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Humanized Mice for the Study of Infectious Diseases

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Abstract

Many of the pathogens that cause human infectious diseases do not infect rodents or other mammalian species. Small animal models that allow studies of the pathogenesis of these agents and evaluation of drug efficacy are critical for identifying ways to prevent and treat human infectious diseases. Immunodeficient mice engrafted with functional human cells and tissues, termed “humanized” mice, represent a critical pre-clinical bridge for *in vivo* studies of human pathogens. Recent advances in the development of humanized mice have allowed *in vivo* studies of multiple human infectious agents providing novel insights into their pathogenesis that is otherwise not possible.

INTRODUCTION

Over 600 million people worldwide are infected with *Plasmodium* species that cause malaria, human immunodeficiency virus type 1 (HIV-1), or hepatitis, accounting for more than 5 million deaths annually (World Health Organization). Mice have provided a platform for investigating many infectious agents leading to insights into the pathogenesis of disease, efficacy of drugs, and evaluation of potential vaccines [1-4]. However, the immune systems of rodents and humans differ greatly [5;6] and a number of infectious agents of most interest do not infect other species [7;8]. Moreover, the recognition of drug-resistant “superbugs”, the threat of bioterrorism, and emerging new infectious agents has accelerated the critical need for small animal models of human infectious diseases.

Since the discovery of the CB17-*Prkdc^{scid}* (CB17-*scid*) mouse in 1983 [9], investigators have strived to engraft human cells into immunodeficient mice to develop models for studies of human infectious agents. In 1988, it was reported that human hematopoietic and immune systems could be engrafted in CB17-*scid* mice [10;11]. These mice supported infection with HIV-1, providing the first animal models of this human-specific viral infection [12;13]. Since 1988, technological and genetic efforts have focused on enhancing human cell engraftment (reviewed in [14]), with a major breakthrough in the early 2000’s describing the

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development of *scid*, *Rag1^{null}*, and *Rag2^{null}* mice bearing targeted mutations in the gene encoding IL2 receptor common gamma chain (*Il2rg*, known as γ_c and CD132) [15-17]. These T and B cell deficient mice lack adaptive immunity, have severe impairments in innate immunity, and completely lack natural killer cells.

Available Strains and Immune Models of Humanized Mice

The most widely available immunodeficient strains for engraftment with human cells and tissues are NOD.Cg-*Prkdc^{scid}Il2rg^{tm1Wjl}* (NOD-*scid* *Il2r^{null}* or NSG) [16;18], NOD.Cg-*Prkdc^{scid}Il2rg^{tm1Sug}* (NOG) [15], and C.129(cg)-*Rag2^{tm1Fwa}Il2rg^{tm1Cgn}* (BALB/c-*Rag2^{null}Il2r^{null}* or BRG) mice [17].

These strains have been engrafted with human hematopoietic and immune cells and tissues to establish four different human immune models, the **Hu-PBL-SCID**, **Hu-SRC-SCID**, **SCID-Hu** and **BLT** models (Figure 1) [14;19;20]. As described in Figure 1, each model has advantages and disadvantages that must be considered to select the most appropriate mouse for a specific scientific investigation.

Humanized Mouse Models of Infectious Agents

HIV

Humanized mice have been used to study infectious agents such as HIV-1, that do not infect other species [21;22], with the exception of chimpanzees [23;24]. Although HIV-1 infection of chimpanzees can lead to viremia, the pathogenesis of HIV-1 infection in these non-human primates differs in many respects from that of humans [23;24]. Furthermore, use of chimpanzees for biomedical research is banned in Europe and the National Institutes of Health has terminated most research on chimpanzees in the United States and recommended that these non-human primates should be permanently retired to sanctuaries (http://dpcpsi.nih.gov/council/pdf/FNL_Report_WG_Chimpanzees.pdf). Thus, it is unlikely that HIV-1 (and other infectious disease) research in chimpanzees will be a feasible approach in the future. Therefore, investigators have turned to the only other available *in vivo* model for the study of HIV-1, humanized mice.

All four models of human immune system engraftment (Figure 1) have been used to study HIV-1, and these have been recently reviewed [7;25;26]. One major advantage of using NOD-*scid* and NSG mice is the robust immune systems that develop, including a mucosal immune system in the BLT model [19;20;26]. This permits investigation of the mucosal transmission route, effect of HIV-1 on mucosal immunity, and analyses of microbicides as pre-exposure prophylaxis therapy [27;28]. Recently, it was shown that NSG-BLT mice infected with HIV-1 generate human CD8 T cell responses that closely resemble cellular immune responses observed in infected humans. The virus undergoes a rapid, immune driven sequence evolution that leads to a reproducible escape from host immunity, recapitulating that observed in infected individuals [29]. BLT mice can also be infected with HIV-1 via the oral, rectal and vaginal routes, providing models for the study of these common routes of HIV-1 transmission [30-32]. HIV-1 infection of humanized mice leads to rapid depletion of peripheral and gastrointestinal CD4⁺ T cells [30] and an influx of human macrophages into the brain leading to neuropathogenesis [33;34] documenting the fidelity of the pathogenesis of disease with that of humans. A recent study done with NOD-*scid* BLT mice used intravital microscopy to demonstrate HIV-1 infected human CD4 T cells function as vehicles for dissemination of virus. The study showed that HIV-1 infected CD4 T cells within lymph nodes are highly motile, form multinucleated syncytia and establish long membrane tethers, which all may enhance cell to cell spread of HIV-1 [35].

Epstein Barr Virus (EBV)

In the original BRG Hu-SRC-SCID report, productive infection following EBV inoculation was demonstrated [17]. Using the NSG Hu-SRC-SCID model, infection with an EBV deficient in the nuclear oncogene EBNA3B, which *in vitro* is dispensable for B cell transformation, leads to aggressive diffuse large B cell human lymphomas *in vivo* [36]. This surprising finding led to identification of unique EBNA3B mutations in individuals with lymphomas, providing new insights into EBV-induction of B cell lymphomas [36]. A rare but devastating syndrome termed hemophagocytic lympho-histiocytosis has been modeled in EBV-infected humanized mice [37;38], and a model of erosive arthritis following EBV infection has been described [39]. Using HLA-A2 transgenic (Tg) mice engrafted with HLA-A2 HSC, EBV-restricted human CD8⁺ T cell responses have been detected as well as protective CD4⁺ and CD8⁺ T cell-mediated immunity [40-42]. Humanized mouse models are now allowing investigators to study pre-symptomatic stages of EBV infection, identify new mechanisms by which EBV infection can induce disease and EBV lymphomas, and provide model systems in which drugs can be tested for efficacy.

Dengue Virus (DENV)

Humans infected with DENV can develop a mild fever to acute febrile illness or progress to a severe capillary leakage syndrome (dengue hemorrhagic fever) following a second infection with a different DENV serotype [43]. However, little is known about the mechanisms by which this occurs [44]. Immunocompetent mice can be infected with DENV using intracranial high dose injections of virus or with mouse-adapted DENV. Mice deficient in both interferon α and β receptors can also be infected but these models have not provided optimal tools for studies of dengue pathogenesis (reviewed in [44;45]). To address this need, humanized mice have been infected with DENV [42;46-51]. Key advances have been the recapitulation of clinical signs of dengue fever [50], the ability to infect humanized mice through infected *Aedes aegypti* mosquito bites [51], and the generation of HLA-A2-restricted human T cell responses in NSG-HLA-A2 Tg mice engrafted with HSC from an HLA-A2 positive donor [47] or in NSG mice engrafted with HLA-A2⁺ fetal tissues [42]. In humanized mice infected by mosquito bites, the human innate immune responses and disease symptoms in infected mice were more pronounced than following simple injection of the virus due to the activation of human innate immunity by factors in the mosquito bite [51].

Hepatitis C (HCV) and B (HBV)

More than 2 billion people worldwide are infected with HCV or HBV resulting in over 600,000 deaths each year (<http://www.who.int/mediacentre/factsheets/fs204/en/>). HCV and HBV are hepatocyte-specific viruses and infect only humans (and chimpanzees) [52]. Human liver/chimeric mouse models have been engineered to develop small animal models of hepatitis. These models have in common mutations or transgenes that result in murine hepatocyte cell death making “room” for the engraftment of human hepatocytes (Table 1). These human liver/chimeric models have been used to study hepatitis B and C pathogenesis as well as the efficacy of anti-viral drugs [53-58]. In these models, levels of human hepatocyte engraftment and virus replication correlate with the severity of the immunodeficiency of the recipient, ultimately leading to development of *scid* or *Rag2^{null}* mice bearing mutated *IL2r^{null}* genes and expressing the mouse hepatocyte defect. Engraftment of these mice with human hepatocytes can result in up to 95% human hepatocyte chimerism [54;55]. Liver pathology, including inflammation, hepatitis and fibrosis were observed in these liver/chimeric/immune system-engrafted mice. Co-injection of human hepatocytes and HSC from the same donor into BRG mice expressing a fusion protein of the FK506 binding domain and caspase 8 under control of the albumin promoter

led to the development of human hepatocytes and an autologous immune system in which an immune response to HCV infection could be investigated [55].

Additional Humanized Mouse Models of Virus Infection

A number of additional virus infection models are at early stages of development in humanized mice. These include cytomegalovirus (CMV), herpes simplex virus type 2 (HSV-2), human T-cell lymphotropic virus (HTLV-1), influenza, and Kaposi's sarcoma-associated herpesvirus (KSHV) (reviewed in [26]).

Humanized Mouse Models of Bacterial Infection

Salmonella enteric serovar Typhi (*S. typhi*)

This infectious bacterium is highly adapted to humans, fails to produce progressive infection in immunocompetent or unmanipulated (non-engrafted) immunodeficient mice, and causes typhoid fever, leading to an estimated 21 million cases and ~200,000 deaths a year [59]. Recently the first humanized mouse models of *S. typhi* have been described [60-62]. Using NSG Hu-SRC-SCID mice infected with *S. typhi*, granulomatous inflammation with mononuclear cell infiltration in the spleen was followed by rapid fatality. Screening of transposon pools in infected mice revealed previously unrecognized *Salmonella* virulence determinants [60]. Using BRG Hu-SRC-SCID mice, prolonged survival was associated with innate and adaptive human immune responses [61], and in some cases, neurological symptoms [62]. These reports highlight the differences in pathogenesis and lethality following *S. typhi* infection in the different strains of humanized mice providing novel models for the study of this infectious agent.

Humanized Mouse Models of Protozoan Infection

Plasmodium falciparum

More than 200 million new cases of malaria are diagnosed each year, resulting in over 650,000 deaths (WHO World Malaria Report, 2011). For the life cycle of the human (and chimpanzee [63]) specific infectious agent *Plasmodium falciparum*, both human hepatocytes and erythrocytes are required. Human liver/chimera models have been established (Table 1), but few human RBCs circulate in the blood of human HSC-engrafted mice despite the presence of erythroid progenitors in the BM [19;20]. To model the erythrocyte portion of the life cycle, repeated injection of packed RBCs into NSG mice preceding infection with *P. falciparum* allowed the therapeutic efficacy of selective inhibitors of *P. falciparum* to be assessed [64]. For study of liver-stages of *Plasmodium falciparum*, liver/chimera models were used (Table 1) [65;66]. In the Vaughan *et al* study, liver/chimera mice were injected with RBCs, permitting transition of liver stage infection to blood stage infection [65]. Mice co-engrafted with human hepatocytes and HSC from the same donor [55], along with injection of human RBCs could provide a model where all stages of the life cycle of *P. falciparum* can be studied in the presence of a human immune system.

Conclusion

Humanized mice are rapidly becoming experimental tools of choice to investigate human infectious agents that do not infect other species, or poorly recapitulate infection, pathogenesis, and immune responses. However, it must be cautioned that there remain a number of limitations in the use of humanized mice for infectious disease studies. Many of the limitations inherent in humanized mice are due to mouse versus human-specific differences in the molecules required for human hematopoietic and immune system development (reviewed in [19;20]). Expression of human transgenes and targeted

inactivation of mouse genes encoding factors that control the development and function of human cells are currently being used to address these remaining limitations. Studies of emerging infectious human diseases drive the need of basic research that supports development and optimization of humanized mouse models for engraftment with a range of human tissues that can support infection with emerging pathogens. Optimized models will need to be able to mount vigorous humoral and cell-mediated human immune responses and permit the rapid testing of experimental vaccines. Moreover the threat of bioterrorism mandates optimization of existing humanized mouse model systems and technological improvements to ensure appropriate humanized mouse models that will support infection with bio-engineered human pathogens. In conclusion, humanized mice are promising pre-clinical bridges for the study of the pathogenesis and efficacy of drugs on human infectious agents prior to their entry into clinical trials.

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Highlights

- Many human infectious diseases are caused by pathogens that do not infect rodents
- Humanized mice serve as a preclinical bridge for in vivo studies of human pathogens
- Next generation humanized mouse models will accelerate drug discovery
- Limitations of humanized mice are being addressed using new technologies

Figure 1. Four major methods of engrafting NSG mice with human hematopoietic cells and tissues

NSG and other strains of immunodeficient mice have been engrafted with human hematopoietic and immune cells or tissues to establish four different immune system engraftment models. **Hu-PBL-SCID:** This model is established by engraftment of human Peripheral Blood Leukocytes (PBL). Most of the engrafting cells are human T cells that express an activated phenotype while few B cells or myeloid cells engraft. This model, described in 1988 [10], is ideal for the study of agents that infect mature effector T cells such as HIV-1 [67]. One caveat is that these mice will develop a xenogeneic graft-versus-host disease (xeno-GVHD) that results in death, but xeno-GVHD can be delayed using immunodeficient mice lacking mouse MHC class I or class II [68]. **Hu-SRC-SCID:** This model, termed the Human Stem Repopulating Cell model, is established by engraftment of human hematopoietic stem cells (HSC) derived from bone marrow, umbilical cord blood, fetal liver, or mobilized peripheral blood HSC. Engrafting mature adult immunodeficient *IL2r^{null}* mice with HSC permits the generation of multiple hematopoietic cell lineages but few T cells [69] while human T cells are readily generated following engraftment of newborn or 3-4 week-old NSG and NOG mice with HSC [15;69]. **SCID-Hu:** This model is established by implantation of human fetal liver and thymus fragments under the renal capsule of immunodeficient mice [14]. Although this was one of the first models available for the study of human immunodeficiency virus-1 (HIV-1), a major limitation is the paucity of human hematopoietic and immune cells in the peripheral tissues. **BLT:** This model (Bone marrow, Liver, Thymus) is established by implantation of human fetal liver and thymus fragments under the renal capsule of sublethally irradiated immunodeficient mice accompanied by intravenous injection of autologous fetal liver HSC [11]. Use of immunodeficient NOD-*scid* mice to establish the BLT model led to human immune system engrafted mice [70;71], which is further enhanced by the engraftment of NSG mice [27]. A

complete hematopoietic and immune system develops, and the human T cells are educated on a human thymus and are HLA-restricted [19;20]. This model has become the model of choice for studies of many infectious agents due to the robust human immune system and the generation of a human mucosal immune system.

Table 1

Humanized Hepatocyte Models for Infectious Disease

Mouse strain	Common Name	Characteristics	Pathogen	Ref
STOCK <i>Prkdc^{scid}</i> Tg(Alb1Plau)144Bri	Alb-uPA/SCID	Expression of urokinase-type plasminogen activator (uPA) driven by the albumin promoter results in hemorrhaging associated with uPA's fibrinolytic and fibrinogenolytic activities and accelerated hepatocyte death. Loss of transgene results in clonal repopulation of the liver with normal mouse hepatocytes	Hepatitis B	[53]
STOCK <i>Prkdc^{scid} Lyst^{bg}</i> Tg(Alb1Plau)144Bri	Alb-uPA/SCID BEIGE	6677 6677 6677 6677 6677	Hepatitis B Hepatitis C <i>Plasmodium falciparum</i>	[56-58;66]
(B6;129)- <i>RagZ^{tm1Fwa}</i> <i>Fah^{tm1Mgo} Il2rg^{tm1Wjl}</i>	FAH, FRG	A targeted deletion of the fumarylacetoacetate hydrolyase (<i>Fah</i>) gene models human tyrosinemia type I. Treatment of mice with 2-(2-nitro-4-trifluoromethylbenzoyl)-1, 3-cyclohexanedione (NTBC) is required prior to and during early stages of hepatocyte engraftment. High incidence of lethality prior to engraftment	Hepatitis B & C	[54]
6677 6677 6677 6677 6677	FAH, FRG	6677 6677 6677 6677 6677	<i>Plasmodium falciparum</i>	[65]
C.Cg <i>RagZ^{tm1Fwa} Il2rg^{tm1Cgr}</i> Tg(Alb-FKBP12/CASP8)#Lsu	AFC8	A fusion protein of the FK-506 binding domain (FKBP) and caspase 8 under control of the albumin promoter. Treatment with the FKBP dimerizer results in inducible hepatocyte suicide. Mice can be repopulated with human hepatocytes and HSC	Hepatitis C	[55]