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Review

The human liver-uPA-SCID mouse: A model for the evaluation of antiviral compounds against HBV and HCV

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ABSTRACT

The study of the hepatitis B virus (HBV) and the hepatitis C virus (HCV) has long been hampered by the lack of a suitable small animal model. Both viruses could only be studied in humans or in chimpanzees. Recently, a new chimeric mouse model was developed that was permissive for HBV and HCV infection. In this model, uPA$^{+/+}$-SCID mice, suffering from a transgene-induced liver disease, are transplanted early after birth with primary human hepatocytes. These human hepatocytes integrate in the parenchyma and progressively repopulate the diseased mouse liver without losing their normal metabolic functions. Successfully transplanted mice can then be infected with HBV and HCV. In this review, we describe the characteristics of this chimeric mouse model in more detail and give an overview of how this model has already contributed to the development of new antiviral compounds for the treatment of viral hepatitis.

Contents

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1. Introduction

The hepatitis B virus (HBV) and hepatitis C virus (HCV) are hepatotropic viruses that represent a serious global health problem. The number of chronically infected subjects is estimated at 360 million for HBV and 170 million for HCV and annually these pathogens kill more than 1.5 million people worldwide (Alter, 2003; Shepard et al., 2005). In the past decade substantial progress has been made in treating these chronic infections but no definitive cure is yet available and today's standard treatment for HCV causes severe side effects. Therefore huge research efforts are being made to develop new antiviral compounds that are more efficacious and better tolerated.

The efficacy of new antiviral compounds is now primarily evaluated in cell culture (Lindenbach et al., 2005; Lohmann et al., 1999; Wakita et al., 2005; Zhong et al., 2005; Zoulim, 2006). However, before the toxicity and activity of investigational new drugs are assessed in humans, preclinical evaluation needs to be performed in relevant animal models. Because both HBV and HCV have a very narrow tropism, preclinical in vivo studies can only be performed in chimpanzees. For obvious financial and ethical reasons, pharmaceutical companies have not always been able to take this important step. In the last decades a variety of animal species have been examined for their permissiveness for HBV and HCV, and several transgenic and chimeric animal models have been developed. In this review, we will give a detailed description of the "human liver-uPA-SCID" mouse, which appears currently to be the most valuable small animal model for the study of viral hepatitis, and compare it to other animal models. In addition, we will provide an overview of how this model has already contributed to the development of new antiviral strategies against HBV and HCV.

2. Animal models for the study of HBV and HCV

For decades chimpanzees have been used to study the immunobiology as well as candidate therapies for both HBV (Prince and Brotman, 2001) and HCV infection (Bukh, 2004). Although humans and chimpanzees share more than 98% of their genome sequences, there are some marked differences between these two species that have an influence on disease pattern and outcome (Muchmore, 2001). Chimpanzees and humans do not share any HLA class I alleles; e.g. HLA-A2 alleles are totally absent in chimps but overall they have more sequence diversity in the MHC region. In addition, clear differences in the MHC class II region are observed. The natural history of HCV in chimpanzees also differs from that observed in man: (1) HCV infections evolve less frequently towards chronicity in chimpanzees and (2) animals that become chronically infected show no signs of overt liver disease and tissue damage, precluding their use for the study of liver cirrhosis and hepatocellular carcinoma. For obvious financial and ethical reasons, alternative animal models were sought. Tree shrews (*Tupaia*) were first proposed as an alternative species to study HBV (Walter et al., 1996; Yan et al., 1996) and HCV (Xie et al., 1998) infections. Unfortunately, after infection with either HBV of HCV tree shrews almost always experience an acute self-limited infection with only low and intermittent viremia. Other primates like the cynomolgus monkey, the green monkey, the Japanese monkey, tamarins and baboons do not seem to be permissive for HCV infection (Abe et al., 1993; Garson et al., 1997; Sithebe et al., 2002).

In 1995, an HBV transgenic mouse was created in which HBV replicated to levels comparable to those observed in chronic patients (Guidotti et al., 1995). However, cccDNA has never been detected in the liver of these transgenic mice and these animals were immune tolerant to the virus. In addition, viral entry cannot be studied since mouse hepatocytes lack the necessary receptors. Nevertheless, these mice have been used to evaluate the antiviral activity of lamivudine (Weber et al., 2002), adefovir dipivoxil (Julander et al., 2002), entecavir (Julander et al., 2003), and HBV-specific siRNAs (Uprichard et al., 2005).

A first chimeric model was developed by transplanting under the kidney capsule of immune deficient mice human liver fragments that were infected ex vivo with HBV or HCV. Because this model required constituents from three different origins (the recipient mouse, the immune deficient bone marrow donor and human liver tissue) it was called the "Trimera" mouse (Ilan et al., 1999, 2002). Major drawbacks of this model that have limited its use are the low viral titers and major histological changes within the transplanted tissue such as ischemia, fibrosis, loss of lobular architecture and necrosis. The occurrence of these histological abnormalities is not surprising since the liver fragments are transferred to an extrahepatic location. In addition, it is impossible to use this model to study viral entry and neutralization since de novo infections of Trimera mice transplanted with healthy human hepatocytes have been unsuccessful until now.

3. The chimeric uPA-SCID mouse

In 1990, Dr. Brinster's team developed a transgenic mouse to study the pathophysiology of plasminogen hyperactivation and to evaluate new therapeutic protocols for bleeding disorders (Heckel et al., 1990). This transgenic mouse carried the mouse urokinase-type plasminogen activator (uPA) gene under the control of the mouse albumin enhancer/promotor. The overexpression of the uPA gene in the liver resulted in high plasma uPA levels and hypofibrinogenemia, which led to severe and sometimes fatal intestinal and abdominal bleeding soon after birth. The hepatocyte-specific expression of the uPA-transgene also induced extensive liver toxicity leading to chronic hepatic insufficiency (Sandgren et al., 1991). This functional liver deficit created a supportive niche for liver regeneration by transplanted hepatocytes of murine origin (Rhim et al., 1994). Important to note is the fact that only animals homozygous for the uPA-transgene turned out to be good recipients for transplanted liver cells. Soon after birth, heterozygous animals experience somatic deletion of the transgene in a minor fraction of diseased hepatocytes (Sandgren et al., 1991). These hepatocytes with a restored normal phenotype have a selective growth advantage over the transgene-expressing cells and therefore compete directly with heterologous transplanted hepatocytes.

To allow liver repopulation by xenogeneic cells, the Alb-uPA transgenic mouse had to be backcrossed onto a genetically immunodeficient mouse strain. This approach led to an almost complete repopulation by rat hepatocytes of the diseased mouse liver in Swiss athymic (nu/nu) Alb-uPA mice (Rhim et al., 1995), while woodchuck hepatocytes repopulated up to 90% of the liver of uPA-RAG2$^{-/-}$ mice (Dandri et al., 2001; Petersen et al., 1998). In 2001, Dandri et al. (2001) were the first to report the successful transplantation of human hepatocytes into uPA-RAG2$^{-/-}$ mice. The number of human hepatocytes present in the mouse liver was rather low (up to 15%) compared to the transplantation efficiencies previously reported with rat and woodchuck hepatocytes. This was probably due to the use of heterozygous animals. Transplantation of primary human hepatocytes in homozygous uPA-SCID mice resulted in a more pronounced and stable engraftment, achieving almost complete replacement of the diseased mouse tissue (Meuleman et al., 2003, 2005). The transplantation of homozygous uPA animals is technically more difficult but the engraftment rate is much higher. The uPA homozygosity requires that cell transfer be performed soon after birth (within the 2nd week of life), which leads to an intervention on a very small creature that, in addition, is prone to severe hemorrhage during or immediately after the operation.

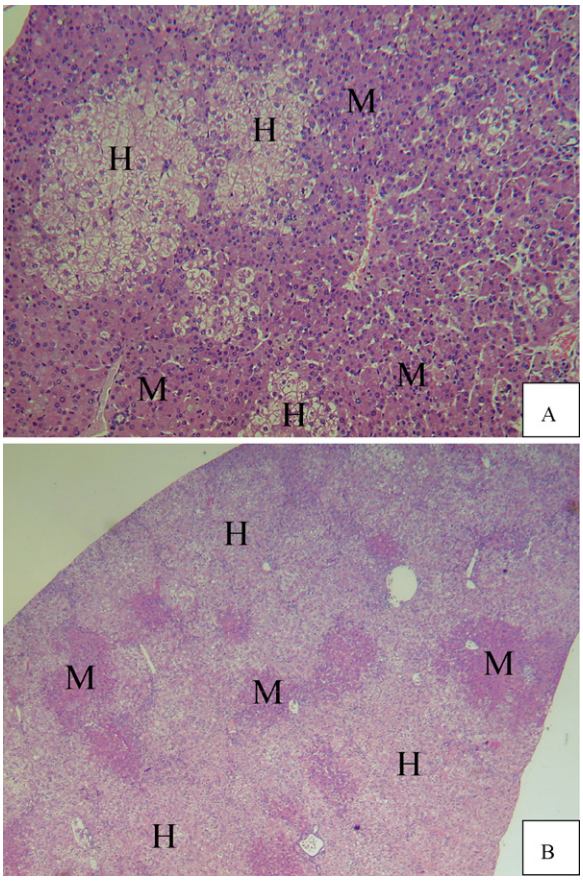

Fig. 1. Histology of a chimeric mouse liver. Hematoxylin and eosin staining of the liver of a chimeric uPA-SCID mouse 1 month (A) and more than 2 months (B) after transplantation with human hepatocytes. The human hepatocytes (H) can be easily discriminated from the mouse hepatocytes (M) by their larger size and pale color.

Following the injection of human hepatocytes into the mouse spleen, the cells migrate via the splenic vein and the portal vein into the liver where they diffusely spread into the hepatic sinusoids. However, only a small proportion of transplanted cells are able to translocate to the liver plate and integrate in the liver parenchyma (Gupta et al., 1999). In the first days to weeks after transplantation, the solitary human cells that reside in the mouse liver start dividing, eventually forming nodules of varying sizes (unpublished data; Fig. 1). Besides mature hepatocytes, hepatic progenitor cells also contribute to the repopulation process (Fig. 2a and b). Remarkably, this occurs in a well-organized fashion: (1) active proliferation is only seen in the human cells located at the periphery of the xenogeneic nodules that make contact with the diseased mouse tissue and (2) human bile canalicular structures make functional connections with the mouse biliary system, indicating communication with the mouse environment (Fig. 2c) (Meuleman et al., 2005).

Human hepatocytes in the mouse liver are functional and secrete a variety of hepatic proteins like albumin, α-1 antitrypsin, apolipoprotein A, apolipoprotein E and several clotting factors and complement proteins that can be detected in plasma (Meuleman et al., 2005, 2006a). The transplanted human hepatocytes also retain their normal pharmacological responses (Tateno et al., 2004) which makes the chimeric mouse model useful to study the "human-type" metabolism of compounds that cannot easily be administered to human volunteers. We have recently evaluated in chimeric uPA-SCID mice the metabolism of different steroids, such as androstenedione, norandrostenedione and methandienone. Analysis of urine indicated a metabolic profile that completely matches that seen in human studies (Lootens et al., 2008). The chimeric mouse model is a major improvement in comparison with the current in vitro cultures of human hepatocytes in which the phenotype and biochemical qualities of the cells rapidly change due to the artificial culture matrices.

4. HBV and HCV infection of human liver chimeric mice

Dandri et al. (2001) showed that chimeric mice were susceptible to HBV infection. Although the livers of the infected animals contained less than 15% human hepatocytes, an HBV infection was established after injection of serum from a chronic HBV carrier. Active replication of the virus was demonstrated by the presence of viral DNA in the serum of the infected mice (4.5 to 10×10^8 genome equivalents/ml), and was confirmed by immunohistochemical detection of HBcAg in liver sections. We have confirmed that chimeric mice can be infected with HBV and upon long-term follow-up (>80 days) we noticed that in mice displaying a high level of viral replication, the HBV became directly cytopathic for the human hepatocytes (Meuleman et al., 2006b). Histological, ultrastructural and molecular analysis of the liver indicated very high viral replication and protein expression up to a point where the majority of human hepatocytes acquired a ground glass appearance and showed signs of severe damage. These lesions closely resemble the pathological changes observed in immune suppressed chronic HBV patients and indicate that HBV can be directly cytopathic in the absence of an adaptive immune response.

A research team at the University of Alberta (Canada) was the first to demonstrate that homozygous uPA$^{+/+}$-SCID/Beige mice transplanted with primary human hepatocytes can be infected with HCV (Mercer et al., 2001). We noticed that unlike HBV, HCV requires a high degree of human chimerism in order for a mouse liver to become infected. Chimeric mouse plasma must contain at least 1 mg/ml of human albumin before the animals can be reproducibly infected with HCV (unpublished data). Once an infection is established, a rapid increase in viral load is observed and within a few weeks levels are reached that frequently exceed 10^7 IU/ml (Meuleman et al., 2005). From then on, only minimal variation in viremia is observed. Sometimes the viral load slowly decreases over a period of several months. In contrast to our observations in long-term HBV infections, we have never noticed signs of hepatotoxicity after a long-term HCV infection. Importantly, the biophysical characteristics of the viral particles produced in the chimeric mouse closely resemble those of particles isolated from infected humans and chimpanzees, but differ from viral particles produced in cell culture (HCV_{cc}) (Lindenbach et al., 2006). Moreover, HCV_{cc} that have acquired cell culture-adaptive changes in their genomes tend to revert to the wild-type sequence after inoculation in chimeric mice (Kaul et al., 2007).

5. Evaluation of strategies to prevent viral infections

5.1. Neutralizing antibodies against HCV

Using different cell culture models, neutralizing antibodies have been detected in the plasma of both acute and chronic HCV patients (Logvinoff et al., 2004; Yu et al., 2004) but their exact role in disease outcome remains unclear (Kaplan et al., 2007; Lavillette et al., 2005; Pestka et al., 2007). Using the chimeric mouse model we have shown that neutralizing antibodies can prevent an HCV infection in vivo (Vanwolleghem et al., 2008). Polyclonal antibodies were isolated from serum collected in 2003 from a chronic HCV

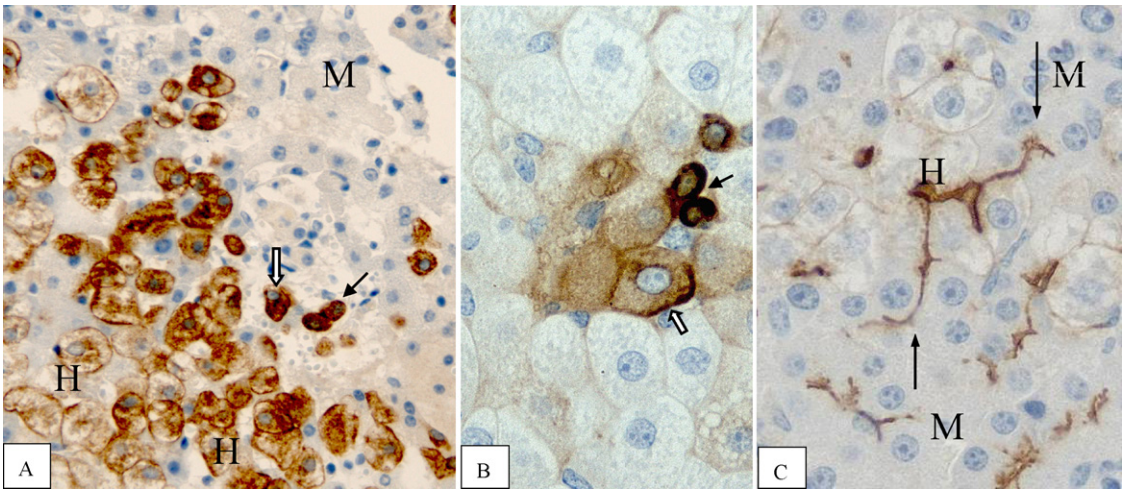

Fig. 2. Immunohistochemical analysis of a chimeric mouse liver. (A) Human hepatic progenitor cells (HPCs) were identified at the interface between human (H) and mouse (M) hepatocytes. These small cells with scanty cytoplasm and a relatively large oval nucleus are strongly immunoreactive for pan-cytokeratin (black arrow). Intermediate hepatocyte-like cells (white arrows) could be recognized as polygonal cells with a size and immunophenotype intermediate between that of HPCs and hepatocytes. (B) Staining with human CK7-specific antibodies confirms the presence of both these cell types, which are surrounded by mature hepatocytes that do not stain for CK7. (C) Staining of the liver with a polyclonal anti-CEA antibody shows connections between mouse canaliculi (arrows) and canaliculi that are somewhat bigger and that are formed by the surrounding human hepatocytes. (Meuleman et al., 2005.) Reprinted with permission of Wiley-Liss Inc., a subsidiary of John Wiley & Sons Inc.

patient (patient H). These antibodies are able to prevent HCV infections of different genotypes in vitro (Scheel et al., 2008). Three days before challenge with H77C virus, all chimeric animals were passively immunized with a low dose of these purified IgG (0.2 mg/g body weight; 4 animals) or a high dose (1 mg/g body weight; 4 animals). H77C is a prototype virus that originally infected patient H in 1977 (Feinstone et al., 1981; Yanagi et al., 1997). Two weeks later, all animals that received the highest antibody dose were completely protected while one animal from the low dose group already contained viral particles in its plasma. Four weeks after challenge, two animals in the low dose group were HCV positive, but also one animal in the high dose group turned out to be infected. The virus isolated from the latter animal contained a mutation in the E1 protein (R231H) but additional analysis demonstrated that this mutation did not have an impact on its neutralization by the polyclonal antibodies used. Our data show that it is possible to protect animals from an autologous HCV infection with polyclonal neutralizing antibodies. We are currently investigating whether these antibodies can also convey protection against infections with HCV strains of different genotypes, as was previously shown in vitro (Scheel et al., 2008). Recently two monoclonal antibodies against the HCV E2 protein were isolated from a phage-display library generated from a donor who suffered from a genotype 1a chronic HCV infection (Law et al., 2008). Chimeric mice were passively immunized with 200 mg/kg of these monoclonal antibodies and challenged with heterologous patient serum of the same genotype. Six weeks later 5 out of 9 chimeric mice turned out to be protected from infection. However, two weeks later, two more animals became HCV positive below the limit of quantification. Although these data are encouraging for vaccine development and for the use of monoclonal antibodies for HCV prophylaxis, more potent antibodies will need to be induced in order to convey full protection.

5.2. Blockade of the HCV (co-)receptors

Instead of targeting viral envelope proteins it may be more effective to design and/or produce compounds that bind to the different membrane (glyco)proteins involved in binding and entry of the virus in the host-cell. CD81, SR-B1 or claudin-1 are the most important host-cell receptors or ligands that have been reported to interact with HCV (reviewed by Dubuisson et al., 2008). These receptors are well conserved and unlike HCV envelope proteins, E2 in particular, are less prone to mutational escape mechanisms. In a "proof of concept" experiment we have evaluated whether antibodies against CD81 are able to prevent a de novo HCV infection in chimeric mice (Meuleman et al., Hepatology, in press). In a dose-range experiment we observed complete protection from infection with 100% infectious doses of HCV of two different genotypes (gt1 and gt4) injected on day 0 when 400 μg of anti-CD81 antibody (clone JS81) was administered on day −1 and day +1. This opens perspectives to prevent graft reinfection after orthotopic liver transplantation in chronically infected HCV patients.

5.3. Prevention of HBV infection using entry inhibitors derived from a viral protein

Petersen et al. (2008) recently reported that administration of acylated peptides, derived from the HBV large envelope protein could prevent an HBV infection in vivo. Two peptides, HBVpreS/2–48^{myr} (IC_{50} = 8 nM) and HBVpreS/2-48^{stearoyl} (IC_{50} = 250 pM), were first evaluated in uPA-SCID mice transplanted with Tupaia hepatocytes. Intraperitoneal as well as subcutaneous delivery of these peptides was shown to almost completely prevent a subsequent infection by woolly monkey hepatitis B virus (WMHBV), an Orthohepadna virus closely related to HBV. In the next experiment, chimeric mice harboring human hepatocytes received one subcutaneous dose of 2 mg/kg body weight of HBVpreS/2–48^{myr} one hour before inoculation with HBV, and subsequent doses on day 1, 2, 3 and 5. Ten weeks later, all four control animals contained HBV DNA in their plasma, while only one treated animal experienced an infection in which circulating virus levels were barely detectable. After 16 weeks, one additional treated animal became HBV positive, but both intrahepatic HBV DNA and HBcAg were below the limit of detection. Although prevention of HBV infection in all treated animals was not achieved, further fine-tuning of this approach may lead to a novel method to prevent graft reinfection after liver transplantation in chronic HBV patients.

6. Evaluation of compounds for the treatment of HCV and HBV infections

6.1. Interferon alpha

The first antiviral drug that was approved by the FDA for the treatment of chronic HCV patients was interferon alpha. Therefore it was an obvious choice to validate the usefulness of the chimeric mouse model for anti-HCV drug testing with this compound. A 10–14 day treatment of genotype 1 infected mice with 1350 IU/(g.day) of IFN-α2b, a 10-fold higher dose than what is administered to patients, resulted in a 10-fold drop in viral titer (Kneteman et al., 2006). When the treatment was extended to 28 days, a 1.66 $\log_{10}$ drop in viremia was observed. In vehicle-treated animals insignificant variations ($-0.27\ \log_{10}$) were observed. A more pronounced decline in viral titer was observed in animals infected with a genotype 3a virus. This correlates well with the more favorable therapeutic outcome of genotype 3a infected patients (Hadziyannis et al., 2004). After cessation of therapy, a rebound toward pretreatment levels occurred in the majority of treated mice. Since chimeric mice have no functional B and T cells, only the direct antiviral effect of interferon alpha is studied here. The second phase response caused by T cell-mediated elimination of infected hepatocytes is lacking (Neumann et al., 1998).

6.2. HCV protease inhibitors

The current standard therapy of HCV consists of pegylated interferon in combination with ribavirin. This treatment is effective in about 50% of chronic infections with genotypes 1, 4, 5, 6 and in about 80% of genotype 2 and 3 infected patients (Heathcote, 2007). This limited efficacy and the numerous and often serious adverse effects of the current treatment have stimulated the search for new antiviral compounds that inhibit the function of non-structural viral proteins belonging to the replication complex.

Boehringer Ingelheim developed a novel compound, called BILN 2061, that inhibited the activity of the NS3 protease (Lamarre et al., 2003). This product entered into clinical trials in the early 2000s and the first results incited high expectations since administration of BILN 2061 alone for 2 days reduced the viral load 100- to 1000-fold (Hinrichsen et al., 2004). However, further clinical development of BILN 2061 was stopped because long-term toxicity studies in rhesus monkeys revealed the induction of cardiotoxicity (Reiser et al., 2005). We have used BILN 2061 to validate the usefulness of the chimeric mouse model for the evaluation of new antiviral compounds (Vanwolleghem et al., 2007). A 4-day treatment with 10 mg/kg BID (twice a day) resulted in an impressive 2.5 $\log_{10}$ reduction in viral titer in genotype 1b infected mice, while vehicle treatment did not affect viremia (Fig. 3). To our surprise, BILN 2061-treated animals physically deteriorated during dosing and experienced acute respiratory abnormalities after minimal blood sampling. Because of the reported cardiotoxic effects of BILN 2061, mouse organs and tissues were examined using light and electron microscopy. Marked ultrastructural changes were observed in cardiomyocytes, but not in skeletal myocytes. Cardiomyocytes contained an increased number of mitochondria which often showed a swollen, pale matrix with inclusions of fat droplets and disrupted cristae (Fig. 4). Further investigations in non-transplanted uPA-SCID mice and non-transgenic SCID mice showed that both drug accumulation and liver function were identical, but only the uPA-SCID mice displayed the BILN 2061-induced cardiac abnormalities.

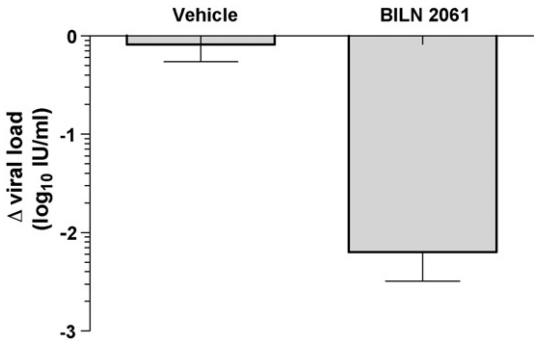

Fig. 3. BILN 2061 treatment of HCV-infected chimeric uPA-SCID mice. Change in HCV viral load after a 4-day treatment with BILN 2061 (right) or vehicle only (left). Error bars indicate the standard deviation ($n = 3$).

6.3. HCV polymerase inhibitors

The RNA-dependent RNA polymerase of HCV (NS5B) is another attractive target for candidate antiviral drugs. Different polymerase inhibitors have been evaluated in vitro and some have been tested in chimpanzees (Carroll et al., 2006; Chen et al., 2007). Currently there are no published data available on the use of the chimeric mouse model for the evaluation of HCV polymerase inhibitors. However, we have recently evaluated the antiviral efficacy of three newly developed non-nucleoside polymerase inhibitors in HCV-infected chimeric mice (unpublished data). After a 4-day BID administration to HCV-infected chimeric mice, an up to 1000-fold decrease in viral load could be observed. In general, HCV plasma levels rebounded to baseline after treatment was stopped. This small survey illustrates that the chimeric mouse model is suitable to test the in vivo efficacy of novel antiviral compounds and provides results that may be of guidance for the further clinical evaluation.

6.4. Cyclophiline inhibitor DEBIO-025

Studies in chronically HCV-infected humans have shown that cyclosporin A (CsA) has a synergistic antiviral effect with interferon (Inoue et al., 2003). However, the immunosuppressive action of CsA can cause severe adverse events. CsA exerts its antiviral effect by inhibiting the interaction between cyclophilin and the viral NS5B protein (Watashi et al., 2005). DEBIO-025 is a molecule derived from CsA that lacks its immunosuppressive effects and is more potent in inhibiting HCV replication in vitro (Paeshuyse et al., 2006). A monotherapy with DEBIO-025 had no effect on viral replication in HCV-infected chimeric mice (Inoue et al., 2007). However, combination therapy of DEBIO-025 with PEG-interferon was more effective than PEG-interferon alone. In animals that received this combined therapy a 100-fold drop in viremia was observed within 1 week, while animals receiving PEG-IFN experienced only a 10-fold drop. After cessation of therapy a rebound in viral titers was observed to baseline levels. DEBIO-025 is currently being evaluated in clinical trials (Flisiak et al., 2008).

6.5. Serine palmitoyltransferase inhibitor

Different reports have indicated that cholesterol and fatty acid biosynthetic pathways have a major influence on HCV replication, virion assembly and infectivity (Aizaki et al., 2008; Kapadia and Chisari, 2005). Umehara et al. (2006) showed that inhibition of the sphingolipid biosynthetic pathway may be used to treat chronically infected HCV patients. Myriomycin is an inhibitor of serine palmitoyltransferase, the enzyme involved in the first step of this

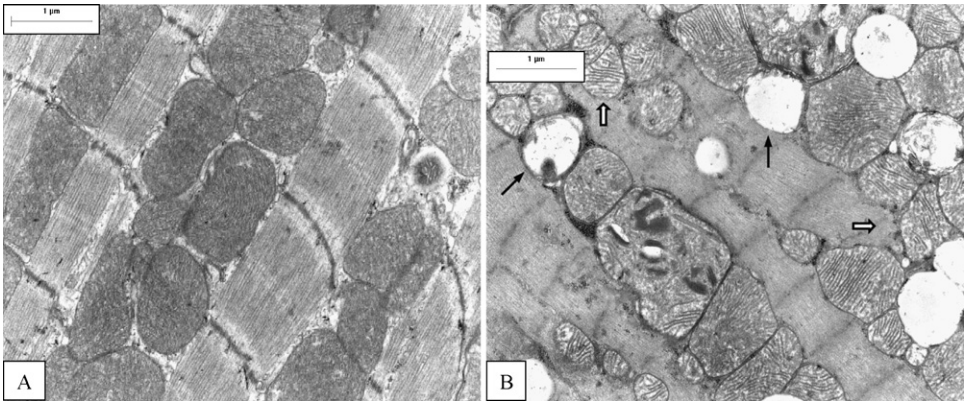

Fig. 4. Ultrastructural analysis of cardiomyocytes after BILN 2061 treatment. (A) Normal ultrastructure of the heart of a vehicle-dosed chimeric mouse. Mitochondria are round to elongated and densely packed, with closely arranged, parallel membranous cristae. (B) BILN 2061 treatment induces diffuse lipid inclusions (black arrow) in the myocardium. Mitochondria are swollen and pale and have disrupted cristae (white arrow).

biosynthetic pathway. Chimeric mice infected with HCV underwent an 8-day treatment of myriomycin monotherapy, pegylated interferon monotherapy or combination therapy. While myriomycin therapy had a comparable effect as PEG-IFN therapy, combination therapy induced a 1000-fold drop in viremia. Fourteen days after cessation of therapy, the viral load in the treated mice rebounded to pretreatment levels.

6.6. Induction of apoptosis in HCV-infected cells

An original and unique way to specifically eliminate HCV-infected cells was proposed by Hsu et al. (2003). An apoptosis-inducing molecule (BH3 interacting domain death agonist, BID) was modified to contain a cleavage site specifically recognized by the HCV NS3/NS4A protease. Cleavage of the BID precursor molecule by the viral protease activated the cell-death pathway, resulting in apoptosis of the HCV-infected target cell. Uninfected cells are resistant to this apoptosis-inducing molecule. The efficacy of this approach was evaluated by injecting three doses of an adenovirus expressing this modified BID into six HCV-infected human liver-uPA mice. This resulted in a 100- to 1000-fold decrease of viral RNA in the serum during treatment, and it even led to clearance of the infection in animals that had a low viral titer at initiation of therapy. Although this procedure cannot immediately be applied to treat infected humans, it might be a promising strategy once safe and effective delivery mechanisms are available.

6.7. Treatment of HBV infected mice

In addition to interferon, different antiviral compounds, mostly polymerase inhibitors, are routinely used to treat chronic HBV-infected patients (Ferir et al., 2008). Lamivudine is a cytidine analogue that leads to premature chain termination upon incorporation into the viral DNA strand. HBV-infected chimeric mice treated with this compound showed a significant reduction in viral replication (Tsuge et al., 2005). Interestingly, a virus isolated from a patient who relapsed from lamivudine therapy was insensitive to treatment in chimeric mice. While a 6-week course of lamivudine induced a 2.8 $\log_{10}$ reduction in HBV DNA in wild-type infected mice, animals infected with the mutant variant only experienced a 0.39 $\log_{10}$ drop. Similarly, treatment with adefovir dipivoxil was shown to be effective in HBV infected chimeric mice. After 6 weeks of adefovir treatment, a more than 100-fold decrease in HBV DNA titer was observed, but a rebound to baseline levels occurred within 3 weeks after cessation of the therapy (Dandri et al., 2005).

7. Conclusions and prospects

The generation of the human liver-uPA-SCID mouse represents a major step forward for the study of human hepatotropic viruses. The transplanted human hepatocytes reside in their natural environment (the liver) and maintain normal functions. More importantly, these animals can be infected in vivo with natural HBV and HCV in a reproducible manner and these infections always show a chronic evolution. We have succeeded in infecting animals with HCV of all six genotypes (unpublished data). The model can therefore be utilized to evaluate in vivo the efficacy of newly developed antiviral compounds that target any possible step in the viral life cycle. In addition, by using hepatocytes from different donors, the possible influence of the genetic background of the donor on the efficiency of antiviral therapy can be investigated. The use of patient serum as viral inoculum allows for the study of the impact of quasispecies diversity on drug efficiency.

However, there are also a few downsides. The transplantation of newborn mice is technically very challenging because of the bleeding disorder of the mice. An even more important factor is the availability of good quality primary human hepatocytes. This requires excellent coordination with the surgeons from, preferably, a hospital with a large liver center. Alternatively, commercially available hepatocytes could be used but these are very costly and certainly not always a guarantee for success. A more difficult problem to get a grasp on is the variability in acceptance of the graft by the recipient mice. Even among littermates, a large difference in repopulation can be observed. Overall, even in our experienced hands the number of animals that can be used for infection studies is less than 50% of those that have been transplanted with good quality hepatocytes.

Since these chimeric animals are genetically immune deficient they are not suited for the evaluation of adaptive immune responses towards HBV and HCV. Vaccine studies are therefore currently impossible. To circumvent this problem, chimeric mice need to be equipped with a functional human immune system. This could be realized by transplanting stem cells or immune competent human cells. However, to create a useful model the hepatocyte and lymphoid cell donor need to be partially or fully haploidentical. Direct transfer of human peripheral blood mononuclear cells (PBMC) into the human liver chimeric mice is impossible because this action will be immediately followed by an acute and lethal graft-versus-host reaction. The development of a human liver-uPA-SCID mouse with a functional human immune system is a major challenge that requires further research. Once this has been achieved, the ultimate small

animal model for the study of viral hepatitis will have been created.

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