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Can Humanized Mice Reflect the Complex Pathobiology of HIV-Associated Neurocognitive Disorders?

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Abstract

There is a rebirth of humanized mouse models in reflecting human immunodeficiency virus (HIV) pathobiology. This has allowed new investigations of viral diversity, immunity and developmental therapeutics. In the past, HIV infection and disease were, in part, mirrored in immune deficient mice reconstituted with human hematopoietic stem cells. What remained from early studies reflected the ability to mirror central nervous system (CNS) disease. As the wide spread use of combination antiretroviral therapies has changed the severity, but not prevalence, of HIV-associated neurocognitive disorders (HAND), mimicking such virus-induced CNS morbidities in humanized animals is essential for HIV/AIDS research activities. To this end, we now review the evidence for how and under what circumstances humanized mice may be utilized for studies of HIV-1 neuropathogenesis.

Keywords

HIV-1; Mouse model; Brain; Neuroinflammation; Microglia; HIV-associated neurocognitive disorders

Humanization of mice

One strategy used to study the pathogenesis of human immunodeficiency virus type one (HIV-1), a species-specific infection of the human immune system, is to recreate a human immune system in rodents. HIV-1 infection of primary human cells was made possible in mice through the discovery of the severe combined immunodeficiency (scid) mutation in CB-17 mice (Bosma et al. 1983). A spontaneous mutation in the *Prkdc* gene encoding the catalytic subunit of DNA-activated protein kinase (DNA-PK, an 83 amino acid truncation from the catalytic C-terminus) occurred in a colony of BALB/c-*Ighb* (C.B-17) mice

maintained at the Institute for Cancer Research in Philadelphia, PA (Bosma et al. 1989). Mice homozygous for the *prkdc* mutation (*scid/scid*) were unable to efficiently rearrange their immunoglobulin and T-cell receptor genes (Schuler et al. 1986; Fulop and Phillips 1990). Animals with DNA-PK deficiency showed both irradiation-induced DNA damage and profound deficiencies in mature T and B lymphocytes (Hendrickson et al. 1991). This severe deficiency of adaptive immunity enabled engraftment of human lymphocytes and hematopoietic tissues into rodents.

In September 1988, two landmark publications sparked the use of humanized mice in studies of HIV-1 disease. One transplanted human peripheral blood lymphocytes (hu-PBL) (Mosier et al. 1988). The transplantation of a human fetal thymus–liver tissues under the kidney capsule (SCID-hu Thy/Liv) was a second approach leading to local human thymocyte production and differentiation (McCune et al. 1988; Namikawa et al. 1988). However, these models were not true humanization. The leakiness of the rodent immune system lead to subsequent graft versus host disease and incomplete reconstitution (Rizza et al. 1996; Roncarolo et al. 1996; Garcia et al. 1997). The life span of these animals was also limited to 7–8 months due to the spontaneous development of lymphomas and thymomas. Moreover, SCID-hu Thy/Liv transplantation did not provide systemic repopulation of mouse body by human T cells, B cells and monocyte/macrophages.

A substantive step towards creating a true humanized mouse came as a result of the transfer of the *scid* mutation to the non-obese diabetic (NOD/LtSz) NOD/LtSz-*scid* (Greiner et al. 1995; Hesselton et al. 1995; Serreze et al. 1995; Shultz et al. 1995) or NOD/Shi-*scid* (Koyanagi et al. 1997) mouse background. These strains differed in the degree of functional impairments of mouse NK cells, and the resultant engraftment of human PBL in non-irradiated, CD122 antibody (beta chain of the three-chain [α , β , γ] IL-2 receptor complex)-treated animals. Addition of the common cytokine receptor gamma (γ) chain mutation to the IL-2R complex, which deletes the intracytoplasmic domain of the γ chain, to the mouse genome (Ohbo et al. 1996) and transfer of the mutation to the NOD/Shi-*scid* background corrected the NK cell functional limitations for human cell engraftment. These modifications produced NOD/Shi-*scid* IL2r^{null} (NOG) mice (Ito et al. 2002). Transfer of the γ chain *null* mutation (DiSanto et al. 1995) from B6.129S4-*IL2rg^{tmWjl}/J* mice (B6-*IL2R^{null}*) to NOD/LtSz-*scid* mice generated a similar strain named NOD.Cg-*Prkdc^{scid} Il2rg^{tm1Wjl}/SzJ* (NSG) (Ishikawa et al. 2005). Because the common cytokine receptor chain is an indispensable component of receptor heterodimers, for a large number of lymphoid-related cytokines (for example, interleukin-2 [IL-2], IL-7, IL-9, IL-12, IL-15, and IL-21), NOG and NSG mice lacked a fully functional adaptive immune system or NK-dependent innate immunity. These animals also have underdeveloped lymphatic tissues. The expression of two lymphoid-specific recombination-activating genes (RAG1 and RAG2) proved critical for cells displaying V (D)J activity (Oettinger 1996). A genetic cross of γ ^{-/-} and RAG2 deficiency on a Balb/c background produced mice doubly homozygous for the γ and RAG2 alleles (γ ^{-/-}/RAG2^{-/-}) (Goldman et al. 1998). These defects facilitated engraftment of human cells and tissues, and revitalized the use of such mice for a wide range of applications, including studies of HIV-1 pathogenesis (Traggiai et al. 2004; Gorantla et al. 2007; Pearson et al. 2008; Brainard et al. 2009; Legrand et al. 2009; Brehm et al. 2010; Denton et al. 2010, 2011; Choudhary et al. 2012; Olesen et al. 2011).

In the past decade and a half, NOD/*scid* mice have been shown to be a unique host for human hematopoietic stem cell (HSC) transplantation (Pflumio et al. 1996; Dick et al. 1997). The NOD/*scid* xenotransplantation model is the “gold standard” for assaying HSC activity (McDermott et al. 2010). Marked increases in engraftment success was linked to reduced NK cell activity and loss of complement lytic activity. The latter was due to a two base-pair deletion in a 5' exon of the murine C5 gene (Baxter and Cooke 1993). Defects in

macrophage and dendritic cell function, including defective regulation of colony-stimulating factor-1, interferon- receptors, and reduced secretion of IL-1 in response to lipopolysaccharide (LPS), also improved engraftment NOD/scid model (Shultz et al. 1995; Ito et al. 2002).

What underlied the improvements observed on the NOD background was determined only 5 years ago (Takenaka et al. 2007; Takizawa and Manz 2007). It occurred as a consequence of a murine polymorphism in signal regulatory protein- (SIRP- , CD172); a region in the insulin-dependent diabetes 13 (*Idd13*) locus on chromosome 2 contains a coding sequence polymorphism for *Sirpa*. NOD-derived SIRP- interacts with the widely expressed cell surface transmembrane glycoprotein CD47 (also called integrin-associated protein, IAP). The interaction of these proteins on the surface of mouse macrophages and human HSCs blocks xenogeneic human cell losses, including HSCs (Ide et al. 2007). Transgenic expression of human SIRP in 129/BALB/c Rag2^{-/-} c^{-/-} mice improved the frequency of engraftment, and increased levels of engrafted human cells, resulted in an improved functional human immune system (Strowig et al. 2011). Moreover, the expression of mouse CD47 in human HSCs demonstrates the sensitivity of human T-cell homeostasis to CD47/SIRP signaling. In lymphoid organs, effective CD47/SIRP interactions lead to T cell enhancement (~45-fold), and B-cell and monocyte generation (a~15-fold increase in spleen). Human mCD47⁺ T cells selectively accumulated in the peripheral lymphoid organs, mostly as naive, resting cells with improved function, as evidenced by increased total plasma IgM and IgG. Similar to T cells, selective accumulation of NK cells in the periphery was observed in the presence of appropriate mCD47/mSIRP interactions. Improved lymph node organogenesis was also consistently observed both in mCD47/Rag2^{-/-} c^{-/-} and NOD.*sirpa*-Rag2^{-/-} c^{-/-} mice (Legrand et al. 2011).

HIV-1 infection of humanized mice

The combination of stable human hematopoiesis with the ability to support replication of HIV-1 created a means to study HIV-1-associated immunopathology in mice. Recent studies have employed Balb/c-Rag2^{-/-} c^{-/-} mice transplanted with cord blood-derived CD34⁺ HSC injected intrahepatically, NSG/NOG mice transplanted with CD34⁺ cells derived from different sources, including cord blood, fetal liver, and cells mobilized from peripheral blood, and finally the combination of fetal liver-thymus transplantation under the kidney capsule with intravenous infusion of CD34⁺ HSC isolated from the liver (summarized in Table 1).

In most cases, with significant viral intraperitoneal inoculums [Yu-2, NL4-3 viruses 2×10⁶ tissue culture infective dose (TCID)₅₀], viremia reached a plateau by 2–6 weeks post-infection and was sustained throughout the observation period (up to 62 weeks). After intraperitoneal delivery of HIV-1_{ADA} (a macrophage-tropic strain), in a majority of mice, viremia peaked at 4–6 weeks (10⁴–10⁶ copies/ml) post-infection, similar to what has been observed for other macrophage-tropic (Bal, JR-CSF) and dual-tropic (Yu-2) viral strain used in humanized mice (Baenziger et al. 2006; Gorantla et al. 2007; Nie et al. 2009; Akkina et al. 2011). Similar to the observations of other researchers, the decline in CD4⁺ cells in peripheral blood and lymphoid tissues was not rapid in animals infected with macrophage-tropic viruses compared to those infected with lymphocyte-tropic strains, that included NL4-3.

These experimental conditions are in contrast to HIV-1 and simian immunodeficiency virus (SIV) infections; after the first wave of viremia, an adaptive immune response was realized and able to tightly control viral replication. This cannot be accomplished by the immature human immune system of humanized mice in response to a high dose of virus infection. Low level control of viral replication may be related to the absence of innate factors

(complement) able to eliminate viral particles (Koyanagi et al. 1997) or to the weak adaptive immune responses of these mice. We found that HIV-1_{ADA} administered at a 10⁴ TCID₅₀ infected ~50% of Balb/c-Rag2^{-/-} c^{-/-} humanized mice and 90–100% of humanized NSG mice (Gorantla et al. 2007, 2010b). This dose of infection in NSG mice allowed viral load to peak at 5 weeks and a reduction of peripheral viral load by 8 weeks post infection. The depletion of CD8⁺ cells using chimeric cM-T807 monoclonal antibodies after infection increased the peripheral viral load set point to ~1 log₁₀, and was not associated with significant depletion of CD4⁺ cells (Gorantla et al. 2010a). The viral load peak and drop to a set point usually seen in humanized mice was not observed after CD8⁺ cell depletion. A low titer of anti-viral IgM could be detected (Gorantla et al. 2010b), as well as the IgM/IgG transition, and significant levels of anti-viral IgG were detected (Watanabe et al. 2007; Sato et al. 2010). The degree and speed of the depletion of CD4⁺ cells in peripheral blood and lymphoid tissues were variable, but were consistent with published observations of macrophage-tropic strain-infected mice [reviewed in (Sato and Koyanagi 2011; Berges and Rowan 2011)].

The common cytokine receptor gamma chain knockout in NSG/NOG significantly compromised overall development of the secondary lymphoid tissues (Cao et al. 1995; Ikebe et al. 1997). These animals have mesenteric, cervical lymph nodes, which can be populated by human cells, but all other groups of lymph nodes, Peyer's patches, lung- and nasal-associated lymphoid tissues remained underdeveloped (van de Pavert and Mebius 2010). The presence of human B cells and activation of lymphotoxin- / mediated signaling facilitate the development of lymph nodes, but their architecture remain immature (Vondenhoff et al. 2009). Under such circumstances, B cells in humanized NSG/NOG mice do not regulate their development, maturation and receptor editing as well as regulate, immunoglobulin class switching, plasma cell differentiation, and IgG specific antibody responses (Manz 2007; Willinger et al. 2011). The weak adaptive immune response also associated with the poor maintenance of some human cell populations (NK and to some degree CD8⁺ cells) (Andre et al. 2010). Balb/c-Rag2^{-/-} c^{-/-} and NSG/NOG mice are widely accepted as the best model for the studies of human-specific HIV-1 infection, antiretroviral treatment (Sango et al. 2010), virus latency and eradication strategies (Choudhary et al. 2009; Choudhary and Margolis 2011) events involved in mucosal virus transmission and microbicide development, and novel gene therapy approaches (Denton et al. 2011; Neff et al. 2011a, b; Olesen et al. 2011; reviewed in Berges and Rowan 2011).

Human macrophages in humanized mice

HIV-1 infects mononuclear phagocytes including brain perivascular blood-derived macrophages and resident microglial cells (Koenig et al. 1986; Navia et al. 1986; Wiley et al. 1986). The pathologic hallmarks of neurologic disease are include infected multinucleated giant cells and microglial nodules and a diffuse white matter pallor (Michaels et al. 1988; Gray et al. 1996).

An important component of HIV-1-associated neurocognitive disorders (HAND) is the presence of virus-infected human macrophages and microglia. Mouse microglia are primarily derived from primitive yolk sac-derived monocytes (Hirasawa et al. 2005; Ginhoux et al. 2010) after limited expansion *during fetal liver hematopoiesis* independent of classic hematopoiesis [reviewed in (Chan et al. 2007; Soulet and Rivest 2008; Graeber and Streit 2010; Kettenmann et al. 2011)]. Although NOD/*scid* mice have been widely used to study human hematopoiesis, very little attention has been paid to the ability of human macrophages to populate the mouse brain in humanized mice. In 2003 and 2004, two important experimental observations were made in NOD/*scid* mice. The first was the successful demonstration of the ability of human macrophages to populate the mouse brain and deliver the therapeutic enzyme -glucuronidase to correct a lysosomal storage disease

(Hofling et al. 2003). NOD/*scid* mice with a mucopolysaccharidosis type VII phenotype (-glucuronidase mutant) and non-mutant NOD/*scid* and were irradiated (3 Gy) and intravenously transplanted with human CD34⁺ cells. The HSCs were derived from cord blood or mobilized peripheral blood. Daniplestim, an agonist of the human interleukin 3 (IL-3) receptor, was administered twice a week throughout the study to support HSC survival and differentiation. At 6 and 12 weeks all engrafted mice had 4%–10% human CD14⁺ cells (among the CD45⁺ cell population) in bone marrow and peripheral blood (some B cells, but no CD3⁺ T cells, were detected). Spleen reconstitution by human macrophages did not exceed 0.6%. Surprisingly, scattered -glucuronidase-positive cells were found in both control and -glucuronidase mutant mice. Positive cells were abundant in the meninges, and were found in close association with the retinal pigment epithelial layer. Additionally, -glucuronidase was detected in a few cells at other sites. Differences were not seen in animals analyzed at 6 or 12 weeks after transplantation. These findings highlight the broad distribution of human macrophages in these mice, including in bone marrow. Moreover, the functional enzyme delivered by human cells had therapeutic effects and, depending on the level of engraftment of human macrophages, was able to facilitate the reduction of stored material in several host organs (Hofling et al. 2003).

The migration, differentiation, and distribution of human CD34⁺ cells purified either from umbilical cord blood or from mobilized peripheral blood in the brain of NOD/*scid* mice were also investigated (Asheuer et al. 2004). In these studies, prior to transplantation, CD34⁺ cells were pre-incubated with recombinant human stem cell factor, Flt3-ligand, IL-3, and pegylated megakaryocyte growth and differentiation factor. Sublethal irradiation (3 Gy) was performed on adult mice before intravenous infusion of CD34⁺ cells. Eighteen weeks post transplantation, the authors found that approximately half of all animals had <5% human cell bone marrow engraftment, and the rest ranged from 5% to 90%. Bone marrow humanization (based on the presence of CD45⁺ cells only) was correlated with the number of human microglia in brain (from 0.5% to 4.7% of total microglia) (Asheuer et al. 2004). Overall, these results demonstrated that human hematopoiesis occurs in the bone marrow of NSG or NOG mice over a 6–18 week period resulted in the presence of human macrophages and microglia in brain meninges, perivascular spaces, and to some degree, the parenchyma.

Successful human macrophage migration into the NOD/*scid* mouse brain may be associated with mouse NOD. *Sirpa*-human CD47/mouse CD47-human SIRP- interaction. SIRP is indeed involved in the transmigration of monocytes across the brain endothelium through interaction with its counterligand CD47, a molecule that upon engagement induces signaling events in the endothelium (de Vries et al. 2002; Stefanidakis et al. 2008). Species restrictions in immune cell/endothelial cell interactions have been demonstrated for both in Ag-specific responses of T lymphocytes and the phenomenon of natural attachment (Johnston et al. 1990). Human lymphocyte function associated antigen-1 CD11a/CD18 (LFA-1) integrin can bind with mouse intracellular adhesion molecule-1 (ICAM-1, CD54), but murine LFA-1 do not interact with human CD54. Nevertheless, efficient engraftment of HSC in NSG/NOG mice is clear evidence that there are functional overlap between adhesion/integrins that govern migration of human cells in xenogeneic mouse environment.

Other aspects regarding human cell homing, migration and the xenocompatibility of cytokine/chemokine and their receptors (Mestas and Hughes 2004) are explored in Cytokines & Cells Online Pathfinder Encyclopedia (<http://www.copewithcytokines.de/cope>).

The new strategies were recently utilized to improve monocytic lineage development in Balb/c-Rag2^{-/-} γ ^{-/-} and NSG mice. To facilitate the development of this lineage, three human growth factors were knocked in (added) to NSG mice: human stem cell factor,

granulocyte-macrophage colony-stimulating factor, and interleukin-3, and the resulting mice were termed NSG-SGM3 mice. Human myeloid cells, and specifically myeloid dendritic cells, were elevated in the bone marrow of these mice compared with non-transgenic NSG mice. The most significant finding was an increase in the CD4⁺FoxP3⁺ regulatory T-cell population in all compartments analyzed. Unfortunately, human immune system development in these mice was observed for only 12 weeks post transplantation (Billerbeck et al. 2011). Longer observation of the behavior of human cells will provide a more complete picture of the effects of sustained expression of human granulocyte macrophage colony-stimulating factor (GM-CSF), which is not only a growth factor, but also polarizes human monocytes. Such observations were made in Balb/c-Rag2^{-/-} γ ^{-/-} mice with a similar human cytokine gene knock-in (Willinger et al. 2011). Without transplantation of human HSCs, these animals developed pulmonary alveolar proteinosis due to the loss of mouse GM-CSF. Human-derived alveolar macrophages partially rescued the pulmonary alveolar proteinosis syndrome (Willinger et al. 2011). Human macrophages in human GM-CSF-expressing animals produced significant levels of TNF- α , IL-6, and IFN- γ in response to intranasal infection with influenza A compared to non-transgenic humanized animals. When expression of human CSF-1 was established on a Balb/c-Rag2^{-/-} γ ^{-/-} background (Rathinam et al. 2011), it showed no substantial effect on the microenvironment of the spleen, liver, or lungs of 20-week-old animals, as indicated by organ architecture. Analysis of humanized CSF-1 mice engrafted with human CD34⁺ cells indicated increased frequencies of human monocytes and macrophages in several tissues compared to control mice (the brain was not analyzed). Human monocytes and macrophages obtained from these mice exhibited enhanced functional properties compared to those obtained from control mice. In conclusion, humanized mice with human cytokines knocked in may represent a useful model to study polarization of human effector cells and human regulatory T-cell development *in vivo*, as well as other specific aspects of human immune cell development and function. However, the lineage skewing observed in human GM-CSF-expressing mice also highlights the importance of adequate selection and spatiotemporal expression of human cytokines for future xenorecipient strain development.

Humanized mice and HAND

The human specificity of HIV-1 precludes extensive study of neuropathogenesis due to limited access to brain biopsies and cerebrospinal fluid (CSF) samples; postmortem evaluation is currently the only option. Neuroimaging studies are complicated by secondary infections, tumors, and drug toxicities (Boska et al. 2004). SIV monkey models are expensive, and SIV or chimeric SHIV viruses require significant adjustments to be used for anti-retroviral (Balzarini et al. 1997; Giuffre et al. 2003), vaccine, and adjunctive therapeutic studies. The absence of a humanized small rodent model is a critical barrier to develop and screen therapeutics for prevention and treatment of cognitive impairments in the HIV-1-infected population. A rodent model that uses human cells to characterize the therapeutic and neuroprotective properties of these compounds would support their application in human trials and unmask their possible side effects. In addition, detection of the dynamics of neuropathology/neuroprotection by neuroimaging technologies will provide translation to human noninvasive correlates.

There have been studies of HIV-1-associated neurodegeneration in rodent models. Prior approaches have used direct injection of human HIV-1-infected macrophages in the mouse brain to reflect components of human disease (Tyor et al. 1993; Persidsky et al. 1996). These studies have not looked at the complexities of cell migration across the nervous system, progressive neurodegeneration, neuroinflammation, ongoing productive or restricted viral infection, or blood-brain barrier compromise. Before the development of humanized NSG/NOG mice, hu-PBL mice were used to investigate HAND, including investigation of

the effects of HIV-1-infected lymphocytes on brain pathobiology. Lymphocyte egress into the meninges, the choroid plexus, and perivascular spaces leads to vasculitis, neuroinflammation, and neuronal death with parallel inhibition of neurogenesis (Koyanagi et al. 1997; Poluektova et al. 2002, 2004a, 2005; Miura et al. 2003). Generation of HIV-1-specific cellular immune responses has also been observed in the brain after intracranial injection of HIV-1-infected monocyte-derived macrophages (Poluektova et al. 2002). However, severe inflammation due to xenoreaction inhibited the ability of the model to truly reflect human disease. Variability, functional immune deficits, and xenoreactions have all limited the use of adult human lymphocyte-reconstituted mice to mimic human disease (Poluektova et al. 2004a, b).

The long-term immune reconstitution of NSG by human HSC provides a new approach to study HAND. HAND includes two major components: sustained viral replication, and immune activation of human macrophages in the periphery and of brain resident macrophages and microglial cells in the presence or absence of detectable levels of viral replication [reviewed in (Clifford 2002)]. The mechanisms of neuronal dysfunction, which manifests as impaired cognitive (learning, memory, and executive function) and motor function, remain elusive. An association of the nadir CD4 cell count with neurocognitive impairment has been found regardless of antiretroviral treatment (Heaton et al. 2010), and the initial impact of systemic viremia may trigger progressive neurodegenerative processes in the central nervous system.

Prior HIV-1 studies in humanized mice have not focused on the brain, but rather have focused on viral immunopathogenesis. This may be because levels of bone marrow reconstitution and monocyte production in the Balb/c-Rag2^{-/-} γ ^{-/-} strain, which has been widely used for studies of the pathogenesis and treatment of HIV-1 infection, are lower than in the NSG and NOG strains. Thus, it is expected that in the Balb/c-Rag2^{-/-} γ ^{-/-} strain, the number of human cells in the brain will be significantly limited, which was confirmed by our studies on Balb/c-Rag2^{-/-} γ ^{-/-} mice (Gorantla et al. 2007, 2010a).

Irradiation has been used to provide a niche for HSC in mouse bone marrow in the majority of studies performed in humanized mice. This procedure is well known to be detrimental to brain development and function [reviewed in (Abayomi 1996)]. Studies of cognitive impairment require evaluation of changes in behavior (such as in memory tests). Combinations of genetic abnormalities (*scid* mutation, common cytokine gamma chain knockout) with low-dose irradiation at birth or higher doses in adult mice, complicate the interpretation of such tests. However, brain inflammatory responses presumably should be present and to some degree should reflect the observations made in postmortem HIV-1-infected human brains.

In NSG and NOG mice, as in NOD/*scid* mice, it is possible to achieve significant colonization of human macrophages in the bone marrow, *meningeas*, and perivascular spaces, and at lower levels in the parenchyma. In our studies, regardless of age (animals in our studies ranged from 18 to 52 weeks), human macrophages were seen along the meningeas, in some menigeal and parenchymal vessels. Some of these cells had dendritic morphology and some resembled endothelial cells. Among human cells, CD45⁺ (total) and CD163⁺ (macrophage lineage) activated HLA-DR-positive cells were detected. Repopulation did not induce glial activation in surrounding mouse brain tissue. As expected, chronic HIV-1 infection resulted in mild to significant inflammation in humanized mice (Gorantla et al. 2010a; Dash et al. 2011). The formation of microglial nodules in white matter tracks, especially in the brainstem and perivascular cuffs, and activation of surrounding astrocytes was observed in animals sacrificed 9–15 weeks after infection. Human activated lymphocytes were rarely found in uninfected animals. After HIV-1

infection, some egress of activated lymphocytes and macrophages was noted and in ~20% of animals, slight accumulation of activated human cells was observed along with a small number of HIV-1 p24 antigen-positive cells in the meninges (Gorantla et al. 2010a). The presence of glial activation in HIV-1 infected animals suggests that changes in neuronal integrity (Dash et al. 2011).

Non-invasive evaluation of brain integrity by MRI in young and old unmanipulated NSG mice, both uninfected and HIV-1 infected animals (observed up to 15 weeks) was performed (Dash et al. 2011). Chronic HIV-1 infection induced spectroscopic imaging changes. Decreased N-acetylaspartate (NAA) concentration in the cerebral cortex was observed in a small cohort of HIV-1-infected humanized mice; this change was correlated with reduced neuronal specific dendritic structural microtubule-associated protein 2 and synaptophysin expression. The extent of structural changes was associated with immunopathology (loss of CD4⁺ cells and high viral load), and axonal neurofilament protein expression was also reduced in the two mice with the highest levels of viral infection. In contrast, changes in astrogliosis, as measured by GFAP expression, was not uniform. The diminished NAA concentration seen in the cortex of HIV-1-infected humanized mice may not be directly related to neuronal apoptosis, but rather may be an indicator of neuronal abnormalities; this is supported by the finding that the biosynthetic enzyme for NAA, N-acetyl transferase, is localized to the neuronal mitochondrial and synaptosomal cell fractions (Moffett et al. 2007). In addition to these biochemical changes, brain structural alterations were evaluated by diffusion tensor imaging (DTI). Fractional anisotropy (FA) is linked to diffusivity in the gray matter regions and was seen in animals with sustained high viral loads and immunopathology. FA is highly sensitive to microstructural changes but is not specific to the type of pathology. The changes observed at earlier stages of HIV-1 infection in humanized mice could be associated with structural alteration of synaptic and dendritic processes. Further study is required to develop a thorough understanding of brain metabolism in the animals. Efforts are now underway to discern the relationships between viral replication, antiretroviral therapy, and altered systemic metabolism as it is linked to chronic infection and immune compromise.

The reproducibility and durability of virus infection within the CNS of humanized mice depend on the number of human cells of macrophage lineage in brain. Unfortunately, existing model of humanized mice for neuroAIDS is limited. Reconstitution with HSC at birth do not provide sufficient numbers of human microglia. The modeling of transplanted mouse microglia *in vivo* as ramified cells with tissue trophic and vasculature development properties is being developed from multiple sources, but not yet successful. The passive approaches to repopulate brain cells by transplantation of bone marrow or HSC in autologous mouse-to-mouse (Chan et al. 2007; Soulet and Rivest 2008), human-to-human (Cogle et al. 2004), or xenogeneic human-to-mouse (Asheuer et al. 2004) were not yet efficient. The intracranial injection of “microglial” cells derived from different sources did not reconstruct the needed cell populations. Reconstitution of human microglia remains critical for studies of HIV-1 infection in the CNS as well as other neurodegenerative disorders. Peripheral-blood monocytes/macrophages are important, but not a complete substitute for modeling of HIV-1 infection (Dickson et al. 1993; Langford and Masliah 2001). The source of microglia should be resident precursors and, to a lesser extent, blood-derived monocytes/macrophages transformed to microglia-like cells. In HIV-1 infection, productive viral replication in perivascular macrophages has divergent outcomes than in microglia; thus, the model that will mimic infection of parenchymal microglia will be a powerful additional tool to study such differences.

Conclusions

NSG/NOG mice accept a high number of human HSCs. This results in a stable cell population within the mouse bone marrow. The demonstration of human thymocyte maturation, and occupancy of the murine thymus, complete reconstitution of residual murine lymph nodes with human T, B and dendritic cells, formation of splenic white pulp, and the infiltration of brain meninges and perivascular spaces by human macrophages make the model more appealing for studies of human disease. Indeed, these “humanized” mice support chronic HIV-1 replication and partially recapitulate the course of human disease progression with loss of CD4⁺ cells and the development of HIV-1-specific cellular and humoral immune responses (Watanabe et al. 2007; Brainard et al. 2009; Gorantla et al. 2010b). Continuous progression of HIV-1-associated disease is observed in the central nervous system in humanized mice (Gorantla et al. 2010a; Dash et al. 2011). Nonetheless, additional studies are needed to quantify the extent of neuronal damage and to validate biomarkers of HIV-1 virus-associated neurodegenerative disease in this model system.

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Table 1
Establishment and investigation of HIV-1-infected humanized mouse models [modified from (Sato and Koyanagi 2011)]

Reference	Designation *	Recipient mouse			Brief observations					*** Immunological	**** PMD
		Strain [†]	Age [‡]	Route [§]	Source of hHSCs ^{**}	HIV-1 strain ^{††}	Virological ^{‡‡}	Pathological ^{§§}	Immunological		
(Baenziger et al. 2006)	–	BALB/c-Rag2 ^{−/−} c ^{−/−}	Pl-3	i.h.	CB	YU-2 (R5), NL4-3 (X4)	A, C	F	M (partial)		17038503
(Nie et al. 2009)	NOG-hCD34 mice	NOG	Pl-3	i.h.	CB	JR-CSF (R5), NL4-3 (X4)	A, C	F, G	n/a		19744686
(Sato et al. 2010)	NOG-hCD34 mice	NOG	Pl-3	i.h.	CB	JR-CSF (R5)	A, C	F	K, M (partial)		20510741
(Watanabe et al. 2007)	hNOG mice	NOG	6–10 w	i.v.	CB	JR-CSF (R5), MNp (X4)	A, B	F	M		16954502
(Watanabe et al. 2007)	hNOG mice ^{†††}	NOG	6–10 w	i.v.	CB	JR-CSF (R5), MNp (X4), NL4-3 (X4)	A, B, C	F, G	n/a		17881441
(Sun et al. 2007)	BLT mice	NSG		Refer to the footnote ^{§§§}		LAI (X4)	A, C, D	F, G, I	K, M		17389241
(Brainard et al. 2009)	BLT mice	NSG		Refer to the footnote ^{§§§}		ADA (R5), JR-CSF (R5)	A, C	F	K, L, M		19420076
(Zhang et al. 2007)	DKO-hu HSC mice	BALB/c-Rag2 ^{−/−} c ^{−/−}	Pl-3	i.h.	CB or FL	JR-CSF (R5), NL4-3 (X4), NL-R3A (R5X4)	A, C, E	F	n/a		17132723
(Berges et al. 2006)	RAG-hu mice	BALB/c-Rag2 ^{−/−} c ^{−/−}	Pl-3	i.h.	FL	BaL (R5), NL4-3 (X4)	A, B, C, E	F, G	n/a		17078891
(Gorantla et al. 2007)	HIS mice	BALB/c-Rag2 ^{−/−} c ^{−/−}	Pl-3	i.h.	CB	ADA (R5), C1157 (R5)	C, D	F, H	n/a		17182671
(An et al. 2007)	HIS-Rag2 ^{−/−} c ^{−/−} mice	H-2 ^d Rag2 ^{−/−} c ^{−/−}	Pl-3	i.p.	FL	NFN-SX SL9 (R5)	E	F	M (no)		17314230
(Gorantla et al. 2010a)	NSG/hCD34	NSG	Pl-3	i.h.	CB	ADA (R5)	A, C	F, G, H	K (CD8 depletion), M (partial)		20495069
(Gorantla et al. 2010b)	NSG/hCD34	NSG	Pl-3	i.h.	CB	ADA (R5)	A, C	F, J (brain)	K (CD8 depletion)		21088215
(Dash et al. 2011)	NSG/hCD34	NSG	Pl-3	i.h.	FL	ADA (R5)	A, B, C	F, J (brain)	F		21368026

CB cord blood; FL fetal liver; n/a not applicable

* Designation of the constructed humanized mice model by the authors

[†] Strain of recipient mouse. NOG, NOD.Cg-Prkdc^{scid} Il2rg^{tm1Wjl}/Jic (established in Central Institute for Experimental Animals, Kawasaki, Kanagawa, Japan); NSG, NOD.Cg-Prkdc^{scid} Il2rg^{tm1Wjl}/J (established in the Jackson Laboratory, Bar Harbor, Maine, USA)

[‡] The age of mice at the time of transplant

[§] Route of transplantation. *i.h.* intrahepatic; *i.v.* intravenous; *i.p.* intraperitoneal

^{**} Source of human CD34⁺ hematopoietic stem cells (hHSCs)

^{††} HIV-1 strains used in each study. Co-receptor utilization for each HIV-1 strain is indicated in parenthesis. R5, CCR5-tropic; X4, CXCR4-tropic; R5X4, CCR5/CXCR4 dual-tropic

^{‡‡} Virological observations: **A**, HIV-1 RNA in plasma; **B**, HIV-1 DNA in tissue(s); **C**, HIV-1-infected cells; **D**, HIV-1 replication by *ex vivo* co-culture with the cells susceptible for HIV-1

^{§§} Pathological observations: **F**, loss of CD4⁺ T-cells in peripheral blood (PB) or decrease of CD4⁺/CD8⁺ ratio in PB; **G**, thymopathy; **H**, lymphadenopathy; **I**, HIV-1 infection and depletion of CD4⁺ T-cells in gut-associated lymphoid tissues; **J**, non-lymphoid tissue pathology—brain

^{***} Immunological observations: **K**, expansion/activation of CD8⁺ T-cells in PB; **L**, detection of HLA-restricted HIV-1 antigen-specific CD8⁺ T-cells; **M**, analysis of anti-HIV-1 antigen antibodies in plasma/serum. Note that the extent of the detected antibodies was indicated with parenthesis

^{†††} PMID of each paper in PubMed (<http://www.ncbi.nlm.nih.gov/pubmed/>)

^{‡‡‡} hNOG mice did not undergo irradiation before transplantation of hHSCs

^{§§§} Fetal liver and thymus were surgically implanted under the kidney capsule of the recipient mouse at 6–8 weeks old. Three weeks after implantation, autologous FL-derived hHSCs were transplanted by *i.v.* injection