Editorial

A mouse model for hepatitis C virus infection: are we there yet?

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Collectively, viral hepatitis remains a global epidemic causing an estimated 1.4 million deaths per year, of which hepatitis C virus (HCV) is the leading cause (1). Despite the availability of new antivirals that are capable of effective cure, the number of HCV infections and re-infections continue to rise worldwide. In addition to progressive liver disease and hepatocellular carcinoma (HCC), HCV is associated with metabolic disorders and co-morbidities including obesity, insulin resistance, type 2 diabetes mellitus, cardiovascular disease, mixed cryoglobulinemia among others (2-4)—all adding to the morbidity and mortality associated with this disease. HCV continues to be a challenge to control, due, at least in part, to the lack of a tangible animal model. Chimpanzees are susceptible to HCV and played a major role in understanding the natural history of the disease. But with limitations on the use of chimpanzees in biomedical research and an attenuated disease course, a tangible animal model could provide information on several gaps in HCV knowledge such as viral pathogenesis and persistence, immune correlates of protection, and importantly vaccine development and testing.

In the absence of a tractable HCV animal model, homolog hepacivirus infections in their natural hosts can be useful surrogates to study antiviral immunity and virus clearance. GB virus B (GBV-B) infection of new world primates is a well-established surrogate model, which has the advantage of being in a primate species. GBV-B typically only causes acute hepatitis, although some instances of chronicity have been reported (5,6). Notably, only a small number of other HCV-related hepaciviruses have been identified in dogs (canine hepacivirus, CHV), horses (equine hepacivirus, EqHV), bats (bat hepacivirus, BHV), rodents (rodent hepacivirus, RHV), and cattle (bovine hepacivirus, BovHepV) (7,8). However, most of these hepaciviruses lack pathogenicity or have altered *in vivo* characteristics (*Table 1*), such as CHV which primarily replicates in the lungs rather than in the liver. The rodent analog of HCV, RHV/NrHV (Norway rat hepacivirus) was first identified in Norway rats of New York in 2014 (9). While rats are the natural hosts for NrHV, a recent study by Billerbeck *et al.* (10) adapted the virus to murine models. With a total of only eight acquired mutations in the viral envelope proteins E1 and E2 of mouse adapted viral strains, NrHV established hepatotropic infection in immune-compromised and immune-competent mice strains. Thus, these investigators have developed a potential surrogate system that could be amenable to genetic and molecular manipulation for a better understanding of HCV immunology and pathogenesis.

Development of viral persistence has been a major deficit in previous surrogate models of HCV infection. Almost 80% of HCV-infected patients develop chronic infection and therefore an animal model that recapitulates chronicity is essential. Unfortunately, HCV infection of chimpanzees results in mixed pathologic chronicity (11), as does GBV-B infection of common marmosets (12,13). NrHV is also cleared acutely in immune-competent mouse strains (C57BL/6J and BALB/c) by 3 to 5 weeks post infection (p.i.), but chronic infection with minimal to mild inflammation was reported in immune-compromised mice lacking adaptive immunity (NRG, NOD-Rag1$^{-/-}$IL-2R$\gamma^{-/-}$), type I (A129, IFNR$\alpha\beta^{-/-}$) and type I/II (AG129, IFNR$\alpha\beta^{-/-}$IFNR$\gamma^{-/-}$) interferon signaling (10). Interestingly, chronic infection lasting 210 days p.i. was demonstrated in immune-competent mice that were CD4$^+$ T cell-depleted prior to infection. It is also important to point out that pre-depletion of NK cells and CD8$^+$ T cells did not lead to chronic infection in this

Table 1 HCV-related hepaciviruses

Virus	Host	Disease characteristics
GBV-B	Tamarins, marmosets and squirrel monkeys	Hepatotropic; mostly acute infection leading to liver inflammation and fibrosis
CHV	Dogs	Dual tropism to respiratory system and liver; however not associated with liver disease
EqHV	Horses	Hepatotropic; causes both acute and chronic infection with hepatic inflammation
BovHepV	Cattle	Hepatotropic; few cases of chronic infection reported
BHV	Bats	Apathogenic; potential natural reservoir for hepaciviruses
RHV	Rats; mice	Hepatotropic; acute infection and mild liver pathology in mice; persistence in rats

GBV-B, GB virus B; CHV, canine hepacivirus; EqHV, equine hepacivirus; BovHepV, bovine hepacivirus; BHV, bat hepacivirus; RHV, rodent hepacivirus.

study, indicating that $CD4^+$ T cells play primary roles in viral clearance. This is consistent with HCV and GBV-B studies that showed association of early and vigorous $CD4^+$ T cell responses with viral clearance (14-17). While NrHV is highly infectious and hepatotropic in mice, severe pathology and fibrosis were not obvious in the liver. Serum alanine aminotransferase (ALT) levels were elevated in $CD4^+$ T cell-depleted, chronically infected mice, however only mild liver inflammation was observed. Similarly, in acute infection minimal to mild hepatic inflammation was observed, and hepatic injury was associated with intrahepatic effector $CD44^+$ T cells expressing high T-bet and producing IFN-γ. In contrast, in GBV-B infection NK cells were identified as a major cause of liver pathology in infected marmosets (13), and NK cells correlated with liver inflammation in chronically infected HCV patients (18). The accumulation of intrahepatic NK cells in NrHV-infected mice was not found to be associated with liver pathology, thus suggesting immunopathogenesis may vary among hepacivirus infection models.

There are multiple gaps in our understanding of immunity to HCV, including, and most importantly, the antiviral immune responses associated with spontaneous clearance and pre-existing immunity involved in rapid clearance following HCV re-infections. Although NrHV infection of mice may not fully recapitulate HCV pathology, it could serve as an excellent model for mechanistic immunological studies. In acute NrHV infection, an early expansion of $Ly6c^+$ monocytes, $NKp46^+$ NK cells and proliferating $CD4^+$ and $CD8^+$ T cells was reported predominantly in the liver tissue of infected mice. Antiviral IFN-γ-secreting NK cells and $CD8^+$ T cells were involved in the early immune responses even though they were not essential for viral clearance. Similar to HCV and GBV-B antiviral immunity, the NS3 and NS4 peptides of NrHV induced dominant IFN-γ^+ T cell responses. In chronic NrHV infection, accumulation of intrahepatic Tregs and exhausted $CD8^+$ T cells characterized as PD-1^{high} $CD44^{low}$ $2B4^+$ Tim-3^+ Eomes^{high} T cells recapitulates T cell exhaustion reported in chronic HCV infection (19). However, PD-1: PD-1 ligand blockade in chronic NrHV infection did not reduce viremia. In re-infected mice, rapid clearance with low viral titers similar to HCV re-infections (20) was reported and was mediated by NrHV specific $CD8^+$ T cell recall response, but pre-existing neutralizing antibody titers were insufficient to prevent re-infection. Further studies will be needed to delineate immune mechanisms and elucidate immune correlates of protection.

The HCV field has long suffered from the lack of an immune-competent animal model capable of chronic infection, which has limited serious progress in vaccine development and immunotherapeutics. NrHV infection of mice model has the potential to be an exciting model to study basic viral immunity, virus host interactions and to evaluate vaccine candidates and therapeutic interventions. Generation of chimeric viruses would further enhance the scope of this model and allow the study of HCV specific immunopathogenesis. However, the biggest limitation with this model is the absence of severe hepatopathology, the most serious complication and hallmark of chronic HCV disease. Further, it is not known if quasispecies variation exists in NrHV-infected mice, and mice are not the natural host for NrHV thus requiring pre-depletion of $CD4^+$ T cells to establish chronic infection. Recently, the same virus

was reported to induce hepatitis and steatosis in chronically infected rats (21), but it remains unclear if this could serve tangibly as a model. Overall, NrHV infection of both mice and rats could be highly valuable in HCV research and may help fill the knowledge gap leading to a successful HCV vaccine.

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