Humanized mice in translational biomedical research

Leonard D. Shultz, Fumihiko Ishikawa‡ and Dale L. Greiner§*

Abstract | The culmination of decades of research on humanized mice is leading to advances in our understanding of human haematopoiesis, innate and adaptive immunity, autoimmunity, infectious diseases, cancer biology and regenerative medicine. In this Review, we discuss the development of these new generations of humanized mice, how they will facilitate translational research in several biomedical disciplines and approaches to overcome the remaining limitations of these models.

Complex biological processes often require *in vivo* analysis, and many important research advances have been made using mice as a model for the study of various biological systems. However, mice are not humans, and the study of human biology *in vivo* is severely limited by ethical and technical constraints. There is a growing need for animal models to carry out *in vivo* studies of human cells, tissues and organs, without putting individuals at risk. Humanized mice, or mouse–human chimaeras, have been developed to overcome these constraints and are now an important research tool for the *in vivo* study of human cells and tissues. Humanized mice are defined in this Review as immunodeficient mice engrafted with haematopoietic cells or tissues, or mice that transgenically express human genes. The development of mice that are 'humanized' by engraftment of human tissues, haematopoietic stem cells (HSCs) or peripheral-blood mononuclear cells (PBMCs) provides an opportunity to study human biological processes *in vivo* that would otherwise not be possible. This Review discusses the new generations of humanized mice that are proving to be powerful tools in pre-clinical testing and in the investigation of many human biological processes, and it also highlights some of the remaining limitations on the development of the optimal humanized mouse.

The development of humanized mice

Advances in the ability to generate humanized mice have depended on a systematic progression of genetic modifications to develop immunodeficient host mice. Three main breakthroughs have occurred in this field (TIMELINE). First, the discovery of the *Prkdc^{scid}* (protein kinase, DNA activated, catalytic polypeptide; severe combined immunodeficiency, abbreviated *scid*) mutation in CB17 mice[1] was soon followed by the observation that human PBMCs[2], fetal haematopoietic tissues[3] and HSCs[4] could engraft in these mice. However, engraftment occurred at only a very low level, and the engrafted human cells failed to generate a functional human immune system. Limitations impeding human-cell engraftment in CB17-*scid* mice include the spontaneous generation of mouse T and B cells during aging (known as leakiness) and high levels of host natural killer (NK)-cell and other innate immune activity, which limit the engraftment of the human haematopoietic compartment[5]. The *scid* mutation also results in defective DNA repair and, consequently, an increase in radiosensitivity[6]. Targeted mutations at the recombination-activating gene 1 (*Rag1*) and *Rag2* loci prevent mature T- and B-cell development in the mice but do not cause leakiness or radiosensitivity. However, these mice retained high levels of NK-cell activity and had limited engraftment of human HSCs[5,7,8].

The second breakthrough was the development of immunodeficient non-obese diabetic (NOD)-*scid* mice[9]. Crossing the *scid* mutation onto different strain backgrounds led to the observation that NOD-*scid* mice supported higher levels of engraftment with human PBMCs than did any of the other strains that were tested, including C3H/HeJ-*scid* and C57BL/6-*scid* mice[10]. Furthermore, it was observed that NK-cell activity, which is one of the main impediments to the engraftment of human haematopoietic cells[11], was lower in NOD-*scid* mice than in CB17-*scid* mice[9]. NOD-*scid* mice also have additional defects in innate immunity[9] that allow higher levels of human PBMC[10] and HSC[12,13] engraftment. Incremental improvements in the extent of human-cell engraftment as a result of the development of new genetic variations of NOD-*scid* mice occurred over the following 10 years (TIMELINE), but the use of humanized NOD-*scid* mice as a model for human immunity remains limited by their relatively short life span, and the residual activity

Severe combined immunodeficiency
(*scid*). Mice homozygous for the *scid* mutation at the protein kinase, DNA activated, catalytic polypeptide (*Prkdc^{scid}*) locus have a complete absence of mature T and B cells.

Leakiness
The spontaneous rearrangement of T- and B-cell receptors in *scid* mice, leading to the generation of mature T and B cells.

Recombination-activating gene 1 (*Rag1*) and *Rag2*
Two linked genes in which targeted mutations result in the complete inability to generate T and B cells expressing antigen-specific receptors.

**The Jackson Laboratory, 600 Main Street, Bar Harbor, Maine 04609, USA. ‡RIKEN, Research Center for Allergy and Immunology, Yokohama Institute, 1-7-22 Suehiro-cho, Tsurumi-Ku, Yokohama, Kanagawa, 230-0045, Japan. §University of Massachusetts Medical School, 373 Plantation Street, Biotech 2, Suite 218, Worcester, Massachusetts 01605, USA. Correspondence to L.D.S. e-mail: lds@jax.org*
doi:10.1038/nri2017

© 2007 Nature Publishing Group

Nude mice
Mice homozygous for a mutation in the forkhead box N1 (*Foxn1*) gene, which causes both hairlessness and impaired development of the thymus, resulting in an inability to generate mature T cells.

SCID-hu mice
Severe combined immunodeficiency (*scid*) mice engrafted with human fetal liver and thymus tissue under the renal capsule.

Hu-SRC-SCID mice
Severe combined immunodeficiency (*scid*) mice that have been sublethally irradiated and injected with human haematopoietic stem cells (HSCs). The HSCs are obtained from three main sources: bone marrow, umbilical cord blood or granulocyte colony-stimulating factor (G-CSF)-mobilized peripheral blood, which contains large numbers of human HSCs.

of NK cells and other components of innate immunity, which impedes the engraftment of the human lymphoid compartment (TABLE 1).

The third breakthrough was the humanization of immunodeficient mice homozygous for targeted mutations at the interleukin-2 receptor (IL-2R) γ-chain locus (*Il2rg*; also known as the common cytokine-receptor γ-chain, γc)[14–17]. These mice support greatly increased engraftment of human tissue, HSCs and PBMCs compared with all previously developed immunodeficient humanized mouse models (TABLE 2). The IL-2R γ-chain is a crucial component of the high-affinity receptors for IL-2, IL-4, IL-7, IL-9, IL-15 and IL-21, and it is required for signalling through these receptors[18]. The absence of the IL-2R γ-chain leads to severe impairments in T- and B-cell development and function, and completely prevents NK-cell development[19–21].

Mice with targeted mutations in the *Il2rg* locus were produced independently as early as 1995, with the nomenclature that was used to describe these mice reflecting the origin of the strain and the genetic mutation used to generate that strain[19–22]. An important step forward was the generation of immunodeficient $Il2rg^{-/-}$ mice, which allowed their humanization after engraftment with human cells and tissues. These immunodeficient strains of $Il2rg^{-/-}$ mice include NOD.Cg-$Prkdc^{scid}Il2rg^{tm1Wjl}$ mice (REFS 16,17; abbreviated as NOD/LtSz-*scid* $Il2rg^{-/-}$ mice), NODShi.Cg-$Prkdc^{scid}Il2rg^{tm1Sug}$ mice (REFS 14,23; abbreviated as NOD/Shi-*scid* $Il2rg^{-/-}$, and often referred to as NOG, mice), C.Cg-$Rag2^{tm1Fwa}Il2rg^{tm1Sug}$ mice (REF. 15; abbreviated as BALB/c-$Rag2^{-/-}Il2rg^{-/-}$ mice) and Stock ($H2^d$)-$Rag2^{tm1Fwa}Il2rg^{tm1Krf}$ mice (REF. 24; referred to by us as $H2^d$-$Rag2^{-/-}Il2rg^{-/-}$ mice). Please note that in this Review, both NOD/LtSz-*scid* $Il2rg^{-/-}$ mice and NOD/Shi-*scid* $Il2rg^{-/-}$ mice are referred to as NOD-*scid* $Il2rg^{-/-}$ mice (FIG. 1).

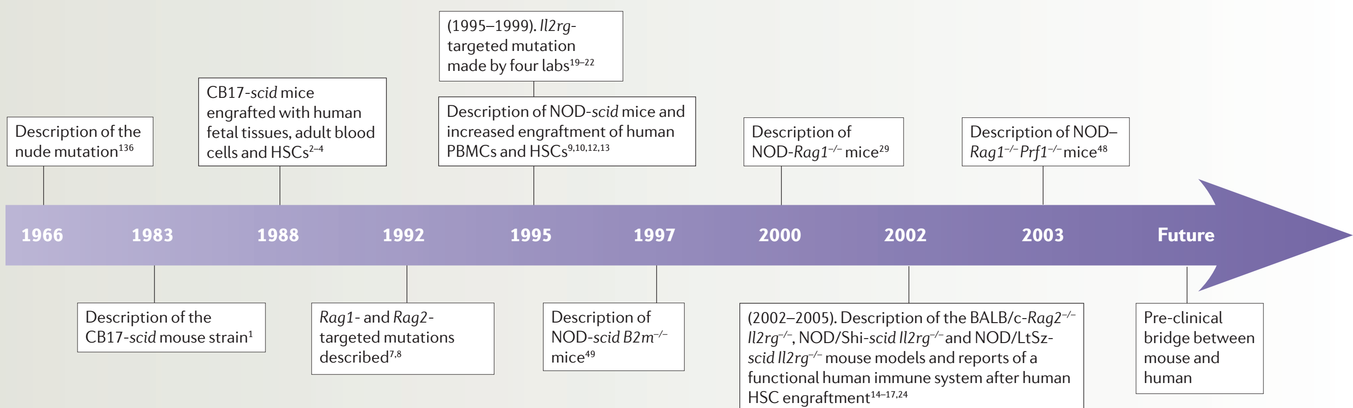
Engraftment of human HSCs and PBMCs in these immunodeficient strains of mice bearing the *Il2rg*-targeted mutations (TABLE 2) is greater than in all previously described humanized mouse strains (TABLE 1). However, these $Il2rg^{-/-}$ strains differ in terms of both the *Il2rg*-targeted mutation (leading to a complete absence of IL-2R γ-chain[19,20,22] or to a truncated γ-chain lacking the intracytoplasmic domain[21]) and the inbred strain background. Strain background is known to affect human-cell engraftment and function markedly in *scid* mice[10], and the ability of each of these strains to support human HSC and PBMC engraftment might differ. Here, we discuss the use of humanized mice in various areas of biomedical research, the remaining limitations to their use and ongoing efforts for the improvement of humanized mice.

Haematopoiesis

A true pluripotent HSC is defined as a long-term self-renewing stem cell that can repopulate the complete haematopoietic system of graft recipients and sustain long-term haematopoiesis after engraftment[25]. Owing to the ethical constraints on studying the ability of HSCs to repopulate the haematopoietic system in humans, investigators have relied on animal models for the *in vivo* study of human HSC function. Originally, these models included the use of fetal sheep[26] and heavily irradiated[27] or nude[28] mice. Very low levels of human stem-cell engraftment were observed using these models, which therefore required sensitive molecular approaches for the detection of human-cell engraftment. The discovery of CB17-*scid* mice led to the development of two powerful models for the study of human haematopoiesis in mice engrafted with human HSCs. The first model, known as SCID-hu mice, was developed by engrafting human fetal tissues into CB17-*scid* mice[3]. The second model, known as Hu-SRC-SCID mice, was developed by injecting human HSCs into irradiated CB17-*scid* mice[4].

The most recent breakthrough in this field followed the generation of immunodeficient mouse recipients with targeted mutations of *Il2rg*. These mice have been used extensively in the Hu-SRC-SCID model for the

Timeline | **Important events in the development of humanized mice**



B2m, β_2-microglobulin; HSC, haematopoietic stem cell; *Il2rg*, interleukin-2 receptor γ-chain; NOD, non-obese diabetic; PBMC, peripheral-blood mononuclear cell; *Prf1*, perforin 1; *Rag*, recombination-activating gene; *scid*, severe combined immunodeficiency.

© 2007 Nature Publishing Group

Table 1 | **Immunodeficient mouse hosts for human cells and tissues**

Mutant allele	Common strain name	Strain nomenclature	Phenotype	Advantages	Disadvantages	Refs
$Foxn1^{nu}$	C57BL/6-*nu*	B6.Cg-$Foxn1^{nu}$	• Athymic	• Lacks T cells	• High NK-cell activity • Intact humoral immunity • No engraftment of human haematopoietic cells	28,136
$Prkdc^{scid}$	CB17-*scid*	C.BKa Igh^{b}-$Prkdc^{scid}$/IcrSmn	• No mature T and B cells • Radiation sensitive (DNA-repair defect, cannot survive high doses of radiation)	• Lacks mature T and B cells	• High level of innate immunity and NK-cell function • Leaky • Very low level of engraftment of human cells	1
$Prkdc^{scid}$	NOD-*scid*	NOD.CB17-$Prkdc^{scid}$	• No mature T and B cells • Radiation sensitive • Decreased innate immunity	• Low level of innate immunity • Low NK-cell function • Increased engraftment of human HSCs and PBMCs	• Residual innate immunity • Low but present NK-cell activity • Decreased lifespan owing to thymic lymphomas	9
$Prkdc^{scid}$ $Lyst^{bg}$	BALB/c-*scid bg*	C.Cg-$Lyst^{bg}Prkdc^{scid}$	• No mature T and B cells • Radiation sensitive • Decreased NK-cell activity	• Low NK-cell function	• High level of innate immunity but low level of NK-cell killing • Low level of engraftment of human HSCs	137
$Prkdc^{scid}$ $Lyst^{bg\text{-}J}$	C57BL/6-*scid bg*	B6.Cg-$Lyst^{bg\text{-}J}$ $Prkdc^{scid}$/J	• No mature T and B cells • Decreased NK-cell activity	• Low NK-cell function	• High level of innate immunity but low level of NK-cell killing • Very low level of human-cell engraftment	11
$Prkdc^{scid}$ $B2m^{tm1Unc\text{-}J}$	NOD-*scid* $B2m^{-/-}$	NOD.Cg-$Prkdc^{scid}$ $B2m^{tm1Unc}$/J	• No mature T and B cells • Radiation sensitive • No β_2m, leading to lack of MHC class I expression	• Very low NK-cell function • Increased engraftment of human HSCs and PBMCs	• Short lifespan owing to rapid development of thymic lymphomas • Haemachromatosis	49,138
$Prkdc^{scid}$ Tg(CMV-IL3,CSF2,KITLG)1Eav	NOD-*scid* IL-3-, GM-CSF- and SCF-transgenic	NOD.Cg-$Prkdc^{scid}$ Tg(CMV-IL3,CSF2,KITLG)1Eav/YgyJ	• No mature T and B cells • Radiation sensitive • Transgenic human cytokine production	• Transgenic expression of human haematopoietic factors	• Low level of engraftment of human HSCs in bone marrow • Expanded terminal myelopoiesis	139
$Rag1^{tm1Mom}$	NOD-$Rag1^{-/-}$	NOD.129S7(B6)-$Rag1^{tm1Mom}$/J	• *Rag1* mutation leading to lack of mature T and B cells	• Radiation resistant (can survive high doses of radiation)	• Residual innate immunity • Low but present NK-cell activity • Low and variable level of engraftment	29
$Rag1^{tm1Mom}$ $Prf1^{tm1Sdz}$	NOD-$Rag1^{-/-}$ $Prf1^{-/-}$	NOD.Cg-$Rag1^{tm1Mom}$ $Prf1^{tm1Sdz}$/SzJ	• *Rag1* mutation leading to lack of mature T and B cells • Lack of perforin	• Radiation resistant • Very low NK-cell cytotoxicity • High level of engraftment of human PBMCs	• Very low NK-cell killing but NK cells still present • Limited engraftment of human HSCs	48
$Prkdc^{scid}$ Tg(HLA-A2.1)Enge	NOD-*scid* HLA-A2.1-transgenic	NOD.Cg-$Prkdc^{scid}$ Tg(HLA-A2.1)1Enge/J	• Transgenic expression of human HLA-A2.1	• Transgenic expression of human MHC molecules	• Immunodeficient $Il2rg^{-/-}$ strain under development	41

B2m, β_2-microglobulin; *bg*, beige; CMV, cytomegalovirus; *Foxn1*, forkhead box N1; GM-CSF, granulocyte/macrophage colony-stimulating factor; HSC, haematopoietic stem cell; IL, interleukin; *Il2rg*, interleukin-2 receptor γ-chain; *Lyst*, lysosomal trafficking regulator; NK, natural killer; NOD, non-obese diabetic; *nu*, nude; PBMC, peripheral-blood mononuclear cell; *Prf1*, perforin 1; *Prkdc*, protein kinase, DNA activated, catalytic polypeptide; *Rag*, recombination-activating gene; SCF, stem-cell factor; *scid*, severe combined immunodeficiency; Tg, transgenic.

© 2007 Nature Publishing Group

Table 2 | **Immunodeficient hosts for human cells and tissues based on targeted mutations of the IL-2 receptor γ-chain**

Mutant allele	Common strain name	Strain nomenclature	Phenotype	Advantages	Disadvantages	Refs
Prkdc^{scid} *Il2rg*^{tm1Wjl}	NOD/LtSz-*scid* *Il2rg*$^{-/-}$	NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl}/SzJ	• No mature T and B cells • Radiation sensitive • IL-2R γ-chain deficiency; no high-affinity signalling through multiple cytokine receptors leading to many innate-immune defects	• Long lifespan • Further reduction in innate immunity • NK cells absent • Higher level of engraftment of human cells • Develop functional human immune system • Complete absence of *Il2rg* gene	• Lack appropriate MHC molecules for T-cell selection in the mouse thymus • Seem to lack some human-specific cytokines required for human cell development and survival • Low and variable level of T-cell-dependent antibody responses	16,17
Prkdc^{scid} *Il2rg*^{tm1Sug}	NOD/Shi-*scid* *Il2rg*$^{-/-}$	NODShi.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Sug}/Jic	• Similar to NOD/LtSz-*scid Il2rg*$^{-/-}$ mice	• Similar to NOD/LtSz-*scid Il2rg*$^{-/-}$ mice except IL-2R γ-chain is truncated, not absent, and can still bind cytokines	• Similar to NOD/LtSz-*scid Il2rg*$^{-/-}$ mice	14,37
Rag2^{tm1Fwa} *Il2rg*^{tm1Sug}	BALB/c-*Rag2*$^{-/-}$ *Il2rg*$^{-/-}$	C.Cg-*Rag2*^{tm1Fwa} *Il2rg*^{tm1Sug}/Jic	• Similar to NOD/LtSz-*scid Il2rg*$^{-/-}$ mice	• Similar to NOD/LtSz-*scid Il2rg*$^{-/-}$ mice except they are radiation resistant	• Similar to NOD/LtSz-*scid Il2rg*$^{-/-}$ mice	15
Rag2^{tm1Fwa} *Il2rg*^{tm1Krf}	H2^{d}-*Rag2*$^{-/-}$*Il2rg*$^{-/-}$	Stock (H2^{d})-*Rag2*^{tm1Fwa} *Il2rg*^{tm1Krf}/Brn	• Similar to NOD/LtSz-*scid Il2rg*$^{-/-}$ mice	• Similar to NOD/LtSz-*scid Il2rg*$^{-/-}$ mice except they are radiation resistant	• Similar to NOD/LtSz-*scid Il2rg*$^{-/-}$ mice	22,24

Il2rg, interleukin-2 receptor γ-chain; NK, natural killer; NOD, non-obese diabetic; *Prkdc*, protein kinase, DNA activated, catalytic polypeptide; *Rag*, recombination-activating gene; *scid*, severe combined immunodeficiency.

study of human haematopoiesis, and this model is the most common use of these new strains of *Il2rg*$^{-/-}$ mice. A common feature of all of the strains of immunodeficient *Il2rg*$^{-/-}$ mice is that the host must be conditioned with sublethal γ-radiation before HSC injection for optimal human HSC engraftment, although the level of irradiation differs on the basis of whether the host is newborn or adult, and expresses the *scid* or the *Rag1*$^{-/-}$ or *Rag2*$^{-/-}$ mutations. Newborn mice are more sensitive to irradiation than are adult mice[16,17], and the *scid* mutation, which causes defects in DNA repair, leads to further radiosensitivity compared with either the *Rag1*$^{-/-}$ or *Rag2*$^{-/-}$ mutations[9,29].

Several injection routes have also been used to attempt to direct the human HSCs to a supportive microenvironmental 'niche', which is important for the development and differentiation of HSCs. Human HSCs have been injected intravenously into irradiated adult[16] or newborn[17] recipients. Irradiated newborn mice have also been engrafted by intrahepatic[15] and intraperitoneal[24] injection. Other engraftment protocols developed in NOD-*scid* mice that increase the level of engraftment include injection into the bone marrow of adult mice[30] and *in utero* injection[31], but these protocols have not yet been tested in immunodeficient *Il2rg*$^{-/-}$ mice. Intravenous injection increases the engraftment of HSCs compared with intraperitoneal injection. Intrahepatic and intra-bone-marrow injection of newborns and *in utero* injection have been used on the basis that these routes of injection should bypass homing requirements and place the HSCs directly into a supportive microenvironment. However, the optimal host strain, age and route of injection are not yet known and await direct comparative analysis of the various model systems.

These various models of immunodeficient *Il2rg*$^{-/-}$ mice (TABLE 2) have allowed more reproducible and increased human-cell engraftment, using smaller numbers of HSCs, compared with all of the immunodeficient mouse models described previously (TABLE 1). The successful differentiation of human HSCs and distinct progenitor-cell populations into many lineages of haematopoietic cells has been observed in *Il2rg*$^{-/-}$ mice, including the generation of platelets[17], red blood cells[17] and T-cell populations[14–17,24] that did not reproducibly develop in previous models (TABLE 1). In addition, analyses of short- and long-term repopulating stem cells[32], as well as analyses of complex cell interactions such as those found in the immune system, are now possible using the *Il2rg*$^{-/-}$ mice.

A particularly attractive use for these *Il2rg*$^{-/-}$ models is in gene-therapy research. The development of new vectors that enable the genetic transduction of HSCs[33] has provided a means to investigate therapies for the correction of human haematological defects *in vivo*[34]. The relatively long lifespan of NOD/LtSz-*scid Il2rg*$^{-/-}$ mice (more than 90 weeks[16]) compared with NOD-*scid* mice (37 weeks[9]) enables the long-term efficacy, as well as safety, of gene therapy to be determined. These *Il2rg*$^{-/-}$ models promise to generate new insights into human haematopoiesis and provide pre-clinical models for gene therapy. Experimental gene therapy has

© 2007 Nature Publishing Group

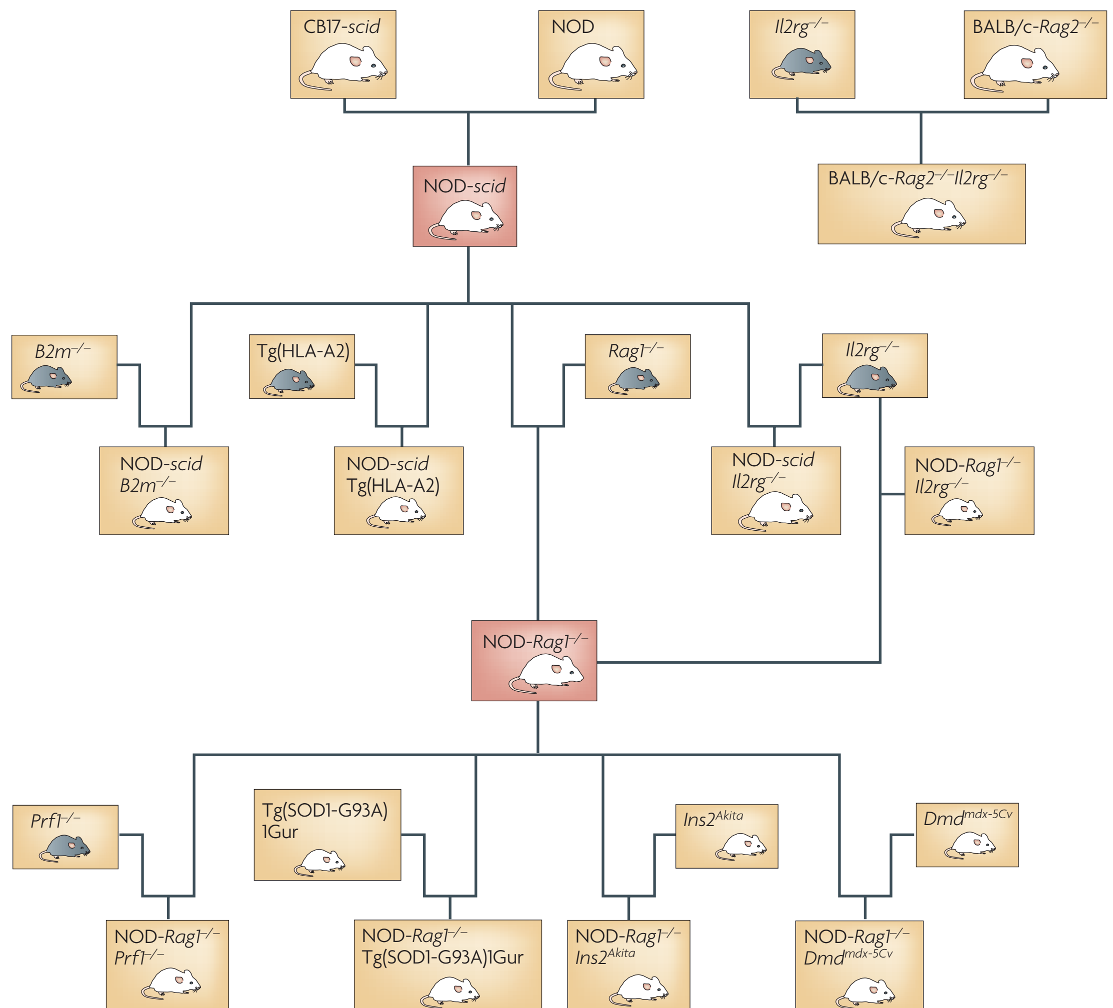


Figure 1 | **A schematic diagram of the development of various immunodeficient mouse models that are used as hosts for human cells and tissues.** Progress in the development of humanized mouse models was advanced by the discovery of the severe combined immunodeficiency (*scid*) gene mutation, which occurs spontaneously on the CB17 strain background. The CB17-*scid* mouse was the first immunodeficient mouse model shown to engraft with human haematopoietic cells. Another important advance in the development of humanized mice came from crossing mice with the *scid* mutation onto the non-obese diabetic (NOD) strain, which led to improved engraftment of human haematopoietic cells owing to decreased natural killer (NK)-cell activity and decreased innate immunity. In the figure, the NOD strain nomeclature denotes both the NOD/LtSz and NOD/Shi sublineages. A breakthrough in the effectiveness of humanized mice came from crossing immunodeficient mice homozygous for targeted mutations at the interleukin-2 receptor (IL-2R) γ-chain locus (*Il2rg*; also known as the common cytokine receptor γ-chain, γc) onto the NOD/LtSz-*scid*, NOD/Shi-*scid*, NOD-*Rag1*$^{-/-}$ (recombination-activating gene-1-deficient) and BALB/c-*Rag2*$^{-/-}$ strain backgrounds. This resulted in mouse strains with a complete absence of NK-cell activity, further decreases in innate immunity and a greatly increased ability to support the engraftment of human haematopoietic cells and tissues. Additional crosses have generated *scid* or *Rag1*$^{-/-}$ mice expressing transgenes or mutant alleles, to produce mouse models of human diseases for use in studying regenerative medicine. The red boxes indicate the NOD-*scid* and NOD-*Rag1*$^{-/-}$ strains that have provided the starting material for many subsequent genetic stocks. *B2m*, β_2-microglobulin; *Dmd*, Duchenne muscular dystrophy; *Ins2*, insulin II; *Prf1*, perforin 1; SOD1, superoxide dismutase 1; Tg, transgenic.

recently been examined in BALB/c-*Rag2*$^{-/-}$*Il2rg*$^{-/-}$ mice that were engrafted as newborns with human HSCs. Transfer of short interfering RNA (siRNA) targeting the gene encoding the human tumour-suppressor p53 (*TP53*) into human CD34^{+} HSCs resulted in the transgenic expression of the siRNA in multiple cell lineages, accompanied by a >95% decrease in the level of expression of p53 (REF. 24).

Development and function of the immune system

An important advance for immunological research would be the establishment of a functional human immune system in mice that generates robust primary and secondary immune responses. Attempts to achieve this goal are based on three model systems. One model relies on the transgenic expression of human molecules such as HLA (human MHC) molecules or

© 2007 Nature Publishing Group

immunoglobulins in immunocompetent mice. HLA-transgenic mice are used to identify antigens presented to T cells by HLA molecules, whereas human immunoglobulin-transgenic mice are used mainly to generate human monoclonal antibodies, such as the epidermal growth factor receptor (EGFR)-specific antibody[35], which are being developed for therapeutic applications.

Two additional humanized mouse models rely on the engraftment of immunodeficient mice with human haematopoietic cells: the Hu-SRC-SCID model, which involves the engraftment of human HSCs[4]; and the Hu-PBL-SCID model, which involves the adoptive transfer of PBMCs to CB17-*scid* mice[2]. Before the development of immunodeficient *Il2rg*$^{-/-}$ mice, the engraftment of human PBMCs and HSCs in immunodeficient mice was variable and often occurred only at a low level. Furthermore, engraftment of human HSCs routinely failed to generate human T cells[5], although B cells with diverse immunoglobulin repertoires were observed[36].

The development of immunodeficient *Il2rg*$^{-/-}$ mice led to significant improvements in the ability to generate a functional human immune system in the Hu-SRC-SCID model. In irradiated NOD-*scid Il2rg*$^{-/-}$ recipients, HSC engraftment leads to the generation of both T and B cells[14,16,17,37] and the T cells that develop progress through the expected stages of intrathymic development. Similarly, adult irradiated BALB/c-*Rag2*$^{-/-}$*Il2rg*$^{-/-}$ mice depleted of host macrophages by treatment with liposome-encapsulated dichloromethylene-bisphosphonate (clodronate) and engrafted with human HSCs generate human T cells *de novo*[38], as do irradiated newborn BALB/c-*Rag2*$^{-/-}$*Il2rg*$^{-/-}$ mice injected intrahepatically[15]. By contrast, there are no reports of successful engraftment of human HSCs in C57BL/6-*Rag2*$^{-/-}$*Il2rg*$^{-/-}$ mice. In NOD-*scid Il2rg*$^{-/-}$ and BALB/c-*Rag2*$^{-/-}$*Il2rg*$^{-/-}$ mice engrafted with human HSCs, human myeloid and plasmacytoid dendritic cells (DCs) also develop. Antibody responses after immunization with T-cell-dependent antigens can be observed[15,17], functional CD5$^+$ B cells develop, and IgM antibodies are produced after immunization with a T-cell-independent antigen[39]. Moreover, human CD4$^+$CD25$^+$FOXP3 (forkhead box P3)$^+$ regulatory T cells develop in the thymus[15]. Injection of BALB/c-*Rag2*$^{-/-}$*Il2rg*$^{-/-}$ mice, that were engrafted as newborns with human CD34$^+$ HSCs from fetal liver, with an agonistic CD28-specific antibody induces intrathymic expansion of T cells and regulatory T cells[40]. This is accompanied by a transient accumulation of T cells in the periphery followed by T-cell depletion[40], which indicates that these mice might be a useful model for pre-clinical analyses of immunomodulatory antibodies.

However, limitations remain even with these advanced Hu-SRC-SCID models. Although T-cell-dependent anti-tetanus antibody responses are generated, the antibody titres are low[15], and human allograft rejection or T-cell-mediated responses *in vivo* (such as delayed-type hypersensitivity) have not been reported. The lack of a robust immune response might be due, in part, to the relative absence of HLA expression on mouse thymic stromal cells, which are important for the positive selection of HLA-class-I-restricted human T cells. Although human HLA-expressing cells of haematopoietic origin are present in the thymus in these mice, there would be few, if any, human HLA-class-I-expressing stromal cells. Human T cells selected on mouse MHC (H2) antigens would not be able to recognize antigens presented by HLA-expressing human antigen-presenting cells (APCs) in the periphery.

It has been reported that human HLA-restricted T-cell clones can be generated from NOD-*scid Il2rg*$^{-/-}$ mice engrafted with human HSCs, which indicates that at least some positive selection on human HLA$^+$ HSC-derived cells in the thymus is possible[17]. In addition, negative selection on mouse MHC antigens seems to occur as there have been no reports of human T-cell xenoreactivity against mouse tissues, which would lead to the development of a xenograft-versus-host disease. However, more efficient positive and negative selection should occur in the thymus of HLA-transgenic mice[41,42], which are currently being generated in the NOD-*scid Il2rg*$^{-/-}$ strain. Another limitation of the Hu-SRC-SCID models that are based on the *Il2rg*$^{-/-}$ mouse strains is the absence of Peyer's patches and lymph nodes that can support a human immune system[19–21]. This constrains the development of a peripheral human immune system[43]. Future advances in the development of synthetic lymphoid-like organoids[44] might partially overcome this limitation.

Human adaptive and innate immune responses have also been generated in a model that involves engraftment of human fetal liver and thymus tissue under the renal capsule of NOD-*scid* mice, followed by sublethal irradiation and intravenous injection with CD34$^+$ cells from the same fetal liver source. This model, known as NOD/SCID-hu BLT mice (abbreviated as BLT mice), was shown to support the generation of human T cells, B cells, monocytes, macrophages and DCs[45]. These BLT mice mount a T-cell response to toxic shock syndrome toxin 1 (TSST1) superantigens and generate HLA-class-I- and HLA-class-II-restricted T-cell responses to Epstein–Barr virus (EBV) infection.

The Hu-PBL-SCID model is used for studies of infectious agents, vaccines, alloimmunity and autoimmunity[46,47]. As for the Hu-SRC-SCID models based on CB17-*scid* and NOD-*scid* mice, the levels of engraftment and function of human PBMCs are constrained by host NK-cell activity in Hu-PBL-SCID mice. To overcome this, targeted mutations of the genes encoding the pore-forming protein perforin 1 (*Prf1*) or β_2-microglobulin (*B2m*) were backcrossed onto NOD-*Rag1*$^{-/-}$ or NOD-*scid* mice[48,49], respectively. Perforin is the main mediator of NK-cell cytotoxicity and NOD-*Rag1*$^{-/-}$*Prf1*$^{-/-}$ mice lack NK-cell cytotoxic function[48]. β_2-microglobulin is required for the expression of MHC class I molecules and the lack of MHC class I molecules in NOD-*scid B2m*$^{-/-}$ mice prevents NK-cell development. As expected, NOD-*scid B2m*$^{-/-}$ mice are deficient in functional NK cells[49].

Even with the development of these two new strains, the consistency of PBMC engraftment remained a problem. Few B cells or myeloid cells engraft. Almost all

Xenoreactivity
An immune reactivity of cells or antibody from one animal directed against cells or tissues of a different species.

NOD/SCID-hu BLT mice
(BLT mice). Non-obese diabetic (NOD)-severe combined immunodeficiency (*scid*) mice engrafted with human fetal liver (L) and thymus (T) under the renal capsule. Three weeks later, mice are irradiated and then injected with a suspension of CD34$^+$ cells from the same human fetal liver sample. The injected fetal liver cells seed to the mouse bone marrow (B).

© 2007 Nature Publishing Group

of the engrafted T cells acquire an activated phenotype, perhaps owing to xenoreactivity[50], which often leads to T-cell anergy and lethal xenograft-versus-host disease[51]. Although secondary immune responses have been reported in these mice, primary immune responses have been variable and difficult to reproduce[46,47]. However, Hu-PBL-SCID mice have been useful for studying allograft rejection, the *in vivo* evaluation of human T-cell-specific reagents[52], and studying human-specific infectious agents (see next section).

In early Hu-PBL-SCID mouse models, incomplete rejection of human skin[53] and human islet allografts[41] was observed, although histological evidence of inflammation and islet necrosis was evident[41]. Modifications using new genetic strains led to a model that had complete rejection of allogeneic HLA-transgenic mouse islet grafts[41], but limitations to this model remained, including the variability of human PBMC engraftment. In addition, limited production of human myeloid cells and B cells was observed in these mice, the latter possibly being due to the lack of human species-specific cytokines that support B-cell survival and development. We recently observed that administration of human B-lymphocyte stimulator (BLYS; also known as TNFSF13B) to NOD-*Rag1*$^{-/-}$ *Prf1*$^{-/-}$ mice engrafted with human PBMCs leads to increased levels of human B-cell engraftment (D.L.G., unpublished observations). BLYS is required for both the differentiation and survival of human B cells, as well as the survival, differentiation and activation of monocytes[54,55].

The EBV-associated B-cell lymphoma that is observed in CB17-*scid* mice engrafted with human PBMCs[56] does not occur in NOD-*scid* recipients[57]. The lack of EBV-associated lymphomas has been hypothesized to result from increased engraftment of functional human cytotoxic $CD8^+$ T cells in NOD-*scid* mice[57], which prevents the development of EBV-associated lymphomas.

It has been reported that high levels of human PBMC engraftment are observed in BALB/c-*Rag2*$^{-/-}$ *Il2rg*$^{-/-}$ mice[58], but in this report, sublethal irradiation and macrophage depletion by clodronate-containing liposomes were used as host pre-conditioning agents. Pre-conditioning BALB/c-*Rag2*$^{-/-}$*Il2rg*$^{-/-}$ mice before PBMC engraftment also accelerates the development of xenograft-versus-host disease, thereby precluding studies of other functional capabilities of the engrafted cells.

We have also observed high levels of human PBMC engraftment in NOD/LtSz-*scid Il2rg*$^{-/-}$ recipients, but the engraftment did not require host pre-conditioning and accelerated xenograft-versus-host disease was not observed (D.L.G., unpublished observations). Human PBMC engraftment is greatly increased in NOD/LtSz-*scid Il2rg*$^{-/-}$ recipients compared with NOD-*scid* mice. Highly reproducible engraftment is achieved with the injection of small numbers of human cells, and consistent rejection of human allogeneic islets is obtained in these mice, which indicates that this new immunodeficient recipient is likely to provide a robust Hu-PBL-SCID model for the study of human allograft rejection and of therapies that might modulate this rejection process.

Remaining limitations of the Hu-PBL-SCID mouse models include the xenoreactivity of the human cells against mouse antigens[50]. Genetic modification to eliminate mouse MHC expression is currently underway in our laboratories and this could reduce xenoreactivity, as much of the xenoreactivity seems to be directed against mouse MHC molecules[50]. Alternatively, genetic modification of immunodeficient mice using the recently described transgene encoding the simian diphtheria-toxin receptor, driven by the DC-specific *Cd11c* promoter, provides a method to eliminate mouse DCs specifically[59]. Mouse cells express a low-affinity diphtheria-toxin receptor and only the transgene-positive DCs are susceptible to the toxic effects of administered diphtheria toxin[59]. The elimination of host DCs should decrease xenoreactive responses of the engrafted human PBMCs.

Infectious diseases

Many infectious diseases of humans are caused by organisms that do not infect mice or other laboratory animal species, which precludes the study of such diseases in animal models. This includes the agents that cause AIDS, malaria, filariasis and Dengue haemorrhagic fever. Humanized mouse models now provide an opportunity to study the pathogenesis of these and other human-specific agents and to test potential vaccines in small animal models.

More than 15 years ago, CB17-*scid* mice engrafted with human PBMCs[60] or HSCs[61] were shown to support HIV infection, but owing to the low and variable level of human-cell engraftment, these models had limited use. As new strains of humanized mice were developed, additional models of infectious diseases have been described[62]. These include models for infection with Dengue virus[63], EBV[64], hepatitis C virus[65], *Brugia malayi*[66], *Mycobacterium tuberculosis*[67] and the liver[68] and erythroid[69] stages of *Plasmodium falciparum* infection. In addition, the use of xenografts of human fetal intestine has facilitated studies of enteric bacteria and protozoa[70]. Because the new generation of humanized mice based on the *Il2rg*$^{-/-}$ mutation support engraftment with human immune systems of increasing function, these models might be useful for studying the efficacy of HIV vaccines as well as other anti-viral agents[71–73].

Recent studies have shown high levels of infection with CXC-chemokine receptor 4 (CXCR4)- and CC-chemokine receptor 5 (CCR5)-tropic HIV in humanized mice. In two studies, BALB/c-*Rag2*$^{-/-}$*Il2rg*$^{-/-}$ mice were engrafted intrahepatically with human cord-blood $CD34^+$ cells[74] or with fetal-liver $CD34^+$ cells[75]. In both studies, long-term persistence of HIV viraemia was observed after infection of the humanized mice. Both studies reported $CD4^+$ T-cell depletion, but found little evidence of humoral antibody responses to HIV. By contrast, infection of human cells that had developed from cord-blood-derived $CD34^+$ HSCs engrafted to newborn NOD/Shi-*scid Il2rg*$^{-/-}$ mice resulted in the production of both HIV Env gp130-specific and Gag24-specific antibodies in mice with high levels of viraemia[76].

© 2007 Nature Publishing Group

Autoimmunity

The study of autoimmunity in humans is limited by restraints on the interventions that can be carried out, as well as by access to the target organs and tissues. Autoimmunity cannot be deliberately induced or adoptively transferred in humans, and in diseases such as type 1 diabetes mellitus, the target organ is unavailable for biopsy. To overcome these constraints, two humanized mouse models have been developed. First, autoantibody production or the cellular effector phases of many autoimmune diseases can be studied using Hu-PBL-SCID mice into which PBMCs from individuals with the disease are transferred. Second, HLA-transgenic immunocompetent mice have been used to identify potential autoantigenic targets of T cells in autoimmune disorders such as multiple sclerosis and type 1 diabetes mellitus.

Historically, researchers investigating autoimmunity in the early 1990s adoptively transferred PBMCs from individuals with thyroid diseases (such as Graves' disease) or type 1 diabetes to CB17-*scid* mice. In the case of PBMCs from patients with type 1 diabetes, they observed production of islet-antigen-specific autoantibodies in the recipient mice, but no evidence of disease[77]. When CB17-*scid Rag2*$^{-/-}$ mice on a segregating strain background were co-transplanted with thyroid organoids and PBMCs from patients with Graves' disease, the production of thyroid-peroxidase-specific autoantibodies was observed[78]. Comparable studies were carried out using cells from patients with rheumatoid arthritis; *scid* mice that received synovial cells or PBMCs produced antibodies specific for rheumatoid factor[79,80]. However, the engraftment of human cells recovered from inflamed synovia in CB17-*scid* mice was low, and cell infiltration and target-organ destruction were not observed in the host mice. This was probably due to the well-described inefficient engraftment of human PBMCs in this immunodeficient host. It will be important to investigate these systems using the new humanized mouse models based on the *Il2rg*$^{-/-}$ mutation, as these mice provide increasingly reliable levels of engraftment. Further improvements in these models could include the expression of HLA transgenes that are associated with the human autoimmune disorder being studied[81,82]. These new models might also be used to detect circulating autoreactive T cells, providing a biomarker for disease progression.

In the second model, the use of immunocompetent HLA-transgenic mice is accelerating the identification of autoantigens. For example, HLA-A2.1-restricted T cells from immunocompetent NOD-*HLA-A2.1*-transgenic mice have been used to identify autoantigens of potential clinical relevance in type 1 diabetes[83]. Furthermore, spontaneous diabetes[84,85] and multiple sclerosis[86], respectively, can develop in NOD and C57BL/6 HLA-transgenic mice, which provides new models to study the pathogenesis of autoimmunity.

In the future, new humanized mouse models of autoimmunity could be developed based on the generation and transfer of human autoreactive T-cell clones[87,88], the use of retroviral technology enabling the transduction of HSCs[89], and the availability of immunodeficient strains of *Il2rg*$^{-/-}$ mice that support the generation of a human immune system from HSCs. By combining these new technologies, it might be possible to generate human T-cell receptor (TCR)-transgenic (retrogenic[89]) mice, which would be engrafted with a human immune system enriched in circulating autoreactive T cells specific for the transgenic TCR. These mice could be used to investigate the development and function of human autoreactive T cells at various stages of the disease process.

Graves' disease
A type of autoimmune disease in which autoantibodies produced by the immune system overstimulate the thyroid gland, causing hyperthyroidism.

Retrogenic
A term used for T-cell receptor (TCR)-transgenic mice generated by retrovirus-mediated transduction of haematopoietic stem cells (HSCs) with a vector carrying a TCR transgene. These transduced HSCs are then injected into conditioned mice to reconstitute the mice with T cells expressing the TCR transgene.

Cancer

The study of cancer in humans is impeded by access to tissues and to the site of tumour growth, ethical concerns and the confounding effects of therapy on tumour growth and biology. To overcome these restrictions, human tumour biology, growth, angiogenesis and metastasis have been evaluated in immunodeficient mouse models, including nude, *scid*, *Rag1*$^{-/-}$ and *Rag2*$^{-/-}$ mice. Immunodeficient mice have also been important for investigating carcinogenesis, cancer therapy and imaging of tumour growth and metastasis.

The first immunodeficient mouse model of cancer to be developed was based on nude mice, which support the growth of solid human tumours[90]. CB17-*scid* mice were shown to support the engraftment of some transplantable human haematological neoplasms, but tumour growth was limited by the high levels of host NK-cell activity[91]. NOD-*scid* mice allowed the growth of transplantable lymphomas and leukaemias that grew poorly in CB17-*scid* mice[91], and NOD-*scid* mice therefore became the preferred host for studies of primary human acute myeloid leukaemia (AML)[92] and other primary human haematological neoplasms. Recently, the use of newborn NOD-*scid B2m*$^{-/-}$ mice as recipients has enabled the growth of human adult T-cell leukaemia caused by human T-cell leukaemia virus type 1 (HTLV1)-infected human $CD4^+$ T cells[93]. The development of NOD-*scid Il2rg*$^{-/-}$ strains that lack host NK-cell activity and are deficient in innate immune function might allow the growth of additional primary human tumours that previously have not grown in immunodeficient mice[94].

It has been hypothesized recently that tumours arise from a tumour stem-cell population. This hypothesis is based on the observation that a rare fraction of cells with stem-cell properties can initiate tumour growth[95,96]. Support for this concept comes from the ability of human tumour stem cells to grow tumours in immunodeficient mice. The use of the NOD-*scid* strains is helping to define human tumour stem cells phenotypically and functionally, in particular the stem cells that might be responsible for AML[97], myeloma[98], and breast[99], colon[100] and brain[101] tumours. The recent observation that primary human AML stem cells grow in newborn NOD-*scid Il2rg*$^{-/-}$ mice[102] highlights the potential of this new immunodeficient mouse model for the investigation of human tumour stem cells.

The engraftment of human tissue in immunodeficient mice has also been used to study carcinogenesis.

© 2007 Nature Publishing Group

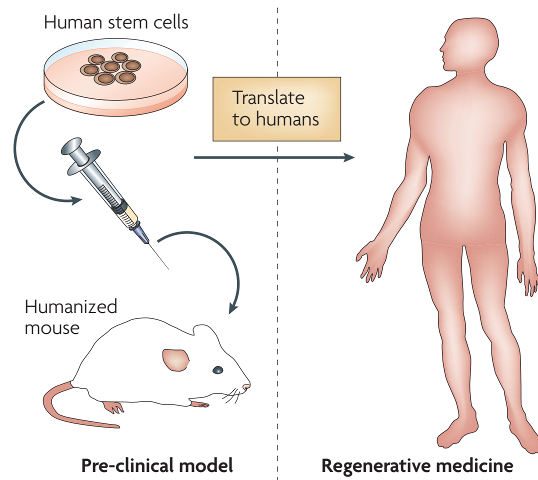


Figure 2 | **The use of severe combined immunodeficient mice in regenerative medicine.** This figure shows schematically the proposed use of humanized mice as a pre-clinical bridge between basic *in vitro* studies and the *in vivo* analysis of the efficacy of human stem-cell transplantation in regenerative medicine. On the left (top) are shown cultures of human stem cells (embryonic stem-cell-derived and adult stem-cell-derived populations) that have been generated *in vitro* and are available for the analysis of *in vivo* efficacy in humanized mice. Depicted bottom left is a humanized mouse engrafted with human stem cells by various routes of injection or implantation. The optimal route of transplantation depends on the stem- or progenitor-cell population under study and the proposed experimental design (see main text). Humanized mice can be used to link *in vitro* analyses of stem cells to the clinic by providing pre-clinical *in vivo* evaluation of the ability of the stem cells to engraft and their potential therapeutic efficacy. Shown on the right is the use of human stem cells for regenerative medicine in the clinic, for the repair or replacement of human cells and tissues, after their 'proof of principle' in humanized mice.

Transdifferentiation
Refers to the ability of a non-stem cell to transform into a different type of cell lineage, or when an already partly differentiated stem cell creates cells of different lineages or cell types.

Teratoma
A tumour that derives from pluripotent germ cells, comprising disorganized tissues derived from all three embryonic germ layers (ectoderm, mesoderm and endoderm). It can arise spontaneously in the human gonads.

For example, engrafted human skin exposed to ultraviolet irradiation, which causes mutations in genes such as *TP53* and *RAS*, can be used to study the development of squamous-cell carcinoma[103]. In addition, recent advances in imaging technologies have allowed human tumour growth and metastasis to be visualized in immunodeficient mice, leading to the development of imaging techniques that have been translated to the clinic[104]. Humanized mice can also be used for the evaluation of therapeutic approaches for the inhibition of human tumour growth, including the use of angiogenesis inhibitors[105], cell-based therapies[106], humanized antibodies[107], traditional immunosuppressive and immunotherapeutic protocols[108], and tumour-growth inhibitors[109,110].

What does the future hold for the use of humanized mice in cancer biology? Human tumour stem cells and the molecular and signalling cascades that lead to carcinogenesis can now be studied *in vivo* in humanized mice. This could lead to the development of patient-specific treatment options. Alternatively, the development of improved viral vectors for the transduction of human HSCs with specific oncogenes[111] might allow researchers to study the entire process of carcinogenesis *in vivo*, from stem cell to tumour. These studies would facilitate the identification of crucial checkpoints in carcinogenesis and provide targets for therapeutic intervention in the clinic.

Regenerative medicine

The promise of regenerative medicine is to transplant stem cells or their progeny into the human body to restore lost function. Humanized mice will allow pre-clinical evaluation of such cell-based therapies before translation to the clinic. This is particularly important as humanized mice can be used to evaluate not only the ability of the cells to engraft, but also their potential therapeutic efficacy.

Studies of regenerative medicine over the past few years have focused on stem-cell therapy, in particular using adult[112] and embryonic[113] stem cells, for the treatment of damaged tissues and organs in humans (FIG. 2). In the haematopoietic system, human bone-marrow- and cord-blood-derived adult stem cells have been shown to generate pancreatic islets, hepatocytes[114,115], cardiac myocytes[116,117], skeletal muscle[118], gastrointestinal epithelium[119], endothelium[120] and nerve cells[121] in humanized mice.

The human stem cells used in these studies might have undergone transdifferentiation or acquired the differentiated phenotype through fusion with host cells, or a combination of both processes[122]. Stem cells might also promote tissue repair through angiogenesis[121] or by providing a supportive environment for cell repair[123]. In the transdifferentiation model, identifying the relevant stem-cell population will be important for cell therapy. In the cell-fusion model, identifying the host target cell involved in the fusion process and elucidating the mechanism by which the donor cells restore host-cell function will be of interest. Transdifferentiation[124] and cell fusion[125] might both have some clinical efficacy[126], and in the field of regenerative medicine, the efficacy of the cell therapy in terms of reversing pathology, irrespective of the mechanism, is the ultimate goal.

In most studies, limited clinical efficacy of stem-cell transplantation for tissue repair is observed, although in models of heart injury, improved cardiac function has been reported after intravenous or intracardial injection of bone-marrow cells and cord blood into NOD-*scid* mice[117]. Pilot clinical trials using transfusion of cord blood in patients with cardiac ischaemic injury have been initiated on the basis of these results[127].

Mouse models have also shown that human embryonic stem cells can generate insulin-producing cells *in vivo*[128] and might be useful as a source of pancreatic islets for transplantation into patients with type 1 diabetes. However, embryonic stem cells commonly generate teratomas when transplanted into nude or *scid* mice[129,130], and this carcinogenic potential of embryonic stem cells is an important obstacle to the initiation of clinical trials. Attempts to generate human HSCs from

© 2007 Nature Publishing Group

Box 1 | **Optimizing humanized mouse models in biomedical research**

Further decrease of host innate immunity

Current mouse models that incorporate mutation of the interleukin-2 receptor γ-chain locus (*Il2rg*$^{-/-}$) together with the severe combined immunodeficiency (*scid*), recombination-activating gene 1 (*Rag1*$^{-/-}$) or *Rag2*$^{-/-}$ mutations lack mature T cells, B cells and natural killer (NK) cells. The non-obese diabetic (NOD)/LtSz and NOD/Shi strain backgrounds confer additional defects in innate immunity, including decreased macrophage function and an absence of haemolytic complement. The engraftment of human haematopoietic stem cells (HSCs) and peripheral-blood mononuclear cells (PBMCs) should be increased by the transient elimination of macrophages, dendritic cells and granulocytes. This can be accomplished by the transgenic expression of the diphtheria-toxin receptor driven by cell-specific promoters, by liposome-encapsulated dichloromethylene-bisphosphonate (clodronate) or by lineage-specific monoclonal antibodies.

Transgenic expression of human HLA and elimination of mouse H2 expression

Intrathymic expression of HLA molecules should support positive and negative selection of human T cells after HSC engraftment and human T-cell survival in the periphery. Elimination of mouse H2 molecules should reduce xenoreactivity and consequent T-cell anergy after PBMC engraftment.

Human cytokine support

The species specificity of haematopoietic growth factors and other cytokines limits the growth and differentiation of human HSCs in humanized mice. Transgenic expression or administration of certain human cytokines should promote human HSC engraftment and differentiation, and facilitate the function of mature human lymphocytes, in mice engrafted with human immune systems.

Availability of humanized lymphoid tissues

With the exception of the SCID-hu model (which was developed by engrafting human fetal tissues into CB17-*scid* mice[3]), the absence of human thymic tissue limits human T-cell development in humanized mice. The absence of mature lymph nodes and Peyer's patches containing human stromal elements limits the induction of human adaptive immune responses. Implantation of synthetic lymphoid-like organoids using human stromal cells embedded in biocompatible scaffolds could provide the lymphoid-organ structure that is required for the normal development of an immune response. New genetic models that eliminate NK-cell activity and decrease innate immunity, but retain lymph-node and Peyer's-patch development, await development.

Homing of human cells to haematopoietic tissues

Direct injection of human HSCs into the bone marrow has circumvented the loss of cells due to impaired homing. Direct injection of human HSCs into the thymic rudiment might improve human T-cell development. This might also be improved by the transgenic expression of human adhesion molecules, which should improve the homing and trafficking of human cells.

embryonic stem cells have met with differing outcomes when transplanted into immunodeficient mice. In one study, pulmonary embolisms were observed[131]. In two additional reports, HSCs were differentiated from human embryonic stem cells by culturing with mouse bone-marrow stroma[132,133]. These HSCs were found to engraft successfully in immunodeficient mice. However, ongoing debate continues to focus on the ethics of the use of human embryonic stem cells in experimental research that produces 'chimeric' humanized mice[134].

An underlying theme in experimental models of regenerative medicine is the requirement to injure the target tissue to create either a niche or appropriate regenerative signals necessary for the induction of stem-cell differentiation into the particular required cell type. Tissue damage can be accomplished in mouse models by surgical (such as myocardial infarction[117]), chemical (such as streptozotocin, a pancreatic β-cell cytotoxic agent used to induce diabetes[41]) or genetic modifications. Numerous genetic mouse models of tissue injury have been developed (see The Jackson Laboratory in Online links box) and many of these have been bred onto immunodeficient backgrounds. For example, the urokinase-type plasminogen activator (*Plau*)-transgenic *scid* mouse model of hepatic injury is used to facilitate the generation of human liver tissue from adult liver stem cells[115]. In our laboratory, genetic mutations that model Duchenne muscular dystrophy (*Dmd*$^{mdx-5Cv}$), amyotrophic lateral sclerosis (transgenic(SOD1-G93A)1Gur) and type 1 diabetes (*Ins2*^{Akita}) have been crossed with immunodeficient mice as models to study the clinical efficacy of stem-cell therapy (L.D.S., unpublished observations). Using genetic mutations to induce tissue injury provides reproducible, well-characterized models to study stem-cell plasticity and obviates the potential confounding effects of chemical toxicity or surgical trauma on the transplanted stem cells. In addition, because human HSCs, embryonic stem cells and mesenchymal stem cells are all sensitive to NK-cell-mediated killing[132,135], it will be important to establish these models in immunodeficient *Il2rg*$^{-/-}$ mice, which completely lack NK cells.

Myocardial infarction
An episode of acute cardiac ischaemia that leads to death of heart-muscle cells. It is usually caused by a thrombotic atherosclerotic plaque.

Future prospects and remaining limitations

The potential for new advances in our understanding of human biological systems provided by studies in humanized mice remains promising. Humanized mice can provide insights into *in vivo* human biology that would otherwise not be possible owing to ethical, logistical and/or technical constraints. The recent development of humanized mice based on immunodeficient *Il2rg*$^{-/-}$ hosts has overcome many of the limitations and constraints of previously available models. However, care should be taken when interpreting the existing literature on humanized mice, as many immunodeficient mouse strains, cell sources and routes of transplantation have been used, each with their own unique caveats and characteristics.

Remaining constraints include the need for genetic modifications to humanize the host strain further (BOX 1). For example, the expression of HLA molecules will facilitate proper intrathymic selection of human T cells during thymocyte differentiation and their survival in the periphery. HLA expression will also be useful for appropriate antigen presentation by host APCs in the peripheral tissues. Transgenic expression of human-specific cytokines might be required for the proper development and function of the transplanted cells. Human-specific adhesion molecules to facilitate proper trafficking of human cells might also be required, in particular for proper immune function. The low level of T-cell-dependent antibody responses observed in the currently available humanized mice might be the result of these remaining limitations. Despite these constraints, humanized mice offer great promise as models for the pre-clinical testing of drugs and human-cell-based therapeutics before their advancement to the clinic (FIG. 2).

© 2007 Nature Publishing Group

1. Bosma, G. C., Custer, R. P. & Bosma, M. J. A severe combined immunodeficiency mutation in the mouse. *Nature* **301**, 527–530 (1983).
2. Mosier, D. E., Gulizia, R. J., Baird, S. M. & Wilson, D. B. Transfer of a functional human immune system to mice with severe combined immunodeficiency. *Nature* **335**, 256–259 (1988).
The original description of the use of CB17-*scid* mice as hosts for human PBMCs, known as the Hu-PBL-SCID model.
3. McCune, J. M. *et al.* The SCID-hu mouse: murine model for the analysis of human hematolymphoid differentiation and function. *Science* **241**, 1632–1639 (1988).
The original description of the use of CB17-*scid* mice as hosts for human haematopoietic fetal liver and thymus, resulting in the development of the SCID-hu model. This model is still used, in particular for testing HIV therapeutics.
4. Lapidot, T. *et al.* Cytokine stimulation of multi-lineage hematopoiesis from immature human cells engrafted in SCID mice. *Science* **255**, 1137–1141 (1992).
The original description of the use of CB17-*scid* mice as hosts for human HSCs and the development of the Hu-SRC-SCID model.
5. Greiner, D. L., Hesselton, R. A. & Shultz, L. D. SCID mouse models of human stem cell engraftment. *Stem Cells* **16**, 166–177 (1998).
6. Fulop, G. M. & Phillips, R. A. The *scid* mutation in mice causes a general defect in DNA repair. *Nature* **347**, 479–482 (1990).
7. Mombaerts, P. *et al.* RAG-1-deficient mice have no mature B and T lymphocytes. *Cell* **68**, 869–877 (1992).
8. Shinkai, Y. *et al.* RAG-2-deficient mice lack mature lymphocytes owing to inability to initiate V(D)J rearrangement. *Cell* **68**, 855–867 (1992).
9. Shultz, L. D. *et al.* Multiple defects in innate and adaptive immunologic function in NOD/LtSz-*scid* mice. *J. Immunol.* **154**, 180–191 (1995).
10. Hesselton, R. M. *et al.* High levels of human peripheral blood mononuclear cell engraftment and enhanced susceptibility to HIV-1 infection in NOD/LtSz-*scid/scid* mice. *J. Infect. Dis.* **172**, 774–782 (1995).
11. Christianson, S. W. *et al.* Role of natural killer cells on engraftment of human lymphoid cells and on metastasis of human T-lymphoblastoid leukemia cells in C57BL/6J-*scid* mice and in C57BL/6J-*scid bg* mice. *Cell. Immunol.* **171**, 186–199 (1996).
12. Lowry, P. A. *et al.* Improved engraftment of human cord blood stem cells in NOD/LtSz-*scid/scid* mice after irradiation or multiple-day injections into unirradiated recipients. *Biol. Blood Marrow Transplant.* **2**, 15–23 (1996).
13. Pflumio, F. *et al.* Phenotype and function of human hematopoietic cells engrafting immune-deficient CB17-severe combined immunodeficiency mice and nonobese diabetic-severe combined immunodeficiency mice after transplantation of human cord blood mononuclear cells. *Blood* **88**, 3731–3740 (1996).
14. Ito, M. *et al.* NOD/SCID/γ_c^{null} mouse: an excellent recipient mouse model for engraftment of human cells. *Blood* **100**, 3175–3182 (2002).
This paper describes the development of a human immune system in NOD/Shi-*scid Il2rg*$^{-/-}$ mice engrafted as adults with human CD34$^+$ cord-blood HSCs.
15. Traggiai, E. *et al.* Development of a human adaptive immune system in cord blood cell-transplanted mice. *Science* **304**, 104–107 (2004).
This paper shows the development of a complete human immune system in BALB/c-*Rag2*$^{-/-}$*Il2rg*$^{-/-}$ mice engrafted intrahepatically as newborns with human cord-blood CD34$^+$ HSCs.
16. Shultz, L. *et al.* Human lymphoid and myeloid cell development in NOD/LtSz-*scid IL2rg^{null}* mice engrafted with mobilized human hematopoietic stem cells. *J. Immunol.* **174**, 6477–6489 (2005).
One of the first studies to show the development of a complete human immune system in NOD-*scid Il2rg*$^{-/-}$ mice engrafted with human mobilized peripheral-blood CD34$^+$ stem cells.
17. Ishikawa, F. *et al.* Development of functional human blood and immune systems in NOD/SCID/IL2 receptor γ-chain^{null} mice. *Blood* **106**, 1565–1573 (2005).
The first paper to show that engraftment of newborn NOD/LtSz-*scid Il2rg*$^{-/-}$ mice with human CD34$^+$ HSCs leads to the generation of a complete haematopoietic system, including red blood cells and platelets.
18. Sugamura, K. *et al.* The interleukin-2 receptor γ-chain: its role in the multiple cytokine receptor complexes and T cell development in XSCID. *Annu. Rev. Immunol.* **14**, 179–205 (1996).
19. Cao, X. *et al.* Defective lymphoid development in mice lacking expression of the common cytokine receptor γ-chain. *Immunity* **2**, 223–238 (1995).
20. DiSanto, J. P., Muller, W., Guy-Grand, D., Fischer, A. & Rajewsky, K. Lymphoid development in mice with a targeted deletion of the interleukin 2 receptor γ-chain. *Proc. Natl Acad. Sci. USA* **92**, 377–381 (1995).
21. Ohbo, K. *et al.* Modulation of hematopoiesis in mice with a truncated mutant of the interleukin-2 receptor γ-chain. *Blood* **87**, 956–967 (1996).
22. Jacobs, H. *et al.* PIM1 reconstitutes thymus cellularity in interleukin-7- and common γ-chain-mutant mice and permits thymocyte maturation in Rag- but not CD3γ-deficient mice. *J. Exp. Med.* **190**, 1059–1068 (1999).
23. Yahata, T. *et al.* A highly sensitive strategy for SCID-repopulating cell assay by direct injection of primitive human hematopoietic cells into NOD/SCID mice bone marrow. *Blood* **101**, 2905–2913 (2003).
24. Gimeno, R. *et al.* Monitoring the effect of gene silencing by RNA interference in human CD34$^+$ cells injected into newborn *Rag2*$^{-/-}$*γc*$^{-/-}$ mice: functional inactivation of p53 in developing T cells. *Blood* **104**, 3886–3893 (2004).
25. Bryder, D., Rossi, D. J. & Weissman, I. L. Hematopoietic stem cells: the paradigmatic tissue-specific stem cell. *Am. J. Pathol.* **169**, 338–346 (2006).
26. Zanjani, E. D., Almeida-Porada, G. & Flake, A. W. Retention and multilineage expression of human hematopoietic stem cells in human–sheep chimeras. *Stem Cells* **13**, 101–111 (1995).
27. Clutterbuck, R. D. *et al.* Studies on the development of human acute myeloid leukaemia xenografts in immune-deprived mice: comparison with cells in short-term culture. *Leuk. Res.* **9**, 1511–1518 (1985).
28. Ganick, D. J., Sarnwick, R. D., Shahidi, N. T. & Manning, D. D. Inability of intravenously injected monocellular suspensions of human bone marrow to establish in the nude mouse. *Int. Arch. Allergy Appl. Immunol.* **62**, 330–333 (1980).
29. Shultz, L. D. *et al.* NOD/LtSz-Rag1^{null} mice: an immunodeficient and radioresistant model for engraftment of human hematolymphoid cells, HIV infection, and adoptive transfer of NOD mouse diabetogenic T cells. *J. Immunol.* **164**, 2496–2507 (2000).
30. Mazurier, F., Doedens, M., Gan, O. I. & Dick, J. E. Rapid myeloerythroid repopulation after intrafemoral transplantation of NOD-SCID mice reveals a new class of human stem cells. *Nature Med.* **9**, 959–963 (2003).
31. Schoeberlein, A. *et al.* Engraftment kinetics of human cord blood and murine fetal liver stem cells following *in utero* transplantation into immunodeficient mice. *Stem Cells Dev.* **13**, 677–684 (2004).
32. Glimm, H. *et al.* Previously undetected human hematopoietic cell populations with short- term repopulating activity selectively engraft NOD/SCID-β2 microglobulin-null mice. *J. Clin. Invest.* **107**, 199–206 (2001).
33. Miyoshi, H., Smith, K. A., Mosier, D. E., Verma, I. M. & Torbett, B. E. Transduction of human CD34$^+$ cells that mediate long-term engraftment of NOD/SCID mice by HIV vectors. *Science* **283**, 682–686 (1999).
34. Cohen-Haguenauer, O. *et al.* *In vivo* repopulation ability of genetically corrected bone marrow cells from Fanconi anemia patients. *Proc. Natl Acad. Sci. USA* **103**, 2340–2345 (2006).
35. Bleeker, W. K. *et al.* Dual mode of action of a human anti-epidermal growth factor receptor monoclonal antibody for cancer therapy. *J. Immunol.* **173**, 4699–4707 (2004).
36. Kolar, G. R., Yokota, T., Rossi, M. I., Nath, S. K. & Capra, J. D. Human fetal, cord blood, and adult lymphocyte progenitors have similar potential for generating B cells with a diverse immunoglobulin repertoire. *Blood* **104**, 2981–2987 (2004).
37. Yahata, T. *et al.* Functional human T lymphocyte development from cord blood CD34$^+$ cells in nonobese diabetic/Shi-*scid*, IL-2 receptor-γ-null mice. *J. Immunol.* **169**, 204–209 (2002).
38. Legrand, N., Weijer, K. & Spits, H. Experimental models to study development and function of the human immune system *in vivo*. *J. Immunol.* **176**, 2053–2058 (2006).
39. Matsumura, T. *et al.* Functional CD5$^+$ B cells develop predominantly in the spleen of NOD/SCID/γc^{null} (NOG) mice transplanted either with human umbilical cord blood, bone marrow, or mobilized peripheral blood CD34$^+$ cells. *Exp. Hematol.* **31**, 789–797 (2003).
40. Legrand, N. *et al.* Transient accumulation of human mature thymocytes and regulatory T cells with CD28 superagonist in 'human immune system' *Rag2*$^{-/-}$*γc*$^{-/-}$ mice. *Blood* **108**, 238–245 (2006).
41. Banuelos, S. J. *et al.* Rejection of human islets and human HLA-A2.1-transgenic mouse islets by alloreactive human lymphocytes in immunodeficient NOD-*scid* and NOD-*Rag1*^{null}*Prf1*^{null} mice. *Clin. Immunol.* **112**, 273–283 (2004).
42. Camacho, R. E. *et al.* Intra-thymic/splenic engraftment of human T cells in HLA-DR1 transgenic NOD/scid mice. *Cell. Immunol.* **232**, 86–95 (2004).
43. Bajenoff, M. *et al.* Stromal cell networks regulate lymphocyte entry, migration, and territoriality in lymph nodes. *Immunity* **25**, 989–1001 (2006).
44. Suematsu, S. & Watanabe, T. Generation of a synthetic lymphoid tissue-like organoid in mice. *Nature Biotechnol.* **22**, 1539–1545 (2004).
45. Melkus, M. W. *et al.* Humanized mice mount specific adaptive and innate immune responses to EBV and TSST-1. *Nature Med.* **12**, 1316–1322 (2006).
46. Ifversen, P. & Borrebaeck, C. A. SCID-hu-PBL: a model for making human antibodies? *Semin. Immunol.* **8**, 243–248 (1996).
47. Murphy, W. J., Taub, D. D. & Longo, D. L. The huPBL-SCID mouse as a means to examine human immune function *in vivo*. *Semin. Immunol.* **8**, 233–241 (1996).
48. Shultz, L. D. *et al.* NOD/LtSz-*Rag1^{null}Pfp^{null}* mice: a new model system to increase levels of human peripheral leukocyte and hematopoietic stem cell engraftment. *Transplantation* **76**, 1036–1042 (2003).
49. Christianson, S. W. *et al.* Enhanced human CD4$^+$ T cell engraftment in β2-microglobulin-deficient NOD-*scid* mice. *J. Immunol.* **158**, 3578–3586 (1997).
50. Tary-Lehmann, M., Lehmann, P. V., Schols, D., Roncarolo, M. G. & Saxon, A. Anti-SCID mouse reactivity shapes the human CD4$^+$ T cell repertoire in hu-PBL-SCID chimeras. *J. Exp. Med.* **180**, 1817–1827 (1994).
51. Greiner, D. L. & Shultz, L. D. in *NOD Mice and Related Strains: Research Applications in Diabetes, AIDS, Cancer and Other Diseases* (eds Leiter, E. & Atkinson, M.) 173–203 (Landes Bioscience, Austin, 1998).
52. May, K. F. Jr *et al.* Anti-human CTLA-4 monoclonal antibody promotes T-cell expansion and immunity in a hu-PBL-SCID model: a new method for preclinical screening of costimulatory monoclonal antibodies. *Blood* **105**, 1114–1120 (2005).
53. Murray, A. G. *et al.* Human T-cell-mediated destruction of allogeneic dermal microvessels in a severe combined immunodeficient mouse. *Proc. Natl Acad. Sci. USA* **91**, 9146–9150 (1994).
54. Chang, S. K., Arendt, B. K., Darce, J. R., Wu, X. & Jelinek, D. F. A role for BLyS in the activation of innate immune cells. *Blood* **108**, 2687–2694 (2006).
55. Woodland, R. T. & Schmidt, M. R. Homeostatic proliferation of B cells. *Semin. Immunol.* **17**, 209–217 (2005).
56. Mosier, D. E. *et al.* EBV-induced human B cell lymphomas in hu-PBL-SCID mice. *AIDS Res. Hum. Retroviruses* **8**, 735–740 (1992).
57. Wagar, E. J. *et al.* Regulation of human cell engraftment and development of EBV-related lymphoproliferative disorders in Hu-PBL-scid mice. *J. Immunol.* **165**, 518–527 (2000).
58. van Rijn, R. S. *et al.* A new xenograft model for graft-versus-host disease by intravenous transfer of human peripheral blood mononuclear cells in *Rag2*$^{-/-}$*γc*$^{-/-}$ double-mutant mice. *Blood* **102**, 2522–2531 (2003).
59. Jung, S. *et al.* *In vivo* depletion of CD11c$^+$ dendritic cells abrogates priming of CD8$^+$ T cells by exogenous cell-associated antigens. *Immunity* **17**, 211–220 (2002).
60. Mosier, D. E. Human immunodeficiency virus infection of human cells transplanted to severe combined immunodeficient mice. *Adv. Immunol.* **63**, 79–125 (1996).
61. McCune, J. *et al.* The SCID-hu mouse: a small animal model for HIV infection and pathogenesis. *Annu. Rev. Immunol.* **9**, 399–429 (1991).
62. Davis, P. H. & Stanley, S. L. Jr Breaking the species barrier: use of SCID mouse–human chimeras for the study of human infectious diseases. *Cel. Microbiol.* **5**, 849–860 (2003).

© 2007 Nature Publishing Group

63. Bente, D. A., Melkus, M. W., Garcia, J. V. & Rico-Hesse, R. Dengue fever in humanized NOD/SCID mice. *J. Virol.* **79**, 13797–13799 (2005).
64. Islas-Ohlmayer, M. *et al.* Experimental infection of NOD/SCID mice reconstituted with human CD34+ cells with Epstein–Barr virus. *J. Virol.* **78**, 13891–13900 (2004).
65. Kneteman, N. M. *et al.* Anti-HCV therapies in chimeric scid-Alb/uPA mice parallel outcomes in human clinical application. *Hepatology* **43**, 1346–1353 (2006).
66. Nelson, F. K., Greiner, D. L., Shultz, L. D. & Rajan, T. V. The immunodeficient *scid* mouse as a model for human lymphatic filariasis. *J. Exp. Med.* **173**, 659–663 (1991).
67. Guirado, E. *et al.* Passive serum therapy with polyclonal antibodies against *Mycobacterium tuberculosis* protects against post-chemotherapy relapse of tuberculosis infection in SCID mice. *Microbes Infect.* **8**, 1252–1259 (2006).
68. Morosan, S. *et al.* Liver-stage development of *Plasmodium falciparum*, in a humanized mouse model. *J. Infect. Dis.* **193**, 996–1004 (2006).
69. Moreno, A. *et al.* The course of infections and pathology in immunomodulated NOD/LtSz-SCID mice inoculated with *Plasmodium falciparum* laboratory lines and clinical isolates. *Int. J. Parasitol.* **36**, 361–369 (2006).
70. Macchiarini, F., Manz, M. G., Palucka, A. K. & Shultz, L. D. Humanized mice: are we there yet? *J. Exp. Med.* **202**, 1307–1311 (2005).
71. Lapenta, C. *et al.* Pertussis toxin B-oligomer inhibits HIV infection and replication in hu-PBL-SCID mice. *Int. Immunol.* **17**, 469–475 (2005).
72. Mosier, D. E. Viral pathogenesis in hu-PBL-SCID mice. *Semin. Immunol.* **8**, 255–262 (1996).
73. Ichiyama, K. *et al.* A duodenally absorbable CXC-chemokine receptor 4 antagonist, KRH-1636, exhibits a potent and selective anti-HIV-1 activity. *Proc. Natl Acad. Sci. USA* **100**, 4185–4190 (2003).
74. Baenziger, S. *et al.* Disseminated and sustained HIV infection in CD34+ cord blood cell-transplanted *Rag2*$^{-/-}\gamma c^{-/-}$ mice. *Proc. Natl Acad. Sci. USA* **103**, 15951–15956 (2006).
75. Berges, B. K., Wheat, W. H., Palmer, B. E., Connick, E. & Akkina, R. HIV-1 infection and CD4 T cell depletion in the humanized *Rag2*$^{-/-}\gamma c^{-/-}$ (RAG-hu) mouse model. *Retrovirology* **3**, 76 (2006).
76. Watanabe, S. *et al.* Hematopoietic stem cell-engrafted NOD/SCID/*Il2rγ*^{null} mice develop human lymphoid system and induce long-lasting HIV-1 infection with specific humoral immune responses. *Blood* **109**, 212–218 (2007).
77. Petersen, J. S. *et al.* Transfer of type 1 (insulin-dependent) diabetes mellitus associated autoimmunity to mice with severe combined immunodeficiency (SCID). *Diabetologia* **36**, 510–515 (1993).
78. Martin, A. *et al.* Characteristics of long-term human thyroid peroxidase autoantibody secretion in *scid* mice transplanted with lymphocytes from patients with autoimmune thyroiditis. *Int. Arch. Allergy Immunol.* **98**, 317–323 (1992).
79. Davis, L. S. *et al.* Inflammation, immune reactivity, and angiogenesis in a severe combined immunodeficiency model of rheumatoid arthritis. *Am. J. Pathol.* **160**, 357–367 (2002).
80. Tighe, H. *et al.* Autoantibody production by severe combined immunodeficient mice reconstituted with synovial cells from rheumatoid arthritis patients. *Eur. J. Immunol.* **20**, 1843–1848 (1990).
81. Gregersen, J. W., Holmes, S. & Fugger, L. Humanized animal models for autoimmune diseases. *Tissue Antigens* **63**, 383–394 (2004).
82. Friese, M. A., Jensen, L. T., Willcox, N. & Fugger, L. Humanized mouse models for organ-specific autoimmune diseases. *Curr. Opin. Immunol.* **18**, 704–709 (2006).
83. Takaki, T. *et al.* HLA-A*0201-restricted T cells from humanized NOD mice recognize autoantigens of potential clinical relevance to type 1 diabetes. *J. Immunol.* **176**, 3257–3265 (2006).
84. Wen, L., Chen, N. Y., Tang, J., Sherwin, R. & Wong, F. S. The regulatory role of DR4 in a spontaneous diabetes DQ8 transgenic model. *J. Clin. Invest.* **107**, 871–880 (2001).
85. Marron, M. P., Graser, R. T., Chapman, H. D. & Serreze, D. V. Functional evidence for the mediation of diabetogenic T cell responses by HLA-A2.1 MHC class I molecules through transgenic expression in NOD mice. *Proc. Natl Acad. Sci. USA* **99**, 13753–13758 (2002).
86. Ellmerich, S. *et al.* High incidence of spontaneous disease in an HLA-DR15 and TCR transgenic multiple sclerosis model. *J. Immunol.* **174**, 1938–1946 (2005).
87. Mallone, R. *et al.* Differential recognition and activation thresholds in human autoreactive GAD-specific T cells. *Diabetes* **53**, 971–977 (2004).
88. Kent, S. C. *et al.* Expanded T cells from pancreatic lymph nodes of type 1 diabetic subjects recognize an insulin epitope. *Nature* **435**, 224–228 (2005).
89. Holst, J., Vignali, K. M., Burton, A. R. & Vignali, D. A. Rapid analysis of T-cell selection *in vivo* using T cell-receptor retrogenic mice. *Nature Methods* **3**, 191–197 (2006).
90. Fogh, J., Fogh, J. M. & Orfeo, T. One hundred and twenty-seven cultured human tumor cell lines producing tumors in nude mice. *J. Natl Cancer Inst.* **59**, 221–226 (1977).
91. Hudson, W. A., Li, Q., Le, C. & Kersey, J. H. Xenotransplantation of human lymphoid malignancies is optimized in mice with multiple immunologic defects. *Leukemia* **12**, 2029–2033 (1998).
92. Dick, J. E. & Lapidot, T. Biology of normal and acute myeloid leukemia stem cells. *Int. J. Hematol.* **82**, 389–396 (2005).
93. Kawano, N. *et al.* Efficient engraftment of primary adult T-cell leukemia cells in newborn NOD/SCID/β2-microglobulin^{null} mice. *Leukemia* **19**, 1384–1390 (2005).
94. Nakamura, Y. *et al.* Engraftment of NOD/SCID/γc^{null} mice with multilineage neoplastic cells from patients with juvenile myelomonocytic leukaemia. *Br. J. Haematol.* **130**, 51–57 (2005).
95. Pardal, R., Clarke, M. F. & Morrison, S. J. Applying the principles of stem-cell biology to cancer. *Nature Rev. Cancer* **3**, 895–902 (2003).
96. Reya, T., Morrison, S. J., Clarke, M. F. & Weissman, I. L. Stem cells, cancer, and cancer stem cells. *Nature* **414**, 105–111 (2001).
97. Bonnet, D. & Dick, J. E. Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. *Nature Med.* **3**, 730–737 (1997).
98. Pilarski, L. M. & Belch, A. R. Clonotypic myeloma cells able to xenograft myeloma to nonobese diabetic severe combined immunodeficient mice copurify with CD34+ hematopoietic progenitors. *Clin. Cancer Res.* **8**, 3198–3204 (2002).
99. Al-Hajj, M., Wicha, M. S., Benito-Hernandez, A., Morrison, S. J. & Clarke, M. F. Prospective identification of tumorigenic breast cancer cells. *Proc. Natl Acad. Sci. USA* **100**, 3983–3988 (2003).
100. O'Brien, C. A., Pollett, A., Gallinger, S. & Dick, J. E. A human colon cancer cell capable of initiating tumour growth in immunodeficient mice. *Nature* **445**, 106–110 (2007).
101. Singh, S. K. *et al.* Identification of human brain tumour initiating cells. *Nature* **432**, 396–401 (2004).
102. Yoshida, S. *et al.* Long-term engraftment and self-renewal of AML stem cells in the newborn NOD-scid/*Il2rg*^{null} immunodeficient mouse model. *Blood* **106**, A1261 (2005).
103. Nomura, T. *et al.* Induction of cancer, actinic keratosis, and specific p53 mutations by UVB light in human skin maintained in severe combined immunodeficient mice. *Cancer Res.* **57**, 2081–2084 (1997).
104. Mitsiades, C. S. *et al.* Fluorescence imaging of multiple myeloma cells in a clinically relevant SCID/NOD *in vivo* model: biologic and clinical implications. *Cancer Res.* **63**, 6689–6696 (2003).
105. O'Reilly, M. S., Holmgren, L., Chen, C. & Folkman, J. Angiostatin induces and sustains dormancy of human primary tumors in mice. *Nature Med.* **2**, 689–692 (1996).
106. Siegler, U. *et al.* Activated natural killer cells from patients with acute myeloid leukemia are cytotoxic against autologous leukemic blasts in NOD/SCID mice. *Leukemia* **19**, 2215–2222 (2005).
107. Flavell, D. J. *et al.* The anti-CD20 antibody rituximab augments the immunospecific therapeutic effectiveness of an anti-CD19 immunotoxin directed against human B-cell lymphoma. *Br. J. Haematol.* **134**, 157–170 (2006).
108. Trieu, Y. *et al.* Soluble interleukin-13Rα2 decoy receptor inhibits Hodgkin's lymphoma growth *in vitro* and *in vivo*. *Cancer Res.* **64**, 3271–3275 (2004).
109. Dewan, M. Z. *et al.* Rapid tumor formation of human T-cell leukemia virus type 1-infected cell lines in novel NOD-SCID/γc^{null} mice: suppression by an inhibitor against NF-κB. *J. Virol.* **77**, 5286–5294 (2003).
110. Watanabe, M. *et al.* A novel NF-κB inhibitor DHMEQ selectively targets constitutive NF-κB activity and induces apoptosis of multiple myeloma cells *in vitro* and *in vivo*. *Int. J. Cancer* **114**, 32–38 (2005).
111. Chalandon, Y. *et al.* BCR–ABL-transduced human cord blood cells produce abnormal populations in immunodeficient mice. *Leukemia* **19**, 442–448 (2005).
112. Serakinci, N. & Keith, W. N. Therapeutic potential of adult stem cells. *Eur. J. Cancer* **42**, 1243–1246 (2006).
113. Solter, D. From teratocarcinomas to embryonic stem cells and beyond: a history of embryonic stem cell research. *Nature Rev. Genet.* **7**, 319–327 (2006).
114. Kakinuma, S. *et al.* Human umbilical cord blood as a source of transplantable hepatic progenitor cells. *Stem Cells* **21**, 217–227 (2003).
115. Tateno, C. *et al.* Near completely humanized liver in mice shows human-type metabolic responses to drugs. *Am. J. Pathol.* **165**, 901–912 (2004).
116. Ishikawa, F. *et al.* Purified human hematopoietic stem cells contribute to the generation of cardiomyocytes through cell fusion. *FASEB J.* **20**, 950–952 (2006).
117. Ma, N. *et al.* Umbilical cord blood cell transplantation for myocardial regeneration. *Transplant Proc.* **38**, 771–773 (2006).
118. Torrente, Y. *et al.* Human circulating AC133+ stem cells restore dystrophin expression and ameliorate function in dystrophic skeletal muscle. *J. Clin. Invest.* **114**, 182–195 (2004).
119. Ishikawa, F. *et al.* Human cord blood- and bone marrow-derived CD34+ cells regenerate gastrointestinal epithelial cells. *FASEB J.* **18**, 1958–1960 (2004).
120. Droetto, S. *et al.* Vasculogenic potential of long term repopulating cord blood progenitors. *FASEB J.* **18**, 1273–1275 (2004).
121. Taguchi, A. *et al.* Administration of CD34+ cells after stroke enhances neurogenesis via angiogenesis in a mouse model. *J. Clin. Invest.* **114**, 330–338 (2004).
122. Pauwelyn, K. A. & Verfaillie, C. M. Transplantation of undifferentiated, bone marrow-derived stem cells. *Curr. Top. Dev. Biol.* **74**, 201–251 (2006).
123. Hess, D. *et al.* Bone marrow-derived stem cells initiate pancreatic regeneration. *Nature Biotechnol.* **21**, 763–770 (2003).
124. Yoshida, S. *et al.* Human cord blood-derived cells generate insulin-producing cells *in vivo*. *Stem Cells* **23**, 1409–1416 (2005).
125. Vassilopoulos, G., Wang, P. R. & Russell, D. W. Transplanted bone marrow regenerates liver by cell fusion. *Nature* **422**, 901–904 (2003).
126. Zhang, S. *et al.* Both cell fusion and transdifferentiation account for the transformation of human peripheral blood CD34+ cells into cardiomyocytes *in vivo*. *Circulation* **110**, 3803–3807 (2004).
127. Stamm, C., Liebold, A., Steinhoff, G. & Strunk, D. Stem cell therapy for ischemic heart disease: beginning or end of the road? *Cell Transplant.* **15** (Suppl. 1), 47–56 (2006).
128. Brolen, G. K., Heins, N., Edsbagge, J. & Semb, H. Signals from the embryonic mouse pancreas induce differentiation of human embryonic stem cells into insulin-producing β-cell-like cells. *Diabetes* **54**, 2867–2874 (2005).
129. Cooke, M. J., Stojkovic, M. & Przyborski, S. A. Growth of teratomas derived from human pluripotent stem cells is influenced by the graft site. *Stem Cells Dev.* **15**, 254–259 (2006).
130. Przyborski, S. A. Differentiation of human embryonic stem cells after transplantation in immune-deficient mice. *Stem Cells* **23**, 1242–1250 (2005).
131. Wang, L. *et al.* Generation of hematopoietic repopulating cells from human embryonic stem cells independent of ectopic HOXB4 expression. *J. Exp. Med.* **201**, 1603–1614 (2005).
132. Tian, X., Woll, P. S., Morris, J. K., Linehan, J. L. & Kaufman, D. S. Hematopoietic engraftment of human embryonic stem cell-derived cells is regulated by recipient innate immunity. *Stem Cells* **24**, 1370–1380 (2006).
133. Galic, Z. *et al.* T-lineage differentiation from human embryonic stem cells. *Proc. Natl Acad. Sci. USA* **103**, 11742–11747 (2006).
134. Robert, J. S. The science and ethics of making part-human animals in stem cell biology. *FASEB J.* **20**, 838–845 (2006).
135. Sotiropoulou, P. A., Perez, S. A., Gritzapis, A. D., Baxevanis, C. N. & Papamichail, M. Interactions

© 2007 Nature Publishing Group

between human mesenchymal stem cells and natural killer cells. *Stem Cells* **24**, 74–85 (2006).
136. Flanagan, S. P. '*Nude*', a new hairless gene with pleiotropic effects in the mouse. *Genet. Res.* **8**, 295–309 (1966).
137. Mosier, D. E., Stell, K. L., Gulizia, R. J., Torbett, B. E. & Gilmore, G. L. Homozygous *scid/scid;beige/beige* mice have low levels of spontaneous or neonatal T cell-induced B cell generation. *J. Exp. Med.* **177**, 191–194 (1993).
138. Lapidot, T. Mechanism of human stem cell migration and repopulation of NOD/SCID and B2m^{null} NOD/SCID mice. The role of SDF-1/CXCR4 interactions. *Ann. NY Acad. Sci.* **938**, 83–95 (2001).
139. Nicolini, F. E., Cashman, J. D., Hogge, D. E., Humphries, R. K. & Eaves, C. J. NOD/SCID mice engineered to express human IL-3, GM-CSF and Steel factor constitutively mobilize engrafted human progenitors and compromise human stem cell regeneration. *Leukemia* **18**, 341–347 (2004).

Acknowledgements
We thank E. Leiter, D. Serreze, M. Berry and A. Rossini for valuable discussions. We are supported by the β-Cell Biology Consortium and Autoimmunity Prevention Centers of the National Institutes of Health (NIH; USA), the Juvenile Diabetes Research Foundation (International), the American Diabetes Association, and the Diabetes Endocrinology Research Center, the National Cancer Institute Center, the National Institute of Allergy and Infectious Diseases and the National Heart, Lung and Blood Institute of the NIH. The contents of this publication are solely the responsibility of the authors and do not necessarily represent the official views of the NIH.

Competing interests statement
The authors declare competing financial interests: see web version for details.

DATABASES
The following terms in this article are linked online to:
Entrez Gene:
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene
B2m | CD34 | *Il2rg* | *Prf1* | *Prkdc* | *Rag1* | *Rag2* | *TP53*

FURTHER INFORMATION
The Jackson Laboratory: http://www.jax.org/
Leonard Shultz's homepage:
http://www.jax.org/staff/leonard_shultz.html
Access to this links box is available online.

© 2007 Nature Publishing Group