

Serum Interleukin-6 and Interferon-gamma Levels in Patients with Hepatitis B-Associated Chronic Liver Disease

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Hepatitis B virus (HBV) infection is associated with a variety of clinical consequences, ranging from acute self-limited hepatitis to fulminant hepatitis during the acute phase; and from asymptomatic carrier state to chronic hepatitis, cirrhosis and hepatocellular carcinoma (HCC) during the chronic phase.¹ The factors and mechanisms determining such varying developments are unclear. However, it has been well recognized that the virus itself is not directly cytopathic, and that the cellular immune response plays an important role in both resolution and disease progression.² Hence, the diversity with respect to immune responsiveness to HBV infection may account for the vastly differing clinical outcomes. For example, as a consequence of immune tolerance in newborns, about 90% of those infected will become chronic carriers of HBV and usually harbor the virus throughout their lives. In contrast, the risk of chronicity in immunocompetent adults is relatively low, ranging between 5 and 10%.³

SUMMARY Hepatitis B virus (HBV) infection can elicit a variety of clinical sequelae ranging from acute self-limited hepatitis to hepatocellular carcinoma, which are not attributable to a direct cytopathic effect of the virus but rather to the individual host's immune response. Cytokines, low-molecular-weight proteins with a broad range of activity, have been shown to be involved in the regulation of hepatocyte functions, as well as in the pathogenesis leading to liver damage. In the present study, we investigated the correlation between serum interleukin 6 (IL-6) and interferon gamma (IFN- γ) in altogether 75 patients chronically infected with HBV. They comprised 15 asymptomatic carriers, 15 chronic persistent hepatitis (CPH) and 15 chronic active hepatitis (CAH) patients, 15 cases of cirrhosis and 15 patients with hepatocellular carcinoma (HCC) previously diagnosed by serology and histology, respectively. IL-6 and IFN- γ levels in their sera were determined using a commercially available kit. Our results showed various concentrations of serum IL-6 detectable in 6.7% of asymptomatic carriers, 13.3% of patients with CPH, 20% of patients with CAH, 33.3% in cirrhotic patients and 66.7% in HCC. In contrast, serum IFN- γ was only found in 13.3% of asymptomatic carriers and CAH, but could not be detected in the other groups. Our data demonstrated a positive correlation between serum IL-6 and clinical severity of chronic HBV infection, whereas the IFN- γ levels appeared not to be correlated. From this we conclude that among chronic hepatitis patients IFN- γ is mostly not expressed at a level detectable by serology, whereas according to other authors it is involved in the immediate immune response triggered by acute hepatitis. IL-6 on the other hand, might rather be responsible for liver inflammation and regeneration in chronic liver disease.

Cytokines are low-molecular-weight proteins that bind to specific cell-surface receptors and possess a wide spectrum of activity such as inflammation regulation, tissue repair, hematopoiesis and im-

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immune response.⁴ In the context of chronic liver disease, it has been demonstrated that cytokine networks play an important role in regulating hepatocyte functions as well as in the pathogenesis leading to liver damage.⁵ An increase of certain cytokine levels in serum may be considered an index of necroinflammatory activity. For example in patients with hepatitis C virus (HCV) infection, increased circulating interleukin-6 (IL-6) is correlated with hepatic inflammatory activity and serum HCV-RNA levels.⁶ In addition, there is a significant correlation between the IL-6 and aminotransferase levels in patients with chronic active hepatitis (CAH) due to HBV infection.⁷ Likewise, serum interferon-gamma (IFN- γ) is detected at a significantly higher frequency in patients with CAH than in those with chronic persistent hepatitis (CPH) due to HBV infection.⁸

In the present study, we investigated the correlation between serum IL-6 and IFN- γ and various stages of chronic HBV infection ranging from the asymptomatic carrier state over chronic persistent hepatitis (CPH) and

chronic active hepatitis (CAH) to cirrhosis and HCC.

MATERIALS AND METHODS

Patients

Seventy-five patients affected by chronic HBV infection who sought treatment at Chulalongkorn Hospital were enrolled in this study. They were divided into 5 groups based on the stage of liver disease. The first group comprised 15 HBV carriers negative for HBeAg and exhibiting normal liver function test results. The second and third groups, respectively, consisted of 15 patients each with CPH (10 with HBeAg and 5 with anti-HBe) and CAH (12 with HBeAg and 3 with anti-HBe) ascertained on histological grounds. The fourth group consisted of 15 patients with histologically proven cirrhosis (7 with HBeAg and 8 with anti-HBe). The remaining group comprised 15 patients with a diagnosis of HCC based on histology and serum alpha-fetoprotein (AFP) levels above 400 pg/ml. We excluded patients with acute infection or febrile illness. Details of the studied patients are summarized in Table 1.

Laboratory methods

Hepatitis B marker and liver function test

The three relevant hepatitis markers, HBsAg, HBeAg and anti-HBe, were determined by enzyme-linked immunosorbent assay (ELISA) (Abbott Laboratories, North Chicago, IL). Serum transaminase (AST and ALT) levels were determined using an automated chemical analyzer (Hitachi 911) at the Central Laboratory, Chulalongkorn Hospital. All patients were informed as to the objective of the study and they subsequently provided their consent. Blood was obtained in the course of examinations performed in the carrier group and during liver biopsy carried out in the other groups. Sera were separated by centrifugation and stored at -70°C until subjected to the respective tests.

Measurement of IL-6 and IFN- γ levels

We determined the respective levels of IL-6 and IFN- γ using a commercially available kit (Diacclone Research, France) ac-

Table 1 Clinical characteristics of patients in this study

Group	N	Sex (M/F)	Age (years)	ALT (IU/l)	HAI score
Asymptomatic carriers	15	11/4	39.9 \pm 11.6	29.6 \pm 4.4	
Chronic persistent hepatitis	15	12/3	39.5 \pm 9.4	93.7 \pm 69.2	4.7 \pm 1.0
Chronic active hepatitis	15	11/4	31.7 \pm 10.2	159.7 \pm 74.0	10.4 \pm 1.9
Cirrhosis	15	13/2	38.4 \pm 9.4	118.4 \pm 66.1	12.4 \pm 1.7
Hepatocellular carcinoma	15	12/3	50.9 \pm 8.7	109.7 \pm 64.7	

HAI score: Histology activity index (HAI) score (Knodell score)
Quantitative variables were expressed as mean \pm SD.

Table 2 IL-6 and IFN- γ detected in serum samples of patients with chronic HBV infection in relation to categorial groups

Group	No. of positive samples (%)	
	IL-6	IFN- γ
Asymptomatic carriers	1/15 (6.7)	2/15 (13.3)
Chronic persistent hepatitis	2/15 (13.3)	0/15 (0)
Chronic active hepatitis	3/15 (20)	2/15 (13.3)
Cirrhosis	5/15 (33.3)	0/15 (0)
Hepatocellular carcinoma	10/15 (66.7)	0/15 (0)

According to the manufacturer's instructions. The protein contents discernible by this method ranged from 6.25 to 200 pg/ml for IL-6, and from 12.5 to 400 pg/ml for IFN- γ , respectively.

Statistical analysis

Data were presented as mean \pm standard deviation or expressed in percent related to the total number of patients, as appropriate.

RESULTS

In our study, elevated IL-6 was more frequently found in patients with HCC than in the other groups. Using the minimal detectable level (6.25 pg/ml) as the cut-off point, various concentrations of serum IL-6 were detected in 1 out of 15 (6.7%) asymptomatic carriers (112.6 pg/ml), 2 out of 15 patients (13.3%) with CPH (range 46.8-144.7 pg/ml), 3 out of 15 patients (20%) with CAH (range 8.5-300.9 pg/ml), 5 out of 15 patients (33.3%) with cirrhosis (range 72.2-622.0 pg/ml) and in 10 out of 15 patients (66.7%) with HCC (range 6.4-304.9 pg/ml) (Table 2). Thus, our study clearly demonstrated a positive correlation between serum IL-6 and the clinical severity of chronic HBV infection. Fig. 1 illustrates the distribution of IL-6 levels per group.

In contrast, serum IFN- γ above the minimal detectable level (12.5 pg/ml) was found only in 2 out of 15 (13.3%) asymptomatic carriers (range 75.1-144.2 pg/ml) and in 2 out of 15 patients (13.3%) with CAH (one HBeAg-positive and one HBeAg-negative; range 32.4-96.5 pg/ml), whereas none of CPH, cirrhosis and HCC patients, had detectable IFN- γ in serum samples (Table 2). The distribution of

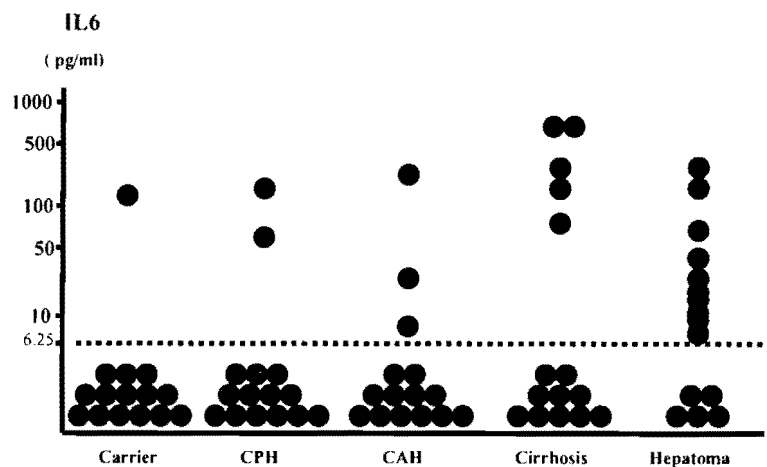


Fig. 1 Serum IL-6 levels in asymptomatic HBV carriers and patients with HBV related chronic liver diseases.

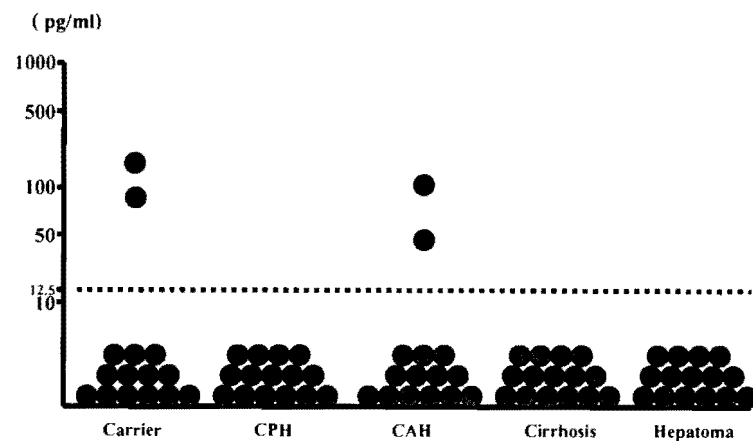


Fig. 2 Serum IFN- γ levels in asymptomatic HBV carriers and patients with HBV related chronic liver diseases.

IFN- γ levels per group is demonstrated in Fig. 2.

DISCUSSION

The existence of HBV carriers with normal liver histology suggests that the virus is not directly cytopathic for hepatocytes. Instead, most studies indicate that cellular and humoral immune responses are involved in the process of acute and chronic hepatitis.⁹ Patients who successfully clear the virus during an episode of acute viral hepatitis develop strong, polyclonal class-I and class-II restricted T cell responses to HBV, whereas these responses are weak and may be rather oligoclonal in patients who fail to clear the virus and subsequently develop chronic liver disease of varying severity.² Indeed, the HLA class-I restricted cytotoxic T cell (CTL) response is not only an important component of recovery from HBV, but also contributes to the pathogenesis of hepatocellular damage in most cases. *In vitro* studies have indicated that hepatocytes are destroyed by CTL upon recognition of HBV-encoded core antigen (HBcAg) on the hepatocytes.¹⁰ Furthermore, CTL also produce several cytokines, which trigger the recruitment of antigen nonspecific inflammatory cells such as macrophages and natural killer (NK) cells. Tissue damage during acute and chronic HBV infections is likely the result of a complex interaction of CTL and these inflammatory mediators, including the cytokines.¹¹ In fact, various cytokines are considered to play important roles at every stage of HBV infection, ranging from the onset of intrahepatic immune response to liver regeneration, fibrogenesis and cirrhotic transformation.¹¹

IFN- γ is secreted predominantly by activated T cells and interacts with other cytokines forming a complex mediator network during immune reactions. In particular, IFN- γ promotes the differentiation of T helper type 0 (Th0) lymphocytes into T helper type 1 (Th1) cells and Th1 cells themselves secrete IFN- γ , along with IL-2, IL-3 and IL-6.¹² These Th1-type cytokines are involved principally in promoting the CTL response and play a crucial role in protecting against viral infection. Yet, Keskinen *et al.*,¹³ in a recent experiment conducted in order to investigate the antiviral activity of IFN- γ against influenza A, Sendai, and vesicular stomatitis viruses (VSV) in human hepatoma cell lines, found that IFN- γ displayed some antiviral effect against influenza A virus but appeared to be ineffective against VSV and Sendai virus. The results suggest hepatoma cells to have an intrinsically poor ability to produce and respond to type I IFNs, which may contribute to their inefficient capacity to resist viral infections.¹³ In the liver, although IFN- γ is an important cytokine that provokes inflammation, its effects on fibrogenesis are suppressive by down-regulating stellate cell activation.¹⁴

In our study, nearly 15% of asymptomatic carriers and CAH patients had serum levels of IFN- γ detectable by ELISA. However, none of the patients in the other remaining groups had IFN- γ detectable in serum samples. These findings agree with previous reports, in that IFN- γ was expressed at more easily traceable levels in patients with acute hepatitis.⁸ In those with chronic active hepatitis, serology might not represent a method sensitive enough to trace

the small amounts of IFN- γ produced by the infiltrating mononuclear cells of the liver in the systemic circulation. In these cases, an alternative measure, *i.e.* extraction and purification of the mRNA specific for IFN- γ from liver tissue would prove necessary,¹⁵ which, due to the invasive method of liver biopsy required, can hardly be justified on the basis of research alone.

IL-6 is a 184-amino acid pleiotropic cytokine produced by a wide variety of cells that mediate inflammatory reactions and immune-mediated processes.¹⁶ In the liver, it is produced by fibroblasts, Kupffer cells and endothelial cells.¹⁷ IL-6 regulates the synthesis of a broad spectrum of acute-phase proteins and is also involved in the process of hepatic regeneration and fibrosis.¹⁸⁻²⁰ High levels of IL-6 have been detected in sera of patients with acute and chronic liver diseases caused by viral hepatitis and alcohol.^{6,7,20} In that context, it is noteworthy that IL-6 has been reported to protect hepatoma cells from apoptosis induced by transforming growth factor-beta (TGF-beta), a well known inducer of apoptosis in liver cells. This anti-apoptotic effect of IL-6 against TGF-beta is apparently mediated by concomitant activation of the PI 3-kinase/Akt and the STAT3 pathways.²¹ Moreover, a study conducted in search for genes abundantly expressed in human primary HCC discovered one of those genes to encode the human hepatocyte growth factor-like protein (HGFLP), a protein structurally homologous to hepatocyte growth factor. Investigation of the effects of cytokines on HGFLP expression revealed IL-6 to cause increase the expression of HGFLP mRNA in Hep G2 cells

whereas IL-1 α , IL-1 β and TNF- α had no effect.²²

In our study, we have demonstrated that patients with various stages of chronic HBV infection exhibited different levels of serum IL-6. For example, in patients with HCC, elevated IL-6 was detectable more frequently than among those of the other forms of hepatic diseases. In addition, patients affected by cirrhosis tended to have more frequently detectable IL-6 levels than patients with chronic hepatitis and asymptomatic carriers. These findings were in accordance with previous reports in that IL-6 correlated with clinical course and disease severity in patients with chronic HBV infection.^{7,23} The potential pathologic role of elevated IL-6 as to hepatocyte differentiation, growth and repair is unclear. However, a recent study has demonstrated that IL-6 can be induced by the HBV-X gene.²⁴ Studies with transgenic mice carrying the HBV-X gene have shown HBV-X to trigger chronic hepatitis.²⁵ Furthermore, the ability of HBV-X to function as a transactivator has led to the suggestion that its properties may be important in hepatic oncogenesis.²⁶ Thus, it is conceivable that the up-regulation of IL-6 by HBV-X protein could induce hepatic inflammation and might play an important role in the pathogenesis of fibrosis, which can eventually lead to cirrhosis and HCC²⁴. Further investigations, performed on a larger sample size, will certainly be required in order to shed light on the potential correlation between HBV-X transactivation, IL-6 up-regulation and liver carcinogenesis.

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