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.

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-2Rγ−), type I (A129, IFNARβ−) and type I/II (AG129, IFNARβ−IFNRγ−) 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 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 CD4+ 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-1high CD44low 2B4+ Tim-3+ Eomeshigh 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

### Table 1 HCV-related hepacviruses

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

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

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**References**


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