Clinical Immunology (2008) **128**, 400–408

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Expression profiles and function of Toll-like receptors 2 and 4 in peripheral blood mononuclear cells of chronic hepatitis B patients

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Received 5 October 2007; accepted with revision 19 April 2008
Available online 20 June 2008

KEYWORDS
Toll-like receptors;
Hepatitis B virus;
Peripheral blood mononuclear cells;
Cytokine

Abstract Toll-like receptors (TLRs) play a central role in sensing and initiating innate antiviral response. In this study, we first investigated the expression of TLR1-10 mRNA transcripts in peripheral blood mononuclear cells (PBMCs) from chronic HBV-infected (CHB) patients and healthy donors by quantitative real-time PCR. The expression of TLR1, TLR2, TLR4 and TLR6 transcripts was significantly lower in PBMCs from CHB patients, and the down-regulation of TLR2 was related to HBV genotype C. Flow cytometric analysis showed that the expression of TLR2 on PBMCs was significantly decreased in CHB patients. Furthermore, impaired cytokine production was observed in PBMCs from CHB patients after challenged with TLR2 and TLR4 ligands and was correlated with the levels of plasma hepatitis B virus surface antigen (HBsAg). In conclusion, our study reveals a possible interaction between HBsAg, TLR signaling and the innate immune response, which may partially explain the mechanism of HBV infection induced immuno-tolerance.

Introduction

Chronic hepatitis B caused by hepatitis B virus (HBV) infection is one of the most prevalent liver diseases in the world, affecting more than 350 million people. Chronic HBV infection (CHB) usually leads to fulminant hepatic failure, liver cirrhosis, and primary hepatocellular carcinoma, which results in more than 500,000 deaths per year [1,2]. During HBV infection, the immune responses are crucial for viral clearance. Most studies have underlined the importance of adaptive immunity in HBV infections. A vigorous and sustained polyclonal Cytotoxic T lymphocyte (CTL) response to HBV was observed in patients with self-limited acute hepatitis, whereas it was significantly lower or undetectable in patients with chronic HBV infection [3,4]. In addition, Thimme et al. demonstrated in a

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doi:10.1016/j.clim.2008.04.006

Table 1 Characteristics of patients with hepatitis B virus and healthy donors

Parameter	Patients	Healthy donors
Number	59	30
Age	37 (19–64)	27 (21–52)
Male/Female (P=0.075)	46/13	18/12
ALT(IU/L)-mean	542 (44–2359)	<40
HBsAg (S/N)-mean	218.12 (7.37–357.98)	Negative
HBeAg +/−	30/29	Negative
HBV genotype (B/C)	25/35	Negative
Autoimmune diseases	None	None

Selection criteria
1. 16 to 70 years old;
2. Consistent with the diagnostic criteria of chronic hepatitis B virus infection according to the guidelines of prevention and treatment for chronic hepatitis B in China;
a) The clinical history of chronic hepatitis B;
b) Persistence of HBsAg in blood for 6 months;
c) Increased liver enzymes, ALT>40 IU/L;
d) Serum HBV DNA>10^5 copies/ml in HBeAg positive and >10^4 copies/ml in HBeAg negative patients;
3. Have not received antiviral treatment or immunotherapy such as nucleoside analogue and interferon in the latest 6 months;
4. Negative for other viral infections, including HCV, HIV, CMV and EBV;
5. B-type ultrasonic inspection showed non-cirrhotic and non-hepatocellular carcinoma patients.

chimpanzee model that intrahepatic HBV-specific $CD8^+$ T cells were required for viral clearance during acute HBV infection [5]. However, the precise role of the innate immune response in HBV infection is still not well understood.

For the role of TLRs in innate immune response against HBV, due to the lack of suitable small animal models reflecting the immune-pathological response in human, there are only a few *in vivo* and *in vitro* studies and the results are not consistent. Stephen Locarnini et al. found that TLR2 was down-regulated in hepatocytes in chronic HBV patients and this down-regulation was related to the precore protein [6]. George Lau et al. reported that up-regulation of TLR7 played an essential role in serological clearance of HBsAg in patients with chronic HBV infection [7]. These results suggested that HBV interacted with the TLR signaling cascade and the alteration of TLR-mediated signals might be one of the escape mechanisms of virus-induced immune modulation.

In the present study, we aimed at determining the effect of HBV infection on the profiles of TLR expression as well as their function in PBMC. To this end, expression of TLRs in PBMC from CHB patients was compared with those from healthy donors. Furthermore, an unconditional multivariate logistic analysis was performed to investigate the independent effect of associated variables on TLR expression. To study its functional consequences, PBMCs were stimulated with TLR-specific ligands and the cytokine profile was then examined.

Materials and methods

Human subjects

Patients with chronic HBV infection, who did not receive antiviral treatment or immunotherapy for the latest 6 months, followed up at Shanghai Public Health Clinical Center were recruited in the present study. Blood samples of healthy donors were from Shanghai Blood Center which were tested negative for human immunodeficiency virus (HIV), HBV, and hepatitis C virus (HCV). Further exclusion criteria

Table 2 Primer sequence used for real-time RT-PCR

Oligo name	Accession code	Sequence (5′–3′)	Expected length
TLR1-for	NM_003263	TTCACAGTGTCTGGTACACGCAT	101 bp
TLR1-rev		ACCGTGTCTGTTAAGAGATTATTGGA	
TLR2-for	NM_003264	GCCTCTCCAAGGAAGAATCC	144 bp
TLR2-rev		TCCTGTTGTTGGACAGGTCA	
TLR3-for	NM_003265	ACAACTTAGCACGGCTCTGGA	124 bp
TLR3-rev		ACCTCAACTGGGATCTCGTCA	
TLR4-for	NM_138554	AATCTAGAGCACTTGGACCTTTCC	116 bp
TLR4-rev		GGGTTCAGGGACAGGTCTAAAGA	
TLR5-for	NM_003268	CCATAGATTTTTCCTCCAACCAAATA	141 bp
TLR5-rev		TCATACATTTTCCCCAGTCCACT	
TLR6-for	NM_006068	CATCCTATTGTGAGTTTCAGGCAT	121 bp
TLR6-rev		GCTTCATAGCACTCAATCCCAAG	
TLR7-for	NM_016562	GGAGGTATTCCCACGAACACC	141 bp
TLR7-rev		TGACCCCAGTGGAATAGGTACAC	
TLR8-for	NM_016610	AAACTTGACCCAACTTCGATACCTAA	101 bp
TLR8-rev		GATCCAGCACCTTCAGATGAGG	
TLR9-for	NM_017442	GGACCTCTGGTACTGCTTCCA	151 bp
TLR9-rev		AAGCTCGTTGTACACCCAGTCT	
TLR10-for	NM_030956	AAGAAAGGTTCCCGCAGACTT	131 bp
TLR10-rev		TGTTATGGCATAGAATCAAAACTCTCA	
GAPDH-for	NM_002046	GGTATCGTGGAAGGACTCATGAC	188 bp
GAPDH-rev		ATGCCAGTGAGCTTCCCGTTCAGC	