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Case Report

Concurrent HBsAg and anti-HBs induced by a preS deletion mutant in a chronic hepatitis B patient

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Abstract: This study analyzed the origins of concurrent hepatitis B surface antigen (HBsAg) and HBsAg antibodies (anti-HBs) in a patient with chronic hepatitis B virus (HBV) infection. The levels of serological markers of HBV infection were determined by enzyme-linked immunosorbent assay (ELISA). The preS/S gene was analyzed by gene amplification and sequencing. The tests revealed that HBsAg and anti-HBs coexisted in this patient with mixed infections of the full-length preS/S virus strain and preS1 183 bp deletion mutant, and both the mutant and the anti-HBs were no longer present after one year, which means that the mutant strain was cleared by the detected antibodies. Thus, it is speculated that anti-HBs antibodies targeted specifically to the preS1 deletion mutant strain instead of the strain with the full-length large S protein were produced. This mechanism is quite different from other immunopathogenic mechanisms for concurrent HBsAg and anti-HBs.

Keywords: Hepatitis B virus, chronic hepatitis B, HBsAg, anti-HBs, preS deletion mutant

Introduction

Hepatitis B virus (HBV) infection is a serious global health problem. It can result in a self-limiting acute infection or chronic hepatitis B (CHB) depending on the interaction between the host's immune system and the virus [1]. HBV encodes three hepatitis B surface antigen (HBsAg) proteins (large, middle, and small). The large multifunctional HBsAg protein surrounding the hepatitis B virion is encoded by the preS1/preS2/S region of the genome. The myristoylated N-terminal portion of the preS1 domain contains a ligand that binds to the sodium taurocholate cotransporting polypeptide (NTCP) receptor found on human hepatocytes that is necessary for infection. Specific antibody responses targeting HBsAg play an important role in neutralizing HBV infectivity and clearing noninfectious HBsAg particles from peripheral blood. Thus, the presence of antibodies against HBsAg (anti-HBs) indicates viral clearance and is often a sign of recovery from HBV infection. In contrast, once the disease progresses to chronic hepatitis B (CHB), anti-HBs generally do not coexist with HBsAg.

In those cases where concurrent HBsAg and anti-HBs occur in patients with CHB, atypical serological characteristics are observed. This coexistence of HBsAg and anti-HBs is generally considered to be the result of immune-associated escape mutations, which include deletions and point mutations within the preS/S region of the genome [2]. However, no significant or identical differences have been reported in the incidence of amino acid substitutions in the small S gene region of the HBV genome from CHB patients with and without anti-HBs [3]. This appears to contradict the hypothesis that HBV immune escape mutants are selected by concurrent anti-HBs.

This report describes a case in which full-length large HBsAg and a deletion mutant coexisted with anti-HBs in the serum of a patient with chronic HBV infection. After one year, both the anti-HBs and mutant HBsAg were no longer present. The correlation between immunity and

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mutations in this case warrants further investigation.

Case presentation

Peripheral blood samples were collected from a 43-year-old Chinese male patient with genotype C CHB who had never been vaccinated and who was admitted to the 960th Hospital of the PLA Joint Logistics Support Force for regular physical examination. Routine screening revealed that he had concurrent HBsAg and anti-HBs. Peripheral blood samples (5 mL), identified as Sample 1 and Sample 2, were collected on two separate occasions at an interval of one year. This study was approved by the hospital ethics committee.

Serological markers of HBV infection were determined by commercially available enzyme-linked immunosorbent assay (ELISA) kits (Xiamen Xinchuang, Shanghai Kehua, China). Positive and negative controls were included in each test. The tests were carried out according to the instructions of each kit.

QIAamp MinElute Virus Spin Kits (Qiagen, Hilden, Germany) were used to extract DNA from the serum samples. DNA was aliquoted and stored at -80°C until use. Commercially available HBV real-time polymerase chain reaction (PCR) kits (Piji Biotec, Shenzhen, China) and a Light Cycler 2.0 Real-Time PCR system (Roche Diagnostics, Basel, Switzerland) were utilized to quantify the viral load of HBV DNA. The preS/S gene regions were amplified on a thermal cycler (Bio-Rad MyCycler, Bio-Rad, CA) using PrimeStar HS high-fidelity DNA polymerase (Takara, Dalian, China). The PCR products from Sample 1 and Sample 2 were recovered from agarose gel and purified using Agarose Gel DNA Purification kits (Takara, Dalian, China) prior to being sequenced directly. The products of Sample 2 were further cloned and inserted into the pMD18-T vector system (Takara, Dalian, China), and ten clones were selected for sequencing and analysis.

Results

Serological markers of HBV infection and quantification of HBV DNA

The virological tests of Sample 1 were positive for HBsAg, anti-HBs, hepatitis B e antigen (HBeAg), and hepatitis B core antibody (anti-HBc). The HBV DNA viral load was 2.16×10^5 IU/mL. The results for Sample 2 revealed reactivity against HBsAg, HBeAg, and anti-HBc, with an HBV DNA viral load of 1.29×10^4 IU/mL, but anti-HBs was not detected.

PCR amplification of the preS/S gene and DNA sequencing results

The PCR product was approximately 1,410 bp long and spans nucleotide positions 2825-1019. Electrophoresis after preS/S PCR amplification of Sample 1 showed two distinguishable bands located at approximately 1,200 bp and 1,400 bp. Sequencing results of the products recovered from the two bands in Sample 1 revealed that one of the bands featured a large deletion mutation in preS1 (**Figure 1**) compared to a full-length preS/S segment in the other band, implying that there were two HBV variants present in the same sample. Comparison of the nucleotide sequence of this mutant with the amino acid (aa) sequence of the wild-type virus showed that the 183 bp deletion in the preS1 region was located at aa 58-118 of the large HBsAg polypeptide (**Figure 2**).

In contrast, only one band located at approximately 1,400 bp was identified in Sample 2 by electrophoresis. The PCR products and clones were subsequently sequenced, and the results showed that only the full-length large surface protein was present. No preS1 deletion mutant was found by sequencing.

Discussion

Although concurrent HBsAg and anti-HBs have been reported in patients with chronic HBV infection, the clinical and virological characteristics of these patients were not well described. Atypical serological profiles suggest distinct disease stages, immune response variations, and viral mutations [4]. Two immunopathogenic mechanisms have been proposed to explain the circumstances leading to the coexistence of HBsAg and anti-HBs. First, frequent studies support the hypothesis that concurrent HBsAg and anti-HBs are associated with virus strains with HBsAg mutations that have escaped anti-HBs neutralization [5]. PreS deletions and “a” determinant mutations can lead to antigenic changes in HBsAg. This results in the concurrence of HBsAg and anti-HBs in the blood since

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                2848 pre-S1
AB014381        ATGGGAGGTTGGTCTTCCAAACCTCGACAAGGCATGGGGACAAATCTTTCTGTTCCCAAT
Sample 1-1      ATGGGAGGTTGGTCTTCCAAACCTCGACAAGGCATGGGGACAAATCTTTCTGTTCCCAAT
Sample 1-2      ATGGGAGGTTGGTCTTCCAAACCTCGACAAGGCATGGGGACAAATCTTTCTGTTCCCAAT
Sample 2        ATGGGAGGTTGGTCTTCCAAACCTCGACAAGGCATGGGGACAAATCTTTCTGTTCCCAAT
                2908
AB014381        CCTCTGGGATTCTTTCCCGATCACCAGTTGGACCCTGCGTTCGGAGCCAACTCAAACAAT
Sample 1-1      CCTCTGGGATTCTTTCCCGATCACCAGTTGGACCCTGCGTTCGGAGCCAACTCAAACAAT
Sample 1-2      CCTCTGGGATTCTTTCCCGATCACCAGTTGGACCCTGCGTTCGGAGCCAACTCAAACAAT
Sample 2        CCTCTGGGATTCTTTCCCGATCACCAGTTGGACCCTGCGTTCGGAGCCAACTCAAACAAT
                2968
AB014381        CCAGATTGGGACTTCAACCCCAACAAGGATCACTGGCCAGAGGCAAATCAGGTAGGAGCG
Sample 1-1      CCAGATTGGGACTTCAACCCCAACAAGGATCATTGGCCAGAGGCAAATCAG--------
Sample 1-2      CCAGATTGGGACTTCAACCCCAACAAGGATCATTGGCCAGAGGCAAATCAGGTAGGAGCG
Sample 2        CCAGATTGGGACTTCAACCCCAACAAGGATCATTGGCCAGAGGCAAATCAGGTAGGAGCG
                3028
AB014381        GGAGCATTCGGGCCAGGGTTCACCCCACCACACGGCGGTCTTTTGGGGTGGAGCCCTCAG
Sample 1-1      ------------------------------------------------------------
Sample 1-2      GGAGCATTCGGGCTAGGGTTCACCCCACCACACGGCGGTCTTTTGGGGTGGAGCCCTCAG
Sample 2        GGAGCATTCGGGCTAGGGTTCACCCCACCACACGGCGGTCTTTTGGGGTGGAGCCCTCAG
                3088
AB014381        GCTCAGGGCACATTGACAACAGTGCCAGTAGCACCTCCTCCTGCCTCCACCAATCGGCAG
Sample 1-1      ------------------------------------------------------------
Sample 1-2      GCTCAGGGCATATTGACAACAGTGCCAGTAACACCTCCTCCTGCCTCCGCCAATCGGCAG
Sample 2        GCTCAGGGCATATTGACAACAGTGCCAGTAACACCTCCTCCTGCCTCCGCCAATCGGCAG
                3148                                                pre-S2
AB014381        TCAGGAAGACAGCCTACTCCCATCTCTCCACCTCTAAGAGACAGTCATCCTCAGGCCATG
Sample 1-1      ------------------------------------------------GCCATG
Sample 1-2      TCAGGAAGACAGCCTACTCCCGTCTCTCCACCTCTAAGAGACAGTCATCCTCAGGCCATG
Sample 2        TCAGGAAGACAGCCTACTCCCGTCTCTCCACCTCTAAGAGACAGTCATCCTCAGGCCATG
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Figure 1. Nucleotide sequences of the pre-S1 regions obtained from Sample 1 and Sample 2. Sample 1 showed two clearly distinguishable bands located at approximately 1,200 bp and 1,400 bp after preS/S amplification. "Sample 1-1" represented a deletion mutant located at approximately 1,200 bp, and "Sample 1-2" represented a full-length strain in the pre-S1 region located at approximately 1,400 bp. Sample 2 showed only one band located at approximately 1,400 bp by electrophoresis. The genotype C reference sequence (AB014381) is shown at the top. The positions of the nucleotide sequences of the pre-S1 region and the start codon of the pre-S1 and pre-S2 genes are indicated above the reference sequence. "-----" indicates deletion sequences in Sample 1-1 compared to the reference.

preS/S mutations may alter HBsAg binding properties [6], thereby masking antibody recognition of HBsAg [7]. A second hypothesis is that HBV immune escape mutants are not selected by anti-HBs that coexist with HBsAg in CHB patients. Rather, the coexistence of HBsAg and

Figure 2. The amino acid sequences of the Pre-S1 regions corresponding to the sequencing results of Sample 1 and Sample 2. The genotype C reference amino acid sequence (BAA32914.1) is shown at the top. The positions of the amino acid sequences of the pre-S1 region and the start codons of the pre-S1 and pre-S2 genes are indicated above the reference sequence. Different sequences are marked in red. "-----" indicates deletion sequences in Sample 1-1 compared to the reference.

anti-HBs in this situation may be explained as anti-HBs that have unmatched specificities for HBsAg, rendering it unable to bind to the antigen [8].

In this study, the analysis of Sample 2 showed that the mutant and the anti-HBs were no longer present, which we propose means that the mutant strain was cleared by the detected antibodies. Thus, we speculate that the state of concurrent HBsAg and anti-HBs in Sample 1 resulted from the production of anti-HBs targeted specifically to the preS1 deletion mutant strain and not to the strain with full-length large HBsAg. Moreover, it did not lead to the selection of escape mutants. This mechanism is quite different from the other immunopathogenic mechanisms mentioned above. The virological findings in this case remain interesting. It is possible that the preS1 deletion strain induced by immune pressure generated anti-HBs with high affinity and specificity for the mutant. Moreover, host-related factors, such as immunocompetence, may have contributed to the abnormal response pattern observed in this patient.

In a similar study that did not include HBV DNA sequencing [9], concurrent HBsAg and anti-HBs were detected in 18 of 1,462 CHB patients (1.2%), 15 of whom (83.3%) were born in Asia or Australia. During a median follow-up of 4 years in 12 subjects, anti-HBs became undetectable in half of the subjects after approximately 1.9 years. Independently, we have presented HBV DNA sequencing data from the preS region of the S gene in a genotype C CHB patient in which anti-HBs and a preS1 deletion variant strain became undetectable after one year, suggesting that the patient was able to generate an HBV mutant-specific immune response. It will be interesting to follow the patient to elucidate whether the ability to generate an HBV-specific immune response positively affects the clinical course, which may have implications for HBV outcome. These findings may also stimulate additional studies on the relevance of preS1 deletions in this process since most of the previous investigations have focused on the small polypeptide of the S gene instead of the preS region, which has many immune epitopes and functional domains [10].

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Disclosure of conflict of interest

None.

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