Antiviral Research 97 (2013) 285–292

Contents lists available at SciVerse ScienceDirect

Antiviral Research

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

IL-17A but not IL-22 suppresses the replication of hepatitis B virus mediated by over-expression of MxA and OAS mRNA in the HepG2.2.15 cell line

Bing Wang [a,b], Xin-Ping Zhao [c], Yu-Chen Fan [a,b], Jian-Jun Zhang [a], Jing Zhao [a], Kai Wang [a,b,*]

[a] Department of Hepatology, Qilu Hospital of Shandong University, Wenhuaxi Road 107#, Jinan 250012, China
[b] Institute of Hepatology, Shandong University, Jinan 250012, China
[c] The Third People's Hospital of Zaozhuang, Zaozhuang 277100, China

ARTICLE INFO

Article history:
Received 8 June 2012
Revised 14 December 2012
Accepted 17 December 2012
Available online 27 December 2012

Keywords:
Interleukin-17A
Interleukin-22
Hepatitis B virus
Myxovirus resistance A
Oligoadenylate synthetase

ABSTRACT

Interleukin-17A (IL-17A) and interleukin-22 (IL-22), mainly secreted by interleukin-17-producing T help cells (Th17), are pleiotropic cytokines that regulate the biological responses of several target cells, including hepatocytes. Th17 frequency was reported to negatively correlate with plasma hepatitis B virus (HBV) DNA load in patients with HBV infection. Several studies have indicated that cytokines, such as IL-6 and IL-4, are involved in the noncytopathic suppression of HBV replication. We therefore hypothesized that IL-17A and IL-22 might have a potent suppressive effect on HBV replication. In our present study, we analyzed the suppressive effect of IL-17A and IL-22 on HBV replication in the hepatocellular carcinoma cell line HepG2.2.15. IL-17A did not inhibit the proliferation of HepG2.2.15 cells. It decreased the levels of HBV s antigen (HBsAg) and HBV e antigen (HBeAg) in culture medium and the levels of intracellular HBV DNA. By contrast, blockage of IL-17 receptor (IL-17R) increased the levels of HBsAg and extracellular HBV DNA in culture medium and the levels of intracellular HBV DNA. The expression of antiviral proteins, including myxovirus resistance A (MxA) and oligoadenylate synthetase (OAS), was enhanced by IL-17A. IL-22 and anti-human IL-22 receptor (IL-22R) antibody did not change any indexes. We demonstrated that IL-17A effectively suppressed HBV replication in a noncytopathic manner and the over-expression of MxA and OAS mRNA was involved in the suppression of HBV replication by IL-17A.

1. Introduction

Hepatitis B virus (HBV) is a hepatotropic, noncytopathic DNA virus with a 3.2 kb partially double-stranded DNA that causes acute and chronic hepatitis. Patients with a persistent HBV infection are at a high risk of developing chronic hepatitis, cirrhosis, and/or hepatocellular carcinoma (Seeger and Mason, 2000). The pathogenesis of HBV-induced liver diseases involves complicated mechanisms related to viral replication and the body's immune responses against HBV infection, including HBV-specific cell-mediated immunity and inflammatory cytokines. The interactions between HBV replication and immune responses against HBV infection play an important role in determining the outcome of viral infection (Rehermann, 2003; Rehermann and Nascimbeni, 2005). Substantial evidence obtained from studies in chimpanzees with acute HBV infection and in HBV transgenic mice indicates that HBV clearance occurs prior to the destruction of infected cells. It has also been shown that cytokines are likely to be involved in both the regulation of the immune responses and the direct inhibition of HBV replication (Guidotti et al., 1999; Guidotti and Chisari, 2001). Several cytokines effectively suppress HBV replication in a noncytopathic manner in a cell culture system and in HBV transgenic mice. Interleukin-6 effectively suppresses HBV replication and prevents the accumulation of HBV covalently closed circular DNA by preventing the formation of genome-containing nucleocapsids in a human hepatoma cell line (Kuo et al., 2009). Interleukin-12, interleukin-18 and intrahepatic induction of alpha/beta interferon (IFN-α/β) effectively inhibit HBV replication in the liver of transgenic mice (Cavanaugh et al., 1997; Kimura et al., 2002; Wieland et al., 2000). Furthermore, interleukin-4 and transforming growth factor beta-1 (TGF-β1) suppress HBV replication in hepatoma cells

Abbreviations: HBV, hepatitis B virus; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; TH17, T helper 17; IL-17A, interleukin-17A; IL-22, Interleukin-22; Ab, antibody; MxA, myxovirus resistance A; OAS, oligoadenylate synthetase; ISFG3, IFN-stimulated gene factor3; STAT1, signal transducer and activator of transcription 1; STAT2, signal transducer and activator of transcription 2.

* Corresponding author at: Department of Hepatology, Qilu Hospital of Shandong University and Institute of Hepatology, Shandong University, Wenhuaxi Road 107#, 250012 Jinan, China. Tel.: +86 531 82169596.
E-mail addresses: wangdoc876@126.com, wangdoc876@yahoo.com.cn (K. Wang).

0166-3542/$ - see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.antiviral.2012.12.018

through the transcriptional regulation of HBV RNA (Chou et al., 2007; Lin et al., 2003). These studies suggest that inflammatory cytokines play an important role in the antiviral response against HBV infection.

Interleukin-17A (IL-17A) and interleukin-22 (IL-22) are mainly secreted by interleukin-17-producing T help cells (Th17). Th17 is a newly identified subset of T helper cells that participate in the disease progression and pathogenesis of liver injury in HBV infected patients (Sun et al., 2012; Wang et al., 2012; Wu et al., 2010; Ye et al., 2010). The functions of Th17 cells are mediated by the production of several cytokines including IL-17A and IL-22 (Ye et al., 2011). The IL-17 receptor (IL-17R) is expressed nearly on all types of liver cells, and IL-22 receptor (IL-22R) expression is restricted to epithelial cells including hepatocytes (Estelle et al., 2008). Th17 cells were reported to increase rapidly with entecavir-induced suppression of HBV replication (Zhang et al., 2010). Xue-song et al. found that plasma IL-17A levels and Th17 frequency negatively correlated with plasma HBV DNA load in patients with chronic HBV infection (Xue-Song et al., 2012). The increased IL-22 in CHB patients inversely correlated with the histological activity index (Xiang et al., 2011), which was correlated with serum HBV DNA (Ke et al., 2011). We therefore hypothesize that IL-17A and IL-22 might have a potent suppressive effect on HBV replication.

HepG2.2.15 cells are derived from the hepatocellular carcinoma cell line HepG2 and are characterized by having stable HBV expression and replication in the culture system (Zhao et al., 2011; Zhang et al., 2012). In this study, we found that IL-17A could effectively suppress HBV replication in a noncytopathic manner in HepG2.2.15. We also demonstrated myxovirus resistance A (MxA) and oligoadenylate synthetase (OAS) were involved in IL-17A-mediated suppression of HBV.

2. Materials and methods

2.1. Cell culture and reagents

HepG2.2.15 cell lines was cultured in DMEM medium (Hyclone, Logan, UT, USA), supplemented with 10% fetal bovine serum (Invitrogen, Carlsbad, CA, USA), in 5% CO2 at 37 °C. A final concentration of 380 mg/L G418 (Invitrogen) was added into the medium for the maintenance of HepG2.2.15 cells. Recombinant human IL-17A, IL-22, anti-human IL-17 R antibody (Ab) and anti-human IL-22 R Ab were purchased from R&D systems. Adefovir was purchased from Shandong LuKang pharmaceutical factory. MxA small interfering RNA (siRNA), OAS siRNA and scramble siRNA was purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA).

2.2. Cell proliferation assay

Cell proliferation was determined with the Cell Counting Kit-8 (CCK-8) assay (Dojindo, Japan). Cells were suspended at a final concentration of 2×10^4 cells/well and cultured in 96-well plates. After overnight culture, IL-17A, IL-22, anti-human IL-17R Ab, anti-human IL-22R Ab or adefovir was respectively added at specific concentration for 72 h. Then CCK-8 reagent (10 μl) was added

Table 1
Primer pairs for RT-PCR.

Gene	Primer	Sequence
OAS	Forward primer	5′-AGGTGGTAAAGGGTGGCT-3′
	Reverse primer	5′-TGCTTGACTAGGCGGATG-3′
STAT1	Forward primer	5′-GCGCTGCCTTTTCTCCTGCCGG-3′
	Reverse primer	5′-CTGGTGAACCTGCTCCAGGAAT-3′
STAT2	Forward primer	5′-CGACCAGACCATTGGAGGGCG-3′
	Reverse primer	5′-TCATCTCAGCCACTGGGTAGG-3′
ISGF3	Forward primer	5′-TGGCATCAGGCAGGGCACGCTG-3′
	Reverse primer	5′-GAACTGTGCTGTCGCTTTGATGG-3′
MxA	Forward primer	5′-ACAATCAGCCTGGTGGTGGTC-3′
	Reverse primer	5′-CCTCCCCTACAGTTTCCTCTCC-3′
Beta-actin	Forward primer	5′-TCACCAACTGGGACGACAT-3′
	Reverse primer	5′-GCACAGCCTGGATAGCAAC-3′

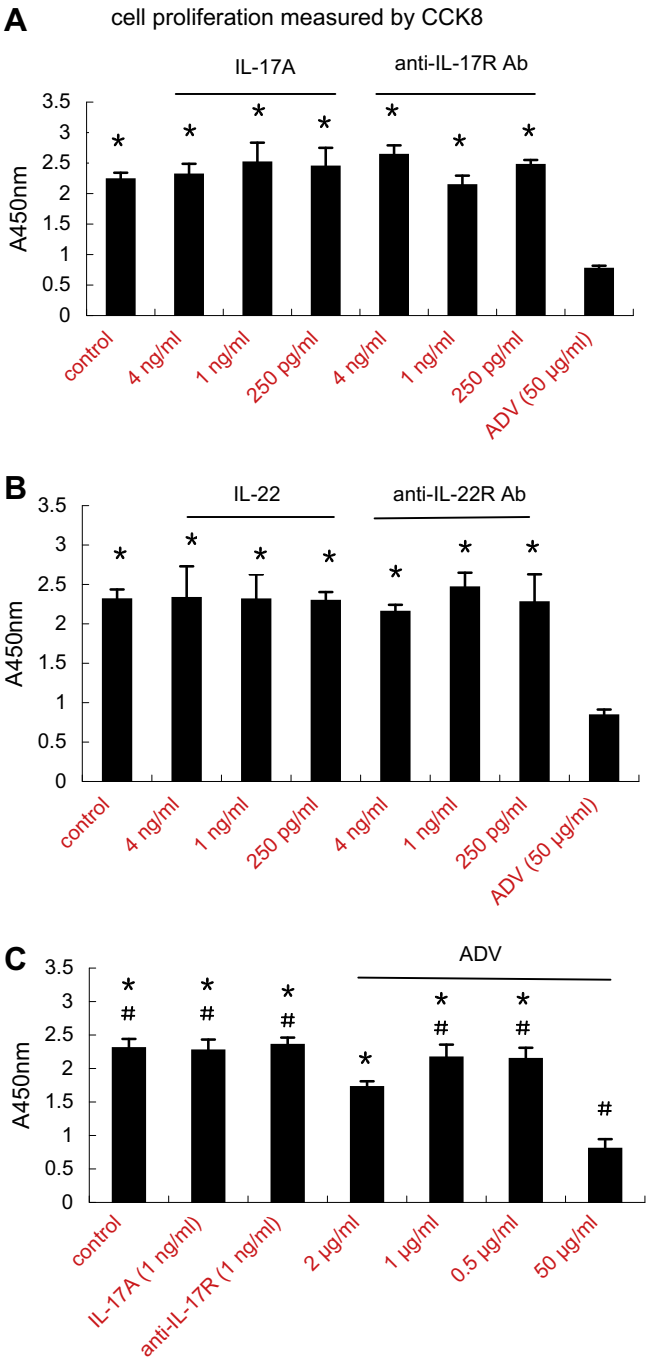

Fig. 1. The effect of IL-17A, anti-human IL-17R Ab, IL-22, anti-human IL-22R Ab or adefovir on the proliferation of HepG2.2.15. Cells were suspended at a final concentration of 2×10^4 cells/well and cultured in 96-well plates. After overnight culture, IL-17A, anti-human IL-17R Ab, IL-22, anti-human IL-22R Ab or adefovir (ADV) were added at the specific concentration for 72 h. Cell proliferation was determined with the CCK-8 assay. (A) The proliferation of HepG2.2.15 cells treated with IL-17A or anti-human IL-17R Ab was not significantly different from the proliferation of the control cells. (B) The proliferation of HepG2.2.15 cells treated with IL-22 or anti-human IL-22R Ab was not significantly different from the proliferation of the control cells. (C) The proliferation of HepG2.2.15 cells treated with 1 μg/ml adefovir was not significantly different from that of control or cells treated with 1 ng/ml IL-17A or anti-human IL-17R Ab. Data ($n = 3$) are expressed as mean ± SEM. $*P < 0.05$ vs. 50 μg/ml adefovir, $\#P < 0.05$ vs. 2 μg/ml adefovir.