Hepatitis B virus persistence and reactivation

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ABSTRACT

Hepatitis B virus (HBV) infection causes chronic hepatitis and has long term complications. Individuals ever infected with HBV are at risk of viral reactivation under certain circumstances. This review summarizes studies on HBV persistence and reactivation with a focus on the definitions and mechanisms. Emphasis is placed on the interplay between HBV replication and host immunity as this interplay determines the patterns of persistence following viral acquisition. Chronic infections exhibit as overt persistence when a defective immune response fails to control the viral replication. The HBV genome persists despite an immune response in the form of covalently closed circular DNA (cccDNA) and integrated DNA, rendering an occult state of viral persistence in individuals whose infection appears to have been resolved. We have described HBV reactivation that occurs because of changes in the virus or the immune system. This review aims to raise the awareness of HBV reactivation and to understand how HBV persists, and discusses the risks of HBV reactivation in a variety of clinical settings.

Introduction

HBV infection is a global health issue. Despite long term control of virus replication, current antiviral therapies are unable to eradicate HBV in patients with chronic infection.1 Studies show that HBV may persist even after patients with acute or chronic infections have completely recovered.2 6 To better understand HBV persistence, studies are under way to investigate the viral replication cycle,5 improve the sensitivity of assays for detecting viral genome,6 8 and to analyze data on HBV reactivation and transmission in patients who test negative for hepatitis B surface antigen (HBsAg).9 10 Although HBV is known potentially to persist once an individual is infected, the breadth of HBV persistence and thereby risk of reactivation may not be well recognized by physicians treating patients with comorbidities such as solid and hematologic malignancies, organ transplants, and rheumatoid and inflammatory diseases, or patients who are prone to HBV reactivation. Better recognition of the risk of HBV reactivation is essential to optimize specialty care.

This review assesses the evidence on HBV persistence and reactivation, with a focus on the underlying mechanisms. It aims to increase the awareness of HBV persistence and to understand the risks of reactivation from the persistent state.

Global epidemiology of HBV infection

An estimated two billion individuals alive worldwide have been infected by HBV at some time in their life.11 Individuals ever infected with HBV are seropositive for anti-hepatitis B core antigen (anti-HBc). A study using a national database of 47 484 individuals aged 2 or older indicated that the prevalence of anti-HBc during the period 1999-2008 in the US was 4.6%, highest in individuals over 70 (7.6%).12 A cross sectional study in 2006 reported anti-HBc prevalence of 34.1% in 81 775 residents aged 1 to 59 across China.17 Two studies published in 2016 that enrolled more than two million men and 764 460 women in rural China showed that the anti-HBc prevalence was 9.08% in men aged 21-49 and 13.24% in women of childbearing age.14 15 Another seroprevalence study in 10 European countries indicated that the prevalence of anti-HBc is 1.3–20.5%.14 Approximately 250 million individuals are chronically infected with HBV.17 The 2017 Global Burden of Disease Study estimated that HBV infection accounted for 799 000 deaths annually, and that most cases of cirrhosis and liver cancer are HBV related.18 Geographic variation in the prevalence of chronic HBV infection ranges from <2% in low prevalence areas (eg, US, Canada, western Europe) to 2-7% in intermediate prevalence areas (eg, Mediterranean countries, Japan, China, Central Asia, and parts of South America) to >8% in high prevalence areas (eg, western Africa).

Sources and selection criteria

We searched PubMed for studies published in English between January 1998 and December 2019. We used the following search terms in combination with the term “HBV” or “hepatitis B”: “life-cycle”, “metabolism”, “epidemiology”, “natural history”, “cure”, “cccDNA”, “integrated DNA”, “clearance”, “innate immunity”, “adaptive immunity”, “physiopathology”, “mortality”, “pathogenesis”,

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Cite this as: BMJ 2020;370:m2200
http://dx.doi.org/10.1136/bmj.m2200

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“persistence”, “occult infection”, “reactivation”, and “guidelines”. When evaluating the potential risk of HBV reactivation caused by a specific drug or therapy, we searched for the non-proprietary or proprietary name of the agent or therapy in combination with the term “HBV” or “hepatitis B.” Regarding mechanisms, we prioritized studies with replicated data in humans, chimpanzees, or humanized chimeric mice when available, but also included those performed in other animal models such as woodchucks and mice or in vitro primary hepatocytes or cell lines, especially in terms of life cycle. We also cited perspectives from high quality reviews published in peer reviewed journals and influential original studies published before 1998. For clinical evidence, we prioritized guidelines, technical reviews, randomized controlled trials, or high quality prospective observational studies or their meta-analysis when available. However, for clinicians’ interest, we included observational studies or case reports about HBV reactivation in certain drugs or therapies, even when the causal relations are uncertain.

HBV structure, genome, and life cycle
HBV is a prototypical member of the Hepadnaviridae family. As shown in figure 1, a complete HBV virion comprises an outer envelope, an inner nucleocapsid, and a 3.2 kb partially double stranded DNA, also known as relaxed circular DNA (RC-DNA), linked to the DNA polymerase inside. The HBV genome contains four overlapping open reading frames: preS1/S2/S, pre-core/core, polymerase, and X domains, which encode seven viral proteins (fig 1).19

After transmission, HBV arrives at the liver through the bloodstream and infects the hepatocyte. The HBV virions bind to hepatocytes by interacting with heparan sulfate proteoglycans for virus docking20 and subsequently with a functional receptor, sodium taurocholate co-transporting polypeptide (NTCP).21 An internalization mechanism that likely results from endocytosis then mediates the entry of HBV virions into the host cell cytoplasm.22 Following viral entry, the nucleocapsid is released into the cytoplasm and transported to the nucleoplasm,33 where the RC-DNA is released and converted into a “plasmid-like” cccDNA molecule34 and forms a viral mini chromosome wrapped around histones and non-histone proteins.25 26 When RC-DNA fails to convert to cccDNA, the aberrant DSL DNA of HBV can be a substrate for viral integrations into the host genome.27 Though not required for completion of the HBV replication cycle, integrated viral DNA may produce viral antigens such as HBsAg and truncated HBxAg via cccDNA independent pathways.29

The episomal cccDNA serves as the template for transcriptional production of pgRNA and several subgenomic RNAs necessary for protein production and viral replication. In the cytoplasm, pgRNA is encapsidated with the polymerase protein and reverse transcribed into progeny RC-DNA.5 The mature RC-DNA containing nucleocapsids are then either coated with HBsAg and released from infected hepatocytes via the multivesicular body pathway30 or directed to the nucleus to establish a cccDNA pool.31 HBsAg can be produced in excessive amounts and released from cells as empty subviral particles through the cell secretory pathway7

Course of HBV infection and reactivation
HBV is transmitted by perinatal, percutaneous, or sexual exposure, and potentially other close person-to-person contact.32 Exposure to HBV can result in acute infection, manifesting from subclinical to icteric hepatitis, and even fulminant hepatitis in rare cases. In adults, more than 95% of HBV infections are self-limited,32 with the disappearance of serum HBV DNA, appearance of hepatitis B core antibody (anti-HBc), HBeAg to anti-HBe serocconversion, and, finally, HBsAg to hepatitis B surface antibody (anti-HBs) serocconversion during recovery. Despite the serologic resolution, traces of HBV DNA may persist in the liver for years or decades.2 33

In contrast, perinatal transmission leads to chronic infection in 90% of newborns in the absence of appropriate HBV immunoprophylaxis and is responsible for most chronic infections in endemic regions.32 Figure 2 shows the course of chronic HBV infection, which is classified into four phases based on biochemical, serologic, and histologic assessments. These phases reflect the dynamic interplay between viral replication and the host immune response:

1. Immune tolerant HBeAg positive phase
2. Immune active or clearance HBeAg positive phase
3. Inactive HBeAg negative phase
4. HBeAg negative chronic hepatitis B16-36 (fig 2).

A minority of individuals with chronic infection may eventually achieve HBsAg clearance with or without the appearance of anti-HBs. This occurs either spontaneously or rarely by antiviral therapy, similar to individuals who naturally recover from acute infection, and has been classified as the “HBsAg negative phase” in the course of chronic infection.34 Likewise, HBV DNA may persist in the liver, with undetectable or fluctuating very low level viremia.34

In summary, HBV may persist after serologic resolution and thereby confers the potential to reactivate (fig 2). HBV reactivation can occur spontaneously or as a complication of therapy for a concomitant medical condition, especially when an immunosuppressive therapy is given for malignancies, bone marrow or solid organ transplant, and inflammatory or autoimmune diseases. HBV reactivation starts with viral replication, followed by liver injury that results from a delayed immune reconstitution.37 The severity of liver injury varies greatly among individuals, ranging from an asymptomatic rise in alanine transaminase levels to severe hepatitis, or even liver failure.37

doi: 10.1136/bmj.m2200 | BMJ 2020;370:m2200 | the bmj
Fig 1 | Molecular biology of HBV. (A) Virion structure: a complete HBV virion comprises an outer envelope with the lipid embedded small (S), middle (M), and large (L) surface proteins, an inner nucleocapsid consisting of a core protein, and the RC-DNA covalently linked to the DNA polymerase inside. (B) Viral genome: the highly compact HBV genome (shown as the two outer DNA strands) encompasses four overlapping open reading frames (ORFs, depicted in the center): preS1/S2/S, pre-core/core, polymerase, and X domains. The transcription of ORFs is regulated by promoters (green arrows), enhancer I/II, direct repeat (DR)1/2 and ribonucleic acid (RNA) primer on DNA (wavy red line). (C) The life cycle of HBV: the solid lines and arrows indicate the steps necessary for viral replication as follows: HBV virion enters the hepatocyte (a) and releases the nucleocapsid into the cytoplasm (b). The nucleocapsid is then transported to the nucleoplasm and releases P linked RC-DNA (c), where it is converted into cccDNA and forms a viral mini chromosome (d). Viral messenger RNAs are transcribed (e) and translated into HBV proteins (f). The polymerase and pre-genomic (pg)RNA are packaged into newly forming nucleocapsids (g) where the first (h) and second strand DNA are synthesized to generate new RC-DNA (i). The mature RC-DNA containing nucleocapsids are coated with envelop proteins (j) and the new virions are either exported from hepatocytes through the multivesicular body (MVB) machinery (k) or directed to the nucleus to establish a cccDNA pool (l). The broken lines and arrows represent viral activities not needed for replication: HBx and hepatitis B e antigen (HBeAg) are produced (f). HBeAg and a large number of subviral particles (SVP) are released via the general secretory pathway (m). Double stranded linear (DSL) DNA is synthesized (n), exported into the nucleus (o), and integrated into host genome (p). The transcription of integrated HBV DNA (q) may produce HBsAg and truncated HBxAg (r). Adapted and modified from Nassal19.
### Definition of HBV persistence

Clinical recovery does not necessarily indicate a complete cure of HBV infection, as a small amount of HBV genome, cccDNA as well as integrated viral DNA, may persist in the nucleus of hepatocytes. Therefore, HBV persistence can be regarded as a shift from an “overt” to an “occult” state, depending on the detection of viral markers (box 1). The overt state, known as chronic infection, is defined as persistence of HBsAg for six months or more after acute infection with HBV. The occult infection is defined as the presence of HBV genome in the liver, with the loss of detectable serum HBsAg.

The presence of cccDNA that is fully replication competent is essential to establish occult HBV infection. Theoretically, a complete cure is possible when HBsAg for six months or more after acute infection with HBV. The occult infection is defined as the presence of HBV genome in the liver, with the loss of detectable serum HBsAg.

### Mechanisms of HBV persistence

#### Immune control after viral acquisition

Identifying patients with acute HBV infection at the earliest stage is challenging, so this review includes studies on human acute infection at relatively later stages, and animal studies using chimpanzees, woodchucks, HBV humanized or transgenic mice, and in vitro cell culture.

Upon HBV infection, the virus does not induce intracellular antiviral interferon responses in the hepatocytes, as seen in HBV inoculum of primary human hepatocytes and hepatic cell culture systems. Genomic analysis of the liver in chimpanzees acutely infected with HBV, and mice engrafted with human hepatocytes, show that the type I interferons in pre-symptomatic patients are undetectable in blood. HBV acts as a “stealth virus” that escapes recognition by the innate immunity of infected hepatocytes. This could be attributed to the replication of HBV, which uses a transcriptional template localized within the nucleus with a host resemble chromatinised structure and produces new viral progenies with the protection of

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**Fig 2 | Phases of HBV persistence and reactivation**

The shaded left column reflects the level of HBV replication. The solid red curve indicates evolution of viral replication activity in acute, chronic, and occult infection. The blue dotted line indicates the hypothetical phase of complete cure. The red dotted curve represents viral replication activity in the phase of reactivation. HBeAg=hepatitis B e antigen, HBsAg=hepatitis B surface antigen, cccDNA=covalently closed circular DNA.

<table>
<thead>
<tr>
<th>Acute infection</th>
<th>Chronic infection</th>
<th>Reactivation</th>
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<tr>
<td>HBsAg positive chronic infection</td>
<td>HBsAg negative chronic infection</td>
<td>Detection threshold of serum HBsAg</td>
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<tr>
<td>HBsAg positive chronic infection</td>
<td>HBsAg negative chronic infection</td>
<td>Detection threshold of serum HBV DNA</td>
</tr>
<tr>
<td>Complete cure</td>
<td>Rejection</td>
<td>Eradication of cccDNA and integrated HBV DNA</td>
</tr>
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Ultra-sensitive, standardized, and valid assays for HBV cccDNA detection in the liver that can differentiate cccDNA from other forms of viral genome are lacking.

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Box 1: Definitions of HBV clearance and persistence

- **Resolution of HBV infection**—sustained, undetectable HBsAg and HBV DNA in serum with or without seroconversion to anti-HBs after resolution of acute infection, or recovery from chronic infection either spontaneously, or following antiviral therapy (also known as “functional cure” in this setting).
- **Complete cure**—complete eradication of HBV cccDNA and integrated DNA from each hepatocyte.
- **Overt infection**—detectable HBsAg in serum
- **Chronic infection**—sustained, detectable HBsAg for at least six months in serum
- **Occult infection**—the presence of replication competent HBV DNA (i.e., episomal HBV cccDNA) in hepatocytes in absence of detectable serum HBsAg

HBV cccDNA is stable in quiescent hepatocytes, as inferred from in vitro experiments measuring hepadnavirus cccDNA in non-dividing primary woodchuck hepatocytes. Furthermore, cccDNA amplification is HBV independent, because duck HBV neutralizing antibodies do not block cccDNA amplification in cultured cells. However, a sharp decrease in the volume of intra-hepatic cccDNA in the pool also occurs during the HBV clearance phase of acute infection, as shown in infected chimpanzees. This is likely the result of multifactorial mechanisms, including the killing of infected hepatocytes, cccDNA dilution or loss via cell division, or antiviral cytokines that inhibit HBV nucleocapsid formation and thereby prevent cccDNA recycling. It is also reported that IFN-α, TNF-α, and IFN-γ can upregulate APOBEC3 deaminases, which target cccDNA for depurination and degradation. However, IFN-α does not reduce cccDNA at the concentrations of inhibiting viral antigens and DNA replication in primary human hepatocytes. Although the intra-hepatic cccDNA pool can be substantially reduced during the clearance phase of acute infection, a low level of residual cccDNA may persist in a small fraction of hepatocytes. The exact lifetime of the remnant cccDNA is unclear, but is expected to be long, based on modeling analysis of cccDNA decline kinetics in patients undergoing treatment with nucleoside analogues; however, the transcriptional activity is tightly controlled by the host’s immunologic and presumably epigenetic mechanisms. Although HBsAg can be cleared, HBV may still keep replicating at a low level in some patients. Furthermore, integrated viral DNA may survive immune clearance in patients with resolved acute HBV infection. Woodchuck experiments further showed that integrated viral DNA persists in liver tissues from recovered animals at essentially undiminished levels of one viral genome per 1000 to 3000 liver cells for weeks.

### Failure of immune control during chronic infection

Chronic HBV infection can be regarded as a prolonged state of overt infection. A defective antiviral immune response, particularly an adaptive immune response, is responsible for the failure of HBV control and subsequent establishment of chronic infection. In contrast to a vigorous and polyfunctional peripheral T cell response—as observed in patients with resolved acute HBV infection—the CD4 and CD8 T cell responses in chronic infection are weak and narrowly focused. HBsAg specific B cells are impaired differentially into antibody producing cells and thereby produce an undetectable amount of anti-HBs in patients with chronic infection. Bone marrow transplants from donors with pre-existing anti-HBV immunity may enable reconstitution of HBV specific adaptive immunity in patients with chronic HBV infection. Likewise, liver transplantation from donors with pre-existing anti-HBV immunity can enable control of HBV infection in recipients who are chronically infected.
Host and viral factors both contribute to the failure to induce an adequate HBV-specific immune response to acute HBV infection. Anti-HBV immunity in newborns may be induced by the HBV precore protein HBeAg, which has the capacity to cross the placenta and dampen T cell function. Infants and children are unable to mount an adequate HBV specific immune response because of the differences in their immune response compared with adults. The concept is supported by experiments which show that adoptive transfer of naive immune cells—that encode replicating viral particles from adult, but not young, transgenic mice—into adult transgenic mice results in adequate T and B cell priming and HBV control with HBsAg seroconversion. The gut microbiota, which modulate local and systemic immune responses, may also contribute to the age dependent effect on the conversion from acute to chronic HBV infection. Duration of antigen exposure might also have an influence, as evidenced by the more preserved T cell function in children and young adults with chronic HBV infection when compared with that of older adults.

To further support this theory, two randomized controlled trials reported that early initiation of antiviral therapy results in high clearance of serum HBsAg in children under 1 and highest in infants under 1. However, such recovery remains frequently incomplete and transient in adult patients, suggesting the possibility that T cells carry permanent functional changes as a result of...
long term contact with antigens. These functionally defective T cells are characterized by partially altered human leukocyte antigen/peptide tetramer reactivity, impaired receptor signaling, downregulation of mitochondrial function, altered cell metabolism, and an increased expression of negative co-stimulatory and apoptotic molecules on the cell surface. Evidence also shows that T cells within the liver are more dysfunctional than those in the periphery, suggesting that the hepatic micro-environment in patients with chronic infection contributes to the exacerbation and maintenance of T cell dysfunction.

**Definition of HBV reactivation**

Broadly, reactivation refers to significant disturbances in the balanced state between host immune control and viral replication, either from the occult to overt state of infection, or an acute exacerbation of overt state of infection owing to any cause. HBV reactivation can occur spontaneously, as a result of inappropriate antiviral therapy, or in response to medications given for comorbidities. However, HBV reactivation is frequently interpreted in the setting of immunosuppression.

As shown in table 1, various definitions of reactivation have been used in previous studies in different clinical scenarios, most of which are proposed in the setting of immunosuppression. These proposed definitions adopt either virologic or serologic criteria, or both. The adopted diagnostic elements as well as specific values of HBV DNA cut-off points are different. Although little consensus exists on a standardized definition of HBV reactivation, two categories have been established: 1) HBV reactivation in chronic infection is defined as an increase in the HBV DNA level in individuals with viremia and reappearance of HBV DNA in individuals without detectable viral DNA; and 2) Reverse seroconversion is defined as reappearance of serum HBsAg and HBV DNA in patients with occult infection. However, the application of any criteria in clinical practice requires highly sensitive HBV DNA and HBsAg assays, which confer the ability to detect HBV reactivation.

Another consideration is whether clinical aspects other than serologic and virologic profiles should be considered in the definition of HBV reactivation. A recent scoring system has been proposed, though yet to be validated, by incorporating the following two additional aspects: 1) hepatic outcomes range from an asymptomatic increase in serum transaminases to severe hepatitis with jaundice, ascites, coagulopathy to fulminant hepatitis, and liver related death; and 2) therapeutic consequences range from continuation, interruption, or change of previously intended immunosuppressive therapy, or cessation of any type of immunosuppression.

**Mechanisms and risks of HBV reactivation**

The virologic basis of HBV reactivation is the presence of replication competent cccDNA in the nucleus of infected cells. Theoretically, even if only one copy of cccDNA remains, the replication can lead to a detectable viremia within a sufficient period upon HBV reactivation. Based on the presence of cccDNA, HBV reactivation can occur with any modulation to the virus or the immune system that may disrupt the balance between the virus and host through the following three possible mechanisms:

**Immunosuppression mediated weakening of host immune control**

With immunosuppression owing to any cause, immune mediated control of HBV replication is impaired and reactivation can then occur. Severe immunosuppressive therapies, including B or T cell depleting agents, biologics, cancer chemotherapeutic agents, corticosteroids, traditional immunosuppressants, and emerging novel cell therapy, can cause immune dysfunctions and the suppression of anti-HBV immunity. The intensity of immunosuppression is determined by the types of immunosuppressive therapies and their doses, duration, or even administration routes, which vary depending on the indication and response to treatment. A retrospective study evaluated HBV reactivation in 198 HBsAg positive patients with asthma or chronic obstructive pulmonary disease and found that continuous systemic corticosteroid treatment for three months resulted in HBV reaction in 11.1% of cases, substantially higher than the reported 3.2% receiving inhaled corticosteroids. Among those receiving systemic corticosteroids, a further higher risk of reactivation has been reported in patients with a dose of ≥20 mg daily of prednisolone or equivalent. Concomitant use of immunomodulatory agents may also increase the intensity of immunosuppression. A randomized controlled trial that comprised 50 patients (25 for the ACE (epirubicin, cyclophosphamide, and etoposide) and 25 for PACE (prednisolone plus ACE)) showed that a glucocorticoid-free chemotherapy regimen significantly reduces the risk of HBV reactivation in HBsAg positive patients with lymphoma (cumulative incidence of HBV reactivation at 9 months after starting chemotherapy was 38% and 73% for the ACE and the PACE arms, respectively, relative risk 2.36, 95% confidence interval 1.05 to 5.29). However, the incorporation of rituximab leads to an increased rate of HBV reactivation. A meta-analysis of case series of 697 patients with lymphoproliferative diseases showed that 8.2% of patients who tested positive for hepatitis B core antibody (HBcAb (+)) but negative for HBsAg (HBsAg (-)) had HBV reactivation following rituximab containing chemotherapy, significantly higher than those with non-rituximab therapy (0.6%), with an odds ratio of 5.6. The highest rate of HBV reactivation was reported in patients undergoing hematopoietic stem cell transplantation, who typically receive intense chemotherapy for the underlying malignancy to induce remission, followed by additional chemotherapy and radiation.
therapy to ablate bone marrow. The HBsAg reverse seroconversion rate is reported to be approximately 10% in patients who test HBcAb (+)/HBsAg (-) after transplantation.160,161

On the other hand, the risk of HBV reactivation depends on the extent of immune control of HBV replication before the use of immunosuppressive therapies (table 3). Several studies have confirmed that patients with chronic infection have substantially higher risk of HBV reactivation than those who are HBsAg (-) in response to the same immunosuppressive therapy, as shown in table 2. Besides, the presence of anti-HBs, which suggest a more potent humoral anti-HBV immunity, further decreases the risk of HBV reactivation. A prospective study showed a doubled 2 year cumulative rate (68.3%) of HBV reactivation in patients with negative baseline anti-HBs compared with those with positive anti-HBs (34.4%) after receiving a rituximab containing chemotherapy for lymphoma.122 The extent of immune control may reflect the susceptibility of HBV specific adaptive immunity to quantity depletion and functional impairment caused by immunosuppressive therapies. Alternatively, it may reflect the quantity of cccDNA pool prepared for HBV reactivation.

Moreover, genetic changes to the virus may alter the risk of reactivation. Several observational studies revealed the presence of a high degree of S gene variability in reactivated HBV isolates, especially in HBsAg (-) patients, with or without anti-HBs antibodies.40,162 Such HBV isolates may be evade anti-HBV immunity and confer risk of reactivation. Patients infected with non-A genotype HBV are more prone to reactivation than those with genotype A infection.163 Studies show that immunodeficient, human hepatocyte chimeric mice displayed slower replication kinetics of genotype A than other genotypes.164 Viruses that replicate more slowly have been reported to create weaker cellular responses,91 which may enhance the likelihood of chronicity, but also reduce the risk of reactivation.

Additional risk factors are identified by epidemiological studies. A three times increase of HBV reactivation risk in men has been reported in a prospective study of more than 600 patients with chronic infection who received chemotherapy for cancers.165 The sex difference in the risk of HBV reactivation may be attributed to the effect of estrogen or androgen on HBV replication.166 Older age has been found to be another risk factor for HBV reactivation,167 as aging is a well known factor associated with compromised global immunity. Cases of spontaneous reactivation of HBV infection have also been reported in recovered older people, even in the absence of known triggers for reactivation.168 Underlying diseases may also affect the risk. HBV reactivation is most commonly reported in hematologic diseases, especially lymphoma;67 however, whether the reactivation is attributed to the disease itself or the use of therapies remains unclear.

<table>
<thead>
<tr>
<th>Studies</th>
<th>Rise of serum HBV DNA to baseline level</th>
<th>Absolute increase of serum HBV DNA</th>
<th>Reappearance of HBV DNA</th>
<th>Reverse HBsAg seroconversion</th>
<th>Reverse HBeAg seroconversion</th>
<th>Reappearance of Hbc IgM</th>
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### Table 2 | Immunosuppressive drugs or therapies associated with HBV reactivation

<table>
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<th>Types</th>
<th>Drugs</th>
<th>Potential diseases for treatment</th>
<th>Potential mechanisms of HBV reactivation</th>
<th>Risk of HBV</th>
<th>Chronic infection</th>
<th>Resolution</th>
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<tr>
<td>B cell or T cell depleting agents</td>
<td>Anti-CD20 antibodies</td>
<td>Rituximab, ofatumumab, obinutuzumab, ocrelizumab</td>
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<td>High (B) 127 138</td>
<td>High (A) 120 132 133</td>
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<tr>
<td></td>
<td>Anti-CD52 antibodies</td>
<td>Alemtuzumab</td>
<td>Refractory chronic lymphocytic leukemia, lymphoma, stem cell transplantation, solid organ transplantation, rheumatoid arthritis</td>
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<td>Cancer, chemotherapy agents</td>
<td>Anthracycline derivatives (doxorubicin epirubicin)</td>
<td>Systemic use for solid cancers and hematologic malignancies, TACE for HCC</td>
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<td>Others</td>
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<td>Psoriasis</td>
<td>Low (C) 138 139</td>
<td>Low (C) 146</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Others</td>
<td>Ustekinumab</td>
<td>Psoriasis and inflammatory bowel diseases</td>
<td>High (B) 155 156</td>
<td>Moderate (B) 157</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Calcineunhibitors</td>
<td>Ciclosporin, tacrolimus</td>
<td>Solid organ transplantation, rheumatoid arthritis, psoriasis, aplastic anemia</td>
<td>Low (C) 138 139</td>
<td>Low (C) 146</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mammalian target of rapamycin (mTOR)</td>
<td>Everolimus</td>
<td>Breast cancer, renal cell carcinoma, neuroendocrine tumors</td>
<td>Unclassifiable (D) 141 142</td>
<td>Data not available</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Chemokine inhibitors</td>
<td>Mogamulizumab</td>
<td>Refractory adult T cell leukemia/lymphoma</td>
<td>Data not available</td>
<td>High (C) 148</td>
<td></td>
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<td></td>
<td>Integer inhibitors</td>
<td>Natalizumab, vedolizumab</td>
<td>Psoriasis, inflammatory bowel disease, multiple sclerosis</td>
<td>Low (B) 145 146</td>
<td>Low (B) 147</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Co-stimulation inhibitors</td>
<td>Abatacept, belatacept</td>
<td>Psoriasis, inflammatory bowel disease</td>
<td>High (C) 148</td>
<td>Low (C) 148</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Proteasome inhibitors</td>
<td>Bortezomib</td>
<td>Multiple myeloma</td>
<td>Moderate (C) 137 148</td>
<td>Unclassifiable (D) 150</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tyrosine kinase inhibitors</td>
<td>Imatinib, nilotinib, dasatinib, erlotinib, ibutinib</td>
<td>Lung cancer, renal cell carcinoma, gastrointestinal stromal tumor, lymphoma, chronic lymphocytic leukemia</td>
<td>Moderate (B) 150 151</td>
<td>Low (B) 152 153</td>
<td></td>
</tr>
<tr>
<td></td>
<td>JAK inhibitors</td>
<td>Tofacitinib, ruxolitinib</td>
<td>Rheumatoid arthritis, myelofibrosis, polycythemia vera</td>
<td>High (C) 149</td>
<td>Low (C) 157</td>
<td></td>
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<tr>
<td></td>
<td>Traditional Immunosuppressants</td>
<td>Azathioprine, 6-mercaptopurine, methotrexate</td>
<td>Inflammatory bowel disease, psoriasis, sarcoidosis, autoimmune liver disease, arthritis</td>
<td>Low (D) 155 156</td>
<td>Low (D) 157</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Corticosteroid</td>
<td></td>
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| State of the art review       |                                                                 |                                                                                                                                                                                                 |                                                                                     |            |                   |            |

**Notes:**
- TACE=transarterial chemoembolization; HCC=hepatocellular carcinoma; FOLFOX-/FOLFIRI=folinic acid, fluorouracil, and oxaliplatin/folinic acid, fluorouracil, and irinotecan hydrochloride; CART=chimeric antigen receptor T cell; Jak-Janus kinase; STAT=signal transducers and activators of transcription. Grade A=high quality evidence from several randomized controlled trials or high quality observational studies with consistent results; Grade B=moderate quality evidence from several observational studies with some limitations but consistent results, one high quality study, or high quality studies with inconsistent results; Grade C=low quality evidence from several observational studies with severe limitations or several observational studies with some limitations but inconsistent results; Grade D=very low quality evidence from case reports or expert opinion. The grading system is established according to Grading of Recommendations Assessment, Development, and Evaluation. Drugs that are in the same class but without relevant report of HBV reactivation were considered to have equal risk. *Data not sufficient to classify risk of reactivation.
Increases in HBV viral replication

Several treatments or drugs can have a direct impact on HBV replication, and thereby cause HBV reactivation.

In vitro studies suggest that immunomodulatory agents such as corticosteroids not only suppress cytotoxic T cell function but also induce direct activation of regulatory elements within HBV genes in cultured human hepatoma cells,\textsuperscript{169} thereby increasing HBV replication and reactivation. In addition, the mTOR inhibitor, everolimus, can feedback-suppress HBsAg synthesis at the transcriptional level,\textsuperscript{170} and thereby can activate HBV replication.

Histone deacetylase inhibitors (HDIs) that target histone deacetylase (a histone modifying enzyme) for cancer treatment, have been reported to induce HBV reactivation in a case report.\textsuperscript{171} Histones are an essential part of the cccDNA minichromosome complex, regulating cccDNA transcription\textsuperscript{25} and acetylation of the cccDNA associated H3 and H4 histones, and have been associated with low activity of transcription and replication both in vivo and in vitro.\textsuperscript{172} Thus HDIs, which reverse the histone deacetylation, result in active HBV transcription and then HBV reactivation.

Co-infection with hepatitis C virus (HCV) presents an unusual setting for HBV replication, and the use of direct acting antivirals targeting HCV can result in HBV reactivation. A meta-analysis of 17 observational studies of 1621 patients showed that HBV reactivation occurs in 24% of patients with chronic HBV infection and 1.4% of those with resolved HBV infection (HBsAg negative but HBCAb (+)) after direct acting antiviral treatment.\textsuperscript{123} HBV replication can be suppressed by HCV co-infection. Previous in vitro studies have shown that the presence of HCV directly inhibits HBV replication.\textsuperscript{173} However, recent co-transfection experiments fail to show direct interference between the two viruses.\textsuperscript{174} Instead, it has been hypothesized that HBV replication is suppressed by host antiviral response induced by HCV infection. This is supported by recent evidence that a high level of interferon γ induced protein 10 (IP-10) is induced in HCV dominant HBV/HCV co-infected patients, and is consistent with the decline of HBsAg.\textsuperscript{175} On the other hand, no current evidence exists to show direct effects of HCV on HBV replication. Furthermore, antitroviral (ART) regimens for patients with HBV/HIV co-infection are recommended to contain nucleoside with anti-HBV activity (eg, tenofovir or tenofovir alafenamide) and thus control HBV replication during lifelong ART treatment.

Unknown mechanisms

Immune checkpoint inhibitors are increasingly used in the treatment of a broad spectrum of solid cancers by restoring anti-tumor T cell functions. A retrospective study reported that six of 114 (5.3%) cancer patients with chronic HBV infection experienced HBV reactivation at a median of 18 weeks from the onset of anti-PD-1 or PD-L1 immunotherapy.\textsuperscript{176} This was unexpected because the immunotherapy overcomes T cell exhaustion and then impairs control of viral replication. Therefore, the underlying mechanism may not be parallel with immunosuppression induced reactivation.

Transarterial chemoembolization (TACE)\textsuperscript{177} and other local therapies for hepatocellular carcinoma, including radiotherapy,\textsuperscript{178} radiofrequency ablation,\textsuperscript{179} or hepatic resection,\textsuperscript{180} can also lead to HBV reactivation to a different extent. While the effect of TACE can be attributed to the use of anthracyclines (doxorubicin or epirubicin), the mechanism of HBV reactivation underlying other therapies remains unclear.

Emerging treatments

Much academic and industrial effort has gone into identifying HBV drug targets and drug discovery. Several therapeutic targets have been identified and the relevant drugs are being assessed in pre-clinical studies or clinical trials. Although in depth discussion of novel anti-HBV therapies is beyond the scope of this review (see reviews\textsuperscript{181-183} for a detailed introduction and discussion of emerging therapies), we have listed these novel drugs or therapies.

A variety of drugs under development target multiple steps or elements during a viral replication life cycle, including viral entry, cccDNA formation, viral gene expression, capsid assembly, and HBsAg secretion. Among these, degrading cccDNA is an attractive strategy that is likely to cure occult HBV infection and abolish the risk of HBV reactivation. All candidates targeting cccDNA editing strategies, including zinc finger nucleases,\textsuperscript{184} transcription activator-like effector nucleases,\textsuperscript{185} and CRISPR/Cas9 systems\textsuperscript{186,187} are effective in pre-clinical studies but have not yet been evaluated by clinical trials.

Alternatively, HBV cure may be achieved by inducing adequate immune control. One approach is to activate innate immunity. The potential to control HBV by doing this has been shown by the known efficacy of IFN-α in clinical practice.\textsuperscript{32,34,35} The novel inducers of innate immunity that are assessed by clinical trials include the agonists of TLR-7, TLR-8, and RIG-1. A randomized controlled trial showed that GS-9620, a TLR7 agonist, augmented the function of natural killer cells but failed to diminish serum HBsAg levels in 28 patients with chronic HBV infection who were treated with nucleotide analogs.\textsuperscript{188} Other agents are being assessed in phase II trials (NCT03491553, NCT02751996).
Another approach is to boost or correct defective adaptive immune response. Strategies include novel therapeutic vaccines, engineering HBV specific T cells, re-directing T cells or cytokines to infected hepatocytes via TCR-like antibodies, and blocking checkpoint molecules on T cell surface. New therapeutic vaccines alone or in combination with antivirals did not show statistically significant effects on HBsAg decline in phase II or III trials. Nivolumab, a programmed death receptor 1 (PD-1) inhibitor, showed potential to reverse defective T cell immune response and to induce HBsAg decline in a phase Ib study. Other strategies have not yet been evaluated by clinical trials.

**Selected guidelines and high quality reviews**

The most up-to-date clinical practice guidelines of chronic hepatitis B by the American Association for the Study of Liver Diseases (AASLD), European Association for the Study of the Liver (EASL), and Asian Pacific Association for the Study of the Liver (APASL) provide detailed information on the staging of chronic HBV infection development. The AASLD guideline combines “HBeAg-positive and negative chronic hepatitis B” as the “immune-active phase.” The EASL guideline extends the “HBsAg-negative stage” with an additional phase in the course of chronic HBV infection. The American Gastroenterological Association Institute Guideline on the Prevention and Treatment of Hepatitis B Virus Reactivation During Immunosuppressive Drug Therapy provides evidence based information on screening strategies and preventive measures for HBV reactivation by immunosuppressive therapies. The EASL guideline on chronic hepatitis B and APASL’s HCV Guideline of Virus-eradicated Patients by Direct-acting Antiviral (DAA) provide consistent recommendations on preventing and monitoring HBV reactivation in patients co-infected with HCV/HBV and treated with direct acting antivirals. A more detailed discussion on the molecular biology of HBV, and innate and adaptive immunity in HBV infection, can be found in references. In depth discussions of HBV cure strategies are available in special reviews.

**Conclusion**

HBV can persist once infection has taken place. Persistence can occur in an overt state with the presence of serum HBsAg owing to induction and maintenance of defective immune response, especially an adaptive immune response resulting from the interaction between viral and host factors. Or it can persist in a long term occult state with the absence of serum HBsAg, owing to the long life of the HBV genome, including cccDNA and integrated DNA within the nucleus of hepatocytes, despite immune control. With the presence of cccDNA, HBV has the potential for reactivation, indicated by an increase or reappearance of serum HBV DNA in chronic infection, and HBsAg in occult infection. Reactivation can result from any modulation to the virus or immune system that may disrupt the interaction between the virus and the host, which can stem from broad therapies for comorbidities.

Therefore, greater awareness is needed among clinicians that HBV persistence may not be completely eradicated in infected individuals.

**Acknowledgment:** We thank Fujie Xu and Hongqun Liu for valuable discussion, and Shereen Ayub for assistance in writing the manuscript. The work was supported by the 13th 5 State S&T Projects of China (2018ZX10302-206) and the Natural Science Foundation of China (81871646 and 81870425).

**Competing interests:** The BMJ has judged that there are no disqualifying financial ties to commercial companies. The authors declare the following other interests: none.

**QUESTIONS FOR FUTURE RESEARCH**

- How can we develop ultra-sensitive, standardized, and valid assays to quantify serum HBsAg (and detect HBV S variants and differentiate from HBsAg fragments), intra-hepatic HBV cccDNA, and other forms of the viral genome to diagnose HBV occult infection more accurately?
- What is the prevalence of occult HBV infection in the general population?
- What are the molecular mechanisms underlying formation, regulation, and metabolism of cccDNA and integrated DNA as the virologic basis of persistence?
- What is the role of innate immunity and B cells in immune control of HBV? How is immune control surveillance of HBV impaired by specific drugs or therapies, leading to HBV reactivation? How can we better design clinical studies to provide more accurate evidence for the risk of any specific drug or therapy that has ever been linked to HBV reactivation? Can research on the interactions between virus, host, and drug provide a precise and individualized risk prediction for patients who receive any specific drug or therapy which has the potential to induce HBV reactivation?
- How can emerging antiviral and immunomodulatory drugs be optimized to achieve functional cure, or even complete cure?

**PATIENT INVOLVEMENT**

We invited review of our manuscript from a 55 year old man who experienced HBV reactivation following immunosuppressive therapy for diffuse large B cell lymphoma, having previously recovered from HBV infection. The patient identified the sections that were most and least relevant to his personal history. He was interested in learning about HBV reactivation after recovering from previous infection. Additionally, he reminded us of the importance of evaluating the risk of HBV reactivation when choosing an immunosuppressive drug or therapy for patients who have been exposed to HBV infection. His input led us to provide a detailed list and evaluation of potential drugs or therapies associated with risk of HBV reactivation (table 2).
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Provenance and peer review: commissioned; externally peer reviewed.

Contributorship statement and guarantor: Both authors contributed to conceiving the idea, undertaking the literature search, and writing the manuscript. MZ is the guarantor.

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