HUMANIZED MOUSE MODELS AND HUMAN VIRUSES

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Abstract
Well-developed mouse models are important for understanding the pathogenesis and progression of immunological response to viral infections in humans. Moreover, to test vaccines, anti-viral drugs and therapeutic agents, mouse models are fundamental for preclinical investigations. Human viruses, however, seldom infect mice due to differences in the cellular receptors used by the viruses for entry, as well as in the innate immune responses in mice and humans. In other words, a species barrier exists when using mouse models for investigating human viral infections. Developing transgenic (Tg) mice models expressing the human genes coding for viral entry receptors and knock-out (KO) mice models devoid of components involved in the innate immune response have, to some extent, overcome this barrier. Humanized mouse models are a third approach, developed by engrafting functional human cells and tissues into immunodeficient mice. With an increase in the advancement of modern techniques used for genetic manipulation, humanized mice have become an important asset. They are becoming indispensable for analyzing human viral diseases since they nearly recapitulate the human disease. These mouse models also serve to test the efficacy of vaccines and antiviral agents. The development of humanized mouse models offers a preclinical in vivo platform for further characterization of human viral infections and human immune responses triggered by these virus particles. This review highlights recent progress in utilizing humanized mice to decipher human specific immune responses against viral tropism.
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1. Introduction

The use of small animal models such as mice and rats has contributed greatly to the understanding of disease pathogenesis and development of therapeutic approaches. Basically, these animals act as surrogates in representing human biology due to the limitations and ethical restrictions of obtaining tissue samples directly from human donors for research purpose. Moreover, these mammalian model systems are often easier to maintain and handle due to their nature of being small, have a high reproductive turnover, and share similar genomic and physiological characteristics with that of a human. Despite utilizing these amazing properties for basic biology, a fine line still separates mice studies from humans as they lack an integral component required in the human microenvironment herein, the immune system. For instance, it is known that the innate immune responses differ between man and mouse whereby mice lack a functional Toll-like-receptor 10 (TLR10) whereas TLR11, TLR12, and TLR13 which are expressed in mice are actually absent in the human genome (1, 2). Moreover, immune responses in wild-type mice infected with murine-adapted viruses are completely different to human immune responses triggered by human-specific pathogens due to inter-species diversity although they are used in studying the same virology. "Humanized" mice with functional human cell/tissue engraftment have garnered some interest lately and are gradually being recognized as an in vivo prerequisite in bridging the gap from bench-to-cage-to-bedside. However, it was not until the early 2000s when immunodeficient mice bearing mutations at the interleukin-2 receptor common gamma chain (*IL-2ry^{null}*) were used for efficient human cells and tissues engraftment (3,4). This proved to be a major breakthrough as the absence of *IL-2rγ* led to severe impairments in multiple cytokine complexes involving IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21 signalling and ultimately profound T cell defect. When these *IL-2ry^{null}* mice were backcrossed with either the protein kinase DNA activated catalytic polypeptide mutation (*Prkdc^{scid}/scid*) or with recombination activating gene

(*Rag*) 1 or 2 (*Rag1*^{null} or *Rag2*^{null}) mutations, murine adaptive (T and B cells) and innate natural killer (NK) cells immunity were completely compromised including defects in mouse macrophages and some dendritic cell subsets (5).

2. Models of Human Diseases Established on Humanized Mice

The introduction of humanized mice provides immeasurable opportunities to advance medical research. These increazingly important pre-clinical models are not only easy to handle due to their small sizes, but they also have short reproductive cycles, an exceptional ability to produce a large number of young and are relatively affordable to maintain in animal facilities as they do not require highly specialized infrastructures that are used by NHPs. In addition, humanized mice allow human-specific pathogens to infect and replicate within them and are able to develop functional human-specific immune responses to an array of diseases.

2.1. Flaviviruses

Members of the virus family Flaviviridae other then DENV include yellow fever virus (YFV), west Nile virus (WNV), Japanese encephalitis virus (JEV), tick-borne encephalitis virus (TBEV), and Zika virus. Although non-human primates (NHPs) like Rhesus and/or cynomolgus macaques remain the best model for hosting DENV, YFV and Zika viruses naturally for vaccine development, the lack of resources in assessing immune responses such as detection of antigen-specific T cell responses after vaccination proved to be a limitation in NHP models. Therefore, humanized mice were utilized not only for antibody production but also immunophenotyping of specific immune subsets. In fact, humanized monoclonal antibodies have been reported for viral neutralization but not clearance against YFV and JEV in vivo. On the other hand, both WNV and Zika virus tackled the advantages of the human immune system in humanized BLT mice for further viral characterization and antiviral therapeutics. Notably, these BLT mice displayed persistence Zika viremia of up to 7 months post-infection which was attenuated with neutralizing antibody.

2.2. Hantavirus

Hantavirus, a negative-sense RNA virus in the Hantaviridae family, is another type of infectious disease that can cause haemorrhagic fever with renal syndrome (HFRS) and hantavirus cardiopulmonary syndrome (HCPS) which give rise to increased vascular permeability and loss of platelets. Rodents generally serve as natural hosts for hantaviruses but it can be detrimental in humans. Hantavirus replication was observed in cell culture but does not have cytopathic consequences suggesting that the human immune system is required for induction of HFRS or HCPS (6, 7).

2.3. Influenza

The flu virus that makes up part genera of the family *Orthomyxoviridae* is one of the most common causes of human respiratory infections (8). Although vaccines and antiviral drugs are available to prevent or treat influenza respectively, there is no guarantee that one could escape infection entirely due to the constant evolution of different viral strains. Some of the mild symptoms include high fever, runny nose, coughing, sneezing, sore throats, etc. but complications may lead to more severe outcomes like gastroenteritis, pneumonia and even deaths. The use of small animal models like humanized mice would allow further understanding of influenza viral life cycle as well as viral replication which ultimately led to some of these symptoms. Indeed, humanized mice represent the best model for studying the flu virus but its poor development in the myeloid compartment remains a major drawback for triggering an immune response at mucosal surfaces such as the lungs. Many reports have demonstrated that human cytokines, interleukin-3 (IL-3) and granulocyte-macrophage colony-stimulating factor (GM-CSF) are essential for pulmonary homeostasis, myeloid cell development and host defense against pathogens. Thus,

immunodeficient mice transplanted with $CD34^+$HSCs were generated with human cytokines knock-in of IL-3 and GM-CSF to compete against mouse cytokines. Substantial improvements in the development of alveolar macrophages triggered effective innate responses when challenged with influenza virus. These humanized mice consistently express high amounts of GM-CSF, tumor necrosis factor alpha (TNF-α), and IL-6 mRNA in the lungs after infection. In addition, M-CSF treatment following influenza infection in NSG Hu-SRC-SCID mice similarly displayed decreased viral transcripts which was associated with overproduction of proinflammatory cytokines, TNF-α and IL-6.

3. Hepatotropic Pathogens in Human Liver Chimeric Mouse Models

The immune system is essential for human immune surveillance for viral replication and providing specific immune biomarkers for viral clearance of all human pathogens. Listed above are some of the human-associated viruses known to have been investigated in humanized mice. However, the study of hepatotropic pathogens remained elusive due to the lack of human hepatocytes in the mouse liver. As mentioned earlier in this review, the establishment of two commonly used human liver chimeric mouse models, Alb-uPA transgenic mice and *FRG* KO mice, fully support the viral life cycle which led to further characterization of infectious pathogens (9,10).

3.1. Hepatitis C Virus (HCV)

Hepatits C virus (HCV) is a single-stranded enveloped flavivirus that binds to cell surface in order to release virus particles into cells by receptor-mediated endocytosis. The restriction of HCV tropism to humans proved to be a major obstacle in understanding viral–host interactions, HCV-specific immune responses, disease progression, and identification of novel drug candidates. The Alb-uPA and *FRG* KO chimeric mice mentioned earlier were first models to exhibit localization of HCV viral proteins in human hepatocytes nodules. HCV from mouse serum was also serially passaged and infected into 3 generations of mice confirming both synthesis and release of infectious viral particles. Transgenic mice expressing these key human-specific factors facilitated viral uptake and replication which was suppressed by both innate and adaptive immunity in vivo. To better understand the role of the human immune system in targeting HCV, humanized mice generated by co-transplantation of $CD34^+$HSCs and hepatic progenitors into hAlb-FKBP-Caspase 8 (*AFC8*)$^+$ transgenic mice supported HCV-induced immune responses and liver diseases. HCV-infected mice displayed elevated levels of human $CD45^+$ leukocytes including $CD68^+$ macrophages and $CD3^+$ T cells infiltration in the liver. These mice also developed severe liver fibrosis but not in immunocompromised Alb-uPA and *FRG* KO chimeric mice after HCV infection indicating the importance of having a fully functional immune system to trigger liver damage.

3.2. Hepatitis B Virus (HBV)

Like HCV infection, chronic HBV (a member of the *Hepadnaviridae* family; genus *Orthohepadnavirus*) can also cause liver inflammation/fibrosis which gives rise to liver cirrhosis and/or ultimately HCC in patients. Although HBV vaccines are available, it is not a solution for established infections. The majority of antiviral therapies could suppress viral replication but not eradicate HBV entirely due to the stability of the covalently closed circular DNA (cccDNA). Indeed, human liver chimeric mice remain the gold standard for supporting hepatotropic infections; however, the highly immunocompromised status of these engrafted mice precludes liver pathogenesis mediated by human immune surveillance. Hence, dual humanization of liver and the immune system was established in immunodeficient mice to study immune responses and liver disease progression in the context of HBV infection. his dual humanized mouse model system allowed persistent HBV infection over several months followed by liver inflammation and fibrosis facilitated by M2-like macrophage infiltration suggesting a critical role for macrophage

polarization in HBV-induced impairment and liver pathology. Similarly, another group demonstrated dual humanization of liver and immune system by syngeneic engraftment of human hepatoblasts and HSCs in *Fah* KO NOD *Rag1*^{null}*/IL-2ry*^{null} (FNRG) mice which also portrayed rapid and sustained viremia upon HBV infection. Similarly, another group demonstrated dual humanization of liver and immune system by syngeneic engraftment of human hepatoblasts and HSCs in *Fah* KO NOD *Rag1*^{null}*/IL-2ry*^{null} (FNRG) mice which also portrayed rapid and sustained viremia upon HBV infection. Due to the limited access to fetal tissues, an alternative method of dual humanization of the liver as well as the immune system was established via transplantation of mature hepatocytes and HSCs from different donors. Here, HBV-infected humanized mice exhibited partial immune control over viral life cycle as evidenced by presence of antigen-specific IgGs and liver-infiltrating Kupffer cells, NK cells (CD69^{+}) and PD-1^{+} effector memory T cells which was in line with immunopathology observed in patients with chronic HBV. Plasma from these infected mice also displayed elevated levels of inflammatory and immune-suppressive cytokines, C-X-C motif chemokine ligand 10 (CXCL10) and IL-10 which correlated with the intrahepatic CD4^{+} T cells subset (11, 12, 13).

3.3. Hepatitis E Virus (HEV)

Hepatitis E virus (HEV) is a single-stranded, non-enveloped RNA icosahedral virus comprising a positive-sense, single stranded RNA genome which transmits viruses via the faecal–oral route. Although acute HEV infection is quite common, patients often undergo full recovery following antiviral treatments. Animal studies of HEV infection are limited but human liver chimeric mice remain the best model for further viral characterization. Since only the mouse liver is humanized, administration of HEV had to be performed intravenously or via the mouse spleen rather than orally due to species-specific intestinal host restrictions. A full viral life cycle was established in HEV-infected mice as evidenced by detection of viral RNA in faeces, bile and liver. Mice with humanized liver harbored the virus over several months without hepatotoxicity but displayed increased expression of innate genes like CXCL9, CXCL10, HLA class 1, and ISGs which may be mediated by the immune response. However, just like HDV research, human-specific immune responses targeting HEV infection are yet to be explored in vivo. Nevertheless, antiviral therapeutics using Ribavirin have shown efficient reduction of virus titer in both plasma and faeces of HEV-infected human liver chimeric mice (14).

4. Conclusions

The evolution of humanized mouse models has contributed to better characterization of human infectious diseases and immune responses. Only in recent times have humanized mice been utilized more frequently as a preclinical platform for in vivo validation, but key improvements in specific areas are required. Firstly, the study of murine-adapted viruses in wild-type mice may not necessarily recapitulate an infectious phenotype observed in clinical settings, suggesting that the source of species-specific virus inoculum is critical for fully understanding viral life cycles and replication/production capabilities in humanized mice. Therefore, the establishment of a fully functional human immune system and/or liver system in vivo is required to accommodate such human-specific infectious pathogens. Reconstitution of human immune cells permits the study of blood-borne viruses while repopulation of human hepatocytes in human liver chimeric mice is essential for the study of liver-associated infectious diseases like viral hepatitis. Most importantly, dual humanization of liver and immune system was able to recapitulate liver pathogenesis and trigger immune responses similarly observed in patients. Secondly, although human immune profiling can be investigated in humanized mice when challenged with infectious pathogens, the less optimal cell reconstitution and responses of the human innate immune components in

humanized mice remain a major limiting factor. New strains of immunocompromised mice have been generated by further replacement of murine immune counterpart components with human histocompatibility markers and other human specific molecules e.g. human cytokines via transgenic knock-in approaches to improve engraftment and maturation of human immune subsets. Other efforts to improve human immune reconstitution in mice have been enforced through various methods like expansion and differentiation of $CD34^+$ HSCs in vitro prior to mice injection, gene editing technology, packaging and delivery of human cytokines.

Hence, technological advancements in humanized mouse models have allowed more robust in vivo characterization to further elucidate viral–host interactions and identify novel immunotherapies and/or vaccine strategies.

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