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Humanized Mouse Models to Study Human Diseases

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Abstract

Purpose of review—Update on humanized mouse models and their use in biomedical research.

Recent findings—The recent description of immunodeficient mice bearing a mutated IL-2 receptor gamma chain (*IL2ry*) facilitated greatly the engraftment and function of human hematolymphoid cells and other cells and tissues. These mice permit the development of human immune systems, including functional T and B cells, following engraftment of hematopoietic stem cells (HSC). The engrafted functional human immune systems are capable of T and B cell-dependent immune responses, antibody production, anti-viral responses, and allograft rejection. Immunodeficient *IL2ry*^{null} mice also support heightened engraftment of primary human cancers and malignant progenitor cells, permitting *in vivo* investigation of pathogenesis and function. In addition, human-specific infectious agents for which animal models were previously unavailable can now be studied *in vivo* using these new generation humanized mice.

Summary—Immunodeficient mice bearing an *IL2ry*^{null} mutated gene can be engrafted with functional human cells and tissues, including human immune systems, following engraftment with human hematolymphoid cells. These mice are now used as *in vivo* models to study human hematopoiesis, immunity, regeneration, stem cell function, cancer, and human-specific infectious agents without putting patients at risk.

Keywords

IL2ry; NOD-SCID; NSG; Humanized mice

Introduction

Studies of human cell and tissue function have traditionally been limited to *ex vivo* analyses, non-invasive procedures, or clinical trials that are costly and severely limited due to ethical constraints. Small animal models of human cell and tissue function would overcome these limitations. A major breakthrough in the generation of humanized mice was the development of immunodeficient mice bearing a targeted *IL2ry* mutation. These mice permit functional *in vivo* studies of human cells and tissues [1–3]. This report summarizes recent progress in the use of humanized mice for the study of human diseases.

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History of Humanized Mice

Human cell and tissue functions in murine hosts have been investigated since the first description of athymic (nude) mice [4]. Humanization of immunodeficient mice advanced greatly with the discovery of the severe combined immune deficiency (*scid*) mutation [5] and the knockout of recombination activating genes 1 and 2 [6;7]. Additional manipulation of the mouse genome by knockout and transgenic technology led to increased engraftment and function of human cells and tissues [1]. The recent description of immunodeficient mice bearing a mutated IL-2 receptor gamma chain (*IL2ry*^{null}) facilitated greatly the *in vivo* engraftment and function of human cells. The history of humanized mice in biomedical research and the tremendous advances in this field resulting from the generation of immunodeficient mice with a mutated *IL2ry* gene has been reviewed recently [1–3]. The present review briefly summarizes the different models of humanized mice available for experiments, highlights recent advances in the model systems, and summarizes new findings that have emerged over the last year.

Models of Humanized Mice

We use a simple definition of humanized mice as "mice engrafted with functional human cells or tissues or expressing human transgenes." Depending on the experimental question, different models of immunodeficient humanized mice are utilized (Table 1). Hu-PBL-SCID mice are engrafted with peripheral blood mononuclear cells [PBMC, 8]. Hu-SRC-SCID mice are engrafted with human hematopoietic stem cells [HSC, 9]. SCID-hu mice are engrafted with human fetal liver and thymus [10]. These models represent humanized mice engrafted with functional human immune systems. Immunocompetent mice expressing human transgenes also provide insights into human biology [11–13]. This review will focus on humanized mice engrafted with functional human cells and tissues.

Continuing Improvements in Humanized Mouse Technology

Human cell and tissue function was enhanced greatly by the generation of immunodeficient *IL2ry*^{null} mice [1–3], but there remain a number of limitations that are continuously being recognized and overcome. One of the first questions in the field arose from the bewildering number of available mouse strains and engraftment protocols. Recognizing this problem, standardized methodologies for establishment of humanized mice were recently published [14;15]. Optimal approaches for engraftment of newborn and adult NOD-*scid IL2ry*^{null} mice with HSC (Hu-SRC-SCID) as well as engraftment of human PBMC (Hu-PBL-SCID) are detailed by Pearson *et al* [14]. Optimal approaches for engraftment of BALB/c-*Rag2*^{null} *IL2ry*^{null} mice with human HSC (Hu-SRC-SCID) are described by Legrand *et al* [15]. It is recommended that investigators working with humanized mouse models initially establish the models based on these standardized guidelines.

The second question is the optimal recipient strain. HSC engraftment in different strains of immunodeficient mice following intrahepatic injection into newborns has been compared [16]. NOD-*scid IL2ry*^{null} mice and BALB/c-*Rag2*^{null} *IL2ry*$^{null\ l}$ mice are equivalent in their generation of human B cells, dendritic cells (DC), and platelets whereas NOD-*scid IL2ry*^{null} mice are superior in supporting human T cell development. Fetal liver and umbilical cord blood (UCB) HSC supported higher percentages of human engraftment than G-CSF-mobilized peripheral blood HSCs. Bone marrow HSC was not tested in this report. We have confirmed that UCB HSC-engrafted newborn NOD-*scid IL2ry*^{null} mice are superior to BALB/c-*Rag1*^{null} *IL2ry*^{null} mice in their ability to support human T cell development, and further found that intrahepatic and intracardiac (i.v.) injections are equivalent (Brehm *et al*, submitted). The NOD vs. BALB/c support of T cell engraftment is not based on the *scid vs. Rag1/2*^{null} mutations as NOD-*scid IL2ry*^{null} mice and NOD-*Rag1*^{null} *IL2ry*^{null} mice are equivalent in their support

of human HSC engraftment [17]. These data suggest that investigators establishing humanized mice requiring a fully functional human immune system in the Hu-SRC-SCID model should consider basing their work on newborn engraftment of NOD-*scid IL2ry*^{null} or NOD-*Rag1*^{null} *IL2ry*^{null} mice.

Another limitation being addressed is the species-specificity of a number of molecules. Examples include species-specific human cytokines. Transgenic expression of human IL15 enhanced human NK cell development and differentiation in HSC-engrafted BALB/c-*Rag2*^{null} *IL2ry*^{null} mice, and documented the critical role that IL15 trans-presentation has in regulating human NK cell homeostasis [18*]. A second cytokine, B Lymphocyte Stimulating factor (BLyS also termed BAFF) is important in B cell survival and differentiation [19;20]. Mouse BLyS cannot support human B cell survival, and administration of human recombinant BLyS to NOD-*Rag1*^{null} *Prf1*^{null} mice engrafted with human PBMC increased human B and, surprisingly, T cell engraftment [21*]. Generation of BLyS transgenic NOD-*scid IL2ry*^{null} mice should enhance human B and T cell immune function in humanized mice and creation of these transgenic mice as well as other human-specific cytokine transgenic mice is underway [22].

Human Immune Function in Humanized Mice

Humanized mice have been used in the past year to investigate multiple types of human immune responses and to test potential therapeutics that modulate human immunity. The Hu-PBL-SCID model has an ~30 day window of analysis due to development of xenogeneic graft-versus-host disease [GVHD, 23]. Investigators have used this observation to establish an *in vivo* model of human immune-mediated GVHD. Using the Hu-PBL-SCID model based on NOD-*scid* mice, activation of human regulatory T cells by HIV-1 envelope protein gp120 delayed development of GVHD [24]. A similar delay of GVHD was observed in the Hu-PBL-SCID model based on NOD-*scid IL2ry*^{null} mice following treatment with soluble Fas ligand [25]. King *et al* examined kinetics of engraftment and development of GVHD in the Hu-PBL-SCID model based on NOD-*scid IL2ry*^{null} mice [26*]. They observed that most of the GVHD was directed against mouse MHC class I and II and mice deficient in MHC class I exhibited delayed GVHD. TNF inhibitors are used in the clinic to treat GVHD [27;28], and similarly, etanercept, a TNF inhibitor delayed the development of GVHD in this model system [26*].

Additional analyses of human T and B cells in Hu-SRC-SCID mice generated following engraftment of adult NOD-*scid IL2ry*^{null} mice revealed that although human B cells develop, they are developmentally blocked [29], likely due to the inability of mouse BLyS to signal human B cells [21*]. The authors further suggested that human T cells selected in the thymus on mouse MHC class II is at least partially responsible for decreased human T cell immune responses.

One approach to enhance human T cell selection in the mouse thymus is to provide a human thymus autologous with the human HSC. This model, termed SCID-hu (Table 1), has been used extensively in the study of infectious agents (see below), and was recently used to establish a porcine islet xenograft rejection model [30] and an approach for induction of xenograft tolerance [31*]. Human HSC model systems supporting rejection of human allografts have to date not been reported. However, SCID-beige immunodeficient mice engrafted with human HSC generate multiple hematopoietic cells but not T cells [16;32]. Macrophages infiltrated human skin allografts in these mice, but produced little injury. However, when combined with adoptive transfer of autologous T cells to activate the infiltrating macrophages, the macrophages produced intimal expansion and calcification, reminiscent of atherogenesis or end-stage renal disease [33;34]. Regarding T cell-mediated allograft rejection, Racki *et al*

demonstrated using the Hu-PBL-SCID model based on NOD-*scid IL2ry*^{null} mice that either purified human CD4 or CD8 T cells can mediate human skin allograft rejection [35].

Additional human immune responses have been described in humanized mice. A model for asthma was used to identify a role for DC-derived CCL17 and CCL22 in attraction of Th2 cells and induction of airway inflammation [36]. In a humanized mouse model of sepsis, human lymphocyte apoptosis and cytokine production recapitulated the findings in patients with septic shock [37]. As new models are generated, investigation of multiple aspects of both immune and autoimmune responses of human immune systems will be possible.

Human-Specific Infectious Disease in Humanized Mice

One of the most prevalent uses of humanized mice is the study of human-specific infectious agents, particularly HIV [38;39]. Using the SCID-hu system, robust virus-specific immune responses following HIV infection were observed [40*]. Despite these robust responses, HIV viral load remained high and correlated with increased PD1 expression on human T cells, an observation also made in humans [41–43]. In a model based on NOD-*scid Jak3*^{null} mice, a nucleoside reverse transcriptase inhibitor blocked HIV infection in Hu-PBL-SCID mice [44]. Using the Hu-SRC-SCID model based on BALB/c-*Rag1*^{null} *IL2ry*^{null} mice, *in vivo* RNAi gene therapy against HIV-1 was investigated [45]. Human HSC were transduced with a lentiviral vector expressing a shRNA against HIV-1 *nef* gene and engrafted into newborn BALB/c-*Rag1*^{null} *IL2ry*^{null} mice. Evidence was obtained that the mature human CD4 T cells recovered from the HSC-engrafted mice exhibited an inhibition of virus replication, confirming efficacy of the shRNA therapy.

In a key series of experiments using both the Hu-PBL-SCID and Hu-SRC-SCID models based on NOD-*scid IL2ry*^{null} mice, Kumar *et al* validated a novel new drug for the prevention and treatment of HIV infection [46**]. They used a modified single chain antibody (scFv) to the human T cell marker CD7 to deliver siRNA *in vivo* against CCR5 and viral *Vif* and *Tat* genes to human CD4 T cells in humanized mice. They documented that HIV infection could be controlled in a prophylactic setting in both model systems when viral challenge was performed after initiation of siRNA treatment, as well as in a post-infection setting, where mice were engrafted with PBMC from an HIV-infected subject.

A number of reports have described Dengue virus humanized mouse models for which no animal model system previously existed. Using newborn HSC-engrafted NOD-*scid IL2ry*^{null} mice infected with eight different viral strains representing the four genotypes of Dengue viruses, viremia, a thrombocytopenia, increase in body temperature and erythema were observed corresponding to clinical characteristics in Dengue-infected humans [47;48].

Another approach in addition to the SCID-hu model to enhance human T cell selection during development in the thymus is to transgenically express human HLA in the mouse recipient. In a report using Hu-SRC-SCID mice based on NOD-*scid IL2ry*^{null} HLA-A2 transgenic mice engrafted with HLA-A2 HSC, Jaiswal *et al* documented the development of virus-specific HLA-A2-restricted human T cell responses to Dengue virus infection [49*]. This is one of the first two reports using HLA-transgenic mice to document a human T cell HLA-restricted immune response. The other report used Epstein Barr Virus (EBV) infection in a model also based on NOD-*scid IL2ry*^{null} HLA-A2 transgenic mice engrafted with HLA-A2 HSC [50**]. HLA-A2-restricted cytotoxic and IFNy-producing human T cells against multiple EBV HLA-A2 epitopes were observed exhibiting similar patterns of reactivity to that detected in human EBV carriers. These two reports document that HLA expressed transgenically in mouse thymus can positively select developing T cells and lead to HLA-restricted immune responses in mice engrafted with human HSC. Development of additional HLA-transgenic immunodeficient mice is currently underway [2;39].

NOD-*scid IL2ry*^{null} mice engrafted as newborns with HSC were used to document a novel approach for enhancing immune responses following immunization. Targeting EBV antigen to human DC *in vivo* stimulated human T cell responses to EBV and induced anti-EBV antibody responses [51]. Similarly, NOD-*scid* β*2m*^{null} mice engrafted with human HSC and autologous mature T cells and then infected with live attenuated trivalent influenza virus generated human T cell responses to influenza [52]. The authors proposed this as a model for investigating antigen-presenting cells in immune responses as the response was completely dependent on reconstitution of the human myeloid compartment.

Finally, the first humanized mouse model for the study of *Plasmodium falciparum* was recently reported [53*]. NOD-*scid IL2ry*^{null} mice were injected repeatedly with human red blood cells, which could then support productive infection with *P. falciparum*. Therapeutic efficacy of a number of anti-malarial agents was tested, permitting determination of the protective ED_{90} of the drugs against infection.

Overall, infectious disease studies in humanized mice are providing important pre-clinical model systems for the investigation of the pathogenesis of human-specific infectious agents, evaluation of therapeutics, and platforms to understand mechanisms of human immune responses following vaccination.

Humanized Mice and Cancer

Immunodeficient *IL2ry*^{null} mice permit engraftment with a number of primary human tumors [1]. Comparing growth of human melanoma lung metastases in NOD-*scid*, NOD-*scid* β*2m*^{null}, and NOD-*scid IL2ry*^{null} mice [54] the absolute NK deficiency in NOD-*scid IL2ry*^{null} mice appeared to be a major factor in its enhanced support of primary tumor growth [54]. The enhanced engraftment of NOD-*scid IL2ry*^{null} mice was confirmed in a report showing that human acute leukemia cells generate a faster and more efficient disease as compared to that observed in NOD-*scid* and NOD-*scid* β*2m*^{null} mice [55]. Human immune responses via antibody-dependent cellular cytotoxicity against primary adult T cell leukemia/lymphomas, Hodgkin lymphoma, and cutaneous T cell lymphoma in NOD-*scid IL2ry*^{null} mice could be potentiated by defucosylated anti-CCR4 antibody [56;57*], suggesting a novel approach to enhance anti-tumor immunity. Confirming the utility of humanized mice for studies of cancer, it was shown that primary lung tumors transplanted into NOD-*scid IL2ry*^{null} mice recapitulated *in vivo* tumor characteristics, including maintenance of stroma and passenger leukocytes that could, when activated with IL12, become tumor effector CTLs [58].

Humanized Mouse Models for Human Cell Therapy

Cellular therapy for treatment of multiple human diseases, particularly diabetes, is a promising approach. Successful human islet transplantation [59], has expanded into a number of clinical trials (www.clinicaltrials.gov). Because sufficient islets are not available for the tremendous need, focus has turned to development of beta cells from stem cells (http://www.betacell.org/). However, animal models for testing the safety and efficacy of this and other forms of human cellular therapy, such as regulatory T cells [60] or embryonic stem cells [61], are needed and humanized mice are being developed to address these critical needs. For example, immunodeficient NOD-*Rag1*^{null} *Prf1*^{null} *Ins2*^{Akita} mice that spontaneously develop a non-autoimmune hyperglycemia [62–65] can be engrafted with human islets [66]. This model can be used to test the *in vivo* function of human beta stem cells. Backcrossing of the *Ins2*^{Akita} mutation to the NOD-*Rag1*^{null} *IL2rγ*^{null} strain will permit human beta stem/progenitor cells to be transplanted into mice bearing a functional human immune system [17; 67], similar to the situation that occurs during transplantation in the clinic.

Conclusion

Humanized mice as pre-clinical models for the *in vivo* study of human cells and tissues have been under development for over 30 years. With the recent generation of immunodeficient *IL2rγ*^{null} mice, the ability of humanized mice to serve as preclinical models is becoming a reality, but not yet ideal. Additional modifications of the model systems and genetic manipulation of the host continue to improve the ability of humanized mice to more accurately recapitulate the *in vivo* function of human cells and tissues. Novel insights into human disease are now possible due to the availability of humanized mice wherein human cells and tissues can be studied *in vivo* without putting patients at risk.

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Table 1

Models of humanized mice

Model	Approach	Characteristics	Use
Hu-PBL-SCID (Human Peripheral Blood Lymphocyte)	Engraftment of mature PBMC obtained from blood, spleen or lymph nodes	Predominately engrafts human CD3 T cells. Study of mature T cell function	Study of mature immune cell function
Hu-SRC-SCID (Human Scid Repopulating Cell)	Engraftment of hematopoietic stem cells	Develops human hematopoietic and naïve immune systems	Study of complete hematopoietic system and naïve immune system
SCID-Hu	Engraftment of human fetal liver and thymus	Develops human hematopoietic and immune systems	Study of complete hematopoietic system and naïve immune system
Hu-Tg Mice	Transgenic expression of human genes	Expresses specific human genes *in vivo*	*In vivo* study of human gene function