Impaired Quality of the Hepatitis B Virus (HBV)-Specific T-Cell Response in Human Immunodeficiency Virus Type 1-HBV Coinfection

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Hepatitis B virus (HBV)-specific T cells play a key role both in the control of HBV replication and in the pathogenesis of liver disease. Human immunodeficiency virus type 1 (HIV-1) coinfection and the presence or absence of HBV e (precore) antigen (HBeAg) significantly alter the natural history of chronic HBV infection. We examined the HBV-specific T-cell responses in treatment-naive HBeAg-positive and HBeAg-negative HIV-1–HBV-coinfected (n = 24) and HBV-monoinfected (n = 39) Asian patients. Peripheral blood was stimulated with an overlapping peptide library for the whole HBV genome, and tumor necrosis factor alpha and gamma interferon cytokine expression in CD8+ T cells was measured by intracellular cytokine staining and flow cytometry. There was no difference in the overall magnitude of the HBV-specific T-cell responses, but the quality of the response was significantly impaired in HIV-1–HBV-coinfected patients compared with mono-infected patients. In coinfected patients, HBV-specific T cells rarely produced more than one cytokine and responded to fewer HBV proteins than in monoinfected patients. Overall, the frequency and quality of the HBV-specific T-cell responses increased with a higher CD4+ T-cell count (P = 0.018 and 0.032, respectively). There was no relationship between circulating HBV-specific T cells and liver damage as measured by activity and fibrosis scores, and the HBV-specific T-cell responses were not significantly different in patients with either HBeAg-positive or HBeAg-negative disease. The quality of the HBV-specific T-cell response is impaired in the setting of HIV-1–HBV coinfection and is related to the CD4+ T-cell count.

There are 40 million people worldwide infected with human immunodeficiency virus type 1 (HIV-1), and 6 to 15% of HIV-1-infected patients are also chronically infected with hepatitis B virus (HBV) (13, 20, 35, 38, 40–42, 47, 50, 61, 69). The highest rates of coinfection with HIV-1 and HBV are in Asia and Africa, where HBV is endemic (33, 68). Following the introduction of highly active antiretroviral therapy (HAART), liver disease is now the major cause of non-AIDS-related deaths in HIV-1-infected patients (12, 13, 38, 59, 65).

Coinfection of HBV with HIV-1 alters the natural history of HBV infection. Individuals with HIV–1–HBV coinfection seroconvert from HBV e (precore) antigen (HBeAg) to HBV e antibody less frequently and have higher HBV DNA levels but lower levels of alanine aminotransferase (ALT) and milder necroinflammatory activity on histology than those infected with HBV alone (18, 26, 49). Progression to cirrhosis, however, seems to be more rapid and more common, and liver-related mortality is higher, in HIV–1–HBV coinfection than with either infection alone (47, 59). HBeAg is an accessory protein of HBV and is not required for viral replication or infection; however, chronic HBV infection typically is divided into two distinct phases: HBeAg positive and HBeAg negative (reviewed in reference 15). Most natural history studies of HIV–1–HBV coinfection to date have primarily focused on HBeAg-positive patients from non-Asian countries (23, 44, 46).

We previously developed an overlapping peptide library for the HBV genome to detect HBV-specific CD4+ and CD8+ T-cell responses to all HBV gene products from multiple HBV genotypes (17). In a small cross-sectional study of patients recruited in Australia, we found that in coinfected patients, HBV-specific CD4+ T-cell responses, as measured by gamma interferon (IFN-γ) production, were diminished compared to those seen in HBV-monoinfected patients (17). However, patients had varying lengths of exposure to anti-HBV-active HAART at the time of analysis. In this study, therefore, we aimed to characterize the HBV-specific T-cell response in untreated HBeAg-positive and HBeAg-negative HIV–1–HBV-coinfected patients and to determine the relationship between

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the HBV-specific immune response, HBeAg status, and liver disease.

MATERIALS AND METHODS

**Patient population.** HIV-1–HBV-coinfected patients (n = 24) were recruited from Chulalongkorn Hospital and the Thai Red Cross HIV Research Centre, Bangkok, Thailand. All of the patients had been referred for consideration of initiation of anti-HBV and/or anti-HIV-1 treatment. ALT was not an inclusion criterion, given previous reports of lower ALT in the setting of HIV-1–HBV coinfection (18, 26, 47, 49). Patients in Bangkok were recruited as part of two prospective randomized clinical trials for initiation of HBV-active HAART (Te- notovir in Coinfection [n = 13] and HIV-NAT 023 [n = 11]; both investigator-initiated studies were funded by Gilead Sciences, San Francisco, CA). Participation was obtained with hospital ethics committee approval and signed consent. Patients who were hepatitis C virus (HCV) antibody positive were excluded from the study. All patients were anti-HBV therapy and HAART naive.

HBV-specific T cells were also evaluated in HBV-monoinfected patients. Asian patients with chronic HBV infection (defined as HBV surface antigen [HBeAg] positive on at least two occasions more than 6 months apart) and HBV DNA of $>$357 IU ml$^{-1}$ who were HIV-1 and HCV antibody negative (n = 35) were recruited from hospital outpatient clinics at Royal Melbourne Hospital, St. Vincent’s Hospital, and Alfred Hospital, Victoria, Australia.

**Measuring cytokine production by ICS.** Intracellular-cytokine staining (ICS) was performed as previously described by stimulating fresh blood with an overlapping peptide library for the HBV proteome, including genotypes A, B, C, and D (16, 17) and HIV-1 Gag (obtained through the AIDS Research and Reference Reagent Program, Division of AIDS, National Institute of Allergy and Infectious Diseases, National Institutes of Health), to define cytokine-producing HBV-specific and HIV-1 Gag-specific CD8$^+$ T cells. Dimethyl sulfoxide with costimulatory molecules (CD28 and CD49d monoclonal antibodies) was used as a negative control, and pokeweed mitogen (Sigma) and staphylococcal enterotoxin B (Sigma) were used as positive controls. Fresh whole blood was stimulated for a total of 7 hours, with brefeldin A (Sigma) added after the first 2 hours of stimulation. The blood was stained with anti-CD8–peridinin chlorophyll protein, anti-CD4–phycoerythrin, and anti-CD3–fluorescein isothiocyanate (all from BD Biosciences) for 30 min at room temperature. After 30 min of incubation in the dark at room temperature, the blood was treated for 10 min at room temperature with FACS Lysing Solution (BD Biosciences) for red blood cell lysis. The cells were permeabilized with FACS Permeabilizing Buffer (BD Biosciences) and incubated in the dark at room temperature for 10 min. The cells were then stained intracellularly with anti-IFN-γ–fluorescein isothiocyanate and anti-tumor necrosis factor alpha (TNF-α)–allophycocyanin (both from BD Biosciences) for 30 min at room temperature. In the dark at room temperature and fixed with 1% paraformaldehyde before acquisition.

**Flow cytometry analysis.** All ICS data were acquired on a FACSCalibur within 18 h of staining and were analyzed using CellQuest (BD Biosciences) or Weseal v2 (Walter Eliza Hall Institute, Parkville, Australia). The cells were gated initially on lymphocytes, as determined by forward and side scatter, and where possible, at least 100,000 small lymphocytes were collected (Fig. 1). In most cases, 10,000 to 30,000 CD8$^+$ T cells were collected for analysis. Antigen-specific cells were calculated as a percentage of cytokine$^+$ CD8$^+$ T cells. A positive response was considered to be $>$0.05% cytokine$^+$ CD8$^+$ T cells above background (the response to stimulation with dimethyl sulfoxide and costimulatory molecules alone) and also at least twofold above background. (C) Representative positive responses are shown for production of TNF-α and IFN-γ from CD8$^+$ T cells in HBV-monoinfected (left) and coinfected (right) individuals.

**Statistical analysis.** Statistical analysis was performed using SPSS for Windows version 11.5.0 (Lead Technologies, Inc., Chicago, IL) and Prism for Windows version 5.01 (GraphPad Software, Inc., La Jolla, CA). Comparisons of HBV DNA, ALT, and the magnitude and breadth of responses between two groups were performed using the Mann-Whitney U test. Nominal values were compared by the Fisher exact test if the sample contained a subpopulation that was less than four or by the χ$^2$ test if all subpopulations sizes were not less than four. Correlations were examined using Spearman’s rho test for nonparametric values. All comparisons among three groups were analyzed using one-way analysis of variance.

**RESULTS**

**Patient characteristics.** Demographic and clinical details of the HIV-1–HBV-coinfected (n = 24) and HBV-monoinfected (n = 35) patients studied are summarized in Tables 1 and 2. All patients were of Asian ethnicity and were mainly infected with HBV genotypes B and C. However, coinfect patients were...
more commonly infected with HBV genotype C than B compared to HBV-monoinfected patients \( (P < 0.001) \), were significantly younger \( (P = 0.046) \), and had significantly higher HBV DNA levels \( (P < 0.002) \). The major mutation observed in both HBV-monoinfected and HIV-1–HBV-coinfected HBeAg-negative patients was the G1896A mutation. Other mutations in the basal core promoter, including A1762T plus G1764A, G1764A plus C1766G, and G1764A mutation combinations, were also identified with similar frequencies in both HBV-monoinfected and HIV-1–HBV-coinfected patients. In the infected patients, the median (range) CD4\(^+\) T-cell count was 60 (6 to 359) cells \( \mu l^{-1} \), and HIV-1 RNA was 6.26 \( \times 10^4 \) (1.19 \( \times 10^2 \) to 5.00 \( \times 10^5 \)) copies \( ml^{-1} \).

### Impaired quality, but not magnitude, of HBV-specific CD8\(^+\) T-cell responses in HIV-1–HBV coinfecion

HBV-specific CD8\(^+\) T-cell responses were low and infrequent in both uninfected infected and monoinfected patients, with no significant difference (Fig. 2A and B). Although the coinfected patients had very low CD4\(^+\) T-cell counts, IFN-\( \gamma \) CD8\(^+\) T-cell responses to Gag peptides were frequently detected and were significantly greater in magnitude and frequency than responses to the full HBV genome (magnitude: \( P = 0.007 \) and \( P = 0.08 \), respectively) (Fig. 2A). The quality of the HIV-1 Gag-specific response differed from that of the HBV-specific response, with greater magnitude and frequency of IFN-\( \gamma \) than of TNF-\( \alpha \) in the Gag-specific T cells \( (P = 0.001 \) and 0.006, respectively) (Fig. 2A and B). These data demonstrate that coinfected individuals were able to make a robust antigen-specific T-cell response to HIV-1 peptides, but not to HBV peptides. Therefore, the absence of an HBV-specific T-cell response in the coinfected patients was not explained by a global inability to respond to all foreign antigens.

To examine the specificity of the HBV-specific response, we calculated the mean response of all patients to each gene product (Fig. 3). The specificity of the response was quite different in HBV-monoinfected patients than in coinfected patients. Overall, HBV-specific T-cell responses were equally distributed across the four major proteins, and similar breadths and frequencies of responses to all proteins were observed in both HBeAg-positive and HBeAg-negative HBV-monoinfected patients. The HBV-specific T-cell response in coinfected patients was predominantly directed to the surface and precore peptide pools, with infrequent responses to polymerase and X protein peptide pools (Fig. 3). The specificities of the HBV-specific T-cell responses were different in HBeAg-positive and HBeAg-negative HBV-monoinfected patients. The HBV-specific T-cell response in coinfected patients was predominantly directed to the surface and precore peptide pools, with infrequent responses to polymerase and X protein peptide pools (Fig. 3).
**TABLE 2.** Demographic details of the cohort when separated according to HBeAg status

<table>
<thead>
<tr>
<th>Parameter</th>
<th>HIV-1–HBV coinfected</th>
<th></th>
<th>HIV-1–HBV coinfected</th>
<th></th>
<th>HIV-1–HBV coinfected</th>
<th></th>
<th>HBV monoinfected</th>
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<th>HBV monoinfected</th>
<th></th>
<th>P value&lt;sup&gt;a&lt;/sup&gt;</th>
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<tbody>
<tr>
<td>No. of patients</td>
<td>14</td>
<td>10</td>
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<td></td>
<td>14</td>
<td>25</td>
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<tr>
<td>Gender (no. male/female)</td>
<td>8/6 (21.5)</td>
<td>8/2 (22.2)</td>
<td>NS</td>
<td></td>
<td>7/7 (46.2)</td>
<td>22/2 (63.6)</td>
<td>0.006&lt;sup&gt;f&lt;/sup&gt;</td>
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<tr>
<td>Age [median (range)] (yr)</td>
<td>31 (21–44)</td>
<td>34 (25–56)</td>
<td>NS</td>
<td></td>
<td>30 (22–39)</td>
<td>47 (26–75)</td>
<td>&lt;0.001&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>Ethnicity (% Asian)</td>
<td>100.0</td>
<td>100.0</td>
<td>NS</td>
<td></td>
<td>85.7</td>
<td>92.0</td>
<td>NS</td>
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<td>ALT [median (range)] (U/liter)</td>
<td>39 (11–97)</td>
<td>46.5 (25–157)</td>
<td>NS</td>
<td></td>
<td>90 (19–2579)</td>
<td>56 (25–484)</td>
<td>NS</td>
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<tr>
<td>HBV DNA [median (range)] (IU/ml)</td>
<td>3.00 × 10^6 (1.70 × 10^5–5.00 × 10^6)</td>
<td>5.41 × 10^7 (1.79 × 10^3–6.00 × 10^10)</td>
<td>0.036&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>2.00 × 10^6 (5.22 × 10^5–9.00 × 10^5)</td>
<td>4.52 × 10^5 (1.26 × 10^4–3.00 × 10^5)</td>
<td>&lt;0.001&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>HBV genotype (no. positive/no. tested) (%)</td>
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<tr>
<td>Genotype A</td>
<td>0/14 (0.0)</td>
<td>0/9 (0.0)</td>
<td>NS</td>
<td></td>
<td>1/13 (7.7)</td>
<td>1/22 (4.5)</td>
<td>NS</td>
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<tr>
<td>Genotype B</td>
<td>3/14 (21.4)</td>
<td>2/9 (22.2)</td>
<td>NS</td>
<td></td>
<td>6/13 (46.2)</td>
<td>14/22 (63.6)</td>
<td>NS</td>
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<td>Genotype C</td>
<td>11/14 (78.6)</td>
<td>7/9 (77.8)</td>
<td>NS</td>
<td></td>
<td>5/13 (38.5)</td>
<td>2/22 (9.1)</td>
<td>NS</td>
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<td>Genotype D</td>
<td>0/14 (0.0)</td>
<td>0/9 (0.0)</td>
<td>NS</td>
<td></td>
<td>1/13 (7.7)</td>
<td>4/22 (18.2)</td>
<td>NS</td>
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<td>Genotype E</td>
<td>0/14 (0.0)</td>
<td>0/9 (0.0)</td>
<td>NS</td>
<td></td>
<td>0/15 (0.0)</td>
<td>1/22 (4.5)</td>
<td>NS</td>
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<td>Precore/BCP mutants (no. positive/no. tested) (%)</td>
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<td>Precore mutant&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0/12 (0.0)</td>
<td>4/5 (80.0)</td>
<td>2/12 (16.7)</td>
<td>16/20 (80)</td>
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<td>BCP mutant&lt;sup&gt;e&lt;/sup&gt;</td>
<td>3/13 (23.1)</td>
<td>4/5 (80.0)</td>
<td>2/12 (16.7)</td>
<td>11/21 (52.4)</td>
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<td>Liver biopsy</td>
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<td>A score [median (range)]</td>
<td>1.0 (0.0–3.0)</td>
<td>1.0 (1.0–2.0)</td>
<td>NS</td>
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<td>1.0 (1.0–3.0)</td>
<td>1.0 (0.0–3.0)</td>
<td>NS</td>
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<tr>
<td>F score [median (range)]</td>
<td>1.0 (0.0–4.0)</td>
<td>2.0 (0.0–3.0)</td>
<td>NS</td>
<td></td>
<td>1.0 (0.0–3.5)</td>
<td>1.0 (0.0–4.0)</td>
<td>NS</td>
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<tr>
<td>HIV RNA [median (range)] (copies/ml)</td>
<td>6.26 × 10^4 (2.18 × 10^4–5.00 × 10^5)</td>
<td>6.32 × 10^5 (1.19 × 10^5–5.00 × 10^5)</td>
<td>NS</td>
<td>NA&lt;sup&gt;a&lt;/sup&gt;</td>
<td>NA</td>
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<td>CD4&lt;sup&gt;+&lt;/sup&gt; T-cell count [median (range)] (cells/μl)</td>
<td>45 (6–359)</td>
<td>94 (9–325)</td>
<td>NS</td>
<td>NA</td>
<td>NA</td>
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<sup>a</sup> NS, not significant.

<sup>b</sup> Mann-Whitney U test.

<sup>c</sup> The number of patients sequenced for precore and basal core promoter (BCP) are not identical. In some patients, we were unable to amplify DNA for precore sequencing despite adequate amplification of the BCP sequencing.

<sup>d</sup> Known precore mutations associated with reduced or lacking HBeAg expression (G1896A mutation).

<sup>e</sup> Known BCP mutations associated with reduced or lacking HBeAg expression (including A1762T plus G1764A, A1762T plus G1764A plus T1753A, G1764A plus C1766G, and G1764A mutation combinations).

<sup>f</sup> Fisher's exact test.

<sup>g</sup> NA, not applicable.
IFN-γ and TNF-α responses in HBeAg-positive coinfected patients compared with HBeAg-positive monoinfected patients ($P = 0.006$ and $P = 0.018$, respectively) (data not shown).

Given that functional antiviral T cells are associated with the production of more than one antiviral cytokine (2, 4, 11), we then compared the percentages of patients that produced one or both cytokines (i.e., both IFN-γ and TNF-α) in response to HBV-specific stimulation. There was no significant difference in the proportions of patients who had no response to any HBV peptides in the HBV-monoinfected and HIV-1–HBV-coinfected patients ($n = 46\%$ and $58\%$, respectively; $P = 0.43$). However, when we compared the cytokine profiles of patients who responded to HBV peptides, HBV-monoinfected patients were more likely to produce both IFN-γ and TNF-α rather than a single cytokine ($P < 0.001$) (Fig. 4A), in contrast to coinfected patients, who rarely produced more than one cytokine ($P = 0.29$).

**Association between the CD4+ T-cell count and the HBV-specific response.** When we examined the relationship between clinical parameters and HBV-specific T cells in coinfected patients, we found that CD4+ T-cell counts were significantly higher in individuals who had a detectable HBV-specific cytokine response (for IFN-γ+ CD8+ T-cell responders, $P = 0.018$) (Fig. 4B), but there was no difference in either HIV-1 RNA, HBV DNA, or ALT in those with or without an HBV-specific T-cell response (data not shown). Individuals with a higher CD4+ T-cell count were also more likely to produce both IFN-γ and TNF-α than either cytokine alone ($P = 0.032$) (Fig. 4B). Taken together, these data suggest that immunocompetence, as assessed by the CD4+ T-cell count, was directly related to the detection and quality of the HBV-specific T-cell response in coinfected patients.

**Relationship of HBV-specific T cells to the presence of HBeAg and liver disease.** Given the known effects of HBeAg as an immunotoleragen (45) and the significant impact of HBeAg on the natural history of HBV disease, we also investigated the relationship of the presence or absence of HBeAg to the HBV-specific T-cell response. In coinfected patients, we found a significantly lower magnitude of HBV-specific TNF-α-positive CD8+ T cells in HBeAg-positive individuals than in HBeAg-negative patients ($P = 0.029$) (Fig. 5A), but there was no difference between HBeAg-positive and HBeAg-negative patients in the magnitude or frequency of the IFN-γ responses to HBV peptides ($P = 0.133$) (Fig. 5). In monoinfected patients, the HBV-specific T-cell responses were no different in HBeAg-positive and -negative patients (Fig. 5).

As liver disease is thought to be secondary to infiltration of both HBV-specific and non-HBV-specific T cells and/or the production of proinflammatory cytokines, we next examined the relationship between liver histology and the frequency of circulating HBV-specific T cells. Necroinflammatory activity (A score) and fibrosis (F score) were both positively correlated in all patients who had a liver biopsy performed (coinfected, $n = 16$, $R = 0.5499$, $P = 0.0273$; HBV monoinfected, $n = 35$, $R = 0.5795$, $P < 0.001$). We did not find any association between the A or F score and the magnitude of HBV-specific T-cell responses in either monoinfected ($P > 0.1$ for both A and F scores) or coinfected ($P > 0.1$ for both A and F scores) patients.

**DISCUSSION**

This is the first detailed study of HBV-specific immunity in HBeAg-positive and HBeAg-negative HIV-1–HBV-coinfected Asian patients. Both monoinfected and coinfected patients had weak and infrequently detected HBV-specific responses. There was a significant difference in the quality and breadth of the response but little difference in the magnitude of the response between mono- and coinfected patients. Overall, the
FIG. 3. Specificity of the HBV-specific T-cell response. (A) The mean magnitude of the HBV-specific T-cell responses to each HBV gene (precore/core, surface, X protein, and polymerase) for all coinfected and HBV-monoinfected patients (left graph) and the specificity of the response for HBeAg-positive and HBeAg-negative monoinfected (middle graph) and coinfected (right graph) patients. *, $P = 0.006$, and **, $P = 0.018$ for comparisons of the breadth of response in HBeAg-positive coinfected and HBV-monoinfected patients. (B) The mean magnitude of the HBV-specific response for each HBV gene separately.
magnitude, frequency, and quality of the HBV-specific T-cell responses decreased with a lower CD4 T-cell count. There was no relationship between circulating HBV-specific T cells and liver damage as measured by A and F scores, and there was little difference in the HBV-specific T-cell responses in the presence or absence of HBeAg. The quality of the HBV-specific T-cell response is impaired in the setting of HIV-1–HBV coinfection and is related to the degree of immunosuppression.

HBV-specific CD8 T-cell responses were infrequent and of small magnitude in both coinfected and HBV-monoinfected patients. In addition, detection of an HBV-specific T-cell response was significantly associated with a higher CD4 T-cell count, but with no other clinical parameter. A decrease in magnitude and altered quality of antigen-specific CD8 T-cell responses has been described in many chronic viral infections, including HIV-1, HCV, and HBV (11, 14, 25, 28, 51–53, 70). The low frequency of an HBV-specific response in coinfected patients was not explained by a limited capacity to mount an antigen-specific response, as Gag-specific IFN-γ responses were always present and were significantly greater in magnitude than HBV-specific responses, consistent with our previous findings in coinfected patients on HAART (17).

More recently, polyfunctionality, or the production of up to five cytokines from the same antigen-specific cell, has been associated with significantly better control of HIV-1 infection and slower disease progression (2, 4, 11). In this study, we were able to measure the production of only IFN-γ and TNF-α from HBV-specific T cells, because we had access to only a four-color flow cytometer in Thailand. We selected these two cytokines in preference to others, such as interleukin 2 (IL-2), because in our previous study of HBV-specific T cells in the blood and livers of HBV-monoinfected patients, we found that IFN-γ and TNF-α were produced at greater magnitude and frequency than either IL-2 or IL-10 by HBV-specific CD8 T cells (16). In addition, both IFN-γ and TNF-α have been dem-
onstrated to be important in the cytolytic and noncytolytic clearance of HBV (30). Finally, TNF-α is a proinflammatory cytokine, and therefore, in addition to its role as an antiviral cytokine, it may be important in facilitating fibrosis and liver disease progression (43, 54, 55). We found that in coinfected patients, HBV-specific CD8⁺ T cells rarely produced more than one cytokine. Future studies should incorporate the assessment of other cytokines, such as IL-2, RANTES, and CD107a, to fully assess whether HBV-specific T cells are polyfunctional.

Chronic HBV infection is generally associated with a narrow HBV-specific response, with the dominant response often being to precore protein (24, 48, 66). We were therefore surprised to find such a broad, although weak, HBV-specific CD8⁺ T-cell response in chronic HBV monoinfection. The earlier studies used either tetramers, whole antigens, limited pools of overlapping peptides, or known epitopes to identify HBV-specific CD8⁺ T cells as opposed to the complete genomic overlapping peptide library used in this study (24, 48, 66). In the coinfected patients, a T-cell response to fewer HBV proteins was detected than in monoinfected patients. As CD4⁺ T-cell help is critical for the maintenance of a broad and functional antigen-specific CD8⁺ T-cell response (9, 34, 39), a skewed CD8⁺ T-cell response in HIV-1-infected patients to many pathogens, including Mycobacterium tuberculosis, cytomegalovirus, and HCV, has been described (1, 27, 31, 32, 36, 56, 67). The loss of CD4⁺ T-cell help may therefore explain the skewed response to HBV antigens that we observed in this cohort of HIV-1–HBV-coinfected patients. The biological significance of this finding, and whether there is any relationship with clinical progression, remains to be determined.

In HBeAg-positive coinfected patients, responses were significantly less frequent, and nearly all responses were directed to the surface protein only. HBeAg has been antigens, limited described as an “immunotolerogen” and can suppress an active immune response to HBV. Previous mouse studies have shown that HBeAg can selectively deplete HBeAg and HBV core antigen (HBeAg)-specific CD4⁺ T cells via fatty acid synthase-mediated apoptosis (45). HBeAg can also suppress the innate immune response via downregulation of specific toll-like receptors (TLR), such as TLR2, as well as other costimulatory molecules (63). Therefore, HBeAg may potentially add to the suppressive effect of HIV-1 on the function of HBV-specific CD4⁺ T cells (17), leading to a more deregulated or skewed CD8⁺ T-cell response to HBV (29, 60), as is also observed in HIV-1–HCV coinfection (32, 36).

We found no relationship between the number or quality of circulating HBV-specific T cells and liver histology, which may have several explanations. First, overall liver disease activity was mild in both patient cohorts, with a median of 1 for both A and F scores. This may have limited the likelihood of finding a relationship, given the very few patients with significant liver disease. Second, recruitment of non-HBV-specific T cells (43, 57, 58) and activated NK cells (22) may be more important in the generation of liver damage than recruitment of HBV-specific T cells to the liver. In this study, we were unable to evaluate intrahepatic T cells or NK cells due to limited access to liver biopsy material. Accelerated disease progression in HIV-1–HBV coinfection may be related to other factors unrelated to an impaired HBV-specific T-cell response, including immune activation, which have recently been reported as important in liver disease progression in HIV-1–HCV coinfection (6) or direct HIV-1 infection of intrahepatic cells, including stellate cells and Kupffer cells (7, 64). Finally, the intrahepatic HIV-1-specific response, not the HBV-specific response, may be important in mediating liver damage, as was recently demonstrated in HIV-1–HCV coinfection (62).

There were several limitations in this study. First, although all patients were treatment naïve and were Asian, the two cohorts were significantly different with respect to age and HBV genotype and, in addition, were recruited in different countries. These factors may have confounded our findings. Second, we were unable to collect accurate data on the time of acquisition of HBV in both cohorts, potentially leading to unknown differences in the duration of HBV infection between the HBV-monoinfected and coinfected patients. However, based on previous epidemiological studies of HBV infection in Asia, it is highly likely that most of the individuals in this study acquired HBV at birth (68). Third, we examined HBV-specific T-cell responses only in blood and not in the intrahepatic compartment, the primary site of HBV infection. However, our recent work showed similar magnitudes of HBV-specific T-cell response in blood and liver (16). Finally, due to the low CD4⁺ T-cell count in the coinfected patients, we were not able to quantify HBV-specific CD4⁺ T-cell responses. The effect of HIV-1 on HBV-specific CD4⁺ T-cell responses may have been even more profound, as demonstrated in HIV-1–HCV coinfection (32, 36, 37), but we were unable to assess this in the current study (17). More sensitive methods, such as major histocompatibility complex class II tetramers, may ultimately be needed to dissect this.

In conclusion, although there was little difference in the magnitudes of the HBV-specific T-cell responses in monoinfected and coinfected patients, the quality of the HBV-specific T-cell response was impaired in HIV-1–HBV-coinfected patients, and detection of a response was significantly correlated with the CD4⁺ T-cell count. In contrast to HBV-monoinfected patients, HBV-specific T-cell responses in coinfected patients were not evenly targeted to all HBV proteins, and the HBV-specific T cells detected rarely produced more than one cytokine, consistent with a less functional phenotype. Further studies are required to determine if immune recovery post-HAART can augment HBV-specific immunity and whether recovery is associated with control of HBV replication and/or liver disease progression.

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REFERENCES


in liver damage and viral control during persistent hepatitis B virus infection. J. Exp. Med. 191:1269–1280.


