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Humanized mice for the study of type 1 and type 2 diabetes

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Abstract

The availability of immunodeficient mice engrafted with functional human immune systems and islets permits *in vivo* study of human diabetes without putting patients at risk.

Keywords

diabetes; humanized mice; animal models; islets

Introduction

Diabetes encompasses a group of diseases that have in common high glucose levels due to an absolute or relative deficiency in insulin production and/or insulin action. Diabetes is classified as type 1 (T1D), an absolute deficiency of insulin, requiring administration of exogenous insulin, or type 2 (T2D), a relative deficiency of insulin and defects in insulin action. Diabetes in the US afflicts 8.3% of the population representing ~26 million Americans at an annual cost of ~\$200 billion/yr (http://www.unitedhealthgroup.com/hrm/UNH_WorkingPaper5.pdf).

Rodent models of diabetes have facilitated our understanding of the disease's cause and identified potential treatments. However, mice and humans differ significantly with regard to their immune systems and pancreatic islet cellular composition, function, and gene expression (1). Moreover, investigating *human* T1D pathogenesis has been difficult due to the inaccessibility of the pancreas for study and the inability to analyze the interaction of immune cells with islets *in vivo*. Despite decades of study with rodent models of T1D, therapies that prevent or cure *human* T1D continue to elude us.

Our laboratories have focused on developing humanized mice to study diabetes pathogenesis and to test potential therapies. We define humanized mice as immunodeficient mice that are engrafted with functional human cells and tissues. This encompasses the transplantation of human islets as well as engraftment of human hematopoietic and immune systems. The need for humanized mice is clearly evident since current mouse models have not effectively predicted clinical trial outcomes in humans. In addition, there is an urgent need to investigate human-specific therapies on human cells and tissues *in vivo*. The availability of humanized mice enables clinically relevant *in vivo* studies of human cells, tissues, and immune systems without putting patients at risk.

Humanized SCID mice—a paradigm shift in diabetes research

We have created novel immunodeficient mouse strains that support engraftment with functional human tissues including hematopoietic stem cells (HSC), mature lymphocytes, and islets. These unique strains are based on NOD-*scid* mice expressing a targeted mutation in the IL2 receptor common gamma chain (*IL2r γ ^{null}*) (2). NOD-*scid* *IL2r γ ^{null}* (NSG) mice are severely immunodeficient and permit high engraftment of functional human cells and tissues (2). This makes NSG mice ideal to investigate human immune system function *in vivo* and to identify therapeutic intervention's mechanisms of action.

How can we use humanized mouse models to study diabetes?

Humanized mice can be used to address the critical questions that are difficult or impossible to study in patients. First, whereas in patients the disease process can only be studied after T1D is well established, the humanized mouse can be developed to identify the disease's initiating factors. Second, the humanized mouse permits access to the immune cells not just from peripheral tissues, but also from the target organ (the pancreas) so that those immune effector populations can be studied. It is simply not possible and is unethical to biopsy the pancreas for the study pancreatic autoreactive cells in patients with T1D. Third, humanized mice will identify potential therapeutic targets and allow testing of novel therapeutics *in vivo*, without putting patients at risk.

Considerations for creating humanized mouse models of diabetes

Meaningful humanized mouse T1D models will require several limitations to be addressed. First, T1D is a T cell-mediated disease, so the islet-associated antigenic targets need to be identical between human and mouse, or in the case of human-specific diabetogenic autoantigens, transgenic expression of the autoantigen or other approaches must be used. Second, the immune cell donor and the islets within the recipient mice need to be matched at the major histocompatibility complex (MHC, in humans termed HLA). Third, a number of murine cytokines and factors do not signal through the corresponding human receptors. The models we have created have been designed to address these limitations.

Models of hyperglycemic humanized mice

Although immunodeficient mice have been used for over 3 decades for the study of human islets *in vivo*, the earlier models have several drawbacks. First, these early models of immunodeficient mice have natural killer (NK) cell activity (2). Human islets are highly sensitive to NK cell killing. Second, these early models did not support engraftment with a functional human immune system. In contrast, NSG mice are completely NK cell deficient (2) and support engraftment with human islets, beta stem/progenitor cells as well functional human immune systems.

The most commonly used approach to study human islet function is to perform transplants into hyperglycemic mice and monitor blood glucose levels. Although injecting streptozotocin (STZ) will render the mice hyperglycemic, the response is quite variable, STZ can be toxic, and endogenous mouse beta cells sometimes recover, all of which conspire to complicate interpreting human islet graft function.

To address these concerns, we are developing new models of immunodeficient hyperglycemic mice. Mice bearing the *Ins2^{Akita}* mutation (so called Akita mice) develop spontaneous hyperglycemia at 3–6 weeks of age due to insulin 2 protein mis-folding, which induces endoplasmic reticulum stress and beta cell apoptosis. We have described NOD-*Rag1^{null} IL2r γ ^{null} Ins2^{Akita}* (NRG-Akita) mice bearing the *Ins2^{Akita}* mutation. These mice spontaneously develop hyperglycemia, and normoglycemia can be restored by transplanting human islets (3). NRG-Akita mice can also be engrafted with functional human immune

systems that can reject human islet allografts (3). We have also used NRG-Akita mice to investigate human beta cells' proliferative capacity *in vivo* under normoglycemic and hyperglycemic conditions (4).

A second model is based on the Tg(*RIP-HuDTR*) mouse. Mouse cells do not bind diphtheria toxin (DT) with high affinity. Administration DT to mice expressing the human DT receptor (DTR) under the control of the rat insulin promoter will specifically kill the mouse beta cells and induce hyperglycemia. We have backcrossed the DTR transgene onto NSG mice. The initial characterization of NSG Tg (*RIP-HuDTR*) mice is underway. An advantage is that the hyperglycemia can be induced at will, is not associated with STZ toxicity, and is irreversible.

In some experimental designs, it may be advantageous to induce hyperglycemia, and when desired, permit the mouse beta cell function to recover. For this, we are developing the NSG Tg(*Ins-rtTA*) Tg (*TET-DTA*) strain. These mice express DT A chain (DTA) in beta cells when the *TET-DTA* gene is activated by adding doxycycline ("doxy") to the animals' drinking water (5). Since expression of a single DTA molecule will kill the beta cell, and since most but not all of the beta cells are activated to express DTA when doxy is administered, so long as doxy is given, the mice remain hyperglycemic. And yet stopping the doxy permits the remaining beta cells to proliferate and restore normoglycemia (5). We are currently generating these mice by speed congenic backcrossing the *Ins-rtTA* and *TET-DTA* transgenes onto the NSG strain.

While it is not currently possible, practical approaches to permit islet imaging is of considerable interest for the diagnosis and prognosis of pre-diabetes, to determine a therapeutic intervention's efficacy, and to follow transplanted islets' fate. Humanized mice can be used to test approaches for non-invasive *in vivo* imaging of human islets. For example, we have used a novel "pre-targeting" approach to image human islets engrafted in NSG mice (6).

Immune engraftment models of NSG mice

NSG models that can be used to study human immune cell function include the Hu-PBL-SCID, the Hu-SRC-SCID, and the BLT (fetal hematopoietic stem cell, liver, thymus) models (2).

Hu-PBL-SCID mice are used to examine allo-immunity, auto-immunity and viral-immunity (2). However, mature human T cells retain xeno-reactivity, and upon engraftment, generate a potent graft-versus-host disease (GVHD) (7). Newer genetic modifications of NSG mice deficient in MHC class I and/or class II reduce GVHD, permitting investigation of immunity by minimizing the confounding effects of xeno-GVHD (7).

Hu-SRC-SCID mice are established by engrafting newborn or adult NSG mice with human hematopoietic stem cells (HSC). The advantage of this model is that essentially all of the cells of both adaptive and innate immune system engraft, and the engrafted immune system is naive. The disadvantage is that the human T cells develop in the mouse thymus and are therefore educated on mouse, not human, MHC.

BLT mice have fragments of human fetal liver and thymus engrafted under the renal capsule, and are then given an intravenous injection of fetal liver HSC derived from the same donor (2). BLT mice develop functional innate and adaptive immune systems, including all hematopoietic lineage cells. Moreover, since HSC are educated on self-MHC, they are HLA-restricted. Both the Hu-SRC-SCID and the BLT model systems have been used to study many aspects of human immunobiology (2).

We have shown that NRG-Akita mice engrafted with either PBL or with HSC will develop a functional human immune system that can reject human islet allografts (3,8). The next step is to engraft functional human autoimmune systems into NSG mice for the study of human autoimmunity.

New HLA genetic models of NSG mice

We have developed several HLA-transgenic (Tg) strains on the NSG strain background, including HLA class I and class II alleles representing up to 80% HLA-locus genes associated with T1D. We have also shown that NSG HLA-A2 transgenic mice engrafted with umbilical cord blood HLA-A2⁺ HSC can support the development of HLA-A2 restricted CD8⁺ T cells following infection with Dengue (9) or Epstein Barr virus (10). We have also developed NSG mice deficient in mouse MHC class I and class II that eliminate education of human T cells on mouse MHC (7).

We currently are engrafting NSG-HLA Tg mice with spleen cells obtained from T1D donors through the JDRF nPOD program (<http://www.jdrfnpod.org/>). We have also engrafted human CD8⁺ T cell clones derived from a HLA-A2 T1D donor that had undergone an islet transplant. The CD8⁺ T cell clone is directed against the islet-specific glucose-6-phosphatase catalytic-subunit related protein (IGRP) peptide that has an identical sequence in mice and humans.

To address the lack of species cross-reactivity between mouse and human cytokines needed for optimal human immune function, we are generating human cytokine transgenic NSG mice. The initial human Tg mice being generated express human BAFF/BLyS, a B cell survival and differentiation factor. We have shown that administration of recombinant human BLyS to Hu-PBL-SCID mice increases human B-cell survival and immunization-induced antibody production. Additional human cytokine transgenic mice are being made using bacterial artificial constructs so that appropriate human regulatory elements for the genes are provided in the mouse.

Finally, so that we might optimally generate new transgenic, knockout, and knockin NSG mice, we have available embryonic stem cells (ESC) from NOD, NSG, and NRG mice (<http://research.jax.org/collaboration/escell.html>). These ESC can generate chimeras, and we are currently using them to create a knockout on the NOD background.

One of the approaches being taken to study T1D in humanized mice is to combine the novel induced pluripotent stem (iPS) cell technology with the new models of humanized mice. Our goal is to use iPS cells derived from T1D donors to generate the critical cells and tissues to recapitulate a donor's T1D following engraftment into humanized mice. This would permit detailed analyses of the disease while it develops and would be a platform to not only identify the causes of T1D, but also permit therapeutic manipulation of the developing human immune system to prevent or cure T1D.

In summary, the humanized mouse model represents a powerful platform to study diabetes pathogenesis and to develop better treatments. Genetic manipulation of the NSG strain has generated mice that become spontaneously hyperglycemic due to a genetic mutation or that can be readily and reversibly induced to become hyperglycemic. Into these mice, we can transplant *human* tissues to evaluate *human* islets or beta stem/progenitor cells *in vivo*. Moreover, without putting patients at risk, these mice can be engrafted with functional human immune systems, permitting the *in vivo* analysis of human beta cells during allo- and/or auto-immune attack. These mouse models will be valuable for investigation of diabetes pathogenesis, identification of potential therapeutic targets, and for *in vivo* evaluation of drugs prior to entering clinical trials.

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