HHS Public Access

Author manuscript

Antiviral Res. Author manuscript; available in PMC 2016 September 01.

Published in final edited form as:

Antiviral Res. 2015 September ; 121: 1–8. doi:10.1016/j.antiviral.2015.06.012.

Modeling hepatitis B virus infection, immunopathology and therapy in mice

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Abstract

Despite the availability of a preventive vaccine, chronic hepatitis B virus (HBV) infection-induced liver diseases continue to be a major global public health problem. HBV naturally infects only humans and chimpanzees. This narrow host range has hindered our ability to study the characteristics of the virus and how it interacts with its host. It is thus important to establish small animal models to study HBV infection, persistence, clearance and the immunopathogenesis of chronic hepatitis B. In this review, we briefly summarize currently available animal models for HBV research, then focus on mouse models, especially the recently developed humanized mice that can support HBV infection and immunopathogenesis *in vivo*. This article is part of a symposium in *Antiviral Research* on “From the discovery of the Australia antigen to the development of new curative therapies for hepatitis B: an unfinished story.”

Keywords

Hepatitis B virus; Immunopathogenesis; antiviral therapy; mouse model

1. Introduction

Approximately 350 million people are chronically infected with hepatitis B virus (HBV), putting them at high risk of developing liver fibrosis/cirrhosis and eventually developing hepatocellular carcinoma (HCC) over several decades (Scaglione and Lok, 2012). The impaired immune response to viral antigens during chronic HBV infection is associated with persistent liver inflammation which leads to liver diseases (Guidotti and Chisari, 2006). The lack of robust animal models has hindered our understanding of how HBV interacts with host restriction factors to establish chronic infection (Bility et al., 2013). Moreover, our knowledge about how chronic infection leads to liver injury, cirrhosis and cancer, is also limited.

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HBV can only naturally infect humans and chimpanzees. To overcome this experimental limitation, several small animal models, such as HBV transgenic mice, the hydrodynamic-based HBV DNA transfection model, the viral vector-based HBV genome transduction model and the human-mouse liver/immune system chimeric model of HBV replication and infection have been developed. These models should help us to understand the immunopathogenesis of chronic hepatitis B and should greatly facilitate the development of new treatments. In this review, we first briefly introduce the contribution of the chimpanzee and tree shrew models, then focus on progress made in the development of mouse models, especially the recently developed human-murine chimeric mouse models, for studying hepatitis B biology and therapy.

2. Contributions of the chimpanzee and tree shrew models to HBV research

The chimpanzee is susceptible to HBV infection, and has been employed to study pathogenesis, host immune responses and the evaluation of potential therapies since the 1970s. Inoculation of chimpanzees with serum from human HBV carriers induced acute infection and hepatitis (Barker et al., 1973; Berquist et al., 1975). Chimpanzees played an essential role in evaluating the efficacy and safety of the yeast-produced recombinant HBsAg vaccine (McAleer et al., 1984).

The chimpanzee model has also contributed substantially to our understanding of HBV virus-host interactions. Genomic analysis of virus-induced and immune response-related genes in the liver of acutely infected chimpanzees indicated that HBV does not significantly induce an innate immune response during entry and expansion (Wieland et al., 2004). In contrast, a large number of T cell-derived IFN-γ-regulated genes are detected in the liver during viral clearance, reflecting the importance of an adaptive immune response in the control of HBV infection (Wieland et al., 2004). The immunological priming and outcome of infection was also dependent on the size of the HBV inoculum (Asabe et al., 2009). *In vivo* depletion experiments demonstrated that CD8 T cells, but not CD4 T cells were the main effector cells responsible for HBV clearance and pathogenesis during acute HBV infection in chimpanzees (Thimme et al., 2003). Viral clearance was shown to be mediated by both cytolytic and noncytolytic effector functions of the effector CD8 T cells (Guidotti and Chisari, 2001; Guidotti et al., 1999).

Chimpanzees can also be chronically infected with HBV, and chronic infections are associated with persistent inflammation in the liver, although the degree of the disease appears to be much milder than in humans (Shouval et al., 1980). This chronic infection model provides a platform for testing antiviral drugs. A recent example is the toll-like receptor 7 agonist (GS-9620), which activates signaling in immune cells of chronically HBV-infected chimpanzees to induce clearance of HBV-infected cells (Lanford et al., 2013). Though the chimpanzee model provides a unique platform for HBV research, the use of this model for basic research and drug testing is restricted for ethical and also economic reasons. The necessity to use chimpanzees for preclinical research was recently reassessed in the USA, where the Institute of Medicine concluded that recent advances in cell-based models and in small animal models with human cells rendered the use of chimpanzees unnecessary (Wadman, 2011).

In addition to higher primates, the tree shrew is the only other animal that can be experimentally infected with HBV (Walter et al., 1996; Yan et al., 1996). HBV infection in tree shrew results in viral replication in the liver, HBsAg secretion in the serum and subsequent appearance of anti-HBe and anti-HBs antibodies, recapitulating many aspects of self-limited acute hepatitis in humans (Walter et al., 1996). HBV infection can also result in chronic infection in tree shrew and can lead to development of hepatocellular carcinoma by week 160(Yan et al., 1996). Primary hepatocytes isolated from tree shrew are susceptible to HBV infection and serve as a valuable tool to study HBV infection *in vitro* (Glebe et al., 2003; Glebe et al., 2005; Walter et al., 1996). Recently, a study using tree shrew hepatocytes found that sodium taurocholate cotransporting polypeptide (NTCP) is a functional receptor for human hepatitis B and D virus (Yan et al., 2012, 2015). However, experimental infection of tree shrews *in vivo* with HBV is not highly efficient, and there is currently a lack of both genetically uniform shrew strains as well as research tools for this model. These factors have hampered further adoption of this system for studying HBV infection.

Surrogate animal models for hepatitis B virus study, such as the woodchuck and duck models, have also contributed a lot to our understanding of hepadnavirus biology, and these models are still helpful for testing new drugs and treatments. We will not introduce these models in detail in the present article. Readers can refer to (Kosinska et al., 2015; Kulkarni et al., 2007) for reviews of WHV-infected woodchucks and (Cova and Zoulim, 2004) for DHBV-infected ducks.

3. HBV transgenic mouse models

Transgenic mice expressing either the complete HBV genome or individual genes provide valuable tools to study HBV replication, pathogenesis and therapies (Babinet et al., 1985; Chisari et al., 1986; Chisari et al., 1989; Chisari et al., 1985; Farza et al., 1988; Guidotti et al., 1995; Kim et al., 1991). When transgenically expressed in mice, small surface antigen (HBsAg) can be readily detected in the serum (Babinet et al., 1985; Chisari et al., 1985). These mice were tolerant to HBsAg and did not develop liver disease (Babinet et al., 1985; Chisari et al., 1985). However, transgenic mice that overexpressed HBsAg along with large surface antigen could retain the proteins in the ER, leading to its accumulation in the liver (Chisari et al., 1986), hepatocellular injury, chronic inflammation and eventually to hepatocellular carcinoma (HCC) (Chisari et al., 1987; Chisari et al., 1986; Chisari et al., 1989).

Although mice that expressed high levels of the HBx antigen in the liver did not have obvious signs of liver injury and inflammation, they nevertheless developed HCC (Kim et al., 1991; Koike et al., 1994). These results indicate that HBx is oncogenic. Mice transgenically expressing the 1.3 copy over-length HBV genome (HBV1.3), which includes the viral promoters and regulatory elements, produce HBsAg, HBcAg and HBeAg (Guidotti et al., 1995). These mice produce levels of infectious virus in the blood that are comparable to those in chronically infected humans(Guidotti et al., 1995). High levels of HBV replication in the liver of mice does not induce hepatocellular injury, which further confirms that HBV is not cytopathic (Guidotti et al., 1995).

Adoptive transfer of HBV-specific CTLs into HBV transgenic mice resulted in the clearance of HBsAg, accompanied by elevated serum alanine transaminase (ALT) (Ando et al., 1993; Moriyama et al., 1990). The CTLs induced an acute hepatitis and lysed HBsAg-expressing hepatocytes, and these effects were dependent on major histocompatibility class I (Ando et al., 1993; Moriyama et al., 1990). Besides killing the infected hepatocytes, the activated, adoptively transferred CTLs produced IFN-γ, which then stimulated the production of chemokines and recruited the infiltration of antigen-nonspecific lymphocytes and neutrophils. The infiltrated cells in the liver amplified the cytopathic effect of the CTLs (Ando et al., 1993). On the other hand, CTLs can also inhibit viral replication by noncytolytic mechanisms that involve secretion of IFN-γ and tumor necrosis factor-α (TNF-α) (Guidotti et al., 1996). Thus, the immune system controls the virus through both cytolytic and noncytolytic mechanisms (Guidotti and Chisari, 2001).

The major clinical problem of HBV infection is HBV-induced chronic hepatitis. However, transgenic mice are immunologically tolerant to HBsAg and do not develop chronic liver disease. To model chronic hepatitis B, transgenic expression of the HBV genome was established in severe combined immunodeficient (SCID) mice. Adoptive transfer of unprimed, syngeneic splenocytes into HBV-transgenic SCID mice lead to partial clearance of the virus from both liver and serum and the development of chronic liver disease (Feitelson et al., 2004; Larkin et al., 1999). This model will permit identification of viral and host factors that contribute to chronic liver disease in the absence of tolerance.

HBV1.3 transgenic mice are not only suitable for the study of hepatitis B pathogenesis, but they also allow for the evaluation of therapeutic stratagems to inhibit viral replication. HBV replication in transgenic mice was shown to be inhibited by various cytokines such as IFN-γ, TNF-α, type I interferon and IL-18 (Kakimi et al., 2000; Kimura et al., 2002; McClary et al., 2000). The efficacy of various nucleoside analogues, such as adefovir dipivoxil (Julander et al., 2002), lamivudine(Weber et al., 2002) and entecavir (Julander et al., 2003), were also tested in HBV-replicating transgenic mice.

In conclusion, the transgenic HBV mouse model contributes substantially to our understanding of hepatitis B immunopathogenesis in vivo and represents a suitable model to investigate novel therapeutic strategies for chronic infection. However, there are important limitations of this model. The study of viral entry and spread, as well as the development of drugs to inhibit these steps, are not possible using this model. Furthermore, these mice are not suitable for monitoring viral elimination, because the HBV genome is integrated in the mouse genome. All viral RNAs in this system are produced from the integrated viral genome and not from cccDNA, which is apparently absent in transgenic mouse hepatocytes. Finally, the tolerance induced by the transgenic viral proteins, probably starting from the embryonic stage, makes the study of therapeutic vaccines difficult. To study the mechanisms of HBV-induced liver disease and to develop therapeutic strategies for viral clearance, alternative mouse models were developed using hydrodynamic injection or viral vector-mediated transduction of the HBV genome.

4. Hydrodynamic-based transfection of HBV DNA in mice

Hydrodynamic injection is an efficient method to transfect genetic materials into the mouse liver in vivo (Liu et al., 1999). Transfection of hepatocytes *in vivo* by hydrodynamic injection with a replication-competent HBV genome is an alternative way to study viral replication and HBV-host interactions in mice, and this can circumvent the problem of central tolerance. After hydrodynamic injection of HBV DNA, HBV antigens and replicative intermediates are synthesized and virus can be detected in the blood (Yang et al., 2002). The transfected immunocompetent mice displayed acute self-limiting hepatitis. The mice developed an HBV-specific antibody response and specific CTLs, which were associated with the disappearance of HBV serum antigens and HBV-positive hepatocytes. In contrast, expression of HBV antigens persisted in immunocompromised NOD-Scid mice (Yang et al., 2002), indicating that the host immune response may contribute to virus clearance.

The immune effectors which contribute to the clearance of HBV DNA were explored using a panel of immune-deficient mouse strains that were hydrodynamically transfected with the HBV genome (Yang et al., 2010). Results showed that CD4 and CD8 T cells, but not B cells, play a major role in HBV clearance. Interestingly, the innate immune effectors, such as type I interferon and TNF-α mediated pathways and NK cells, also contribute to the elimination of transfected HBV DNA (Yang et al., 2010). However, it should be noted that the persistence of HBV transgenes in mouse liver by hydrodynamic transfection is dependent on the mouse genetic background and the plasmid backbone (Huang et al., 2006). HBV surface antigenemia persisted for >6 months in around 40% of C57BL/6 mice after hydrodynamic injection of pAAV/HBV1.2 plasmids but not the pGEM4Z/HBV1.2 plasmids (Huang et al., 2006). It is likely that specific sequences in the AAV backbone could regulate the expression of the transgenes and lead to persistent expression of HBV surface antigen. Unlike in C57BL/C mice, serum HBsAg level dropped quickly 1 week after hydrodynamic injection of pAAV-HBV1.2 into Balb/C mice (Huang et al., 2006). This result indicates that genetic backgrounds indeed influence HBV clearance in mice. Thus, for the study of HBV tolerance, C57BL/6 mice hydrodynamically injected with pAAV/HBV1.2 plasmids can serve as a useful model to investigate mechanisms and therapies.

Experiments using the pAAV-HBV1.2 hydrodynamic injection model have shown that the HBV nucleocapsid determines HBV persistence in both C57BL/6 and Balb/C mice (Lin et al., 2010). The capsid structure of HBV, but not the free core protein, seems important for the induction HBV-specific antibody and CTL responses. In addition to the viral factors, host factors also affect virus persistence. HBV clearance in humans is heavily dependent on the age at the time of exposure. A recent report suggests that the gut microbiota contribute to the age-dependence of HBV clearance, using a hydrodynamic transfection mouse model (Chou et al., 2015).

The pAAV-HBV1.2 hydrodynamic injection model also provides a platform for testing therapies for HBV infection. Liver-infiltrating T cells from mice with HBV persistence expressed higher level of programmed death 1(PD-1), and blockade of the PD-1 pathway not only reversed T cell dysfunction but also reduced HBV persistence (Tzeng et al., 2012).

Another report showed that IL-15 treatment can suppress HBV replication in pAAV-HBV1.2 transfected mice (Yin et al., 2012). The HBV regulatory protein X (HBx) has been shown to enhance viral replication in vivo (Keasler et al., 2007). Treatment with bifunctional 5'-triphosphate short interfering RNAs targeting X (3p-HBx-siRNAs) not only directly reduced HBx and HBV replication, but also triggered type I IFN signaling through the RIG-I pathway to inhibit HBV in HBV carrier mice (Han et al., 2011).

5. Transduction of HBV DNA in mice

Another route to deliver HBV genomes into the liver of mice is by adenoviral vector or adeno-associated virus vector. Adenoviral vectors are efficient for transduction of liver cells of immunocompetent mice(Bramson et al., 1995), and HBV-specific T cell and antibody responses can be detected after high dose infection (10^9 infectious units per mouse) of Ad-HBV transduction (von Freyend et al., 2011). However, transduction of large amounts of HBV genome using high doses of adenovirus results in only transient HBV replication in the liver (Huang et al., 2012) because adenoviral infection induces a strong immune response in mice, resulting in HBV clearance (Hartman et al., 2007). Only infection of mice with low doses of adenoviral vector (10^8 infectious units per mouse) results in persistent HBV infection. Mice transduced with a low dose neither develop a strong HBV-specific T-cell response nor produce antibodies against HBV. This model could be used to study the pathogenesis of chronic HBV infection and develop new therapeutic strategies. However, it should be noted that high levels of HBV, which are commonly detected in chronic hepatitis B patients, could not be recapitulated in this model.

Compared to adenovirus transduction, adeno-associated virus (AAV) infection does not induce an obvious immune response (Mingozzi et al., 2003). In addition, *in vivo* hepatic gene transfer through AAV vectors can induce immune tolerance to the transgene (Mingozzi et al., 2003). Thus, AAV vectors should be an ideal vehicle to deliver HBV DNA into the liver. Recently, several groups successfully used AAV vectors to transfer replication-competent HBV genomes (Dion et al., 2013; Dong et al., 2010; Wang et al., 2012; Yang et al., 2014). Yang et al. reported that AAV/HBV induces sustained viremia in both neonatal and adult mice. Similar to chronically infected patients, mice infected with AAV/HBV were negative for antibodies against HBsAg, and the AAV8/HBV-infected mice failed to elicit an HBV-specific immune response upon immunization with conventional HBV vaccine. In the other report, HLA-A2/DR1 mice transduced with 5×10^{10} viral genome equivalents of an AAV serotype 2/8 chimera lead to persistence of HBV DNA, HBsAg and HBeAg in serum for at least 1 year (Dion et al., 2013). While HBV replication intermediates and transcripts were detected in the livers of the AAV2/8/HBV infected mice, no significant inflammation was observed in the liver, and T cells were tolerant to HBV antigens (Dion et al., 2013).

In order to identify a vaccine that can potentially circumvent the immune tolerance induced by AAV/HBV infection, Yang et al. used TLR9 agonist CpG-B as an adjuvant, and found that AAV/HBV-infected mice vaccinated with HBsAg/CpG induced strong HBV-specific antibody production and T-cell responses, leading to clearance of viremia (Yang et al., 2014). Furthermore, both HBV DNA and protein were significantly reduced in the livers of AAV/HBV-infected mice (Yang et al., 2014). Martin et al. reported that vaccination of

AAV/HBV infected mice with TG1050, a non-replicative adenovirus serotype 5 encoding a large HBV fusion protein composed of two HBV envelope domains, a truncated HBV core and a modified HBV polymerase, induced both splenic and intrahepatic functional T cells specific to HBV and a significant reduction of circulating viral markers (Martin et al., 2014).

In conclusion, HBV genome transfer by hydrodynamic injection or by AAV vector transduction results in sustained viremia and immune tolerance, which resemble clinical HBV carriers. This nontransgenic mouse model is therefore particularly suited to develop therapeutic interventions to clear HBV from transfected or transduced cells. The major limitations of this model are that there is no natural infection and, most importantly, there is no re-infection of mouse hepatocytes. Since HBV clearance in humans entails not only the elimination of virus from infected hepatocytes, but also control of HBV spread by new infection, a model permissive for true HBV infection is an important research need.

6. Human-murine liver chimeric mouse models

Currently, three kinds of human liver chimeric models are available to study HBV infection and test therapeutics. The first is based on transgenic mice with the urokinase plasminogen activator (uPA) gene (Rhim et al., 1994), expressed specifically in the liver under the control of the mouse albumin promoter. However, over-expression of the uPA gene in an uncontrolled manner leads to high lethality of newborn pups, so that newborn uPA mice must be rapidly transplanted with healthy hepatocytes. Handling of uPA mice therefore became expensive and time-consuming (Vanwolleghem et al., 2010).

A big improvement was made by using a non-liver specific major urinary protein (MUP) promoter to generate MUP-uPA SCID/Beige mice, which are generally healthier than Alb-uPA mice and therefore provide a longer window for engraftment with human hepatocytes(Tesfaye et al., 2013). The second mouse model is based on the fumaryl acetoacetate hydrolase (Fah) knockout mice, including the FRG (Azuma et al., 2007), NOD-FRG (Vaughan et al., 2012) and NRG-FAH (Li et al., 2014). Knockout of Fah results in the hepatic accumulation of toxic tyrosine metabolitic intermediates and the death of mouse hepatocytes. The advantage of Fah knockout mice, compared to uPA mice, is that the mice are much healthier and easier to handle, and liver injury can be controlled by administration and withdrawal of 2-(2-nitro-4-fluoromethylbenzoyl)-1,3-cyclohexanedione (NTBC), which blocks the accumulation of toxic intermediates caused by Fah knockout(Azuma et al., 2007; Bissig et al., 2010). Maintenance of the Fah knockout colony only requires supplementation of the water with NTBC.

The third mouse model is based on the recently-generated TK-NOG mice (Hasegawa et al., 2011), which carry the herpes simplex virus (HSV) thymidine kinase (TK) transgene under the control of hepatic specific Alb promoter. Similar to Fah knockout mice, the TK-NOG mouse liver damage occurs in a controlled manner. Specific mouse hepatocyte depletion can be achieved by administration of a pro-drug, gancyclovir (GCV). One disadvantage of TK-NOG mice is that male mice are sterile, and each batch of mice should be genotyped for TK positivity.

The above three mouse models all have high levels of human hepatocyte repopulation, as evidenced by the high human serum albumin level (Bissig et al., 2010; Dandri et al., 2001; Hasegawa et al., 2011; Kosaka et al., 2013; Vanwolleghem et al., 2010). The surgical procedure to generate human hepatocyte chimeric mice is rather simple, and involves intrasplenic injection of between one and several million primary hepatocytes. Usually, it will take 2–3 months to reach workable reconstitution of human hepatocytes, which can vary from a few percent to over 95 percent of hepatocytes within the mouse liver (Bissig et al., 2010; Hasegawa et al., 2011; Kosaka et al., 2013).

Human chimeric liver mice have been used to evaluate HBV antivirals in vivo, including polymerase inhibitors such as lamivudine, entecavir and adefovir (Bissig et al., 2010; Dandri et al., 2005; Tsuge et al., 2005) and entry inhibitors such as Myrcludex-B, which is derived from the HBV large S protein (Oehler et al., 2014; Petersen et al., 2008). This model is also used to study HBV covalently closed circular DNA (cccDNA) biology *in vivo* (Lutgehetmann et al., 2010). It was shown that, in the absence of antiviral drugs, cell division in the setting of liver regeneration induced strong destabilization of the cccDNA reservoir, leading to cccDNA clearance in the great majority of chronically infected hepatocytes.

Because the chimeric mice are genetically immune deficient, the immune-mediated inhibition and/or clearance of HBV-infected hepatocytes cannot be evaluated in these models. However, in the absence of an adaptive immune system, these models can allow for the investigation of the antiviral effect of some innate immunomodulators, such as IFN-α (Belloni et al., 2012; Lutgehetmann et al., 2011; Tsuge et al., 2011). IFN-α treatment was shown to inhibit HBV transcription in this system by promoting epigenetic repression of the cccDNA template(Belloni et al., 2012). HBV, in turn, was found to counteract the inhibition by down-regulating the IFN-stimulated genes or blocking the IFN signaling pathway, by inhibiting STAT1 translocation into the nucleus (Lutgehetmann et al., 2011; Tsuge et al., 2011). Thus, human chimeric mice are useful for the study the HBV and host cell interactions, but only in the context of innate immunity.

It is still technically challenging to study HBV infection using cell culture models, because these do not support secondary rounds of infection. Human liver chimeric mice, on the other hand, are permissible for spreading infection, allowing for more relevant investigation of the biology of chronic hepatitis B. Experimentation with HBV bearing different mutations, or from different genotypes (Sugiyama et al., 2006; Tanaka et al., 2008; Tsuge et al., 2010; Tsuge et al., 2005), has furthered our understanding of HBV genes. For example, HBeAg was demonstrated to be unnecessary for viral infection and replication (Tsuge et al., 2005). However, HBx is indispensable for viral replication in vivo (Tsuge et al., 2010).

Ectopic transplantation of human liver tissue under the kidney capsule can also support HBV replication (Bocher et al., 2000; Eren et al., 2000; Ilan et al., 1999; Ohashi et al., 2000). These models were used to assess some antivirals in the early years. However, because the human liver engraftment in this model does grow and is relatively small in volume compared to the chimeric livers in the above models, the HBV titer in the blood was

lower and viremia duration was short. This model is now seldom used, as human liver chimeric mice are widely available.

It is well known that HBV is non-cytopathic, and HBV infection induced-liver diseases are mediated by host immune responses. While the human-mouse liver chimeric model contains humanized liver, it does not have a human immune system. Therefore this model is not suitable to study how human immune cells interact with HBV and cause liver disease. Chimeric mice with both human liver and immune system are necessary to study the immune-pathogenesis of HBV and to develop immuno-therapeutic treatments.

7. Humanized mouse models with both human liver and immune cells

To elucidate the mechanism of HBV-induced liver diseases and enable immunotherapeutic testing, we recently developed the novel NSG/Jo2 model, which is an immunodeficient mouse model that enables human liver cells and functional immune system development following transplantation with human fetal liver-derived hematopoietic stem cells and liver progenitor cells (Bility et al., 2014a).The NSG/Jo2 model is derived from the NOD-scid$^{-/-}$-gamma chain$^{-/-}$ (NOD.Cg-Prkdcscid Il2rgtm1Wjl/SzJ) mouse with the human HLA-A2 transgene, and it utilizes the Jo2 antibody (mouse CD95 activating antibody) as a means of selectively inducing apoptosis in mouse hepatocytes (Bility et al., 2014a). Intrahepatic transplantation of immunodeficient mice with human CD34^{+} hematopoietic stem cells and human hepatic progenitor cells purified from 15–18 weeks old human fetal liver tissue into newborn immuno-deficient mice results in hematopoietic cell development. Conditional induction of mouse hepatocyte apoptosis then results in human hepatocyte regeneration (Bility et al., 2014a; Washburn et al., 2011). A human immune system is developed at 12 weeks post-transplantation, along with human hepatocyte regeneration, as measured by human CD45^{+} cells and sub-lineages and human albumin levels in the blood, respectively (Bility et al., 2014a; Washburn et al., 2011).

Most importantly, the double-humanized immune system and liver-reconstituted mice exhibit normal liver physiology and structure, with no sign of liver disease (Bility et al., 2014a; Washburn et al., 2011). The A2/NSG-hu HSC/Hep humanized mouse model supported persistent HBV infection, human immune responses, chronic liver inflammation and fibrosis. HBV-mediated liver immune impairment and liver disease were associated with high level of infiltrated human immunosuppressive/pro-fibrogenic macrophages. The results of this study suggest a critical role for macrophage activation in hepatitis B-induced liver diseases, thus providing a novel therapeutic target. This novel humanized mouse model provides a valuable platform for studying HBV infection, human immune response and liver diseases. (Bility et al., 2014a).

In addition, we reported another novel humanized mouse model, namely the AFC8 model, which is derived from the Balb/C-RAG2-γC-null immuno-deficient mouse (double-knockout [DKO]) carrying a liver-specific transgene with inducible apoptotic activity, that enables human liver cells and immune system development and supports HCV infection following transplantation with human fetal liver-derived hematopoietic stem cells and liver progenitor cells (Bility et al., 2014b; Bility et al., 2012; Washburn et al., 2011).

Although the recently reported humanized mouse models with human liver cells and immune system have enabled the study of the immunopathogenic mechanisms of chronic HBV-induced liver disease, these models have several limitations. The human immune system in the DKO mouse background is not fully developed, with deficiencies in antigen-specific T- and B-cell responses (Aryee et al., 2014; Shultz et al., 2011; Washburn et al., 2011). The NSG mice have improved antigen-specific T and B cell responses, compared to the DKO mice, but they still do not generate a completely functional human immune system (Aryee et al., 2014; Bility et al., 2014a; Shultz et al., 2011). Additionally, the human liver cell repopulation in the hepatic progenitor cell-reconstituted animals is relatively reduced, compared to adult hepatocyte-reconstituted mice (Bissig et al., 2010; Washburn et al., 2011).

Furthermore, the differentiation state of hepatic progenitor cells is also relatively immature (Washburn et al., 2011). HBV/HCV infection of humanized mice with human liver and immune system results in relatively lower viral replication, when compared to adult hepatocyte-transplanted mice (Bility et al., 2014a; Bissig et al., 2010; Washburn et al., 2011), which is most likely due to the low hepatocyte repopulation level and the immature state of the hepatocytes. However, the human immune system could also be playing a role in controlling viral replication, albeit inefficiently (Bility et al., 2014a; Washburn et al., 2011). The development of humanized mouse models with high reconstitution of fully-differentiated human hepatocytes, along with a completely competent human immune system, remains the goal for developing small animal models for studying chronic hepatitis B and associated inflammatory liver diseases (Bility et al., 2013; de Jong et al., 2010; Legrand et al., 2009; Strick-Marchand et al., 2015).

8. Summary and Perspectives

HBV naturally only infects human and chimpanzees; this narrow host range has hindered our understanding of HBV biology. Thus, a well-defined small animal model which supports robust infection is essential for us to understand the infection, replication and pathogenesis of chronic hepatitis B and to develop therapeutics.

Several mouse models have been developed for HBV study, each with its own advantages and disadvantages (summarized in Table 1). Transgenic mice expressing either the complete HBV genome or single viral genes have allowed investigators to study the replication, gene expression, and immunopathogenesis of HBV infection. However, the immune system of transgenic mice is tolerant to the virus, and the mice don’t show signs of hepatitis. Chronic hepatitis can be achieved by the adoptive transfer of anti-HBV CTLs into immunocompetent HBV transgenic mice, or by the transfer of syngeneic spleen cells into HBV transgenic SCID mice. From these models, we now know that the host immune response serves as a double-edged sword toward HBV infection, inhibiting replication while also contributing to the development of chronic liver disease.

Although HBV-transgenic mice have been used to test numerous drugs, the model cannot be used for the study of HBV entry spread, or to test drugs that inhibit these steps. Furthermore, these mice are not suitable for monitoring viral elimination, because the HBV genome is integrated into the host chromosome.. The immune system also exhibits central tolerance to

the transgenic gene products, making the study of therapeutic vaccines in this model difficult. Alternative models were developed by transfecting HBV DNA into the liver cells by hydrodynamic-based gene delivery methods or by transduction using AAV vectors. However, none of these models allow us to investigate real HBV infection and its interaction with a natural host immune system.

Recent progress in human-mouse liver chimeric models allows us to study HBV virus infection of human hepatocytes and to test therapeutics. Even more importantly, the chimeric mouse model with both human liver and immune system provides a unique platform to study how the virus interacts with the human immune system *in vivo* and to manipulate the human immune system to treat HBV infection.

Many preclinical studies based on immunotherapy are now ongoing to cure chronic hepatitis B. However, most such studies are performed in HBV-transgenic or transfected/transduced mice. Differences between the mouse and human immune system will probably make translation difficult. The humanized mouse with both human liver and human immune system should serve as a unique model for the development of immunotherapeutic strategies for HBV infection. Efforts are needed to improve the reconstitution of both the human liver and human immune system in the same mouse, to make this model more efficient and more applicable for immuno-intervention.

Acknowledgment

This work was supported in part by grants from UNC UCRF innovation grant, from NIH: DK095962, DK100664, CA164029, AI095097 (L.S.), and UNC Lineberger Comprehensive Cancer Center and UNC Infectious Disease Pathogenesis Postdoctoral Training Grants (M.T.B.).

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Highlights

- It is important to establish small animal models to study HBV infection, persistence and immunopathogenesis.
- We first review the contribution of chimpanzee and tree shrew models of chronic hepatitis B.
- We then review transgenic mouse models, hydrodynamic-based transfection of HBV DNA and transduction of HBV DNA.
- We then review human-murine liver chimeric mouse models and humanized mice with both human liver and immune cells.

Table 1

Mouse models for studying hepatitis B virus infection, immunopathology and therapy.

	Applicability for study of:					
Model	**Infection**	**Replication**	**cccDNA**	**Immuno-pathogenesis**	**Pros**	**Cons**
Transgenic mouse	No	Yes	No	Yes (upon immune cell transfer)	Convenient and stable system	No infection; no HBV clearance; central tolerance
Hydrodynamicbased transfection	No	Yes	No	Yes	Study both acute and chronic infection; study different HBV strains/mutants	No re-infection; induce damage signal by hydrodynamic injection
AAV-based transfer	No	Yes	No	Yes (induce immunotolerance)	Consistent; immunotolerance resembles clinical carriers; study viral clearance	No re-infection
Human-murine liver chimeric mouse	Yes	Yes	Yes	No	HBV infection/replication in human hepatocyte; testing of drugs that block infection/inhibit cccDNA	Complex system; cannot study viral-host immune system interaction
Humanized mouse with both human liver and immune system	Yes	Yes	Yes	Yes	HBV infection/ replication in human hepatocyte; study human immune response; develop immunotherapeutics	Complex system; not easy to establish