Humanized SCID Mouse Models for Biomedical Research

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Abstract There is a growing need for effective animal models to carry out experimental studies on human hematopoietic and immune systems without putting individuals at risk. Progress in development of small animal models for the in vivo investigation of human hematopoiesis and immunity has seen three major breakthroughs over the last three decades. First, CB17-*Prkdc*^{scid} (abbreviated CB17-*scid*) mice were discovered in 1983, and engraftment of these mice with human fetal tissues (SCID-Hu model) and peripheral blood mononuclear cells (Hu-PBL-SCID model) was reported in 1988. Second, NOD-*scid* mice were developed and their enhanced ability to engraft with human hematolymphoid tissues as compared with CB17-*scid* mice was reported in 1995. NOD-*scid* mice have been the "gold standard" for studies of human hematolymphoid engraftment in small animal models over the last 10 years. Third, immunodeficient mice bearing a targeted mutation in the IL-2 receptor common gamma chain (*IL2rγ*^{null}) were developed independently by four groups between 2002 and 2005, and a major increase in the engraftment and function of human hematolymphoid cells as compared with NOD-*scid* mice has been reported. These new strains of immunodeficient *IL2rγ*^{null} mice are now being used for studies in human hematopoiesis, innate and adaptive immunity, autoimmunity, infectious diseases, cancer biology, and regenerative medicine. In this chapter, we discuss the current state of development of these strains of mice, the remaining deficiencies, and how approaches used to increase the engraftment and function of human hematolymphoid cells in CB17-*scid* mice and in previous models based on NOD-*scid* mice may enhance human hematolymphoid engraftment and function in NOD-*scid IL2rγ*^{null} mice.

Abbreviations AML: acute myelogenous leukemia; asialoGM1: asialoganglioside ganliotetraosylceramide; BAFF: B cell activating factor; Blys: B lymphocyte stimulator factor; BM: bone marrow; DTR: diphtheria toxin receptor; ES: embryonic stem; FLT-3L: fms-related tyrosine kinase 3 ligand; EPO: erythropoietin; GM-CSF: granulocyte-macrophage colony-stimulating factor; HSC: hematopoietic stem cell; IL: interleukin; IC: intracardiac; IP: intraperitoneal; IV: intravenous; mAb: monoclonal antibody; MGDF: megakaryocyte growth and development factor; MHC: major histocompatibility complex; MSC: mesenchymal stem cell; NK: natural killer; PBMC: peripheral blood mononuclear cells; RBC: red blood cells; SCF: stem cell factor; SDF-1: stromal cell-derived factor-1; TCR: T cell receptor; TNFα: tumor necrosis factor-α; TPO: thrombopoietin; USSC: unrestricted somatic stem cells; UCB: umbilical cord blood

1 Introduction

1.1 Need for Small Animal Models for Preclinical Research

Animal models, particularly mice and rats, have provided important fundamental insights into biological and immunological processes that are common between species. However, direct translation of these results from rodents to humans often

fails because of species-specific differences. Furthermore, in vivo experimentation on humans is constrained by ethical and technical concerns. There is a critical need to develop animal models for the study of human immunity and other complex human biological processes without putting individuals at risk. Many of these processes involve complex biological systems that cannot be modeled in vitro or ex vivo. Furthermore, our understanding of biological process in humans is not sufficient to permit in silico modeling of the complex traits. Based on the discovery of immunodeficient CB17-*scid* mice in 1983, investigators have attempted to "humanize" mice to model complex human immunological and biological processes in small animals. Improvements in the generation of humanized mice have led to their growing use in the investigation of human hematopoiesis, innate and adaptive immunity, autoimmunity, infectious diseases, cancer biology, and, more recently, regenerative medicine.

1.2 Humanized Mice

Transplantation of human cells or tissues and transgenic expression of human molecules such as major histocompatibility complex (HLA) antigens all fall under the umbrella of the generic term "humanized" mice. Humanization by transgene expression can be used in immunocompetent mice to identify, for example, the antigenic epitopes presented by a human HLA molecule to an immune system [120]. Alternatively, engraftment of immunodeficient mice with human hematopoietic stem cells (HSC) or human peripheral blood mononuclear cells (PBMC) permits investigation of the development and function of a human immune system in vivo. We first provide a historical perspective on the development of humanized mice, approaches that have been used to enhance the engraftment and function of the transplanted human cells and tissues, and the exciting opportunities that are now possible based on the development of immunodeficient mice with targeted mutations in the IL-2 receptor common gamma chain.

1.3 Historical Perspective

Initial attempts to engraft human hematolymphoid cells in *Foxn1*^{nu} (abbreviated nude) mice were disappointing, even when the *Lyst*^{bg} (beige) and *Btk*^{xid} (xid) mutations were crossed onto nude mice [25]. The first major breakthrough in the ability of mice to be engrafted with human hematolymphoid cells was the discovery of CB17-*scid* mice in 1983 [14]. This was followed by the demonstration in 1988 that human fetal tissues and PBMC could engraft in CB17-*scid* mice [71, 74] (Fig. 1). Limitations of this model included high levels of host NK cell activity and the development of murine T and B cells on aging ("leakiness"), resulting in their ability to support only very low levels of human hematolymphoid engraftment [41].

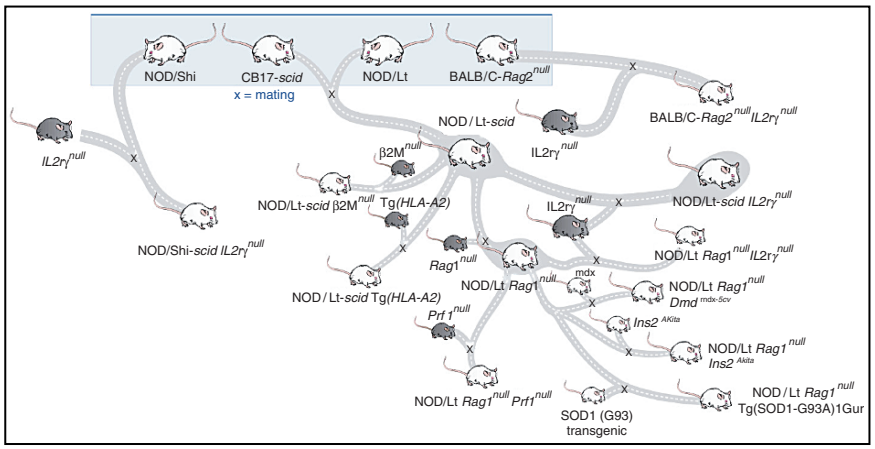

Fig. 1 A road map showing many of the diverse immunodeficient mouse stocks used for engraftment of human cells and tissues. The generation of these stocks of immunodeficient mice for humanization was based on the discovery of the *scid* mutation in 1983. The second major breakthrough was based on the development of the NOD-*scid* strain of mice. The most recent breakthrough in humanization is based on the generation of immunodeficient mice bearing a targeted mutation in the IL-2 receptor gamma chain gene. At the Jackson Laboratory, we have used the NOD-*scid* and NOD-*Rag1*^{null} strains as the base stocks of mice for generating immunodeficient *IL2rγ*^{null} mice and all future genetic modifications of these mice. These modifications leading to improvements in humanization of the model system will be useful for targeted research applications for diseases such as diabetes, muscular dystrophy, and various neurological disorders

Attempts to reduce innate immunity and increase the engraftment and function of human hematolymphoid cells included backcrossing the *scid* mutation onto other strains of mice with defects in innate immunity such as C3H/HeJ mice that express macrophage abnormalities or mice bearing the beige mutation that leads to defects in NK cell cytotoxicity [44]. However, these genetic manipulations led to only incremental improvements in human hematolymphoid cell engraftment [110].

The second major breakthrough was the development of NOD/LtSz-*Prkdc*^{scid} (abbreviated NOD-*scid*) mice in 1995 (Fig. 1). NOD-*scid* mice have reduced levels of NK cell activity and additional deficiencies in innate immunity [107] and support heightened levels of human hematolymphoid cell engraftment as compared to CB17-*scid* mice [44, 65, 86]. Since its development in 1995, this model has undergone many genetic modifications in attempts to improve the engraftment and function of human hematolymphoid cells and tissues. These modifications included efforts to further decrease innate immunity by generating NOD-*scid* mice homozygous for a targeted mutation at the β2-microglobulin (*B2m*) locus that results in NK cell deficiency [23] or the null mutation in the perforin (*Prf1*) locus) that markedly reduces NK cell-mediated cytotoxicity [109]. Additionally, a more radioresistant immunodeficient stock of NOD mice was generated by introducing a null mutation in the recombination activating gene 1 (*Rag1*) locus [108]. Finally,

mice expressing human HLA molecules and cytokines via transgenesis have been generated. Together, these genetic modifications improved human lymphohematopoietic cell engraftment over CB17-*scid* mice, but models based on NOD-*scid* mice remained limited by their short life span due to the early development of thymic lymphomas and the lack of development of a fully functional human immune system after human HSC engraftment.

The third and most recent breakthrough in the field was the development of immunodeficient mice carrying a targeted mutation at the interleukin 2 receptor common gamma (*Il2rg*) chain (hereafter abbreviated *IL2rγ*^{null}) (Fig. 1). Deficiency of the *IL2rg* chain causes X-linked SCID in humans [60]. This molecule is utilized by a number of cytokine receptors and is indispensable for IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21 high-affinity ligand binding and signaling [118]. Thus, mice bearing an *IL2rγ*^{null} mutation have severe impairments in innate and adaptive immunity. Mice harboring an *IL2rγ*^{null} mutation in combination with the *scid*, *Rag1*^{null}, or *Rag2*^{null} mutations show a major increase in their ability to support engraftment of functional human hematolymphoid cells.

IL2rγ targeted mutations have been produced independently by four different groups [17, 29, 51, 82], and these *IL2rγ*^{null} genetic stocks including *Il2rg*^{tm1Wlj}, *Il2rg*^{tm1Sug}, and *Il2rg*^{tm1Krf}, and *Il2rg*^{tm1Cgn} have been bred to *scid*, *Rag1*^{tm1Mom} (*Rag1*^{null}), or *Rag2*^{tm1Fwa} (*Rag2*^{null}) mice to develop models for human hematolymphoid engraftment [110]. However, there remain limitations on the engraftment and function of human hematolymphoid cells even in these new immunodeficient *IL2rγ*^{null} genetic stocks. Lessons learned over the last 10 years from approaches used to enhance the ability of previous generations of immunodeficient mice to engraft with human hematolymphoid cells may provide insights into approaches that will facilitate engraftment and function of human hematolymphoid cells in NOD-*scid* *IL2rγ*^{null} mice.

2 Manipulations Used to Improve Human Hematolymphoid Cell Engraftment in Immunodeficient Mice

2.1 Manipulations to Depress Innate Immunity

Mice bearing the *scid* mutation or the *Rag1*^{null} or *Rag2*^{null} targeted mutations lack adaptive immunity. However, depending on the strain background, these immunodeficient mice retain robust innate immunity. This innate immunity poses a significant barrier to the engraftment of xenogeneic hematopoietic tissues [22]. Beginning with the CB17-*scid* strain, which expressed high levels of host innate immune activity, many methods have been used to depress innate immunity to increase human hematolymphoid cell engraftment (Table 1). Initially, these reagents targeted NK cells, which are known to be a major obstacle to engraftment of hematopoietic cells [42]. The original approach used antibody against asialoganglioside ganli-

Table 1 Experimental approaches used to decrease innate immunity in SCID mice

Target Cells	Treatment	Reference
NK cells	Anti–asialo–GM–1 antibody	99, 105
NK cells	Anti–CD122 (TMβ1) mAb	8, 72, 121
NK cells	Anti–NK1.1 (PK-136) mAb	22
Granulocytes	Anti–GR1 (RB6–8C5) mAb	100
Macrophages	Liposome–encapsulated clodronate	34, 97, 104, 125, 131

otetraosylceramide (asialoGM1), but this antibody is highly cross-reactive between species and also targets human NK cells, activated $CD8^+$ T cells [119], and macrophages [73] that are present in the human cell inoculum. In strains such as C57BL/6-*scid* mice that express the NK1.1 allele, anti-NK1.1 monoclonal antibody (mAb) has been used to deplete NK cells [22]. However, even in anti-NK1.1 mAb-treated C57BL/6 mice, human hematolymphoid cell engraftment remained low [22]. Interestingly, this also demonstrates the importance of mouse strain background in the support of human cell engraftment in immunodeficient hosts. The more recent use of anti-CD122 (IL-2 receptor beta chain) mAb, which targets a molecule that is highly expressed on murine NK cells and does not cross-react with human CD122 has facilitated human hematopoietic cell engraftment in NOD-*scid* mice. Finally, other approaches have targeted neutrophils with anti-Gr1 mAb, and macrophages have been depleted with liposome-encapsulated clodronate (Table 1).

2.2 Exogenous Administration of Human Hormones, Growth Factors, and Cytokines

An additional obstacle to human cell engraftment and function, recognized early in the course of investigations using humanized mice, is the lack of species cross-reactivity of many mouse hormones, growth factors, and cytokines that are required for development, survival, and function of human hematolymphoid cells (http://www.copewithcytokines.de/cope.cgi?key=Cytokine%20Inter-species%20Reactivities). For example, mouse and human type 1 interferon are species specific and do not cross-react [128]. It has also been reported that granulocyte monocyte colony-stimulating factor (GM-CSF) and IL-3 are species specific and are not cross-reactive between mice and humans, meaning that these mouse cytokines will not support the growth of engrafted human cells [5]. In contrast, factors such as erythropoietin (EPO), granulocyte colony-stimulating factor (G-CSF), and stem cell factor (SCF or kit-ligand) are cross-reactive between human and mouse, and host production of these molecules can support human cell growth [5]. In addition, many necessary human-specific factors are not produced by the human hematolymphoid cells, but rather by nonhematopoietic stromal cells not present in the human HSC inoculum. An example of this is the B cell cytokine B lymphocyte stimulator (BLyS, also termed B cell

activating factor or BAFF, official gene nomenclature, *TNFSF13B*) [136]. BLyS, produced by stromal cells and dendritic cells, is required for B cell differentiation and survival. Our collaborators (Drs. Woodland and Schmidt at the University of Massachusetts) have found that murine BLyS fails to promote human B cell survival ex vivo or in humanized mice. These observations suggest that human BLyS may need to be provided, either by exogenous administration or by transgenic expression, for robust B cell engraftment and function in humanized mice.

In an attempt to overcome these obstacles, investigators have provided exogenous human factors to human hematolymphoid cell-engrafted mice. These factors comprise three main categories. Those important for (1) the engraftment of human HSC, (2) the differentiation of human HSC into various hematopoietic lineages, and (3) the function of the differentiated cells in the immunodeficient murine host. Exogenous administration of cytokines and factors required for early HSC homing to the bone marrow and survival have been identified and used to improve engraftment of human HSC. These factors targeted homing molecules such as stromal cell-derived factor-1 (SDF-1) and molecules required for HSC expansion and differentiation such as IL-3, FMS-related tyrosine kinase 3 ligand (FLT-3 ligand), and GM-CSF (Table 2).

A second series of exogenous human factors administered to engrafted mice included those targeted toward differentiation and function of specific human hematopoietic lineages. The inability of NOD-*scid* mice engrafted with human HSC to generate human T cells prompted attempts to enhance T cell differentiation. Important advances in this area include the administration of TNF-α [98] or IL-7 to

Table 2 Treatments used to promote human hematolymphoid cell survival, function, or trafficking in SCID mice

Treatment	Reference
Human growth hormone	75, 123
SDF–1 peptide agonist	84
Flt3 ligand	19, 53
Erythropoietin	19, 53
NIP–004 human TPO receptor agonist	78
TNF–α	98, 132
IL–2	7, 96
IL–3, GM–CSF, PIXY 321	7, 19, 59
IL–4	18
IL–6	19, 53, 56
IL–7	53, 79, 106
IL–12	102, 135
IL–15	96
IL–18	102
Type 1 interferon	58, 101
Anti–CD40 mAb	76
Anti–CTLA–4 mAb	69

human HSC-engrafted mice [106]. Additional lineage-specific factors such as EPO for red blood cell (RBC) development have also been used to enhance human RBC generation. Exogenous administration of EPO may be needed to enhance human RBC development in HSC-engrafted immunodeficient mice, as reports suggest that engrafted HSC generate only low levels of human RBC [48]. Finally, additional cytokines required for differentiation and function of human lymphocytes have been used to drive HSC differentiation toward the lymphocyte lineage, as well as molecules that provide costimulatory signals to cells of the developing immune system (Table 2). A third series of factors includes those directed at regulating the function or maturation of the engrafted human hematolymphoid cells. These include factors such as thrombopoietin, anti-CD40 mAb, anti-CTLA-4 mAb, and cytokines such as IL-12, which is known to be important in cell immune function (Table 2).

3 Transgenic Expression of Human Hormones, Growth Factors, Cytokines and HLA Molecules

A potentially more efficient approach to providing human molecules required for human HSC engraftment and function is through transgenic expression of growth factors. In addition, other noncytokine human molecules that are important for human cell engraftment of lineage development can be introduced via transgenesis. Examples include human HLA class I and class II molecules, which are discussed next.

3.1 Transgenic Expression of Human Hormones, Growth Factors, and Cytokines

One of the first attempts to use transgenic immunodeficient mice to enhance engraftment of human hematolymphoid cells was the transgenic expression of IL-3, GM-CSF, and SCF in CB17-*scid* mice [11]. However, human HSC engraftment was not improved, and subsequently transgenic expression of these three factors was determined to be detrimental to the engraftment and differentiation of human HSC [80]. This effect was hypothesized to result from either the increased mobilization of stem cell progenitors into the blood, effectively preventing their seeding into the bone marrow, or the induced terminal differentiation of the stem cells in the presence of high levels of these cytokines.

3.2 Transgenic Expression of Human HLA Molecules

A second class of human transgenes that have been expressed in mice are HLA class I and class II molecules. The use of human HLA class I [32] and class II [35] immunocompetent transgenic mice for the study of immune function and

Table 3 Examples of transgenic expression of human HLA molecules and hematopoietic growth factors

Human transgene expression	Reference
Human transgenes in SCID mice	
HLA–A2	8
HLA–DR1	16
IL3, GM–CSF, SCF	11, 80
Human HLA transgenes in immunocompetent mice	
HLA–A24	39
HLA–B27	57, 124
HLA–DR3	67
HLA–DR4	122, 137
HLA–DQ8	64, 77, 91

autoimmunity has been extensively reviewed. In immunodeficient humanized mice, expression of HLA molecules on host thymic epithelium is required for appropriate thymic selection and antigen-specific restriction by human T cells. To address this issue, numerous transgenic mice expressing human HLA molecules have been created (Table 3). However, only a few of these transgenes have been backcrossed onto immunodeficient mouse stocks for use in human HSC engraftment experiments. In the earlier generations of non-HLA-transgenic immunodeficient mice that have been used in humanization experiments, variable levels of human T cell development have been observed [16]. The generation of NOD-*scid Il2r*γ^{null} mice expressing human HLA transgenes should now permit the development of HLA-restricted human T cells in these mice.

4 Ex Vivo Modifications of Human HSC Before Engraftment

An alternative approach to the treatment or modification of the immunodeficient host to improve human hematolymphoid cell engraftment and differentiation is to perform ex vivo manipulations of the human HSC inoculum (Table 4). Indeed, there has been intense interest in the ex vivo expansion of human HSC. This is due in part to the need for sufficient HSC numbers to achieve efficacy in the clinic for human stem cell engraftment from umbilical cord blood (UCB), based on a recommended dose of 2.0×10^7 UCB nucleated cells per kilogram of recipient body weight [13].

Most approaches have relied on manipulations of cultured HSC with cocktails containing multiple human cytokines. These approaches have been hampered by the difficulty in achieving ex vivo expansion of human $CD34^+$ stem cells without inducing their differentiation and consequent loss of in vivo stem and progenitor cell repopulating capacity [113].

Noncytokine approaches have also been used in ex vivo manipulations to modify human HSC to facilitate their in vivo engraftment. For example, surface fucosylation of CD34$^+$ UCB stem cells has been reported to generate selectin ligands that enhance the initial interactions with microvessels following injection [45, 138]. Selectins contribute to homing of adult CD34$^+$ cells, but their ligands are absent on a subset of UCB CD34$^+$ cells. This deficiency has been associated with reduced α1,3-fucosyltransferase expression and activity. Exogenous treatment of UCB CD34$^+$ stem cells to introduce α1,3-linked fucose to cell surface glycans on their surface has been used successfully to enhance their engraftment in vivo [45, 138].

A third approach has used lineage-specific differentiation cytokines to drive ex vivo human stem and progenitors cells into distinct lineages, with the goal being in vivo replacement of particular hematopoietic cell lineages. This approach has recently been used to correct the delayed platelet recovery following UCB stem cell transplantation. Ex vivo culture of CD34$^+$ UCB cells with thrombopoietin (TPO) accelerates platelet production following engraftment into NOD-*scid* mice [130], and this may be a useful approach for improving the production of human platelets in HSC-engrafted NOD-*scid IL2r*γ^{null} mice [48] (Table 4).

Table 4 Examples of ex vivo manipulations of human hematolymphoid cells carried out to improve human engraftment

Treatment	Reference
HSC expansion or upregulation of homing molecules using cytokines	
IL–3, IL–6, SCF, FLT–3 ligand, and IL–11	43
FLT–3 ligand, SCF, megakaryocyte growth development factor (MGDF), and G–CSF	55
G–CSF and MGDF	12
SCF	142
Notch ligand delta1	26
IL–3 and SCF	115
SDF–1 peptide analog, SCF, TPO, Flt–3 ligand, and BM stromal cell culture supernatant	63
Coculture with human brain endothelial cells	24
SCF, GM–CSF, IL–3, IL–6, EPO, and TPO	37
FLT–3 ligand, SCF, and TPO	30
FLT–3 ligand, IL–7, and TPO	61
SCF, FLT–3 ligand, G–CSF, GM–CSF, IL–3, IL–6, MGDF, EPO, and TPO	87
FLT–3 ligand, IL–6, MGDF, and SCF	95
SCF and FLT–3 ligand	141
Non–cytokine–mediated HSC expansion	
Surface fucosylation of HSC	138
Chromatin–modifying agents	4
HSC differentiation	
Myeloid and B lymphoid lineage using hepatocyte growth factor and TPO	40
Megakaryocyte expansion using TPO	130

5 Coinjection of HSC with Mesenchymal Stem Cells and Other Stromal Cell Populations

Engraftment of immunodeficient mice with human HSC provides the potential to generate a full human hematopoietic system, including the immune system. However, as discussed above, numerous human-specific factors required for human hematolymphoid cell engraftment, differentiation, and survival are not produced by human hematopoietic cells. To attempt to overcome this limitation, investigators have coinjected human HSC with human mesenchymal stem cells (MSC), unrestricted somatic stem cells (USSC), or human cytokine-transduced stromal cells in attempts to provide a "stromal" human cell population capable of facilitating human HSC engraftment (Table 5). The hypothesized mechanisms are thought to be establishment of an appropriate stromal microenvironment of human origin, secretion of human-specific factors that are not produced by human hematopoietic cells, or both. Of particular interest has been the relative success of coinjection of allogeneic human MSC plus HSC. Coinjection of these allogeneic cell populations does not appear to lead to a graft-versus-host reaction between these two tissues [94]. MSC are thought to be immunosuppressive [92] and may in fact suppress both the cord blood T cell response to the allogeneic MSC as well as the host innate immune system, effectively facilitating HSC engraftment.

6 The IL2 Receptor Gamma Common Chain Knockout Mouse

Investigators attempting to create humanized mice with functional human immune systems have been re-energized with the recent development of immunodeficient mice bearing targeted mutations at the IL-2 receptor common gamma chain locus (*IL2rg*^{null}). Four independent groups have generated immunodeficient mice bearing *IL2r*γ^{null} mutations on four different strain backgrounds. These are the NOD/Lt-*scid IL2rg*^{null} [48, 106], NOD/Shi-*scid IL2rg*^{null} [50, 139], BALB/c-*Rag2*^{tm1Fwa}*IL2rg*^{null} [127], and *H2*^{d} *Rag2*^{tm1Fwa} *IL2rg*^{null} strains [38]. In these strains of immunodeficient mice, human HSC have been shown to generate complete human immune systems, including human thymocytes and peripheral mature T and B cells, myeloid cells,

Table 5 Human stromal cell populations coinjected with human hematolymphoid cells

Cell population(s) coinjected with HSC	Reference
Mesenchymal stem cells	3, 47, 81
Adipose stromal cells	54
IL–7–transduced stromal cells	27
Unrestricted somatic stem cells	21

myeloid and plasmacytoid dendritic cells, platelets, and RBC [110]. T cells that develop in these mice have a diverse TCR repertoire and mount antigen-specific IgM and IgG antibody responses after immunization with T-dependent antigens (for recent review see [110]). These studies have used different strains, newborn and adult recipients, and different routes of injection.

In the newborn engraftment model, four different routes of human HSC injection have been reported (Fig. 2). These routes of human HSC injection include intraperitoneal (IP), intravenous (IV) via intracardiac (IC) or facial vein, and intrahepatic (IH). The IP route of human HSC engraftment appears to be suboptimal for achieving high engraftment of human HSC whereas the IC and IH routes appear to be equivalent in engraftment of human HSC (T. Pearson, unpublished observations). Direct comparison of the four different strains of immunodeficient *IL2rγ*^{null} mice and their engraftment by the various routes of HSC injection has not been reported, leaving the optimal strain and route of injection in newborns undetermined.

In the adult engraftment model, two main routes of human HSC injection into immunodeficient mice have been reported. The most commonly used route of injection is the IV route via the tail vein [110]. More recently described routes of injection involve the direct intrafemoral [70] or intratibial [133] injection of human HSC. These latter routes of injection overcome the bone marrow homing requirements of human HSC injected intravenously and have been reported to facilitate human HSC engraftment in NOD-*scid* recipients [70, 133]. The engraftment of human HSC following intrafemoral or intratibial injection into NOD-*scid Il2rγ*^{null} mice has not been reported.

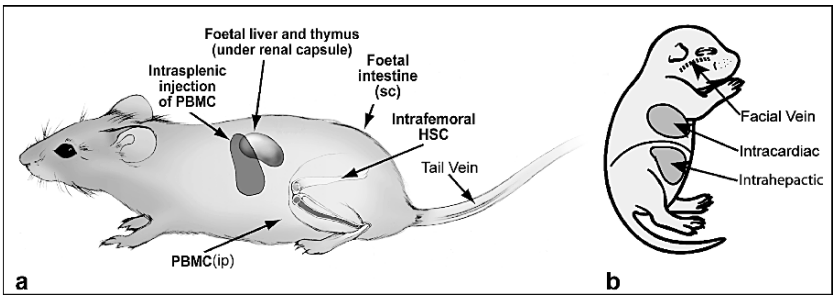

Fig. 2 Major routes of engraftment of human hematopoietic stem cells and tissues in adult and newborn immunodeficient mice. **a** In the adult, numerous routes of stem cell engraftment have been reported depending on the experimental research protocol. Hematopoietic stem cells and been injected using intravenous, intrafemoral, and intratibial injection routes. For fetal tissue engraftment into adults, the traditional site is under the renal capsule. For PBMC engraftment, intraperitoneal, intravenous, and intrasplenic injection routes have been described. **b** For newborn engraftment with HSC, intravenous injection via the facial vein or intracardiac routes have been used, as well as an intrahepatic route of HSC injection

7 Current Research Uses of Humanized Mice

The availability of humanized immunodeficient *Il2rγ*^{null} mice harboring a functional human immune system following engraftment with human HSC has reinvigorated the hope that these humanized mice can be used for the investigation of a number of complex human hematological and immunological processes, including infectious disease, autoimmunity, cancer biology, and regenerative medicine [110]. This aspect of humanized mice has been reviewed extensively since 2005 and is considered only briefly here.

7.1 Infectious Disease

One of the more exciting uses of humanized mice is the investigation of infectious diseases that are human specific [110]. These include viral infections such as human immunodeficiency virus (HIV), Epstein-Barr virus (EBV), hepatitis C, dengue, and the protozoan *Plasmodium falciparum*, the causative agent of malaria and the leading global disease that has killed more individuals than all the wars and other plagues combined (http://en.wikipedia.org/wiki/Plasmodium_falciparum). Because these agents infect human but not mouse cells, no suitable small animal models are currently available for the study of the pathogenesis of these diseases. Humanized mice are also being used to develop immunization protocols that can then be functionally tested by determining their resistance to reinfection with the test organism, a practice that is unethical in humans. Currently, vaccine development requires extensive preclinical testing in nonhuman primates prior to entry into the clinic. Exciting examples of progress in this area are the recent reports on HIV infection in humanized immunodeficient *IL2rγ*^{null} models [6, 10, 134]. These humanized mice have long-lasting infection of human cells by CXCR4- and CCR5-tropic HIV isolates as well as HIV-specific human immune responses.

7.2 Autoimmunity

Animal models have been used extensively to study the pathogenesis of autoimmune diseases. Reliance on animal models is in part recognition of the importance of patient safety-first do no harm. Unlike humans, mice and rats can be experimentally manipulated to study the disease process. Disease can be deliberately induced, the diseased tissue can be biopsied, and the animals can be necropsied at various stages of the autoimmune disease process for study. The genetic basis for the autoimmune disease can be identified, the genome can be altered with knockout, knockin, or transgenic technology, and the genome can be fixed by inbreeding. Importantly, therapies to prevent or reverse the autoimmune disorder can readily be tested without the ethical concerns associated with clinical research in humans.

Humanized mice have been used for the study of autoimmune type 1 diabetes, autoimmune thyroiditis, and rheumatoid arthritis [110]. In diabetes, PBMC from diabetic individuals adoptively transferred to CB17-*scid* mice led to the detection of autoantibodies to islet components, but no infiltration or beta cell destruction was observed [85]. More recently, the development of human T cell clones with specificities to islet autoantigens has permitted the study of the adoptive transfer of diabetes into NOD-*scid* mice. In these studies, infiltration, but not islet cell destruction, was detected [129]. The use of newer models of immunodeficient mice based on the *IL2rγ*^{null} mutation and transgenic expression of human HLA molecules (Table 3) may provide new models that will permit the direct study of human autoreactive T cells in vivo.

7.3 Cancer

Immunodeficient mice have been used to study the growth of tumor cells for over 3 decades [110]. The first studies were the implantation of solid tumors in nude mice [33], followed by the observation that some human lymphomas could grow in CB17-*scid* mice [46]. The development of NOD-*scid* mice permitted the growth of many primary human lymphomas and leukemias that could not grow in CB17-*scid* mice [46], in part because of the decreased innate immunity and lower NK cell activity in NOD-*scid* as compared with CB17-*scid* mice [107]. More recently, primary acute myelogenous leukemia (AML) cells that did not grow in NOD-*scid* mice have been observed to grow in NOD/Lt-*scid IL2rγ*^{null} mice [140], suggesting that the in vivo study of primary human leukemias and lymphomas not feasible in previous generations of immunodeficient mice will now be possible.

An important concept that has been validated with immunodeficient mice is the existence of a "tumor stem cell" [83, 93]. This concept has been tested by transplantation of small numbers of tumor cells with stem cell characteristics into immunodeficient mice to document that they self-renew, differentiate, and give rise to the tumor [110]. This concept has translated into important clinical considerations, as the primary target in tumor therapy has previously been reduction of tumor mass, not targeting of tumor stem cells. Furthermore, for tumors such as AML, one treatment option is to isolate CD34^{+} cells from the patient's bone marrow for autologous transplantation after the disease has been driven into remission. However, the rate of recurrence is high after this procedure, and it has recently been shown that AML tumor stem cells also express CD34 [1, 28, 140], leading to their enrichment, not depletion, in autologous stem cell transplantation protocols,. Indeed, with the use of NOD-*scid* mice as hosts, human tumor stem cells have been isolated for a number of additional malignancies, including myeloma [88] and breast [2], brain [111], and pancreatic [62] cancers. Future studies of human tumor stem cells using immunodeficient *IL2rγ*^{null} mice as the in vivo testing ground for functional analyses could lead to individualized and focused new therapies that specifically target the tumor stem cell on a patient-by-patient basis.

7.4 Regenerative Medicine

Regenerative medicine is a rapidly growing field that is based on using stem cell therapy to replace damaged or destroyed cells and tissue [103, 112]. Important for rapid advancement of this field will be the ability to test the regenerative potential of the stem cells in an in vivo setting before translation of the cell-based therapy to the clinic. To address this need, immunodeficient animal models with genetic or induced tissue injury are being used for in vivo evaluation of the regenerative capacity of human stem cells. The need for tissue damage or injury for stem cells to repair tissue via regeneration, transdifferentiation, or cell fusion is a common feature particularly amenable for modeling in animals as they can easily be experimentally manipulated for study. This approach is being applied for the preclinical testing of human embryonic stem (ES) cell-derived populations, with particular interest focused on the use of ES and HSC [110] as stem cell sources for generating insulin-producing beta cells (http://www.betacell.org/).

Recognition that transdifferentiation and/or cell fusion of transplanted HSC also occurs in damaged tissues has led to intense investigation in this area [90]. Although not a form of "true regeneration of endogenous tissues," clinical efficacy has been observed in animal models in which HSC transplantation leading to cell fusion has been used for the treatment of diseased or damaged tissues [110]. The use of stem cell therapy has also been found to have efficacy in treating heart damage in experimental models [49, 66] and in humans [116]. The use of humanized mouse models to study the regenerative capacity for human stem cells to repair damaged or lost tissues presents the exciting possibility that humanized mice may serve as a "preclinical" bridge for translating data from animal models to human cells and tissues before their application in the clinic (Fig. 3). Important in these experiments will be

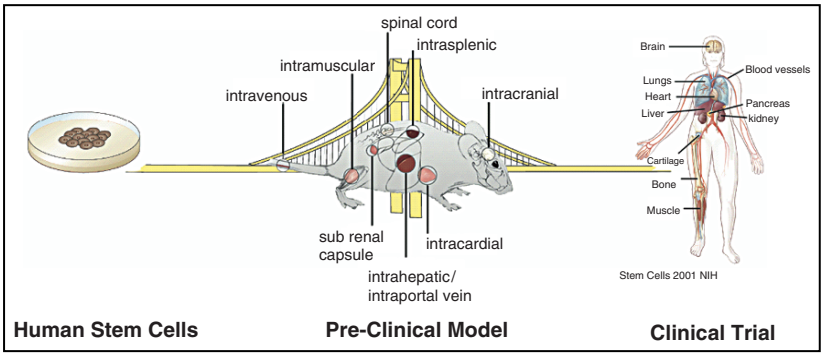

Fig. 3 Humanized SCID mice serve as a preclinical bridge between studies in laboratory animals and clinical research on humans. Stem cells are defined as long-term self-renewing cells that can repopulate the complete hematopoietic system and sustain long-term multilineage hematopoiesis. Functional analyses of these cells can only be accomplished with in vivo model systems. Humanized mouse models are used to define the functional activity of human stem cells and can function as a preclinical bridge between in vitro analyses of stem cells and the potential clinical use of stem cell therapy for a variety of human diseases in the clinic

the use of immunodeficient *IL2rγ*^{null} mice engrafted with human embryonic or adult stem cells. It has recently been reported that ES cells and their progeny, HSC and MSC, are highly susceptible to NK cell-mediated killing [41, 114, 126]. The use of immunodeficient *IL2rγ*^{null} mice that are completely NK cell deficient may permit study of the regenerative potential of stem and progenitor cell populations not possible in previous immunodeficient humanized mouse models.

8 Remaining Hurdles and Future Directions

There are a number of remaining limitations in immunodeficient *IL2rγ*^{null} humanized mouse models, comprising three broad categories. First, there are many human-specific molecules required for proper human immune system function that are not expressed in mouse tissues. Second, there are issues of remaining innate immunity that present obstacles to human stem cell engraftment. Third, the architecture of the lymphoid system remains undeveloped, and lymph nodes and secondary lymphoid organs in unmanipulated immunodeficient *IL2rγ*^{null} mice are exceedingly small and their stromal cell structure is very poorly developed. Overcoming these limitations should be achievable in the future with additional modifications, some of which are outlined below.

8.1 Human-Specific Molecule Expression

Genetic manipulation of mice is a powerful tool that can be used to knock out genes, knock in genes, and transgenically express human genes. In the latter case, expression of human-specific genes required for proper human immune system development and function can easily be accomplished. Of great importance is the expression of human HLA transgenes (Table 3) that will permit proper thymic selection of developing human T cells in the immunodeficient *IL2rγ*^{null} host. Additional molecules, such as cytokines and growth factors including IL-2, IL-7, and BLyS, will facilitate the development, differentiation, and survival of a functional human immune system. IL-2 may be particularly important, as it is a key cytokine in T lymphocyte development and function. Exogenous administration of IL-2 could not be performed in previous generations of immunodeficient mice, particularly those on the NOD-*scid* background, because of IL-2-dependent acceleration of thymic lymphoma development and early mortality [41, 107]. In immunodeficient *IL2rγ*^{null} mice, IL-2 signaling cannot occur and exogenous administration of IL-2 should not induce thymic lymphomas and premature death. Additional molecules, such as adhesion and homing molecules expressed on tissues in the host, may overcome some of the issues associated with homing of human immune cells. Engraftment of human lymphocytes may similarly facilitate restoration of the structure of the secondary lymphoid organs in HSC-engrafted immunodeficient *IL2rγ*^{null} mice.

8.2 New Approaches for Reducing Residual Innate Immunity

Although much improved as recipients of human stem cells and PBMC, immunodeficient *IL2rγ*^{null} mice still have residual innate immune function that impedes engraftment. The residual innate immune compartment, while functionally depressed in the absence of the *IL2rγ* signaling, still includes macrophages, granulocytes, dendritic cells, and Langerhans cells capable of mediating resistance to engraftment with human hematolymphoid cells. Both genetic approaches and exogenous administration of specific reagents can be used to further depress innate immunity to improve human cell engraftment. For example, transgenic simian diphtheria toxic receptor expression on dendritic cells, macrophages, and other cell populations, driven by the *Itgax* (CD11c) [52], *Itgam* (CD11b) [31], (Langerin) [9], or *Cre* [68] promoters, has been generated. Because mouse cells are relatively insensitive to the effects of diphtheria toxin [20], cells transgenically expressing the diphtheria toxic receptor can be selectively depleted in immunodeficient *IL2rγ* ^{null} mice by exogenous administration of diphtheria toxin before engraftment of human hematolymphoid cells. Finally, anti-Gr1 mAb may prove useful to deplete host neutrophils before engraftment of human cells [100].

8.3 Artificial Organoids

One of the remaining concerns relating to the humanized immunodeficient *IL2rγ*^{null} mice model is the lymphoid architecture and poor lymph node and secondary peripheral lymphoid organ development. Appropriate lymphoid architecture requires interaction of follicular dendritic cells (FDC) with lymphocytes and other nonlymphoid cells for full development [36]. In immunodeficient *IL2rγ*^{null} mice that lack lymphocytes, it is not surprising that lymphoid architecture is underdeveloped. This may have functional consequences, as secondary lymphoid organs are the primary site for antigen recognition and generation of immune responses. Reconstitution of immunodeficient *IL2rγ*^{null} mice with a human immune system may in part overcome this limitation, as immunization of these mice leading to the population of the lymphoid structures with human lymphocytes may promote lymphoid architecture development and function [134].

Additionally, progress is being made in the development of artificial lymphoid structures. Artificial thymic stroma [89] and biocompatible scaffolding [117] are being developed as approaches for providing the appropriate architectural environment for proper lymphocyte development and function. In addition, transduction of bone marrow or thymic stromal cells with factors that are needed for hematopoiesis or lymphocyte development may provide a source for those factors that are not provided by the murine stromal environment. For example, a recent report suggests that the use of a CXCR4-containing viral vector to drive CXCR4 expression of human HSC enhances homing and engraftment of the stem cells in immunodeficient mice [15].

9 Conclusions

The ability to study complex human biological processes in a small animal model that can be experimentally manipulated offers great promise for rapid advances in many areas of scientific research. The pathway to achieving this goal started approximately 40 years ago, and has progressed in a manner similar to that in other scientific disciplines: a handful of breakthrough advances interspersed in years of hard work. The scientific community has just experienced a significant breakthrough in the field of humanized mice, with the development of immunodeficient *IL2rγ*^{null} mice that support robust human hematolymphoid engraftment. However, limitations remain in the generation of a fully functional human immune system in these mice that recapitulates immune responses observed in immunocompetent individuals. Applying approaches pioneered to improve HSC engraftment and function in earlier generations of immunodeficient hosts (i.e., the steady hard work done between the big leaps in progress) may prove to be a fertile field for clearing the remaining hurdles in today's immunodeficient murine hosts. As these remaining limitations are overcome and improvements in the models are achieved, the use of humanized mice as robust preclinical models for bridging the route from bench to bedside will become increasingly important (Fig. 3).

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