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Humanized mouse model for vaccine evaluation: an overview

Animal models are essential in medical research for testing drugs and vaccines. These models differ from humans in various respects, so their results are not directly translatable in humans. To address this issue, humanized mice engrafted with functional human cells or tissue can be helpful. We propose using humanized mice that support the engraftment of human hematopoietic stem cells (HSCs) without irradiation to evaluate vaccines that influence patient immunity. For infectious diseases, several types of antigens and adjuvants have been developed and evaluated for vaccination. Peptide vaccines are generally used for their capability to fight cancer and infectious diseases. Evaluation of adjuvants is necessary as they induce inflammation, which is effective for an enhanced immune response but causes adverse effects in some individuals. A trial can be done on humanized mice to check the immunogenicity of a particular adjuvant and peptide combination. Messenger RNA has also emerged as a potential vaccine against viruses. These vaccines need to be tested with human immune cells because they work by producing a particular peptide of the pathogen. Humanized mice with human HSCs that can produce both myeloid and lymphoid cells show a similar immune response that these vaccines will produce in a patient.

Keywords: Humanized mice, Peptide vaccines, mRNA vaccines, Immune response antigens, Hematopoietic stem cells

Introduction

Developing a new vaccine is a very time-consuming and expensive process in which preclinical trials play a crucial role. Preclinical testing is done to collect sufficient data indicating the vaccine's safety, potential efficacy, toxicity, and pharmacokinetic properties. Therefore, the selection of suitable animal models is very important. Using animals such as rodents and non-primates is unsuitable for preclinical studies due to the numerous species differences [1]. Immunodeficient mice implanted with human hematopoietic stem cells (HSCs), i.e., hu-HSC and fetal bone marrow-liver-thymus (BLT), have the capacity to generate dendritic cells, B cells, T cells, macrophages, and natural killer (NK) cells. They show both humoral and cellular immune responses after infection with antigens [2]. So, humanized mice are being explored to evaluate new vaccines as they are more faithful than conventional rodent models [1].

In this review, we summarize the reports on various humanized mouse models developed till now (Table 1, Fig. 1) and how their modification affects the production and proliferation of HSCs in them. We also include various studies on how humanized mouse have been used to study various immune responses and vaccine trials.



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Table 1. Humanized mice models across the years

	Baseline strains		Primary models		Second generation models		Recent/emerging models	
	Strain	Year	Strain	Year	Strain	Year	Strain	Year
SCID derived	SCID	1983	CB17-SCID	1992	SGM3	2003, 2010	NBSGW	2015
	bg/mu/xid	1988	NOD-SCID	1995	BLT	2006, 2013	pROR γ t- γ c	2018
	IL2R γ null	1995	NOD-SCID IL2R γ null	2002	SCF	2012	BLT-lung	2019
RAG1/2 -/- derived					Ossicles	2012		
					IL-3/GM-SCF	2012		
	RAG1/2-/-	1992	(BALB-c)-RAG2-/-IL2R γ null	1998–2005	CSF-1	2011	BRGWv	2014
	ILR γ null	1995	NOD-RAG2-/-IL2R γ null	2013	TPO	2011	MISTRG	2014
					IL-3/GM-CSF	2011	BRGF	2016
					Ossicles	2012	BRGSF	2017
							SRG-15	2017
						IL-6	2017	
						BRGST	2018	
						NFA2	2018	

SCID, severe combined immunodeficient; NOD, non-obese diabetic; BLT, bone marrow-liver-thymus; IL-2R γ , interleukin-2 receptor gamma; IL, interleukin; GM, granulocyte-macrophage; SCF, stem cell factor; CSF, colony-stimulating factor.

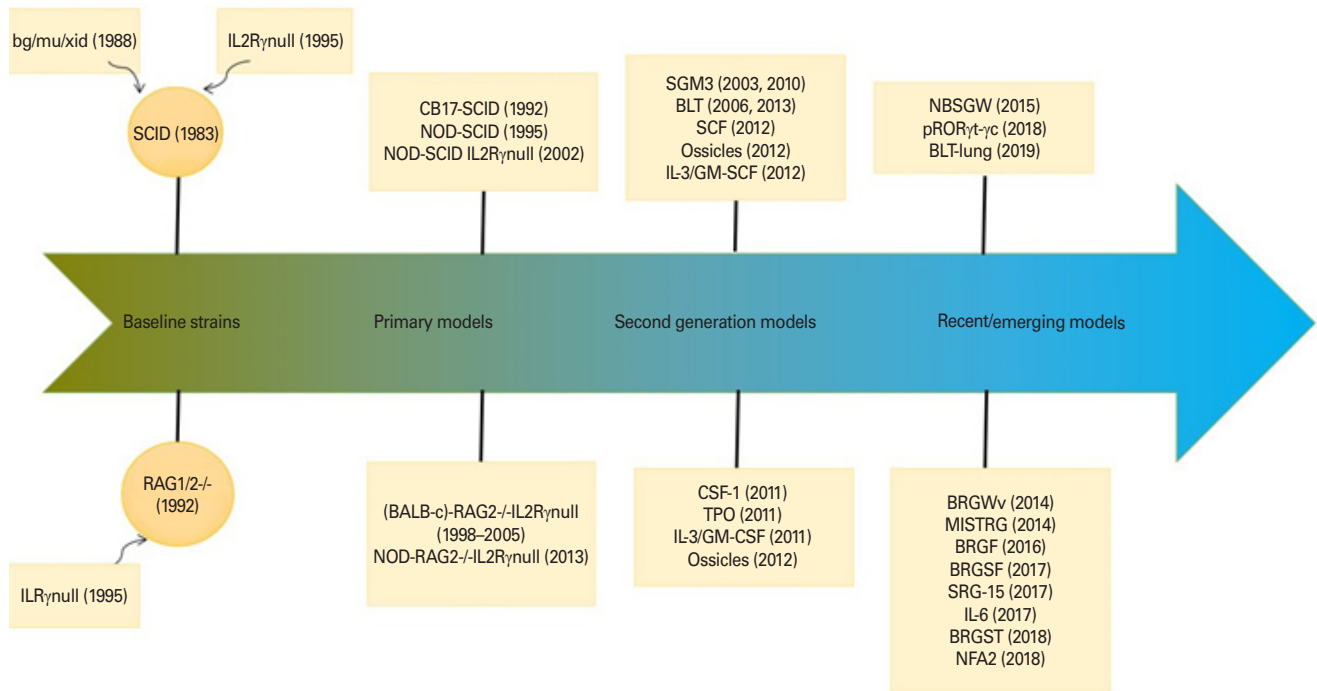


Fig. 1. Timeline showing the evaluation of humanized mice models. SCID, severe combined immunodeficient; NOD, non-obese diabetic; IL-2R γ , interleukin-2 receptor gamma; BLT, bone marrow-liver-thymus; SCF, stem cell factor; GM, granulocyte-macrophage; CSF, colony-stimulating factor; IL-6, interleukin-6.

mRNA Vaccine

Messenger RNA (mRNA) vaccines have high safety and can induce balanced cellular and humoral immunity without being subject to major histocompatibility complex (MHC) hap-

lotype restriction [3]. mRNA shows many advantages over subunit, killed, and live attenuated virus and DNA-based vaccines regarding safety, efficacy, and production [4]. mRNA can encode any protein, enabling therapeutic vaccines to fight diverse infectious diseases and cancer [3]. The working

of the mRNA vaccine has been depicted in Fig. 2.

Various mRNA vaccines have recently been validated for their immunogenicity and efficacy [5,6]. Synthetic mRNA is more translatable than ever due to RNA sequence engineering [4]. For achieving therapeutic relevance, efficient *in-vivo* mRNA delivery is essential. Two basic approaches for the delivery are, first, *ex-vivo* mRNA loading in dendritic cells and then reinfusion of the transfected cells [7]. Second, mRNA direct parenteral injection with or without a carrier [4].

Several mRNA vaccines induce robust CD8+ T-cell responses in addition to potent CD4+ T-cell responses [8]. With only one or two low-dose immunizations, mRNA vaccines have the potential to generate strong neutralizing antibody responses [9,10]. It is shown that mRNA vaccines have elicited immunity in animal models against various infectious diseases [11,12]. In both cases, immunogenicity was more modest

in humans than expected in animal models [4]. Therefore, humanized mice models came into play for more predictable results.

Till now, only one mRNA vaccine has come in use, i.e., against coronavirus disease 2019 (COVID-19). Human immune system (HIS)-humanized mouse model (“DRAGA”: HLA-A2.HLA-DR4.Rag1KO.IL-2RgcKO.NOD) is used to research COVID-19 showing the immunogenic effects of the virus-like that in humans [13].

Peptide Vaccines

Peptide vaccines use short peptide fragments to induce targeted immune responses in individuals. Accordingly, 20–30 amino acid sequences form an immunogenic peptide molecule that acts as an antigen determinant to activate sufficient

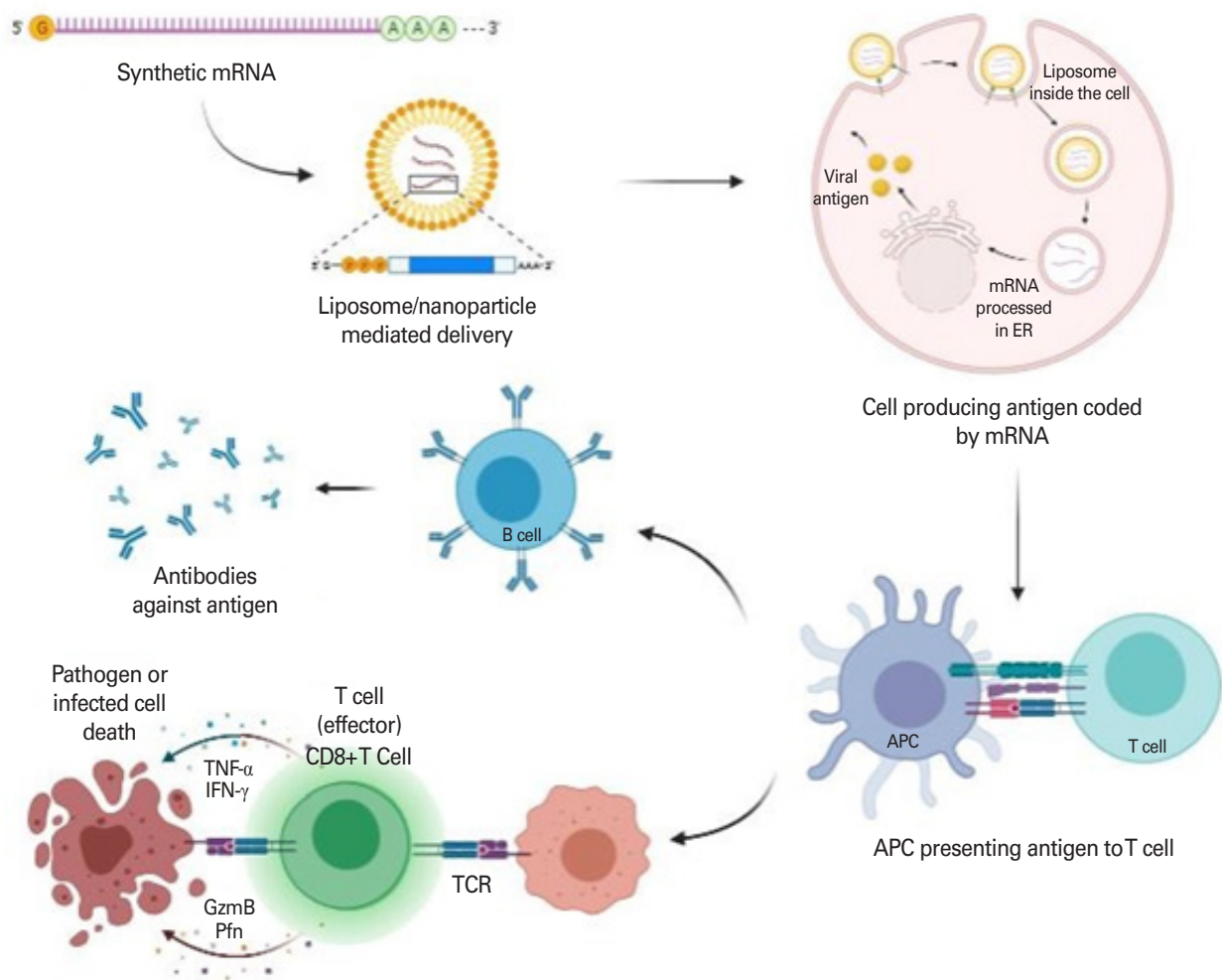


Fig. 2. Liposome/nanoparticle mediated delivery of messenger RNA (mRNA) vaccine and its working. Increased antibody production, and more effective T-cells killing infected cells after vaccination. ER, endoplasmic reticulum; TNF- α , tumor necrosis factor-alpha; IFN- γ , interferon-gamma; Pfn, perforin; TCR, T-cell receptor; APC, antigen-presenting cells.

cellular and humoral immunity. Thus, eliminating the induction of allergenic or reactogenic responses [14,15]. The peptides produced are generally small; as a result, they produce a low immunogenic effect without a carrier molecule [16,17]. Compared to conventional vaccines, manufacturing peptide vaccines are safe and cost-effective [14]. The peptides are presented on patient MHC class I for cytotoxic T-cell activation and MHC class II for antibody production. The prediction of peptide presentation for HLA and mouse MHC is made using available algorithms [18-20]. However, even if experimental mouse MHC presents the peptide, the same peptide does not need to be successfully presented on HLA [14]. So, a humanized mouse model is needed to provide a human immune environment for more accurate predictions.

For a vaccine to be successful, it should protect against the disease and elicit a potent and prolonged memory humoral and cellular immune response. The working of the peptide vaccine has been depicted in Fig. 3.

B-cell response

The primary protection mechanism for many vaccines is the induction of epitope-specific antibodies. The targeted epitope often binds to the immunoglobulin G (IgG) antigen-binding fragment (Fab) region for infectious diseases. This results in certain effector functions that inhibit the infection by blocking host cell attachment or inducing pathogen-antibody complexes that are systemically cleared (i.e., by agglutination/opsonization) [21]. These effector functions include clearance or destruction of pathogen or pathogen-infected cells by complement activation or antibody-dependent cell-mediated cytotoxicity.

Monomeric peptides are often poorly immunogenic as elicitation of protective antibodies requires affinity maturation, which is stimulated by cross-linking B-cell receptors (BCRs). The pathogen surface has multiple copies of the epitope that efficiently cross-links BCRs, stimulating affinity maturation. To improve the immunogenicity of desired peptide epitopes, they

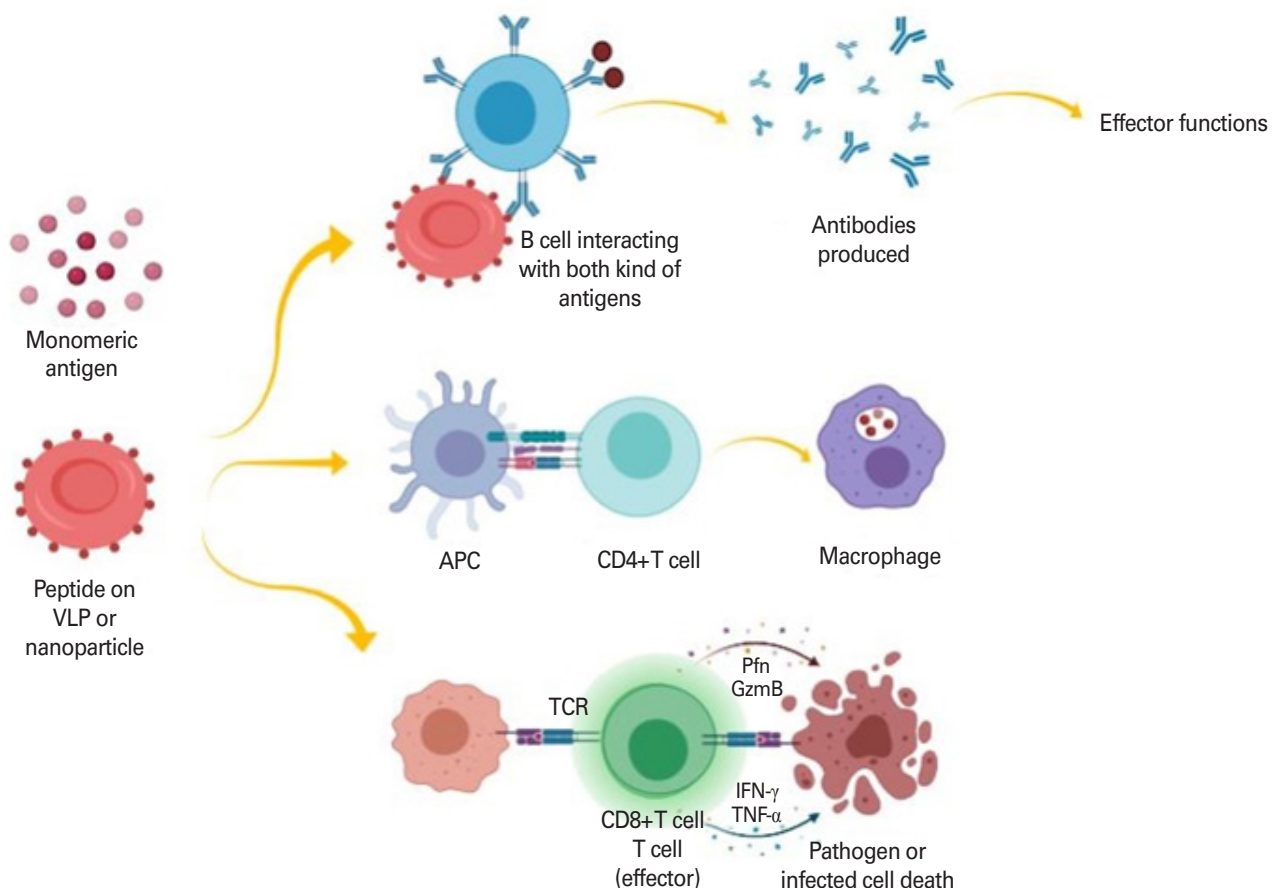


Fig. 3. Working of peptide vaccine. Increased antibody production, activation of macrophages, and more effective T-cells killing infected cells after vaccination. APC, antigen-presenting cells; VLP, virus-like particle; TCR, T-cell receptor; Pfn, perforin; IFN- γ , interferon-gamma; TNF- α , tumor necrosis factor-alpha.

can be linked to virus-like particles or nanoparticles that can more efficiently cross-link BCRs [21]. Certain microorganisms' pathogenesis is linked to secreted or released factors like toxins (e.g., tetanus toxoid, anthrax toxin, or *Staphylococcus aureus* enterotoxin B) against which antibodies are produced. Thus, fragments and inactive variants of toxins can be used for vaccine production [22-24].

T-cell response

In the context of infectious disease, stimulation of epitope-specific T-cells clears and destroys the pathogen itself or infected host cells, thereby stopping the spread of infection [25]. In immuno-oncology, immunotherapies that upregulate cancer-specific T-cells have shown great promise against blood cancers [15].

Epitope specific for T-cells are presented in the peptide binding groove of class I or class II MHCs on antigen-presenting cells (APCs), which is mediated by the T-cell receptors (TCRs).

Antigens' presentation and processing can occur via exogenous or endogenous pathways.

Peptides presented in class I and class II MHC follow a sequence pattern that contains anchor position and, thus, is in the interior of the peptide binding groove and away from TCR [26]. The remaining residues interact with TCR and mediate epitope specificity. The loaded peptides into MHCs must have the sequence requirement, but that does not mean that the particular epitope will be immunogenic. To check the presentation of immunogenic sequences, the peptide should be repeatedly loaded onto APCs such as dendritic cells [27]. For immuno-oncology, this can be the tumor-infiltrating lymphocyte expansion method, which can then be infused for adoptive transfer cell therapy in patients [27]. In other situations, for stimulation of T-cell expansion, systemic delivery of peptide or DNA encoding the epitopes is sufficient [28]. Furthermore, the structural features of the epitope-MHC-TCR ternary complex are essential for T-cell targeted vaccines as antigen

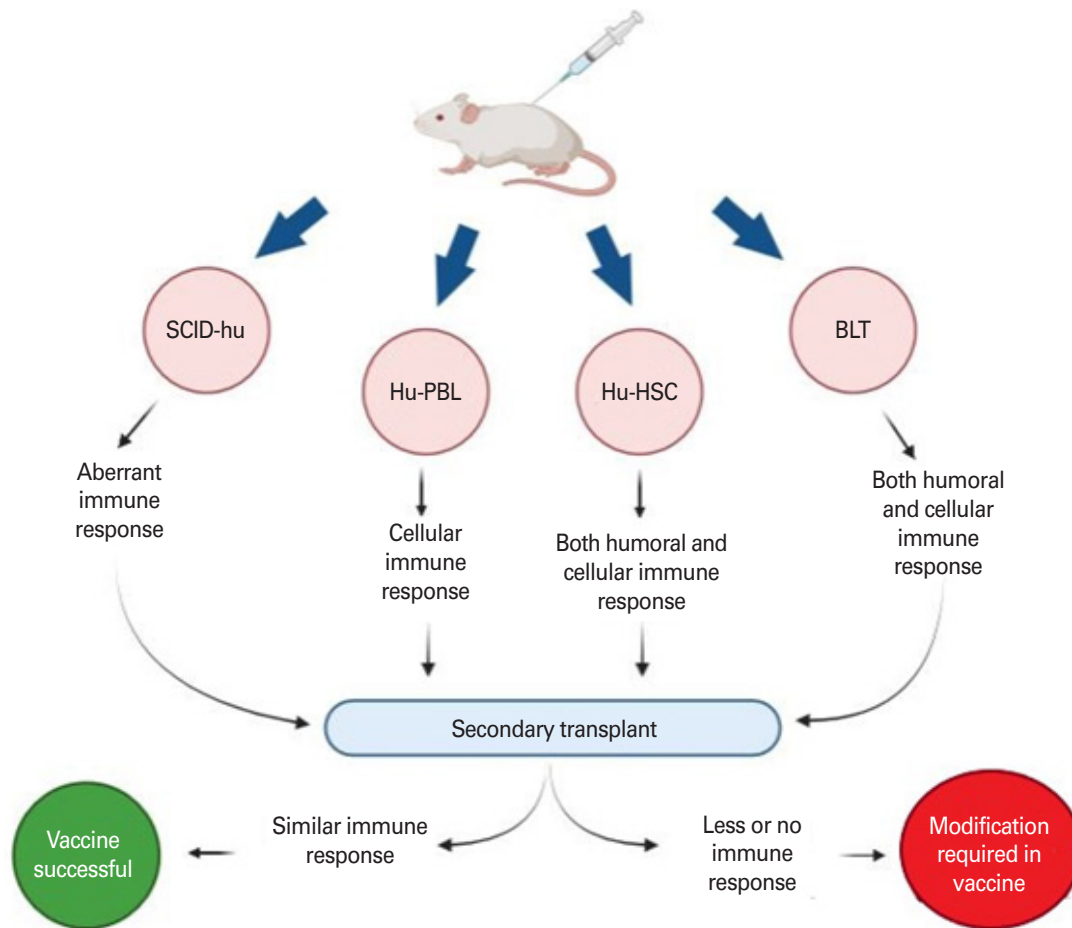


Fig. 4. Representation of humanized mice utilization in vaccine trial. SCID-hu, severe combined immunodeficiency-human; Hu-PBL, human peripheral blood leukocyte; Hu-HSC, human hematopoietic stem cell; BLT, bone marrow-liver-thymus.

specificity is dependent on it [21].

Humanized Mice and Immunity

The prediction of the development of protective immunity by vaccination is difficult as the immune condition is different for different patients (Fig. 4). A humanized mouse system reconstituted with the patient's immune cells may help determine the immune condition of each patient [29]. This system may not only show the effect of the specific vaccine, but it may also provide information regarding the patient's immune response against the pathogen/cancer.

Reconstitution of Human Immune System in Humanized Mice with Hematopoietic Cells

Mice with HIS mice provide valuable tools for *in-vivo* study of human immunity [30,31]. These mice are made by transplantation of human HSCs into non-obese diabetic (NOD) severe combined immunodeficient (SCID) mouse model that led to the development of human lymphocytes and myeloid cells [32,33]. Completely human-type antibody production in these mouse models has also been attempted with the transplantation of various types of HSCs [34].

Current HIS Mouse Models

Various humanized mice models with advantages and disadvantages are available for specific experimental needs. The mouse models are described below and summarized in Table 2.

SCID-Human Mouse

SCID mice are characterized by T and B lymphocyte impairment [35]. Therefore, they have severe combined immunodeficiency, unable to mount an effective humoral or cellular immune response to foreign antigens [36]. Usually, these mice show high susceptibility to infections from bacteria, fungi, and viruses that lead to death within the first 2 years of life if not treated by stem cell transplant [37]. C.B-17 strain with autosomal recessive mutation that causes impaired lymphopoiesis became the first mouse model of SCID [38].

SCID mouse is unable to reject HSCs and human thymus when introduced together, enabling T cell development and maturation that mimics human physiology [36]. Sub-lethally irradiated mice ensure complete reconstitution [39]. Co-implantation of the thymus and liver in SCID mice shows prolonged reconstitution of human immune cells with minimum graft-versus-host disease [40].

Table 2. Humanized mouse models and immune responses

Model	Mouse strain	Transplanted tissue	Immune response	Isotypes	Advantages	Disadvantages	Reference
SCID-hu	SCID	Co-implantation of human fetal liver and thymic fragments under kidney capsule	No primary immune response	IgG	Abundant T cell lymphopoiesis	Surgical implantation needed; requires human fetal tissue; no multilineage hematopoiesis; poor peripheral T cell engraftment	[36,40]
Hu-PBL	SCID; NOD-SCID; NOG; NSG; BRG; NCG	Intraperitoneal injection of human PBMCs	No primary immune response	IgM; IgG	Easy preparation; immediate use; good T cell engraftment	No multilineage hematopoiesis; graft versus host disease	[41,48-50]
Hu-HSC	SCID; NOD-SCID; NOG; NSG; BRG; NRG; DRAG	Injection of HSC and CD34+ cells from cord blood/fetal liver/bone marrow/mobilized peripheral blood	Antigen specific humoral and cellular responses	IgM; IgG	Easy to prepare; multilineage hematopoiesis; mucosal human cell engraftment	No human HLA restriction with the exception of HLA class I transgenic mice	[56,66,68]
BLT	SCID; NOD-SCID; NSG; NRG	Human fetal liver and thymic fragments co-implantation with injection of CD34+ cells from fetal liver	Antigen specific humoral and cellular responses	IgM; IgG	Multilineage hematopoiesis; T cell maturation in autologous thymus; HLA restriction; mucosal human cell engraftment	Surgical implantation needed; human fetal tissue required	[69-71,78]

SCID, severe combined immunodeficient; SCID-hu, SCID-human; IgG, immunoglobulin G; Hu-PBL, human peripheral blood leukocyte; NOD, non-obese diabetic; NOG, NOD/Shi-scid/IL2R γ null; NSG, NOD/SCID/IL2R γ -null; BRG, BALB/c-Rag2null IL-2R γ null; NCG, NOD CRISPR Prkdc Il2r gamma; PBMCs, human peripheral blood mononuclear cells; IgM, immunoglobulin M; HSC, hematopoietic stem cell; HLA, human leukocyte antigen.

SCID-human mice can provide great stride for immune response research but have certain limitations. Mature T cells are primarily restricted to implanted thymus/liver organoids. Also, functional immune responses that recapitulate the human immune response are not produced in these mice [35].

Human Peripheral Blood Leukocyte Mouse

First, human peripheral blood mononuclear cells (PBMCs) were transferred into SCID-human in 1988 [41]. To a certain extent, effector functions are displayed as human immune cells persist for many weeks [2]. Human peripheral blood leukocyte-SCID mice infected with hematopoietic cell tropic viruses such as human immunodeficiency virus (HIV)-1 have led to productive infection and a significant decrease in CD4+ cell numbers [42,43].

Further, NOD mice showed better engraftment when crossed with SCID mice due to defects in the innate immune system [44,45]. NOD/SCID mice with interleukin-2 receptor gamma (IL-2R γ)-chain null mutation completely abrogate murine natural killer cell development and function [46], negatively affecting human lymphoid cell engraftment in mice [47]. NOD/SCID/IL2r γ -null (NSG) or BALB/c-Rag2null IL-2R γ null (BRG) backgrounds provide better reconstitution [48-50]. These mice lack a primary immune response as de novo-multilineage hematopoiesis is absent [41,51-53]. Graft versus host disease is a significant limitation in this model [50,54,55].

Hematopoietic Stem Cell Mouse

SCID and bg/nu/xid mice were intravenously infused with hematopoietic cells from human bone marrow, and it was found that bg/nu/xid mice showed higher levels of human progenitors. T cells, B cells, and macrophage progenitors can be isolated and cultured *in vitro* [56]. HSC mice, when provided with human IL-3, erythropoietin, and human mast cell growth factor, showed increased differentiation of immature human bone marrow cells into erythroid, myeloid, and lymphoid lineages [57].

Human HSCs showed both short-term [58] and long-term [59] colony-forming potential *in vitro* and differentiation into lymphoid and myeloid cell lineages in NOD/SCID mice [60]. CD34+ cells (widely accepted human HSC marker) can be isolated from fetal liver, liver, and umbilical cord blood, but they show varying levels of erythroid, lymphoid, and myeloid progeny [61].

Various backgrounds have been used for reconstitution with HSCs, including NOD/SCID [62], NOD/Shi-scid mice [63], NOD/Shi-scid/IL2R γ null (NOG) [64], and NSG [65,66], and comparative analysis has been performed [67]. NSG and NOG mice had more significant engraftment than NOD/Lt-scid and NOD/Shi-scid in the thymus and spleen [67]. When limiting doses of HSCs were given, female NSGs showed superior engraftment than males [67]. Lack of antibody class switching due to lack of donor-matched HLA molecules in the mouse thymus is a significant drawback in HSC humanized mice [35].

DRAG Mouse

For the development of CD4+ T cells and B cells with antibody class switching, NOD.Rag1KO.IL2RccKO mice expressing HLA-DR4 (DRAG) were intravenously injected with CD34+ HSCs isolated from HLA-DR*0401 positive umbilical cord blood [9]. DRAG showed higher levels of reconstitution of CD4+ T cells when compared against HLA mismatched recipients. However, there was no drastic increase in CD8+ cells [9].

On stimulation with either CD3/28 or phorbol myristate acetate/ionomycin, vigorous responses were shown by T cells isolated from DRAG mice similar to PBMCs from healthy volunteers [9]. Immunoglobulin M levels were significantly higher, but B cell reconstitution was similar to control mice. The immunoglobulin class switching was confirmed as substantial levels of IgG reconstitution were seen in DRAG mice. Moreover, all human IgG subclasses were seen in DRAG mice plasma, with IgG2 being the most prevalent [9]. This makes DRAG mouse a suitable model system for long-term vaccination studies.

Bone Marrow-Liver-Thymus Mouse

Co-implantation of fetal thymus/liver with simultaneously transplanting CD34+ fetal liver cells in NOD/SCID mice was first performed in 2006 [68], later termed BLT for bone marrow-liver-thymus humanized mice [10]. Due to an autologous human thymic environment, BLT allows the development of MHC-restricted T cells. Potent *in vivo* immune responses were seen in this mouse model and repopulate multiple organs, like spleen, lymph nodes, bone marrow, thymus, liver, lung, reproductive and digestive tracts with multilineages of immune cells, including neutrophils, monocytes, T cells, B cells, NK cells, and dendritic cells [10,68-74].

NSG background BLT mice showed superior reconstitution than NOD/SCID BLT mice [75]. However, another study

showed NOD/SCID BLTs having higher levels of intraepithelial lymphocytes in the large and small intestines [70].

In BLT mice, B cell reconstitution is considered primarily immature, and antibody class switching is defective. BLT mouse is proposed as a model for hypogammaglobulinemia because of inadequate antibody response [75].

Application of Vaccines in Humanized Mice

Mouse models have been of great advantage to understanding human immunology and drug testing for years. Even so, these models have several limitations. Humanized mouse models have the potential to overcome the current limitations of conventional mouse and animal models. New generation hu-mice have been used to investigate several vaccines against human pathogens, including dengue, malaria, HIV-1, hepatitis C virus (HCV) and Epstein-Barr virus. A study showed that the Hu-SPL-NSG model, when immunized with LSA3-FL or LSA3-729 (montanide ISA720 adjuvant), triggered T-helper-type 1 cellular immune response and production of human antibodies for LSA3-729 only. This result was similar to the clinical findings obtained [1]. In another study, hu-HSC mice showed a neutralizing antibody response against dengue viral infection, while BLT and class-II transgenic mice showed HLA-restricted cellular responses [2]. For HIV-1 infections, BLT mice showed weak antibody production and HLA-restricted cellular responses, while hu-HSC mice demonstrated varied antibody responses [2]. HIS-DRA-GA mice have also evaluated specific T and B cell responses after immunization with other pathogens like Zika, influenza, scrub typhus, malaria protozoans, and HIV [13]. PBMC-transplanted NOG-hIL-4Tg mice also showed IgG production when vaccinated with keyhole limpet hemocyanin or HER2 multiple antigen peptide (CH401MAP) [76]. Hu-HSC-liver mice demonstrated immune responses against HCV and hepatitis B virus infection [2].

Conclusion

In conclusion, the findings suggests that humanized models could be cost-saving and relevant alternative for early evaluation of vaccine candidates before clinical trials and thus, require further studies.

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