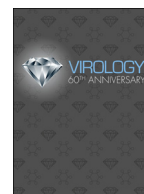




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## Review

## Studies of retroviral infection in humanized mice

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## ABSTRACT

Many important aspects of human retroviral infections cannot be fully evaluated using only *in vitro* systems or unmodified animal models. An alternative approach involves the use of humanized mice, which consist of immunodeficient mice that have been transplanted with human cells and/or tissues. Certain humanized mouse models can support robust infection with human retroviruses including different strains of human immunodeficiency virus (HIV) and human T cell leukemia virus (HTLV). These models have provided wide-ranging insights into retroviral biology, including detailed information on primary infection, *in vivo* replication and pathogenesis, latent/persistent reservoir formation, and novel therapeutic interventions. Here we describe the humanized mouse models that are most commonly utilized to study retroviral infections, and outline some of the important discoveries that these models have produced during several decades of intensive research.

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## Introduction

Infectious agents exact a devastating daily toll of morbidity and mortality worldwide (WHO, 2014). This not only creates millions of personal tragedies each year, but also deeply affects wider communities as the economic and social impact of infectious disease is

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borne by society as a whole. Fortunately, the application of basic and translational scientific research coupled with careful clinical studies has produced a range of vaccines and therapeutic agents that can prevent, treat, or cure previously common human diseases. These successes are built on a foundation of studying the causative organism in relevant biological systems that are suitable for investigating its replication and pathogenesis, and which in turn may be used to evaluate new therapies.

Researchers in the biological sciences are continually striving to develop improved *in vitro* approaches that mimic natural hosts more completely. However, *in vitro* systems represent isolated situations that cannot fully emulate the complexity of an *in vivo* environment. In some cases unmodified animals have proven to be suitable models for studying human pathogens, but this method carries the limitations that the microorganism must be able to replicate efficiently and ideally should cause disease within the animal host species, and that interaction with animal rather than human cells and tissues are being studied.

A powerful complementary approach to *in vitro* or unmodified animal studies is the use of humanized mice. These are mice that have either been engineered to express human genes or (as will primarily be discussed in this review) immunodeficient mice that have been reconstituted with human cells and/or tissues. One particular area of research in which humanized mice have proven useful is in the field of retrovirology. Retroviruses are important human pathogens, and humanized mice have provided important insights into many aspects of retrovirus biology, including transmission, *in vivo* replication and pathogenesis, latent/persistent reservoir formation, and evaluation of novel therapeutic interventions. The purpose of this review is to provide a background on retroviral studies in humanized mice, explain the main types of humanized mouse models currently in use, and provide some examples of the many important areas of retrovirus research that have benefited from the use of humanized mice.

#### *Exogenous retroviruses in humans*

Currently, four exogenous retroviruses have been identified that are endemic in certain human populations: human immunodeficiency virus type 1 and 2 (HIV-1 and HIV-2), and human T cell lymphotropic virus type 1 and 2 (HTLV-1 and HTLV-2). Additional retroviruses including HTLV-3, HTLV-4, and human foamy virus (Mahieux and Gessain, 2011; Meiering and Linial, 2001) are occasionally found in rare individuals, but this is generally the result of infrequent zoonotic transmission of related simian viruses. Consequently, the majority of humanized mouse studies of retroviruses have focused on HIV, and to a much lesser extent on HTLV.

HIV is a lentivirus which infects and depletes CD4<sup>+</sup> T cells, leading to the development of acquired immune deficiency syndrome (AIDS), which typically manifests approximately 10 years after primary infection with the virus (Barre-Sinoussi et al., 1983; Gallo et al., 1983). HIV originated in nonhuman primates in West Africa. The most common strains of HIV type 1 (HIV-1) are derived from a related simian immunodeficiency virus (SIV) SIV<sub>CPZ</sub> present in chimpanzees (*Pan troglodytes troglodytes*), whereas HIV-2 is derived from SIV<sub>SMM</sub> in sooty mangabeys (*Cercocebus atys*) (Hirsch et al., 1989; Keele et al., 2006; Sharp and Hahn, 2011). Over the past century, more than 10 different strains of either HIV-1 or HIV-2 have jumped into humans through independent zoonotic transmission events. The most prevalent of these (HIV-1, group M) was transmitted into humans approximately 100 years ago and is responsible for well over 90% of global HIV infections (Worobey et al., 2008). HIV-2 also causes AIDS but is associated with lower viral loads and reduced person-to-person transmission rates, and has not spread substantially from West Africa (Reeves and Doms, 2002; Sharp and Hahn, 2011). Overall, approximately 35 million people are currently

infected with HIV and a similar number have already died of AIDS (UNAIDS, 2013).

HTLV is likely to also have originated in non-human primates, which carry related simian T cell lymphotropic viruses (Proietti et al., 2005). However HTLVs have potentially been present in humans for thousands of generations (Weiss, 1996). HTLV-1 infects around 10–20 million people worldwide and is endemic in regions of Japan, Caribbean islands, South America, and equatorial Africa. The majority of HTLV-1-infected individuals remain asymptomatic, but approximately 5% of carriers go on to develop adult T cell leukemia (ATL) or HTLV-associated myelopathy/tropical spastic paraparesis (HAM/TSP) (Proietti et al., 2005). More rarely, other conditions including infective dermatitis and HTLV-associated uveitis are also caused by HTLV-1 (Proietti et al., 2005). HTLV-2 has not been causatively linked to any disease.

These retroviral infections of humans, particularly the devastating HIV epidemic, provided a strong impetus for the development of animal models that would allow their study in relevant *in vivo* systems.

#### *Animal models of retroviral infection*

Many aspects of retroviral infection cannot be adequately investigated in infected humans. This is in part because detailed studies of *in vivo* retroviral replication and pathogenesis necessitate that infections be performed under controlled conditions with pre-defined viral variants. Invasive sampling of tissues at specific post-infection timepoints is also often required. Furthermore, the risks associated with experimental treatments are often too great to perform early efficacy testing in infected patients, and instead can only be reasonably performed in relevant animal models.

Early virus challenge experiments showed that HIV cannot infect unmodified small animals (Morrow et al., 1987). Consequently, most *in vivo* modeling of HIV has focused on alternative approaches. HIV-1 can infect chimpanzees but ethical concerns and their endangered status, coupled with other issues including infrequent and slow development of disease (Keele et al., 2009) now effectively preclude the use of chimpanzees in laboratory HIV research. Certain SIVs can infect and cause AIDS-like disease in non-human primates such as rhesus (*Macaca mulatta*), cynomolgus (*Macaca fascicularis*), or pigtailed (*Macaca nemestrina*) macaques. These models have therefore been used to investigate replication and pathogenesis of a virus that is closely related to HIV, with many of the findings subsequently translated to HIV (Gardner and Luciw, 2008; Hatziioannou and Evans, 2012; Letvin et al., 1985). A variety of modified chimeric SIV/HIV viruses termed simian-human immunodeficiency viruses (SHIVs) have also been created, which can replicate in nonhuman primates (Hatziioannou and Evans, 2012). This allows, for example, antiretroviral drugs or vaccine candidates that are directed towards HIV rather than SIV proteins to be evaluated in simian models. Non-human primate models have limitations in that they are expensive and individual studies are usually restricted to relatively small numbers of animals. The required use of an SIV or SHIV strain of virus also means that some aspects of the interaction between wild-type HIV and human cells or tissues cannot be adequately studied in these models.

Mice have been used to model human disease for many years. Studies with mouse models benefit from their small size, relatively short generation time, and the fact they share many features of physiology and immune function with humans. However mice cannot be infected by HIV, and no murine lentivirus that might serve as a natural model for HIV infection of mice has been found. Transgenic mice that have increased susceptibility to HIV infection through the engineered expression of human HIV entry receptors (CD4 and CCR5) have produced useful information, but are limited

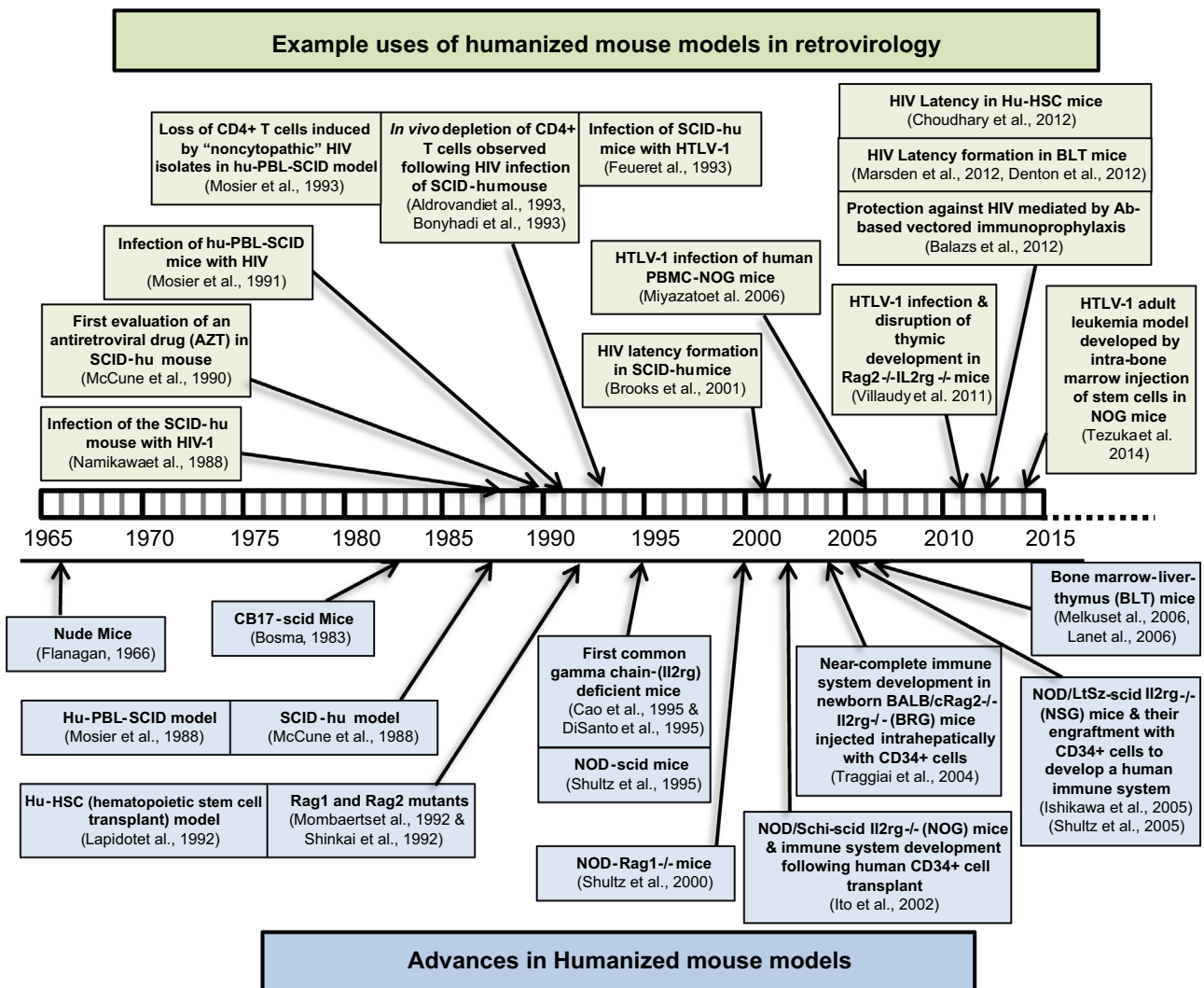


Fig. 1. Timeline describing some significant advances in humanized mouse models and examples of their application in retrovirology research.

by the fact that murine cells harbor multiple post-entry blocks that severely limit HIV replication (Bieniasz and Cullen, 2000; Browning et al., 1997). Transgenic expression of the HIV genome or individual HIV gene products in mice has also provided insights into aspects of HIV disease progression (Hanna et al., 1998; Leonard et al., 1988; Sun et al., 2008) but are similarly limited by restricted HIV expression and lack of HIV replication in mouse cells.

In summary, while these models have proved extremely useful in the study of HIV, small animal models that allow complementary information to be gained using wild-type HIV infecting human cells in an *in vivo* experimental setting are still important. While rabbit, non-human primate, and rat models of HTLV-1 infection also exist, they similarly have limitations that make complementary studies with humanized mice valuable (reviewed in Dodon et al. (2012)). For these reasons humanized mouse models have been extensively utilized to study many aspects of pathogenesis and treatment of retroviruses which infect humans.

#### A brief overview of immunodeficient mice

Mice with an intact immune system will rapidly clear transplanted human cells through a vigorous innate and adaptive immune response. It was thus only with the identification and selection of immunodeficient mouse strains that maintenance of transplanted human cells in the mouse became possible. The history of humanized

mouse development is thus closely linked to the sequential development of increasingly immunodeficient mouse strains that can serve as recipients for xenografts with human cells or tissues. Some significant developments in the history of humanized mouse models and a few of the many examples of their use in retrovirus research are provided (Fig. 1). It is worth noting that the nomenclature surrounding immunodeficient mouse strains and humanized mouse models is rather tangled. This is partly due to the fact that specific genes related to immune function have in some cases been disrupted in different ways, leading to different genotypes of mice showing similar phenotypes. Crossing of previously separate immunodeficient mouse strains along with further targeted disruption of immune-related genes has also increased the diversity of available immunodeficient strains. Finally, the common names for the humanized mouse models themselves are not necessarily applied universally, and might refer to either identical or broadly similar procedures performed in any one of several recipient mouse strains (Shultz et al., 2012, 2007).

Nude mice were first described in the 1960s (Flanagan, 1966). These mice carry a mutation in the gene encoding the forkhead box protein N1 (*Foxn1<sup>nu</sup>*) which leads to abnormal hair growth and an athymic phenotype due to defects in the development of thymic stroma (Nehls et al., 1994). Nude mice thus do not produce mature CD4<sup>+</sup> and CD8<sup>+</sup> T cells and have severely impaired B cell function due to a lack of T cell help. They proved useful for transplant of

human cells, particularly in the study of cancer (Fu et al., 1991). However, nude mice retain substantial immune function, making them inadequate to serve as the basis for a robust humanized mouse model that would allow effective engraftment with primary human cells or tissues. This became possible with the description of severe combined immunodeficient (*scid*) CB17 mice (Bosma et al., 1983). These mice have a mutation that developed spontaneously in the protein kinase, DNA activated, catalytic polypeptide gene (*Prkdc<sup>scid</sup>*), which encodes a DNA repair enzyme that is required for T cell and B cell receptor gene rearrangement. Consequently SCID mice have no functional T or B cells, and can be used in long-term transplant of primary human cells and tissues. Limitations of SCID mice included the fact that in older animals some production of B and T cells is observed (*i.e.* "leakiness"). The deficiencies in DNA repair in SCID mice also leads to them being more sensitive to radiation and some chemotherapy drugs than other mouse strains. Furthermore, CB17-*scid* mice retain innate immune responses including NK cell function. This problem could be partially ameliorated by using a non-obese diabetic (NOD) background strain, which resulted in NOD-*scid* mice with lower levels of innate immunity including reduced complement activity and NK cell function (Shultz et al., 1995).

T-cell and B-cell receptor rearrangement also requires the action of recombination-activating gene 1 (*Rag 1*) and *Rag 2* gene products. Targeted disruption of these genes individually has also led to the production of mouse strains that produce no T or B cells and are more radiation resistant than *scid* strains (Mombaerts et al., 1992; Shinkai et al., 1992). Similarly to NOD-*scid* mice, these *Rag* knockout mice strains lack adaptive immune responses but still harbor significant innate immune responses that hinder long-term, systemic reconstitution with human cells.

It was only when the targeted disruption of the coding sequence for the interleukin (IL)-2 receptor common gamma chain (*Il2rg*, also referred to as  $\gamma c$ ) was achieved by several different laboratories that substantial systemic reconstitution of immunodeficient mice with human cells became possible (Cao et al., 1995; DiSanto et al., 1995; Jacobs et al., 1999; Ohbo et al., 1996). The common gamma chain is shared by the receptors for IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21, and its disruption profoundly affects innate and adaptive immune function, and importantly results in a complete lack of NK cells, primarily due to the absence of IL-15 signaling (Kennedy et al., 2000; Ranson et al., 2003).

The most common mouse strains currently in use as platforms for humanized mouse experiments are the NOD.Cg-*Prkdc<sup>scid</sup>Il2rg<sup>tm1Wjl</sup>* (NSG), the NODShi.Cg-*Prkdc<sup>scid</sup>Il2rg<sup>tm1Sug</sup>* (NOG) and various strains similar to C;129S4-*Rag2<sup>tm1Flv</sup>Il2rg<sup>tm1Flv</sup>* (BRG) as described in more detail elsewhere (Ito et al., 2012; Shultz et al., 2012). NSG mice do not express *Il2rg* and thus their cells cannot bind the relevant cytokines, while in NOG mice the common gamma chain receptor has a deleted cytoplasmic domain, which can bind cytokines but does not signal. BRG mice have strain-dependent disruptions in *Il2rg*, which are either non-expressed or incapable of intracellular signaling (Shultz et al., 2012).

Researchers are continuously striving to generate new mouse strains with an improved capacity to reconstitute with human cells. For example, one limitation of some humanized mouse models is the development of graft-versus-host-disease (GVHD), where the implanted cells mount an immune response against those of the host. This can be reduced through disruption of important murine immune-related genes including  $\beta 2$  microglobulin, resulting in a lack of cell surface major histocompatibility complex (MHC) class I expression

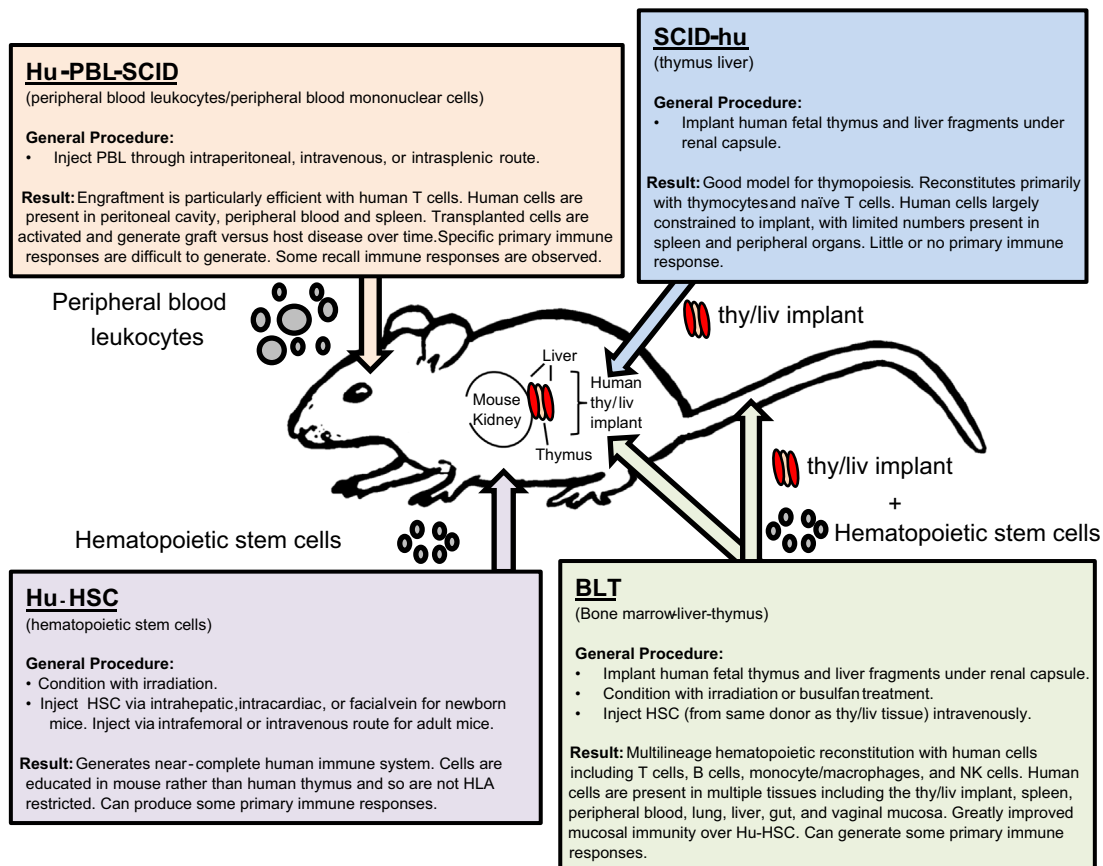


Fig. 2. Overview of the major humanized mouse models used in retrovirus research.

(Christianson et al., 1997), or MHC class II (King et al., 2009). Other advances in immunodeficient mice have included the transgenic expression of human HLA molecules (Danner et al., 2011) or expression of human cytokines including macrophage colony-stimulating factor, granulocyte-macrophage colony-stimulating factor, and IL-3 (Rathinam et al., 2011; Willinger et al., 2011), resulting in improved reconstitution or enhanced functionality of particular hematopoietic lineages of human cells in the mouse. Efforts to reduce rejection of implanted human cells have included the generation of CD47-knockout mice. The interaction between signal regulatory protein (SIRP)- $\alpha$  and CD47 is required to suppress phagocytosis of cells by macrophages. Murine macrophages do not recognize human CD47, and thus can phagocytose transplanted human HSCs. However, it has been shown that in CD47-knockout BRG mice this requirement is negated, allowing the development of high levels of human multilineage hematopoiesis and reduced GVHD following transplant of human fetal thymus and HSCs (Lavender et al., 2013). Additional techniques for reducing the rejection of transplanted human cells in disease models involving transplant of fetal thymus and allogenic HSCs have also been developed. These include the depletion of endogenous thymocytes present within the transplanted fetal tissue using an anti-human CD2 monoclonal antibody and cryopreserving then thawing the tissue prior to transplantation (Kalscheuer et al., 2012).

#### Common humanized mouse models used in retroviral research

In parallel with the development of immunodeficient mouse models, methods for engrafting these animals with primary human cells also improved. Four basic models for humanization of immunodeficient mice have been developed (Fig. 2), although these have each been further adapted in various ways to optimize them for particular applications. Early pioneering studies in mouse humanization involved the injection of human peripheral blood mononuclear cells into the intraperitoneal cavity of CB17-*scid* mice (Mosier et al., 1988). In the original description of this hu-PBL-SCID model, the cells persisted for over six months and could produce some specific immune responses *in vivo* (Mosier et al., 1988). Importantly, the hu-PBL-SCID model could also be infected with HIV, with virus recoverable from the spleen, peripheral blood, peritoneal cavity, and lymph nodes for up to 16 weeks after infection (Mosier et al., 1991). The hu-PBL-SCID model benefits from its relative simplicity, strong engraftment with T cells, and the fact that it can be used for infection soon after the human cells are injected (Rizza et al., 1996). However, this model is somewhat limited by the development of GVHD, which generally is evident within three to four weeks after transplant of human cells. Levels of reconstitution in the CB17-*scid* mouse were also relatively poor. This was improved 5–10-fold by the use of NOD-*scid* mice (Hesselton et al., 1995), and further enhanced with the use of NSG recipient mice, which also allowed substantial engraftment following intravenous injection rather than only through an intraperitoneal route (King et al., 2008; Kumar et al., 2008).

A further breakthrough approach in modeling human hematopoiesis in immunodeficient mice was the development of the SCID-hu model, also referred to as the “SCID-hu (Thy/Liv)” model (McCune et al., 1988). This model was created by implanting fragments of human fetal liver as a source of hematopoietic stem cells (HSCs) along with human fetal thymus tissue under the kidney capsule of SCID mice, resulting in the formation of a new conjoint organ that is structurally and functionally similar to a human thymus. In this implant, human T cells undergo normal thymopoiesis through a CD4+CD8+ (double-positive) phase before differentiating into single-positive naïve CD4+ or CD8+ T cells. Human cells can be maintained in this model for over 1 year. The SCID-hu model proved invaluable for studying T cell development (Namikawa et al., 1990), and could readily be infected with HIV by

direct injection of virus into the implant, thereby providing a robust *in vivo* model for studying HIV replication (Namikawa et al., 1988). Early SCID-hu studies involved co-implantation of human lymph node tissues, which allowed intravenous infection and could be used to model virus interaction with human secondary lymphoid organs (Kaneshima et al., 1991). However the majority of reported SCID-hu studies involve only implantation of the thymus and liver tissue. Implants can also be placed under the capsules of both kidneys, resulting in greater numbers of human T cells in the peripheral blood, spleen, and lymph nodes (Kollmann et al., 1994). However, even with this modification, SCID-hu mice have poor peripheral lymphoid compartment reconstitution with human cells and produce little or no primary cellular or humoral immune responses. Additional limitations of the SCID-hu model include the need for surgery during implantation and the requirement for human fetal tissues.

A third model for humanizing mice has variously been termed human-hematopoietic stem cell (hu-HSC), human-SCID reconstituting cell-SCID (hu-SRC-SCID), or sometimes human immune system (HIS) mice (Lapidot et al., 1992). In this model multilineage human hematopoiesis can be generated in various strains of immunodeficient mice by subjecting them to sublethal irradiation to clear space in the bone marrow then injecting human hematopoietic stem cells (HSCs). Experiments were originally conducted using CB17-*scid* mice but improved reconstitution can be achieved with NSG or NOG mice (Lepus et al., 2009; Manz, 2007). A wide range of different parameters have been evaluated for this model, all of which can affect the levels and quality of human cell engraftment. These include the individual mouse strain used, the age of the animal, the specific radiation source or chemotherapy agent used for ablation, the source of human HSCs, and the route of HSC administration to the mice (Brehm et al., 2010; Hayakawa et al., 2009; Ito et al., 2012; Lepus et al., 2009). HSCs for this model are typically sourced from fetal liver or cord blood, but can also be obtained from bone marrow, or human peripheral blood from individuals who have received granulocyte colony-stimulating factor to mobilize CD34+ cells (Ito et al., 2012). Reconstitution is particularly effective in newborn mice, which are already undergoing vigorous hemato-lymphoid expansion (Brehm et al., 2010). Primary immune responses are generated in this model, but are limited in some respects. For example antibody responses are often weak and largely restricted to IgM due to inefficient class switching (Akkina, 2013; Ishikawa et al., 2005; Manz, 2007; Traggiai et al., 2004; Watanabe et al., 2007) presumably due to a lack of helper T cell function. It has also been noted that an autoantibody-like response can be generated in hu-HSC mice, suggesting that in some cases antibody responses can be skewed in this mouse model (Chang et al., 2012).

Finally, a combination of the hu-HSC and SCID-hu models termed the bone marrow-liver-thymus (BLT) model has been developed. This model involves the implantation of adult immunodeficient mice with fetal thymus and liver tissue followed by sublethal irradiation then injection of HSCs (Lan et al., 2006; Melkus et al., 2006). This provides the opportunity for T cells to be educated on human thymic stroma and results in reconstitution with all lineages of immune cells including excellent T cell reconstitution. Human immune cells are present in multiple organs including the peripheral blood, thy/liv implant, lymph nodes, spleen, gut, lungs, bone marrow, brain and liver (Merkus et al., 2006; Shimizu et al., 2010). This model has also been modified in various ways, including the use of NSG recipient mice rather than the originally described NOD/SCID strain. Other alterations have included the use of busulfan rather than irradiation to induce myelosuppression, surgical implantation under the renal capsule of both kidneys to increase T cell numbers, and the disaggregation of liver tissue followed by reassembly using a matrigel scaffold prior to implantation (Shimizu et al., 2010). This last modification allows HSCs

to be extracted from the liver tissue, transduced with a gene of interest, and then transplanted along with thymus tissue, resulting in a higher frequency of transgene-expressing cells. The BLT model has proven useful in studying some primary immune responses to HIV, and produces particularly efficient mucosal reconstitution with human cells compared with other available models. Limitations of this model include the requirement for surgery, the need for fetal tissue, and an approximately 12 week waiting period between transplantation and full reconstitution.

### HIV studies in humanized mouse models

Soon after the development of each major humanized mouse model they were also tested and found to be susceptible to infection with HIV. These models have therefore been invaluable in both improving our understanding of HIV biology and as systems for evaluation of novel anti-HIV therapeutics. The examples outlined below are by no means exhaustive, but are instead intended to illustrate some important uses of both the older hu-PBL-SCID and SCID-hu models and the more recently developed and versatile hu-HSC and BLT mice. Historically, studies of HIV in humanized mice have primarily focused on HIV-1 because this virus is responsible for the vast majority of global HIV infections (Sharp and Hahn, 2011).

#### *Pathogenesis and depletion of HIV target cells*

When the hu-PBL-SCID and SCID-hu models were developed in the late 1980s, the HIV coreceptors (primarily CXCR4 and CCR5) had not yet been discovered and the relationship between a virus clone's ability to replicate effectively or induce syncytia formation *in vitro* and its capacity to replicate and cause disease *in vivo* was not clear. Humanized mice provided an *in vivo* environment to address these issues.

Indeed, soon after the hu-PBL-SCID model was shown to be capable of supporting HIV replication (Mosier et al., 1991) it was demonstrated that so called “noncytopathic” (R5-tropic) HIV could induce extensive CD4+ T cell depletion in this model, illustrating that *in vitro* cytopathicity does not predict the ability of a viral strain to deplete CD4+ cells *in vivo* (Mosier et al., 1993).

HIV infection of the SCID-hu mouse also results in depletion of HIV target cells, with the initial loss of CD4+CD8+ (double positive) thymocytes followed by selective loss of CD4+ (single-positive) cells, resulting in an inversion of normal CD4/CD8 ratios (Aldrovandi et al., 1993; Bonyhadi et al., 1993). The length of time before severe depletion of HIV target cells is evident varies according to viral strain, but typically occurs at 3–9 weeks post-infection. This hypocellularity is accompanied by further pathological changes in the implant which are visible histologically, including thymic involution and loss of cortical–medullary junctions (Jamieson et al., 1996). The presence of additional lineages of cells in the SCID-hu mouse also allowed the evaluation of indirect effects of HIV on cells that are not generally considered hosts for the virus. For example, HIV infection is well known to affect multiple different hematopoietic lineages in addition to CD4+ T cells, resulting in anemia, thrombocytopenia, granulocytopenia, and myelodysplastic alterations of the bone marrow (Calenda and Chermann, 1992). One potential contributing factor to this was identified following HIV infection of SCID-hu mice, where virus infection was found to disrupt *ex vivo* myeloid and erythroid colony-forming activity of progenitor cells through indirect mechanisms (without HIV infection of CD34+ cells), potentially through perturbation of cytokine levels (Jenkins et al., 1998; Koka et al., 2003, 1998, 1999). More recently studies using BLT mice also demonstrated direct HIV infection of human intermediate hematopoietic progenitor cells

(Nixon et al., 2013), thus a direct killing mechanism may also contribute to the hematopoietic abnormalities seen in untreated HIV disease.

In some respects HIV infection of the original hu-PBL-SCID and SCID-hu models emulate the early stages of HIV infection in humans, with exponential virus replication accompanied by profound CD4 depletion within weeks of infection in the absence of a specific antiviral immune response. In contrast, some of the newer hu-HSC and BLT models provide an environment that is more typical of later stages of HIV infection in humans, with specific primary cellular and (albeit limited) humoral immune responses present and slower CD4 declines. For example, studies of HIV infection of a hu-HSC model based on BRG mice showed that these mice can be infected with both X4 and R5 HIV variants, and that plasma viremia can be sustained for over 4 months and in some cases longer than 1 year (Berges et al., 2006, 2008; Zhang et al., 2007). Hu-HSC based on NOG mice was similarly found to be susceptible to X4 and R5 viruses and produced an antibody response to HIV Env and Gag epitopes, with no significant CD4 depletion evident following infection with the R5 virus for over 40 days (Watanabe et al., 2007). This difference between models is further supported by the fact that little selective viral evolution is observed during HIV infection of SCID-hu mice (Jamieson et al., 1995), whereas BLT mouse infection is associated with the development of HIV-specific CD8+ T cell immune responses and rapid viral evolution, predominantly in CD8+ T cell epitopes (Dudek et al., 2012). Rates of viral evolution of the HIV *env* gene in hu-HSC mouse infections lasting up to 44 weeks were also found to be similar to those in infected humans over similar periods of time (Ince et al., 2010), indicating that these models can be used to study HIV evolution and adaptation in a complex host environment.

HIV can also infect cells in the brain (primarily parenchymal microglia and perivascular microglia/macrophages) and cause both encephalitis and HIV associated dementia (Anthony and Bell, 2008). Humanized mice have the potential to emulate various aspects of HIV neuropathogenesis, but many questions remain (Honeycutt et al., 2014). In particular, the application of newer models such as the BLT mouse could improve our understanding of the mechanisms underlying virus-induced central nervous system damage and whether the brain contains a reservoir of HIV that can persist despite suppression of HIV replication by antiretroviral drugs (Honeycutt et al., 2014).

Humanized mouse models are still utilized to answer fundamental questions about the ability of HIV to replicate and deplete target cells in a relevant *in vivo* system. For example, BLT mice have recently been employed to evaluate the *in vivo* replication capacity of viruses isolated from untreated HIV+ patients who have undetectable viral loads (elite controllers/suppressors), demonstrating that these viruses are replication-competent and capable of inducing CD4+ T cell depletion *in vivo*. This study shows that the viral strains in these individuals are not obviously attenuated and that elite suppressors can control infection with apparently fully pathogenic viruses (Salgado et al., 2014).

#### *Function of different HIV gene products and other viral genetic elements*

HIV has been intensively studied for over three decades and we arguably know more about this virus than any other human pathogen. However, the vast body of accumulated research, including extensive biochemical and *in vitro* analysis of HIV proteins and non-coding sequences is often difficult to connect directly to HIV replication and pathogenesis *in vivo*. Humanized mouse models have proven useful as one measure for evaluating the biological relevance of *in vitro* findings in a more physiologically relevant environment.

An illustrative example of the use of humanized mouse models to better understand the determinants of HIV pathogenesis is the case of the HIV Nef protein. HIV negative effect factor (Nef) was originally found in some cases to reduce HIV expression in culture (Ahmad and Venkatesan, 1988; Luciw et al., 1987). However there was also evidence that Nef is required for efficient replication in primary cells and therefore the actual role of Nef in HIV replication and pathogenesis was unclear (Kirchhoff and Hunsmann, 1992). Using the SCID-hu model it was demonstrated that *nef* was required for *in vivo* replication and pathogenicity (CD4 depletion), even in cases where *nef*-defective mutants replicated similarly to wild-type virus levels *in vitro* (Jamieson et al., 1994). Similar studies were conducted using the hu-PBL-SCID model, which showed that *nef* deletion in several virus strains delays increases in plasma viremia and slows but does not prevent CD4+ T cell depletion, reinforcing the importance of Nef for *in vivo* HIV replication (Gulizia et al., 1997). More detailed analysis of Nef function *in vivo* was then performed using a panel of *nef*-deficient viruses, which identified key regions of the viral protein that are important for replication and CD4 depletion, including the myristolation site and the region encoding amino acids 41–49 of Nef. Conversely, two PXXP SH3 binding domains were not found to be important for *in vivo* replication, suggesting that some functions of Nef identified by *in vitro* testing may not influence *in vivo* pathogenesis (Aldrovandi et al., 1998). This was further characterized using additional mutant viruses in the SCID-hu mouse to show that it is the capacity of Nef to downregulate CD4 that correlates with Nef-mediated enhancement of pathogenicity, rather than other Nef functions such as MHC class I downregulation, (Pxx)4-associated enhancement of infectivity, or HCK binding (Stoddart et al., 2003). Later studies in the BLT mouse model similarly showed that *nef*-deleted virus results in an infection characterized by lower viral loads and reduced CD4 depletion. However the extensive tissue repopulation with human cells in BLT mice made it possible to demonstrate reduced depletion of CD4+ cells by the *nef*-deleted virus in a wider range of tissues including peripheral blood, lymph nodes, spleen, Thy/Liv implant, bone marrow, lung, and liver (Zou et al., 2012). In summary, the use of various humanized mouse models has clarified our understanding of the *in vivo* function of Nef, and helped bridge the gap in knowledge between suggestive and in some cases contradictory *in vitro* experimental studies and observations of HIV in infected patients, where natural infection with *nef*-defective viruses has been associated with slower disease progression (Deacon et al., 1995).

Studies utilizing additional HIV-1 accessory gene mutants tested in the SCID-hu mouse demonstrated that these viruses differed profoundly in their infectivity, replicative capacity, and ability to deplete CD4+ cells (Aldrovandi and Zack, 1996). In these assays *nef*-minus virus proved the most attenuated, with *vpu* and *vif* mutant viruses also showing significantly reduced infectivity and modestly reduced pathogenicity. In contrast *vpr*-deleted virus had little or no effect on any of these parameters (Aldrovandi and Zack, 1996). Analysis of mutant HIV viruses in humanized mice has not been restricted to only protein-coding sequences. HIV has an array of non-coding elements within its genome that are evolutionarily conserved but whose contribution to HIV replication and pathogenesis is difficult to resolve using only *in vitro* assays. For example, the modest *in vivo* reduction in HIV replication and pathogenesis observed in the SCID-hu mouse infected with a virus encoding a disrupted HIV “central DNA flap” helped resolve differing reports concerning the relative importance of this element based on *in vitro* observations of mutant virus replication (Marsden and Zack, 2007).

#### Efficacy of antiretroviral drugs

Evidence that humanized mice would become a powerful tool in the preclinical evaluation of antiretroviral drugs came when the

nucleoside analog reverse transcriptase inhibitor 3'-azido-3'-deoxythymidine (AZT) was found to be effective at suppressing virus replication in SCID-hu mice (McCune et al., 1990b). Follow-up studies with 2',3'-dideoxinosine (ddI) yielded similar results, and the doses used (after the application of allometric scaling to account for higher drug metabolism in the mouse) were similar to those found effective in patients (McCune et al., 1990a). Additional studies using different classes of antiretroviral drugs have further validated the use of the SCID-hu model in the comparative evaluation of antiretroviral drug efficacy (Stoddart et al., 2007, 2012).

In the early days after combination antiretroviral therapy (ART) became available it was not clear whether inhibition of virus replication would be sufficient to fully restore immune competency, or conversely whether virus-induced damage to primary and secondary lymphoid organs would prove irreversible even in further virus replication was inhibited by ART. The SCID-hu model was employed to address this issue, and evaluate how suppression of virus replication with antiretroviral therapy affects T cell generation. Treatment of infected SCID-hu mice with combination ART resulted in renewed thymopoiesis with a normal distribution of T-cell receptor variable gene families, demonstrating that HIV-induced damage to thymic tissue did not prevent generation of new T cells if virus replication was prevented by antiretroviral drugs (Kitchen et al., 2000; Withers-Ward et al., 1997).

The *in vivo* consequences that accompany the development of resistance to antiretroviral drugs are also difficult to predict from exclusively *in vitro* testing. Humanized mouse models have also proved useful in this regard, including the use of SCID-hu mice to demonstrate that protease inhibitor resistant viruses have impaired replication *in vivo* (Stoddart et al., 2001).

ART effectively inhibits HIV replication in hu-HSC and BLT mouse models (Choudhary et al., 2009; Denton et al., 2012; Halper-Stromberg et al., 2014; Marsden et al., 2012) and results in viral rebound if ART is stopped or drug resistant viruses evolve *in vivo* (Choudhary et al., 2012; Denton et al., 2012). Recent studies in BLT mice have further showed that the inclusion of an anti-HIV immunotoxin along with antiretroviral therapy diminished productively infected cells not eliminated by the antiretroviral drugs alone (Denton et al., 2014). Use of these newer models will therefore not only be useful in determining the *in vivo* efficacy of new antiretroviral drugs, but also in the investigation of antiretroviral drug resistance development and exploring the reservoirs of virus that persist during ART.

Humanized mice are also suitable for evaluation of naturally occurring agents with putative antiviral activity. For example the interferon-inducible cholesterol-25-hydroxylase converts cholesterol into the soluble antiviral factor 25-hydroxycholesterol (25HC), and it has recently been shown that 25HC can inhibit the growth of numerous enveloped viruses (Blanc et al., 2011; Liu et al., 2013). The *in vivo* functionality of 25HC against HIV was demonstrated with the administration of 25HC to hu-HSC humanized mice prior to HIV infection, which resulted in reduced viral loads, fewer HIV-infected cells, and protection of CD4+ cells from depletion (Liu et al., 2013).

#### Transmission and prophylaxis

The early virologic and immunologic events accompanying HIV transmission are difficult to study in infected people for many reasons. Individuals are generally unaware of their infected status until symptoms appear and much of the early virus replication occurs in tissues that are not easily accessible using clinical sampling. Clinical trials directed towards preventing transmission of virus are also expensive, logistically challenging, and require a large number of participants. Humanized mice have therefore been applied to the study of HIV transmission and evaluation of methods for preventing it.

Several early studies using the hu-PBL-SCID model showed that passive immunization with neutralizing monoclonal antibodies could protect against infection with HIV (Parren et al., 1995; Safrit et al., 1993), and protection could also be achieved in this model even when Ab was administered several hours after challenge with primary HIV isolates (Gauduin et al., 1997). More recently, inhibition of HIV transmission with new generations of broadly-neutralizing antibodies that recognize and neutralize diverse HIV strains have represented a particularly promising area of research that is very amenable to study using humanized mice. For example, a hu-HSC model was used to show that a mixture of 3 broadly-neutralizing antibodies can prevent HIV transmission (as assessed by the presence of detectable plasma viremia) in some cases when added beginning 4 days post-infection (Halper-Stromberg et al., 2014).

Studies of mucosal HIV transmission and the use of topical microbicides to inhibit transmission *via* this route have also benefited greatly from humanized mouse studies. Because hu-PBL-SCID and SCID-hu models do not produce significant human immune cells in the vaginal mucosa, early studies were performed by infecting PBL with HIV and then performing vaginal administration, resulting in the infection of animals challenged in this way (Di Fabio et al., 2001). The advent of Hu-HSC models allowed the study of mucosal transmission of HIV through vaginal and rectal routes in a more physiologic setting (Berges et al., 2008), and with the extensive mucosal reconstitution with human immune cells achieved in the BLT mouse model the systematic testing of HIV transmission and intervention studies became possible (Denton et al., 2008; Stoddart et al., 2011). These studies of mucosal transmission in BLT mice have yielded a variety of clinically relevant observations regarding the biology of HIV transmission across intact mucosal surfaces and the efficacy of different intervention strategies. For example, the BLT mouse has been used to demonstrate the efficacy of antiretroviral pre-exposure prophylaxis in preventing vaginal (Denton et al., 2008) and rectal (Denton et al., 2010) HIV transmission. Inhibition of vaginal HIV transmission by the topical administration of an antiretroviral drug (Denton et al., 2011), and the ability of human breast milk to reduce oral HIV transmission have also been demonstrated in this model (Wahl et al., 2012). In summary, these humanized mouse models have proved to be useful in the evaluation of mucosal HIV infection, and provide relevant systems for evaluation and optimization of future therapeutics intended to prevent the transmission of HIV.

#### Gene therapy/engineered immunity

Gene therapy approaches represent attractive opportunities to prevent or treat HIV infection (reviewed in Marsden and Zack (2013)) and humanized mice have been invaluable in the assessment of these efforts. The new generations of humanized mice are particularly conducive to stem cell gene therapies, since the protocols for manufacture of these mice already require the enrichment of HSCs. If the HSC are genetically modified before transplant, then progeny cells that differentiate in the mouse will also harbor the modified genome. Indeed, humanized mice were used to first demonstrate lentiviral vector-mediated gene delivery into human long-term repopulating HSCs (Miyoshi et al., 1999). This strategy has been used to evaluate various stem cell gene therapy approaches for inhibiting HIV.

Approximately 1% of Caucasians are homozygous for a 32 base pair deletion in the gene for CCR5, a chemokine receptor that also functions as the HIV coreceptor (CCR5- $\Delta$ 32). This results in a truncated CCR5 protein that is not expressed on the cell surface. These individuals are highly resistant to HIV infection but otherwise healthy. Furthermore, the bone marrow transplant from a CCR5- $\Delta$ 32 homozygous donor to an HIV positive individual undergoing aggressive treatment for acute myeloid leukemia (the “Berlin Patient”) represents the only reported case of an apparent HIV cure (Hutter et al., 2009)

(reviewed in Marsden and Zack (2013)). This has provided a strong impetus for the use of corrective gene therapy to emulate a CCR5-deficient protective phenotype in autologous cells from HIV+ patients. Promising research in this area has included the development of an RNAi approach to knock-down CCR5 RNA *via* lentiviral vector delivery (Qin et al., 2003). shRNA knockdown was then shown to be effective at inhibiting R5 HIV infection *ex vivo* after differentiation of the resistant cells in the BLT mouse (Shimizu et al., 2010). Direct disruption of the CCR5 gene in HSC using zinc-finger nucleases has also been achieved, resulting in control of R5 HIV infection in hu-HSC mice (Holt et al., 2010). CD4+ T cells can also be disrupted for CCR5 or CXCR4 (an alternative HIV coreceptor) using zinc-finger nucleases, and then transplanted into immunodeficient mice to validate function (Perez et al., 2008; Wilen et al., 2011; Yuan et al., 2012).

It is likely that similarly to what is seen with antiretroviral drugs, for genetic therapies against HIV to be truly efficacious they must also involve a combination approach targeting multiple viral or cellular targets. This would allow enhanced virus suppression while making the evolution of viral variants that are resistant to the gene therapy less likely. Therefore more complex approaches involving multiple therapeutic genes are also being explored. For example a Hu-PBL model was used to show that a lentiviral vector encoding a multiplexed construct expressing seven anti-HIV shRNAs (one targeting CCR5 and six targeting the HIV genome) could suppress *in vivo* HIV replication and protect from CD4+ T cell depletion when HIV infected cells were transduced with a lentiviral vector before transfer into NSG mice (Choi et al., 2014). Similarly a combination shRNA approach targeting CCR5 (as described above) as well as the R region of the HIV long terminal repeat, protected cells from HIV-induced depletion by R5- and X4-tropic HIV in BLT mice (Ringpis et al., 2012).

Additional gene therapy approaches that involve modulating anti-viral immune responses have also been evaluated in humanized mice. These include the generation of HIV-specific cytotoxic T lymphocytes *via* transgenic expression of a T cell receptor delivered to hematopoietic progenitor cells (Kitchen et al., 2012), and the use of broadly-neutralizing antibodies encoded by adeno-associated virus vectors, which are expressed following intramuscular injection and result in secretion of antibody that protects against intravenous or vaginal challenge with HIV (Balazs et al., 2012, 2014).

In summary, a wide array of novel gene therapy approaches have been tested in humanized mice. Importantly, while the studies above are directed towards HIV, the broad approaches are also applicable to other infectious agents (Balazs et al., 2013; Deal et al., 2014). Therefore the importance of these humanized mouse studies goes beyond HIV research and therapeutics.

#### Additional therapeutics

Humanized mouse models for HIV have also been crucial in the evaluation of new delivery systems or therapeutic approaches targeting HIV. Both Hu-PBL mice and Hu-HSC mice were used to show that a CD7-specific single chain antibody conjugated to oligo-9-arginine peptide (scFvCD7-9R) could be used to deliver siRNA specifically to T cells *in vivo*. Delivery of anti-CCR5 siRNA or other antiviral siRNAs in this manner resulted in suppression of HIV viremia in the mice (Kumar et al., 2008). A dendrimer-based nanoparticle-mediated delivery of combinatorial dicer substrate short-interfering RNAs (dsiRNAs) targeting HIV or cellular transcripts has also been tested in humanized mice. These novel particles suppressed HIV viral loads and prevented CD4+ T cell depletion after intravenous administration to Hu-HSC mice (Zhou et al., 2011). Similar inhibition of HIV replication was reported using an RNA aptamer designed to bind to HIV envelope gp120 protein and neutralize virus, either with or without a conjugated



siRNA targeting the *tat/rev* common exon of HIV (Neff et al., 2011). Humanized mouse models have also been used to evaluate novel preparations of antiviral agents, including long acting nanoformulated ART (Dash et al., 2012). These diverse studies show the versatility of humanized mouse models in evaluating a wide range of novel therapeutics targeting HIV.

#### Viral persistence and latency

Combination ART is capable of inhibiting HIV replication and often results in undetectable plasma viral loads in treated individuals. However, replication-competent HIV is still present in rare latently-infected cells, which harbor integrated, non-expressing viral genomes that can be induced to produce infectious virus if stimulated (Chun et al., 1997b) (reviewed in Marsden and Zack (2010, 2013)). Based on *ex vivo* viral outgrowth assays, approximately 1 million of these latently-infected cells are present in each infected patient at a frequency of around 1 per million resting CD4+ T cells (Chun et al., 1997a), although this is likely a minimal estimate (Ho et al., 2013). These cells decay very slowly *in vivo* with a half-life of approximately 44 months, and are capable of persisting for decades (Finzi et al., 1999). Therefore if the patient stops taking ART then the presence of this reservoir allows virus to rapidly re-emerge (Chun et al., 1999). Consequently, while other reservoirs of persistent HIV might also exist, latently-infected cells are considered to be one of the main barriers to an HIV cure.

Humanized mice have been particularly useful in the study of latent HIV. HIV can productively infect activated cells but not resting cells (Stevenson et al., 1990; Zack et al., 1990). It is therefore believed that one means by which HIV latency forms *in vivo* is when an activated cell becomes infected by HIV but transitions to a resting CD4+ T cell before it can be killed by the virus or immune response. This may happen during memory formation, when an activated CD4+ T cell becomes a resting memory CD4+ T cell. However a similar reduction in cellular activation also occurs during thymopoiesis when a CD4+CD8+ double-positive cell transitions to a resting naïve CD4+ single-positive T cell. Indeed, it was found that when CD4+CD8+ (double positive) cells are infected by HIV in the SCID-hu mouse and then transition to CD4+ (single positive) cells, this can be associated with establishment of latency (Brooks et al., 2001). The high frequency of infected cells in this model coupled with the rapid differentiation of T cells results in a relatively large percentage (in some cases > 5%) of the CD4+ T cells in HIV infected implants harboring latent virus. This model was subsequently used to evaluate various aspects of latent HIV infection. It was found that the non-tumor inducing phorbol ester prostratin (Korin et al., 2002) and interleukin 7 (Scripture-Adams et al., 2002) could each activate expression of latent HIV *ex vivo*. Furthermore these recently activated latently-infected cells could be depleted with the addition of an anti-HIV envelope immunotoxin, which can bind to and kill cells expressing HIV envelope proteins on their surface (Brooks et al., 2003b). Without stimulation, latently infected cells showed no obvious distinguishing features that might be used to differentiate them from their uninfected counterparts (Brooks and Zack, 2002). These latently-infected cells were also found to be most responsive to stimulants that function *via* protein kinase C or nuclear factor of activated T cells (NF-AT) (Brooks et al., 2003a), and HIV transcription was initiated very rapidly upon stimulation of the host cell, with significant increases in intracellular HIV mRNA evident within 2 h of stimulation (Arlen et al., 2006). Latently-infected cells from the SCID-hu model were also used to evaluate novel methods for inducing expression of latent HIV. For example, lipid nanoparticles loaded with the protein kinase C activating compound bryostatin 2 were used to activate HIV expression from latently-infected CD4+ SCID-hu thymocytes *ex vivo*,

validating a novel delivery method for HIV latency activating compounds (Kovochich et al., 2011).

HIV latency has also been shown to form following infection of BLT (Denton et al., 2012; Marsden et al., 2012) and hu-HSC (Choudhary et al., 2012) mice. In these studies the mice were infected and then treated with antiretroviral therapy to suppress plasma viral loads and emulate the situation in infected patients. A modification of the SCID-hu mouse model where thy/liv implants are inserted under the kidney capsule of NSG mice (as opposed to the standard NOD/SCID) was recently shown to generate substantial systemic reconstitution with human T cells, but not other immune lineages including monocyte/macrophages, B cells, or dendritic cells. This model, termed a T cell only mouse (TOM) also permits the formation of HIV latency (Honeycutt et al., 2013). These models are now being utilized to test methods for eliminating persistent HIV reservoirs. For example, broadly neutralizing anti-HIV envelope antibodies were used to suppress viral loads in HIV-infected hu-HSC mice and then followed with administration of a combination of three different HIV latency activating reagents (Halper-Stromberg et al., 2014). This resulted in a lack of viral rebound in over half of the treated mice once antibody levels waned, suggesting that the combination of neutralizing antibodies and viral inducers had reduced the levels of underlying latent or persistent virus that would otherwise contribute to viral rebound (Halper-Stromberg et al., 2014; Marsden and Zack, 2014). These models should continue to serve as useful frameworks for experiments designed to investigate HIV latency and persistence during therapy, and in the evaluation of new agents intended to eliminate the latent reservoir of HIV.

#### HTLV

HTLV has not been studied extensively in humanized mice, but some humanized mouse models for investigating HTLV-1 infection have been developed, which broadly parallel those used for the study of HIV. These models have provided complementary information to data obtained from other animal models of HTLV, which do not adequately model all aspects of viral pathogenesis, particularly the complex mechanisms underlying the process of leukemogenesis (Dodon et al., 2012). Early humanized mouse studies with HTLV focused on the use of SCID mice as recipients in a hu-PBL-SCID model, where PBL from asymptomatic HTLV-1 positive individuals or patients diagnosed with ATL or HAM/TSP were introduced to the mice (Feuer et al., 1993). This resulted in a persistent infection with HTLV characterized by a proliferative advantage for the HTLV-infected cells, and the development of lymphoblastic lymphomas in animals injected with PBL from two ATL patients (Feuer et al., 1993). Experiments involving either the injection of HTLV+ cell lines, or alternatively ATL patient cells obtained from either peripheral blood or lymph node coupled with daily injection of interleukin 2, yielded comparable results (Imada et al., 1995; Kondo et al., 1993). As was identified with other humanized mouse experiments based on CB17-*scid* mice, the presence of mouse NK cells in these animals was problematic and prevented engraftment of HTLV-1 *in vitro* transformed cell lines (Stewart et al., 1996). This could be partially improved by approaches including the pre-treatment of mice with anti-murine IL-2 receptor antibody to deplete NK cells, whole-body irradiation, or treatment of mice with anti-asialo GM1 antibody, which transiently inhibits *in vivo* NK cell activity (Feuer et al., 1995; Kondo et al., 1993). The use of NOD/SCID mice further improved engraftment efficiency, particularly when coupled with sublethal whole-body irradiation (Liu et al., 2002). This approach led to rapid tumor development within 2–3 weeks of engraftment in all of the tested animals. The neoplastic cells were widely distributed, invading and displacing organs including the spleen, liver, abdominal

wall musculature, kidney, pancreas, and intestine. All of these tumors contained HTLV-1 tat/rex sequences and human  $\beta$ -globin sequences, indicating that they were derived from the engrafted transformed cell line (Liu et al., 2002).

A hu-PBL-SCID model system based on NOG mice has also been successfully used to study HTLV-1 infection by co-inoculating mice with human lymphocytes and the HTLV-1 producing cell line MT-2, resulting in the presence of proviral DNA in both CD4+ and CD8+ T cells (Miyazato et al., 2006). This model was also suitable for testing inhibition of infection with antiretroviral drugs. The reverse transcriptase inhibitors AZT and tenofovir prevented primary infection when administered soon after infection, but did not affect proviral loads if administered 1 week post-infection suggesting that after the early stages of infection, clonal proliferation of infected cells was primarily responsible for increases in proviral DNA (Miyazato et al., 2006). More recently, a humanized mouse model based on bone marrow injection of human CD133+ stem cells into NOG mice has been used to study HTLV. Upon infection of this model, an increase in CD4+ T cells was observed in the periphery, which was eventually dominated by a limited number of CD25+ cells. Importantly, this model also produced HTLV-specific immune responses allowing the study of tumor formation in the face of an adaptive human immune response (Tezuka et al., 2014). This should pave the way for future studies of HTLV infection in humanized mice that focus on the complex interplay between virus proliferation and the host immune response.

## Summary

Humanized mice have provided numerous insights into retroviral replication and pathogenesis and represent versatile systems for testing novel therapeutics targeting retroviral infections. As these models are further optimized to more completely replicate the human immune system they will only become more useful in future retroviral studies, with broad applications in the evaluation of vaccine candidates, gene therapy approaches, and small molecule therapeutics intended to achieve the difficult task of either preventing initial infection or permanently eliminating these deadly viruses from infected individuals.

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