Journal of Virus Eradication 2015; **1**: 4–12

Towards the elimination and eradication of hepatitis B

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Abstract

Despite the introduction of vaccination, chronic hepatitis B remains a major cause of liver-related morbidity and mortality including cirrhosis, decompensated cirrhosis and hepatocellular carcinoma. Maintenance antiviral therapy is required for most people, as low rates of cure occur. The stated aim of therapy presently is HBV DNA suppression; effective suppression of viral replication is associated with significant reductions in morbidity from end-stage liver failure and to an extent, hepatocellular carcinoma. Unfortunately, major barriers to cure, such as a reservoir of episomal covalently closed circular DNA (cccDNA) (the HBV minichromosome), and a dysfunctional immune response, pose challenges. These barriers will need to be overcome to ensure higher rates of cure than can be achieved presently.

Quantitative and diagnostic testing for HBV DNA is not generally available, hampering effective monitoring and treatment in low-income countries. The majority of patients in resource-constrained countries are not identified before the onset of cirrhosis. Without coordinated action, and transfer of new diagnostic technologies and treatments to low-income countries, recent therapeutic advances will have little effect on the global burden of disease.

A shift to curative treatment for the majority would be a major advance in the elimination of hepatitis B. New and improved molecular therapeutics and immunological strategies for the treatment of chronic hepatitis are emerging, however. A number of promising lines of development are in progress. A curative regimen may require a combination of viral suppression via nucleoside analogue therapy to prevent cccDNA amplification and viral propagation, safe selective cccDNA inhibitors to deplete, silence or degrade cccDNA, agents to block the entry of HBV into the hepatocyte plus compounds to prevent capsid assembly and cccDNA interactions. Targeted immune activation could restore the exhausted immune cell repertoire.

Keywords: Hepatitis, chronic hepatitis B, antiviral therapy, nucleoside analogues, interferon

Introduction

Type B hepatitis is caused by the hepatitis B virus (HBV), a small, enveloped DNA. HBV infection can be either acute or chronic, and can range in severity from being inapparent and asymptomatic to severe or fulminant. The chronic disease may be asymptomatic, until progressive and ultimately fatal illness occurs. Acute hepatitis B is defined as a self-limiting disease marked by acute inflammation, and hepatocellular necrosis in association with a transient HBV infection. Chronic hepatitis B is defined as persistent HBV infection accompanied by evidence of hepatocellular injury, inflammation and fibrosis. The diagnosis of chronic hepatitis B is based upon the finding of abnormal concentrations of serum aminotransferases (ALT) and hepatitis B surface antigen (HBsAg) in serum for 6 months or more.

The identification of HBV led to the development of recombinant DNA-derived vaccines that are widely used throughout the world. However, despite the introduction of vaccination, chronic hepatitis B remains a major cause of liver-related morbidity and mortality including cirrhosis, decompensated cirrhosis and hepatocellular carcinoma (HCC). Some countries have developed effective national plans and the World Health Organization (WHO) has developed a Framework for Global Action based on specific interventions ranging from raising awareness to increasing access to care and treatment [1].

Treatment has been restricted to interferon, pegylated interferon or five nucleoside analogues: lamivudine, adefovir, telbivudine, entecavir and tenofovir. Maintenance therapy is required for most people, as low rates of cure occur. In many regions treatment is governed by international guidelines. Long-term suppression of HBV DNA is the achievable endpoint for most patients. Hepatitis B e antigen (HBeAg)-positive patients may lose HBeAg and subsequently HBsAg, but HBsAg loss occurs in a minority. In anti-HBe-positive persons, sustained low levels of replication are induced by nucleoside analogue therapy. Thus, the stated aim of therapy presently is HBV DNA suppression; effective suppression of viral replication is associated with significant reductions in morbidity from end-stage liver failure and to an extent, hepatocellular carcinoma (HCC).

Unfortunately, major barriers to cure such as a reservoir of stable episomal covalently closed circular DNA (cccDNA) and a dysfunctional immune response, pose challenges. These barriers will need to be overcome to ensure higher rates of cure than can be achieved presently. New and improved molecular therapeutics and immunological strategies for the treatment of chronic hepatitis are emerging, however.

Epidemiology and prevention

Over 400 million people are chronically infected with hepatitis B virus. HBV is thus a major cause of liver-related morbidity. Most persons who acquire chronic HBV have been infected at birth or in early childhood, usually during the first 5 years of life [2–4]. Worldwide, up to 650,000 people die from the complications of chronic HBV, cirrhosis and HCC each year [5]. The incidence of HCC and cirrhosis is low before the age of 35, but rises in mid- and later life [6]. Although, in Africa a higher incidence of HCC has been reported in young male adults.

WHO recommends that all infants receive hepatitis B vaccine as soon as possible after birth, preferably within 24 hours, and that the birth dose is followed by two or three subsequent doses [7]. The vaccine is effective in 95% of infants and children but protection may fail in infants born to highly viraemic mothers. By 2012, 183 countries vaccinated infants against hepatitis B as part of primary vaccination schedules [8]. Unfortunately new infections are still occurring and vaccination, while effective in reducing incident chronic disease in endemic regions, will not have the desired impact on the rates of end-stage liver disease and HCC

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in the extant, chronically infected population. Thus, a policy to identify and treat persons at risk with chronic HBV is important.

Transmission

Most persons who are infected at birth or in the first 5 years of life develop chronic hepatitis B [2–4]. HBsAg has been found in blood and in various body fluids (e.g. saliva, menstrual and vaginal discharges, seminal fluid, colostrum and breast milk, serous exudates) and these have been implicated as vehicles of transmission of infection. Transmission of the infection may result from accidental inoculation of minute amounts of blood or fluid contaminated with blood during medical, surgical and dental procedures; immunisation with inadequately sterilised syringes and needles; intravenous and percutaneous drug abuse; tattooing; body piercing; acupuncture; laboratory accidents; and accidental inoculation with razors and similar objects that have been contaminated with blood. The virus may be infective by mouth. There is considerable evidence for the transmission of hepatitis B by intimate contact and by the sexual route. Sexually promiscuous unvaccinated individuals, particularly men who have sex with men, are at high risk; however, infection in adulthood leads to chronic HBV in <5% of cases.

Viraemic mothers, who are seropositive for HBeAg, almost invariably transmit the infection to their infants at the time of, or shortly after, birth in the absence of prophylaxis. Individuals infected at an early age exhibit high levels of viraemia, and a limited or dysfunctional immune response, and may remain viraemic for decades. Perinatal transmission has been an important factor in maintaining the reservoir of the infection in some regions, particularly in China and Southeast Asia. The mechanism of perinatal infection is uncertain, but it probably occurs during or shortly after birth. However, mother-to-infant transmission only accounts for half of the infections in children and horizontal transmission between children is an important, if poorly defined, route of infection. The probability of a childhood infection becoming persistent declines with age, from around 90% in neonates to less than 5% if infection is acquired in adolescence or later.

A proportion of infants born to HBsAg mothers acquire hepatitis B despite prophylaxis. Research has suggested that the babies most likely to become infected are those born to mothers with very high viraemia, defined as $>10^7$ IU/mL [9,10], but estimates of the risk of transmission despite HBV vaccination and HBIG vary [11].

Virology

The HBV genome is 3,200 base pairs in length. Analysis of protein coding reveals four conserved open reading frames (ORFs) encoding: HBsAg; HBcAg; the viral polymerase; and the HBx protein. Identification of animal viruses resembling human HBV has led to the characterisation of the replication cycle of the hepadnaviruses. The genomes of a variety of isolates of hepatitis B virus have been cloned and the complete nucleotide sequences determined. There is some variation in sequence (up to 12% of nucleotides) between these isolates and up to nine genotypes (A to I) have been described on the basis of more than 8% nucleotide sequence divergence. HBV replicates largely in the liver. The hepadnaviruses are unique among animal DNA viruses in that they replicate through an RNA intermediate. HBV exists as a 42-nm, double-shelled particle found in serum. HBV has an outer envelope component of HBsAg and an inner nucleocapsid component of HBcAg. In addition to complete virions, incomplete viral particles, 20-nm spheres and tubules, which consist entirely of HBsAg without HBcAg or nucleic acid, are present in serum and outnumber virions. HBsAg can be detected in the liver. The nucleocapsid, HBcAg, is not found free in serum, but within HBV virions and can be detected by histochemical staining in the liver. HBV DNA can be detected in serum and is used to monitor viral replication. HBeAg, unlike HBsAg and HBcAg, is not particulate, but rather is detectable as a soluble, 17-kDa protein in serum. The HBV surface proteins (HBs) are composed of three proteins: large, middle and small surface proteins, and include the preS1, preS2 and S regions. The large HBs includes the preS1, preS2 and S regions; the middle HBs comprises the preS2 and S; and the small HBs comprises the S region [12,13]. The large envelope glycoprotein on the surface of HBV (and HDV) particles has been shown to play a pivotal role in virus entry. Virus entry incorporates binding of virions to heparan sulphate proteoglycans at the hepatocyte surface and subsequent binding of the myristoylated N-terminal preS1-domain of the L-protein to the sodium taurocholate co-transporting polypeptide – the recently identified HBV (and HDV) entry receptor [13].

On infection of the hepatocyte, the viral DNA is uncoated and converted to a covalently closed circular (supercoiled) form in the nucleus (cccDNA), which is the template for transcription of the viral RNAs including the pre-genomic RNA. There are at least four viral promoters; mRNAs for the synthesis of HBeAg, HBcAg, the viral polymerase and progeny genomes have been identified. Binding of the polymerase to a secondary structure at the 5′ end (epsilon signal, ε) of the pre-genome leads to packaging into immature viral cores in the cytoplasm. The amino terminal domain of the viral polymerase acts as the primer for minus strand DNA synthesis. Minus strand synthesis proceeds by reverse transcription of the pre-genome by the viral polymerase with concomitant degradation of the template. The capsid of HBV is formed by copies of the 22-kDa core protein, and assembled into core particles. HBV capsids (core) are coated with HBsAg to form mature virus particles [14]. HBV DNA is integrated into the genome of hepatocytes and HBsAg can be produced following transcription of integrated viral DNA [15]. Expansion of clones of such cells may be a stage in progression to neoplasia, but integration of the viral genome is not believed to be required for replication of the virus. Integrations of HBV DNA into the host genome seem to be random but ostensibly contribute to the development of HCC.

A number of naturally occurring mutations in the pre-core region preventing HBeAg synthesis have been identified in HBeAg-negative carriers. These variants of hepatitis B virus are found in serum of anti-HBe and HBV DNA-positive persons who have HBcAg in hepatocytes and histological evidence of active chronic hepatitis but lack HBeAg in serum. The C gene has two initiation codons upstream of two regions (pre-core and core) and two potential molecular forms (HBcAg and HBeAg) can be produced. Initiation of translation at the first site (nucleotide 1814) produces a 312-amino acid polypeptide (p25) that has a signal peptide directing it to the endoplasmic reticulum. There a signal piece is removed by signal peptidase to cleave the N-terminal 19 amino acid residues as well as the C-terminal 34 residues; the resultant polypeptide of 150 amino acids is secreted as HBeAg (p15–18), a soluble protein that is the product of 10 residues coded by the pre-core region and 149 residues coded by the C gene. Translation from the second initiation codon (nucleotide 1901) results in unprocessed polypeptides (p23, 183 amino acids), which are assembled into core particles within the liver (p21).

Amplification by polymerase chain reaction and subsequent sequencing of DNA from virions in serum of patients lacking

HBeAg has revealed one or more nucleotide substitutions in the pre-core region of the HBV genome and an HBV variant with a G–A mutation at nucleotide 83 in the pre-core region accounts for most cases of HBeAg-negative hepatitis B. A point mutation from G to A creating an in-frame TAG stop codon, with or without additional point mutations in succeeding codons, has been described. This mutation induces a Trp at codon 28, and explains the serological absence of HBeAg. During disease, HBeAg-defective virus may be selected for by immune selection pressure. There is an influence of HBV genotype on the prevalence of pre-core mutations. For example, the pre-core mutation is relatively uncommon in HBsAg persons of North American and Western European origin who are infected with genotype A of HBV and who carry a cytosine at position 1858 [rather than a thymine (uracil) at this position]. Uracil at position 1858 may form a base pair with either G or A in nucleotide 1896, but a cytosine at position 1858 cannot pair with the G–A mutation in nucleotide 1896, and this reduces the efficiency of encapsidation and replication.

In the areas where HBV genotype C is common, particularly East Asia, perinatal transmission accounts for more than half the cases of chronic HBV in unvaccinated infants of HBsAg-positive mothers. However, in areas where genotype C is rare, such as Africa, the Middle East and Europe, seroconversion from HBeAg to anti-HBe occurs more frequently in childbearing women. Higher rates of HCC have been found in persons infected with genotypes C and F compared with those infected with genotypes B or D. HCC occurs at a younger age in patients infected with genotypes F and in those infected with subtypes of genotype A found in southern Africa, although aflatoxin exposure may play a role in parts of sub-Saharan Africa [8]. Fortunately, both HBV nucleoside analogue therapy and HBV vaccine are equally effective against all HBV genotypes.

Natural history

The natural history of HBV is complex, not linear and is incompletely understood. Older patients in endemic regions frequently present for the first time with complications of cirrhosis, or even HCC. Several phases of chronic hepatitis B are recognised. The terms 'immune tolerant,' 'immune active,' 'immune escape' and 'inactive carrier' phases have been commonly used to describe sequential stages of the disease (Table 1), but it is increasingly recognised that these descriptions are not fully supported by immunological data [16]. Typically, in early childhood and young adults, serum HBsAg and HBeAg are detectable; serum HBV DNA levels are high (usually greater than 10^6 IU/mL) and serum ALT may be normal or only minimally elevated. High levels of HBsAg are found. This pattern is common in young individuals who are infected in the neonatal period and whose infection may last for 10–30 years afterwards. This phase has been referred to as the 'immune tolerant' phase, although the concept of true immune tolerance is being challenged. In HBeAg-positive patients, progression to cirrhosis occurs at an annual rate of 2–5.5% with a cumulative 5-year incidence of progression of 8–20%. The high replicative phase may be followed by a phase of active inflammatory disease when symptoms of hepatitis may be present, and serum ALT may become elevated. During this HBeAg-positive phase, exacerbations in serum ALT are observed, accompanied by variable and fluctuating changes in HBV DNA concentrations. Severe histological hepatitis and fibrosis may ensue during this 'immune active' phase.

A proportion of patients with chronic hepatitis B may undergo spontaneous seroconversion from HBeAg to anti-HBe (the 'inactive carrier' state). The inactive phase is characterised by prolonged and persistent normalisation of ALT and suppression of HBV DNA levels to under 2,000 IU/mL. Lower quantities of HBsAg are present in serum. Some of these patients will eventually lose HBsAg at a rate of 0.5–2.0% per year resulting in a decreased risk of complications but there will still be a risk of HCC. A spontaneous remission in disease activity may occur in approximately 10–15% of HBeAg-positive persons per year. The prognosis for these patients, if stable and without pre-existing advanced disease, is good. HBeAg seroconversion rates are higher in those with raised serum aminotransferases and in patients with genotype D and (in Asia) genotype B infection. Once HBeAg is cleared, the disease can remit and serum aminotransferases become normal. This may confer a good prognosis if seroconversion occurs at a young age, prior to the onset of significant liver disease.

Active chronic hepatitis can occur in HBsAg-positive, HBeAg-negative, anti-HBe-positive persons with serum HBV DNA concentrations >2,000 IU/mL and raised aminotransferases (the previously termed 'immune escape' phase). HBeAg is undetectable in these persons because of selection for HBV virions not expressing HBeAg (pre-core mutant HBV). Individuals with anti-HBe-positive chronic hepatitis B tend to be older, and may present with progressive necro-inflammatory change or cirrhosis. HBeAg-negative chronic hepatitis has a variable course, often with fluctuating serum aminotransferases and HBV DNA levels. Severe exacerbations may occur. Progression to cirrhosis is generally more rapid in anti-HBe-positive disease and occurs at an annual rate of 8–20%. Levels of HBsAg are generally 1 log lower than in HBeAg-positive patients.

The immunological profile underlying hepatic inflammation is not fully explained. The concept that the establishment of persistent infection and induction of 'immune tolerance,' that is the inability to mount a virus-specific immune response, are linked is being challenged. Studies suggest that young adolescents exhibit a normal Th1 T cell response and harbour hepatitis B-specific T cells that are functionally active. It is possible that this phase is triggered by hepatitis B-specific CD8+ T cells but the hepatic inflammation is not proportional to the quantity of hepatitis B-specific CD8 T cells [17,18]. Other factors such as chemokines and natural killer cell activation may be important. Exacerbations in serum ALT may be observed accompanied by variable decreases in HBV DNA concentrations and can be followed by HBeAg to anti-HBe seroconversion.

Recent retrospective studies have examined survival in compensated cirrhosis due to hepatitis B. The reported yearly incidence of hepatic decompensation is about 3% with a 5-year cumulative incidence of 16%. In a European multicentre longitudinal study to assess the survival of 366 cases of HBsAg-positive compensated cirrhosis, death occurred in 23% of patients mainly due to liver failure or HCC. The cumulative probability of survival in this cohort was 84% and 68% at 5 and 10 years, respectively [19–22]. The worst survival was in HBeAg-negative but HBV DNA-positive subjects. Chinese patients remaining HBeAg positive are more likely to develop HCC.

Diagnosis and pathology

Acute or chronic hepatitis B is usually diagnosed by the detection of HBsAg in serum. Many persons can be detected through routine screening for HBsAg or the presence of abnormal serum aminotransferases. Detection of viral DNA is the optimal method of quantitating hepatitis B viraemia and standardised quantitative

Table 1. Commonly defined phases of HBV disease

Stage	HBEAG serological status	Pattern	Current treatment	Potential curative treatment
Immune tolerant	HBeAg positive	HBeAg positive High levels of HBV replication (high HBV DNA concentrations) Minimal histological disease Stage seen in many children	Interferon generally ineffective Maintenance therapy nucleosides required	Benefit if HBsAg loss at early stage Obviates progression Direct inhibition of HBV replication? Immune activation?
Immune active	HBeAg positive; may develop anti-HBe	Raised ALT Histological activity HBeAg to anti-HBe seroconversion possible; normalisation of ALT Lobular hepatitis, bridging fibrosis and fibrosis can be present May progress to anti-HBe-positive disease	Suitable for treatment with nucleosides or interferon	Direct inhibition Immune activation feasible
Immune escape	HBeAg negative, anti-HBe positive	HBeAg-negative disease HBeAg negative, anti-HBe positive Ongoing HBV replication HBV DNA >20,000 IU/mL Exacerbations of ALT Older persons Progressive disease	Nucleoside analogue treatment Less commonly, interferon	Direct inhibition Immune activation feasible
Reactivation of acute or chronic hepatitis	HBeAg positive or negative	HBV DNA elevated Serum ALT elevated Seroreversion to HBeAg can occur if HBeAg negative High risk of decompensation if cirrhosis Can be precipitated by immunosuppression	Nucleoside analogue treatment required	Curative treatment would prevent Urgent intervention required
Inactive carrier	HBeAg negative anti-HBe positive	HBV DNA <2,000 IU/mL Risk of cirrhosis declines HCC risk lower Can develop anti-HBe-positive disease	Monitoring only	Functional cure HBsAg loss obviates frequent monitoring

assays are valuable for monitoring virus loads during antiviral therapy [23]. Quantitative assays for HBV DNA were previously limited by a lack of standardisation but a WHO standard has been developed [23]. Unfortunately HBV DNA testing is not widely available in low-income countries.

HBeAg is a marker of viraemia but anti-HBe does not necessarily indicate clearance of virus replication. The levels of aminotransferases may fluctuate with time. Usually, the levels of ALT are higher than those of aspartate aminotransferase (AST). However, with progression of the disease to cirrhosis, the AST/ALT ratio may be reversed. Elevation of these enzymes may be the only abnormality to be found in individuals with asymptomatic and anicteric infections. A progressive decline in serum albumin concentrations and prolongation of the prothrombin time are characteristically observed after decompensated cirrhosis has developed.

Single measures of ALT do not indicate disease status in a disease as dynamic as hepatitis B, and there is a controversy regarding the level below which HBV DNA concentrations are indicative of 'inactive' disease, or provide a threshold for initiating treatment [24]. Longitudinal measures, over at least a few months are required. A full staging of the disease includes measures of serum albumin, platelet count, prothrombin time, and assessment of cirrhosis, including measures to determine the presence or absence of oesophageal varices. Ultrasonography is usually used to screen patients for HCC, as part of regular surveillance for HCC. Alpha-fetoprotein (AFP) is usually monitored in patients with cirrhosis. Non-invasive methods are supplanting liver biopsy and have been validated in hepatitis B. Markers for fibrosis, including APRI and FIB-4 as well as commercial markers can be performed or a FibroScan, to ascertain for advanced fibrosis. Their validation should be encouraged in resource-poor regions [25–27]. Liver ultrasound has fair specificity but low sensitivity for cirrhosis, but may be helpful. Moderate fibrosis may be more difficult to detect by any non-invasive test.

Many clinicians would consider a liver biopsy helpful for ascertaining the degree of necro-inflammation and fibrosis. Hepatic morphology can assist the decision to treat. There are several established methods of scoring histology, measuring activity (necro-inflammation) separately from stage (fibrosis). There are, however, several limitations of biopsy including sampling error, subjectivity and reproducibility, and of course costs, risks and discomfort to the patient, and lack of training opportunities and infrastructure in low-income countries. The activity of hepatitis B can vary over time but ultimately the degree of hepatic fibrosis determines the prognosis. Assessment of fibrosis measures how far the disease has progressed. Progression of disease in hepatitis B is not linear, but is influenced by episodic activity and injury to the liver.

The pathological features of chronic hepatitis B depend upon the stage of the disease, the host immune response and the degree of virus replication. In chronic hepatitis B with mild activity, only rare piecemeal necrosis is seen. Characteristic hepatocytes with eosinophilic ground-glass cells are relatively common in anti-HBe-positive patients with low levels of virus replication. Lobular hepatitis is more common in patients with active virus

replication, and raised serum aminotransferases. CD8+ cells predominate in areas of piecemeal necrosis. HBsAg and HBcAg can be detected by immunoperoxidase staining in routinely fixed liver biopsy sections. Patients with high levels of viraemia may have minimal hepatitis [28].

Co-infections with hepatitis B

HBV and hepatitis delta virus (HDV)

Delta hepatitis (HDV) was first recognised following detection of a novel protein delta antigen, HDAg, by immunofluorescence staining in the nuclei of hepatocytes from patients with hepatitis B [29]. HDV is now known to require HBsAg for its propagation and transmission. HDV is coated with HBsAg, which is required for release from hepatocytes and for entry and propagation. The virus consists of a particle measuring 35–37 nm in diameter with an internal nucleocapsid comprising the genome surrounded by the delta antigen and envelope composed of HBsAg. The genome consists of a single standard circular RNA of around 1,700 nucleotides; the delta antigen is encoded by anti-genomic RNA [30,31].

Two major forms of delta hepatitis infection are known. In the first, a susceptible individual is co-infected simultaneously with both HBV and HDV, which can lead to a more severe form of acute hepatitis. In the second, an individual infected chronically with HBV becomes superinfected with HDV. This may accelerate the course of the chronic disease and cause overt disease in asymptomatic HBsAg carriers. HD antigen has been observed in individuals post liver transplant as HDV replication persists in isolated hepatocytes.

Limited studies indicate a worldwide distribution of hepatitis D infection. The infection is important in southern Europe, Turkey, the Middle East, Japan, Taiwan and parts of Africa including the horn of Africa, West Africa and Saudi Arabia. The disease is encountered in South America. It has been estimated that 5% of HBsAg-positive carriers worldwide, approximately 15 million people, are co-infected with HDV. In areas of low prevalence those at risk of hepatitis B, particularly intravenous drug users, are also at risk of HDV infection. There are eight reported genotypes. Genotype 1 is prevalent worldwide. Genotype 2 is found in Japan, Taiwan and Russia. Genotype 3 is common in the Amazon basin with genotype 4 being found in Taiwan and Japan. Genotypes 5–8 have been detected in Africans [31].

The range of clinical presentation is wide, varying from mild disease to fulminant liver failure. The prevalence of HDV is increasing in northern and central Europe because of immigration. A test for antibody to hepatitis delta is mandatory in all HBsAg-positive persons. Specific serological tests detect antibody to HDV (anti-HD IgM and anti-HD IgG) as well as HDV RNA. Hepatitis D antigen can be detected by histochemical staining. Superinfection of HBV carriers with HDV frequently results in persistent HDV infection. Hepatitis D viraemia is followed by anti-HD IgM and subsequently IgG anti-HD. Markers of HBV replication may be suppressed during chronic hepatitis D.

Treatment of HDV is with pegylated interferon α; however, response rates are poor. Novel therapeutic targets for chronic viral hepatitis are urgently required. The mainstay of treatment of chronic HDV remains long-term pegylated interferon. Newer agents such as prenylation or HBV entry inhibitors may prove useful [32]. Patients with decompensated liver disease are candidates for transplantation. Prevention and control measures of HDV are similar to those for HBV: immunisation with hepatitis B vaccine protects against HDV infection.

HIV and HBV

HIV and HBV co-infection is problematic in resource-poor settings. HBV has little effect on the natural history of HIV infection. However, HIV, and its treatment, profoundly affects the natural history of HBV. Appropriate management of both diseases is required. In high-income countries therapy is supported by appropriate diagnostic tests. Antiretroviral therapy in high-income countries usually encompasses tenofovir, which effectively suppresses both HIV and HBV. Monitoring has been simplified. Unfortunately the prior use of lamivudine, previously a key drug in first-line HIV treatment regimens, has led to high rates of resistant hepatitis B in low-income countries. HBV resistance occurred in more than 90% of co-infected individuals after 4 years of lamivudine therapy [33].

Routine testing for HBsAg in HIV-positive individuals has not been widely applied and thus the prevalence of co-infection is not fully established in Asia or sub-Saharan Africa. A test for HBsAg in all HIV-positive individuals is justified and should be mandated. It has become apparent that in many parts of the world, persons with HIV have better access to HBV treatment than HIV-negative HBV mono-infected persons.

In some countries 6–13% of persons with HIV are co-infected with HBV [34,35]. Persons with HIV co-infection can experience a more rapid progression to cirrhosis. Persons in the inactive phase of HBV can experience reactivations in the setting of HIV co-infection with a decline in CD4 cell count [36,37]. Furthermore, when reconstitution of the immune system occurs in patients treated with antiretroviral therapy, flares of hepatitis can occur with elevation of ALT and even fulminant hepatitis if ART therapy does not adequately cover both HIV and HBV [38].

Recent longitudinal cohort studies have found that co-infection with HBV also can lead to progression of AIDS-related outcomes and death in HIV-infected persons [37,39]. The all-cause mortality has been found to be higher in co-infected individuals. The increase in mortality has followed a large decrease in AIDS-related mortality, which declined after the introduction of HAART. The highest rates of liver-related mortality have been observed in persons co-infected with HIV and HBV. Co-infected men are eight times more likely to have died from liver disease than those infected with HIV alone. Thus, comprehensive management of both diseases is required [38].

ALT elevations in co-infected patients may be the result of opportunistic infections, HAART hepatotoxicity, mitochondrial toxicity, HBV clearance, immune reconstitution, emergence of drug resistance, reactivation after withdrawal of therapy, or superinfection with HDV, HAV or HCV. Other general causes of active disease include alcohol or drugs.

HBV and HCV

A European concerted action study provided a comprehensive analysis of hepatitis B and C infections in primary liver cancer in Europe. A high prevalence of co-infection between hepatitis B and C infections has been found in patients with HCC. Persons with HBV who are also co-infected with HCV have a much higher risk of developing HCC in several cohort studies [40]. However, the role of co-infection to progression to cirrhosis is less clear since, in many instances, HCV is the dominant virus and may suppress levels of HBV DNA [41]. Persons with HCV and HBV infection may be more likely to develop an infiltrating and aggressive HCC and are younger than those with nodular HCC, perhaps suggesting accelerated hepatocarcinogenesis [42]. Although hepatitis B virus plays a predominant role in the aetiology of HCC in Africa, co-infection with both viruses may increase the risk [43,44].

Treatment of HBV

Although the disease can be prevented by vaccination, chronic hepatitis B is still a cause of considerable morbidity. Morbidity is linked to ongoing HBV replication. Thus, treatment of existing carriers forms an important part of the control of the disease. There are international, national and association guidelines [45–56] for the treatment of hepatitis B. Some controversies and discordant decisions remain.

The choice of therapy depends upon a number of factors. The major goals of therapy are to prevent disease progression to cirrhosis and to prevent end-stage liver disease or HCC. If HBV replication can be suppressed, the accompanying reduction in histological chronic hepatitis reduces the risk of cirrhosis and HCC. Extrahepatic manifestations of hepatitis B such as glomerulonephritis or polyarteritis nodosa require treatment. In general, treatment should be targeted at patients with active disease and viral replication, preferably before the signs and symptoms of cirrhosis or significant injury have occurred.

Treatment rates have improved over the past three decades with the advent of interferon α and more recently of nucleoside analogues. Interferon α has remained a benchmark therapy for chronic hepatitis B. Interferon α is a naturally occurring intracellular signalling protein that induces an antiviral state in cells, inhibits cellular proliferation and induces immunomodulation, although its mechanism of action is not fully understood. Lucifora *et al*. have recently demonstrated that interferon α can induce specific degradation of the nuclear viral DNA; they proposed that interferon α and lymphotoxin β receptor activation upregulated APOBEC3A and APOBEC3B cytidine deaminases, respectively, in HBV-infected cells, primary hepatocytes and human liver tissue specimens [57].

The main advantages of treatment with interferon α over nucleoside analogues are the absence of resistance and the possibility of immune-mediated clearance of hepatitis B. Pre-treatment factors predictive of response to interferon α have been identified. These include low virus load, high serum ALT levels and increased activity scores on liver biopsy and shorter duration of infection. A number of relative and absolute contraindications to interferon exist and these include Child's B or C cirrhosis and hypersplenism, autoimmune hepatitis, severe coronary artery disease, renal transplant disease, pregnancy, seizures, concomitant drugs, retinopathy, thrombocytopenia or leukopenia. Side-effects of interferon are common. Pegylated interferon add-on to entecavir therapy may result in greater suppression of HBsAg than entecavir alone [58]. Combinations of, or sequential therapy with, pegylated interferon and nucleoside analogues probably induce HBsAg synthesis but the effects on cure are marginal. The efficacy of interferon α is restricted: only a proportion will respond. However, sustained suppression of viral replication can be achieved and HBeAg or even HBsAg seroconversion can be attained. Thus, finite courses of interferon α can be successful.

HBV DNA and HBsAg levels can guide treatment although the positive predictive value of HBsAg concentrations at treatment week 12 is suboptimal. HBsAg concentrations of HBsAg >20,000 IU/mL at treatment week 12 have a high negative predictive value irrespective of HBV genotype and can be used to stop therapy in HBeAg-positive patients [59]. Side-effects of treatment with interferon α preclude its use in a proportion of patients and its utility in resource-poor countries is restricted. Eradication is only possible in a minority of patients.

Nucleoside analogues have similar structures to the natural nucleotides and compete at the HBV polymerase catalytic site during synthesis of HBV DNA. They prevent the formation of a covalent bond with the adjoining nucleotide causing chain termination of the elongating DNA. Although all nucleotide analogues act on HBV polymerase, their mechanism differs. Adefovir inhibits the priming of reverse transcription. Lamivudine, emtricitabine and tenofovir inhibit the synthesis of the viral minus strand DNA. Entecavir inhibits three major stages of HBV replication. Nucleosides are less effective against cccDNA formation and thus residual viral replication persists during antiviral treatment [60]. Nucleoside analogues act to inhibit HBV DNA strand synthesis via inhibition of priming or inhibition of chain elongation and are thus effective inhibitors of HBV replication but seldom result in cure, as they have little effect on cccDNA formation or maintenance and the HBV DNA minichromosome. They may have some effect on the immune response after prolonged suppression. Thus, low cure rates and loss of HBsAg are observed. The long-term effects of nucleoside analogues for hepatitis B are unknown.

New treatments

Tenofovir alafenamide fumarate (TAF) is an orally bioavailable phosphonoamidate prodrug of tenofovir. By comparison with tenofovir, TAF enables enhanced delivery of the parent nucleotide and its active diphosphate metabolite into lymphoid cells and hepatocytes. The enhanced delivery is attributed to an improved plasma stability and differential intracellular activation mechanism for TAF relative to tenofovir. TAF is in phase 3 trial and may offer effective and safer treatment for HIV and HBV, and offer economic advantages, but is unlikely to enhance rates of cure [61–63]. Other agents in the pipeline include besifovir [64]. Newer pro-drugs are in development that may bypass the non-productive first phosphorylation step. It is proving possible to deliver potent antiviral drugs encapsulated within biodegradable organic nanoparticles. Early compounds have exhibited a significantly prolonged blood circulation time, decreased plasma elimination rate, and enhanced area under the curve.

Higher rates of cure are being sought by new treatment approaches. Cure of hepatitis B will require several steps for eradication or functional cure in the host. Some of the problems that prevent cure are the maintenance of capsid RT–cccDNA interaction and assembly, and maintenance of HBV replication during nucleoside analogue therapy. The S protein may play a role in tolerance resulting in a poor antibody response. A dysfunctional T cell response and T cell exhaustion also permit chronic infection. Thus, a cure might require prolonged suppression of HBV replication, degradation or silencing of cccDNA and restoration of the innate and adaptive immune response.

New molecules under investigation include entry inhibitors and short interfering RNA (siRNAs), or capsid inhibitors [65]. The sodium taurocholate co-transporting polypeptide has been identified as the HBV (and HDV) receptor [13]. A synthetic acylated pre-S peptide derived from the large protein of HBV that blocks the entry of HBV in susceptible cells (Myrcludex B) is being studied in both chronic HBV and HDV infection [66]. A formulation for subcutaneous application has been developed and initial, promising phase 1 studies with nucleosides and interferon are in progress.

Cai and co-workers have identified two structurally related disubstituted sulphonamides (DSS), CCC-0975 and CCC-0346, which act as inhibitors of cccDNA production [67–69]. Epigenetic regulation may also limit transcription from cccDNA. Current models of chromatin indicate that transcription from cccDNA

could be reduced by post-translational modification of histones; and acetylation, phosphorylation, methylation and ubiquitylation reactions could be targeted. Two groups of enzymes, histone deacetylases (HDACs) and histone acetyltransferases (HATs), determine the acetylation status of histones; relaxed chromatin is associated with activation of gene expression whereas compacted chromatin is associated with repression of gene expression. The acetylation status of the HBV minichromosome (cccDNA-bound H3 and H4 histones) regulates HBV transcription replication and is reflected in viral load. It may also be possible to mediate degradation of cccDNA in infected hepatocytes. Lucifora *et al*. have proposed that lymphotoxin β receptor activation upregulated APOBEC3A and APOBEC3B cytidine deaminases [57].

It is not clear whether all cccDNA chromatin would need to be cleared for a cure of hepatitis B or whether low threshold levels would result in slowing of the disease (as may be the case in inactive carriers). Can 'epigenetic' and immunological control achieve the same effect? Unfortunately there are no proven surrogates for cccDNA that can be studied in early phase experiments, and trials in patients may require liver tissue and validated tools to quantify cccDNA. HBsAg quantitation may serve this purpose but phase 1 dose-finding studies would probably require quantitation of cccDNA with liver tissue.

Methylation of HBV DNA may influence high and low replication phenotypes of HBV. Recent studies suggested that cccDNA contains methylation-prone CpG islands, and that the minichromosome structure of cccDNA is epigenetically regulated by DNA [67]. Epigenetic silencing of cccDNA transcriptional activity could prove an antiviral strategy in cccDNA eradication or silencing [70–73].

The HBV nucleocapsid may be a crucial target because of the interaction between the HBV capsid and cccDNA. HBV core protein mediated the interaction with nuclear cccDNA, an interaction that resulted in cytidine deamination, and cccDNA degradation; thus lymphotoxin receptor activation could prove a therapeutic target.

Mutational inactivation is another avenue being explored. Mobile genetic elements in bacteria are neutralised by clustered regularly interspaced short palindromic repeats (CRISPRs) and CRISPR-associated (Cas) proteins. The CRISPR/Cas9 system is potentially a powerful tool for site-specific cleavage of HBV DNA targets, after direction by a synthetic guide RNA (gRNA) base-paired to the target HBV DNA sequence. Lin *et al*. and Schiffer *et al*. have examined whether these molecules could cleave HBV genomes, by designing gRNAs against HBV [74,75]. The CRISPR/Cas9 system reduced the production of HBV core and surface proteins in Huh-7 transfected cells, suggesting that the CRISPR/Cas9 system could disrupt HBV *in vivo*.

Nucleic acid technologies

Several new antisense, siRNA targeting technologies are being examined. These include targeting with ARC 520, which is in phase 2 trial. ARC 520 is an RNA interference (RNAi)-based, liver-targeted antiviral molecule. The compound comprises an equimolar combination of two cholesterol-conjugated siRNA molecules (AD0009 and AD0010) that target HBV RNA transcripts with the aim of triggering a sequence-specific downmodulation of gene expression, reducing viral load and viral proteins [76–79].

HBV replication depends upon the assembly of the core particle composed of the capsid protein polymerase and pre-genomic RNA. A number of new compounds could inhibit or dysregulate encapsidation; capsid disassembly, impeding the entry of RC DNA into the nucleus, could inhibit conversion of rcDNA to cccDNA. Pre-packaging inhibitors of packaging with compounds active against the core are in development, for example BAY41-4109 a heteroaryldihydropyrimidine compound. Other compounds include phenylpropenamide derivatives. Control of HBsAg secretion could potentially reduce tolerance owing to high HBsAg levels, and several compounds are being investigated.

Regulation of immunity

Restoration of adaptive immunity may be difficult. Chronic hepatitis B is associated with functional exhaustion of HBV-specific CD8 T cells, the result of prolonged exposure to large quantities of HBsAg and HBeAg. The antigen-specific cells express inhibitory molecules such as PD-1, and thus lose their effector function. Blocking these immune regulatory receptors, which may be driving T cell dysfunction, could restore functional T cell activity to exhausted T (and B) cells. In a woodchuck study, blockade of the PD-1 pathway with woodchuck PD-L1 antibody, therapeutic DNA vaccination and treatment with entecavir enhanced virus-specific T cell immunity and led to the resolution of chronic infection in some woodchucks.

Experimental toll-like receptor agonists suggest that HBV replication can be controlled by the activation of innate immune responses in the liver. The effects of immune activation with GS-9620, a selective orally active small molecule agonist of toll-like receptor 7 in chimpanzees with chronic HBV infection, has been investigated. GS-9620 administered to chimpanzees thrice weekly for 4 weeks resulted in a 2-log reduction in HBV DNA, but levels of HBsAg in serum were not altered. The molecule also induced production of interferon α and other cytokines. These pharmacodynamic effects are being further studied in humans [80].

Other strategies

Other new therapeutic strategies and novel immunological therapies include the possible application of therapeutic vaccines to boost HBV-specific T cell responses or offset the dysfunctional immune response and restore an intrahepatic innate immune response. A tarmogen, GS4774, is a genetically modified yeast expressing HBV antigens to activate T cells. Other therapeutic vaccines including adenovirus fusion proteins are in human trials.

A full review of potential curative strategies is beyond the scope of this article. However, cure of hepatitis B is the next goal of therapy of hepatitis B. Cure could be considered if patients are HBsAg negative and have undetectable HBV DNA in blood and in liver, have no relaxed circular DNA, no detectable (or functionally silent) cccDNA and are HBcAg negative.

A number of promising lines of development are in progress. A curative regimen may require a combination of viral suppression via nucleoside analogue therapy to prevent cccDNA amplification and viral propagation; safe selective cccDNA inhibitors to deplete, silence or degrade cccDNA; immune activation to activate an immune response or restore an exhausted T cell repertoire; and agents to block the entry of HBV into the hepatocyte, cell spread, or compounds to prevent capsid assembly and cccDNA interactions.

Conclusions

Universal vaccination has fortunately limited the transmission of hepatitis B and limited the incidence of chronic infections in previously high-prevalence regions. In low-income countries diagnostic testing has lagged behind, and therapy has been

limited. Treatment will need to form part of the control of the disease. HBV DNA suppression is effective in preventing progression to cirrhosis and to hepatic decompensation and can reverse advanced fibrosis if present. Treatment may (to a degree) reduce the incidence of hepatocellular carcinoma. However HBsAg loss is infrequent, and the prospect of lifelong maintenance suppressive therapy deters policymakers. Quantitative and diagnostic testing for HBV DNA is not generally available, hampering effective monitoring and treatment. Without coordinated action, and transfer of new diagnostic technologies and treatments to low-income countries, recent therapeutic advances will have little effect on the global burden of disease. A shift to curative treatment for the majority would be a major advance in the elimination of hepatitis B. The relationship between intrahepatic cccDNA and viral replication, and immunological control during chronic hepatitis B is being actively studied; new cell lines that support the entire life cycle of hepatitis B virus are being developed and will provide powerful tools for study. Broadly curative antiviral strategies are the next goal for the worldwide management of hepatitis B.

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