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Review

Involvement of Interleukin 6 in Hepatitis B Viral Infection

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Key Words
Interleukin 6 • Hepatitis B • HBx • SNP

Abstract
Hepatitis B is a major global health problem and a potentially life-threatening liver infection caused by hepatitis B virus (HBV). Many cytokines including interleukin 6 (IL-6) have been shown to be involved in the HBV infection process. IL-6 is a typical cytokine made up of 184 amino acids, and the gene is located in chromosome 7p21. For healthy people, serum IL-6 levels are usually too low to be detected. However, dysregulated synthesis of IL-6 has been discovered in chronic inflammatory diseases such as hepatitis B, Crohn's disease and rheumatoid arthritis. IL-6 also plays an important role in HBV replication and in the development of hepatitis B disease. This review aims to present the latest discoveries concerning the role of IL-6 in hepatitis B disease progression, and HBV entry and replication, and evaluate polymorphisms that are associated with the development of hepatitis B disease.

Introduction

Hepatitis B is a major global health problem and is a potentially life-threatening liver infection caused by hepatitis B virus (HBV). HBV infection can lead to a great number of clinical consequences such as acute self-limited hepatitis, chronic hepatitis, liver failure, liver cirrhosis (LC) and hepatocellular carcinoma (HCC) [1]. These complications do not result from a direct cytopathic effect of the virus itself, but from the immune response of the host that affects both outcome and disease progression [2, 3]. Cytokines have been shown to be engaged in regulating hepatocyte functions, and play a fundamental role in HBV infection immunopathogenesis [4]. Among these cytokines [5], increasing importance has been

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attached to the involvement of interleukin 6 (IL-6) in HBV infection. IL-6 is a typical cytokine that is composed of 184 amino acids, and its gene is located in chromosome 7p21 [6]. As a pleiotropic cytokine, it is produced by various cell types and regulates a variety of biological processes [7].

IL-6 exerts its biological functions through two receptors: IL-6 receptor (IL-6R) and gp130 [8]. IL-6R occurs in two forms, transmembrane IL-6R and soluble IL-6R (sIL-6R). Transmembrane IL-6R is only present on few cells in the body including hepatocytes and some leukocytes. In contrast, sIL-6R without the cytoplasmic region is present in human serum, and gp130 is ubiquitously expressed [9]. The generation of sIL-6R includes two major mechanisms: proteolytic cleavage of the transmembrane IL-6R and transcription of an alternatively spliced IL-6R mRNA lacking the transmembrane and cytosolic domains [10]. Thus, the presence of sIL-6R and features of gp130 enables the expansion of the repertoire of cells responsive to IL-6 (Fig. 1). When IL-6 binds to IL-6R, homodimerization of gp130 is induced and activates the Janus kinase (JAK)-signal transducer and activator of transcription 3 (STAT3) signaling pathway [11]. This is a classical signaling pathway through transmembrane IL-6R or a trans-signaling pathway through sIL-6R. It has been reported that regenerative or anti-inflammatory activities of IL-6 are mediated by classic signaling, whereas pro-inflammatory responses of IL-6 are mediated by trans-signaling [12].

This review aims to present and discuss the latest discoveries concerning the role of IL-6 in hepatitis B disease progression, HBV entry and replication, as well as IL-6 polymorphisms that affect HBV infection.

IL-6 levels in hepatitis B

Various conditions including autoimmune and chronic inflammatory diseases have been reported to be associated with high levels of IL-6 [13]. A number of clinical studies have shown that serum IL-6 levels in hepatitis B patients are higher than those in healthy people. Other reports have stated that HBV proteins such as hepatitis B virus X protein (HBx) could up regulate IL-6 expressions via the NF-κB pathway [14]. Additionally, up regulated IL-6 expressions further activates a corresponding inflammation- or tumor-associated signaling pathway, which leads to hepatitis B progression to cirrhosis or HCC [15]. In this section, we focused on the effects of HBV proteins on IL-6 and the relationship of IL-6 levels on hepatitis B progression.

Up regulation of IL-6 in hepatitis B patients

In general, pathogen-associated molecular patterns (PAMPs) recognized by pathogen recognition receptors (PRRs) in infected lesions [16] and damage-associated molecular patterns (DAMPs) released from damaged or dying cells in non-infectious inflammation [17] could promote IL-6 synthesis in various types of cells such as immune-competent cells, mesenchymal cells, endothelial cells, fibroblasts and epithelial cells [7, 18]. IL-6 initiates warning signals to the entire body, and many experiments have shown that serum IL-6 levels are elevated in patients with hepatitis B. Hence, IL-6 is also a good marker for HBV-related disease progression [19]. IL-6 levels are significantly higher in chronic hepatitis B (CHB) patients than in healthy people [20], and IL-6 is also expressed at significantly higher levels in patients with advanced liver disease (LC or HCC) compared to the CHB groups [21].

Recently, the role of IL-6 in acute exacerbation (AE) of chronic HBV infection has been reported. Patients with low serum IL-6 levels have a high frequency of hepatitis B e antigen (HBeAg) seroconversion after AE of CHB [22]. HBeAg seroconversion, the loss of serum HBeAg, and the development of anti-HBe antibodies often indicate that a patient has changed from the immune-active phase to the inactive carrier state of the disease [23]. Moreover, a combination of HBV genotype, HBV DNA levels, IL-10, and ALT levels in addition to IL-6 has been shown to be useful for predicting AE outcomes of CHB [24].

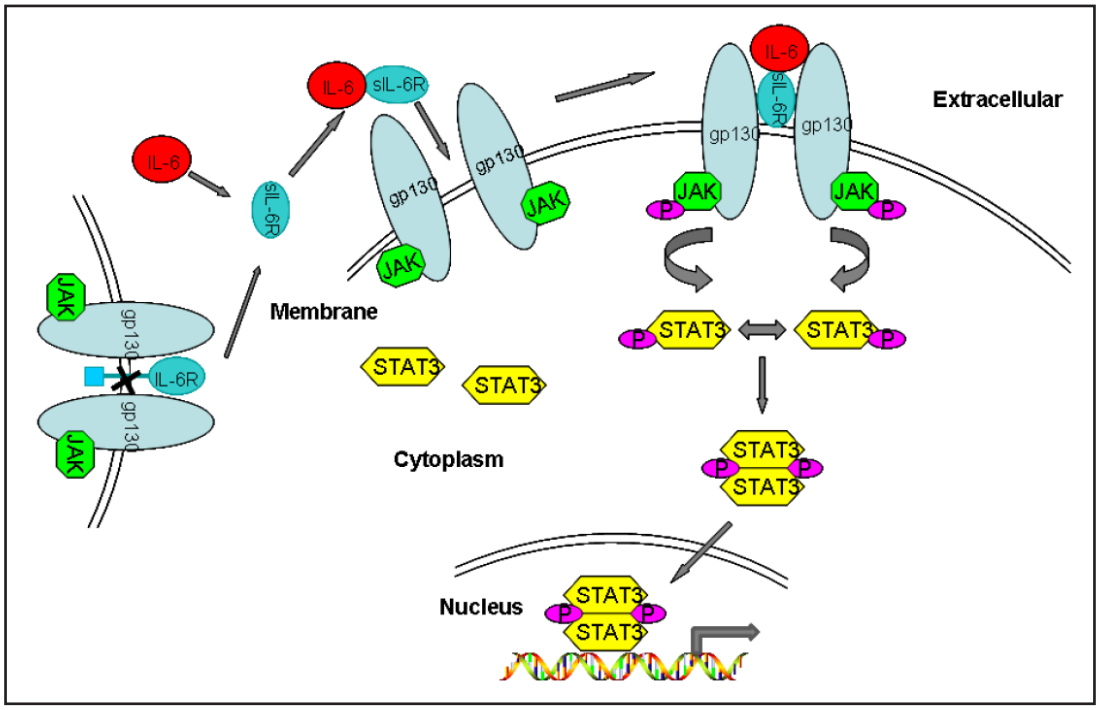

Fig. 1. Trans-signaling of IL-6. Proteolytic cleavage of the membrane bound precursor produces sIL-6R, which binds IL-6 with comparable affinity as the membrane bound form and mediates gp130 activation. Dimerization of gp130 leads to the activation of specific members of the JAK family. Activated JAKs then mediate the phosphorylation and activation of STAT3. Phosphorylated STATs dimerize and translocate into the nucleus to regulate target gene transcription.

HBx regulates IL-6 expression

IL-6 synthesis is tightly regulated both transcriptionally and post-transcriptionally [25], and transcriptional regulation of IL-6 has been reported in CHB patients [26]. IL-6 gene transcription is regulated by a variety of transcription factors. In the human IL-6 gene 5′-flanking region, functional cis-regulatory elements consist of binding sites for NF-κB and nuclear factor IL6 (NF-IL6) [27]. Many factors such as IL-1 and tumor necrosis factor (TNF) can activate cis-regulatory elements, which results in the activation of the IL-6 promoter [25, 28]. HBx enhances IL-6 gene transcription by increasing NF-κB and/or NF-IL6 DNA binding activity [26]. Lu et al. revealed that HBx gene transfection induced IL-6 expressions [29].

In addition, the study of Quétier et al. has shown that both IL-6 mRNA and circulating IL-6 levels are higher in HBx transgenic mice compared to their wild-type littermates after partial hepatectomy (PH) [30]. A recent study further verified that IL-6 levels increase with HBx expressions in hepatocytes and hepatoma cells, and discovered that the underlying mechanism for the HBx-induced production of IL-6 occur in a MyD88-dependent manner. It has been further concluded that parenchymal liver cells are an additional source of high IL-6 levels in a HBV-infected liver microenvironment [31]. Therefore, HBx is considered as an upregulator of IL-6 levels.

Effects of IL-6 on the progression of hepatitis B

Clinical and epidemiological studies have shown that inflammation plays a significant role in the initiation and progression of tumors [32]. HCC is an example of an inflammation-related cancer that could be partly triggered by exposure to infectious agents such as hepatotropic viruses [33]. Furthermore, IL-6 is considered as one of the molecules that links inflammation with cancer [34].

MyD88-dependent IL-6 production was studied in mice using a chemical carcinogen, diethylnitrosamine (DEN) [35]. Reduction in IL-6 levels leads to an almost complete inhibition of DEN-induced liver tumors. It was found that hepatocyte necrosis caused by DEN results in the production of various macromolecules, which activates macrophages through toll-like receptors (TLRs). In turn, TLRs activate MyD88 followed by NF-κB translocation to the nucleus and IL-6 induction. Therefore, the TLR-Myd88-NF-κB-IL-6 signaling pathway has a key role in liver tumor generation [32].

In addition, IL-6-IGF1R-OCT4/NANOG signaling was found to be associated with early tumor recurrence and poor prognosis in patients with HCC [36]. Transcription factors OCT4 and NANOG are pluripotency-related genes, and HCC recurrence is associated with tumor stemness properties [37]. Patients with HBV-HCC have higher serum IL-6 levels that increase autocrine IGF1 and IGF1R expressions, and increase OCT4 and NANOG expressions in a STAT3-dependent manner. Furthermore, HBx proteins upregulate IL-6 expressions and has been shown to promote HCC development through an IL-6-mediated increase in microRNA-21 expression [38].

Thus, attention should be paid to high levels of IL-6 in hepatitis B patients.

Effects of IL-6 on Hepatitis B Infection

HBV has been shown to affect serum IL-6 levels [21]. Conversely, IL-6 can also mediate HBV entry into hepatocytes [39]. However, there are also conflicting reports that IL-6 inhibits HBV entry through the down-regulation of an HBV-specific receptor [40]. IL-6 also induces an inhibitory effect on HBV replication [41]. In addition, IL-6 polymorphisms have been shown to have a close relationship to HBV infection [42]. Therefore, in this section, we focused on the influence of IL-6 and its polymorphisms on HBV infection.

IL-6-mediated HBV entry

Major target cells for HBV infection are hepatocytes. Although a number of studies on the pathogenesis and treatment of HBV infection have been carried out, the mechanism on how the pathogen enters hepatocytes remains unelucidated [43]. The main processes of HBV infection into host hepatocytes are as follows: first, HBV binds to host cell surface proteoglycans with low affinity [44]; then, more specific receptors with higher affinities mediate the early entry step [45]; and finally, the virus fuses with the cellular membrane compartment after endocytosis-mediated internalization [46]. However, cellular factors include high-affinity binding, and the early entry process has only been recently clarified.

By using human-mouse chimeras, Galun et al. demonstrated that human IL-6 promotes the HBV infection process, and they suggested that IL-6 might be a potential mediator for HBV entry into hepatocytes [39]. The preS (21-47) segment of the HBV envelope protein has been shown to bind to cell receptors [47]. Furthermore, a study carried out by Neurath et al. revealed that most (92.9%) of the IL-6 adsorbs to preS (21-47)-cellulose derivatives and play a role in HBV-cell interactions [48]. The primary interaction of HBV with cells seems to be mediated by the interaction between IL-6 and the preS1 region of the HBV envelope. IL-6 functions as an HBV receptor anchored to the cell surface through other PI-PLC releasable component(s), and at least one of which is a proteoglycan.

However, immunoprecipitation was performed to determine whether a direct or indirect interaction is present between IL-6 and HBV envelope proteins. Results reveal that there is no direct binding of human IL-6 with the preS region of the large HBV envelope protein. It was also found that soluble IL-6 receptor component sIL-6R and soluble gp130 (sgp130) do not bind with preS, either alone or in combination with IL-6 [49]. However, other studies have arrived at contradictory conclusions. One study reported that IL-6 strongly inhibited HBV entry [40], which is inconsistent with previous studies [39]. Sodium taurocholate cotransporting polypeptide (NTCP) is a multiple transmembrane transporter predominantly expressed in the liver [50], and is recognized as an HBV-specific receptor that binds to the N-terminal part of the pre-S1 region of the large envelope protein [51]. NTCP is

known to be strongly downregulated by IL-6 [52]. Thus, IL-6 prevents HBV entry into cells through the downregulation of NTCP. This finding supports the significant indirect role of IL-6 in mediating HBV entry.

Therefore, previously proposed mechanisms by which IL-6 and HBV interact with each other are not consistent with the current study. The exact role of IL-6 in HBV infection requires further research. In addition, two *in vitro* HBV infection models that consist of primary human hepatocytes (PHH) and the HepaRG cell line can provide more persuasive data in clarifying the mechanism of HBV entry [53, 54].

IL-6-mediated HBV replication

Some cytokines have been demonstrated to effectively suppress HBV replication in a non-cytopathic manner in a cell culture system (IFN-α/β, IFN-γ and TNF-α) and in HBV transgenic mice (IL-12, IL-18 and intrahepatic induction of IFN-α/β). In addition, serum IL-6 levels have been reported to be elevated in patients with HBV-related diseases. Based on these data, the study of Kuo et al. revealed that IL-6 suppresses HBV replication in an HBV-replicating cell line [41].

There are two major mechanistic classes of cytokines involved in non-cytopathic suppression of hepatitis B virus replication. The first class including IL-4 [55] and TGF-β1 [56] inhibits viral gene expression, and subsequently suppresses HBV replication. The second class (Types I and II IFN [57] and TNF-α [58]) destabilizes viral genome-containing capsids or prevents their assembly.

IL-6 blocks HBV replication through a moderate reduction of viral transcripts as IL-4 and TGF-β1, and a more dramatic reduction of viral genome-containing nucleocapsids similar to the effect seen with interferon [41]. Thus, IL-6 inhibits HBV replication using both mechanisms. In addition, IL-6 also inhibits the accumulation of HBV cccDNA [41]. Since the formation of HBV genome-containing nucleocapsids is a complicated process, determining the exact mechanism of the antiviral effect of IL-6 requires further investigation.

Hosel et al. [59] reported findings that were similar to those of Kuo et al. The former found that non-parenchymal liver cells, most probably Kupffer cells, recognizes HBV patterns, and results in the IL-6-mediated control of HBV infection at a transcriptional level. The recognition of HBV patterns by non-parenchymal liver cells activates NF-kappa B, and subsequently induces the release of IL-6. IL-6 mediates and controls HBV transcription by binding to its receptors (IL-6R and gp130). IL-6 activates mitogen-activated protein kinases exogenous signal-regulated kinase (ERK) 1/2 and c-jun N-terminal kinase (JNK), which in turn downregulates the expression of hepatocyte nuclear factor (HNF) 1 alpha and HNF 4 alpha. These two transcription factors control HBV gene expression and replication in a concerted action, demonstrating a new mechanism by which cytokines control HBV transcription.

Therefore, if IL-6 levels are therapeutically decreased, HBV-infected patients should be carefully monitored.

Relationship between IL-6 polymorphisms and the development of hepatitis B infection

The virus, immune response and genetic diversity are three main factors that affect the outcome of HBV infections [60, 61]. Many researchers favor the idea that gene polymorphism has an important effect on the outcome of HBV infection. Single nucleotide polymorphisms (SNPs) play a role in causing the diversity of HBV clinical courses. Recent studies have demonstrated that IL-6 polymorphisms are involved with chronic HBV infection progression [42].

Cytokines are important aspects of the immune response to HBV, and are involved in determining whether an HBV infection is self-limited, persistent or progressive. IL-6 polymorphisms have been studied to explore the possible relationship among IL-6 promoter variants, chronic hepatitis B evolution, and risk of HCC progression. An association between IL-6 gene polymorphisms -174, -572 and the clinical course of chronic HBV infection has been reported [62].

Park et al. [63] conducted a study of the IL-6-174 site and attempted to evaluate the correlation between chronic hepatitis B progression and IL-6 promoter variants. They found that Koreans and Caucasians have different genetic backgrounds with regard to allele frequencies of the IL-6 promoter SNPs. In their study, the allele frequency in Koreans (0.002) is much lower than those reported in Caucasians (range, 0.40-0.45) [64]. Therefore, as frequencies of IL-6 174 G/C are very low, there appears to be no significant relationship between IL-6 genetic variants and the development of LC and HCC from chronic HBV infection. Thus, these data is not conclusive, although IL-6 may yet have an important role in chronic HBV infection progression. Another attempt to characterize cytokine gene polymorphisms in chronic HBV infection was made in a Japanese population. However, Migita et al. [65] were unable to show conclusive data on the role of IL-6-174, because no significant difference was found between chronic hepatitis B patients and controls at this position.

Similarly, Ribeiro et al. [66] studied the relationship between cytokine genetic polymorphisms and hepatitis B infection progression in a Brazilian population. However, this study found no definitive evidence pointing to the association of IL-6 -174 polymorphisms in the chronic HBV patient group versus the self-limited infection group. They concluded that more chronic HBV patients should have been involved in the study.

Regarding the -572 site, some studies have reported that there is no significant association between IL-6-572 polymorphism and the outcome of chronic hepatitis B disease [63]. However, the chance of HCC is higher with the G allele in male subjects than among patients with the CC genotype when all subjects are adjusted based on gender [67]. In females, there is no correlation between HBV-related HCC and the G allele of IL-6-572. HBV-related HCC risk is only associated with the G allele in males.

Furthermore, one study discovered that the IL-6-572 site is associated with spontaneous clearance of HBV [68]. Through a case-control study that included 219 cases with chronic HBV infection and 212 controls that had spontaneously recovered from HBV infection, Lu et al. found that controls have significantly higher allele G and GG genotype frequencies for IL-6 -572 C/G than patients with chronic HBV infection. Thus, they concluded that IL-6-572 allele G is associated with the natural elimination of hepatitis B virus infection.

In conclusion, the role of IL-6-174 is not involved in the development of LC and HCC from chronic HBV infection. However, the G allele in the IL-6-572 polymorphism site is associated with the increased risk of HBV-related HCC, which is observed only in males. The relationship between IL-6 polymorphisms and the development of hepatitis B viral infection needs to be further studied.

Conclusions and Outlook

IL-6 levels are frequently elevated in patients with hepatitis B, and persistent exposure to IL-6 could result in liver injury, which finally leads to HCC. Thus, abolishing IL-6 signal transduction may be an innovative therapeutic strategy for hepatitis B-related HCC. Humanized neutralizing IL-6R monoclonal antibody tocilizumab [69] has been used for treating systemic juvenile idiopathic arthritis [70], rheumatoid arthritis [71] and Castleman disease [72]. A greater clinical benefit than conventional therapy has been shown for various intractable inflammatory autoimmune diseases. Diseases such as large-vessel vasculitis are being considered as candidate diseases for off-label applications of tocilizumab [73]. Currently available treatments for CHB include antiviral agents (nucleos(t)ide analogues and NAs) and immune-based therapies (IFN-α or pegylated-IFN-α). Some NAs are susceptible to drug resistance development and have poor sustained virological response rates [74]. Interferon-based therapies are frequently accompanied by numerous side effects [75]. Therefore, various alternative immunotherapeutic interventions have been tried as adjuvants to inhibit HBV replication. TNF-α and IFN-γ are two important cytokines that have been considered for immunotherapeutic intervention [76]. Based on available data, IL-6 should also be taken into consideration.

However, several studies have shown that IL-6 could suppress HBV replication and inhibit HBV entry. IL-6 can have regenerative and protective characteristics during infection and inflammation, which is needed for the activation of the immune system [77]. Thus, the use of IL-6 as a therapeutic strategy for hepatitis B is not simple, and it is important to keep in mind that neutralization of IL-6 may present a risk for hepatitis B patients. Tocilizumab blocks IL-6 classic- and trans-signaling pathways, and blocks both pro- and anti-inflammatory activities of IL-6. In recent literatures on IL-6 classic- and trans-signaling pathways, studies on antibodies have shown that specifically blocking trans-signaling pathways may be a better solution. This may provide the basis for new specific drugs [12].

Abbreviations

IL-6 (interleukin 6); HBV (hepatitis B virus); LC (liver cirrhosis); HCC (hepatocellular carcinoma); IL-6R (IL-6 receptor); sIL-6R (soluble IL-6R); JAK (Janus kinase); STAT3 (signal transducer and activator of transcription 3); SHP-2 (Src-homology domain-containing protein tyrosine phosphatase 2); MAP (mitogen-activated protein); HBeAg (hepatitis B e antigen); PAMPs (pathogen-associated molecular patterns); PRRs (pathogen recognition receptors); DAMPs (damage-associated molecular patterns); CHB (chronic hepatitis B); AE (acute exacerbation); NF-IL6 (nuclear factor IL6); TNF (tumor necrosis factor); PH (partial hepatectomy); DEN (diethylnitrosamine); TLRs (toll-like receptors); IGF1 (insulin-like growth factor-1); IGF1R (insulin-like growth factor-1 receptor); OCT4 (octamer-binding transcription factor 4); sgp130 (soluble gp130); NTCP (sodium taurocholate cotransporting polypeptide); PHH (primary human hepatocytes); ERK (exogenous signal-regulated kinase); JNK (c-jun N-terminal kinase); HNF (hepatocyte nuclear factor); SNPs (single nucleotide polymorphisms); NAs (nucleos(t)ide analogues); HBx (hepatitis B virus X protein).

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Disclosure Statement

The authors have no conflicts of interest to disclose.

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