CD8⁺ Cell Responses to Hepatitis C Virus (HCV) in the Liver of Persons with HCV-HIV Coinfection versus HCV Monoinfection

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Objective. Cellular immune responses are difficult to detect in the peripheral blood of persons with chronic hepatitis C virus (HCV) infection. We sought to determine whether T cell responses were present in the liver of patients with human immunodeficiency virus (HIV) and HCV coinfection.

Methods. T cells were expanded from liver-biopsy samples from 10 patients coinfected with HIV and HCV (median CD4⁺ cell count, 456 cells/mm³) and 8 patients infected with HCV alone. CD8⁺ cell responses were detected by use of a modified enzyme-linked immunospot (ELISpot) assay with recombinant vaccinia virus, and CD4⁺ cell responses were detected by use of ELISpot with recombinant HCV proteins core, nonstructural (NS) 3, and NS5.

Results. Intrahepatic CD8⁺ cell responses to HCV were detected in 7 of 10 patients coinfected with HCV and HIV (median frequency, 638 spot-forming cells [sfc]/10⁶ cells) and were similar to those observed in patients singly infected with HCV (7/8; median, 647 sfc/10⁶ cells). Intrahepatic HCV-specific CD4⁺ cell responses were also comparable in both groups and correlated with the intrahepatic CD8⁺ cell responses (r = 0.59; P = .03).

Conclusion. HCV-specific CD8⁺ cell responses are present in the liver of persons with chronic HCV infection even when they are coinfected with HIV; these correlate with intrahepatic HCV-specific CD4⁺ cell responses.

Because of the shared routes of transmission, coinfection with hepatitis C virus (HCV) and HIV is common and is of increasing clinical relevance [1–3]. Coinfection with HIV leads to an increased risk of chronic HCV infection [4], increased HCV loads [5, 6], and an increased risk of progression to cirrhosis [7]. This increased risk of progression to cirrhosis is correlated with the degree of immunodeficiency—the fibrosis progression rate is highest in those individuals with a CD4⁺ cell count of <200 cells/mm³ [8], and the progression of liver disease may slow with immune reconstitution after the administration of antiretroviral therapy (ART) [9, 10].

Vigorous and polyclonal immune responses are clearly important in the spontaneous recovery from acute HCV infection [11–14] and probably play an important role in clearance after interferon (IFN) and ribavirin therapy [15, 16]. However, the role of the immune response in the progression of liver injury during HCV infection is, at present, controversial. Although clinical evidence in persons who are coinfected with HIV and HCV and those with other immunodeficiencies all of whom have more rapid disease progression, suggests that cellular immune responses play a role in limiting liver injury, the classic understanding of the pathogenesis of liver disease caused by HCV infection is that it is mediated by the cellular immune response [17–19]. Model systems that have used the constitutive expression of HCV in transgenic animals have suggested that HCV proteins are not directly cytotoxic [20, 21] and have demonstrated an absence of liver injury in the absence of a host immune response against the virus [22], with liver injury becoming apparent only after the adoptive transfer of HCV-specific cells into the animals [23].
In individuals with chronic HCV infection, HCV-specific CD4+ and CD8+ cell responses in the peripheral blood are weak and barely detectable, even by sensitive tests, such as those based on tetramers [24, 25]. However, numerous studies have shown that HCV-specific CD4+ and CD8+ cell responses are present in the liver in chronic HCV infection at much higher frequency than in the peripheral blood [26–30]. To date, a limited number of studies have examined the HCV-specific immune response in the setting of coinfection. HCV-specific responses are detectable only in the periphery in HIV-infected long-term non-progressors [31, 32]; in those with progressive HIV infection, even when they are receiving ART, responses in the peripheral blood are of very low magnitude and of much lower frequency than HIV-specific responses [33]. We have recently characterized intrahepatic cytokine production by HCV-specific CD4+ cells in the setting of coinfection [34], but, to our knowledge, there are no data available on intrahepatic CD8+ cell responses. In the present study, we sought to determine whether HCV-specific CD8+ cell responses were present in the liver of coinfected persons and whether there were qualitative differences in the intrahepatic CD4+ and CD8+ cell responses, compared with those in patients infected with HCV alone.

PATIENTS, MATERIALS, AND METHODS

Patients and samples. Liver and blood samples were obtained from 18 patients with chronic HCV infection who were undergoing routine liver biopsies for diagnostic purposes before receiving anti-HCV treatment. Eight patients were singly infected with HCV, and 10 patients were coinfected with HCV and HIV (table 1). All patients were HCV RNA positive, and none had evidence of clinical liver decompensation, including ascites, encephalopathy, jaundice, bleeding varices, or coagulopathy (prothrombin time, >3 s over control). Persons with other forms of liver disease—including hepatitis B virus (HBV) infection and alcoholism—and other immunosuppressive conditions—including malignancy, chronic renal failure requiring hemodialysis, organ transplant, and other comorbid diseases requiring immunosuppressive therapy—were excluded. The protocol was reviewed by the investigational review boards of the University of Cincinnati College of Medicine and the Beth Israel Deaconess Medical Center, and all patients gave informed consent for the collection of samples.

Vaccinia-HCV recombinant viruses, recombinant HCV proteins, and a control peptide pool. Vaccinia constructs (vv), which were used for the CD8 assays, were provided by Michael Houghton (Chiron) and Charles Rice (Rockefeller University, New York, NY). Six vaccinia-HCV recombinant viruses were constructed to express the HCV proteins spanning the entire HCV-1a genome: vv-core/E1 (aa 1–339), vv-E1(NS1)/NS2 (aa 347–906), vv-E2/NS2/NS3 (aa 364–1619), vv-NS4 (aa 1590–2050), vv-NS5A (aa 2005–2396), and vv-NS5B (aa 2396–3011). A vv expressing only Escherichia coli β-galactosidase gene (vv-lac) was used as a control. As a control to assess immune responses against recall antigens, the CD8+ cell response to other viruses were evaluated by use of a pool of 23 major histocompatibility class I–restricted T cell epitopes from human cytomegalovirus (CMV), Epstein Barr virus (EBV), and influenza virus (CEF; AIDS Reagent Program, National Institutes of Health [NIH]). The recombinant HCV proteins used for the CD4 assays were derived from HCV genotype 1b and included core (aa 1–115) and nonstructural (NS) proteins NS3 (aa 1007–1534) and NS5 (aa 2622–2868; Mikrogen).

Preparation of peripheral blood mononuclear cells (PBMCs) and liver-infiltrating lymphocytes. Liver-tissue samples were

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<th>Table 1. Characteristics for the two groups of studied persons coinfected with hepatitis C virus (HCV) and HIV and those singly infected with HCV.</th>
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<td>Characteristic</td>
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<td>Age, median (range), years</td>
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<td>Male/female sex</td>
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<td>CD4+ cell count, median (range), cells/mm^3</td>
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<td>HIV RNA, median (range), copies/mL</td>
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<td>Liver histological results, median (range)^b</td>
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NOTE. ALT, liver enzyme alanine transaminase; NS, not significant.
* All coinfected persons were treated for HIV infection.
^b Scored by use of the Metavir scoring system or the modified Ishak system, then converted to Metavir scores for comparison.
cut into 2 parts, and each part was cultured in 1 well of a 24-well plate in R-10-100 or R-10 medium (RPMI 1640 medium plus 10% heat-inactivated fetal calf serum, antibiotics, and HEPES buffer) supplemented with 100 IU/mL of recombinant interleukin-2 (AIDS Reagent Program, NIH). Bispecific monoclonal antibodies (MAbs) CD3,4B or CD3,8 (gift from Johnson Wong, Massachusetts General Hospital, Boston) were added to clonal antibodies (MAbs) CD3,4B or CD3,8 (gift from Johnson Wong, Massachusetts General Hospital, Boston) as a polyclonal stimulus of T cell proliferation. At no time during the expansion process were liver-infiltrating lymphocytes exposed to exogenous HCV antigens. Cells were cultured for ~5–6 weeks, then cryopreserved for further assays. PBMCs were isolated from blood by Ficoll-Hypaque (Amersham Bioscience) density-gradient centrifugation and were cryopreserved. These PBMCs were used to prepare the autologous antigen-presenting cells. For the CD8 assays, EBV-transformed B cell lines were established, as described elsewhere [26].

**IFN-γ enzyme-linked immunospot (ELISpot) assays.** For ELISpot assays, 96-well polyvinylidene difluorid plates (Millipore) were precoated with 100 μL of primary MAb anti–IFN-γ (Endogen) at a concentration of 5 μg/mL in PBS and incubated overnight at 4°C. Excess antibody was removed by 3 successive washes with PBS. Remaining binding sites on the wells were then saturated with R-10 for 30 min at 37°C. Enriched lymphocytes were plated in triplicate at 0.5 × 10^5 cells/well. The CD8-enriched liver-infiltrating lymphocytes were cocultured for 20 h with an equal number of autologous EBV-transformed B cell lines that had been infected for 1 h at 37°C with recombinant vaccinia-HCV vectors at an MOI of 5 pfu/cell or in presence of the CEF peptide pool (2 μg/mL). The CD4-enriched liver-infiltrating lymphocytes were cocultured for 48 h with an equal number of irradiated (3000 rads) autologous PBMCs in the presence of the recombinant HCV proteins (1 μg/mL). Positive control wells consisted of phytohemagglutinin (5 μg/mL; Sigma). Negative control wells consisted of EBV-transformed B cell lines alone or infected with vv-lac for the CD8 assays and buffer alone for the CD4 assays. After 20 h for the CD8 assays and 48 h for the CD4 assays, cells were removed by successive washes: 3 times with PBS, 3 times with PBS that contained 0.05% Tween 20, and 3 times with PBS. Then, 50 μL of biotin-conjugated secondary MAb anti–human IFN-γ (Endogen) was added to each well at a concentration of 0.2 μg/mL and incubated for 2 h at 37°C. The plates were rinsed 3 times with PBS; then, 100 μL of streptavidin alkaline phosphatase (1:1000 dilution; Sigma) was added for 1 h at 37°C. Plates were washed 3 times with PBS, then 50 μL of substrate (5-bromo-4-chloro-indolyl-phosphate/4-nitrobluetetrazolium liquid; Sigma) was added and incubated at room temperature until the appearance of blue spots, at which point they were rinsed with tap water. Antigen-specific spot-forming cell frequencies were measured on an automated microscope (Zeiss) and are expressed as values obtained after background subtraction. For the experiments with vaccinia, this background consisted of results observed with EBV-transformed B cell lines infected with lac. For the analysis of the CD8+ cell response to CEF, the background consisted of EBV-transformed B cell lines alone. For the CD4 assays, the background was the result observed with buffer alone.

Overall results were considered to be positive if a minimum of 50 sfc/1 × 10^6 cells were detected above background. This positive threshold was arbitrarily defined according to the positive threshold used for the HIV ELISpot experiments in peripheral blood [35] and is >3 SDs above any response observed in the peripheral blood of healthy donors or in the liver of 1 HCV-negative patient with HBV infection (data not shown), because it is not possible to obtain fresh, healthy human liver for comparison.

**Fluorescence-activated cell-sorting (FACS) analysis.** Analysis of the enriched T cells for CD8+ (CD3+CD8+) and CD4+ (CD3+CD4+) cell content were performed on 100,000 cells by use of fluo escent MAb anti–CD3 (Endogen) at a concentration of 5 μg/mL in 1× PBS and incubated overnight at 4°C. Excess antibody was removed by 2 successives washes with PBS. Remaining binding sites on the wells were then saturated with R-10 for 30 min at 37°C. Enriched lymphocytes were plated in triplicate at 0.5 × 10^5 cells/well. The CD8-enriched liver-infiltrating lymphocytes were cocultured for 20 h with an equal number of autologous EBV-transformed B cell lines that had been infected for 1 h at 37°C with recombinant vaccinia-HCV vectors at an MOI of 5 pfu/cell or in presence of the CEF peptide pool (2 μg/mL). The CD4-enriched liver-infiltrating lymphocytes were cocultured for 48 h with an equal number of irradiated (3000 rads) autologous PBMCs in the presence of the recombinant HCV proteins (1 μg/mL). Positive control wells consisted of phytohemagglutinin (5 μg/mL; Sigma). Negative control wells consisted of EBV-transformed B cell lines alone or infected with vv-lac for the CD8 assays and buffer alone for the CD4 assays. After 20 h for the CD8 assays and 48 h for the CD4 assays, cells were removed by successive washes: 3 times with PBS, 3 times with PBS that contained 0.05% Tween 20, and 3 times with PBS. Then, 50 μL of biotin-conjugated secondary MAb anti–human IFN-γ (Endogen) was added to each well at a concentration of 0.2 μg/mL and incubated for 2 h at 37°C. The plates were rinsed 3 times with PBS; then, 100 μL of streptavidin alkaline phosphatase (1:1000 dilution; Sigma) was added for 1 h at 37°C. Plates were washed 3 times with PBS, then 50 μL of substrate (5-bromo-4-chloro-indolyl-phosphate/4-nitrobluetetrazolium liquid; Sigma) was added and incubated at room temperature until the appearance of blue spots, at which point they were rinsed with tap water. Antigen-specific spot-forming cell frequencies were measured on an automated microscope (Zeiss) and are expressed as values obtained after background subtraction. For the experiments with vaccinia, this background consisted of results observed with EBV-transformed B cell lines infected with lac. For the analysis of the CD8+ cell response to CEF, the background consisted of EBV-transformed B cell lines alone. For the CD4 assays, the background was the result observed with buffer alone.

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**Statistical analysis.** The coinfected and HCV singly infected groups were compared by use of the Mann-Whitney U test for continuous data and Fisher’s exact test for categorical data. Spearman rank tests were performed for the correlations. Calculations were performed by use of STATview SAS PC software (version 6.01; SAS Institute), and P ≤ .05 was considered to be significant.

**RESULTS**

**Patient characteristics.** Patient characteristics at the time of study entry are shown in table 1. All the coinfected patients were treated for HIV, and most had undetectable HIV loads (median, <50 copies/mL; range, <50–74,073 copies/mL). CD4+ cell counts from the time of the liver biopsy were done only for the coinfected group and were relatively high in this cohort (median, 456 cells/mm3; range, 298–539 cells/mm3). Nadir CD4 values were not available. As expected, there was a trend toward higher HCV loads in the coinfected group, with median HCV loads of >1000 × 10^3 IU/mL, versus 218 × 10^3 IU/mL in the group in-
Intrahepatic CD8+ Cells in HCV and HIV

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Comparison of IFN-γ-producing intrahepatic CD8+ cell responses to HCV and CEF. Because too few T cells are recovered from typical liver specimens to directly measure virus-specific populations, we nonspecifically expanded CD8+ cells from the liver tissue. FACS analysis demonstrated that median percentages of the CD8+ cells enriched in this manner were 98% in both the HIV-HCV coinfected and HCV singly infected groups (data not shown). We first studied the intrahepatic CD8+ cell responses to HCV antigens that span the entire HCV genome using a modified ELSpot assay, with autologous EBV-immortalized B cell lines infected with 6 recombinant vaccinia-HCV viruses used to present the entire HCV genome. Seven of 10 HIV-HCV coinfected patients positively responded (>50 sfc/1 × 10^6) to at least 1 HCV construct, versus 7 of 8 HCV singly infected persons. CD8+ cells producing IFN-γ in response to each region of HCV were detected in both groups with highly variable frequencies; surprisingly, there was no significant difference in the median number of HCV-specific CD8+ cells between the 2 groups (NS5A, P = .08; for all other HCV antigens, P > .1) (figure 1). When we compared the summed values for all HCV proteins of spot-forming cells secreting IFN-γ, the median total number of intrahepatic CD8+ cells responding to HCV remained similar in both groups: 632 sfc/1 × 10^6 cells (range, 14–5700 sfc/1 × 10^6 cells) in HIV-HCV coinfected patients, versus 647 sfc/1 × 10^6 cells (range, 10–1941 sfc/1 × 10^6 cells) in HCV singly infected patients (P = .9) (figure 2). Intrahepatic CD8+ cell responses to the EBV-transformed B cell lines alone were not significantly different between the 2 groups. In contrast, the intrahepatic CD8+ cell responses to CEF, the pool of peptides from other viruses, were significantly higher in the HIV-HCV coinfected group (median, 1927 sfc/1 × 10^6 cells; range, 47–4000 sfc/1 × 10^6 cells) than in the HCV singly infected group (median, 392 sfc/1 × 10^6 cells; range, 0–1433 sfc/1 × 10^6 cells) (P = .04). Furthermore, overall intrahepatic CD8+ cell responses to CEF were not correlated with the total intrahepatic CD8+ cell responses to HCV (r = 0.15; P = .56).

Comparison of IFN-γ-producing intrahepatic CD4+ cell responses to HCV. We also nonspecifically expanded CD4+ cells from the liver tissue using the bi-specific MAb CD3,8. Median CD4+ cell percentages according to FACS analysis were 77% in both the HIV-HCV coinfected and HCV singly infected groups (data not shown). We studied the intrahepatic CD4+ cell responses to 3 HCV antigens, including the core and NS3 and NS5, using autologous irradiated PBMCs as antigen-presenting cells. One-half of the patients in each group responded (>50 sfc/1 × 10^6) to at least 1 HCV antigen. As with the CD8+ cell responses, the CD4+ cells producing IFN-γ in response to each of the 3 studied HCV proteins were detected with highly variable frequencies; medians and 75th and 90th percentiles. Results were compared by use of the Mann-Whitney U test.

Figure 1. Hepatitis C virus (HCV)-specific intrahepatic CD8+ cell responses evaluated by interferon (IFN)-γ enzyme-linked immunospot (ELISpot) assay for each HCV protein in the 2 groups of patients: 10 HIV-HCV coinfected and 8 HCV singly infected. Briefly, CD8+ cells were polyclonally expanded from the intrahepatic lymphocytes and tested for IFN-γ production in a modified ELISpot assay by use of autologous Epstein-Barr virus (EBV)-transformed B cell lines infected with recombinant vaccinia viruses expressing HCV core/E1, E2 (nonstructural [NS] 1)/NS2, E2/NS2/NS3, NS4, NS5a, and NS5b or a control gene (lac). HCV-specific IFN-γ production was determined by ELISpot assay, and results are expressed in no. of spot-forming cells (sfc) per 1 × 10^6 cells over background (result observed with vaccinia expressing the control gene lac). Individual analysis for each HCV protein are shown as medians and 75th and 90th percentiles. Results were compared by use of the Mann-Whitney U test.
frequencies in both groups, and there was no significant difference in the median numbers of HCV-specific CD4+ cells between the 2 groups ($P > .1$) (figure 3). Summed values per person of spot-forming cells secreting IFN-γ to core, NS3, and NS5 were also not significantly different between the 2 groups: 32 sfc/1 $\times 10^6$ cells (range, 0–450 sfc/1 $\times 10^6$ cells) in HIV-HCV coinfected patients, versus 81 sfc/1 $\times 10^6$ cells (range, 0–1066 sfc/1 $\times 10^6$ cells) in HCV singly infected patients ($P = .8$) (figure 3).

**Correlations between the HCV-specific intrahepatic CD8+ and CD4+ cell responses.** Given that the activity of CD8+ cells is based on CD4+ cells, we sought to determine whether there was a relationship between these 2 populations. Interestingly,
Figure 4. Correlation between total intrahepatic hepatitis C virus (HCV)-specific CD8+ and CD4+ cell responses in 8 HIV-HCV coinfected and 6 HCV singly infected patients. Total HCV-specific CD8+ and CD4+ cell responses were measured by use of an interferon (IFN)-γ enzyme-linked immunospot (ELISpot) assay, as described in Patients, Materials, and Methods. Correlation was assessed between the total HCV-specific intrahepatic CD8+ and CD4+ responses in all patients by use of Spearman’s rank test. There was a significant positive correlation between CD8+ and CD4+ cell responses.

sfc, spot-forming cells.

overall total numbers of HCV-specific intrahepatic CD8+ cells were positively correlated with the total number of HCV-specific intrahepatic CD4+ Th1 cells \((r = 0.596; P = .034)\) (figure 4), but neither HCV-specific intrahepatic CD8+ nor CD4+ cell responses were correlated with the peripheral CD4+ cell counts \((r = -0.1; P > .8)\) in persons with HIV-HCV coinfection (data not shown). No correlations were found either with the intrahepatic IFN-γ HCV-specific CD8+ or CD4+ cell responses and plasma HCV loads or liver disease (histology or liver enzymes) in this small cohort.

DISCUSSION

In the present study, our objective was to determine whether there were qualitative differences in the intrahepatic T cell response between patients with HIV-HCV coinfection and those infected with HCV alone. We measured the HCV-specific T cell response in liver lymphocytes using a modified IFN-γ ELISpot assay to detect CD8+ cell responses against the entire HCV genome and CD4+ cell responses against 3 recombinant HCV proteins—core, NS3, and NS5. We found that HCV-specific CD8+ and CD4+ cell responses were present and recognized multiple antigens in the liver of patients with HCV-HIV coinfection. We then compared the intrahepatic T cell responses between the HCV-HIV coinfected and HCV singly infected patients. Despite the presence of HIV, there was no difference in the HCV-specific CD8+ or CD4+ cell response in our cohort. Therefore, we have confirmed that HCV-specific responses are not absent in HCV-HIV coinfection but, rather, appear to compartmentalize to the liver, as they do in HCV infection alone. Although we did not specifically compare the response in the liver and peripheral blood in the present study, we and others have previously demonstrated an attenuated response in the peripheral blood of coinfected patients [34, 36, 37]. Moreover, we found a positive correlation between the HCV-specific CD4+ Th1 cell responses and the CD8+ cell responses but not with the peripheral CD4+ cell counts.

One of the limitations of measuring T cell responses in the liver is the low number of cells obtained in clinically achievable amounts of liver tissue, which required us to expand cells. We believe that our expansion method does not prime naïve T cells, given that we were unable to expand HCV-reactive cells from the peripheral blood of patients naïve for HCV or from the liver of 1 patient who was HCV negative but had HBV infection. Because of limitations on the numbers of cells that can be directly isolated from liver tissue, a comparison of T cell responses before and after the expansion of intrahepatic cells was not possible. However, we previously expanded CD4+ cells from PBMCs and found that HCV-specific CD4+ cell responses were quantitatively expanded, as would be expected for the CD4-enriched PBMCs, and there were no significant qualitative differences in antigenic specificity before and after expansion [34]. The finding that responses against a pool of recall antigens was higher in the coinfected patients was a surprise, and we speculate that this higher frequency is due to a number of factors related to HIV itself or the care of HIV, such as more frequent CMV viremia or better rates of immunization.
against influenza either of which could have contributed to the higher response against this pool of peptides. It is possible that some aspect of CD8+ cells in HIV allows for the easier expansion of CEF-reactive cells, although this is less likely, because the intrahepatic CD8+ cell responses to EBV-transformed B cells alone that reflect the responses to EBV (one of the CEF components) were similar in the 2 groups of patients.

In the present cohort, the presence of HIV-related immunodeficiency had no effect on HCV-specific immune responses. However, all coinfected patients in our study were receiving ART; as a consequence, most of them had undetectable HIV loads and relatively high, although not normal, CD4+ cell counts. In this limited study, we cannot address the issue of whether the same results would be found in persons with profound immunodeficiency (CD4+ cell counts <200 cells/mm3). One of the difficulties with this particular type of study is that patients with more profound immunodeficiency are often not considered to be appropriate candidates for the treatment of HCV and do not routinely undergo liver biopsy. However, as more data on treatment response rates in patients with coinfection become available and as HIV providers realize the importance of liver biopsies in the determination of the stage of liver disease, we hope to extend these studies to patients with more severe immunodeficiency.

An interesting finding here is that HCV-specific CD8+ cell responses in the liver were positively correlated with intrahepatic CD4+ Th1 cell responses to HCV but not to the peripheral CD4+ cell count, which is consistent with the finding of a report that described the same correlation in the peripheral blood of HIV-infected long-term nonprogressors who were coinfected with HCV [31]. Together, this emphasizes the importance of Th1 responses in the maintenance of CD8+ cell activity in chronic HCV infection. Studies in chimpanzee models have indicated that HCV replication is prolonged in the absence of intrahepatic memory CD8+ cells [38] and that the capacity of the CD8+ cells to terminate infection is limited in the absence of adequate CD4+ cell help [39]. In HIV monoinfection, a positive association between HCV-specific CD8+ and CD4+ Th1 cell responses that inversely correlated with HIV loads has been described in untreated HIV-infected patients [40] as well as in patients receiving ART, who, despite detectable HIV viremia, maintained persistently low HIV loads and stable CD4+ cell counts ([41]). Of note, no correlation between the HCV-specific CD8+ cell responses and the peripheral CD4+ cell counts has been observed in ART-treated patients who have persistently low viral loads (N.A. et al., unpublished data). This lack of correlation with the CD4+ cell counts has also been described in untreated HIV-infected patients monitored before the advent of highly active ART [42], which suggests that CD4+ cell depletion does not directly influence the loss of virus-specific CD8+ cell responses.

One of the interesting questions raised by the present and other studies that suggest the compartmentalization of immune responses to the liver during chronic infection is why this should happen. One simple explanation might be that the major site of HCV replication is within hepatocytes, so the virus-specific cells are homing to the major site of antigen. However, HCV might also specifically up-regulate chemokines or their receptors that are involved in the homing of activated T cells to areas of inflammation [43]. For example, IFN-γ-inducible protein-10, a chemokine that recruits activated T cells, has recently been shown by in situ hybridization to be expressed in the liver during chronic HCV infection, with a concomitant increase of the ligand CXCR3 on infiltrating liver lymphocytes [44]. Similarly, the expression of HCV may specifically drive the expression of the CC chemokines RANTES and monocyte chemotactic protein–1 [45]. Other groups have reported high expression of both CXC and CC chemokines in activated T cells within the liver during HCV infection and the correlation of these markers with severity of liver injury [46, 47]. Costimulatory molecule interactions such as CD28/B7, Fas/Fas-ligand, and CD40/CD154 may also play an important role in the pathogenesis of HCV infection. Whether the presence of HIV alters chemokine or costimulatory expression in the HCV-infected liver represents an interesting area of future study.

In summary, the present results demonstrate the presence of broadly directed HCV-specific CD8+ cell responses in the liver of patients with chronic HCV infection, even in the presence of HIV coinfection, and suggest the importance of the CD4+ cell help for the maintenance of these CD8+ cell responses. Future studies will be needed to determine the role that these cells play in liver injury.

References