CASE REPORT

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A YIDD Mutation in a Case of Recurrent Hepatitis B after Liver Transplantation Induced by an S-escape Mutant

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A 47-year-old woman underwent orthotopic liver transplantation (OLT) for hepatitis B virus (HBV)-related end-stage liver cirrhosis. The patient received hepatitis B immunoglobulin prophylaxis after OLT. Despite the protective level of the serum anti-hepatitis-B surface antibody, HBV recurred at 22 months post-OLT and induced subacute hepatic failure. The pre-OLT HBV genome contained a complex mutation pattern in overlapping frame regions of the surface (S) and polymerase (P) genes, which is the same mutation pattern as seen in post-OLT HBV DNA. G145R and K141R mutations in the "a" determinant were detected only in the post-OLT sample. Clevudine (30 mg once daily) was administered for recurrent hepatitis B. Hepatitis B was reactivated with a flare-up, and a M204I mutation (YIDD mutant type) appeared with a higher viral load at 9 months after clevudine treatment. We report here a case of a YIDD mutation that developed in recurrent hepatitis B after OLT induced by an S-escape mutant. **(Gut Liver 2010;4:253-257)**

Key Words: G145R; G1896A; Recurrent hepatitis B; Liver transplantation; rtM204I

INTRODUCTION

Hepatitis B virus (HBV)-induced end-stage liver disease is one of the indications of orthotopic liver transplantation (OLT).[1-3] The use of hepatitis B immune globulin (HBIG) and/or nucleoside analogues has led to a significant decrease in the incidence of recurrent hepatitis B of allografts.[1,2,4-7] However, some recurrent HBV strains with certain mutations in the S gene enable viral persistence in spite of an adequate anti-HBs titer. These S-escape mutants include substitution(s) of single or double amino acid(s) in the "a" determinant that is a major cluster of antigenic epitopes of HBsAg, which results in a conformational change of the immunogenic major hydrophilic loop of small S antigen and decreases the affinity of HBsAg to anti-HBs. Therefore, recurrent hepatitis B develops even in the presence of a sufficient titer of anti-HBs.[8-11] Some of the "a" determinant mutations documented in association with post-OLT recurrence of HBV are as follows: S gene codons sM133T,[12] sD144E,[13] sX144G,[14] sG145K, sG145E, sG145R[9,15] and sG145A.[9,12-15] S-escape mutations occur frequently under selective immune pressure imposed by HBIG after OLT,[15-17] and the duration of HBIG therapy is significantly correlated with the development of mutations in the "a" determinant.[15]

Lamivudine frequently induces substitutions of isoleucine and valine for methionine in the YMDD (tyrosine, methionine, aspartate, aspartate) motif (domain C) of the polymerase protein, resulting in YIDD and YVDD mutations, respectively. Moreover, mutants with changes in the YMDD motif and "a" determinant of the S gene show enhanced replication *in vitro* in the presence of lamivudine as well as induced resistance to lamivudine therapy.[18]

Recently, we experienced a case of recurrent hepatitis B with subacute hepatic failure (SHF) after OLT, which developed even with sufficient titer of anti-HBs achieved by regular administration of HBIG. This case occurred due to

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the presence of a HBV mutant strain that contained mutations in the "a" determinant (K141R and G145R), basal core promoter (BCP) mutations (A1762T and G1764A) and a precore stop codon mutation (G1896A). Clevudine successfully induced not only the resolution of hepatitis B, but also recovery from SHF. However, a YIDD mutant was found with a higher viral load and hepatic failure rapidly redeveloped. We describe here, the clinical course and virological findings of the patient.

CASE REPORT

A 47-year-old Korean woman underwent OLT for HBV-related end-stage liver cirrhosis. The patient was seropositive for HBsAg and HBeAg one month before OLT (294 S/N [AxSYM; Abbott Diagnostics, Abbott Park, IL, USA] and 126 S/CO, respectively), and the serum HBV DNA titer was 1.44×10^7 copies/mL (COBAS Taqman 48; Roche Diagnositcs, Indianapolis, IN, USA). The presence of anti-hepatitis C virus antibody (anti-HCV) was negative. After OLT, level of both HBsAg and HBeAg became negligible and the amount of HBV DNA in serum was below the detection limit for the use of a real-time polymerase chain reaction (<500 copies/mL). The level of serum anti-HBs was measured monthly, and the anti-HBs titer was maintained over 500 mIU/mL by administration of 4,000 to 10,000 mIU/mL of HBIG (Green Cross Pharmaceutical, Yongin, Korea). Lamivudine (Zeffix; GlaxoSmithKline Pharamaceuticals, Brentford, Middlesex, UK), 100 mg once daily, was prescribed; however, the drug was withdrawn 12 months after OLT because of the insurance coverage limit and HBIG was solely administered .

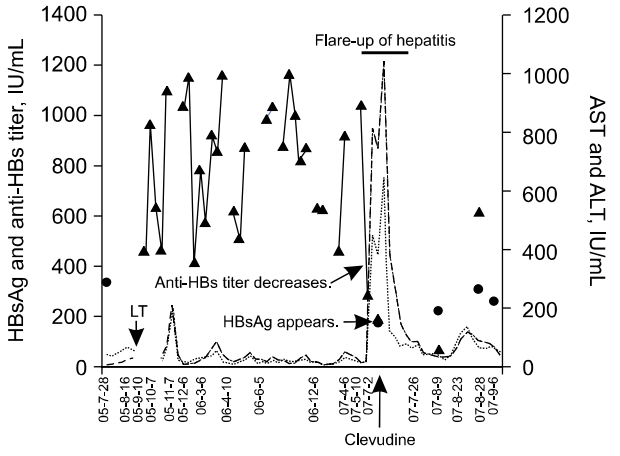

Fig. 1. Summary of the clinical course of the patient. Two lines without any dot markers show the levels of alanine and aspartate aminotransferases (AST and ALT), respectively. Triangle with black lines indicate the serum level of anti-HBs (IU/mL). The circle shows the level of HBsAg. Acute hepatitis-like flare-up developed together with a rapid decrease of the anti-HBs titer and the appearance of HBV with an HBe-minus strain, which was HBeAg-positive prior to liver transplantation.

As shown in Fig. 1 and Fig. 2, the clinical course showed that the patient developed acute hepatitis flare-up with hepatic failure suddenly, presenting with fatigue, dyspepsia and jaundice at 22 months after OLT. The serum alanine aminotransferase (ALT) level increased to 954 IU/L and the level of total bilirubin and the prothrombin time in serum were 1.36 mg/dL and 49.4% (INR 1.48), respectively. HBsAg was detected at a level of 156 S/N and the anti-HBs titer decreased to 165 mIU/mL. The presence of HBeAg was still negative, but the serum HBV DNA titer was 1.33×10^8 copies/mL. Clevudine (Levovir; Bukwang Pharmaceutical, Seoul, Korea), 30 mg once daily, was started immediately, but hepatic failure was aggravated for 10 days; during that period, the peak level of total bilirubin and prothrombin time prolongation in serum were 20.6 mg/dL and 37.8% (INR 1.78), respectively.

To investigate HBV reinfection, DNA sequencing analysis was performed in pre-and post-OLT sera, which were collected one month before and 22 months after OLT, respectively. S gene mutations in and around the "a" determinant were identified by the use of automated DNA sequencing and the presence of mutations in the YMDD motif and precore-core promoter regions were determined by the use of matrix-assisted laser desorption/ionization time-of flight mass spectrometry (MALDI-TOF MS) based restriction fragment mass polymorphism (RFMP) analysis.

The HBV strain at the time of post-OLT hepatitis B flare-up contained double mutations identified in the "a"

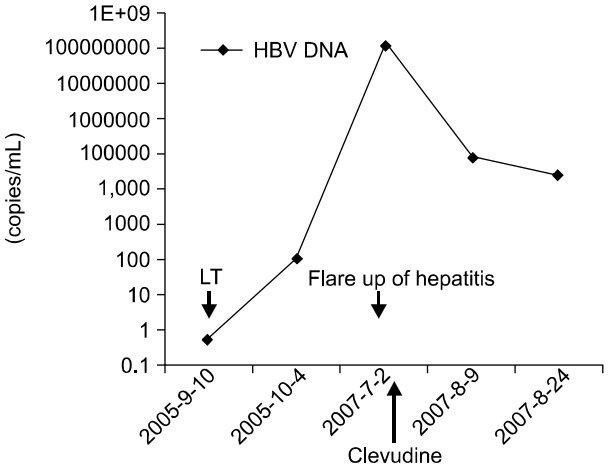

Fig. 2. Serum hepatitis B virus (HBV) DNA titers in the clinical course of the patient.

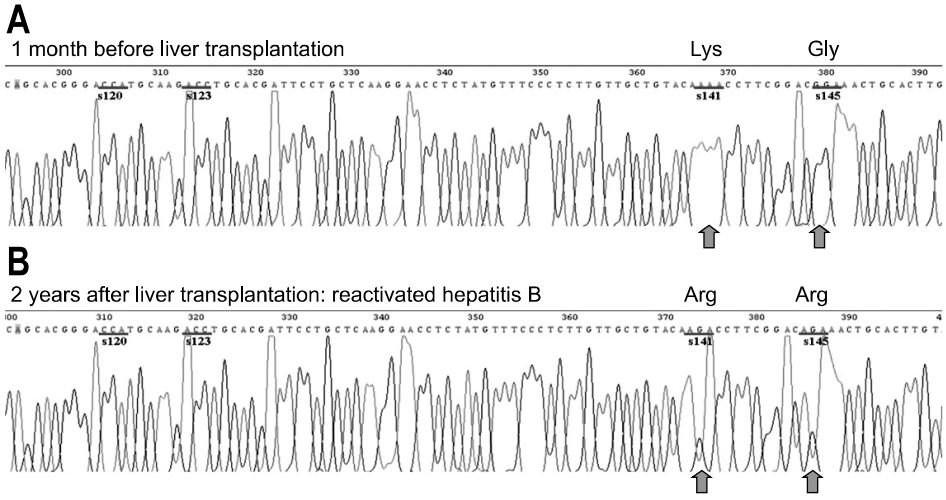

Fig. 3. DNA sequencing analysis reveales two mutations identified in the "a" determinant regions of the S gene. There are mutations in codons 141 (L141a, lys-to-arg) and 145 (G145A, gly-to-arg) in hepatitis B virus (HBV) DNA from serum after post-orthotopic liver transplantation (OLT) flare-up hepatitis B (B) relative to a wild-type sequence in HBV DNA prior to OLT (A).

Table 1. Summary of the Automated DNA Sequencing Analysis for the Overlapping Frame Region of the S and P Genes, and MALDI-TOF MS RFMP Analysis Used to Detect the BCP (A1762T and G1764A), Precore Stop Codon (G 1896A), and YMDD (rtM204I/V) Mutations

Mutation sites	Samples	
	Dated on 2005.06.29	Dated on 2007.07.04
sP120T	Pro(CCA)	Pro(CCA)
sT123N	Thr(ACC)	Thr(ACC)
sK141N	Lys(AAA)	Arg(AGA)
sG145R	Gly(GGA)	Arg(AGA)
sV177A	Ala(GCG):Val(GTG)=2:1	Val(GTG)
sV180A	Val(GTT):Ala(GCT)=5:1	Ala(GCT)
sW182Stop	Trp(TGG):Stop(TGA)=5:1	Trp(TGG)
sS204R	Arg(AGA):Ser(AGT)=2:1	Arg(AGA)
sI213L	Leu(TTA):Ile(ATA)=3:1	Leu(TTA)
rtM2041/V	M	M
BCP, A1762T	T	T
BCP, G1764A	A	A
Precore, G1896A	G:A(3:1)	A

MALDI-TOF MS, matrix-assisted laser desorption/ionization time-of flight mass spectrometry; RFMP, restriction fragment mass polymorphism; BCP, basal core promoter.

determinant of the S gene, codons K141R and G145R, indicating S-escape mutations, while no mutations were found in the "a" determinant before OLT (Fig. 3). According to the RFMP analysis for pre-core mutations (G1896A), the patient was co-infected with wild (G1896) and mutant (A1896) strains before OLT, but the only mutant type viral strain was detected during post-OLT hepatitis. In addition, this HBe-minus variant carried BCP dual mutations (A1762T and G1764A), simultaneously (Table 1). Although there was no mutation in the YMDD motif in HBV DNA from pre-OLT and post-OLT sera, a mixture of wild and mutant strains was detected in overlapping frame regions of the S and P genes in HBV DNA from pre-OLT serum, including codons 177, 180, 204, and 213, based on the use of automated sequencing analysis. However, the only mutant strain having codons A177V, V180R, S204R, and I213L was identified in HBV DNA from post-OLT serum (Table 1).

Liver function recovered gradually revealing a serum total bilirubin of 0.76 mg/dL and prothrombin time of 79.6% (INR 1.13) at five months after clevudine therapy, and serum HBV DNA titers at one and two months after clevudine therapy decreased to 7.83×10^4 and 2.22×10^4 copies/mL, respectively (Fig. 2). Six months after clevudine therapy, HBV DNA was not detectable by real-time PCR. However, a M204I mutation (YIDD mutant type) appeared in serum with a higher viral load nine months after clevudine therapy, thereby leading to hepatitis flare-up. Serum HBV DNA titer was 4.78×10^7 copies/mL, the level of total bilirubin and prothrombin time in serum were 5.29 mg/dL and 23.7% (INR 2.58), respectively. The use of clevudine was switched to 1mg once daily entecavir (Baraclude; Bristol-Myers Squibb Pharmaceutical, Seoul, Korea). However, the patient rapidly deteriorated over one month and subsequently died of hepatic failure.

DISCUSSION

In this case, we showed that an HBV S-escape mutant with dual mutations, K141R and G145R in the "a" determinant, had an etiologic role for recurrent hepatitis B leading to SHF long after OLT. As the presence of HBeAg was positive prior to OLT, we supposed at first that the re-infected HBV acquired both S-escape and HBe-minus mutations simultaneously. However, RFMP analysis for

precore and BCP complex mutations clearly showed that the recurrent HBV originated from a mutant HBV strain with a precore mutation (G1896A) and BCP mutations (A1762T and G1764), which pre-existed in a mixture of wild and mutant types before OLT.

Additional changes at the overlapping frame region of the P gene corresponding to the S gene were also found homogeneously in HBV DNA from post-OLT serum as follows: rtV191, rtT213 and rtF221. However, heterogeneous mixtures of rtV191 and rt191I, rtS213 and rtT213 and rtF221 and rtY221 were found in HBV DNA from pre-OLT serum, together with the same precore stop codon and BCP mutations as found in post-OLT serum. These findings suggest that the post-OLT HBV S-escape mutant derived from a pre-OLT precore mutant rather than from the co-existing wild type.

Most patients who have undergone OLT for HBV-related liver disease are re-infected with the same virus population that existed prior to OLT. In rare cases, new mutants emerge after OLT or pre-existing mutants are lost.[8] Patients with a more advanced stage of liver disease before OLT have an accumulation of HBV S-gene mutations, resulting in a heterogeneous viral population, and the average number of amino acid changes is higher in anti-HBe-positive patients as compared to HBeAg-positive patients.[19] Changes in the HBV population occur during the observation period for recurrent hepatitis B in OLT recipients with frequent accumulation of precore stop codon mutations.[20]

HBV reinfection with escape variants causes graft failure in 44% of cases.[14] The type of viral population does not determine the severity of hepatitis B in the graft;[20] therefore, even milder precore mutants disappear and wild type HBV becomes the predominant virus strain in the majority of patients.[8] However, in a small group of patients, post-OLT hepatitis B accompanies a desperate clinical outcome, so-called "fibrosing cholestatic hepatitis (FCH)" in which a peculiar variant of HBV infection in immunocompromised patients is characterized by rapid viral replication.[21,22] FCH can be caused by an HBe-minus variant as well as the wild type.[23-25] On literature review there has been no reported data about the rate of lamivudine-resistant escape mutants (YIDD and YVDD mutant) in post-OLT HBV reinfections.

In summary, this case shows that a YIDD mutation occurred in HBV S-escape strain with double mutations in the "a" determinant that emerged following OLT. The acquisition of the S-escape mutant derived from a pre-existing HBe-minus mutant that was numerically weaker than the co-infected wild type.

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