Immune Tolerance and Hepatitis B

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Abstract

Chronic hepatitis B (CHB) infection is the major cause of liver cirrhosis and hepatocellular carcinoma. Current treatment for CHB aims to suppress viral replication with little emphasis on viral eradication, and lifelong therapy is required in the majority of infected patients. CHB infection is, particularly in Asia, the result of virus transmission from HBV^+ mothers to their infants/children. HBV is thought to exploit the immaturity of the host immune system by inducing a state of immune tolerance that facilitates HBV persistence. Consequently, treatment is generally not recommended in "immunotolerant" children or young patients due to the presumably lack of disease/immune activity and poor treatment responses. However, recent advances in our understanding of the immunopathological manifestations of the disease challenge the concept of a generic immunotolerant state in CHB-infected children/young patients, with immunological, histological, and virological evidence supporting an underlying active disease. Thus, we propose a need to redefine the major phases of CHB infection, with the "high replicative, low inflammatory" phase replacing the classical "immune tolerant" phase. With many new and exciting HBV therapeutic strategies in the development pipeline, together with our changing perceptions of the disease, we address the potential to consider earlier therapeutic intervention in young patients to better harness their immune system to achieve a "functional cure."

Keywords

Hepatitis B · Vertical transmission · Immune tolerance · HBV-specific T cells · Trained immunity · Antiviral therapy · Immunomodulator

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8.1 Global Importance of Hepatitis B

The World Health Organization (WHO) estimates that approximately 240 million people, or about 4% of the global population, are chronically infected with hepatitis B virus (HBV) representing a major public health problem [1, 2]. About half of the world's population with chronic

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HBV lives in areas of high endemicity, including Asia and sub-Saharan Africa, and the development of chronicity, particularly in Asia, is usually the result of virus transmission from HBV^+ mothers to their infants. Despite the development of an effective HBV prophylactic vaccine since the 1980s [3, 4], the prevalence of the virus continues to increase, and liver-related morbidity and mortality due to chronic hepatitis B (CHB) including cirrhosis and hepatocellular carcinoma account for more than 600,000 deaths per year [5].

Hepatitis B virus is a hepatotropic, non-cytopathic, DNA virus that causes acute or chronic liver diseases characterized by different levels of liver inflammation and viral replication. An acute HBV infection is often self-limited and resolves without any clinical symptoms or with acute liver inflammation (acute hepatitis). Other patients fail to clear the virus and develop chronic infection. Persistent HBV infection can cause minimal pathological manifestations or trigger chronic liver inflammation that develops into liver cirrhosis or cancer [2]. These different clinical and virological profiles are determined by a complex interplay of host and viral factors including host genetic background, dose or route of infection, viral genotype, and age of the patient at the time of infection [6, 7]. The natural history of chronic HBV is considered to evolve through a number of distinct disease phases reflecting different points in the host-virus relationship. Thus, understanding the concept of these different phases not only sheds light on the pathogenesis of chronic HBV infection but also helps determine the optical timing and strategy of antiviral therapy toward the ultimate goal of HBV cure.

8.2 Revisiting the Natural History of Chronic Hepatitis B

Classically, the natural history of CHB is divided into four distinct chronological phases: immune tolerance, immune clearance, inactive carrier, and reactivation phases. In recent years, our understanding of HBV immunopathogenesis and virology has improved considerably. This has prompted researchers into redefining the phases of infection during the natural course of CHB, given that the classical definition of disease phases may not accurately reflect the true immunological status of patients in each phase. The course of HBV infection depends largely on the age of the patient at the time of infection, with more than 90% chronicity following vertical or perinatal transmission, 20–30% between the ages of 1–5 years, and less than 5% in immunocompetent adults [8, 9]. Based on our newfound knowledge on the immunopathogenesis and virology of the disease, we propose an alternative interpretation of the natural history of CHB: high replicative low inflammatory or HRLI (previously known as "immune tolerance"), low replicative high inflammatory or LRHI (previously known as "immune clearance"), non-replicative or NR (previously known as "inactive carrier"), and reactivation phase (Fig. 8.1). It is important to note that CHB is a dynamic disease and the stages of infection are not always sequential, with the possibility of transition from one phase to another in any direction [10]. It should also be noted that not all patients will go through all four phases, and arguments against such new definitions have raised an interesting debate [11].

Nevertheless, what we proposed to define as the initial "high replicative, low inflammatory" or HRLI phase (previously termed "immune tolerance") is characteristically associated with perinatal infection or infection acquired during early childhood, the predominant mode of HBV transmission in Asia or Africa [4]. In patients infected perinatally or during early childhood, there is commonly a prolonged period of high serum HBV DNA levels, positive hepatitis B e antigen (HBeAg) in the sera, normal or low serum alanine aminotransferase (ALT) levels, and minimal or no liver inflammation that usually lasts from a few years to several decades without disease progression [6, 8, 12]. It is thought that the establishment of persistent infection is due to immaturity of the neonatal immune system, i.e., the inability to mount a virus-specific immune response [7], or deletion/exhaustion of HBV-specific T cells *in utero* due to high levels of viral antigenemia from the mother [13]. CHB is believed to run a benign

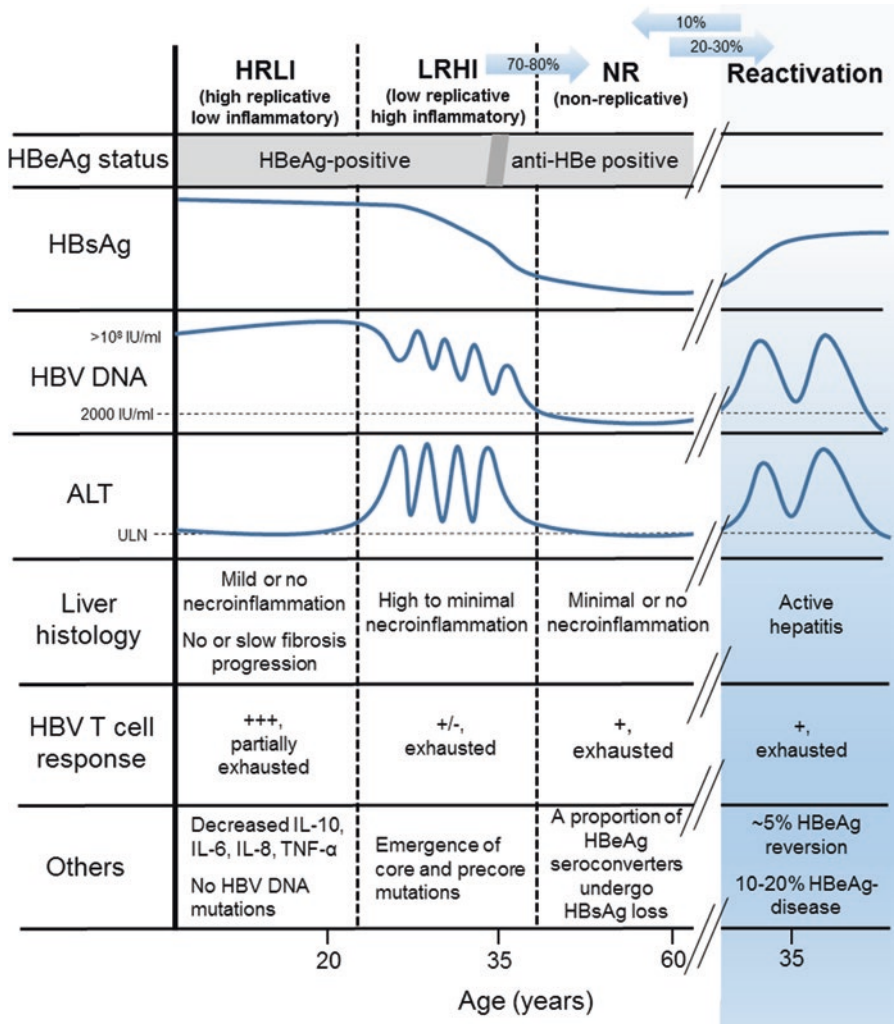

Fig. 8.1 Proposed new representations of the major phases of chronic hepatitis B infection. We propose to redefine the natural history of CHB into four major phases: high replicative, low inflammatory (HRLI); low replicative, high inflammatory (LRHI); non-replicative (NR); and reactivation. The corresponding virological, serological, biochemical, histological, and immunological characteristics are shown in the figure. These phases do not occur in all patients, and transitions between them are dynamic and can be non-sequential. ULT – upper limit of normal

course in children and young adults [14, 15], and therefore antiviral therapy is generally not recommended during this phase. However, the concept that this disease phase remains quiescent in children and young adults is now increasingly being challenged (refer next Sects. 8.3 on **Vertical Transmission of Hepatitis B: Does It Induce Immune Tolerance?** and 8.4 **Immunological and Virological Parameters During the HRLI Phase of HBV Infection**).

The transition from HRLI phase to the low replicative, high inflammatory phase or LRHI (previously known as "immune clearance") is characterized by HBeAg positivity at the outset and fluctuating levels of serum HBV DNA and ALT. ALT levels are persistently or intermittently elevated, reflecting immune activity against the virus. Consequently, HBV DNA levels fluctuate, decreasing from high (>20,000 IU/ml) to low or undetectable levels (>2000 IU/ml). Similarly,

Alt levels eventually decline to normal levels (<25 U/l for males and < 22 U/l for females or < 33 U/l for males and < 24 U/l for females according to the NHANES-derived normal values [16] and CALIPER study [17], respectively). There is high to minimal necroinflammation in the liver and the emergence of core and precore mutations in some patients. The mechanism(s) involved in the transition from the HRLI phase to the LRHI phase remains largely unknown, but it is thought to represent an "awakening" or activation of the immune response to actively "combat" HBV infection [7] and is therefore associated with immune-mediated liver injury leading to liver damage, progressive disease, and the development of fibrosis [18]. Following the LRHI phase, the majority of patients will eventually undergo HBeAg seroconversion, defined by the loss of HBeAg and the appearance of anti-HBe antibody. The remaining 10% of patients encounter recurrent hepatic flares and remain positive for HBeAg and often require antiviral therapy.

Following HBeAg seroconversion, about 70–80% of patients then enter a residual "non-replicative" or NR phase (previously known as "inactive carrier"), reflecting immune control of the virus. Transition from LRHI to NR phase is not a definite sequence of events, since it depends on the individual host's ability to control the virus. This phase is characterized by sustained low (<2000 IU/ml) or undetectable HBV DNA levels, normalization of ALT, and minimal or no hepatic necroinflammation. A small proportion of HBeAg seroconverters will eventually undergo spontaneous HBsAg loss, which may indicate either (1) the resolution of CHB, and such patients usually have good clinical outcome provided there is no underlying cirrhosis, or (2) occult HBV infection, in which there is loss of HBsAg but intrahepatic persistence of the entire viral genome as free episomal forms and, in particular, the persistence of viral cccDNA as stable chromatinized episomes. There is detectable HBV DNA in the liver and very low or undetectable HBV DNA in the serum, and in certain cases all serum HBV markers are negative [19].

A proportion of patients with HBeAg seroconversion may develop disease reactivation, with 5% experiencing HBeAg reversion [2, 20] and 10–20% of them encounter reactivation of hepatitis with elevated serum HBV DNA level (>2000 IU/ml) and fluctuations in ALT despite remaining negative for HBeAg. This phase of disease is referred as HBeAg-negative CHB, and these patients usually have low rates of spontaneous remission, with high risk of HBV complications if left untreated [21–23].

8.3 Vertical Transmission of Hepatitis B: Does It Induce Immune Tolerance?

Mother-to-child transmission (or vertical transmission) of viruses is often associated with higher levels of viral replication, a greater risk of persistent or chronic infection, and more severe disease outcome compared to those acquired during adulthood [24]. Chronic HBV infection, particularly in Asia, is caused by mother-to-child transmission of the virus. HBV infection in infants or young children is usually asymptomatic until late adulthood, when it causes liver pathologies (cirrhosis and hepatocellular carcinoma) [2]. To explain this dichotomy, HBV vertical infection is thought to induce an "immunotolerant phase" of disease, characterized by high level of HBV replication and low incidence of liver inflammatory events.

This immunotolerance hypothesis is mainly supported by data from experimental animal models (i.e., HBV transgenic animals) that showed the presence of immunological defects which impair HBV-specific T- and B cell priming in neonatal animals [13, 25, 26], thus predisposing to HBV chronicity. A recent study in 2016 by Tian et al. [27] investigated the immunological mechanisms contributing to the ability of HBV to establish chronicity after vertical infection using a mouse model of HBV persistence. The authors found qualitative and quantitative defects in HBV-specific $CD8^+$ T cells in mice born to HBeAg-transgenic mothers, and these defects were associated with the expression of the co-inhibitory ligand PD-L1 on HBeAg-conditioned macrophages. As a result, mice born to $HBeAg^+$

mothers failed to clear HBV from the liver following hydrodynamic transfection. While these data are methodologically robust, their significance in relation to HBV pathogenesis should be taken with caution, since the data supporting such immunological features are derived exclusively from HBV transgenic animals that do not support natural HBV infection. Instead, HBV virions are produced from HBV transgenes introduced into the mouse genome under the control of hepatocyte-specific promoter and as such cannot fully recapitulate the natural course of HBV infection. Therefore, a lack of appropriate animal models represents a major hurdle to study immunotolerance in HBV [28].

The concept of immunological tolerance, the basis of which the disease is managed and treatment decisions are made, is increasingly being challenged. Although the immunological data both during and after natural vertical HBV infection is limited, several epidemiological and experimental evidences can be used to challenge this concept of immunotolerance during vertical HBV infection. For example, the functionality of dendritic cells, immune cells important for the presentation and maturation of HBV-specific T cells, are intact or minimally altered in neonates of HBV^+ mothers [29–31]. Furthermore, a better analysis of T cells during natural vertical HBV infection has demonstrated that both core- and polymerase-specific T cells can be detected in HBsAg-negative children born to HBV^+ mothers in two independent studies [32, 33]. This shows that neonates born to HBV^+ mothers do not necessarily harbor defects in T cell priming and that they have the ability to mount HBV-specific T cell responses. Analysis of HBV quasispecies in children with a clinical profile labeled as immunotolerant showed a high HBV diversity [34], a virological profile that is compatible with the presence of an active immune pressure and not with complete immune tolerance during this initial phase of infection.

Furthermore, the efficacy of HBV vaccination at birth in HBV^+ children [35, 36] raises doubts that the state of complete HBV immune tolerance and the broad defects in T- and B cell interaction detected in murine models exist during natural infection. The dogma of immunotolerance in vertical HBV-infected children is also in contrast to epidemiological observations showing that HBV-related fulminant hepatitis is more frequent in infants <1 year of age compared to older subjects [37] or with the observations obtained from malaria-HBV coinfected young patients in whom reduced parasitemia [38] and increased incidences of cerebral malaria [39], a Th1-mediated malaria complication, have been reported. Such observations are more in line with the possibility of an alternative relationship between HBV and humans during early life.

The concept that the neonatal immune response is somehow "defective" or "immature" is also changing, and there is mounting evidence showing that the neonatal immune responses defy such simple categorization. Recent findings have provided new insights that the immune effectors as well as regulatory responses are already in place during early fetal life [40, 41]. Newborns have also been shown to have the ability to mount virus-specific T cell response toward viral infections in early life [42–44]. Besides, exposure of the newborn immune system toward microbes at birth can also alter the maturation status of the newborn infant. For instance, epidemiological and experimental evidences have shown that exposure to bacterial or viral infections after birth and vaccination with live vaccines can protect infants against unrelated pathogens by inducing an increased functional efficiency of their innate immune system. Such nonspecific enhancement of innate immune functionality against reinfection has been termed "trained immunity" [45]. All these earlier reports show that the immune system of newborns and infants is not "immature" or "defective" per se. Rather, it appears to be less prone to trigger a full-blown pro-inflammatory reaction, likely as an evolutionary adaptation to prevent undesirable immune reactions *in utero*.

We have recently performed a detailed characterization of the immunological parameters in the cord blood of newborns of HBV^+ mothers. Contrary to the dogma of generic immunotolerance, we found that HBV exposure *in utero* triggers a state of "trained immunity," characterized

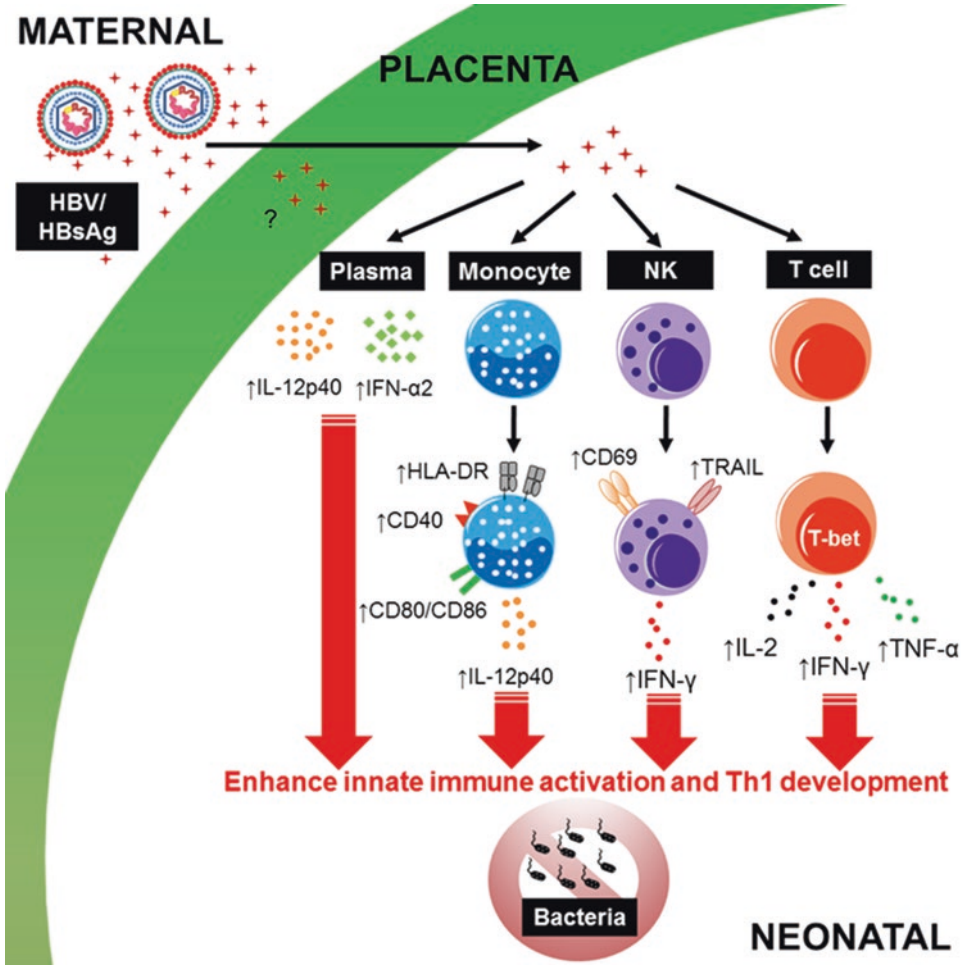

Fig. 8.2 Trained immunity in neonates of HBV⁺ mothers. HBsAg⁺ cells could be detected in the neonatal cord blood of HBV-infected mothers, demonstrating *in utero* exposure to viral products. These HBsAg⁺ cells could be due to transplacental passage of maternal HBsAg⁺ cells or active uptake of serum HBsAg by neonatal cells. HBV exposure *in utero* was associated with significantly elevated plasma levels of the antiviral cytokine IL-12p40 and, in some cases, IFN-α2. Exposure to HBV *in utero* also induced innate immune cell (including monocytes and NK cells) maturation/activation, and enhanced Th1 T cell development. Importantly, this heightened state of innate immune functionality results in a stronger ability of neonatal immune cells to respond to unrelated pathogen challenge, in a process known as "trained immunity"

by increased innate immune cell activation and Th1 development, which in turn enhances the ability of HBV-exposed cord blood immune cells to respond to bacterial infection *in vitro* [46] (Fig. 8.2). These training effects are associated with alterations in the cytokine environment. Specifically, cytokine analysis in the sera of neonates born to HBV⁺ mothers revealed a cytokine signature compatible with a Th1-like response with higher production of IL-12p40, and in some cases IFN-α2, and lower production of IL-10 and pro-inflammatory cytokines (such as IL-6, IL-8, TNF-α). This Th1 cytokine signature is more suggestive of a symbiotic relationship between HBV and humans during early life, similar to that already demonstrated in murine models of herpesvirus persistent infection [47] than to the induction of a tolerogenic response. Various

attempts to detect HBV-specific T cells in cord blood of HBV$^+$ mothers were unsuccessful, supporting the hypothesis that HBV vertical infection can preferentially tolerize HBV-specific immunity. Nonetheless, whether the absence of HBV-specific T cells in cord blood is really linked to genuine features of immunotolerance is questionable since it is difficult to study, due to ethical constraints, whether these neonates are infected by HBV (i.e., HBV replication in the hepatocytes) or are only exposed to it (i.e., HBV is present in the circulation but is not able to establish a productive infection in hepatocytes). Nevertheless, the induction of a trained immunity profile with a general Th1 response and suppression of pro-inflammatory events in HBV-exposed neonates show that the neonatal immune system can be "trained" by HBV exposure and further activated to possibly counteract unrelated pathogens during early life.

8.4 Immunological and Virological Parameters During the HRLI Phase of HBV Infection

Most of the evidence supporting an immunotolerance disease phase of HBV infection during early childhood is based on clinical and virological parameters. HBV is not directly cytopathic, and HBV-specific CD8$^+$ T cells control virus replication by recognizing and killing HBV-infected hepatocytes [48]. As a consequence, ALT is released from dying hepatocytes leading to a rise in serum ALT levels. Therefore, serum ALT is interpreted as a marker of immune activity, i.e., the presence or absence of ALT fluctuations correlates with the presence or absence of HBV-specific T cells. In this context, normal or minimal alterations in ALT levels, detectable in the majority of HBV-infected children, have been perceived as an indication of lack of HBV-specific T cell response. On the other hand, fluctuations in the levels of ALT and HBV DNA replication, more commonly observed during adulthood, are interpreted as an "awakening" of HBV-specific immunity.

In reality, both experimental data in animal models and in humans during natural HBV infection have shown that ALT measurement cannot be used as a reliable surrogate of a virus-specific T cell response. Studies performed in adenovirus-infected mice revealed that T cell immunity against hepatocytes could occur without elevation in serum ALT level [49]. Furthermore, adoptive transfer of HBV-specific T cells can lead to substantial inhibition of HBV replication without increase in serum ALT through cytokine-mediated non-cytopathic effects [50]. Direct quantification of HBV-specific T cells in the blood and liver of CHB patients has shown that, in contrast to patients with acute hepatitis B [51], the quantity of HBV-specific T cells does not correlate with ALT levels [52, 53]. Instead, robust inflammatory events in the liver causing fluctuations in ALT levels, demonstrated both in adult mice and in patients, are associated with intrahepatic recruitment of granulocytes, monocytes, and non-antigen-specific T cells [52, 54, 55].

The concept of immune tolerance phase being a quiescent disease phase with an absence of virus-specific T cells and minimal changes in liver histology is now increasingly being challenged. Our recent study of chronic hepatitis B-infected children and young adults with a clinical and virological profile labeled as "immunotolerant" showed the existence of HBV-specific T cell responses that is less compromised than that observed in CHB-infected adult patients in the "immune clearance" phase [56]. A further demonstration that these patients do not display any generic state of immunotolerance was exemplified by the superior ability of circulating T cells from young IT patients with CHB to produce type 1 T-helper cytokines including TNF-α and IFN-γ, compared to age-matched healthy controls. Besides, the production of immunosuppressive cytokines including IL-10 and IL-4 was not increased in these patients. Detailed analysis of the phenotype of T cells in these patients showed that young IT patients with CHB have increased frequency of T cells with an exhausted/inhibitory phenotype, characterized by the expression of the co-inhibitory receptor programmed death 1 (PD-1). This expression of

PD-1 is interpreted as a marker of immune activity since T cell exhaustion is a consequence of repetitive activation of reactive T cells. The frequency of exhausted T cells ($CD8^+$ T cells that are PD-1^+ and $CD127^{lo}$) increases with age in CHB patients, reflecting a progressive state of T cell exhaustion over time during the course of CHB infection. In this context, children and young adults were found to exhibit a partially exhausted T cell profile, compared to adult patients with a more exhausted T cell profile. This could explain why older patients have a less favorable response to therapy and suggest that earlier therapeutic intervention may be more advantageous in young people who lack a fully exhausted T cell profile.

In addition, there is mounting evidence that surrogate markers such as ALT that suggest quiescent disease might not reflect the true disease status and what constitutes a normal or healthy ALT level has been the subject of much discussion. The new cutoff value for adults recommended as healthy by Prati et al. [57], i.e., ≤30 U/l for men and ≤ 19 U/l for women, is lower than the traditional cutoff value of 40 U/l. For children, the cutoff values for ALT are <25 U/l for males and < 22 U/l for females according to the NHANES-derived normal values [16] or < 33 U/l for males and < 24 U/l for females according to the CALIPER study [17]. Indeed, a study by Seto et al. reported that substantial fibrosis and necroinflammatory activity already exist in the liver biopsy of some patients in the HRLI phase with the traditional normal ALT value of ~40 U/l [58]. Similarly, a recent study by Mason et al. further strengthens the concept that ongoing disease activity exists in the liver of CHB patients during the HRLI phase [59]. In this study, the immunopathological profiles of patients with HRLI disease were compared to patients with $HBeAg^+$ disease or those in the immune clearance phase. The authors showed by inverse polymerase chain reaction (PCR) that an unexpectedly high number of HBV DNA integration sites were randomly distributed across the human chromosome in all three groups of patients. These results imply that HBV DNA integration occurs not only in the later active disease phase but also during the initial, presumably quiescent, disease phase. Viral DNA integration and the resulting genomic instability are associated with the risk of developing hepatocarcinogenesis, with 80% of HBV-related HCC demonstrating clonal integrated HBV sequences [60]. Therefore, these results signify that genetic alterations promoting HCC development already exist in the initial HRLI phase, which is in agreement with previous findings suggesting the presence of viral integration even earlier at an acute phase of infection [61] or in $HBsAg^+$ children with hepatocellular carcinoma [62].

In addition to HBV DNA integration, clonal hepatocyte expansion was also detected in patients with HRLI disease at an unexpectedly high rate [59]. Clonal expansion of hepatocytes, a risk factor for the development of HCC, probably occurs in response to hepatocyte turnover mediated by HBV-specific T cell killing of infected hepatocytes, since HBV-specific T cells were detected in the peripheral blood of these patients. Furthermore, the maximum hepatocyte clone size did not differ between patients with HRLI disease and those with $HBeAg^+$ disease or in the immune clearance phase. All these findings demonstrate that promoters of oncogenesis exist in all phases of CHB infection, even in patients at the early stage of CHB infection traditionally considered "immunotolerant." Collectively, these recent findings do not support the notion that the initial HRLI phase is completely devoid of markers of disease progression or that there is a lack of immune response during this initial disease phase.

8.5 Current Treatment Recommendations for Chronic HBV Patients (Please also Read Chap. 10)

Treatment objectives in CHB are constantly evolving. Historically, the goals of therapy for CHB patients are the reduction of viremia and amelioration of hepatic dysfunction, with the hope that this would delay progression to cirrhosis and the subsequent development of HCC [12]. While a "sterilizing cure" of HBV with the

removal of cccDNA and integrated virus is difficult or impossible to achieve, most experts now agree that a "functional cure," whereby patients achieve sustained suppression of HBV viremia and loss of HBsAg after a defined course of therapy and returned to a state of health equivalent to a person who has recovered spontaneously from HBV infection, should at least be the goal of next wave of therapies [12].

Current first-line therapies for CHB remain limited to nucleos(t)ide analogues (NUCs) and pegylated IFN-α (pegIFN-α). NUCs target the reverse transcriptase function of the HBV polymerase and prevent the synthesis of viral DNA from pregenomic RNA. These antiviral agents are highly effective in suppressing viral replication, leading to ALT normalization, and more recently shown to reverse fibrosis [63]. However, they have limited effect on HBeAg seroconversion and rarely lead to HBsAg loss in the majority of patients. Furthermore, since NUCs do not directly target cccDNA, the chance of relapse after drug withdrawal is high. Consequently, lifelong therapy is usually required [64]. Another concern with long-term NUC therapy is the development of drug resistance mutations, which could lead to exacerbation of liver disease.

Conversely, pegIFN-α exhibits pleiotropic effects with antiviral, antiproliferative, and immunomodulatory properties, with the ability to halt the progression of fibrosis [65, 66]. Moreover, pegIFN-α offers the advantage of finite treatment duration (48 weeks) with the absence of antiviral resistance. Although pegIFN-α leads to slower clearance of HBV viremia, higher rates of HBeAg and HBsAg loss with anti-HBe and anti-HBs seroconversion, even though at modest levels, could be achieved [67]. However, the main disadvantages of pegIFN-α are the need for parenteral administration and the frequent side effects. Besides, pegIFN-α is contraindicated in patients with decompensated cirrhosis [68] or those undergoing immunosuppressive or cancer chemotherapy. Combination or sequential therapies with NUCs and pegIFN-α are rapidly evolving and may offer the promise of achieving higher rates of HBeAg seroconversion and HBsAg decline.

8.6 Novel Treatment Strategies in Chronic HBV Patients (Please also Read Chap. 16)

The key challenges in achieving sustained virologic control or functional cure of CHB are the persistence of nuclear HBV cccDNA and the ability of HBV to evade the host immune response recognition [69]. Exciting progress has been made in the preclinical development of new class of antivirals with novel mechanisms of action with the focus on strategies targeting cccDNA and the development of novel immune-based strategies to better harness the immune system for effective off-treatment responses.

8.6.1 Novel Antivirals Against Chronic Hepatitis B

Putative new antivirals targeting various steps of the HBV life cycle are under investigations, including viral entry inhibitors, core/capsid inhibitors, targets against cccDNA (including rcDNA-cccDNA conversion inhbitors, DNA cleavage enzymes, and small interfering RNA or siRNA-based strategies), apoptosis inducers, and HBV secretion inhibitors (Table 8.1). Viral entry inhibitor such as the well-known pre-S1-derived lipopeptide Myrcludex B targets the HBV entry receptor NTCP, thus preventing viral entry and viral replication [70–72]. Furthermore, Myrcludex B was shown, in a humanized mouse model, to reduce cccDNA formation in hepatocytes without overt cytotoxicity and pathology [73]. This makes Myrcludex B a promising candidate in targeting both active viral replication and persistence. Myrcludex B is currently being evaluated in a phase II clinical trial in Russia.

Multiple capsid inhibitors are in the pipeline, and they work by interfering with HBV RNA packaging and capsid assembly, resulting in lower intracellular capsids and ultimately undetectable HBV DNA [74, 75]. Some capsid inhibitors have been shown to interfere directly with cccDNA transcription and stability or indirectly with cccDNA formation by preventing the recycling of capsids into the nucleus to replenish cccDNA [12].

Table 8.1 Novel antivirals against chronic HBV

Type	Target	Compounds	Stage of development	References
Entry inhibitor	NTCP	HBV preS1-derived lipopeptide	Myrcludex B in phase II	[70–73]
		Cyclosporine A, ezetimibe	FDA approved but not tested for HBV	
Capsid inhibitor	HBV capsid	Phenylpropenamide derivatives	Preclinical and early clinical phase	[74, 75]
		Heteroaryl-dihydropyrimidines	Morphothiadine mesilate (GLS4) in phase II	
	rcDNA-cccDNA conversion	Disubstituted sulfonamide	Preclinical	[76]
	cccDNA	DNA cleavage enzymes	Preclinical	[77–85]
	HBV RNA	siRNA	ARC-520 in phase II	[86]
		Antisense	ISIS-HBVRx in phase I	
Apoptotic inducer	Cellular inhibitor of apoptosis proteins	Birinapant	Preclinical	[87]
Secretion inhibitor	HBV secretion and budding	Benzimidazole BM601	Preclinical	[88]

Novel therapies directly targeting cccDNA are the focus of current therapeutics, which may show promise in the treatment of CHB. Some examples include disubstituted sulfonamide (DSS) compounds that inhibit the conversion of rcDNA to cccDNA [76] and DNA cleavage enzymes such as zinc finger nucleases, homing endonucleases, and transcription activator-like effector nucleases that directly degrades cccDNA [77–80]. A therapeutic strategy that has generated great interest is the development of the CRISPR-Cas9 genome editing tool, which has shown promise in removing cccDNA *in vitro* [81, 82] and recently in HBV-infected mice [83–85]. However, both theoretical and practical considerations have to be taken into consideration for future therapeutic application in CHB patients. Another direct antiviral strategy against cccDNA is based on RNA interference (RNAi). A phase II study performed in HBeAg- CHB patients showed that the combination of entecavir and the HBV siRNA ARC-520 resulted in 50% reduction in HBsAg levels in treated patients compared to placebo [86] and similar success has been demonstrated with other siRNA platforms in preclinical models.

In addition to interfering with cccDNA formation and stability, novel drugs targeting other host cell pathways are being developed. This includes a new class of antivirals that target the apoptotic mechanism of infected hepatocytes, leading to cytolysis and clearance of HBV. An example is birinapant (TL32711), a second mitochondrial-derived activator of caspases (SMAC) mimetic that antagonizes the cellular inhibitor of apoptosis proteins. Birinapant has been shown to improve TNF-mediated killing of HBV-infected hepatocytes and reduce HBV DNA load and HBsAg production in a mouse model [87], suggesting the potential to translate this drug from treating cancers to CHB. Lastly, several inhibitors of HBV secretion have been described that could decrease HBV DNA levels and interfere with HBsAg release, thereby restoring antiviral immunity. The benzimidazole BM601 has been reported to selectively inhibit HBsAg relocalization to the Golgi, thus decreasing HBsAg release, HBV maturation, and secretion [88]. However, the drawback of such inhibitors is the accumulation of HBsAg leading to storage diseases, and the blockage of mature virion release could increase intracellular cccDNA pools.

8.6.2 Immunomodulators

A different therapeutic area for CHB is the development of immunotherapies that (1) activate

Table 8.2 Immunomodulators against chronic HBV

Type	Target	Compounds	Stage of development	References
Innate immune system modulator	TLR7 activation	GS-9620	Phase II	[90–92]
	RIG-I and NOD2 activation	SB-9200	Phase II	[94, 95]
	T cell, NK activator, cytokine production	Thymosin-α1	Phase IV	[96, 97]
	Protein kinase activation	Nitazoxanide	Phase I	[98]
Therapeutic vaccine	T cell	Recombinant HBsAg/HBcAg	ABX 203 in phase II/III	[99]
		Recombinant HBsAg	Engerix-B in phase I/IV	
		Fusion X-S-Core proteins	GS-4774 in phase II	[104]
		HBV CTL epitope	CY-1899 in pilot study	[100]
		HBV DNA	DV-601 in phase I	[101–103]
			DNA vaccine pCMVS2.S in phase I/II	
		Autologous monocytes	*In vitro*	[105]
Adaptive immune system modulator	PD-1 blockade	Nivolumab	Phase II	[107]
	Genetically modified T cells	Engineered HBV-specific T cells using CAR or HLA-restricted TCR	CAR T cells in preclinical	[109–111]
			HLA-restricted TCR-redirected T cells in phase I	
Inflammation modulator	Antiplatelet	Aspirin/clopidogrel	Preclinical	[113]

intrahepatic antiviral immunity and (2) restore HBV-specific T- and B cell immunity. Immunomodulatory compounds exhibiting activity against HBV in preclinical or clinical development include TLR agonists, therapeutic vaccines, immune checkpoint inhibitors, and engineered T cells (Table 8.2).

Agonists of TLRs 3, 8, 7, and 9 have been shown to have anti-HBV effects in animal models [89]. Of these, GS-9620, an oral TLR7 agonist, has been shown to reduce HBV DNA levels in serum and livers of chronic HBV-infected woodchucks and chimpanzees [90, 91]. Upon stimulation of TLR7, plasmacytoid dendritic cells produce high levels of IFN-α and other cytokines, resulting in activation of natural killer cells and cytotoxic T lymphocytes. Despite its success in preclinical setting, a recent clinical trial testing the efficacy of low-dose TLR7 agonist in CHB patients showed lack of clinical efficacy, even though treatment was safe and well tolerated [92]. The use of another TLR agonist, such as TLR8, may be a potential new therapeutic target since it has been reported that activation of TLR8 efficiently triggered IFN-ɣ production in human intrahepatic environment [93]. In addition to TLR agonists, other small molecule modulators of innate immunity are being tested for their anti-HBV effects: SB-9200, which activates the RIG-I/NOD2 pathway [94, 95], and thymosin α1 [96, 97] and nitazoxanide [98], which induce the production of IFN and/or activation of T- and B cells.

Numerous therapeutic vaccine approaches for CHB have been explored, including antigen-based vaccines [99], CTL epitope vaccine [100], and DNA-based vaccines [101–103]. Although these strategies demonstrated good safety and tolerability profiles with robust immunogenicity, they do not show significant clinical benefit in

chronic hepatitis B patients [104]. One likely explanation is the exhaustion of HBV-specific T cells caused by prolonged exposure to high levels of soluble HBV antigens. Therefore, factors that are known to impair the effectiveness of therapeutic vaccines need to be reversed, at least partially, to increase the efficacy of therapeutic vaccines. As an alternative to vaccine therapy, an interesting approach to consider is the use of autologous monocytes to present personalized HBV antigens in CHB patients [105].

T cell exhaustion is a hallmark of human chronic viral infections and is characterized by the increased expression of various inhibitory receptors such as programmed death-1 (PD-1) and cytotoxic T-lymphocyte antigen-4 (CTLA-4), among others [106]. PD-1 is strongly upregulated in circulating HBV-specific T cells in CHB patients, and blockade of this inhibitory signal restores HBV-specific T cell functionality. A recent phase I/II trial of nivolumab, an anti-PD-1 monoclonal antibody, in HCC patients infected with HBV showed some clinical benefit, suggesting the potential to use such therapy in patients with HBV-related HCC [107]. Another underlying molecular defect of exhausted HBV-specific T cells was recently reported in the mitochondria, which showed defects in depolarization [108]. The cytokine IL-12 was shown to recover mitochondrial potential and oxidative phosphorylation of HBV-specific T cells *in vitro*, highlighting the prospect of targeting mitochondrial or other metabolic defects as novel therapeutic approaches to restore antiviral T cell responses.

Another exciting approach to immunotherapy is to engineer patients' immune cells, such as T cells to eliminate HBV-infected hepatocytes. This could be achieved via the expression of chimeric antigen receptors (CARs) or HLA-restricted T cell receptors (TCRs) on T cells that enable HLA-independent or HLA-dependent recognition and killing of HBV-infected hepatocytes. CAR-engineered T cells were recently shown to have potential promise in mouse models of HBV [109], but no human data are available, whereas T cells engineered to overexpress HBV-specific TCR have been shown to recognize HBV-infected hepatoma cells *in vitro* and *in vivo* [110] and can significantly reduce HBsAg levels in a patient with HBV-associated hepatocellular carcinoma [111]. Newer approaches utilizing mRNA electroporation, instead of retroviral vectors, to overexpress HBV-specific TCRs are underway. These electroporated T cells have been successful in reducing tumor growth in HCC mouse models [112] and have unique antiviral effects *in vitro* without overt cytotoxicity. Thus, this highly individualized therapy could become a feasible option in advancing therapies for CHB patients.

Finally, CHB can be viewed not only as a viral disease but also a necroinflammatory disease. To this end, anti-inflammatory agents designed to inhibit liver inflammatory events such as antiplatelet therapy to inhibit the development of hepatocellular carcinoma in transgenic mice [113] may prove to be important in controlling CHB infection. Thus, the goals of immunotherapy are to increase antiviral immunity and to reduce the chronic inflammatory process with the ultimate aim of achieving a reliable cure for HBV.

8.7 Future Treatment Strategies for Chronic Hepatitis B: Can We Treat Earlier or at a Younger Age?

With major advances in HBV therapeutics in recent years, together with our increased scientific insights into the pathogenesis of CHB, it is now an exciting moment for the treatment of CHB patients. However, it remains debated whether young CHB patients in the initial HRLI disease phase are indicated for treatment. Current guidelines from the international liver associations recommend treatment for CHB patients only when they show signs of clinically active disease or development of fibrosis, typically after the age of 30 years old. Yet, symptoms of advanced disease often appear later in life, at a stage when little can be done to alter the disease course. This could explain the poor response rate to therapies observed in adult patients, thus highlighting the limitations of current practice and the

need to better define the optimal timing for treatment. The recent new findings discussed above challenge the concept of immunotolerance from HBV-exposed newborn infants to children and young adults and provide further support for considering earlier therapeutic treatment in young patients with CHB, a patient cohort currently excluded from treatment consideration.

A point to note is that there is a paucity of data on the treatment of children and young adults, the patient population which is highly viremic and infectious with the highest risk of disease progression and HCC development [114]. A small pilot study by D'Antiga et al. [115] investigated the effect of combination therapy with lamivudine and pegIFN-α in children with HRLI disease. The results were encouraging, evidenced by a beneficial response to early treatment in a proportion of young patients. These beneficial responses were associated with an increase in HBV-specific T cell proliferation, reduction in HBV DNA levels, and notable increase in HBsAg seroconversion, thus providing further support for the potential benefit of early treatment in CHB patients [115, 116]. Similar studies performed in CHB-infected children have demonstrated that IFN-α was well tolerated and children less than 5 years of age may have an enhanced response to IFN-α [117]. Likewise, the efficacy of tenofovir disoproxil fumarate was comparable between CHB-infected adolescents (<18 years old) with that observed in adult subjects [118]. Nonetheless, extended longitudinal follow-up observations of these young patients will undoubtedly provide more insights on the rates of HBeAg and HBsAg loss as well as the rates of seroconversion, in order to determine whether earlier treatment is genuinely associated with better treatment outcomes.

The emerging concept of "trained immunity" discussed above [46] also has significant therapeutic implications. Rather than being immature or tolerized, we should keep in mind that the immune system of neonates or even young children is already "trained" or "matured" following birth and is actually capable of responding immunologically with broad cross-protective responses toward viral antigens. In this context, therapies that reduce viral protein expression may "release the brakes" on the immune system of these young patients, allowing it to be fully competent to achieve long-term suppression of HBV. On the other hand, our findings that young CHB patients in the initial HRLI phase have a less compromised HBV-specific T cell response compared to adult patients in the LRHI phase [56] suggest that therapeutic interventions aimed at enhancing HBV-specific immunity are likely to be more effective in young CHB patients compared to adult patients.

8.8 Summary

CHB infection is a complex dynamic disease where the timing and most appropriate treatments continue to be debated. We have shown that CHB-infected children and young adults, a patient population historically considered to be "immunotolerant," are less likely to run a benign course of disease since liver histology, T cell responses, DNA integration, and hepatocyte clonal expansion all point toward underlying disease and immune activity. A better understanding of trained immunity and how HBV establishes a permissive state in the host may pave the way for better development of therapies that is targeted toward these group of patients currently not indicated for treatment. This could potentially result in expanding therapeutic options to more patients, including treating at a younger age and at a much earlier stage of disease. If earlier treatment is advocated, careful consideration should be taken into account for pediatric patients, in whom the safety, efficacy, and adverse effect profiles of NUCs have not been well established compared to the adult population.

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