Virology 444 (2013) 317–328

Contents lists available at ScienceDirect

Virology

journal homepage: www.elsevier.com/locate/yviro

Hepatitis B virus (HBV) induces the expression of interleukin-8 that in turn reduces HBV sensitivity to interferon-alpha ☆

Teresa Pollicino [a,c,*], Luigi Bellinghieri [b], Agnese Restuccia [b,c], Giuseppina Raffa [b,c], Cristina Musolino [b,c], Angela Alibrandi [d], Diana Teti [b], Giovanni Raimondo [b,c]

[a] Department of Pediatric, Gynecologic, Microbiological, and Biomedical Sciences, University of Messina, Messina, Italy
[b] Department of Clinical and Experimental Medicine, University of Messina, Messina, Italy
[c] Division of Clinical and Molecular Hepatology, University of Messina, Messina, Italy
[d] Department of SEAM, University of Messina, Messina, Italy

ARTICLE INFO

Article history:
Received 4 April 2013
Returned to author for revisions
2 May 2013
Accepted 25 June 2013
Available online 23 July 2013

Keywords:
Hepatitis B virus chronic infection
Interleukin-8
IL-8 promoter
Transcription factors
Hepatitis B X viral regulatory protein
Signal pathways
Chromatin immunoprecipitation
Chromatin modifying enzymes
IL-8 siRNA
Interferon-alpha

ABSTRACT

High levels of serum interleukin-8 (IL-8) have been detected in chronic hepatitis B (CHB) patients during episodes of hepatitis flares. We investigated whether hepatitis B virus (HBV) may directly induce IL-8 production and whether IL-8 may antagonize interferon-alpha (IFN-α) antiviral activity against HBV. We showed that CHB patients had significantly higher IL-8 levels both in serum and in liver tissue than controls. In HBV-replicating HepG2 cells, IL-8 transcription was significantly activated. AP-1, C/EBP and NF-kB transcription factors were concurrently necessary for maximum IL-8 induction. Moreover, HBx viral protein was recruited onto the IL-8 promoter and this was paralleled by IL8-bound histone hyperacetylation and by active recruitment of transcriptional coactivators. Inhibition of IL-8 increases the antiviral activity of IFN-α against HBV. Our results indicate that HBV activates IL-8 gene expression by targeting the epigenetic regulation of the IL-8 promoter and that IL-8 may contribute to reduce HBV sensitivity to IFN-α.

Introduction

Hepatitis B virus (HBV) infection is a major health problem worldwide with estimates of nearly 400 million people currently infected (Dienstag, 2008). HBV infection may associate with a large spectrum of clinical forms, ranging from very mild and asymptomatic clinical pictures to the most severe liver diseases, including fulminant hepatitis, cirrhosis, and hepatocellular carcinoma (HCC) (Dienstag, 2008).Chronic HBV infection is a dynamic condition in which the interaction between virus and the host immune response influences the outcome of the disease. Its natural history is complex and variable. It includes different – not always sequential – phases that are distinguishable on the basis of the presence in the serum of HBV "e" antigen (HBeAg) or its antibody (anti-HBe), of the HBV DNA serum levels, of alanine aminotransferases (ALT) values and of the liver histology (EASL, 2012). The anti-HBe positive phase may be characterized by persistently low viremia levels < 2000 UI/ml), normal ALT and minimal histological lesions (inactive chronic HBV carrier state) (EASL, 2012) or by stable or fluctuating high levels of viral replication and ALT values, with active liver necroinflammation (HBeAg-negative chronic hepatitis). Patients with HBeAg-negative chronic hepatitis B (CHB) may undergo recurrent, spontaneous "hepatic flares," characterized by wide fluctuations of viremia levels, liver inflammation and a propensity to a rapid progression towards cirrhosis (EASL, 2012).

The HBV replication cycle is not directly cytotoxic to cells and the pathogenesis of liver disease has conventionally been attributed to cytolytic killing of infected hepatocytes by virus-specific T cell response (Chisari and Ferrari, 1995; Perrillo, 2001). However, important data have shown that the high frequencies of HBV-specific CD8+ T cells in patients with chronic HBV infection are associated with HBV control rather than with hepatic injury

* Corresponding Author. Division of Clinical and Molecular Hepatology, Department of Pediatric, Gynecologic, Microbiological, and Biomedical Sciences, University of Messina, via Consolare Valeria, 98124 Messina, Italy. Fax: +39 090 221 3594.
E-mail addresses: tpollicino@unime.it (T. Pollicino), bellinghieri.luigi@email.it (L. Bellinghieri), restuccia.a@gmail.com (A. Restuccia), graffa@unime.it (G. Raffa), musolinocristina@gmail.com (C. Musolino), aalibrandi@unime.it (A. Alibrandi), dteti@unime.it (D. Teti), raimondo@unime.it (G. Raimondo).

0042-6822/$ - see front matter © 2013 The Authors. Published by Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.virol.2013.06.028

Table 1
Characteristics of patients with HBV-related chronic liver disease (CLD) and control subjects.

Parameter	Patients with HBV-related CLD ($n=26$)	Control subjects[a] ($n=22$)	*P* value
Age (years), median (range)	41 (29–84)	42 (20–60)	NS (0.987)
Male sex, n (%)	21 (80.8)	13 (59)	NS (0.150)
ALT (IU/L), median (range)	100 (50–260)	18 (10–38)	< 0.0001
Intrahepatic IL-8 mRNA Rel.C. (fold), median (range)	0.71 (0.01–4.9)	0.06 (0.01–0.07)	0.003
Serum IL-8 (pg/mL), median (range)	29.5 (7.7–278.3)	10.5 (2–5)	< 0.0001

Abbreviations: ALT, alanine aminotransferases; Rel. C., Relative Concentration; HBV, hepatitis B virus.

[a] None of the 22 control subjects had any sign of liver disease, seven of them were inactive HBV carriers and 15 were individuals negative for all HBV serum markers.

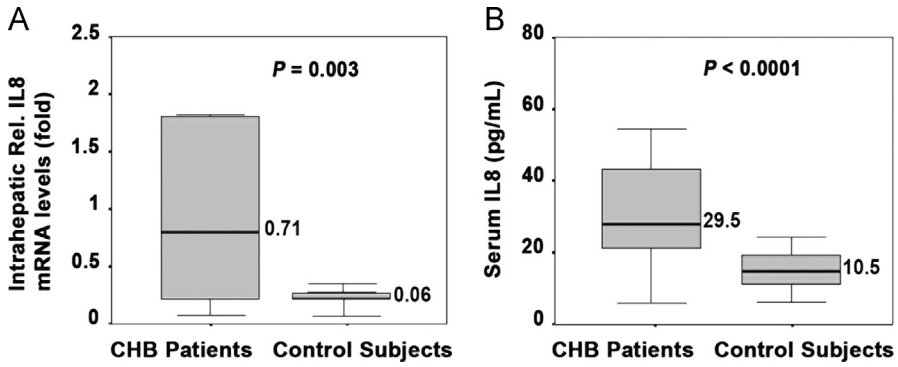

Fig. 1. Chronic hepatitis B (CHB) patients have significantly higher amounts of IL-8 mRNA in the liver and of IL-8 chemokine in the serum than control subjects. (A) Intrahepatic amounts of IL- 8 mRNA. (B) Serum IL-8 levels. Median relative amounts of IL-8 mRNA in the liver of CHB patients ($n=14$) and of control subjects ($n=5$) were 0.71 (range: 0.01–4.9) and 0.06 (range: 0.01–0.079), respectively, whereas median levels of IL-8 chemokine in the serum of CHB patients ($n=26$) and of control subjects ($n=22$) were 29.5 pg/mL (range: 7.7–278.3 pg/mL) and 10.5 pg/mL (range: 2–20 pg/mL), respectively.

(Bertoletti et al., 2010; Maini et al., 2000; Rehermann, 2003). In addition, it has been demonstrated that adoptive transfer of HBsAg-specific CD8+ T cells in HBV transgenic mice results in the rapid recruitment of HBV-non-specific bystander lymphocytes and that liver damage becomes manifest when non-specific, chemokine-mediated recruitment of neutrophils, natural killer (NK) cells and activated T-cells occurs (Kakimi et al., 2001; Sitia et al., 2004). Of interest, it has been recently shown that in chronic HBV infected patients flares of liver inflammation (either spontaneous or induced by anti-viral withdrawal) are preceded by a parallel increase of interleukin-8 (IL-8) production and serum HBV DNA levels (Dunn et al., 2007; Tan et al., 2010). IL-8 may synergize with interferon- alpha (IFN-α) to activate NK cells, (Dunn et al., 2007) and very recent data have shown that HBV-specific T cells maturing in the intrahepatic inflammatory environment can produce IL-8, which in turn can contribute to the development of liver damage through the recruitment of granulocytes (Gehring et al., 2011; Zimmermann et al., 2011).

IL-8 is a CXC chemokine able to elicit granulocytes, NK cells and T cell chemotaxis at the inflammatory site (Mukaida, 2003; Taub et al., 1996). It is a principal mediator of the inflammatory response to many viruses and bacteria (Girard et al., 2002; Green et al., 2006; Hisatsune et al., 2008; Polyak et al., 2001a, 2001b; Rajaiya et al., 2008; Venza et al., 2007; Wagoner et al., 2007; Yu et al., 2011; Zheng et al., 2008) and it may interfere with the antiviral effect of IFN-α (Girard et al., 2002; Khabar et al., 1997a; Lee et al., 2011; Polyak et al., 2001a, 2001b). Previous data had demonstrated that the HBV-encoded regulatory HBX protein is able to transactivate the IL-8 promoter (Mahe et al., 1991). However, the exact mechanisms through which HBV is able to induce IL-8 gene expression remain mostly unknown.

In the present study we investigated IL-8 in the course of HBV infection and for this purpose we quantified the IL-8 amounts in paired serum and liver tissue samples of chronic HBV infected individuals either CHB or inactive carriers. Moreover, by using a cell-based HBV replication system that makes it possible to recapitulate the HBV replication cycle including the nuclear generation of covalently closed-circular DNA (cccDNA) molecules (Pollicino et al., 2006), we studied the molecular mechanisms implicated in the activation of IL-8 gene expression and verified whether IL-8 might reduce HBV sensitivity to IFN-α.

Results

IL-8 amounts in liver tissues and sera of CHB patients, inactive HBV carriers and HBV-neg subjects

Real-time PCR analysis on liver tissue specimens showed that CHB patients had significantly higher median amounts of IL-8 mRNA (0.71 [range: 0.01–4.9] versus 0.06 [range: 0.01–0.07]; $P=0.003$) compared with the subjects of the control group, including both inactive HBV carriers (IBC) and HBV-neg subjects (Table 1 and Fig. 1A). Moreover, among CHB patients intrahepatic IL-8 mRNA levels were significantly higher in HBeAg-negative patients (1.7 [range: 0.5–4.7] versus 0.1 [range: 0.01–4.9]; $P=0.028$) than in HBeAg-positive ones. Both HBeAg-negative and HBeAg-positive CHB patients had much higher amounts of IL-8 mRNA in the liver than controls ($P=0.004$ and $P=0.015$, respectively). Concentrations of circulating IL-8 chemokine, assayed by a quantitative ELISA kit, were also significantly increased in CHB patients compared with controls (median, 29.5 pg/mL [range: 7.7–278.3 pg/mL] versus 10.5 pg/mL [range: 2–20 pg/mL], respectively; $P<0.0001$) (Table 1 and Fig. 1B) and this statistical significance was maintained also when serum IL-8 amounts in CHB patients were separately compared with the amounts in IBC (median, 9.78 pg/mL; range, 4.2–19.9 pg/mL; $P<0.0001$) and in HBV-neg