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New Strategy Treating Hepatitis B Virus (HBV) Infection: A Review of HBV Infection Biology

Yong-Yuan Zhang

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Abstract

Chronic hepatitis B virus (HBV) infection affects 240 million people worldwide and represents a significant burden on public health. Current antiviral treatment of chronic hepatitis B mainly focuses on inhibiting viral replication. A main deficiency of the current treatment is unable to protect uninfected liver cells or hepatocytes that cleared HBV from next rounds of infection. HBV infection biology shows that natural clearance of HBV cccDNA from infected cells frequently occurs, HBV infection including chronic HBV infection is established and maintained by multiround infection, and the course of HBV infection is largely determined by the number of round of infection. Thus, an effective treatment of HBV infection must block new rounds of infection. A proposed new strategy for treating chronic HBV infection aims to immediately interrupt infection course and to achieve HbsAg seroconversion as early as possible. Under this strategy, a main target of antiviral treatment is extracellular viruses, and an effective therapeutics is specific neutralizing (anti-HBs) antibodies. A difference in tempo and efficiency of treating HBV infection between current antivirals and neutralizing antibody is that the antivirals inhibit viral infection only after cells are virus infected while the neutralizing antibody clears viruses before the infection of cells takes place.

Keywords: hepatitis B virus, acute hepatitis B, chronic HBV infection, HBV infection biology, antivirals, neutralizing antibody, anti-HBs

1. Introduction: why we need a better understanding of HBV infection biology?

Chronic hepatitis B virus (HBV) infection affects 240 million people worldwide [1]. Estimated 4.5 million of new HBV infection occurs each year [2]. A significant portion of new HBV infections occurs in infants borne to HBV-positive mothers despite a fully scheduled HBV immunization. More than 90% of HBV-infected infants will become chronic [3–5], resulting in constant expansion of chronic HBV-infected population.

Chronic HBV infection can induce severe or repeated liver injury, which can lead to advanced liver diseases including cirrhosis [6, 7] and hepatocellular carcinoma [8]. The disease burden of HBV infection is enormous, and WHO reports that 780,000 people die of HBV-related liver diseases or complications annually [9]. In addition, as many as 70% of chronic HBV-infected patients who have persistently normal ALT already experienced significant alterations in liver histology [10–13]. We need to provide more effective treatment to chronic HBV-infected patients.

Current antiviral treatment is recommended for chronic HBV-infected patients who have evidence of liver injury related to HBV infection [14, 15]. Antivirals that mainly consist of nucleos/tide analogues can potently inhibit HBV replication, mitigate liver injury and slow-down progression of necroinflammation in the liver [16, 17]. However, the current treatment rarely clears chronic HBV infection. Majority of chronic HBV-infected patients are not suitable for current antiviral treatment. Untreated patients, despite normal ALT history, can experience unpredictable flare-ups of liver injury [18, 19]. Exacerbation insults, if occurred in patients with chronic liver injury or significant alterations in liver histology despite normal ALT, can trigger acute chronic liver failure with up to 70% mortality [20, 21].

Clearly, the current antiviral treatment strategy and available antivirals do not meet clinical needs.

Here, we briefly discuss a number of limitations of current antiviral strategy and antivirals.

1.1. Current treatment strategy does not protect virus-cleared cells or uninfected cells from new rounds of infection

Current antiviral therapy is guided by belief and strategy that a viral infection can be cleared by directly inhibiting viral replication. This approach certainly mitigates viral diseases associated with viral replication, even clears viral infection under certain circumstances, for instance, a significant portion of chronic HCV infection can be cleared with a relatively short course of direct-acting antiviral agents [22, 23]. However, it does not take consideration of viral infection biology, which is featured with multiround infection (see below). This is why current antiviral strategy cannot clear chronic HBV infection. The NA-based antiviral therapy for chronic HBV infection, even with early generation of NAs like lamivudine, can clear HBV from HBV-infected cells, evidence includes wild-type (WT) virus, or early viral population was eliminated and replaced with drug-related mutant (MT) virus [24, 25]. Additional evidence consists of 5–6 logs reduction of serum HBV DNA level [26, 27–29] and 100-fold reduction of

intracellular total HBV DNA and cccDNA levels in treated animals and patients [30–32]. However, during the same period, serum HBsAg level is only reduced by two- to three fold [33], suggesting HBsAg level remains constantly high. The enduring high level of HBsAg keeps depleting the limited amount of endogenous neutralizing antibodies and leaves HBV virions that are produced by infected cells in the same livers, unneutralized and infectious, which continue to cause new rounds of infection. Under the current antiviral strategy, virus-cleared cells can immediately become infected again, gains in clearing viral infection are continuously reversed, and it is extremely difficult to establish permanent and complete viral clearance under current antiviral strategy because virus-cleared cells and uninfected cells are not protected.

1.2. Antivirals target viral replication in infected cells and do not directly act against extracellular viruses

Two events occur simultaneously during antiviral therapy. One is viral replication is inhibited intracellularly, and the other is new rounds of infection continue as long as there are unneutralized extracellular viruses and susceptible cells that are not protected. Antivirals only suppress viral replication in infected cells, reducing production of new viruses that will lower level of extracellular viruses, which eventually lead to reducing new rounds of infection. Once no more viruses available for new rounds of infection, the infection course is actually interrupted, which brings the infection course to the end. Thus, the direct impact of treatment with antivirals is to reduce viral replication while an indirect impact is leading to limiting spread of infection, which really matters in containing and clearing viral infection. However, the antivirals do not directly act against extracellular viruses that may continuously cause new rounds of infection and compromise antiviral efficacy. This feature determines relatively ineffectiveness of direct antivirals in treating chronic HBV infection.

1.3. Effectiveness of antivirals depends on HBV replication efficiency

The third issue is that antivirals do not inhibit or kill viruses that were already produced. They only inhibit producing new viruses upon replication. Antivirals will not function if there was no active viral replication. Actual effectiveness of an antiviral in treating hepatitis B is largely determined by HBV replication efficiency. In clinic, a link between HBV replication level and antiviral response has been indicated. For instance, in patients with long-term entecavir therapy, a full virological response rate was 59, 84, 90, 93, and 95% after 48, 96, 144, 192, and 240 weeks of therapy, respectively [27]. Clearly, 59% of treated patients achieved the full virological response to entecavir in the first 48 weeks, but only 16, 6, 3 and 2% of net increased response were, respectively, achieved when the therapy was extended from 48 to 96, 144, 192 and 240 weeks. The net increased response rate was progressively declined along with extending therapy period since the viral replication was increasingly depressed to very low level. The replication-dependent effectiveness determines the direct antivirals are not effective against HBV infection with inactive replication, for instance in anti-HBe–positive patients with low HBV DNA level or only HBsAg positive patients.

1.4. Ineffective against severe acute HBV diseases

Direct antivirals would not be effective in treating severe acute hepatitis B since they take a few days or longer to significantly reduce viral replication or serum viral load (a log or greater) [34–37]. They would take much longer time to mitigate pathologic injury and to improve clinical manifestations [38]. Relatively slow action and no direct inhibition of extracellular viruses highlight potential ineffectiveness of antivirals in treating fulminant hepatitis B and acute severe exacerbation of chronic HBV infection-induced liver injury, both of which induce rapidly deteriorated clinic course and high mortality [39–41].

1.5. Antivirals alone cannot completely clear viral infection

Antivirals may be no longer effective once the viral replication was inhibited to low level. Residual viruses can be secreted out, or released by turnovers of infected cells, leading to clearing infection or infected cells. The released residual viruses are small in quantity and can be completely neutralized if there is relatively sufficient amount of endogenous neutralizing antibodies, which lead to stopping the course of viral infection by blocking new rounds of infection. This scenario likely happens to clearing chronic hepatitis C virus infection with direct antivirals.

The released residual viruses following potent inhibition of virus production will still be capable of causing new rounds of infection if there were no sufficient neutralizing antibodies, prolonging the course of chronic infection. This scenario likely happens to antivirals-treated HBV-infected patients.

Clearly, antivirals alone cannot complete clearing viral infection,which requires the presence of sufficient neutralizing antibodies.

What we need is new treatment strategies and new therapeutics that can improve current treatment approach and antivirals in treating chronic HBV infection.

We believe we can find effective solutions to current problems in treating chronic HBV infection through a better understanding of HBV infection biology, which will also accelerate developing new prophylactics and therapeutics.

2. Understanding HBV infection biology

2.1. Natural course of HBV infection

Infection biology is science illustrating fundamentals of infection, which is centered on understanding how a productive infection is established and maintained. Understanding of infection biology starts with understanding natural course of infection. In this review, we only focus on the natural course of HBV infection that causes hepatitis. The natural course of hepatitis B consists of initial infection, incubation period and clinical phase (**Figure 1**).
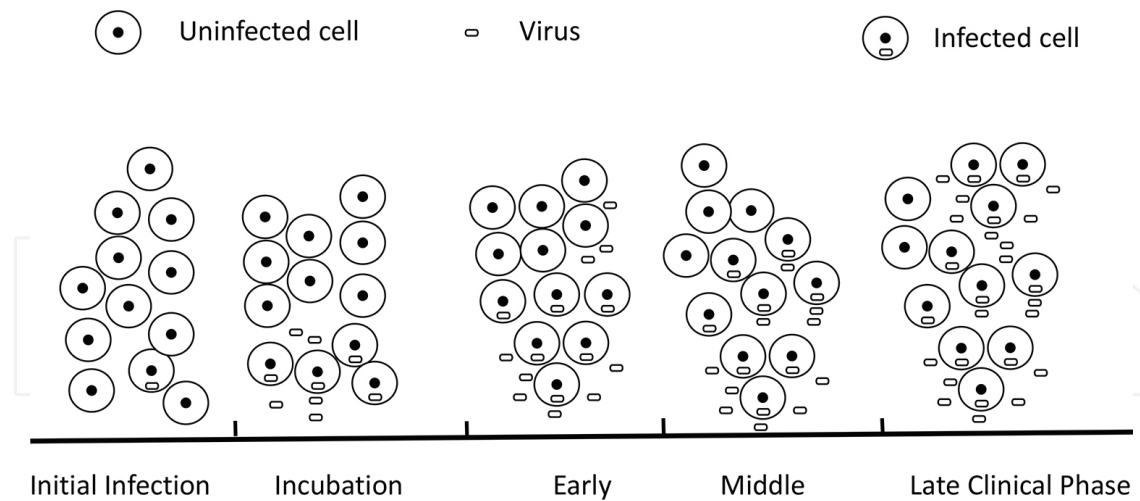
Figure 1. Schematic illustration of a typical course of infection that consists of initial infection, incubation and clinical phases. Progression of infection course is directly driven by new rounds of infections. It also suggests the infection course can be ended at any stage as long as extracellular pathogens are completely neutralized.

2.1.1. Initial HBV infection

Initial infection represents beginning of an infection and it provides seed of infection and usually consists of a few or small clusters of infected cells in the liver. The initial infection is important, but it cannot become a full-blown infection without incubation period.

2.1.2. Incubation period

The incubation refers to a period between initial infection and appearance of clinical symptoms. Length of the incubation varies considerably from individual to individual, but it is required for every HBV infection that causes hepatitis, which suggests the number of virus involved in the initial infection is so small that cannot immediately cause significant injury or clinical manifestations. Likely sequential events during the incubation include that infected HBV replicates in initial infected cells, progeny viruses are released through secretion or/and cytopathic destruction of infected cells, resulting in new rounds of infection of more cells, most likely in neighboring areas because short distances between viruses and susceptible cells favor higher efficiency of infection. Continuously released viruses keep initiating new rounds of infections, infecting more cells and extending the scope of infection. When the number of destructed cells reached an extent that causes clinical symptoms, the incubation is progressing to clinical phase.

Requirement of incubation period in full-blown HBV infection provides evidence that more than one round infection is required to establish and maintain a full-blown infection or hepatitis B (**Figure 1**). Thus, acute hepatitis B is caused by multiround of infection.

A main factor that allows multiround of infection is that there is no sufficient amount of endogenous neutralizing antibodies to neutralize the released viruses.

New rounds of infections inevitably occur during the infection course as long as there are unneutralized extracellular viruses and susceptible cells.

2.1.3. Clinical phase of acute HBV infection

Acute HBV infection in adults is a self-limiting disease, and 95% of them will be recovered without treatment [42]. A unique kinetics of serum HBV DNA level during acute HBV infection includes a rapid fall of HBV DNA level after the peak (**Figure 2**), suggesting HBV infection is being progressively cleared from the liver, as evidenced by rising ALT level (a result of destructing the infected cells) at the same time frame. Critically, it also suggests no new rounds of HBV infection, implying a block of new rounds of infection is required for clearing HBV infection. A mechanism behind these changes is called "HBsAg seroconversion," a hallmark for resolving HBV infection.
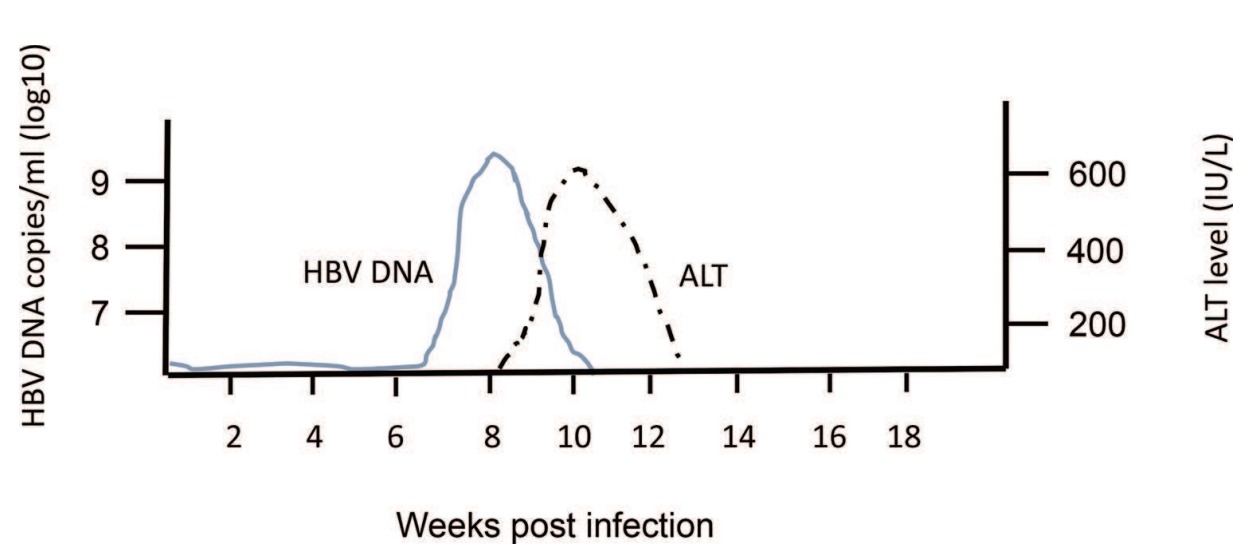

Figure 2. Kinetics of serum HBV DNA and ALT levels during acute hepatitis B. HBV DNA quickly falls after the peak, which coincides with rising ALT level. The rapid fall of HBV DNA suggests no new round infection.

When destruction of the infected cells is started during acute HBV infection, it will initially cause transit elevation of serum HBsAg and HBV DNA levels by releasing viral particles. However, the serum HBsAg and HBV DNA levels will then keep falling because the number of infected cells that supply and replenish serum HBsAg and HBV virion is progressively reduced by the liver injury (the supply reduced) while the removal or the half-life of viral particles from/in circulation should be at a constant rate, contributing to rapidly significant reduction of serum HBsAg and HBV levels. At certain point, the relative ratio between amounts of HBV particles (both viral and subviral particles) and anti-HBs antibodies will be reversed from the former greatly exceeding the latter, to the latter exceeding the former (HBsAg seroconversion). This conversion creates a sufficient anti-HBs capacity to clear serum HBsAg and to block new round infection, which allows uninfected cells and cells that cleared HBV infection permanently stay infection free. This process highlights an indispensable require-

ment of anti-HBs antibodies in blocking new rounds of HBV infection and resolving HBV infection.

Different impacts of liver injury on outcomes of infection are expected if occurred at different frequencies. For instance, continuous or progressive liver injury at moderate or medium scale, as is in acute hepatitis B, or at massive scale as is in fulminant hepatitis can lead to complete clearance of HBV infection because of successful reversal of the ratio of serum HBsAg and anti-HBs. On the other hand, if occurred intermittently, it unlikely leads to a complete viral clearance as is in chronic hepatitis B because of no reversal of the ratio of HBsAg and anti-HBs. The different outcomes once again emphasize that HBsAg seroconversion is critically required for completely clearing HBV infection.

A HBV infection can be ended at any stage if subsequently released viruses were completely neutralized by endogenous neutralizing antibodies. Most of HBV infections are aborted during the incubation period without developing into a full-blown infection because relative amount of infected viruses is still low. Those individuals who ended the HBV infection before clinical stage only show detectable anti-HBs and anti-HBc antibodies without clinical manifestations and noticeable HBV infection history [43, 44].

Thus, a natural strategy to clear viral infection or end infection course utilized by the host is to include producing neutralizing antibodies to block new rounds of infection with newly released viruses [45]. However, in minority cases, the infection will advance to clinical stage or viral disease will be aggravated or clinical stage will be prolonged if the amount of endogenous neutralizing antibodies produced in the host is not high enough to neutralize all specific viruses during the incubation or clinical stage of infection. This is how acute and chronic infections are established.

Cellular immunity consists of two major functional mechanisms in controlling viral infection [46–48], one is to kill infected cells by cytotoxic T cells that not only contribute to pathological changes of viral disease, but also release viruses for new rounds of infection if not neutralized. The impacts of killing infected cells by the specific immunity are similar to cytopathic effects of viruses. The other is to inhibit replication of viruses in the infected cells by cytokines, which will reduce the number of released viruses. From controlling infection point of view, the cellular immunity can be counterproductive or helpful dependent on net effect of two actions (the amount of virions released) as well as the level of specific neutralizing antibodies. A difference in tempo of clearing viral infection between cellular and humoral immunity is that the cellular immunity clears viral infection only after cells are virus infected while the neutralizing antibody clears viruses before the infection takes place, one step ahead of the cellular immunity (**Figure 3**). Humoral immunity of neutralizing antibody is direct, decisively effective and required for controlling viral infection. Once new rounds of infection were completely blocked by sufficient amount of neutralizing antibodies, the viral infection in the already infected cells will be cleared by virus secretion, cytopathic effects of viral replication, cell turnover or the cellular immunity. Alternatively, the viral infection will be restricted to those already infected cells if the infected cells were long-lived, implying that viral infection has been brought under control with the neutralizing antibody.
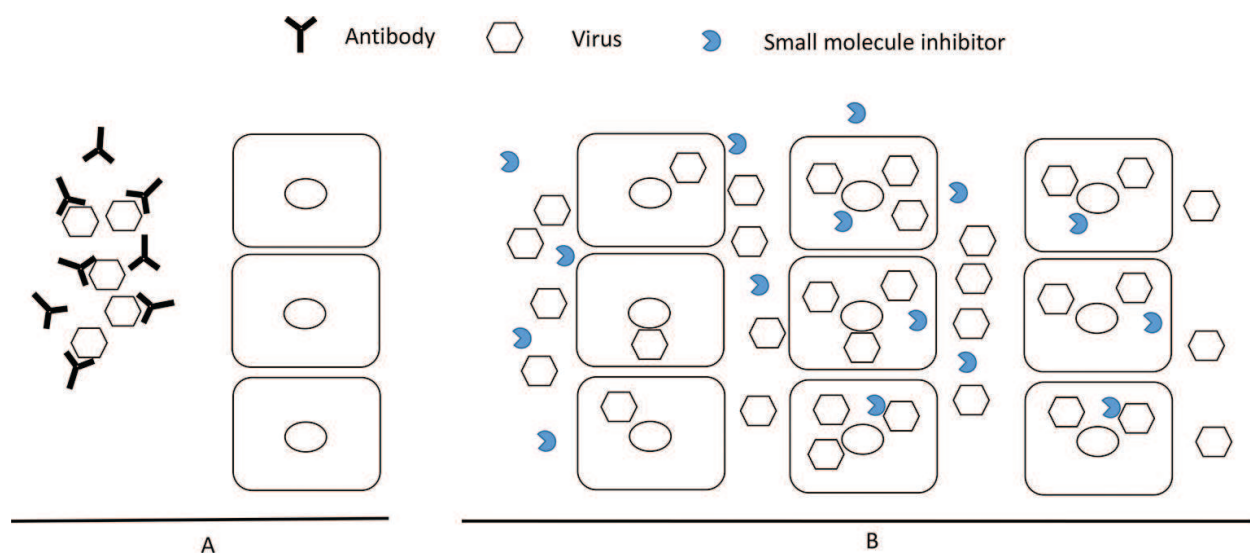
Figure 3. Differences in tempo and efficiency of clearing viral infection between neutralizing antibody and antivirals. (A) Administrated neutralizing antibody immediately neutralizes extracellular viruses and protect uninfected cells; (B) administrated small molecule inhibitor first allows virus to infect cells then starts inhibiting viral replication in infected cells. It cannot immediately and completely remove viremia.

A chronic HBV infection is usually maintained by new rounds of infection. The chronic infection can be spontaneously cleared if the level of endogenous neutralizing antibodies is high and can neutralize all released viruses to stop new rounds of infection. For instance, spontaneous clearance of chronic HBV infection occurs at annual rates of 1–2% and is featured with seroconversion of HBsAg to anti-HBs antibody [49, 50].

2.1.4. A main risk that will prolong HBV infection course is extracellular viruses, and a main target in treating viral diseases is extracellular viruses

Clearly, to cure HBV infection, it is essential and also efficient to interrupt infection course that consists of repeated rounds of infection.

There are three consequent scenarios during the course of hepatitis B: one is the infected cells become lost, resulting in releasing more virions as a consequence of cytopathic effects of infected HBV, cell turnover or destruction by cellular immunity; the second one is the infection is spontaneously cleared from infected cells by virus secretion or/and cellular immunity that reduces production and release of viruses, a same outcome as inhibiting viral replication by antivirals, and the third one is the infected cells are relatively long-lived (non-cytopathic infection) and producing and releasing low or high level of viruses. A shared main consequence produced by each scenario is releasing viruses. Thus, a main target in treating HBV infection should be the extracellular viruses, which cause new rounds of infection. Direct antivirals like NAs are not agents that can directly counter the extracellular viruses. Overall effectiveness of treating chronic HBV infection will be significantly improved if neutralizing antibodies are employed.

2.2. HBV infection course and persistence

HBV infection is known for long incubation period that may last up to 6 months (average 2–3 months) [51, 52] before occurrence of clinical symptoms of acute HBV infection. We believed that multiround infection occurs during the incubation period. Duck hepatitis B virus (DHBV) experimental infection was utilized to verify this understanding of HBV infection dynamics during the incubation period. Ducklings were inoculated with high DHBV inoculation dose, and three animals were daily sacrificed for 7 days after inoculation. Viral core protein was stained on liver sections, and DHBV DNA was detected in liver tissues [45]. As shown in **Figure 4A** and **B**, DHBV infection rapidly expanded from a few clusters of initially infected cells in the infected livers and reached a full-blown infection in 7 days, showing there were repeatedly new rounds of infection that expanded the infection scope in the livers, even under the circumstances of experimental infection that used a very large inoculum.
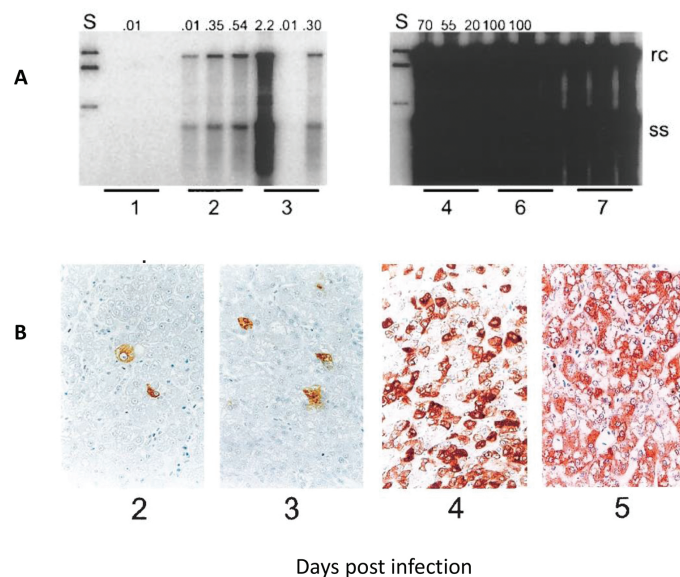

Days post infection

Figure 4. Multiround infection during the incubation period of DHBV infection. Viral replicative intermediates (A) and antigen-staining cells (B) were detected in livers of DHBV-infected ducks. Three ducks were sacrificed at the indicated days postinfection, and replicative intermediates were extracted from the liver. The viral DNA was detected by hybridization. The percent antigen-staining hepatocytes in some of the samples is indicated at the top of the lanes.

Are there continuously new rounds of infections after the full-blown HBV infection was established?

Current theory views that chronic HBV infection is a consequence of the host's insufficient T-cell immunity that cannot kill all HBV-infected cells or cannot clear HBV infection from infected cells [53, 54], implying chronic HBV infection is a simple extension of the initial or early HBV infection; the hepatocytes, once infected with HBV, are long-lived and constantly infected with the same initial viruses during chronic course.

However, such view is not supported by experimental and clinical evidence.

To investigate whether DHBV infection and DHBV-infected cell populations are stable after liver was fully infected, 3-day-old ducklings were inoculated with a large inoculum containing both DHBV 3 and 16 viruses at 1:1 ratio. The first biopsy was conducted at day 11 by which the liver was fully infected, and then, series of liver biopsies were performed every 3 weeks. Distinct genetic markers in two viral genomes allowed us to monitor kinetic changes in DHBV 3 and 16 singly and dually infected populations by determining cccDNA genotypes at single nucleus level. It was found that majority of hepatocytes were singly infected and nearly 20% of cells exhibited dual infection with both viruses at day 11 postinfection (p.i.) (**Figure 5**). We detected new rounds of infections at each of 5 time points after day 11 because the fraction of DHBV3-infected cells was expanding, but the expansion occurred in only singly infected fashion, suggesting that already infected cells resisted superinfection while the new rounds of infection were occurring. It also suggested occurrence of viral clearance in DHBV-infected liver, which generated uninfected cells for new rounds of infection. This viral clearance conclusion is consistent with data showing that the fraction of DHBV16-infected cells was decreased from 80% at day 32, to about 40% at day 131 p.i., and at least 40% of DHBV16-infected cells either cleared the infection or were eliminated, which triggered regeneration of hepatocytes. Either of two scenarios would produce uninfected cells targeted by new rounds of infection. The results suggest DHBV infection even after the full infection was established in the liver remains dynamic and is featured with new rounds of infections.
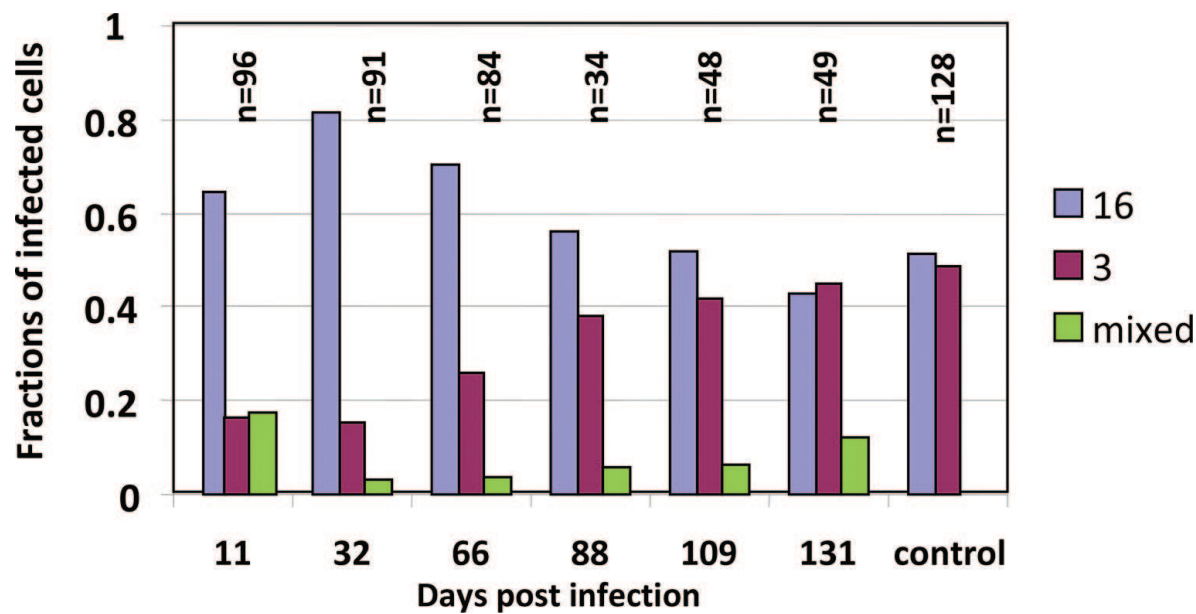

Figure 5. Repeated new rounds of infection after a liver was fully infected. Three-day-old ducklings (n = 20) were infected with an inoculum containing both DHBV3 and 16. Six biopsies were performed on one animal at day 11, 32, 66, 88, 109 and 131 days postinfection (shown in this figure). cccDNA genotypes were determined by sequencing PCR products amplified from cccDNA released from single individual nuclei. DHBV3-infected cells were expanding, while DHBV16-infected cells were decreasing during the period of six times of biopsies, suggesting ongoing viral clearance and new rounds of infection. As a quality control for our procedure, the same number of nuclei isolated from DHBV3 and DHBV16 singly infected livers was mixed and single individual nucleus was sorted into individual well of 96-well plates for PCR amplification and sequencing. No dual infections were detected in 128 mixed nuclei, suggesting that our procedure for detection is valid.

The results on clearing of DHBV16 cccDNA and new establishing of DHBV3 cccDNA pool from new rounds of infection are consistent with the early reports, in which a replication defective pre-core mutant or revertants successfully spread infection and became the predominant viral population following elimination of wild type (WT) or the initially inoculated viruses (**Figure 6**) [55, 56]. Taken together, all results suggest that a chronic DHBV infection is not a simple extension of the initial infection because the initial infection was cleared and the early cccDNA genotypes were replaced. Rather chronic DHBV infection course is dynamic and consists of viral clearance and new rounds of infection. Thus, repeatedly new rounds of infection maintain the persistence of viral cccDNA and prolong the course of chronic infection.
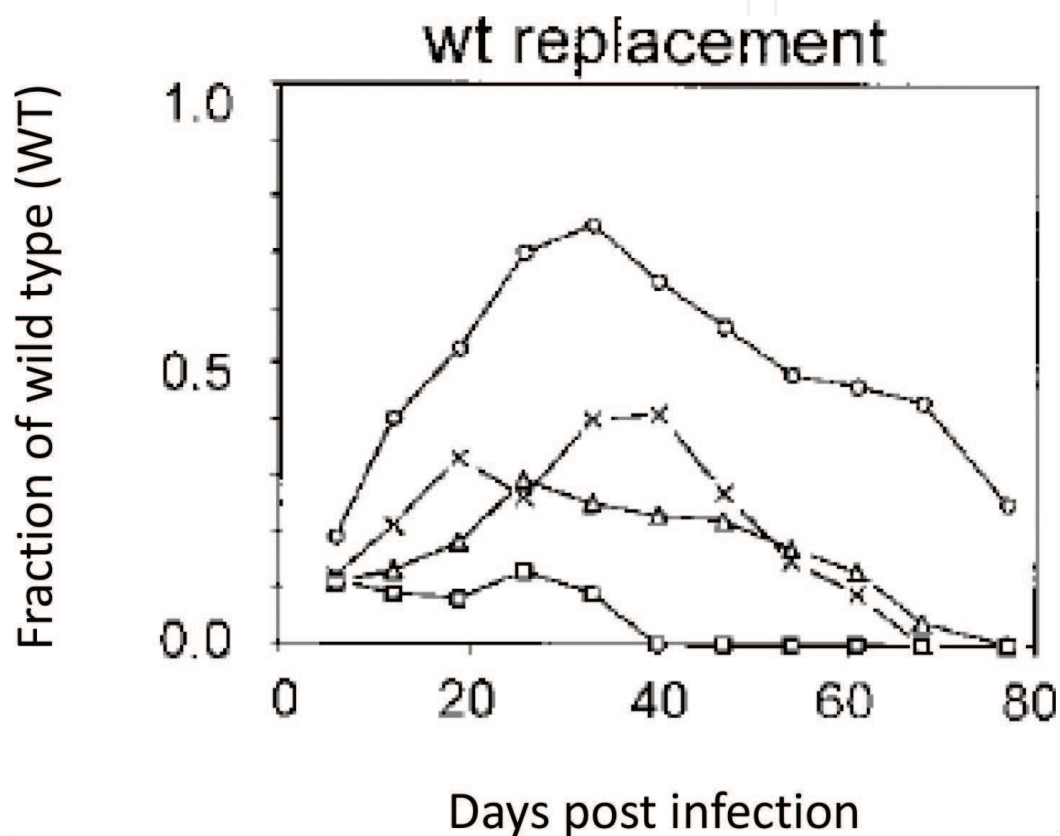

Figure 6. WT DHBV was being replaced over time. The fraction of WT DHBV DNA in serum of four DHBV-infected ducks was determined by PCR sequencing assay and plotted against the time postinfection. The kinetics of replacing WT DHBV infection suggests DHBV infection in fully infected livers remains dynamic and featured with clearing WT and new rounds of infection with MT.

These conclusions are supported by clinical HBV infection data.

It is well known that WT HBV infection is frequently replaced with mutant (MT) infection [25, 57–82] in untreated chronic HBV-infected patients. For instance, naturally occurring pre-core, core, pre-S and S mutants became only or dominant viral population following the WT was being eliminated from infected livers during natural course of chronic HBV infection. The data strongly imply that WT or early HBV infection is frequently cleared at cccDNA level during chronic HBV infection. It is notable that such cccDNA clearance naturally occurs without

intervention. This understanding is consistent with cccDNA clearance in adult patients with acute HBV infection in which HBV infection is naturally resolved, and no antiviral treatment is needed.

Available data also suggest that HBV WT is replaced with HBV MT in NAs-treated chronic HBV-infected patients as evidenced by emergence of drug resistant mutant infection [25, 82]. The drug mutant infection that may not bear drug resistant phenotypes was also frequently detected in new generation of NAs-treated patients. A recent report shows that approximately 50% of patients treated with tenofovir (TDF) who remained viremic, developed pol/RT mutant infection though none of patients showed the drug resistance phenotypes [83]. The frequency of mutant infection in this report may be still underestimated because only the pol/RT sequence was analyzed. The observation that drug-related mutants spread following elimination of WT during NAs treatment suggests frequent viral clearance at cccDNA level and new rounds of infection with mutants.

3. New strategy and therapeutics for treating HBV infection guided by the viral infection biology

We propose a new strategy for treating HBV infection. This strategy directly aims to establish HBsAg seroconversion as early as possible through administrating sufficient amount of specific neutralizing antibodies, which will constantly and completely neutralize extracellular viruses to block repeated rounds of infection. This new strategy represents a paradigm shift in treating HBV infection, which has been treated primarily by inhibiting viral replication.

3.1. Directly dealing with the huge pool of HBsAg in chronic HBV infection

As the evidence points out, chronic HBV infection is not a simple extension of the initial infection, but is established and maintained by new rounds of infection. A unique situation in HBV infection is that it produces a huge pool of subviral particles (HBsAg) that are 1000–10,000 fold higher than virions [84]. The HBsAg primarily depletes the limited amount of endogenous neutralizing antibodies and leaves virions unneutralized and infectious. Current treatment strategy and approved antivirals are not designed to deal with new rounds of infection, almost impossibly deliver HBsAg seroconversion, and this is why current treatment rarely cures chronic HBV infection. The key to curing chronic HBV infection is to establish HBsAg seroconversion and to interrupt the infection course as early as possible by providing and maintaining high level of HBV neutralizing antibodies until the amount of endogenous HBV neutralizing antibodies exceeds the amount of HBV particles or all infected cells cleared HBV.

Advantages of the new strategy:

1. It directly immediately targets extracellular viruses and blocks the spread of infection;

2. It facilitates permanent and complete viral clearance in the liver;

3. It significantly reduces side effects of treating HBV infection. Unlike NAs and interferon that function intracellularly, the neutralizing antibody is to replenish a normal component of the immunity, which is relatively deficient in face of a huge pool of HBsAg, and it mainly functions extracellularly;

4. Neutralizing efficacy does not depend on efficiency of viral replication in infected cells.

4. Conclusions

In this paper, we analyze the deficiencies of current HBV treatment strategy and antivirals and the reason why they cannot cure chronic HBV infection. We also review the viral infection biology to fresh our understanding of general phases and natural course of HBV infection. We conclude that a full-blown HBV infection is established and maintained through multiround infection. We propose a new strategy for treating HBV infection. The core of this new strategy is that we must achieve HBsAg seroconversion naturally or interventionally to effectively clear HBV infection. Under the proposed strategy, a main target of the treatment is extracellular viruses, and an effective therapeutics is specific neutralizing antibodies.

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