Available online at www.sciencedirect.com
ScienceDirect
Infection, Genetics and Evolution 8 (2008) 20–25
Infection, Genetics and Evolution
www.elsevier.com/locate/meegid

Association of TNF-α −308 polymorphism with the outcome of hepatitis B virus infection in Turkey

Bilkay Basturk [a,*], Zeki Karasu [b], Murat Kılıc [c], Sezgin Ulukaya [d], Sedat Boyacioglu [e], Barbaros Oral [f]

[a] Department of Immunology, Gazi University Faculty of Medicine, Besevler 06500, Ankara, Turkey
[b] Department of Gastroenterology, Ege University Faculty of Medicine, Bornova, İzmir, Turkey
[c] Department of General Surgery, Ege University Faculty of Medicine, Bornova, İzmir, Turkey
[d] Department of Anesthesiology and Reanimation, Ege University Faculty of Medicine, Bornova, İzmir, Turkey
[e] Departments of Internal Medicine, Baskent University Faculty of Medicine, Besevler, Ankara, Turkey
[f] Microbiology and Infectious Diseases Department, Immunology Unit, Uludag University Faculty of Medicine, 16080 Bursa, Turkey

Received 3 November 2006; received in revised form 14 August 2007; accepted 7 September 2007
Available online 14 September 2007

Abstract

Background and aim: Cytokines play important roles in the regulation of immune response. The aim of the study was to investigate the association of the cytokine gene polymorphisms with persistence of hepatitis B virus (HBV) infection and the development of end-stage liver disease (ESLD) due to HBV infection.

Methods: The study involved 27 patients with end-stage liver disease due to HBV infection, 23 HBV carriers and 60 healthy controls. All genotyping (TNF-α, TGF-β, IL-10, IFN-γ) experiments were performed using sequence specific primers (PCR-SSP) by using commercial kit according to manufacturers' instructions.

Results: The frequencies of TNF-α −308 G/G and TGF-β1 codon 10–25 T/C–G/G polymorphisms were significantly higher in HBV-infected individuals (patients + carriers) when compared with those of healthy controls (p: 0.02 and p: 0.004, respectively). The frequency of TNF-α −308 G/G polymorphism was significantly higher in the patients than those of the healthy controls (p: 0.02), whereas the frequency of TGF-β1 codon 10–25 T/T–G/G polymorphism was lower (p: 0.028). On the other hand, TNF-α −308 G/G and TGF-β codon 10–25 T/C–G/G polymorphisms were significantly more common in HBV carriers than the control group (p: 0.017 and p: 0.018, respectively). In addition, TNF-α −308 G allele frequency was significantly more common in HBV-infected individuals (patients + carriers) than those of healthy controls (p: 0.0007). TNF-α −308 G allele frequency was also found to be higher in patients or carriers when compared with those of healthy controls (p: 0.01 and p: 0.01, respectively). Statistically significant differences were still kept after Bonferroni correction of the p-values for only TNF-α −308 G allele frequency in patients or carriers (Pc).

Conclusion: Our study suggests that TNF-α gene polymorphism in patients infected with HBV would result in relatively inefficient inhibition of HBV and development of ESLD, and therefore, may be valuable predictor determinants for the development of ESLD in patients with chronic HBV infection.

Keywords: Cytokines; Gene polymorphisms; Hepatic failure; Hepatitis B

1. Introduction

Hepatitis B (HBV) infection is a major global health problem with approximately 400 million people chronic carriers worldwide. In endemic areas, chronic HBV eventually leads to death due to end-stage liver disease (ESLD) including liver cirrhosis (LC) and hepatocellular carcinoma (HCC). HBV infection is mainly transmitted through exposure to contaminated blood, saliva, or semen in adults (Befeler et al., 2000). Individuals with an inadequate primary immune response to hepatitis B are under high risk for developing chronic HBV, and 5–10% of infected individuals are not able to clear the virus. The risk of chronic infection is related to age at time of exposure. Newborns of actively infected mothers become

* Corresponding author. Tel.: +90 312 202 4743; fax: +90 312 212 7572.
E-mail address: bilkayy@superonline.com (B. Basturk).

1567-1348/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.meegid.2007.09.001

long-term carriers in over 90% of cases. Immunocompetent adults have a risk of chronicity of 5%. Older infants and children have intermediate rates of chronic infection. Nearly all infants and most adults who progress to chronic infection have no symptoms during the acute phase (Tassopoulos et al., 1987). The major feature of chronic HBV infection is the long-term (more than 6 months) presence of hepatitis B surface antigen (HBsAg) in the blood.

Complete clearance of HBV by the immune response depends on the destruction of infected cells by the effector cells of the innate and adaptive immune system. Much of the antiviral effects of these cells are mediated by their ability to produce antiviral cytokines such as interferon (IFN)-γ and tumor necrosis factor (TNF)-α at the site of the infection. These cytokines can clear viruses from infected cells noncytopathically as long as the cell is able to activate antiviral mechanisms and the virus is sensitive to them. The same cytokines also control viral infections indirectly, by modulating the induction, amplification, recruitment and effector functions of the immune response and by upregulating antigen processing and display of viral epitopes at the surface of infected cells Terms (Koziel, 1999; Akalin and Murphy, 2001). The balance among three distinct T cell cytokine patterns is extremely important for the development of immune response to viral infections. T helper 1 (Th1) cytokines, interleukin (IL)-2, IFN-γ and TNF-α are associated with resistance to infection, whereas Th2 cytokines, IL-4 and IL-13, are associated with progressive disease (Sugimoto et al., 2005). In addition, IL-10, one of the T regulatory cytokines, seems to play a pivotal role during the chronic/latent stage of hepatitis B infection. Another T regulatory cytokine transforming growth factor (TGF)-β, mainly produced by Th3 cells, may be beneficial or detrimental (Alves Oliveira et al., 2006; Breitkopf et al., 2005). Of fundamental immunologic importance are the factors that influence the nature of cytokine response, such as polymorphisms of cytokine genes. Polymorphisms in several cytokine genes have been described and demonstrated to influence gene transcription, leading to interindividual variations in cytokine production (Turner et al., 1997; Kroeger et al., 1997; Wilson et al., 1997). Cytokine gene polymorphisms have been shown to be involved in the susceptibility, severity and clinical outcome of several diseases including infectious ones (Cipriano et al., 2005).

Several studies have reported that individuals who recover from HBV infection are characterized by type 1 cytokine release (Penna et al., 1997), whereas T cell clones from patients with chronic HBV infection produce predominantly type 2 cytokines (Bertoletti et al., 1997).

In this study, we aimed to investigate the association of the cytokine gene polymorphisms with the development of ESLD following HBV infection.

2. Methods

The study involved 27 (7 female, 20 male) patients with ESLD following HBV infection, 23 HBV carriers (11 female, 12 male) and 60 (34 female, 26 male) healthy controls. The control group was composed of geographically and racially matched adult healthy blood-donor volunteers who were negative for hepatitis B surface antigen (HBsAg) and positive for anti-HBs (antibody to HBsAg). The diagnostic criteria for chronic HBV infection were seropositivity for HBsAg over a 6-month period. We excluded subjects who were positive for anti-HBs without antibody to hepatitis B core antigen (anti-HBc), that is serological status by vaccination, and patients who were positive for anti-HCV or anti-HIV. The patients who had any other types of liver disease such as autoimmune hepatitis, toxic hepatitis, primary biliary cirrhosis or Budd-Chiari Syndrome were also excluded.

Genomic DNA was extracted from whole ethylenediamine tetraacetate (EDTA)-treated blood with the Machery Nagel DNA isolation kit (Duren, Germany) according to the manufacturer's instructions.

Single nucleotide polymorphisms were analyzed in five cytokines for genotype assignment. The presence of a G or A nucleotide in position −308 of the promoter region was analyzed for TNF-α. Two single nucleotide mutations in coding region were surveyed for TGF-β1: codon 10 can be either T or C, and codon 25, either C or G. Three different polymorphisms were analyzed for the IL-10 promoter region: position −1082 (G vs. A), position −819 (C vs. T) and position −592 (A vs. C). The presence of a single nucleotide modification in position −174 was examined for IL-6 promoter. An additional coding sequence mutation (T vs. A) at position + 874 was analyzed for IFN-γ.

Cytokine genotypes were determined using PCR-sequence-specific primers (SSP) method by a commercially available kit (One lambda, Inc., Canoga Park, CA, USA) according to the manufacturer's instructions. Briefly, after the addition of the master mix provided by the manufacturer, the DNA samples were subjected to 30 cycles of PCR as follows: 1 cycle of 130 s at 96 °C, dropping to 63 °C for an additional 60 s; 9 cycles of 10 s at 96 °C and 60 s at 63 °C and the final 20 cycles, which included a three-temperature ramp-annealing for 10 s at 96 °C, hybridization for 50 s at 59 °C and an extension step of 30 s at 72 °C. PCR products were then loaded onto an agarose gel and visualised by using an ultraviolet transilluminator. The DNA extractions and PCR amplifications were performed by a technician blinded to the study groups.

3. Statistical analysis

Statistical analysis was performed by Epi Info Version 3.2.2 (Centers for Disease Control and Prevention, USA). The distribution of cytokine genes polymorphisms were compared between patients with cirrhosis due to HBV, HBV carriers and healthy controls by the χ^2 or Fisher's exact test. p-Values smaller than 0.05 were considered significant. Odds ratios (OR) and 95% confidence intervals (CI) were calculated in case of that χ^2 or Fisher's exact test was significant. Also, significant probability values obtained were corrected for multiple testing (Bonferroni correction; Pc).

4. Results

We evaluated the frequencies of cytokine gene polymorphisms in HBV carriers and patients with ESLD due to HBV infection and in healthy volunteers.