Advanced Research in Gastroenterology & Hepatology
ISSN: 2472-6400

Juniper Publishers
key to the Researchers

Mini Review
Volume 9 Issue 1 – February 2018
DOI: 10.19080/ARGH.2018.09.555754

Adv Res Gastroentero Hepatol

Development of Humanized Mice for the Study of Liver-Associated Diseases

Fritz Shien Choong Lai[1] and Qingfeng Chen[1,2]

[1]*Institute of Molecular and Cell Biology, Agency for Science, Technology and Research (A*STAR), Singapore*

[2]*Department of Microbiology and Immunology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore*

Submission: February 16, 2018; **Published:** February 19, 2018

***Corresponding author:** Qingfeng Chen, Institute of Molecular and Cell Biology, Proteos, 61 Biopolis Drive, Singapore 138673; Fax: 65-67791117; E-mail: qchen@imcb.a-star.edu.sg

Mini Review

Viral hepatitis such as HBV and HCV remain the leading cause of chronic liver inflammation/fibrosis which may give rise to liver cirrhosis and/or ultimately hepatocellular carcinoma (HCC). A recent comprehensive study reported that the burden of deaths due to chronic viral infections have almost doubled between 1990 (~0.89 million) and 2013 (~1.45 million) [1,2]. In fact, viral hepatitis is currently ranked seventh highest in mortality worldwide. Although the introduction of prophylactic HBV vaccination in infants and anti-HBV treatments have reduced the prevalence of HBV dramatically, virus clearance and virologic control was not sustainable after therapy withdrawal [2]. However, there were encouraging data indicating that boosting the immune system has proven to be effective in the long-term control of HBV as shown in the majority of infected adults with resolved infection. Nevertheless, immune-based therapies have yielded poor outcomes which led to the importance of small animal models in understanding the complexity between virus and host interactions as well as development of antiviral immunity [3,4]. Indeed, considerable success was achieved in the generation of humanized mice by engrafting human hematopoietic stem cells (HSCs) [5,6]. In addition to depleting γ-chain of the interleukin 2 receptor, non-obese diabetic (NOD)-severe combined immunodeficiency (SCID) mice which lacked mouse T, B and Natural Killer (NK) cells reduced graft rejection considerably.

We have previously demonstrated that adoptive transfer of $CD34^+$ cells isolated from human fetal liver into sublethally irradiated NSG pups gave rise to not only human immune system reconstitution but also human hepatocyte-like cells in the liver of the recipient mice [7]. Importantly, we showed that $CD34^{hi}CD133^{hi}$ population in particular, contains majority of hematopoietic stem cells which would differentiate into mature human T cells, B cells, NK cells, Dendritic cells (DCs), and monocytes/macrophages. On the other hand, ~4% of human hepatocyte-like cells which expressed CD29 and albumin but not CD45 in mouse liver originated from the $CD34^{lo}CD133^{lo}$ population. Despite both HSCs and hepatic progenitors sharing a similar transcriptome, our findings suggested that these cell types were committed to separate lineages upon differentiation. In fact, we had shown that our humanized mouse model could support HCV infection, liver inflammation/fibrosis, HCV-specific human immune responses, as well as blockade of HCV-associated liver pathogenesis by interferon alpha-2a (IFNα-2a) [8]. We detected a significant increase in liver immune cell infiltration in HCV-infected humanized mice especially hepatic T cells and macrophages which was consistent with HCV patients [9]. Likewise, most of human leucocytes residing in spleens of HCV-infected mice were dominated by $CD3^+CD4^+$ and $CD3^+CD8^+$ T cells whereas majority of splenocytes in mock-infected mice were primarily $CD19^+$ B cells suggesting that HCV-specific human immune responses in humanized mice were similarly observed in patients. In addition, antibody-based depletion of human $CD4^+$ and $CD8^+$ T cells in humanized mice prior to HCV infection dramatically reduced leukocyte infiltration, human interferon-gamma (IFN-γ), human IL-6, and liver fibrosis [8]. In terms of therapeutic interventions, we were able to recapitulate antiviral activity in humanized mice with the clinically proven drug, IFNα-2a known to suppress HCV replication and disease progression in HCV-infected patients successfully [10]. Like the T cells depletion assays, significantly reduced leukocyte infiltration and liver fibrosis were detected in the IFNα-2a-treated mice cohorts [8]. The humanized mouse model not only provides a good platform for characterization of human liver pathogenesis but may also be useful in the discovery of novel therapeutics and/or improved modes of drug delivery. In collaboration with Motoichi Kurisawa's group, we have demonstrated that microstructured dextran hydrogels substantially prolong the circulation half-life

of PEG (polyethylene glycol)ylated-IFNα-2a (PEG-IFNα-2a) for the treatment of chronic HCV-infected humanized mice [10,11]. We reported that a one-time subcutaneous implantation of PEG-IFNα-2a-loaded Dex-Tyr/PEG hydrogels displayed comparable anti-HCV effects with 8 weekly injections of PEG-IFNα-2a formulation [11]. Although both delivery vehicles significantly reduced alanine aminotransferase (ALT) and IFN-γ *in vivo*, Dex-Tyr/PEG hydrogels enabled long-term sustained release of the incorporated PEG-IFNα-2a resulting in effective treatment of HCV with less frequency of drug administration. This approach paves ways for improved sustained protein delivery systems which will be advantageous in biomedical and pharmaceutical applications.

Chronic viral infection such as HBV is not considered cytopathic as activation of antigen-specific T cells are usually responsible in recognition and clearance of infected and/or dead hepatocytes [12]. Unlike in an acute infection scenario, virus-specific T cells are generally low in number and may not even be detectable at late-stage liver disease presumably due to majority of them being functionally exhausted [13]. Although the mechanism by which chronic liver inflammation is sustained remains to be fully understood, sub-population of myeloid cells particularly monocytes and macrophages have been gaining wide interest of late. In fact, elevation of both $CD14^+16^+$ monocytes and $CD68^+$ macrophages were reported in livers of patients with chronic liver diseases according to severity [14,15]. Multiple liver-injury animal models have portrayed the role of liver-infiltrating monocytes in angiogenesis, liver fibrosis and HCC development [12]. On the other hand, liver cirrhosis is caused by translocation of bacteria and bacterial products from the gut microbiota to the liver which ultimately led to liver inflammation and hepatocarcinogenesis [16,17]. Our HBV-infected humanized mice exhibited a large accumulation of pro-inflammatory $CD14^+HLA\text{-}DR^{hi}CD206^+$ cells which were highly liver-specific and that treatment with oral antibiotics abrogated these myeloid residents suggesting that both $CD14^+HLA\text{-}DR^{hi}CD206^+$ cells and intestine-derived bacterial products may contribute to the pathogenic sustainability of liver inflammation [12]. The increase of serum lipopolysaccharide (LPS) (measurement of serum sCD14) was a clear reflection of circulating bacterial products in HBV-infected humanized mice [18]. Antibiotic treatment not only abolished intestinal microbiota expectedly but more intriguingly, intrahepatic accumulation of pro-inflammatory $CD14^+CD206^+$ myeloid cells and subsequent liver inflammation were diminished almost completely [12].

Indeed, human chimeric mouse models offer a solid *in vivo* platform to study a wide range of human diseases. Although the current development of humanized mice may provide us a better understanding of human liver pathogenesis and human immune system responses to liver-associated injuries, the low reconstitution of human hepatocytes proved to be a limitation. In the last decade, two models have been established to overcome such drawback. The first model involved urokinase plasminogen activator (uPA) transgenic mouse whereby constitutive expression of uPA in liver causes hepatic injury to allow expansion of mouse/human hepatocytes upon transplantation [5,19]. The second mouse model was built on fumarylacetoacetate hydrolase (*Fah*) gene knockout background [20]. Since Fah is an enzyme required for normal liver metabolism, a defect in this pathway resulted in accumulation of toxic metabolites and ultimately hepatocellular injury to similarly permit human hepatocytes repopulation. However, Fah KO mice are advantageous as liver injury can be controlled by administration of 2-(2-nitro-4-trifluoro-methylbenzoyl)-1,3-cyclohexanedione (NTBC) which is a small molecule that blocks tyrosine catabolism [5,20]. In addition to depletion of *Fah*, combination of immunodeficient alleles such as *Rag2* and *Il2rγ* could lead to extensive liver humanization [20]. Although multiple research groups have already demonstrated the success of such mice models in supporting HBV and HCV, the lack of human immune responses could not mirror the microenvironment of patients infected with viral hepatitis [21-25]. Therefore, it is vital to address the importance of incorporating high levels of human liver chimeras and HLA-matched humanization of the immune system to further characterize immune cells responses to hepatotropic infections *in vivo*.

Conflict of Interest

The author declares no conflict of interest.

Acknowledgement

This work is supported by the Eradication of HBV TCR Program: NMRC/TCR/014-NUHS/2015, National Medical Research Council, Singapore. Q.C. is supported by Singapore National Research Foundation Fellowship NRF-NRFF2017-03.

References

1. Stanaway JD, Flaxman AD, Naghavi MC, Fitzmaurice T, Vos I, et al. (2016) The global burden of viral hepatitis from 1990 to 2013: findings from the Global Burden of Disease Study 2013. Lancet 388(10049): 1081-1088.
2. Maini MK, Bertoletti A (2017) HBV in 2016: Global and immunotherapeutic insights into hepatitis B. Nat Rev Gastroenterol Hepatol 14(2): 71-72.
3. Tian Y, Kuo CF, Akbari O, Ou JH (2016) Maternal-Derived Hepatitis B Virus e Antigen Alters Macrophage Function in Offspring to Drive Viral Persistence after Vertical Transmission. Immunity 44(5): 1204-1214.
4. Zeng Z, Li L, Chen Y, Wei H, Sun R, et al. (2016) Interferon-gamma facilitates hepatic antiviral T cell retention for the maintenance of liver-induced systemic tolerance. J Exp Med 213(6): 1079-1093.
5. Grompe M, Strom S (2013) Mice with human livers. Gastroenterology 145(6): 1209-1214.
6. Ramírez M, Rottman GA, Shultz LD, Civin CI (1998) Mature human hematopoietic cells in donor bone marrow complicate interpretation of stem/progenitor cell assays in xenogeneic hematopoietic chimeras. Exp Hematol 26(4): 332-344.
7. Chen Q, Khoury M, Limmon G, Choolani M, Chan JK, et al. (2013) Human fetal hepatic progenitor cells are distinct from, but closely related to, hematopoietic stem/progenitor cells. Stem Cells 31(6): 1160-1169.

How to cite this article: Fritz Shien C L, Qingfeng C. Development of Humanized Mice for the Study of Liver-Associated Diseases. Adv Res Gastroentero Hepatol 2018; 9(1): 555754. DOI: 10.19080/ARGH.2018.09.555754.

8. Keng CT, Sze CW, Zheng D, Zheng Z, Yong KS, et al. (2016) Characterisation of liver pathogenesis, human immune responses and drug testing in a humanised mouse model of HCV infection. Gut 65(10): 1744-1753.

9. Khakoo SI, Soni PN, Savage K, Brown D, Dhillon AP, et al. (1997) Lymphocyte and macrophage phenotypes in chronic hepatitis C infection. Correlation with disease activity. Am J Pathol 150(3): 963-970.

10. Heathcote EJ, Shiffman ML, Cooksley WG, Dusheiko GM, Lee SS, et al. Peginterferon alfa-2a in patients with chronic hepatitis C and cirrhosis. N Engl J Med 343(23): 1673-1680.

11. Bae KH, Lee F, Xu K, Keng CT, Tan SY, et al. (2015) Microstructured dextran hydrogels for burst-free sustained release of PEGylated protein drugs. Biomaterials 63: 146-157.

12. Tan-Garcia A, Wai LE, Zheng D, Ceccarello E, Banu N, et al. (2017) Intrahepatic CD206(+) macrophages contribute to inflammation in advanced viral-related liver disease. J Hepatol 67(3): 490-500.

13. Wedemeyer H, He XS, Nascimbeni M, Davis AR, Greenberg HB, et al. Impaired effector function of hepatitis C virus-specific CD8+ T cells in chronic hepatitis C virus infection. J Immunol 169(6): 3447-3458.

14. Liaskou E, Zimmermann HW, Li KK, Oo YH, Suresh S, et al. (2013) Monocyte subsets in human liver disease show distinct phenotypic and functional characteristics. Hepatology 57(1): 385-398.

15. McGuinness PH, Painter D, Davies S, McCaughan GW (2016) Increases in intrahepatic CD68 positive cells, MAC387 positive cells, and proinflammatory cytokines (particularly interleukin 18) in chronic hepatitis C infection. Gut 46(2): 260-269.

16. Dapito DH, Mencin A, Gwak GY, Pradere JP, Jang MK, et al. (2012) Promotion of hepatocellular carcinoma by the intestinal microbiota and TLR4. Cancer Cell 21(4): 504-516.

17. Seki E, De Minicis S, Osterreicher CH, Kluwe J, Osawa Y, et al. (2007) TLR4 enhances TGF-beta signaling and hepatic fibrosis. Nat Med 13(11): 1324-1332.

18. Sandler NG1, Koh C, Roque A, Eccleston JL, Siegel RB, et al. (2011) Host response to translocated microbial products predicts outcomes of patients with HBV or HCV infection. Gastroenterology 141(4): 1220-1230.

19. Rhim JA, Sandgren EP, Degen JL, Palmiter RD, Brinster RL (1994) Replacement of diseased mouse liver by hepatic cell transplantation. Science 263(5150): 1149-1152.

20. Azuma H, Paulk N, Ranade A, Dorrell C, Al-Dhalimy M, et al. (2007) Robust expansion of human hepatocytes in Fah-/-/Rag2-/-/Il2rg-/- mice. Nat Biotechnol 25(8): 903-910.

21. Bissig KD, Wieland SF, Tran P, Isogawa M, Le TT, et al. (2010) Human liver chimeric mice provide a model for hepatitis B and C virus infection and treatment. J Clin Invest 120(3): 924-930.

22. Strick-Marchand H, Dusséaux M, Darche S, Huntington ND1, Legrand N, et al. (2015) A novel mouse model for stable engraftment of a human immune system and human hepatocytes. PLoS One 10(3): e0119820.

23. Winer BY, Huang T, Low BE, Avery C, Pais MA, et al. (2017) Recapitulation of treatment response patterns in a novel humanized mouse model for chronic hepatitis B virus infection. Virology 502: 63-72.

24. Lütgehetmann M, Mancke LV, Volz T, Helbig M, Allweiss L, et al. (2012) Humanized chimeric uPA mouse model for the study of hepatitis B and D virus interactions and preclinical drug evaluation. Hepatology 55(3): 685-694.

25. Xia Y, Carpentier A, Cheng X, Block PD, Zhao Y, et al. (2017) Human stem cell-derived hepatocytes as a model for hepatitis B virus infection, spreading and virus-host interactions. J Hepatol 66(3): 494-503.

DOI: 10.19080/ARGH.2018.09.555754