Keywords: HIV-1 infection; Antiviral therapy; Immune system; HIV-1 cure; Immune cells

Abbreviations: HPBMC: Human Peripheral Blood Mononuclear Cells; HSC: Human Stem Cells; NK: Natural Killer; GvHD: Graft-Versus-Host Disease; SCID: Severe Combined Immunodeficiency; NOD: Non-obese diabetic; ART: Antiretroviral Therapy; ARV: Antiretroviral

Discussion

Small animal models that successfully and closely mimic the human immune system are acutely needed in biomedical research. In this regard, the NOD.Cg-PrkdcsnullIL2rgnull/null mouse strain (NSG) is better suited for in vivo research purposes than other SCID/NOG strains [3] because; they harbor a scid mutation and an interleukin 2 (IL-2) receptor common gamma chain-targeted mutation (IL2rgtm1Wjl). As a result, these NSG mice lack mature T-, B- cells and functional natural killer (NK) cells. These mice are not only deficient in the above listed lineages, but also in cytokine signaling, and they permit the development of the above listed lineages of human origin, as well as in the development of human macrophages [1,2].

The IL2rgtm1 mutation overcame some previous limitations including minimal donor chimerism and poor expansion of lymphoid cells [4,5]. The model supports the development of a functional human immune system, allowing detailed analyses of human immune physiology and function [5]. Furthermore, the genotype does not lead to development of thymomas, which occur in 30% of classic NSG mice by the age of 6 months. Clearly this model is a good option for short term experiments of up to 4 weeks following HIV-1 infection [5,6]. The model was used to engraft human PBMCs into adult mice to study the effects of HIV-1 entry blockade by INK128, a prototype TOR-KI (the catalytic inhibitor; in dual targeting of mTORC-1/2) [7]. It was also reported that in comparison with all immunodeficient strains, the NSG mouse model has highest human HSC engraftment levels [8]. All together, it makes this xenotransplantion model as the “gold standard” for in vivo studies.

Our previous studies demonstrated that after intraperitoneal (i.p.) injection with PBMCs, NSG adult mice were able to support robust HIV-1 replication, developing high serum p24 levels and progressively lower CD4+T cell levels, as measure by flow cytometry. Specifically, we showed that when the mice were injected i.p. with 20,000 of 50% tissue culture infective dose (TCID50) of HIV-1 Bal, they developed a productive infection [6]. Quantifying CD4+ T cells, CCR5 + cells, and CCR5 expression levels to evaluate and validate the NSG adult mouse model, we showed that levels of both CD4+ and CCR5+ cells from within the CD4+ T cell population remained stable in non-infected mice up to 4 weeks post engraftment [6]. As expected, infected mice showed a steep decline in CD4+ T cells and CCR5+ cells, as well as somewhat reduced CCR5 expression levels [6].

A second approach, based upon the injection of HSC into newborn mice supports longer-term experiments and allows multilineage development of human immune cells. Mice transplanted with fetal liver and thymus tissues are useful to replicate HIV-1 induced pathology and to test hematopoietic stem cell-based gene therapy. That and the utilization of the human adaptive immune system in cord blood cell-transplanted mice led to the development of a mouse model in which CD34+ cells were transplanted into the liver of newborn mice. This results in the development of a lymphoid-like system of human origin with T- and B- cells, monocytes, plasmacytoid and conventional dendritic
cells- DCs, lymph and thymus nodes. In this case, more than 40% of T cells are of a naive phenotype [9]. Lymphoid tissue contains key latent reservoirs established by HIV-1 during acute infection, and the availability of this model opens new options in HIV-1 latency research. The ability to reconstitute a relatively representative human immune system in newborn NSG mice engrafted with HSC will surely help facilitate a better understanding of processes of greater duration, such as HIV-1 latency. An additional advantage of the neonatal model compared with the shorter term adult model for long term studies is due to a longer life span (for at least 12 months) [4,10].

Choudhary et al. [11] recently showed that replication-competent HIV-1 persists in CD4+ T cells in mice treated with antiretrovirals (ARVs) creating an attractive system for studying viral reservoirs. ARVs suppress HIV-1 replication to undetectable levels within weeks after therapy starts, but none of the currently available treatments result in full eradication of virus from long-lived reservoirs in resting memory CD4+ T cells [12]. Upon termination of ARVs, HIV-1 infection reappears within weeks to months. Clearly, an available hu-mouse model for exploring the potency of novel compounds for targeting HIV-1 latency is a must.

There are a few crucial considerations for using hu-HSC mice to study persistent HIV-1 latency. First, infected mice must recapitulate the essential aspects of HIV-1 pathogenesis, which includes immune dysfunction, and subsequent recovery of the host immune system as a result of ARVs. Next, any interruption of ongoing ART must result in a rebound of virus replication from the original latent reservoirs. Lastly, for an effective hu-mouse model, long term ARVs should be non-toxic and well accepted by the mice [12]. There are numerous studies reporting relatively beneficial effects of ART in HIV-1 infected hu-mice with different anti-viral reagents or combination of ARV drugs that act synergistically [13-16]. There are, for example, reports of broadly neutralizing Abs combined with viral transcription inducers (thus acting by independent mechanisms) that synergistically reduced reservoirs, as measured by viral rebound [17].

Together, these studies offer a promising preclinical proof of principle for these specific anti-viral approaches and indicate that these model systems have the potential to fuel current efforts and to generate future milestones in HIV-1 treatment. Hu-HSC mice have also been used to show the feasibility of suppressing HIV-1 infection by targeting CCR5 in zinc-finger nuclease gene editing experiments [18]. Introduction of CD34+ cells treated with a CCR5-specific zinc finger nuclease into HIV-1 infected hu-HSC mice resulted in selection of CCR5-negative cells, reduction of HIV-1 viral loads (VLs), and preservation of CD4+ T cells. The same model was used to show that VLs were 30 fold lower in mice treated with human CD4+ T cells in which HIV-1 was targeted with siRNA small interfering RNA constructs, (attractive as antiviral therapy-related constructs as they can deliver anti-CR4, -CCR5, -vif- and -tat siRNAs). In general, delivering siRNAs to HIV-1 infected cells in vivo can be highly effective [10]. Although technically challenging, it becomes feasible in mouse models when the siRNAs are conjugated with an HIV-1 gp120-specific aptamer [19].

The most effective model currently for HIV-1 studies is the BLT (bone marrow-liver-thymus) mouse model, as these mice can recapitulate a primary immune response against HIV-1 infection, and can be used to better understand the ability of ART to prevent secondary HIV-1 transmission [20,21] with the strengths such as:

A. mice are highly susceptible to HIV-1 infection;
B. VLs can be readily controlled with intensive ART regimens;
C. VLs rebound upon ARV drugs cessation, with the additional advantage that PBMCs can be purified from mice and induced to express virus in vivo; and
D. robust mucosal human immune systems render these animals susceptible to rectal and vaginal HIV-1 transmission [4,21]. The susceptibility of BLT mice to mucosal HIV-1 transmission permits exploring HIV-1 preventive approaches that include use of topical and systemically applied HIV-1 inhibitors [22]. This model has yielded significant progress in testing CCR5-targeted siRNA (in combination with ARV), which provided a protective effect against HIV-1 replication [23]. Lastly, engineered mice have been developed to contain exclusively T lymphocytes or macrophages and these can be utilized to accomplish in vivo identification and mapping of HIV-1 reservoirs. However, the main disadvantage when compared with the NSG adults and neonatal models is the technical and surgical expertise that the engrafment of BLT model requires [4]. Based on our experience both NSG adult and neonatal models have certain limitations:

- a. not all animals have successful engrafment and remaining innate immunity impairs engrafment;
- b. adults and neonatal mice can develop lethal xenogeneic graft-versus-host disease (GvHD) syndrome;
- c. some animals in the NSG newborn model can develop more B-cells than T-cells rendering these mice unsuitable for HIV-1 infection studies;
- d. mice have impaired lymph node development and poorly developed germinal centers [10];
- e. many human cytokines are species specific [10];
- f. in the NSG neonatal model, a long time period is required following HSC engrafment to determine whether the mice have an adequate percentage of CD4+ T cells (3 months), making it difficult to rely on the number of available animals for all the control experiments; and
- g. costs for pre-made hu-mice are high.

A further caution is that after establishing an effective drug dose in hu-mice, the optimal animal dose cannot be extrapolated to a human equivalent dose by a simple conversion based on a body weight. Instead, parameters such as body surface normalization method must be considered when a translational dose is to be used in humans for Phase I and II clinical trials [24].

Conclusion
Simple ART approach does not provide a virologic HIV-1 cure. However, it was recently demonstrated in principle that the HIV-1 reservoir can be successfully altered by combination therapy with antibodies and viral transcription inducers, providing some new insights into the possibility of developing better options for a
cure. Hu-mice are an increasingly important tool in translational research, addressing both mechanistic questions and issues related to innate and adaptive immunity, autoimmunity, cancer biology, and pathology of increasing number of infectious diseases, by drug therapy and by vaccines. The advantages of using a poor Hu-mice model that tolerates combinations of ARVs with other synergistic anti-viral reagents together with a relatively low maintenance cost compared with non-human primate models are enormous. In addition, a possibility of exploring approaches or alternatives to "shock and kill", such as sooth and snooze in Hu-NSG mice, represents a more promising avenue in future efforts towards an HIV-1 cure.

Acknowledgement
The authors especially thank Drs. Marvin Reitz and Alonso Heredia for critical reading and valuable inputs for this manuscript.

References