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Mutations in the S gene and in the overlapping reverse transcriptase region in chronic hepatitis B Chinese patients with coexistence of HBsAg and anti-HBs

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ABSTRACT

Background: The mechanism underlying the coexistence of hepatitis B surface antigen and antibodies to HBsAg in chronic hepatitis B patients remains unknown.
Aims: This research aimed to determine the clinical and virological features of the rare pattern.
Methods: A total of 32 chronic hepatitis B patients infected by HBV genotype C were included: 15 carrying both HBsAg and anti-HBs (group I) and 17 solely positive for HBsAg (group II). S gene and reverse transcriptase region sequences were amplified, sequenced and compared with the reference sequences.
Results: The amino acid variability within major hydrophilic region, especially the "a" determinant region, and within reverse transcriptase for regions overlapping the major hydrophilic region in group I is significantly higher than those in group II. Mutation sI126S/T within the "a" determinant was the most frequent change, and only patients from group I had the sQ129R, sG130N, sF134I, sG145R amino acid changes, which are known to alter immunogenicity.
Conclusions: In chronic patients, the concurrent HBsAg/anti-HBs serological profile is associated with an increased aa variability in several key areas of HBV genome. Additional research on these genetic mutants are needed to clarify their biological significance for viral persistence.

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Introduction

Hepatitis B virus (HBV) infection is a serious global public health problem, and its prevalence varies considerably from region to region. Worldwide, more than 350 million people are chronic carriers of HBV, and have a risk of dying from development of cirrhosis and hepatocellular carcinoma.[1] Clinically, the appearance of circulating hepatitis B surface antigen (HBsAg) heralds HBV infection, whereas the presence of the antibody to HBsAg (anti-HBs) usually indicates resolution of infection and is considered indicative of immunity to HBV infection.[2] It is generally believed that anti-HBs can neutralize and clear HBsAg. Therefore, the concomitant presence in the same serum of HBsAg and anti-HBs could be possible. However, the persistence of HBsAg associated with anti-HBs in patients with chronic HBV infection has been reported in previous studies.[3–5] So far, the mechanism underlying the concurrent detection of HBsAg and anti-HBs remains largely controversial.

HBV genome contains four partially overlapping open reading frames (ORF), and the "a" determinant is located at codon positions 124–147 within the major hydrophilic region (MHR) of the HBsAg. This determinant is the main target of recognition of HBsAg by anti-HBs and immune response cells during the course of the initial immune response in acute hepatitis B. HBV has been classified into eight genotypes, designated as A–H, and the most prevalent genotypes in China are genotypes B and C.[5] Notably, previous reports documented that, in comparison to genotype B, genotype C takes a more aggressive disease course and has a lower response rate to antiviral therapy.[6–9]

Several reports showed the paradoxical coexistence of HBsAg and anti-HBs might be associated with the selection of HBV immune escape mutants during chronic carriage.[10–12] These studies documented increased amino acid (aa) mutations in and around the "a" determinant, which is responsible for the stability and immunogenicity of HBsAg.[13] Nonetheless, a later report rejected this hypothesis and instead suggested that the pattern of simultaneous appearance of HBsAg and anti-HBs was related to the weak binding of anti-HBs to HBsAg.[12] As the reverse transcriptase (RT) region of HBV polymerase gene overlaps the HBsAg ORF, mutations within HBsAg gene might result in structural and functional alterations in the HBV reverse transcriptase with potential influence on viral replication capacity and efficacy of antiviral drugs.[14,15]

Therefore, the aims of this study were to investigate the prevalence of the coexistence of anti-HBs in HBsAg-positive CHB patients infected with genotype C HBV, and explore the relationship between the variability of the HBV S gene and the paradoxical serological profile.

Materials and methods

Patients

From January to December 2013, 1194 patients with CHB recruited from Wuhan, Huangshi, and Yichang were enrolled in the study. The inclusion criteria were HBsAg carriage for more than six months, HBV DNA concentration of $>1 \times 10^4$ IU/mL, serum alanine aminotransferase (ALT) >2 ULN (upper limit of normal), and associated symptoms. Exclusion criteria included having received passive immunization, previous antiviral therapy, coinfection with other hepatitis viruses, or HIV. Sera were collected and stored at −70 °C. All individuals provided written informed consent before entering the study.

Serologic testing

HBV serological markers were determined by using the chemiluminescent microparticle immunoassay (CMIA) technique with an Architect-i2000 automatic analyzer (Abbott Laboratories, USA). Commercially available kits were purchased from Abbott Laboratories. The analytical threshold of anti-HBs was defined as 10 mIU/mL. Serum was diluted according to the Manual Dilution Procedure if HBsAg concentration value >250,00 IU/mL. ALT was assayed with an ADVIA automatic biochemical analyzer (Siemens, Germany).

HBV DNA quantification

HBV DNA was quantified in serum using a commercially available real time fluorescence quantitative kit (Da An, China) with a lower detection threshold of 500 copies/mL.

Serum DNA extraction

Viral DNA was extracted from 200 μL of serum using QIAamp DNA blood mini kit (Qiagen, Germany) according to the manufacturer's instructions.

PCR amplification and sequencing of PCR fragment

Amplification and sequencing of the full-length S gene and the overlapping RT regions were performed using the protocol as follows. The primer sequences were synthesized by Beijing SBS Biotechnology Company. PCR was carried out in a 50-μL reaction mixture containing 10 μL of HBV DNA template, 0.2 μM each primer, 0.2 mM of each dNTP, 2 mM $MgCl_2$, and 1 μL of high-fidelity Taq polymerase (Takara, China). Besides, PCR was performed with hot start and denaturation at 94 °C for 5 min, 35 cycles at 94 °C for 1 min, at 50 °C for 1 min, and at 72 °C for 2 min, then incubated at 72 °C for 5 min. In order to obtain a complete description of HBV quasispecies, sequence analysis was performed with the powerful ultra-deep sequencing (UDS) approach, using the primers according to the reference sequence X04615 (the upstream primer: 5′-GTCACCATATTCTTGGGAAC-3′ nt2818-2837; the downstream primer: 5′-CATATCCCATGAAGTTAAGG-3′ nt 888–869).[12]

Sequence analysis

Genomic sequences obtained for the S gene and the overlapping RT regions were translated into aa sequences and compared with HBV reference sequences used on the NCBI Website (http://www.ncbi.nih.gov/projects/genotyping/view.cgi?db=2). For analysis, the full-length S protein was divided into three regions corresponding to structural and/or functional domains: the N terminal region (aa 1–99), the MHR