

New perspective on the natural course of chronic HBV infection

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Abstract Chronic hepatitis B virus (HBV) infection is a significant threat to public health and an enormous burden on society. Mechanisms responsible for chronic HBV infection remain poorly understood. A better understanding of the natural course of chronic HBV infection may shed new light on the mechanisms underlying this disease and help in designing new antiviral strategies. Natural course of chronic HBV infection is conventionally viewed as an uninterrupted process that is usually marked by HBV e antigen (HBeAg) seroconversion or characterized by different phases associated with assumed host responses to HBV infection. However, none of these descriptions captures or highlights the core events that determine the natural course of chronic HBV infection. In this review, we briefly present the current knowledge on this subject and explain the significance and implication of events that occur during infection. A pre-core mutant becomes predominant in the viral population following elimination of the wild-type virus in duck hepatitis B virus-chronically infected animals. The coupled events in which first there is viral clearance that clears wild-type virus and then there is the reinfection of wild-type virus cleared livers with mutant virus are highly relevant to understanding of the natural course of chronic HBV infection under both treated and untreated conditions. In our new perspective, a general natural course of chronic HBV infection comprises cycles of viral clearance and reinfection, and such cycles prolong the chronic HBV infection course. Reviewing published data on the natural course of chronic HBV infection can reduce the possibility of missing important points in the initial data interpretation.

Keywords hepatitis B virus; chronic HBV infection; natural course; hepatitis B; seroconversion

Why we need new perspectives on the natural course of chronic HBV infection?

Approximately 350 million people worldwide are chronically infected with hepatitis B virus (HBV), which can lead to cirrhosis and hepatocellular carcinoma [1–4]. Current antiviral therapy with nucleoside/nucleotide analogs (NAs) can significantly inhibit HBV replication, normalize alanine aminotransferase (ALT), and improve liver histology [3,5–7]. The overall antiviral response is further improved among patients treated with new generation NAs [8–11]. However, the fundamental problem is that antiviral therapy fails to completely clear chronic HBV infection even after long-term treatment and the financial and social burden remains huge [12]. Such inability to clear HBV infection is associated with the persistence of covalently closed circular DNA (cccDNA) in the livers of patients. A new antiviral strategy that can

effectively clear chronic HBV infection is urgently needed. A better perspective of the natural course of chronic HBV infection can help devise new antiviral strategies, which are often impeded by the complexities of chronic HBV infection course.

Currently accepted perspectives in the field include marking HBV infection with different phases [6]. The natural course of chronic HBV infection is traditionally defined with changes in the HBV serology profile. For example, chronic HBV infection is often distinguished by an early HBeAg positive phase and, subsequently, by an HBeAg negative/anti-HBeAg antibody positive phase [6,13,14]. HBeAg seroconversion-based characterization of the natural course of chronic HBV infection is useful in guiding the clinical management of chronic hepatitis B because the HBeAg positive phase is usually associated with high serum HBV DNA levels. Elevation of ALT activity is observed (more than one cycle of ALT elevation can occur) prior to the switch to the anti-HBe positive phase. The anti-HBe positive phase manifests a significant decrease in serum HBV DNA levels and normal ALT levels. Anti-HBe positive patients are often

referred to as inactive HBsAg carriers. However, what is missed by dividing chronic HBV infection course into HBe⁺ and anti-HBe⁺ phases is without highlighting the events of viral clearance and reinfection that take place in both phases. There is viral clearance and reinfection during HBeAg positive period as is demonstrated by eliminating WT or early viral population and the virus cleared livers are reinfected with mutant viruses (MT) [15]. The viral clearance and reinfection also occur after HBeAg seroconversion. For instance, a portion of anti-HBe positive patients manifest increases in serum HBV DNA levels followed by flares of liver disease [6,8]. Flares of liver inflammation are frequently associated with emerging HBV mutant infections, such as pre-core or/and core MTs [15–17]. Spreading of MT infection that follows clearing of WT or early infected viral population from infected livers illustrates the cyclic nature of the natural course of chronic HBV infection after the initial round of HBV infection. Clearly, viral clearance and reinfection occur regardless of HBeAg status.

The emergence of HBV MT infection following elimination of the wild-type (WT) virus from infected livers does not always result in liver injury. Emerging of MT infection, which is preceded by declining of WT viral infection, can be detected in anti-HBe-positive asymptomatic or inactive HBsAg carriers [18–27]. MT infection may remain undiagnosed, and the frequency of occurrence may be underestimated in patients who fail to exhibit liver injury symptoms since determination of MT infection in asymptomatic HBsAg carriers has not been as widely carried out as it does in patients with flares of liver disease, and often only certain types of mutations, not all mutations were analyzed in studied patients. All circumstances suggest that MT infection may occur more frequently than we thought despite the lack of association with flares of liver inflammation during the chronic HBV infection course. The increase in pre-core stop codon (G1896A) MT is related to certain HBV genotypes, but no evidence has yet suggested that the appearance of other HBV MT infections is HBV genotype-dependent [28]. A description of the natural course of chronic HBV infection using HBeAg or anti-HBe positive phases does not characterize and highlight recurring key events that contribute to prolonging chronic HBV infection course and are independent of HBeAg seroconversion.

What does the presence of HBeAg or anti-HBe positivity mean? HBeAg or anti-HBe positivity is an indirect indicator of relative HBV lifecycle replicating activity mainly reflecting efficiency and level of transcribing pre-core mRNA and possible pregenomic RNA from cccDNA template and a relative measure of virion infectivity in serum. The efficiency and level of the pre-core mRNA and pregenomic RNA are significantly reduced when chronic HBV infection is extended and this is why the natural HBeAg seroconversion occurs. A critical message delivered by the seroconversion is that HBV lifecycle replicating activity is partially (because HBsAg level remains relatively stable) significantly reduced

in the infected liver, but it does not disclose occurrence of cycles of viral clearance and reinfection. Thus, classifying the natural course of chronic HBV infection as HBeAg and anti-HBe positive phases is insufficient and incomplete because it does not cover occurring significant events of viral clearance and reinfection. The ongoing MT infection replaces the WT infection that is being cleared. Such MT infection is a characteristic of chronic HBV infection but may or may not cause liver injury. The significance of HBV MT infection (particularly its relationship with initial WT HBV infection) is not well understood and has not been highlighted in the context of the natural course of chronic HBV infection despite the availability of numerous studies that nearly exclusively focus on characterizing genotypic and phenotypic profiles of detected MTs [17–20, 28–59]. To understand the natural course of chronic HBV infection, the following question must be answered: what is the implication of HBV MT infection following elimination of the initial WT virus?

Another description of the natural course of chronic HBV infection in the field involves evaluating of serum HBV DNA and ALT levels and the assumed mechanisms underlying their changes during the course of chronic HBV infection. Chronic HBV infection is suggested to undergo immune tolerance, clearance, reactivation, and inactive replication phases [60]. Such descriptions of the natural infection course, similar to the depictions with HBe and anti-HBe phases, basically define chronic HBV infection as an uninterrupted process, also implying that initially infected WT virus is persistent for the entire infection course. Such characterizations do not consider and highlight the impact and consequence of HBV MT infections that occur after the elimination of the WT infection as well as the cyclic nature of the infection that contributes to the natural course of chronic HBV infection.

What is the new perspective on the natural course of chronic HBV infection?

The natural course of chronic HBV infection comprises viral clearance and reinfection, and it is established and maintained through these cyclic processes. This new perspective is formed after reviewing and understanding the implication of the available data, especially data on the complexity of chronic HBV course. One of us observed a reversal in the fate of a replication-defective pre-core MT after initial near-elimination in competition with the WT virus in livers of duck hepatitis B virus (DHBV)-infected animals. The declining MT fraction accompanied by increasing WT population at early time points was a result of the replication deficiency of the MT. However, the first author later observed an opposite outcome, in which the pre-core MT population was progressively expanded after near-extinction to become the predominant population, whereas the WT population was being depleted [61]. This finding recaptures similar events that occur among chronically HBV infected patients, namely,

pre-core or core or other MTs frequently become predominant population after eliminating the WT. Our initial interpretation of the data is that the host's immune pressure selectively eliminates the WT and allows the spread of the pre-core MT population despite its defective replication. We believe that our perspective explains why the WT is eliminated, but our understanding of observed data is lacking because we were unable to highlight the significance and implication of sequential events in the context of the natural course of chronic HBV infection. These sequential events include first depleting of the WT viral population and then increasing of the MT population in infected livers. Re-reviewing these data led to appreciation of the implication of sequential events observed in DHBV-infected animals, realizing that the nature of the sequential events is that there is frequent viral clearance (i.e., elimination of WT) occurred in chronically infected livers. However, such viral clearance was not permanent and subsequently reversed because MT reinfection immediately spread and predominated in the same livers. DHBV MT reinfections following frequent viral clearance extend the duration of chronic DHBV infection. Viral clearance and reinfections are not isolated events observed in a single animal experiment but rather frequent events identified in multiple DHBV infection experiments whenever changes in viral populations are observed [62]. Thus, viral clearance is ubiquitous in chronic DHBV infection. Viral clearance occurs in chronically infected animals at a frequency as high as 60% [47]. This percentage may be underestimated because our assay was unable to detect WT or other MT reinfections. We also determined why viral clearance precedes reinfection. Infected cells resist superinfection with WT or MT virus, and regeneration of uninfected hepatocytes is required for reinfection to occur (unpublished data).

What occurs in HBV chronically infected livers? Substantial evidence supports data obtained from DHBV-infected animals. HBV DNA became undetectable or was significantly reduced in patients in which seroconversion from HBeAg to anti-HBe positive [6,16,30] was observed, and cccDNA levels were significantly reduced during conversion [63]. However, significant gains in viral clearance were frequently lost because of reinfections. Pre-core or core or other MT infections developed and predominated in a large percentage of patients who may or may not have liver injury in anti-HBe positive phase [10–45]. Reinfections occurred following depletion of WT [9,28,30–32,44,64,65], which indicates spontaneous, ongoing viral clearance, as demonstrated by depletion of the WT population in chronic HBV infection. However, such viral clearance usually paves the way for MT and/or WT reinfection, which prolongs the chronic HBV infection course (Fig. 1). Information on the frequency of MT reinfection during the course of chronic HBV is incomplete because the majority of studies investigating MT infection have focused on pre-core/core regions or other known mutations during a particular period when HBeAg serocon-

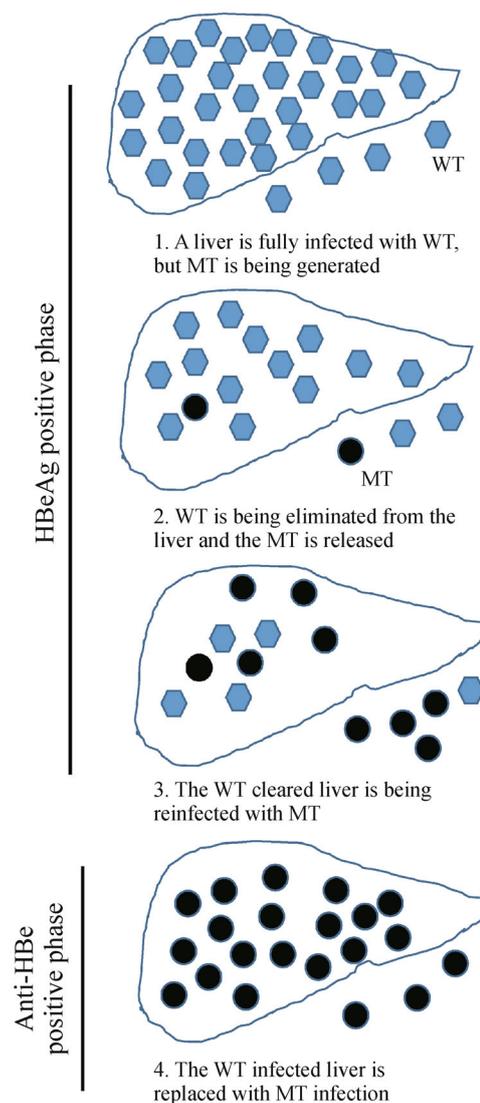


Fig. 1 Main scenario of the natural course of chronic HBV infection, during which the WT-infected liver is cleared but reinfected with an MT, thereby prolonging the course of chronic HBV infection.

version occurs. MT infection may occur as early as 32 days post-inoculation in animals chronically infected with DHBV (unpublished data).

Reinfection also occurs during antiviral therapy. HBV DNA replication can be significantly inhibited, but chronic HBV infection is rarely terminated in patients treated with NAs. Chronic HBV infection is not eliminated by NAs because cccDNA is still present in infected cells [6]. NAs do not directly inhibit cccDNA amplification, but they can impact replenishing of the cccDNA pool indirectly by reduced relaxed circular DNA level in treated cells. This indirect effect on cccDNA level was confirmed by a carefully conducted study involving NA-treated, chronically infected woodchucks; cccDNA levels were reduced by 80% to 90% in

livers after 30 weeks of treatment [66]. Clinical observations also confirmed this finding [63,67–71]. However, significantly reduced viral replication fails to improve the odds of newly produced viruses no longer causing infection because the production of subviral particles remains high and relatively stable; such production will continue to deplete the limited amount of endogenous neutralizing antibodies available, leading to WT or MT reinfection in the WT cleared livers. MTs can also be detected as major viral populations in approximately 50% patients who were treated with new NA like tenofovir and did not have drug-resistant breakthrough infection [10]. Significant viral clearance by antiviral therapy can be reversed by MT infection, thereby prolonging the infection course and requiring long-term therapy.

This new perspective on the natural course of chronic HBV infection is primarily based on the DHBV animal infection model and clinical observations of human HBV infection. Further experiments are required to reach valid conclusions. We are in the process of generating evidence to verify this new perspective.

Summary

Events that lead to the emergence of a MT population that replaces WT infection during HBeAg seroconversion or during NAs treatment have been well observed and described in a variety of publications [10–45]. However, these reports often focus only on the viral genotypic and clinical phenotypic profiles of MTs. Important sequential events, including the depletion of early infected viruses and expansion of MT populations, have not yet been recognized as indications of frequently occurring viral clearance and reinfection, which prolong the course of chronic HBV infection. Diverse responses and variable manifestations in chronic HBV infection create very complicated pictures for full comprehension. Fortunately, decades of research have accumulated high volume of very valued data and new studies provide us new insights. Our understanding of natural course of chronic HBV infection will keep improving. We believe that our proposed new perspective will incite new discussions on HBV-related pathogenesis and the natural course of chronic HBV infection and contribute to the development of new antiviral strategies.

Compliance with ethics guidelines

Yong-Yuan Zhang, Ke-Qin Hu, and Zhongping Duan declare that they have no conflicts of interest. This manuscript is a review article and does not involve a research protocol requiring approval from a relevant institutional review board or ethics committee.

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