Towards an HBV cure: state-of-the-art and unresolved questions—report of the ANRS workshop on HBV cure

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ABSTRACT

HBV infection is a major cause of liver cirrhosis and hepatocellular carcinoma. Although HBV infection can be efficiently prevented by vaccination, and treatments are available, to date there is no reliable cure for the >240 million individuals that are chronically infected worldwide. Current treatments can only achieve viral suppression, and lifelong therapy is needed in the majority of infected persons. In the framework of the French National Agency for Research on AIDS and Viral Hepatitis 'HBV Cure' programme, a scientific workshop was held in Paris in June 2014 to define the state-of-the-art and unanswered questions regarding HBV pathobiology, and to develop a concerted strategy towards an HBV cure. This review summarises our current understanding of HBV host-interactions leading to viral persistence, as well as the roadblocks to be overcome to ultimately address unmet medical needs in the treatment of chronic HBV infection.

INTRODUCTION

HBV infection is a major public health problem with >240 million chronically infected individuals worldwide. These subjects are at high risk of developing liver cirrhosis and hepatocellular carcinoma (HCC). Whereas effective and safe vaccines exist to prevent HBV infection, there is no cure for the majority of patients with chronic infection. Several antiviral agents are approved for the management of these patients, including interferon (IFN)-α or pegylated (PEG) -IFN-α and nucleoside analogues (NUC). A 48-week (PEG) -IFN-α-based therapy leads to a sustained virological response (SVR) and HBsAg loss off-treatment in only 3–7% of patients.[1 2] Adverse effects and contraindication to (PEG) -IFN-α administration represent major drawbacks of this treatment. Long-term oral treatment with NUC is better tolerated, but the duration of the treatment is unpredictable and the chance to achieve an SVR and HBsAg loss off-treatment is very low (approximately 1% per year).[3 4] The main goal of current antiviral therapies is sustained suppression of HBV viraemia (ie, circulating HBV), yet without decreasing intrahepatic replication, thereby only slowing down progression of liver disease. Recent antiviral agents can induce reduction of viraemia below threshold of detection in 95–100% of cases with virtually no emergence of resistance (reviewed in ref. [5]). However, as these treatments do not eradicate the intrahepatic replication of the virus, they require lifelong and costly administration that is not affordable in most middle and underdeveloped countries with endemic HBV infection. Given these issues, there is an unmet medical need for an efficient HBV cure.[6]

The French ANRS (National Agency for Research on AIDS and Viral Hepatitis) recently created the 'HBV Cure' programme to (i) promote basic and translational science studies, (ii) shape the organisation of HBV research in France and (iii) foster international collaborations in the field of HBV, similarly to what has been implemented with the HIV Cure Initiative.[7] A coordinated action has been launched in 2014, and a first scientific workshop was organised on 17 June 2014 in Paris to bring together researchers, clinicians and pharmaceutical companies to define the current state-of-the-art and unanswered questions in HBV pathobiology in order to develop a concerted strategy towards an HBV cure.

In this review, we summarise key unanswered questions both at clinical and basic research levels as well as our current understanding of HBV host-interactions leading to viral persistence (boxes 1 and 2). We also highlight the roadblocks that were addressed during the workshop, which need to be overcome in order to fulfil unmet needs in the treatment of chronic HBV infection.

THE CLINICAL NEEDS FOR CHRONIC HEPATITIS B: DEFINING A CURE FOR HBV

HBV is an enveloped DNA virus of the *Hepadnaviridae* family comprising a partially double-stranded relaxed circular DNA (rcDNA) genome. Following entry into human hepatocytes, this rcDNA is converted into a covalently closed circular DNA (cccDNA) in the host cell nucleus. Around 10% of the virions contain double-stranded linear DNA, which are the major precursors for integration of viral DNA into host DNA[8 9] (1:10 000 hepatocytes). But contrary to retroviruses, HBV DNA integration

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Box 1 Unanswered questions in the clinical management of hepatitis B

- Advance our understanding of viral and host factors involved in the pathogenesis of chronic HBV infection in order to improve strategies for chronic hepatitis B management and treatment.
- Identify biomarkers of disease progression to efficiently prevent cirrhosis and hepatocellular carcinoma (HCC).
- Assess the impact of treatment of immune-tolerant patients or inactive carriers on the prevention of cirrhosis and HCC.
- Identify appropriate short-term and long-term endpoints to define an HBV cure.
- Develop new classes of antivirals and therapeutic strategies, including combination therapies, to ultimately cure the majority of patients with chronic hepatitis B.

into the host DNA is not required for completion of the HBV replication cycle; however, this phenomenon may lead to carcinogenesis.[10] As linearisation leads to disruption of at least one open reading frame, only cccDNA serves as a template for the transcription of all four viral RNAs that function as mRNAs for translation of the seven HBV proteins: the envelope protein consisting of three separate surface proteins (large (L), middle (M) and small (S) proteins—HBsAg); the DNA polymerase (P protein); the core protein or capsid protein, also termed hepatitis B c antigen (HBcAg); the hepatitis B x protein (HBx); and the hepatitis B e antigen (HBeAg). The latter is a secreted proteolytically modified form of the capsid protein, which is derived from translation from a start codon upstream the core AUG resulting in frame translation of the pre-core region targeting the protein to the secretory pathway. Furthermore, one of these RNAs, which also encodes for HBcAg and the polymerase, represents the template for viral replication via reverse transcription and is accordingly termed pregenomic RNA (pgRNA). This RNA, but also the HBeAg encoding mRNA, is transcribed under the control of the basal core promoter. HBV RNAs can also be spliced allowing additional proteins to be produced such as hepatitis B spliced protein (HBSP).[11 12] HBV is classified into 10 different genotypes (A–J) with distinct geographical distributions. HBV genotypes influence disease severity, risk of HCC development and response to IFN therapy.[13 14]

The natural history of HBV infection is variable, and the outcome of infection is mostly dependent on the age of individuals at the time of infection: while infection with HBV during adulthood results in acute self-resolving infection in the vast majority of individuals, infection at birth by perinatal transmission or during childhood usually progresses to chronic hepatitis B. Chronic HBV carriers can pass through different disease phases that correlate with immunopathology. These often include an 'immune-tolerant' phase, an 'immune-reactive' phase, an 'inactive carrier' stage and a 'reactivation' phase, mainly based on clinical markers such as serum levels of HBV DNA (ie, viraemia) and transaminases (reviewed in ref.[15]). Diagnosis of ongoing HBV infection is based on the detection of serum HBV DNA and HBsAg. The presence of HBsAg-specific antibodies in the serum (HBsAg seroconversion) testifies either to recovery from HBV infection or to immunisation. In rare cases, HBsAg and anti-HBs antibodies can be detected in parallel, particularly during fulminant acute hepatitis. HBeAg can also be detected in the serum of HBV-infected patients. Loss of HBeAg and development of anti-HBeAg antibodies (HBeAg seroconversion) correlates with resolution of active disease and, often but not always, of the infection. However, chronic carriers can be classified into either HBeAg-positive or HBeAg-negative chronic hepatitis B. The latter are characterised by a low viral load and HBV genome harbouring nucleotide substitutions in the pre-core regions generating a stop codon, thereby abrogating production of HBeAg or mutations in the basal core promoter region leading to decreased HBeAg expression. HBeAg-positive patients can achieve HBeAg loss and seroconversion to anti-HBeAg antibodies during antiviral therapy, but they remain at a higher risk of HBV reactivation than patients with spontaneous HBeAg seroconversion.[16] Noteworthy, although the 'immune-tolerant' phase—related to perinatal transmission and clinically defined as HBeAg-positive with high serum HBV DNA levels but normal aminotransferases—is usually asymptomatic and characterised by low incidence of liver inflammation and fibrosis. However, accumulating evidence challenges this concept.[17] Indeed, newborns are not devoid of virus-specific T cell responses and preserved T cell functions have been reported in patients with immune-tolerant HBV.[18] It has been suggested that absence of liver inflammation in these patients may reflect a reduced proinflammatory cytokine reaction characteristic of young individuals.[17 19] Moreover, a recent study in mice where HBV persistence was modelled by hydrodynamic injection of an HBV genome-containing plasmid suggested that HBV persistence may induce hepatocyte-intrinsic immunotolerance that leads to an HBV-specific systemic adaptive immune tolerance.[20]

Box 2 Unanswered questions in basic HBV research

- Uncover the molecular mechanisms of HBV entry.
- Characterise the molecular mechanisms underlying formation and regulation of covalently closed circular DNA.
- Determine the exact role of the hepatitis B X protein.
- Develop novel *in vitro* models for the study of viral dissemination.
- Assess the role of innate immunity in HBV clearance.
- Identify virus-specific determinants of HBV-specific T cell exhaustion involved in viral persistence.
- Understand the role of B cells in the control of HBV infection.
- Characterise the mechanisms involved in HBV-induced hepatocellular carcinoma.

Cohort studies are very valuable to better understand the natural course of HBV infection. Before the implementation of a mass vaccination programme, Taiwan had one of the highest rates of HBV infection in the world with a 15–20% HBsAg carrier rate, liver cancer representing the second leading cause of mortality in this country.[21 22] This nationwide vaccination programme led to a dramatic decrease in HBsAg prevalence and incidence of liver cancer.[23 24] Furthermore, within the past years, the clinical outcomes of Taiwanese patients with HBV have been thoroughly studied in three large cohorts of patients (REVEAL-HBV, SEARCH-B and ERADICATE-B). This led to the identification of several factors associated with disease progression. The risk factors for a greater risk of HCC include male gender, age, high alanine aminotransferase (ALT) levels, high HBV DNA levels, high HBsAg levels and HBV genotype C.[25] First, HBV viral load is a strong independent risk predictor for cirrhosis and HCC in patients aged 30 or older, patients with persistently high HBV DNA levels having the highest risk of liver disease progression;[26 27] the risk of HCC

development in patients with high HBV levels and normal ALT remains to be determined. In clinical practice, the close monitoring of HBV viral load may help define which HBV carriers aged 30 or older are at high risk of developing cirrhosis and HCC. Second, in spontaneous HBeAg seroconverters with HBV genotype B or C infection, a low serum HBsAg level at the early HBeAg-negative phase was associated with a higher HBsAg loss rate. However, serum HBV DNA levels were a better predictor than HBsAg levels of disease progression in spontaneous HBeAg seroconverters.[28 29] Third, despite the lack of correlation between pre-core/core promoter HBV variants and HBeAg-negative hepatitis, a major proportion of basal core promoter mutants was associated with an increased risk of cirrhosis for patients with high viral load.[30] Thus, persons with high frequency of core promoter mutants should be considered to receive early therapy. Taken together, the findings from these cohort studies suggest that HBV DNA and HBsAg are complementary markers for the risk of disease progression.[31] Moreover, these results led to the definition of an algorithm to categorise disease progression in Asian HBV carriers. The algorithm provides a risk score for development of HCC, which may thereby improve the clinical management of patients with chronic HBV.[31 32] Nevertheless, to date the costs for assessing the different risk parameters remain an economic issue and thus more cost-effective risk calculators are currently being developed. Moreover, it was recently described that the risk of HCC cannot be confidently predicted using HCC risk scores at baseline nor during therapy in Caucasians.[33] Currently, international liver societies recommend antiviral treatment only during the inflammatory phase of the disease. The goal of antiviral therapy is the prevention of disease progression towards end-stage liver disease and HCC. Nevertheless, even subjects in 'inactive carrier' stage can develop liver cancer in the long term, and this may be an issue for individuals that have been infected very early in life.[34] A better understanding of time course of HBV pathogenesis is required to better predict disease progression and improve the clinical management of chronic hepatitis B. With this in mind, ANRS launched CO22 HEPATHER, a large French cohort that aims to include 10 000 patients with viral hepatitis B from 32 clinical centres and gather clinical and therapeutic data, biological collections and quality-of-life criteria. Subjects will be followed for 8 years and about one million biological samples will constitute an unprecedented biobank. This cohort will enable scientists to better describe the progression of chronic viral hepatitis in the long term and identify associated prognosis factors, including biomarkers. It will also allow evaluation of clinical effectiveness and safety of treatments in 'real-life' situation in order to identify treatments that will most likely improve overall patient health while limiting the emergence of escape variants and viral breakthroughs. Moreover, this cohort will define cost-effective strategies for the management and treatment of chronic viral hepatitis. Finally, it will provide resources for clinical trials or research on biomarkers in tailored subpopulations.

To achieve a cure for chronic hepatitis B, it is important to discuss the definition of the concept of 'HBV cure' and endpoints of antiviral treatment. In theory, the virological definition of cure would be the eradication of cccDNA (as the ultimate goal), but in practice the clearance of HBsAg would be more easily achievable in a shorter term. A definition of a functional cure would be HBsAg seroconversion even in case of liver cccDNA persistence, along with cessation of liver disease[35] (table 1). When viral eradication is not achievable, lowering of liver cccDNA levels, inactivation of cccDNA-directed transcription to prevent viral replication and induce a remission of liver disease could be a realistic endpoint. However, aiming for an inactive carrier status is probably not ambitious enough given that these subjects remain at risk for developing HCC.[33 34] Moreover, the long-term consequences of HBV genome integration will have to be taken into account even following viral eradication or control. It is worth noting that persons who clear HBV infection after a chronic infection have a greater risk of developing HCC than individuals who have not been infected with HBV.[36 37] Moreover, patients that resolved an infection may see virus reactivation, with attendant liver disease, in case of immunosuppressive therapy for cancer, autoimmune disease or organ transplantation.[38] Therefore, in the near future, the aims of the field are to (i) advance our understanding on the viral and host factors involved in HBV pathogenesis, including innate and adaptive antiviral immune responses; (ii) uncover biomarkers of disease progression in order to identify patients with minimal hepatitis who are at risk of developing cirrhosis and HCC and (iii) define new targets for antiviral therapy to achieve an HBV cure.

Table 1 Definitions of HBV cure

	HBsAg	Anti-HBs Ab	Viraemia	cccDNA
Functional cure	–	+	–	+
Complete cure	–	+	–	–

Ab, antibodies; cccDNA, covalently closed circular DNA.

Furthermore, it is important to take into account that between 5% and 10% of chronic HBV carriers are also chronically infected with the hepatitis delta virus (HDV), an infectious agent that needs HBV for its production. In HBV/HDV coinfected patients, HDV virions are produced in coinfected liver cells, along with HBV particles. HDV virions are coated with the envelope proteins of the helper HBV and contain an inner ribonucleoprotein (RNP) consisting of the HDV RNA genome and HDV-encoded proteins. The HDV RNA can replicate to very high levels in hepatocyte nucleus, leading to the production of HDV RNPs that can egress only in the presence of HBV envelope proteins and after assembly of HDV virions. The latter can subsequently infect human hepatocytes using the same entry pathway as the one used by HBV (figure 1) to propagate infection throughout the liver. The result of chronic HBV/HDV coinfection significantly worsens the course of the liver disease.[39 40]

STATE-OF-THE-ART AND UNANSWERED QUESTIONS IN HBV BASIC RESEARCH

Model systems to study HBV

Within the past years, researchers have used various model systems (table 2) to characterise the HBV replication cycle (figure 1) and identify novel antiviral targets as well as to study HBV pathogenesis and assess the efficacy of novel therapeutic strategies. HBV particles are taken up by hepatocytes through a receptor-mediated internalisation mechanism that is not well understood yet. After internalisation, viral capsids are released and subsequently directed to the nucleus where HBV genomes are liberated. In the nucleus, rcDNA genomes are converted into cccDNA that may persist in the nucleus of infected cells as minichromosomes and serve as template for viral RNA transcription. In the cytoplasm, together with the viral polymerase, pgRNA is encapsidated and reverse transcribed within the nucleocapsid into progeny rcDNA. Mature nucleocapsids are

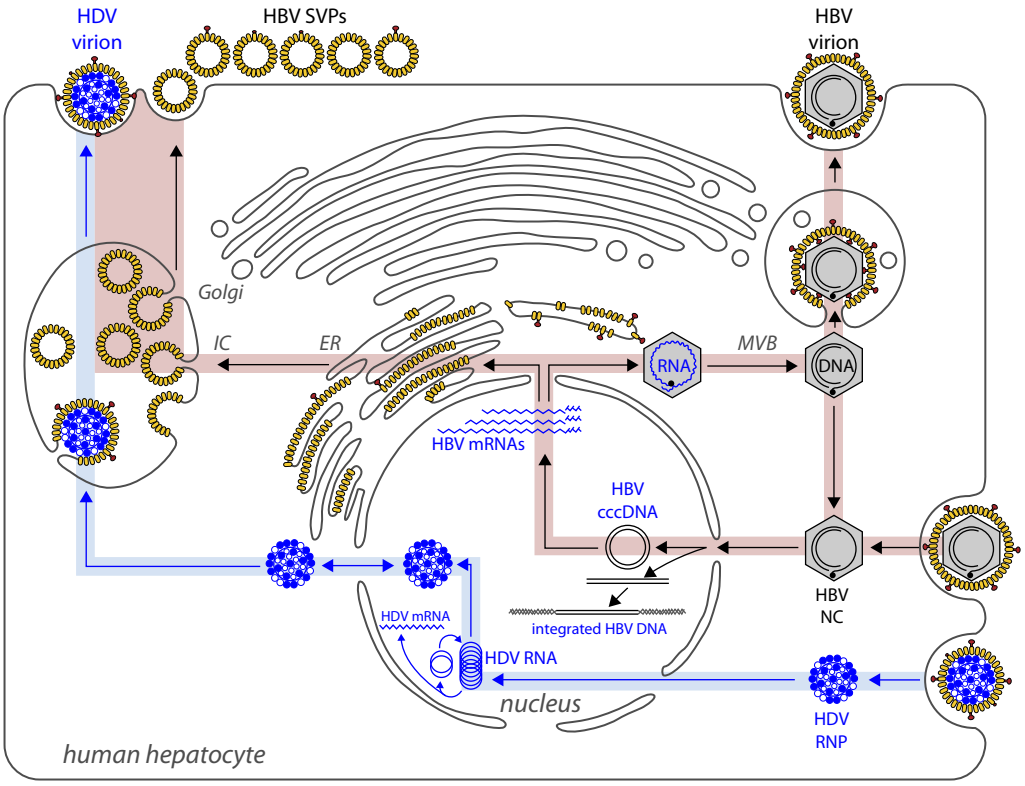

Figure 1 Schematic overview of the HBV/hepatitis delta virus (HDV) life cycles. cccDNA, covalently closed circular DNA; MVB, multivesicular bodies; RNP, ribonucleoprotein; SVP, subviral particles.

then either directed to the multivesicular body pathway for envelopment with HBV envelope proteins or directed to the nucleus to establish a cccDNA pool.[41]

Several steps of the HBV life cycle are now known in detail, but the mechanisms of viral entry, cccDNA formation and regulation, intracellular trafficking and morphogenesis, as well as interaction with the host immune system, are still poorly understood. This lack of information is due to the difficulties encountered in obtaining a robust tissue culture system (table 2) and the lack of practical animal models recapitulating the HBV life cycle and pathogenesis (table 3).

Human hepatocytes are the natural target cells of HBV and HDV. These cells can be isolated from liver resections and retain susceptibility to HBV infection for a short period in culture.[42] However, the accessibility to fresh human liver resections, the quality and the variability of the individual preparations limit their use. In the mid-1990s, several laboratories showed that primary hepatocytes of *Tupaia belangeri* were also susceptible to HBV infection (reviewed in refs. [43 44]). Although primary tupaia hepatocytes are valuable to study HBV infection, the difficulty to rear these animals and the absence of tupaia-specific reagents for functional studies limit their use. To bypass the hurdles to using primary cell cultures, human hepatoma Huh7 and HepG2 cell lines were used for many years to perform in vitro experiments on HBV. Although those cells are permissive to HBV replication and viral particle assembly, they are not susceptible to infection due to the lack of expression of the receptor(s) and thus only allow study of post-transcriptional steps of the HBV life cycle after plasmid transfection. Alternatively, the HepaRG cell line, described in 2002, can be used for in vitro studies. HepaRG cells are liver progenitors that become susceptible to HBV and HDV infection after differentiation in culture.[45] However, infection rates are low and virus spread within the cultures was never observed. Since the recent discovery of sodium taurocholate co-transporting polypeptide (NTCP) as an HBV/HDV receptor,[46 47] HepG2 and Huh7 cell lines (over)-

Table 2 Human cells for HBV study in vitro

	Immortalisation	Transformation	Availability	Variability	Rate of infection	DMSO for infection	cccDNA levels*	HBV propagation	Innate immunity	Maintenance
Primary human hepatocytes	–	–	+	+++	20–100%	1.8–2%	1–2 copies per nuclei	–	+++	2–3 weeks
Differentiated HepaRG cell line	+	–	+++	++	5–20%	1.8–2%	0.2–0.5 copies per nuclei	–	+++	>6 months
HepG2/Huh7 cell lines	+	+	+++	+	0%	0%	–	–	–	–
NTCP-HepG2 cell line	+	+	+++	+	50–100%	2.5–3.5%	1–5 copies per nuclei	–	–	10 days

*After HBV infection with a multiplicity of infection of 1000 vge/mL (viral genome equivalent per mL).
cccDNA, covalently closed circular DNA; DMSO, dimethyl sulfoxide; NTCP, sodium taurocholate co-transporting polypeptide.

Table 3 In vivo models for HBV study

	Chimpanzees	Macaques	*Tupaia belangeri*	HuHep mice	His-HuHep mice	Ad-HBV or AAV-HBV mice
HBV entry	+	?	+	+	+	–
HBV production	+	+	+	+	+	+
cccDNA establishment	+	?	+	+	+	–
Chronic HBV infection	–	?	+	+	?	+
HCC development	–	?	?	?	?	?
Adaptive immune responses	+	?	?	–	+	+
HBV tolerance	–	?	?	–	+	+
Antiviral drug testing	+	?	+	+	+	+
Therapeutic vaccine development	+	?	?	–	+	+

cccDNA, covalently closed circular DNA; HCC, hepatocellular carcinoma.

expressing NTCP have been generated. These cells are susceptible to HBV and HDV infection, but their capacity to allow virus propagation remains to be determined as does their relevance, because of their transformed nature, for studies of virus–host cell interactions. Finally, a recent study showed that micropatterning and co-culturing of primary human hepatocytes or induced-pluripotent stem cells differentiated into hepatocyte-like cells with fibroblasts maintains prolonged HBV infection,[48] a model eventually amenable to study virus–host interactions and antiviral drugs affecting early infection steps.

While cell culture models are very valuable to characterise defined aspects of the viral life cycle, in vivo models are necessary to study HBV pathogenesis and new antiviral strategies including immunotherapies (table 3). HBV has an extremely narrow host range since it only infects hominoid apes including chimpanzees. The latter have been used in pivotal studies deciphering host responses during acute HBV infection[49 50] but are no longer available for experimental studies.[51] Therefore, macaques, which have a 93% sequence identity with humans and are highly used in toxicology, have been considered as an alternative model to study viral hepatitis. In addition, a naturally occurring, transmissible, chronic HBV infection has been found in cynomolgus macaques from Mauritius with a prevalence of 53%. The viraemia lasted up to 9 months and interspecies transmission was possible to the sylvanus macaques of Morocco.[52] It was found that the preS1 binding site in sylvanus macaques NTCP is identical to that of *Macaca fascicularis* in bearing substitutions at positions 157–158 (Isabelle Chemin, personal communication) that were described as detrimental to an HBV receptor function.[46 53] Further studies are required to improve the robustness of the model before using macaques as an alternative to chimpanzees and for developing immunotherapeutic approaches. There are also various HBV-related viruses such as duck HBV, woodchuck HBV and ground squirrel HBV that have been invaluable models to study HBV infection. The woodchuck is one of the best models available and has been used to explore many aspects of *Hepadnaviridae* biology such as the pathogenesis of the infection, new vaccines, therapeutic vaccination, drug toxicity and antiviral drugs.[54] However, this is an expensive model with a very limited number of animals available from commercial sources. In addition, there is a series of limitations: cancer development is strikingly different to humans as woodchucks integrate linearised genomes close to N-myc; the metabolism changes with their hibernation limiting the time during which studies can be performed; and sophisticated immunological tools that would allow an examination of the virus-specific immunological responses are lacking. Mice are naturally not susceptible to HBV infection, but they can be humanised to study HBV infection in vivo. Four murine models have been used to generate human liver-chimeric (HuHEP) mice: urokinase-type plasminogen activator-severe combined immunodeficiency (uPA-SCID), FRG (Fah–/– Rag2–/– IL2Rγc–/–), thymidine kinase (NOD/Shi-scid/IL-2Rγ^{null}) (TK-NOG) and AFC8.[55] These mice are characterised by a progressive degeneration of mouse liver cells and immunodeficiencies, thereby allowing engraftment of human hepatocytes. Re-population of the liver by human hepatocytes is monitored by determination of the human albumin levels in the serum of the mice. Recently, an 'ANRS Consortium on Humanized Mouse Models for Viral Hepatitis' was created to develop, compare, optimise and master HuHEP models in France and for share with the 'viral hepatitis' research community. The ANRS consortium focuses on three main models: FRG, uPA-SCID and BRGS (BALB/c Rag2–/– IL-2Rγc–/– SIRPα.NOD) uPA models. FRG mice have a triple knock out: fumaryl acetoacetate hydrolase (FAH)–/–, Rag2–/– and IL2Rγc–/–. The knock-out of FAH induces liver toxicity, which can be controlled by administration of NTBC 2-(2-nitro-4-trifluoromethylbenzoyl) cyclohexane-1,3-dione to control the degree of liver damage and the engraftment of human hepatocytes postinfusion.[56] Inoculation of human liver-chimeric FRG mice with HBV leads to a sustained production of HBV particles in the serum of these mice for up to 10 weeks.[57] uPA-SCID mice express the uPA transgene under the control of the albumin promoter, which induces liver damage and allows subsequent repopulation with human hepatocytes.[56] Inoculation of liver humanised uPA-SCID mice with HBV also leads to productive infection,[58] and this model has been used for proof-of-concept studies assessing the efficacy of novel antiviral strategies (refs. [59 60 61] and reviewed in ref.[62]). Furthermore, human liver-chimeric uPA-SCID mice have already been used to study HBV/HDV coinfection.[63 64] Nevertheless, the absence of a functional immune system and human liver microenvironment in these models precludes the study of defined aspects of HBV/HDV infection. To allow assessment of viral pathogenesis in the context of a functional human immune system and to test immunotherapies, a double humanised mouse, carrying both a humanised immune system and human hepatocytes (HIS-HuHEP mice), was created in BRGSuPA mice (Strick-Marchand, personal communication). Following infection with HBV, the cross-talk between the immune system and the infected hepatocytes was analysed, and a balance between proinflammatory and immunosuppressive modulators was observed in HIS-HuHEP mice (Strick-Marchand, personal communication), suggesting that this model may recapitulate key aspects of chronic viral hepatitis in patients. Finally, two immunocompetent mouse models of chronic HBV infection have been established recently by using low doses of adenovirus-associated[65] or adeno-associated virus-mediated[66] gene transfer of HBV. The further development of such small animal models will allow testing of novel therapies

combining direct-acting antivirals (DAAs) with immunomodulatory drugs, as well as the consequences of coinfection by hepatic viruses and HIV.

The HBV replication cycle: from molecular mechanisms to antiviral targets

HBV/HDV entry into hepatocytes: the first steps of virus–host interactions

HBV entry into hepatocytes requires both viral and host factors. Given that HBV and HDV share the same envelope proteins, HDV has been widely used as a surrogate model to study HBV entry. While the viral determinants of HBV/HDV entry have been extensively characterised over the past years, the host factors involved in HBV/HDV entry remained elusive until the recent discovery of NTCP as an HBV/HDV receptor.[46] The viral membrane contains three forms of the viral envelope protein: large (L), middle (M) and small (S). They are translated from their own start codons but share the same C-terminal amino acids, called the S domain. As a consequence, the M protein contains an extra domain called the pre-S2 domain compared with the S protein, and the L protein contains two extra domains called the pre-S2 and pre-S1 domains. It has been known for a long time that the M protein[67 68] as well as glycosylation of the envelope protein is dispensable for HBV/HDV assembly/infectivity.[69] On the contrary, pre-S1 is crucial to infectivity of HBV and HDV[70] and needs a minimal spacer between pre-S1 and the transmembrane domain 1 (TMD1) for activity in viral entry.[71] It was also shown that the antigenic loop (AGL) in the S protein—but not in the L protein—is essential for infectivity and that this AGL and the pre-S1 infectivity determinants work independently of each other.[72 73] AGL mediates attachment to cell surface heparan sulfate proteoglycans (HSPGs).[74 75] Noteworthy, hepatocytes synthesise very liver-specific HSPG sequences that may contribute to the tissue specificity of the infection. TMD1 was also shown to be instrumental in infectivity.[76] However, it is not yet known whether it contains a fusion peptide like other enveloped viruses. Identification of a fusion peptide essential for viral entry is a fundamental unresolved issue about HBV. Finally, cholesterol in the viral membrane (but not in the target cells) is required for infectivity.[77] Very importantly, it was shown that a myristoylated pre-S1-specific peptide (2–48 Myr also known as antiviral candidate Myrcludex) is a potent inhibitor of viral entry,[78 79] working within nanomolar ranges. The Wenhui Li Beijing group recently used this peptide as a bait to pull down the HBV/HDV receptor and identified NTCP,[46] a member of the solute carrier protein family, as a functional HBV entry factor. It is expressed at the basolateral membrane of the hepatocytes for uptake of bile acids but also for transport of hormones and several xenobiotics. Two determinants of HBV receptor function in NTCP (amino acids 84–87 and 157–165) have been identified.[46 53] Interestingly, a number of polymorphisms have been reported in human NTCP, which alter bile acid transporter activity, but it remains to be determined whether they all correlate to a lack of HBV receptor activity. That is, for instance, the case for the S267F mutation observed in 7.5% of Chinese Americans, leading to a loss of bile acid transport and a loss of susceptibility to HBV infection.[80] Altogether, the available data on HBV/HDV entry allow proposing a relatively simple model involving HSPGs as low-affinity receptors for virus docking at the human hepatocyte surface, and NTCP as high-affinity, liver-specific receptor.[81] However, our knowledge of the HBV/HDV entry mechanism is still limited, despite recent progress. Indeed, additional host cell factors, functioning as co-receptors, are probably involved in the HBV entry process, either prior to NTCP binding or post-NTCP binding, to participate in an eventual fusion mechanism. Subviral particles may also play a role in viral entry because they are fully equipped to interact with HSPGs and theoretically also with NTCP. Indeed, it remains to be determined whether the amount of L proteins in subviral particles is too low for efficient interaction with NTCP or whether an alternative mechanism prevents them from competing with Dane particles. It also remains to be determined whether entry involves clathrin-dependent endocytosis or raft/caveolar endocytosis.[77 82 83] Using microscopy and pharmacological inhibitions, it was demonstrated that HBV capsids are transported to the nucleus through a microtubule-dependent mechanism[84] involving direct interaction with specific dynein light chains (Michael, Kann, personal communication). Once arriving in the nuclear periphery the capsids interact with importin α/β receptors,[85] HBV capsids pass the nuclear pores intact and arrive in the nuclear basket. It was shown that capsids interact with one specific protein of the nuclear basket, called Nup153, that arrests them.[85] While immature capsids stay arrested, mature capsids (containing rcDNA) disintegrate, leading to viral genome release.[85]

cccDNA formation and regulation: key steps for viral persistence

cccDNA is responsible for HBV persistence in the liver and even a single copy could theoretically reactivate full infection. Despite >30 years of molecular biology study on HBV, little is known about how cccDNA is formed and regulated. A better understanding of these mechanisms will probably be instrumental to curing HBV infection. cccDNA is exclusively produced from rcDNA, either from incoming virions or from neoformed nucleocapsids, probably by a multistep process including (i) the removal of the polymerase (P) protein covalently linked to the minus strand of rcDNA, (ii) removal of the RNA primer covalently linked to the plus strand of rcDNA, (iii) generation of exactly one unit length double-stranded DNA and (iv) ligation of the ends of both strands. It is believed that most of these activities are provided by the host cell. A very recent study uncovered the mechanism of P protein removal from rcDNA.[86] Indeed, it was demonstrated that tyrosyl-DNA-phosphodiesterase (TDP)2 can specifically cleave the Tyr–DNA bond and release P protein from authentic HBV rcDNA in vitro. Moreover, interfering RNA (RNAi)-mediated TDP2 depletion in human cells significantly slowed down the conversion of rcDNA to cccDNA, while ectopic TDP2 expression in the same cells restored conversion kinetics.[86] These data strongly suggest that TDP2 is one but likely not the only host DNA-repair factor involved in HBV cccDNA biogenesis. Once formed, cccDNA persists as a minichromosome in the nucleus of infected cells[87 88] and the regulation of its transcription occurs through epigenetic modulations. Using chromatin immunoprecipitation assays (ChIP), it has been shown that modification of histones bound to cccDNA regulates its transcriptional activity. Indeed, HBV replication parallels the acetylation status of HBV cccDNA-bound H3 and H4 histones in HBV replicating cells and in patients.[89] A number of transcription factors, chromatin-modifying enzymes as well as viral proteins (ie, HBc and HBx), were identified as cccDNA-bound and involved in its modulation[88 90] HBx was shown to be necessary for the transcription from cccDNA through epigenetic regulation.[90 91] However, studies of the epigenetic regulations of cccDNA are still limited in current HBV replication models. Indeed, the detection limit of ChIP assays is 0.2 cccDNA copies per cell and 0.5–1 cccDNA copies per cell are necessary to assess multiple parameters in the same biopsy sample, but it also depends on the number of infected cells in the sample.

Assembly and dissemination of virions

Following cccDNA transcription by host RNA polymerase II into HBV mRNAs in the nucleus and translation of the latter to

viral proteins, assembly of core protein subunits with pgRNA into nucleocapsids in the cytoplasm represents the initial step in the assembly of progeny virions. Neoformed nucleocapsids then interact with the HBV envelope proteins at a postendoplasmic reticulum, pre-Golgi compartment, before being released from the cells as mature enveloped virions through the multivesicular body pathway,[92] whereas the huge excess of HBV envelope proteins that is a characteristic of an HBV-infected cell is exported as empty subviral particles through the cell secretory pathway. Some key aspects of viral assembly and dissemination of virions remain unanswered. For instance, mechanisms that are favouring secretion of neoformed nucleocapsids rather than recycling towards the nucleus and vice versa are still unclear. Virus assembly in the setting of antiviral-induced inhibition of viral DNA synthesis (for instance, by NUC) has not been studied either. Indeed, for instance, the fate of pgRNA-containing nucleocapsids (degradation or secretion) is unknown.

HBV pathogenesis: interplay between the virus and the immune system

The outcomes of HBV infection are highly dependent on interactions between the virus and the host immune system. Indeed, whereas 95% of immunocompetent adults will clear the infection, only 5–10% of children will be able to do so. Infection of hepatocytes is non-cytopathic in the short term. But as hepatocytes are long lived (half-life of ~6 months) and have self-renewing properties, the liver loses genetic complexity over time. By increasing cell death, hepatitis increases this loss of complexity and increases hepatocyte clonality. For instance, in a healthy 50-year-old individual, with one turnover per year, liver complexity will drop down to about 2% of what it was at the start of life. For a patient with active liver disease, turnover is elevated 5-fold to 10-fold. A 50-year-old patient could therefore have a 500-year-old liver. Chronic infection will thus have a major impact on complexity loss by raising the daily rate of hepatocyte turnover. The loss of hepatocyte complexity and/or the increase of DNA damage in proliferating hepatocytes could trigger hepatocyte transformation and tumour development. Transient infection causes one liver turnover and does not have a significant impact in the long term. When HBV enters the liver, it is confronted with many different cell types. Indeed, the liver is a complex and structured organ that contains hepatocytes (parenchymal cells), non-parenchymal cells such as liver sinusoidal endothelial cells, stellate cells and numerous resident immune cells, including Kupffer cells (KC), dendritic cells (DCs), natural killer (NK)/NKT, CD4+ T cells, CD8+ T cells, regulatory T cells (Treg) and B cells. These cells are organised according to a very particular and unique architecture. The importance of the whole liver microenvironment is often underestimated, and one should be cautious with in vitro experiments using hepatocytes that may behave differently when studied outside this microenvironment. Because it contains so many immune cells, the liver is considered as a secondary lymphoid organ, with crucial immune functions.[93] However, the liver is also associated with the induction of immune tolerance,[94] as exemplified by transplantation tolerance. Acute self-limited HBV infection involves coordinated immune responses. The immune response is delayed by 4–6 weeks postinfection, during which time HBV DNA is detectable in the serum. Strong polyclonal CD8+ T and CD4+ T cell responses are then activated, which respectively result in the destruction of infected hepatocytes and antibody production.[95] It has been shown that HBV replication early in the immune clearance phase is suppressed by non-cytopathic mechanisms involving cytokines.[49] In contrast, during chronic HBV infection, there is an uncontrolled viral replication and ongoing liver damage, with a strong influx of non-HBV-specific T cells in the liver. Chronic HBV infection is characterised by (i) a low frequency of HBV-specific CD8+ T cell responses that have an exhausted phenotype characterised by overexpression of PD-1, CTLA-4, CD244, Tim3, and so on; (ii) an impaired production of interleukin (IL)-2 and impaired proliferation of CD4+ T cells and (iii) an increase in the number of Treg in the liver and in the number of IL-10-secreting T cells.[96] For a long time, HBV has been considered as a 'stealth' virus since there was no gene modulation following the onset of viraemia in HBV-infected chimpanzees[50] and very low cytokine production in patients with HBV.[97] However, it was also shown that NK/NKT[98] as well as KC[99] play a role in the early control of infection and that a strong replication of HBV in vitro led to a productive IFN response in hepatocytes.[100] These conflicting data suggest that the virus could be detected by innate immunity sensors, but it probably sets up strategies to escape from the nascent response. Indeed, it has been shown that HBV can block innate immunity at several steps in hepatocytes (for review, see ref.[101]), as well as in other liver cells. In particular, HBV has been shown to alter KC functions by blocking the secretion of IL-1β,[102] preventing dsRNA-mediated type-1 IFN gene expression[103] and inducing IL-10 production.[104] HBV is also able to block pDC functions,[105 106] as well as the cross-talk between pDC and NK cells.[107] Moreover, HBV has been reported to downregulate TLR2 expression in chronically infected patients[108] and Taqman Low-Density Array data confirmed significant impairment of innate immune pathways in chronically infected patients compared with non-infected controls.[109] Thus, the exact role played by innate immunity in HBV clearance is still unclear and, for instance, pathogen recognition receptors involved in HBV sensing and detection remain unidentified. Understanding interactions between HBV and its host is crucial to achieve an HBV cure. Indeed, experience from the 'HIV Cure' initiative launched in 2010 clearly showed the importance of a detailed understanding of the relationship between host and virus to achieve HIV cure. For instance, recent studies allowed the identification of four host restriction factors (TRIM5, APOBEC3G, Tetherin and SAMHD1) that bind to target viral compounds and inhibit viral replication at a specific point in its replication cycle (for review, see ref.[110]). Moreover, a study on a cohort of patients with HIV showed a very strong genetic bias in long-term non-progressors and elite controllers for whom the levels of the HIV reservoir remained highly stable over 10 years. The exceptional elite controller status is usually not driven by virus gross genetic defects, despite some virus attenuation resulting from immune selective pressure, but is frequently determined by host's genetic factors permitting robust cell-mediated immunity to control the virus replication and reservoirs. For instance, the only genetic marker associated with non-progression was found in chromosome 6 in the major histocompatibility complex locus and is particularly associated with human leucocyte antigen (HLA)-B27 and the HLA-B57.[111] Transcriptomic analysis also identified three specific signatures involving overexpression of T cell receptor and costimulation signalling pathways, overexpression of the PRDM-1/Blimp-1 transcriptional repressor, and downmodulation of type I IFN-related genes. Among subsets, the PRDM1/Blimp-1 upregulation was associated with lower levels of both cellular HIV-DNA and HIV mRNA levels.[112]

DRUG DISCOVERY: TOWARDS AN HBV CURE

To broaden the therapeutic landscape in chronic hepatitis B management and to ultimately achieve a reliable HBV cure,

novel antivirals with original mechanisms of action are needed. Drug development thus focuses on strategies targeting cccDNA either by preventing cccDNA formation, eliminating cccDNA or silencing cccDNA transcription. Control of cccDNA should be achievable by either capsid disassembly, inhibition of rcDNA entry into the nucleus, inhibition of conversion of rcDNA to cccDNA, physical elimination of cccDNA, inhibition of cccDNA transcription (epigenetic control) or inhibition of viral or cellular factors contributing to cccDNA stability/formation. Drugs with such activity could be DAAs targeting the virus or host-targeting agents (HTAs), including inhibitors of key host factors required for the viral replication cycle and immunomodulatory agents (table 4).

Among the emerging DAAs against HBV currently in the pipeline are novel polymerase inhibitors, capsid inhibitors, rcDNA–cccDNA conversion inhibitors, DNA cleavage enzymes and small interfering RNA (siRNA)-based strategies.[113–115] While the prospects of novel polymerase inhibitors remain to be determined, capsid inhibitors, inhibitors of cccDNA formation and DNA cleavage enzymes have great potential as novel antiviral strategies that could directly impact cccDNA pools. The recent achievement to produce recombinant HBV polymerase at a large scale may be instrumental to design novel improved polymerase inhibitors.[116] However, these novel inhibitors, whether they inhibit the priming, the RNA-dependent or DNA-dependent DNA synthesis, or the RNAseH activity of the polymerase, will have to show significant advantage over the existing nucleos(t)ide analogues with a high antiviral potency and a high barrier to resistance.[117 118] The HBV capsid plays a central role in the viral life cycle. It is essential for HBV genome packaging, reverse transcription, intracellular trafficking and maintenance of chronic infection as encapsidated HBV genomes are imported into the nucleus. The first family of nucleocapsid inhibitors, phenylpropenamide derivatives, were shown to interfere with HBV RNA packaging, leading to the formation of empty capsids and reducing the amount of intracellular immature capsids, mature viral particles and intracellular cccDNA pools, without affecting HBcAg levels.[119 120] Interestingly, a synergistic antiviral activity between phenylpropenamide derivatives and polymerase inhibitors, as well as a lack of cross-resistance, was reported in vitro, highlighting the potential of combining capsid inhibitors with other antivirals for therapy.[119 121 122] Heteroaryldihydropyrimidines (HAPs) constitute another family of capsid inhibitors.[123 124] HAPs were shown to bind to core particles in a specific but reversible manner and to reduce both HBV DNA and HBcAg levels, the latter due to degradation by the proteasome pathway.[124] In addition to inducing capsid disassembly, HAPs have been shown to enhance viral assembly and to favour assembly of aberrant particles, indicating that HAPs interfere with capsid formation/stability in a complex manner.[125–127] Similar to phenylpropenamide derivatives, HAPs are able to efficiently inhibit viral variants that are resistant to current polymerase inhibitors.[122] Moreover, a short-term study using HBV-infected human liver-chimeric mice demonstrated an HAP-induced reduction of viral load, with virus production increasing again following discontinuation of the drug.[61] Morphothiadine mesilate (GLS4) is the first member of this family of compounds having entered early clinical development; phase I and II clinical trials have been conducted in China.[128 129] Furthermore, HBV capsid proteins can traffic to the nucleus of infected cells and exert additional biological functions by repressing the transcription of several

Table 4 Emerging drugs against HBV

	Targets	Compounds	Stage of development	References or ClinicalTrials.gov Identifier
DAAs	HBV capsid	Phenylpropenamide derivatives	Preclinical and early clinical phase	119, 120
		Heteroaryldihydropyrimidines	Morphothiadine mesilate (GLS4) in phase II	123, 124, 128
	rcDNA-cccDNA conversion	Disubstituted sulfonamide	Preclinical	133
	cccDNA	DNA cleavage enzymes	Preclinical	114, 134, 135, 136
	HBV RNA	siRNA	ARC-520 in phase II	NCT02065336
		antisense	ISIS-HBVRx in phase I	170, 171
HTAs	NTCP	HBV preS1-derived lipopeptide	Myrcludex-B in phase II	149
		cyclosporine A, ezetimibe	FDA approved but not tested for HBV	146, 147, 148
	Host factors involved in HBV secretion and budding	Iminosugar derivatives of butyldeoxynojirimycin and related glycolipids	Preclinical	145
		α-glucosidase inhibitors	Preclinical	145
		triazol-o-pyrimidine derivatives	Preclinical	150
		benzimidazole derivative	Preclinical	151
		phosphorothioate oligonucleotides	REP 9 AC in phase II	152
	Innate immune responses	LTβR agonists	Preclinical	153
		TLR7 agonists	Phase II	NCT02166047
		thymosin α1	Phase IV	NCT00291616
		Nitazoxanide	Phase I	156, 157
		interleukin-7	Phase I/II	NCT01027065
		IFN-λ	Phase II	NCT01204762
	Adaptive immune responses	PD1 blockade	Phase I/II for HCC	NCT01658878
				172, 173
		X-S-Core proteins (antigen-based vaccine)	GS-4774 in phase II,	159, 160
		HBV DNA (DNA-based vaccine)	DV-601 in phase I	
			DNA vaccine pCMVS2.S in phase I/II	NCT00536627 161, 162, 164

Molecules and compounds are currently moving rapidly from one development phase to another. Updated information can be found on http://www.hepb.org/professionals/hbf_drug_watch.htm.
cccDNA, covalently closed circular DNA; DAA, direct-acting antiviral; FDA, US Food and Drug Administration; HCC, hepatocellular carcinoma; HTA, host-targeting agent; IFN, interferon; LTβR, lymphotoxin-β receptor; NTCP, sodium taurocholate co-transporting polypeptide.

IFN-stimulated genes[103 130 131] or activating the transcription of viral genes from cccDNA;[132] these nuclear functions may represent additional targets for drug development. Interestingly, it was also suggested that HAPs might directly affect cccDNA stability.[132]

A complementary approach to the development of capsid inhibitors with direct impact on cccDNA pools is the design of enzymes targeting cccDNA formation or decay. Recently, a small-molecule library screen was conducted to uncover compounds inhibiting cccDNA synthesis. This led to the discovery of disubstituted sulfonamide (DSS) compounds as inhibitors of cccDNA in cell-based assays.[133] DSS did not appear to directly promote the degradation of rcDNA or cccDNA but rather to inhibit de novo cccDNA formation by interfering with rcDNA conversion into cccDNA.[133] Furthermore, DNA cleavage enzymes, including homing endonucleases or meganucleases, zinc-finger nucleases, TAL effector nucleases and CRISPR-associated system 9 proteins, specifically targeting the cccDNA are currently being engineered.[114 134–136] These enzymes can be delivered as genes within viral vectors to target hepatocytes.[137] Computational modelling studies suggested that several enzymes may have to be administered concomitantly in order to avoid selection of resistant viruses.[114] Of note, enzyme-based strategies are currently also being evaluated in other viral infections, including HIV infection, where this approach is used in order to modify the viral receptors CCR5 and CXCR4 on T cells ex vivo (reviewed in ref.[138]). Further studies are needed to evaluate the potential of these novel antiviral strategies against HBV infection. Noteworthy, cccDNA transcription can be silenced to some extent using small molecules targeting different classes of chromatin-modifying enzymes, similar to the epigenetic silencing of cccDNA by IFN-α,[139 140] which would lead to functional, although transient, HBV cure.

Silencing HBV gene expression using RNAi constitutes another original antiviral approach against HBV. Polymer formulations enable efficient delivery of siRNA to hepatocytes.[141] ARC-520 is a combination of siRNAs directed against conserved HBV RNA sequences and efficiently knocks down HBV RNA, proteins and DNA levels. ARC-520 is currently being evaluated in a phase II clinical trial (ClinicalTrials.gov identifier NCT02065336). Other siRNAs are also at the preclinical stage. Interestingly, HBV gene silencing could also be combined with IFN induction in the liver. Indeed, 5′-triphosphate (3p) siRNAs directed against HBV can bind and activate cytosolic helicase retinoic acid-inducible protein I to induce expression of type I IFNs. The antiviral activity of these bifunctional, HBV-specific, 3p-siRNAs was more efficient and sustained for a longer time than 3p-RNAs without silencing capacity or siRNAs that targeted identical sequences but did not contain 3p.[142]

In addition to interfering with cccDNA formation and stability, future drugs aiming at curing HBV infection may target other host cell pathways to interfere with the viral replication cycle and/or restore anti-HBV immune responses (reviewed in ref.[143]). The recent clinical development of HTAs for the treatment of chronic hepatitis C highlights the promise of this approach to address unmet needs in the treatment of virus-induced liver disease (reviewed in ref.[144]). In contrast to HCV, few HTAs targeting the HBV replication cycle have been described. These include inhibitors of the recently uncovered HBV receptor NTCP and inhibitors of HBV envelope protein maturation and secretion (reviewed in refs.[81 113 145]). Small-molecule compounds binding to NTCP, including cyclosporine A and ezetimibe, have been shown to inhibit HBV/HDV entry in cell culture models, and although licensed for other clinical settings, none of these compounds has so far been tested in vivo against HBV.[146–148] In contrast, the well-known HBV pre-S1-derived lipopeptide Myrcludex-B that competes with HBV/HDV for binding to NTCP efficiently prevents HBV/HDV entry both in vitro and in human liver-chimeric uPA-SCID mice.[64 79 149] Furthermore, this lipopeptide was also able to impair viral dissemination when administered subsequent to viral inoculation in this mouse model.[59] Myrcludex-B is currently being evaluated in a phase II clinical trial in Russia.

The HBV secretory pathway is another potential target for novel antivirals, as inhibiting HBV secretion and budding should decrease the release of progeny subviral particles and virions. This could not only decrease HBV DNA levels but also interfere with HBsAg-mediated immunosuppression, thereby restoring antiviral immunity. Several inhibitors of HBV secretion have been described so far, including iminosugar derivatives of butyldeoxynojirimycin and related glycolipids, α-glucosidase inhibitors, triazol-o-pyrimidine derivatives and a benzimidazole compound.[145 150 151] The benzimidazole BM601 has recently been reported to selectively inhibit intracellular re-localisation of the HBV surface protein to the Golgi apparatus. Thereby, it decreases HBsAg and HBV release without affecting HBeAg secretion or induces the release of cellular proteins with an original mechanism of action compared with previously described inhibitors of HBV maturation and secretion.[151] Moreover, amphipathic DNA polymers such as phosphorothioate oligonucleotides have been shown to inhibit HBsAg release, thereby contributing to immunological control of HBV infection.[152] Noteworthy, such compounds exhibit broad antiviral activities and are also evaluated as HIV and HCV fusion inhibitors (reviewed in ref.[144]). As potential disadvantages, HBsAg accumulation could lead to storage diseases and the block of mature virion synthesis could increase cccDNA copy number.

In order to circumvent the systemic side effects of IFN-α that limit its clinical use, efforts are ongoing to uncover other means of inducing intrahepatic antiviral immune responses in the infected host. Most recently, an original mechanism to activate antiviral immune responses has been described using antibodies directed against the lymphotoxin-β receptor (LTβR).[153] Similarly to members of the IFN or TNF family of cytokines, antibodies activating LTβR reduced HBV DNA, HBsAg and cccDNA levels in relevant cell culture models in the absence of detectable hepatotoxicity. Antibody-mediated activation of LTβR appeared to have a dual mechanism of action, targeting both HBV replication and cccDNA stability via induction of deamination and apurinic/apyrimidinic site formation in cccDNA and upregulation of the expression of nuclear APOBEC3 deaminases.[153] This approach may however imply a significant problem as the increased mutation rate may support generation of resistant variants as it was shown for HIV. The potential synergistic effect of combinations of LTβR agonists and current polymerase inhibitors remains to be assessed.

Other immunomodulatory compounds exhibiting activity against HBV in clinical development include TLR7 agonists (ClinicalTrials.gov identifier NCT02166047), thymosin α1 (ClinicalTrials.gov identifier NCT00291616) and nitazoxanide, which induce the production of IFN and/or activation of B and T cells.[154–158] Furthermore, recombinant IL-7 and IFN-λ have also been considered to enhance immune functions against HBV (ClinicalTrials.gov identifier NCT01027065 and NCT01204762). Interestingly, the immunomodulatory properties of some of these compounds have also been suggested to be of interest in treating other infectious diseases, including chronic HCV infection. However, it remains to be demonstrated whether they can clear

viral infection. Finally, therapeutic vaccines designed to trigger both humoral and cellular immune responses against HBV are also currently being evaluated in clinical trials[159–164] (ClinicalTrials.gov identifier NCT01943799, NCT01023230 and NCT00536627) and may be potentiated with the use of NUC[165] and/or TLR-9 agonists that induce the formation of intrahepatic myeloic cell aggregates involved in T cell expansion and support HBV clearance by favouring local cytotoxic T lymphocyte expansion, at least in mouse models of HBV infection.[166–168]

CONCLUSIONS AND PERSPECTIVES

The recent progress in HCV therapy with novel DAAs allowing to cure chronic HCV infection[169] has created expectations of a cure for other chronic viral infections. Although HCV and HBV both target human hepatocytes and induce chronic liver disease and HCC, they are fundamentally different in terms of genomic structure and virus replication cycle. The ability of HBV to persist as cccDNA and to integrate into the host genome hampers its eradication. The recent development of novel in vitro infection models opened a new era for the study of HBV, and the time seems right to develop a concerted strategy to achieve HBV cure and reduce the burden of HBV-induced liver disease and HCC. The main challenges towards an HBV cure and the new concepts to be explored have been discussed during the ANRS workshop. The main conclusions were that the primary aims of the field are to (i) develop novel model systems to further characterise the molecular mechanisms of the HBV replication cycle, particularly the formation and regulation of cccDNA; (ii) advance our understanding on the viral and host factors involved in HBV pathogenesis, including innate and adaptive antiviral immune responses; (iii) uncover biomarkers of disease progression to better identify patients who are at risk of developing cirrhosis and HCC; and (iv) define new targets for antiviral therapy to achieve an HBV cure. A concerted action of academic centres and pharmaceutical industries will also be warranted to hasten the development of new antiviral strategies to combat HBV infection.

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