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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

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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.

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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′

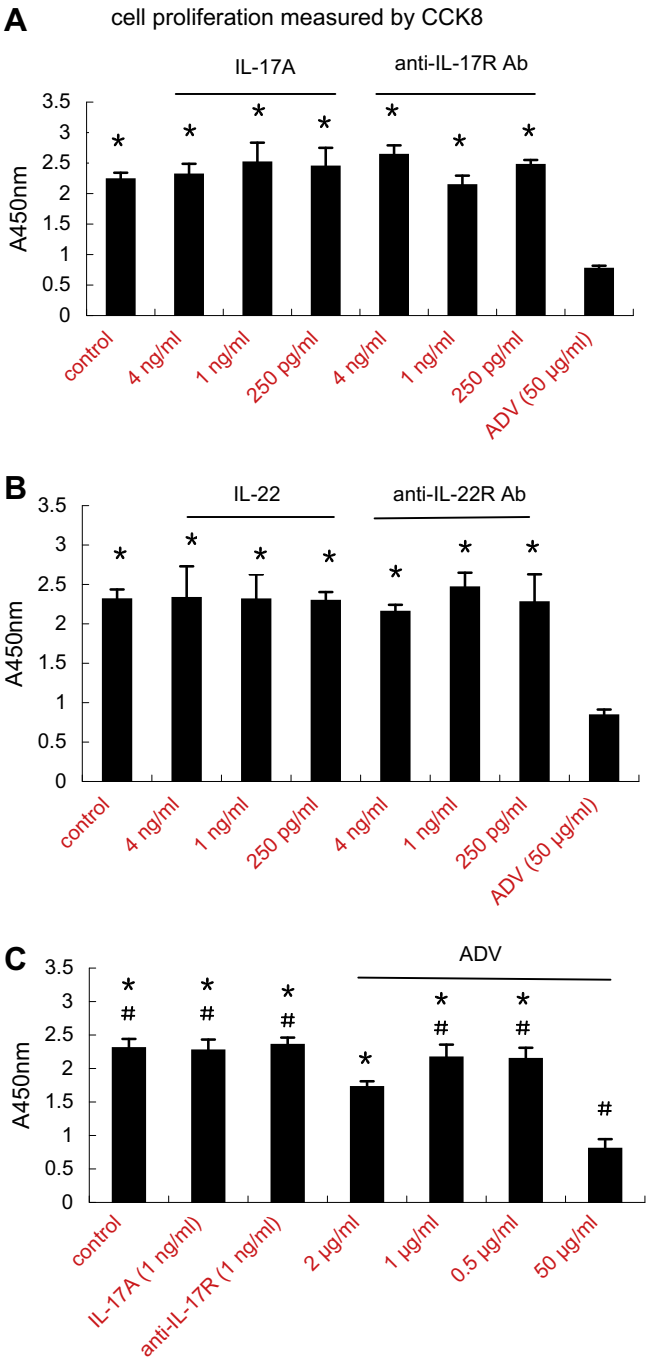

Fig. 1. The effect of IL-17A, anti-human IL-17R Ab, IL-22, anti-human IL-22R Ab or adefovir on the proliferation of HepG2.2.15. Cells were suspended at a final concentration of 2×10^4 cells/well and cultured in 96-well plates. After overnight culture, IL-17A, anti-human IL-17R Ab, IL-22, anti-human IL-22R 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.

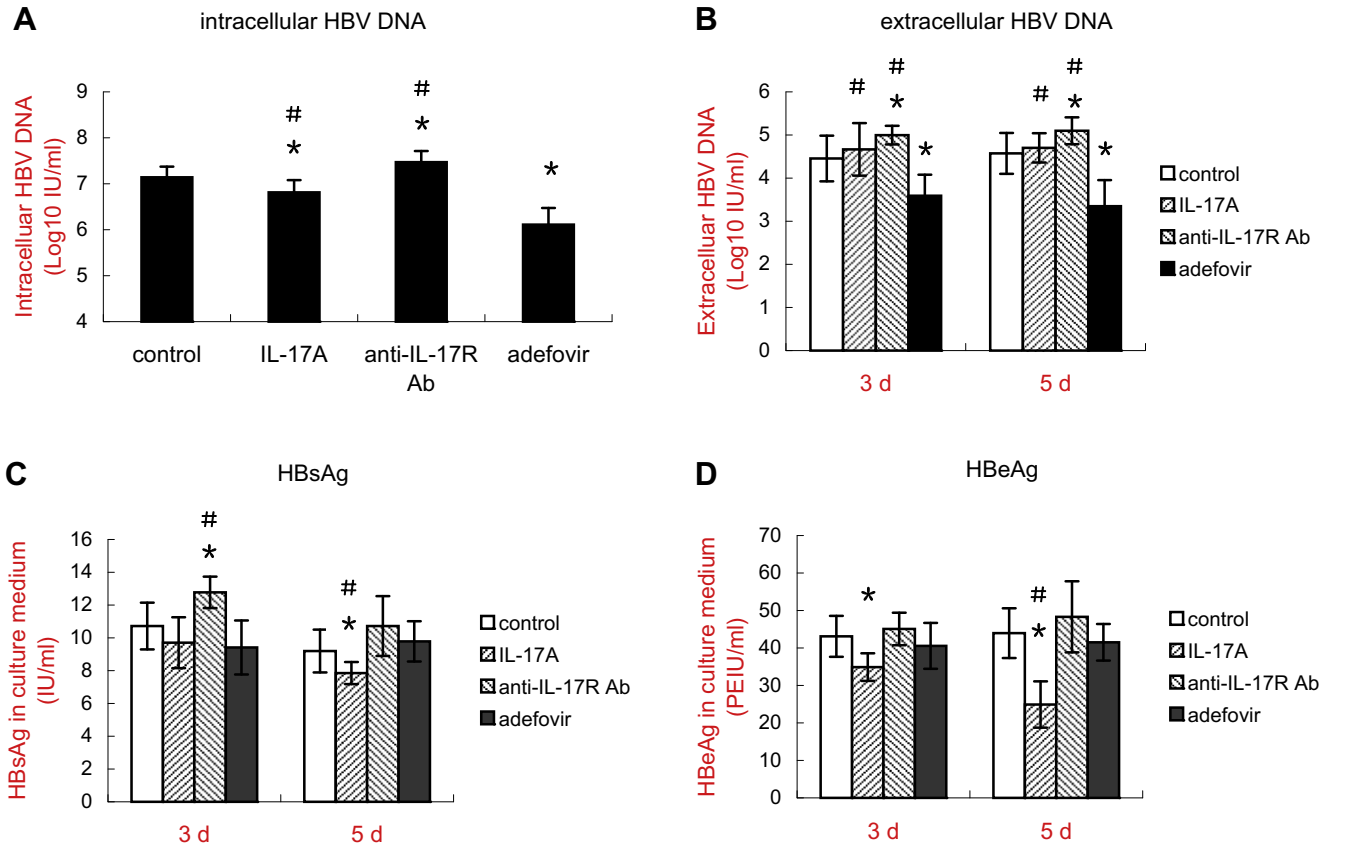

Fig. 2. IL-17A suppressed HBV replication in HepG2.2.15. HepG2.2.15. cells were treated with IL-17A (1 ng/ml), anti-human IL-17R Ab (1 ng/ml) or adefovir (1 μg/ml) for 5 days. (A) The amount of intracellular HBV DNA at day 5 was measured by real-time PCR. (B) The amount of extracellular HBV DNA in culture medium at day 3 and day 5 was measured by real-time PCR. (C) The amount of HBsAg in culture medium at day 3 and day 5 was measured using the electrochemiluminescence assay. (D) The amount of HBeAg in culture medium at day 3 and day 5 was measured using the electrochemiluminescence assay. Data ($n = 6$) are expressed as mean ± SEM. $*P < 0.05$ vs. control, $\#P < 0.05$ vs. adefovir.

to each well of a 96-well plate containing 100 μl culture medium and the plate was incubated for 2 h at 37 °C. Viable cells were evaluated by absorbance measurements at 450 nm.

2.3. Measurement of HBsAg and HBeAg

HepG2.2.15 cells were seeded in 25-cm^2 cell culture flasks at a concentration of 2×10^5 cells/ml. The culture was respectively treated with IL-17A (1 ng/ml), IL-22 (1 ng/ml), anti-IL-17R Ab (1 ng/ml), anti-IL-22R Ab (1 ng/ml) or adefovir (1 μg/ml). After 72 h incubation, the culture medium was collected and a new culture medium was added with IL-17A (1 ng/ml), IL-22 (1 ng/ml), anti-IL-17R Ab (1 ng/ml), anti-IL-22R Ab (1 ng/ml) or adefovir (1 μg/ml) for another 48 h of incubation. We collected the culture medium again and harvested the cells. The concentrations of hepatitis B virus s antigen (HBsAg) and hepatitis B virus e antigen (HBeAg) in culture medium were measured by electrochemiluminescence assay (Roche Diagnostics Ltd., Germany) as previously reported (Lee et al., 2012; Wursthorn et al., 2011). The units for HBsAg and HBeAg are IU/ml and Paul Ehrlich Institute (PEI) U/ml.

2.4. Analysis of intracellular and extracellular HBV DNA

After incubation with 5 days, HepG2.2.15 cells were harvested. All samples were stored at −80 °C. Total DNA was extracted from the harvested cells and the culture medium according to the method described by Klintschar and Neuhuber (Klintschar and Neuhuber, 2000). The HBV PCR fluorescent quantitative detection kit (Da An Gene, Guangzhou, China) was used according to the manufacturer's protocols.

2.5. RNA extraction

HepG2.2.15 cells were seeded into 6-well plates at a concentration of 2×10^5 cells/ml and treated with IL-17A (1 ng/ml). Total cellular RNA was extracted 72 h later using the RNeasy Mini kit (Qiagen, Tokyo, Japan) according to the manufacturer's instructions. RNA samples were then stored at −80 °C until use. RNA quality was examined using the A_{280}/A_{260} ratio (Pharmacia Biotech, Bedford, MA, USA).

2.6. cDNA synthesis and real-time PCR

cDNA synthesis was performed using a RevertAid™ First Strand cDNA Synthesis Kit (Fermentas, Lithuania). Each reaction contained 1 μg of RNA. The RNA levels of MxA, IFN-stimulated gene factor 3 (ISGF3), OAS, signal transducer and activator of transcription 1 (STAT1), and signal transducer and activator of transcription 2 (STAT2) were quantified by real-time PCR with SYBR Green (Wu et al., 2010) using the LightCycler 480 (Roche Diagnostics, Germany). Thermal cycling conditions were 95 °C for 30 s followed by 45 cycles of 95 °C for 5 s, 60 °C for 30 s and 72 °C for 30 s, and finally 65 °C for 15 s and 40 °C for 30 s. All primers (Table.1) for examining gene expression were purchased from Invitrogen. Gene expression was normalized to beta-actin to determine the fold change in gene expression between the test sample and control

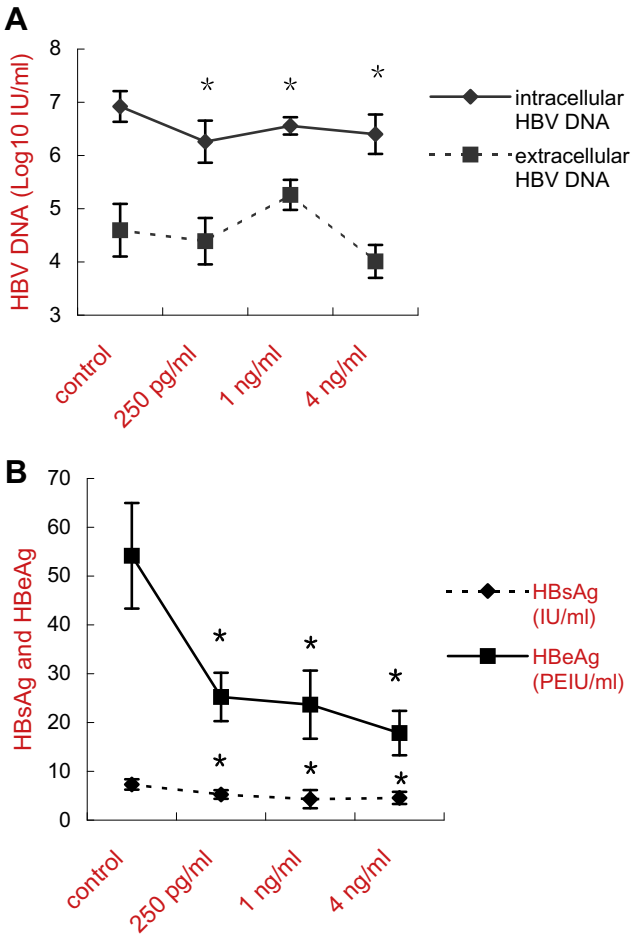

Fig. 3. The suppressive effect of IL-17A was not dose-dependent. HepG2.2.15 cells were treated with various doses of IL-17A (250 pg/ml, 1 ng/ml, and 4 ng/ml) for 5 days. (A) The amount of intracellular HBV DNA and extracellular HBV DNA in culture medium at day 5 was measured by real-time PCR. (B) The amount of HBsAg and HBeAg in culture medium at day 5 was measured using the electrochemiluminescence assay. There is no significant difference among the various dose groups. Data ($n = 6$) are expressed as mean ± SEM. $*P < 0.05$ vs. control.

sample by 2^{-ddCT} (comparative cycle threshold) method (Wu et al., 2010). The genes were annotated by Entrez Gene (NCBI, Bethesda, MD, USA).

2.7. siRNA and transfection

Briefly, HepG2.2.15 cells were seeded in growth media without antibiotics 1 day before transfection. siRNA (80 nM) and siRNA transfection reagent (5 μl) were diluted in 1 ml of transfection medium, mixed, and incubated at room temperature for 45 min. HepG2.2.15 cells were washed two times with transfection medium. The diluted siRNA was added for 7 h. Next 2 × growth medium with or without 1 ng/ml IL-17A was added, the cells were cultured for 48 h and the cells were collected. The depletion of gene expression by siRNA was assessed by real-time RT-PCR and the levels of intracellular HBV DNA were measured.

2.8. Statistical analysis

Data were analyzed by using Statistical Product and Service Solutions (SPSS) 16.0 (SPSS Inc., Chicago, IL, USA). The results were expressed as mean ± SEM. One-way analysis of variance (ANVOA) was used and Dunnett's test was used to further compare treated groups with the control group to assess statistical significance, with values of $P < 0.05$ considered statistically significant.

3. Results

3.1. IL-17A, IL-22, anti-human IL-17R Ab and anti-human IL-22R Ab had no effect on the proliferation of HepG2.2.15 cells

To investigate the effect of IL-17A, IL-22, anti-human IL-17R Ab and anti-human IL-22R Ab 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, IL-22, anti-human IL-17 R Ab or anti-human IL-22 R Ab was added to the cells at the specific concentrations (0.25–4 ng/ml) for 72 h. Cell proliferation was determined using the Cell Counting Kit-8 (CCK-8) assay. The proliferation of HepG2.2.15 cells after stimulation was not significantly inhibited or enhanced (Fig. 1(A) and (B).

To assess the cytotoxicity of adefovir in HepG2.2.15 cells, cells were suspended at a final concentration of 2×10^4 cells/well and cultured in 96-well plates. After overnight culture, adefovir was added to the cells at specific concentration (0.78–100 μg/ml) for 72 h. Cell proliferation was determined using the CCK-8 assay. The TC50 value was 32.15 ± 2.80 μg/ml (mean ± SEM, $n = 3$) calculated by the Reed–Muench method (data not shown). Adefovir which significantly inhibited the proliferation of HepG2.2.15 cells at 50 μg/ml, was chosen as the positive control for cell proliferation experiment. Because there was no significant difference in the proliferation of HepG2.2.15 cells when cells were treated with 1 μg/ml adefovir, 1 ng/ml IL-17A, or 1 ng/ml anti-human IL-17R Ab, 1 μg/ml was chosen as the concentration of adefovir for subsequent experiments.

3.2. IL-17A suppressed HBV replication in HepG2.2.15

To investigate the influence of IL-17A, anti-human IL-17R Ab, or adefovir on HBV replication, HepG2.2.15 cells were seeded in 25 cm^2 cell culture flasks. HepG2.2.15 cells were treated with IL-17A (1 ng/ml), anti-human IL-17R Ab (1 ng/ml) or adefovir (1 μg/ml). The culture medium was collected at day 3 and day 5 and the cells were harvested at day 5.

In the IL-17A-treated cells, the levels of intracellular HBV DNA (Fig. 2(A) decreased, and the levels of HBsAg (Fig. 2(C) and HBeAg (Fig. 2(D) in culture medium decreased. In the anti-IL-17R Ab-treated cells, the levels of intracellular HBV DNA (Fig. 2(A) increased and the levels of extracellular HBV DNA (Fig. 2(B) and HBsAg (Fig. 2(C) in culture medium increased. In the adefovir-treated cells, the levels of intracellular and extracellular HBV DNA (Fig. 2(A) and (B) decreased.

As described previously, the proliferation of HepG2.2.15 cells was not significantly inhibited or enhanced by IL-17A or anti-IL-17R Ab. Thus, IL-17A could effectively inhibit HBV replication in a noncytopathic manner. Anti-IL-17R Ab might block the effect of IL-17 present in fetal bovine serum.

Both IL-17A and adefovir inhibited HBV replication. Both IL-17A and adefovir reduced the levels of intracellular HBV DNA, however adefovir had a more potent effect (Fig. 2(A). IL-17A reduced the levels of HBsAg and HBeAg, but adefovir had no obvious effect on HBsAg and HBeAg. Adefovir reduced the levels of extracellular HBV DNA, but IL-17A had no obvious effect on the levels of extracellular HBV DNA.

To investigate the relationship between the dose of IL-17A and the suppression of HBV replication, HepG2.2.15 cells were treated with various doses of IL-17A (250 pg/ml, 1 ng/ml, and 4 ng/ml) for 5 days. The culture medium was collected and the cells were harvested at day 5. The levels of all indexes did not differ significantly

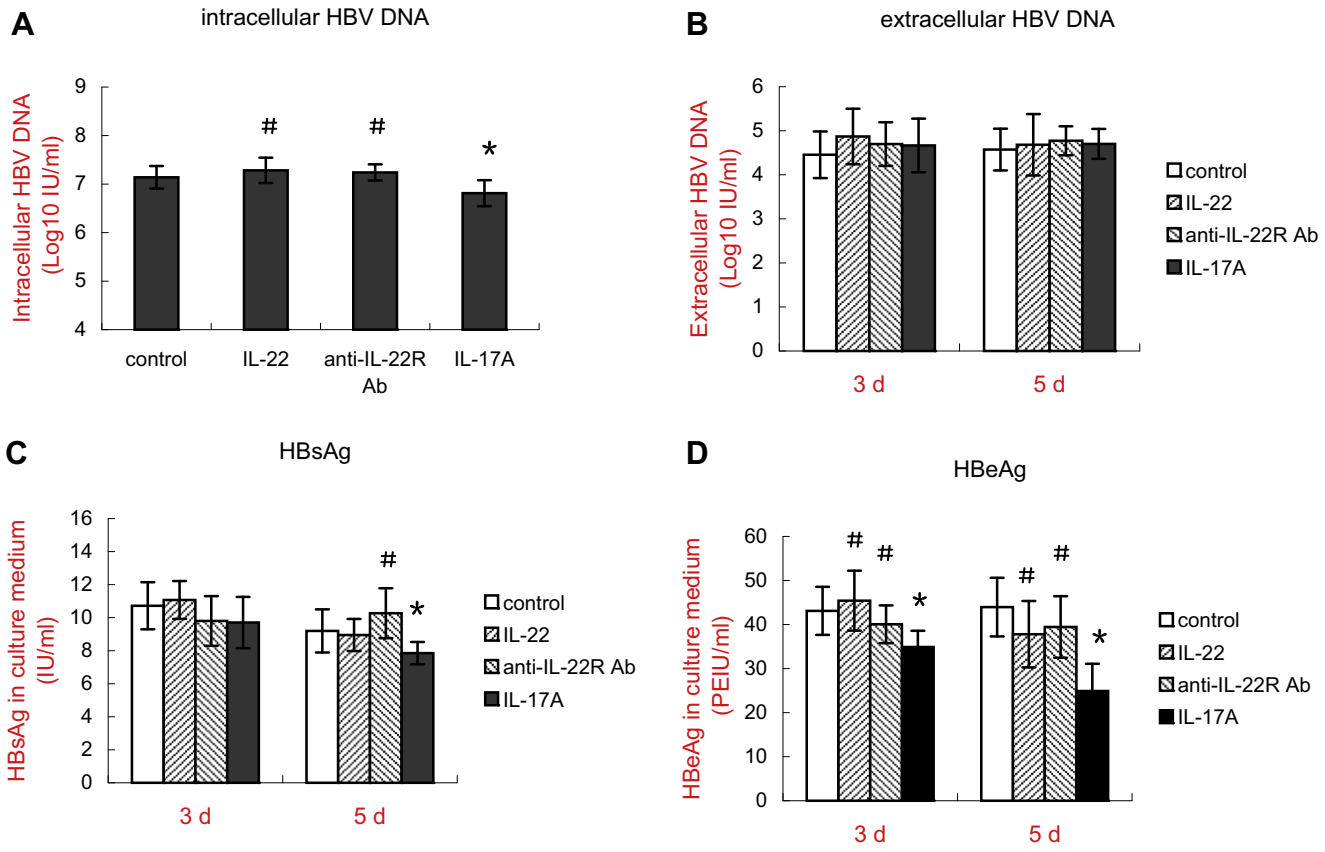

Fig. 4. IL-22 could not suppress HBV replication in HepG2.2.15. HepG2.2.15 cells were treated with IL-22 (1 ng/ml), anti-human IL-22R Ab (1 ng/ml) or IL-17A (1 ng/ml). (A) The amount of intracellular HBV DNA at day 5 was measured by real-time PCR. (B) The amount of extracellular HBV DNA in culture medium at day 3 and day 5 was measured by real-time PCR. (C) The amount of HBsAg in culture medium at day 3 and day 5 was measured using the electrochemiluminescence assay. (D) The amount of HBeAg in culture medium at day 3 and day 5 was measured using the electrochemiluminescence assay. Data ($n = 6$) are expressed as mean ± SEM. $*P < 0.05$ vs. control, $\#P < 0.05$ vs. IL-17A.

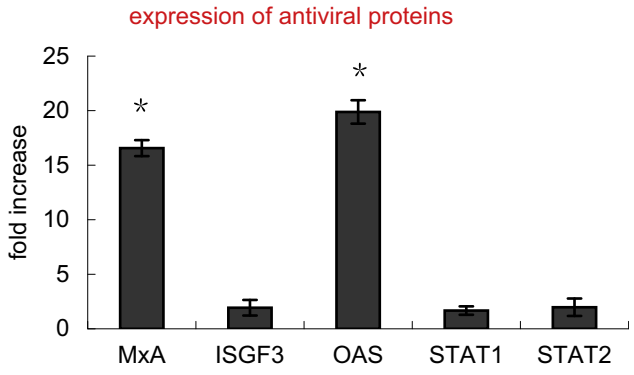

Fig. 5. The expression of MxA and OAS was upregulated by IL-17A treatment in HepG2.2.15. HepG2.2.15 cells were treated with IL-17A (1 ng/ml) for 72 h. The cells were harvested and the expression of MxA, ISGF3, OAS, STAT1 and STAT2 was tested by real-time RT-PCR. Data ($n = 3$) are expressed as mean fold increase ± SEM compared with the expression in untreated cells after normalization with beta-actin. $*P < 0.05$ vs. control.

among various dose groups (Fig. 3). The suppressive effect of IL-17A is not dose-dependent within the concentration range of 250 pg/ml to 4 ng/ml.

At day 5, IL-17A decreased the level of HBsAg, but at day 3, IL-17A did not significantly suppress HBsAg compared with HBsAg in the control (Fig. 2(C). Additionally, IL-17A suppressed HBeAg 43.31% at day 5, but only 19.04% at day 3. Thus, the suppressive effect of IL-17A was time-dependent.

3.3. IL-22 did not suppress HBV replication in HepG2.2.15

To investigate the influence of IL-22 and anti-IL-22R Ab on HBV replication, HepG2.2.15 cells were treated with 1 ng/ml IL-17A or anti-IL-22R Ab for 3 and 5 days. The levels of all indexes did not significantly differ between the IL-22-treated group and the control group or between the anti-IL-22R Ab-treated group and the control group (Fig. 4). By contrast, IL-17A reduced the levels of intracellular HBV DNA, HBsAg and HBeAg. Thus, IL-17A but not IL-22 suppressed HBV replication in HepG2.2.15 cells.

3.4. The enhanced expression of MxA and OAS induced by IL-17A suppressed HBV replication

To investigate the mechanism of IL-17's effect, HepG2.2.15 cells were treated with or without IL-17A (1 ng/ml) for 3 days. The expression of MxA, ISGF3, OAS, STAT1 and STAT2 was tested by real-time RT-PCR. The expression of MxA and OAS increased in the IL-17A-treated group. The expression of ISGF3, STAT1 and STAT2 did not significantly differ between the IL-17A-treated group and the control group (Fig. 5). MxA and OAS are two important antiviral proteins and may be involved in the suppression of HBV replication by IL-17A.

To confirm the suppressive effect of MxA and OAS on HBV replication, we transfected HepG2.2.15 with MxA-targeted siRNA or OAS-targeted siRNA, and treated the cells with 1 ng/ml IL-17A for 48 h. MxA-targeted siRNA and OAS-targeted siRNA greatly reduced the levels of MxA mRNA and OAS mRNA (Fig. 6(A) and (B),

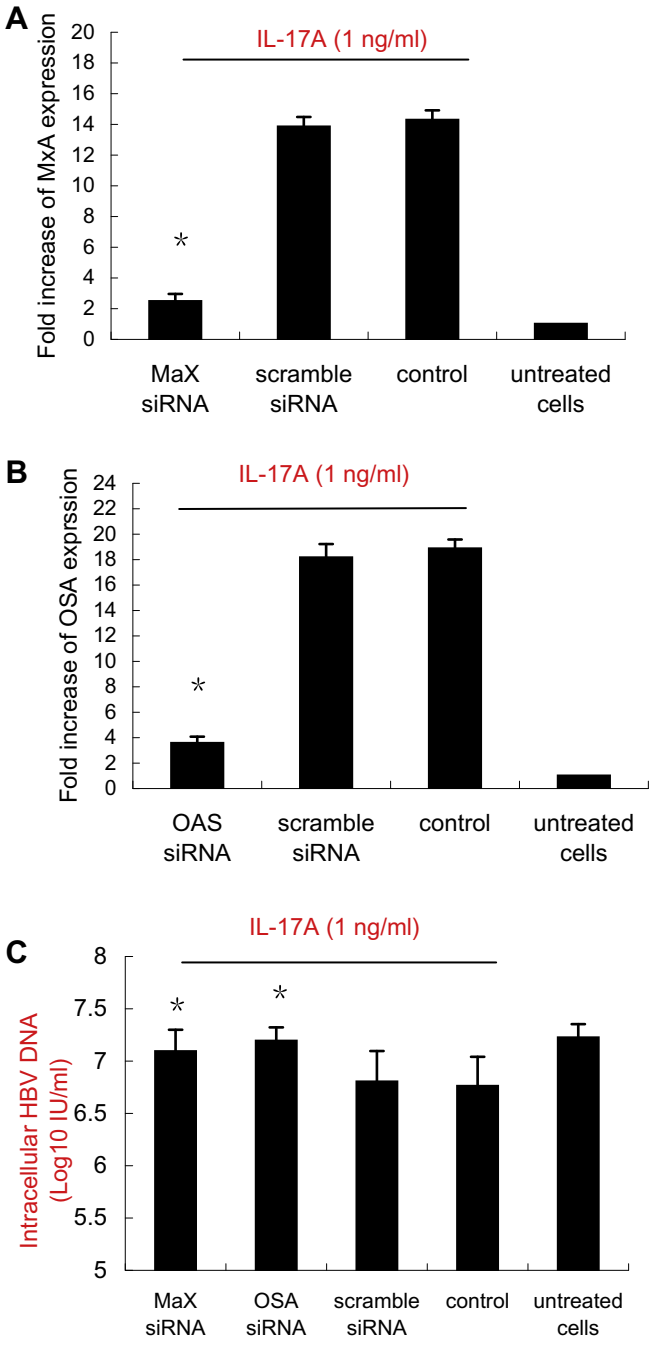

Fig. 6. siRNA-mediated reduction of MxA and OAS abolished the antiviral effect of IL-17A. HepG2.2.15 cells were untreated, treated with IL-17A, transfected with MxA siRNA, OAS siRNA or scramble siRNA and then treated with IL-17A. (A) Real-time RT PCR was used to analyze MxA mRNA expression. Data ($n = 4$) are expressed as mean fold change ± SEM compared with the expression in untreated cells after normalization with beta-actin. (B) Real-time RT PCR was used to analyze OAS mRNA expression. Data ($n = 4$) are expressed as mean fold increase ± SEM compared with the expression in untreated cells after normalization with beta-actin. (C) The amount of intracellular HBV DNA was measured by real-time PCR. Data ($n = 4$) are expressed as mean ± SEM.$*P < 0.05$ vs. control or scramble siRNA.

respectively, and abolished the suppressive effect of IL-17A on HBV replication (Fig. 6(C).

4. Discussion

IL-17A and IL-22 are mainly secreted by Th17 cells (Zhang et al., 2012). Evidence has shown that Th17 cells and Th17-specific effectors cytokines, including IL-17A and IL-22, contribute to the disease progression and pathogenesis of liver injury in HBV infected patients (Huang et al., 2012; Sun et al., 2012; Wu et al., 2010). IL-17A is linked to inflammation and host antimicrobial immunity (Chen and O'Shea, 2008) and can be used as a biomarker for the severity of acute hepatic injury (Wang et al., 2012; Ye et al., 2010). In normal mice, IL-17A reduced the growth of colony-forming unit erythroid progenitors (Jovcić et al., 2001), but the proliferation of HepG2.2.15 cells was not inhibited by IL-17A. IL-17A cannot directly induce hepatic cell apoptosis, but it can mobilize, recruit and activate neutrophils leading to hepatic inflammation. Interestingly, plasma IL-17A levels and Th17 frequency negatively correlate with plasma HBV DNA load in patients with chronic HBV infection (Xue-Song et al., 2012). Our results indicate that in patients with hepatitis B, a higher proportion of peripheral Th17 cells and high level of IL-17A might suppress HBV replication to a certain extent.

Adefovir is a nucleotide analog and is converted intracellularly by cellular kinases to its active metabolite, adefovir diphosphate, which competitively inhibits HBV DNA polymerase. Our results indicate the mechanism of action is different between adefovir and IL-17A. The suppressive efficacy of adefovir on HBV DNA was stronger than that of IL-17A, but IL-17A could reduce the production of HBsAg and HBeAg. IL-2 or IL-12 has been used to treat patients with hepatitis B (Tomova et al., 2009; Rigopoulou et al., 2005). Although the *in vivo* antiviral effect of IL-17A needs to be verified, and other biological effects of IL-17A, such as inducing pro-inflammatory cytokines and chemokines, should be considered, our findings highlight the utility of IL-17A as a novel and potentially effective drug for viral suppression.

The protective role of IL-22 in the liver was confirmed by results from experimental hepatitis models (Ki et al., 2010; Pan et al., 2004; Radaeva et al., 2004; Zenewicz et al., 2007). Xiang et al. determined that the increased IL-22 in CHB patients inversely correlated with the histological activity index (Xiang et al., 2012), which correlated with serum HBV DNA (Ke et al., 2011). Our results indicate that IL-22 could not directly enhance the proliferation of HepG2.2.15 or inhibit HBV replication. Therefore, the protective effect of IL-22 is exerted through another mechanism. A recent study demonstrated that much of the liver damage observed in the mouse HBV transgenic model is mediated by the Th17 type cytokine IL-22, without necessarily playing a role in the noncytolytic control of viral replication (Zhang et al., 2011). These results are similar to those obtained by us.

MxA and OAS are two important antiviral proteins. MxA, an interferon-inducible cytoplasmic dynamin-like GTPase, possesses antiviral activity against multiple RNA viruses and DNA viruses, including HBV (Frese et al., 1996; Gordien et al., 2001; Pavlovic et al., 1990; Peltekian et al. 2005; Yu et al., 2008). OAS, a critical component of the innate immune response to viruses, uses adenosine triphosphate in 2′-specific nucleotidyl transfer reactions to synthesize 2′,5′-oligoadenylate (2′,5′AS), which activates latent ribonuclease, resulting in the inhibition of virus replication (Hovanessian and Justesen, 2007). Both MxA and OAS are associated with the suppression of HBV replication. Our results indicate that the expression of MxA and OAS which might be increased by IL-17A has a suppressive effect on HBV replication.

MxA and OAS proteins are key mediators of antiviral resistance, mainly induced in cells by type I (α/β) and type III (λ) IFNs through the Janus tyrosine kinase (JAK)-signal transducer and activator of transcription (STAT) pathway (Horvath and Darnell, 1996; Heim, 2000; Samuel, 2001). Moreover, IFNs can activate the phosphoinositide 3-kinase (PI3K)-serine/threonine kinase (AKT) and Raf-the mitogen-activated protein kinase (MEK)-extracellular signal-regulated kinase (ERK) signaling pathways in specific cell type and induce the expression of MxA and OAS (Chai et al., 2011). Our data

suggest that IL-17A dose not induce MxA and OAS expression is not through the JAK-STAT pathway, since we could not detect the enhanced expression of other interferon-stimulated genes (ISGs), including STAT1, STAT2 and ISGF3. IL-17A might increase the expression of MxA and OAS through the PI3K-AKT and Raf-MEK-ERK signaling pathways in an IFN-dependent manner. Alternatively, a recent research has shown that MxA can be induced by an endogenous antimicrobial peptide without the activity of IFNs (Mahanonda et al., 2012). Thus, IL-17A might increase the expression of MxA and OAS independent of IFN.

In this study, we found that IL-17A suppressed HBV replication in the hepatocellular carcinoma cell line HepG2.2.15. The inhibitory effect was not a result of cell apoptosis since IL-17A could not inhibit the proliferation of HepG2.2.15 cells. We also demonstrated that IL-17A may exert its inhibitory effect though antiviral proteins, including MxA and OAS. The exact mechanism involved in the antiviral effect of IL-17A remains to be further investigated.

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