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Breaking the species barrier for hepatitis delta virus

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Comment

Hepatitis delta virus (HDV) is a satellite virus that requires the envelope proteins from hepatitis B virus (HBV) to form infectious virions. Co-infections with HBV and HDV cause more exacerbated liver disease as compared to HBV mono-infections and the pathogenesis is frequently more accelerated. Given the dependence of HDV on HBV, it is not surprising that both viruses share the same limited host tropism, robustly infecting only chimpanzees and humans. While the vaccine for HBV also protects individuals from HDV infection, there currently are no direct-acting antiviral therapies for HDV. The development of such antiviral therapies and an in-depth understanding of HDV's interaction with the mammalian host have been hampered by the scarcity of suitable animal models. Addressing this need, Wenhui He *et al.* recently described the development of a transgenic mouse model expressing the HDV and HBV entry receptor, human sodium taurocholate co-transporting polypeptide (hNTCP), which is susceptible to HDV infection.

Hepatitis delta virus (HDV) is one of five known human hepatitis viruses. HDV is a single-stranded, negative-sense RNA virus of about 1600 bp expressing a single gene product, the HDV antigen (HDAg). Since HDV can only propagate in the presence of HBV, it is considered a subviral satellite virus. HBV/HDV co-infections are a global health problem. Of the approximately 350 million chronically infected HBV patients worldwide, 15–20 million are co-infected with HDV. Although the HBV vaccine is highly efficacious in preventing infection and leads to protection against both HBV and HDV, there is no cure and current treatment options utilizing pegylated interferon are costly and rather ineffective. The development of novel therapies has been hampered by the lack of a cell culture system and small animal models with HDV susceptibility.

The discovery of hNTCP as a bonafide HBV receptor was a watershed moment. Overexpression of hNTCP in a human hepatoma cell line rendered the cells susceptible to both HBV and HDV infection (1). This led to a human cell culture system for studying both viruses *in vitro*. However, when hNTCP is overexpressed in murine hepatoma cells, these cells are permissive to HDV, but not HBV, infection, indicating that dominant negative murine factors might restrict HBV entry or that other human host factors are needed for HBV uptake (2). The susceptibility of cultured murine hepatocytes expressing hNTCP to HDV infection raises the possibility that *in vivo* expression of hNTCP in a transgenic mouse's liver could lead to HDV infection of murine hepatocytes.

In the early 1990s, HDV+ serum from a woodchuck chronically infected with woodchuck hepatitis B virus (WHBV) was used to inoculate both CB17 mice and CB17 mice with

severe combined immunodeficiency (CB17-SCID) (3). Interestingly, these HDV virions packaged with WHBV envelope proteins were capable of infecting murine hepatocytes, albeit at very low levels not exceeding 0.5% of cells five days post-infection. HDV RNA was still detected 5–10 days post-infection in CB17-SCID mice but disappeared by day 20. Thus, the presence of HDV may not represent a true infection but rather could be due to trapping of HDV particles in the mouse liver. These data suggest that viral clearance is likely T and B cell independent as CB17-SCID mice lack functional T and B lymphocytes (3).

More recently, host adaptation through transplantation of human hepatocytes into suitable xenorecipients has been explored to establish both HDV mono-infection as well as HBV/HDV co-infection. In the resulting human liver chimeric mice chronically infected with HBV, HDV viremia was observed four weeks after HDV challenge. At this time point, 2% of human hepatocytes were HDAg positive. This number increased to 46% and 80% by weeks 8 and 12, respectively (4).

In human liver chimeric mice inoculated only with HDV, 1.2–1.9% of hepatocytes were HDAg+ six weeks following infection (5). Upon superinfection with HBV, the frequency of viral (HDV and HBV) antigen-bearing hepatocytes increased to over 50% nine-weeks post-superinfection. This study indicates that HDV can persist in hepatocytes for extended periods of time without HBV co-infection and can still lead to a productive infection upon superinfection with HBV.

In their recent work, He *et al.* (6) created a transgenic mouse, in which a murine, hepatocyte-specific albumin promoter drives hNTCP expression. Following infection with a high inoculum (3×10^{10}) of HDV, ~3% of hepatocytes in these mice became HDAg+. Prophylactic administration of antibodies directed against the HBV envelope proteins prevented HDV infection *in vivo*, demonstrating that viral uptake in hNTCP transgenic mice is viral glycoprotein dependent. In accordance with previous data (3), HDV infection was age-dependent. While mice 17 days and younger became infected following challenge with high doses of HDV, older mice challenged with HDV four weeks after birth showed no detectible HDV infection. This age dependence is not observed in humans and suggests that maturation of the murine immune system may be important for viral clearance. This is further supported by the observation that young mice with detectible levels of HDV infection are able to clear the virus two weeks post-infection.

To test what components of the immune system are necessary for viral clearance, the authors crossed the hNTCP transgenic mouse with SCID mice and IFN α/β receptor deficient mice (IFN α/β $R1^{-/-}$), which are severely impaired in their ability to mount type I IFN responses. Despite these severe immune impairments, HDV was still readily cleared within two weeks after infection in both strains. These findings suggest that if innate immunity contributes to viral clearance, the process is IFN-independent. Transcriptomic analysis on whole liver tissue from infected mice identified differential expression of IFN-stimulated genes in addition to other genes that might promote viral clearance (Figure 1).

The work of He *et al.* has raised several interesting questions and possible avenues for further research. Even with a high HDV inoculum (3×10^{10}), only 3% of hepatocytes became

infected in the hNTCP transgenic mice. This is similar to observations in HDV mono-infected human liver chimeric mice, but to attain this level of infection the authors had to use a 1,000 fold higher inoculum suggesting that infection is far less efficient *in vivo* in murine cells expressing hNTCP than in human hepatocytes. It is unclear why only this sub-fraction of hepatocytes became HDAg positive. Previous work demonstrated that the region within the HBV envelope responsible for interaction with NTCP can also bind to mouse hepatocytes (7). However, mouse NTCP does not promote viral entry. It is conceivable that injected HDV competes for binding to both the endogenous murine hepatocytes and transgenic hNTCP hepatocytes. However, this is a remote possibility as the human NTCP transgene is about 10,000 fold overexpressed in the liver. Conceivably, more faithful expression of human NTCP under gene-specific regulatory elements may improve HDV uptake. Furthermore, it cannot be excluded at this point that other human-specific host factors are missing that would boost HDV uptake.

Another interesting finding in this model was the age dependence of HDV infection. Are only young mice expressing hNTCP susceptible to HDV infection because they lack a mature innate immune system for responding to viral replication? To more faithfully recapitulate HDV infection in humans, the factors responsible for this distinct phenotype should be identified so that older mice also become susceptible to HDV infection.

Furthermore, the genes identified through transcriptomic analysis as potentially important in viral clearance were from juvenile mice with productive HDV infection. Would the same genes be important for viral clearance in adult mice? Additionally, how are the pathways that inhibit HDV replication triggered? As noted by the authors, it is possible that HDV may induce production of cytokines and other soluble factors in non-parenchymal murine cells, thus antagonizing HDV replication in murine but not human hepatocytes. The lack of cross-talk between mouse and human factors may also explain the differences observed between HDV monoinfection in humanized mice and hNTCP transgenic mice. Furthermore, what are the mechanisms involved in clearing HDAg-bearing cells? He *et al.* mention that HDAg-bearing cells show signs of apoptosis. This murine model with inbred susceptibility to HDV infection now opens avenues for applying mouse genetics to deciphering the mechanisms of viral clearance. hNTCP transgenic mice deficient in hepatic apoptosis or necrosis pathways could be used to interrogate whether HDV clearance from mouse livers is due to the death of the HDV replicating hepatocytes.

Another caveat is that the RNAseq data was performed on RNA isolated from whole tissue – not just infected hepatocytes. Investigating the genes up-regulated specifically in the small population of HDAg+ cells would present valuable information on viral clearance.

These above comments are not to detract from the importance of the hNTCP transgenic mouse model that He *et al.* have developed. The creation of this model is an important step in understanding both HDV and HBV viral infection *in vivo*. Undoubtedly, additional refinements are needed to model HDV pathogenesis, but host immune responses can now be studied in largely immunocompetent mice with inheritable susceptiblity to HDV infection. Thus, this hNTCP transgenic mouse nicely complements previously established human liver chimeric mice.

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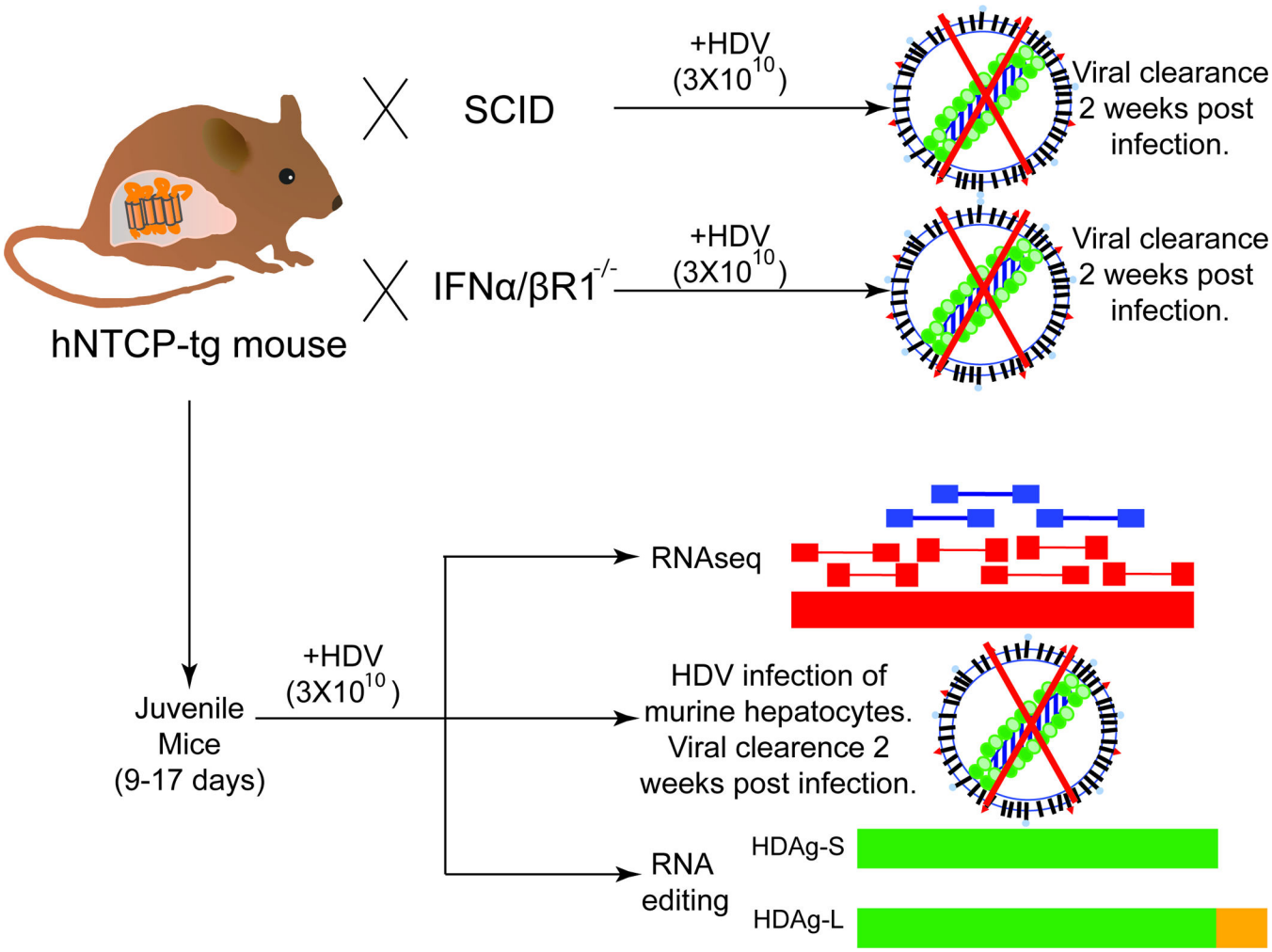

Figure 1. Characterization of HDV infection in hNTCP-tg mice

HDV infection was observed in juvenile mice. Approximately 3% of murine hepatocytes were HDV Ag positive and HDV RNA editing occured suggesting active HDV replication. Transcriptomic analysis was performed on RNA isolated from whole livers to identify genes that were differentially expressed in HDV challenged mice. hNTCP-tg mice were crossed with SCID or IFNα/βR1−/− mice and challenged with HDV. HDV infection was observed but was cleared in both cases two weeks post viral challenge.