, P. P. (1999). Age-related behavioural deficits in transgenic mice expressing the HIV-1 coat protein gp120. *Eur. J. Neurosci.* **11**, 4398-4402.
- Dash, P. K., Gorantla, S., Gendelman, H. E., Knibbe, J., Casale, G. P., Makarov, E., Epstein, A. A., Gelbard, H. A., Boska, M. D. and Poluektova, L. Y. (2011). Loss of neuronal integrity during progressive HIV-1 infection of humanized mice. *J. Neurosci.* **31**, 3148-3157.
- Davis, L. E., Hjelle, B. L., Miller, V. E., Palmer, D. L., Llewellyn, A. L., Merlin, T. L., Young, S. A., Mills, R. G., Wachsman, W. and Wiley, C. A. (1992). Early viral brain invasion in iatrogenic human immunodeficiency virus infection. *Neurology* **42**, 1736-1739.
- de Jong, Y. P., Rice, C. M. and Ploss, A. (2010). New horizons for studying human hepatotropic infections. *J. Clin. Invest.* **120**, 650-653.
- Denton, P. W. and Garcia, J. V. (2011). Humanized mouse models of HIV infection. *AIDS Rev.* **13**, 135-148.
- Dihné, M., Hartung, H. P. and Seitz, R. J. (2011). Restoring neuronal function after stroke by cell replacement: anatomic and functional considerations. *Stroke* **42**, 2342-2350.
- Dou, H., Birusingh, K., Faraci, J., Gorantla, S., Poluektova, L. Y., Maggirwar, S. B., Dewhurst, S., Gelbard, H. A. and Gendelman, H. E. (2003). Neuroprotective activities of sodium valproate in a murine model of human immunodeficiency virus-1 encephalitis. *J. Neurosci.* **23**, 9162-9170.
- Dyson, S. C. and Barker, R. A. (2011). Cell-based therapies for Parkinson's disease. *Expert Rev. Neurother.* **11**, 831-844.
- Eggert, D., Dash, P. K., Gorantla, S., Dou, H., Schifitto, G., Maggirwar, S. B., Dewhurst, S., Poluektova, L., Gelbard, H. A. and Gendelman, H. E. (2010). Neuroprotective activities of CEP-1347 in models of neuroAIDS. *J. Immunol.* **184**, 746-756.
- Eugenin, E. A., Clements, J. E., Zink, M. C. and Berman, J. W. (2011). Human immunodeficiency virus infection of human astrocytes disrupts blood-brain barrier integrity by a gap junction-dependent mechanism. *J. Neurosci.* **31**, 9456-9465.
- Fitting, S., Xu, R., Bull, C., Buch, S. K., El-Hage, N., Nath, A., Knapp, P. E. and Hauser, K. F. (2010). Interactive comorbidity between opioid drug abuse and HIV-1 Tat: chronic exposure augments spine loss and sublethal dendritic pathology in striatal neurons. *Am. J. Pathol.* **177**, 1397-1410.
- Garza, H. H. J., Prakash, O. and Carr, D. J. (1996). Aberrant regulation of cytokines in HIV-1 TAT72-transgenic mice. *J. Immunol.* **156**, 3631-3637.
- Gimeno, R., Weijer, K., Voordouw, A., Uittenbogaart, C. H., Legrand, N., Alves, N. L., Wijnands, E., Blom, B. and Spits, H. (2004). Monitoring the effect of gene silencing by RNA interference in human CD34+ cells injected into newborn Rag2- γ mouse: functional inactivation of p53 in developing T cells. *Blood* **104**, 3886-3893.
- Gorantla, S., Makarov, E., Finke-Dwyer, J., Castaneda, A., Holguin, A., Gebhart, C. L., Gendelman, H. E. and Poluektova, L. (2010). Links between progressive HIV-1 infection of humanized mice and viral neuropathogenesis. *Am. J. Pathol.* **177**, 2938-2949.
- Holman, A. G., Mefford, M. E., O'Connor, N. and Gabuzda, D. (2010). HIVBrainSeqDB: a database of annotated HIV envelope sequences from brain and other anatomical sites. *AIDS Res. Ther.* **7**, 43.
- Ince, W. L., Zhang, L., Jiang, Q., Arrildt, K., Su, L. and Swanstrom, R. (2010). Evolution of the HIV-1 env gene in the Rag2- γ humanized mouse model. *J. Virol.* **84**, 2740-2752.
- Ishikawa, F., Yasukawa, M., Lyons, B., Yoshida, S., Miyamoto, T., Yoshimoto, G., Watanabe, T., Akashi, K., Shultz, L. D. and Harada, M. (2005). Development of functional human blood and immune systems in NOD/SCID/IL2 receptor γ chain(null) mice. *Blood* **106**, 1565-1573.
- Ito, M., Hiramatsu, H., Kobayashi, K., Suzue, K., Kawahata, M., Hioki, K., Ueyama, Y., Koyanagi, Y., Sugamura, K., Tsuji, K. et al. (2002). NOD/SCID/ γ chain(null) mouse: an excellent recipient mouse model for engraftment of human cells. *Blood* **100**, 3175-3182.
- Jantzie, L. L., Rauw, G. A. and Todd, K. G. (2006). The effects of doxycycline administration on amino acid neurotransmitters in an animal model of neonatal hypoxia-ischemia. *Neurochem. Int.* **49**, 717-728.
- Joers, V. L. and Emborg, M. E. (2009). Preclinical assessment of stem cell therapies for neurological diseases. *ILAR J.* **51**, 24-41.
- Johnson, T. and Nath, A. (2011). Immune reconstitution inflammatory syndrome and the central nervous system. *Curr. Opin. Neurol.* **24**, 284-290.
- Jones, M., Olafson, K., Del Bigio, M. R., Peeling, J. and Nath, A. (1998). Intraventricular injection of human immunodeficiency virus type 1 (HIV-1) tat protein causes inflammation, gliosis, apoptosis, and ventricular enlargement. *J. Neuropathol. Exp. Neurol.* **57**, 563-570.
- Kang, Y. J., Digicaylioglu, M., Russo, R., Kaul, M., Achim, C. L., Fletcher, L., Masliah, E. and Lipton, S. A. (2010). Erythropoietin plus insulin-like growth factor-1 protects against neuronal damage in a murine model of human immunodeficiency virus-associated neurocognitive disorders. *Ann. Neurol.* **68**, 342-352.
- Kennedy, D. W. and Abkowitz, J. L. (1997). Kinetics of central nervous system microglial and macrophage engraftment: analysis using a transgenic bone marrow transplantation model. *Blood* **90**, 986-993.
- Kim, B. O., Liu, Y., Ruan, Y., Xu, Z. C., Schantz, L. and He, J. J. (2003). Neuropathologies in transgenic mice expressing human immunodeficiency virus type 1 Tat protein under the regulation of the astrocyte-specific glial fibrillary acidic protein promoter and doxycycline. *Am. J. Pathol.* **162**, 1693-1707.
- Koenig, S., Gendelman, H. E., Orenstein, J. M., Dal Canto, M. C., Pezeshkpour, G. H., Yungbluth, M., Janotta, F., Aksamit, A., Martin, M. A. and Fauci, A. S. (1986). Detection of AIDS virus in macrophages in brain tissue from AIDS patients with encephalopathy. *Science* **233**, 1089-1093.
- Kramer-Hämmerle, S., Rothenaigner, I., Wolff, H., Bell, J. E. and Brack-Werner, R. (2005). Cells of the central nervous system as targets and reservoirs of the human immunodeficiency virus. *Virus Res.* **111**, 194-213.
- Krucker, T. T. S., Mucke, L. and Siggins, G. R. (1998). Transgenic mice with cerebral expression of human immunodeficiency virus type-1 coat protein gp120 show divergent changes in short- and long-term potentiation in CA1 hippocampus. *Neuroscience* **83**, 691-700.
- Lee, M. H., Wang, T., Jang, M. H., Steiner, J., Haughey, N., Ming, G. L., Song, H., Nath, A. and Venkatesan, A. (2011). Rescue of adult hippocampal neurogenesis in a mouse model of HIV neurological disease. *Neurobiol. Dis.* **41**, 678-687.
- Legrand, N., Weijer, K. and Spits, H. (2006). Experimental models to study development and function of the human immune system in vivo. *J. Immunol.* **176**, 2053-2058.
- Legrand, N., Ploss, A., Balling, R., Becker, P. D., Borsotti, C., Brezillon, N., Debarry, J., de Jong, Y., Deng, H., Di Santo, J. P. et al. (2009). Humanized mice for modeling human infectious disease: challenges, progress, and outlook. *Cell Host Microbe* **6**, 5-9.
- Li, W., Li, G., Steiner, J. and Nath, A. (2009). Role of Tat protein in HIV neuropathogenesis. *Neurotox. Res.* **16**, 205-220.

- Lipton, S. A.** (1994). HIV-related neuronal injury. Potential therapeutic intervention with calcium channel antagonists and NMDA antagonists. *Mol. Neurobiol.* **8**, 181-196.
- Marcondes, M. C., Burudi, E. M., Huitron-Resendiz, S., Sanchez-Alavez, M., Watry, D., Zandonatti, M., Henriksen, S. J. and Fox, H. S.** (2001). Highly activated CD8(+) T cells in the brain correlate with early central nervous system dysfunction in simian immunodeficiency virus infection. *J. Immunol.* **167**, 5429-5438.
- Marcondes, M. C., Burdo, T. H., Sopper, S., Huitron-Resendiz, S., Lanigan, C., Watry, D., Flynn, C., Zandonatti, M. and Fox, H. S.** (2007). Enrichment and persistence of virus-specific CTL in the brain of simian immunodeficiency virus-infected monkeys is associated with a unique cytokine environment. *J. Immunol.* **178**, 5812-5819.
- Marra, C. M., Zhao, Y., Clifford, D. B., Letendre, S., Evans, S., Henry, K., Ellis, R. J., Rodriguez, B., Coombs, R. W., Schifitto, G. et al.** (2009). Impact of combination antiretroviral therapy on cerebrospinal fluid HIV RNA and neurocognitive performance. *AIDS* **23**, 1359-1366.
- Nath, A.** (2002). Human immunodeficiency virus (HIV) proteins in neuropathogenesis of HIV dementia. *J. Infect. Dis.* **186**, S193-S198.
- Norman, J. P., Perry, S. W., Reynolds, H. M., Kiebała, M., De Mesy Bentley, K. L., Trejo, M., Volsky, D. J., Maggirwar, S. B., Dewhurst, S., Masliah, E. et al.** (2008). HIV-1 Tat activates neuronal ryanodine receptors with rapid induction of the unfolded protein response and mitochondrial hyperpolarization. *PLoS ONE* **3**, e3731.
- Nottet, H. S., Persidsky, Y., Sasseville, V. G., Nukuna, A. N., Bock, P., Zhai, Q. H., Sharer, L. R., McComb, R. D., Swindells, S., Soderland, C. et al.** (1996). Mechanisms for the transendothelial migration of HIV-1-infected monocytes into brain. *J. Immunol.* **156**, 1284-1295.
- Okamoto, S., Kang, Y. J., Brechtel, C. W., Siviglia, E., Russo, R., Clemente, A., Harrop, A., McKercher, S., Kaul, M. and Lipton, S. A.** (2007). HIV/gp120 decreases adult neural progenitor cell proliferation via checkpoint kinase-mediated cell-cycle withdrawal and G1 arrest. *Cell Stem Cell* **1**, 230-236.
- Patrick, C., Crews, L., Desplats, P., Dumapo, W., Rockenstein, E., Achim, C. L., Everall, I. P. and Masliah, E.** (2011). Increased CDK5 expression in HIV encephalitis contributes to neurodegeneration via tau phosphorylation and is reversed with Roscovitine. *Am. J. Pathol.* **178**, 1646-1661.
- Perry, S. W., Barbieri, J., Tong, N., Poleskaya, O., Pudasaini, S., Stout, A., Lu, R., Kiebała, M., Maggirwar, S. B. and Gelbard, H. A.** (2010). Human immunodeficiency virus-1 Tat activates calpain proteases via the ryanodine receptor to enhance surface dopamine transporter levels and increase transporter-specific uptake and Vmax. *J. Neurosci.* **30**, 14153-14164.
- Persidsky, Y. and Gendelman, H. E.** (1997). Development of laboratory and animal model systems for HIV-1 encephalitis and its associated dementia. *J. Leukoc. Biol.* **62**, 100-106.
- Persidsky, Y., Limoges, J., McComb, R., Bock, P., Baldwin, T., Tyor, W., Patil, A., Nottet, H. S., Epstein, L., Gelbard, H. et al.** (1996). Human immunodeficiency virus encephalitis in SCID mice. *Am. J. Pathol.* **149**, 1027-1053.
- Persidsky, Y., Stins, M., Way, D., Witte, M. H., Weinand, M., Kim, K. S., Bock, P., Gendelman, H. E. and Fiala, M.** (1997). A model for monocyte migration through the blood-brain barrier during HIV-1 encephalitis. *J. Immunol.* **158**, 3499-3510.
- Persidsky, Y., Ghorpade, A., Rasmussen, J., Limoges, J., Liu, X. J., Stins, M., Fiala, M., Way, D., Kim, K. S. and Witte, M. H.** (1999). Microglial and astrocyte chemokines regulate monocyte migration through the blood-brain barrier in human immunodeficiency virus-1 encephalitis. *Am. J. Pathol.* **155**, 1599-1611.
- Poluektova, L. Y.** (2011). Murine models for neuro-AIDS. In *The Neurology of AIDS* (ed. H. E. Gendelman, I. Grant, I. P. Everall, H. S. Fox, H. A. Gelbard, S. A. Lipton and S. Swindells), pp. 414-430. New York: Oxford University Press.
- Ridge, J. P., Fuchs, E. J. and Matzinger, P.** (1996). Neonatal tolerance revisited: turning on newborn T cells with dendritic cells. *Science* **274**, 1723-1726.
- Robertson, K. R., Su, Z., Margolis, D. M., Krambrink, A., Havlir, D. V., Evans, S. and Skiest, D. J.** (2010). Neurocognitive effects of treatment interruption in stable HIV-positive patients in an observational cohort. *Neurology* **74**, 1260-1266.
- Robinson, B., Li, Z. and Nath, A.** (2007). Nucleoside reverse transcriptase inhibitors and human immunodeficiency virus proteins cause axonal injury in human dorsal root ganglia cultures. *J. Neurovirol.* **13**, 160-167.
- Roy, N. S., Cleren, C., Singh, S. K., Yang, L., Beal, M. F. and Goldman, S. A.** (2006). Functional engraftment of human ES cell-derived dopaminergic neurons enriched by coculture with telomerase-immortalized midbrain astrocytes. *Nat. Med.* **12**, 1259-1268.
- Sango, K., Joseph, A., Patel, M., Osiecki, K., Dutta, M. and Goldstein, H.** (2010). Highly active antiretroviral therapy potentially suppresses HIV infection in humanized Rag2-/-gammac-/- mice. *AIDS Res. Hum. Retroviruses* **26**, 735-746.
- Sato, K. and Koyanagi, Y.** (2011). The mouse is out of the bag: insights and perspectives on HIV-1-infected humanized mouse models. *Exp. Biol. Med.* **236**, 977-985.
- Sato, K., Izumi, T., Misawa, N., Kobayashi, T., Yamashita, Y., Ohmichi, M., Ito, M., Takaori-Kondo, A. and Koyanagi, Y.** (2010a). Remarkable lethal G-to-A mutations in vif-proficient HIV-1 provirus by individual APOBEC3 proteins in humanized mice. *J. Virol.* **84**, 9546-9556.
- Sato, K., Nie, C., Misawa, N., Tanaka, Y., Ito, M. and Koyanagi, Y.** (2010b). Dynamics of memory and naïve CD8+ T lymphocytes in humanized NOD/SCID/IL-2Rgamma null mice infected with CCR5-tropic HIV-1. *Vaccine* **28 Suppl. 2**, B32-B37.
- Schneider, J. B. H., Bienzle, U. and Hunsmann, G.** (1985). A glycopolypeptide (gp 100) is the main antigen detected by HTLV-III antisera. *Med. Microbiol. Immunol.* **174**, 35-42.
- Schneider, J., Kaaden, O., Copeland, T. D., Oroszlan, S. and Hunsmann, G.** (1986). Shedding and interspecies type sero-reactivity of the envelope glycopolypeptide gp120 of the human immunodeficiency virus. *J. Gen. Virol.* **67**, 2533-2538.
- Shultz, L. D., Lyons, B. L., Burzenski, L. M., Gott, B., Chen, X., Chaleff, S., Kotb, M., Gillies, S. D., King, M., Mangada, J. et al.** (2005). Human lymphoid and myeloid cell development in NOD/LtSz-scid IL2R gamma null mice engrafted with mobilized human hemopoietic stem cells. *J. Immunol.* **174**, 6477-6489.
- Simioni, S., Cavassini, M., Annoni, J. M., Rimbault Abraham, A., Bourquin, I., Schiffer, V., Calmy, A., Chave, J. P., Giacobini, E., Hirschel, B. et al.** (2010). Cognitive dysfunction in HIV patients despite long-standing suppression of viremia. *AIDS* **24**, 1243-1250.
- Stoddart, C. A., Maidji, E., Galkina, S. A., Kosikova, G., Rivera, J. M., Moreno, M. E., Sloan, B., Joshi, P. and Long, B. R.** (2011). Superior human leukocyte reconstitution and susceptibility to vaginal HIV transmission in humanized NOD-scid IL-2R(-/-) (NSG) BLT mice. *Virology* **417**, 154-160.
- Tamaki, S., Eckert, K., He, D., Sutton, R., Doshe, M., Jain, G., Tushinski, R., Reitsma, M., Harris, B., Tsukamoto, A. et al.** (2002). Engraftment of sorted/expanded human central nervous system stem cells from fetal brain. *J. Neurosci. Res.* **69**, 976-986.
- Tamaki, S. J., Jacobs, Y., Dohse, M., Capela, A., Cooper, J. D., Reitsma, M., He, D., Tushinski, R., Belichenko, P. V., Salehi, A. et al.** (2009). Neuroprotection of host cells by human central nervous system stem cells in a mouse model of infantile neuronal ceroid lipofuscinosis. *Cell Stem Cell* **5**, 310-319.
- Tervo, H. M., Goffinet, C. and Keppler, O. T.** (2008). Mouse T-cells restrict replication of human immunodeficiency virus at the level of integration. *Retrovirology* **5**, 58.
- Thompson, K. A., Cherry, C. L., Bell, J. E. and McLean, C. A.** (2011). Brain cell reservoirs of latent virus in presymptomatic HIV-infected individuals. *Am. J. Pathol.* **179**, 1623-1629.
- Toggas, S. M., Masliah, E., Rockenstein, E. M., Rall, G. F., Abraham, C. R. and Mucke, L.** (1994). Central nervous system damage produced by expression of the HIV-1 coat protein gp120 in transgenic mice. *Nature* **367**, 188-193.
- Toggas, S. M., Masliah, E. and Mucke, L.** (1996). Prevention of HIV-1 gp120-induced neuronal damage in the central nervous system of transgenic mice by the NMDA receptor antagonist memantine. *Brain Res.* **706**, 303-307.
- Traggiai, E., Chicha, L., Mazzucchelli, L., Bronz, L., Piffaretti, J. C., Lanzavecchia, A. and Manz, M. G.** (2004). Development of a human adaptive immune system in cord blood cell-transplanted mice. *Science* **304**, 104-107.
- Vellutini, C., Horschowski, N., Philippon, V., Gambarelli, D., Nave, K. A. and Filippi, P.** (1995). Development of lymphoid hyperplasia in transgenic mice expressing the HIV tat gene. *AIDS Res. Hum. Retroviruses* **11**, 21-29.
- Vogel, J., Hinrichs, S. H., Reynolds, R. K., Luciw, P. A. and Jay, G.** (1988). The HIV tat gene induces dermal lesions resembling Kaposi's sarcoma in transgenic mice. *Nature* **335**, 606-611.
- Wallace, D. R., Dodson, S., Nath, A. and Bozzer, R. M.** (2006). Estrogen attenuates gp120- and tat1-72-induced oxidative stress and prevents loss of dopamine transporter function. *Synapse* **59**, 51-60.
- Wiley, C. A., Schrier, R. D., Nelson, J. A., Lampert, P. W. and Oldstone, M. B.** (1986). Cellular localization of human immunodeficiency virus infection within the brains of acquired immune deficiency syndrome patients. *Proc. Natl. Acad. Sci. USA* **83**, 7089-7093.
- Windrem, M. S., Roy, N. S., Wang, J., Nunes, M., Benraiss, A., Goodman, R., McKhann, G. M., 2nd and Goldman, S. A.** (2002). Progenitor cells derived from the adult human subcortical white matter disperse and differentiate as oligodendrocytes within demyelinated lesions of the rat brain. *J. Neurosci. Res.* **69**, 966-975.
- Windrem, M. S., Nunes, M. C., Rashbaum, W. K., Schwartz, T. H., Goodman, R. A., McKhann, G., 2nd, Roy, N. S. and Goldman, S. A.** (2004). Fetal and adult human oligodendrocyte progenitor cell isolates myelinate the congenitally dysmyelinated brain. *Nat. Med.* **10**, 93-97.
- Windrem, M. S., Schanz, S. J., Guo, M., Tian, G. F., Washco, V., Stanwood, N., Rasband, M., Roy, N. S., Nedergaard, M., Havton, L. A. et al.** (2008). Neonatal chimerization with human glial progenitor cells can both remyelinate and rescue the otherwise lethally hypomyelinated shiverer mouse. *Cell Stem Cell* **2**, 553-565.
- Xiao, H., Neuveut, C., Tiffany, H. L., Benkirane, M., Rich, E. A., Murphy, P. M. and Jeang, K. T.** (2000). Selective CXCR4 antagonism by Tat: implications for in vivo expansion of coreceptor use by HIV-1. *Proc. Natl. Acad. Sci. USA* **97**, 11466-11471.
- Zhang, B., Duan, Z. and Zhao, Y.** (2009). Mouse models with human immunity and their application in biomedical research. *J. Cell. Mol. Med.* **13**, 1043-1058.