MAJOR ARTICLE

Effect of HIV Infection and Antiretroviral Therapy on Hepatitis B Virus (HBV)–Specific T Cell Responses in Patients Who Have Resolved HBV Infection

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Coinfection with hepatitis B virus (HBV) is a common occurrence in human immunodeficiency virus (HIV)–positive patients and an increasing cause of morbidity and mortality. The $CD8^+$ T cell response is critical for long-term control of HBV in patients resolving acute infection. Here, we examine the effect of HIV on HBV-specific $CD8^+$ T cell responses in patients who have resolved HBV infection. A cross-sectional study showed a reduction in HBV-specific $CD8^+$ T cell responses in HIV-positive, HBV-immune patients, compared with those in HIV-negative, HBV-immune patients. A longitudinal study of a subgroup of patients examined whether this attrition could be reversed by effective antiretroviral therapy. The introduction of highly active antiretroviral therapy (HAART) resulted in reconstitution of some HBV-specific $CD4^+$ and $CD8^+$ T cell responses, in association with restoration of $CD4^+$ T cell counts. These data provide a mechanism to account for the observed impairment of control of HBV infection in the setting of HIV infection and support the ability of HAART to reconstitute functionally active T cell responses.

Coinfection with hepatitis B virus (HBV) is a frequent occurrence in HIV-positive patients and an increasing cause of morbidity and mortality in the context of the prolonged survival associated with highly active antiretroviral therapy (HAART) [1–4]. The proportion of HIV-positive patients with serological evidence of previous exposure to HBV ranges from 64% to 84% in published cohorts; 10%–15% of such patients are chronically infected with HBV [5, 6]. HIV-related immunodepletion influences the natural history of HBV infection. Epidemiological studies have revealed that HIV-positive patients are more likely to have a prolonged duration of acute illness after HBV infection and to have lower rates of clearance of hepatitis B e antigen [7]. They have higher circulating levels of HBV DNA and higher rates of reactivation of HBV infection [8]. These findings suggest impaired immune control of HBV infection during HIV infection.

In the present study, we explore, for the first time, the effect of HIV-related immunodepletion on HBV-specific $CD8^+$ T cell responses in patients who have resolved HBV infection. Recent data have directly demonstrated that $CD8^+$ T cells are critical effectors in the control of acute HBV infection [9]. HBV-specific $CD8^+$ T cell responses are difficult to detect in patients chronically infected with HBV [10], making them an unsuitable group in which to study the impact of HIV infection. We therefore studied patients who resolved HBV infection, since, in the absence of HIV, such patients maintain strong $CD4^+$ and $CD8^+$ T cell responses for many years after complete clinical and serological recovery [11, 12]. Patients without a history of jaundice found to have natural immunity to HBV (hepatitis B core antibody [HBcAb] plus/minus hepatitis B surface antibody [HBsAb]) as part of routine screening before HBV vaccination have never previously been studied

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Table 1. Clinical characteristics of HIV-positive patients.

Patient	$CD4^+$ T cell count nadir,[a] cells/μL	$CD4^+$ T cell percentage	HIV load, copies/mL	HAART status/combination at study entry	HAART combination started
P1	320	19.0	54,000	Naive	Stavudine/lamivudine/nevirapine
P2	700 (160)	24.1	<50	Stavudine/lamivudine/nelfinavir	…
P3	540	25.5	11,000	Naive	…
P4	480	12.8	118,600	Naive	…
P5	220	6.7	360,100	Naive	Zidovudine/didanosine/efavirenz
P6	290	22.3	2200	…	…
P7	260	20.0	296,700	Naive	Zidovudine/lamivudine/efavirenz
P8	280	13.2	141,400	Naive	Zidovudine/lamivudine/efavirenz
P9	510	25.0	8800	Naive	…
P10	310	24.2	50,000	Naive	…
P11	320 (230)	13.7	13,000	Naive	…
P12	350 (240)	18.0	500	Zidovudine/lamivudine	…
P13	190	15	426,000	Naive	…
P14	150	21.7	173,400	Naive	…
P15	640	22.0	800	Naive	…
P16	710	19.5	21,600	Naive	…

NOTE. HAART, highly active antiretroviral therapy.

[a] $CD4^+$ T cell count nadirs in parentheses are different from $CD4^+$ T cell counts at study entry.

immunologically, with regard to their HBV-specific immune responses, but are common among HIV cohorts [5, 13]. HBV-specific responses were compared in HIV-positive and HIV-negative patients, with a focus on those who had resolved HBV infection without symptoms. We used the sensitive technique of intracellular cytokine staining for interferon (IFN)–γ, to explore the breadth of functionally active HBV-specific $CD8^+$ T cell responses across a range of previously defined HLA-A2–restricted HBV epitopes [14–16].

To investigate to what extent any loss of HBV-specific immune responses could be reversed by antiretroviral therapy, a group of HBV-immune patients starting HAART were studied prospectively. There is a growing body of evidence suggesting that HAART may lead to successful restoration of specific immune responses to previously encountered pathogens [17–19]. A previous small study of HIV patients chronically infected with HBV suggested that HAART may be associated with restoration of HBV-specific responses once HBV load is reduced [20]. Natural immunity to HBV provided a good model system in which to further explore the potential for functional restoration of HBV-specific $CD4^+$ and $CD8^+$ T cell responses in the setting of controlled HBV replication.

PATIENTS, MATERIALS, AND METHODS

Patients and controls. Patients were recruited from the Mortimer Market Centre and provided written, informed consent, and the local ethics committee approved the study. All patients had HBV serologic test results confirming natural immunity to HBV (hepatitis B surface antigen [HBsAg] negative, HBcAb positive, and HBsAb positive) and were further categorized according to whether they had a history consistent with symptomatic acute hepatitis B.

Thirty-two HIV-positive gay men with natural immunity to HBV were screened, to identify 16 HLA-A2–positive patients. Two had a history of symptomatic acute hepatitis B, and 14 had no history of jaundice or other relevant symptoms. Patients had a median $CD4^+$ T cell count of 320 cells/μL and a median HIV load of 36,000 copies/mL (table 1). Fourteen patients were HAART naive, and 2 patients were already receiving antiretroviral therapy; 4 HLA-A2–positive, HAART-naive patients were followed up longitudinally after starting combination therapy. Twenty-five HIV-negative patients (tested within the previous year) with natural immunity to HBV were screened, to identify 14 HLA-A2–positive patients, of whom 3 had a history of symptomatic acute hepatitis B.

The HIV-positive and HIV-negative groups had similar demographic characteristics, with all but 1 patient who resolved asymptomatic HBV infection being gay men, and with all being white, apart from 1 Asian patient in the HIV-positive group. The HIV-negative group had a mean age of 38 years (median, 39 years), and the HIV-positive group had a mean age of 39 years (median, 39 years).

Tissue typing. 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 fluorescein isothiocyanate–conjugated sheep anti–mouse IgG second-layer MAb and flow-cytometric analysis.

Synthetic HBV peptides and antigens. Peptides corresponding to the sequence of core 18–27; envelope 183–91, 335–43, 338–47, and 348–57; and polymerase 455–63, 502–10, 575–83,

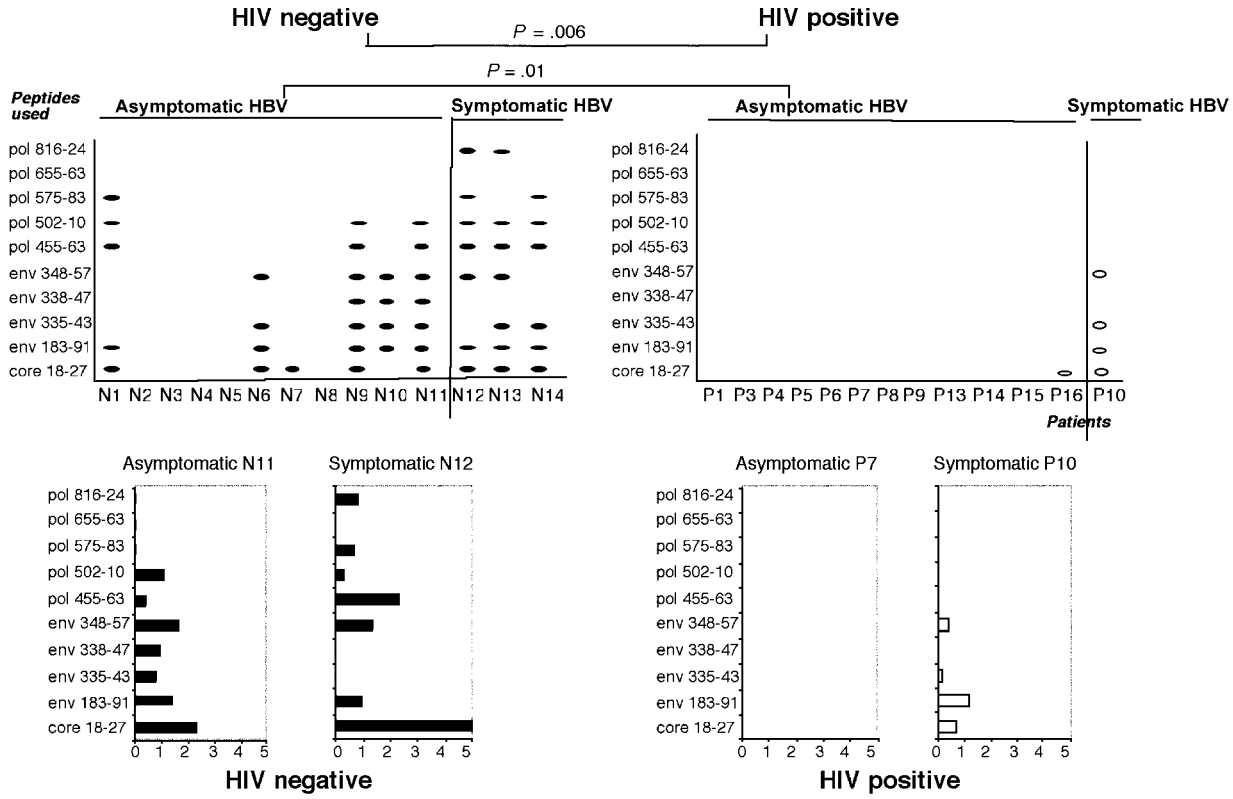

Figure 1. Comparison of $CD8^+$ T cell responses in HIV-positive and HIV-negative patients with symptomatic or asymptomatic resolved hepatitis B virus (HBV) infection. Intracellular cytokine staining for interferon (IFN)–γ after 10 days of in vitro stimulation with 10 peptides representing HLA-A2–restricted HBV epitopes is shown for 14 HIV-negative patients (11 who resolved asymptomatic and 3 who resolved symptomatic HBV infection) and 13 HIV-positive patients (12 who resolved asymptomatic and 1 who resolved symptomatic HBV infection). The black marks indicate epitopes where the response was greater than the background level. In an analysis that took into account the no. and size of $CD8^+$ T cell responses per patient, there were significantly more responses detectable in the HIV-negative group ($P = .006$, for all patients, Mann-Whitney *U* test; $P = .01$, for patients with asymptomatic HBV infection, Mann-Whitney *U* test). The histograms show representative examples of the magnitude of responses after expansion for patients from each category (frequencies for all responses for each patient are listed in table 2). HBV-specific $CD8^+$ T cell responses are expressed as the percentage of total $CD8^+$ T cells producing IFN-γ after stimulation with the appropriate peptide and after subtraction of background production of IFN-γ in unstimulated control wells. env, envelope; pol, polymerase.

655–63, and 816–24 regions of HBV genotype D were synthesized (Chiron Mimotopes). Hepatitis B core antigen (HBcAg) and HBsAg were produced in *Escherichia coli* strain K802 and were 90% pure. HBsAg was supplied by Rhein Biotech, and HBcAg was provided by G. Borisova (University of Latvia).

Production of T cell lines and intracellular IFN-γ staining. PBMCs were seeded at a concentration of 3×10^6 cells/mL and stimulated with 1 μmol/L relevant peptide (the 10 peptides listed above). Recombinant interleukin (IL)–2 (10 IU/mL) (Boehringer Mannheim) was added on day 4 of cell culture. Cells were restimulated on day 10, with 1 μmol/L relevant peptide (5 h), the last 4 h with 10 μg/mL brefeldin A (Sigma-Aldrich). Cells were stained with anti-$CD8^+$ (Pharmingen), permeabilized with Cytofix/Cytoperm (Pharmingen), stained with anti–IFN-γ mAb (R&D Systems), and analyzed by use of a FACScan flow cytometer with CELLQuest software (Becton Dickinson).

For enumeration of HBV-specific $CD4^+$ T cells, PBMCs were suspended at a concentration of 3×10^6 cells/mL in RPMI 1640 and 5% human serum and stimulated with 1 μmol/L HBcAg or HBsAg for 6–16 h, with addition of brefeldin A after 1 h. Cells were washed, stained with anti-$CD4^+$ phycoerythrin (Pharmingen), and subjected to intracellular cytokine staining with anti–IFN-γ or anti–IL-2 mAb (R&D Systems). For all intracellular cytokine staining experiments, responses were calculated by subtracting background production of IFN-γ or IL-2 in a negative control well without peptide or antigen restimulation. Stimulation with anti-CD3 and anti-CD28 MAb (1 μmol/L each) or phorbol 12-myristate 13-acetate (3 ng/mL) and ionomycin (100 ng/mL), for 6 h in the presence of brefeldin A, was used as a positive control.

Statistical analysis. In the cross-sectional study of HIV-negative and HIV-positive patients, the nonparametric Fisher's exact test was used to compare the number of patients with

Table 2. Cross-sectional study of CD8+ T cell responses after 10 days of in vitro stimulation.

Category, infection, patient	core 18–27	env 183–91	env 335–43	env 338–47	env 348–57	pol 455–63	pol 502–10	pol 575–83	pol 655–63	pol 816–24
HIV negative										
Asymptomatic HBV										
N1	0.7	0.3	0	0	0	0.1	0.1	0.1	0	0
N2	0	0	0	0	0	0	0	0	0	0
N3	0	0	0	0	0	0	0	0	0	0
N4	0	0	0	0	0	0	0	0	0	0
N5	0	0	0	0	0	0	0	0	0	0
N6	0.8	0.6	2.0	0	0.1	0	0	0	0	0
N7	0.4	0	0	0	0	0	0	0	0	0
N8	0	0	0	0	0	0	0	0	0	0
N9	14.8	1.5	1.3	0.8	6.3	6.6	0.2	0	0	0
N10	0	0.9	0.1	0.1	0.1	0	0	0	0	0
N11	2.3	1.4	0.8	0.9	1.6	0.4	1.1	0	0	0
Symptomatic HBV										
N12	5.0	1.0	0	0	1.4	2.4	0.3	0.7	0	0.8
N13	20.4	0.9	0.4	0	6.9	12.3	1.3	0	0	0.4
N14	1.1	1.2	0.5	0	0	7.9	0.6	0.5	0	0
HIV positive										
Asymptomatic HBV										
P1	0	0	0	0	0	0	0	0	0	0
P3	0	0	0	0	0	0	0	0	0	0
P4	0	0	0	0	0	0	0	0	0	0
P5	0	0	0	0	0	0	0	0	0	0
P6	0	0	0	0	0	0	0	0	0	0
P7	0	0	0	0	0	0	0	0	0	0
P8	0	0	0	0	0	0	0	0	0	0
P9	0	0	0	0	0	0	0	0	0	0
P13	0	0	0	0	0	0	0	0	0	0
P14	0	0	0	0	0	0	0	0	0	0
P15	0	0	0	0	0	0	0	0	0	0
P16	0.1	0	0	0	0	0	0	0	0	0
Symptomatic HBV, P10	0.6	1.1	0.1	0	0.4	0	0	0	0	0

NOTE. Data are the percentage of CD8+ T cells producing interferon-γ in response to each peptide. env, envelope; HBV, hepatitis B virus; pol, polymerase.

any HBV-specific CD8+ T cell response detectable. The nonparametric Mann-Whitney *U* (Wilcoxon rank sum) test was used to compare the groups, with the number and size of T cell responses for each patient taken into account.

RESULTS

Reduction of HBV-specific CD8+ T cell responses in HIV-positive, HBV-immune patients. We initially conducted a cross-sectional study of HIV-positive and HIV-negative patients with natural immunity to HBV, either with or without a history of acute symptomatic infection. All patients were screened for CD8+ T cells producing IFN-γ in response to a panel of 10 peptides representing frequently recognized HLA-A2–restricted HBV epitopes, and results were calculated after subtraction of background staining in wells without peptide restimulation. In 3 HLA-A2–positive, HIV-negative patients who had successfully resolved acute symptomatic HBV infection 3–5 years before, we found peptide-specific production of IFN-γ for 6 or 7 of the 10 epitopes tested (figure 1 and table 2). We found that HIV-negative patients who had resolved HBV infection without prior symptoms of acute infection also had detectable CD8+ T cell responses to at least 1 HLA-A2–restricted epitope in 6 of 11 cases (figure 1 and table 2). In some patients (e.g., N9, whose last risk exposure to HBV was >10 years before), responses had a level of multispecificity and expansion potential similar to those seen in patients with symptomatic acute infection, who have been the basis of all previous immunological studies of patients resolving HBV infection.

In a similar group of 12 HAART-naive, HIV-positive patients who also had serological evidence of past HBV infection without a history of symptoms, HBV-specific CD8+ T cell responses were markedly diminished. This group was matched with the HIV-negative, HBV-immune group for sex, age, ethnic origin, and likely route of acquisition of HBV infection, and all

Table 3. Cross-sectional study of CD8+ T cell responses directly ex vivo.

Category, infection, patient	core 18–27	env 183–91	env 335–43	env 338–47	env 348–57	pol 455–63	pol 502–10	pol 575–83	pol 655–63	pol 816–24
HIV negative										
Asymptomatic HBV										
N1	0.2	0	0	0.4	0.2	0.2	0	0	0.8	0
N7	0.3	0	0	0	0	0	0	0	0	0
Symptomatic HBV										
N12	0.1	0	0	0	1.2	1.2	0.1	0.5	0	0.7
N13	1.0	0.9	0.2	0.1	0.3	1.3	0	0	0	0
HIV positive										
Asymptomatic HBV										
P5	0	0	0	0	0	0	0	0	0	0
P7	0	0	0	0	0	0	0	0	0	0
Symptomatic HBV										
P10	0.8	0.8	0	0	0.7	0.6	0	0	0	0
P11	0	0	0	0	0	0	0	0	0	0

NOTE. Data are the percentage of $CD8^+$ T cells producing interferon-γ in response to each peptide. env, envelope; HBV, hepatitis B virus; pol, polymerase.

were antiretroviral naive. HBV-specific $CD8^+$ T cell responses were undetectable after 10 days of specific peptide stimulation, by both intracellular cytokine staining (figure 1) and tetramer staining (data not shown), apart from 1 low-level core 18–27 response in P16. In an HIV-positive patient with a history of acute symptomatic hepatitis B (P10; 8 years before), intracellular cytokine staining for IFN-γ after 10 days of in vitro stimulation showed recognition of 4 of 10 epitopes tested. The proportion of patients with any HBV-specific $CD8^+$ T cell response and the total number and magnitude of responses was significantly greater for the whole HIV-negative group than for the whole HIV-positive group ($P = .018$, Fischer's exact test, and $P = .006$, Mann-Whitney U test, respectively). These differences remained significant in a comparison of the closely matched subsets in the HIV-negative and HIV-positive groups who resolved HBV infection without symptoms ($P = .027$, for the proportion of patients with at least 1 response, Fisher's exact test; $P = .01$, for the number and size of responses, Mann-Whitney U test) (figure 1).

Intracellular cytokine staining directly ex vivo, in a subgroup of 2 HBV-immune patients who resolved symptomatic infection and 2 HBV-immune patients who resolved asymptomatic infection, from both the HIV-negative and HIV-positive groups (table 3), confirmed that responses were detectable in all of the HIV-negative patients tested. In the HIV-positive group, responses were detected directly ex vivo in only 1 of the HIV-positive patients (in P10, who had a history of acute symptomatic HBV infection 8 years before, but not in P11, who had acute infection >15 years before, or in the patients with asymptomatic infection). In all patients studied by both methods, the breadth of the HBV-specific immune responses seen after direct ex vivo expansion was similar to that seen after in vitro expansion.

HBV-specific CD8+ T cell responses in HIV-positive patients receiving antiretroviral therapy. To investigate any potential for reconstitution of HBV-specific $CD8^+$ T cell responses, we initially examined responses in 2 patients who had resolved HBV infection without symptoms and were already receiving treatment for HIV infection. P12 started zidovudine and lamivudine at a $CD4^+$ T cell count nadir of 240 cells/μL; at the time that samples were obtained, he had been maintained on this regimen for 3 years (at which time his $CD4^+$ T cell count was 350 cells/μL and his HIV load was 500 copies/mL). P2 had been receiving HAART for 6 months, but lamivudine was substituted for didanosine 1 month before samples were obtained. His $CD4^+$ T cell count increased from a nadir of 160 cells/μL to 700 cells/μL, and his HIV load was suppressed to <50 copies/mL. Both of these patients had $CD8^+$ T cell responses to 3 of the 10 HBV-specific, HLA-A2–restricted epitopes tested (figure 2), which, after 10 days of in vitro expansion, were present at a magnitude similar to that seen in the HIV-negative, HBV-immune patients. This contrasted with the lack of responses detectable in 11 of the 12 untreated HIV-positive patients who had an equivalent pattern of HBV immunity and no history of symptomatic acute infection (figure 2). HBV-specific $CD8^+$ T cell responses were detectable in the treated HIV-positive patients but not in the untreated HIV-positive, HBV-immune patients tested directly ex vivo (figure 2).

Reconstitution of HBV-specific CD8+ T cell responses on longitudinal study of patients starting HAART. The cross-sectional data suggested a decrease in HBV-specific $CD8^+$ T cell responses in HIV-positive, HBV-immune patients and a possible reconstitution of such responses in those receiving HAART. We therefore longitudinally studied the impact of HAART on HBV-specific immune responses. Four patients who had resolved

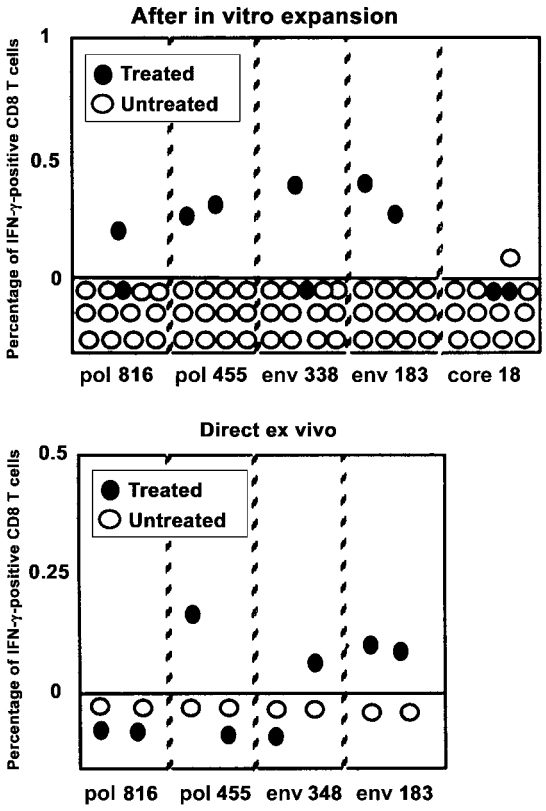

Figure 2. Comparison of breadth of $CD8^+$ T cell responses in HIV-positive patients with immunity to hepatitis B virus (HBV), after asymptomatic infection, with or without antiretroviral treatment. Intracellular cytokine staining for interferon (IFN)–γ after 10 days of stimulation with 10 HLA-A2–restricted peptides is shown for patients with immunity after asymptomatic HBV infection *(upper panel).* Results are shown for 14 HIV-positive patients: 12 highly active antiretroviral therapy (HAART) naive (P1, P3, P4, P5, P6, P7, P8, P9, P13, P14, P15, and P16) and 2 antiretroviral treated (P2 and P12). HBV-specific $CD8^+$ T cell responses are expressed as the percentage of total $CD8^+$ T cells producing IFN-γ after stimulation with the appropriate peptide and after subtraction of background production of IFN-γ in unstimulated control wells. Only the epitopes with any positive response are shown. *Lower panel,* Intracellular cytokine staining directly ex vivo in a subgroup of the above patients (2 HIV-positive, HAART-naive patients [P5 and P7] and 2 HIV-positive, antiretroviral-treated patients [P2 and P12]). env, envelope; pol, polymerase.

HBV infection (without a history of asymptomatic acute infection) and were starting HAART for progressive HIV infection were studied prospectively, and sequential blood samples were obtained before and during therapy. The drug regimens used are shown in table 1 and figure 3*A* and were selected by the clinician independently of participation in the present study. These patients were screened for functionally active HBV-specific $CD8^+$ T cell responses on 2–6 occasions by use of the same panel of 10 peptides applied in the cross-sectional studies.

The temporal relationship between increase in $CD4^+$ T cell count, suppression of HIV load, and reconstitution of HBV-specific $CD8^+$ T cell responses for each of the 4 patients is presented in figure 3*A*. Patients were screened up to 2 years after starting HAART, and, 3 years after starting HAART, samples were again obtained from any patients with reconstitution of HBV-specific $CD8^+$ T cell responses. In 2 patients, the increase in $CD4^+$ T cell count and suppression of HIV load were accompanied by detection of HBV-specific $CD8^+$ T cell responses by 24 weeks of antiretroviral treatment. The epitopes detected were from the envelope group; 1 patient also recovered a response to core 18–27 (figure 3*A*). HBV-specific $CD8^+$ T cell responses were still detectable when samples were again obtained from P1 and P7, 3 years after starting HAART (figure 3*A*), and, in P1, expanded to higher frequencies than at earlier sampling times. This is compatible with the finding of persistent $CD8^+$ T cell responses in the patients studied cross-sectionally 6 months to 3 years after starting antiretroviral therapy (figure 2).

Direct ex vivo analysis of P7 revealed reconstitution of HBV-specific $CD8^+$ T cell responses at week 24 (figure 3*B*), which is consistent in specificity and timing with the in vitro data (figure 3*A*), and a simultaneous decrease in levels of $CD8^+$ specific for an HIV-1–specific, HLA-A2–restricted epitope (gag 77–85; data not shown). This patient was also tested directly ex vivo for HBcAg-specific $CD4^+$ T cell responses, by intracellular IFN-γ staining, which revealed a parallel increase in HBV-specific $CD4^+$ and $CD8^+$ T cell responsiveness (figure 3*B*).

Parallel between reconstitution of HBV-specific $CD4^+$ T cell responses and HAART-induced increases in total $CD4^+$ T cells. To further investigate the potential role that enhanced $CD4^+$ T cell help plays in recovering HBV-specific $CD8^+$ T cell responses in these patients, we screened all the patients being longitudinally studied for HBV-specific $CD4^+$ T cell responses at each time point. In light of recent data highlighting the potential importance of IL-2–producing $CD4^+$ T cells in maintaining adequate antiviral $CD8^+$ T cell responses (reviewed in [21]), patients were tested for the presence of $CD4^+$ T cells able to produce IFN-γ and/or IL-2 on stimulation with HBcAg and HBsAg. The assay used to measure $CD4^+$ intracellular production of IFN-γ and/or IL-2, with or without stimulation with HBcAg or HBsAg, is shown in figure 4*A*. As illustrated by the fluorescence-activated cell sorter dot-plot profiles for this treated patient (P1; week 150), responses to overnight antigenic stimulation were easily detectable in some cases (IL-2–positive $CD4^+$ responding to HBcAg). In other examples (IFN-γ–positive $CD4^+$ responding to HBcAg and HBsAg), the responding populations were only slightly greater than background levels but could be confirmed by their ability to increase after a 10-day antigen-specific in vitro expansion (figure 4*A*).

The temporal relationship between $CD4^+$ T cell responses and changes in $CD4^+$ T cell count and HIV load is shown in figure 4*B*. All 4 HAART-treated patients developed detectable $CD4^+$ T cell responses to both HBcAg and HBsAg from week 6 onward, which persisted for the duration of follow-up (at

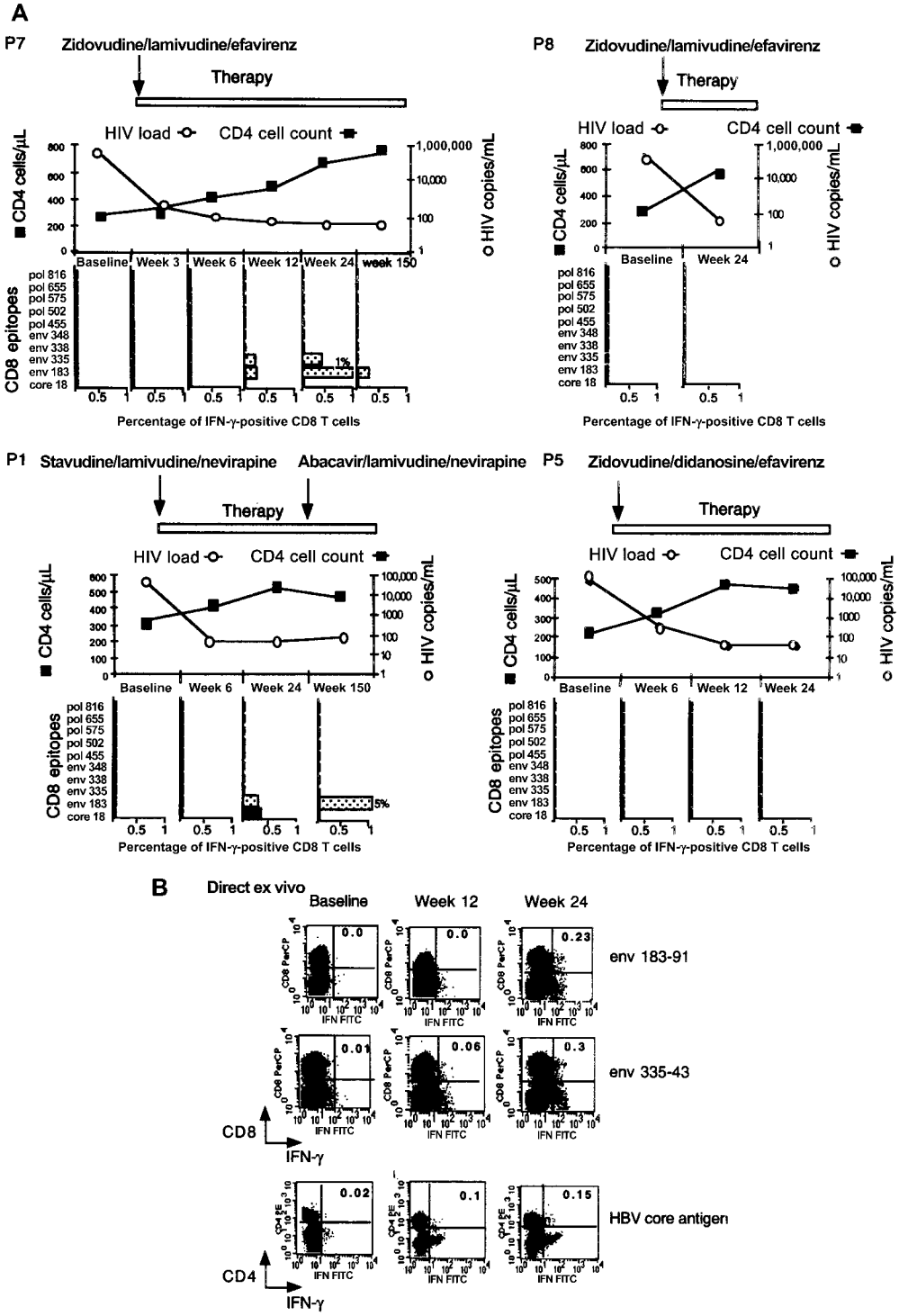

Figure 3. Hepatitis B virus (HBV)–specific $CD8^+$ T cell responses in HIV-positive, HBV-immune patients starting highly active antiretroviral therapy (HAART). *A,* Longitudinal analysis of HBV-specific $CD8^+$ T cell responses in 4 HBV-immune patients (P1, P5, P7, and P8) starting HAART. Drug regimens are shown for each patient above the changes in $CD4^+$ T cell count and HIV load at each time point at which T cell responses were measured. The lower panel for each patient shows the results, for each time point, of intracellular cytokine staining of $CD8^+$ T cells for IFN-γ after 10 days of in vitro stimulation with 10 HBV peptides. *B,* Fluorescence-activated cell sorter dot-plot profiles of intracellular cytokine staining of $CD8^+$ T cells from P7 for IFN-γ, directly ex vivo. The epitopes for which response greater than the background level was detected are represented. Nos. in the right upper quadrants represent the percentage of $CD8^+$ T cells responding to envelope (env) 183–91 *(upper panel)* and env 335–43 *(middle panel). Lower panel,* Flow-cytometric data from the same patient time points, showing intracellular cytokine staining of $CD4^+$ T cells for IFN-γ, directly ex vivo, after stimulation with hepatitis B core antigen. FITC, fluorescein isothiocyanate; PE, phycoerythrin; PerCP; peridin chlorophyll protein; pol, polymerase.

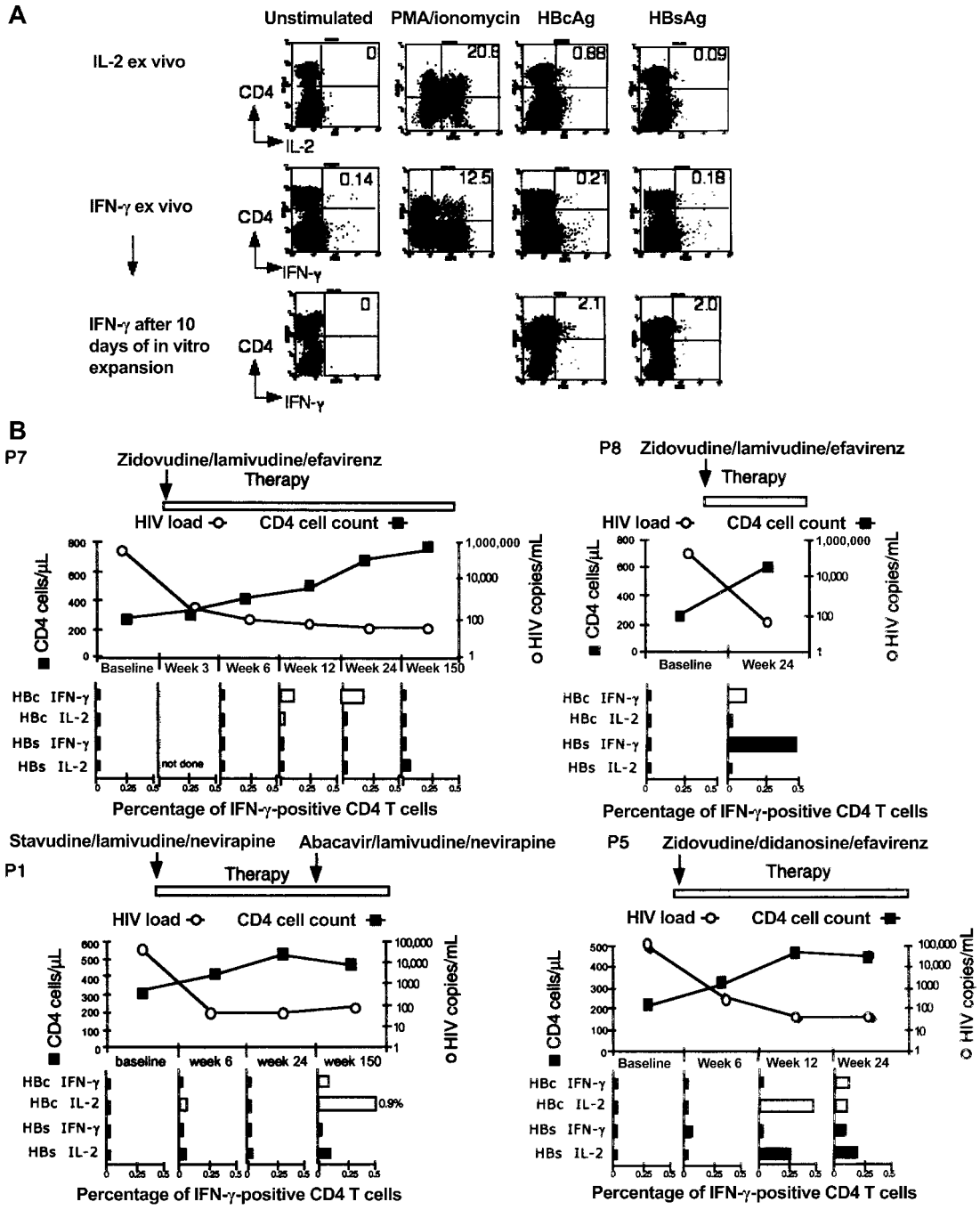

Figure 4. Hepatitis B virus (HBV)–specific $CD4^+$ T cell responses in HIV-positive, HBV-immune patients starting highly active antiretroviral therapy (HAART). *A,* Fluorescence-activated cell sorter dot-plot profiles of intracellular cytokine staining of $CD4^+$ T cells for interleukin (IL)–2 *(upper panel)* and interferon (IFN)–γ *(middle panel)* after overnight stimulation with hepatitis B core antigen (HBcAg) or hepatitis B surface antigen (HBsAg), compared with that of an unstimulated negative control and a phorbol 12-myristate 13-acetate (PMA)/ionomycin positive control (P1; week 150). The lower panel shows intracellular cytokine staining for IFN-γ with the same antigens after 10 days of antigen-specific in vitro expansion. Nos. in the right upper quadrants represent the percentage of $CD4^+$ T cells responding to each stimulus. *B,* Longitudinal analysis of HBV-specific $CD4^+$ T cell responses in 4 HBV-immune patients (P1, P5, P7, and P8) starting HAART. Drug regimens are shown for each patient above the changes in $CD4^+$ T cell count and HIV load at each time point at which T cell responses were measured. The lower panel for each patient shows the direct ex vivo intracellular cytokine staining of $CD4^+$ T cells with IFN-γ and IL-2 in response to HBcAg and HBsAg (not done, $CD4^+$ T cell responses not analyzed at this time point). *C,* Cross-sectional comparison of HBV-specific $CD4^+$ T cell responses in 5 HIV-positive, HAART-naive patients *(left chart),* with the peak of responses seen while receiving HAART treatment in 4 of these patients *(middle chart)* and with the responses in 6 HIV-negative patients *(right chart).* All patients had resolved asymptomatic HBV infection. The no. and size of responses was significantly greater in HIV-negative than in HIV-positive patients ($P = .005$, Mann-Whitney *U* test), and responses in HIV-negative patients were comparable with peak responses while receiving treatment in HIV-positive patients ($P = .67$, Mann-Whitney *U* test).

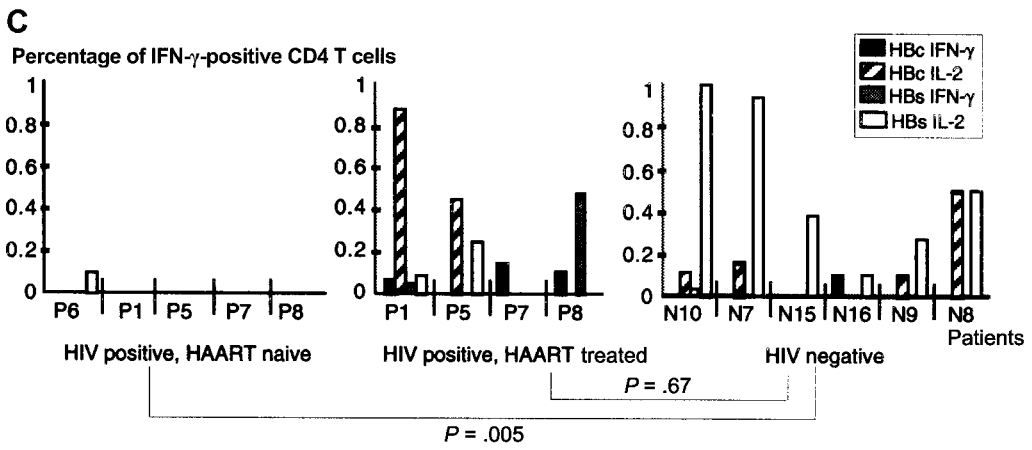

Figure 4. *(Continued.)*

least 6 months in 2 patients and at least 3 years in the other 2). Three of the 4 patients were given lamivudine-containing combinations; P5, who also developed strong HBV-specific $CD4^+$ T cell responses, was not treated with any drug with anti-HBV activity. All patients achieved adequate viral suppression and good total $CD4^+$ T cell count recovery/increases, but the patient with the highest $CD4^+$ T cell count nadir, lowest starting HIV load, and most rapid suppression of HIV load (P1) had the most effective reconstitution of both $CD4^+$ and $CD8^+$ T cell responses (figure 4*B*).

Figure 4*C* summarizes the lack of $CD4^+$ T cell responses to HBcAg and HBsAg in 5 HBV-immune patients with HIV-related $CD4^+$ lymphopenia ($CD4^+$ T cell count <350 cells/μL) before the start of antiretroviral therapy. This contrasts with the HBV-specific $CD4^+$ T cell responses in 6 HIV-negative patients who had also resolved asymptomatic HBV infection. The peak responses seen in the HIV-positive patients receiving HAART were comparable in frequency and magnitude to those seen in the HIV-negative patients (figure 4*C*).

DISCUSSION

In the present study, we explored the effect of HIV immunodepletion and HAART-related immune restoration on reshaping immune responses to HBV infection. We observed a decrease in functionally active HBV-specific $CD8^+$ T cell responses in association with HIV infection in HBV-immune patients. Accumulating data suggest that residual virus is kept under tight control by an ongoing immune response in such patients [11, 12, 22]. Thus, the reduction in HBV-specific T cell responses observed in the present study provides a mechanism that could contribute to the increased risk of reactivation of hepatitis B surface antigenemia seen in HIV infection and other situations of clinical immunodepletion [23, 24].

To date, immunological studies of HBV immunity after acute infection have only included patients presenting with symptomatic infection. However, a large proportion of patients are found to have immunity to HBV without having had any prior symptoms of acute infection. Although the duration since the initial exposure to HBV was not always known, a number of HIV-negative patients in the potentially heterogeneous group in the present study had preserved HBV-specific responses over the course of many years. Although we cannot exclude the possibility that HIV-positive patients had a longer interval between resolution of HBV infection and the time that samples were obtained, the efficient long-term preservation of HBV-specific $CD8^+$ T cell responses makes this unlikely to account for their lack of responsiveness.

Data describing the impact of HIV on responses to other viruses suggest that the persistence of detectable responses is related to the magnitude of antigen-specific response generated by each virus. For example, Epstein-Barr virus (EBV) and cytomegalovirus (CMV) are both associated with stable high-frequency memory cytotoxic T lymphocyte (CTL) populations, which, although reduced by HIV infection, remain easily detectable [18, 25]. Since the responses generated in association with control of HBV infection are typically lower in frequency than those to viruses such as EBV and CMV, it is not surprising that few remain detectable in HIV-positive patients. A recent study [26] including long-term follow-up of HIV-positive patients revealed maintenance of numbers but functional impairment of the EBV-specific $CD8^+$ T cell response associated with subtle increases in EBV load and progression to non-Hodgkin lymphoma. Thus, careful long-term assessment of HIV-positive, HBV-immune patients would be required to examine the virological impact of the impaired HBV-specific $CD8^+$ T cell response in this group of patients. A reduction of HBV-specific $CD8^+$ T cell responses after HIV infection would be consistent with the demonstrated ability of HIV to induce apoptosis of CTLs of unrelated specificities through FasL-mediated [27] and tumor necrosis factor–mediated [28] counterattack. Similarly,

data from murine models show that heterologous viral infections can quantitatively and qualitatively alter the memory pool of existing antiviral $CD8^+$ [29, 30].

Our previous study of HIV-positive patients with ongoing HBV infection revealed the potential to recover some HBV-specific T cell responses in patients receiving HAART, in association with the reduction in HBV load induced by anti-HBV agents [20]. By contrast, in the present study, we investigated a group of patients in whom HBV load was already efficiently suppressed, to dissect the potential contribution of HAART-mediated immune reconstitution to the restoration of HBV-specific T cell responses. There is evidence from studies of the herpes viridae (EBV, CMV, and Kaposi sarcoma–associated herpesvirus) that HAART can restore specific T cell frequencies [17, 18, 31, 32], and this correlates with a decrease in end-organ disease [4, 33, 34]. The 2 patients studied cross-sectionally and 2 of the 4 patients studied longitudinally restored functionally active HBV-specific $CD8^+$ T cell responses that had expansion potential after in vitro stimulation. That $CD8^+$ T cell responses were reconstituted only in a proportion of these HBV-immune patients is in accordance with our findings in HIV-negative patients without a history of symptomatic HBV infection and are in line with those of a study of the impact of HIV infection and antiretroviral therapy on responses to *Mycobacterium avium* complex [35].

An important contribution to the reconstitution of HBV-specific $CD8^+$ T cell responses is likely to be a restoration of $CD4^+$ T cell help associated with the increasing $CD4^+$ T cell counts. T cell help is known to be important for maintaining functionally active $CD8^+$ T cell responses [36], and recent data suggest that IL-2–producing $CD4^+$ T cells play a key role in antiviral immunity [21, 37]. In both of the patients with restoration of HBV-specific $CD8^+$ T cell responses, we observed a concomitant reconstitution of IFN-γ–positive and IL-2–positive $CD4^+$ T cell responses to HBcAg and HBsAg after the start of HAART. These HBV-specific $CD4^+$ T cell responses were similarly observed in a patient receiving an antiretroviral combination that did not include lamivudine or other drugs with anti-HBV activity. Importantly, we demonstrated the potential for long-term maintenance of both $CD4^+$ and $CD8^+$ T cells during prolonged follow-up of 2 patients, in contrast to the rapid but short-lived effects of lamivudine on HBV-specific T cell function reported in patients with ongoing HBV infection [20, 38]. There is a temporal correlation between the reconstitution of T cell responses observed in the present study and that seen in other studies of HAART-mediated reconstitution of T cell function (reviewed in [39]), with initial reconstitution at ~3 months and subsequent reconstitution continuing gradually for at least 2 years [40]. These findings should be extended to a larger group of patients, since the present study was not powered to detect an effect of $CD4^+$ T cell count nadir, magnitude of changes in $CD4^+$ T cell count, or HIV load on the extent of HBV-specific T cell reconstitution. However, it is worth noting that the patient with the most effective reconstitution of HBV-specific $CD4^+$ and $CD8^+$ T cell responses was the one who started HAART with the highest $CD4^+$ T cell count nadir and the lowest HIV load and who achieved the most efficient containment of HIV to undetectable levels.

The findings of the present study could be extrapolated to suggest that, in patients treated for HBV/HIV coinfection, reconstitution of HBV-specific T cell responses may involve 2 distinct components: the reconstitution of responses associated with reduction in HBV load [20], as seen in treatment of HBV monoinfection [41, 42], and the more gradual, sustained reconstitution associated with the prolonged suppression of HIV viremia and reconstitution of HBV-specific $CD4^+$ T cell responses demonstrated here. Thus, antiretroviral therapy can lead to an increase in functional $CD4^+$ and $CD8^+$ T cell responses to HBV infection, supporting the potential of HAART to reconstitute immune responses to clinically important pathogens.

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References

1. Thio CL, Seaberg EC, Skolasky R Jr, et al. HIV-1, hepatitis B virus, and risk of liver-related mortality in the Multicenter Cohort Study (MACS). Lancet **2002**; 360:1921–6.
2. Puoti M, Spinetti A, Ghezzi A, et al. Mortality for liver disease in patients with HIV infection: a cohort study. J Acquir Immune Defic Syndr **2000**; 24:211–7.
3. Martin-Carbonero L, Soriano V, Valencia E, Garcia-Samaniego J, Lopez M, Gonzalez-Lahoz J. Increasing impact of chronic viral hepatitis on hospital admissions and mortality among HIV-infected patients. AIDS Res Hum Retroviruses **2001**; 17:1467–71.
4. Palella FJ Jr, Delaney KM, Moorman AC, et al. Declining morbidity and mortality among patients with advanced human immunodeficiency virus infection. HIV Outpatient Study Investigators. N Engl J Med **1998**; 338:853–60.
5. Hadler SC, Judson FN, O'Malley PM, et al. Outcome of hepatitis B virus infection in homosexual men and its relation to prior human immunodeficiency virus infection. J Infect Dis **1991**; 163:454–9.
6. Scharschmidt BF, Held MJ, Hollander HH, et al. Hepatitis B in patients with HIV infection: relationship to AIDS and patient survival. Ann Intern Med **1992**; 117:837–8.
7. Colin JF, Cazals-Hatem D, Loriot MA, et al. Influence of human immunodeficiency virus infection on chronic hepatitis B in homosexual men. Hepatology **1999**; 29:1306–10.
8. Gilson RJ, Hawkins AE, Beecham MR, et al. Interactions between HIV and hepatitis B virus in homosexual men: effects on the natural history of infection. AIDS **1997**; 11:597–606.
9. Thimme R, Wieland S, Steiger C, et al. $CD8^+$ T cells mediate viral clearance and disease pathogenesis during acute hepatitis B virus infection. J Virol **2003**; 77:68–76.
10. Maini MK, Boni C, Lee CK, et al. The role of virus-specific $CD8^+$ cells in liver damage and viral control during persistent hepatitis B virus infection. J Exp Med **2000**; 191:1269–80.

11. Penna A, Artini M, Cavalli A, et al. Long-lasting memory T cell responses following self-limited acute hepatitis B. J Clin Invest **1996**; 98: 1185–94.
12. Rehermann B, Ferrari C, Pasquinelli C, Chisari FV. The hepatitis B virus persists for decades after patients' recovery from acute viral hepatitis despite active maintenance of a cytotoxic T-lymphocyte response. Nat Med **1996**; 2:1104–8.
13. Francisci D, Baldelli F, Papili R, Stagni G, Pauluzzi S. Prevalence of HBV, HDV and HCV hepatitis markers in HIV-positive patients. Eur J Epidemiol **1995**; 11:123–6.
14. Penna A, Chisari FV, Bertoletti A, et al. Cytotoxic T lymphocytes recognize an HLA-A2–restricted epitope within the hepatitis B virus nucleocapsid antigen. J Exp Med **1991**; 174:1565–70.
15. Nayersina R, Fowler P, Guilhot S, et al. HLA A2 restricted cytotoxic T lymphocyte responses to multiple hepatitis B surface antigen epitopes during hepatitis B virus infection. J Immunol **1993**; 150:4659–71.
16. Rehermann B, Fowler P, Sidney J, et al. The cytotoxic T lymphocyte response to multiple hepatitis B virus polymerase epitopes during and after acute viral hepatitis. J Exp Med **1995**; 181:1047–58.
17. Wilkinson J, Cope A, Gill J, et al. Identification of Kaposi's sarcoma–associated herpesvirus (KSHV)–specific cytotoxic T-lymphocyte epitopes and evaluation of reconstitution of KSHV-specific responses in human immunodeficiency virus type 1–infected patients receiving highly active antiretroviral therapy. J Virol **2002**; 76:2634–40.
18. Dalod M, Dupuis M, Deschemin JC, et al. Broad, intense anti–human immunodeficiency virus (HIV) ex vivo $CD8^+$ responses in HIV type 1–infected patients: comparison with anti–Epstein-Barr virus responses and changes during antiretroviral therapy. J Virol **1999**; 73:7108–16.
19. Kostense S, Otto SA, Knol GJ, et al. Functional restoration of human immunodeficiency virus and Epstein-Barr virus–specific $CD8^+$ T cells during highly active antiretroviral therapy is associated with an increase in $CD4^+$ T cells. Eur J Immunol **2002**; 32:1080–9.
20. Lascar RM, Gilson RJ, Lopes AR, Bertoletti A, Maini MK. Reconstitution of hepatitis B virus (HBV)–specific T cell responses with treatment of human immunodeficiency virus/HBV coinfection. J Infect Dis **2003**; 188:1815–9.
21. Day CL, Walker BD. Progress in defining CD4 helper cell responses in chronic viral infections. J Exp Med **2003**; 198:1773–7.
22. Maini MK, Boni C, Ogg GS, et al. Direct ex vivo analysis of hepatitis B virus–specific $CD8^+$ T cells associated with the control of infection. Gastroenterology **1999**; 117:1386–96.
23. Chazouilleres O, Mamish D, Kim M, et al. "Occult" hepatitis B virus as source of infection in liver transplant recipients. Lancet **1994**; 343: 142–6.
24. Waite J, Gilson RJ, Weller IV, et al. Hepatitis B virus reactivation or reinfection associated with HIV-1 infection. AIDS **1988**; 2:443–8.
25. Komanduri KV, Donahoe SM, Moretto WJ, et al. Direct measurement of CD4+ and CD8+ T-cell responses to CMV in HIV-1–infected subjects. Virology **2001**; 279:459–70.
26. van Baarle D, Hovenkamp E, Callan MF, et al. Dysfunctional Epstein-Barr virus (EBV)–specific $CD8^+$ T lymphocytes and increased EBV load in HIV-1 infected individuals progressing to AIDS-related non-Hodgkin lymphoma. Blood **2001**; 98:146–55.
27. Xu XN, Screaton GR, McMichael AJ. Virus infections: escape, resistance, and counterattack. Immunity **2001**; 15:867–70.
28. Herbein G, Mahlknecht U, Batliwalla F, et al. Apoptosis of $CD8^+$ T cells is mediated by macrophages through interaction of HIV gp120 with chemokine receptor CXCR4. Nature **1998**; 395:189–94.
29. Selin LK, Lin MY, Kraemer KA, et al. Attrition of T cell memory: selective loss of LCMV epitope–specific memory CD8 T cells following infections with heterologous viruses. Immunity **1999**; 11:733–42.
30. McNally JM, Zarozinski CC, Lin MY, Brehm MA, Chen HD, Welsh RM. Attrition of bystander CD8 T cells during virus-induced T-cell and interferon responses. J Virol **2001**; 75:5965–76.
31. Rinaldo CR Jr, Huang XL, Fan Z, et al. Anti–human immunodeficiency virus type 1 (HIV-1) $CD8^+$ T-lymphocyte reactivity during combination antiretroviral therapy in HIV-1–infected patients with advanced immunodeficiency. J Virol **2000**; 74:4127–38.
32. Casazza JP, Betts MR, Picker LJ, Koup RA. Decay kinetics of human immunodeficiency virus–specific $CD8^+$ T cells in peripheral blood after initiation of highly active antiretroviral therapy. J Virol **2001**; 75:6508–16.
33. Komanduri KV, Viswanathan MN, Wieder ED, et al. Restoration of cytomegalovirus-specific $CD4^+$ T-lymphocyte responses after ganciclovir and highly active antiretroviral therapy in individuals infected with HIV-1. Nat Med **1998**; 4:953–6.
34. Ledergerber B, Telenti A, Egger M. Risk of HIV related Kaposi's sarcoma and non-Hodgkin's lymphoma with potent antiretroviral therapy: prospective cohort study. Swiss HIV Cohort Study. BMJ **1999**; 319:23–4.
35. Havlir DV, Schrier RD, Torriani FJ, Chervenak K, Hwang JY, Boom WH. Effect of potent antiretroviral therapy on immune responses to *Mycobacterium avium* in human immunodeficiency virus–infected subjects. J Infect Dis **2000**; 182:1658–63.
36. Kalams SA, Walker BD. The critical need for CD4 help in maintaining effective cytotoxic T lymphocyte responses. J Exp Med **1998**; 188: 2199–204.
37. Younes SA, Yassine-Diab B, Dumont AR, et al. HIV-1 viremia prevents the establishment of interleukin 2–producing HIV-specific memory $CD4^+$ T cells endowed with proliferative capacity. J Exp Med **2003**; 198:1909–22.
38. Boni C, Penna A, Bertoletti A, et al. Transient restoration of anti-viral T cell responses induced by lamivudine therapy in chronic hepatitis B. J Hepatol **2003**; 39:595–605.
39. Carcelain G, Debre P, Autran B. Reconstitution of $CD4^+$ T lymphocytes in HIV-infected individuals following antiretroviral therapy. Curr Opin Immunol **2001**; 13:483–8.
40. Notermans DW, Pakker NG, Hamann D, et al. Immune reconstitution after 2 years of successful potent antiretroviral therapy in previously untreated human immunodeficiency virus type 1–infected adults. J Infect Dis **1999**; 180:1050–6.
41. Boni C, Bertoletti A, Penna A, et al. Lamivudine treatment can restore T cell responsiveness in chronic hepatitis B. J Clin Invest **1998**; 102: 968–75.
42. Boni C, Penna A, Ogg GS, et al. Lamivudine treatment can overcome cytotoxic T-cell hyporesponsiveness in chronic hepatitis B: new perspectives for immune therapy. Hepatology **2001**; 33:963–71.