Reconstitution of Hepatitis B Virus (HBV)–Specific T Cell Responses with Treatment of Human Immunodeficiency Virus/ HBV Coinfection

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Liver-related mortality is an increasing problem in human immunodeficiency virus (HIV)/hepatitis B virus (HBV)–coinfected patients receiving highly active antiretroviral therapy (HAART). In HIV-negative patients, HBV chronicity is associated with a reduction in specific T cell responses that can be partially restored by treatment with lamivudine. We studied 5 HIV/HBV-coinfected patients treated with HAART, either with or without addition of a drug with specific anti-HBV activity. Our data show that reconstitution of some HBV-specific T cell responses can also occur in HIV-positive patients after a reduction in HBV load. This potential to recover T cell responses, which has been thought to be critical for HBV control, provides support for the addition of anti-HBV therapy in the treatment of HIV/HBV-coinfected patients.

A large proportion of patients infected with human immunodeficiency virus (HIV) have also been infected with hepatitis B virus (HBV) because of their shared transmission routes, and at least 10%–15% remain coinfected. Recent evidence has confirmed that HIV/HBV-coinfected patients, especially those with low CD4 cell counts, are at significantly increased risk of mortality due to liver disease [1]. Since the advent of highly active antiretroviral therapy (HAART), the overall improvement in survival of patients [2] has been associated with an apparent increase in liver disease–related mortality in coinfected patients [1, 3]. This increase in mortality has been attributed not only to potential hepatotoxicities of these drugs but also to prolonged survival, which allows time for slowly progressive viral hepatitis to become clinically relevant. Therefore, in the era of HAART, there is a need to reexamine the use of specific anti-HBV drugs in this coinfected population. It is unclear whether the immune reconstitution associated with HAART is sufficient to either restore control of HBV infection (as occurs with several other opportunistic infections) or allow specific anti-HBV therapy to be effective.

An important consideration is the effect that HAART regimens with and without an anti-HBV drug have on HBV-specific T cell responses in coinfected patients. Recent studies of HIV-negative patients have revealed that the hyporesponsiveness of both CD4 and CD8 T cells that is associated with HBV chronicity can be partially overcome by treatment with lamivudine [4, 5]. We studied patients coinfected with HIV and HBV to examine whether HAART with and without an anti-HBV drug is capable of inducing HBV-specific immune responses in the setting of HIV infection.

Patients and methods. Participating patients gave informed consent, the study was approved by the local ethical committee, and the experimentation guidelines of the authors' institution were followed. Twenty patients coinfected with HIV and HBV were screened to identify patients who expressed the HLA-A2 allele and allow assessment of HLA-A2–restricted CD8 cell responses; 11 patients were identified. Five of these patients were studied serially for up to 24 weeks after either the start of HAART or the addition of an anti-HBV drug to the HAART regimen. All patients were HIV-1 antibody–positive high-infectivity HBV carriers with alanine transaminase (ALT) levels elevated to >1.5 times normal. HBV DNA was quantitated by the Digene assay, HIV-1 loads were measured by the Quantiplex HIV RNA assay (bDNA; version 3.0; Chiron), and CD4 cell counts were monitored by flow cytometry by use of the Tritest program (Becton Dickinson) as part of the quality-controlled diagnostic service. Screening for the HLA-A2 haplotype was performed by staining peripheral blood mononuclear cells (PBMCs) with an anti–HLA-A2–positive monoclonal antibody (MAb; Incstar) followed by staining with a fluorescein isothiocyanate–conjugated sheep anti–mouse IgG second-layer MAb and flow cytometric analysis.

Peptides corresponding to the sequence of core 18–27; envelope 183–191, 335–343, 338–347, and 348–357; and polymerase 455–463, 502–510, 575–583, 655–663, and 816–824 regions of HBV genotype D were synthesized by Chiron Mimotopes and were found to be >90% pure. PBMCs were expanded in vitro

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with 1 μmol/L of the relevant HBV peptide for 10 days and then were subjected to peptide restimulation in the presence of brefeldin A for 6 h. Cells were stained with anti-CD8, were permeabilized with Cytofix/Cytoperm (BD Biosciences), and were stained with an anti–interferon (IFN)–γ MAb, as described elsewhere [6], to allow flow cytometric analysis of peptide-specific CD8 cells. HBV-specific CD4 cell responses were measured at baseline and at 24 weeks, by intracellular cytokine staining, after stimulation with either the hepatitis B surface antigen (HBsAg) or hepatitis B core antigen (HBcAg) for 10 days followed by restimulation for 6 h in the presence of brefeldin A. For all intracellular cytokine–staining experiments, HBV-specific responses were calculated by subtracting background IFN-γ production in a negative control well of cells not stimulated with peptide or antigen.

Results. To study HBV-specific CD8 cell responses across a wide range of epitopes, we used intracellular cytokine staining after 10 days of in vitro expansion with a panel of peptides representing commonly recognized HLA-A2–restricted cytotoxic T lymphocyte (CTL) epitopes. This technique allows sensitive detection of circulating HBV-specific T cells on the basis of their essential antiviral function of IFN-γ production [7]. HBV-specific CD8 cell responses were detected, at a low level, in only 2 of the 11 HLA-A2–positive, HIV/HBV–coinfected patients studied at baseline (data not shown), although most were not tested more than once before treatment. Table 1 shows the effect of antiviral therapy on HBV-specific T cell responses in relation to changes in HIV and HBV loads, CD4 cell count, and ALT level, in the 5 patients studied longitudinally. All patients were screened for CD8 cell responses to 10 epitopes at multiple time points for up to 24 weeks after either the start of HAART or the addition of an anti-HBV drug to the antiretroviral regimen. Of the 4 patients in whom a reduction in HBV load was achieved, 3 developed some detectable HBV-specific T cell responses at several time points (table 1). Patient 2 had no response at 24 weeks (the only posttreatment time point available) but could have had a transient earlier response, as reported in some HIV-negative patients treated for HBV infection [5]. Patient 3 reconstituted functional CD8 cell responses to 3 HBV epitopes on starting a lamivudine-containing treatment regimen, despite the presence of advanced immunosuppression and decompensated cirrhosis. By contrast, patient 1, in whom HAART effectively reduced HIV load and increased CD4 cell count in the absence of anti-HBV activity, showed no reconstitution of HBV responses.

These differential effects were dissected further by examining the 2 patients in whom existing antiretroviral drug regimens were augmented by addition of drugs with specific anti-HBV activity but without any effect on HIV load or CD4 cell count (patients 4 and 5). In both cases, HBV reactivity was augmented after the addition of drugs (lamivudine or adefovir dipivoxil) capable of reducing HBV DNA levels. To investigate whether the effect of these drugs was restricted to HBV-specific responses, an Epstein-Barr virus (EBV)–specific CD8 cell response was also studied longitudinally. In patient 4, the response to the HLA-A2–restricted GLCTLVAML epitope from the EBV BMLF1 lytic protein remained at a constant level after the addition of lamivudine (data not shown), excluding a generalized immunostimulatory effect of this drug.

HBV-specific CD4 cell responses were then analyzed in 3 of the patients, of whom 2 had a reduction in HBV load. Intracellular cytokine staining of CD4 cells showed reconstitution of HBV-specific CD4 cell responses in patient 5, in whom there was a marked reduction of HBV DNA level (table 1 and figure 1). These responses were measured by quantitation of IFN-γ production on restimulation with HBcAg and HBsAg, after 10 days of cell culture; ex vivo intracellular cytokine staining and 5-day proliferation assays showed equivalent results (data not shown).

The kinetics of HBV-specific CD8 cell responses were studied (exemplified by the results for patient 5; figure 1) and showed that recovery of T cell reactivity was preceded by a reduction in HBV DNA level and surface-antigen titers; in patient 3, the level of HBeAg also became undetectable. HBV-specific CD8 cell responses were maximal between 12 and 24 weeks after the start of treatment, which is similar to the time course in HIV-uninfected patients starting anti-HBV therapy [5]. Results derived from 10-day in vitro cultures do not necessarily represent circulating frequencies but are a reflection of the proliferative potential of these cells. Intracellular cytokine staining was therefore performed directly ex vivo in patient 5 and confirmed that identical CD8 cell specificities became detectable with the same time course as after 10 days of in vitro expansion (data not shown). Reductions in HBV load were accompanied by reductions in ALT level, and reconstituted CD8 cell responses were not associated with any "flares" of ALT (figure 1).

Discussion. Here we have presented the first analysis of HBV-specific T cell responses in HIV/HBV-coinfected patients in whom the effect of HAART with or without an anti-HBV drug can be examined. We have found evidence for reconstitution of functionally active HBV-specific CD8 cell responses when HIV/HBV-coinfected patients are treated. The fact that low-level responses were detected in only 2 of the 11 HIV/HBV–coinfected patients studied cross-sectionally adds weight to the increase in detectable responses after treatment. Our preliminary findings from this longitudinal study of 5 patients need to be confirmed in larger studies, but, from our sample, it appears that HAART alone may be insufficient for reconstitution of HBV-specific responses. However, some reconstitution of specific T cell responses can certainly occur with reduction of HBV load, even in the context of advanced immunosuppression or ongoing HIV viremia. These observations are consistent with the fact that HBV-specific T cell responses are difficult to detect in HIV-

Table 1. Hepatitis B virus (HBV)–specific T cell responses in human immunodeficiency virus (HIV)/HBV–coinfected patients with changes in CD4 cell count, HIV and HBV load, and alanine transaminase (ALT) level, on starting the antiviral regimens indicated.

Patient	HBV-related clinical disease	HAART regimen (regimen change)	CD4 cell count, cells $\times 10^9$/L		HIV-VL reduction at 24 weeks, $\log_{10}$ copies/mL	HBV DNA–load reduction at 24 weeks, $\log_{10}$ copies/mL	ALT-level reduction	HBV-specific CD8 cell peak response (% of total CD8 cell count)	No. of positive responses/no. of samples after treatment change	HBV-specific CD4 cell count (% of total CD4 cell count)
			At baseline	At 24 weeks						
1	Mildly increased ALT level	ddI/ABC/NFV	100	480	4.28	0	No	None	0/4	None
2	Hepatitis with fibrosis, membranous glomerulonephritis	ZDV/3TC/EFV	80	340	4.1	2.1	Yes	None[a]	0/1	Not tested
3	Decompensated cirrhosis	ZDV/3TC/EFV	70	180	4.15	1.5	Yes	Envelope 183–191 (1.5),[c] 338–347 (0.6), 348–357 (0.3)	3/4	None
4	Recurrent hepatitis flares	ddI/EFV/d4T (d4T switch to 3TC)	500	480	0	1.8	Yes	Envelope 183–191 (0.22), 338–347 (0.25), 348–357 (0.18)	2/3	Not tested
5	Persistently increased ALT level	ZDV/ddI (addition of ADF[b])	360	320	−0.2	4.2	Yes	Envelope 183–191 (1.45), 335–343 (0.26)	3/4	HBcAg (0.7),[d] HBsAg (0.26)

NOTE. 3TC, lamivudine; ABC, abacavir; ADF, adefovir; d4t, stavudine; ddI, didanosine; EFV, efavirenz; HAART, highly active antiretroviral therapy; HBcAg, hepatitis B core antigen; HBsAg, hepatitis B surface antigen; NFV, nelfinavir; VL, virus load; ZDV, zidovudine.

[a] Only tested at 24 weeks.
[b] Ten milligrams once daily (therapeutic dose for HBV, not HIV).
[c] Percentage of interferon (IFN)–γ–positive CD8 cells, out of total CD8 cells.
[d] Percentage of IFN-γ–positive CD4 cells, out of total CD4 cells.

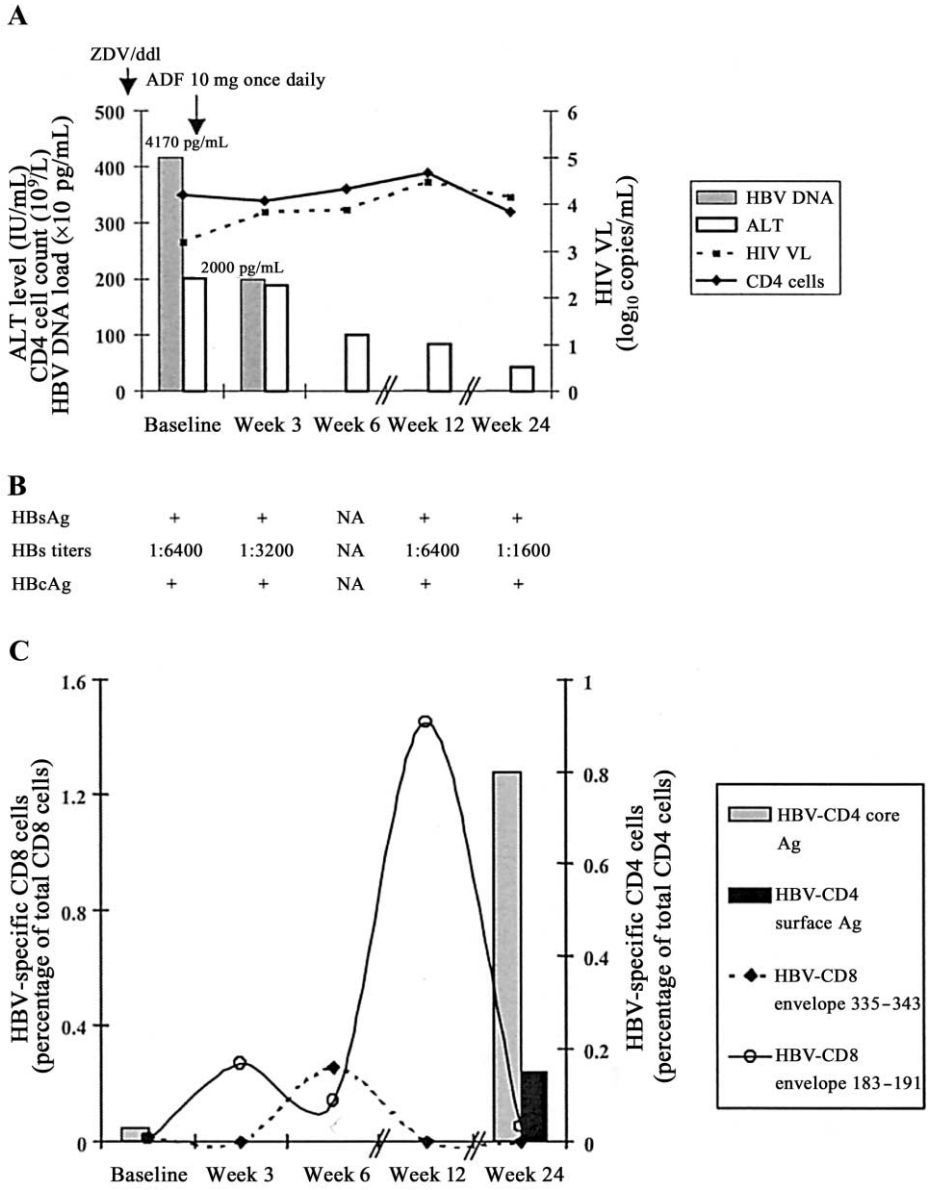

Figure 1. Kinetics of hepatitis B virus (HBV)–specific T cell responses *(A)* in relation to changes in HBV serostatus *(B)* and in human immunodeficiency virus (HIV)/HBV load, CD4 cell count, and alanine transaminase (ALT) level *(C),* for patient 5 after addition of adefovir dipivoxil to existing antiretroviral therapy regimen. ADF, adefovir; ddI, didanosine; HBcAg, hepatitis B core antigen; HBsAg, hepatitis B surface antigen; NA, not applicable; VL, virus load; ZDV, zidovudine.

negative patients with HBV infection [8] until HBV load is reduced [5]. The increase in HBV-specific CD8 cell responses after treatment contrasts with the situation observed for HIV-specific CTLs, which are easily detectable in patients with high virus loads and typically decrease after the reduction in virus load that is induced by HAART [9].

One patient who had a marked reduction in HBV viremia on the addition of the novel anti-HBV drug adefovir dipivoxil [10] showed reconstitution of HBV-specific CD4 cell responses to HBcAg and HBsAg. Although he was receiving only a dual nucleoside regimen and had incomplete suppression of HIV load, he was the only patient who had never been severely immunosuppressed and might therefore be expected to most closely mimic an HIV-negative patient with chronic HBV infection. Such CD4 cell responses are thought to play a critical role in maintaining functionally active CTLs [11], and it is of note that this patient also had the highest peak CD8 cell response while receiving treatment.

The reconstituted HBV-specific CD8 cell responses were mostly low frequency after 10 days of in vitro expansion, and all were envelope specific, even though all patients were screened repeatedly for responses to epitopes within core and

polymerase as well. Reconstituted responses in HIV-negative patients have also been reported to be frequently envelope specific [5], with a notable absence of the core-specific CD8 cells that are immunodominant in response to acute infection associated with viral control [12]. However, these responses were only measured from the peripheral blood; the recent identification of other CD8 cell specificities in secondary lymphoid organs during treatment with lamivudine in HIV-negative patients with HBV infection [6] suggests the potential for a more complete reconstitution of the multispecific response associated with viral control. Recent reports have highlighted the potential benefits of additional anti-HBV drugs, such as adefovir dipivoxil and tenofovir disoproxil fumarate, for HIV/HBV-coinfected patients. Whether more-potent anti-HBV therapy or therapeutic immunization will ultimately be able to further enhance reconstitution of HBV-specific T cell responses, even in the setting of HIV disease, remains to be established.

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