Case report

Possible association of vigorous hepatitis B virus replication with the development of fulminant hepatitis

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Introduction

In Asian countries where hepatitis B virus (HBV) infection is endemic, fulminant hepatic failure (FHF) frequently occurs in persistent HBV infection irrespective of the presence of hepatitis B e antigen (HBeAg) in serum. Serial virological examination of such cases has revealed that highly enhanced HBV replication precedes transaminase elevation.2 Rapid spread of HBV infection throughout the liver and presentation of viral antigens on the surface of infected hepatocytes may trigger a robust host immune response, including specific cytotoxic T lymphocyte (CTL) responses.5

Here we present a clinical case in which acute infection with highly replicative mutant HBV may be associated with the development of fulminant hepatitis.

Key words: hepatitis B virus, fulminant hepatic failure, precore mutation, core promoter mutation

A 49 year-old man was referred to our hospital for fear of developing fulminant hepatic failure. There had been an outbreak of fulminant hepatitis B in a dialysis clinic in the western part of Honshu, Japan, that resulted in four deaths among six patients. After the sixth patient contracted severe hepatitis, all patients in the unit were screened biweekly for hepatitis B surface antigen (HBsAg) to detect newly infected patients as soon as possible. Our patient was the seventh victim, and on the day he gave a positive result for HBsAg, his hepatitis B virus (HBV) DNA level had reached $1.1 \times 10^{11}$ copies/ml as assessed by real time polymerase chain reaction. Sequence analysis of the causal HBV revealed the presence of a mutation in the precore region (nt 1896), two mutations in the core promoter (nt 1762 and nt 1764), and some minor mutations in the P gene that were restricted to the upstream region. These mutations are indicative of a virus with a high replicative rate that cannot secrete HBeAg. Taken together, these findings indicate that it is very likely that the replicative ability of the causal virus was as vigorous as that of HBV in hepatitis B e antigen-positive asymptomatic carriers with markedly high viral titers. The present case report provides clinical evidence of a possible association between the rapid spread of highly replicative HBV before host immunological recognition and the development of fulminant hepatitis.

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Methods

All routine hematological and biochemical examinations were performed using an autoanalyzer. Hepatitis B surface antigen (HBsAg) and the corresponding antibody (anti-HBs), HBeAg, and antibody to HBeAg (anti-HBe), antibody to hepatitis A virus (anti-HAV), and antibody to hepatitis D virus (anti-HDV) were measured by radioimmunoassay. Second-generation antibody to hepatitis C virus (anti-HCV) was measured by enzyme-linked immunosorbent assay (ELISA) (Ortho Diagnostic Systems, Tokyo, Japan). Serum HBV DNA level was measured by TaqMan (Applied Biosystems, Foster City, CA, USA) real-time quantitative polymerase chain reaction (PCR).6

The entire nucleotide sequence of the HBV genome was determined as described previously.7 Briefly, two overlapping regions of HBV DNA were amplified by nested PCR with TaKaRa Ex Taq (TaKaRa Shuzo, Kyoto, Japan) and the appropriate primers derived from conserved areas of the genomes of the eight different strains of HBV (genotypes A to H). The HBV genome was sequenced directly on both strands, using the BigDyeTerminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems). The two overlapping regions (primer sequences at both ends excluded) that were amplified spanned nt 480–2380 [1901 base pairs (bp)] and nt 2328–3215 and 1–667 (1554 bp).

All presently available kits for HBeAg detection are reactive with antigenic determinants on the C-terminus of HBeAg, a protein sequence that is also shared by core protein. Since these kits are also reactive with HBV core protein, we investigated the presence of HBeAg in serum by ELISA with a monoclonal antibody against the precore region (Y0583A). The details of the development of the ELISA were described previously.8

Clinical case

Over a 5-month period in 1999, there was an outbreak of severe acute hepatitis B, including FHB, in a dialysis unit in the western part of Japan. Of the seven patients involved in the outbreak, five died of FHB. After the sixth patient developed severe hepatitis, a biweekly HBsAg screening assay was established for all patients receiving hemodialysis in order to identify newly infected patients as early as possible. A seventh patient became positive for HBsAg, and 5 days later his transaminase levels began to rise. The patient was then referred to our hospital, some 600 km away, for prevention of FHF. The viral load on the day HBsAg was first detected in his serum was $1.1 \times 10^{11}$ copies/ml, as ascertained by an in-house real-time detection PCR assay.6 The patient was placed on a combination of interferon, lamivudine, and famciclovir for antiviral treatment. A large dose of methyl prednisolone and cyclosporin A was administered to suppress a presumably enhanced host immune response.9,10 The efficacy of these treatments has been described previously.11 Artificial liver support (ALS) comprising plasma exchange and hemodiafiltration was initiated immediately after the patient fell into hepatic coma. After three sessions of ALS, the patient regained full consciousness. HBV DNA level decreased to $1.9 \times 10^6$ copies/ml, and alanine aminotransferase (ALT) levels declined from 1740 U/l to 509 U/l during the 2-week ALS treatment. In spite of intensive medical treatment, the patient contracted acute bacterial meningitis, developed sepsis, and died of multiorgan failure (Fig. 1).

The partial HBV DNA sequence in each of the seven patients involved in the outbreak was compared with that of HBV carriers receiving hemodialysis in the same dialysis unit. Sequence analysis revealed that the HBV sequence was identical between one of the HBV carriers receiving dialysis and each of the seven patients. The full genomic sequence of an HBV isolate from the present case was determined (DDBJ/GenBank/EMBL accession number AB205152) and compared with five full HBV sequences from HBeAg-positive asymptomatic carriers of the same genotype (genotype C) whose viral titer was over $10^8$ copies/ml. A comparative analysis was performed, and the results are summarized in Table 1. HBeAg was detected in four of the seven fulminant cases involved in the outbreak, including the present case, by a commercially available kit, although there was a stop codon mutation in the precore region (nt 1896). Core promoter mutations at nt 1762 and 1764, also implicated in the development of FHF,4 were detected in all cases, including the present case. We tried to confirm the presence of HBeAg in serum by ELISA with a specific monoclonal antibody against the precore region of HBeAg,8 but HBeAg was not detected by this assay.

Discussion

Patients with chronic renal failure who are maintained on hemodialysis are immunologically compromised12 and usually carry mild chronic hepatitis B. Severe hepatitis is rare in Japan, with only a single report of an outbreak of FHB in a dialysis unit in Tokyo.13 In common with the previous outbreak in Tokyo, the causal HBV in the present report had a mutation in the precore region (nt 1896) (Table1). However, in contrast to the Tokyo outbreak, in which all cases were positive...