Review Article

IL-6 Plays a Crucial Role in HBV Infection

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Abstract

Interleukin-6 (IL-6), a cytokine mainly produced by activated monocytes, has broad pleiotropic actions that affect the functions of a variety of lymphoid cells. The roles of IL-6 in regulating immunity to infections are currently being defined. Remarkably, IL-6-mediated cellular and humoral immune responses play a crucial role in determining the outcome of viral infection. This article reviews the current knowledge on the critical role of IL-6 in hepatitis B virus (HBV) infection. As a competent intermediary, IL-6 derived from activated monocytes plays an important role in promoting lymphocytes responses that are essential for effective viral control. However, as a mediator of inflammation, IL-6 is also involved in the development of HBV-induced liver cirrhosis and exacerbating liver injury. Overall, the current data point to IL-6 as an immunoregulatory cytokine in HBV infection. Immunotherapeutic strategies aimed at optimizing the beneficial effects of IL-6 in HBV infection may prove to be an ordeal in the future, as they should foster the strengths of IL-6 while circumventing potential drawbacks.
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Introduction

Hepatitis B virus (HBV) is the most common virus with the potential to cause human liver disease, including self-limiting acute hepatitis, chronic hepatitis, fulminant hepatic failure, liver cirrhosis and hepatocellular carcinoma (HCC). The latter three diseases often result in death. Worldwide, HBV causes an estimated 250,000 deaths per year and is a significant public health problem. Hepatitis B is an immune-related disease, and the hepatocellular injury caused by HBV is indirect and is induced by an immune response that results in hepatocellular degeneration and necrosis and hyperplasia of hepatic fibrous tissue. A complete and specific immune response against HBV can completely eradicate the virus in infected hepatocytes.[1] However, mounting an inappropriate immune system response results in one of the primary clinical presentations of HBV-related diseases.[2] The mechanisms underlying these persistent and progressive HBV infections remain unknown. A number of factors, such as virus subtype, host condition, and environmental and genetic factors, affect the outcome of HBV infections. In addition, host immunological and genetic factors play important roles in the pathogenesis of hepatitis B.[3] The current consensus is that cytokines and regulatory molecules are involved in generating an appropriate immune response to eradicate an HBV infection. Interleukin (IL)-6 is a multifunctional, potent, pleiotropic inflammatory cytokine and is a key immune response regulator.[4] IL-6 inhibits macrophages to produce IL-1 and tumor necrosis factor (TNF), which has been shown to mediate protective and anti-inflammatory effects in endotoxin-induced lung injury. According to many studies, IL-6 expression is increased differentially in response to inflammation, infection, and the presence of certain tumors.[5−7] Thus, IL-6 is a sensitive index for disease severity and prognosis. It seems that IL-6 can play crucial roles in the induction of immune-tolerance against HBV antigens, and its activity is likely to be involved in the determination of outcomes of hepatitis B patients. Here we review the most recent information concerning the relationship between IL-6 and HBV infection.

Introducing IL-6

Human IL-6 is a glycosylated protein with a molecular weight of 26 kD. The gene is located on chromosome 7 and contains 5 exons and 4 introns. The IL-6 precursor peptide is composed of 212 amino acids (aa). The IL-6 gene promoter region contains important transcriptional control elements that are regulated by nuclear factor kappa B (NF-κB) and activating protein-1 (AP1), among other proteins.

The IL-6 receptor is composed of the IL-6 binding receptor protein (IL-6R) and a universally expressed 130 kD signal-transducing β-receptor (gp130).[8] An important marker of IL-6 activity, gp130 is a glycopeptide expressed on the surface of most cells and is capable of trans-membrane signaling. In contrast, IL-6R expression is restricted largely to hepatocytes, leukocytes and megakaryocytes,[9] and the soluble form of IL-6R (SIL-6R) has been found in a variety of bodily fluids.[10] A study by Matthews identified two modes

of SIL-6R production: direct release into extracellular fluids upon synthesis and catabolic release from the membrane following limited proteolysis.[11]

IL-6 transmembrane signal transduction can be divided into two types: classical signaling pathways and a trans-signaling pathway. In the classical signaling pathways, binding of IL-6 to IL-6R activates the receptor-associated gp130 to transmit secondary signals in cells.[12] However, in the trans-signaling pathway, IL-6 first combines with the subunit IL-6Ra, which exists unbound in the extracellular fluid, to form the IL-6/IL-6Ra complex. This complex can then be coupled to the gp130 subunit on the surface of the plasma membrane to complete signal transduction.[13]

IL-6 was originally prepared and purified by Hirano *et al*. from the culture supernatant of mitogen- or antigen-stimulated human T-lymphotropic virus type 1 (HTLV-1)-transfected T lymphocytes.[14] These authors elucidated the N-terminal amino acid sequence and successfully cloned the cDNA encoding human IL-6.

Activated monocytes are the main source of IL-6 in blood. When inflammation occurs, monocytes and macrophages are the first reactive cells that produce IL-6.[15] Thus, IL-6 was initially thought to promote T lymphocyte population expansion and activation, promote B lymphocyte differentiation, and regulate the acute-phase response.[16] Fibroblasts and some tumors, such as cardiac myxoma, cervical carcinoma, and myeloma, spontaneously produce IL-6.[17–19] *In vitro*, dendritic cells secrete IL-6. Therefore, almost all stromal and immune cells are capable of producing IL-6. IL-1b and TNF are major activators of IL-6 expression. Other pathways, such as toll-like receptors, prostaglandins, adipokines, and cytokines, can also regulate IL-6 synthesis. We now know that IL-6 has hormone-like functions that contribute to cardiovascular disease, lipid metabolism, insulin resistance, mitochondrial activity, and neuroendocrine regulation.[20,21] However, the most important biological activity of IL-6 is immunoregulation, as IL-6 deficiency can lead to dysfunctional innate and adaptive immunity against many infections.[22,23] Puel *et al*. reported a case in which a child with autoimmunity against IL-6 developed recurring staphylococcal cellulitis and subcutaneous abscesses.[24] Similarly, patients with Job's syndrome, who harbor mutations in the gene encoding signal transducer and activator of transcription-3 (STAT3), exhibited impaired IL-6 activity and were susceptible to almost all types of recurrent infections. According to the literature, IL-6 exhibits both pro- and anti-inflammatory functions in innate immunity.[25] Due to its bidirectional capabilities, the effect of IL-6 is dependent on its expression level in a given tissue. Normal levels of IL-6 expression are conducive to immunological homeostasis, whereas excessive production can cause a series of inflammatory lesions, leading to such diseases as rheumatoid arthritis, Crohn's disease, and glomerulonephritis.[26–28] Transgenic mice overexpressing IL-6 developed a variety of disorders, including pulmonary fibrosis, hypertension, multiple myeloma, plasmacytosis, and neurological disease.[29–31]

As a lymphocyte-stimulating factor, IL-6 can induce B cells to differentiate into antibody-secreting cells and induce the latter to transcribe mRNA encoding secretory immunoglobulin (SIg), thereby increasing the secretion of IgM, IgG, and IgA antibodies.[32] Muraguchi *et al*. demonstrated that B lymphocytes activated by staphylococcal protein A (SPA) or poke weed mitogen (PWM) could synthesize immunoglobulin.[33] The addition of anti-IL-6 monoclonal antibody prevented this effect, suggesting that IL-6 is required for the production of antibodies by B lymphocytes. Previous studies have demonstrated the role of IL-6 as a central link between T cell and B cell responses.[34,35] Human IL-6 is a terminal cofactor of cytotoxic T lymphocytes (CTL), and the ability of IL-6 to promote adaptive immunity has been linked to helper T cells and regulatory T cells through the interaction with other cytokines.[36]

IL-6 and HBV infection

Given the role of IL-6 in balancing the differentiation of pro- and anti-inflammatory cells, it follows that it may play an important role in the progression of HBV infections. A number of studies have shown that IL-6 serum levels are increased in HBV-infected patients and are significantly higher in patients with severe, acute infections than patients with a chronic active infection. Chronically affected patients exhibited significantly higher IL-6 levels during the acute jaundice stage.[37–39] HBV can infect peripheral blood monocytes, and can actively replicate in these cells.[40] In chronic active hepatitis (CAH), the decrease in the processing of antigens by Kupffer cells and the polyclonal activation of intestinal antigens increased serum IL-6 levels.[41] In chronic severe hepatitis (CSH), massive necrosis of hepatocytes, loss of Kupffer cell phagocytosis, and endotoxemia, which is attributed to adecrease in intestinal mucosa function and default in endotoxin elimination due to liver injury, stimulated the mononuclear phagocyte system to produce more IL-6. In addition, since the liver is the major organ responsible for the elimination of IL-6, severe injury of the liver will impair removal of IL-6, leading to an increase in plasma levels of IL-6. Simultaneously, high levels of IL-6 can induce proliferation and differentiation of cytotoxic T-cells,[42] causing liver inflammation and the destruction of immunological cells. In patients with chronic hepatitis B, IL-6 levels in hepatitis B early antigen (HBeAg) (+) and HBV-DNA (+) patients were significantly higher than HBeAg (−) and HBV-DNA (−) patients. After treatment with interferon, IL-6 serum levels decreased significantly in HBeAg (+) and HBV-DNA (+) patients, indicating that HBV replication is related to IL-6 levels and that high IL-6 levels have a synergistic antiviral effect.[43] Therefore, IL-6 may be a useful marker for monitoring disease activity and therapeutic efficacy in patients with hepatitis B. Undetectable serum IL-6 levels (<3 pg/mL) during the early stage of acute exacerbation (AE) in patients with chronic hepatitis B can signify patients who will have favorable clinical outcomes, indicating that IL-6 may be a useful clinical predictor of prognosis.[44] Furthermore, Zhang *et al*. detected the serum concentrations of IL-6 in 18 patients with chronic virus hepatitis B (CH), 14 patients with hepatitis cirrhosis (HC) without ascites, and 22 HC patients with ascites (HC). In the 18 patients with CH, serum IL-6 concentrations were the lowest. IL-6 level in the 22 HC patients with ascites was significantly higher than that in the 14 HC patients without ascites, indicating that IL-6 may participate in the pathological process of CH and that cirrhosis could play an important role in ascites formation.[45]

Nevertheless, the mechanisms of adhesion and invasion of human hepatocytes by HBV virus particles remain unclear. De Meyer *et al*. hypothesized that IL-6 participates in the interaction between the HBV viral particle and the hepatocyte plasma membrane. HBV can bind the preS1 domain, which is likely an important attachment site on human hepatocytes
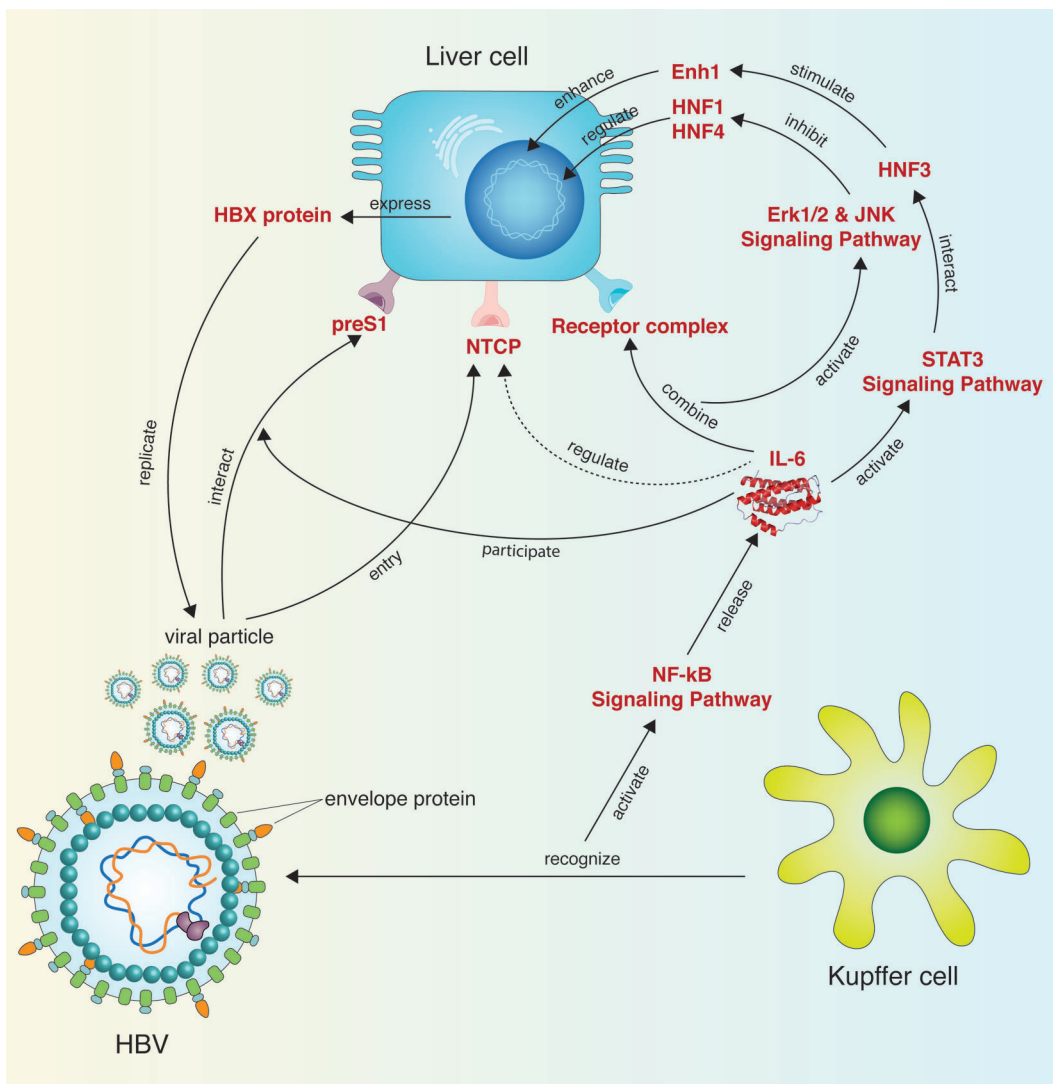
Fig. 1. Potential mechanism of IL-6 on the HBV infection.

that mediates HBV infection (Fig. 1).[46] Another study indicated that the HBV envelope protein is critical during the infection process and is recognized by nonparenchymal liver cells (predominantly liver macrophages (Kupffer cells), although they are not infected).[47]

By activating certain signaling pathways, IL-6 can increase the activity of the HBV enhancer 1 (Enh1) to control the expression of HBV X protein (HBx) and the replication of HBV (Fig. 1).[48] A study by Chou *et al*. identified IL-6 as the main bystander mediator of radiotherapy (RT)-induced HBV replication. HBV transgenic mice were treated with whole liver RT and IL-6. HBV core protein staining confirmed the augmentation of intrahepatic HBV replication. Simultaneously, in HepG2 hepatoma cells that received the same treatment *in vitro*, a similar conclusion was reached. Furthermore, RT of the liver and longer, sustained IL-6 levels induced HBV reactivation through the interaction of phosphorylated STAT3/hepatocyte nuclear factor (HNF)-3 complex with HBV Enh1 (Fig. 1).[49] In turn, HBx expression in human hepatocytes and hepatoma

cells has been shown to activate the IL-6 gene and stimulate IL-6 protein synthesis, a process that requires the participation of IL-1 receptor associated protein kinase (IRAK)-1, p38/extracellular signal regulated kinase (ERK), or NF-κB (Fig. 1).[50–52] These results indicated that the sensitivity of hepatocytes and hepatoma cells to HBx is considerably different. Hepatocytes synthesize and secrete much more IL-6 than hepatoma cells, and they react differently than hepatoma cells to IL-6 stimulation during the regulation of HBV replication. It has been reported that IL-6 in hepatoma cells may stimulate HBV transcription by activating STAT-3, which interacts with HNF3 bound to the HBV enhancer (Fig. 1).[49] In contrast, Kuo *et al*. demonstrated that IL-6 effectively suppressed HBV replication and prevented the accumulation of HBV covalently closed circular DNA (cccDNA) in HepG2 hepatoma cells.[53] Furthermore, Hosel *et al*. found that recognition between the HBV envelope proteins and Kupffer cells led to activation of NF-κB and the release of IL-6, which activated mitogen-activated protein kinases, including ERK 1/2 and

c-jun N-terminal kinase. These kinases inhibited the expression of the transcription factor HNF1 and HNF4, which are essential for HBV gene expression and replication (Fig. 1).[47] The authors posit that IL-6 ensures early virus control, limits the activation of the adaptive immune response, and prevents the death of HBV-infected hepatocytes. Similarly, the effect of IL-6 on hepatocytes is also controversial. IL-6 levels increase significantly during severe hepatitis, and IL-6 is involved in the activation of natural killer (NK) cells and CTLs that induce the killing of hepatocytes, indicating that IL-6 plays an important role in liver cell necrosis and apoptosis.[42] However, according to Klein *et al*., IL-6 is beneficial during liver injury.[54] The expression of IL-6–gp130–STAT3–dependent genes can protect hepatocytes from injury via regulatory T cells, which promote the regeneration of liver cells and reduce the effect of various damaging factors, such as alcohol and carbon tetrachloride.[54]

Clinically, although serum IL-6 levels are positively related to disease severity and HBV-DNA load, therapeutic neutralization of IL-6 as a treatment for certain diseases may be risky if the patient is infected with HBV.[47]

The HBx protein plays a critical role in the development of HBV-related HCC.[55] Previous studies have shown that numerous HBx-altered genes and signaling pathways contribute to tumorigenesis via hepatocytes.[56–58] Li *et al*. observed that the HBx-miR-21 pathway was up-regulated in HCC cells and that HBx expression in Hep3B and PLC/PRF/5 cells significantly suppressed miR-21 expression, which is mediated by the HBx-induced IL-6 pathway with subsequent activation of the STAT3 transcription factor.[59] Another study demonstrated that high IL-6 serum levels were significantly correlated with high OCT4/NANOG expression in HBV-related HCC, and early tumor recurrence was regulated by IL6-induced insulin-like growth factor (IGF)/IGF 1 receptor (IGFIR) activation.[60]

Recently, the human liver bile acid transporter Na(+)/taurocholate cotransporting polypeptide (NTCP) was identified as an HBV specific receptor, and silencing NTCP was shown to inhibit HBV and HDV infection (Fig. 1).[61] Another study demonstrated that IL-6 inhibited HBV entry by regulating NTCP expression in a dose- and time-dependent manner.[62]

IL-6 is important for the progression of chronic forms of hepatitis B infection and plays a critical role in HBV-induced fibrosis, liver cirrhosis, and HCC. Thus, IL-6 can be regarded as a risk factor for hepatitis B and its associated complications. Additional research is required to elucidate the mechanisms of IL-6 in the process of HBV infection.

IL-6 gene polymorphism and HBV infection

IL-6 is a highly polymorphic gene, and several regions have been identified that may be responsible for its variable protein expression, including three variations upstream of its coding sequence at positions -174, -572, and -597.[63] Furthermore, genotype and allele frequency vary widely among different races. Among Caucasian and Indian populations, the gene frequency of the C allele (-174G>C) ranges from 0.4–0.55, whereas among the African American population, the gene frequency of this allele ranges from 0.5–0.9.[64] Early work posited that IL-6 gene polymorphism was not significantly correlated with susceptibility to HBV infection or the progression of HBV-related diseases.[63] However, these studies were small in scale, and the data were not convincing. A subsequent investigation demonstrated that the -174G/C single

nucleotide polymorphism (SNP) in the IL-6 promoter region increased the transcription and expression of IL-6, and serum IL-6 level was found to be associated with the progression of HBV-related HCC.[65] A study by Lu *et al*. indicated that the IL-6 -572 G allele may be beneficial for spontaneous HBV clearance, but the allele and genotype frequencies of -597G/A were not significantly different between patients with chronic HBV infections and patients who spontaneously recovered.[66] Similarly, Dai *et al*. observed the genotypes -174G/C, -572G/C and -597G/A in 160 Chinese patients infected with HBV and 212 healthy blood donors.[67] The authors found that there was no link between HBV resistance and the -174G/C and -597G/A alleles, but a significant difference at the -572G/C loci was observed between the HBV infected patients and healthy controls. A hospital-based, case control study of SNPs in the IL-6 promoter region involving 381 cases of HBV-related HCC, 340 hepatitis B surface antigen (HBsAg) carriers, and 359 non-tumor controls[68] revealed significant differences among the three groups in the -572G/C allele of the IL-6 gene. Compared to the CC genotype, the GG genotype was correlated with an increased risk of HBV infection but was not associated with HCC. A recent study found that among Indian people, the IL-6 (-572G>C) GC genotype was positively associated with hepatitis among controls and was negatively associated with cirrhosis and consequent HCC development among carriers. Furthermore, the IL-6 (-597G>A) GA genotype was potentially protective against hepatitis, cirrhosis, and subsequent HCC development in carriers.[69] In conclusion, these data illustrate how SNPs in the IL-6 gene promoter fundamentally determine low and high risk among HBV-infected patients.

Conclusions

IL-6 is a key mediator of inflammation and the acute phase response of the liver. IL-6 has been shown to prevent apoptosis during HBV infections, and serum levels of IL-6 are increased in HBV-infected patients. In addition, serum IL-6 levels are positively correlated with disease severity. Thus, IL-6 may be a useful indicator of disease activity and therapeutic efficacy in patients with hepatitis B. It is currently thought that IL-6 increases the activity of Enh1 to control HBx expression and HBV replication through activation of the IL-6R/gp130/STAT-3 signaling pathway. In contrast, HBx (in combination with IRAK-1, p38/ERKs, or NF-κB) can activate the IL-6 gene and stimulate IL-6 protein synthesis. However, the protective effect of IL-6 on hepatocytes during liver injury is controversial. Numerous studies have suggested that the SNP -572G/C in the IL-6 gene promoter region is significantly correlated with susceptibility to HBV infection and progression of HBV-related diseases.

Recently, IL-6 has become a target of therapeutic interventions aimed at reducing the progression of inflammatory autoimmune diseases and cancers. One popular strategy is a combination therapy consisting of IL-6 blockade and conventional drugs; this approach has resulted in improved treatment efficacy and patient response compared with monotherapy.[70,71] As IL-6 is closely linked to the development of HBV infection, which is accompanied by immune disorders and tumorigenesis, we speculate that IL-6 blockade could boost treatment efficacy in patients with HBV-related diseases. Therefore, similar clinical trials are urgently needed to elucidate the pathogenesis of IL-6 function and

confirm the role of IL-6 in the progression of HBV-related diseases.

Conflict of interest

None.

Author contributions

Drafting the manuscript (TL), revising the manuscript (LC), designing the figure (LW), conceiving this work, giving critical comments and editing the manuscript (YFY).

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