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Development of Novel Major Histocompatibility Complex Class I and Class II-Deficient NOD-SCID IL2R Gamma Chain Knockout Mice for Modeling Human Xenogeneic Graft-Versus-Host Disease

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Abstract

Immunodeficient mice have been used as recipients of human peripheral blood mononuclear cells (PBMC) for *in vivo* analyses of human xeno-graft-versus-host disease (GVHD). This xeno-GVHD model system in many ways mimics the human disease. The model system is established by intravenous or intraperitoneal injection of human PBMC or spleen cells into unconditioned or irradiated immunodeficient recipient mice. Recently, the development of several stocks of immunodeficient *Prkdc^{scid}* (*scid*), or recombination activating 1 or 2 gene (*Rag1* or *Rag2*) knockout mice bearing a targeted mutation in the gene encoding the IL2 receptor gamma chain (*IL2rγ*) have been reported. The addition of the mutated *IL2rγ* gene onto an immunodeficient mouse stock facilitates heightened engraftment with human PBMC. Stocks of mice with mutations in the *IL2rγ* gene have been studied in several laboratories on NOD-*scid*, NOD-*Rag1^{null}*, BALB/c-*Rag1^{null}*, BALB/c-*Rag2^{null}*, and Stock-H2^d-*Rag2^{null}* strain backgrounds. Parameters to induce human xeno-GVHD in H2^d-*Rag2^{null}* *IL2rγ^{null}* mice have been published, but variability in the frequency of disease and kinetics of GVHD were observed. The availability of the NOD-*scid* *IL2rγ^{null}* stock that engrafts more readily with human PBMC than does the Stock-H2^d-*Rag2^{null}* *IL2rγ^{null}* stock should lead to a more reproducible humanized mouse model of GVHD and for the use in drug evaluation and validation. Furthermore, GVHD in human PBMC-engrafted *scid* mice has been postulated to result predominately from a human anti-mouse major histocompatibility complex (MHC) class II reactivity. Our recent development of NOD-*scid* *IL2rγ^{null}* *β2m^{null}* and NOD-*scid* *IL2rγ^{null}* *Ab^{null}* stocks of mice now make it possible to investigate directly the role of host MHC class I and class II in the pathogenesis of GVHD in humanized mice using NOD-*scid* *IL2rγ^{null}* stocks that engraft at high levels with human PBMC and are deficient in murine MHC class I, class II, or both classes of MHC molecules.

Keywords

scid; *Rag*; humanized mice; GVHD; immunodeficient mice; MHC class I; MHC class II

INTRODUCTION

1.0 Hematopoietic Stem Cell Transplantation and Graft-versus-Host Disease

Hematopoietic stem cell (HSC) transplantation has the potential to be curative for a variety of human diseases. These include diseases of the blood, metabolic disorders, autoimmune diseases, and solid tumors (1-5). Of particular importance is the use of HSC transplantation for the treatment of hematological malignancies (6-8). In this setting, the beneficial graft-versus-leukemia (GVL) effects versus the detrimental GVHD effects are commonly intertwined (9). The beneficial effects of GVL appear to correlate directly with the severity of chronic GVHD and these two phenomena are difficult to study as independent phenomena in patients (10-12). A small animal model of human immunity that accurately mimics the clinical presentation of human GVHD would provide a model for the studies of GVL as well as GVHD and for testing the efficacy of human-specific therapeutics prior to their use in the clinic. Such a model system would also provide a tool for investigating the underlying mechanisms involved in the pathogenesis of human GVHD and to discover ways to dissociate GVHD from GVL effects.

1.1 Immunodeficient Mice Engrafted with Human Lymphohematopoietic Cells

The first description of engrafting immunodeficient mice with human PBMC was reported in 1988 (13). In those studies, CB17 mice harboring the *Prkdc^{scid}* (*scid*) mutation were injected intraperitoneally with $10\text{--}100 \times 10^6$ human PBMC, and low levels of human PBMC engraftment were observed. However, instead of developing GVHD, these mice developed EBV-associated lymphomas (14). Most of the human T cells that did engraft became anergic (15, 16) and few engrafted mice developed GVHD (17). Over the ensuing two decades, genetically altered immunodeficient mice were found to support heightened engraftment with human lymphohematopoietic cells, with a major advance being the generation of NOD-*scid* mice (18). Additional heritable modifications included the generation of NOD-*scid* mice genetically deficient in beta-2 microglobulin ($\beta 2m^{tm1Unc}$, abbreviated as $\beta 2m^{null}$) (19). In the absence of $\beta 2m$, natural killer (NK) cell numbers and activity are severely depressed due to the lack of MHC class I expression (19). This removed a major impediment to human lymphohematopoietic cell engraftment. Although NOD-*scid* $\beta 2m^{null}$ mice support higher levels of human PBMC engraftment than do CB17-*scid* or NOD-*scid* mice (19), development of symptoms of GVHD remained variable (20). In this and other model systems developed during this period, the low frequency and poor engraftment observed, and the high numbers of human PBMC required for engraftment and disease expression, precluded widespread use of these mice for studies of xeno-GVHD.

1.2 Immunodeficient Stocks of *IL2r γ ^{null}* Mice

A major breakthrough in modeling human immunity and human xeno-GVHD reactivity in immunodeficient mice followed the development by several research groups of immunodeficient mice bearing a targeted mutation in the gene encoding the IL2 receptor gamma chain (*IL2rg*), abbreviated as (*IL2r γ*) (21). The IL2 receptor gamma chain is important for high affinity signaling through the IL2, IL4, IL7, IL9, IL15, and IL21 cytokine receptors. Without these receptor signals, both adaptive and innate immunity are severely impaired (22). We and others are now building on the foundation of

immunodeficient *IL2r γ ^{null}* mice that are available to knockout additional genes and to introduce new genes that will facilitate the engraftment of functional human immune systems. On the BALB/c strain background, this is being pursued using BALB/c embryonic stem cells to genetically manipulate the genome. For example, knocking in human genes into the BALB/c ES cells can rapidly introduce human genes of particular importance for the enhancement of human hematolymphopoietic cell engraftment without requiring 2 or more years of backcrossing the mutated genes made in B6/129 ES cells onto the BALB/c strain background. Development of NOD ES cells, currently underway in a number of laboratories, when available will provide an important tool for rapidly manipulating the NOD genome, facilitating the development of new stocks of NOD-*scid* *IL2r γ ^{null}* mice for human cell engraftment.

Using a Stock-*H2^d* mouse strain bearing both the *Rag2^{null}* and *IL2r γ ^{null}* targeted mutations, it was shown that there was improved engraftment of human PBMC as compared with NOD-*scid* mice. Unfortunately, even extensive preconditioning of the Stock-*H2^d*-*Rag2^{null}* *IL2r γ ^{null}* recipient with total body irradiation and macrophage depletion using chlodronate-containing liposomes did not lead to reproducible development of GVHD symptoms in all of the mice (23).

1.3 Engraftment of NOD-*scid* *IL2r γ ^{null}* Mice with Human PBMC

The independent development of NOD-*scid* mice bearing a targeted mutation in the *IL2r γ* gene was reported by two separate groups. The laboratory of Ito and colleagues developed a stock bearing a truncated *IL2r γ* (*IL2rg^{tm1Sug}*, abbreviated as *IL2r γ ^{trunc}*) gene (24). We have developed a stock bearing a complete null *IL2r γ* mutation (*IL2rg^{tm1Wjll}*, abbreviated as *IL2r γ ^{null}*) (25). We have recently described the parameters of human PBMC engraftment into unconditioned NOD-*scid* *IL2r γ ^{null}* mice (26). We observed that injection of as few as $1-5 \times 10^6$ human PBMC led to engraftment, and that all mice were successfully engrafted when injected with 10×10^6 or more human PBMC. At about 30-40 days after PBMC engraftment, we consistently observed that all mice were losing weight and developing symptoms of GVHD (26). Our observations here are consistent with the recent report of the gradual development of phenotypic changes resembling GVHD over the course of 4 to 8 weeks observed following injection of 10×10^6 human PBMC into unconditioned NOD-*scid* *IL2r γ ^{null}* mice (27). These data suggest that heightened engraftment of functional human PBMC in NOD-*scid* *IL2r γ ^{null}* mice, particularly that of CD8⁺ T cells, leads to the development of xenoreactivity, aggression against host tissues, and development of GVHD.

More recently, we have shown that the NOD-*scid* *IL2r γ ^{null}* mouse model supports higher levels of human hematolymphoid engraftment than does the CBy.Cg-*Rag1^{tm1Mom}* *Il2rg^{tm1Wjl}/Sz* (abbreviated as BALB/c-*Rag1^{null}* *IL2r γ ^{null}*) stock of mice (28). We have subsequently shown that NOD.129S7(B6)-*Rag1^{tm1Mom}* *Il2rg^{tm1Wjll}* (abbreviated as NOD-*Rag1^{null}* *IL2r γ ^{null}*) mice also engraft with human PBMC at high levels that are comparable to those observed in NOD-*scid* *IL2r γ ^{null}* mice (29). These latter data document that the difference in human PBMC engraftment in NOD-*scid* *IL2r γ ^{null}* and BALB/c.129P2-*Rag2^{tm1Mnz}* (abbreviated as BALB/c-*Rag2^{null}* *IL2r γ ^{null}*) mice is not due to the effects of the *scid* vs. *Rag1^{null}* or *Rag2^{null}* genes but rather to other polymorphic background modifiers.

One of these important background modifiers has been reported to be the *Sirpa* (also termed *Shps-1* and *Ptpns1*) gene. The SIRP- α protein is a potent regulator of interactions between hematopoietic cells and the bone marrow microenvironment. The NOD SIRP- α protein is more similar to the human SIRP- α protein than that of the C57BL/6 SIRP- α protein, shows enhanced binding to the human CD47 ligand, and its expression on mouse macrophages leads to better support of human hematopoiesis (30).

2.0 Role of Mouse MHC Class I and Class II in GVHD Following Engraftment of NOD-*scid* *IL2r γ ^{null}* Mice with Human PBMC

It has been suggested that mismatches at the human HLA class I loci are the most important predictor of human GVHD following hematopoietic stem cell transplantation (9). In humanized mice, there is contradictory evidence on the role of MHC class I and class II in the pathogenesis of GVHD. Human CD4 T cell clones derived from CB17-*scid* mice engrafted with PBMC exhibited reactivity against host MHC class II (15). However, the predominant human cell population engrafting in CB17-*scid* mice was the CD8 T cell population (31), which would more likely be driven to proliferate by mouse MHC class I rather than MHC class II. To re-examine the role of mouse MHC class I and II in the engraftment and development of xeno-GVHD following human PBMC engraftment, we developed two new stocks of NOD-*scid* *IL2r γ ^{null}* mice.

2.1 Development of NOD-*scid* *IL2r γ ^{null}* Mice That Are Also Deficient in Mouse MHC Class I or MHC Class II

The development of the NOD.Cg-*B2m^{tm1Unc} Prkdc^{scid} Il2rg^{tm1Wjl}/Sz* (abbreviated as NOD-*scid* *IL2r γ ^{null} β 2m^{null}*) genetic stock was initiated by crossing NOD-*scid/scid* *IL2r γ ^{null}* females with NOD-*scid/scid* *β 2m^{null}* males. The NOD-*scid* *IL2r γ ^{null}/Y +/ β 2m^{null}* F1 males were backcrossed to NOD-*scid/scid* *IL2r γ ^{null}* females. The NOD-*scid/scid* *IL2r γ ^{null} +/ β 2m^{null}* female offspring were mated with their NOD-*scid/scid* *IL2r γ ^{null}/Y +/ β 2m^{null}* sibs to produce homozygous mice at each of the mutated genes. The homozygous NOD-*scid* *IL2r γ ^{null} β 2m^{null}* offspring were then intercrossed to establish and maintain this strain.

The development of the NOD.Cg-*Prkdc^{scid} H2-Ab1^{tm1Gru} Il2rg^{tm1Wjl}/Sz* (abbreviated as NOD-*scid* *IL2r γ ^{null} Ab^{null}*) genetic stock was initiated by first crossing NOD-*scid/scid* *IL2r γ ^{null}* females with NOD-*scid/scid* *Ab^{null}* males. The NOD-*scid/scid* *IL2r γ ^{null}/Y +/Ab^{null}* F1 males were backcrossed to NOD-*scid/scid* *IL2r γ ^{null}* females. The NOD-*scid/scid* *IL2r γ ^{null} +/Ab^{null}* female offspring were mated with their NOD-*scid/scid* *IL2r γ ^{null}/Y +/Ab^{null}* sibs to produce homozygous mice at each of the mutated genes. The homozygous NOD-*scid* *IL2r γ ^{null} Ab^{null}* offspring were intercrossed to establish and maintain this strain. Both NOD and 129 strains of mice have a genetic deficiency in I-E α expression. The crossing of the *Ab^{null}* allele, made using 129 strain ES cells, onto the NOD strain background therefore results in a deficiency of both I-A and I-E expression and complete lack of MHC class II expression.

2.2 Phenotype of Cells Developing in NOD-*scid* *IL2r γ* ^{null} Mice Deficient in Mouse MHC Class I or MHC Class II

We have previously shown that NOD-*scid* *IL2r γ* ^{null} mice lack NK cells, mature T lymphocytes and mature B lymphocytes are excellent recipients of human PBMC (26). To determine if deletion of either the mouse MHC class I or class II altered the phenotype of the murine cell subsets in NOD-*scid* *IL2r γ* ^{null} mice, we performed flow cytometry analyses. As shown in **Tables 1 and 2**, and **Figure 1, Panels A and B**, there were no MHC class I positive cells in the bone marrow and spleens of NOD-*scid* *IL2r γ* ^{null} $\beta 2m$ ^{null} mice. Similarly, there were no MHC class II positive cells in the bone marrow and spleens of NOD-*scid* *IL2r γ* ^{null} *Ab*^{null} mice. We also confirmed the absence of mature mouse CD4 and CD8 T cells, mature cell-surface Ig-positive B cells, and LGL⁺/CD122⁺ NK cells in the spleens and bone marrow of NOD-*scid* *IL2r γ* ^{null} $\beta 2m$ ^{null} and NOD-*scid* *IL2r γ* ^{null} *Ab*^{null} mice. NOD-*scid* *IL2r γ* ^{null} $\beta 2m$ ^{null} mice also exhibited decreased percentages of splenic Gr-1⁺/Mac1⁺ granulocytes, Gr-1⁺/Mac1⁺ macrophages, and Ter-119⁺ erythroid cells compared with NOD-*scid* *IL2r γ* ^{null} mice. There were also reduced percentages of Ter-119⁺ erythroid cells in the bone marrow of NOD-*scid* *IL2r γ* ^{null} $\beta 2m$ ^{null} mice compared with NOD-*scid* *IL2r γ* ^{null} mice.

2.3 Absence of Thymic Lymphomas in NOD-*scid* *IL2r γ* ^{null} $\beta 2m$ ^{null} Mice

NOD-*scid* and NOD-*scid* $\beta 2m$ ^{null} mice develop thymic lymphomas and die at an early age (19). NOD-*scid* mice have a median lifespan of 36 weeks (18) while in NOD-*scid* $\beta 2m$ ^{null} mice the median lifespan is shortened to 24 weeks (19). In contrast, NOD-*scid* *IL2r γ* ^{null} mice do not develop thymic lymphomas (25). The absence of thymic lymphoma development suggests that signaling through *IL2r γ* receptor-dependent cytokines such as IL-2 or IL-7 is required for the thymic lymphomagenesis. Because of the increased incidence and accelerated kinetics of thymic lymphoma development in NOD-*scid* $\beta 2m$ ^{null} mice, we investigated whether NOD-*scid* *IL2r γ* ^{null} $\beta 2m$ ^{null} mice developed thymic lymphomas and die at an early age. We first examined the histology of the thymus from 10 week old NOD-*scid* *IL2r γ* ^{null} $\beta 2m$ ^{null} mice and compared it with age-matched NOD-*scid* $\beta 2m$ ^{null} mice. As shown in **Figure 2A and B**, the thymus of young adult NOD-*scid* *IL2r γ* ^{null} $\beta 2m$ ^{null} mice is a hypoplastic, degenerate thymic rudiment with a cystic formation. In contrast, the thymus of age matched NOD-*scid* $\beta 2m$ ^{null} mice display thymic lymphomas showing a highly proliferative thymus containing numerous mitotic cells. In addition, although NOD-*scid* $\beta 2m$ ^{null} mice do not survive beyond 8 months of age due to thymic lymphomas, NOD-*scid* *IL2r γ* ^{null} $\beta 2m$ ^{null} mice show an increased lifespan with no gross or histological evidence of thymic lymphomas at 8 months, with mice surviving past 14 months of age (unpublished observations). A cohort of NOD-*scid* *IL2r γ* ^{null} $\beta 2m$ ^{null} retired breeders is being aged for evaluating thymic lymphoma development. Four of these mice euthanized at 12-18 months of age were found to have no histological evidence of thymic lymphoma.

2.4 Development of Hemochromatosis in NOD-*scid* *IL2r γ* ^{null} $\beta 2m$ ^{null} Mice

We have previously reported that the lack of $\beta 2$ -microglobulin led to iron accumulation in the liver of NOD-*scid* $\beta 2m$ ^{null} mice (19), consistent with previous reports showing that $\beta 2$ -

microglobulin is required for appropriate regulation of iron absorption (32, 33). To determine whether the addition of the *IL2r γ ^{null}* gene to NOD-*scid* β 2m^{null} mice altered this phenotype, we stained livers from 10-week-old NOD-*scid* *IL2r γ ^{null}* β 2m^{null} for the presence of iron. As shown in **Figure 2C**, we observed the accumulation of iron deposition in the liver. In contrast, there was no detectable iron accumulation in the liver of age-matched NOD-*scid* *IL2r γ ^{null}* mice (**Figure 2D**). These observations document that the accumulation of iron in the liver of β 2-microglobulin knockout mice does not require common gamma chain-dependent receptor signaling.

2.5 Engraftment of NOD-*scid* *IL2r γ ^{null}* β 2m^{null} or NOD-*scid* *IL2r γ ^{null}* *Ab^{null}* Mice with Human PBMC

The next logical step in the development of this model system is to determine the proliferative response of human PBMC to antigen-presenting cells obtained from NOD-*scid* *IL2r γ ^{null}* deficient in MHC class I or class II. In preliminary studies, we have observed that the deficiency of MHC class I severely reduces the ability of mouse antigen-presenting cells to stimulate *in vitro* proliferation of human CD8 T cells and similarly, the deficiency of MHC class II reduces their ability to stimulate *in vitro* proliferation of human CD4 T cells. These data suggest that xeno-GVHD in immunodeficient mice may be dependent on both MHC class I and class II expression of the murine immunodeficient host. Furthermore, the *in vitro* proliferative data suggest that the development of GVHD in NOD-*scid* *IL2r γ ^{null}* mice will likely be mediated by both human CD4 and CD8 T cells targeting MHC class II or class I, respectively. If so, these MHC class I or class II deficient recipients may permit the ability to study the role of each cell subset in the absence of the confounding contribution of the other cell subset to the development of the disease. This hypothesis is currently being tested. This would make these excellent model systems to test the efficacy of therapeutics to modulate the activity of either cell subset in the absence of the functional contribution of the other cell subset to the disease process.

2.6 Human Immune Responses in PBMC Engrafted Mice

Previous attempts to measure immune function of human cells following PBMC engraftment of immunodeficient mice have been met with limited success (34). The lack of a vigorous primary immune response mediated by human PBMC engrafted into earlier humanized mouse models was associated with host murine NK cell activity and other innate immune function as well as an overwhelming xenogeneic GVHD response of the human T cells. The development of NOD-*scid* *IL2r γ ^{null}* mice provided an immunodeficient mouse lacking host NK cells, facilitating human PBMC engraftment. Although NOD-*scid* *IL2r γ ^{null}* mice support survival and improved function of engrafted human T cells, the human cells mount a severe xenogeneic GVHD following engraftment into these NK cell-deficient hosts. Our findings that removal of mouse MHC class I activity greatly reduces human *in vitro* T cell proliferative responses to murine cells should also lead to reduced xenogeneic GVHD following engraftment with human PBMC, an hypothesis we are currently testing. This may now allow immunization of PBMC engrafted NOD-*scid* *IL2r γ ^{null}* β 2m^{null} mice with experimental vaccines to provide a model system for the assessment of the ability of these vaccines to elicit a protective response against infection with human pathogens in the absence of an overwhelming xeno-GVHD.

3.0 CONCLUSIONS

The use of immunodeficient mice for the *in vivo* study of human xeno-GVHD holds the promise of understanding the pathogenesis of human GVHD *in vivo* in a small animal model without putting patients at risk. This model will be critical for understanding the role of host MHC class I and class II in the pathogenesis of GVHD, as well as opening the possibility of setting up an allogeneic GVHD model system with the emergence of NOD-*scid* *IL2r*^{null} mice deficient in MHC class I or II that also transgenically express human HLA molecules (35). Of particular importance will be the use of this model system for evaluating the efficacy of human-specific therapeutics on the pathogenesis of GVHD *in vivo*. This model will permit the *in vivo* study of human-specific drugs to test their ability to ameliorate or prevent the disease process without putting patients at risk. NOD-*scid* *IL2r*^{null} mice have also been engrafted with blood obtained from patients with human acute myelogenous leukemia and chronic myelogenous leukemia as well as other hematopoietic tumors. This may provide “personalized” animal models of primary human tumors that can be used to screen drugs for their *in vivo* effectiveness on the tumor and tumor stem cells prior to their use in the patient. The combination of optimal immunodeficient recipients and the analyses of human immunity and xeno-GVHD reactions *in vivo* should provide insights into the mechanisms of human immunity and the pathogenesis of GVHD and other human diseases and their response to drugs in ways not possible in the clinic.

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REFERENCES

1. Alderuccio F, Siatskas C, Chan J, Field J, Murphy K, Nasa Z, Toh BH. Hematopoietic stem cell gene therapy to treat autoimmune disease. *Curr.Stem Cell Res.Ther.* 2006; 1:279–287. [PubMed: 18220873]
2. Passweg J, Tyndall A. Autologous stem cell transplantation in autoimmune diseases. *Semin.Hematol.* 2007; 44:278–285. [PubMed: 17961728]
3. Ringden O, Le Blanc K. Allogeneic hematopoietic stem cell transplantation: state of the art and new perspectives. *APMIS.* 2005; 113:813–830. [PubMed: 16480452]
4. Welniak LA, Blazar BR, Murphy WJ. Immunobiology of allogeneic hematopoietic stem cell transplantation. *Annu.Rev Immunol.* 2007; 25:139–170. [PubMed: 17129175]
5. Demirer T, Barkholt L, Blaise D, Pedrazzoli P, Aglietta M, Carella AM, Bay JO, Arpaci F, Rosti G, Gurman G, Niederwieser D, Bregni M. Transplantation of allogeneic hematopoietic stem cells: an emerging treatment modality for solid tumors. *Nat.Clin.Pract.Oncol.* 2008; 5:256–267. [PubMed: 18398414]
6. Bhatia M, Walters MC. Hematopoietic cell transplantation for thalassemia and sickle cell disease: past, present and future. *Bone Marrow Transplant.* 2008; 41:109–117. [PubMed: 18059330]
7. Lebensburger J, Persons DA. Progress toward safe and effective gene therapy for beta-thalassemia and sickle cell disease. *Curr.Opin.Drug Discov.Devel.* 2008; 11:225–232.

8. Pinto FO, Roberts I. Cord blood stem cell transplantation for haemoglobinopathies. *Br.J.Haematol.* 2008; 141:309–324. [PubMed: 18307566]
9. Kawase T, Matsuo K, Kashiwase K, Inoko H, Saji H, Ogawa S, Kato S, Sasazuki T, Kodera Y, Morishima Y. HLA mismatch combinations associated with decreased risk of relapse: Implications for molecular mechanism. *Blood.* 2008 On line publication, December, 2008.
10. Gale RP, Horowitz MM, Butturini A. Autotransplants in acute leukaemia. *Br.J.Haematol.* 1991; 78:135–137. [PubMed: 2043473]
11. Porter D, Levine JE. Graft-versus-host disease and graft-versus-leukemia after donor leukocyte infusion. *Semin.Hematol.* 2006; 43:53–61. [PubMed: 16412789]
12. Foss FM. The role of purine analogues in low-intensity regimens with allogeneic hematopoietic stem cell transplantation. *Semin.Hematol.* 2006; 43:S35–S43. [PubMed: 16549113]
13. 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]
14. Mosier DE, Baird SM, Kirven MB. EBV-associated B-cell lymphomas following transfer of human peripheral blood lymphocytes to mice with severe combined immune deficiency. *Curr.Top.Microbiol.Immunol.* 1990; 166:317–323. [PubMed: 1963581]
15. Tary-Lehmann M, Lehmann PV, Schols D, Roncarolo MG, Saxon A. Anti-SCID mouse reactivity shapes the human CD4⁺ T cell repertoire in hu-PBL-SCID chimeras. *J.Exp.Med.* 1994; 180:1817–1827. [PubMed: 7964463]
16. Tary-Lehmann M, Saxon A. Human mature T cells that are anergic in vivo prevail in SCID mice reconstituted with human peripheral blood. *J.Exp.Med.* 1992; 175:503–516. [PubMed: 1346272]
17. Hoffmann-Fezer G, Gall C, Zengerle U, Kranz B, Thierfelder S. Immunohistology and immunocytology of human T-cell chimerism and graft-versus-host disease in SCID mice. *Blood.* 1993; 81:3440–3448. [PubMed: 8099506]
18. Shultz LD, Schweitzer PA, Christianson SW, Gott B, Schweitzer IB, McKenna S, Mobraaten L, Rajan TV, Greiner DL, Leiter EH. Multiple defects in innate and adaptive immunologic function in NOD/LtSz-scid mice. *J.Immunol.* 1995; 154:180–191. [PubMed: 7995938]
19. Christianson SW, Greiner DL, Hesselton RM, Leif JH, Wagar EJ, Schweitzer IB, Rajan TV, Gott B, Roopenian DC, Shultz LD. Enhanced human CD4⁺ T cell engraftment in β 2-microglobulin-deficient NOD-scid mice. *J.Immunol.* 1997; 158:3578–3586. [PubMed: 9103418]
20. Nervi B, Rettig MP, Ritchey JK, Wang HL, Bauer G, Walker J, Bonyhadi ML, Berenson RJ, Prior JL, Piwnicka-Worms D, Nolta JA, DiPersio JF. Factors affecting human T cell engraftment, trafficking, and associated xenogeneic graft-vs-host disease in NOD/SCID beta2mnull mice. *Exp.Hematol.* 2007; 35:1823–1838. [PubMed: 17764813]
21. Shultz LD, Ishikawa F, Greiner DL. Humanized mice in translational biomedical research. *Nat.Rev Immunol.* 2007; 7:118–130. [PubMed: 17259968]
22. Cao X, Shores EW, Hu-Li J, Anver MR, Kelsall BL, Russell SM, Drago J, Noguchi M, Grinberg A, Bloom ET, Paul WE, Katz SI, Love PE, Leonard WJ. Defective lymphoid development in mice lacking expression of the common cytokine receptor gamma chain. *Immunity.* 1995; 2:223–238. [PubMed: 7697543]
23. van Rijn RS, Simonetti ER, Hagenbeek A, Hogenes MC, de Weger RA, Canninga-van Dijk MR, Weijer K, Spits H, Storm G, van Bloois L, Rijkers G, Martens AC, Ebeling SB. A new xenograft model for graft-versus-host disease by intravenous transfer of human peripheral blood mononuclear cells in RAG2^{-/-} gammac^{-/-} double-mutant mice. *Blood.* 2003; 102:2522–2531. [PubMed: 12791667]
24. 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]
25. Shultz LD, Lyons BL, Burzenski LM, Gott B, Chen X, Chaleff S, Gillies SD, King M, Mangada J, Greiner DL, Handgretinger R. Human lymphoid and myeloid cell development in NOD/LtSz-scid IL2r γ null mice engrafted with mobilized human hematopoietic stem cell. *J.Immunol.* 2005; 174:6477–6489. [PubMed: 15879151]

26. King M, Pearson T, Shultz LD, Leif J, Bottino R, Trucco M, Atkinson MA, Wasserfall C, Herold KC, Woodland RT, Schmidt MR, Woda BA, Thompson M, Rossini AA, Greiner DL. A new Hu-PBL model for the study of human islet alloreactivity based on NOD-scid mice bearing a targeted mutation in the IL-2 receptor gamma chain gene. *Clin.Immunol.* 2008; 126:303–314. [PubMed: 18096436]
27. Golovina TN, Mikheeva T, Suhoski MM, Aqui NA, Tai VC, Shan X, Liu R, Balcarcel RR, Fisher N, Levine BL, Carroll RG, Warner N, Blazar BR, June CH, Riley JL. CD28 costimulation is essential for human T regulatory expansion and function. *J.Immunol.* 2008; 181:2855–2868. [PubMed: 18684977]
28. King M, Pearson T, Rossini AA, Shultz LD, Greiner DL. Humanized mice for the study of type 1 diabetes and beta cell function. *Ann.N.Y.Acad.Sci.* 2008; 1150:46–53. [PubMed: 19120266]
29. Pearson T, Shultz LD, Miller D, King M, Laning J, Fodor W, Cuthbert A, Burzenski L, Gott B, Lyons B, Foreman O, Rossini AA, Greiner DL. Non-obese diabetic-recombination activating gene-1 (NOD-Rag1 null) interleukin (IL)-2 receptor common gamma chain (IL2r gamma null) null mice: a radioresistant model for human lymphohaematopoietic engraftment. *Clin.Exp.Immunol.* 2008; 154:270–284. [PubMed: 18785974]
30. Takenaka K, Prasolava TK, Wang JC, Mortin-Toth SM, Khalouei S, Gan OI, Dick JE, Danska JS. Polymorphism in Sirpa modulates engraftment of human hematopoietic stem cells. *Nat.Immunol.* 2007; 8:1313–1323. [PubMed: 17982459]
31. Mosier DE. Adoptive transfer of human lymphoid cells to severely immunodeficient mice: models for normal human immune function, autoimmunity, lymphomagenesis, and AIDS. *Adv.Immunol.* 1991; 50:303–325. [PubMed: 1950798]
32. Rothenberg BE, Volland JR. beta2 knockout mice develop parenchymal iron overload: A putative role for class I genes of the major histocompatibility complex in iron metabolism. *Proc.Nat'l.Acad.Sci.* 1996; 93:1529–1534. [PubMed: 8643666]
33. Junghans RP, Anderson CL. The protection receptor for IgG catabolism is the β_2 -microglobulin-containing neonatal intestinal transport receptor. *Proc.Nat'l.Acad.Sci.USA.* 1996; 93:5512–5516. [PubMed: 8643606]
34. Torbett BE, Picchio G, Mosier DE. hu-PBL-SCID mice: a model for human immune function, AIDS, and lymphomagenesis. *Immunol.Rev.* 1991; 124:139–164. [PubMed: 1666627]
35. Banuelos SJ, Shultz LD, Greiner DL, Burzenski LM, Gott B, Lyons BL, Rossini AA, Appel MC. Rejection of human islets and human HLA A2.1 transgenic mouse islets by alloreactive human lymphocytes in immunodeficient NOD-scid and NOD-Rag1nullPrf1null mice. *Clin.Immunol.* 2004; 112:273–283. [PubMed: 15308121]

Fig. 1A and Fig 1B.

Flow cytometric analysis of MHC class I and II expression. *Panel A.* Analysis of MHC class I (H-2 K^d) and MHC class II (I-A^{b7}) in spleen and bone marrow cells. While nearly all NOD +/+ splenocytes and bone marrow cells express MHC class I (H-2 K^d), <1% of NOD-scid IL2r^γ^{null} β2m^{null} bone marrow cells or splenocytes are MHC class I positive. Approximately 50% of NOD +/+ splenocytes and 13% of NOD +/+ bone marrow cells express MHC class

II. NOD-*scid IL2 γ ^{null}* as well as NOD-*scid IL2 γ ^{null} β 2m^{null}* mice have reduced percentages of MHC class II positive cells, consistent with a lack of mature B cells. *Panel B.* Analysis of MHC class I (H-2 K^d, NOD-derived and H-2D^b, C57BL/6-derived) and MHC class II (IA^{g7}, NOD-derived and I-A^b, C57BL/6-derived) expression in splenic cells. Different sets of antibodies were used because genetic backcrossing of the MHC class II targeted mutation in 129 ES cells crossed onto the C57BL/6 J strain and subsequently crossed onto the NOD strain results in the crossing of the entire MHC region onto NOD. Thus, NOD MHC class II (I-A)^{null} mice express the 129/C57BL/6 class I haplotype (H-2^b) and fail to express either the NOD MHC class I (H-2 K^d) or the NOD MHC class II (I-A^{g7}) antigens. NOD +/+ mice express H-2 K^d and H-2D^b MHC class I alleles. They also express I-A^{g7} and do not express the I-A^b allele. NOD-*scid IL2 γ ^{null}* mice express the H-2 K^d and H-2D^b MHC class I alleles. Expression is duller because of the absence of MHC class I-bright lymphocytes. These mice have low numbers of I-A^{g7} expressing cells and lack expression of I-A^b on their cells. NOD-*scid IL2 γ ^{null} Ab^{null}* mice lack MHC class II expression and exhibit dull H-2D^b expression with no expression of H-2 K^d.

Fig. 2.

Histopathological sections of tissues from NOD-scid $IL2r\gamma^{null}$ $\beta 2m^{null}$ and control mice. *Panel A.* Thymic lobe from 10-week-old NOD-scid $IL2r\gamma^{null}$ $\beta 2m^{null}$ female. Thymus is composed of epithelial and stromal cells and is completely devoid of lymphocytes. Note the cyst lined by a single layer of occasionally ciliated epithelial cells present within the thymic parenchyma (branchial arch remnant). *Panel B.* Thymic lobe from 10-week-old NOD-scid $\beta 2m^{null}$ male. A thymic lymphoma is present. Thymic architecture is replaced by dense sheets of neoplastic lymphocytes. Note large atypical lymphocytes with abundant mitotic figures (insert). *Panel C.* Liver from 10-week-old NOD-scid $IL2r\gamma^{null}$ $\beta 2m^{null}$ male. Hepatocytes show intracytoplasmic deposition of iron. *Panel D.* Liver from 20-week-old NOD-scid $IL2r\gamma^{null}$ male. There are no detectable iron depositions (**A** and **B**, H&E; **C** and **D**, Gomori's iron) (**A** and **B**, $\times 100$, insert $\times 400$; **C** and **D**, $\times 200$).

Table 1

Flow cytometry analyses of spleen cells from NOD-scid *IL2rg^{null}* $\beta 2M^{null}$ and NOD-scid *IL2rg^{null}* *Ab^{null}* mice showing percentages of leukocyte populations^a

Spleen Surface Marker	NOD +/+ (N=1)	NOD-scid <i>IL2rg^{null}</i> (N=5)	NOD-scid <i>IL2rg^{null}</i> <i>p2M^{null}</i> (N=6)	NOD-scid <i>IL2rg^{null}</i> <i>Ab^{null}</i> (N=5)
CD3 ⁺ /CD4 ⁺	26.1	0.0±0.0	0.0±0.0	0.0±0.0
CD3 ⁺ /CD8 ⁺	10.6	0.0±0.0	0.0±0.0	0.0±0.0
B220 ⁺ /IgK, light chain ⁺	45.0	0.2±0.1	0.0±0.0	0.0±0.0
B220 ⁺ /IgK, light chain-	1.3	3.1±0.7	0.9±0.2	1.6±0.5
GR-1 ⁺ /Mac-1 ⁺	6.3	17.3±1.0	10.9±2.0*	21.1±0.9
GR-1 ⁺ /Mac-1 ⁻	5.1	9.6±0.9	5.9±0.4*	11.0±1.2
Ter 119 ⁺	2.8	45.4±2.6	25±1.8*	44.3±1.7
DX5 ⁺ /CD122 ⁺	4.2	1.3±0.1	1.2±0.1	1.5±0.1
LGL ⁺ /CD122 ⁺	0.7	0.1±0.0	0.3±0.1	0.1±0.0
H-2K ^{d+}	94.2	65.3±2.2	1.0±0.1**	0.2±0.0**
H-2D ^{b+}	83.6	28.8±3.5	N/A	36.8±1.6
I-A ^{g7+}	50.9	3.6±0.4	6.4±0.0	0.0±0.0**

^aData are expressed as mean ± SE

* $p < 0.05$ NOD-scid $IL2rg^{null} \beta 2M^{null}$ or NOD-scid $IL2rg^{null} Ab^{null}$ mice versus NOD-scid $IL2rg^{null}$

** $> p < 0.001$ NOD-scid $IL2rg^{null} \beta 2M^{null}$ or NOD-scid $IL2rg^{null} Ab^{null}$ mice versus NOD-scid $IL2rg^{null}$

Table 2

Flow cytometry analyses of bone marrow cells from NOD-scid *IL2rg^{null}* $\mu 2M^{null}$ and NOD-scid *IL2rg^{null}* *Ab^{null}* mice showing percentages of leukocyte populations^a

Bone Marrow Surface Marker	NOD +/+ (N=1)	NOD-scid <i>IL2rg^{null}</i> (N=5)	NOD-scid <i>IL2rg^{null}</i> $\beta 2M^{null}$ (N=6)	NOD-scid <i>IL2rg^{null}</i> <i>Ab^{null}</i> (N=5)
CD3 ⁺ /CD4 ⁺	0.5	0.0±0.0	0.0±0.0	0.0±0.0
CD3 ⁺ /CD8 ⁺	0.5	0.0±0.0	0.0±0.0	0.0±0.0
B220 ⁺ /IgK, light chain ⁺	5.0	0.0±0.0	0.0±0.0	0.0±0.0
B220 ⁺ /IgK, light chain ⁻	14.9	1.6±0.1	0.8±0.0	1.3±0.2
GR-1 ⁺ /Mac-1 ⁺	48.8	57.9±0.3	67.7±1.4	52.1±2.8
GR-1 ⁻ /Mac-1 ⁺	6.9	9.6±0.4	5.9±0.4	9.2±0.3
Ter 119 ⁺	10.4	14.7±0.5	6.5±0.7 [*]	18.3±1.0
DX5 ⁺ /CD122 ⁺	1.8	1.9±0.1	1.3±0.0	1.4±0.1
LGL ⁺ /CD122 ⁺	0.41	0.2±0.0	0.1±0.0	0.2±0.0
H-2K ^{d+}	92.8	85.8±2.0	0.3±0.0 ^{**}	0.3±0.1 ^{**}
H-2D ^{b+}	68	72.2±0.6	N/A	69.2±1.2
I-A ^{g7+}	12.6	2.9±0.2	4.3±0.6	0.3±0.0 ^{**}

^a Data are expressed as mean ± SE

^{*} $p<0.05$ NOD-scid *IL2rg^{null}* (32M^{null}) or NOD-scid $\beta 2M^{null}$ mice versus NOD-scid *IL2rg^{null}*

^{**} $p<0.001$ NOD-scid $\beta 2M^{null}$ or NOD-scid $\beta 2M^{null}$ *Ab^{null}* mice versus NOD-scid $\beta 2M^{null}$