VIRAL HEPATITIS

The bumpy road to animal models for HBV infection

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Cell culture infection models help to develop antiviral agents, but animal models are required to understand complex virus–host interactions and the development of immune therapies. Although identification of the HBV uptake receptor enabled establishing cell lines that replicate HBV from its natural transcription template, animal models supporting the full HBV life cycle are still lacking.

Refers to Lempp, F. A. *et al.* Sodium taurocholate cotransporting polypeptide is the limiting host factor of hepatitis B virus infection in macaque and pig hepatocytes. *Hepatology* http://dx.doi.org/10.1002/hep.29112 (2017) | Yan, Z. *et al.* HBVcircle: a novel tool to investigate hepatitis B virus covalently closed circular DNA. *J. Hepatol.* http://dx.doi.org/10.1016/j.jhep.2017.02.004 (2017)

In a new study published in *Hepatology,* Lempp *et al.*[1] report that, in macaque and pig hepatocytes, the sodium taurocholate cotransporting polypeptide (NTCP) is the key host factor limiting HBV infection. In contrast to rodent and dog, pig and macaque hepatocytes became susceptible to HBV upon expression of human NTCP, indicating that — except for an appropriate NTCP molecule — they provide all other essential host factors.

In a different approach reported in the *Journal of Hepatology*, Yan *et al.*[2] used artificial HBV DNA circles as substitutes for the nuclear HBV transcription template, the so-called covalently closed circular DNA (cccDNA). The cccDNA-like molecules, termed HBVcircle, were introduced into hepatocytes by hydrodynamic injection and supported high levels and persistent replication of HBV *in vivo*[2]. These two studies help us move a step closer to much-needed new animal models that will further our understanding of the virus–host interaction in HBV infection and facilitate the development of novel therapeutics.

Despite the availability of a prophylactic vaccine, an estimated 240 million individuals are chronically infected with HBV, at risk of developing liver cirrhosis or hepatocellular carcinoma and accounting for 680,000 deaths per year (Hepatitis B fact sheet, The WHO, 2016). Co-infection with the hepatitis delta virus (HDV) occurs in ~10% of HBV-infected individuals, resulting in the most severe and rapidly progressing form of viral hepatitis. HBV is a small, enveloped DNA virus, whereas HDV is a viroid that co-opts the HBV envelope. Both viruses attach to heparan sulfate proteoglycans and are taken up upon binding to the hepatocyte-specific bile acid transporter, NTCP, which was identified as the *bona fide* HBV (and HDV) receptor[3]. After fusion of the viral envelope with endosomal membranes, HDV can readily start to replicate in the cytoplasm, whereas the HBV capsid needs to be transported to the nucleus where it releases the viral DNA genome. In the nucleus, the incomplete DNA genome is converted, or 'repaired', by cellular enzymes to cccDNA, which then serve as HBV transcription templates.

Although nucleoside and nucleotide analogues can control HBV replication, these drugs do not affect cccDNA persistence and have no effect on HDV infection. Thus, as HBV cccDNA persists, it drives a relapse when therapy stops or when resistant viral variants develop. In addition, a substantial hepatocellular carcinoma risk remains despite treatment. Although the development of liver cirrhosis is mainly due to immune pathogenesis of HBV infection, hepatocarcinogenesis seems to be a multifactorial process, including inflammation and increased hepatocyte turnover, but also insertional mutagenesis and direct effects of HBV proteins as driving forces. Thus, curative treatments would be very important but require a better understanding of the pathogenesis of HBV and/or HDV infection and their effects in hepatocarcinogenesis. However, such progress is currently hindered by the lack of appropriate animal models.

Among the animal models used for HBV infection (TABLE 1), chimpanzees are the only natural hosts of HBV besides humans. Chimpanzees have been instrumental to understanding important aspects of HBV–host interaction and immune biology[4], however, their use has always been restricted and now is completely banned in many countries, including the USA in 2011. Tree shrews are the only other animal permissive for HBV infection, but only at a very low level that restricts their applicability as an animal model[4]. Peking ducks and woodchucks have also been used as surrogate models, as they can be infected with animal viruses related to HBV. However, both animals show a different disease pathogenesis and a number of treatment approaches are HBV-specific and not applicable.

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Immunodeficient mice crossbred with genetic variants that are toxic for the mouse hepatocyte can be repopulated with human hepatocytes that support HBV infection[5]. These 'humanized' mice, however, are unsuitable for determining immune pathogenesis, for modifications to host cell genetics or for testing curative therapeutic approaches relying on immune reconstitution. Thus, an immune-competent animal infection model for HBV is lacking.

Taking advantage of the convenience of mouse models, the broad range of genetically variant mouse strains and the elaborate toolsets available, a number of surrogate models have been developed in mice. Replication-competent, linear HBV genomes have been used to generate transgenic mice that were instrumental to understand important aspects of the immune control of HBV[6]. In these mice, however, a high level of immune tolerance is

Table 1 | **Overview of animal models of HBV infection**

Animal model	Virus	Viral persistence	'Cure' possible	Immune competence	Nuclear cccDNA	Genetic host variants	Testing of viral variants
Chimpanzee	HBV	Yes	Yes	Yes	Yes	No	Yes
Mice with 'humanized' liver	HBV	Yes	Yes	No	Yes	No	Yes
HBV1.3 transgenic mice	HBV	Integrate	No	Strong tolerance for HBV	No	Yes	No
Adeno-HBV-infected mice	HBV	Dose-dependent	Yes	Yes	No	Yes	Yes
AAV-HBV-infected mice	HBV	Yes	Yes	Yes	No	Yes	Yes
Hydrodynamic injection of HBV plasmids	HBV	No	Yes	Yes	No	Yes	Yes
Hydrodynamic HBVcircle injection	HBV	Yes	Yes	Yes	Yes, but substitute format	Yes	Yes
Woodchuck	WHV	After neonatal infection	Yes	Yes	Yes	No	Only WHV
Peking duck	DHBV	After neonatal infection	Yes	Yes	Yes	No	Only DHBV
NTCP-expressing macaque	HBV	Not known	Yes	Yes	Yes	No	Yes
NTCP-expressing pig	HBV	Not known	Yes	Yes	Yes	Yes	Yes

AAV, adeno-associated virus; cccDNA, covalently closed circular DNA; DHBV, duck hepatitis B virus; NTCP, sodium taurocholate cotransporting polypeptide; WHV, woodchuck hepatitis virus.

operative and, therefore, HBV cannot be eliminated. As an alternative, adenoviral[7] as well as adeno-associated viral (AAV) vectors[8] have been used to transfer HBV genomes into the mouse liver and initiate HBV replication. AAV-HBV vectors, in particular, have enabled the establishment of persistent HBV replication over months. Hydrodynamic injection has been used introduce plasmid DNA with HBV genomes into hepatocytes[9], but this process is associated with hepatocyte damage and HBV replication is transient in nature[10]. All these HBV genome transfer approaches enable working with fully immune-competent animals, studying elimination of a (surrogate) HBV transcription template and taking advantage of all the genetic mouse variants available, but fail to permit the study of the authentic transcription template, the HBV cccDNA.

To establish a mouse model that supports "cccDNA-dependent" transcription, Yan *et al.*[2] developed HBVcircle, taking advantage of minicircle technology that enables production of circular DNA devoid of plasmid DNA fragments in genetically engineered *Escherichia coli*. The HBVcircle is a close mimic of cccDNA and persists for months in hepatocytes if applied appropriately in suitable mouse strains[2]. Hence, it represents a novel tool for addressing questions on the biology of nuclear HBV-DNA and for cccDNA-targeting anti-HBV drug discovery. However, one should not forget that this model relies on hydrodynamic injection of DNA in a large volume over a short time, which unlike natural infection, leads to an enormous increase in alanine transaminase levels shortly after injection[10]. In addition, it disregards the nuclear import of partially double-stranded HBV-DNA and its repair to cccDNA — an important part of the HBV lifecycle.

Uptake via NTCP, in contrast, should enable HBV and HDV to sneak into a hepatocyte and initiate virus replication. Lempp *et al.*[1] show that complementation of mouse, rat or dog hepatocytes with the human NTCP conferred susceptibility of the respective hepatocytes to HDV but not to HBV, indicating the requirement of additional HBV-specific host factors. Hepatocytes from pigs as well as cynomolgus and rhesus macaques, by contrast, supported the full HBV replication cycle[1]. Most likely, the host factors needed in addition to NTCP are required for the import of incomplete HBV genomes into the nucleus or their repair to the persistent cccDNA form. Thus, the findings of Lempp *et al.*[1] pave the way for the development of macaques or pigs as an immunocompetent infection model supporting the full HBV life cycle that might enable studying the immune pathogenesis of a natural HBV infection, testing novel antiviral strategies and, in particular, examining immune stimulatory approaches *in vivo* in well-characterized and fully immune competent animals. Whether macaques or pigs permit HBV infection to persist over weeks and months as AAV-HBV or HBV DNA circles do in mice needs to be investigated once animals carrying the human NTCP on their hepatocytes become available.

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Competing interests statement
The author declares no competing interests.

FURTHER INFORMATION
Hepatits B fact sheet, The Who 2016: http://www.who.int/mediacentre/factsheets/fs204/en/
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