
Pathogenesis of Hepatitis B Virus Associated Chronic Liver Disease

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Abstract

Hepatitis B virus (HBV) infection is associated with chronic liver diseases (CLD), which progress from hepatitis to fibrosis, cirrhosis, and finally hepatocellular carcinoma (HCC) over 30–50 years. The pathogenesis of CLD is immune mediated, which is characterized by persistent immune responses against virus infected hepatocytes. During bouts of CLD, the virus gene encoding the hepatitis B x antigen (HBx) is increasingly found integrated at multiple sites within the human genome. Many of these integrated templates express HBx, which is a *trans*-regulatory protein that supports virus gene expression and replication on one hand, but also alters patterns of gene expression in the infected cell. HBx alters gene expression by constitutively activating signal transduction pathways in the cytoplasm and promoting epigenetic mediated changes in the expression of cellular genes. In doing so, HBx contributes to the persistence of virus infected cells and to the pathogenesis of CLD by triggering multiple hallmarks which are characteristic of cancer.

Keywords: hepatitis B virus, chronic liver disease, hepatocellular carcinoma, hepatitis B x, immune mediated liver disease, epigenetics, hallmarks of cancer

1. Introduction

Hepatitis B virus (HBV) is a blood-borne virus that infects the liver. Until the discovery of the virus in the 1960s [1], it was transmitted sexually and by transfusion of contaminated blood and blood fractions. Today, the virus has been virtually eliminated from the blood supply by a simple blood test while infection has been prevented by a highly efficacious vaccine [2, 3]. Prior to establishment of vaccination programs in various countries, infants born to infected mothers replicating virus often acquired the virus at birth by exposure to contaminated maternal blood.

More than 90% of these children became HBV carriers, characterized by the persistence of virus or virus antigens in their blood for years to decades. These children were a high risk for the development of chronic liver disease (CLD), which progressed from hepatitis, to fibrosis, cirrhosis, and hepatocellular carcinoma (HCC) [4]. Fortunately, newborns in many countries receive the HBV vaccine at birth, which helps to prevent mother-to-infant transmission as well as protect from exposure later in life. Among unvaccinated adults engaging in unprotected sex, roughly 5–10% become carriers, and these individuals are also at high risk for the development of CLD and HCC. Although estimates vary, there are ~300–350 million carriers of HBV worldwide [5]. HCC is the sixth most prevalent cancer worldwide, with about 600,000 newly diagnosed cases annually, and the second leading cause of cancer deaths [6]. Interferon, and in more recent years, powerful nucleoside analogs, have successfully treated patients with chronic hepatitis B, but presently there is no cure [7, 8]. HCC is curable by surgical resection, but this is often accompanied by relapse. Dozens of drugs, alone or in combination, have been evaluated in clinical trials for patients with advanced HCC, but only the multi-kinase inhibitors, Sorafenib and regorafenib, and the immune checkpoint inhibitor nivolumab, have been useful in modestly extending the lifespan of such patients [9, 10]. Given that the carrier state and CLD are the major risk factors for HCC [11], there is strong rationale to better understand the host-virus relationship that contributes to the pathogenesis of chronic infection.

2. Variations in pathogenesis

A hallmark in the pathogenesis of HBV infection is its' variability. Among acutely infected adults, up to 65% develop a subclinical infection characterized only by the appearance of one or more viral antibodies in the blood, while another 25% develop acute resolving infection, which may or may not include a bout of hepatitis. The remaining 10% of patients develop chronic infection (i.e., the persistence of virus and virus antigens in the blood for more than 6 months). In chimpanzees [12] and woodchucks [13], acute infections are characterized by the nearly complete clearance of virus from the blood and liver followed by seroconversion from surface antigen to corresponding antibody. In this case, virus is mostly cleared by non-cytolytic cytokines (e.g., interferon gamma [IFN γ] and tumor necrosis factor alpha [TNF α]) prior to the appearance of T and other inflammatory cells in the liver, suggesting that most virus clearance occurs prior to the development of acute hepatitis. Further work showed that CD4⁺ and CD8⁺ T cells, natural killer (NK) cells, Fas, various IFNs and corresponding receptors, and the TNF receptor 1 participate in virus clearance, suggesting redundant pathways inhibit HBV replication in the liver [14]. The subsequent contribution of a T cell response appears to clear virus infected cells by cytolytic mechanisms involving Fas and granzymes. In this context, CD4⁺ T cells are required to prime CD8⁺ T cells to facilitate virus elimination in acute infection [14]. When this happens in acute, resolving infection, the T cell response to HBV is vigorous, polyclonal and multi-specific, while among those who go on to develop chronic infection, adaptive immunity is relatively weak and narrowly focused, suggesting that clearance of HBV is T cell dependent. When T cell responses are not adequate, CLD may develop and progress to cirrhosis and HCC. However, CLD may spontaneously resolve at any of these stages. While the origin of this variability is not completely characterized, it is clear that the ability of the host to mount adaptive immune responses is a key element to limiting virus spread.

3. Contributions of hepatitis B surface, core and e antigens to the pathogenesis of chronic infection

3.1. Hepatitis B surface antigen (HBsAg)

HBV is a small virus consisting of only four open reading frames (ORF) [15]. One ORF encodes a family of envelope polypeptides (**Figure 1**). The major envelope polypeptide, HBsAg, triggers neutralizing antibody which is central to virus clearance after acute exposure and is the major component of the HBV vaccine [2]. HBsAg polypeptides are transmembrane proteins and glycoproteins that are on the envelope of virus particles, and are also secreted as small, spherical and variably long filamentous subviral particles that lack the virus nucleocapsid and HBV DNA. It is thought that these subviral particles, which are produced at concentrations several logs above that of infectious virus particles, absorb neutralizing antibody and trigger immunological tolerance, both of which promote virus persistence in the blood. Moreover, in patients with CLD, there does not seem to be any correlation between intrahepatic HBsAg expression patterns and inflammatory infiltrates [16, 17], nor have HBsAg specific T cell clones been isolated from such patients [18]. In addition, T cell sensitization to HBsAg in acute and chronic HBV infection is usually undetectable [19],

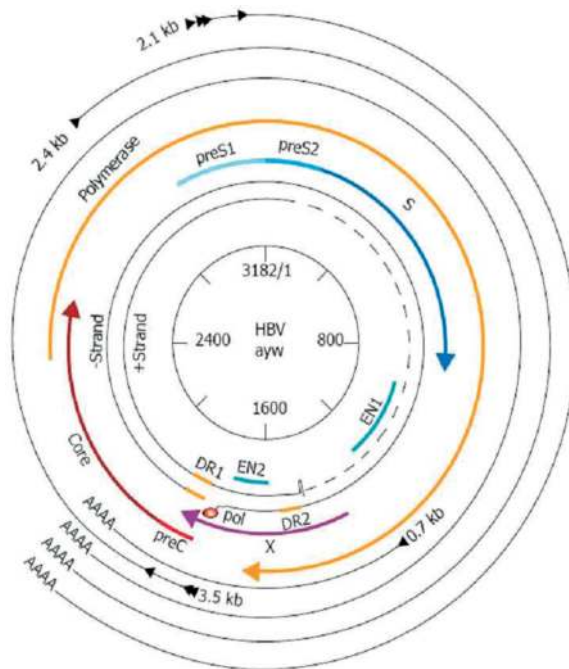


Figure 1. Genetic organization of HBV showing the ORFs (in color). The positions of enhancer 1 (EN1) and 2 (EN2) are also shown. The direct repeat 1 (DR1) and 2 (DR2) sequences at the ends of the long and short DNA strands are also indicated. The pregenomic RNA (3.5 kb) is greater than genome length, while the 2.1 and 2.4 kb subgenomic mRNAs encode surface antigen polypeptides, and the 0.7 kb mRNA encodes the X protein. Reproduced from [20] with permission.

so while HBsAg clearance occurs in acute, resolving infections, it is not clear that it is an immunological target in established infections.

3.2. Hepatitis B core antigen (HBcAg)

The second ORF, or core gene, encodes the hepatitis B core antigen (HBcAg) or nucleocapsid protein that polymerizes as an icosahedron around the virus replication complex, the latter of which consists of the virus nucleic acid and HBV encoded polymerase [20]. The fact that the pregenomic RNA and the reverse transcribed viral DNA product are sequestered within a nucleocapsid means that they are not readily detected by pattern recognition receptors, (e.g., toll-like receptors, retinoic acid inducible gene 1 [RIG-1], and mitochondrial anti-viral signaling [MAVS]) that trigger innate immunity [21]. Moreover, innate immune responses do not develop in the liver of acutely infected chimpanzees [22], suggesting that HBV replication and spread may be conducted in “stealth” mode with virus nucleocapsids upon infection and again during virus replication. If so, then this may explain why up to 70% of acutely infected adults who become carriers do not develop CLD. However, carriers who develop CLD also have intrahepatic core antigen, suggesting that HBcAg may be an important immunological target in CLD [23]. Alternatively, patients with acute, resolving hepatitis show a vigorous peripheral blood mononuclear cell response to HBcAg that is temporally associated with the clearance of HBsAg, while in patients with chronic infection, T cell responsiveness to HBcAg is relatively weak, providing an opportunity for HBV to spread in the liver and establish a chronic infection [19].

3.3. Hepatitis B e antigen (HBeAg)

A proteolytic fragment of HBcAg, known as HBeAg, is secreted into the circulation and serves as a surrogate marker of virus replication. Seroconversion from HBeAg to anti-HBe is usually accompanied by a significant decrease in virus replication in both the liver and blood and resolution of CLD [24]. The detection of HBcAg specific cytotoxic T lymphocytes (CTL) is associated with the clearance of virus replication, often a transient exacerbation of CLD, and seroconversion to anti-HBe during the natural history of infection [24], suggesting that HBcAg is an important virus target in CLD. HBcAg specific T cells have been detected in the peripheral blood and liver [18, 25] of patients with CLD, suggesting that HBcAg is an immunological target in chronic hepatitis B. Interestingly, HBeAg in serum may attenuate immune responses against virus infected liver, because some patients who develop mutations in HBV that no longer express HBeAg, continue to support high levels of virus replication and ongoing, CLD [26, 27]. In fact, HBeAg appears to be a T cell tolerogen that down-regulates immune responses against HBcAg [28]. HBeAg may also stimulate the appearance of regulatory dendritic cells, which would also suppress virus specific immunity and promote virus persistence [29] by up-regulating the expression of suppressor of cytokine signaling 2 (SOCS2), which in turn represses IFN signaling, thereby blunting innate anti-viral responses and promoting virus persistence [30]. Thus, HBeAg polypeptides, like subviral HBsAg particles, promote chronicity by acting as tolerogens.

3.4. Hepatitis B polymerase

The HBV encoded polymerase, encoded by a third ORF, has DNA dependent and RNA dependent DNA polymerase (DNAP) activities, and RNase H activity. Upon infection, the partially

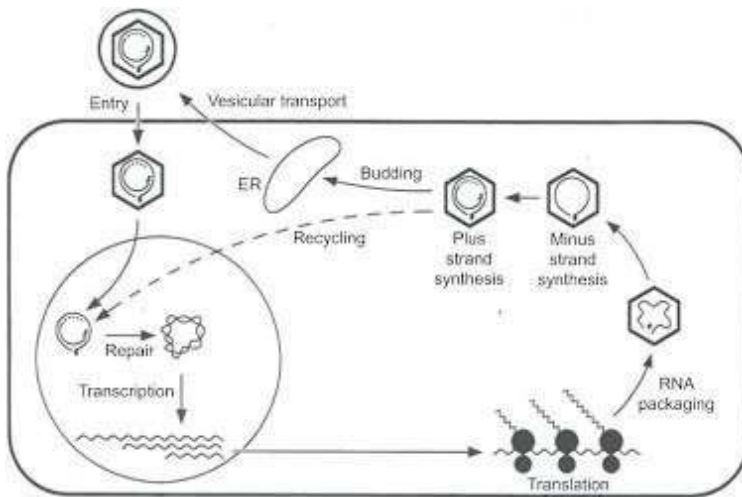


Figure 2. General scheme of HBV replication. See the text for additional details. Reproduced from [20] with permission.

double stranded viral DNA is made fully double stranded by the endogenous DNAP activity [20] (**Figure 2**). The HBV genome then appears as a supercoiled mini-chromosome in the nuclei of infected cells, and this acts as a template for the transcription of subgenomic RNAs and a greater than genome length pre-genomic RNA. The latter then migrates into the cytoplasm, where it is packaged with the virus polymerase into nascent (“immature”) core (or nucleocapsid) particles, where the pregenomic RNA is reverse transcribed into minus strand DNA, with the latter then being used as a template for partial plus strand synthesis just prior to the budding and secretion of progeny virus (**Figure 2**). Some immature core particles are recycled into the nucleus to replenish the pool of covalently closed circular (ccc) HBV DNA. Although the HBV polymerase triggers antibody responses [31], there is no evidence that immune responses against the polymerase directly impact pathogenesis or virus persistence. However, HBV polymerase inhibits RIG-1 and nuclear factor kappa B (NF- κ B) induction of IFN β , suggesting that the polymerase could block innate signaling [32, 33], thereby contributing to virus persistence.

4. Relationship between persistent virus replication, integration of HBV DNA, and the risk for the development of HCC

There is evidence to suggest that persistent, high levels of HBV replication correlate with the progression of CLD to HCC [34]. However, independent work showed an elevated risk for HCC among patients with CLD but low virus titers [35, 36]. Other observations have shown no correlation between HBV DNA levels in serum ($>10^5$ copies/ml) and histological grade or stage of liver disease in carriers [37, 38]. In addition, it is controversial as to whether long term nucleoside analog therapy resulted in a decreased risk for the development of HCC [6, 35]. Given that HBV is not directly cytopathic [39], that carriers with high levels of HBV DNA in serum are often asymptomatic, and that the pathogenesis of CLD is immune mediated [17,

40], a correlation between virus replication and CLD may contribute to, but not by itself, determine disease progression. Moreover, most carriers with CLD who develop cirrhosis and HCC have long since seroconverted from HBeAg (reflecting high levels of virus replication) to anti-HBe (reflecting low or undetectable virus replication), indicating that disease progression may occur at low virus titers [36]. Among patients with sustained high levels of HBV replication and successive bouts of CLD, there is a wave of liver regeneration following each episode of hepatitis to restore full liver function. At these times, fragments of HBV DNA, mostly encoding the HBx ORF (and sometimes the HBx plus preS/S ORFs), become integrated at multiple sites within host DNA [41, 42] (**Figure 3**). Over time, these integration events result in increased intrahepatic expression levels of HBx that alter patterns of host (and support virus) gene expression (**Figure 3**). HBV integrates early after infection, not only in permissive liver cell lines, but also in non-replicating primary human hepatocytes [43]. Many fragments of integrated HBV DNA encode HBx that is capable of *trans*-activation [44]. Although the relatively low levels of HBx made from the virus mini-chromosome support virus gene expression and replication, it is hypothesized that as intrahepatic levels of HBx increase [45] (**Figure 3**), it epigenetically alter the expression patterns of selected host genes [46] that contribute to both virus persistence and to malignant transformation. Thus, the changing intrahepatic levels of HBx promote virus persistence and ultimately, contribute to malignant transformation [47].

4.1. Covalently closed circular HBV (ccc) DNA

Given that the current treatment of chronic hepatitis B with nucleoside analogs is not curative, there has been a major effort to eliminate ccc DNA [47], especially since ccc DNA is the template for all virus transcripts. Since nucleoside analogs do not eliminate integrated HBV templates or the HBV mini-chromosome, continued virus gene expression from these templates will drive pathogenesis toward HCC. Formation of ccc DNA is a complex process that involves a variety of host proteins, including several DNA polymerases [48] that could potentially be therapeutic targets, although this approach may be accompanied by toxicity. As outlined below, HBx regulates the formation, function and intracellular copy number of ccc DNA by several epigenetic mechanisms that involve altered expression of histone methyltransferases and histone deacetylases, by promoting degradation of the anti-viral restriction factor Smc5/6, and by increasing expression of DNA methyltransferases [48]. Anti-viral immune responses in which selected cytokines mediate non-cytolytic degradation of ccc DNA have also been documented *in vitro* [48, 49]. Among these, IFN alpha up-regulated expression of APOBEC3

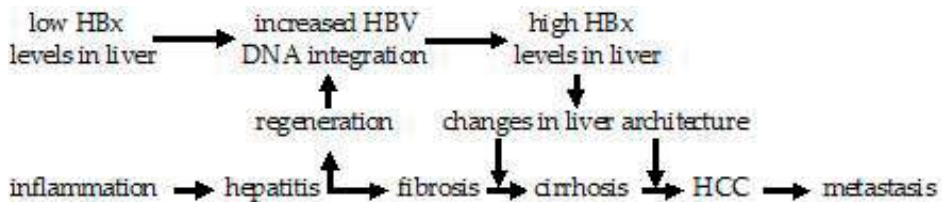


Figure 3. Natural history of chronic hepatitis B featuring the progressive lesions that develop in CLD compared to increased number of integration events, many of which produce functional HBx (modified from [53] with permission).

nuclear deaminase resulted in a modest reduction in ccc DNA copy number via deamination [50]. Gene editing approaches, such as CRISPR/Cas9 have also been demonstrated to work *in vitro* and *in vivo* [51], but off-target effects, ability to access and act on all susceptible cells, and recognition of all HBV genotypes, remain to be addressed. In addition, the recent finding of ccc host DNA in both normal and tumor cells, as a mechanism whereby host cells regulate gene expression [52], implies that targeting ccc DNA may also have toxic effects on the treated cells whether or not they are virally infected. Thus, it is not clear whether this approach in a liver which is already damaged will exacerbate that damage and/or have an anti-tumor effect.

5. Contribution of HBx to pathogenesis of CLD by regulation of HBV replication

HBx, the *trans*-activation protein of HBV, *trans*-activates virus gene expression and replication *in vitro* [54, 55]. The contribution of this regulatory protein to virus persistence in the carrier state was shown in woodchucks experimentally infected with the HBV-like virus, woodchuck hepatitis virus (WHV). Wild type WHV readily establishes a chronic infection, characterized by persistent virus replication and CLD that progresses to HCC [56]. However, experimental infection with a mutant of WHV that does not encode woodchuck hepatitis x (WHx) antigen yielded no carrier state and no CLD [57, 58], suggesting that *trans*-activation of virus gene expression and replication is central to the establishment of the carrier state. Among infected woodchucks, there was co-staining between WHV core antigen (where virus replication takes place) and WHx [59, 60], while in human infection, HBx often co-existed with HBe in serum [61] and replication complexes (i.e., with HBcAg) in the liver [62]. Thus, HBx expression is associated with virus replication.

5.1. Mechanisms regulating HBV replication

At the molecular level, HBx regulates HBV replication by binding to various cellular proteins. For example, HBx binds to jumonji C-domain-containing 5 (JMJD5), a arginyl-hydroxylase, which promotes the expression of transcription factors (e.g., such as hepatocyte nuclear factors 3 gamma and 4 alpha [HNF3G and HNF4A] and CCAAT/enhancer-binding protein alpha) that facilitate hepatocyte differentiation [63]. Given that HBV replicates in differentiated hepatocytes, the binding of HBx to JMJD5 facilitates HBV replication via epigenetic alterations in host gene expression. In addition, HBx promotes the formation of ccc DNA by recruiting the transcriptional scaffold, p300; the cAMP response element binding protein CREB; the CREB transcription factor binding protein, CBP; the histone acetyltransferase p300/CBP-associated factor, as well as the histone deacetylases HDAC1, Sirt1 [48] and Sirt2 [12]. Once ccc DNA is formed, HBx up-regulates HBV replication, in part, by binding to cullin4-damage specific DNA binding protein (CUL4-DDB1) ubiquitin ligase [64, 65], suggesting that HBx may function, at least in part, at the level of the proteasome. HBx modulates proteasome activity by direct binding to the 26S proteasomal subunit [66], which is responsible for degradation of HBx and several anti-viral proteins. One of the latter is Smc5/6, which is involved in the structural maintenance of chromosomes (i.e., genome stability) and DNA repair [67]. Smc5/6 and HBx bind to the HBV mini-chromosome [67, 68],

resulting in epigenetic changes of virus gene expression. HBx binding to CUL4-DDB1 triggers altered enzymatic activity of the E3 ligase CRL4, which then stimulates the ubiquitination and subsequent proteasomal degradation of Smc5/6 [68–70], thereby promoting virus replication. Other anti-viral systems, such as IFN induced APOBEC3A [50], may also be similarly degraded. In this context, HBV is not very good in triggering innate immunity, which may underscore why there are hundreds of millions of carriers worldwide [71]. As mentioned above, sequestration and reverse transcription of pregenomic HBV RNA in immature nucleocapsids (**Figure 2**) may block the induction of innate immunity. In addition, although HBV replication is exquisitely sensitive to inhibition by IFNs, HBx appears to block IFN expression and signaling [72–74], suggesting that both innate and adaptive immunity could be compromised, thereby permitting virus persistence. Under these circumstances, CLD would continue to damage the liver while being unable to resolve the virus infection. HBx also regulates HBV replication by stimulating the expression of DNA methyl-transferases (DNMTs), which suppresses HBV transcription via DNA methylation [75]. DNMTs also methylate tumor suppressor genes, thereby down-regulating their expression, and permitting the accumulation of mutations and chromosomal instability that contribute importantly to HCC. Thus, HBx regulates the activity of ccc DNA in both positive and negative ways, and in doing so, impacts upon the pathogenesis of CLD. The reason why it is important to regulate the intrahepatic levels of ccc DNA is because when virus antigens are greatly overproduced, they could trigger cytopathic effects (CPE), thereby limiting virus replication. For example, mutations in the preS region of the S gene prevent secretion of surface antigen and complete virus particles, and eventually CPE. Pre-S mutations also promote recycling of viral DNA into the nucleus where it results in increased levels of viral ccc DNA, which potentially promotes virus persistence [76] (**Figure 2**). In transgenic mice overproducing HBsAg, CPE develops and eventually evolves into HCC [77]. Although the latter is not characteristic of HCC pathogenesis among human carriers, it does underscore that selected HBV mutants that may arise during chronic infection potentially contribute to pathogenesis via CPE.

5.2. Oxidative damage and inflammation

Although HBV is not cytopathic, HBx strongly activates NF- κ B [78], which promotes the expression of many pro-inflammatory cytokines and chemokines that attenuate virus replication and contribute to the pathogenesis of CLD and HCC. For example, HBx stimulates the expression of IFN inducible proteins, such as the CXC chemokine IP-10 [79] which promotes leukocyte chemotaxis. HBx also stimulates production of interleukin-23 (IL-23) [79], which contributes to the maintenance and expansion of pro-inflammatory Th17 cells. Among others, IL-6 is up-regulated by HBx in a MyD88 manner [80], which indicates that HBx is activating a pro-inflammatory environment via innate immune pathways early on after infection. The repressive effect of IL-6 upon HBV replication is demonstrated by the fact that IL-6 treatment of infected cells results in the loss of HNF1a and HNF4a, both of which bind to ccc DNA. IL-6 also redistributes signal transducers and activators of transcription 3 (STAT3) signaling from ccc DNA to IL-6 target genes [49]. HBx targets up-regulation of IL-18, which up-regulates FasL [81], which in this case blocks the killing of infected cells by CTLs. HBx also up-regulates tumor TNF α [82], which was shown to suppress HBcAg expression [83], thereby inhibiting virus replication. In addition, the pro-inflammatory IL-32 was up-regulated by HBx in a NF- κ B

dependent manner [84]. This is not an exhaustive list. Many of these molecules are turned on as a result of HBx stimulating multiple signal transduction pathways in the cytoplasm (in addition to NF- κ B), but the bigger question is trying to understand how a non-cytopathic virus is mediating these and other related changes in infected cells.

The fact that HBx plays a central role in HBV replication suggests that intracellular conditions that stimulate HBx activity would also promote the carrier state, which would be evolutionally selected for because it would provide a large window of time for virus to be transmitted to other hosts. In this context, the expression and activity of HBx is stimulated in an oxidative environment, since the addition of anti-oxidants to cells expressing HBx strongly diminish HBx *trans*-activation activity [85, 86]. An oxidative environment (accompanied by oxidative stress of cellular organelles) could be created in the infected cell by virtue of the association of HBx with mitochondria [87]. HBx interacts with the voltage dependent anion channel on the outer mitochondrial membrane, altering transmembrane potential [88], resulting in diminished electron transport, increased free radical accumulation, including elevated lipid peroxidation products [89], release of calcium into the cytosol [55], and under specific circumstances, cell death [90]. Release of calcium into the cytosol, resulted in the activation of the protein tyrosine kinase 2 and Src kinase families, leading to stimulation of ras, raf, mitogen activated protein kinase, and Jun, which stimulate HBV transcription and replication [55]. HBx also induces oxidative stress in the endoplasmic reticulum, which activates the unfolded protein response and expression of pro-inflammatory cyclooxygenase-2 through the activating transcription factor 4 pathway [91]. Free radicals are also characteristic of immune responses aimed at damaging and destroying infected cells that are replicating HBV. In addition, mitochondrial associated HBx induces oxidative stress, which activates selected transcription factors, such as NF- κ B, STAT3 and activating protein 1 [86]. However, HBx is also known to block mitochondrial triggered cell death, not only by activation of survival [21, 92] and hepato-protective pathways such as NF- κ B that over-ride apoptosis signaling, but also by blocking key caspases and promoting autophagy [93] and mitophagy [94]. The maintenance of mitochondrial and cellular homeostasis by mitophagy acts to attenuate virus induced apoptosis, so that on the one hand, autophagy and mitophagy promote cell survival and virus persistence, while simultaneous mitochondrial damage may contribute to CLD [94].

5.3. HBx and inflammation

In this chronic pro-inflammatory environment, one would expect to see a correlation between HBx staining and the intensity of CLD. In fact, WHx staining has been observed around inflammatory foci in chronically infected woodchuck livers [95], and among human carriers, relatively low levels of intrahepatic HBx staining was observed in patient biopsy samples from people with low grade hepatitis, while intense and widespread HBx staining was observed in patient biopsies from those with advanced fibrosis and cirrhosis [45, 96], suggesting a direct correlation between HBx staining and liver damage. Independent work also showed low levels of HBx mRNA in the livers of patients with mild CLD (e.g., mild hepatitis), and much higher levels among patients with severe lesions in the liver (advanced fibrosis and cirrhosis) [97]. The relationship of HBx expression to disease severity is also consistent with the idea that when the liver regenerates following each bout of hepatitis, fragments of HBV

DNA encoding the HBx region (and sometimes part of the preS/S encoding gene as well) increasingly integrate into multiple regions of the host genome during normal host DNA replication, resulting in increasing accumulation of intrahepatic HBx as CLD progresses. In contrast, the copy number of ccc DNA per cell decreases with regeneration.

The relationship between HBx expression and CLD has been recapitulated in HBx transgenic mice, where the presence, frequency and distribution of HBx in the liver increase with age, as does liver pathology, which progressively develops from hepatitis and steatosis, to dysplasia and microscopic nodules of HCC, and finally to multi-nodular macroscopic HCC with age [98]. In this model, HBx is expressed from its own enhancer and promoter, which is not active until after birth when appropriate transcription factors in the liver begin to appear. HBx expression triggers immune responses in the absence of other HBV gene products, so it is likely that the pathogenesis observed is due to the impact of increasing levels of HBx upon host gene expression combined with immune responses directed against virus infected cells. There is no ccc DNA in this system, just as it is difficult to detect HBV replication among patients with advanced stages of CLD (i.e., cirrhosis). Thus, it is possible that early in chronic infection, and immune responses to virus antigens emanating from ccc DNA templates play an important role in triggering and sustaining immune mediated pathogenesis, but following bouts of CLD and liver regeneration, where the levels of virus replication decrease at the same time that integration of virus DNA fragments increase, pathogenesis appears to be increasingly driven by one or more antigens made from integrated HBV DNA. Although *cis*-acting mechanisms have been postulated to contribute importantly to the pathogenesis of HCC in selected cases, the broadly distributed integration events of the HBx ORF into most chromosomes [99], suggests that the HBx proteins encoded by most integration events promote CLD and HCC in *trans* [47]. In this model, integration of HBV sequences would accumulate in areas of euchromatin and fragile sites much more frequently than at or within specific genes [100].

The model above suggests that targeting ccc DNA in HBeAg carriers with CLD may be an important therapeutic goal to bring about a functional (but not sterilizing) cure due to the presence of integrated virus DNA that express one or more virus proteins. Among anti-HBe carriers with advanced CLD, targeting the much lower levels of ccc DNA may not be effective in preventing progression to cirrhosis and HCC, because at this stage, most of the HBx made probably comes from integrated templates. Under these circumstances, ccc DNA may persist in a transcriptionally inactive form, which is consistent with the absence of HBV DNA in the blood, even after treatment with direct acting anti-viral agents or therapy aimed at stimulating immune responses against virus infected cells [101, 102]. In fact, early work already pointed out that seroconversion to anti-HBe is sometimes associated with the progression of CLD [103, 104], even though later work showed that disease progression was associated with continued replication of HBV DNA carrying one or more mutations in the core gene that blocks production of HBeAg [27]. These mutations were probably selected for during the natural history of infection by immune responses targeting HBcAg [105]. Although these findings suggest that CLD progresses in the liver supporting replication of selected virus mutants, it has also recently been suggested that linear HBV DNA, and not ccc DNA, is the template for integration into host DNA [43], from which one or more virus gene products are made, and contribute to pathogenesis. Thus, persistent inflammation in a chronically damaged liver may result in the development of HCC despite low levels or undetectable levels of virus replication.

6. Conclusions

HBV encodes polypeptides from four ORFs that trigger corresponding immune responses during acute and chronic infections. When these responses are rapid, strong and multi-specific, acute, resolving infection can be achieved. When these immune responses are weak and of limited specificity (against few virus epitopes), the carrier state may develop. Although the pathogenesis of HBV is variable in different hosts, the virus encodes proteins that blunt innate immunity, and as a consequence, adaptive immunity is not triggered at all or to a limited extent. The latter causes liver damage over many years without eliminating the virus. Even though available treatments suppress virus replication, none are curative, and the persistence of viral ccc DNA sustains infection. Production of HBx regulates virus gene expression and replication, but over time, increased integration of HBV DNA fragments encoding HBx results in high levels of HBx expression that epigenetically alter the expression of numerous host genes that up- or down-regulate HBV replication and impact disease activity. For example, HBx activation of AKT decreased HBV replication, but this was accompanied by an inhibition of apoptosis, suggesting that HBx balances HBV replication and cell survival by stimulating signaling that enhance hepatocyte survival at the expense of higher levels of HBV replication [106]. The generation of free radicals by immune responses against virus infected cells, combined with HBx mediated alterations in mitochondrial function, promote HBx activity. These events result in the activation of signaling pathways (e.g., AP-1 and NF- κ B) that over-ride apoptosis and/or directly block the activation of critical caspases, so that whether HBx stimulates or block apoptosis depends upon whether the liver is experiencing inflammation and oxidative stress. It also depends upon whether HBx is being expressed in normal hepatocytes, where apoptotic pathways could be triggered, or whether HBx is expressed at high levels in cells where apoptotic pathways are compromised. In addition to being pro-inflammatory, activated NF- κ B protects infected cells against immune elimination. Thus, the dichotomy of HBx activity may be a reflection of the environment wherein HBx is expressed. Importantly, the epigenetic mechanisms whereby HBx regulates virus replication also have an impact on cell growth and survival, and many of these same alterations in host gene expression are also hallmarks of cancer [107], which may explain why there is such a high risk of HCC among carriers with CLD [11]. The common denominator is that many of the pathways and molecules that support HBV gene expression and replication also protect infected cells from elimination, and contribute centrally to malignant transformation.

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Conflict of interest

The author declares no conflict of interest.

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