Published in final edited form as:
Nat Rev Immunol. 2012 November ; 12(11): 786–798. doi:10.1038/nri3311.

Humanized mice for immune system investigation: progress, promise and challenges

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Abstract

Preface—Significant advances in our understanding of the *in vivo* functions of human cells, tissues and immune systems have resulted from the development of mouse strains that are based on severely immunodeficient mice expressing mutations in the interleukin-2 (IL-2) receptor common γ-chain locus. These mouse strains support the engraftment of a functional human immune system and permit detailed analysis of human immune biology, development and functions. In this Review, we discuss recent advances in the development of humanized mice, the lessons learned, the remaining challenges and the promise of using humanized mice for the *in vivo* study of human immunology.

Introduction

A fundamental understanding of many biological processes in humans has stemmed from experimental studies in animal models, particularly in rodents. Using these models, key aspects of the development and regulation of the haematopoietic and immune systems have been elucidated at the cellular and molecular levels. However, many aspects of mammalian biological systems, particularly their immune systems, are species specific. Moreover, rodents are refractory to certain human specific infectious agents, and many of the new therapeutic and immunomodulatory reagents that have been developed are human specific.

For many years, investigators have relied on chimpanzees to bridge the final gap between rodent models and humans. However, the use of chimpanzees for biomedical research has been banned in Europe, and a recent NIH directive has stopped all new research on chimpanzees in the United States (http://www.nih.gov/news/health/dec2011/od-15.htm). Based on these restrictions it is unclear whether experimentation in chimpanzees will be a feasible approach in the future for moving discoveries made in rodents through the pre-clinical phase to human trials.

Therefore, to address the limitations of translating discoveries in rodents into clinical applications, sophisticated small animal models that more closely recapitulate human biological systems, termed “humanized” mice, are more acutely required. We define humanized mice in this review as immunodeficient mice that have been engrafted with

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Competing interest statement: D.L.G. and L.D.S. are consultants for ViaCord Inc and Biogen/IDEC Inc. M.A.B. receives grant support from Biogen/IDEC Inc. J.V.G. is a consultant for Calimmune, GSK,and Boehringer Ingelheim.

human primary haematopoietic cells and tissues that generate a functional human immune system. We[1] and others[2, 3] have reviewed the history of humanized mice. . There has been a rapid growth in use of humanized mice that emanated from breakthroughs following the development of immunodeficient mice with a mutation in the interleukin-2 (IL-2) receptor γ-chain locus (*Il2rg*; also known as γ_c and CD132) in the mid 2000's[4-6]. The *Il2rg* mutation leads to severe impairments in B and T cell development and function, and completely prevents natural killer (NK) cell development. In addition, this mutation affects the development of lymph node anlagen, resulting in poor lymph node development and organization. The focus of this Review is on the progress in the generation and use of new models of immune engrafted humanized mice that has occurred over the last few years, the limitations present in the earlier models that have recently been overcome, the challenges yet to be conquered and the promise that humanized mice provide for the future of biomedical research.

Model systems and technologies

To understand the progress, promise and remaining challenges in the use of humanized mice, it is important to recognize the variety and complexity of the model systems being used in a particular study to appropriately interpret the results. There are many and quite diverse strains of immunodeficient mice with a mutant *Il2rg* gene that have been developed, as well as a complex nomenclature surrounding the various strains and models (Text Box 1). Any one specific model will not be optimal for addressing the myriad of questions that might be considered, and it is important to not only choose the appropriate model system for the specific question at hand, but to also be innovative in designing questions and experiments that can provide valid data that can be properly interpreted using the individual models.

There are three main platforms of mouse strains that are used: NOD.Cg-*Prkdc*^{scid}*Il2rg*^{tm1Wjl} (NSG), NOD.*cg-Prkdc*^{scid}*Il2rg*^{tm1Sug} (NOG), and C;129S4- *Rag2*$^{tm1.1Flv}$ (BRG), Text Box 1). Several of the strains of NSG mice and BRG mice are available from The Jackson Laboratory (http://www.jax.org/), whereas NOG mice are available from Taconic (http://www.taconic.com/wmspage.cfm?parm1=16). The derivation and unique characteristics of each of the immunodeficient mouse models derived from immunodeficient *Il2rg*^{null} mice have been reviewed in detail[1].

Recent evidence shows that these mouse models differ in their ability to support the engraftment of functional human immune systems. For example, NSG and NRG mice support higher levels of human haematopoietic stem cell (HSC) engraftment and T cell development than do BRG mice[7] and NSG mice support higher levels of human HSC engraftment in bone marrow than do NOG mice[8]. Choice of the specific mouse model will impact the engraftment levels of the transplanted human haematopoietic and immune systems, the types of cells that can be successfully engrafted, and the functional capabilities of specific immune cells.

There are four different technological approaches for the engraftment of a functional human immune system in these immunodeficient mouse models, each with distinct advantages and caveats (Text Box 2, Figure 1). Depending on the question, the investigator will need to choose the appropriate human immune system engrafted mouse for their studies.

Overcoming limitations in humanized mice

There were previously several limitations inherent in humanized mice due, in part, to mouse-versus human-specific differences in the growth factors and cytokines required for human haematopoietic and immune system development (Figure 2), and due to the presence

of mouse MHC versus human HLA molecules (reviewed in REF [1]). Additional limitations included deficiencies in the development of lymph nodes, poorly organized lymphoid architecture and impaired affinity maturation and class switching of antibodies following immunization in the humanized mice[1]. In addition, species-specificity of homing molecules may impede the appropriate trafficking of human immune cells. Finally, remaining mouse innate immunity in the humanized mouse models represented a continuing impediment to human cell engraftment and investigation of human immune function[1].

Expression of human transgenes and targeted inactivation of mouse genes encoding factors that control the development and function of the innate immune system have recently addressed many of these limitations, resulting in increased human immune system function in the humanized mice. The technological approaches for developing these "next generation" immunodeficient *Il2rg*^{null} mice are quite diverse, and differences in the recipient strain and levels of functional engraftment of human immune systems differ depending on the technology used.

Technologies to deliver human species-specific factors to enhance human haematopoiesis and immune system development and function are also diverse. The simplest approach is to provide the needed factors by injection of recombinant proteins (reviewed in REF [9]). Other technologies include the transduction of human HSCs with genes encoding factors such as mouse CD47 [10], which regulates phagocytic uptake of cells (that is, a do not eat me signal), and delivery of plasmid DNA encoding human cytokines by hydrodynamic tail vein injection [11]. In addition, lentiviral vectors have been used to deliver human IL7 into BRG mice, promoting homeostatic proliferation of both adoptively transferred and endogenously generated T cells[12].

As described below, three main genetic engineering technologies have been adopted: transgenic expression of cDNA constructs driven by tissue-specific or ubiquitous promoters; transgenic expression of bacterial artificial chromosomes (BACs); and knock-in technology.

New tools

Strains of transgenic immunodeficient *Il2rg*^{null} mice have been developed using cDNA constructs expressing human genes under the control of host tissue specific or ubiquitous promoters. However, this approach often leads to transgene expression at non-physiological levels or lacking temporal controls leading to adverse effects on the development and function of the human immune system in humanized mice. A second approach is the use of human bacterial artificial chromosomes (BACs) that often contain critical regulatory elements for gene expression. The use of BACs to create transgenic mice facilitates gene expression at physiological levels, when appropriate during development, and when functionally needed. Using these transgenic approaches, the homologous mouse gene is left intact and therefore both the mouse and human gene of interest are expressed. A third approach is to use knock-in technology in which the mouse gene is replaced by the corresponding human gene, resulting in the expression of the human gene product in the absence of the mouse gene product. This strategy has been successfully used for multiple genes in C;129RG mice due to the availability of embryonic stem (ES) cells of the C;129RG strain[3]. These ES cells can also be used to develop knockout mice in which a targeted mouse gene is specifically inactivated. The development of knock-in and knockout mice on the NOD strain was previously hindered by the lack of available NOD ES cells, but this limitation has recently been overcome by the generation of ES cells based on the NOD[13] and NSG strain (http://research.jax.org/collaboration/escell.html) and will result in the development of knock-in and knockout NSG and NRG mice.

Additional emerging technologies that can alter the genome of mice have been described, including zinc finger technology (ZFN) and the transcription activator like effector nuclease (TALEN) technology. Zinc finger nucleases are engineered DNA-binding proteins that cause double stranded DNA breaks at specific locations and enables targeted editing of the genome[14]. TALENS utilize transcription activator-like effectors, secreted by plant bacteria that can be designed to bind to specific DNA sequences[15]. ZFN and TALEN technology can be applied directly in embryos and thus can be used for the development of new strains with increasing genetic complexity that cannot easily be generated using traditional crosses. Although, neither ZFN nor TALEN technology have yet been applied to the field of humanized mice, these technologies will likely make an important impact on future humanized mouse model development.

New models

Many genetically modified strains of immunodeficient *Il2rg*^{null} mice are now available for the study of human immunobiology (Table 1) and it is likely that many new stocks will soon be available.

Using knock-in technology, three new strains of BRG mice expressing human haematopoietic growth factors that are important in myeloid cell development have been developed[3]. As expected, each human growth factor enhanced the development of the human cell lineage targeted by that specific factor. For example, transgenic expression of macrophage colony-stimulating factor (CSF1) increased the frequencies of human monocytes and macrophages following human HSC engraftment and enhanced the production of pro-inflammatory cytokines following challenge with the Toll-like receptor 4 (TLR4) agonist lipopolysaccharide[16]. However, adverse events were also observed. For example, in the thrombopoietin (TPO) knockin strain, increased myelopoiesis and HSC self-renewal was observed, but thrombocytopaenia developed[17]. Although human TPO can support murine thrombocytopoiesis, the levels of human TPO that were expressed in the mouse was ~10 fold lower than the normal levels of mouse TPO. This led to decreased numbers of mouse platelets, and because only very low levels of human platelets circulate in the blood of humanized mice, thrombocytopaenia developed. In IL3 and granulocyte macrophage colony-stimulating factor (GM-CSF) knockin mice, human HSC engraftment resulted in enhanced development of human alveolar macrophages, but non-engrafted mice developed pulmonary alveolar proteinosis caused by the absence of mouse GM-CSF[18].

Transgenic expression of three hematopoietic growth factors (IL3, GM-CSF and SCF) under the control of a ubiquitous promoter led to increased frequencies of human myeloid cells in the bone marrow of NSG mice engrafted with human HSC[19]. However, earlier studies with these co-integrated transgenes showed that GM-CSF-mediated HSC mobilization resulted in reduced frequencies of human HSCs in the bone marrow compared with non-transgenic NOD-*scid* mice[20]. Increased levels of $CD4^{+}Foxp3^{+}$ T cells in the peripheral tissues were also apparent following HSC engraftment of triply transgenic NSG mice, suggesting that non-physiological exposure to cytokines during T cell development can alter the cell population generated[19]. This deficiency might be overcome using the knockin approach in which physiological levels of cytokines would be expected. In contrast, the transgenic expression of membrane-bound human SCF (mbSCF) driven by a ubiquitous promoter results in increased human mast cell levels and enhanced HSC engraftment in NSG mice with no reported pathological changes in the murine host[21]. Moreover newborn NSG mice expressing mbSCF support high levels of human HSC engraftment without the need for X-irradiation conditioning[22]. A caveat associated with expressing human factors in immunodeficient mice is that although some murine cytokines do not function on human cells, many of the human factors cross-react with murine cells[23]. This could cause unexpected phenotypic changes. For example, transgenic expression of a factor used to

increase levels of human innate immunity following human HSC engraftment could upregulate mouse innate immunity and reduce levels of human engraftment. In addition, even cross-reactive mouse cytokines that work *in vitro* may have lower *in vivo* potency than their corresponding human cytokines.

Additional immunodeficient mouse strains have been developed to minimize reactivity of human immune cells against host tissue and include NSG MHC class I- and MHC class II-deficient mice[24, 25]. These mice were specifically designed to reduce xenogeneic GVHD, and when combined with transgenic expression of human HLA molecules, they permit the development of HLA-restricted human T cells in the absence of H2-resticted human T cells in Hu-SRC-SCID mice[25].

Although the current models are appropriate for addressing many questions of human immune system development and function, recognition of remaining deficiencies in the models continues to evolve. These deficiencies are systematically being addressed using genetic approaches to improve human immune system engraftment and function. Continued development of mice expressing human-specific growth factors and HLA molecules, combined with additional approaches to reduce murine H2 expression and decrease host innate immunity, promise to provide models with increased levels of human immune system engraftment and improved functional activity.

Lessons learned from humanized mice

Haematopoiesis

To generate a functional human immune system that contains multiple cell lineages required for innate and adaptive immunity, immunodeficient *Il2rg*^{null} mice are engrafted with human HSCs. For many years, it was known that NOD-*scid* mice engrafted at higher levels with human HSCs than did immunodeficient CB17, BALB/c and C57BL/6 mice[1]. Of interest was the report that the signal regulatory protein α(*Sirpa*) gene expressed in NOD mice has a polymorphism that is very similar to the human gene, and that the SIRPα expressed by C57BL/6 and BALB/c mice have much less homology to human SIRPα[26]. Appropriate interaction of SIRPα on host macrophages with human CD47 on engrafted haematopoietic cells is important for hematopoietic cell survival and therefore expression of human or NOD (human-like) SIRPα is a major determinant for engraftment and survival of human haematopoietic cells in mice[26]. BRG mice transgenically expressing a human SIRPα increase engraftment to levels achieved in NSG and NRG mice, indicating that SIRPα is a causal factor in controlling engraftment levels[27]. A NSG mouse strain transgenically expressing human SIRPα is currently under development to identify additional strain-specific factors important in human HSC engraftment.

Key insights into the biology of human immune system development from HSCs have been gained using humanized mice. HSCs derived from human fetal liver, fetal bone marrow and adult bone marrow were used to engraft immunodeficient mice[28]. Fetal liver and fetal bone marrow generated higher numbers of $CD4^+CD25^+Foxp3^+$ regulatory T (Treg) cells in the thymus than did adult HSCs. The data suggest a “layering” of immune system development during ontogeny in humans that is due to intrinsic differences in HSC populations, leading to distinct immune systems in fetal versus adult individuals[28]. Another report using humanized mice showed that human myeloid and plasmacytoid dendritic cells can arise from a common precursor that is independent of conventional myeloid and lymphoid haematopoietic pathways[29]. Humanized mice have also been used to analyze the *in vivo* function of HSCs derived from human induced pluripotent stem (iPS) cells. Although the human iPS cell-derived $CD34^+$ HSC had bipotential ability to generate both hematopoietic and endothelial lineages *in vitro*, these studies demonstrated preferential differentiation into endothelial cells

in vivo[30]. Investigations into potential mechanisms of human immune system development and function following engraftment of HSCs in humanized mice will continue to provide insights in human immune cell differentiation and function.

Immune system

Approaches for studying human immunity in humanized mice include engraftment of mature lymphoid cells (the Hu-PBL-SCID model) or stem cells (the Hu-SRC-SCID and the BLT models).

The use of Hu-PBL-SCID mice to study effector mechanisms in a xenogeneic GVHD system has focused on the efficacy of drugs and Treg cells in preventing disease[24]. Recent modifications of the Hu-PBL-SCID model have permitted investigation of the infiltration and rejection of human islet, skin and arterial allografts and the cell populations involved in the immune rejection process[31-33]. The transfer of human Treg cells that were expanded *ex vivo* prevented rejection of human skin and arterial grafts in the Hu-PBL-SCID model[34, 35]. These observations demonstrate that human Tregs can modulate transplantation rejection and that humanized mice provide both a platform for testing the efficacy of Tregs as a cellular therapy as well as a tool with which to identify the mechanisms underlying their in vivo regulatory activity.

Of interest was the demonstration that survival of adoptively transferred antigen-specific central memory T cells is IL-15 dependent, and that this T cell population is superior to effector memory T cells in their ability to survive in adoptive recipients, providing guidance for clinical trials of adoptive T cell therapy[36]. Although rejection of allografts and the development of xenogeneic GVHD have been readily demonstrated in Hu-PBL-SCID mice, antigen-specific activation of T cells has been much harder to document, perhaps due to the limited engraftment of human APCs in this model[24]. To address this, donor-matched human dendritic cells have been transferred with PBLs to NSG mice. Following immunization, adenovirus antigen-specific T cells developed[37], and this model therefore provides an opportunity to test vaccines and investigate mechanisms underlying antigen-specific T cell immune responses.

One caveat of interpreting results following engraftment of human effector T cells from PBL or other sources in the Hu-PBL-SCID model is the confounding effect of the xenogeneic GVHD response, due primarily to human T cell reactivity against mouse MHC molecules[24]. To address this, NSG mice lacking MHC class I and class II molecules (NSG $B2m^{-/-}$ mice and NSG $I\text{-}A^{-/-}$ mice, respectively) have been developed and these mice have reduced xenogeneic GVHD[24]. Xenogeneic GVHD is further reduced following engraftment of NSG $IA^{-/-}$ mice with purified human $CD4^+$ T cells[25]. When human HLA-$DR4^-CD4^+$ T cells are engrafted into NSG-HLA-DR4 transgenic mice lacking mouse MHC class II molecules (NSG $I\text{-}A^{-/-}$ Tg(HLA-DR4), an allogeneic GVHD response develops in the absence of xenogeneic GVHD, providing for the first time a model system for the study of human allogeneic GVHD[25].

Although all lineages of immune cells (including T cells) are generated in Hu-SRC-SCID and BLT mice, the immune system generated is not as robust as desired[1]. Splenic germinal centers are poorly organized, preventing the development of a robust antigen-specific antibody response[23]. Antigen-specific human IgM antibody responses and IgM hybridomas are generated following immunization of HSC-engrafted mice, but achievement of affinity maturation and class switching from IgM to IgG isotype has been particularly difficult[38]. A recent study has suggested that most human B cells that develop in HSC-engrafted humanized mice are immature $CD5^+$ B cells[39]. However, antibody repertoires in human B cells generated in HSC-engrafted NSG mice are very similar to the repertoire observed in B

cells in humans[38, 40]. Also, mouse B cell activating factor (BAFF; also known as BLyS), which is a critical B cell differentiation and survival factor, is ineffective on human B cells[41]. Administration of recombinant human BAFF enhances B cell engraftment and survival in the Hu-PBL-SCID model, in which human B cells usually fail to efficiently engraft[41]. Use of recombinant human BAFF during immunization of humanized mice may support affinity maturation of the immune response and class switching from IgM to IgG isotype and analysis of immunodeficient *Il2rg*^{null} mice transgenically expressing BAFF is underway. Furthermore, providing both human BAFF and IL-7 may further enhance the antibody response in humanized mice[41, 42].

Another critical factor may be the requirement for HLA expression in the mouse host. Engraftment of HLA-DR4$^+$ HSCs into NSG or NOG Tg(HLA-DR4) mice permitted generation of human IgG antibodies following tetanus toxoid vaccination, although antibody levels were still much lower than observed in immunized individuals[43, 44].

In contrast to difficulties in generating antigen-specific B cell responses in Hu-SRC-SCID and BLT humanized mice, they readily generate antigen-specific T cell responses. NSG mice develop a highly diverse T cell repertoire following engraftment with cord blood HSCs[45] and human CD4$^+$, CD8$^+$ and Treg cells develop in both the Hu-SRC-SCID and BLT models[7, 46]. Delayed type hypersensitivity (DTH) responses can be elicited in humanized mice, and IL-17-specific antibody can block the DTH response to collagen V, suggesting a role for IL-17 in human DTH responses[47].

Moreover, a human T cell-mediated immune response to the superantigen toxic shock syndrome toxin 1 (TSST1) has been demonstrated in BLT mice, permitting the mechanisms underlying this response to be investigated and effective therapies to be tested[48]. The development of functional human T cells in humanized mice can be improved by administration of recombinant human IL-7[5, 42]. However, insights into the role of IL-7 in human T cell development and function using humanized mice suggests that the timing of administration of human IL-7 is critical, as exposure to IL-7 prior to the entrance of T cell progenitors into the thymus impedes rather than enhances human T cell development and function[49]. Of great interest is the use of humanized mice to study the mechanisms underlying human-specific drugs that target T cells. For example, mechanisms underlying the beneficial effects of Teplizumab (a CD3-specific antibody) in type 1 diabetics was shown using humanized mice to induce gut-tropic Treg cells, and this observation was then confirmed in patients treated with Teplizumab[50].

Human allograft rejection has been studied in the Hu-SRC-SCID model. Using BRG mice, islet allograft rejection was not observed, likely due to the low levels of human T cells that are generated in this model[51]. In contrast, using NSG mice that develop higher levels of human T cells[7], islet allograft rejection was observed[52]. A variation combining the Hu-PBL-SCID and Hu-SRC-SCID models has been used to study human allograft rejection.

A Hu-SRC-SCID model generated by engraftment of mobilized peripheral blood stem cells was used to generate B cells and macrophages in the absence of naive T cells. In this study CD34$^+$ enriched circulating mononuclear cells isolated by leukapheresis following G-CSF mobilization were injected along with adoptive transfer of PBMCs autologous to the HSC donor. This treatment led to rejection of human artery allograft endothelial cells[33]. Additional studies using this model demonstrated that effector memory CD4+ T cells directly recognize and destroy allogeneic endothelial cells in the tissue graft, and that treatment with a neutralizing human IL-6-specific antibody enhances human Treg cells and prevents rejection[53].

Humanized mice can also be used to study cells of the innate immune system. NK cells develop in HSC-engrafted mice[1]. However, their ability to kill targets is not robust. Pre-activation of NK cells or trans-presentation of recombinant human IL-15 enhances human NK cell development in humanized mice, and they begin to acquire cytotoxic activity[54, 55]. However, complete "licensing" of NK cells will require human HLA transgenic expression by the murine host to permit fully functional human NK cell development. An additional component of the human immune system that has only recently been able to be studied in rodents due to species-specific differences is the CD1 antigen presenting system. Using NSG BLT mice, it was shown that a functional human CD1-dependent cellular compartment can develop in these mice[56]. This was confirmed by CD1d tetramer staining of human invariant NKT (iNKT) cells, secretion of IFNγ by CD1-restricted iNKT cells, and rapid production of IFNγ and IL-4 following injection of α-galactosylceramide[56]. This now permits for the first time investigation of the human CD1 system in a small animal model.

Infectious diseases

A major experimental use of humanized mice over the last few years has been for the study of human infectious agents (Table 2). An example of the utility of Hu-SRC-SCID mice for infectious disease experiments was the study of *Salmonella enteric* serovar Thyphi infection[57, 58]. *S.* Typhi is highly adapted to humans and fails to cause progressive infection in mice. Much of the current understanding of *Salmonella* pathogenesis resulted from studies of *S.* Typhimurium in mice, but many differences exist between *S.* Typhi and *S.* Typhimurium. In a report using NSG Hu-SRC-SCID mice infected with *S.* Typhi, granulomatous inflammation with mononuclear cell infiltration in the spleen, which mimics human pathology, was observed, and infections induced rapid fatality. Screening of transposon pools in infected NSG Hu-SRC-SCID mice revealed previously unrecognized *Salmonella* virulence determinants[57]. In another report using BRG Hu-SRC-SCID mice, prolonged survival following *S.* Typhi infection was associated with innate and adaptive human immune responses[58]. These differences in pathogenesis and lethality following *S.* Typhi infection highlight the differences in the different strains of humanized mice (NSG versus BRG mice), each having its own advantages and constraints.

A NOG HSC-engrafted model of Epstein-Barr virus was described that closely mimicked the pathological conditions observed following infection of humans with EBV, resembling a syndrome termed hemophagocytic lympho-histiocytosis, a rare but devastating disorder in EBV-infected individuals.[59] The availability of HLA-A2- transgenic immunodeficient *IL2rg*^{null} mice permitted extension of infection models to the study of HLA-A2-restricted human CD8$^+$ T cell responses to infectious agents[60-62]. Infection of HSC engrafted immunodeficient NSG-HLA-A2 transgenic mice with Epstein–Barr virus (EBV) led to the development of HLA-A2-restricted human CD8$^+$ T cell responses and protective CD4$^+$ and CD8$^+$ T cell-mediated immunity[60, 61]. A humanized mouse model of EBV infection is particularly important, as pre-symptomatic stages of EBV infection in humans are largely unexplored but can now be investigated in humanized mice. HLA-A2-restricted human T cell responses have also been observed following dengue virus infection of NSG-HLA-A2 transgenic mice engrafted with HSCs or in BLT mice[62]. Infected humans can develop severe capillary leakage syndrome (dengue hemorrhagic fever) following a second infection with a different dengue virus serotype, although little is known about the immune response or mechanisms by which this occurs[63]. Humanized mice may become a useful model for studying the role of prior immunity to dengue virus in contributing to this disease[63].

One of the greatest impacts of humanized mice has been in the field of HIV research. HIV infection of humanized mice has utilized all four models (Hu-PBL-SCID, Hu-SRC-SCID, SCID-Hu, and BLT) of immune system engraftment, and have been extensively reviewed

elsewhere[64-67], therefore only key recent advances are discussed here. Numerous studies have tested HIV therapeutic interventions that limited viral replication and $CD4^+$ T cell depletion. These therapeutic interventions include: systemic antiretroviral therapy[68], systemic delivery of HIV-neutralizing antibodies[69, 70], transplantation of genetically modified HSCs[71, 72] and novel approaches for delivering small inhibitory RNA to either cellular or viral targets[73-75]. In addition, recent studies using antiretrovirals to suppress viremia demonstrated that HIV latency in $CD4^+$ T cells occurs in humanized mice, highlighting the value of these models for evaluating HIV eradication strategies[76-78]. In a related study, Hu-SRC-SCID mice were used to examine the potential of HSCs to serve as a reservoir of latent HIV[79]. In the BLT model, robust mucosal human immune systems render these animals susceptible to rectal and vaginal HIV transmission[80, 81]. The susceptibility of BLT mice to mucosal HIV transmission permits evaluation of HIV prevention strategies using topically or systemically applied viral inhibitors[75, 80, 81]. Importantly, infection of BLT mice results in rapid systemic dissemination of the virus and peripheral $CD4^+$ T cell depletion including gastrointestinal $CD4^+$ T cells, similar to what is observed in humans[80]. Together these studies demonstrate the broad applicability of humanized mice in pre-clinical HIV research.

Both the function and HIV-associated dysfunction of the human immune system have been described in HIV infected humanized mice. In BLT mice, the production of low levels of HIV-specific IgG was detected by 8 weeks post-infection[82] and by 16 weeks all infected mice were producing human HIV-specific immunoglobulin[83]. Furthermore, HIV-epitope restricted cytotoxic T-lymphocyte (CTL) responses were detected in HIV-infected BLT mice[83]. Additional evidence of CTL function against HIV in humanized mice include the finding that depletion of human $CD8^+$ T cells accelerated HIV-induced immunopathology, suggesting a role of these cells in controlling infection[84]. Extensive sequencing analysis showed that immune selective pressure drove evolution of the HIV env gene in Hu-SRC-SCID mice[85], and functional innate immune responses mediated by the antiretroviral cytidine deaminase APOBEC3 toward HIV have been demonstrated[86]. Immune dysfunction in HIV disease is, in part, characterized by abnormal immune activation[87] and a similar activated T cell phenotype has been recently described in BLT mice[88]. Hu-SRC-SCID mice have been utilized to demonstrate that HIV infection activates and impairs plasmacytoid DC function[89]. Finally, the influx of human macrophages into the brain of Hu-SRC-SCID mice exhibiting progressive HIV infection was linked with viral neuropathogenesis[90, 91]. Although immune responses generated in humanized mice are not protective against infection, these studies reveal functional human immune responses in humanized mice and show that known and new HIV-induced immune dysfunctions can be studied in these models.

Autoimmunity

There are only a few reports of successfully recapitulating autoimmune diseases in humanized mice. In one study using the Hu-PBL-SCID model engrafted with PBLs from individuals with systemic lupus erythematosus (SLE), mice developed microthrombi and infiltration of B and T cells into the glomeruli, which are features of SLE[92]. In another report, HLA-A2-matched PBLs from individuals with type 1 diabetes (T1D) transferred to NSG-HLA-A2 mice allowed recovery of islet-associated T cells, suggesting that this might provide a model for studying diabetogenic T cells[93]. Using the Hu-SRC-SCID model, bone marrow-derived HSCs from patients with osteoarthritis transferred into anti-CD122-treated NK cell-depleted NOD-*scid* mice developed synovial inflammation following intra-articular injection of *Chlamydia trachomatis* to induce arthritis[94]. To date, studies of the immunological mechanisms of autoimmunity have yet to be performed using these models. However, as the next generation of humanized mice becomes available, modeling of

autoimmune disorders and investigation into the immunological mechanisms underlying autoimmune diseases will follow.

Tumour immunology

Humanized mice have permitted new types of human tumours to be grown and studied in vivo[1]. These new models have been used to characterize cancer stem cells and other facets of tumour biology [95, 96], and reports are now appearing on features of the human anti-tumor immune response. Transplantation of primary lung tumors revealed the existence of tumor infiltrating effector memory T cells that could be activated by administration of human IL-12[97]. Primary human epithelial ovarian tumors engrafted in NSG mice also contain human T cells and efforts are underway to activate the intra-tumor T cells for anti-ovarian tumour immunity[98].

Humanized mice have also been used to study tumour therapies. CD47 is expressed on most solid human tumours, and administration of human CD47-specific antibody to human tumour-bearing NSG mice leads to tumour cell phagocytosis and elimination, demonstrating that tumour-engrafted NSG mice can be used to evaluate antibody-mediated cancer therapies[99]. Engraftment of BLT mice using HSCs transduced with anti-tumor $CD8^+$ T cell receptors have been shown to control human melanomas[100]. This model will be important for optimizing human immune responses to melanomas for cellular adoptive therapies. Efficacy of adoptive transfer of human T cells genetically engineered to express melanoma antigen-specific T cell receptors has been evaluated in NOG mice[101]. In a novel approach, newborn NSG mice were co-engrafted with human HSCs and human breast cancer cells. The tumours grew in the presence of the immune system, and the generation of tumour-specific T cells was observed, as well as NK cell accumulation at the tumour site[102]. Understanding the resistance of the tumour to the engrafted immune system and how to modulate the immune system to overcome this resistance will provide new approaches for cancer immunotherapy.

Future promise and remaining challenges

The new models that have been described and used to study human immunity are only the beginning of the next generation of humanized mice that will soon be available (Figure 2). Remaining challenges are to generate new strains of mice needed for further enhancement of human immunity with reduced mouse innate immunity (Table 3)[1, 2].

A critical issue remaining is the limited development of lymph nodes in immunodeficient *Il2rg*^{null} mice. Although mesenteric lymph nodes develop, other peripheral lymph nodes are small with little to no lymphoid organization[103]. Current approaches to address this include administration of human IL-7, which is critical in neonatal maturation of lymph nodes[104] and provision of human agonist anti-lymphotoxin β receptor during a critical early maturation step in lymph node development[105]. One of the cell populations deficient in *Il2rg*^{null} mice is the lymphoid tissue-inducer cell, which is required for lymph node and gut-associated lymphoid tissue formation. Efforts to identify approaches to enhance development of these cell populations are continuing.

Another limitation being addressed is the generation of immunodeficient *Il2rg*^{null} HLA transgenic mice that represent many of the common HLA class I superfamilies[106]. For the study of autoimmunity, HLA class II transgenic NSG mice are being developed. These new models will facilitate investigations of T1D, multiple sclerosis and rheumatoid arthritis, autoimmune disorders strongly associated with specific HLA class II alleles. For the study of human NK cells, NSG-HLA transgenic mice expressing HLA-B27 or HLA-Cw*0304, which are important in NK cell licensing, are under study. Use of HLA transgenic mice co-

expressing IL-7 and/or BAFF may increase T and B cell function, respectively, and enhance immune responses. Overcoming poor adaptive humoral immune responses in humanized mice will result in more effective models for vaccine testing.

Additional modifications are needed for studies of infectious agents that require circulating human RBCs or resident hepatocytes as part of their life cycle, such as *Plasmodium falciparum*, which has obligate stages in both hepatocytes and RBCs. Mouse macrophages are a significant impediment to the survival of human RBCs in NSG mice and depletion of mouse macrophages allows enhanced development and survival of peripheral human RBCs in BLT mice[107]. Alternatively, the study of hepatitis B and C virus infections in the presence of an immune system requires the presences of both human hepatocytes and HSCs in the humanized mouse. One approach to achieve this has been to engraft human fetal liver hepatocytes and HSCs into an immunodeficient recipient with additional genetic modifications that induced host liver cell damage. Both human liver and immune systems developed, and the mice supported infection with hepatitis C virus[108].

For proper immune function, appropriate trafficking of human immune cells to non-lymphoid tissues is required. Due to species-specific differences in adhesion molecules and potential incompatibilities of receptor-ligand pairs, human lymphocyte homing may not occur properly in immunodeficient mice. For example, there are known species-specific differences in the mouse/human lymphocyte function-associated antigen-1 (LFA-1) and intercellular adhesion molecule-1 (ICAM-1). Mouse LFA-1 can bind to human ICAM-1, but murine LFA-1 does not bind to human ICAM-1[109].

One of the final obstacles in establishing a full human chimera is the inability of a number of human hematopoietic cell lineages, including granulocytes, platelets and RBCs, to circulate and traffic appropriately in the murine host. When the murine host is conditioned sufficiently to replace essentially all of the murine bone marrow with human hematopoietic cells, the mice die, likely due to a severe anemia and/or greatly reduced levels of circulating platelets and granulocytes[21]. Although human RBCs, platelet and granulocyte progenitors are detected in the bone marrow, few of these cell populations are found in the circulation, likely due to their removal in the circulation by murine macrophages[110].

Conclusion and perspective

The diversity of humanized model strains, differences in approaches for engraftment of human cells and tissues, and the various technological approaches being used to generate the next generation of immunodeficient *Il2rg*^{null} mice leads to the key caveat in the use of humanized mice for the study of human immunology. Experiments using humanized mice, or any animal model system, need to be designed to address a mechanistic question rather than attempting to fully recapitulate the human biological process or pathology. With these caveats in mind, humanized mice are becoming increasing important tools in translational biomedical research, permitting insights into our understanding of human hematopoiesis, innate and adaptive immunity, autoimmunity, infectious disease and cancer immunology.

Acknowledgments

We thank D. Serreze and R. Maser for critical review of the manuscript. The authors are supported by the US National Institutes of Health (grants AI46629, UO1 DK089572, DK092758 and AI073146), an institutional Diabetes Endocrinology Research Center grant (DK32520), a Cancer Core Grant (CA034196), the Juvenile Diabetes Research Foundation International, and the Leona M. and Harry B. Helmsley Charitable Trust. The contents of this publication are solely the responsibility of the authors and do not necessarily represent the official views of the US National Institutes of Health.

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Glossary terms

γc A type I cytokine receptor chain that is shared by the receptors for the interleukins IL-2, IL-4, IL-7, IL-9, IL-15 and IL-21.

Severe combined immunodeficiency	(SCID). Humans or mice with this rare genetic disorder lack functional B and T cells owing to a mutation in a gene that is involved in B cell and/or T cell development, and consequently they suffer from recurrent infections. Several forms of SCID have been described, including mutations in the IL-2 receptor γ-chain, which is shared by several interleukin receptors, protein kinase, DNA activated, catalytic polypeptide (PRKDC), Janus activated kinase 3 (JAK3) deficiency and adenosine deaminase deficiency.
Graft-versus-host disease	(GVHD). Tissue damage in a recipient of allogeneic tissue (usually a bone-marrow transplant) that results from the activity of donor cytotoxic T lymphocytes recognizing the tissues of the recipient as foreign. GVHD varies markedly in extent, but it can be life threatening in severe cases. Damage to the liver, skin and gut mucosa are common clinical manifestations.
Bacterial artificial chromosome	(BAC). A cloning vector derived from a single-copy F-plasmid of *Escherichia coli* that carries the F replication and partitioning systems that ensure low copy number and faithful segregation of plasmid DNA to daughter cells. Large genomic fragments can be cloned into such vectors and they are faithfully replicated, which makes BACs useful for constructing genomic libraries.
Knock-in technology	The introduction of a transgene into a precise location in the genome, rather than a random integration site. Knocking-in uses the same technique of homologous recombination as a knockout strategy but the targeting vector is designed to allow expression of the introduced transgene under control of the regulatory elements of the targeted gene.
thrombocytopaenia	A reduced number of circulating platelets, owing to either the failure of production from bone-marrow megakaryocytes or increased clearance from the circulation, predominantly in the spleen.
Central memory T cells	Antigen-experienced T cells that lack immediate effector function but can mediate rapid recall responses. They also rapidly develop the phenotype and function of effector memory T cells after restimulation with antigen. Central memory T cells retain the migratory properties of naive T cells and therefore circulate through the secondary lymphoid organs.
Effector memory T cells	Memory T cells that home to peripheral tissues and plasma cells that home to the bone marrow and secrete antibodies. They are responsible for immediate protection to a re-infection.
Germinal centre	A lymphoid structure that arises within follicles after immunization with, or exposure to, a T cell-dependent antigen. It is specialized for facilitating the development of high-affinity, long-lived plasma cells and memory B cells.

Delayed-type hypersensitivity	A cellular immune response to antigen that develops over 24–72 hours with the infiltration of T cells and monocytes, and is dependent on the production of T_H1 cell-specific cytokines.
α-Galactosylceramide	(α-GalCer). A synthetic or marine-sponge-derived glycolipid containing an a-anomeric glycosidic linkage of the galactose residue to the sphingosine base. This lipid, and structurally related ones, potently activates CD1d-restricted natural killer T cells that express the semi-invariant Vα214–Jα18 T cell receptor in mice (and the Vα24–Jα18 equivalent receptor in humans).
Systemic lupus erythematosus	(SLE). An autoimmune disease in which autoantibodies specific for DNA, RNA or proteins associated with nucleic acids form immune complexes. These complexes damage small blood vessels, especially in the kidneys. Patients with SLE generally have abnormal B and T cell function.
lymphoid-tissue inducer cell	A cell that is present in developing lymph nodes, Peyer's patches and nasopharynx-associated lymphoid tissue (NALT). Lymphoid-tissue inducer cells are required for the development of these lymphoid organs. The inductive capacity of these cells for the generation of Peyer's patches and NALT has been shown by adoptive transfer, and it is generally assumed that they have a similar function in the formation of lymph nodes.

Text Box 1

Platforms for Creating Human Immune System Engrafted Mice (reviewed in REF[1])

Platform Stocks of Immunodeficient Mice	Common Abbreviation	Characteristics
NOD.Cg-*Prkdc*^{scid}*Il2rg*^{tm1Wjl}	NSG	The *Il2rg* targeted mutation is a complete null, is not expressed and will not bind cytokines
NOD.*cg-Prkdc*^{scid}*Il2rg*^{tm1Sug}	NOG	The *Il2rg* chain lacks the intracytoplasmic domain. It Is expressed and will bind cytokines but will not signal
C;129S4- *Rag2*$^{tm1.1Flv}$	BRG	On a mixed BALB/c x 129 *Rag2*^{null} strain. *Il2rg* is complete null, is not expressed and will not bind cytokines
Platform Genes to Create Immunodeficient Mice		
protein kinase, DNA activated, catalytic polypeptide	*Prkdc*^{scid} (often abbreviated as *scid*)	Double strand DNA repair defect that prevents TCR and BCR recombination
recombination activating gene 1 recombination activating gene 2	*Rag1*$^{-/-}$*Rag2*$^{-/-}$	T and B cell receptor recombination defects T and B cell receptor recombination defects

Text Box 2

Human immune system engraftment models

Human Immune System Models	Common Abbreviati on	Establishment of Model	Advantages	Limitations
Human Peripheral Blood Lymphocyte engrafted immunodeficient (SCID) mouse	Hu-PBL-SCID	Injection of peripheral lymphoid cells	• Easy to establish • Good T cell engraftment • Engrafts an effector/ memory T cell immune population • Model of xeno-graft-versus-host disease (GVHD)	• Predominately T cells engraft, B an myeloid cells do not engraft well • All T cells engrafted are activated • Limited window for experimentation due to GVHD • Hard to generate primary immune responses • Xeno-GVHD confounds induce human immune responses • Host APCs do not express HLA and do not interact wit engrafted T cells
Human Scid-Repopulating Cell SCID	Hu-SRC-SCID	Injection of newborn or adult mice with hematopoietic stem cells derived from fetal liver, cord blood, bone marrow or from granulocyte colony-stimulating factor (G-CSF)-mobilized peripheral blood	• Development of multiple lineages of hematopoietic cells, ncluding T and B cells, PCs, myeloid cells and NK cells. • Engraftment of newborns leads to higher levels of CD3 T cells[7] • Generates a naïve human immune system	• Human T cells are educated onmouse thymic epithelium and areH2-restricted, not HLA-restricted12 • Human-derived polymorphonuclea leukocytes, red blood cells (RBCs),and megakaryocytes are present in bon marrow but only low levels circulat in the blood
SCID-Human	SCID-Hu	Co-implantation of human fetal liver and thymus under the renal capsule	• Human T cells are educated on autologous thymic epithelium and are LA-restricted • Robust thymus and T cell development is observed in the thymus • Excellent model for study of HIV	• Low levels of haematopoietic ce lineages other tha T cells are generated • Low levels of haematopoietic engraftment in bone marrow and peripheral tissues • Poor human immune system function
Bone marrow, Liver, Thymus	BLT	Human fetal liver and autologous thymus fragments are co-implanted under the	• Complete human immune system	• Technical and surgical expertise required for

	renal capsule of an immunodeficient mouse, and following preconditioning human HSCs isolated from the same fetal liver are then injected intravenously to engraft the bone marrow[48]	engrafted, including antigen-presentingcells • T cells are HLA-restricted • Higher levels of total human haematopoietic cell engraftment than in Hu-SRC-SCID model • Only model that leads to the generation of a mucosal human immune system	establishment of the model system • Fetal tissue availability may b limiting • Long-term BLT mice begin to display a “wasting like diseasewith multi-organ infiltration and inflammation and eventual death • Immune responses although present are weak and vaccinations for viruses are not protective • Predominately IgM antibody response to immunization o virus infection with little IgG antibodygenerated

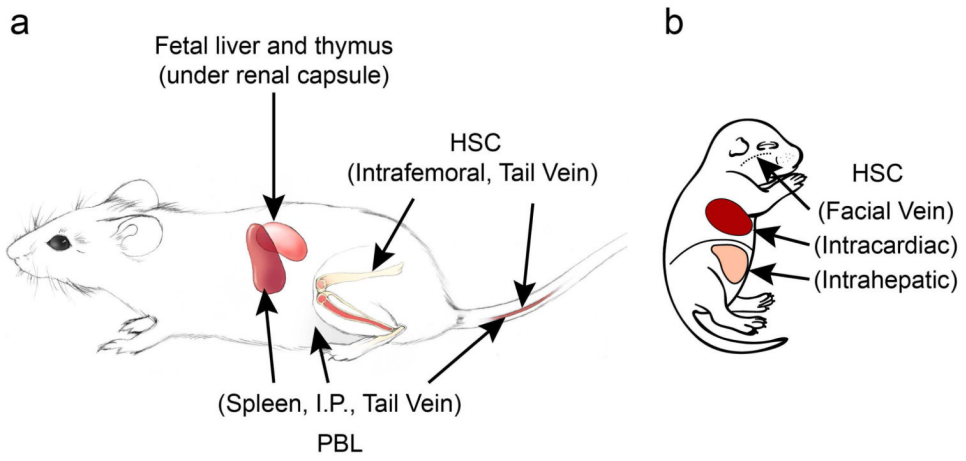

Figure 1. Approaches for engraftment of human immune systems in immunodeficient mice

Legend to Figure 1: a: To establish Hu-PBL-SCID mice, injection routes include IV, IP, and intrasplenic into adult recipients. To establish Hu-SRC-SCID mice, injection routes of HSC in adult recipients include IV and intrafemoral. b: Injection routes into newborn mice include facial vein, intracardiac, and intrahepatic. IP injection of HSC into newborn or adult recipients results in very low engraftment. To establish SCID-Hu mice, fetal liver and thymus fragments are implanted under the renal capsule into adult mice. To establish BLT mice, fetal liver and thymus fragments are implanted under the renal capsule into irradiated adult mice, and HSC derived from the same fetal liver are injected IV. HSC, haematopoietic stem cell; IP, intraperitoneal; IV, intravenous; PBL, peripheral blood lymphocytes.

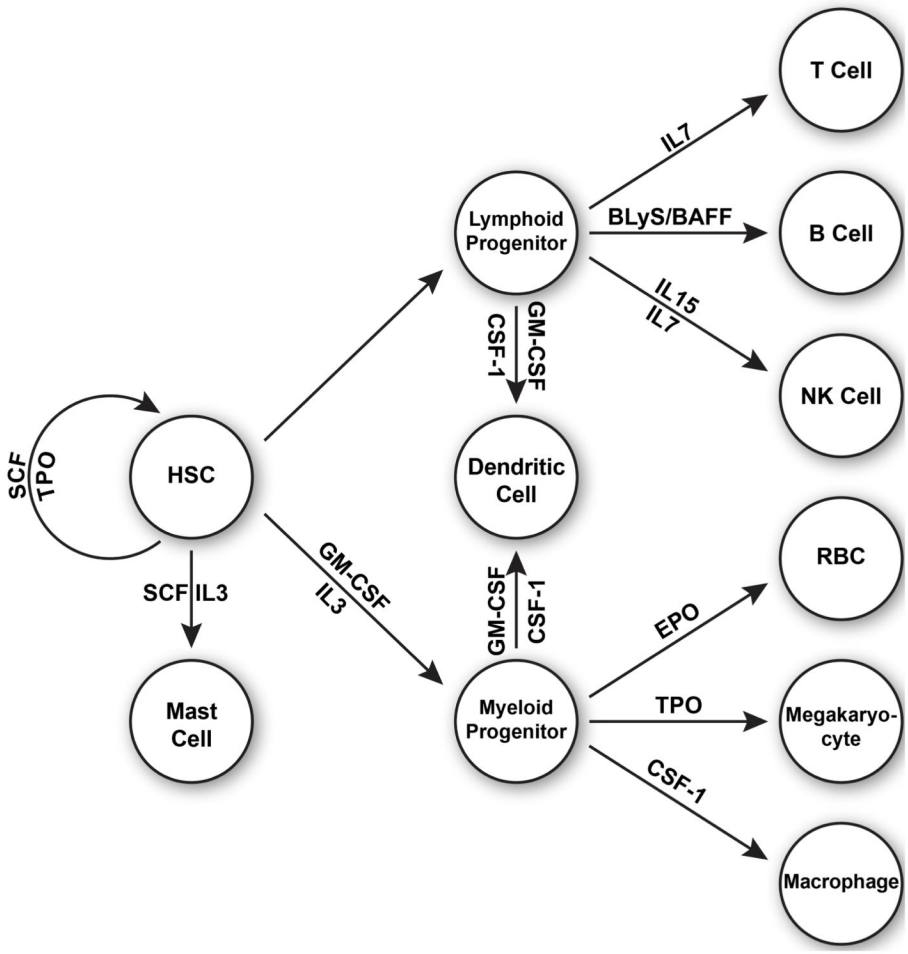

Figure 2. Cytokines expressed transgenically in immunodeficient *IL2rγ*^{null} strains

Legend to Figure 2: Human cytokines that have been, or are currently under development, expressed transgenically in immunodeficient *IL2rγ*^{null} strains to enhance human hematopoietic cell engraftment and immune system development and function. SCF, stem cell factor, TPO, thrombopoietin, HSC, hematopoietic stem cell, GM-CSF, granulocyte macrophage colony stimulating factor, IL3, interleukin 3, EPO, erythropoietin, CSF-1, colony stimulating factor-1, BLyS/BAFF, B lymphocyte stimulator/B cell activating factor, IL7, interleukin 7, IL15, interleukin 15.

Table 1

Immunodeficient *Il2rg*^{null} mouse strains genetically engineered to enhance immunity in humanized mice.

Immunodeficient mouse strain	Commonly used strain abbreviation	Human factors/ targeted mutations	Technology used for human factor expression	Expression and effect on host mouse	Effect on engraftment of human HSCs, PBMCs and fetal tissues	Ref.
C;129S4- *Rag2*$^{tm1.1Flv}$*Thpo*$^{tm1.1(TPO)Flv}$*Il2rg*$^{tm1.1Flv}$	C;129 Rag2G TPO-KI	Thrombopoietin	Knockin	Thrombocytopenia Decreased HSC numbers	Increased human myelopoiesis; decreased lymphopoiesis; Increased HSC self-renewal capacity	17
C;129S4- *Rag2*$^{tm1.1Flv}$*Csf1*$^{tm1.1(CSF 1)Flv}$*Il2rg*$^{tm1.1Flv}$	C;129 Rag2G CSF1-KI	Macrophage colony-stimulating factor	Knockin	No pathologic changes Normal hematopoiesis	Increased frequencies of human monocytes/ macrophages; augmented macrophage function	16
C;129S4- *Rag2*$^{tm1.1Flv}$*Csf2/IL3*$^{tm1.1(CSF2,IL3)Flv}$*Il2rg*$^{tm1.1Flv}$	C;129 Rag2G (GM-CSF, IL-3)-KI	IL-3 and GM-CSF	Knockin	(PAP)	Enhanced development of human alveolar macrophages; Partial rescue of PAP	18
C;129S4- *Rag2*$^{tm1.1Flv}$*Il2rg*$^{tm1.1Flv}$Tg*(*SIRPA*)*1Flv	C;129 Rag2G Tg(Hu-SIRPA)	Signal regulatory protein α	BAC transgenic	No pathological changes Normal hematopoiesis	Enhanced human HSC engraftment and maintenance in BM; Enhanced antigen-specific IgM Ab responses	27
NOD.Cg-*Prkdc*^{scid}*Il2rg*^{tm1Wjl}Tg(CMV-IL3,CSF2,KITLG)1Eav	NSG Tg(Hu-GM-CSF, IL-3, SCF)	IL-3, GM-CSF, SCF	Transgene construct under control of a CMV promoter/enhancer	No pathological changes	Reduced frequencies of human HSC; Increased frequencies of human myeloid cells in BM; Increased frequencies of CD4+ FoxP3+ cells; Decreased B cell frequencies	19
NOD.Cg-*Prkdc*^{scid}*Il2rg*^{tm1Wjl}Tg(PGK1-KITL*220)441Daw	NSG Tg(Hu-bSCF)	Membrane-bound human SCF	Transgenic construct	No pathological changes	Supports neonatal HSC engraftment without host irradiation; Increased human mast cell numbers	21, 22
NOD.Cg-*Prkdc*^{scid}*Il2rg*^{tm1Wjl}*B2m*^{tm1Unc}	NSG *B2m*$^{-/-}$	MHC class I KO	Targeted mutation	Prevents MHC class I expression; Hemachromatosis	Decreases severity of GVHD mediated by allo anti-MHC class 1 responses following PBL engraftment	24
NOD.Cg-*Prkdc*^{scid}*I2rg*^{tm1Wjl}*H2-Ab1*^{tm1Gru}	NSG *IA*$^{-/-}$	MHC class II KO	Targeted mutation	No pathological changes	Decreases severity of GVHD mediated by allo anti-MHC class II following PBL engraftment	24
NOD.Cg-*Prkd c*^{scid}*Il2rg*^{tm1Wjl}Tg(HLA- A/H2-D/B2M)1Dvs/SzJ	NSG Tg(HLA-A2, B2M)	HLA-A2, Huβ2M	Transgene construct	No pathological changes	Supports development of human HLA-A2-restricted T cells following human HSC engraftment	61, 62
NOD.Cg-*Prkdc*^{scid}*Il2rg*^{tm1Wjl}Tg(HLA-A2.1)1Eng	NSG Tg(HLA-A2)	HLA-A2	Transgene construct	No pathological changes	Supports development of human HLA-A2-restricted T cells following human HSC engraftment	60
NOD.Cg-*Prkdc*^{scid}*Il2rg*^{tm1Wjl}*H2-Ab1*^{tm1Gru} Tg(HLA-DRB1)31Dmz	NSG *I-A*$^{-/-}$ Tg(HLA-DR4)	HLA-DR4 Mouse Class II KO	Transgene construct	No pathological changes	Target for allo-GVH following PBMC engraftment	25
NOD-Cg-*Rag1*^{tm1Mom}*IL2rg*^{nulI}Tg(HLA-DRB1)31Dmz	NSG Tg(HLA-DR4)	HLA-DR4	Transgene construct	No pathological changes	Enhanced human immune function	43

KI, knock-in; TPO, thrombopoietin; CSF1, macrophage colony-stimulating factor 1; GM-CSF, granulocyte macrophage colony-stimulating factor; IL-3, interleukin-3; SIRP, signal regulatory protein-α; SCF, stem cell factor; BAC, bacterial chromosomal construct; CMV, cytomegalovirus; BM, bone marrow; PAP, pulmonary alveolar proteinosis; HSC, haematopoietic stem cell; mbSCF, membrane bound human stem cell factor; KO, knockout; GVHD, graft-versus-host disease; Tg, transgenic; PBMCs, peripheral blood mononuclear cells; C;129 Rag2G, C;129S4- *Rag2*$^{tm1.1Flv}$; NSG, NOD-*scid Il2rg*^{null}; NOG, NOD-*scid Il2rg*^{trunc}; B2M, beta 2-microglobulin; HSC, hematopoietic stem cell, PBL, peripheral blood lymphocytes; BLT, bone marrow, liver, thymus; RBC, red blood cells.

Table 2

Pathogenic microorganisms studied in humanized *Il2rg*^{null} mice.

Infectious agent	Mouse strain	Engraftment	Ref.
HIV	Many	Multiple models HSC,PBL,SCID/Hu, BLT	64, 65
HTLV1	BRG	HSC	111
EBV	NSG Tg(HLA-A2, B2M)	HSC	60, 61
EBV	NOG	HSC	59, 60, 112
Ebola virus	NSG	PBL	113
Dengue virus	NSG	HSC	114, 115
Dengue virus	NSG Tg(HLA-A2, B2M)	HSC, BLT	62
Dengue virus	BRG	HSC	116
Hepatitis C virus	BRG FK506 Caspase 8	HSC and fetal hepatocytes	108, 117-119
Hepatitis B virus	STOCK *Prkdc*^{scid} Tg(Alb1-Plau)Bri	Adult hepatocytes	118-120
Salmonella enteric serovar Thyphi	NSG, C;129RG	HSC	57, 58
Borrelia hermsii	NSG	HSC	121
Plasmodium falciparum	NSG	Hu RBC	122
Cytomegalovirus virus	NSG	HSC	123

HIV, human immunodeficiency virus; HTLV1, human T-lymphotropic virus 1; EBV, Epstein–Barr virus; Alb1-Plau, human urokinase-type plasminogen activator gene under control of the albumin promoter

Table 3
Remaining challenges in humanized mice and approaches to overcome these challenges

Challenges	Opportunities
Impaired mature B cell development and function following PBL or HSC engraftment	Provide human cytokines that promote B cell development (BAFF, IL-6)
Low IgG antibody responses	Enhance T cell help for class switch Enhance lymph node germinal center development
Develop HLA-restricted T cells for multiple HLA class I and II alleles	Develop a panel of HLA transgenic mice
Engraftment with mature T cells results in GVHD	Targeting of mouse MHC class I and II genes
Infection with human host-specific pathogens	Engraft with human infectable cells
Modeling human autoimmune diseases	Engraft with human target cells and with human autoreactive immune system
Rapid clearance of human RBCs from circulation	Deplete mouse macrophages and express human SIRPα protein
Engraft with human tumor cells that grow poorly in existing models	Provide needed human growth factors, hormones, etc; Engraft tumors in orthotopic site
Development of HSC and other functional cell populations from human ES cells or iPS cells	Provide needed microenvironment and growth factors
Lack of appropriate trafficking of human immune cells	Generate human adhesion molecule transgenic mice to facilitate appropriate trafficking of human immune cells