Journal of Immunological Methods 410 (2014) 3–17

Contents lists available at ScienceDirect

Journal of Immunological Methods

journal homepage: www.elsevier.com/locate/jim

Review

Generation of improved humanized mouse models for human infectious diseases

Michael A. Brehm [a,1], Michael V. Wiles [b,2], Dale L. Greiner [a,3], Leonard D. Shultz [b,*]

[a] The University of Massachusetts Medical School, 368 Plantation Street, Worcester, MA 01605, United States
[b] The Jackson Laboratory, 600 Main Street, Bar Harbor, ME 04609, United States

ARTICLE INFO

Article history:
Received 14 January 2014
Accepted 18 February 2014
Available online 4 March 2014

Keywords:
NSG
Humanized mice
Immunodeficient mouse
Immune response
Infectious agents
Animal model

ABSTRACT

The study of human-specific infectious agents has been hindered by the lack of optimal small animal models. More recently development of novel strains of immunodeficient mice has begun to provide the opportunity to utilize small animal models for the study of many human-specific infectious agents. The introduction of a targeted mutation in the IL2 receptor common gamma chain gene (IL2rg^{null}) in mice already deficient in T and B cells led to a breakthrough in the ability to engraft hematopoietic stem cells, as well as functional human lymphoid cells and tissues, effectively creating human immune systems in immunodeficient mice. These humanized mice are becoming increasingly important as pre-clinical models for the study of human immunodeficiency virus-1 (HIV-1) and other human-specific infectious agents. However, there remain a number of opportunities to further improve humanized mouse models for the study of human-specific infectious agents. This is being done by the implementation of innovative technologies, which collectively will accelerate the development of new models of genetically modified mice, including; i) modifications of the host to reduce innate immunity, which impedes human cell engraftment; ii) genetic modification to provide human-specific growth factors and cytokines required for optimal human cell growth and function; iii) and new cell and tissue engraftment protocols. The development of "next generation" humanized mouse models continues to provide exciting opportunities for the establishment of robust small animal models to study the pathogenesis of human-specific infectious agents, as well as for testing the efficacy of therapeutic agents and experimental vaccines.

Abbreviations: APC, antigen-presenting cell; BLT, bone marrow/liver/thymus; BRG, C.129(cg)-*Rag2*^{tm1Fwa}*9Il2rg*^{tm1Cgn}; CMV, cytomegalovirus; CRISPR, Clustered Regularly Interspaced Short Palindromic Repeats CRISPR/Cas9; EBV, Epstein–Barr Virus; ESC, embryonic stem cell; FDC, follicular dendritic cell; G-CSF, granulocyte-colony stimulating factor; GM-CSF1, granulocyte/macrophage-colony stimulating factor; GVHD, graft-versus-host disease; HIV-1, human immunodeficiency virus-1; HSC, hematopoietic stem cell; *IL2rg*^{null}, IL2 receptor common gamma chain gene; JCV, JC virus; LT, lymphotoxin; LTi, lymphoid tissue inducer; MERS-CoV, Middle East respiratory syndrome coronavirus; NCF1, neutrophil cytosolic factor 1; NHEJ, non-homologous end joining; NK, natural killer; NOG, NOD.Cg-*Prkdc*^{scid}*Il2rg*^{tm1Sug}; NSG, NOD.Cg-*Prkdc*^{scid}*Il2rg*^{tm1Wjl}; NSG-(*K*^{b}*D*^{b})^{null}, NOD.Cg-*Prkdc*^{scid}*Il2rg*^{tm1Wjl} *H2-K1*^{tm1Bpe} *H2-D1*^{tm1Bpe}/Sz; PAMP, pathogen-associated molecular patterns; PBL, peripheral blood lymphocytes; PML, progressive multifocal leukoencephalopathy; PRR, pattern recognition receptors; SIRPa, signal regulatory protein alpha; SRC, scid repopulating cell; TALEN, transcription activator-like effector nuclease; TLR, Toll-like receptors; TNF, tumor necrosis factor; ZFN, zinc finger nucleases.

* Corresponding author. Tel.: +1 207 288 6405.
E-mail addresses: michael.brehm@umassmed.edu (M.A. Brehm), michael.wiles@jax.org (M.V. Wiles), dale.greiner@umassmed.edu (D.L. Greiner), lenny.shultz@jax.org (L.D. Shultz).
[1] Tel.: +1 508 856 3130.
[2] Tel.: +1 207 288 6766.
[3] Tel.: +1 508 856 1911.

http://dx.doi.org/10.1016/j.jim.2014.02.011
0022-1759/

Contents

1. Introduction

There are a number of human-specific infectious agents for which small animal models are critically needed to permit efficient and cost-effective evaluation of disease pathogenesis, therapeutic responses in vivo, and for the development of new vaccines, all without putting individuals at risk. Since many of these agents only infect human cells and tissues (Baumler and Fang, 2013; Wolfe et al., 2007), traditional small animal models such as mice and rats cannot be used as hosts for infection. In addition to the human-specific nature of many infectious agents, there are also cell and tissue-specific requirements for infection (Baumler and Fang, 2013; Wolfe et al., 2007). For example, *Neiserria gonorrhoeae* infects only human epithelial cells due to their requirement for binding to human CEACAM1 glycoprotein to enter the cell, a protein that differs between humans and other species (Voges et al., 2012). Thus, development of new small animal models for the study of these human-specific and cell and tissue-specific agents requires engraftment into animals of multiple types of human cells and tissues, including those from human hematopoietic and immune systems. The development of "next generation" humanized mice will accelerate investigation of currently known human-specific infectious agents including, for example, human immunodeficiency virus type 1 (HIV-1) and will support rapid identification and study of new emerging human-specific infectious agents for example the Middle East respiratory syndrome coronavirus (MERS-CoV, http://www.who.int/csr/don/2013_05_22_ncov/en/index.html).

2. Human immune system engrafted humanized mice

For the engraftment of functional human immune system in immunodeficient mice, three major model systems, described below, are commonly used. The protocols for establishing each of these models have been reviewed recently (Shultz et al., 2012; Rongvaux et al., 2013; Ito et al., 2012). Each of the model system has its strengths and limitations for the study of human immunobiology. It is these limitations that provide fresh opportunities for improvements in the models for the study of human infectious diseases and for the evaluation of vaccines.

2.1. Hu-PBL-SCID

The simplest approach to engraft a human immune system is by injection of human peripheral blood lymphocytes (PBLs) into adult immunodeficient mice, and is termed the Hu-PBL-SCID model (Mosier et al., 1988). In this system, PBLs are injected intraperitoneally or intravenously into non-irradiated or conditioned, usually sublethally-irradiation conditioning, recipients. The primary population of engrafting cells is the T cell (Mosier et al., 1988; King et al., 2009; Ito et al., 2002). All introduced T cells rapidly acquire an activated phenotype after one week, and few B cells, myeloid cells or other immune cells can be detected (Ito et al., 2002; King et al., 2009). This model is used to study effector T cell activity, and resulting Hu-PBL-SCID mice have been shown to be capable of mediating human skin and islet allograft rejection (King et al., 2008; Racki et al., 2010). However, the model is limited with the window for experimental observation being relatively short as all engrafted mice will develop a lethal xenogeneic graft-versus-host disease (GVHD) within a few weeks (Ito et al., 2002; King et al., 2009).

2.2. Hu-SRC-SCID

A second model, known as Hu-SRC-SCID, is established by the injection of human CD34^{+} hematopoietic stem cells (HSCs), defined functionally as scid-repopulating cells (SRCs), into newborn or adult immunodeficient recipients (Lapidot et al., 1992). Human HSCs are usually obtained from the bone marrow, umbilical cord blood, granulocyte-colony stimulating factor (G-CSF) mobilized peripheral blood or fetal liver, with fetal liver and cord blood being the most commonly used as sources as they are more efficient in repopulating immunodeficient mice than adult HSCs (Matsumura et al., 2003; Lepus et al., 2009). In the Hu-SRC-SCID model complete human hematopoietic and immune systems develop, however human T cells undergo thymic education through positive and negative selection on mouse thymus and are mouse MHC (H2)-restricted, precluding appropriate HLA-restricted interaction of human antigen-presenting cells (APCs) and human T cells in peripheral tissues (Watanabe et al., 2009). The Hu-SRC-SCID model has been used extensively for the study of human hematopoiesis, cell-mediated immunity, as well as infectious diseases such as HIV and Epstein–Barr Virus (EBV) (Shultz et al., 2012; Rongvaux et al., 2013; Ito et al., 2012).