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Development of Humanized Mice in the Age of Genome Editing

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Abstract

Mice are the most commonly used model organisms to study human disease. Many genetic human diseases can be recapitulated by modifying the mouse genome, which permits testing of existing and novel therapeutics, including combinatorial therapeutics, without putting humans at risk. Specifically, the development of "humanized" mice, i.e., severely immunodeficient mice engrafted with functional human hematopoietic and immune cells and tissues, has revolutionized our ability to study and model human diseases in preclinical *in vivo* systems. Until recently it has been challenging to develop strains of humanized mice with targeted mutations or that transgenically express human genes with site-specific mutations, permitting optimal growth of functional human cells and tissues. However, recent advances in targeted nuclease-based genetic engineering have enabled precise modification and development of humanized mouse models at an unprecedented pace. These modifications permit optimal growth of functional human cells and tissues and can replicate human genetically determined diseases.

Keywords

CRISPR/Cas9; immunodeficient; humanized; site-specific endonucleases; NSG; transgenic mice

Introduction

The human genome contains more than 20,000 protein-coding genes, with a total of at least five times that number of gene products, microRNAs, and long non-coding RNAs (Pan et al., 2008). A major effort in biomedical research has been the identification of these genes, determination of the gene products, and evaluation of their complex interactions in the context of human disease. To probe such complex systems directly in humans is challenging both in the biological complexity of the systems and because of ethical constraints. A working compromise is a surrogate mammalian species with physiology similar to that of humans, permitting modeling of complex human diseases. The most obvious choice is the study of non-human primates (Vallender and Miller, 2013). However, the use of such species is often not acceptable; for example, NIH has phased out funding of chimpanzee research using invasive approaches (Grimm, 2016). Therefore, biomedical research has turned to non-primate mammals, especially mice.

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Advantages of the mouse as a model of human diseases include its small size, high fecundity, and minimal housing requirements (Vandamme, 2015). Although the mouse, with these combined attributes, is a powerful model organism for understanding the complexity of various human physiological processes, certain limitations must be overcome; for instance, mice are not susceptible to certain human-specific infectious agents, and mouse models do not faithfully recapitulate human tumor immunology or hematopoietic cell differentiation and maturation. Moreover, novel therapeutics developed to treat human diseases are highly human-specific and not effective in mice.

A sophisticated approach for overcoming the challenge of species differences is to "humanize" those biological systems we wish to understand in the mouse (Ito et al., 2002; Shultz et al., 2005), i.e., replacing selected components of a mouse genome with the corresponding human genes such that the desired biological changes are effected. This process begins with selection of a strain that has been genetically modified, with minimal selected modifications, to generate an immunocompromised mouse with the capacity to serve as a functional model for engraftment of human cells or tissues without rejection (Ito et al., 2012; Shultz et al., 2007). Such a base background model platform can then be reiteratively/sequentially modified by gene ablation and/or the addition of human genes to provide functional support for the maintenance of human cells, tissues, and organ fragments without rejection (Brehm et al., 2014; Shultz et al., 2012).

A crucial advance in the development of humanized mice was the generation of immunodeficient strains carrying a targeted mutation of the *Il2rg* gene. These *Il2rg*^{null} strains facilitated the *in vivo* examination of human immune system development by supporting functional human hematopoietic stem cell (HSC) reconstitution (Ito et al., 2002; Shultz et al., 2005). Subsequently, human immune system-engrafted immunodeficient *Il2rg*^{null} mouse strains have enabled functional examination of human hematopoiesis (Ishikawa et al., 2005). Humanized mice have also been used extensively to study tumor progression and metastasis, regeneration, type I and type II diabetes, and infectious diseases (Brehm et al., 2014; Greiner et al., 2011; Walsh et al., 2017; Wege et al., 2011; Zhou et al., 2014). To understand the development and glimpse the future potential of such humanized **immunodeficient mouse models**, we focus here on the methodologies and technologies that have facilitated their development to become powerful tools in many areas of biomedical research.

Development of immunodeficient *Il2rg*^{null} mice

The most commonly used mouse strains used to support the development of a human hematopoietic and immune system *in vivo* are NOD.Cg-*Prkdc*^{scid}*Il2rg*^{tm1wl} (NSG), NODShi.Cg-*Prkdc*^{scid}*Il2rg*^{tm1Sug} (NOG), and C;129S4-*Rag2*^{tm1Flv}*Il2rg*^{tm1Flv} (BRG) (Ito et al., 2002; Shultz et al., 2005; Song et al., 2010; Traggiai et al., 2004). Importantly, all three strains carry a targeted mutation in the *Il2rg* gene, leading to severe impairments in not only murine B and T cell development and function, but also natural killer (NK) cell development (Ito et al., 2012; Shultz et al., 2012). Here, we focus on the development of humanized mice through the lens of the immunodeficient mouse strain NSG, the most widely used immunodeficient *Il2rg*^{null} mouse model, and how this was achieved through progressive

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refinement and incorporation of genetic modifications using a succession of major genetic engineering breakthroughs.

The history of the strain can be traced to the discovery of the spontaneous *Prkdc*^{scid} (*scid*) mutation in CB17 mice, which leads to severe combined immunodeficiency. The *scid* mutation blocks the development of functional T and B cells (Bosma et al., 1983; Mosier et al., 1988) while maintaining a normal hematopoietic microenvironment. Subsequent studies showed that CB17-*scid* mice could also support the engraftment of human peripheral blood mononuclear cells (PBMCs) and HSCs (Lapidot et al., 1992; McCune et al., 1988). However, HSC engraftment and subsequent hematopoietic reconstitution levels remained low, with engrafted cells failing to generate a functional human immune system (Greiner et al., 1998). This poor HSC engraftment was attributed to remaining innate immunity, including NK cells and macrophages that destroy human HSC xenografts (Christianson et al., 1996; Greiner et al., 1998).

A major development was the creation of an immunodeficient non-obese diabetic (NOD)-*scid* mouse, which involved backcrossing of the CB17 *scid* mutation onto the NOD/ShiLtSz background, a polygenic model for type 1 diabetes (Shultz et al., 1995). The resulting strain NOD.CB17-*Prkdc*^{scid}/J now combined desired additional attributes, showing deficits in its innate immune system, i.e., decreased NK cell activity and compromised macrophage function. This combination of mutations and polygenic modifiers resulted in support of higher levels of human HSC and PBMC engraftment compared with CB17-*scid* mice (Hesselton et al., 1995; Lowry et al., 1996; Pflumio et al., 1996).

Although the development of humanized mice had initially relied primarily upon spontaneous mutations, this changed with the ability to target genes in validated germ-line-potential mouse embryonic stem (ES) cells. This technology eventually led to the development of immunodeficient mice carrying a disrupted interleukin-2 receptor gamma (*IL2rg*) chain locus (Ishikawa et al., 2005; Ito et al., 2002; Shultz et al., 2005; Traggiai et al., 2004). The *IL2rg* chain was targeted because it is a component of the IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21 receptors that is necessary for their function, and its absence leads to defective signaling through these receptors, resulting in severe immune-system impairment and prevention of NK cell development. Targeting of the *IL2rg* chain was achieved via *in vitro* modification of 129P2/OlaHsd-derived ES cells by homologous recombination targeting and disruption of the *IL2rg* locus. This eventually led to the creation of the B6.129S4-*Il2rg*^{tm1Wjl}/J strain (Cao et al., 1995). Subsequently, the *Il2rg*^{tm1Wjl} mutated allele was extensively backcrossed onto the NOD.CB17-*Prkdc*^{scid}/J strain, followed by genetic selection for the required alleles, i.e., homozygous for the *Prkdc*^{scid} allele and homozygous (females) or hemizygous (males) for the *Il2rg*^{tm1Wjl} targeted allele. The resulting strain—NOD-*scid IL2rg*^{null} (NSG)—supports heightened levels of human hematopoietic and lymphoid cell engraftment compared with previously described humanized mouse strains (Ishikawa et al., 2005; Shultz et al., 2005).

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Humanization of NSG mice

Three basic methods are currently used for producing a functional human immune system in NSG mice (Ito et al., 2012; Rongvaux et al., 2013; Shultz et al., 2012). A simple approach for generating a human immune system is by intraperitoneally or intravenously injecting PBMCs (Mosier et al., 1988) into sublethally irradiated adult NSG mice. Although within one to two weeks human T and B cells are detected in the circulation, a major limitation is that engrafted mice succumb to a xenogeneic graft-versus-host disease (GVHD) (King et al., 2009). Another approach involves injection of human $CD34^+$ HSCs, obtained from fetal liver, umbilical cord blood, or bone marrow, or following mobilization into sublethally irradiated newborn or adult NSG mice (Lapidot et al., 1992). Despite successful development of a human immune system, a limitation of this model is that human T cells are educated in the mouse thymus in the context of mouse major histocompatibility complex (MHC) antigens (Watanabe et al., 2009). A third method, termed the bone marrow, liver, thymus (BLT) approach, involves subrenal capsule transplantation of fragments of human fetal liver and thymus into sublethally irradiated adult NSG mice, followed by intravenous injection of $CD34^+$ HSCs isolated from the same fetal liver (Lan et al., 2006; McCune et al., 1988; Melkus et al., 2006). In contrast to the second approach, where human T cells undergo selection on the mouse thymus, in the BLT model human T cells are educated on an autologous human thymus (Rongvaux et al., 2013; Shultz et al., 2012). The third approach is superior to the other two, as it provides the most robust engraftment of a human immune system; however, engrafted mice succumb to a wasting syndrome similar to GVHD (Covassin et al., 2013; Greenblatt et al., 2012).

Limitations of NSG mice

The murine immune system is severely compromised in NSG mice, enabling generation of a functional human immune system following injection of human $CD34^+$ HSCs or peripheral blood lymphocytes. Yet, the remaining innate immunity mediated by macrophages and other myeloid cell populations interfere significantly with survival and expansion of engrafted human cells (Rongvaux et al., 2013; Shultz et al., 2012). Additionally, many human cytokine receptors are not activated by their mouse cytokine equivalents, leading to poor development of the xenografts (Brehm et al., 2014). These limitations provide opportunities to further refine and optimize NSG mice using novel genetic engineering technologies.

Optimization of humanized mice

Knock-in technologies and transgenic expression of either cDNA constructs expressing human genes or human-gene-containing bacterial artificial chromosomes (BACs) have been utilized to advance the development of humanized mice (Rathinam et al., 2011; Rongvaux et al., 2011; Willinger et al., 2011). A simple approach for expressing human genes in immunodeficient mice is to utilize human cDNA constructs. For instance, to supply needed human growth factors, novel NSG strains have been developed using cDNA constructs that transgenically express the human cytokines interleukin-3 (IL3), granulocyte-macrophage colony-stimulating factor (GM-CSF), and *stem cell factor* (SCF, or c-kit ligand) (Brehm et al., 2012). The resulting triple-transgenic NSG-SGM3 mouse strain combines the phenotype

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of the immunodeficient NSG mouse with human cytokines, facilitating robust engraftment of multiple human hematopoietic lineages (Billerbeck et al., 2011; Nicolini et al., 2004). However, this approach is not optimal for development and function of engrafted human cells, owing to the expression of human genes at non-physiological levels (Shultz et al., 2012).

A second approach is to utilize large human-gene-containing fragments (10 to 100 kb) derived from BACs. Such larger constructs often contain appropriate locus control regions (LCR) enabling copy-dependent, tissue-appropriate expression of human genes at physiological levels (Shultz et al., 2012). Nonetheless, in both of the above-mentioned approaches, if the mouse homolog of a human cytokine transgene is intact, the expressed mouse cytokine may interfere or compete with the transgenically expressed human cytokine.

A third approach, knock-in technology, circumvents the competition issue, as it replaces the mouse gene with the equivalent human gene. For instance, transgenic expression of human granulocyte-macrophage colony-stimulating factor via the use of knock-in technology to insert the human *CSF1* gene, which encodes this cytokine, increases the frequencies of human monocytes in BRG mice engrafted with human HSCs (Rathinam et al., 2011). On the other hand, knock-in of human thrombopoietin (*THPO*) in BRG mice resulted in reduced numbers of mouse platelets, as the levels of human THPO were too low to support murine thrombocytopoiesis (Rongvaux et al., 2011).

Because many strains of genetically modified immunodeficient *Il2rg*^{null} mice have been developed and made available using the above-mentioned technologies (Shultz et al., 2012), and several methods exist for humanization of immunodeficient *Il2rg*^{null} mice, researchers must exercise prudence in selecting the appropriate model system for their experimental needs.

Further refinement of next-generation NSG mice using targeted nucleases

In recent years the development and use of exquisitely controllable targeted nucleases has led to a major transformation in the creation of genetically modified animals. This began with the advent of zinc finger nucleases (ZFN), followed by transcription activator-like effector nucleases (TALEN) and, most recently, clustered regularly interspaced short palindromic repeats (CRISPR) with its CRISPR-associated system 9 (CRISPR-Cas9) (Gaj et al., 2013). ZFN and TALEN are based on the use of customized modular DNA-binding proteins that can be assembled to bind to desired DNA target sequences. Upon their binding, a double-stranded DNA (dsDNA) break is created at the target site by an associated nuclease. In contrast, CRISPR-Cas9 comprises a non-specific endonuclease (Cas9) and an RNA molecule that contains 17–20 nucleotides of the matching target sequence that guides and activates the nuclease. Upon binding to its genomic target, the complex initiates a dsDNA break (Jinek et al., 2012). The CRISPR-Cas9 system has become the simplest of three to design and use. In all three approaches, the dsDNA breaks are repaired rapidly in cells by an error-prone process, nonhomologous end-joining (NHEJ) repair, which leads to the creation of indels (+/− 1 bp additions, with 10s to 100s of base pairs deleted), often leading to gene disruption. If the DNA break occurs in the presence of donor DNA with

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homology to the targeted region, a slower, homology-directed repair (HDR) process can occur that leads to incorporation of donor DNA, including any contained nested nonhomologous sequences, into the genome (Gaj et al., 2013). The crucial and revolutionary element of all three approaches is the ability to use them directly in zygotes; i.e., the gene-editing components can be microinjected, for example, as RNAs (plus/minus donor DNA), directly into a zygote isolated from any mouse strain, yielding a high targeting frequency in the mice (Shen et al., 2013; Wang et al., 2013). The approach is also rapid, as animals with homozygous modification can be obtained in about six months.

Our research group began using targeted nucleases to modify immunodeficient mouse strains by microinjection into zygotes in 2012 with a simple knockout of the *Fah* gene in NOD-*Rag1*^{null} *IL2rg*^{null} (NRG) mice by NHEJ. Subsequently, we progressed to using TALEN and CRISPR for both gene knockout and knock-in (see Table for summary). From these data it is obvious that NHEJ and HDR occur at high efficiency in the NSG/NRG strains, opening their genomes to rapid genetic modifications. Genes chosen for targeting were selected either on the basis of ideas to improve HSC reconstitution (e.g., BAFF is also known as B lymphocyte stimulator (BLyS)); for the study and use of xenografts derived from normal tissues and tumors (e.g., *Fah*, *Hprt*); or to further increase the versatility of the strains by enabling larger-scale genetic modifications. Knock-ins have focused on subtle gene-editing events where 1 to ~50 bp are changed, enabling seamless repair and modification of the loci to generate gain-of-function or hypomorphic alleles. Importantly, all the above approaches permit sidestepping of complex breeding strategies and working with ES cells, which prevents random transgenesis while facilitating rapid sequential refinement of complex models.

Alternative approaches for generation of next-generation NSG models

Site-specific recombinases, regarded as tools for ‘cut-and-paste’ genome editing, are enzymes that mediate precise integration between two DNA strands (donor and host) containing recombination sites by bringing them into close proximity via covalent interactions (Hollis et al., 2003). Such recombinases have been used for precise integration of DNA of interest into the mouse genome with high efficiency. The only components required for site-specific recombination are a circular donor vector with a recombination site, a target sequence with a recombination site, and the recombinase mRNA or protein. Therefore, genetically modified mice can be generated by pronuclear injection of these components into mouse zygotes. For instance, bacteriophage integrase φC31, which, together with all other integrases is a type of site-specific recombinase, has been used to generate transgenic mice via pronuclear injection with up to 40% efficiency (Tasic et al., 2011). Importantly, this process does not trigger cellular repair and enables unidirectional insertion of tens of kilobase pairs of DNA, which is currently not possible using nucleases (Olorunniji et al., 2016). A drawback of the use of integrases is that, in contrast to nuclease-mediated one-step insertion of DNA into mouse zygotes, integrase-mediated integration of recombinant DNA is a two-step process. It requires prior insertion of a recombination site into specific loci in the mouse genome, though this integration can be easily achieved using the CRISPR/Cas9 system. Furthermore, although the use of site-specific recombinases can result in precise integration of DNA, there are known drawbacks including low efficiency,

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off-target activity, and toxicity (Olorunniji et al., 2016). Nevertheless, integrases can be a potential alternative strategy for precise and efficient integration of large chunks of DNA (>10 kb).

Although ES cells have fallen from favor in the direct modification of mouse strains, they may still have potential uses. Because targeted nucleases demonstrate limited efficiency for insertion of large fragments of DNA, NSG ES cells may be useful for this purpose, as they allow selection of rare modification events and their complete characterization before attempting germ-line transmission. NSG ES cell lines have been made and validated (Landel et al., 2013) (Laura Reinholdt, The Jackson Laboratory, personal communication), and may eventually enable very large-scale modification of the NSG genome.

Conclusions

A mouse is not a human. Nevertheless, over the years mice have been invaluable tools for modeling human diseases and identifying the underlying mechanisms. To overcome certain limitations of mouse models in studying human tumor immunology, infectious diseases, autoimmunity, and hematopoietic cell differentiation and maturation, immunodeficient mouse models bearing mutations in the IL2 receptor common gamma chain ($IL2rg^{null}$) have been developed. The most widely used immunodeficient $IL2rg^{null}$ strains are NSG, NOG, and BRG mice, which enable engraftment of not only human hematopoietic stem cells but also normal as well as malignant human tissues. These immunodeficient mice, when engrafted with a human immune system, resulting in what are commonly known as humanized mice, faithfully recapitulate human biological systems and serve as valuable preclinical tools in biomedical research. Furthermore, these small animal models are replacing non-human primates for the study of human diseases. Until recently it has been challenging to modify the genomes of humanized NSG, NOG, and BRG mice; however, the availability of site-specific nucleases and recombinases is enabling the development of next-generation humanized mice to progress at an unparalleled pace. With the continued development of new gene-editing technologies and other new approaches, future generations of humanized mice will undoubtedly make an even greater impact on the understanding of human diseases and the development of new therapies.

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References

Billerbeck E, Barry WT, Mu K, Dorner M, Rice CM, Ploss A. Development of human CD4+FoxP3+ regulatory T cells in human stem cell factor-, granulocyte-macrophage colony-stimulating factor-, and interleukin-3-expressing NOD-SCID IL2Rgamma(null) humanized mice. Blood. 2011; 117:3076–3086. [PubMed: 21252091]

Bosma GC, Custer RP, Bosma MJ. A severe combined immunodeficiency mutation in the mouse. Nature. 1983; 301:527–530. [PubMed: 6823332]

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Brehm MA, Racki WJ, Leif J, Burzenski L, Hosur V, Wetmore A, Gott B, Herlihy M, Ignotz R, Dunn R, Shultz LD, Greiner DL. Engraftment of human HSCs in nonirradiated newborn NOD-scid IL2rgamma null mice is enhanced by transgenic expression of membrane-bound human SCF. Blood. 2012; 119:2778–2788. [PubMed: 22246028]

Brehm MA, Wiles MV, Greiner DL, Shultz LD. Generation of improved humanized mouse models for human infectious diseases. Journal of immunological methods. 2014; 410:3–17. [PubMed: 24607601]

Cao X, Shores EW, Hu-Li J, Anver MR, Kelsall BL, Russell SM, Drago J, Noguchi M, Grinberg A, Bloom ET, et al. Defective lymphoid development in mice lacking expression of the common cytokine receptor gamma chain. Immunity. 1995; 2:223–238. [PubMed: 7697543]

Christianson SW, Greiner DL, Schweitzer IB, Gott B, Beamer GL, Schweitzer PA, Hesselton RM, Shultz LD. 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. Cellular immunology. 1996; 171:186–199. [PubMed: 8806787]

Covassin L, Jangalwe S, Jouvet N, Laning J, Burzenski L, Shultz LD, Brehm MA. Human immune system development and survival of non-obese diabetic (NOD)-scid IL2rgamma(null) (NSG) mice engrafted with human thymus and autologous haematopoietic stem cells. Clinical and experimental immunology. 2013; 174:372–388. [PubMed: 23869841]

Gaj T, Gersbach CA, Barbas CF 3rd. ZFN, TALEN, and CRISPR/Cas-based methods for genome engineering. Trends in biotechnology. 2013; 31:397–405. [PubMed: 23664777]

Greenblatt MB, Vrbanac V, Tivey T, Tsang K, Tager AM, Aliprantis AO. Graft versus host disease in the bone marrow, liver and thymus humanized mouse model. PloS one. 2012; 7:e44664. [PubMed: 22957096]

Greiner DL, Brehm MA, Hosur V, Harlan DM, Powers AC, Shultz LD. Humanized mice for the study of type 1 and type 2 diabetes. Annals of the New York Academy of Sciences. 2011; 1245:55–58. [PubMed: 22211980]

Greiner DL, Hesselton RA, Shultz LD. SCID mouse models of human stem cell engraftment. Stem cells (Dayton, Ohio). 1998; 16:166–177.

Grimm D. ANIMAL RESEARCH. Chimpanzee sanctuaries open door to more research. Science (New York, NY). 2016; 353:433–434.

Hesselton RM, Greiner DL, Mordes JP, Rajan TV, Sullivan JL, Shultz LD. High levels of human peripheral blood mononuclear cell engraftment and enhanced susceptibility to human immunodeficiency virus type 1 infection in NOD/LtSz-scid/scid mice. The Journal of infectious diseases. 1995; 172:974–982. [PubMed: 7561218]

Hollis RP, Stoll SM, Sclimenti CR, Lin J, Chen-Tsai Y, Calos MP. Phage integrases for the construction and manipulation of transgenic mammals. Reproductive biology and endocrinology : RB&E. 2003; 1:79. [PubMed: 14613545]

Ishikawa F, Yasukawa M, Lyons B, Yoshida S, Miyamoto T, Yoshimoto G, Watanabe T, Akashi K, Shultz LD, Harada M. Development of functional human blood and immune systems in NOD/SCID/IL2 receptor {gamma} chain(null) mice. Blood. 2005; 106:1565–1573. [PubMed: 15920010]

Ito M, Hiramatsu H, Kobayashi K, Suzue K, Kawahata M, Hioki K, Ueyama Y, Koyanagi Y, Sugamura K, Tsuji K, Heike T, Nakahata T. NOD/SCID/gamma(c)(null) mouse: an excellent recipient mouse model for engraftment of human cells. Blood. 2002; 100:3175–3182. [PubMed: 12384415]

Ito R, Takahashi T, Katano I, Ito M. Current advances in humanized mouse models. Cellular & molecular immunology. 2012; 9:208–214. [PubMed: 22327211]

Jinek M, Chylinski K, Fonfara I, Hauer M, Doudna JA, Charpentier E. A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. Science (New York, NY). 2012; 337:816–821.

King MA, Covassin L, Brehm MA, Racki W, Pearson T, Leif J, Laning J, Fodor W, Foreman O, Burzenski L, Chase TH, Gott B, Rossini AA, Bortell R, Shultz LD, Greiner DL. Human peripheral blood leucocyte non-obese diabetic-severe combined immunodeficiency interleukin-2 receptor gamma chain gene mouse model of xenogeneic graft-versus-host-like disease and the role of host

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major histocompatibility complex. Clinical and experimental immunology. 2009; 157:104–118. [PubMed: 19659776]

Lan P, Tonomura N, Shimizu A, Wang S, Yang YG. Reconstitution of a functional human immune system in immunodeficient mice through combined human fetal thymus/liver and CD34+ cell transplantation. Blood. 2006; 108:487–492. [PubMed: 16410443]

Landel CP, Dunlap J, Patton JB, Manser T. A germline-competent embryonic stem cell line from NOD.Cg-Prkdc (scid) Il2rg (tm1Wjl)/SzJ (NSG) mice. Transgenic research. 2013; 22:179–185. [PubMed: 22767020]

Lapidot T, Pflumio F, Doedens M, Murdoch B, Williams DE, Dick JE. Cytokine stimulation of multilineage hematopoiesis from immature human cells engrafted in SCID mice. Science (New York, NY). 1992; 255:1137–1141.

Lowry PA, Shultz LD, Greiner DL, Hesselton RM, Kittler EL, Tiarks CY, Rao SS, Reilly J, Leif JH, Ramshaw H, Stewart FM, Quesenberry PJ. Improved engraftment of human cord blood stem cells in NOD/LtSz-scid/scid mice after irradiation or multiple-day injections into unirradiated recipients. Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation. 1996; 2:15–23.

McCune JM, Namikawa R, Kaneshima H, Shultz LD, Lieberman M, Weissman IL. The SCID-hu mouse: murine model for the analysis of human hematolymphoid differentiation and function. Science (New York, NY). 1988; 241:1632–1639.

Melkus MW, Estes JD, Padgett-Thomas A, Gatlin J, Denton PW, Othieno FA, Wege AK, Haase AT, Garcia JV. Humanized mice mount specific adaptive and innate immune responses to EBV and TSST-1. Nature medicine. 2006; 12:1316–1322.

Mosier DE, Gulizia RJ, Baird SM, Wilson DB. Transfer of a functional human immune system to mice with severe combined immunodeficiency. Nature. 1988; 335:256–259. [PubMed: 2970594]

Nicolini FE, Cashman JD, Hogge DE, Humphries RK, Eaves CJ. 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. 2004; 18:341–347. [PubMed: 14628073]

Olorunniji FJ, Rosser SJ, Stark WM. Site-specific recombinases: molecular machines for the Genetic Revolution. The Biochemical journal. 2016; 473:673–684. [PubMed: 26965385]

Pan Q, Shai O, Lee LJ, Frey BJ, Blencowe BJ. Deep surveying of alternative splicing complexity in the human transcriptome by high-throughput sequencing. Nature genetics. 2008; 40:1413–1415. [PubMed: 18978789]

Pflumio F, Izac B, Katz A, Shultz LD, Vainchenker W, Coulombel L. 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. 1996; 88:3731–3740. [PubMed: 8916937]

Rathinam C, Poueymirou WT, Rojas J, Murphy AJ, Valenzuela DM, Yancopoulos GD, Rongvaux A, Eynon EE, Manz MG, Flavell RA. Efficient differentiation and function of human macrophages in humanized CSF-1 mice. Blood. 2011; 118:3119–3128. [PubMed: 21791433]

Rongvaux A, Takizawa H, Strowig T, Willinger T, Eynon EE, Flavell RA, Manz MG. Human hemato-lymphoid system mice: current use and future potential for medicine. Annual review of immunology. 2013; 31:635–674.

Rongvaux A, Willinger T, Takizawa H, Rathinam C, Auerbach W, Murphy AJ, Valenzuela DM, Yancopoulos GD, Eynon EE, Stevens S, Manz MG, Flavell RA. Human thrombopoietin knockin mice efficiently support human hematopoiesis in vivo. Proceedings of the National Academy of Sciences of the United States of America. 2011; 108:2378–2383. [PubMed: 21262827]

Shen B, Zhang J, Wu H, Wang J, Ma K, Li Z, Zhang X, Zhang P, Huang X. Generation of gene-modified mice via Cas9/RNA-mediated gene targeting. Cell research. 2013; 23:720–723. [PubMed: 23545779]

Shultz LD, Brehm MA, Garcia-Martinez JV, Greiner DL. Humanized mice for immune system investigation: progress, promise and challenges. Nature reviews. Immunology. 2012; 12:786–798.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Shultz LD, Ishikawa F, Greiner DL. Humanized mice in translational biomedical research. Nature reviews. Immunology. 2007; 7:118–130.

Shultz LD, Lyons BL, Burzenski LM, Gott B, Chen X, Chaleff S, Kotb M, Gillies SD, King M, Mangada J, Greiner DL, Handgretinger R. Human lymphoid and myeloid cell development in NOD/LtSz-scid IL2R gamma null mice engrafted with mobilized human hemopoietic stem cells. Journal of immunology (Baltimore, Md: 1950). 2005; 174:6477–6489.

Shultz LD, Schweitzer PA, Christianson SW, Gott B, Schweitzer IB, Tennent B, McKenna S, Mobraaten L, Rajan TV, Greiner DL, et al. Multiple defects in innate and adaptive immunologic function in NOD/LtSz-scid mice. Journal of immunology (Baltimore, Md: 1950). 1995; 154:180–191.

Song J, Willinger T, Rongvaux A, Eynon EE, Stevens S, Manz MG, Flavell RA, Galan JE. A mouse model for the human pathogen Salmonella typhi. Cell host & microbe. 2010; 8:369–376. [PubMed: 20951970]

Tasic B, Hippenmeyer S, Wang C, Gamboa M, Zong H, Chen-Tsai Y, Luo L. Site-specific integrase-mediated transgenesis in mice via pronuclear injection. Proceedings of the National Academy of Sciences of the United States of America. 2011; 108:7902–7907. [PubMed: 21464299]

Traggiai E, Chicha L, Mazzucchelli L, Bronz L, Piffaretti JC, Lanzavecchia A, Manz MG. Development of a human adaptive immune system in cord blood cell-transplanted mice. Science (New York, NY). 2004; 304:104–107.

Vallender EJ, Miller GM. Nonhuman primate models in the genomic era: a paradigm shift. ILAR journal. 2013; 54:154–165. [PubMed: 24174439]

Vandamme TF. Rodent models for human diseases. European journal of pharmacology. 2015; 759:84–89. [PubMed: 25823811]

Walsh NC, Kenney LL, Jangalwe S, Aryee KE, Greiner DL, Brehm MA, Shultz LD. Humanized Mouse Models of Clinical Disease. Annual review of pathology. 2017; 12:187–215.

Wang H, Yang H, Shivalila CS, Dawlaty MM, Cheng AW, Zhang F, Jaenisch R. One-step generation of mice carrying mutations in multiple genes by CRISPR/Cas-mediated genome engineering. Cell. 2013; 153:910–918. [PubMed: 23643243]

Watanabe Y, Takahashi T, Okajima A, Shiokawa M, Ishii N, Katano I, Ito R, Ito M, Minegishi M, Minegishi N, Tsuchiya S, Sugamura K. The analysis of the functions of human B and T cells in humanized NOD/shi-scid/gammac(null) (NOG) mice (hu-HSC NOG mice). International immunology. 2009; 21:843–858. [PubMed: 19515798]

Wege AK, Ernst W, Eckl J, Frankenberger B, Vollmann-Zwerenz A, Mannel DN, Ortmann O, Kroemer A, Brockhoff G. Humanized tumor mice–a new model to study and manipulate the immune response in advanced cancer therapy. International journal of cancer. 2011; 129:2194–2206. [PubMed: 21544806]

Willinger T, Rongvaux A, Takizawa H, Yancopoulos GD, Valenzuela DM, Murphy AJ, Auerbach W, Eynon EE, Stevens S, Manz MG, Flavell RA. Human IL-3/GM-CSF knock-in mice support human alveolar macrophage development and human immune responses in the lung. Proceedings of the National Academy of Sciences of the United States of America. 2011; 108:2390–2395. [PubMed: 21262803]

Zhou Q, Facciponte J, Jin M, Shen Q, Lin Q. Humanized NOD-SCID IL2rg–/– mice as a preclinical model for cancer research and its potential use for individualized cancer therapies. Cancer letters. 2014; 344:13–19. [PubMed: 24513265]

Low BE, Kutny PM, Wiles MV. Simple, Efficient CRISPR-Cas9-Mediated Gene Editing in Mice: Strategies and Methods. Methods in molecular biology. 2016; 1438:19–53. [PubMed: 27150082]

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Table

Endonuclease-mediated gene editing in immunodeficient NSG/NRG mice

Mouse Strain	Year	Loci	Endonuclease[1]	Strategy	Efficiency[2]
NRG[3]	2012	*Fah*	ZFN	Knockout	up to 20%
NSG	2013	*BAFF (BLyS)*, and other loci	TALEN	Knockout	up to 16%
NSG	2013	*Hprt*	CRISPR/Cas9	Knockout	up to 18%
NSG	2014	Multiple loci	CRISPR/Cas9	Knockout	14% – 85%
NSG	2015	*Rhbdf2*	CRISPR/Cas9	Knock-in (<100 bp insert)	up to 50%
NSG	2015	Multiple loci	CRISPR/Cas9	Knockout	13% – 81%
NSG	2015	*Rosa26*	CRISPR/Cas9	Knock-in (<100 bp insert)	up to 60%
NSG	2016	Multiple loci	CRISPR/Cas9	Knockout	7% – 50%

Note: A typical project consists of 100 microinjected zygotes yielding on average 22 live-born pups that are screened by PCR and Sanger sequencing for desired genetically modified events. The founder animals are backcrossed to the appropriate strain, and offspring are genotyped for the required events. Subsequently, heterozygous animals are bred to generate homozygous animals [for our detailed methodology see (Low et al., 2016)].

[1] The high degree of variation in efficiency is linked to microinjection conditions and the targeting of different chromosomal loci.

[2] CRISPR/Cas9 endonuclease-mediated gene editing has significantly improved the targeting efficiency.

[3] NOD.Cg-*Rag1*^{tm1Mom} *Il2rg*^{tm1Wjl}/SzJ, also known as NOD-*Rag1*^{null} *IL2rg*^{null}, abbreviated as (NRG).