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In vivo models of hepatitis B and C virus infection

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Abstract

Globally, more than 500 million individuals are chronically infected with hepatitis B (HBV), delta (HDV), and/or C (HCV) viruses, which can result in severe liver disease. Mechanistic studies of viral persistence and pathogenesis have been hampered by the scarcity of animal models. The limited species and cellular host range of HBV, HDV, and HCV, which robustly infect only humans and chimpanzees, have posed challenges for creating such animal models. In this review, we will discuss the barriers to interspecies transmission and the progress that has been made in our understanding of the HBV, HDV, and HCV life cycles. Additionally, we will highlight a variety of approaches that overcome these barriers and thus facilitate in vivo studies of these hepatotropic viruses.

1. Introduction

Worldwide, over 500 million people are persistently infected with hepatitis B virus (HBV), hepatitis delta (HDV) and/or hepatitis C virus (HCV). HBV and HCV infect the liver and can lead to a broad spectrum of disease outcomes. Between 15% and 40% of individuals chronically infected with HBV or HCV develop serious liver disease, including cirrhosis and/or hepatocellular carcinoma (HCC). In contrast, HDV is a satellite virus that needs HBV envelope proteins in order to form infectious virions. Approximately 15–20 million individuals worldwide are co-infected with HBV/HDV [1]. These patients experience a faster progression of liver disease and also have the highest mortality rate (20%) of any of the viral hepatitises [2–4]. It is widely believed that the outcome of these viral infections and the pathogenesis of the associated liver diseases are determined by host-virus interactions mediated by the immune response.

A safe and effective vaccine for HBV is available and protects against both HBV and HDV infections. For infected individuals, the standard treatment of care is pegylated interferon (IFN)-α in combination with nucleos(t)ide analogs. Although these treatments can suppress viremia, they rarely result in a cure [5]. The scarcity of experimental systems for studying HBV, particularly tractable small animal models [6], has been a bottleneck for testing the efficacy of novel, potentially curative HBV treatments.

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Unlike HBV, there is no vaccine for HCV. Over the last few years, a combination of direct-acting antivirals (DAAs), including NS3-4A inhibitors, NS5A inhibitors and NS5B nucleos(t)ide or non-nucleos(t)ide inhibitors, have demonstrated strong potency as treatments, resulting in a sustained virologic response (SVR) in ca. 90–100% [7, 8] of treated individuals. Patients can thus now be cured of HCV infection, but this antiviral treatment is still poorly accessible due to its high costs [9]. Moreover, patients who are cured of their HCV infection but have advanced liver disease still remain at significant risk for developing HCC, making a prophylactic or preventive HCV vaccine an urgent need to significantly impact HCV spread worldwide [10].

2. Molecular virology of hepatitis B, C, and delta viruses

HBV is a small, double-stranded DNA virus belonging to the *Hepadnaviridae* family. Its genome is ~3.2 kb in length, with four open reading frames (ORFs) encoding for four gene products [11, 12]: the core protein, X protein, viral polymerase, and the surface polypeptides which in turn encode for three proteins: the small (S), medium (M), and large (L) surface antigens [13, 14]. The N-terminal section of the L antigen (preS1) is known to be essential for HBV infection [15]. Following productive entry into human cells, the HBV genome is uncoated. It has been proposed that capsid proteins interact with the nuclear basket, leading to genome release [16]. Afterwards, the relaxed circular genome (rcDNA) is inserted into the nucleus. Here, covalently closed circular DNA (cccDNA), which serves as the transcriptional template for all four viral gene products, is then formed via poorly defined mechanisms. Pre-genomic RNA (pgRNA), one of the four transcripts produced from cccDNA, has the viral polymerase attached. The viral polymerase then interacts with the core protein to facilitate packaging. Once packaged in a nucleocapsid, the pgRNA is reverse transcribed into rcDNA that can be imported into the nucleus to replenish the cccDNA pool or the nucleocapsid-encased rcDNA can enter the ER, be enveloped by a lipid bilayer containing the HBV envelope proteins, and then egress from the host cell as a fully formed, infectious virion [11]. In addition to infectious virions, several non-infectious sub-viral filaments are also released [17, 18].

HDV is a satellite virus that uses the HBV envelope proteins to form infectious spherical particles 36 nm in diameter [19–23]. The HDV genome is a single-stranded, circular RNA comprised of 1679 bp. This genome has one ORF encoding for the delta antigen (HDAg), of which there are two isoforms: the large HDAg (27 kDa, 214 aa) and the small HDAg (24 kDa, 195 aa).

HCV is an enveloped, positive-sense, single-stranded RNA virus of the family *Flaviviridae* with a 9.6-kb genome [24]. Translation of the genome initiates at an internal ribosome entry site (IRES) in the 5′ non-translated region (NTR) and produces a single polyprotein of ca. 3000 amino acids in length which is cleaved into three structural proteins (Core, E1 and E2) and seven nonstructural (NS) proteins (p7, NS2, NS3, NS4A, NS4B, NS5A and NS5B) by cellular and viral proteases [25]. Following translation, the HCV proteins are associated with membranes derived from the endoplasmic reticulum. NS3 through NS5B comprise the replication machinery, which replicates the positive-sense RNA genome through a negative strand intermediate. Nascent RNA genomes serve as new/additional RNA templates for

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further RNA replication, are translated to produce new viral proteins, and are assembled into infectious virions. The HCV replication complex is subcellularly located in the so-called membranous web, an accumulation of ER-derived membrane vesicles and lipid droplets (LDs). HCV assembly is intricately connected to lipid metabolism, and both structural and non-structural proteins of HCV interact with components of lipid metabolism at different levels (reviewed in [26, 27]). Following accumulation of the viral components near LDs, virion assembly is initiated - nucleocapsid formation, budding and, finally, maturation of the infectious particle [28]. Importantly, the very low-density lipoprotein (VLDL) assembly pathway is closely related to the production of infectious particles, which are released from cells as so-called lipoviral particles.

3. HBV and HCV have a limited host range

The study of HBV, HCV, and HDV pathogenesis as well as host responses to infection have been hindered by the limited species and cellular tropism of these viruses, which robustly infect only the hepatocytes of humans and chimpanzees. In the past, the use of chimpanzees as a model for HBV and HCV infections was limited due to the high cost and low availability of these animals. In recent years, further ethical considerations have led to a moratorium on federal funding for research using chimpanzees. As a result of this moratorium, a small tractable animal model is a pressing need.

4. Surrogate systems for studying viral hepatitis *in vivo*

4.1. Animal models for investigating human HBV and HCV

Chimpanzees are the only existing immunocompetent models for HBV(/HDV) and HCV infection and have thus served as an important platform for studying immune responses to these viruses as well as disease progression [29–34]. For example, much of our knowledge concerning the mechanisms used by the innate and adaptive immune system to combat HCV infection was acquired from such experimental infections in chimpanzees [35, 36]. However, as mentioned above, the chimpanzee has fallen out of favor as an experimental model due to limited availability, high associated costs and ethical concerns. As a result, the motivation for developing a small animal model for studying hepatotropic viral infections has greatly increased [37, 38].

Another organism considered for studying HCV and HBV infections *in vivo* is the tupaia (*Tupaia belangeri*), also known as the tree shrew. Tupaias are small, non-primate mammals, but genomic analysis has revealed that the *Tupaia* genus is more closely related to humans than to rodents. Tupaia hepatocytes are susceptible to both HBV and HCV infection *in vitro* and *in vivo* [39–41]. A longitudinal analysis of HCV-infected tupaias showed that HCV inoculum caused mild hepatitis and intermittent viremia during the acute phase of infection. Histological analysis revealed that HCV caused chronic hepatitis that worsened over time as well as liver steatosis, cirrhotic nodules, and tumorigenesis [42]. However, viremia following HBV infection in tupaia is quite low, suggesting that innate and adaptive immune responses help to control the virus in this organism. Together, these data suggest that tupaias have the potential to serve as an animal model for experimental studies of HBV or HCV infection. However, the difficulties of genetically manipulating its genome, the scarcity of tupaia-

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specific reagents, and the genetic heterogeneity of this outbred species limit its application for a mechanistic study of HBV or HCV pathogenesis.

4.2. Hepatitis C and related hepaci- and pegiviruses

With chimpanzees no longer an option for studying HCV infection and the logistical difficulties inherent to research with tupaias, viruses closely related to HCV have been proposed as surrogate models. One such example is the hepacivirus George Barker virus B (GBV-B), which has a genome organized identically to HCV [43]. More recently, a virus highly similar genetically to HCV was identified in dogs and thus termed canine hepacivirus (CHV). It remains unclear whether CHV replicates in the canine liver and whether it induces hepatitis [44]. Other related viruses of either the *Pegivirus* genus, another member of the *Flaviviridae* family, or *Hepacivirus* genus have also been identified in horses [45, 46], wild mice [47] and rats [48]. Of the non-primate hepaciviruses (NPHV), those observed in horses are the most genetically similar to HCV [49]. However, it remains to be shown whether these viruses indeed cause hepatitis in experimentally inoculated animals before it can be determined whether they might be surrogates for modeling HCV. Importantly, there is currently no experimental evidence of NPHV transmission between horses and humans [50].

4.3. Hepatitis B and related hepadnaviridae

HBV is part of the *Hepadnaviridae* family. Hepadnaviruses are divided into two genera, the orthohepadnaviruses and the avihepadnaviruses, which infect mammals and birds, respectively.

The two most important surrogate viruses are duck hepatitis B virus (DHBV) and woodchuck hepatitis virus (WHV). DHBV has been instrumental in understanding the hepadnavirus life cycle, specifically the formation of covalently closed circular DNA (cccDNA), which is the stable template for all viral transcripts. DHBV was utilized to investigate how the DNA intermediate precursor of HBV cccDNA, rcDNA, is synthesized [51–53]. The virus has also aided in our understanding of the mechanism by which hepadnaviruses increase the pool of cccDNA within the host nucleus [54–56].

Woodchuck hepatitis virus (WHV) was discovered at the Penrose Zoo in Philadelphia where animals showed liver pathogenesis similar to that observed in chronic HBV patients [57, 58]. The virus isolated had ~70% nucleotide identity to human HBV [59]. Woodchucks infected with WHV have been instrumental in the study of nucleos(t)ide analog drugs and their effect on viral suppression and the emergence of resistance [58, 60, 61]. However, there are still limitations to performing research in woodchucks as they are outbred, genetically diverse organisms, and few reagents are available for investigating their immune response to viral infection. Additionally, the nucleotide differences between HBV and WHV pose considerable challenges for evaluating the efficacy of drug and vaccine candidates directed against the human virus [59].

Several other hepadnaviruses have been identified, such as woolly monkey hepatitis B virus (WMHBV), ground squirrel hepatitis B virus (GSHBV), heron hepatitis B virus (HHBV), and, recently, tent bat hepatitis B virus (TBHBV) [62, 63]. All of these viruses have a highly limited tropism and have not been extensively utilized as surrogate models. The

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identification of TBHBV has raised the possibility that bats might be a zoonotic reservoir for HBV, but this virus and its tropism must be investigated more thoroughly before any conclusions can be made. Recently, it was reported that a hepadnavirus was isolated from the livers of *Macaca fascicularis,* a small Old World monkey, living in the Mauritius Islands. The isolated virus appears to be highly similar to HBV genotype D [64]. This virus and the *M. fascicularis* population should be studied further in order to determine (1) if chronic HBV infections exist in the population and (2) what is the tropism and pathogenesis of this newly identified hepadnavirus.

5. Barriers of interspecies transmission of hepatitis B virus

The HBV life cycle is blocked at multiple steps in rodent cells (Figure 1 *left*). Recently, the sodium taurocholate co-transporting polypeptide (hNTCP) was identified as a receptor for HBV and HDV [65] [66]. HepG2 hepatoma cells overexpressing hNTCP are susceptible to both viruses, albeit at low levels. When hNTCP is overexpressed in murine hepatoma lines, these cells become susceptible to HDV infection but not HBV [66], suggesting that either additional human host factors are needed to facilitate HBV uptake or there are dominant negative murine factors that restrict HBV. However, dominant negative murine factors seem unlikely because heterokaryons formed between human and mouse hepatoma cells become infected when challenged with HBV [67]. HBV can bind to a variety of NTCP orthologues from species including mice and dogs. Human-murine NTCP chimeric proteins with the loop region (84–87 aa) of murine NTCP replaced with the corresponding human sequence results in HBV and HDV infection when expressed in HepG2 [68]. A block may also exist at the level of HBV replication. In 1.3x HBV transgenic mice that stably express the HBV genome, HBV cccDNA [69] cannot be detected, indicating blocks at the level of HBV cccDNA formation and maintenance within murine hepatocytes.

Overexpression of hNTCP in murine hepatoma lines renders these cells susceptible to HDV infection but not HBV infection [68]. This raises the possibility of creating an hNTCP transgenic mouse model susceptible to HDV infection. Recently, a transgenic mouse model was developed with hNTCP expression driven by the mouse albumin promoter. When challenged with high amounts of HDV virions, these mice had detectable HDV virus in their livers, with approximately 3% of their hepatocytes infected. The infection was age-dependent in the hNTCP transgenic mice as only juvenile mice under the age of 17 days had observable levels of HDV infection in the liver. In addition, these young mice were able to clear HDV two weeks post-infection. This study also tested components of the immune system by crossing the hNTCP transgenic mouse with severe combined immunodeficient (SCID) mice and IFN α/β receptor deficient mice (IFNα/β $R1^{-/-}$). Despite these severe immune system impairments, these mice were still able to clear an HDV infection within two weeks. This suggests that viral clearance is due to the innate immune response and is IFN-independent [70].

6. Barriers of interspecies transmission of hepatitis C virus

The narrow species tropism of HCV, which is limited to humans and chimpanzees, is incompletely understood [37]. Mouse cells do not support viral entry [71] and replicate

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HCV RNA inefficiently, but they do support virion assembly and release (Figure 1, *right*) [72, 73]. HCV entry is a highly coordinated process, involving numerous host cellular factors, some of which contribute to the tissue and host tropism of HCV [74]. It was previously reported that CD81 and occludin (OCLN) represent the minimal set of human factors needed for HCV uptake into mouse cells *in vitro* [75]. Recently, two successive studies reported the first genetically humanized mouse model with inheritable susceptibility to HCV via transient expression of these two human factors [76] or stable expression of all four canonical HCV entry factors (CD81, OCLN, SR-BI and claudin-1) [77]. A large number of host cellular factors are required for HCV replication, but mouse hepatoma cell lines and embryonic fibroblasts can harbor replicating HCV RNA, albeit at a very low frequency [73, 78, 79]. This suggests that all host factors necessary for HCV replication are present in mouse cells, but the viral RNA replication machinery cooperates more efficiently with the human cellular factors. Identifying these human-specific factors will facilitate the development of additional mouse models for HCV infection. While it is possible that cell-intrinsic, species-specific dominant restriction factors pose an important barrier to prevent cross-species viral transmission, heterokaryons of mouse and human cells support the entire HCV lifecycle, suggesting these restriction factors do not exist [80]. All this evidence increases the hope for an immunocompetent mouse model for HCV infection by transgenic expression of the essential human host factors.

7. HBV and HCV transgenic mouse models

Several groups have generated transgenic mice expressing transgenically the HCV proteins either individually or together as a polyprotein to study the effect of these proteins on liver pathology – ultimately steatosis and even liver cancer were observed in these mice [81–86]. However, as the expression level and profile of HCV proteins in these transgenic mice is very different from a natural infection, it is unclear if these findings are directly applicable to the pathogenesis of HCV infection *in vivo*.

In the absence of a mouse model that has inheritable susceptibility to HBV infection, transgenic mice expressing a 1.3x over length HBV genome have contributed significantly to our understanding of the immunobiology and pathogenesis of HBV, especially with regards to the role of cytotoxic T lymphocytes in viral pathogenesis [87]. In this 1.3x HBV tg model, HBV replication persists, generating infectious virions. However, HBV cccDNA is not observed, indicating that the murine hepatocyte environment is most likely not amenable to HBV cccDNA formation/maintenance [87, 88]. Persistence is possible when a synthetic HBV cccDNA is delivered to the nucleus of an immortalized mouse hepatoma cell line [89]. However, natural primary murine hepatocytes are unable to support HBV cccDNA formation/maintenance [89]. In addition to the full HBV genome, subgenomic fragments have also been transgenically expressed in mice, providing great insight into the role of these proteins in HBV viral pathogenesis. For example, overexpressing the large (L) envelope protein in mice led to the discovery that L expression results in retention of the small (S) envelope protein in the ER [90, 91]. The X protein has also been transgenically expressed in a CD1 mouse and led to the development of HCC [92].

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While transgenic expression of HBV proteins has proven very helpful to study HBV *in vivo*, the generation of such models on different genetic backgrounds is rather slow and cumbersome and since the HBV genome is already expressed pre- or neo-natally, the mouse's immune system is tolerized to the viral antigens. Alternatively, an over-length, replication-competent HBV genome can be delivered into mice either by adeno-associated-(AAV) or adenovirus (AdV) vectors, resulting in persistent HBV antigen expression for extended periods of time [93–95]. These models have been important in assessing HBV immunopathogenesis as the HBV/AdV-or-AAV can be delivered into any genetic mouse background, including mutant strain [94, 95]. For example, vector-mediated delivery of an HBV genome to the liver of wild-type mice lead to detectable HBV DNA, HbsAg, and HbeAg and resulted in fibrosis [93][94]. However, cccDNA was undetectable which is consistent with previous observations in HBV transgenic mice expressing a 1.3x HBV genome. Immune tolerance of HBV has also been observed in the AAV-HBV model. However, HBV-specific T cells could be stimulated in peripheral tissue and could migrate to the liver and lead to clearance of the virus [94]. Additional work demonstrated that C57BL/6 mice infected with an AAV-HBV were seronegative for HBsAg, as also observed in chronic HBV patients. These same mice, upon immunization with the conventional HBV vaccine plus an albumin adjuvant, failed to mount an immune response. When the vaccine was administered with CpG, a Toll-like receptor 9 agonist, a strong antibody and T cell response were induced, leading to clearance of the virus [95].

These models are a reasonable proxy for studying the immune responses and pathogenesis of HBV and, to a degree, HBV replication. However, a major caveat of this approach is that HBV replication does not occur via the native mechanism. This is born out by the fact that HBV cccDNA is not formed in either the AdV-HBV, AAV-HBV, or 1.3x HBV tg mouse models. This indicates that there is a block at the level of HBV cccDNA formation/ maintenance in murine hepatocytes, which may present a block in the native replication mechanisms that HBV utilizes to establish and persist in hepatocytes.

9. Humanized xenotransplantation models for the study of hepatitis B and C viruses

9.1. Human liver chimeric mice are a versatile model for studying human hepatotropic pathogens

As an alternative to studying surrogate viruses or using transgenic animals expressing viral genes, HBV or HCV can also be investigated by adapting the murine environment to support the uptake and replication of either virus. Liver chimeric mice that harbor human tissue are permissive to HBV, HCV, and HDV infections [96]. These mice are generated by the engraftment of human hepatocytes into a suitable xenorecipient strain. To facilitate engraftment, the xenorecipient must be immunodeficient to avoid graft rejection, and liver injury must be inducible in the endogenous murine hepatocyte population [97, 98]. The best-characterized recipient strain is the transgenic mouse expressing urokinase plasminogen activator (uPA) in hepatocytes driven by the hepatocyte-specific albumin promoter (Alb-uPA mice). uPA overexpression is toxic in the native murine hepatocytes [99], so the transplanted human hepatocytes possess a selective growth advantage and expand within the liver [37].

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The fragility of Alb-uPA mice has limited the speed at which human liver chimeric animals can be generated. In search for more robust liver injury models, mice deficient in fumarylacetoacetate hydrolase (FAH) have been explored as alternatives [100–102]. FAH is the terminal enzyme in the catabolic pathway of tyrosine, and its absence results in the accumulation of toxic tyrosine metabolites leading to hepatic failure [103]. However, the administration of 2-(2-nitro-4-trifluoro-methylbenzoyl)-1,3-cyclohexanedione (NTBC) inhibits the activity of an enzyme upstream of FAH and thus abrogates the accumulation of toxic metabolites to maintain $FAH^{-/-}$ mice in a healthy state [104]. Thus, the timing of the toxic insult applied to native murine hepatocytes to provide a selective advantage to transferred human hepatocytes in the repopulation of the liver can be controlled by switching on and off NTBC administration. TK-NOG humanized mice have also been developed and proven successful in studying HBV and HCV infection. In this model, mouse liver cells express herpes simplex virus type-1 thymidine kinase (HSVtk) and can thus be selectively ablated following exposure of the mice to ganciclovir (GCV), leaving transplanted human hepatocytes with a distinct growth advantage [105].

These and other liver injury models can be engrafted to very high levels with human hepatocytes when crossed to immunodeficient backgrounds. These resultant human liver chimeric mice are susceptible to HBV and HCV infection and are a versatile model to study basic virology and for preclinical applications (Figure 2) [106–109]. When injected with cell culture- or patient derived HCV, highly engrafted human liver chimeric mice become readily viremic and can sustain persistent HCV infection for several months [96, 102, 106, 109, 110]. Similarly, following infection of these mice with HBV, viral intermediates have been detected in the engrafted human hepatocytes, including HBV cccDNA and pgRNA [108]. HDV can infect human hepatocytes in human liver chimeric mice, persisting for up to six weeks post-infection. Upon super-infection with HBV, HDV viremia is observed due to the fact that co-infection with HBV facilitates packaging of HDV virions to infect other human hepatocytes in the liver [111]. These models have also been utilized to test viral suppression drugs [112] and to help ascertain the metabolic and toxicological responses to drugs in engrafted donor hepatocytes [113].

9.2. Challenges and improvements of humanized mouse models

Even with all their advantages, there are several limitations to the use of human liver chimeric mice. First, due to their immunodeficient background, pathogenesis and immune response studies are limited in this model. However, several groups are working to overcome this by developing protocols for the co-engraftment of human liver cells and components of the human immune system in a single murine xenorecipient [114, 115]. Donor matching is desirable but can only currently be achieved with hematopoietic stem cells (HSCs) and hepatoblasts derived from a fetal donor or potentially through the use of stem cell-derived hepatocyte-like cells (HLCs) and HSCs. However, fetal hepatoblasts do not engraft robustly in Alb-uPA mice [116]. Engraftment efficiency appears to be strain-dependent, as more robust engraftment of fetal progenitor cells has been reported in the AFC8 background [117] and in NOD SCID $IL2R\gamma^{NULL}$ mice treated with an anti-Fas antibody to ablate mouse hepatocytes [118]. Likewise, a recent study demonstrated that HLCs could also be engrafted reasonably well in a specific, less frequently used strain, MUP-uPA mice, and support

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persistent HCV infection [119]. While these data are encouraging, protocols need to be refined further to improve dual chimerism and minimize inter- and intravariability of experiments. More recent reports have demonstrated that extensive double humanization of both the liver and immune system can be achieved with mature hepatocytes and HSCs [120, 121]. Dually engrafted mice, when challenged with HBV or HCV, mount a virus-specific immune response resulting in human-specific liver fibrosis (Figure 2) [118].

Secondly, the generation of these chimeric mice requires substantial infrastructure and advanced technical skills and the scarcity of adequate primary human material remains a significant logistical challenge. Finally, due to different donor backgrounds, there is substantial variation in the levels of human chimerism in these models. Donor background and variability could possibly be addressed by utilizing HLCs. Unfortunately, HLCs do not engraft as well into xenorecipients, most likely due to their incompletely differentiated state. However, MUP-uPA mice were successfully engrafted recently with HLCs and were able to become infected following challenge with HCV [119].

10. Conclusions and Perspectives

HBV, HCV, and HBV/HDV co-infections are a major global health burden. While there is an effective vaccine for HBV to protect against HBV mono-infections and HBV/HDV co-infections and treatment available to suppress viremia, there is still no cure. For HCV, very effective treatments have recently come on the market that effectively cure chronic HCV patients, but there is still no vaccine to prevent infection. Due to the limited species and cellular tropism of these viruses, they have been very difficult to study. The use of surrogate viruses has aided in understanding many steps of the HBV life cycle. Additionally, transgenic animals models have helped to shed light on the role individual, sub-genomic components play in the viral pathogenesis of both HBV and HCV. The use of human liver chimeric mouse models has been instrumental in understanding the life cycle of all three viruses. With the continued improvement of human hepatocyte engraftment and the possibility of co-engraftment with a functional human immune system, these models will lend greater insight into viral pathogenesis in the future. The widespread use of these xenotransplantation models is limited, however, by their high costs and limited output. The creation of mouse models with inheritable susceptibility to HBV, HBV/HDV co-infection and HCV infection is a pressing need. An improved understanding of the barriers restricting the HCV and HBV life cycles in murine cells will facilitate the further development of genetically humanized mice. Mice with inheritable susceptibility to HCV infection hold promise to contribute to a better understanding of HCV immunopathogenesis. Lastly, the identification of hNTCP as a receptor for HBV and HDV leads to the tantalizing possibility that a mouse model with inheritable susceptiblity can be created once other additional human host factors necessary for the early stages of HBV infection are identified.

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References

1. Rizzetto M, Ciancio A. Epidemiology of hepatitis D. Semin Liver Dis. 2012; 32:211–9. [PubMed: 22932969]
2. Farci P, Niro GA. Clinical features of hepatitis D. Semin Liver Dis. 2012; 32:228–36. [PubMed: 22932971]
3. Buti M, Homs M, Rodriguez-Frias F, Funalleras G, Jardi R, Sauleda S, Tabernero D, Schaper M, Esteban R. Clinical outcome of acute and chronic hepatitis delta over time: a long-term follow-up study. J Viral Hepat. 2011; 18:434–42. [PubMed: 20546496]
4. Grabowski J, Wedemeyer H. Hepatitis delta: immunopathogenesis and clinical challenges. Digestive diseases. 2010; 28:133–8. [PubMed: 20460901]
5. Kwon H, Lok AS. Hepatitis B therapy. Nature reviews Gastroenterology & hepatology. 2011; 8:275–84. [PubMed: 21423260]
6. Dandri M, Lutgehetmann M. Mouse models of hepatitis B and delta virus infection. J Immunol Methods. 2014; 410:39–49. [PubMed: 24631647]
7. Liang TJ, Ghany MG. Current and future therapies for hepatitis C virus infection. N Engl J Med. 2013; 368:1907–17. [PubMed: 23675659]
8. Pawlotsky JM. New antiviral agents for hepatitis C. F1000 Biol Rep. 2012; 4:5. [PubMed: 22403588]
9. Hoofnagle JH, Sherker AH. Therapy for hepatitis C--the costs of success. N Engl J Med. 2014; 370:1552–3. [PubMed: 24725236]
10. Man John Law L, Landi A, Magee WC, Lorne Tyrrell D, Houghton M. Progress towards a hepatitis C virus vaccine. Emerg Microbes Infect. 2013; 2:e79. [PubMed: 26038445]
11. Seeger C, Mason WS. Hepatitis B virus biology. Microbiol Mol Biol Rev. 2000; 64:51–68. [PubMed: 10704474]
12. Dienstag JL. Hepatitis B virus infection. N Engl J Med. 2008; 359:1486–500. [PubMed: 18832247]
13. Levrero M, Pollicino T, Petersen J, Belloni L, Raimondo G, Dandri M. Control of cccDNA function in hepatitis B virus infection. Journal of hepatology. 2009; 51:581–92. [PubMed: 19616338]
14. Belloni L, Pollicino T, De Nicola F, Guerrieri F, Raffa G, Fanciulli M, Raimondo G, Levrero M. Nuclear HBx binds the HBV minichromosome and modifies the epigenetic regulation of cccDNA function. Proceedings of the National Academy of Sciences of the United States of America. 2009; 106:19975–9. [PubMed: 19906987]
15. Petersen J, Dandri M, Mier W, Lutgehetmann M, Volz T, von Weizsacker F, Haberkorn U, Fischer L, Pollok JM, Erbes B, Seitz S, Urban S. Prevention of hepatitis B virus infection in vivo by entry inhibitors derived from the large envelope protein. Nat Biotechnol. 2008; 26:335–41. [PubMed: 18297057]
16. Schmitz A, Schwarz A, Foss M, Zhou L, Rabe B, Hoellenriegel J, Stoeber M, Pante N, Kann M. Nucleoporin 153 arrests the nuclear import of hepatitis B virus capsids in the nuclear basket. PLoS Pathog. 2010; 6:e1000741. [PubMed: 20126445]
17. Watanabe T, Sorensen EM, Naito A, Schott M, Kim S, Ahlquist P. Involvement of host cellular multivesicular body functions in hepatitis B virus budding. Proceedings of the National Academy of Sciences of the United States of America. 2007; 104:10205–10. [PubMed: 17551004]
18. Jiang B, Himmelsbach K, Ren H, Boller K, Hildt E. Subviral hepatitis B virus filaments are released like infectious viral particles via multivesicular bodies. Journal of virology. 2015
19. Taylor JM. Virology of hepatitis D virus. Semin Liver Dis. 2012; 32:195–200. [PubMed: 22932967]
20. Chang J, Gudima SO, Tarn C, Nie X, Taylor JM. Development of a novel system to study hepatitis delta virus genome replication. Journal of virology. 2005; 79:8182–8. [PubMed: 15956563]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

21. Hughes SA, Wedemeyer H, Harrison PM. Hepatitis delta virus. Lancet. 2011; 378:73–85. [PubMed: 21511329]
22. Taylor J. Hepatitis delta virus. Journal of hepatology. 1991; 13(Suppl 4):S114–5. [PubMed: 1822502]
23. Taylor J, Pelchat M. Origin of hepatitis delta virus. Future Microbiol. 2010; 5:393–402. [PubMed: 20210550]
24. Moradpour D, Penin F, Rice CM. Replication of hepatitis C virus. Nat Rev Microbiol. 2007; 5:453–63. [PubMed: 17487147]
25. Lindenbach BD, Rice CM. The ins and outs of hepatitis C virus entry and assembly. Nat Rev Microbiol. 2013; 11:688–700. [PubMed: 24018384]
26. Targett-Adams P, Boulant S, Douglas MW, McLauchlan J. Lipid metabolism and HCV infection. Viruses. 2010; 2:1195–217. [PubMed: 21994676]
27. Popescu CI, Rouille Y, Dubuisson J. Hepatitis C virus assembly imaging. Viruses. 2011; 3:2238–54. [PubMed: 22163343]
28. Gastaminza P, Cheng G, Wieland S, Zhong J, Liao W, Chisari FV. Cellular determinants of hepatitis C virus assembly, maturation, degradation, and secretion. J Virol. 2008; 82:2120–9. [PubMed: 18077707]
29. Rizzetto M, Canese MG, Gerin JL, London WT, Sly DL, Purcell RH. Transmission of the hepatitis B virus-associated delta antigen to chimpanzees. J Infect Dis. 1980; 141:590–602. [PubMed: 6989929]
30. Bukh J. A critical role for the chimpanzee model in the study of hepatitis C. Hepatology. 2004; 39:1469–75. [PubMed: 15185284]
31. Thimme R, Wieland S, Steiger C, Ghrayeb J, Reimann KA, Purcell RH, Chisari FV. CD8(+) T cells mediate viral clearance and disease pathogenesis during acute hepatitis B virus infection. J Virol. 2003; 77:68–76. [PubMed: 12477811]
32. Fields HA, Govindarajan S, Margolis HS, Schable CD, Ebert JW, Maynard JE. Experimental transmission of the delta virus to a hepatitis B chronic carrier chimpanzee with the development of persistent delta carriage. Am J Pathol. 1986; 122:308–14. [PubMed: 3511725]
33. Govindarajan S, Fields HA, Humphrey CD, Margolis HS. Pathologic and ultrastructural changes of acute and chronic delta hepatitis in an experimentally infected chimpanzee. Am J Pathol. 1986; 122:315–22. [PubMed: 3511726]
34. Billerbeck E, de Jong Y, Dorner M, de la Fuente C, Ploss A. Animal models for hepatitis C. Current topics in microbiology and immunology. 2013; 369:49–86. [PubMed: 23463197]
35. Guidotti LG, Chisari FV. Immunobiology and pathogenesis of viral hepatitis. Annu Rev Pathol. 2006; 1:23–61. [PubMed: 18039107]
36. Bukh J. Animal models for the study of hepatitis C virus infection and related liver disease. Gastroenterology. 2012; 142:1279–1287 e3. [PubMed: 22537434]
37. Ploss A, Rice CM. Towards a small animal model for hepatitis C. EMBO Rep. 2009; 10:1220–7. [PubMed: 19834510]
38. Altevogt, BM.; Pankevich, DE.; Shelton-Davenport, MK.; Kahn, JP., editors. Chimpanzees in Biomedical and Behavioral Research: Assessing the Necessity. Institute of Medicine and National Research Council of the National Academies; Washington, D.C: 2011.
39. Xu X, Chen H, Cao X, Ben K. Efficient infection of tree shrew (Tupaia belangeri) with hepatitis C virus grown in cell culture or from patient plasma. The Journal of general virology. 2007; 88:2504–12. [PubMed: 17698660]
40. Walter E, Keist R, Niederost B, Pult I, Blum HE. Hepatitis B virus infection of tupaia hepatocytes in vitro and in vivo. Hepatology. 1996; 24:1–5. [PubMed: 8707245]
41. Kock J, Nassal M, MacNelly S, Baumert TF, Blum HE, von Weizsacker F. Efficient infection of primary tupaia hepatocytes with purified human and woolly monkey hepatitis B virus. Journal of virology. 2001; 75:5084–9. [PubMed: 11333889]
42. Amako Y, Tsukiyama-Kohara K, Katsume A, Hirata Y, Sekiguchi S, Tobita Y, Hayashi Y, Hishima T, Funata N, Yonekawa H, Kohara M. Pathogenesis of hepatitis C virus infection in Tupaia belangeri. J Virol. 2009; 84:303–11. [PubMed: 19846521]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

43. Bukh J, Apgar CL, Yanagi M. Toward a surrogate model for hepatitis C virus: An infectious molecular clone of the GB virus-B hepatitis agent. Virology. 1999; 262:470–8. [PubMed: 10502525]
44. Kapoor A, Simmonds P, Gerold G, Qaisar N, Jain K, Henriquez JA, Firth C, Hirschberg DL, Rice CM, Shields S, Lipkin WI. Characterization of a canine homolog of hepatitis C virus. Proc Natl Acad Sci U S A. 2011; 108:11608–13. [PubMed: 21610165]
45. Burbelo PD, Dubovi EJ, Simmonds P, Medina JL, Henriquez JA, Mishra N, Wagner J, Tokarz R, Cullen JM, Iadarola MJ, Rice CM, Lipkin WI, Kapoor A. Serology enabled discovery of genetically diverse hepaciviruses in a new host. J Virol. 2012
46. Kapoor A, Simmonds P, Cullen JM, Scheel TK, Medina JL, Giannitti F, Nishiuchi E, Brock KV, Burbelo PD, Rice CM, Lipkin WI. Identification of a pegivirus (GB virus-like virus) that infects horses. J Virol. 2013; 87:7185–90. [PubMed: 23596285]
47. Kapoor A, Simmonds P, Scheel TK, Hjelle B, Cullen JM, Burbelo PD, Chauhan LV, Duraisamy R, Sanchez Leon M, Jain K, Vandegrift KJ, Calisher CH, Rice CM, Lipkin WI. Identification of rodent homologs of hepatitis C virus and pegiviruses. mBio. 2013; 4:e00216–13. [PubMed: 23572554]
48. Firth C, Bhat M, Firth MA, Williams SH, Frye MJ, Simmonds P, Conte JM, Ng J, Garcia J, Bhuva NP, Lee B, Che X, Quan PL, Lipkin WI. Detection of zoonotic pathogens and characterization of novel viruses carried by commensal Rattus norvegicus in New York City. mBio. 2014; 5:e01933–14. [PubMed: 25316698]
49. Pfaender S, Cavalleri JM, Walter S, Doerrbecker J, Campana B, Brown RJ, Burbelo PD, Postel A, Hahn K, Kusuma A, Riebesehl N, Baumgartner W, Becher P, Heim MH, Pietschmann T, Feige K, Steinmann E. Clinical course of infection and viral tissue tropism of hepatitis C virus-like non-primate hepaciviruses. Hepatology. 2014
50. Pfaender S, Walter S, Todt D, Behrendt P, Doerrbecker J, Wolk B, Engelmann M, Gravemann U, Seltsam A, Steinmann J, Burbelo PD, Klawonn F, Feige K, Pietschmann T, Cavalleri JM, Steinmann E. Assessment of cross-species transmission of hepatitis C virus-related non-primate hepacivirus in a population of humans at high risk of exposure. J Gen Virol. 2015; 96:2636–42. [PubMed: 26041875]
51. Molnar-Kimber KL, Summers J, Taylor JM, Mason WS. Protein covalently bound to minus-strand DNA intermediates of duck hepatitis B virus. Journal of virology. 1983; 45:165–72. [PubMed: 6823008]
52. Molnar-Kimber KL, Summers JW, Mason WS. Mapping of the cohesive overlap of duck hepatitis B virus DNA and of the site of initiation of reverse transcription. Journal of virology. 1984; 51:181–91. [PubMed: 6328037]
53. Summers J, Mason WS. Replication of the genome of a hepatitis B--like virus by reverse transcription of an RNA intermediate. Cell. 1982; 29:403–15. [PubMed: 6180831]
54. Summers J, Smith PM, Horwich AL. Hepadnavirus envelope proteins regulate covalently closed circular DNA amplification. Journal of virology. 1990; 64:2819–24. [PubMed: 2335817]
55. Tuttleman JS, Pourcel C, Summers J. Formation of the pool of covalently closed circular viral DNA in hepadnavirus-infected cells. Cell. 1986; 47:451–60. [PubMed: 3768961]
56. Wu TT, Coates L, Aldrich CE, Summers J, Mason WS. In hepatocytes infected with duck hepatitis B virus, the template for viral RNA synthesis is amplified by an intracellular pathway. Virology. 1990; 175:255–61. [PubMed: 2155510]
57. Summers J, Smolec JM, Snyder R. A virus similar to human hepatitis B virus associated with hepatitis and hepatoma in woodchucks. Proceedings of the National Academy of Sciences of the United States of America. 1978; 75:4533–7. [PubMed: 212758]
58. Korba BE, Wells FV, Baldwin B, Cote PJ, Tennant BC, Popper H, Gerin JL. Hepatocellular carcinoma in woodchuck hepatitis virus-infected woodchucks: presence of viral DNA in tumor tissue from chronic carriers and animals serologically recovered from acute infections. Hepatology. 1989; 9:461–70. [PubMed: 2465987]
59. Galibert F, Chen TN, Mandart E. Nucleotide sequence of a cloned woodchuck hepatitis virus genome: comparison with the hepatitis B virus sequence. Journal of virology. 1982; 41:51–65. [PubMed: 7086958]

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60. Jacob JR, Korba BE, Cote PJ, Toshkov I, Delaney WEt, Gerin JL, Tennant BC. Suppression of lamivudine-resistant B-domain mutants by adefovir dipivoxil in the woodchuck hepatitis virus model. Antiviral Res. 2004; 63:115–21. [PubMed: 15302140]
61. Korba BE, Cote P, Hornbuckle W, Tennant BC, Gerin JL. Treatment of chronic woodchuck hepatitis virus infection in the Eastern woodchuck (Marmota monax) with nucleoside analogues is predictive of therapy for chronic hepatitis B virus infection in humans. Hepatology. 2000; 31:1165–75. [PubMed: 10796894]
62. Kidd-Ljunggren K, Miyakawa Y, Kidd AH. Genetic variability in hepatitis B viruses. J Gen Virol. 2002; 83:1267–80. [PubMed: 12029141]
63. Drexler JF, Geipel A, Konig A, Corman VM, van Riel D, Leijten LM, Bremer CM, Rasche A, Cottontail VM, Maganga GD, Schlegel M, Muller MA, Adam A, Klose SM, Carneiro AJ, Stocker A, Franke CR, Gloza-Rausch F, Geyer J, Annan A, Adu-Sarkodie Y, Oppong S, Binger T, Vallo P, Tschapka M, Ulrich RG, Gerlich WH, Leroy E, Kuiken T, Glebe D, Drosten C. Bats carry pathogenic hepadnaviruses antigenically related to hepatitis B virus and capable of infecting human hepatocytes. Proceedings of the National Academy of Sciences of the United States of America. 2013; 110:16151–6. [PubMed: 24043818]
64. Lucifora J, Arzberger S, Durantel D, Belloni L, Strubin M, Levrero M, Zoulim F, Hantz O, Protzer U. Hepatitis B virus X protein is essential to initiate and maintain virus replication after infection. Journal of hepatology. 2011; 55:996–1003. [PubMed: 21376091]
65. Yan H, Zhong G, Xu G, He W, Jing Z, Gao Z, Huang Y, Qi Y, Peng B, Wang H, Fu L, Song M, Chen P, Gao W, Ren B, Sun Y, Cai T, Feng X, Sui J, Li W. Sodium taurocholate cotransporting polypeptide is a functional receptor for human hepatitis B and D virus. eLife. 2012; 1:e00049. [PubMed: 23150796]
66. Yan H, Peng B, Liu Y, Xu G, He W, Ren B, Jing Z, Sui J, Li W. Viral entry of hepatitis B and D viruses and bile salts transportation share common molecular determinants on sodium taurocholate cotransporting polypeptide. Journal of virology. 2014; 88:3273–84. [PubMed: 24390325]
67. Lempp FA, Mutz P, Lipps C, Wirth D, Bartenschlager R, Urban S. Evidence that hepatitis B virus replication in mouse cells is limited by the lack of a host cell dependency factor. Journal of hepatology. 2015
68. Yan H, Peng B, He W, Zhong G, Qi Y, Ren B, Gao Z, Jing Z, Song M, Xu G, Sui J, Li W. Molecular determinants of hepatitis B and D virus entry restriction in mouse sodium taurocholate cotransporting polypeptide. J Virol. 2013; 87:7977–91. [PubMed: 23678176]
69. Raney AK, Eggers CM, Kline EF, Guidotti LG, Pontoglio M, Yaniv M, McLachlan A. Nuclear covalently closed circular viral genomic DNA in the liver of hepatocyte nuclear factor 1 alpha-null hepatitis B virus transgenic mice. Journal of virology. 2001; 75:2900–11. [PubMed: 11222715]
70. He W, Ren B, Mao F, Jing Z, Li Y, Liu Y, Peng B, Yan H, Qi Y, Sun Y, Guo JT, Sui J, Wang F, Li W. Hepatitis D Virus Infection of Mice Expressing Human Sodium Taurocholate Co-transporting Polypeptide. PLoS Pathog. 2015; 11:e1004840. [PubMed: 25902143]
71. Ploss A, Evans MJ. Hepatitis C virus host cell entry. Current opinion in virology. 2012; 2:14–9. [PubMed: 22440961]
72. Long G, Hiet MS, Windisch MP, Lee JY, Lohmann V, Bartenschlager R. Mouse hepatic cells support assembly of infectious hepatitis C virus particles. Gastroenterology. 2011
73. Vogt A, Scull MA, Friling T, Horwitz JA, Donovan BM, Dorner M, Gerold G, Labitt RN, Rice CM, Ploss A. Recapitulation of the hepatitis C virus life-cycle in engineered murine cell lines. Virology. 2013; 444:1–11. [PubMed: 23777661]
74. Ding Q, von Schaewen M, Ploss A. The impact of hepatitis C virus entry on viral tropism. Cell Host Microbe. 2014; 16:562–8. [PubMed: 25525789]
75. Ploss A, Evans MJ, Gaysinskaya VA, Panis M, You H, de Jong YP, Rice CM. Human occludin is a hepatitis C virus entry factor required for infection of mouse cells. Nature. 2009; 457:882–6. [PubMed: 19182773]
76. Dorner M, Horwitz JA, Robbins JB, Barry WT, Feng Q, Mu K, Jones CT, Schoggins JW, Catanese MT, Burton DR, Law M, Rice CM, Ploss A. A genetically humanized mouse model for hepatitis C virus infection. Nature. 2011; 474:208–11. [PubMed: 21654804]

77. Dorner M, Horwitz JA, Donovan BM, Labitt RN, Budell WC, Friling T, Vogt A, Catanese MT, Taniguchi T, Satoh T, Kawai T, Akira S, Law M, Rice CM, Ploss A. Completion of the entire hepatitis C virus life-cycle in genetically humanized mice. 2013 in revision.

78. Zhu Q, Guo JT, Seeger C. Replication of hepatitis C virus subgenomes in nonhepatic epithelial and mouse hepatoma cells. J Virol. 2003; 77:9204–10. [PubMed: 12915536]

79. Uprichard SL, Chung J, Chisari FV, Wakita T. Replication of a hepatitis C virus replicon clone in mouse cells. Virol J. 2006; 3:89. [PubMed: 17069661]

80. Frentzen A, Huging K, Bitzegeio J, Friesland M, Haid S, Gentzsch J, Hoffmann M, Lindemann D, Zimmer G, Zielecki F, Weber F, Steinmann E, Pietschmann T. Completion of Hepatitis C Virus Replication Cycle in Heterokaryons Excludes Dominant Restrictions in Human Non-liver and Mouse Liver Cell Lines. PLoS Pathog. 2011; 7:e1002029. [PubMed: 21552323]

81. Moriya K, Fujie H, Shintani Y, Yotsuyanagi H, Tsutsumi T, Ishibashi K, Matsuura Y, Kimura S, Miyamura T, Koike K. The core protein of hepatitis C virus induces hepatocellular carcinoma in transgenic mice. Nat Med. 1998; 4:1065–7. [PubMed: 9734402]

82. Moriya K, Nakagawa K, Santa T, Shintani Y, Fujie H, Miyoshi H, Tsutsumi T, Miyazawa T, Ishibashi K, Horie T, Imai K, Todoroki T, Kimura S, Koike K. Oxidative stress in the absence of inflammation in a mouse model for hepatitis C virus-associated hepatocarcinogenesis. Cancer Res. 2001; 61:4365–70. [PubMed: 11389061]

83. Lerat H, Honda M, Beard MR, Loesch K, Sun J, Yang Y, Okuda M, Gosert R, Xiao SY, Weinman SA, Lemon SM. Steatosis and liver cancer in transgenic mice expressing the structural and nonstructural proteins of hepatitis C virus. Gastroenterology. 2002; 122:352–65. [PubMed: 11832450]

84. Machida K, Tsukiyama-Kohara K, Seike E, Tone S, Shibasaki F, Shimizu M, Takahashi H, Hayashi Y, Funata N, Taya C, Yonekawa H, Kohara M. Inhibition of cytochrome c release in Fas-mediated signaling pathway in transgenic mice induced to express hepatitis C viral proteins. J Biol Chem. 2001; 276:12140–6. [PubMed: 11278624]

85. Disson O, Haouzi D, Desagher S, Loesch K, Hahne M, Kremer EJ, Jacquet C, Lemon SM, Hibner U, Lerat H. Impaired clearance of virus-infected hepatocytes in transgenic mice expressing the hepatitis C virus polyprotein. Gastroenterology. 2004; 126:859–72. [PubMed: 14988840]

86. Alonzi T, Agrati C, Costabile B, Cicchini C, Amicone L, Cavallari C, Rocca CD, Folgori A, Fipaldini C, Poccia F, Monica NL, Tripodi M. Steatosis and intrahepatic lymphocyte recruitment in hepatitis C virus transgenic mice. J Gen Virol. 2004; 85:1509–20. [PubMed: 15166435]

87. Chisari FV. Hepatitis B virus transgenic mice: models of viral immunobiology and pathogenesis. Curr Top Microbiol Immunol. 1996; 206:149–173. [PubMed: 8608715]

88. Guidotti LG, Matzke B, Schaller H, Chisari FV. High-level hepatitis B virus replication in transgenic mice. Journal of virology. 1995; 69:6158–69. [PubMed: 7666518]

89. Cui X, Guo JT, Hu J. Hepatitis B Virus Covalently Closed Circular DNA Formation in Immortalized Mouse Hepatocytes Associated with Nucleocapsid Destabilization. Journal of virology. 2015; 89:9021–8. [PubMed: 26085156]

90. Heermann KH, Goldmann U, Schwartz W, Seyffarth T, Baumgarten H, Gerlich WH. Large surface proteins of hepatitis B virus containing the pre-s sequence. Journal of virology. 1984; 52:396–402. [PubMed: 6492255]

91. Chisari FV, Filippi P, McLachlan A, Milich DR, Riggs M, Lee S, Palmiter RD, Pinkert CA, Brinster RL. Expression of hepatitis B virus large envelope polypeptide inhibits hepatitis B surface antigen secretion in transgenic mice. Journal of virology. 1986; 60:880–7. [PubMed: 3783819]

92. Kim CM, Koike K, Saito I, Miyamura T, Jay G. HBx gene of hepatitis B virus induces liver cancer in transgenic mice. Nature. 1991; 351:317–20. [PubMed: 2034275]

93. Ye L, Yu H, Li C, Hirsch ML, Zhang L, Samulski RJ, Li W, Liu Z. Adeno-Associated Virus Vector Mediated Delivery of the HBV Genome Induces Chronic Hepatitis B Virus Infection and Liver Fibrosis in Mice. PLoS One. 2015; 10:e0130052. [PubMed: 26075890]

94. Dion S, Bourgine M, Godon O, Levillayer F, Michel ML. Adeno-associated virus-mediated gene transfer leads to persistent hepatitis B virus replication in mice expressing HLA-A2 and HLA-DR1 molecules. Journal of virology. 2013; 87:5554–63. [PubMed: 23468504]

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95. Yang D, Liu L, Zhu D, Peng H, Su L, Fu YX, Zhang L. A mouse model for HBV immunotolerance and immunotherapy. Cell Mol Immunol. 2014; 11:71–8. [PubMed: 24076617]
96. Bissig KD, Wieland SF, Tran P, Isogawa M, Le TT, Chisari FV, Verma IM. Human liver chimeric mice provide a model for hepatitis B and C virus infection and treatment. J Clin Invest. 2010; 120:924–30. [PubMed: 20179355]
97. Brehm MA, Jouvet N, Greiner DL, Shultz LD. Humanized mice for the study of infectious diseases. Curr Opin Immunol. 2013; 25:428–35. [PubMed: 23751490]
98. Billerbeck E, de Jong Y, Dorner M, de la Fuente C, Ploss A. Animal models for hepatitis C. Current topics in microbiology and immunology. 2013; 369:49–86. [PubMed: 23463197]
99. Heckel JL, Sandgren EP, Degen JL, Palmiter RD, Brinster RL. Neonatal bleeding in transgenic mice expressing urokinase-type plasminogen activator. Cell. 1990; 62:447–56. [PubMed: 1696178]
100. Azuma H, Paulk N, Ranade A, Dorrell C, Al-Dhalimy M, Ellis E, Strom S, Kay MA, Finegold M, Grompe M. Robust expansion of human hepatocytes in Fah–/–/Rag2–/–/Il2rg–/– mice. Nat Biotechnol. 2007; 25:903–10. [PubMed: 17664939]
101. Bissig KD, Le TT, Woods NB, Verma IM. Repopulation of adult and neonatal mice with human hepatocytes: a chimeric animal model. Proc Natl Acad Sci U S A. 2007; 104:20507–11. [PubMed: 18077355]
102. de Jong YP, Dorner M, Mommersteeg MC, Xiao JW, Balazs AB, Robbins JB, Winer BY, Gerges S, Vega K, Labitt RN, Donovan BM, Giang E, Krishnan A, Chiriboga L, Charlton MR, Burton DR, Baltimore D, Law M, Rice CM, Ploss A. Broadly neutralizing antibodies abrogate established hepatitis C virus infection. Sci Transl Med. 2014; 6:254ra129.
103. Grompe M. The pathophysiology and treatment of hereditary tyrosinemia type 1. Semin Liver Dis. 2001; 21:563–71. [PubMed: 11745044]
104. Al-Dhalimy M, Overturf K, Finegold M, Grompe M. Long-term therapy with NTBC and tyrosine-restricted diet in a murine model of hereditary tyrosinemia type I. Mol Genet Metab. 2002; 75:38–45. [PubMed: 11825062]
105. Hasegawa M, Kawai K, Mitsui T, Taniguchi K, Monnai M, Wakui M, Ito M, Suematsu M, Peltz G, Nakamura M, Suemizu H. The reconstituted 'humanized liver' in TK-NOG mice is mature and functional. Biochem Biophys Res Commun. 2011; 405:405–10. [PubMed: 21238430]
106. Mercer DF, Schiller DE, Elliott JF, Douglas DN, Hao C, Rinfret A, Addison WR, Fischer KP, Churchill TA, Lakey JR, Tyrrell DL, Kneteman NM. Hepatitis C virus replication in mice with chimeric human livers. Nat Med. 2001; 7:927–33. [PubMed: 11479625]
107. Kosaka K, Hiraga N, Imamura M, Yoshimi S, Murakami E, Nakahara T, Honda Y, Ono A, Kawaoka T, Tsuge M, Abe H, Hayes CN, Miki D, Aikata H, Ochi H, Ishida Y, Tateno C, Yoshizato K, Sasaki T, Chayama K. A novel TK-NOG based humanized mouse model for the study of HBV and HCV infections. Biochemical and biophysical research communications. 2013; 441:230–5. [PubMed: 24140055]
108. Dandri M, Burda MR, Torok E, Pollok JM, Iwanska A, Sommer G, Rogiers X, Rogler CE, Gupta S, Will H, Greten H, Petersen J. Repopulation of mouse liver with human hepatocytes and in vivo infection with hepatitis B virus. Hepatology. 2001; 33:981–8. [PubMed: 11283864]
109. Meuleman P, Libbrecht L, De Vos R, de Hemptinne B, Gevaert K, Vandekerckhove J, Roskams T, Leroux-Roels G. Morphological and biochemical characterization of a human liver in a uPA-SCID mouse chimera. Hepatology. 2005; 41:847–56. [PubMed: 15791625]
110. Lindenbach BD, Meuleman P, Ploss A, Vanwolleghem T, Syder AJ, McKeating JA, Lanford RE, Feinstone SM, Major ME, Leroux-Roels G, Rice CM. Cell culture-grown hepatitis C virus is infectious in vivo and can be recultured in vitro. Proc Natl Acad Sci U S A. 2006; 103:3805–9. [PubMed: 16484368]
111. Giersch K, Helbig M, Volz T, Allweiss L, Mancke LV, Lohse AW, Polywka S, Pollok JM, Petersen J, Taylor J, Dandri M, Lutgehetmann M. Persistent hepatitis D virus mono-infection in humanized mice is efficiently converted by hepatitis B virus to a productive co-infection. Journal of hepatology. 2014; 60:538–44. [PubMed: 24280293]
112. Belloni L, Allweiss L, Guerrieri F, Pediconi N, Volz T, Pollicino T, Petersen J, Raimondo G, Dandri M, Levrero M. IFN-alpha inhibits HBV transcription and replication in cell culture and in

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

humanized mice by targeting the epigenetic regulation of the nuclear cccDNA minichromosome. J Clin Invest. 2012; 122:529–37. [PubMed: 22251702]

113. Strom SC, Davila J, Grompe M. Chimeric mice with humanized liver: tools for the study of drug metabolism, excretion, and toxicity. Methods Mol Biol. 2010; 640:491–509. [PubMed: 20645070]

114. Strick-Marchand H, Dusseaux M, Darche S, Huntington ND, Legrand N, Masse-Ranson G, Corcuff E, Ahodantin J, Weijer K, Spits H, Kremsdorf D, Di Santo JP. A novel mouse model for stable engraftment of a human immune system and human hepatocytes. PLoS One. 2015; 10:e0119820. [PubMed: 25782010]

115. Shultz LD, Brehm MA, Garcia-Martinez JV, Greiner DL. Humanized mice for immune system investigation: progress, promise and challenges. Nature reviews Immunology. 2012; 12:786–98.

116. Haridass D, Yuan Q, Becker PD, Cantz T, Iken M, Rothe M, Narain N, Bock M, Norder M, Legrand N, Wedemeyer H, Weijer K, Spits H, Manns MP, Cai J, Deng H, Di Santo JP, Guzman CA, Ott M. Repopulation efficiencies of adult hepatocytes, fetal liver progenitor cells, and embryonic stem cell-derived hepatic cells in albumin-promoter-enhancer urokinase-type plasminogen activator mice. Am J Pathol. 2009; 175:1483–92. [PubMed: 19717639]

117. Washburn ML, Bility MT, Zhang L, Kovalev GI, Buntzman A, Frelinger JA, Barry W, Ploss A, Rice CM, Su L. A Humanized Mouse Model to Study Hepatitis C Virus Infection, Immune Response, and Liver Disease. Gastroenterology. 2011

118. Bility MT, Cheng L, Zhang Z, Luan Y, Li F, Chi L, Zhang L, Tu Z, Gao Y, Fu Y, Niu J, Wang F, Su L. Hepatitis B virus infection and immunopathogenesis in a humanized mouse model: induction of human-specific liver fibrosis and M2-like macrophages. PLoS Pathog. 2014; 10:e1004032. [PubMed: 24651854]

119. Carpentier A, Tesfaye A, Chu V, Nimgaonkar I, Zhang F, Lee SB, Thorgeirsson SS, Feinstone SM, Liang TJ. Engrafted human stem cell-derived hepatocytes establish an infectious HCV murine model. J Clin Invest. 2014; 124:4953–64. [PubMed: 25295540]

120. Gutti TL, Knibbe JS, Makarov E, Zhang J, Yannam GR, Gorantla S, Sun Y, Mercer DF, Suemizu H, Wisecarver JL, Osna NA, Bronich TK, Poluektova LY. Human hepatocytes and hematolymphoid dual reconstitution in treosulfan-conditioned uPA-NOG mice. Am J Pathol. 2014; 184:101–9. [PubMed: 24200850]

121. Wilson EM, Bial J, Tarlow B, Bial G, Jensen B, Greiner DL, Brehm MA, Grompe M. Extensive double humanization of both liver and hematopoiesis in FRGN mice. Stem cell research. 2014; 13:404–412. [PubMed: 25310256]

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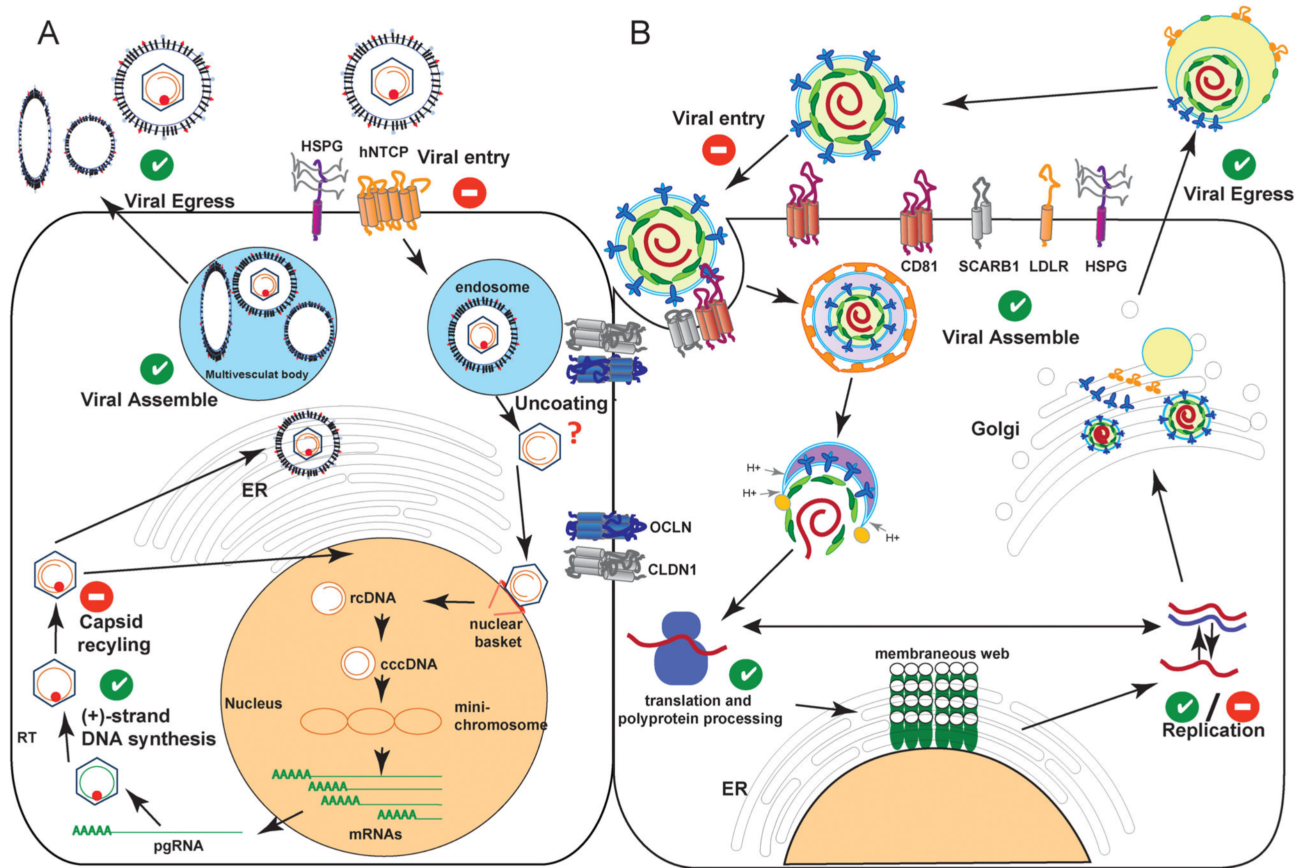

Figure 1.

A. Barriers to HBV species tropism. Mouse cells do not support the HBV life cycle. The expression of hNTCP renders mouse hepatocytes susceptible to HDV infection but not HBV infection. Blocks may/do occur (red question marks or dashes, respectively) at the level of nucleocapsid un-coating and also in formation/maintenance of HBV cccDNA. However, the mechanisms of HBV virion assembly and egress are functional in murine hepatocytes (green check marks). **B. Barriers to HCV species tropism.** Mouse cells inefficiently support the HCV life cycle. The expression of human CD81 and OCLN can facilitate the entry of viral particles into mouse cells, and HCV RNA translation is supported, whereas HCV RNA replication occurs inefficiently. Virion assembly and release can take place in mouse cells. hNTCP, human sodium taurocholate co-transporting polypeptide; rcDNA, relaxed circular DNA; cccDNA, covalently closed circular DNA; pgRNA, pre-genomic RNA; RT, reverse transcription; HSPG, heparan sulfate proteoglycan; LDLR, low density lipoprotein receptor; CLDN1, claudin 1; OCLN, occludin.

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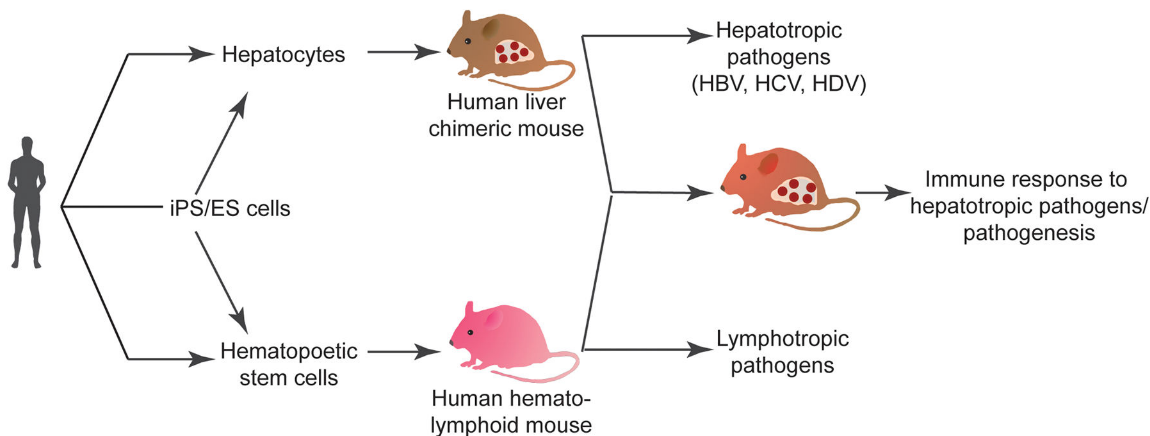

Figure 2. Xenotransplantation approaches for studying pathogenesis of hepatotropic viruses

Hepatocytes and HSCs can be isolated from human subjects. The transplantation of these cells into appropriate xenorecipient mice gives rise to human liver chimeric mice or to human hemato-lymphoid mice, respectively. Alternatively, directed differentiation approaches allow the generation of hepatocyte-like cells and HSCs from human iPS or ES cells. However, these cells engraft considerably less efficiently in commonly used xenerecipient strains. Hemato-lymphoid mice can be used to study a variety of human (lympho-) tropic viruses. Human liver chimeric mice can be used to study hepatotropic pathogens. Dually engrafted mice, which harbor both a chimeric human-mouse liver and a human immune system, can be utilized to investigate the immune response of hepatotropic viruses. HSC, hematopoietic stem cells; iPS, induced pluripotent stem cells; ES, embryonic stem cells.