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Therapeutic Direction and Issues Regarding HBV Infection

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ABSTRACT

With up to 400 million affected people worldwide, chronic hepatitis B virus (HBV) infection is still a major health care problem. During the last decade, several novel therapeutic approaches have been developed and evaluated. In most regions of the world, interferon-α (IFN-α), and nucleos(t)ide analogues are currently approved. Despite major improvements, none of the existing therapies is optimal since viral clearance is rarely achieved. HBV establishes a stable nuclear covalently closed circular DNA (cccDNA). Interferon-α treatment can clear HBV but is limited by systemic side effects. Up-regulation of APOBEC3A and APOBEC3B enzymes by use of IFN-α or lymphotoxin-β (LT βR) was found to result in cytidine deamination, apurinic/apyrimidinic site formation, and finally cccDNA degradation that prevented HBV reactivation, while genomic DNA was found to remain intact. As such, development of new therapeutics in combination with existing antivirals, may cure hepatitis B. With respect to the selectivity observation on the activation of LTβR, however, more studies are necessary on the potential utility of LTβR agonists for clearance of cccDNA in chronic hepatitis B (CHB). HBV is a DNA virus that can integrate DNA into host genome thereby increases the yield of trans-activator protein HBxAg that may deregulate many pathways involving in metabolism of cells causing Hepatocellular Carcinoma (HCC) development. This review aimed at therapeutic direction and issues regarding HBV infection.

Keywords: Hepatities B, Interferon, lymphotoxin-β, Antiviral, Carcinoma, cccDNA

INTRODUCTION

Chronic hepatitis B (CH-B) is characterized by inflammatory liver disease of variable severity driven by persistent replication of the hepatitis B virus (HBV) [1]. In the HBV life cycle, the DNA containing nucleocapsids fulfill two functions. First, they can be either re-imported into the nucleus to form additional cccDNA or second, they can be enveloped for secretion *via* the endoplasmic-reticulum (ER). After budding into the ER lumen, the envelope proteins are secreted by the cell either as small, non-infectious subviral spherical or filamentous particles (SVPs) of 22 nm diameter or as infectious virions of 42 nm (Dane particles). Usually, the non-infectious SVPs are produced in a 1,000 to 1,000,000-fold excess over virions [1]. The development of a safe and effective hepatitis B surface antigen recombinant vaccine was an important milestone towards achieving control of CH-B, and its widespread implementation has dramatically reduced the incidence of infection [2]. However, for those chronically infected with HBV, antiviral chemotherapy represents the best prospect of controlling active replication and thereby preventing life-threatening hepatic disease [3].

Existing therapies either approved or in clinical trial, still have the disadvantage of low response rates and selection of resistance [4]. The complex interplay between the HBV-infected hepatocyte and the host immune response greatly influences the clinical course of disease and, consequently, strategies for clinical management [5]. As morbidity and mortality in CH-B are linked to the development of cirrhosis and hepatocellular carcinoma, the goals of antiviral therapy are to induce disease remission, to arrest disease progression to cirrhosis, and to block the liver failure and/or hepatocellular carcinoma [6].

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More than 2000 million people alive today have been infected with HBV at some time in their lives (Fig. 1). It is estimated that there are more than 300 million carriers of the hepatitis B virus in the world, with over 500,000 dying annually from hepatitis B-related liver disease [7]. Most people with acute hepatitis B may recover [8]. However, in about 5 percent of adults the virus stays in the liver and it continues to make copies of itself for many years. People who continue to harbor the virus are referred to as carriers [8]. If liver damage develops because of longstanding infection, the person is said to have chronic hepatitis [8]. Chronic hepatitis B develops more commonly in people who are infected with the virus at an early age. Many people with chronic hepatitis B have no symptoms at all; other people have symptoms of ongoing liver inflammation, such as fatigue and loss of appetite.

In some parts of the world, such as in Southeast Asia, China, and sub-Saharan Africa, as many as 1 in 10 people have chronic hepatitis B infection [9]. Specific treatment for acute hepatitis B is usually not needed since in about 95 percent of adults, the immune system controls the infection and gets rid of the virus within about six months [10]. In people who develop chronic hepatitis, an antiviral medication might be recommended to reduce or reverse liver damage and to prevent long-term complications of hepatitis B [11].

Current antiviral agents can control but not eliminate hepatitis B virus (HBV), because HBV establishes a stable nuclear covalently closed circular DNA (cccDNA) [12,13]. Nucleoside or nucleotide analogs are efficient antiviral agents but only control and do not cure HBV infection owing to the persistence of HBV cccDNA. Therefore, long-term treatment is required, which is expensive and may lead to concomitant resistance [14]. Interferon-α treatment can clear HBV but is limited by systemic side effects [15]. Interferon-α can induce specific degradation of the nuclear viral DNA without hepatotoxicity and proposed lymphotoxin-β receptor activation as a therapeutic alternative [13].

Interferon-α and lymphotoxin-β receptor activation up-regulated APOBEC3A and APOBEC3B [16] cytidine deaminases, respectively, in HBV-infected cells. HBV core protein mediated the interaction with nuclear cccDNA, resulting in cytidine deamination, apurinic/apyrimidinic site formation, and finally cccDNA degradation that prevented HBV reactivation. Genomic DNA was not affected. Thus, inducing nuclear deaminases for example, by lymphotoxin-β receptor activation allows the development of new therapeutics that, in combination with existing antiviral compounds, may cure hepatitis B. In addition, cccDNA degradation is possible and can be induced without side effects on the infected host cell. Therefore, efficient

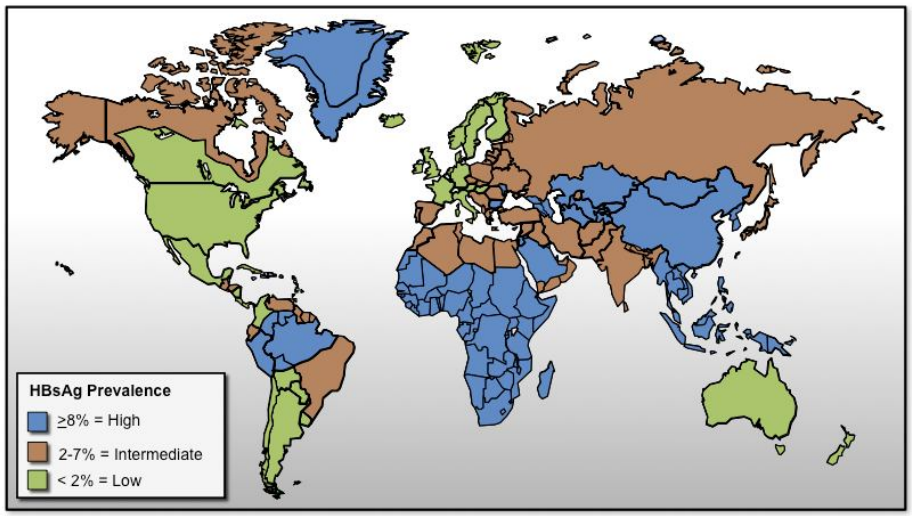


Fig. 1. Global prevalence of chronic hepatitis B virus infection.

and nontoxic elimination of cccDNA in hepatocytes is a major goal of HBV research. It has been shown that HBV replication in particular, the cccDNA content of the liver can be affected by noncytopathic mechanisms involving cytokines such as interferons and tumor necrosis factor (TNF), which influence RNA and capsid stability [16-19]. Interestingly, recently, an antiviral mechanism that interferes with cccDNA stability was described, which found to be distinct from influences of antiviral cytokines on cccDNA activity [13,20].

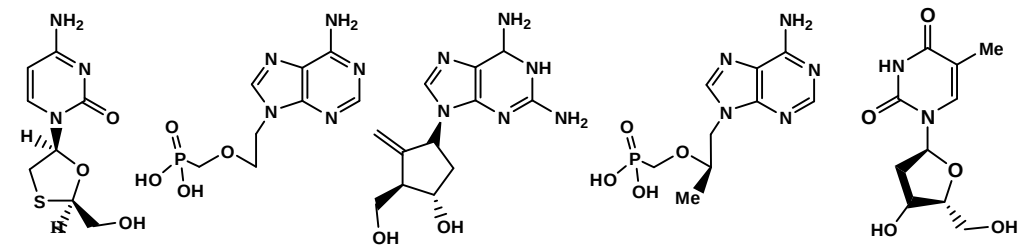
Herein, we will review the important facts and findings in combating HBV infections.

VIRAL THERAPY AGAINST HBV

While viral therapies against DNA viruses including herpes viruses (HSV) are quite successful; yet chemotherapy against hepatitis virus (HBV) is quite challenging [21]. In an effort to eradicate hepatitis B transmission, governments must adopt proactive strategy including HBsAg screening of pregnant women; then to potentially infectious mothers immunoprophylaxis should be given to infants born to prevent the infection. In addition, hepatitis B vaccination should be integrated into current childhood immunization schedules in high-risk populations. This practice provides immunity to teens and adults before they become at risk for hepatitis B infection.

However, the primary treatment goals for patients with hepatitis B (HBV) infection are to prevent progression of the disease, particularly to cirrhosis, liver failure, and hepatocellular carcinoma (HCC) [21]. Risk factors for progression of chronic HBV include the persistently elevated levels of HBV DNA and, in some patients, alanine aminotransferase (ALT), as well as the presence of core and pre-core mutations [21]. A synergistic approach of suppressing viral load and boosting the patients' immune response with immunotherapeutic interventions is needed for the best prognosis [22]. Therapy is currently recommended for patients with evidence of chronic active hepatitis B disease. Various algorithms have been proposed, such as that by the American Association for the Study of Liver Diseases (AASLD) [23], the European Association for the Study of the Liver (EASL) [24], the Asian Pacific Association for the Study of the Liver (APASL) [25], the Canadian Association for the Study of the Liver (CASL) [26] and the National Institute for Health and Clinical Excellence (NICE) [27].

Never the less, hitherto described antiviral therapies include the use of Lamivudine (Zeffix, Epivir-HBV®) (100 mg/day by mouth) [28], which is effective in decreasing hepatitis B virus activity and ongoing liver inflammation. It is safe in patients with liver failure and long-term treatment can decrease the risk of liver failure and liver cancer. The major problem with lamivudine is that a resistant form of hepatitis B virus (referred to as an YMDD mutant) frequently develops after the long term use. Adefovir (Hepsera®), (10 mg/day by mouth) [29], is an alternative choice for people who have detectable hepatitis B virus activity with liver inflammation. An advantage of adefovir compared to lamivudine is that resistance to adefovir is less likely to develop. In addition, adefovir can suppress lamivudine-resistant HBV. Entecavir (Baraclude®), (0.5 to 1.0 mg/day by mouth) [30], is generally more potent than lamivudine and adefovir. Resistance to entecavir is uncommon in people who have never been treated with antivirals, but occurs in up to 50 percent of people who have used lamivudine. Tenofovir (Viread®), (300 mg/day by mouth) [31], is more potent than adefovir. Resistance to tenofovir is rare. Tenofovir is effective in suppressing hepatitis B virus that is resistant to lamivudine, telbivudine, or entecavir. Tenofovir is not as effective in patients with adefovir-resistant hepatitis B. Telbivudine (Sebivo. Tyzeka®) (600 mg/day by mouth) [32] with more potency relative to lamivudine and adefovir. Resistance to telbivudine is common, and hepatitis B virus that is resistant to lamivudine is also resistant to telbivudine.



Lamivudine **Adefovir** **Entecavir** **Tenofovir** **Telbivudine**

On the other hand, interferon-α is also an appropriate treatment for people with chronic hepatitis B infection who have detectable virus activity and ongoing liver inflammation [33]. Both conventional interferon and pegylated interferon are approved by US FDA [34]. However, the disadvantages of interferon- α are that it must be taken by injection and it can cause many side effects [33].

In successful antiviral therapy of hepatitis B drugs in combinations without cross-resistance, can delay or prevent the emergence of drug-resistant mutants [35]. Combining drugs may achieve synergistic or additive antiviral effects compared with single drug therapy [36,37]. Potentially harmful effects of combination therapy include higher rates of side effects, reduced efficacy due to drug competition and the risk of multidrug-resistant hepatitis B virus (HBV) if therapy is insufficient to prevent resistance. Combination therapy has been shown to reduce the rate of drug resistance in chronic hepatitis B, but only when drugs with a low barrier to resistance are used (lamivudine & adefovir). Combination therapies may achieve greater degrees of HBV DNA suppression, but this has not been associated with higher rates of sero-conversion (hepatitis Be antigen or hepatitis B surface antigen) compared to single drug therapy [38]. The benefit of combination therapy has yet to be demonstrated with agents that are associated with a high barrier to resistance (tenofovir & entecavir). The use of combination therapy is recommended in specific patient groups: those with decompensate cirrhosis, those co-infected with human immunodeficiency virus, those who are on antiretroviral therapy or have undergone liver transplantation, and those with drug-resistant HBV infection. There is insufficient evidence to recommend combination therapy as first-line therapy for all patients with chronic hepatitis B.

In pregnant women with HBV who require treatment for their own health, therapy selection should be based on antiviral efficacy, risk of resistance, human safety data, and pregnancy class of the drug. In women with HBV who become pregnant while on therapy, consider whether to continue or stop treatment on an individual basis. Decision making should be based on pregnancy stage, severity of liver disease, the risk adversity of the mother to medications during pregnancy, and the risk of flares when stopping medications [39].

It should be noted that liver transplantation, however, may be the only option for people who have developed advanced cirrhosis [40]. The liver transplantation process involves an extensive screening process to ensure that a person is a good candidate.

According to the National Viral Hepatitis Roundtable (NVHR) [41], the proposed 2011 annual budget for CDC's Division of Viral Hepatitis is only $21 million, just two percent of the overall budget for the National Center for HIV/AIDS, viral hepatitis, sexually transmitted diseases, and tuberculosis prevention (NCHHSTP). Shockingly, this is less than the Division's budget of $25 million from ten years ago.

According to the law, the insurance industries discriminatory practice of withholding coverage from people with pre-existing conditions will be banned by 2014 in USA. This means that people with chronic HBV can finally gain access to what will hopefully be affordable healthcare. However, implementation of the new legislation is expected to be an uphill battle, and will take a few years [41].

An institute of medicine report highlighted the lack of provider awareness about HBV contributes to the problem. According to one survey, 44 percent of primary care providers did not know HBV can be controlled with treatment [41].

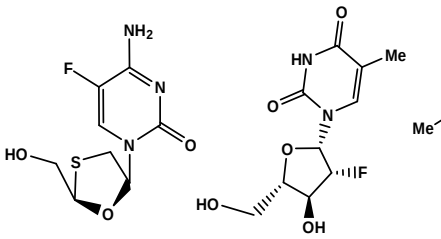
ANTI-HBV EXPERIMENTAL AGENTS IN THE PIPELINE

Although antiviral drugs can control HBV, drug resistance may develop over time, leaving people with no treatment options. This is of particular concern as resistance can develop faster for people co-infected with HIV and HBV. Long-term toxicity is likely to be an issue. These concerns will not be resolved until there is a robust drug pipeline. Advances in basic science are needed to better understand HBV pathogenesis, identify new drug targets and to stop HBVs destructive track by finding drugs with better potency and higher resistance barriers. Table 1 illustrates the anti-HBV experimental compounds in the pipeline.

Table 1. Oral anti-HBV Experimental Agents in the Pipeline

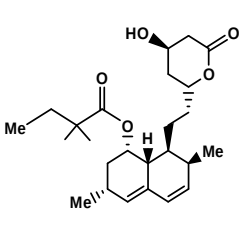
Agent	Manufacturer	Stage of development	Class
Emtricitabine/ Tenofovir (Truvada) [42]	Gilead	Phase III/IV	NRTI*
Clevudine [43]	Bukwang/Eisai	Phase III	NRTI
LB80380 [44]	LG Life Sciences	Phase IIb	NRTI
MIV-210 (Lagociclovir valactate) [45]	Medivir/ Daewoong	Phase II	NRTI
Simvastatin [46]	University of Oklahoma Health Sciences Center/VA Medical Center	Phase I	3-Hydroxyl-3-methyl-glutaryl coenzyme A (HMG CoA) reductase inhibitor
Bay 41-4109 [47]	AiCuris	Pre-clinical	Heteroaryldihydro-pyrimidine

*Nucleoside reverse transcriptase inhibitor (NRTI).

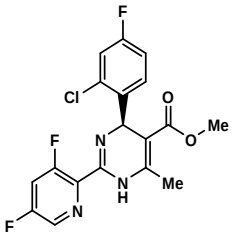


Emtricitabine

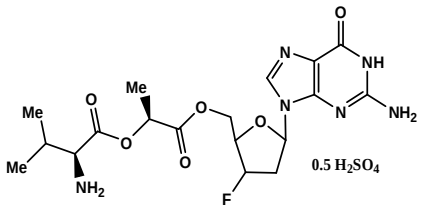
Clevudine



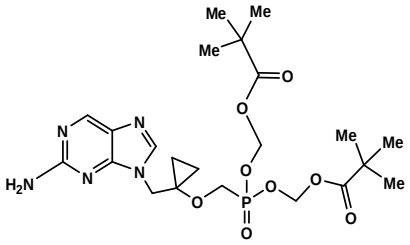
Simvastatin



Bay 41-4109



MIV-210



LB80380 (Prodrug)

IMMUNE BASED THERAPIES AGAINST HBV

Immunotherapeutic approaches primarily designed to control viral replication through the boosting of antiviral immunity or that aim to inhibit the liver inflammatory processes linked with cirrhosis and HCC development [48]. Although, there are no investigational new drugs in late stage of development for HBV; yet some hope is coming from immune-based therapies. Never the less, the treatment of chronic hepatitis B virus (HBV) infection has greatly improved over the last 10 years, but alternative treatments are still needed. Therapeutic vaccination is a promising new

strategy for controlling chronic infection. However, this approach has not been as successful as initially anticipated for chronic hepatitis B. General impairment of the immune responses generated during persistent HBV infection, with exhausted T cells not responding correctly to therapeutic vaccination, is probably responsible for the poor clinical responses observed to date. Intensive research efforts are now focusing on increasing the efficacy of therapeutic vaccination without causing liver disease. In order to overcome the inhibitory mechanisms impairing immune responses, new approaches to use therapeutic vaccination (innovative strategies for generating functional immune responses) described [48]. Figure 2 illustrates a general Scheme for antiviral immune responses.

Hepatitis B immunoglobulin (HBIG) is also an example among antibodies that are able to attach to the hepatitis B viruses and cause them to be destroyed. When have not been immunized against HBV, an injection of HBIG may help to prevent HBV infection, if it is given within 14 days of patients exposure to the virus of someone who has hepatitis B (*i.e.*, contact with the blood, semen or vaginal fluids including menstrual blood) [48]. HBIG is also useful for the one being immunized against HBV but have not yet received all three shots in the vaccination series; yet was exposed to the virus. In most cases, HBIG will prevent infection until the vaccine takes effect.

The current immunotherapeutic strategies designed to suppress and control HBV replication have strong scientific support. However, they are restricted by our limited knowledge, and further understanding of the relationship with the virus in the unique environment of the human liver will almost certainly provide opportunities to enhance therapeutic agents or perhaps develop totally novel approaches.

The expression of viral antigens or danger molecules on the surface of infected cells also activates these cells. NK cell can also be indirectly activated by DC- or macrophage-derived cytokines. Activated, NK cells can kill infected cells and secrete IFN-g and TNF-α which can exert direct anti-viral effects, or IL-2 which can promote T cell proliferation. The activated DC can present antigens to $CD4^+$ and $CD8^+$ T cells and also produces IFN-g, TNF-α and IL-2 which provide signals that stimulate expansion and subsequent cytotoxic T cell responses. The production of

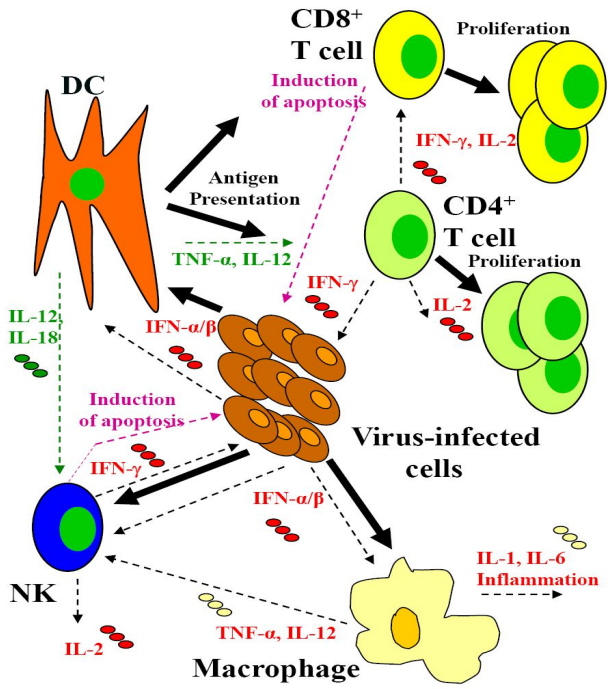


Fig. 2. General Scheme for Anti-viral Immune Response: Virus-infected cells release IFN-α/β which activate DC, macrophages and NK cells.

IFN-g and IL-2 by CD4+ T cells can sustain and amplify the anti-viral immune responses. Macrophages can also produce IL-1 and IL-6 to initiate inflammation.

Immune therapy, however, are in the early stage of development (Table 2). Complete list of agents in development for chronic hepatitis B, updated on September 2013 (Hepatitis B Foundation Drug Watch) [49]. Those include interferon [49], non interferon immune enhancers, nucleosides and nucleotides, non-nucleosides and post-exposure and/or post-liver transplant treatment [49].

Interferon-α (IFN-α) also found to inhibit viral replication in vitro and *in vivo*. Pegylated IFN-α is a commonly administered treatment for individuals infected with HBV [59]. The HBV genome contains a typical IFN-stimulated response element (ISRE), but the molecular mechanisms by which IFN-α suppresses HBV replication has not been established in relevant experimental systems. It was shown that IFN-α inhibits HBV replication by decreasing the transcription of pregenomic RNA (pgRNA)

Table 2. Immune-based Therapy against HBV in the Pipeline

Agent	Manufacturer	Stage of Development	Class
Thymosin alpha (zadaxin) [50]	SciClone Pharmaceuticals	Phase IV	Immunomodulator
Interferon gamma 1b (Actimmune) [51]	InterMune	Phase II	Immunomodulator
CYT107 (recombinant human interleukin-7) [52]	Cytheris	Phase I/IIa	Immunomodulator
DNA vaccine pCMVS2.S [53]	ANRS (French Agency for Research on AIDS and Viral Hepatitis)	Phase I/II	Therapeutic vaccine
DNA vaccine (HB-110) [54]	Genexine	Phase I	Therapeutic vaccine
Hepatitis B vaccine (Synthesized peptide PA-44) [55]	Chongqing Jiachen Biotechnology	Phase I	Therapeutic vaccine
HBV DNA plasmid pdpSC18 vaccine [56]	PowderMed/Pfizer	Phase I	Therapeutic vaccine
DV-601 [57]	Dynavax	Phase I	Therapeutic vaccine
Heplisav [58]	Dynavax	Phase III	Preventive vaccine

and sub-genomic RNA from the HBV covalently closed circular DNA (cccDNA) [60].

In contrast to nucleoside analogues, interferon has direct immunomodulatory properties (Fig. 3). Interferon enhances human leukocyte antigen (HLA) class I antigen expression on the surface of infected hepatocytes and augments $CD8^+$ cytotoxic T-lymphocyte activity. This could be operatively important in reducing the amount of HBV DNA (the genomic template for viral transcription) during the second phase of viral clearance. More sustained reduction of HBV DNA, perhaps even clearance of cccDNA, may explain the loss of HBsAg that occurs in approximately 5 to 8% of interferon-treated patients.

The inhibitory activity of IFN-α was found to be linked to the IFN-stimulated response element (IRSE), as IRSE-mutant HBV transcribed less pgRNA and could not be repressed by IFN-α treatment.

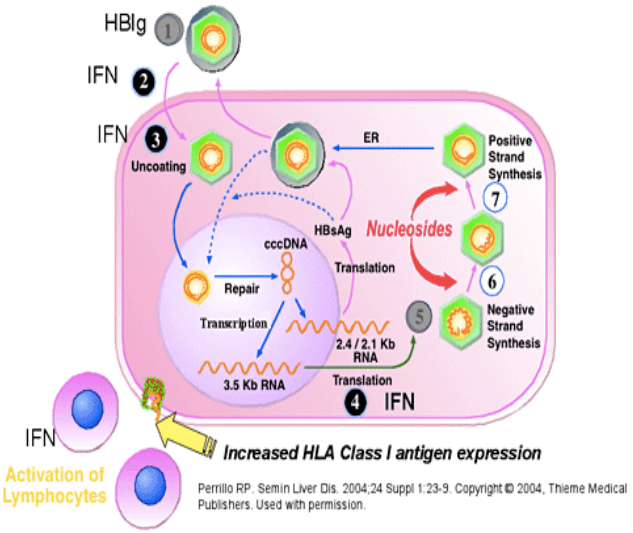


Fig. 3. Schematic of interferon's proposed mechanisms of action. Interferon action on suppression of viral replication involves mechanisms that are complementary to those of nucleoside analogues.

PATHOGENESIS OF HEPATITIS B VIRUS INFECTION

Unlike that of retroviruses, integration of viral genomic DNA into host cellular chromosomes is not an obligatory step in the HBV life cycle [61,62]. Activation of TLRs in NPCs induces the production of pro-inflammatory cytokines and chemokines (type I IFNs) [63]. Then type I IFNs bind

to their receptors on hepatocytes to trigger JAK-STAT signaling pathway and induce the expression of ISGs, which limit HBV replication *via* inhibition of cccDNA transcription and encapsidation of HBV pre-genomic RNA [63].

Generally, the antibody response contributes to the clearance of circulating virus particles and prevention of viral spread within the host; yet the cellular immune response eliminates infected cells [63]. The T cell response to the hepatitis B virus (HBV) is vigorous in acutely infected patients who successfully clear the virus [64]. This action is relatively weak in chronically infected patients. Thus, the clearance of HBV is T cell dependent. The pathogenetic and antiviral potential of the cytotoxic T lymphocyte (CTL) response to HBV has been proven by the induction of a severe inflammatory liver disease following the adoptive transfer of HBsAg specific CTL into HBV transgenic mice [65]. The CTLs also purge HBV replicative intermediates from the liver by secreting type 1 inflammatory cytokines thereby limiting virus spread to uninfected cells and reducing the degree of immunopathology required to terminate the infection [66]. Persistent HBV infection is characterized by a weak adaptive immune response due to inefficient $CD4^+$ T cell priming early in the infection and subsequent development of an ineffective $CD8^+$ T cell response [63,67]. These are expected to result in chronic liver cell injury, regeneration, inflammation, widespread DNA damage and insertion deregulation of cellular growth control genes, which could lead to cirrhosis of the liver and hepatocellular carcinoma [68].

The pathogenesis of chronic hepatitis B is characterized by a dynamic equilibrium between viral production and clearance [4]. The introduction of antiviral therapy can upset this equilibrium by inhibiting virus production and causing a decline in the viral load. Indeed, the rate of decline in the viral load is a measure of the rate of viral clearance and by inference, must be equivalent to the rate of virus production before therapy. The various models proposed to describe the fall in serum HBV differ principally in their underlying assumptions regarding at least three factors: (1) the nature and efficacy of the inhibition of viral replication that is imposed on the virus-host system by nucleoside analogue therapy, (2) the behavior of the infected cell population after the commencement of therapy, and (3) ultimately, the residual effect of this population on the level of viremia [69].

VIRAL LIFE CYCLE: WHERE ARE THE NEW TARGETS

The life cycle of hepatitis B virus is complex (Fig. 4). Hepatitis B is one of a few known *para*-retroviruses: non-retroviruses that still use reverse transcription in their replication process. The virus enters the cell by binding to Na^+taurocholate cotransporting polypeptide (NTCP) on the surface by endocytosis mechanism [70]. Because the virus multiplies *via* RNA made by a host enzyme, the viral genomic DNA has to be transferred to the cell nucleus by host proteins called chaperones [71].

The partially double stranded viral DNA is then made fully double stranded and transformed into covalently closed circular DNA (cccDNA) that serves as a template for transcription of four viral mRNAs [72] (Fig. 5).

The largest mRNA, (which is longer than the viral genome), is used to make the new copies of the genome and to make the capsid core protein and the viral DNA polymerase [73]. These four viral transcripts undergo additional processing and go on to form progeny virions that are released from the cell or returned to the nucleus and re-cycled to produce even more copies [74]. The long mRNA is then transported back to the cytoplasm where the virion P protein (the DNA polymerase) synthesizes DNA *via* its reverse transcriptase activity [74] (Fig. 6).

The virus is divided into four major serotypes (adr, adw, ayr, ayw) based on antigenic epitopes presented on its envelope proteins, and into eight genotypes (A-H) according to overall nucleotide sequence variation of the genome [75,76]. The genotypes have a distinct geographical distribution and are used in tracing the evolution and transmission of the virus [9,77].

The review of the HBV life-cycle reveals that, apart from reverse transcription, most viral processes are dependent on host-cell machinery [78]. The most important of these are the generation and persistence of cccDNA [79]. It was found that cccDNA persists throughout the natural history of chronic hepatitis B, even in patients with serologic evidence of viral clearance [79]. Long-term

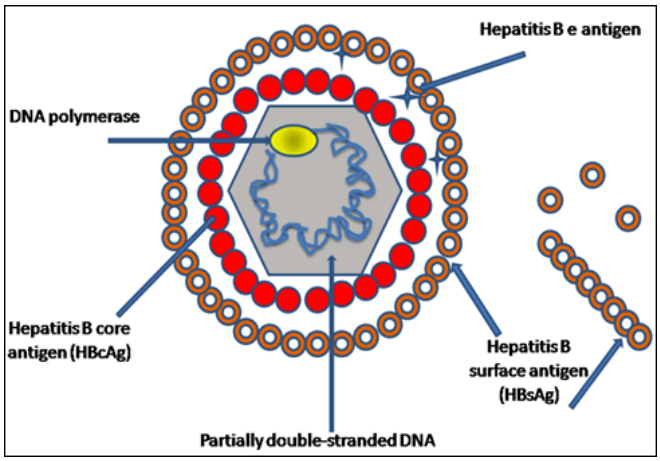


Fig. 4. Hepatitis B Virus and its Components.

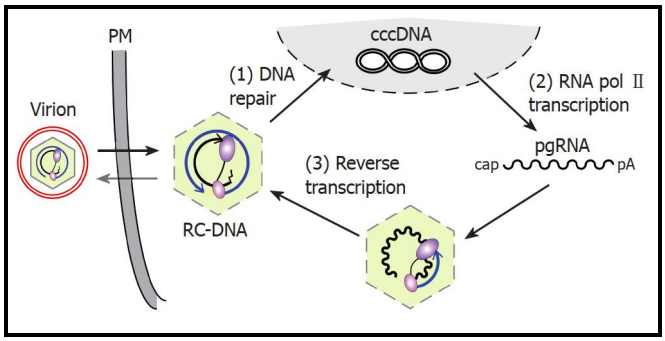


Fig. 5. Covalently closed circular DNA (cccDNA).

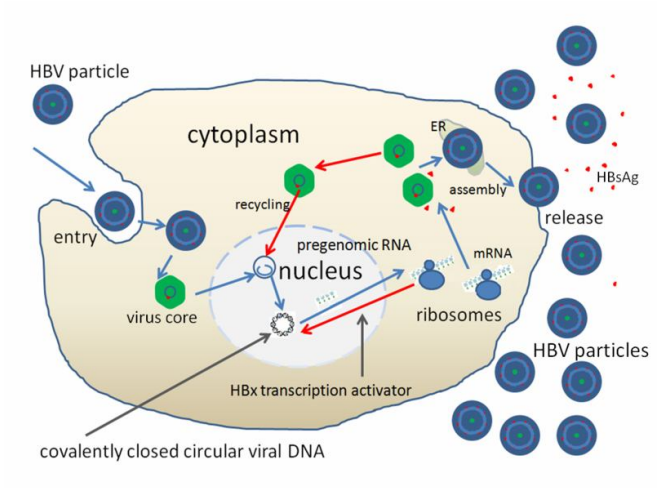


Fig. 6. Hepatitis B virus replication.

adefovir dipivoxil (ADV) therapy significantly decreased cccDNA levels by a primarily noncytolytic mechanism [80]. However, long term monotherapy may result in a limited virological response [81]. Thus, combination strategies including potent antiviral agents should be recommended for patients with resistant mutations.

Conventional antiviral inhibitors of viral DNA synthesis such as nucleoside/nucleotide analogues can prevent or reduce the development of new molecules of cccDNA [82]. However, successful elimination of the existing pool of hepadnaviral cccDNA has only been achieved by either a non-cytolytic Th1 immune response or immune-mediated cell killing followed by hepatocyte cell division [83]. In this context, it is important to note that treatment of CH-B with nucleoside analogues may result in the (partial) restoration of (specific) immune-responsiveness, which appears necessary for durable host-mediated control of infection [84]. Collectively, the concept of successful therapy for CH-B is converging on the use of both antiviral and immune-modulating approaches [85].

New data presented at the International Liver Congress™ 2013 on targeting covalently closed circular DNA (cccDNA) [86]. In chronic hepatitis B infection, the viral genome forms a stable mini-chromosome - the covalently closed circular DNA (cccDNA) - which can persist throughout the lifespan of the hepatocyte. Current treatments focus on suppression of HBV, and discovery of compounds directly targeting cccDNA has been one of the major challenges to curing HBV infection [86]. Recently, based on the mechanism of APOBEC-dependent degradation of HBV cccDNA (Fig. 7), a new strategy targeting cccDNA was suggested [13].

APOBECs can deaminate cccDNA that is transiently rendered single-stranded during transcription. Uracils, resulted from deamination of cytosine, in HBV cccDNA are recognized and excised by cellular DNA glycosylases, leading to the formation of AP sites, which are then recognized by cellular AP endonucleases [88], and leading in turn to cccDNA digestion (Fig. 8).

AP site (apurinic/apyrimidinic site), also known as an abasic site, is a location in DNA that has neither a purine nor a pyrimidine base, either spontaneously or due to DNA damage [88]. As shown in Fig. 9, DNA ligase is a specific type of enzyme that facilitates the joining of DNA strands

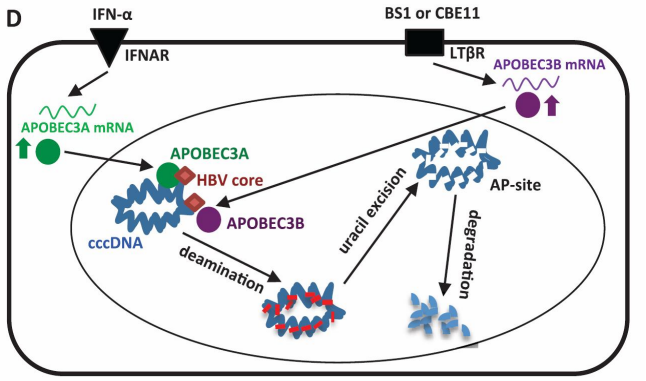


Fig. 7. Model of cccDNA degradation induced by IFN-α [33,34] treatment or LTβR activation [87].

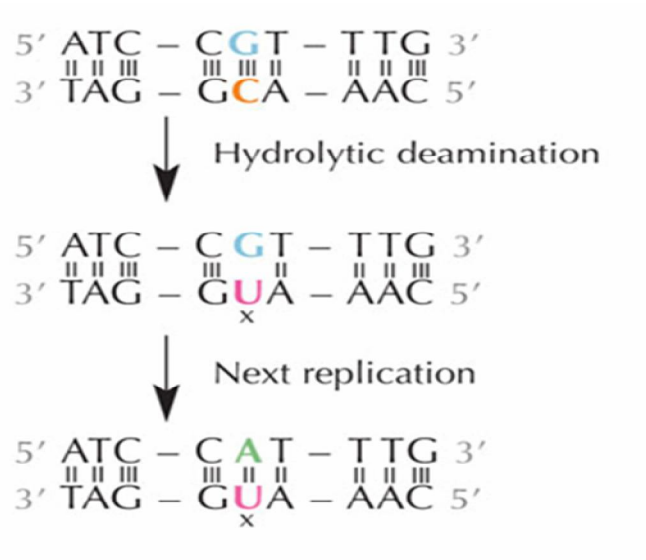


Fig. 8. Deamination of cytosine to uracil and the consequence of its replication leading in turn to cccDNA digestion.

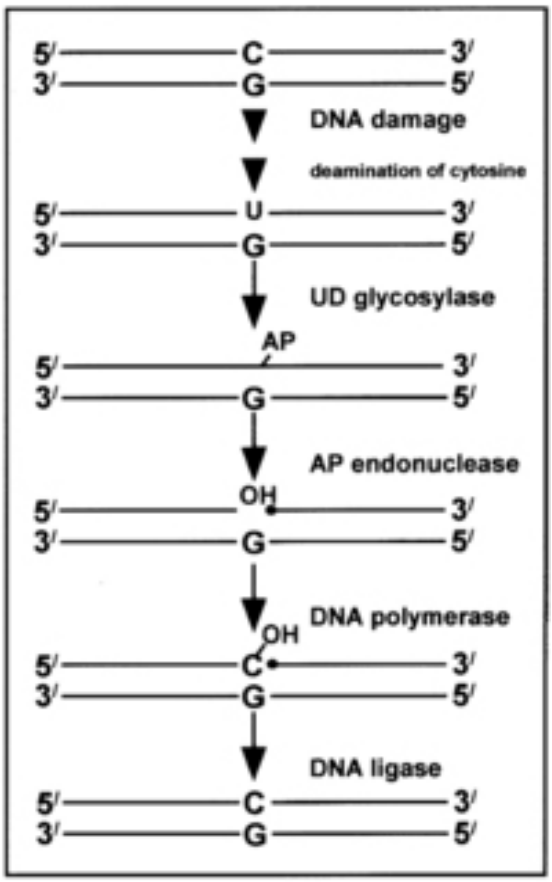


Fig. 9. DNA damage; AP site formation and DNA repair by use of a DNA ligase.

together by catalyzing the formation of a phosphodiester bond (fully repair the DNA) [88]. AP site formation in cccDNA upon IFN-α treatment and LTβR activation will occur [13,86].

Since a cure for HBV infection needs to eliminate cccDNA, induction of an additional factor (future drugs) promoting DNA degradation may shift the equilibrium from cccDNA repair [89] to degradation. As such, it was proposed [13] that adoptive T cell therapy [90], Lymphotoxin β Receptor (LTβR) agonists [91] or cytokines or cytokine receptor agonists that can trigger HBV cccDNA deamination and degradation [13,92] can be interesting antiviral candidates, particularly in combination with nucleoside or nucleotide analogs. However, while LTβR signaling supported liver regeneration [93,94]; yet its

inhibition has already been used for anti-cancer therapy in some experimental models [95] (Fig. 10). Therefore, particular care must be taken in the use of agonists and antagonist as therapeutic agents.

Among the APOBEC3 family members only A3A and A3B located into the nucleus, where they can gain access to cccDNA [96]. Recently, Lucifora and colleagues [13] elucidated that the deamination and degradation of the cccDNA in HBV are mediated through up-regulation of APOBEC3 [16] in the absence of adequate DNA repair activity. It seems that the elevated level of deamination is specific and restricted to the episomal cccDNA. Data [13] suggested that the association of APOBEC3 A and C with the viral core (C) protein results in its specific recruitment to viral specific chromatin, sparing the host genome. IFN-α treatment induces mainly A, F and G APOBEC 3 family members, whereas LTβR agonist induces APOBEC 3B. A deletion of the A3B allele has been associated with poor prognosis for HBV clearance in patients. They suggested [13] that A3A may be targeted to cccDNA by interaction with the HBV core; yet not such targeting to genomic DNA has been described so far. Because APOBEC3 deaminases act on single-stranded DNA, binding of A3A to genomic DNA was not detected. Human tribbles 3 protein also prevents A3A direct binding to DNA [97,98]. As such, whether APOBEC3A binds to genomic DNA? Is the reason for cccDNA degradation, rather than being repaired, due to the high number of AP sites introduced after treatment, which exceeds the capacity of the cellular repair machinery? Does LTβR agonist would be used only for a limited period of time; minimizing the risk of inflammatory liver disease and hepatocellular carcinoma [99,100]?

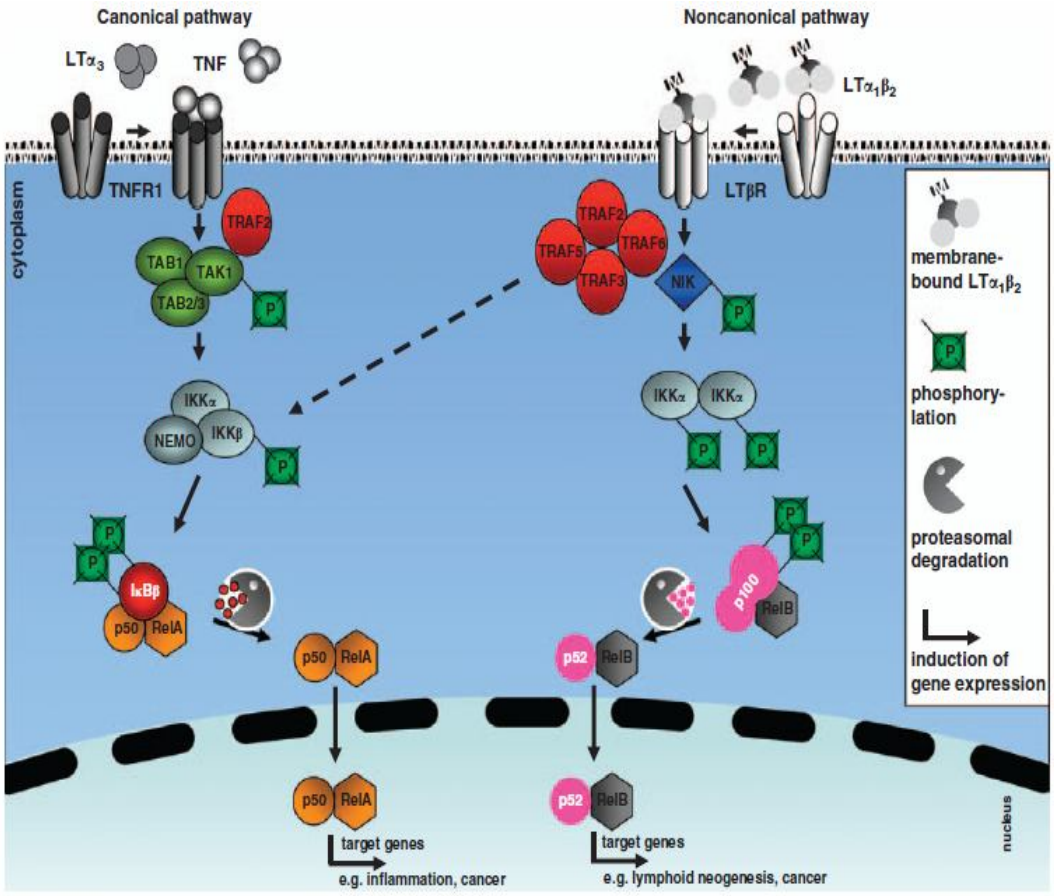


Fig. 10. LTβR signaling pathways.

PATHWAYS ACTIVATED BY HBV INFECTION

Many pathways of cellular immune system are activated during HBV infection. Autophagy is a catabolic process by which cells remove long-lived proteins and damaged organelles for recycling. Viral infections may also induce autophagic response. HBV can enhance autophagic response in cell cultures, mouse liver, and during natural infection [101]. Further analysis indicates that autophagy enhances HBV DNA replication [101].

Deregulation of signaling pathways by HBV were also found to be closely related with development of hepatocellular carcinoma (HCC). These signaling cascades mostly lead to down-regulation of tumor-suppressor gene and up regulation of tumor-causing genes [102]. It has been studied that both cytokine lymphotoxin (LT) α and β and their receptor (LTβR) are up regulated in HBV-induced HCC. Sustained LT signaling is another channel involved in HBV-induced HCC [102]. Many signal transduction processes that were important for stem cell differentiations and proliferation also deregulated during hepato-carcinogenesis [103]. The results obtained indicates that CHD1L-ARHGEF9- Cdc42- EMT might be a novel pathway involved in metastasis and HCC progression [104]. The level of IL6 was found to be increased in HCC cells which proved that IL6 and inflammatory cytokines play a significant role in HCC development [105]. Level of IL6 may also predict the shift from viral hepatitis to HCC in humans due to Hh signal activation [106]. It has been documented that the expression of HBx and Hh is highly correlated in human liver cancer cell lines [107-112]. The protein, vimentin, and scaffold protein, IQGAP1, mRNA expression levels increased significantly throughout hepatotumorigenesis provide another target to treat HCC [113]. Targeting the key molecules in the oncogenic signaling pathway might be a promising strategy for HCC therapy (Fig. 11) [114].

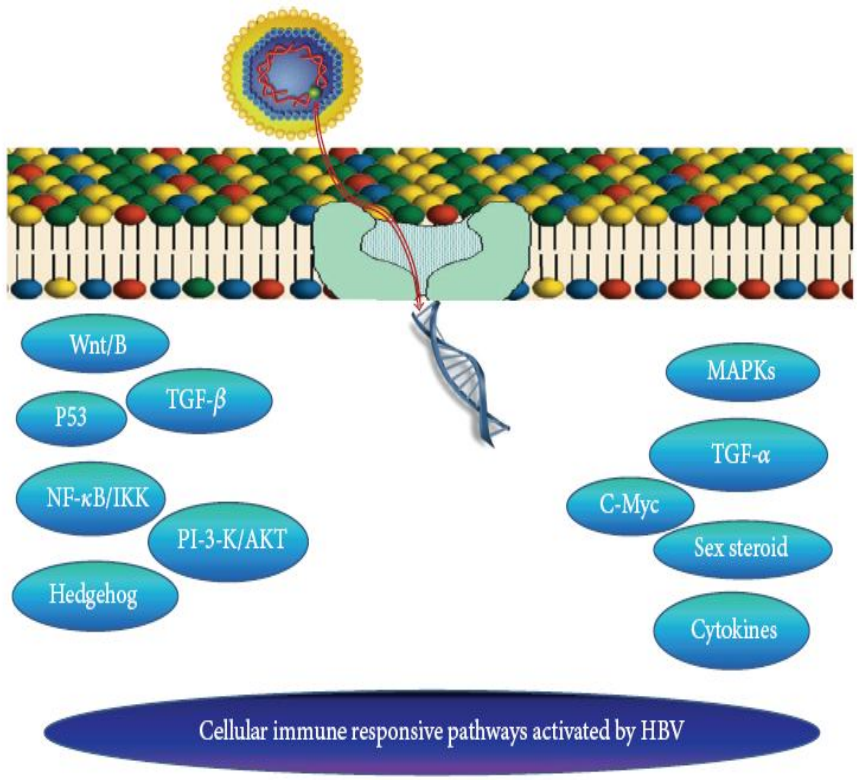


Fig. 11. Pathways Involve in HBV Induced HCC.

ROLE OF CCCDNA IN HBV-RELATED HEPATOCELLULAR CARCINOMA (HCC) RECURRENCE

HBV enters the hepatocyte, and the envelope is subsequently removed. Within the nucleus, the partially double-stranded DNA is repaired to form a cccDNA, which serves as the stable template for the transcription of the viral mRNA necessary for productive viral replication. This cccDNA template remains in the nucleus during chronic viral infection and may persist in the liver for the lifetime of the patient [115].

Chronic liver injury and increased cell turnover confer a predisposition to hepatocyte transformation, HBV DNA is integrated into the DNA of the hepatocyte, the HBx protein is expressed as a transcriptional transactivator, and mutations in the core promoter region increase viral replication and thus the risk of hepatocellular carcinoma.

HBV cccDNA serves as a template for the production of HBV pre-genomic RNA (pgRNA) that is responsible for the persistent HBV infection in hepatocytes [116-118]. The cccDNA level increases unexpectedly in the initial phase of proliferation and then its level decreases dramatically during cell division due to the loss of extra-chromosomal plasmid DNA [119]. The pegylated interferon α-2b (Peg-IFN) and adefovir dipivoxil (ADV) antiviral therapy led to considerable decrease in cccDNA level by a primarily non-cytolytic mechanism [116,120-124]. HBcrAg is a predictor of the post-treatment recurrence of HCC during antiviral therapy. Serum HBcrAg and intrahepatic cccDNA suppression by nucleot(s)ide analogues (NAs) may be important to prevent HCC recurrence [125].

CONCLUSIONS

Although none of the available HBV drugs can clear the infection, they can stop the virus from replicating, thus minimizing liver damage. As of 2008, there are seven medications licensed for treatment of hepatitis B infection. These include antiviral drugs lamivudine (Epivir), adefovir (Hepsera), tenofovir (Viread), telbivudine (Tyzeka) and entecavir (Baraclude), and the two immune system modulators, interferon α-2b and Pegylated interferon α-2b. The treatment reduces viral replication in the liver, thereby reducing the viral load (the amount of virus particles as measured in the blood). Interferon treatment may produce an “e” antigen seroconversion rate of 37% in genotype A but only a 6% seroconversion in type D. Genotype B has similar seroconversion rates to type A while type C seroconverts was found to be only in 15% of cases. Sustained “e” antigen loss after treatment is ~45% in types A and B but only 25-30% in types C and D

The emergence of drug-resistant HBV as a consequence of long-term antiviral therapy and the low sustained/durable responses of existing therapies signal the need for new approaches. Viral dynamic studies have pointed to a problem with the second phase of HBV decline that follows on from the initial rapid response to chemotherapy. This will be a major challenge to over-come, but new insights into HBV pathogenesis and the development of novel immune-therapies may provide useful ways forward. Whether this is by using additional nucleoside/nucleotide analogues or combining existing immune-modulators to nucleoside/nucleotide analogue warrants further clinical investigation. The next logical step may be to initiate trials of triple therapy with two nucleoside/nucleotide analogues in combination with Pegylated IFN-α.

In the meantime, the development of the future generation of anti-HBV antiviral agents including non-nucleoside analogues such as packaging inhibitors and immune-therapies such as dendritic cell (antigen-presenting cells) and vaccines may well be needed to finally achieve “a cure” for chronic HBV. Also it is indicated that cccDNA degradation is possible and can be induced without side effects on the infected host cell. An important task will be the testing of combinations of nucleoside or nucleotide analogs with novel antiviral strategies such as use of LTβR agonists or adoptive T cell therapy to activate A3A or A3B to cure hepatitis B. With respect to the selectivity observation on the activation of LTβR, more investigations are necessary on the potential utility of LTβR agonists for clearance of cccDNA in chronic hepatitis B.

Unfortunately, HBV infection is one of the major causes of HCC development. HBV protein S, C, P and X are responsible for viral replication and activation of several cell signaling pathways such as NF-κB, PI-3K and Hedgehog that eventually may lead to HCC. Targeting the key elements involve in different signaling pathways that

are activated by HBV infection as well as the genetic factors will be helpful to achieve a way to cure from HCC.

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