Research & Reviews: Journal of Microbiology and Biotechnology

Epidemiology, Prevention and Control of Hepatitis B virus Infection in China

Bo Qin[1, 2], Guozhong Zhou[1], Xuejun Dong[1]*

[1]Department of Clinical Laboratory Center, Shaoxing People's Hospital, Shaoxing Hospital of Zhejiang University, Zhenjiang Province, PR China
[2]Shaoxing Centre for Disease Control and Prevention, Zhejiang Province, PR China

Review Article

*For Correspondence

Xuejun Dong, Shaoxing People's hospital, Shaoxing Hospital of Zhejiang University, 568 Zhongxing North Road, Shaoxing, Zhejiang, 312000, People's Republic of China, Tel: +86 575 8822 8576

E-mail: dxj9666@163.com

ABSTRACT

Hepatocellular carcinoma (HCC) is one of the major keys to cause cancer-related mortality in the whole world, with the majority of patients related to persistent infection of hepatitis B virus (HBV), especially in Asian and African, while HCV in Europe and America. HBV is the smallest DNA-containing, enveloped mammal virus known so far. High tissue and species specificity, unique genome and replication mechanism make HBV be shrouded in mystery. A high HBV load is the most significant risk element for HCC recurrence. Individuals chronically infected with HBV are 200 times more inclined to develop HCC than uninfected people. Effective antiviral treatment against HBV could alleviate HBV-associated HCC and improve survival. From several aspects including epidemiology, infection, replication of HBV, immune state, anti-HBV therapy and gut microbiota of patients, we made a summary of currently available knowledge about the epidemiology, prevention and control of HBV infection for the organism as a whole.

INTRODUCTION

Liver disease is an important cause of illness and death, consisting of primarily viral hepatitis, alcoholic liver disease and nonalcoholic fatty liver disease. Among them viral hepatitis resulted from hepatitis B virus is predominant, especially in China. Estimated by The World Health Organization (WHO), chronic infection with HBV is a major cause of chronic hepatitis, cirrhosis and hepatocellular carcinoma, currently affects about 360 million people in worldwide [1-4]. HBV plague the people all over the whole world, especially in China, in past, at present and in the future [4-6].

In the past decade, the prevention and control of Chronic Hepatitis B (CHB) virus infection have significantly improved [4,5]. However, owing to different available anti-HBV reagents and the continuous variable guidelines, the control of HBV becomes more and more complex [7]. Now a days, available therapies popularly used in the whole world are safe, well tolerated, and highly effective in anti-HBV therapy, both reducing HBV viremia and improving clinical course and prognosis [4,5,8-10]. However, due to antiviral resistance and HBV speciality, long-term administration remains a clinical challenge: only long-term virologic control, elimination of HBV and the recovery of CHB patients are not possible [4,11]. HBV had been devoted great deal of scientific research investment, however, understanding HBV chronicity and pathogenesis is hampered by its narrow host range, mostly restricted to human and chimpanzee [12,13]. This paper focuses on summarising the current epidemiology of HBV infection in China, giving emphasis on the use of novel treatment to deal with HBV.

EPIDEMIOLOGY OF HBV

Over past decade, China has made some significant efforts and progresses to shed its "leader in liver diseases" title by paying large amounts of money on funding research, vaccination and drug development, and on recruiting many Western well-

trained hepatologists and experts. Different from the past two decades, liver disease research as shown by the dramatic increase in the number of publications was gradually improved and advanced both in breadth and depth. Nevertheless, many challenges troubled us and need to be tackled collaboratively. Here, we reviewed the epidemiology and characteristics of liver diseases and liver related research in China.

Among the kinds of viral causes to hepatitis, few are of greater importance than HBV, which gives rise to a major public health threat with more than 360 million humans worldwide, over 130 million in China alone [14]. Accumulating reports have shown that HBV is accounted for about one million deaths owing to liver failure, cirrhosis, furthermore, over 75% of the hepatocellular carcinomas (HCC) develop from HBV infection every year, 0.37-0.5 million deaths occur in China [15], in which 65% of deaths are caused by primary hepatic carcinoma [16,17]. Liver cancer, one of the most fatal cancers, is the second most common cancer in China. In China, about 383,000 people die from liver cancer annually, which accounts for more than one half of the deaths from hepatocellular carcinoma worldwide. China was a place with most HBV carrier in the world: nearly 10% of the whole population. The medical and financial burden of HBV infection and HCC is also considered to be the world's largest [18].

The HBV carrier rate varies from low (0.1-2%) in the USA and western-Europe, to in-between (2-8%) in Mediterranean littoral countries and Japan, high (8-20%) in some Asian countries and sub-Saharan Africa [4,9-21]. Based on the result of a national study in early 1979, the overall prevalence of chronic HBV was about 8.75%, and then rose to 9.75% in 1992 [22-24]. Undoubtedly, China had been classified as high HBV prevalence before 2006 [25]. The Expanded Program on Immunization (EPI) for infants was carried out in China since 1992, what uplifting is that the EPI implement led to a significant downtrend in HBV prevalence from 9.75% to 7.18% till 2006 [19,24]. Regionally, according to the seroepidemiology study of HBV in China, the highest HBV infection rate observed in the mid-south, while lowest rate in northern China [14]. Ominously, there are still a large population of newborns of HBsAg-positive mothers at high risk for HBV infection, and they are the main origin of HBV transmission [26,27].

Currently, there are mainly four transmission manners: perinatal transmission, horizontal transmission, sexual contact and parenteral exposure to infected fluids [28]. HBV on any surface can keep its infectivity for less than a week, such as table-tops, razor blades, blood stains and so on. The percutaneous/parenteral contact with infected body fluids, and sexual intercourse were risk factors of HBV transmission [29]. Intrauterine infection of HBV is one of the main reasons for the failure of combined immunoprophylaxis [30]. HBV is not able to cross the skin or the mucous membrane barrier and the placenta unless via amniocentesis which breaks in the maternal-fetal barrier. The biggest risk for babies to infect HBV from their mothers is during birth [31]. Thus it can be seen that prevention of perinatal infection and routine infant vaccination are of the same importance with antiviral therapy.

HBV genotype and serotype

Subtypes of HBV with specific geographic distributions can serve as epidemiological markers [32]. Due to the established serological heterogeneity, HBV isolates could be classified into nine different serological subtypes by using subtype-specific antibodies against Hepatitis B surface antigen (HBsAg) including ayw1, ayw2, ayw3, ayw4, ayr, adw2, adw4, adrq- and adrq+ [33,34]. These serological subtypes defined by two mutually exclusive determinant pairs, d/y and w/r and a common determinant a can reflect the genetic variability of HBV.

Genetic classification based on the comparison of HBV genomes has defined 10 genotypes, from A to J, exhibiting a minimum divergence of 8% in the complete genome sequences [35]. Genotypes A and D are widely distributed in all the continents, but A is predominant in northern Europe, D prevails in the Mediterranean area. Genotypes B and C are found mainly in eastern Asia and the Far East, the Near and Middle East and south Asia, while genotype E is indigenous to sub-Saharan West Africa, genotype F is likely to be existed in populations with origins on the American continent, and genotype G is found in the USA and Mexico and Europe, genotype H is closely related to F and prevails in Central and North America [32]. Different genotypes have shown diverse characteristics [36]. The dominant genotypes of HBV in China are B and C. Comparing with the patients infected with HBV genotype B, people with C genotype of HBV infection showed higher HBV DNA level and activity of hepatic histology, longer immune clearance phases [37], more frequently or persistently fluctuating alanine transarninase (ALT), and lower response to interferon, nucleoside analog [38]. There is also a legible and definite association between HBV genotypes and mutations of precore and basal core promoter (BCP). Genotype C has a higher frequency of substitution mutations (A1762T/G1764A) in BCP, pre-S deletion and is related with higher HBV load than B. Similarly, genotype D has a higher appearance of BCP mutations (A1762T/G1764A) than A genotype. Both Genotype C and D are correlated with severer liver disease, such as cirrhosis and HCC [39]. Infection with genotype A and B shows better responses to IFN-based therapy than C and D, but there is no significant difference for NAs [37]. What is more, genotype C might be a risk factor for HBV transmission from mother to child [40].

Both serotype and genotype can be served as epidemiological markers of HBV infection. HBV isolates of the same serotype/genotype are believed to have evolutionary relation generally, and can be used for tracing the route of HBV transmission and geographic migration of HBV carriers. The correlation of the nine serotypes with the ten genotypes has been studied previously [15]. Genomes encoding *adw* are found in genotypes A, B, C, F and G, while the genomes encoding both *adr* and *ayr* occur in genotype C, along with *adw* [41]. However, these results are still incomplete and inadequate because the HBV isolates analyzed were small in number and were limited to certain geographic areas. There is no causal relationship between them, only some tiny connections.

HBV genome organization

HBV is a member of the Hepadnaviridae family and an enveloped virus with a partially double stranded DNA in 3200-bp size, which is covalently linked to the viral polymerase, it is remarkably compact and achieves its genomic economy by relying on an efficient strategy of encoding proteins from four overlapping genes, including S, C, P, and X [42]. HBV shares similar reverse transcription with HIV, replicates through an RNA intermediate, namely, the pregenomic RNA [5]. Despite this genetic relationship to retroviruses, there are fundamental differences between them; Hepadnavirions contain viral DNA genome instead of RNA [43]. Due to lack of proofreading function of HBV polymerase, error-prone replication of HBV (error rate of 10^{-4} to 10^{-5}) leads to an accumulation of a pool full with heterogeneous HBV genomic sequences, also called quasi species [5].

HBV virions combined hepatocyte-surface receptors, such as Na$^+$/taurocholate Cotransporting Polypeptide (NTCP) expressed at the basolateral membrane of hepatocytes and responsible for the uptake of Na$^+$-dependent bile acid in liver, it was proved to be a functional receptor of HBV [44,45]. After membrane fusion, nucleocapsids are presented to the cytosol, RC DNA is releasing and then transported to the nucleus, in where with the assistance of the host DNA polymerase it is converted to a covalently closed circular DNA (cccDNA), which serves as the transcriptional template for host RNA polymerase II to generates a series of genomic and subgenomic transcripts necessary for protein production and viral replication, including 3.5 kb (over-length pregenomic RNA), 2.4 kb, 2.1 kb and 0.7 kb HBV mRNA. All viral mRNAs which are coterminal, polyadenylated, and capped are transported to the cytoplasm and translated into viral proteins, containing envelope, core, and polymerase proteins, as well as the X and pre-C poly-peptides. Once the viral RNA is encapsidated, reverse transcription begins to synthetize minus DNA chain using encapsidated RNA as template, during or after the production of this strand, the RNA template is degraded and the synthesis of the complementary plus-strand DNA initiate, using the newly minus DNA strand as template to form the rcDNA. A part of nucleocapsids with mature genome are transported back to the nucleus, took part in the new production cycle to maintain a stable intranuclear pool of transcriptional templates [46]. However, overwhelming majority of nucleocapsids bud into intracellular membranes in where they can acquire the viral envelope proteins, and then exported from the cell [47].

Clinical Features and Laboratory Diagnosis

The course of HBV infection may be extremely variable, and has different clinical manifestations rests with the age at infection, immune status and the disease stage of patient [48]. Incubation phase of HBV infection varies from 6 to 24 weeks, during which patients may feel unwell, usually with gastrointestinal symptoms including nausea, vomiting, diarrhea, anorexia and headaches [29]. Accidental patients with no obvious symptoms can be identified by detecting biochemical or HBV-specific serologic alterations. Most adult patients could recover completely from HBV infection, while about 5 to 10% of patients can't clear HBV and will become asymptomatic carriers who constitute a reservoir for further transmission to others, or develop into CHB with high possibility of resulting in cirrhosis and/or HCC [49]. Biochemical assessment of liver function consisting of total and direct bilirubin, ALT, AST, alkaline phosphatase, prothrombin time, total protein, albumin, serum globulin, complete blood count, and coagulation studies can be the hint and assist of HBV diagnosis [50]. Serum microRNA profiles were proved to be novel biomarkers for HBV Infection and Hepatocarcinoma result from HBV [51].

The conventional HBV diagnosis is administrated based on viral specific antigens and/or antibodies [52]. There are five clinical useful serum markers have been identified for laboratory Diagnosis of HBV, including HBsAg, anti-HBs, HBeAg, anti-HBc and anti-HBe. HBsAg can be found in the serum from 2-6 months after HBV infection [53]. HBsAg is present in serum during acute infections and persists in chronic infections. Its presence indicates that the person is potentially infectious. In the early incubation period, pre-S1 and pre-S2 are present [54]. HBV DNA, DNA polymerase, HBeAg could be detected in the following. HBeAg found in serum is associated with relatively high infectivity and severity of hepatitis except for the appearance of basal core promoter mutation [55]. The first antibody of HBV appeared in serum is anti-HBc which indicates current or past viral infection. Anti-HBe follows in anti-HBc's step, its presence correlates to a weakened infectivity. Anti-HBe replaces HBeAg in the resolution of the disease [56]. Neutralizing anti-HBs is substituting for HBsAg as acute hepatitis is resolving and normally persists for a lifetime in most patients. If constant serum HBsAg concentration or persistent HBeAg for 8 to 10 weeks after symptoms have resolved, acute hepatitis patients possibly become HBV carriers and develop chronic liver disease [3]. HBV DNA is the most directive, specifically and sensitive marker for HBV infection diagnosis. The higher load means more active viral replication and higher infectiousness. RT-PCR and DNA hybridization assays could be used for the detection of HBV DNA [57].

HBV chronicity and immunotherapy

The immunopathogenesis of HBV depends on a complex interplay of host and viral factors, such as age, gender, immune status and so on [58]. Primary HBV infection in about 90% of perinatal and childhood, 20%-50% of children will develop into chronic infection, while 95% of immunocompetent adults with acute HBV spontaneously clear the infection [59]. Due to unique genomic organisation and replication strategy, HBV will persist in infected hepatocytes of CHB patients. The natural procedure of CHB infection can be divided into four phases: immune tolerance, immune clearance (HBeAg-positive CHB), immune control (low or non-replicative) and immune escape [60]. These four phases are identified based on specific biochemical, serological, virological characteristics, including serum ALT levels, HBeAg serostatus, HBsAg level and HBV DNA titre [61]. The CHB patients in immune clearance or immune escape phases are potential candidates for antiviral and immune modulatory treatments [62].

HBV whole virions and other component containing HBs/e/c/x/p protein and HBV DNA can impair the function of dendritic cells (DC), T- and B-cells, immune tolerance will take place afterwards [63-67]. HBeAg was shown to act as an immune decoy for the core antigen by depleting e-specific and c-specific T-helper cells and exerting a tolerogenic effect in utero [68]. Furthermore, HBeAg has been proved to latently downregulate genes involved in RNA transport and processing, cell signalling, cytosol-nuclear trafficking and innate immune responses [69]. Distinct virus-mediated mechanisms may account for the limited effectiveness of the innate immune responses and subsequent adaptive immune responses in HBV infection and thereby may significantly contribute to viral persistence [70].

It is very important to break the immune tolerance through immunoregulation [7]. *In vitro* activation of DC and loading with HBV sub-viral particles can overcome tolerance against HBV and reactivate B- and T-cell responses in HBV transgenic mice [71]. Till now, there are several immune-based anti-HBV drugs as follows. Zadaxin, found naturally in the circulation and synthesized in the body's thymus gland, is a synthetic peptide in 28 amino acids size. Due to promising immunomodulatory function and related curative potential, zadaxin has been used to treat CHB through triggering lymphocyte maturation, enhancing T-cell function, and reconstituting immune defects [72]. NOV-205 (BAM 205), a hepatoprotective agent owning well-tolerated, immunomodulating and anti-inflammatory properties [73], was approved to greatly reduce/eliminate viral loads of HBV and HCV, significantly improve liver function, and mainly used in the Russian Federation [74].

The innate immune system, invoked by the interactions between pathogen recognition receptors (PRR)-pathogen associated molecular pattern (PAMP), represents an immediate, first-line defence against foreign pathogens and plays a fundamental role in regulating the adaptive immune response [75]. PRRs includes Toll-like receptors (TLRs), RIG-I like receptors (RLRs), NOD-like receptors (NLRs), and others triggering signals to activate intracellular pathways, production of antiviral and immune regulatory effector molecules. As the crucial parts of innate immune system, TLRs activation could develop into novel therapeutic strategy to treat HBV infection [76]. GS-9620, an effective selective TLR-7 agonist, was designed for anti-HBV therapy [77]. In chronically-HBV-infected chimpanzees, the pharmacokinetics of GS-9620, viral load of HBV, ISG expression, cytokine, chemokine levels, lymphocyte, NK cell activation, safety and tolerability parameters were all assessed after oral administration, it turned out that GS-9620 could suppress HBV DNA of serum and liver, serum levels of HBsAg and HBeAg, and the numbers of HBsAg-positive hepatocytes [78,79]. GI-13020 was engineered to express a chimera of HBV X, S, and C antigens based on yeast expression system. In consideration of its founction of eliciting HBV specific T cell responses, GI-13020 will possibly be used together with anti-HBV reagents to enhance HBsAg seroconversion rate of CHB patients in the future [80].

HBV control and current problem

The key goal for current HBV treatment is to improve and reverse the progression of liver fibrosis, to reduce the occurrence of HCC. In order to reduce the spread of HBV, prevention of transmission by immunization were carried out [18]. Treatments for HBV infection include interferon and NAs. Interferon (IFN)-α is licensed for HBV treatment and can result in HBV clearance in a proportion of patients, but its efficacy is limited and not well-tolerated because of systemic side effects [5]. Recombinant IFN-α has been approved and successfully used as a standard treatment for chronic HBV infection. Treatment with PEG-IFN results in a prolonged clinical remission with durable anti-HBV effect in HBeAg-positive CHB patients, enhancive rate of HBsAg seroconversion and improved liver histology [81]. Furthermore, long-term therapy also resulted in decrease in fibrosis and regression of cirrhosis.

Until now, five kinds of NAs, powerful inhibitors of HBV replication, including lamivudine (LMV), adefovir dipivoxil (ADV), entecavir (ETV), telbivudine (LdT) and tenofovir (TDF), have been approved for CHB treatment [6]. They were well tolerated but the efficacy of these agents is heavily discounted by emergence of antiviral resistance. *In vivo* and *in vitro*, NAs mimic physiological nucleosides in terms of uptake and metabolism and are incorporated into newly synthesized DNA chains leading to synthesis inhibition through termination of DNA replication [82]. In CHB patients, HBV exists in the form of quasi species due to the error-prone HBV reverse transcriptase (RT), which is responsible for the rich variety of drug-resistant mutants. Under antiviral pressure, HBV variants will contribute to the production and further selection of replication-competent resistance mutants, which will spread to other hepatocytes, eventually may replace the wild-type cccDNA molecules in the hepatocytes nucleus [83].

HBV RT is subdivided into 7 domains (A-E, G and F), in which nearly all resistance mutations are located [82]. NA-resistant mutations inhibit the anti-HBV effects of NAs and induce virological advancement and hepatopathological progression. A number of mutations have been reported to account for drug resistance. Mutations of rtM204I or rtM204V in the YMDD motif within the RT domain of the HBV polymerase lead to LMV resistance (LMVr) and LdT resistance (LdTr). ETV resistance (ETVr) has also been observed in patients with LMVr [5]. Under the background of rtM204I/V, a combination of mutations in the B, C or D domains of RT may result in ETVr, such as rtI169T, rtL180M, rtS184G, rtA186T, rtS202I, rtM204V, rtM250V [5,6]. The substitution mutations rtA181V/T, rtN236T, rtN238R, rtT240Y and rtN248H can reduce the anti-HBV effects of ADV, while rtP177G and rtF249A have been reported to induce TDF resistance, apart from rtV214A, rtQ215S and rtA194T which all need further confirmation [5]. Usually, when LMVr occurs, ADV remains effective. Due to a higher genetic barrier to HBV resistance than LMV and ADV, ETV and TDF are high potency drugs for the therapy of CHB patients, even those with LMVr and ADVr [84]. Compensatory mutations that increase viral replication levels can be found in other domains of the HBV Polymerase, such as rtL80V/I, rtI163V, rtI169T, rtV173L, rtT184S/G, rtS202I, rtQ215S and rtQ267H [85].

The HBV transmission from mother to child is another serious problem for HBV prevention and control. There is no doubt that HBV plague the people all over the whole world, especially in China, the greatest suffered country with at least 130 million CHB carriers, 30%-50% of whom are considered to infect HBV from mother [86,87]. About 90% of infants infected during 1-year-old and 30% to 50% of children infected between 1 to 5 years old likely develop into chronic infection [88]. The virus with mutation on "a" region of surface protein located from 121 to 149 amino acids may escape vaccine-elicited antibodies or hepatitis B immune globulin (HBIG) and may be transmitted from mother to infant [87]. Therefore effective cutting off the mother-to-child transmission route is the only way to decrease HBV infection and relieve the HBV disease burden [88]. About 90% of HBV mother-to-child transmission can be blocked by the administration of immunoprophylaxis with the HB vaccine and HBIG in China. It has been confirmed that the immunoprophylaxis failure of a child is mainly associated with his mother with seropositivity for HBeAg and high HBV DNA viral load. Hence the pregnant women with a high HBV DNA viral load were recommended to take anti-HBV therapy in late pregnancy to inhibit virema [89].

Prophylactic and therapeutic vaccine for HBV

HBV was discovered for about half a century ago, since 1966, but finding a cure for CHB remains a challenging task. Thankfully, hepatitis B is a vaccine-preventable disease, till now the vaccine is the first and the only one towards a major human cancer [90]. HBV vaccine which is a most effective tool in preventing HBV and HDV transmission is composed of HBsAg (plasma derived or recombinant DNA expression). A safe and effective HBV vaccine has been used for more than 20 years, and 95% of recipients were protected after being administered properly [18]. Due to the contribution of preventive vaccine, the incidence of HBV carriers worldwide was induced significantly [49]. Nonetheless, a great population is still HBV-suffered. Besides currently approved antiviral treatment with NAs and INF, therapeutic vaccine is a hopeful new strategy for the control of CHB, including the peptide/protein-based, DNA-based, cell-based and antigen-antibody-based therapeutic vaccines [91]. There are three groups of people should receive HBV vaccination: newborns with incomplete immunity and no incomplete against HBV, preschoolers who are also susceptible for HBV, people with HBV-infected spouse or who have jobs in risk of HBV infection, people exposed to HBV [18]. In China, the whole course of HBV vaccination includes three injections with one and six months interval [24].

Unfortunately, conventional antiviral therapy directly target HBV to inhibit its replication and protein expression, the virus and virus-infected cells may not be eliminated [92]. Therapeutic vaccines are considered to be able to reverse the dysfunctional immune state of chronic hepatitis B and thus hold the promise for HBV cure. Unlike antiviral treatment, immunotherapy shows indirect therapy through triggering host immune responses to repair the immunologic defects and supplement necessary immune factors to inhibit or clear the persisting virus and virus-infected cells [93]. Currently, several therapeutic vaccines for CHB are in experimental or clinical studies, including autologous monocyte-derived DCs, hepatitis B DNA vaccine, HBsAg-HBIG immunogenic complex and so on [90,94-97]. Wen et al. have developed an HBsAg-HBIG immunogenic complex therapeutic vaccine candidate, aimed at breaking immune tolerance to HBV through modulating HBV antigen processing and presentation, it shows a satisfying effect and needs in-depth test in further clinical trials [98,99]. Sung etc. evaluated the safety and immunogenicity of HB-110, one kind of HBV DNA vaccine, in mice and CHB patients, the result showed that HB-110 was safe and tolerable in CHB patients [100]. Xu et al. developed a novel therapeutic vaccine of HBV consisting of HBs and c antigen, using CpG as adjuvant, which elicits forceful humoral responses directed against s and c antigen, promotes a Th1/Th2 balance response against HBs and a Th1-biased response against HBc in mice model [101]. Another novel adenovirus-based therapeutic vaccine named TG1050, induced long-lasting anti-HBV CD8+ T-cell immunity in mouse models [102].

Novel strategies to achieve sustained virologic remission of HBV

Potential anti-HBV drug targeting viral entry, as described above, Na+/taurocholate Cotransporting Polypeptide (NTCP) is a functional receptor of HBV, and opens the door for HBV infection. HBV has the tendency to bind and infect hepatocytes and the following HBV entry is mediated by the specific interactions between HBV membrane proteins and cellular receptors [44]. There are two kinds of agents which can specifically target the NTCP, including Myrcludex-B (a synthetic lipopeptide derived from pre-S1 of HBV) and Vanitaracin A (a novel tricyclic polyketide), they all can efficiently inhibit the entry of HBV [103-105]. NTCP antagonists targeting viral entry will be new strategy to treat HBV [106]. Furthermore, HBV infection could only infect freshly isolated primary hepatocytes of humans, chimpanzees or northern treeshrews in the past, but recently some NTCP-expressing cells could be infected by HBV, it will be a powerful platform for anti-HBV drug screening [107].

Potential anti-HBV strategy targeting viral assembly and/or encapsidation

The HBV capsid composed of capsid protein (Cp), polymerase, and pregenomic RNA plays an important role in HBV life cycle, is involved in genome packaging, reverse transcription, intracellular trafficking, and maintenance of HBV stable infection [108]. Assembly is a key step of HBV replication and dominates HBV persistence and transmission. To perturb HBV assembly and thereby alter either the timing or the geometry of capsid formation will be a promising antiviral strategy [109]. Multiple classes of compounds could inhibit HBV assembly and encapsidation, such as Heteroaryldihydropyrimidines (HAPs) which could inhibit capsid formation through triggering assembly inappropriately, misleading assembly, reducing the stability of normal HBV capsids. The most important is that they are effective to both wild-type and NAs-resistance HBV mutants [92]. In the HAPs family, Bay 41-4109 can induce inappropriate assembly and yield an aberrant virus particle which is incompetent for HBV whole life cycle. GLS4,

another member of HAP family, is a small molecular compound with the effect to inhibit the replication of both wild-type and ADVr HBV mutants *in vitro* [110].

There is another phenylpropenamide family w shown to suppress HBV replication *in vitro* including AT-61 and AT-130 [111]. These two compounds have specific antiviral effect only to HBV (both wild-type and LMVr), but no activity against other hepadnaviruses, such as DHBV, WHBV and HIV-1 [112]. Based on previous studies, they inhibit HBV replication through interfering with pregenomic RNA encapsidation, yielding pseudo HBV particles which are in normal appearance, but lack genetic material [113].

Potential anti-HBV policy targeting HBsAg secretion

Due to ineffective anti-HBV immune response towards HBV, the infection will progress and persist. High level of HBsAg is considered to play a crucial role in impairing the HBV-specific immunity [114]. The impaired innate immunity can be regulated by direct interaction between HBV antigens and host. Therefore, control of HBsAg secretion could be a potential anti-HBV method [92]. Several classes of drugs containing Nitazoxanide (NTZ) and tizoxanide (TIZ) have been studied to reduce HBsAg secretion and HBV replication [115]. Phosphorothioated oligonucleotides (PS-ONs) with sequence-independent antiviral activity is a novel class of compounds with promising microbicide pathway, and is described as amphipathic DNA polymers (APs) with broad spectrum antiviral activity, against a wide range of viruses, including HBV, HIV-1, HCV, herpes simplex virus, arenavirus and lymphocytic choriomeningitis virus (LCMV) [116]. REP 9 AC which is currently in phase Ⅰ/Ⅱ clinical trial is a polycytidine amphipathic DNA polymer in 40 nucleotides with the effect to inhibit HBsAg release from HBV-infected hepatocytes and allows CHB patients to reestablish durable immunity through eliminating the immunosuppression mediated by HBsAg [117]. Clinical trial results suggest that REP 9AC possesses promising effect of HBsAg clearance and HBV DNA inhibition with no pro-inflammatory side effects and may develop into an effective new tool to treat HBV [118].

Potential new NAs targeting HBV replication

Besides these five inhibitors of HBV DNA polymerase, Emtricitabine was originally approved for anti-HIV therapy and found to be also powerful anti-HBV effect in both HBeAg positive and negative patients, and was applied in combination with TDF in HIV/HBV coinfected patients [119]. Clevudine [1-(2-deoxy-2-f luoro-β-L-arabinofuranosyl) thymine, L-FMAU] has effective antiviral activities against both HBV and Epstein-Barr virus (EBV) but not HIV, approved in South Korea and Philippines [92]. MIV-210 (Lagociclovir valactate) is a prodrug of 3'-fluoro-2', 3'-dideoxyguanosine (FLG) with high oral bioavailability and potent activity against HBV will be a good candidate for anti-HIV/HBV. Besifovir (LB80380), with chemical similarity to adefovir and tenofovir, is an acyclic nucleotide phosphonate and the prodrug of LB80331, and will be metabolized to the active metabolite with anti-HBV effect after intracellular phosphorylation to triphosphate form [120]. Tenofovir Alafenamide (TAF; GS-7340),a prodrug of TDF, achieves higher metabolite concentrations in peripheral blood mononuclear lymphocytes (PBMCs) and lymphatic tissues [121]. TAF will behydrolyzed to tenofovir-alanine conjugate (TFV-Ala) which is the intermediate and then converted into the parent tenofovir, which is phosphorylated in the following to yield the active tenofovir diphosphate (TFV-DP) metabolite [122]. CMX157 is a hexadecyloxypropyl conjugate of TDF with anti-HIV and HBV, 267-fold more active than TDF against HIV-1 and 4.5-fold more active against HBV *in vitro*. CMX157 is currently in phase Ⅰ clinical study [123]. AGX-1009 is another novel patented prodrug of TDF and exibits ideal efficacy in inhibiting HBV replication, phase Ⅰ trials were carried out in 2013 in China [119]. A class of simple unnatural L-nucleosides which have in common a hydroxyl group in the 3'-position (3'-OH) of the beta-L-2'-deoxyribose sugar were found to own specific anti-HBV effect. The more interesting is that the replacement of the 3'-OH will broaden their activity to other viruses. What's important is that human DNA polymerases and mitochondrial function are not hurt. They can be used alone or in combination to treat CHB [124].

Gene silence targeting HBV antigen

RNA interference (RNAi) induces a sequence-specific degradation of homologous mRNA. This process can be mediated by MicroRNAs (miRNAs) and small interfering RNAs (siRNAs) which are two popular used classes of small RNAs with different origin and early processing pathways [125,126]. HBV is an approving target for an RNAi approach because its high-efficiency, compact and overlapping genome that lacks significant redundancy [92]. There was a breakthrough platform developed for disease treatment named ddRNAi which was designed to specifically treat human diseases through inserting a DNA construct into host cells, triggering the synthesis of dsRNA, subsequently, the dsRNA will be cleaved into siRNA which will play an efficient role in destructing target mRNA and knocking-down or silencing the expression of target gene [127]. Unlike other RNAi-based technologies, treatments of ddRNAi leads to long-term benefit and potentially cure. ARC-520, a siRNA-based therapeutic agent which is targeting HBV sequences, resulted in long-term suppression in serum levels of HBsAg, HBeAg and HBV DNA both in transient and transgenic mouse, and chimpanzee chronically infected with HBV model by intravenous injection [128]. Phase Ⅰ study of ARC-520 in healthy adult volunteers implemented in Melbourne, Australia had been finished in the fourth quarter of 2013, phase Ⅱ trial in chronic HBV patients began in 2014. The satisfactory anti-HBV efficacy of ARC-520 lets it be a new promising anti-HBV drug [98,128]. In future, the nucleic acid-based therapeutics will be improved gradually and put into use increasingly.

Targeting covalently closed circular DNA (cccDNA) for "cure"

Current antivirals aimed towards inhibition of viral replication can inhibit but not eradicate HBV, because of cccDNA, it

steadily exists in hepatocyte nuclear and association with histones and DNA chaperone proteins as a minichromosome that serves as template to generate all RNAs necessary for protein production and viral replication, and also secures HBV persistence avoiding host innate immune responses [129]. As we know, the failure of HBV clearance through anti-HBV therapy with NAs is mostly due to the persistence of the cccDNA in CHB patients. Furthermore, long half-life of HBV-infected hepatocytes allows the maintenance of cccDNA in the hepatocytes nuclei which acts as reservoir for HBV reactivation [130]. CHB infection persistence will permit the virus to manipulate the cellular machinery to meet its replication and propagation requirements, and to evade the antiviral response of the host. So to help host being superior in the war of virus-host interactions is the priority among priorities. Efficient and nontoxic clearance of cccDNA in nuclear is a final goal of anti-HBV treatment. Interferon-α (IFN-α) and lymphotoxin-β-receptor activation were reported to respectively up-regulate APOBEC3A and 3B cytidine-deaminases both in HBV-infected cells and human liver-needle biopsies. It is gratifying that this up-regulation can inhibit HBV replication and lead to cccDNA degradation [131].

The way to cure CHB

In cancer, proteins called the inhibitor of apoptosis proteins (IAPs) are over-expressed and block the apoptosis pathways, causing cancer cells to survive and become resistant to chemotherapies. IAPs impair HBV clearance through preventing TNF-mediated killing of HBV-infected cells. The second mitochondrial-derived activator of caspases (SMAC) is a natural antagonist of IAPs and enables apoptotic cell death [132,133]. Birinapant is a member of SMAC mimetics and can be used as antagonists of IAP. It can specifically target and inhibit IAPs, thus naturally drive tumor cells to apoptosis. The latest studies show that birinapant and other SMAC mimetics are able to rapidly reduce HBsAg and HBV DNA in serum, to promote the clearance of hepatocytes with HBcAg, to enhance the anti-HBV ability of ETV in CHB mouse model. It follows that combination of ETV and birinapant or other SMAC mimetics is a promising weapon to cure CHB patients [134].

Relationship between HBV, innate immunity and gut microbiota

The liver connects and interacts directly with the gut through the hepatic portal and bile secretion. This is the reason why enteric dysbiosis, bacteria translocation and their products are involved in the progression of liver cirrhosis [135]. Nan Qin et al. utilized a unusual system sequencing 16S ribosomal RNA genes of gut microbiota from patients with liver cirrhosis, the change of the gutmicrobiota, invasion of the gut from oral bacterial species, biomarkers specific to liver cirrhosis, genes that differ in abundance between the patients and healthy individuals were analyzed in details, something interesting and important was found and concluded. Their study paves the way to the development of novel probiotics to help combat the aggravation of liver cirrhosis, also to the modulation of microbiota to correct the major dysbioses systems [136].

HBV clearance in patients heavily depends on the age of exposure, but the reason for this remains obscure [137]. More recently, in hydrodynamic transfection mouse model, gut microbiota was reported to correlate with the age dependence of HBV immunity. In young mice, the gut bacteria was not established, immuno-tolerating pathway to HBV which depend on TLR4 was prevailed, while the maturation of gut microbiota could significantly stimulate liver immunity in adult mice, leading to rapid HBV clearance. The above study reveals the close relationship between gut microbiota and immune tolerance, paves the vital way to control HBV infection, transmission [137].

To sum up, the causes and treaments of some clinical diseases including child obesity, intestinal inflammatory diseases, allergic dermatitises, hepatic cirrhosis, tumor and so on, are closely related with intestine mucosal organism barrier and intestinal probiotics [138,139]. The intestinal microbiota plays a key role in maintaining the normal function of the human gastrointestinal tract. Many probiotics are derived from human gut flora, and have been confirmed to be valuable in the management of gastrointestinal diseases [140]. HBV is a key cause to liver cirrhosis, chronic HBV infection must be characterized by similar changes in the gut of CHB patients as reported. To regulate and improve gut microbiota of host may be a hopeful adjuvant therapy for conventional anti-HBV strategy.

CONCLUSION & FUTURE PERSPECTIVE

For a long period of time in the future, due to the large population base with CHB infection, the persistent of HBV will sustain in the whole world, especially in China. But be assured: the affected population will become smaller and smaller in view of successful available vaccines popular used. Neonatal inoculation and appropriate antiviral therapy for CHB patients is the key to control HBV.

It is critical to understand the basis and mechanism of HBV persistence, and design more suitable therapeutic strategies to eradicate HBV. Combination approaches enhancing host immune responses, degrading cccDNA which is a key viral target, improving gut microbiota along with conventional antiviral drugs will offer the most appropriate, pragmatic and effective technique to achieve sustained HBV remission of most CHB patients.

ACKNOWLEDGMENTS

REFERENCES

1. Schulze A, et al. Hepatitis B virus infection initiates with a large surface protein-dependent binding to heparan sulfate proteoglycans. Hepatology 2007; 46: 1759-1768.

2. Chen P, et al. Prevalence of hepatitis B in insular regions of southeast China: a community-based study. PloS one 2013; 8: e56444.

3. Halegoua-De Marzio D and Hann HW. Then and now: the progress in hepatitis B treatment over the past 20 years. World J Gastroenterol 2014; 20: 401-413.

4. Zoulim F and Durantel D. Antiviral therapies and prospects for a cure of chronic hepatitis B. Cold Spring Harb Perspect Med. 2015; 5.

5. Zoulim F and Locarnini S. Hepatitis B virus resistance to nucleos(t)ide analogues. Gastroenterology 2009; 137: 1593-608 e1-2.

6. Qin B, et al. The amino acid substitutions rtP177G and rtF249A in the reverse transcriptase domain of hepatitis B virus polymerase reduce the susceptibility to tenofovir. Antiviral Res 2013; 97: 93-100.

7. Huang Z, et al. Aberrant expression and dysfunction of TLR2 and its soluble form in chronic HBV infection and its regulation by antiviral therapy. Antiviral Res 2015; 118: 10-9.

8. Marcellin P, et al. Peginterferon alfa-2a alone, lamivudine alone, and the two in combination in patients with HBeAg-negative chronic hepatitis B. Gut 2004; 351: 1206-17.

9. Torriani FJ, et al. Peginterferon Alfa-2a plus ribavirin for chronic hepatitis C virus infection in HIV-infected patients. N Engl J Med 2004; 351: 438-50.

10. Lau GK, et al. Peginterferon Alfa-2a, lamivudine, and the combination for HBeAg-positive chronic hepatitis B. N Engl J Med 2005; 352: 2682-95.

11. Ohara T, et al. Efficacy of double filtration plasmapheresis with pegylated interferon/ribavirin therapy for intractable chronic hepatitis C patients and hepatitis C patients with combined liver cirrhosis by HBV, leading to early viral elimination. Hepato-gastroenterology 2011; 58: 133-136.

12. Chen J, et al. Persistent hepatitis C virus infections and hepatopathological manifestations in immune-competent humanized mice. Cell Res 2014; 24: 1050-1066.

13. Dandri M and Locarnini S. New insight in the pathobiology of hepatitis B virus infection. Gut 2012; 61: 16-17.

14. Lu FM, et al. Epidemiology and prevention of hepatitis B virus infection in China. J Viral Hepat. 2010; 17: 4-9.

15. Zhou B, et al. Geographical and ethnic distribution of the HBV C/D recombinant on the Qinghai-Tibet Plateau. PloS one 2011; 6: e18708.

16. Zuo C, et al. Role of antiviral therapy in reducing recurrence and improving survival in hepatitis B virus-associated hepatocellular carcinoma following curative resection (Review). Oncol Lett 2015; 9: 527-34.

17. Rehermann B and Bertoletti A. Immunological aspects of antiviral therapy of chronic hepatitis B virus and hepatitis C virus infections. Hepatology 2015; 61: 712-21.

18. Cui F, et al. Review of hepatitis B surveillance in China: improving information to frame future directions in prevention and control. Vaccine 2013; 31: J79-84.

19. Hou J, et al. Epidemiology and Prevention of Hepatitis B Virus Infection. Int J Med Sci 2005; 2: 50-7.

20. Saikia A, et al. Molecular epidemiology of HBV infection in chronic hepatitis B virus infected patients in northeast India. J Med Virol 2015.

21. Andre F. Hepatitis B epidemiology in Asia, the Middle East and Africa. Vaccine 2000; 18: S20-2.

22. Li H, et al. Submassive hepatic necrosis distinguishes HBV-associated acute on chronic liver failure from cirrhotic patients with acute decompensation. J Hepatol 2015.

23. Montuclard C, et al. Causes of death in people with chronic HBV infection: A population-based cohort study. J Hepatol 2015; 62: 1265-71.

24. Liang X, et al. Epidemiological serosurvey of hepatitis B in China--declining HBV prevalence due to hepatitis B vaccination. Vaccine 2009; 27: 6550-7.

25. Yang X, et al. The impact of anti-HBV treatment on the occurrence and recurrence of hepatocellular carcinoma: focus on Asian studies. Discov Med 2015; 19: 89-99.

26. Khamduang W, et al. Analysis of residual perinatal transmission of hepatitis B virus (HBV) and of genetic variants in human immunodeficiency virus and HBV co-infected women and their offspring. Journal of clinical virology : J Clin Virol 2013; 58: 415-21.

27. Kew MC. Epidemiology of chronic hepatitis B virus infection, hepatocellular carcinoma, and hepatitis B virus-induced hepatocellular carcinoma. World J Gastroenterol 2010; 58: 273-7.

28. Tokita H, et al. Back-ground factors which affect the outcome of steroid withdrawal therapy in patients with chronic type B hepatitis--statistical evaluation of the importance of the mode of HBV transmission. The Japanese journal of gastro-enterology 1987; 84: 2706-13.

29. Gitlin N. Hepatitis B: diagnosis, prevention, and treatment. Clin Chem 1997; 43: 1500-6.

30. Sun H, et al. A preliminary study on the molecular evolution of the two routes of intrauterine transmission of HBV. Arch Virol 2012; 157: 155-64.

31. Wang Z, et al. Quantitative analysis of HBV DNA level and HBeAg titer in hepatitis B surface antigen positive mothers and their babies: HBeAg passage through the placenta and the rate of decay in babies. J Med Virol 2003; 71: 360-6.

32. Liu CJ, et al. Molecular epidemiology of hepatitis B viral serotypes and genotypes in taiwan. J Biomed Sci 2002; 9: 166-70.

33. Kidd-Ljunggren K, et al. Genetic variability in hepatitis B viruses. J Clin Virol. 2002; 83: 1267-80.

34. Kramvis A, et al. Hepatitis B virus genotypes. Vaccine 2005; 23: 2409-23.

35. Pujol FH, et al. Worldwide genetic diversity of HBV genotypes and risk of hepatocellular carcinoma. Cancer Lett 2009; 286: 80-8.

36. Chan HL, et al. Viral genotype and hepatitis B virus DNA levels are correlated with histological liver damage in HBeAg-negative chronic hepatitis B virus infection. Am J Gastroenterol 2002; 97: 406-12.

37. Xibing G, et al. Relationship between HBV genotypes B, C and follicular helper T cells in patients with chronic hepatitis B and its significance. Hepat Mon 2013; 13: e6221.

38. Lin CL, et al. High prevalence of occult hepatitis B virus infection in Taiwanese intravenous drug users. J Med Virol. 2007; 79: 1674-8.

39. Lee MH, et al. Combination of preS deletions and A1762T/G1764A mutations in HBV subgenotype C2 increases the risk of developing HCC. Intervirology 2012; 55: 296-302.

40. Ding Y, et al. Chronic HBV infection among pregnant women and their infants in Shenyang, China. Virol J 2013; 10: 17.

41. Kanagawa H, et al. Hepatitis B surface antigen particles of subtypes adw and adr, and compound subtype (adwr) in symptom-free carriers in Japan. J Med Virol 1992; 37: 288-93.

42. Mondal RK, et al. Immune-driven adaptation of hepatitis B virus genotype D involves preferential alteration in B-cell epitopes and replicative attenuation-an insight from human immunodeficiency virus/hepatitis B virus coinfection. Clin Microbiol Infect 2015.

43. Ostrow KM and Loeb DD. Characterization of the cis-acting contributions to avian hepadnavirus RNA encapsidation. J Virol 2002; 76: 9087-95.

44. Yan H, et al. Sodium taurocholate cotransporting polypeptide is a functional receptor for human hepatitis B and D virus eLife 2014; 3.

45. Tsukuda S, et al. Dysregulation of retinoic acid receptor diminishes hepatocyte permissiveness to hepatitis B virus infection through modulation of sodium taurocholate cotransporting polypeptide (NTCP) expression. J Biol Chem 2015; 290: 5673-84.

46. Beck J and Nassal M. Hepatitis B virus replication. World J Gastroenterol: WJG 2007; 13: 48-64.

47. Phillips S, et al. Alisporivir inhibition of hepatocyte cyclophilins reduces HBV replication and hepatitis B surface antigen production. Gastroenterology 2015; 148: 403-14 e7.

48. Zhang YY, et al. New perspective on the natural course of chronic HBV infection. Front Med 2014; 8: 129-34.

49. Liu J and Fan D. Hepatitis B in China. Lancet 2007; 369: 1582-3.

50. Kumar A, et al. Significance of alanine aminotransferase testing in diagnosis of acute and chronic HBV infection. Asian Pacific journal of cancer prevention: APJCP 2009; 10: 1171-2.

51. Li LM, et al. Serum microRNA profiles serve as novel biomarkers for HBV infection and diagnosis of HBV-positive hepatocarcinoma. Cancer Res 2010; 70: 9798-807.

52. Shi WQ, et al. Investigation of clinical diagnosis and liver biopsy diagnosis in cases of patients with chronic HBV infection. Afr Health Sci 2014; 13: 2931-8.

53. Yang JX, et al. Profile of HBV antiviral resistance mutations with distinct evolutionary pathways against nucleoside/nucleotide analogue treatment among Chinese chronic hepatitis B patients. Antivir Ther 2010; 15: 1171-8.

54. Nair PV, et al. Clinical, serologic, and immunologic effects of human leukocyte interferon in HBsAg-positive primary hepatocellular carcinoma. Cancer 1985; 56: 1018-22.

55. Jardi R, et al. Mutations in the basic core promoter region of hepatitis B virus. Relationship with precore variants and HBV genotypes in a Spanish population of HBV carriers. J Hepatol 2004; 40: 507-14.

56. Chen Y, et al. Comparison of the results for three automated immunoassay systems in determining serum HBV markers. Clin Chim Acta 2006; 372: 129-33.

57. Hsiao CC, et al. High-resolution melting and real-time PCR for quantification and detection of drug-resistant HBV mutants in a single amplicon. Antivir Ther 2012; 17: 291-303.

58. Huang CJ, et al. Impact of genetic heterogeneity in polymerase of hepatitis B virus on dynamics of viral load and hepatitis B progression. PloS one 2013; 8: e70169.

59. Wright TL. Introduction to chronic hepatitis B infection. Am J Gastroenterol 2006; 101: S1-6.

60. Lin C, et al. Hepatitis B e Antigen Seroconversion Is Related with the Function of Dendritic Cells in Chronic Hepatitis B Virus Infection. Gastroenterol Res Pract 2014; 2014: 413952.

61. Hatipoglu I, et al. Hepatitis B virus e antigen (HBeAg) may have a negative effect on dendritic cell generation. Immunobiology 2014; 219: 944-9.

62. Mekky MA. To treat or not to treat the "immunotolerant phase" of hepatitis B infection: A tunnel of controversy. World J Hepato 2014; 6: 226-9.

63. Lutgehetmann M, et al. Hepatitis B virus limits response of human hepatocytes to interferon-alpha in chimeric mice. Gastroenterology 2011; 140: 2074-83, 83 e1-2.

64. Arzumanyan A, et al. Hedgehog signaling blockade delays hepatocarcinogenesis induced by hepatitis B virus X protein. Cancer Res 2012; 72: 5912-20.

65. Vivekanandan P, et al. Hepatitis B virus replication induces methylation of both host and viral DNA. J Virol 2010; 84: 4321-9.

66. Wei C, et al. The hepatitis B virus X protein disrupts innate immunity by downregulating mitochondrial antiviral signaling protein. J Immunol 2010; 185: 1158-68.

67. Xu Y, et al. HBsAg inhibits TLR9-mediated activation and IFN-alpha production in plasmacytoid dendritic cells. Mol Immunol 2009; 46: 2640-6.

68. Chen M, et al. Immune tolerance split between hepatitis B virus precore and core proteins. J Virol 2005; 79: 3016-27.

69. Locarnini S, et al. Cellular response to conditional expression of the hepatitis B virus precore and core proteins in cultured hepatoma (Huh-7) cells. J Clin Virol 2005; 32: 113-21.

70. Bertoletti A and Ferrari C. Innate and adaptive immune responses in chronic hepatitis B virus infections: towards restoration of immune control of viral infection. Gut 2012; 61: 1754-64.

71. Farag MM, et al. Immune tolerance against HBV can be overcome in HBV transgenic mice by immunization with dendritic cells pulsed by HBVsvp. Vaccine 2012; 30: 6034-9.

72. Xia Y, et al. Phyllanthus species versus antiviral drugs for chronic hepatitis B virus infection. The Cochrane database of systematic reviews 2013; 4: CD009004.

73. Tang CM, et al. Management of chronic hepatitis B infection: current treatment guidelines, challenges, and new developments. World J Gastroenterol: WJG 2014; 20: 6262-78.

74. Godzik P, et al. Inhibitors of hepatitis C virus--therapeutic possibilities. Przegl Epidemiol 2010; 64: 479-84.

75. Bertoletti and Ferrari C. Innate and adaptive immune responses in chronic hepatitis B virus infections: towards restoration of immune control of viral infection. Postgrad Med J 2013; 89: 294-304.

76. Kawai T and Akira S. Toll-like receptors and their crosstalk with other innate receptors in infection and immunity. Immunity 2011; 34: 637-50.

77. Menne S, et al. Sustained efficacy and seroconversion with the Toll-like receptor 7 agonist GS-9620 in the Woodchuck model of chronic hepatitis B. J Hepatol 2015; 62: 1237-45.

78. Gane EJ, et al. The oral toll-like receptor-7 agonist GS-9620 in patients with chronic hepatitis B virus infection. J Hepatol. 2015.

79. Lanford RE, et al. GS-9620, an oral agonist of Toll-like receptor-7, induces prolonged suppression of hepatitis B virus in chronically infected chimpanzees. Gastroenterology 2013; 144: 1508-17, 17 e1-10.

80. Gaggar A, et al. Safety, tolerability and immunogenicity of GS-4774, a hepatitis B virus-specific therapeutic vaccine, in healthy subjects: a randomized study. Vaccine 2014; 32: 4925-31.

81. Pei R, et al. Interferon-induced proteins with tetratricopeptide repeats 1 and 2 are cellular factors that limit hepatitis B virus replication. J Innate Immun.2014; 6: 182-91.

82. Qin B, et al. A novel method for the analysis of drug-resistant phenotypes of hepatitis B virus. Int J Mol Med. 2013; 31: 975-81.

83. Yang HC and Kao JH. Persistence of hepatitis B virus covalently closed circular DNA in hepatocytes: molecular mechanisms and clinical significance. Emerging microbes & infections 2014; 3: e64.

84. Menendez-Arias L, et al. Nucleoside/nucleotide analog inhibitors of hepatitis B virus polymerase: mechanism of action and resistance. Curr Opin Virol.2014; 8: 1-9.

85. Qin B, et al. Substitution rtq267h of hepatitis B virus increases the weight of replication and Lamivudine resistance. Hepat Mon 2013; 13: e12160.

86. Navabakhsh B, et al. Hepatitis B Virus Infection during Pregnancy: Transmission and Prevention. Middle East J Dig Dis 2011; 3: 92-102.

87. Han GR, et al. A prospective and open-label study for the efficacy and safety of telbivudine in pregnancy for the prevention of perinatal transmission of hepatitis B virus infection. J Hepatol 2011; 55: 1215-21.

88. Xu YY, et al. Peripheral blood mononuclear cell traffic plays a crucial role in mother-to-infant transmission of hepatitis B virus. Int J Biol Sci 2015; 11: 266-273.

89. Pan CQ, et al. Telbivudine prevents vertical transmission from HBeAg-positive women with chronic hepatitis B. Clin Gastroenterol Hepatol 2012; 10: 520-6.

90. Mancini-Bourgine M, et al. Immunogenicity of a hepatitis B DNA vaccine administered to chronic HBV carriers. Vaccine 2006; 24: 4482-9.

91. Wen Y, et al. Vaccine therapies for chronic hepatitis B: can we go further? Front Med 2014; 8: 17-23.

92. Kapoor R and Kottilil S. Strategies to eliminate HBV infection. Future virology 2014; 9: 565-85.

93. Yang HC and Kao JH. Viral hepatitis. HBV cure--can we pin our hopes on immunotherapy? Nature reviews Gastroenterology & hepatology 2015; 12: 129-31.

94. Carotenuto P, et al. In vitro use of autologous dendritic cells improves detection of T cell responses to hepatitis B virus (HBV) antigens. Journal of medical virology 2009; 81: 332-9.

95. Luo J, et al. Autologus dendritic cell vaccine for chronic hepatitis B carriers: a pilot, open label, clinical trial in human volunteers. Vaccine 2010; 28: 2497-504.

96. Michel ML and Mancini-Bourgine M. Therapeutic vaccination against chronic hepatitis B virus infection. Journal of clinical virology : the official publication of the Pan American Society for Clinical Virology 2005; 34: S108-14.

97. Vandepapeliere P, et al. Therapeutic vaccination of chronic hepatitis B patients with virus suppression by antiviral therapy: a randomized, controlled study of co-administration of HBsAg/AS02 candidate vaccine and lamivudine. Vaccine 2007; 25: 8585-97.

98. Xu DZ, et al. Results of a phase III clinical trial with an HBsAg-HBIG immunogenic complex therapeutic vaccine for chronic hepatitis B patients: experiences and findings. J Hepatol 2013; 59: 450-6.

99. Xu DZ, et al. A randomized controlled phase IIb trial of antigen-antibody immunogenic complex therapeutic vaccine in chronic hepatitis B patients. PloS one 2008; 3: e2565.

100. Yoon SK, et al. Safety and immunogenicity of therapeutic DNA vaccine with antiviral drug in chronic HBV patients and its immunogenicity in mice. Liver Int 2015; 35: 805-15.

101. Li J, et al. Hepatitis B surface antigen (HBsAg) and core antigen (HBcAg) combine CpG oligodeoxynucletides as a novel therapeutic vaccine for chronic hepatitis B infection. Vaccine 2015; 33: 4247-54.

102. Martin P, et al. TG1050, an immunotherapeutic to treat chronic hepatitis B, induces robust T cells and exerts an antiviral effect in HBV-persistent mice. Gut 2014 64:1961-71.

103. Kaneko M, et al. A Novel Tricyclic Polyketide, Vanitaracin A, Specifically Inhibits the Entry of Hepatitis B and D Viruses by Targeting Sodium Taurocholate Cotransporting Polypeptide. J Virol 2015; 89: 11945-53.

104. Uhl P, et al. A liposomal formulation for the oral application of the investigational hepatitis B drug Myrcludex B. Eur J Pharm Biopharm 2016; 103:159-66.

105. Li W and Urban S. Entry of hepatitis B and hepatitis D virus into hepatocytes: Basic insights and clinical implications. J Hepatol 2016; 64: S32-40.

106. Volz T, et al. The entry inhibitor Myrcludex-B efficiently blocks intrahepatic virus spreading in humanized mice previously infected with hepatitis B virus. Journal of hepatology 2013; 58: 861-7.

107. Yan H, et al. NTCP opens the door for hepatitis B virus infection. Antiviral Res 2015; 121: 24-30.

108. Hirsch RC, et al. Polymerase gene products of hepatitis B viruses are required for genomic RNA packaging as wel as for reverse transcription. Nature 1990; 344: 552-5.

109.   Katen SP, et al. Assembly-directed antivirals differentially bind quasiequivalent pockets to modify hepatitis B virus capsid tertiary and quaternary structure. Structure 2013; 21: 1406-16.

110.   Wu G, et al. Preclinical characterization of GLS4, an inhibitor of hepatitis B virus core particle assembly. Antimicrob Agents Chemother. 2013; 57: 5344-54.

111.   Delaney WEt, et al. Phenylpropenamide derivatives AT-61 and AT-130 inhibit replication of wild-type and lamivudine-resistant strains of hepatitis B virus in vitro. Antimicrob Agents Chemother 2002; 46: 3057-60.

112.   Billioud G, et al. The main hepatitis B virus (HBV) mutants resistant to nucleoside analogs are susceptible in vitro to non-nucleoside inhibitors of HBV replication. Antiviral Res 2011; 92: 271-6.

113.   Feld JJ, et al. The phenylpropenamide derivative AT-130 blocks HBV replication at the level of viral RNA packaging. Antiviral Res 2007; 76: 168-77.

114.   Dougherty AM, et al. A substituted tetrahydro-tetrazolo-pyrimidine is a specific and novel inhibitor of hepatitis B virus surface antigen secretion. Antimicrob Agents Chemother 2007; 51: 4427-37.

115.   Korba BE, et al. Nitazoxanide, tizoxanide and other thiazolides are potent inhibitors of hepatitis B virus and hepatitis C virus replication. Antiviral Res 2008; 77: 56-63.

116.   Cardin RD, et al. Amphipathic DNA polymers exhibit antiviral activity against systemic murine Cytomegalovirus infection. Virol J 2009; 6: 214.

117.   Noordeen F, et al. Nucleic acid polymers inhibit duck hepatitis B virus infection in vitro. Antimicrob Agents Chemother 2013; 57: 5291-8.

118.   Guzman EM, et al. Amphipathic DNA polymers are candidate vaginal microbicides and block herpes simplex virus binding, entry and viral gene expression. Antivir Ther 2007; 12: 1147-56.

119.   Wang XY and Chen HS. Emerging antivirals for the treatment of hepatitis B. World J Gastroenterol 2014; 20 :7707-17.

120.   Yuen MF, et al. Two-year treatment outcome of chronic hepatitis B infection treated with besifovir vs. entecavir: results from a multicentre study. J Hepatol 2015; 62: 526-32.

121.   Murakami E, et al. Implications of Efficient Hepatic Delivery by Tenofovir Alafenamide (GS-7340) for Hepatitis B Virus Therapy. Antimicrob Agents Chemother 2015; 59: 3563-9.

122.   Agarwal K, et al. Twenty-eight day safety, antiviral activity, and pharmacokinetics of tenofovir alafenamide for treatment of chronic hepatitis B infection. J Hepatol 2015; 62: 533-40.

123.   Painter GR, et al. Evaluation of hexadecyloxypropyl-9-R-[2-(Phosphonomethoxy)propyl]- adenine, CMX157, as a potential treatment for human immunodeficiency virus type 1 and hepatitis B virus infections. Antimicrob Agents Chemother 2007; 51: 3505-9.

124.   Bryant ML, et al. Anti-HBV specific beta-L-2'-deoxynucleosides. Nucleosides Nucleotides Nucleic Acids 2001; 20: 597-607.

125.   Schramke V, et al. RNA-interference-directed chromatin modification coupled to RNA polymerase II transcription. Nature 2005; 435: 1275-9.

126.   Kennedy S, et al. A conserved siRNA-degrading RNase negatively regulates RNA interference in C. elegans. Nature 2004; 427: 645-9.

127.   Rice RR, et al. Simple, robust strategies for generating DNA-directed RNA interference constructs. Methods Enzymol 2005; 392: 405-19.

128.   Sebestyen MG, et al. Targeted in vivo delivery of siRNA and an endosome-releasing agent to hepatocytes. Methods Mol Biol 2015; 1218: 163-86.

129.   Nassal M. HBV cccDNA: viral persistence reservoir and key obstacle for a cure of chronic hepatitis B. Gut 2015.

130.   Chisari FV, et al. Virology. Comment on "Specific and nonhepatotoxic degradation of nuclear hepatitis B virus cccDNA". Science 2014; 344: 1237.

131.   Lucifora J, et al. Specific and nonhepatotoxic degradation of nuclear hepatitis B virus cccDNA. Science 2014; 343: 1221-8.

132.   Beug ST, et al. Combinatorial cancer immunotherapy strategies with proapoptotic small-molecule IAP antagonists. Int J Dev Biol 2015.

133.   Zhang Y, et al. Role of Smac in apoptosis of lung cancer cells A549 induced by Taxol. Clin Lab 2015; 61: 17-21.

134.   Ebert G, et al. Cellular inhibitor of apoptosis proteins prevent clearance of hepatitis B virus. Proc Natl Acad Sci U S A 2015; 112 :5797-802.

135.   David LA, et al. Diet rapidly and reproducibly alters the human gut microbiome. Nature. 2014; 505: 559-63.

136.   Qin N, et al. Alterations of the human gut microbiome in liver cirrhosis. Nature 2014; 513: 59-64.

137. Chou HH, et al. Age-related immune clearance of hepatitis B virus infection requires the establishment of gut microbiota. Proc Natl Acad Sci U S A 2015; 112: 2175-80.

138. Bischoff SC, et al. Intestinal permeability--a new target for disease prevention and therapy. BMC Gastroenterol 2014; 14:189.

139. Stecher B and Hardt WD. The role of microbiota in infectious disease. Trends Microbiol 2008; 16: 107-14.

140. Backhed F, et al. Host-bacterial mutualism in the human intestine. Science 2005; 307: 1915-20.