Predominance of Pre-S1 Mutated Hepatitis B Virus in a Patient Following Treatment With Adefovir Dipivoxil

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A liver transplant recipient reinfected with a lamivudine-resistant mixed wild-type/pre-S1–deleted hepatitis B virus (HBV) strain and rescued with adefovir dipivoxil was still HBV DNA positive after more than 1 year of therapy. Analysis of serum HBV DNA, amplified by polymerase chain reaction and directly sequenced by dideoxy nucleotide chain-termination method, showed that adefovir inhibited the wild type, but not the pre-S1–deleted HBV. Predominance of the pre-S1–deleted strain over wild type after adefovir treatment suggests that either adefovir inhibited the wild type more effectively or the pre-S1 mutant replicates more efficiently. The normality of liver condition confirms that to exert its pathogenic effect, the pre-S1–deleted strain requires the presence of wild-type virus. (*Liver Transpl 2003;9:188-190.*)

Adefovir dipivoxil is a nucleotide phosphonate analogue that has strong activity against hepatitis B virus (HBV) infection and represents an effective alternative to lamivudine when drug-induced YMDD mutations occur. Inhibition of HBV replication by adefovir dipivoxil is usually rapid, in the range of a 4 log10 reduction in HBV DNA after 12 weeks of treatment.[1] To date, no adefovir-resistant mutation has been reported. We report events associated with its use in a liver transplant recipient with recurrent lamivudine-resistant chronic hepatitis B.

The patient, an anesthesiologist, experienced several needle-stick exposures during his professional lifetime. HBV infection was diagnosed in 1986. In 1990, liver biopsy showed chronic hepatitis with moderate activity. At that time, alanine aminotransferase (ALT) levels constantly ranged between 4 and 6 times greater than the upper limit of normal (ULN). HBV DNA was positive by dot blot hybridization. The patient was started on recombinant interferon (IFN), 9 million units thrice weekly. He initially responded, but after 3 months, he experienced a sharp hepatitic flare. ALT levels increased to 15 times greater than the ULN. These levels were maintained for more than 2 months when IFN therapy was discontinued.

Retrospective sequence analysis, after cloning of the polymerase chain reaction (PCR) products of HBV DNA, showed pure wild-type virus before starting IFN therapy. At the time of hepatitic flare, 6 months after beginning IFN therapy, a mutant HBV population appeared. This mutant population first bore a 42-bp deletion, then a double of 42 and 81 nucleotides in the pre-S1 region, which became predominant and constant. Associated with the deletions, a C→T mutation at the second codon after the ATG of the pre-S2 region occurred, which gave rise to a stop codon preventing pre-S2 transcription and increasing intermediate HBV replicative forms.[2,3] The 123-bp deletion of the pre-S1 region, being multiple of three nucleotides, did not alter the open reading frame of the polymerase region that bore, as single alterations, a YVDD mutation in the samples after lamivudine treatment (Fig. 1).

The mutant population gradually became predominant in comparison to the wild-type one, which nevertheless always was present.

The clinical course was characterized by rapidly progressive liver failure requiring liver transplantation (LT) in 1996. At the time of LT, HBV DNA was positive by PCR (3,430 copies/mL) and weakly by dot blot, with a mixture of wild-type and mutated strain (Fig. 2).

One year after LT, HBV infection recurred despite weekly administration of 5,000 IU of specific antibody to hepatitis B surface antigen (anti-HBs) immunoglobulin intravenously (a dose necessary to keep the anti-HBs titer $>$ 200 IU/L).[3] Serum HBV DNA levels increased to greater than 23,000 copies/mL (Cobas Amplicor; Roche, Milan, Italy); sequence analysis showed pure wild-type HBV sequence. Serum ALT levels increased to more than 20 times the ULN (35 IU/L). Lamivudine (100 mg/d) was able to control recurrence for 8 months. However, M552V mutation

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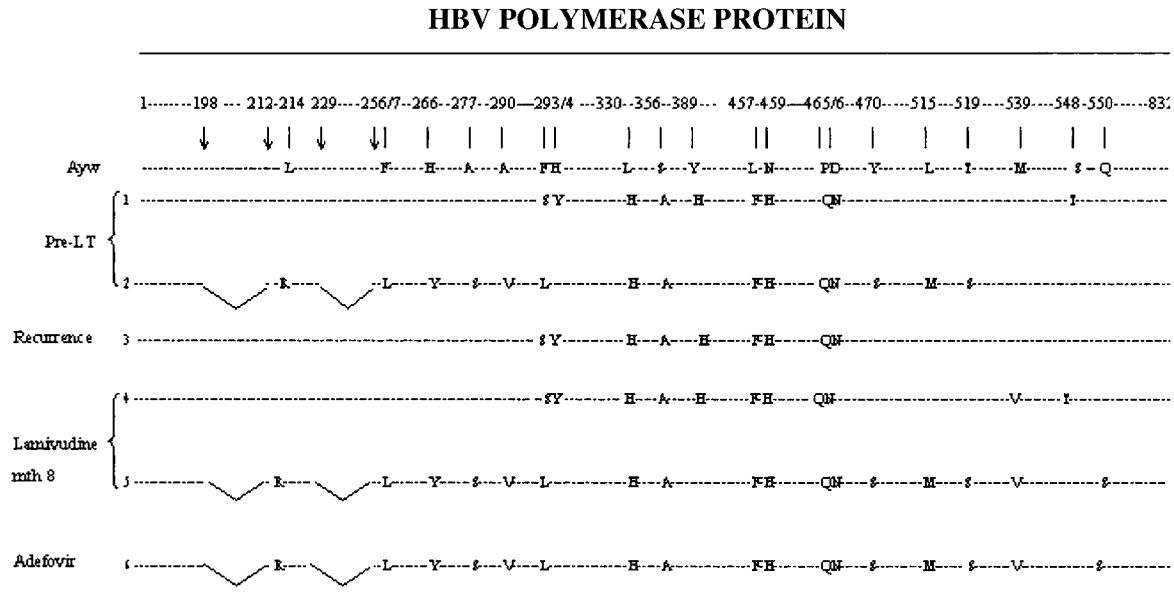

Figure 1. Sequence analysis of HBV polymerase protein before LT, after recurrence, and after lamivudine and adefovir treatment. Arrows indicate the length of the deleted portions of polymerase protein in the pre-S1–deleted strain, shown in sequences as appropriate.

occurred, and HBV DNA (a mixture of wild-type and pre-S1–deleted HBV in a 1:1 proportion) levels became elevated again (31,000 copies/mL). ALT levels increased to more than 15 times the ULN, and prothrombin time rapidly increased to 22 seconds. The patient's clinical condition gradually worsened, and slight ascites developed.

The patient was started on adefovir dipivoxil, 10 mg/d, in May 2000. Before starting adefovir dipivoxil, the patient's serum HBV DNA level reached 37,844 copies/mL. Subsequent serum HBV DNA levels gradually decreased during the next 3 months (10,397 copies/mL at month 3), and his clinical condition improved: ALT levels and prothrombin times gradually returned to normal values.[4] Serum HBV DNA levels steadily decreased during the next 6 months to 1,529

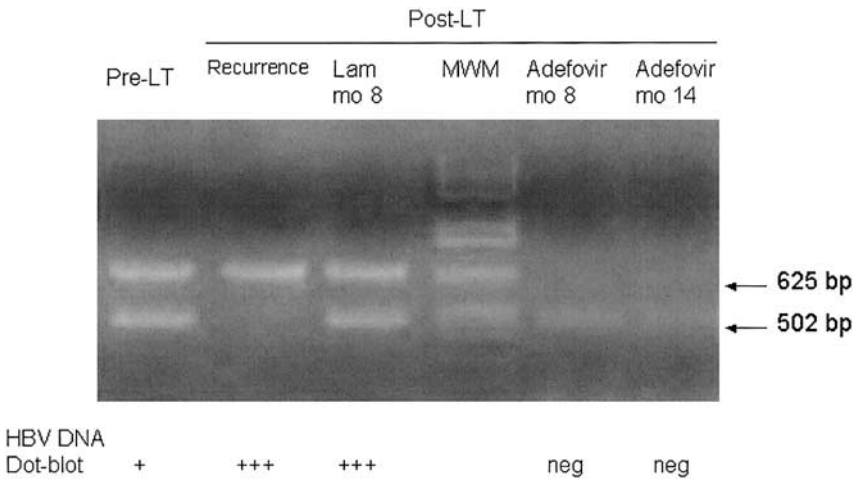

Figure 2. Relative proportion of wild-type (625-bp) and pre-S1–deleted (502-bp) sequences during the course of HBV infection before LT and at recurrence after LT. HBV DNA evaluated semiquantitatively by dot blot was always negative after starting adefovir dipivoxil therapy. Absence of wild-type virus in the samples after starting adefovir therapy also was confirmed by hybridization with ^{32}P-CTP HBV DNA and dideoxy nucleotide chain-termination method of cloned PCR products. (Lam, lamivudine; MWM, molecular weight markers; CTP, cytidine triphosphate).

copies/mL; however, after more than 1 year on adefovir dipivoxil therapy, serum HBV DNA levels still fluctuate between 600 and 1,000 copies/mL, sometimes below the sensitivity limit of the method.

Sequence analysis by the dideoxy nucleotide chain-termination method of PCR products at all times after beginning adefovir dipivoxil therapy showed that residual HBV DNA was constituted by pure pre-S1–deleted strain, whereas wild-type virus had been completely inhibited since the first months of adefovir dipivoxil therapy (Fig. 2).

Predominance of the pre-S1–deleted strain over wild type after adefovir dipivoxil treatment suggests that either adefovir dipivoxil inhibited wild-type virus more effectively or the pre-S1 mutant replicates more efficiently. Certainly, the level of pre-S1–deleted strain after adefovir dipivoxil therapy was decreased in comparison to those measured before and could have contributed to the improvement in clinical situation. However, this case report strongly suggests that to exert its pathogenic effect, the pre-S1–deleted HBV strain requires the presence of wild-type virus.[2] In both the pretransplantation and posttransplantation recurrence periods, the contemporary presence of both strains was associated with progressive liver failure, whereas the presence of the pure deleted mutant strain was compatible with normal clinical situation.

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