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Humanized mice as a preclinical tool for infectious disease and biomedical research

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Abstract

Immunodeficient mice bearing an *IL2 γ* ^{null} gene permit engraftment of a functional human immune system and study of human-specific infectious agents that was not previously possible.

Keywords

SCID; humanized mice; immunity; infectious disease; NSG

Introduction

There is a critical need for the development of new animal models for the *in vivo* study of human immunity. Most experimental work is done in rodent models. However, mice are not humans, and observations made in rodents may not translate to the clinic. Tractable small animal models that can be engrafted with functional human cells, tissues, and immune systems would provide important predictive pre-clinical models to study human immunity. Moreover, there are many infectious agents that are in need of small animal models for investigation of their *in vivo* pathogenesis and for testing new drugs or vaccines that can prevent or ameliorate disease without putting individuals at risk. Many of the infectious diseases of humans are caused by organisms that do not infect mice or other laboratory animal species, precluding the study of these pathogens in traditional animal models. To address this, immunodeficient mice have been developed that can be engrafted with functional human cells, tissues, and a functional human immune system.

History of humanized mouse development

In 1983, discovery of the severe combined immunodeficiency (*Prkdc*^{scid}, abbreviated *scid*) mutation in CB17 mice was followed by observations that human hematopoietic cells, including peripheral blood mononuclear cells (PBMCs) and hematopoietic stem cells (HSCs) could engraft in these mice. However, levels of engraftment were low due to genetic limitations inherent in the strain background and high levels of NK cells. In 1995 NOD-*scid* mice were developed that exhibit low levels of NK cells and other innate immune defects that permitted enhanced engraftment of human hematolymphoid cells (1).

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Conflict of Interest

The authors have no conflict of interest to report

***IL2r γ* ^{null} and *Sirpa*, key improvements in humanized mice**

A major advancement in the field was the development of immunodeficient mice bearing a targeted mutation in the IL-2 receptor common gamma chain (*IL2r γ* ^{null}) (1). These mice support enhanced engraftment of human hematopoietic cells and for the first time a fully functional human immune system.

Another key finding was discovery of the role that signal regulatory protein alpha (*Sirpa*) modulates human hematopoietic cell engraftment in immunodeficient mice (1). Macrophages in NOD mice express a *Sirpa* that closely resembles human *Sirpa* and display reduced levels of phagocytosis of human cells relative to macrophages derived from C57BL/6 and BALB/c mice that have a *Sirpa* with little homology to human *Sirpa*. This observation is likely one of the key reasons that NOD-*scid* *IL2r γ* ^{null} (NSG) and NOD-*Rag1*^{null} *IL2r γ* ^{null} (NRG) mice engraft at higher levels than do BALB/c-*Rag2*^{null} *IL2r γ* ^{null} (BRG) mice (2). Recently, a human *Sirpa* transgene expressed on the BRG background has been reported to exhibit enhanced engraftment of human HSC (3).

Although *Sirpa* is a key component of determining engraftment of human hematopoietic cells in immunodeficient mice, NSG mice also have numerous defects in cells of the innate immune system (1), and it will be important to determine if transgenic expression of human *Sirpa* increases human hematopoietic cell engraftment in NSG mice.

Immune models of humanized mice

A number of human immune model systems have been developed: (1) Hu-PBL-SCID, (2) Hu-SRC-SCID, (3) SCID-Hu, and (4) BLT.

Hu-PBL-SCID

Hu-PBL-SCID mice are established by engraftment of human peripheral blood leukocytes (PBL). This model system is ideal for the study mature effector T cells as this is the primary cell population that engrafts. This system has also been used as a model for the study of xenogeneic graft-versus-host disease (xeno-GVHD) (4). These data document that the engrafted human T cells retain their immune function following engraftment into the immunodeficient recipient. A limitation of this model is the lack of HLA-expression in the recipient. The human T cells are educated on the PBL-donor thymus and are HLA-restricted. The antigen-presenting cells (APCs) in the NSG recipient express the mouse MHC, and function poorly as APCs for human T cells. Second, the mature functional human T cells that engraft mediate xeno-GVHD confounding study of the human immune function in the recipient.

Hu-SRC-SCID

Hu-SRC-SCID mice are established by intravenous or intrafemoral injection of conditioned adults, or intravenous or intrahepatic injection of conditioned newborns with human HSC. Engraftment of adult immunodeficient *IL2r γ* ^{null} mice with HSC leads to development of multiple hematopoietic cell lineages but few T cells (2). In contrast, human T cells are readily generated following engraftment of newborn NSG mice with HSC.

SCID-Hu

The SCID-Hu model is established by implantation of human fetal liver and thymus fragments under the renal capsule (1). This system was one of the first models available for the study of human immunodeficiency virus (HIV) in a small animal model, and was used

extensively to evaluate potential HIV therapeutics. A major limitation of this model is the paucity of human T cells in the peripheral tissues (1).

BLT

The BLT model system (bone marrow, liver, thymus) is established by implantation of fetal human liver and thymus fragments under the renal capsule of irradiated recipients and intravenous injection of fetal liver cells HSC (Fig. 1). A robust human immune system develops, including human T cells that are HLA-restricted. A major advantage of this model system is that a human mucosal immune system is also generated. This model has been used to establish a system for the study of mechanisms of mucosal infection with HIV, a common pathway for transmission of this virus in humans.

Limitations of immune systems in humanized mice

Limitations include: (1) Lack of HLA molecules required for appropriate T cell selection in the Hu-SRC-SCID or lack of appropriate HLA APCs in the BLT model, (2) Species-specificity of growth factors and other molecules, (3) low levels of humoral immune responses, (4) limited development of lymph nodes, (5) few circulating human red blood cells, neutrophils, or platelets, and (6) residual innate immunity of the host.

HLA and T cell education

We and others have developed human HLA-transgenic (Tg) mice. Using NSG-HLA-A2 Tg mice engrafted with HLA-A2 HSC, we have shown that functional human immune systems develop, and that HLA-A2 restricted human CD8⁺ T cells are generated following infection with Dengue (5) or Epstein Barr virus (6).

However, NSG mouse still express murine MHC class I and class II antigens. To address this, we have developed NSG mice deficient in host MHC class I and class II (4). Adoptive transfer of PBL into NSG mice deficient in MHC class I or class II significantly delays the development of xeno-GVHD (4). More recently, we have used these new generation NSG MHC class II deficient mice to establish a novel model for allo-GVHD.

Species-specific growth factors and other molecules

A number of species-specific factors in mice are not cross-reactive with human cells (1). To address this, we and others provide recombinant proteins or use knock-in and transgenic immunodeficient *IL2 γ ^{null}* mice expressing human cytokines, growth factors, and molecules. For example, in the original description of NOD-*scid* *IL2 γ ^{null}* mice, administration of recombinant human IL-7 enhanced human T cell growth (1) and administration of recombinant human IL-7 during the induction of a delayed hypersensitivity reaction increased the human cellular immune response in humanized mice (7). More recently, expression of human *Sirpa* in BRG mice enhanced human cell engraftment (3). Our laboratory has created a panel of human HLA and cytokine transgenic NSG mouse models and has additional models under development.

Human specific infectious agents in humanized mice

HIV

HIV was the first human infectious agent to be studied in humanized mice (1). Since the development of immunodeficient *IL2 γ ^{null}* mice, the use of humanized mice in infectious disease has expanded exponentially. For HIV research, the BLT model permits study of infection via the mucosal route. In these models, numerous therapeutic approaches have

been used to prevent and treat infection. In the Hu-PBL-SCID and Hu-SRC-SCID models, we have used humanized NSG mice to demonstrate the therapeutic activity siRNA therapy to suppress HIV infection (8).

Dengue virus

Humanized mice can support infection with Dengue virus, and both T and B cell specific responses can be elicited (5). Using the Hu-SRC-SCID model, we have shown the induction of anti-Dengue virus antibodies, and generation of HLA-A2 restricted CD8⁺ T cell responses (5). Using Hu-SRC-SCID mice, it has also been shown that administration of siRNA against TNF- α in Hu-SRC-SCID mice infected with Dengue virus suppressed the production of TNF- α by human dendritic cells. Of interest will be the study of dengue virus infection in the BLT model, which appears to generate more robust human immune responses than does the Hu-SRC-SCID or Hu-PBL-SCID model systems.

Malaria

There has been a growing need for an animal model for the study of malaria. Early studies using *Plasmodium falciparum*-infected human erythrocyte-engrafted NOD-*scid* mice showed low levels of parasitemia. Investigators have used NSG mice to engraft human red blood cells, and induce a productive infection with *P. falciparum* for the study of efficacy of anti-malarial drugs.

Epstein Barr Virus

Epstein Barr Virus (EBV) will only infect human cells and tissues, and humanized mice have been used to study the immune response to EBV infection (6). The use of NSG HLA-A2 Tg mice engrafted with HLA-A2 CD34⁺ cells derived from umbilical cord blood permitted the identification of CD8⁺ HLA-A2-restricted T cell responses to EBV epitopes (6).

Salmonella typhi

Using the NSG Hu-SRC-SCID model, humanized mice can be infected with this pathogen, and this induced a lethal outcome with pathological and inflammatory cytokine responses resembling human typhoid (9). This model was used to identify potential *Salmonella* virulence determinants that modulated their *in vivo* infectivity. Another model of *Salmonella typhi* based on the BRG strain in the Hu-SRC-SCID model also supported productive infection, but the infection in this strain was not lethal, permitting the immune response to *S. typhi* to be investigated.

We are also developing humanized mouse models of hemorrhagic fever virus infection that is emerging as a serious biological threat. These examples of how humanized mice are being used to study human-specific infectious agents without putting individuals at risk suggest that there will be a growing interest in these models for such studies. Indeed, we have been able to substantiate the use of these models to accurately determine efficacy of anti-viral agents against highly deadly viruses such as Ebola and Marburg. In these studies, we determined the level of viral clearance in human cells engrafted in NSG mice in presence of specific therapeutics (data not shown). These types of approaches encompass critical components of data packages for FDA consideration of licensing of therapeutics and vaccines against biological agents where human clinical studies may not be feasible or possible.

Regenerative medicine and cancer

There are multiple uses for humanized mice in translational biomedical research. For example, the function of cells and tissues derived from embryonic stem cells or induced pluripotent stem cells can be investigated *in vivo*. Humanized mice are used to identify human tumor stem cells. In particularly intriguing studies on human acute myelogenous leukemia (AML), NSG mice were engrafted with chemotherapy-resistant AML stem cells to identify the precise location of these stem cells in the bone marrow of the recipients. These studies were extended to validate the cell surface phenotype of the AML stem cells, and to determine therapeutic targets for quiescent, chemotherapy-resistant leukemia stem cells. Finally, the humanized mice engrafted with these AML stem cells were used to develop a novel protocol for elimination of the leukemic stem cells, highlighting the potential of humanized mice to identify novel approaches for the clinical treatment of tumors and provide a practical guide for how to treat the disease (10).

Summary

Immunodeficient *IL2rg^{null}* mice permit *in vivo* investigation of functional human cells and tissues. Humanized mice are rapidly becoming important investigative tools as pre-clinical models of human translational medicine. Study of functional human cells and tissues in a small animal model provides a cost-efficient approach that will bridge translation of results obtained using *in vitro* human cultures and *in vivo* studies in rodents to the clinic. Humanized mice permit investigation of human immune responses to infectious disease as well as providing a platform for testing human-specific reagents and drugs. The model systems that have been developed recently set the stage for an increasingly important role humanized mice will have in pre-clinical translational research on human cells and tissues.

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Figure 1.

Establishment of the BLT model. (A) Schematic of the approach for transplantation of fragments of human fetal liver and thymus in the renal capsule followed by light irradiation of the recipient and intravenous injection of human CD34 $^{+}$ HSC isolated from the same fetal liver preparation. (B) and (C) Thymus and T cell development in BLT mice. Representative thymus grafts 16 weeks after transplantation of 1 mm 3 fragments of fetal thymus, B. Development of human CD45 $^{+}$ cells, CD3 $^{+}$ T cells and CD20 $^{+}$ B cells in the blood of NSG-BLT mice, C.