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Human immune responses and potential for vaccine assessment in humanized mice

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Abstract

The new humanized mouse models with a transplanted human immune system have a capacity for de novo multilineage human hematopoiesis and generate T cells, B cells, macrophages, dendritic cells and NK cells. Of the two current leading humanized mouse models, the hu-HSC model is created by human hematopoietic stem cell (HSC) engraftment whereas the BLT mouse model is prepared by cotransplantation of human fetal liver, thymus and HSC. Humoral and cellular immune responses are seen in both models after immunization with antigens or infection with hematotropic pathogens such as EBV, HIV-1 and dengue viruses. While consistent antigen specific IgM production is seen, IgG responses were found to be generally feeble which is attributed to inefficient immunoglobulin class switching. BLT mice permit human HLA restricted T cell responses due to the autologous human thymus contributing to T cell maturation. Use of HLA Class I and II transgenic hu-HSC mice recently demonstrated that the HLA restriction deficiency could be overcome in this model. However, the overall vigor of the immune responses needs further improvement in both the models to approach that of the human. Towards this goal, supplementation with human cytokines and growth factors by transgenesis to improve human cell reconstitution and their homeostatic maintenance are beginning to yield improved mouse strains to create more robust human immune competent mice for immunoprophylaxis studies.

Introduction

While the conventional laboratory mice have long been instrumental in our understanding of the immune system and in developing immune prophylactic approaches, many knowledge gaps exist in translating the results from these to humans [1]. Many factors are responsible for this drawback. Among these are, mice are short lived mammals evolutionarily distant from humans and their immune system is designed to fit their survival needs in the eco niche they occupy. Use of highly inbred mice with multiple homozygous recessive defects also skews the orchestration of their immune responses, and therefore, the immunological epitopes preferentially recognized by these may be different than in the human. Moreover, many native human pathogens such as HIV do not infect mice and cause disease thus making it difficult to assess the immune responses to a replicating human pathogen in vivo in an experimental setting. In this regard humanized mice capable of human immune responses present an excellent opportunity to overcome the limitations posed by the use of ordinary mice [2*]. During the last two decades a number of immunodeficient mice have

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been developed which facilitated transplantation of human immune cells and tissues without rejection and the evolution of more complex humanized mouse models. This opened up new avenues of humanized mice research and their application in many areas of translational medicine/immunology.

Immunodeficient mouse strains for human cell engraftment and maintenance

The early severe combined immunodeficiency (SCID) mice lacking T and B cells permitted human cell engraftment leading to the generation of hu-PBL-SCID and SCID-hu mouse models [3, 4]. Later, NOD-SCID mice with lower levels of NK cells and innate immunity permitted higher levels of human cell engraftments [5]. However these were still far from being ideal for induction of primary immune responses. A subsequent advancement was the targeted disruption of the murine IL-2 receptor common gamma chain (IL2-R γ) gene leading to blocking of the actions of the native cytokines IL-2, IL-4, IL-7, IL-9, IL-15 and IL-21 [5, 6]. This mutation, when bred into mice harboring SCID, NOD, RAG1 or RAG2 gene mutations in various combinations yielded improved immunocompromized mice (Rag2^{-/-} c γ ^{-/-}, Rag1^{-/-} c γ ^{-/-} (RG), NOD/shi-scid/c γ ^{-/-} null (NOG) and NOD/SCID/c γ ^{-/-} (NSG) mice) with more severe restriction of mouse immune cell development [5]. These mice were found to be far superior for human cell engraftment resulting in the generation of all the necessary human immune cell subsets namely T, B, NK cells, macrophages and dendritic cells. With notable successes, ongoing improvements and refinements which focused on introducing human HLA Class I and II immune system and cytokine genes are yielding new transgenic mice to permit even more robust human cellular and humoral immune responses [7*].

Current human immune cell reconstituted mouse models

A number of hu-Mice are currently available for specific experimental needs each with its own advantages and disadvantages [2*]. A major advantage with the new hu-Mouse models over the earlier versions is their capacity to support primary human immune responses. Current models are summarized in Table 1 with a general description below.

Hu-PBL mice

These are created by engrafting with human mature PBMCs by i/p route into SCID, NSG or RG mice. The human immune cells persist for many weeks and display immune effector functions to a certain extent. Hematopoietic cell tropic viruses such as HIV-1 can productively infect these mice [8]. Antibodies from prior antigen exposure are continued to be produced by the human B cells. However, there are no notable primary immune responses seen due to the lack of de novo multilineage human hematopoiesis. Graft versus host disease (GVHD) is a significant drawback [5].

SCID-hu mice

Co-implantation of human fetal thymus and liver fragments under the SCID mouse renal capsule yields mice harboring a functional human thymus (thy/liv organoid) [3]. While there is de novo thymopoiesis with generation of T cells, these mice are deficient in human immune responses due to the lack of a full array of immune cells.

Hu-HSC mice

These are generated by reconstitution with hematopoietic stem cells (HSC) by various routes. New generation IL-2 c γ ^{-/-} mice such as RG, NOG or NSG mice are used [2*, 5]. While injection of HSC into adult irradiated mice gives rise to multiple hematopoietic

lineages, the yield of T cells is poor. However, injection of HSC into conditioned newborn RG, NSG or NOG mice via routes such as intrahepatic results in far superior human cell engraftment with generation of a full complement of T cells, B cells, macrophages, NK cells and dendritic cells. Infection with appropriate human pathogens or immunization with antigens results in human immune responses. Good mucosal human cell engraftment in neonatally transplanted mice permits challenges by mucosal routes with agents such as HIV-1 in protection studies [9–11].

BLT mice

A modification and improvement of the earlier SCID-hu mouse model by additional engraftment with autologous HSC created the BLT mouse model (bone marrow, liver and thymus) [12, 13]. Superior human cell engraftment with multilineage generation of T cells, B cells, macrophages, NK cells and dendritic cells is seen in this model. Presence of a functional autologous human thymus in this model permits appropriate T cell education and human HLA cell restriction. Good mucosal cell engraftment is also seen thus permitting mucosal pathogen challenges [14].

Hu-Liver-HSC mice

Since the above models are limited to human immune cells/tissue reconstitution, immune responses are restricted to infectious agents that only replicate in human hematopoietic cells. To overcome this limitation, hu-Mice incorporating other human tissues are created, such as harboring human liver, with uPA and Fah mice, further broadening their application [15, 16]. Novel transgenic mice with simultaneous human hepatocyte and HSC engraftment (AFC8-hu HSC/Hep) mice were recently developed permitting infection with hepatitis C virus and specific immune response [17**].

Immune responses in hu-Mice

A feature that distinguishes the newer generation hu-HSC and BLT humanized mouse models from those of early hu-PBL and SCID-hu mouse models is the de novo multilineage human hematopoiesis with cells that constitute the essential components of adaptive and innate immune responses. Human immune cell levels and their maturation characteristics are dependent on factors such as the age of the mouse at the time of HSC transplantation (neonatal versus adult), type of HSC used (cord blood versus fetal liver CD34 cells), and whether an autologous thymus is co-transplanted as done in BLT mice [2*]. Analysis of antibody repertoire in hu-HSC RG and NSG mice revealed that the human B cell repertoire was normal, thus indicating no obvious deficiency to generate human antibodies of varied specificities [18–20*]. Antigen-specific IgM and IgG human antibody responses in hu-HSC mice were documented in several reports that either used live pathogens or purified antigens [21–25]. However, IgG responses were found to be suboptimal in a number of these studies, which is attributed to inefficient class switch. With regard to BLT mice wherein human HLA class restriction is known to occur due to the T cells developing in the autologous thymus, early studies have shown both IgM and IgG antigen specific responses albeit with varied robustness [12, 13]. In a recent study even repeated booster immunizations did not result in secondary responses characterized by the production of IgG and an abundance of a “B-1 like” B cell population (CD19+CD5+) was noted in contrast to the human [26]. In general, it has become clear that a number of factors contribute to the inefficient IgG responses, such as a lack of or inadequate T cell help and suboptimal germinal center formation [27*, 28*]. Improved IgG responses could be obtained in MHC Class II transgenic mice transplanted with matching donor HSC [29**]. A more recent study in hu-HSC mice demonstrated efficient B cell maturation and generation of robust antibody

generation. Efficient IgG class switch was shown, coincident with accumulation of higher T cell numbers (as seen in older engrafted mice) prior to antigen challenge [30**].

Cell mediated immune responses

Antigen specific T cell responses against various human pathogens were demonstrated in hu-HSC mice in a number of reports [22, 31, 32] and there was an abrogation of immune control of HIV-1 and EBV infection following depletion of CD8 T cells [33, 34]. However, deficiencies in T cell responses were reported by others [35–37]. Even in reports showing responses, specific CD4 and CD8 responses as measured by cytokine secretion, cell proliferation or cytotoxic assays were found to be weak. Several factors might be responsible for less than optimal responses which include the overall low level of T cells due to a lack of HLA restriction, poor survival in the periphery and functional alterations due to potential lymphopenia induced T cell activation [27*]. Therefore methods that help improve T cell numbers are likely to rectify these limitations. Along these lines a drastic elevation in the numbers of thymic and peripheral T cells was seen in HLA-DR4 Class II transgenic RG mice reconstituted with matching HLA-DR4 CD34 cord blood cells [29**]. Similarly, Class I HLA-A2 transgenic mice reconstituted with matched HSC were shown to be capable of HLA restricted cellular immune responses to EBV and dengue virus [38**, 39]. T cell responses appear to be qualitatively normal in BLT mice due to the presence of an autologous human thymus for proper T cell education thus permitting HLA restriction [13, 40]. Epitope specific anti-viral responses in a class I restricted manner were seen in HIV-1 infected BLT mice [41*]. However, the responses are not robust indicating further improvements are necessary for any future vaccine studies. Moreover it also needs to be reconciled that the overall T cell responses seen in BLT mice are not entirely human HLA restricted due to some T cell progenitors maturing in the pre-existing native murine thymus and thus are H2 restricted.

Human pathogen, vaccine and immune enhancement studies

Both T cell dependent and independent antigens, human vaccine preparations and a number of human pathogens, viruses HIV-1, EBV, dengue and HCV in particular since no vaccines are currently available, have been investigated in new generation hu-Mice. Some examples are presented here. Neutralizing antibody responses were demonstrated in dengue viral infection of hu-HSC mice and HLA restricted cellular responses were documented in and Class II transgenic and BLT mice [25, 39, 42*, 43]. While antibody responses were varied in HIV-1 infections of hu-HSC mice [24, 37, 44], BLT mice showed more consistent, albeit weak antibody and HLA restricted cellular responses [40, 41*]. With human herpes viruses, adaptive and some protective T cell immune responses to EBV and HSV-2 and antibody responses to HSV-2 and KSHV were [13, 31, 32, 34, 45, 46]. Hu-HSC-Liver mice sustained HCV and HBV infection with immune responses being evident [17**, 47, 48]. Work with non-viral pathogens encompassed *Salmonella typhi* [49, 50], malaria parasite *P. falciparum* [51] and adaptive and innate immune responses demonstrated against *Leishmania* [52]. A variety of antigens and human vaccine preparations were also shown to induce cellular and/or human immune responses. These include, DNP(23)-KLH antigen, Toxic shock syndrome toxin 1, tetanus toxoid, DTaP vaccine and HBV vaccine among others [20*, 22, 30**, 53**]. Hu-Mice were also put to use to generate human monoclonal antibodies as recently demonstrated in hu-HSC RG mice following vaccination with TT and HBC vaccines [20*]. Based on their capacity for HIV-1 mucosal transmission, antibody mediated protection against vaginal viral challenge was shown in hu-HSC mice paving the way for future HIV-1 vaccine testing [10]. Other novel approaches tested vector-based antibody production in vivo [54]. In addition to potential immunoprophylaxis testing, hu-Mice can also be employed for immunopotential/enhancement studies. PD-1 receptor is upregulated in

chronic infections thus leading to immune exhaustion. Interfering with PD-1 pathway by administration of monoclonal antibodies against PD-L1, the ligand for PD-1, resulted in decreased HIV-1 viral loads and improved CD4 T cell counts in chronically infected hu-HSC mice demonstrating the merits of this promising approach [55**].

Limitations of the current models and future outlook

Despite many notable recent advances in the generation of humanized mice, several limitations still exist [2*, 56**]. They are residual innate immunity, less than ideal T cell numbers, sub-optimal maturation of B cells, lack of HLA class I and class II restriction in standard hu-HSC mouse models, inadequate levels of HLA APCs in the BLT mouse model, inefficient T and B cell cooperation resulting in inefficient immunoglobulin class switch, and poorly cross-reactive native murine growth factors and cytokines resulting in inefficient human cellular expansion and their homeostatic maintenance. Residual innate clearance in part is addressed by the development of human SIRP α transgenic mice or transduction of SIRP α ligand CD47 into transplanted HSC [57**, 58]. While HLA Class I or Class II expression resulted in improved immune responses in hu-HSC mice, their co-expression to derive doubly transgenic mice is required to realize the full potential [29**, 38**]. Knock-in of the respective human genes in mouse MHC loci will also preclude the unwanted mouse H-2 restricted responses. Supplying essential human cytokines and growth factors via exogenous administration or preferably by transgenesis will permit improved human cell engraftment as demonstrated with GM-CSF, IL-4, M-CSF for monocyte/macrophage lineages, IL-7 for T cells, IL-15 for NK cells, BAFF for B cells and EPO for erythrocytes [59**]. Convergence of the above strategies to derive an ideal composite recipient mouse strain will lead to the generation of newer humanized mouse models that satisfy the criteria for reliable human vaccine testing.

Conclusions

Hu-Mice with human immune systems have evolved considerably during the recent years with incremental advances yielding improved immune responses. Irrespective of the hu-Mouse model employed, robust generation of a full complement of immune cells in correct proportions and desired levels to give rise to protective immune responses comparable to the human and thus for reliable immunoprophylaxis testing remains a foreseeable goal. However, with the current rapid pace of ongoing work, it is not too far in the future to reach this goal.

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References

1. Davis MM. A prescription for human immunology. *Immunity*. 2008; 29(6):835–8. [PubMed: 19100694]
2. Akkina R. New generation humanized mice for virus research: comparative aspects and future prospects. *Virology*. 2013; 435(1):14–28. [PubMed: 23217612] * A recent detailed review on human immune responses and comparison of different humanized mouse models.
3. McCune JM. Development and applications of the SCID-hu mouse model. *Seminars in immunology*. 1996; 8(4):187–96. [PubMed: 8883141]
4. Mosier DE. Evaluation of protective immunity to HIV-1 in human PBL-SCID mice. *Antibiotics and chemotherapy*. 1996; 48:125–30. [PubMed: 8726515]

5. Shultz LD, Ishikawa F, Greiner DL. Humanized mice in translational biomedical research. *Nature reviews. Immunology*. 2007; 7(2):118–30.
6. Ito R, et al. Current advances in humanized mouse models. *Cellular & molecular immunology*. 2012; 9(3):208–14. [PubMed: 22327211]
7. Rongvaux A, et al. Human Hemato-Lymphoid System Mice: Current Use and Future Potential for Medicine. *Annual review of immunology*. 2013* Recent review covering new developments in hu-Mice research.
8. Mosier DE. Human xenograft models for virus infection. *Virology*. 2000; 271(2):215–9. [PubMed: 10860873]
9. Berges BK, et al. Mucosal transmission of R5 and X4 tropic HIV-1 via vaginal and rectal routes in humanized Rag2^{-/-} gammac^{-/-} (RAG-hu) mice. *Virology*. 2008; 373(2):342–51. [PubMed: 18207484]
10. Veselinovic M, et al. Topical gel formulation of broadly neutralizing anti-HIV-1 monoclonal antibody VRC01 confers protection against HIV-1 vaginal challenge in a humanized mouse model. *Virology*. 2012; 432(2):505–10. [PubMed: 22832125]
11. Hur EM, et al. Inhibitory effect of HIV-specific neutralizing IgA on mucosal transmission of HIV in humanized mice. *Blood*. 2012; 120(23):4571–82. [PubMed: 23065154]
12. Lan P, et al. Reconstitution of a functional human immune system in immunodeficient mice through combined human fetal thymus/liver and CD34⁺ cell transplantation. *Blood*. 2006; 108(2):487–92. [PubMed: 16410443]
13. Melkus MW, et al. Humanized mice mount specific adaptive and innate immune responses to EBV and TSST-1. *Nature medicine*. 2006; 12(11):1316–22.
14. Denton PW, Garcia JV. Mucosal HIV-1 transmission and prevention strategies in BLT humanized mice. *Trends in microbiology*. 2012; 20(6):268–74. [PubMed: 22503637]
15. Meuleman P, Leroux-Roels G. The human liver-uPA-SCID mouse: a model for the evaluation of antiviral compounds against HBV and HCV. *Antiviral research*. 2008; 80(3):231–8. [PubMed: 18706933]
16. Zhou XL, et al. Humanized murine model for HBV and HCV using human induced pluripotent stem cells. *Archives of pharmacol research*. 2012; 35(2):261–9. [PubMed: 22370780]
17. Washburn ML, et al. A humanized mouse model to study hepatitis C virus infection, immune response, and liver disease. *Gastroenterology*. 2011; 140(4):1334–44. [PubMed: 21237170] ** Generation of hu-HSC-liver mice that permit HCV infection and human immune response.
18. Chang H, et al. Human B-cell ontogeny in humanized NOD/SCID gammac(null) mice generates a diverse yet auto/poly- and HIV-1-reactive antibody repertoire. *Genes and immunity*. 2012; 13(5):399–410. [PubMed: 22592523]
19. Ippolito GC, et al. Antibody repertoires in humanized NOD-scid-IL2Rgamma(null) mice and human B cells reveals human-like diversification and tolerance checkpoints in the mouse. *PLoS one*. 2012; 7(4):e35497. [PubMed: 22558161]
20. Becker PD, et al. Generation of human antigen-specific monoclonal IgM antibodies using vaccinated “human immune system” mice. *PLoS one*. 2010; 5(10)* Use of hu-Mice for human monoclonal antibody generation.
21. Matsumura T, et al. Functional CD5⁺ B cells develop predominantly in the spleen of NOD/SCID/gammac(null) (NOG) mice transplanted either with human umbilical cord blood, bone marrow, or mobilized peripheral blood CD34⁺ cells. *Experimental hematology*. 2003; 31(9):789–97. [PubMed: 12962725]
22. Traggiai E, et al. Development of a human adaptive immune system in cord blood cell-transplanted mice. *Science*. 2004; 304(5667):104–7. [PubMed: 15064419]
23. Ishikawa F, et al. Development of functional human blood and immune systems in NOD/SCID/IL2 receptor {gamma} chain(null) mice. *Blood*. 2005; 106(5):1565–73. [PubMed: 15920010]
24. Gorantla S, et al. Human immunodeficiency virus type 1 pathobiology studied in humanized BALB/c-Rag2^{-/-}-gammac^{-/-} mice. *Journal of virology*. 2007; 81(6):2700–12. [PubMed: 17182671]
25. Kuruvilla JG, et al. Dengue virus infection and immune response in humanized RAG2^{-/-}-gamma(c)^{-/-} (RAG-hu) mice. *Virology*. 2007; 369(1):143–52. [PubMed: 17707071]

26. Biswas S, et al. Humoral immune responses in humanized BLT mice immunized with West Nile virus and HIV-1 envelope proteins are largely mediated via human CD5+ B cells. *Immunology*. 2011; 134(4):419–33. [PubMed: 22044090]
27. Garcia S, Freitas AA. Humanized mice: Current states and perspectives. *Immunology letters*. 2012; 146(1–2):1–7. [PubMed: 22507217] * Recent review on immune responses in hu-HSC mice and discussion of various deficiencies.
28. Vuyyuru R, Patton J, Manser T. Human immune system mice: current potential and limitations for translational research on human antibody responses. *Immunologic research*. 2011; 51(2–3):257–66. [PubMed: 22038527] * A concise review detailing factors involved in antibody generation in hu-Mice.
29. Danner R, et al. Expression of HLA class II molecules in humanized NOD.Rag1KO.IL2RgcKO mice is critical for development and function of human T and B cells. *PloS one*. 2011; 6(5):e19826. [PubMed: 21611197] ** Evidence that Class II expression results in improved T cell maturation and immunoglobulin class switch.
30. Lang J, et al. Studies of Lymphocyte Reconstitution in a Humanized Mouse Model Reveal a Requirement of T Cells for Human B Cell Maturation. *Journal of immunology*. 2013** Detailed study showing efficient immunoglobulin class switch in hu-HSC mice and factors affecting normal B cell development.
31. Strowig T, et al. Priming of protective T cell responses against virus-induced tumors in mice with human immune system components. *The Journal of experimental medicine*. 2009; 206(6):1423–34. [PubMed: 19487422]
32. Kwant-Mitchell A, Ashkar AA, Rosenthal KL. Mucosal innate and adaptive immune responses against herpes simplex virus type 2 in a humanized mouse model. *Journal of virology*. 2009; 83(20):10664–76. [PubMed: 19656896]
33. Gorantla S, et al. CD8+ cell depletion accelerates HIV-1 immunopathology in humanized mice. *Journal of immunology*. 2010; 184(12):7082–91.
34. Yajima M, et al. T cell-mediated control of Epstein-Barr virus infection in humanized mice. *The Journal of infectious diseases*. 2009; 200(10):1611–5. [PubMed: 19832115]
35. Watanabe Y, et al. The analysis of the functions of human B and T cells in humanized NOD/shi-scid/gammac(null) (NOG) mice (hu-HSC NOG mice). *International immunology*. 2009; 21(7): 843–58. [PubMed: 19515798]
36. Baenziger S, et al. Disseminated and sustained HIV infection in CD34+ cord blood cell-transplanted Rag2–/–gamma c–/– mice. *Proceedings of the National Academy of Sciences of the United States of America*. 2006; 103(43):15951–6. [PubMed: 17038503]
37. An DS, et al. Use of a novel chimeric mouse model with a functionally active human immune system to study human immunodeficiency virus type 1 infection. *Clinical and vaccine immunology : CVI*. 2007; 14(4):391–6. [PubMed: 17314230]
38. Shultz LD, et al. Generation of functional human T-cell subsets with HLA-restricted immune responses in HLA class I expressing NOD/SCID/IL2r gamma(null) humanized mice. *Proceedings of the National Academy of Sciences of the United States of America*. 2010; 107(29):13022–7. [PubMed: 20615947] ** Generation of HLA restricted T cell responses in Class I transgenic hu-HSC mice.
39. Jaiswal S, et al. Dengue virus infection and virus-specific HLA-A2 restricted immune responses in humanized NOD-scid IL2rgammanull mice. *PloS one*. 2009; 4(10):e7251. [PubMed: 19802382]
40. Brainard DM, et al. Induction of robust cellular and humoral virus-specific adaptive immune responses in human immunodeficiency virus-infected humanized BLT mice. *Journal of virology*. 2009; 83(14):7305–21. [PubMed: 19420076]
41. Dudek TE, et al. Rapid evolution of HIV-1 to functional CD8(+) T cell responses in humanized BLT mice. *Science translational medicine*. 2012; 4(143):143ra98.* HIV epitope specific T cell responses in BLT mice and immunological escape.
42. Jaiswal S, et al. Enhanced humoral and HLA-A2-restricted dengue virus-specific T-cell responses in humanized BLT NSG mice. *Immunology*. 2012; 136(3):334–43. [PubMed: 22384859] * Improved HLA restricted T cell responses to dengue virus in BLT mice.

43. Cox J, et al. Mosquito bite delivery of dengue virus enhances immunogenicity and pathogenesis in humanized mice. *Journal of virology*. 2012; 86(14):7637–49. [PubMed: 22573866]
44. Sato Y, Nagata S, Takiguchi M. Effective Elicitation of Human Effector CD8(+) T Cells in HLA-B*51:01 Transgenic Humanized Mice after Infection with HIV-1. *PloS one*. 2012; 7(8):e42776. [PubMed: 22880104]
45. Parsons CH, et al. KSHV targets multiple leukocyte lineages during long-term productive infection in NOD/SCID mice. *The Journal of clinical investigation*. 2006; 116(7):1963–73. [PubMed: 16794734]
46. Smith MS, et al. Granulocyte-colony stimulating factor reactivates human cytomegalovirus in a latently infected humanized mouse model. *Cell host & microbe*. 2010; 8(3):284–91. [PubMed: 20833379]
47. He Z, et al. Liver xeno-repopulation with human hepatocytes in Fah^{-/-}Rag2^{-/-} mice after pharmacological immunosuppression. *The American journal of pathology*. 2010; 177(3):1311–9. [PubMed: 20651238]
48. Robinet E, Baumert TF. A first step towards a mouse model for hepatitis C virus infection containing a human immune system. *Journal of hepatology*. 2011; 55(3):718–20. [PubMed: 21616105]
49. Firoz Mian M, et al. Humanized mice are susceptible to Salmonella typhi infection. *Cellular & molecular immunology*. 2011; 8(1):83–7. [PubMed: 21200387]
50. Libby SJ, et al. Humanized nonobese diabetic-scid IL2rgammanull mice are susceptible to lethal Salmonella Typhi infection. *Proceedings of the National Academy of Sciences of the United States of America*. 2010; 107(35):15589–94. [PubMed: 20713716]
51. Arnold L, et al. Further improvements of the P. falciparum humanized mouse model. *PloS one*. 2011; 6(3):e18045. [PubMed: 21483851]
52. Wege AK, et al. Leishmania major Infection in Humanized Mice Induces Systemic Infection and Provokes a Nonprotective Human Immune Response. *PLoS neglected tropical diseases*. 2012; 6(7):e1741. [PubMed: 22848771]
53. Tonomura N, et al. Antigen-specific human T-cell responses and T cell-dependent production of human antibodies in a humanized mouse model. *Blood*. 2008; 111(8):4293–6. [PubMed: 18270327] ** Overcoming PD-1 mediated T cell exhaustion to enhance immune response.
54. Balazs AB, et al. Antibody-based protection against HIV infection by vectored immunoprophylaxis. *Nature*. 2012; 481(7379):81–4. [PubMed: 22139420]
55. Palmer BE, et al. In vivo blockade of the PD-1 receptor suppresses HIV-1 viral loads and improves CD4⁺ T cell levels in humanized mice. *Journal of immunology*. 2013; 190(1):211–9. ** Transgenic expression of human SIRPα to improve human cell reconstitution in hu-HSC mice.
56. Shultz LD, et al. Humanized mice as a preclinical tool for infectious disease and biomedical research. *Annals of the New York Academy of Sciences*. 2011; 1245:50–4. [PubMed: 22211979] ** Innate factors governing human cell engraftment and facilitating improved human cell reconstitution.
57. Strowig T, et al. Transgenic expression of human signal regulatory protein alpha in Rag2^{-/-}–gamma(c)^{-/-} mice improves engraftment of human hematopoietic cells in humanized mice. *Proceedings of the National Academy of Sciences of the United States of America*. 2011; 108(32):13218–23. [PubMed: 21788509] ** A review detailing transgenic expression of various human cytokines and growth factors necessary for improved generation and maintenance of different human hematopoietic cell sub-sets.
58. Legrand N, et al. Functional CD47/signal regulatory protein alpha (SIRP(alpha)) interaction is required for optimal human T- and natural killer- (NK) cell homeostasis in vivo. *Proceedings of the National Academy of Sciences of the United States of America*. 2011; 108(32):13224–9. [PubMed: 21788504]
59. Willinger T, et al. Improving human hemato-lymphoid-system mice by cytokine knock-in gene replacement. *Trends in immunology*. 2011; 32(7):321–7. [PubMed: 21697012] ** A review detailing transgenic expression of various human cytokines and growth factors necessary for improved generation and maintenance of different human hematopoietic cell sub-sets.

Highlights

- Recent developments and comparative aspects of human immune system mice
- Improved human antibody and cellular immune responses in new generation humanized mice
- New transgenic immunodeficient mice expressing HLA class I and II, human cytokine and growth factors to generate improved humanized mice
- Vaccine testing and generation of human monoclonal antibodies in humanized mice
- Immuno-enhancement approaches based on interfering with PD-1 pathway

Figure 1.**Hu-Mice Generation and Immune Responses**

A schematic describing the generation of the two current leading models of hu-Mice and immune responses. Hu-HSC mice are prepared by transplantation of human CD34+ HSC, whereas the BLT mice are generated by co-transplantation of human fetal liver, thymus and autologous HSC.

Table 1

Current Humanized Mouse Models

Model	Method/Mouse Strains	Primary Immune Response/Comments	Viruses/Antigens Studied	Advantages	Disadvantages
Hu-PBL	Reconstitution with human PBMC. SCID, NOD-SCID, NSG, NOG.	No. Useful for GvHD studies.	HIV, EBV, Measles, HCV	Easy preparation. Immediate use. Good T cell engraftment.	No multilineage hematopoiesis. No primary immune response. Graft versus host disease.
Hu-HSC	CD34+ HSC engraftment into neonates or adults. Rag2-/- yc-/- (RG or DKO), NSG, NOG, (tg mice with HLA Class I, Class II, and various human cytokines also used)	Antigen specific humoral and cellular responses. Consistent IgM but weak IgG response. Improved IgG class switch in HLA Class II tg mice and coincident with higher T cell engraftment. HLA restriction and improved T/B cell cooperation in Class I and II transgenic mice. HSC reconstitution of neonates gives superior & lasting engraftment.	HIV-1, EBV, Dengue, HSV-2, HCV, HBV, Salmonella typhi, Tetanus toxoid, Haem. Influenza B conj. vaccine, Hep. B surface antigen vaccine	Easy to prepare. Multilineage hematopoiesis. Primary immune response. Mucosal human cell engraftment.	No human HLA restriction with the exception of HLA Class I transgenic mice
SCID-hu	Human fetal liver and thymic tissue co-implantation under kidney capsule. SCID or NOD-SCID.	No	HTLV, EBV, KSHV, HIV, CMV, HHV6, HSV-1, VZV	Abundant T cell lymphopoiesis.	Surgical implantation needed. Requires human fetal tissue. No multilineage hematopoiesis. No primary immune response. Poor peripheral T cell engraftment.
BLT	Co-implantation of human fetal liver and thymic fragments under kidney capsule together with injection of autologous CD34+ HSC. Rag2-/-yc-/-, NOD-SCID, NSG.	Antigen specific humoral and cellular responses. Consistent IgM but weak IgG response. Human HLA T cell restriction.	HIV-1, EBV, WNV envelope, Dengue, Toxic shock syndrome toxin 1, 2,4-dinitrophenyl hapten-keyhole limpet hemocyanin	Multilineage hematopoiesis. T cell maturation in autologous thymus. HLA restriction. Mucosal human cell engraftment	Surgical implantation needed. Human fetal tissue requirement.