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Use of humanized severe combined immunodeficient mice for human vaccine development

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Abstract

The severe combined immunodeficient (SCID) mouse has no adaptive immunity, lacking mature T and B cells in the peripheral blood or the lymphoid organs. It has been used extensively in biomedical research as a valuable translational model for xeno-engraftment of human tissues and cells. This review focuses on the engraftment of human peripheral blood cells and tissues in SCID mice, as well as in the newly established and more permissive SCID mice deficient in the IL-2 receptor γ -chain. Human immune responses could be elicited and assessed in these humanized SCID mice upon vaccination or sensitization with allogeneic tissues. A translational model is proposed to attain preclinical data for testing human vaccines.

Keywords

preclinical model; severe combined immunodeficient mice; vaccine development

The severe combined immunodeficient (SCID) mouse has no adaptive immunity, lacking mature T and B cells in the peripheral blood or the lymphoid organs. It was discovered approximately 25 years ago in the laboratory of Melvin Bosma at the Fox Chase Cancer Center (PA, USA). A spontaneous mutation was noted in a colony of Balb/c mice, line CB17, while the investigators were studying allotypes of mouse immunoglobulins [1]. These mice were found to have minimal mouse immunoglobulin and, upon further investigation, they lacked both mature T and B cells, and precursors of T and B cells failed to differentiate into functional immune cells. The point mutation was later identified to affect the catalytic subunit of a mouse DNA-dependent protein kinase [2,3], resulting in severe impairment of the recombination of T- and B-cell receptors. These mice are, however, viable, surviving quite well in a pathogen-free environment, mainly by utilizing their innate immunity of natural killer (NK) cells, myeloid cells and monocytic cells for immune defenses.

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In view of its limited ability to mount an immune response to foreign tissues, immunologists quickly realized the advantage of such a SCID mouse in accepting allografts and xenografts. Indeed, both Mosier *et al.* [4] and McCune *et al.* [5] published their findings of engrafting human peripheral blood mononuclear cells (PBMCs) or human fetal thymus/liver in these mice. The terminologies hu-SCID-PBL or SCID-hu thy/liv were used respectively to distinguish these two experimental systems. The development of the two models in xenografts concurred with the discovery of HIV, which can only propagate in human immune cells. Both systems have been successfully used in testing therapeutics for HIV and in improving understanding of the pathophysiology of the virus [6,7]. It was, however, found that the original CB17/SCID mice could be 'leaky', such that, as the mice get older, they can spontaneously repair the defect and could often have circulating immunoglobulins. Shultz *et al.* later bred the SCID mutation onto the nonobese diabetic (NOD) mouse, creating the NOD/SCID mouse, which has reduced 'leakiness', lower levels of NK cells and a defective complement system [8]. The development and modification of the NOD/SCID mice has been detailed in a recent review by Shultz *et al.* [9]. The most significant advance within the humanized mouse model is the creation of the SCID mouse, with the disrupted IL-2 receptor (IL-2R)- γ chain (c)-knockout (KO), the common cytokine receptor for IL-2, -4, -7, -9, -15 and -21 [10,11]. Four independent laboratories have created or studied four separate lines with IL-2R- γ c-KO mice that are now in use for engraftment of human hematopoietic cells and other tissues [11–15]. Two of the IL-2R- γ c-KO mouse lines are on BALB/c – the Rag2-KO mouse, where the recombination activation gene 2 is deleted; thus, similar to the SCID phenotype, it lacks mature T and B cells. The origin of BALB/c – the Rag2^{-/-} IL-2R- γ c-KO used in Spit's laboratory – was a IL-2R- γ c-KO mouse that was generated by A Berns and P Krimpenfort at The Netherlands Cancer Institute (Amsterdam, The Netherlands) [SPITS H, PERS. COMM.]. The Rag2^{-/-} IL-2R- γ c-KO mouse used in Manz's study came from M Ito at the Central Institute for Experimental Animals (CIEA; Kawasaki, Japan) [13]. The other two SCID lines with IL-2R- γ c-KO on NOD/SCID mice originated from M Ito at CIEA and L Shultz at The Jackson Laboratory (Bar Harbor, ME, USA) [11,15,16] and are designated as NOG and NSG, respectively. These NOD/SCID-IL-2R- γ c-KO (NOG or NSG) mice and the BALB/c-Rag2^{-/-}IL-2R- γ c-KO (DKO for Ito's mice) mice are the most permissive hosts for xenografts and, in particular, human CD34⁺ stem cells, due in part to their lack of NK cells, whose differentiation depends on some of these cytokines. Subsequent to the original publications, many laboratories have used various strategies to achieve engraftment of human CD34⁺ stem cells, succeeding in effective differentiation and development of mature T and B cells, monocytes and NK cells. Human granulocytes and erythrocytes are, however, not well populated in these humanized mice. The data have been extensively described in several reviews, a number of which are summarized in TABLE 1. However, with the exception of using human fetal thymus, the engrafted and emerging human T cells were 'educated' on the mouse thymus epithelium and appeared to exhibit mouse T-cell restriction [14,17]. While the human T-cell receptors were rearranged, the viral epitopes recognized by the immunized T cells were in the context of the mouse MHC molecules, rather than that of the human, in both the Epstein-Barr virus (EBV) and influenza systems [14,17]. It was, thus, hypothesized that providing human MHC HLA class I and II transgenes in these NOG, NSG or DKO mice would allow the engrafted human pre-T cells to be 'educated' and selected on the thymus epithelium, bearing human MHC elements (class I and II transgenes), and developing humanlike T-cell repertoires and responses. Accordingly, we and other investigators are currently establishing SCID or NSG mice with transgenic HLA class I genes, such as the A*0201 allele (A2-NSG) from NOD/SCID-HLA-A*0201 mice [18,19], as well as transgenic HLA class II DR1*0101 from NOD/SCID-DR1 mice [20].

In this review, we shall focus on the various protocols for engrafting human PBMCs or umbilical cord blood mononuclear cells (CBMCs) into SCID or IL-2R- γ c-KO SCID mice, and their use in vaccine development. For simplicity, SCID mice will include NOD/SCID and Rag2^{-/-} mice with or without IL-2R- γ c-KO, and NOG (M Ito's mice) or NSG (L Shultz's

mice) are used for the respective NOD/SCID-IL-2R- γ c-KO mice, where applicable. The advantage of engrafting PBMCs or CBMCs is the ease of obtaining these cells and engrafting them into the SCID mice. Such a humanized mouse model provides unique *in vivo* systems, enabling the study of the effect of antigenic stimulation by vaccination. This is supported by the increasing publications describing the application of such shortterm engraftment of PBMCs, CBMCs and mature T cells in testing vaccines and therapeutics in the SCID mice.

Engraftment of human PBMCs & CBMCs in SCID mice for vaccine development

In the earlier studies by Mosier *et al.*, the CB17/SCID mice were engrafted with human PBMCs, consisting of lymphocytes, monocytes and very few granulocytes [4]. The preclinical model to test therapeutics for the HIV virus was established with 50 million PBMCs per mouse, administered intra peritoneally. The requirement of such high inoculums of PBMCs is in part due to the presence of NK cells, which lyse or inhibit the engraftment of human PBMCs. The use of NOD/SCID mice with lower NK activity marginally improved the engraftment. Depletion of the NK activity with anti-NK antibody, such as anti-AsGm1 antibody, further improved the engraftment [21]. However, a syndrome of graft-versus-host disease (GVHD) often occurred in this humanized mouse model. The engrafted animals lost weight and eventually died within 2–3 months. The humanized SCID mice have contributed immensely to biomedical research, despite the engrafted human cells being within 5% of the spleen or blood populations. TABLE 2 lists some of the studies where PBMCs or T cells have been engrafted into SCID mice for the monitoring and studying of infectious diseases, autoimmune disease of systemic lupus erythematosus (lupus), skin-graft rejection and adoptive T-cell immunotherapy.

Engraftment of PBMCs in SCID-IL-2R- γ c-KO mice

Recently, IL-2R- γ c-KO mice were observed to give superior engraftment of PBMCs. King *et al.* presented data detailing the kinetics of the engraftment of human PBMCs in NSG mice, over 4–5 weeks postengraftment [22]. They compared the engraftment of PBMCs in NOD/SCID versus NSG mice and clearly demonstrated better engraftment of PBMCs in NSG mice. Furthermore, 10–20 million cells intravenously or intraperitoneally were required to achieve engraftment of 50–80% of CD45⁺ cells in the blood and spleen. King *et al.* also tested the immune responsiveness of the engrafted PBMCs in a diabetes model, which was implanted with human islet cells in the spleen of a chemically induced diabetic NSG mouse. Upon engraftment of allogeneic PBMCs, the exogenous human islet cells were destroyed, resulting in subsequent diabetes. Thus, this study demonstrated that the PBMCs in the IL-2R- γ c-KO mice can mount allogeneic responses to the human islet cells.

In a study to test a putative vaccine, Okada *et al.* transfused approximately 10 million PBMCs intraperitoneally into NOG mice and immunized the humanized mice with cDNA constructs of the antigenic proteins of severe acute respiratory corona virus. After three immunizations, they could demonstrate some antibody response as well as a modest cytotoxic T-cell response specific to the virus [23]. These studies showed that the PBMC-engrafted NSG or NOG mice could mount specific immune responses.

Engraftment of PBMCs into transgenic HLA-class I A*0201 NSG mice (A2-NSG)

Based on encouraging results that naive human T cells developed from CD34⁺ stem cells in the various IL-2R- γ c-KO mice can be sensitized to EBV [13], we immunized humanized A2-NSG-PBMC mice with human cytomegalovirus (CMV) antigen-presenting cells. The rationale of our investigation is founded on previous marginal success to obtain *in vitro* CMV antigen-specific cells from PBMCs of CMV-seronegative individuals [24]. The use of A2-NSG mice

is based on the assumption that the antigen-presenting cells in these mice can facilitate improved immune responses. We have, thus, infused PBMCs derived from a series of healthy seronegative, HLA A*0201⁺ and non-HLA A*0201⁺ volunteer donors into nonirradiated A2-NSG mice ($2\text{--}3 \times 10^6$ PBMCs intraperitoneally and $2\text{--}3 \times 10^6$ PBMCs via intravenous routes, totaling 5–6 million cells per mouse). Subsequently, on days 3 and 4, we immunized these mice by subcutaneous inoculation with irradiated HLA A*0201⁺ artificial antigen-presenting cells (AAPCs), transduced to express CMV-pp65 as immunogen. The AAPCs are mouse NIH 3T3 fibroblasts engineered to express a human class I HLA allele, and they can process and efficiently present viral epitopes on the expressed HLA allele, eliciting functional, virus-specific human T-cell responses, as previously demonstrated by Papanicolaou *et al.* [25]. These AAPCs express the human costimulatory molecules ICAM-1, B7.1 and LFA-3 as well as B₂M and a single HLA class I heavy-chain molecule, such as HLA-A*0201. The A2-AAPC–CMV-pp65 cells were, thus, used as a surrogate vaccine.

On day 21, following twice-weekly immunizations, spleen cells were analyzed for the level of human T-cell engraftment and cultured and stimulated *in vitro* for T cells responsive to CMV-pp65. At this time, 40–92% of the spleen cells were human CD45⁺ mononuclear cells, of which 85–90% were CD3⁺. Among the T cells, 13–37% were CD3⁺CD8⁺ cells and 53–87% were CD3⁺CD4⁺ cells. The harvested splenic T cells were subsequently expanded *in vitro* for an additional 10–21 days in the presence of irradiated HLA-matched AAPC–CMV-pp65 cells, and tested for CMV-pp65-specific T-cell responses. These included the quantification of CD8⁺ cells binding to the HLA-A2–CMV peptide tetramers and the production of IFN- γ when the human cells were sensitized with the specific CMV peptides. We found that approximately 2% of the cells were CMV-pp65 epitope-HLA A0201 tetramerpositive T cells. Approximately 1–2% of the CD8⁺ T cells also generated IFN- γ , specifically in response to stimulation with CMV-pp65 peptide-loaded autologous EBV-transformed B cells [25]. Therefore, these studies suggest that *in vivo* sensitization in the humanized NSG mouse may indeed elicit primary CMV-pp65-specific T-cell responses from naive populations in CMV-seronegative individuals. TABLE 3 shows a summary of some of the immune functions of the spleen cells from the humanized mice immunized with A2-AAPC–CMV-pp65 cells. Interestingly, an A1⁺B08⁺ donor also responded to the immunization with A2-AAPC–CMV-pp65 cells (TABLE 3), although we did not have B08-specific peptide tetramers to test for antigen-specific T cells. These results suggest that the pp65 antigen was likely processed by the autologous antigen-presenting cells.

These A2-NSG–PBMC mice, however, eventually lost approximately 10% of their weight in 4–6 weeks and became anemic by the end of the study, with 7% of the mice in the study dying. These mice generally showed more than 50% engraftment of CD45⁺ cells, but they did not exhibit the typical GVHD of skin lesions or enteritis, perhaps consistent with the observation that there was little engraftment of human cells in the mesenteric nodes and Peyer's patches of the humanized mice (Su L, PERS. COMM.) [16], probably due to the IL-2R- γ c mutation. We have also intravenously transfused 5 million A2-CBMCs into A2-NSG mice and observed similar engraftment as when A2⁺ PBMCs were used. Furthermore, we can also elicit an immune response that was restricted to the HLA-A2 allele in the CMV system (data not shown). Presently, we have preliminary data suggesting that the immune response of PBMCs in A2-NSG mice appeared to be better, when comparing engraftment of the same donor in NSG and A2-NSG mice. Therefore, these results substantiate the contention that A2-NSG–PBMC mice could respond to novel antigens and that they represent a unique preclinical translational model for vaccine development.

Engraftment of CBMCs in SCID mice with HLA class II transgenes for vaccine development

Due to the undesirable outcome of GVHD with the engraftment of PBMCs, CBMCs have been used to engraft NOD/SCID mice, with the expectation that the more naive T-cell CBMCs will not mount GVHD as readily as the mature T cells in adult PBMCs. Indeed, there were fewer symptoms of weight loss and anemia related to GVHD, although the engraftment of CBMCs resulted predominantly in B cells in the bone marrow, with little T-cell engraftment observed [26]. As an alternative strategy to improve engraftment of human T cells, Camacho *et al.* developed the NOD/SCID mouse with HLA class DR1*0101 (NOD/SCID-DR1) transgenes [20], where the DR1 transgene was located on chromosome 5. We characterized their immunobiology and the innate immunity, such as NK activity, LPS response and antigen presentation, and they were found to be similar to that of the NOD/SCID mice [27]. We found enhanced engraftment of human T cells in the transgenic mice, compared with NOD/SCID mice, whether the donors were DR1*0101 or not. Engraftment with human CBMCs resulted in slight changes in the architecture/structure of spleens, with no defined germinal centers of a normal spleen. Such chimeric mice were then immunized with an adenovirus cancer vaccine to human carcino-embryonic antigen (CEA). The engrafted human cells responded to this cancer vaccine by producing intracellular interferons when the spleen cells from the immunized chimeric mice were further cultured *in vitro* with CEA peptides [27]. Therefore, engrafted CBMCs can respond to the CEA vaccine in the humanized NOD/SCID-DR1 mice. Presently, we are breeding the DR1 transgene onto the NSG background, rendering the mice more amenable for engraftment of human hematopoietic cells, as elaborated earlier.

Engraftment of tumor cells & tissues in SCID mice as targets for immune responsiveness to vaccination

Severe combined immunodeficient mice have been used extensively in preclinical models of tumor engraftment for understanding the pathophysiology of the tumor or for testing therapeutic agents, such as adoptive T-cell therapy [28,29]. Tumors cells were introduced into the mouse either by subcutaneous, intravenous or intraperitoneal administration to test for the tumor stem cells, as demonstrated by numerous studies in the past decade [28], and these are summarized in TABLE 4. In preclinical models of adoptive T-cell therapy, the engrafted tumor cells could be labeled by the transduction of bioluminescent luciferin, to image and monitor the lytic effect of the tumor-specific T cells [30,31]. When T cells and tumors cells were labeled with differential probes or dyes, the number of T cells required to treat leukemia and tumor cells could be approximated. Similarly, the trafficking pattern of T cells could be assessed, in order to explore the mechanism of homing of adoptively transferred T cells to the tumor site.

While established cell lines could be readily engrafted in SCID mice, engraftment of primary tumors has been more challenging. A number of primary leukemia cells could be propagated in either irradiated or nonirradiated SCID mice [28]. Furthermore, successful secondary transfer of bone marrow containing the leukemia cells to another SCID recipient confirmed that leukemia stem cells were established in these mice. With regards to the successful implant of leukemia cells in NOD/SCID mice, Yan *et al.* reported a correlation between the aggressiveness of the leukemia and the poor outcome of the patients [32]. Kong *et al.* also used the NSG mice in propagating primary leukemia cells to recapitulate leukemogenesis in patients [33]. A recent study by Bankert's group described the use of NSG mice in engrafting primary human lung tumors to study tumor-infiltrating T cells [34]. They identified tumor-derived memory effector T cells, which were anergic but could be activated by IL-12. Therefore, primary human cancer cells are readily engrafted in SCID mice and, in particular, in IL-2R- γ -KO mice.

There are other studies of successful engraftment of normal human skin, prostate gland and pancreatic islets into SCID mice (TABLE 4). Pober's group has extensively studied the

engraftment of human skin in CB17/SCID mice and, lately, in the CB17/SCID-beige mice, in order to explore the mechanism of rejection and the role of endothelial cells in such xenotransplants [35]. More recently, Moffat's group engrafted the NOD/SCID mice with human fetal skin, followed by infection with *Varicella zoster*, in order to study the disease process of the virus [36]. In the NOD/SCID mice, Taylor *et al.* were able to grow human stem cells into human prostate tissue, by providing the microenvironment of rat prostate tissue/mesenchymal cells, engrafted under the kidney capsule [37]. NSG mice were used in a recent study of a human diabetes model of islet transplantation [22]. The host was later engrafted with human allogeneic PBMCs, which became sensitized to the pancreatic islets, resulting in rejection of the islet cells, as described earlier.

Therefore, the engrafted human tissues or tumors appropriately chosen can be used to monitor immune responsiveness of a vaccinated humanized mouse (FIGURE 1). Accordingly, adult PBMCs or CBMCs (5 million/mouse) could be injected either intravenously or via a combination of intravenous and intraperitoneal administration (2.5 million in each route of injection). Recipients could include various NOG, NSG or DKO SCID mice, with or without HLA transgenes. After 3 days postinfusion of human cells, the humanized mice could be immunized two- to three-times with the vaccine of interest. A total of 7–21 days following the second or third immunization, the sensitized mice could be challenged with the specific vaccinated antigen, in the form of tumor cells or tissues, expressing the putative antigen and matched for the HLA haplotype of the donor PBMCs or CBMCs. Ideally, using autologous tumor and tissues of the PBMC/CBMC donors would be the best strategy to avoid allogeneic rejection/reaction. For testing immune responsiveness, *ex vivo* immune functional assays could also be assessed, such as the measurement of the production of IFN- γ after further *in vitro* antigenic stimulation, as is commonly performed in vaccine evaluation.

Such experimental protocols would then mimic the immune state of the immunized humanized mouse by obliterating virus-infected skin tissues [36] or by rejecting allogeneic skin grafts [35] or tumors in an antigen-specific manner [29]. Furthermore, the concomitant expression of human HLA molecules/transgenes, for example HLA-A2, in such A2⁺ CBMC- or PBMC-engrafted SCID mice, will offer the opportunity to test for putative toxicity of immune responses with HLA-A2-specific T cells on HLA-A2-expressing mouse tissues.

Expert commentary

The IL-2R- γ c-KO mice on NOD/SCID or BALB/c *Rag2*^{-/-} background are exceptionally useful models for engrafting human CD34⁺ cells, PBMCs or CBMCs for vaccine development. The published data support the contention that engraftment of PBMCs or CBMCs in these mice, along with the target tissues for the vaccine, present a unique human-like entity. With some caution in avoiding allogeneic reactivity of the PBMCs or CBMCs toward the MHC antigen of the tissues, through the use of autologous or partially matched MHC-expressing tissues, such a system constitutes an exquisite approach to test the efficacy of human vaccines.

Five-year view

The SCID mice have provided valuable models in the study of human immunity, hematopoiesis and disease processes. The SCID-IL-2R- γ c-KO mice are currently the most permissive hosts for engrafting human cells or tissues for exploring the pathophysiology, the effects of therapeutics or vaccine development for infectious diseases or cancer. The advent of stem cell research, where many organs and tissues can now be differentiated and transplanted, is expected to further promote the application of this unique translation model. The direction of research in this area, over the next 5 years, is predicted to include:

- The use of transgenic HLA class I and class II NOG, NSG or other IL-2R- γ -KO mice. These mice have significant potential to establish more refined clinically relevant humanized IL-2R- γ -KO models. Their human-like MHC will impact studies of transplantation of stem cell-derived tissues, as well as studies of the immune responses to infectious agents and cancer. With their presentation of the MHC in their tissues, the transgenic mice could be valuable models for assessing the toxicity of cellular and biologic therapies on different MHC-expressing organ systems;
- Improved engraftment of other subsets of hematopoietic cells, such as the myeloid and erythroid compartments, by supplementing growth factors specific for these cells. These other cell types, which are currently not well represented in the humanized mice, will enable the development of model systems for studying diseases arising from these cell compartments;
- The ability of the immune cells engrafted in SCID-IL-2R- γ -KO mice, using PBMCs or CD34⁺ cells, to respond to antigenic challenges in the humanized mice, being used to validate this system as a translational preclinical model to evaluate and test the efficacy of vaccines and therapeutics.

Key issues

- Severe combined immunodeficient (SCID) mice with disrupted IL-2 receptor- γ -chain-knockout are the most permissive hosts for engraftment of human cells and tissues. Human CD34⁺ stem cells could differentiate in SCID-IL-2 receptor- γ -chain-knockout mice and could reconstitute T and B cells, natural killer cells and monocytic compartments in the humanized mice.
- Humanized SCID mice have been used to study the pathophysiology of infectious agents, such as HIV and EBV and their therapeutics.
- SCID mice engrafted with peripheral blood mononuclear cells could be sensitized to allogeneic skin grafts, pancreatic islets and viral vaccines.
- SCID mice could be engrafted with normal human tissues, such as skin, pancreatic islets and primary tumor cells and cell lines.
- Humanized NOD/SCID-IL-2 receptor- γ -chain-knockout (NOG or NSG) mice, engrafted with peripheral blood mononuclear cells, could be immunized with vaccines and assessed for their specific immune responsiveness with infectious agents or tumor cells, constituting a preclinical small animal translational model.

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- of considerable interest

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Figure 1. Proposed translational SCID-IL-2 receptor- γ -chain-knockout-PBMC model for vaccine development

CBMC: Cord blood mononuclear cell; KO: Knockout; NOG: Central Institute for Experimental Animals NOD/SCID-IL-2 receptor γ -chain-knockout mice; NSG: The Jackson Laboratory NOD/SCID-IL-2 receptor γ -chain-knockout mice; PBMC: Peripheral blood mononuclear cell; SCID: Severe combined immunodeficient.

Table 1Summary of engraftment of CD34⁺ cells in severe combined immunodeficient mice and their applications.

Authors	Mice	Source of CD34 ⁺	Application	Ref.
Ito <i>et al.</i> (2002/2008)	NOD/SCID-IL-2R- γ c-KO (NOG)	Cord blood	Hematopoiesis	[11,16]
Yahata <i>et al.</i> (2002)	NOD/SCID-IL-2R- γ c-KO (NOG)	Cord blood	Hematopoiesis	[12]
Legrand <i>et al.</i> (2006); Gimeno <i>et al.</i> (2004)	BALB- <i>Rag2</i> ^{-/-} IL-2R- γ c-KO	Fetal liver	Hematopoiesis, EBV	[14,38]
Traggiai <i>et al.</i> (2004); Manz (2007)	BALB- <i>Rag2</i> ^{-/-} IL-2R- γ c-KO (DKO)	Cord blood	Hematopoiesis, EBV	[13,17]
Shultz <i>et al.</i> (2005/2008)	NOD/SCID-IL-2R- γ c-KO (NSG)	PBSC*	Hematopoiesis	[15,39,40]
Melkus <i>et al.</i> (2006)	NOD/SCID/J	Fetal thy/liv plus CD34 ⁺	EBV immune responses	[41]
Tonomura <i>et al.</i> (2008)	NOD/SCID/J	Fetal thy/liv plus CD34 ⁺	T- and B-cell responses	[42]
Baenziger <i>et al.</i> (2006)	BALB- <i>Rag2</i> ^{-/-} IL-2R- γ c-KO (DKO)	Cord blood	HIV	[43]
Jiang <i>et al.</i> (2008)	BALB- <i>Rag2</i> ^{-/-} IL-2R- γ c-KO (DKO)	Fetal liver	HIV	[44]
Yajima <i>et al.</i> (2008)	NOD/SCID-IL-2R- γ c-KO (NOG)	Cord blood	EBV	[45]

* Collected after treatment with granulocyte colony-stimulating factor.

BALB: Bagg albino; EBV: Epstein-Barr virus; DKO: Double knockout; KO: Knockout; NOD: Nonobese diabetic; NOG: Central Institute for Experimental Animals NOD/SCID-IL-2 receptor- γ -chain-knockout mice; NSG: The Jackson Laboratory NOD/SCID-IL-2 receptor- γ -chain-knockout mouse; PBSC: Peripheral blood stem cell; SCID: Severe combined immunodeficient.

Table 2

Examples of short-term engraftment of mononuclear cells or T cells in severe combined immunodeficient mice and their applications.

Authors	Mice	Source of MNCs	Application	Ref.
Mosier <i>et al.</i> (1988/1992)	CB17/SCID	PBMCs	HIV	[4,46]
Rossi <i>et al.</i> (2001)	NOD/SCID	Cord blood	Hematopoiesis	[26]
Pober <i>et al.</i> (2003)	CB17/SCID	PBMCs	Skin transplant	[35]
Mauermann <i>et al.</i> (2004)	CB17/SCID	Lupus patients	Lupus therapy	[47]
Koehne <i>et al.</i> (2003)	NOD/SCID	Antigen-specific T cells	EBV lymphoma and leukemia	[29]
King <i>et al.</i> (2008)	NSG	PBMCs	Diabetes	[22]
Okada <i>et al.</i> (2007)	NOG	PBMCs	SARS corona virus vaccine	[23]
Camacho <i>et al.</i> (2007)	DR1-NOD/SCID	Cord blood	CEA vaccine	[27]
Yu <i>et al.</i> (2008)	NOD/SCID- <i>B2m</i> ^{-/-}	CD34 ⁺ and autologous T cells	Influenza vaccine	[48]

CEA: Carcino-embryonic antigen; EBV: Epstein-Barr virus; MNC: Mononuclear cell; NOD: Nonobese diabetic; NOG: Central Institute for Experimental Animals NOD/SCID-IL-2 receptor- γ -chain-knockout mice; NSG: The Jackson Laboratory NOD/SCID-IL-2 receptor- γ -chain-knockout mice; PBMC: Peripheral blood mononuclear cell; SARS: Severe acute respiratory syndrome; SCID: Severe combined immunodeficient.

Table 3
Immune responses of humanized A2-NSG-PBMC mice immunized with A2-AAPC-CMC-pp65 cells.

PBMC donors in A2-NSG mice	CD45 engraftment (%)	Splenic CD45 ⁺ CD3 ⁺ CD4 ⁺ cells (%)	Splenic CD45 ⁺ CD3 ⁺ CD8 ⁺ cells (%)	Tetramer ⁺ in CD8 ⁺ cells (%)	IFN- γ ⁺ in CD8 ⁺ cells (%)
HLA-A2 ⁺	40–50	62–64	27–28	2.08	0.42
HLA-A2 ⁺	60–80	53–72	22–37	1.66	ND
HLA-A1 ⁺ B08 ⁺	60–92	54–87	13–33	ND	1.9

Mice were engrafted with three different normal donors with HLA-A2⁺ or HLA-A1⁺B08⁺ genotypes, negative for detectable CMV antibodies (n = 2 control mice per donor without immunization; n = 4 mice per donor with immunization). By 3 days postinfusion of human PBMCs, mice were immunized with irradiated A2-AAPC-CMV-pp65 cells. At 21 days postimmunization, spleen cells were analyzed for human CD45⁺ cells and subsets of T cells, and they were then further stimulated *in vitro*. Spleens from two mice were pooled. After culturing with HLA-matched irradiated AAPC-CMV-pp65 cells for 10–14 days, the cells were quantified for CD8⁺ cells binding to the HLA-A2-CMV-peptide tetramers from pooled cultures. B08-specific peptide tetramers were not available presently for this study. Some samples from pooled cultures were tested for production of IFN- γ , after incubating with CMV specific pp65 peptides, presented by autologous PBMC- or EBV-transformed B cells [25]. Negative controls included nonspecific tetramer or peptides, with values of less than 0.2%. The percentage of tetramer-positive cells, as well as IFN- γ -positive cells, were similar to that observed in CMV-positive donors, cultured under these conditions [25,49].

* n = 12.

AAPC: Artificial antigen-presenting cell; CMV: Cytomegalovirus; EBV: Epstein-Barr virus; ND: Not detected; NSG: The Jackson Laboratory NOD/SCID-IL-2 receptor- γ -chain-knockout mice; PBMC: Peripheral blood mononuclear cell.

Table 4

Short-term engraftment of tissues and tumor cells in severe combined immunodeficient mice as possible targets for *in vivo* immune responses.

Authors	Mice	Tissue	Application	Ref.
Pober <i>et al.</i> (2003)	CB17/SCID	Skin	Transplant rejection	[35]
Moffat <i>et al.</i> (2004)	NOD/SCID	Skin	<i>Varicella zoster</i>	[36]
Taylor <i>et al.</i> (2006)	NOD/SCID	Prostate tissues	Prostate development	[37]
King <i>et al.</i> (2008)	NSG	Human islets	Diabetes and transplant rejection	[22]
Barabe <i>et al.</i> (2007)	NOD/SCID	Leukemia	Leukemogenesis	[28]
Koehne <i>et al.</i> (2003); O'Reilly <i>et al.</i> (2007)	NOD/SCID	EBV lymphoma	T-cell immunotherapy	[29,50]
Simpson-Abelson <i>et al.</i> (2008)	NSG	Primary lung cancer	T cells in tumor	[34]

EBV: Epstein–Barr virus; NOD: Nonobese diabetic; NSG: The Jackson Laboratory NOD/SCID–IL-2 receptor γ -chain-knockout mice; SCID: Severe combined immunodeficient.