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Altered mRNA Levels of MOV10, A3G, and IFN-α in Patients with Chronic Hepatitis B

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To explore the relationship of the MOV10, A3G, and IFN-α mRNA levels with chronic hepatitis B virus (HBV) infection, Blood samples from 96 patients with chronic hepatitis B (CHB) and 21 healthy individuals as control were collected. HBV DNA load and aminotransferase in the serum were tested using real time PCR and velocity methods, respectively. The MOV10, A3G, and IFN-α mRNA levels in the peripheral blood mononuclear cells (PBMC) were examined through qRT-PCR. The MOV10, A3G, and IFN-α mRNA levels in CHB group was significantly lower than those in the control group ($P<0.01$, $P<0.05$, $P<0.01$, respectively). The A3G mRNA level in the high-HBV DNA load group was lower than that in the low-HBV DNA load group ($P<0.05$). However, no statistical difference was found in the MOV10 and IFN-α mRNA levels between the two HBV DNA load groups. Furthermore, the MOV10 mRNA level showed positive correlation with IFN-α in the control group. These results indicated that the expression of the innate immune factors MOV10, A3G, and IFN-α is affected by chronic HBV infection.

Keywords: hepatitis B virus, innate immune, Moloney Leukemia Virus 10, APOBEC3G, IFN-α

Introduction

Data from the World Health Organization show that approximately two billion people or 1/3 of the world's population is infected with hepatitis B virus (HBV). 5% of which are chronically infected (Weinbaum *et al*., 2008). This finding can be ascribed to various reasons, such as mutation caused by HBV(Olivero *et al*., 2006) and the immune status of the host, including both innate and acquired, is also a factor (Bertoletti and Ferrari, 2013).

The expression and function of innate immune factors have recently attracted considerable attention. Moloney Leukemia Virus 10 (MOV10) protein belongs to an RNA helicase superfamily and has an important function in the anti-retroviral virus(HIV-1, SIV, and MLV) infection of the host (Furtak *et al*., 2010; Arjan *et al*., 2011; Goodier *et al*., 2012). MOV10 inhibits HIV-1 replication and reduces virus infectivity. Apolipoprotein B mRNA editing enzyme catalytic polypeptide-like3G (A3G), which was first reported by Sheehy, is an innate immune factor with cytosine deaminase activity. This enzyme can also resist Vif-deficient human immunodeficiency virus-1 (HIV-1) replication (Sheehy *et al*., 2002; Lecossier *et al*., 2003). Studies have revealed that A3G also influences HBV replication because reverse transcription processes are observed in the life cycle of HBV (Rosler *et al*., 2005). A3G inhibits HBV replication in Huh7 cell lines (Turelli *et al*., 2004). Existing studies have focused on the relationship between A3G and HBV *in vitro*. Only several studies have been conducted *in vivo* (Bonvin and Greeve, 2008; Henry *et al*., 2009; Vartanian *et al*., 2010). In addition, both A3G and MOV10 process body-related proteins that mutually affect the RNA-dependent approach (Wang *et al*., 2010). Given that HBV has a reverse transcription process, MOV10 was proposed to be involved in HBV infection. The MOV10, A3G, and IFN-α mRNA levels were detected in this study to explore the relationship of these innate immune factors with chronic HBV infection.

Materials and Methods

Study population

A total of 96 CHB patients were admitted in the Department of Infectious Diseases, The First Affiliated Hospital of Harbin Medical University from July 2011 to February 2012. All subjects were consistent with the diagnostic criteria for CHB: current HBsAg- and/or HBV DNA-positive patients with a history of hepatitis B or exhibiting positive result in HBsAg test for over six months, persistent or recurrent elevated ALT. In addition, the patients should not have received any anti-HBV therapy in the past six months or suffered from other systemic diseases. A total of 21 healthy individuals that were examined from the physical examination center of the same hospital served as control. Informed consent forms were signed by all participants prior to sample collection.

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Table 1. Primers for real-time PCR

Gene	Primers for real-time PCR
MOV10	Forward: 5′-ACATTCTACATTGCCCGCTTCTTG-3′
	Reverse: 5′-CTCCTTCCTCTATCCGATTGGTCAC-3′
A3G	Forward: 5′-GCTGTGCTTCCTGGACGTGA-3′
	Reverse: 5′-GGTGGTCCACAAAGGTGTCCC-3′
IFN-α	Forward: 5′-GGTGCTCAGCTGCAAGTCAA-3′
	Reverse: 5′-GCTACCCAGGCTGTGGGTT-3′
GAPDH	Forward: 5′-ATCACTGCCACCCAG AAGAC-3′
	Reverse: 5′-TTTCTAGACGGCAGGTCAGG-3′

Detection of HBV-related antigens by ELISA

The levels of HBV-related antigens in the sera were measured by ELISA using diagnostic kits for HBsAg, HBsAb, HBeAg, HBeAb, and HBcAb (Shanghai Shiye Kehua Company, China). According to the manufacturer's instructions, a ratio of sample/negative (S/N) ≥ 2.1 was considered as a positive response to the related antigen.

Detection of HBV DNA by real time PCR

The level of HBV DNA molecules in the sera was detected with Quantitative HBV PCR Fluorogence Diagnostic kit (Shanghai Shiye Kehua Company). An HBV DNA level ≥ 5.0×10^2 copies/ml was considered as a positive response according to the instructions.

Detection of A3G, MOV10, and IFN-α mRNA by quantitative RT-PCR

Ficoll separating medium was used to separate the whole blood peripheral blood mononuclear cells (PBMC). Total tissue RNA from the PBMC was isolated with TRIzol reagent (Invitrogen, USA). RT was performed with 1 μg of total RNA with PrimeScript™ RT reagent kit (TaKaRa, China) to obtain cDNA as the template. Quantitative PCR was performed using 2 μl of the synthesized cDNA and SYBR PrimeScript Ex Taq II (TaKaRa) in a LightCycler 2.0 (Roche, Switzerland). Fold variations between RNA samples were calculated after normalizing to the GAPDH mRNA. Positive controls were plasmids containing MOV10 and A3G provided by our laboratory. Negative controls were template-free plasmids. The primers used are shown in Table 1.

Table 2. Comparison of the information between CHB and control groups

	CHB (n=96)		Control group (n=21)
	High-HBV DNA	Low-HBV DNA	
Gender (m/f)	42/20	22/12	14/7
Age (yr)	36.03±10.60	37.08±10.40	36.14±8.41
HBeAg- (n)	0	34	21
HBeAg+ (n)	62	0	0
HBV DNA (Log10)	7.14 ± 0.72	3.61±0.81	0
ALT (U/L)**	169.40±101.90	138.96±105.04	16.82±8.23
AST (U/L)**	146.13± 68.21	104.96±42.96	20.02±4.31

Note: **, P<0.01, CHB *vs.* Control group

Aminotransferase test with the velocity method

The ALT and AST levels in the sera were detected with Alanine Aminotransferase Assay Kit and Aspartate Transaminase Assay Kit (Beckman Coulter Laboratory Systems (Suzhou) Co., Ltd., USA), respectively. According to the manufacturer's instructions, the reference ranges were from 5 U/L to 35 U/L for ALT and from 8 U/L to 40 U/L for AST. ALT and AST values that are twice as much as the reference values were considered to be of clinical significance.

Statistical analysis

SPSS16.0 was used to analyze the data, and t-test was used to compare two groups. Pearson correlation analysis was also adopted. All data are expressed as means ± standard deviation, and P<0.05 was considered statistically significant.

Results

Study population

The background characteristics of the CHB patients and healthy individuals are listed in Table 2. Among the 96 CHB patients, 62 were HBeAg positive and 34 were HBeAg negative. The ALT and AST levels in patients with chronic hepatitis B were significantly higher than those in the control group (P<0.01). Moreover, no statistically significant differences were found in the age and gender between the two groups.

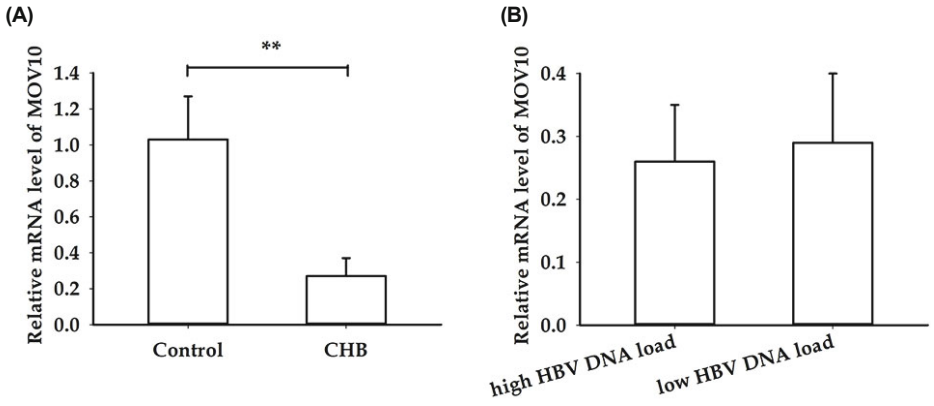

Fig. 1. Comparison of MOV10 mRNA levels among the different groups (, P<0.01).**

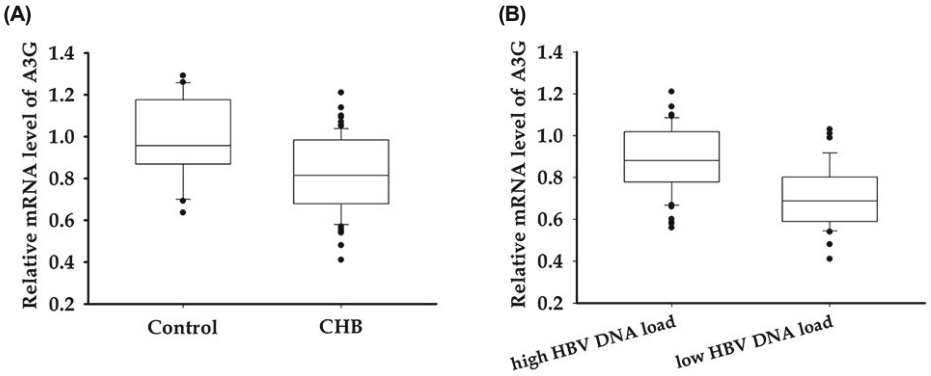

Fig. 2. Comparison of A3G mRNA levels among the different groups.

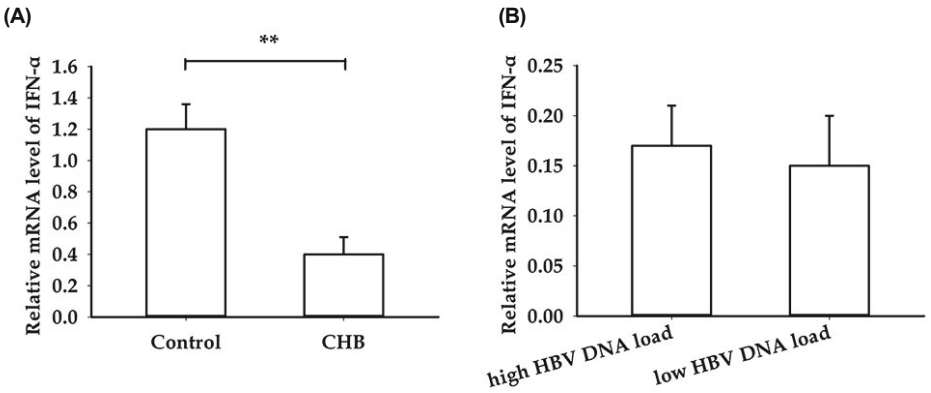

Fig. 3. Comparison of IFN-α mRNA levels among the different groups (**, $P<0.01$).

Comparison of MOV10, A3G, and IFN-α mRNA levels between the CHB group and the control groups

As demonstrated in Fig. 1A, the MOV10 mRNA level in the CHB group (0.27 ± 0.10) was significantly lower ($P<0.01$) than that of the control group (1.03 ± 0.24). The A3G mRNA level in the CHB group (0.82 ± 0.17) was significantly lower ($P<0.05$) than that of the control group (1.00 ± 0.20) (Fig. 2A). The IFN-α mRNA level in the CHB group (0.16 ± 0.11) was significantly lower ($P<0.01$) than that of the control group (1.20 ± 0.40) (Fig. 3A).

Comparison of the A3G, MOV10, and IFN-α mRNA levels between the high- and low-HBV DNA load groups

To explore whether the HBV DNA load could affect the MOV10, A3G, and IFN-α mRNA levels, the CHB patients were divided into two groups based on HBV DNA load: the high (HBV DNA > $1.0e^{+5}$, $n = 62$) and the low (HBV DNA < $1.0e^{+5}$, $n = 34$) HBV load group. As shown in the Fig. 4, the HBV DNA load of the high-HBV DNA load group (7.14 ± 0.72) was significantly higher ($P<0.05$) than that of the low HBV DNA load group (3.61 ± 0.81).

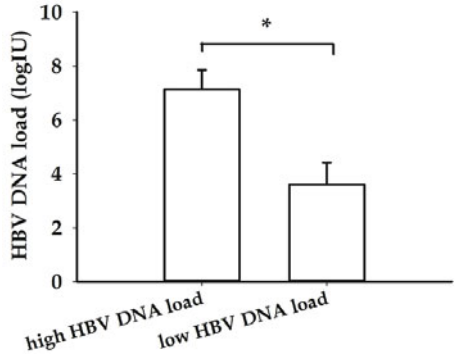

Fig. 4. Comparison of log mean value of HBV DNA between the high- and low-HBV DNA load groups (* $P<0.05$).

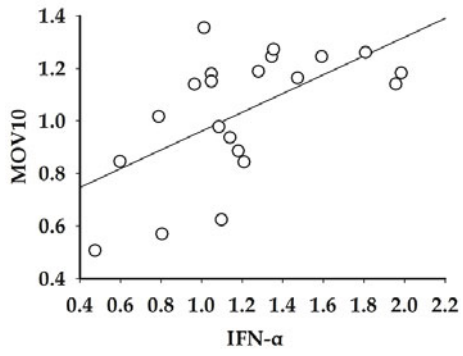

Fig. 5. Correlation analysis of MOV10 and IFN-α in the control group.

Figure 1B illustrates that the MOV10 mRNA level in the high-HBV DNA load group (0.26 ± 0.09) was similar to that of the low-HBV DNA load group (0.29 ± 0.11). The A3G mRNA level in the high-HBV DNA load group (0.88 ± 0.15) was significantly higher ($P<0.05$) than that of the low-HBV DNA load group (0.70 ± 0.14) (Fig. 2B). The IFN-α mRNA level in the high-HBV DNA load group (0.17 ± 0.04) was not significantly higher than that of the low-HBV DNA load group (0.15 ± 0.05) (Fig. 3B).

Correlation analysis

The software *Pearson* was used for the correlation analysis of the A3G, MOV10, and IFN-α mRNA levels in each group. In the control group, the MOV10 mRNA levels showed a positive correlation with the IFN-α mRNA levels ($r=0.588$, $P=0.005$) (Fig. 5). However, correlation tests among immunity indices in the CHB, high-HBV DNA load, and low-HBV DNA load groups were not conducted.

Discussion

When HBV invades the human body, the antiviral immunity of an organism not only protects the body from viruses but also leads to immune pathological damage. In CHB patients, chronic HBV infection can induce lymphocyte death, T lymphocyte subset adjustment disorders and IL-2, as well as other cytokines, resulting in immunocompromised patients (Miseta, 2002; Pollack, 2002).

Recent studies have demonstrated that MOV10 has very broad and potent antiretroviral activities. MOV10 can inhibits HIV-1 replication in multiple stages and mainly interacts with HIV-1 nucleic acid proteins with an RNA-dependent approach, thus suppressing HIV-1 packaging (Burdick *et al*., 2010). A relationship between the MOV10 and HBV infection was assumed because MOV10 and A3G exist concurrently in the P body, MOV10 could interact with A3G, and the inhibitory effects of A3G and MOV10 on HIV-1 were cumulative. Hence, the MOV10 mRNA level was tested in our study. The results showed that the MOV10 mRNA level in the CHB groups was lower than that of the control group. However, no significant difference was found between the HBV DNA load groups. This result indicated that MOV10 expression could be affected by chronic HBV infection, but has no association with HBV DNA load. The IFN-α mRNA level in the CHB group significantly decreased ($P<0.01$) compared with that in the control group, which illustrates the reduced antiviral immune function in CHB patients. This finding also agrees with the results of Lu *et al*. (1997) that IFN-α expression was lower in CHB patients compared with that in healthy individuals. However, no significant difference was found in the IFN-α mRNA levels between the high- and low-HBV DNA load groups. In the control group, MOV10 was positively correlated with IFN-α. MOV10 has extensive antiretroviral activity, and HBV also has a reverse transcriptase process. However, whether MOV10 can affect HBV replication remains unknown. In addition, whether MOV10, as host factor, can be affected by IFN-α has yet to be determined. Further confirmation through in vitro and *in vivo* experiments is needed.

In this study, the A3G mRNA levels in CHB patients and healthy individuals were also detected. The results showed that the A3G mRNA level in the CHB patients was significantly lower than that in the control group, indicating that chronic HBV infection of CHB patients could lead to the accordingly reduced A3G. This result was consistent with the reduction in A3G mRNA level by HBV through the inhibition of A3G promoter activity in vitro (Zhao *et al*., 2010). The A3G mRNA level in the high-HBV DNA load group was significantly higher than that in the low-HBV DNA load group. Moreover, this analysis indicates that HBV DNA load could affect A3G expression. However, studies on the relationship between HBV DNA load and A3G expression are inconclusive. Mohamadkhani *et al*. (2012) used liver biopsy immunohistochemistry to verify the very low A3G expression among 34 CHB patients, which was similar to our results. Noguchi also found that the A3G G-to-A hypermutation in HBV genomes is associated with the reduction in plasma HBV DNA level (Noguchi *et al*., 2009). By contrast, Peng *et al*. (2007) found that the A3G mRNA level was higher in the 27 CHB patients than among the healthy controls. The A3G mRNA level also showed a positive correlation with serum log HBV DNA. These contrasting results may be attributed to the diverse techniques used, number of cases, and types of patients. Thus, further studies are required, especially on the mechanism of participation of A3G in the anti-HBV infection.

In summary, this study verified that the MOV10, A3G, and IFN-α mRNA levels in CHB patients were reduced by chronic HBV infection, which suggests that chronic HBV infection could weaken the innate immune function of the body. The specific functional mechanism of these factors has yet to be determined and needs further research and verification *in vivo* and *in vitro*.

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