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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\(\gamma\)] and tumor necrosis factor alpha [TNF\(\alpha\)]) 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 trans-membrane 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].
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 HBeAg 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. HBeAg 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
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-κB) induction of IFNβ, 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 (>10⁵ 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,
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](image-url). 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 deactylases 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],
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http://dx.doi.org/10.5772/intechopen.79746

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