Review article

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Humanized mouse model for vaccine evaluation: an overview

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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.

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	pRORγt-γc	2018
	IL2Rγnull	1995	NOD-SCID IL2Rγnull	2002	SCF	2012	BLT-lung	2019
					Ossicles	2012		
					IL-3/GM-SCF	2012		
RAG1/2 -/- derived	RAG1/2-/-	1992	(BALB-c)-RAG2-/-IL2Rγnull	1998–2005	CSF-1	2011	BRGWv	2014
	ILRγnull	1995	NOD-RAG2-/-IL2Rγnull	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-2Rγ, interleukin-2 receptor gamma; IL, interleukin; GM, granulocyte-macrophage; SCF, stem cell factor; CSF, colony-stimulating factor.

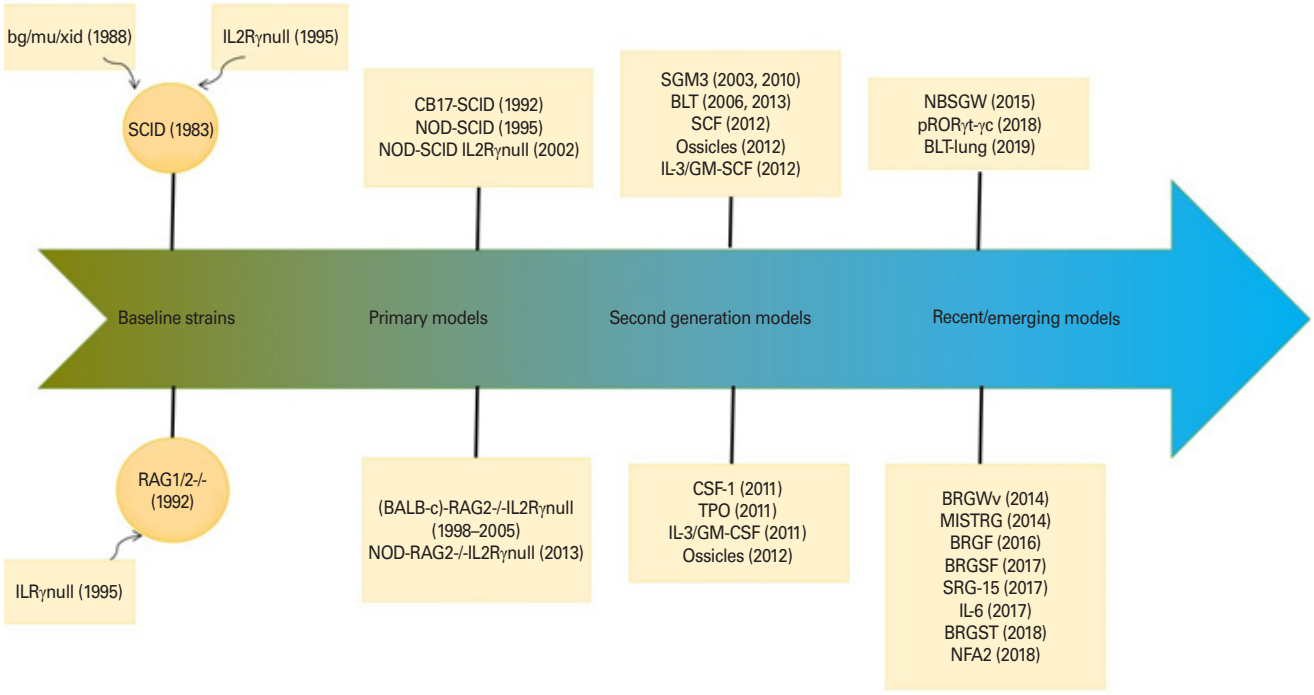

Fig. 1. Timeline showing the evaluation of humanized mice models. SCID, severe combined immunodeficient; NOD, non-obese diabetic; IL-2Rγ, interleukin-2 receptor gamma; BLT, bone marrow-liver-thymus; SCF, stem cell factor; GM, granulocyte-macrophage; CSF, colony-stimulating factor; IL-6, interleukin-6.

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) haplotype 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

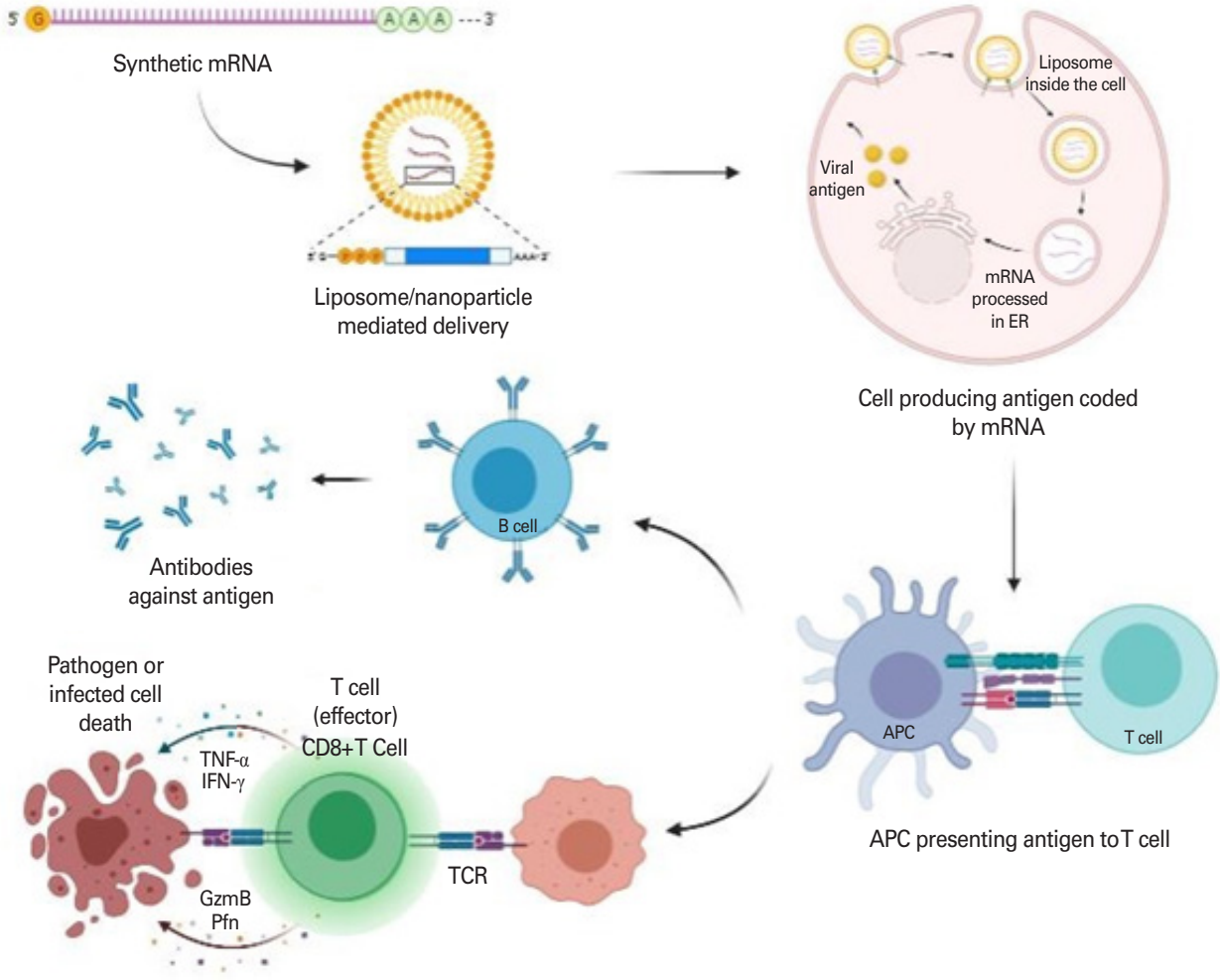

Fig. 2. Liposome/nanoparticle mediated delivery of messenger RNA (mRNA) vaccine and its working. Increased antibody production, and more effective T-cells killing infected cells after vaccination. ER, endoplasmic reticulum; TNF-α, tumor necrosis factor-alpha; IFN-γ, interferon-gamma; Pfn, perforin; TCR,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

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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

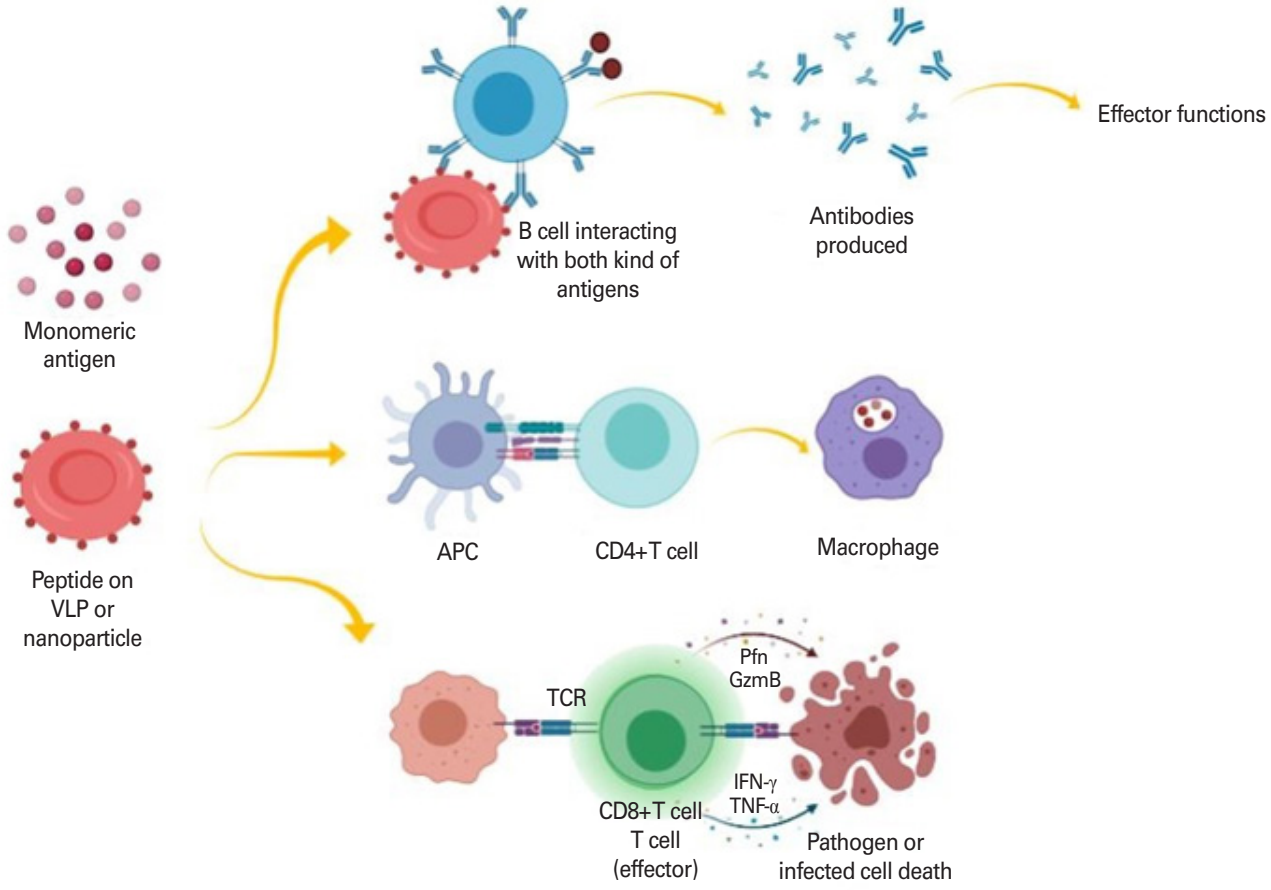

Fig. 3. Working of peptide vaccine. Increased antibody production, activation of macrophages, and more effective T-cells killing infected cells after vaccination. APC, antigen-presenting cells; VLP, virus-like particle; TCR, T-cell receptor; Pfn, perforin; IFN-γ, interferon-gamma; TNF-α, tumor necrosis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

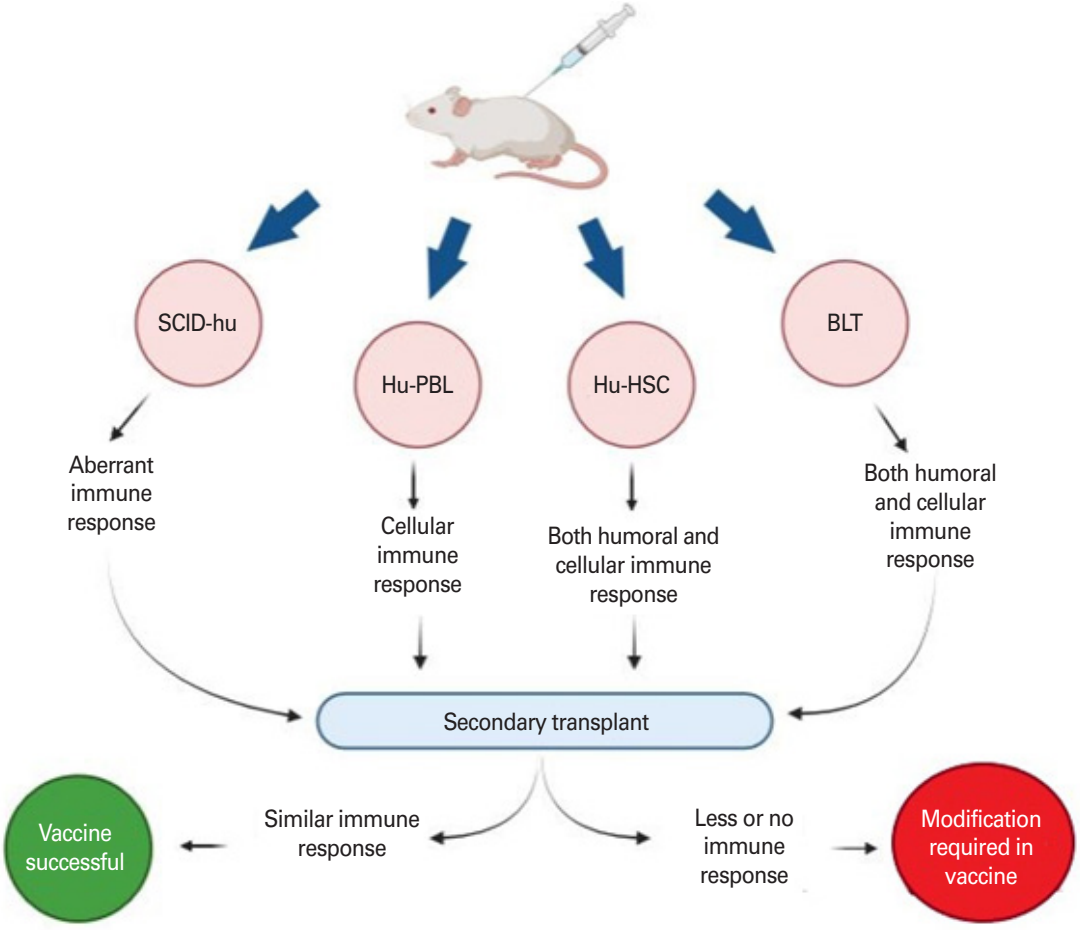

Fig. 4. Representation of humanized mice utilization in vaccine trial. SCID-hu, severe combined immunodeficiency-human; Hu-PBL, human peripheral blood leukocyte; Hu-HSC, human hematopoietic stem cell; BLT, bone marrow-liver-thymus.

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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/IL2Rγnull; NSG, NOD/SCID/IL2rγ-null; BRG, BALB/c-Rag2null IL-2Rγnull; 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-2Rγ)-chain null mutation completely abrogate murine natural killer cell development and function [46], negatively affecting human lymphoid cell engraftment in mice [47]. NOD/SCID/IL2rγ-null (NSG) or BALB/c-Rag2null IL-2Rγnull (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/IL2Rγnull (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

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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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References

1. Ghosn S, Chamat S, Prieur E, Stephan A, Druilhe P, Bouharoun-Tayoun H. Evaluating human immune responses for vaccine development in a novel human spleen cell-engrafted NOD-SCID-IL2rγNull mouse model. Front Immunol 2018;9:601.
2. Akkina R. Human immune responses and potential for vaccine assessment in humanized mice. Curr Opin Immunol 2013;25:403-9.
3. Schlake T, Thess A, Fotin-Mleczek M, Kallen KJ. Developing mRNA-vaccine technologies. RNA Biol 2012;9:1319-30.
4. Pardi N, Hogan MJ, Porter FW, Weissman D. mRNA vaccines: a new era in vaccinology. Nat Rev Drug Discov 2018;17:261-79.
5. Petsch B, Schnee M, Vogel AB, et al. Protective efficacy of in vitro synthesized, specific mRNA vaccines against influenza A virus infection. Nat Biotechnol 2012;30:1210-6.
6. Pardi N, Hogan MJ, Pelc RS, et al. Zika virus protection by a single low-dose nucleoside-modified mRNA vaccination. Nature 2017;543:248-51.
7. Benteyn D, Heirman C, Bonehill A, Thielemans K, Breckpot K. mRNA-based dendritic cell vaccines. Expert Rev Vaccines 2015;14:161-76.
8. Leibman RS, Richardson MW, Ellebrecht CT, et al. Supraphysiologic control over HIV-1 replication mediated by CD8 T cells expressing a re-engineered CD4-based chimeric antigen receptor. PLoS Pathog 2017;13:e1006613.
9. Danner R, Chaudhari SN, Rosenberger J, et al. Expression of HLA class II molecules in humanized NOD.Rag1KO.IL-2RgcKO mice is critical for development and function of human T and B cells. PLoS One 2011;6:e19826.
10. Melkus MW, Estes JD, Padgett-Thomas A, et al. Humanized mice mount specific adaptive and innate immune responses to EBV and TSST-1. Nat Med 2006;12:1316-22.
11. Bahl K, Senn JJ, Yuzhakov O, et al. Preclinical and clinical demonstration of immunogenicity by mRNA vaccines against H10N8 and H7N9 influenza viruses. Mol Ther

2017;25:1316-27.
12. Alberer M, Gnad-Vogt U, Hong HS, et al. Safety and immunogenicity of a mRNA rabies vaccine in healthy adults: an open-label, non-randomised, prospective, first-in-human phase 1 clinical trial. Lancet 2017;390:1511-20.
13. Brumeanu TD, Vir P, Karim AF, et al. A Human-Immune-System (HIS) humanized mouse model (DRAGA: HLA-A2. HLA-DR4. Rag1 KO.IL-2Rγc KO. NOD) for COVID-19. bioRxiv [Preprint] 2021 Jan 29. https://doi.org/10.1101/2020.08.19.251249
14. Li W, Joshi MD, Singhania S, Ramsey KH, Murthy AK. Peptide vaccine: progress and challenges. Vaccines (Basel) 2014;2:515-36.
15. Bijker MS, Melief CJ, Offringa R, van der Burg SH. Design and development of synthetic peptide vaccines: past, present and future. Expert Rev Vaccines 2007;6:591-603.
16. Purcell AW, McCluskey J, Rossjohn J. More than one reason to rethink the use of peptides in vaccine design. Nat Rev Drug Discov 2007;6:404-14.
17. Aguilar JC, Rodriguez EG. Vaccine adjuvants revisited. Vaccine 2007;25:3752-62.
18. Andreatta M, Karosiene E, Rasmussen M, Stryhn A, Buus S, Nielsen M. Accurate pan-specific prediction of peptide-MHC class II binding affinity with improved binding core identification. Immunogenetics 2015;67:641-50.
19. Andreatta M, Nielsen M. Gapped sequence alignment using artificial neural networks: application to the MHC class I system. Bioinformatics 2016;32:511-7.
20. Rasmussen M, Fenoy E, Harndahl M, et al. Pan-specific prediction of peptide-MHC class I complex stability, a correlate of T cell immunogenicity. J Immunol 2016;197:1517-24.
21. Malonis RJ, Lai JR, Vergnolle O. Peptide-based vaccines: current progress and future challenges. Chem Rev 2020;120:3210-29.
22. Verreault D, Ennis J, Whaley K, et al. Effective treatment of staphylococcal enterotoxin B aerosol intoxication in rhesus macaques by using two parenterally administered high-affinity monoclonal antibodies. Antimicrob Agents Chemother 2019;63:e02049-18.
23. Pohl MA, Rivera J, Nakouzi A, Chow SK, Casadevall A. Combinations of monoclonal antibodies to anthrax toxin manifest new properties in neutralization assays. Infect Immun 2013;81:1880-8.
24. Arunachalam B, Ghosh S, Talwar GP, Raghupathy R. A single human monoclonal antibody that confers total protection from tetanus. Hybridoma 1992;11:165-79.
25. Sharma P, Allison JP. Immune checkpoint targeting in cancer therapy: toward combination strategies with curative potential. Cell 2015;161:205-14.
26. Garboczi DN, Ghosh P, Utz U, Fan QR, Biddison WE, Wiley DC. Structure of the complex between human T-cell receptor, viral peptide and HLA-A2. Nature 1996;384:134-41.
27. Yang JC, Rosenberg SA. Adoptive T-cell therapy for cancer. Adv Immunol 2016;130:279-94.
28. Chianese-Bullock KA, Irvin WP Jr, Petroni GR, et al. A multipeptide vaccine is safe and elicits T-cell responses in participants with advanced stage ovarian cancer. J Immunother 2008;31:420-30.
29. Kametani Y, Miyamoto A, Seki T, Ito R, Habu S, Tokuda Y. Significance of humanized mouse models for evaluating humoral immune response against cancer vaccines. Pers Med Universe 2018;7:13-8.
30. Shultz LD, Brehm MA, Garcia-Martinez JV, Greiner DL. Humanized mice for immune system investigation: progress, promise and challenges. Nat Rev Immunol 2012;12:786-98.
31. Rongvaux A, Takizawa H, Strowig T, et al. Human hematolymphoid system mice: current use and future potential for medicine. Annu Rev Immunol 2013;31:635-74.
32. Traggiai E, Chicha L, Mazzucchelli L, et al. Development of a human adaptive immune system in cord blood cell-transplanted mice. Science 2004;304:104-7.
33. Saito Y, Ellegast JM, Manz MG. Generation of humanized mice for analysis of human dendritic cells. Methods Mol Biol 2016;1423:309-20.
34. Villaudy J, Schotte R, Legrand N, Spits H. Critical assessment of human antibody generation in humanized mouse models. J Immunol Methods 2014;410:18-27.
35. Abeynaike S, Paust S. Humanized mice for the evaluation of novel HIV-1 therapies. Front Immunol 2021;12:636775.
36. 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 1988;241:1632-9.
37. Gatti RA, Meuwissen HJ, Allen HD, Hong R, Good RA. Immunological reconstitution of sex-linked lymphopenic immunological deficiency. Lancet 1968;2:1366-9.
38. Bosma GC, Custer RP, Bosma MJ. A severe combined immunodeficiency mutation in the mouse. Nature 1983;301:527-30.
39. Fulop GM, Phillips RA. Full reconstitution of the immune deficiency in scid mice with normal stem cells requires

low-dose irradiation of the recipients. J Immunol 1986; 136:4438-43.
40. Namikawa R, Weilbaecher KN, Kaneshima H, Yee EJ, McCune JM. Long-term human hematopoiesis in the SCID-hu mouse. J Exp Med 1990;172:1055-63.
41. Mosier DE, Gulizia RJ, Baird SM, Wilson DB. Transfer of a functional human immune system to mice with severe combined immunodeficiency. Nature 1988;335:256-9.
42. Rizza P, Santini SM, Logozzi MA, et al. T-cell dysfunctions in hu-PBL-SCID mice infected with human immunodeficiency virus (HIV) shortly after reconstitution: in vivo effects of HIV on highly activated human immune cells. J Virol 1996;70:7958-64.
43. Mosier DE. Human xenograft models for virus infection. Virology 2000;271:215-9.
44. Makino S, Kunimoto K, Muraoka Y, Mizushima Y, Katagiri K, Tochino Y. Breeding of a non-obese, diabetic strain of mice. Jikken Dobutsu 1980;29:1-13.
45. Leiter EH, Serreze DV, Prochazka M. The genetics and epidemiology of diabetes in NOD mice. Immunol Today 1990; 11:147-9.
46. Cao X, Shores EW, Hu-Li J, et al. Defective lymphoid development in mice lacking expression of the common cytokine receptor gamma chain. Immunity 1995;2:223-38.
47. Christianson SW, Greiner DL, Schweitzer IB, et al. Role of natural killer cells on engraftment of human lymphoid cells and on metastasis of human T-lymphoblastoid leukemia cells in C57BL/6J-scid mice and in C57BL/6J-scid bg mice. Cell Immunol 1996;171:186-99.
48. 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. J Infect Dis 1995;172:974-82.
49. van Rijn RS, Simonetti ER, Hagenbeek A, et al. 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: 2522-31.
50. Ali N, Flutter B, Sanchez Rodriguez R, et al. Xenogeneic graft-versus-host-disease in NOD-scid IL-2Rγnull mice display a T-effector memory phenotype. PLoS One 2012; 7:e44219.
51. Torbett BE, Picchio G, Mosier DE. hu-PBL-SCID mice: a model for human immune function, AIDS, and lymphomagenesis. Immunol Rev 1991;124:139-64.
52. Ifversen P, Borrebaeck CA. SCID-hu-PBL: a model for making human antibodies? Semin Immunol 1996;8:243-8.
53. Murphy WJ, Taub DD, Longo DL. The huPBL-SCID mouse as a means to examine human immune function in vivo. Semin Immunol 1996;8:233-41.
54. Mutis T, van Rijn RS, Simonetti ER, et al. Human regulatory T cells control xenogeneic graft-versus-host disease induced by autologous T cells in RAG2-/-gammac-/- immunodeficient mice. Clin Cancer Res 2006;12:5520-5.
55. Gregoire-Gauthier J, Durrieu L, Duval A, et al. Use of immunoglobulins in the prevention of GvHD in a xenogeneic NOD/SCID/γc- mouse model. Bone Marrow Transplant 2012;47:439-50.
56. Kamel-Reid S, Dick JE. Engraftment of immune-deficient mice with human hematopoietic stem cells. Science 1988; 242:1706-9.
57. 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 1992;255:1137-41.
58. Civin CI, Strauss LC, Brovall C, Fackler MJ, Schwartz JF, Shaper JH. Antigenic analysis of hematopoiesis. III. A hematopoietic progenitor cell surface antigen defined by a monoclonal antibody raised against KG-1a cells. J Immunol 1984;133:157-65.
59. Sutherland HJ, Eaves CJ, Eaves AC, Dragowska W, Lansdorp PM. Characterization and partial purification of human marrow cells capable of initiating long-term hematopoiesis in vitro. Blood 1989;74:1563-70.
60. Bhatia M, Wang JC, Kapp U, Bonnet D, Dick JE. Purification of primitive human hematopoietic cells capable of repopulating immune-deficient mice. Proc Natl Acad Sci USA 1997;94:5320-5.
61. Holyoake TL, Nicolini FE, Eaves CJ. Functional differences between transplantable human hematopoietic stem cells from fetal liver, cord blood, and adult marrow. Exp Hematol 1999;27:1418-27.
62. Larochelle A, Vormoor J, Hanenberg H, et al. Identification of primitive human hematopoietic cells capable of repopulating NOD/SCID mouse bone marrow: implications for gene therapy. Nat Med 1996;2:1329-37.
63. Ueda T, Yoshino H, Kobayashi K, et al. Hematopoietic repopulating ability of cord blood CD34(+) cells in NOD/Shi-scid mice. Stem Cells 2000;18:204-13.
64. Yahata T, Ando K, Nakamura Y, et al. Functional human T lymphocyte development from cord blood CD34+ cells in

nonobese diabetic/Shi-scid, IL-2 receptor gamma null mice. J Immunol 2002;169:204-9.
65. Shultz LD, Lyons BL, Burzenski LM, et al. Human lymphoid and myeloid cell development in NOD/LtSz-scid IL2R gamma null mice engrafted with mobilized human hemopoietic stem cells. J Immunol 2005;174:6477-89.
66. Hiramatsu H, Nishikomori R, Heike T, et al. Complete reconstitution of human lymphocytes from cord blood CD34+ cells using the NOD/SCID/gammacnull mice model. Blood 2003;102:873-80.
67. McDermott SP, Eppert K, Lechman ER, Doedens M, Dick JE. Comparison of human cord blood engraftment between immunocompromised mouse strains. Blood 2010;116:193-200.
68. 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;108:487-92.
69. Brainard DM, Seung E, Frahm N, et al. Induction of robust cellular and humoral virus-specific adaptive immune responses in human immunodeficiency virus-infected humanized BLT mice. J Virol 2009;83:7305-21.
70. Denton PW, Nochi T, Lim A, et al. IL-2 receptor γ-chain molecule is critical for intestinal T-cell reconstitution in humanized mice. Mucosal Immunol 2012;5:555-66.
71. Smith DJ, Lin LJ, Moon H, et al. Propagating humanized BLT mice for the study of human immunology and immunotherapy. Stem Cells Dev 2016;25:1863-73.
72. Nikzad R, Angelo LS, Aviles-Padilla K, et al. Human natural killer cells mediate adaptive immunity to viral antigens. Sci Immunol 2019;4:eaat8116.
73. Denton PW, Estes JD, Sun Z, et al. Antiretroviral pre-exposure prophylaxis prevents vaginal transmission of HIV-1 in humanized BLT mice. PLoS Med 2008;5:e16.
74. Sun Z, Denton PW, Estes JD, et al. Intrarectal transmission, systemic infection, and CD4+ T cell depletion in humanized mice infected with HIV-1. J Exp Med 2007;204:705-14.
75. Stoddart CA, Maidji E, Galkina SA, et al. Superior human leukocyte reconstitution and susceptibility to vaginal HIV transmission in humanized NOD-scid IL-2Rγ(-/-) (NSG) BLT mice. Virology 2011;417:154-60.
76. Kametani Y, Katano I, Miyamoto A, et al. NOG-hIL-4-Tg, a new humanized mouse model for producing tumor antigen-specific IgG antibody by peptide vaccination. PLoS One 2017;12:e0179239.