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Aberrant expression and dysfunction of TLR2 and its soluble form in chronic HBV infection and its regulation by antiviral therapy

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ABSTRACT

Toll-like receptor 2 (TLR2) plays an important role in the immunopathogenesis of hepatitis B virus (HBV) infection. The relationship between TLR2 expression and clinical outcome of chronic HBV infection is not yet elucidated in details so far. Here, we employed clinical cohorts to investigate TLR2 expression and function in different phases of HBV infection and dynamic changes of TLR2 expression in HBeAg-positive chronic hepatitis B (CHB) patients during antiviral therapy. TLR2 was mainly expressed in monocytes and its ligand stimulation resulted in TNF-α, IL-6 and IL-10 production. Serum soluble TLR2 (sTLR2) levels were negatively correlated with TLR2 mRNA in PBMCs. As compared with immunotolerant carriers and inactive carriers, CHB patients showed an elevated TLR2 expression and TNF-α, IL-6 induction in PBMC, but had a decreased level of sTLR2 in serum. However, TLR2 expression and TNF-α induction in monocytes of CHB patients remained lower than healthy controls. Furthermore, higher TLR2 expression in PBMCs and lower level of sTLR2 in serum at baseline were predictive of a complete response to 52 weeks of telbivudine (LdT) therapy. Temporal dynamic analysis showed that TLR2 expression was restored with viral suppression and ALT normalization from week 12 to 24. However, peg-IFN-α-2a therapy induced a slightly decline in TLR2 expression. In conclusion, TLR2 expression and function in monocytes were impaired by chronic HBV infection. Higher TLR2 expression in PBMC and lower level of sTLR2 in serum at baseline were associated with a complete response to LdT therapy, and dynamic TLR2 expression was differently regulated by LdT and peg-IFN-α-2a therapy.

1. Introduction

Hepatitis B virus (HBV) infection is a global health problem and about 1 million people die from HBV-associated liver diseases per year (Dandri and Locarnini, 2012). It has been clearly shown that deletion or exhaustion of adaptive immunity leads to HBV persistence in patients (Bertoletti and Ferrari, 2012). However, the exact role of innate immunity in chronic HBV infection remains to be defined. Toll-like receptors (TLRs) play a pivotal role in innate immunity by recognizing and responding to pathogen-associated molecular patterns and the production of a variety of pro-inflammatory cytokines (Akira et al., 2006). Increasing evidence demonstrates that the application of TLR ligands suppresses HBV replication *in vitro* and *in vivo* (Isogawa et al., 2005; Wu et al., 2007; Zhang et al., 2012a, 2009). Therefore, activating TLR-

Abbreviations: ALT, alanine aminotransferase; CHB, chronic hepatitis B; CR, complete response; HBeAg, hepatitis B e antigen; HBV, hepatitis B virus; HC, healthy control; IC, inactive carrier; IFN, interferon; IL, interleukin; IT, immunotolerant; LdT, telbivudine; NCR, non-complete response; PBMC, peripheral blood mononuclear cell; PCR, polymerase chain reaction; peg-IFN-α-2a, pegylated interferon α-2a; sTLR2, soluble Toll-like receptor 2; TLR, Toll-like receptor; TNF-α, tumor necrosis factor α; WHV, woodchuck hepatitis virus.

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Table 1
Clinical characteristic of the cross-sectional study participants.

Group	HC	IT	CHB	IC
n	21	24	27	22
Gender (male/female)	10/11	13/11	20/7	10/12
Age (years)	25 (22–28)	27.5 (18–38)	28 (18–38)	28 (21–45)
HBV-DNA (log_{10} copies/mL)	ND	8.01 (6.72–8.82)	8.59 (6.37–9.48)	<3
ALT (U/L)	15 (6–38)	23.8 (14–41)	113 (55–593)	16.5 (10–39)
HBeAg/anti-HBe	0/0	24/0	27/0	0/22

Values are *n* or median (range).
ALT, alanine aminotransferase; HBeAg, hepatitis B e antigen; HBV, hepatitis B virus; HC, healthy controls; IT, immunotolerant carriers; CHB, chronic hepatitis B patients; IC, inactive carriers; ND, not determined.

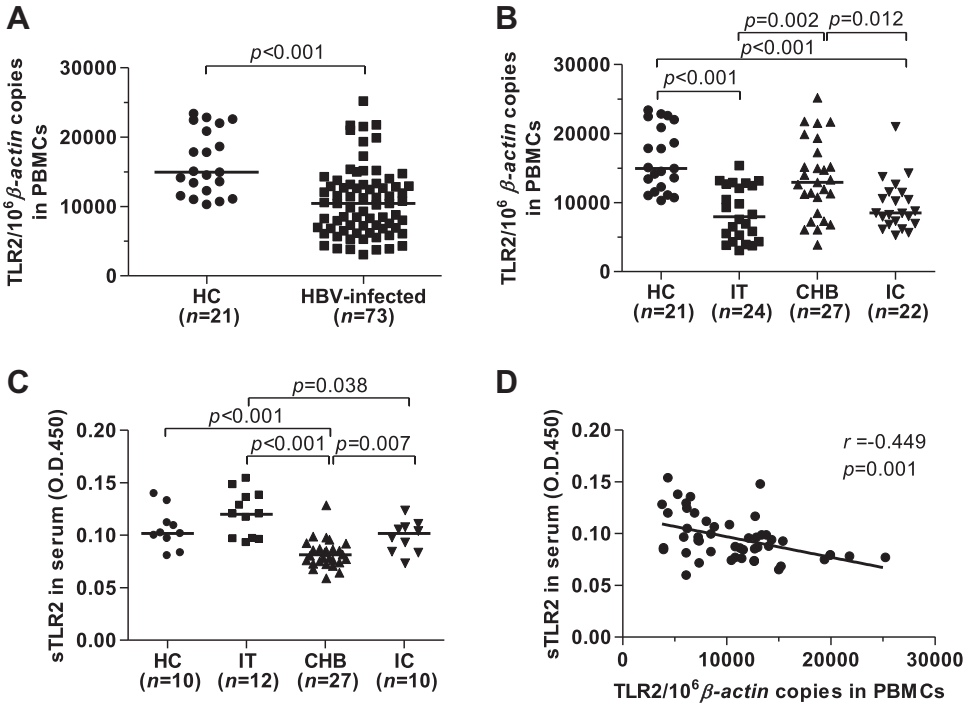

Fig. 1. TLR2 expression in PBMCs and sTLR2 levels in sera from chronic HBV-infected patients and HC. PBMCs and serum samples from patients and HC were taken and TLR2 expression and sTLR2 level were examined by real time RT-PCR and ELISA, respectively. (A) Comparison of TLR2 mRNA in PBMCs from HC and chronic HBV-infection patients. (B) Comparison of TLR2 mRNA in PBMCs from HC, IT, CHB and IC groups. (C) Comparison of sTLR2 in sera from HC, IT, CHB and IC patients. (D) Spearman's correlation of TLR2 mRNA in PBMCs and sTLR2 in sera from all chronic HBV-infected patients. HC, healthy controls; IT: immunotolerant carriers; CHB, chronic hepatitis B patients; IC, inactive carriers; sTLR2: soluble TLR2.

mediated innate immunity might be a new therapeutic option for patients with chronic hepatitis B (CHB) (Durantel and Zoulim, 2012; Zoulim et al., 2013).

In concert with TLR1 or TLR6, TLR2 recognizes various bacterial, viral, fungal, and certain endogenous components, including peptidoglycans, lipopeptides, and lipoproteins (Kawai and Akira, 2007). Previous results have demonstrated that the expression of TLR2 on hepatocytes, Kupffer cells, and peripheral monocytes is significantly reduced in patients with hepatitis B e antigen (HBeAg)-positive CHB, in comparison with HBeAg-negative CHB and controls (Visvanathan et al., 2007). Furthermore, we and others had shown that activation of TLR2-mediated intracellular signaling leads to a reduction in HBV/woodchuck hepatitis virus (WHV) replication and gene expression in human hepatoma cells and primary woodchuck hepatocytes (Thompson et al., 2009; Zhang et al., 2012b). Compared to naïve woodchucks, relatively lower levels of TLR2 expression were found in peripheral blood mononuclear cells (PBMCs) and in the liver tissue from chronic WHV carriers (Zhang et al., 2012b). These findings suggest that the TLR2-mediated innate immune response is involved in the control of hepadnaviral replication, and that hepadnaviruses might interfere with TLR2 expression. However, the relationship between TLR2 expression and natural history of HBV infection and that during antiviral therapy has not been investigated in detail.

In the present study, we aimed to examine TLR2 expression and function at the different phases of chronic HBV infection. In addition, we performed an in-depth prospective analysis of the kinetics of TLR2 expression in CHB patients receiving telbivudine (LdT) or pegylated interferon α-2a (peg-IFN-α-2a) therapy. Moreover, the expression of TLR2 and its association with the response to antiviral therapy were investigated.

2. Patients and methods

2.1. Study participants

Blood samples were obtained from 73 patients with chronic HBV infection, who were recruited at Nanfang Hospital