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Animal models of HBV infection

Maura Dandri, PhD, Professor [a, b, *], Joerg Petersen, MD, Professor [c]

[a] I. Department of Medicine, Center for Internal Medicine, University Medical Center Hamburg-Eppendorf, Hamburg, Germany
[b] German Center for Infection Research (DZIF) Hamburg-Lübeck-Borstel Site, Germany
[c] IFI Institute for Interdisciplinary Medicine at Asklepios Clinic St. Georg, Hamburg, Germany

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A B S T R A C T

The mechanisms determining hepatitis B virus (HBV) persistence and pathogenesis are not fully elucidated, but appear to be multi-factorial. Current medication to repress viral replication is available; however, the unique replication strategies employed by HBV enable the virus to persist within the infected hepatocytes. Consequently, cure is rarely achieved. Progresses in HBV research and preclinical testing of antiviral agents have been limited by the narrow species- and tissue-tropism of the virus, the paucity of infection models available and the restrictions imposed by the use of chimpanzees, the only animals fully susceptible to HBV infection. Mice are not HBV permissive but major efforts have focused on the development of mouse models of HBV replication and infection, such as the generation of humanized mice. By presenting the different animal models available, this review will highlight the most important and clinically relevant findings that have been retrieved from the respective systems.

HBV is a small, enveloped DNA virus causing acute and chronic hepatitis. Even if HBV is not directly cytopathic for the infected cell, the infection leads to a wide spectrum of liver disease, which often progresses to liver cirrhosis and hepatocellular carcinoma. Despite the availability of approved antivirals efficiently suppressing HBV replication, resolution of chronic infection is rarely achieved. This is mostly due to the persistence of the viral genome, the covalently closed circular DNA (cccDNA), in the nuclei of infected hepatocytes [1] and to the inability of the immune system to mount effective HBV-specific immune responses. Thus, the main goal of the current antiviral therapies is to achieve sustained viremia suppression and hinder liver disease progression. Despite decades of research, there is still limited knowledge about the interactions that HBV engages with its host both in the process of establishing and in the process of maintaining infection within the infected human hepatocyte, as well as with the immune system. Such gaps of knowledge are mostly due to the narrow host range and tissue tropism of HBV. Moreover, the virus is characterized by a unique genomic organisation and replication mechanism that, upon cell entry, involves the conversion of the incoming genome into the cccDNA, which associates with histone and non-histone proteins to build a stable minichromosome. The cccDNA serves as template for the transcription of all viral RNAs [2], including the pregenomic RNA (pgRNA), an over-length complement of the viral cccDNA that is reverse transcribed by the viral polymerase within newly formed nucleocapsids, before being enveloped and secreted in the bloodstream as progeny virus.

Because of such unique replication mechanism, a major challenge of novel antiviral approaches aiming to achieve resolution of HBV infection will be to generate strategies targeting the cccDNA in the nucleus of infected hepatocytes, probably in combination with strategies also promoting immune restoration [3]. This will require advancing our knowledge of HBV biology, in particular understanding mechanisms of entry, cccDNA formation, stability and regulation, and further development of experimental models enabling complete recapitulation of the HBV life cycle and analyses of antiviral immune responses.

The only natural cell target of HBV infection and replication is the human hepatocyte. However, primary human hepatocytes (PHHs) are not readily available in most laboratories and their infection susceptibility is timely restricted, since plated PHHs rapidly lose the capability to express hepatocyte-specific factors, which are essential both to establish the infection but also to study the virus-host interplay involved in persistence and HBV-associated pathogenesis. Thus, most basic knowledge in HBV replication have

Abbreviations: HBV, hepatitis B virus; cccDNA, covalently closed circular DNA; pgRNA, pregenomic RNA; DHBV, duck hepatitis B virus; WHV, woodchuck hepatitis virus.

* Corresponding author. I. Department of Internal Medicine, Center for Internal Medicine, University Medical Center Hamburg-Eppendorf, Martinistr. 52, D-20246 Hamburg, Germany. Fax: +49 40 7410 57232.
E-mail address: m.dandri@uke.de (M. Dandri).

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Table 1
Characteristics of the *in vivo* systems available for studies with HBV and HBV-related viruses.

Systems	Virus & vector type	Characteristics & applicability for studies on:				Advantages	Limitations
		HBV replication	cccDNA	Infection	Immunology		
Duck	DHBV	yes	high copies	yes	innate, adaptive, vaccine	High cccDNA copies high infectivity & replication levels;	lowest similarity to human infection outbred animals vertical transmission
Woodchuck	WHV	yes	high copies	yes	innate, adaptive, vaccine	High cccDNA copies; long-term & carcinogenesis studies	outbred animals, limited availability
Tupaia	HBV (WM-HBV)	weak, transient	low copies	weak, transient	innate, adaptive, vaccine	isolated hepatocytes: good alternative for infection studies	outbred animals limited availability weak infection
Chimpanzee	HBV	yes	yes	yes	innate, adaptive, vaccine	well-characterized immune system; highest similarity to human infection	very strong ethical & costs restrains
Mouse models	Transgenic mice integrated HBV genome	yes	no	no	Immune competent adaptive (upon immune cell transfer)	convenient, inbred animals; antiviral studies (except entry/cccDNA)	no-infection cccDNA not built no virus clearance immune tolerance
	HDI- injection Injection naked HBV DNA	transitory	(no)	no	Immune competent acute infection	studies on viral clearance testing of clinical isolates	transient vector-driven replication; short span of replication
	Adeno- AAV-transduced mice Vector-driven HBV-DNA constructs	yes	no	no	Immune competent acute infection	studies on viral clearance (acute immune responses)	transient vector-driven infection; no spreading
	Human liver-chimeric mice HBV	yes	yes	yes	Intrinsic innate responses & (upon immune cell transfer)	infection in natural cell target; long-term studies; testing of clinical isolates	human hepatocytes needed complex system Immune deficiency

been gained using in vitro transfection systems and models based on HBV-related hepadnaviruses (Table 1), such as the duck hepatitis B virus (DHBV) and the woodchuck hepatitis virus (WHV). Only in 2012, the identification of the long searched hepatocyte-specific receptor, the Na^+-taurocholate cotransporting polypeptide (NTCP) [4] has paved the way for the development of in vitro infection systems enabling more efficient anti-viral drug screening and possibly elucidation of still unknown steps in HBV life cycle. However, in spite of the existence of hepatocyte-based infection systems, cultured cells may respond differently to infections and other stimuli than cells in the living organ. Having lost the capability to express various hepatocyte-specific genes, discrepancies between data obtained *in vitro* and *in vivo* may occur. Thus, findings generated *in vitro* need to be confirmed using *in vivo* systems.

Models based on the use of HBV-related viruses

The Duck model

The Duck Hepatitis B Virus (DHBV) is the most distantly related to human HBV (only 40% homology), but duck hepatocyte cultures and ducklings are readily available. Consequently, various antiviral compounds were tested in the duck model [5–7], even though these animals appear to be less sensitive to potential toxic effects than other animal models, like woodchucks. For instance, nucleocapsid inhibitors of the HAP family appeared inactive on DHBV but efficiently suppressed HBV [8,9]. All things considered, the duck model played a pivotal role to elucidate many steps of the replication mechanism adopted by this family of viruses (hepadnaviruses). However, DHBV uses the carboxypeptidase D (DCPD) and not NTCP as a receptor for viral entry and the infection is mostly not associated with liver disease and development of hepatocellular carcinoma (HCC). Therefore, both results of antiviral drug screening and findings related to DHBV biology might be of limited value for human HBV infection due to both virus and host differences.

The woodchuck model

The woodchuck hepatitis virus (WHV) is closer to HBV in terms of genomic organization and studies in naturally and experimentally infected woodchucks, the American *marmota monax*, have been fundamental in the preclinical evaluation of most antiviral drugs now in use for treatment of HBV infection [10–12]. Regarding the screening of immune modulatory substances, therapeutic strategies involving the inhibition of PD-L1 [13] has been employed to study the efficacy of this approach to break immunological tolerance, while treatment with the oral TLR7 agonist GS-9620 was shown to induce sustained antiviral responses and seroconversion in a proportion of treated animals [14].

Experimental infection of newborn woodchucks almost invariably leads to chronic infection, whereas animals infected at older ages generally develop acute hepatitis. Moreover, sequencing of the woodchuck transcriptome indicated that chronic WHV infection is associated with a limited type I interferon response and induction of markers that are associated with T cell exhaustion [15], thus resembling finding determined in HBV chronic infected patients. However, it should be noted that in the woodchuck system WHV DNA integrations frequently lead to the activation of the myc proto-oncogene [16] and nearly all neonatal infected woodchucks develop hepatocellular carcinoma, indicating that important differences exist in the process leading to carcinogenesis between WHV and HBV. Moreover, an increasing body of evidence revealed important differences between animal hepadnaviruses and HBV also regarding their capacities to form the cccDNA and control its