Cell-Mediated Immune Responses and Loss of Hepatitis B e-Antigen (HBeAg) during Successful Lamivudine and Famciclovir Combination Therapy for Chronic Replicating Hepatitis B Virus Infection

Nucleoside analogues have been used as monotherapy for chronic hepatitis B virus (HBV) infection, and in most cases a rapid drop is seen in the viral load shortly after the start of therapy, to a >90% drop after 4–8 weeks [1]. Once therapy is stopped, relapses are common, and during prolonged treatment periods the emergence of resistance is common [2]. Recently it was shown that the combination of lamivudine and famciclovir had a synergistic inhibitory effect in vitro in the duck hepatitis virus model [3].

There have been conflicting reports on the activation of endogenous HBV-specific cellular immune responses during nucleoside analogue monotherapy [4, 5], in contrast to those detected during α-IFN therapy or spontaneous seroconversion from positivity for hepatitis B early antigen (HBeAg) to positivity for antibodies to HBeAg (anti-HBe) [6, 7]. Since it is generally believed that these responses are of importance in resolving HBV infections, the absence of an activated endogenous cellular immune response during nucleoside monotherapy is consistent with low HBeAg–to–anti-HBe seroconversion rates [1]. To benefit from a potential synergistic effect and to delay or inhibit the evolution of resistance to lamivudine and famciclovir, combination therapy can be used.

A 76-year-old man with chronic obstructive pulmonary disease and posttransfusion chronic HBV infection with compensated cirrhosis in a nonreplicative stage after seroconversion from HBeAg- to anti-HBe–positivity in February 1997 was found to have reactivated infection in October 1997. During the reactivation, transaminase concentrations increased, HBeAg reappeared, serum HBV DNA levels increased, and the patient became less well clinically. Treatment with famciclovir (500 mg twice weekly) and lamivudine (150 mg daily) was started on 3 December 1997 and continued until 17 May 1999.

Serum levels of alanine aminotransferase (ALT), bilirubin, creatinine, and hemoglobin, as well as the WBC count and differential counts, were closely monitored. Levels of HBV DNA (tested by Amplicor; Roche Laboratories, Nutley, NJ), hepatitis B surface antigen (HBsAg), HBeAg, and anti-HBe were determined by standard assays (Abbott Laboratories, North Chicago, IL). The proliferative and cytokine responses to hepatitis B core antigen (HBcAg) and HBeAg in peripheral blood mononuclear cells (PBMCs) were monitored by in vitro recall assays, as described elsewhere [8]. Serum levels of apolipoprotein 1/Fas (APO-1/Fas) and IL-12 were determined with use of commercial kits (Zymed Laboratories, San Francisco, and Biosource, Camarillo, CA).

During treatment the HBV DNA level decreased from 1.7×10^7 copies/mL to 1.7×10^5 copies/mL (99%) within the first week and reached the levels noted before the reactivation (3.0×10^3 copies/mL) within 16 weeks. Within 6 weeks ALT levels started to decline, and they normalized within 23 weeks (figure 1). The start of the stable decrease in ALT levels coincided with a transient cellular HBcAg- and HBeAg-specific immune response in PBMCs, evidenced by proliferation, peaking at week 6, and a profound increase in γ-IFN and IL-12 levels, peaking at weeks 4–8 (figure 1).

HBcAg- and HBeAg-specific IL-2 levels were undetectable, the IL-4 levels ranged from 0 pg/mL to 6 pg/mL, and IL-10 levels fluctuated throughout the study (figure 1). The proliferative and cytokine responses in PBMCs returned to pretreatment levels within 19 weeks from the start of therapy, simultaneously with seroconversion from HBeAg- to anti-HBe–positivity. Serum levels of APO-1/Fas and IL-12 showed a minor increase at weeks 4 and 3, respectively (figure 1), possibly reflecting active killing of infected liver cells.

Only a few reports on combination therapy for HBV infection are available. Famciclovir and lamivudine combination therapy was highly effective, since within 1 week the viral load had decreased by 99%. This is comparable or superior to the effectiveness of lamivudine monotherapy [9]. The regimen was well tolerated and caused no notable adverse effects, and the patient's condition improved. Because of the fear of reactivation and the advanced stage of the patient's cirrhosis, treatment was continued for 17 months.

At that time a biopsy showed dramatic improvement of the necroinflammatory and fibrotic changes in the liver; hence, treatment was stopped. During therapy an early, transient, T helper 1–like cell-mediated immune response preceded the seroconversion from HBeAg to anti-HBe positivity, paralleling similar observations during spontaneous and αIFN-induced seroconversion [6]. An interesting observation was that these PBMC responses coincided with important biochemical and serological events.

Thus the start of the stable decrease in ALT levels preceded the peak PBMC proliferative response by 1 week. This implies that the antiviral T-cell population present in the liver may transiently exceed the number of productively infected hepatocytes as the viral load drops. Subsequently, the transiently detectable PBMC responses may reflect either a redistribution or a leakage of antiviral T cells from the liver, which would be consistent with the view that γ-IFN and IL-12 may potentially participate in the liver disease and clearance of the infection [10].

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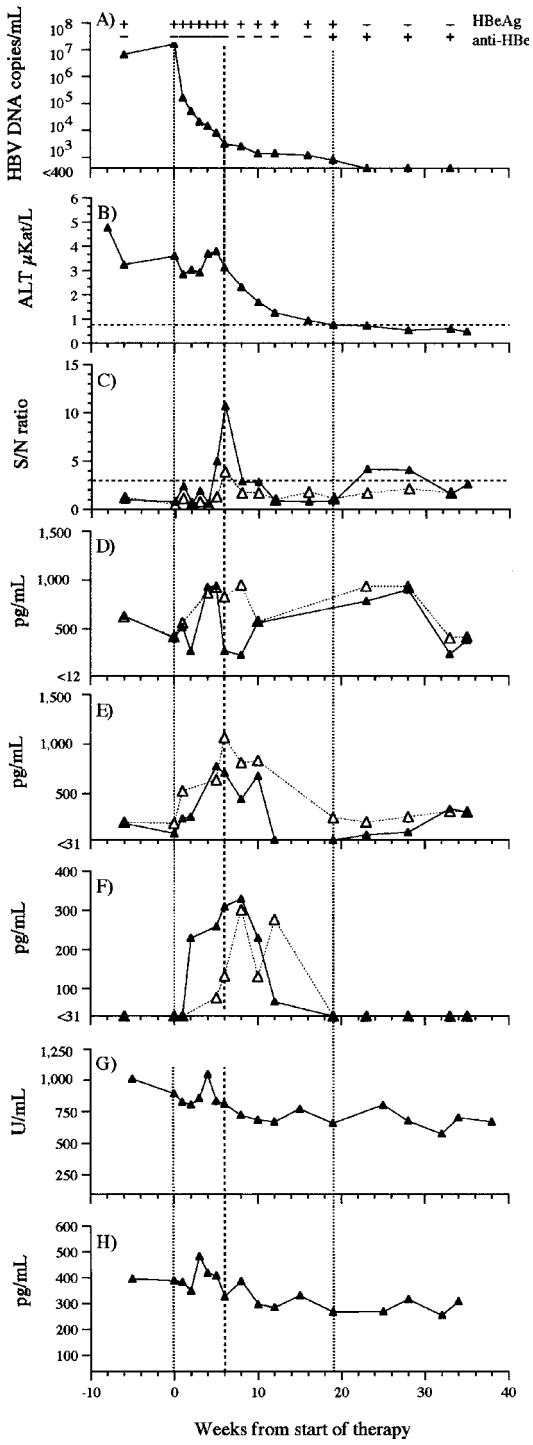

Figure 1. Data from virological, immunologic, and biochemical analyses during lamivudine and famciclovir combination therapy for a patient with reactivated chronic hepatitis B virus (HBV) infection. *A,* Serum status (+ or −) for hepatitis B early antigen (HBeAg) and antibody to HBeAg (anti-HBe) and HBV DNA levels (▲). *B,* Serum alanine aminotransferase (ALT) levels (μkat/L). *C,* Proliferative responses, as sample-to-negative (S/N) ratios, in peripheral blood mononuclear cells (PBMCs) to hepatitis B core antigen (HBcAg; ▲) and HBeAg (△). *D,* HBcAg-specific (▲) and HBeAg-specific (△) IL-10 production in PBMCs. *E,* HBcAg-specific (▲) and HBeAg-specific (△) IL-12 production in PBMCs. *F,* HBcAg-specific (▲) and HBeAg-specific (△) γIFN production in PBMCs. *G,* Serum levels of apolipoprotein 1/Fas. *H,* Serum levels of IL-12. Right and left vertical lines indicate start of therapy at week 0 and HBeAg–to–anti-HBe seroconversion at week 19, respectively. Middle vertical line indicates start of normalization of ALT levels and peak of PBMC proliferation.

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