Reactivation of Hepatitis B Virus Replication Accompanied by Acute Hepatitis in Patients Receiving Highly Active Antiretroviral Therapy

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We describe 2 patients who were initially positive for antibodies to hepatitis B surface antigen and who experienced a strong and sudden increase of hepatitis B virus (HBV) replication during highly active antiretroviral therapy (HAART). We found that reactivation of HBV replication during HAART can occur independently of lamivudine resistance or withdrawal of lamivudine, and in spite of increasing CD4$^+$ cell counts.

Hepatitis B reactivation in patients infected with HIV who were positive for hepatitis B surface antigen (HBsAg) has been reported previously [1]. Even patients who had fully recovered from hepatitis B virus (HBV) infection and developed antibodies to HBsAg may experience recurrence of HBsAg and hepatitis during HIV coinfection [2]. In a number of patients receiving highly active antiretroviral therapy (HAART) for HIV infection, HBV-associated episodes of severe hepatitis have been reported and were explained by HAART-induced immune reconstitution [3–7]. In some patients who were taking lamivudine as part of the antiretroviral treatment, appearance of lamivudine-resistant HBV was observed and may have contributed to the development of hepatitis [8]. A sharp increase in the level of serum HBV DNA during HAART was docu-

mented in only 1 patient, who had been positive for hepatitis B surface antibody (anti-HBs) for a long time, and this increase was attributed to the withdrawal of lamivudine [9].

Within the last 2 years, we observed 5 patients who received HAART and experienced reactivation of hepatitis B. The present study reports on 2 of these patients; they were initially anti-HBs positive. The sudden reactivation of HBV replication and hepatitis during HAART can neither be explained by withdrawal of lamivudine therapy nor by development of resistance to nucleoside analogues.

HBV DNA in serum was quantified by an in-house dot blot hybridization assay (lower detection limit, 0.7 pg HBV DNA, corresponding to $\sim 2 \times 10^5$ molecules per mL of serum). For PCR, HBV DNA from 100 μL of serum was purified by proteinase K digestion and phenol-chloroform extraction, and full-length 3.2-kb HBV genomes were amplified as described previously [10] with primers P1 and P2 (P1, HBV nucleotides 1821–1841, *CCGGAAAGCTTGAGCTCTTC*TTTTTCACCTC-TGCCTAATCA; P2, 1823–1806, *CCGGAAAGCTTGAGCTC-TTC*AAAAAGTTGCATGGTGCTGG; heterologous sequences to facilitate cloning in italics) and the Expand-High-Fidelity system (Roche). The detection limit of this PCR is about 10^3 molecules per mL of serum [11]. The amplified genomes were digested with *Sst*I or *Hin*dIII within the heterologous primer sequences and cloned into vector pUC19. Regions encoding the catalytic YMDD locus and the upstream domain B of the HBV polymerase as well as the major antigenic domain of HBsAg (a-determinant) were sequenced on several cloned HBV genomes from each patient and were compared with corresponding wild-type sequences.

Patient A was a 65-year-old HIV-infected man from Germany who had been positive for anti-HBs and antibodies to HBV core antigen (anti-HBc) and HBV DNA negative by PCR assay since 1983 (figure 1A). Fourteen years after diagnosis of HIV, his CD4$^+$ cell count was 90 cells/μL, and he presented with *Candida* esophagitis. Therapy with zidovudine (500 mg/d), zalcitabine (2.25 mg/d), and indinavir (2400 mg/d) was started. Four months later, HBsAg was detected for the first time, but HBV DNA remained still undetectable by PCR ($<10^3$ molecules/mL). Because the HIV load had decreased only by 1 order of magnitude, HAART was initiated with didanosine (400 mg/d), stavudine (80 mg/d), and indinavir (2400 mg/d).

Three months later, CD4$^+$ lymphocytes had risen to 170 cells/μL and HIV RNA had fallen below the detection limit of the assay (<400 molecules/mL). Didanosine was replaced by la-

Figure 1. Clinical and virological course of patients A *(A)* and B *(B)*. *Top,* parameters of HIV infection (CD4$^+$ cell count and HIV RNA level as determined by HIV Amplicor Monitor; periods of treatment with lamivudine (3TC), antiretroviral therapy (ART), or highly active antiretroviral therapy (HAART) are indicated by bars above the plots. *Bottom,* parameters of hepatitis B virus (HBV) infection, including alanine aminotransferase (ALT) and HBV DNA level as determined by dot blot assay. The results of serological and PCR testing are shown above the plots. Values below the limit of detection are plotted at the limit, with *arrowheads* indicating that the values were lower than shown.

mivudine (300 mg/d) at the patient's request. At the same time, strong HBV replication was retrospectively evident from serum HBV DNA level of 175 pg/mL (5×10^7 molecules/mL), but clinically detectable hepatitis was still absent. Another 4 weeks later, the patient was admitted to the clinic with jaundice and elevated alanine aminotransferase levels. Antiretroviral treatment was interrupted because we suspected drug-induced liver toxicity. Anti-HBc IgM was positive, whereas antibodies to hepatitis C virus (HCV), antibodies to hepatitis D virus, HCV RNA, and hepatitis G virus RNA were negative. After 8 weeks, liver enzymes had normalized and treatment began again. HBV DNA levels dropped rapidly to undetectable levels, and anti-HBs antibodies reappeared 15 months after HBV reactivation.

Patient B, a 35-year-old man from Zaire, was found to be positive for HIV and anti-HBs and anti-HBc antibodies, and negative for HBV DNA by PCR (figure 1*B*) in 1996. HIV therapy was started in 1997 with zidovudine (500 mg/d) and lamivudine (300 mg/d) when CD4$^+$ cell levels had dropped to 40 cells/μL. At this time, HBsAg was positive and, retrospectively, HBV DNA was detectable by PCR but not by hybridi-

zation assay (10^3–10^5 molecules/mL). Because the patient did not respond to treatment, HAART was started in March 1998 with stavudine (80 mg/d), didanosine (400 mg/d), ritonavir (800 mg/d), and saquinavir (800 mg/d). HIV RNA decreased to undetectable levels (<400 molecules/mL) within 1 month.

HIV suppression remained stable for 6 months and was accompanied by an increase of CD4$^+$ lymphocytes to 231 cells/μL; at 6 months, the patient developed acute hepatitis and treatment was interrupted. The HBV DNA level was 6 pg/mL (2×10^6 molecules/mL). Anti-HBc IgM was positive, whereas antibodies to HCV, antibodies to hepatitis D virus, and HCV RNA were negative. Liver enzymes returned to normal within 7 weeks, and HIV treatment was resumed. HBV DNA levels dropped rapidly below the detection limit of the hybridization assay. Anti-HBs antibodies reappeared 19 months after reactivation, and HBV DNA became undetectable by PCR.

We describe 2 patients coinfected with HBV and HIV who had fully recovered from HBV infection, as indicated by anti-HBs and anti-HBc serostatus and absence of liver disease. Both reseroconverted to HBsAg and experienced a strong and sudden

Reverse transcriptase

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                    domain B                        domain C
           |_____|       |_____|
           510      520       530      540      550      560      570
           *        *         *        *        *        *        *
A          WKLHLYSHPIVLGFRKIPMGVGLSPFLLAQFTSAICSVVRRAFPHCLAFSYMDDVVLGAKSVQHRESLYT
10/97 1-6  ...................................................................

B          RKLHLYSHPIIMGFRKIPMGVGLSPFLLAQFTSAICSVVRRAFPHCLAFSYMDDVVLGAKSVQHLESLYT
6/97  1-8  ...................................................................
7/98  1-6  ...................................................................
10/98 1-6  ...................................................................
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HBsAg a-determinant

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                                    major epitope
                             |_____|
           100      110      120      130      140      150      160      170      180
           *        *        *        *        *        *        *        *        *
A          LDYQGMLPVCPLIPGSTTTSTGPCKTCTTPAQGNSMFPSCCCTKPTDGNCTCIPIPSSWAFAKYLWEWASVRFSWLSLLVPFVQWF
10/97  1   .......A...............................................................L.................
       2   .......A...............................................................L.................
       3   ..S.S..................................................................L.................
       4   ..S.S..................................................................L.................
       5   ..S.N..................................................................L.................
       6   ..S.N..................................................................L.................

B          LDYQGMLPVCPLIPGSSTTSTGPCRTCTTLAQGTSMFPSCCCSKPSDGNCTCIPIPSSWAFGKFLWEWASARFSWLSLLVPFVQWF
6/97   1   ...H.............P...................................................................
       2   ...H.............P...................................................................
       3   ...H.............P...................................................................
       4   ...H.............P...................................................................
       5   ...H.............P...................................................................
       6   ...H.............P...................................................................
       7   ...H.............P...................................................................
       8   ...H.............P...............................................................L.....

7/98   1   ...H.............P.......P...............A...........................................
       2   ...H.............P.......P...............A...........................................
       3   ...H.............P...................................................................R..
       4   ...H.............P...................................................................R..
       5   ...H..F..:.......P...................................................................R..
       6   ...H.............P...................................................................

10/98  1   ...H.............P.......P...............A...........................................
       2   ...H.............P.......P...............A...........................................
       3   ...H.............P.......P...............A...........................................
       4   ...H.............P.......P...............A...........................P.........
       5   ...H.............P...................................................................R..
       6   ...H.............P...................................................................
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Figure 2. Amino acid sequences of hepatitis B virus (HBV) polymerase domains B and C (YMDD motif) as well as of hepatitis B surface antigen (HBsAg) a-determinant. A total of 6 to 8 clones per HBV isolate (isolate 10/97 from patient A and isolates 6/97, 7/98, and 10/98 from patient B) were sequenced. The sequences of the patient's isolates were compared with wild-type HBV sequences of the corresponding genotype: that of patient A with a genotype A–specific wild-type sequence (HBVADW2), and those of patient B with a genotype E–specific wild-type sequence (HHVBBAS).

reactivation of HBV replication during HAART (figure 1). In patient A, the HBV DNA level increased by at least 4 orders of magnitude within 3 months after initiation of HAART. In patient B, HBV DNA increased by at least 2 orders of magnitude between the fourth and seventh month of HAART. The peak of hepatitis B viremia occurred in both patients when the HIV load had fallen below the detection limit and CD4^{+} cell counts were increasing (figure 1). This is surprising because there is strong evidence that HBV infection is mainly controlled by cellular immunity [12], and therefore one would have expected a decrease rather than an increase of HBV replication.

A similar clinical course was recently described and was attributed to the withdrawal of lamivudine from HIV treatment a few weeks before HBV reactivation [9]. In patient A, withdrawal of lamivudine cannot account for the increase of HBV replication because treatment with this drug was initiated at the time of reactivation, not before. In patient B, we also consider withdrawal of lamivudine an unlikely reason for HBV

reactivation because the events occurred 7 months apart. Although the absence of lamivudine in the HAART regimen during HBV reactivation argues against the emergence of lamivudine-resistant strains, this possibility was excluded by the results of sequencing that part of the HBV polymerase in which resistance mutations occur.

Resistance to lamivudine is invariably associated with M552→I or M552→V mutations in the catalytic YMDD locus; the latter mutation is usually accompanied by a L528→M mutation in the upstream domain B [13–18]. As expected, in the patients in our study, no mutations were found in these regions when compared with wild-type HBV sequences of the corresponding genotype (figure 2, reverse transcriptase sequences). This finding also largely excludes the purely speculative possibility that any other nucleotide analogue included in the therapy of the patients had an adverse inhibitory effect on HBV and has led to selection and breakthrough of HBV with resistance mutations in the catalytic domain.

Because both patients were initially anti-HBs positive, the appearance of HBsAg and HBV DNA may be at least partially because of selection and accumulation of immune escape mutants with amino acid changes in the main HBsAg B cell epitope (positions 121–147), as has been described after passive and active immunization with anti-HBs and HBsAg, respectively [19–21]. In patient A, this domain was completely conserved; however, several mutations were observed in upstream and downstream regions that also form epitopes [22] (figure 2, HBsAg sequences A). The relevance of mutations in these regions is still unknown.

In patient B, 2 mutations (L127→P and G145→A) occurred within the major epitope that predictably changes HBs antigenicity [19] (figure 2, HBsAg sequences B 7/98 and 10/98). Additional mutations were found in upstream and downstream regions. Both mutations in the major epitope as well as a Q181→R mutation were observed shortly before and at the time of reactivation, but not 13 months earlier (figure 2, HBsAg sequences B 6/97), indicating that corresponding mutants were selected during this period of time. Their emergence may have contributed to HBV reactivation.

Altogether, in contrast to previous interpretations [8, 9], withdrawal of lamivudine as well as development of resistance to nucleoside analogues are either excluded or highly unlikely to be responsible for the strong and sudden increase of HBV replication in the patients in our study. However, escape from anti-HBs may be involved. Alternatively (or in addition), it is conceivable that HAART exerts a positive effect on the HBV replication level in vivo, as suggested by the obvious temporal connection between initiation of HAART and HBV reactivation.

Further observations support this speculation. For example, serum levels of HCV RNA were also found to increase in patients coinfected with HIV and HCV who were receiving HAART [23]. A possible mechanism that may underlie these effects was recently demonstrated in vitro and in mice infected with lymphocytic choriomeningitis virus (LCMV). The protease inhibitor ritonavir was shown to inhibit proteasome activity and antigen presentation and thus to reduce the cytotoxic T-lymphocyte response against LCMV [24]. In analogy to the observations in patients infected with HBV or HCV who were receiving HAART therapy, administration of ritonavir to mice infected with LCMV eventually increased the virus titer.

In the patients in our study, the episode of acute hepatitis was preceded or accompanied by the peak of viremia. This suggests that enhanced HBV replication was the main reason for hepatic inflammation. HAART-induced reconstitution of specific cellular immune functions could have an additional effect. Triggering the HBV-specific immune response may even lead to full recovery in patients with a very low number of CD4+ cells, as indicated by reseroconversion to anti-HBs and clearance of HBV DNA in both patients and in 3 previously reported patients [4, 7, 9]. Also, reactivation of other viral diseases in patients receiving HAART (hepatitis C virus [23] and cytomegalovirus [25]) may not exclusively be explained by immunological recovery but may be interpreted as a complex phenomenon involving reactivation of both the pathogen and the immune response.

In conclusion, reactivation of HBV and hepatitis appears to be an important complication of HAART, and further study is warranted, as are prospective studies. HBsAg-positive and anti-HBs–positive patients should be monitored for HBV reactivation after initiation of HAART, and treatment with lamivudine or adefovir may be considered.

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