Lack of Association between Tumor Necrosis Factor-α -308 and -238 Promoter Polymorphisms and Chronic Hepatitis B Virus Infection

Won Hee Jang1, Young-II Yang2, Youn Jae Lee4, Jin Ho Chun5, Sung Su Yea1, Dae-Hyun Seog1 and Hyeong-In Kim2

1Department of Biochemistry, College of Medicine, Inje University, Busan, Korea
2Paik Institute for Clinical Research, Inje University, Busan, Korea
3Department of Pathology, College of Medicine, Inje University, Busan, Korea
4Department of Internal Medicine, College of Medicine, Inje University, Paik Hospital, Busan, Korea
5Department of Preventive Medicine, College of Medicine, Inje University, Busan, Korea

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The pro-inflammatory cytokine tumor necrosis factor-α (TNF-α) is an important mediator of the immune response in hepatitis B virus (HBV) infection. Since the production of TNF-α is mostly regulated at the transcriptional level and polymorphisms in the TNF-α promoter alter its expression, TNF-α promoter polymorphisms could affect the pathogenesis of chronic HBV infection. In this study, we investigated the potential association of TNF-α promoter polymorphisms with chronic HBV infection. The study included 181 patients with chronic HBV infection, 201 persons who had been spontaneously recovered from hepatitis B, and 170 unrelated healthy controls. The -308G/A and -238G/A polymorphisms in the TNF-α promoter were analyzed by PCR-restriction fragment length polymorphism. The distribution of both the -308 and -238 genotypes in the patient group was not statistically different from that in the spontaneous recovery and control groups (p>0.05). There was also no significant difference in the allele frequency between the groups (p>0.05). The results suggest that the TNF-α -308 and -238 promoter polymorphisms are not associated with the development of chronic HBV infection in the Korean population.

Key words: Tumor necrosis factor-α, polymorphism, chronic hepatitis B

Introduction

Hepatitis B virus (HBV) is a small, hepatotropic DNA virus, which causes acute and chronic hepatitis in humans. HBV infection is a major health problem, with chronic carriers of over 350 million worldwide. Korea is one of the most endemic areas for HBV infection. Persistent HBV infection is implicated in the development of cirrhosis and hepatocellular carcinoma [1]. The clinical outcome of HBV infection depends on several factors such as age at the time of infection, variations in viral sequence, and host genetic factors [17,33].

Genetic studies of infectious diseases have identified a variety of host genetic factors affecting disease susceptibility [14]. Tumor necrosis factor-α (TNF-α) gene has also been intensively studied as a strong candidate gene associated with disease susceptibility in numerous infectious diseases, since TNF-α is a major proinflammatory cytokine and a central mediator of the host response [3] and the TNF-α gene lies within the class III region of the major histocompatibility complex. Indeed, biallelic polymorphisms at positions -308 and -238 in the TNF-α promoter region [3,55], which influence TNF-α expression [20,22], have been found to be associated with several infectious diseases [4,16,24].

TNF-α also has an important role in the pathogenesis of chronic HBV infection. Plasma TNF-α levels and TNF-α production by peripheral blood mononuclear cells are increased in patients with chronic HBV infection [28,36]. In addition, a significant induction of TNF-α production in hepatocytes was observed in patients chronically infected with HBV [11]. The expression of TNF-α receptors is also up-regulated in chronic HBV infection [23,34]. Furthermore, TNF-α mediates the down-regulation of HBV gene expression and replication by HBV-specific cytotoxic T lymphocytes in the liver of HBV transgenic mice [10,12]. Given these data, it is possible to speculate that TNF-α promoter polymorphisms modulating TNF-α production may influ-
ence the outcome of HBV infection. There were several reports on the relationship of TNF-α promoter polymorphisms to chronic HBV infection, but the results are inconsistent [2,5,9,15,21,25,30,32]. Therefore, more studies are needed to clarify the precise role of TNF-α promoter polymorphisms on the pathogenesis of chronic HBV infection.

In the present study, we investigated whether the -308 and -238 TNF-α promoter polymorphisms contribute to susceptibility to chronic HBV infection in Koreans.

Materials and Methods

Subject

The study included 181 patients with chronic HBV infection, 201 patients who had been spontaneously recovered from hepatitis B, and 170 unrelated healthy controls. Patients (150 men, 31 women; mean age 38.6±17.7 yr) had been hepatitis B surface antigen (HBsAg)-positive for more than 6 months and were positive for IgG antibody to hepatitis B core antigen (anti-HBc IgG). Patients with any other types of liver disease, such as alcoholic hepatitis, chronic hepatitis C and autoimmune hepatitis, were excluded. All patients were recruited from Busan Paik Hospital, Korea. Spontaneously-recovered individuals (112 men, 89 women; mean age 48.8±10.3 yr) were negative for HBsAg and positive for antibody to HBsAg (anti-HBs) and anti-HBc. Healthy controls (56 men, 114 women; mean age 43.1±11.63 yr) were negative for HBsAg and anti-HBc with or without anti-HBs. Spontaneously-recovered individuals and healthy controls were recruited from persons who had visited Busan Paik Hospital for a regular medical check-up. All subjects were Korean living in the Busan-Gyeongnam area. The study was approved by the local Ethical Committee and informed consent was obtained from all subjects.

TNF-α promoter genotyping by PCR-restriction fragment length polymorphism

Genomic DNA was isolated from liver biopsy specimens or peripheral blood mononuclear cells by the standard method using proteinase K and phenol-chloroform extraction [27]. Nested PCR amplified a 118-bp fragment including the polymorphic sites at positions -308 and -238 in the TNF-α promoter from the template genomic DNA. Primers TNF-P11 and TNF-P21 were used for the first-round PCR and TNF-Nco3 and TNF-Bg for the second-round, nested PCR. Nucleotide sequences and positions of the primers are shown in Fig. 1. The reaction conditions for the first-round PCR were: 94°C for 5 min; 35 cycles of 94°C for 30 sec, 50°C for 30 sec, and 72°C for 30 sec; and 72°C for 5 min. Two microliters of the first-round PCR products were used for the nested PCR in a 20 μl reaction volume under the same conditions except that the annealing temperature was 55°C. Four microliters of the nested PCR products were digested overnight at 37°C in a 10 μl reaction volume containing 2.5 units of NcoI to detect the -308 polymorphism [35] or 2.5 units of BglII to detect the -238 polymorphism [18], and then analyzed on a 10% polyacrylamide gel. The PCR product containing G at position -308 was cleaved by NcoI yielding a 94-bp fragment, meanwhile BglII digestion resulted in a 95-bp fragment from the PCR product containing A at position -238.

Statistical analysis

Distributions of TNF-α promoter polymorphisms were compared between the investigated groups by the usual χ² test and the χ² test for trend. Odds ratios (OR) and 95% confidence intervals (CI) were also calculated. P-values smaller than 0.05 were considered significant. Statistical analysis was performed using the SAS system, version 6.12 (SAS Institute Inc., Cary, NC, USA).

Results and Discussion

The genotype distribution and allele frequency of the -308 and -238 TNF-α promoter polymorphisms in the 181

Fig. 1. Nucleotide sequence of the human TNF-α promoter. Sequences and positions of PCR primers are shown. The underlined nucleotides of the primers, TNF-Nco3 and TNF-Bg, indicate the mismatches, which were introduced to create restriction sites or minimize the duplex formation of primers.
patients with chronic HBV infection, the 201 spontaneously-recovered subjects, and the 170 healthy controls are shown in Table 1. Genotype frequencies were in Hardy-Weinberg equilibrium. The distribution of both the TNF-α -308 and -238 genotypes in the patient group was not statistically different from that in the spontaneous recovery and control groups. There was also no significant difference in the allele frequency between the groups.

HBV is a noncytopathic virus [6]. Thus, HBV replication itself in hepatocytes does not give rise to liver injury during HBV infection. Instead, the liver injury is thought to be caused by an antiviral cellular immune response that paradoxically, also mediates viral clearance [26]. TNF-α plays an important role in immune response involved in both liver injury and viral clearance [10-12,23,31]. Therefore, polymorphisms in the TNF-α gene influencing its expression could be associated with the pathogenesis of chronic HBV infection. Recently, several groups have examined the possible relationship between polymorphisms in the TNF-α promoter and chronic HBV infection, but the results are contradictory [2,5,9,15,21,25,30,32].

Höhler et al. reported for the first time that the TNF-α promoter polymorphism was associated with the development of chronic HBV infection in a German Caucasian population [24]. They found an association of the TNF-α -238A allele, but not the polymorphism at position -308, with the development of chronic HBV infection. Similarly, other studies showed that the TNF-α -308 genotype was not associated with chronic HBV infection, although they didn’t examine the TNF-α -238 genotype distribution [2,30,32].

Du et al. also found no correlation of the TNF-α -308 genotype with the outcome of HBV infection, but, in contrast to the finding of Höhler et al., they showed that the presence of the TNF-α -238G/G genotype was associated with persistence of HBV infection [9]. On the other hand, several studies found that the TNF-α -308G/G genotype was associated with HBV persistence, while the polymorphism at position -238 was not associated with the outcome of HBV infection [5,21,25].

These conflicting findings including ours may be a result of the difference in genetic background between the populations. The frequency of the TNF-α -308A allele in the control group from Korea was 0.082 (this study), which is much higher than 0.017 in the Japanese population, but lower than 0.14 - 0.20 in German Caucasian, Indian, Taiwanese, Italian, Gambian, Tunisian, American Caucasian, and Swedish populations [7,13,15,29,32]. The frequency of the TNF-α -238A allele in the Korean population was 0.044 (this study), which is higher than 0.03 and 0.02 in Japanese and Swedish populations, respectively, similar to 0.035 in the German Caucasian and lower than 0.063 and 0.09 in Italians and Blacks of South Africa, respectively [13,15,29].

However, despite the discrepancy, the excess of the TNF-α -238A allele [15] is in accordance with the scarcity of the TNF-α -308A allele [5,21,25] in their effects on TNF-α expression. The G to A substitution at position -308 in the TNF-α promoter is associated with increased TNF-α production [22]. In contrast, another common polymorphism, the G to A substitution at position -238, was reported to cause a significant decrease in transcription of the TNF-α gene in human T and B cells [20]. Therefore, both the excess of the TNF-α -238A allele and the scarcity of the TNF-α -308A allele in persistent HBV infection could indicate that inadequate expression of TNF-α due to the possession of the low-producing genotypes may impede the clearance of HBV infection and consequently lead to persistent infection.

The reason for the disparity between our study and two others on Korean populations is not clear [5,21]. Recently, Jin et al. reported that Koreans may have at least two origins [19]. Therefore, one of the possible reasons is the genetic difference in populations between regions (Seoul-Suwon versus Busan-Gyeongnam) within Korea.

Conclusively, the current study has not detected any
correlation between the polymorphisms at positions -308 and -238 in the TNF-α promoter and chronic HBV infection in a Korean population. Nevertheless, the possible implications of polymorphisms influencing TNF-α production in the pathogenesis of HBV infection still remain to be answered. Further studies in other ethnic groups and with larger subjects will be required to unequivocally validate the role of the TNF-α genotypes.

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References