Association of Chronic Hepatitis C Infection With T-Cell Phenotypes in HIV-Negative and HIV-Positive Women

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Background: Hepatitis C virus (HCV) viremia is thought to have broad systemic effects on the cellular immune system that go beyond its impact on just those T cells that are HCV specific. However, previous studies of chronic HCV and circulating T-cell subsets (activation and differentiation phenotypes) in HIV negatives used general population controls, rather than a risk-appropriate comparison group. Studies in HIV positives did not address overall immune status (total $CD4^+$ count).

Methods: We used fresh blood from HIV-positive and at-risk HIV-negative women, with and without chronic HCV, to measure percentages of activated $CD4^+$ and $CD8^+$ T cells, Tregs, and T-cell differentiation phenotypes (naive, central memory, effector memory (EM), and terminally differentiated effector). This included 158 HIV negatives and 464 HIV positives, of whom 18 and 63, respectively, were HCV viremic.

Results: In multivariate models of HIV negatives, HCV viremia was associated with 25% fewer naive $CD4^+$ ($P = 0.03$), 33% more EM $CD4^+$ ($P = 0.0002$), and 37% fewer central memory $CD8^+$ ($P = 0.02$) T cells. Among HIV positives, we observed only 1 of these 3 relationships: higher percentage of EM $CD4^+$ among HCV viremic women. Furthermore, the association with EM $CD4^+$ among HIV positives was limited to individuals with diminished immune status (total $CD4^+$ count $\leq$500 cells/μL), as were associations of HCV viremia with higher percentages of activated $CD4^+$ and Tregs. Among HIV positives with high $CD4^+$ count, no significant associations were observed.

Conclusions: These data suggest that HCV viremia in HIV negatives is associated with accelerated T-cell differentiation, but among HIV positives, the impact of HCV viremia is less straightforward and varies by total $CD4^+$ count.

Key Words: hepatitis C virus, HIV, T cell, phenotype, activation, differentiation

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INTRODUCTION

T cells play an important role in the adaptive immune response to acute hepatitis C virus (HCV) infection. In particular, broad and sustained $CD4^+$ and $CD8^+$ T-cell responses against HCV antigens after acute infection have been prospectively associated with subsequent immune clearance of HCV viremia.[1,2] In individuals with chronic HCV infection, however, T-cell responses against HCV antigens are generally weak or even undetected despite ongoing viral replication.[2,3] Furthermore, a large fraction of HCV-specific $CD8^+$ T cells express cell surface markers of differentiation and exhaustion during chronic HCV infection, and these cells are susceptible to apoptosis.[4] However, the broader impact of chronic HCV infection on overall T-cell differentiation and activation, including T cells that are not specific for HCV antigens, is not well understood. These relationships are important because nonspecific immune activation in individuals with chronic HCV infection has been hypothesized to contribute to the development of extrahepatic conditions, including diabetes and cardiovascular disease (CVD) that are found in excess among these individuals.[5–9]

Three recent studies examined T-cell phenotypes in HCV-viremic individuals and HCV-uninfected controls. All 3 studies reported lower percentages of naive $CD4^+$ T cells, and 1 study also reported a lower percentage of naive $CD8^+$ T cells in HCV-viremic individuals as compared with HCV-seronegative controls.[10–12] The data from these studies were not consistent, although, regarding percentages of central memory (CM), effector memory (EM), and terminally differentiated effector (TE) T cells by HCV viremia status. EM and TE cells have shortened telomeres as compared with naive and CM cells and represent more differentiated T-cell phenotypes, with diminished capacity to replicate in response to antigenic stimulation.[13]

Studies by our group and others have additionally examined T-cell expression of activation markers (eg, CD38 and HLA-DR) in HCV-viremic individuals compared with uninfected controls. Most of these studies found no differences in the percentage of $CD4^+$ and $CD8^+$ T cells that were activated by HCV status,[14–17] although 1 study found that $CD4^+$ T-cell activation was higher in those with HCV viremia.[18] Comparisons of the percentage of regulatory $CD4^+$ T cells (Tregs) between individuals with HCV viremia and HCV-uninfected controls have also been conducted, but the data have conflicted.[10,19–22]

An important limitation of these previous studies is that the HCV-uninfected controls were often healthy individuals from the general population that differed in important ways from the subjects with HCV viremia. For example, cigarette smoking and injection drug use (IDU) are more common in HCV-seropositive individuals than in the general population and may have a strong influence on the immune response in general and on T-cell function in particular.[23–28] Finally, although the association of HCV viremia with T-cell differentiation and activation in HIV-positive individuals has been studied,[10,15–19,21,29] no previous investigation directly tested whether the relation of HCV viremia to T-cell differentiation and activation might differ according to the overall level of immunosuppression as measured by total $CD4^+$ T cell count.

MATERIALS AND METHODS

Study Population

The Women's Interagency HIV Study (WIHS) is a multicenter prospective study of HIV-infected and HIV-uninfected women. Women were recruited using similar methods at 6 US sites during 3 recruitment periods: 1994–1995 (N = 2623), 2001–2002 (N = 1143), and an ongoing recruitment period initiated in 2011. Detailed methods and characteristics of the study population have been described previously.[30,31] At enrollment and then prospectively on a semiannual basis, interviews are conducted, a physical examination performed, and blood specimens collected. The protocol was approved by the Institutional Review Boards at each study site, and all participants provided written informed consent.

Structured interviews are conducted at each semiannual visit to obtain demographic, behavioral, and clinical information. This includes past and current IDU, alcohol use, cigarette smoking, and adherence to prescribed antiretroviral therapy regimens. The definition of highly active antiretroviral therapy (HAART) in WIH6S was guided by the DHHS/Kaiser Panel (DHHS/Kaiser 2008) guidelines and is defined as the reported use of 3 or more antiretroviral medications, 1 of which has to be a PI, an NNRTI, 1 of the NRTIs abacavir or tenofovir, an integrase inhibitor (eg, raltegravir), or an entry inhibitor (eg, maraviroc or enfuvirtide). A clinical examination is also performed at each study visit, which includes measurement of height and weight among other characteristics.

We enrolled 658 WIHS women into a T-cell substudy conducted between October 2011 and March 2013. Specifically, women were enrolled sequentially into the substudy, as they presented for one of their routine semiannual follow-up visits, during which a fresh peripheral blood sample (10 mL) was drawn into a sodium heparinized vacutainer tube, which was then packed in a temperature-controlled shipping container and shipped overnight to Rush University for processing and phenotypic evaluation.

There is not a large body of literature examining the effects of cryopreservation on FACS results, but most studies suggest that the major lymphocyte subset percentages (eg, the total percentage of $CD4^+$ T cells) are similar whether measured in fresh or frozen specimens.[32,33] However, the data are conflicting as to whether more detailed phenotyping of T cells by FACS, including the percentage of naive T cells, Tregs, and other subsets, may be significantly altered by cryopreservation.[32,34–36] Moreover, in many clinical/epidemiologic studies, including the WIHS, there is likely to be some variability in the adequacy cryopreservation, and cell viability is a concern. For these reasons, we measured T-cell phenotypes in fresh unfrozen blood samples in this study.

Flow Cytometry to Assess $CD4^+$ and $CD8^+$ T-Cell Phenotypes

Upon arrival at Rush University, peripheral blood samples were diluted in phosphate-buffered saline (PBS) without Ca++ and Mg++ and processed over Lymphocyte Separation Media (Mediatech Inc., Manassas, VA). The interface containing peripheral blood mononuclear cells (PBMCs)

was collected, washed in PBS without Ca++ and Mg++, and stained for flow cytometric evaluation. PBMCs were stained with Fixable Aqua Dead Cell stain (Life Technologies, Grand Island, NY), washed, and stained with fluorochromeconjugated antibodies against the following cell surface markers: CD3, CD8, CD25, CD38, CD45RA, CCR7, HLA-DR (BD Biosciences, San Jose, CA), and CD4 (Life Technologies, Grand Island, NY). Cells were washed and stained intracellularly with fluorochrome-conjugated FoxP3 (eBioscience) antibody. After staining, the cells were washed, fixed, and acquired on a BD LSR II flow cytometer using FACS Diva v6.1.1 software. T-cell phenotypes were defined using Flow Jo v8.8.7 (Tree Star, Inc.) as the percentage of live (Aqua-) cells that expressed specific markers among total $CD3^+/CD4^+$ or $CD3^+/CD8^+$ T cells, including activated ($CD38^+$ and $HLA\text{-}DR^+$), naive ($CD45RA\ (RA)^+$ and $CCR7\ (R7)^+$), CM ($RA^-/R7^+$), EM ($RA^-/R7^-$), and TE ($RA^+/R7^-$) T-cell markers. T-regulatory cells ($CD3^+/CD4^+/CD25^+/FoxP3^+$) are expressed as a percentage of total $CD4^+$ T cells.

Clinical Laboratory Measurements

HCV serostatus was determined at WIHS enrollment using a commercial second- or third-generation enzyme immunoassay, and HCV viremia was determined in HCV-seropositive women using either the COBAS Amplicor Monitor 2.0, which has a linear range of 600–5.0×10^5 IU/mL, as previously described,[29] or the COBAS Taqman assay, which has a linear range of 10–2.0×10^8 IU/mL (both from Roche Diagnostics, Branchburg, NJ). Follow-up HCV antibody testing was conducted in 1998–1999 in most women who were HCV seronegative at their 1994–1995 enrollment visit. Follow-up HCV RNA testing has also been conducted on most women who were HCV RNA positive at enrollment using the Amplicor or Taqman assay, primarily in 1997–2000 and 2006–2007. Plasma HIV RNA levels were assessed by polymerase chain reaction assays in HIV-infected women using assays that had lower levels of detection of 80 copies per milliliter and total $CD4^+$ and total $CD8^+$ T-cell counts (cells per microliter) were determined in both HIV-infected and HIV-uninfected women by flow cytometry in laboratories participating in the DAIDS Quality Assurance Program at each study visit.[37]

Statistical Methods

In preliminary data analysis, we summarized selected demographic, behavioral, and clinical laboratory measurements by HIV serostatus and the presence of HCV viremia, at the time of specimen collection. Women who were enrolled in the T-cell substudy but lacked HCV serologic data (n = 6) or who were HCV seropositive but lacked HCV RNA data (n = 30) were excluded from the analysis. HCV viremia was defined based on the most recent HCV RNA data—for n = 63 women, the most recent HCV RNA test was in 2006–2007, whereas for n = 18 women, the most recent HCV RNA test was in 1997–2000. Usage of antiretroviral therapies and other medications, including medications used for the treatment of chronic HCV, are reported as a part of standard questionnaires at each semiannual WIHS visit. If a woman reported usage of an HCV medication at any time in the preceding 6 months, she was asked whether she had stopped taking this drug because the therapy was "successful." Six HIV-positive women reported successful HCV antiviral therapy after their most recent HCV RNA test, and we considered the data set with and without inclusion of these women.

Multivariate linear regression models were used to examine associations between HCV viremia and the percentage of $CD4^+$ and $CD8^+$ T cells that were naive, CM, EM, or TE cells, the fraction that were Tregs, as well as the fraction defined as activated. Distributions that were not normally distributed, including percentage of $CD4^+$ activated, $CD8^+$ CM, and $CD8^+$ activated T cells were $\log_{10}$ transformed before analysis. Analyses were conducted separately in HIV-positive and HIV-negative women. Specifically, in the HIV-negative women, we adjusted for age (continuous term), race/ethnicity (non-Hispanic black, Hispanic, non-Hispanic white/other), and other factors known to be associated with inflammation and immune dysregulation: cigarette smoking (current, former/never) and body mass index (BMI—thin/normal, overweight, obese). Only 3 HIV-negative women reported recent IDU, and we assessed the results both including and excluding these individuals.

In HIV-positive women, all multivariate models included adjustment for age, race/ethnicity, cigarette smoking, and BMI but additionally included current and/or nadir $CD4^+$ T-cell counts (<350, 350–500, and >500), HIV RNA viral load (undetectable, lower limit of detection: 4000, 4001–20,000, 20,001–100,000, and $>100{,}000$ copies/mm^3), HAART use (yes/no), and percent self-reported adherence with the prescribed HAART regimen (no HAART, $<75\%$ adherence, 75%–95%, $>95\%$). Finally, because we hypothesized that associations between HCV viremia and T-cell phenotypes might differ by immune status in HIV-positive women, in a second set of models, we stratified by 2 different binary thresholds to define high vs. low total $CD4^+$ count; that is, using a total $CD4^+$ count of ≤ 500 or ≤ 350 $CD4^+$ T cells per microliter.

RESULTS

Selected Characteristics of the Study Population

The data set included 158 HIV-negative and 464 HIV-positive women, of whom 18 and 63, respectively, were HCV viremic. Table 1 shows selected subject characteristics at the time of T-cell phenotyping. In both HIV-negative and HIV-positive groups, the HCV viremic women were older than HCV nonviremic women, and were more likely to have been recruited in 1994–1995 (the first WIHS recruitment period). Cigarette smoking, history of IDU and, among the HIV-positive women, recent IDU, and alcohol use, also differed by HCV viremia (all $P < 0.05$). Of the factors studied only in HIV positives, none differed significantly between those with and without HCV viremia, including current and nadir $CD4^+$ T-cell count, HIV RNA viral load, and recent HAART use (Table 1). The median percentage of live lymphocytes observed in blood samples of study women was 98.4 and the interquartile range was 97.1–99.0.

TABLE 1. Selected Characteristics Among HIV-Negative and HIV-Positive Women by HCV RNA Status

	HIV− (N = 158)			HIV+ (N = 464)		
	HCV− (N = 140)*	HCV+ (N = 18)	*P*†	HCV− (N = 401)*	HCV+ (N = 63)	*P*†
Age, median (IQR)	46 (39–52)	52 (48–59)	<0.01	47 (42–53)	55 (50–58)	<0.01
Race/ethnicity, N (%)			0.34			0.11
Non-Hispanic black	92 (66)	9 (50)		200 (50)	40 (63)	
Hispanic	31 (22)	6 (33)		138 (34)	14 (22)	
Non-Hispanic white/other	17 (12)	3 (17)		63 (16)	9 (14)	
Recruitment wave, N (%)			0.07			<0.01
1994–1995	55 (39)	11 (61)		186 (46)	51 (81)	
2001–2002	58 (41)	7 (39)		144 (36)	12 (19)	
2011–2013	27 (19)	0 (0)		71 (18)	0 (0)	
Cigarette smoking, N (%)			<0.01			0.01
Former/never	81 (58)	3 (18)		266 (67)	31 (49)	
Current	58 (42)	14 (82)		130 (33)	32 (51)	
Alcohol use, N (%)‡			0.28			0.03
None	55 (40)	10 (59)		236 (60)	44 (70)	
Light	46 (33)	3 (18)		125 (32)	10 (16)	
Moderate/heavy	38 (27)	4 (24)		35 (9)	9 (14)	
Ever IDU, N (%)			<0.01			<0.01
No	112 (87)	3 (19)		341 (91)	15 (27)	
Yes	17 (13)	13 (81)		32 (9)	41 (73)	
IDU past 6 mo, N (%)			0.29			0.02
No	137 (99)	16 (94)		396 (100)	61 (97)	
Yes	2 (1)	1 (6)		0 (0)	2 (3)	
Body mass index, N (%)			0.46			0.08
Thin/normal	25 (19)	5 (28)		92 (24)	22 (35)	
Overweight	29 (22)	5 (28)		110 (29)	20 (32)	
Obese	77 (59)	8 (44)		182 (47)	21 (33)	
$CD4^+$ T-cell count, N (%)			§			0.08
>500	§	§		196 (50)	31 (49)	
350–500	§	§		108 (28)	11 (17)	
<350	§	§		87 (22)	21 (33)	
Nadir $CD4^+$ T-cell count, N (%)			§			0.64
>500	§	§		28 (7)	3 (5)	
350–500	§	§		49 (12)	6 (10)	
<350	§	§		324 (81)	54 (86)	
HIV RNA viral load (copies/mm^3), N (%)			§			0.64
Undetectable	§	§		218 (56)	31 (49)	
LLD—4000	§	§		118 (30)	23 (37)	
4001–20,000	§	§		18 (5)	4 (6)	
20,001–100,000	§	§		28 (7)	3 (5)	
>100,000	§	§		9 (2)	2 (3)	
HAART use past 6 mo, N (%)			§			0.73
No	§	§		39 (10)	7 (11)	
Yes	§	§		362 (90)	56 (89)	
HAART compliance (%), N (%)‖			§			0.94
>95	§	§		302 (84)	47 (85)	
75–95	§	§		45 (12)	6 (11)	
<75	§	§		14 (4)	2 (4)	

*The HIV−/HCV− group includes 137 HCVAb− and 3 HCV Ab+/HCV RNA− women, whereas the HIV+/HCV− group includes 364 HCVAb− and 37 HCV Ab+/HCV RNA− women.

†*P* values are from Pearson χ^2 tests, Fisher exact tests, or *t* tests (for continuous variables).

‡Alcohol use was defined as light (<3 drinks/wk), moderate (3–13 drinks/wk), and heavy (>13 drinks/wk).

§Not applicable.

‖Two women who reported HAART use in the past 6 months had missing HAART compliance data.

IQR, interquartile range; LLD, lower limit of detection.

HIV-Negative Women

Table 2 and Table S1 (see **Supplemental Digital Content,** http://links.lww.com/QAI/A557) show the relationship between HCV viremia in HIV-negative women and the percentage of naive, CM, EM, TE, and activated $CD4^+$ and $CD8^+$ T cells and Tregs. In both unadjusted analyses (Table 2 and Fig. 1) and in analyses adjusted for age, race/ethnicity, smoking, and BMI (Table 2), HCV viremic women had lower percentages of naive $CD4^+$ cells (25% lower in adjusted analyses vs. the mean percentage (28.8%) in HCV nonviremics; $P = 0.03$) and higher percentages of EM $CD4^+$ cells (33% higher in adjusted analyses vs. the mean percentage (38.7%) in HCV nonviremics; $P = 0.0002$) than women without HCV viremia. In addition, HCV viremia was associated with a significantly lower percentage of CM $CD8^+$ T cells in multivariate models (37% lower vs. the mean percentage (0.46%) in HCV nonviremics; $P = 0.02$). Excluding the 3 women who reported recent IDU had no impact on the findings (data not shown).

HIV-Positive Women

Table 3 and Table S2 (see **Supplemental Digital Content,** http://links.lww.com/QAI/A557) show similar data in HIV-positive women. HCV viremia was significantly associated with higher percentages of activated $CD4^+$ T cells and Tregs in both unadjusted analyses (Table 3 and Fig. 1) and in models adjusted for age, race/ethnicity, cigarette smoking, BMI, current and nadir $CD4^+$ T-cell counts, HIV viral load, HAART use in the past 6 months, and adherence with the prescribed HAART regimen. For example, in adjusted analyses, activated $CD4^+$ T cells were 26% higher [vs. the mean percentage (0.5%) in HCV nonviremics; $P = 0.01$], whereas Tregs were 17% higher [vs. the mean percentage (5.2%) in HCV nonviremics; $P = 0.02$] in women with vs. without HCV viremia. The percentage of activated $CD8^+$ T cells was also marginally associated with HCV viremia in adjusted analysis ($P = 0.06$). These results were unaffected by additional adjustment for alcohol use, study site, and recruitment wave, or by exclusion of women who reported successful HCV antiviral therapy (data not shown).

Results Stratified by $CD4^+$ T-Cell Count in HIV-Positive Women

To determine whether the associations of HCV viremia with T-cell differentiation and activation might vary by the level of immunosuppression in HIV-positive women, we conducted multivariate analyses stratified by current total $CD4^+$ T-cell count. Among HIV-positive women with diminished immune status (whether defined as $\leq$500 or $\leq$350 $CD4^+$ T cells/μL), HCV viremia was associated with an increased percentage of cells that were Tregs and the fraction of $CD4^+$ cells that were activated (Table 4). The percentage of EM $CD4^+$ cells was also significantly higher in women with vs. without HCV viremia with diminished immune status, regardless of whether immune status was defined as $\leq$500 $CD4^+$ cells per microliter (14% higher EM $CD4^+$ cells vs. the mean percentage (45.7%) in HCV nonviremics; $P = 0.03$) or as $\leq$350 $CD4^+$ cells per microliter (18% higher EM $CD4^+$ cells vs. the mean percentage (49.2%) in HCV nonviremics; $P = 0.01$). Conversely, no significant associations between HCV viremia and $CD4^+$ T-cell phenotypes were seen in HIV-infected women with good immune status (defined as either >500 or >350 $CD4^+$ cells/μL). $CD8^+$ T-cell phenotype percentages were not associated with HCV viremia in any analyses in HIV-positive women. Exclusion of women who reported successful HCV antiviral therapy did not affect the findings, with the exception of associations of HCV viremia with percentage of $CD4^+$ EM cells among women with total $CD4^+$ $\leq$500 cells per microliter ($P = 0.03$ to $P = 0.09$)

TABLE 2. Associations Between HCV Viremia and T-Cell Phenotypes, HIV-Negative Women

	Unadjusted		Adjusted*†	
T-Cell Phenotype‡§	**β (95% CI) (n = 158)**	***P***	**β (95% CI) (n = 148)**	***P***
$CD4^+$ naive	−8.6 (−14.4 to −2.8)	0.004	−7.2 (−13.6 to 0.8)	0.03
$CD4^+$ CM	−2.6 (−5.7 to 0.5)	0.11	−3.0 (−6.3 to 0.4)	0.08
$CD4^+$ EM	13.8 (7.8 to 19.8)	<0.0001	12.7 (6.3 to 19.2)	0.0002
$CD4^+$ TE	−2.6 (−5.7 to 0.5)	0.10	−2.6 (−6.1 to 0.9)	0.15
$CD4^+$ activated	0.0 (−0.2 to 0.1)	0.58	−0.1 (−0.2 to 0.1)	0.46
$CD4^+$ Treg	0.1 (−0.7 to 0.9)	0.86	0.2 (−0.7 to 1.0)	0.74
$CD8^+$ naive	−7.7 (−15.3 to 0.0)	0.05	−3.1 (−11.1 to 4.9)	0.45
$CD8^+$ CM	−0.1 (−0.3 to 0.0)	0.12	−0.2 (−0.4 to 0.0)	0.02
$CD8^+$ EM	4.8 (−1.8 to 11.4)	0.15	4.1 (−3.0 to 11.1)	0.26
$CD8^+$ TE	3.4 (−3.6 to 10.5)	0.33	0.5 (−6.9 to 7.9)	0.89
$CD8^+$ activated	0.1 (−0.1 to 0.3)	0.37	0.0 (−0.2 to 0.2)	0.72

*Adjusted for age (continuous term), race/ethnicity (non-Hispanic black, Hispanic, non-Hispanic white/other), cigarette smoking (current, former/never), and body mass index (thin/normal, overweight, obese).

†Ten women did not have complete covariate information and were not included in adjusted models.

‡T-cell phenotype abbreviations: EM, CM, and TE.

§T-cell phenotype distributions that were not normally distributed, including percentage of $CD4^+$ activated, $CD8^+$ CM, and $CD8^+$ activated T cells were $\log_{10}$ transformed before analysis.

LLD, lower limit of detection.

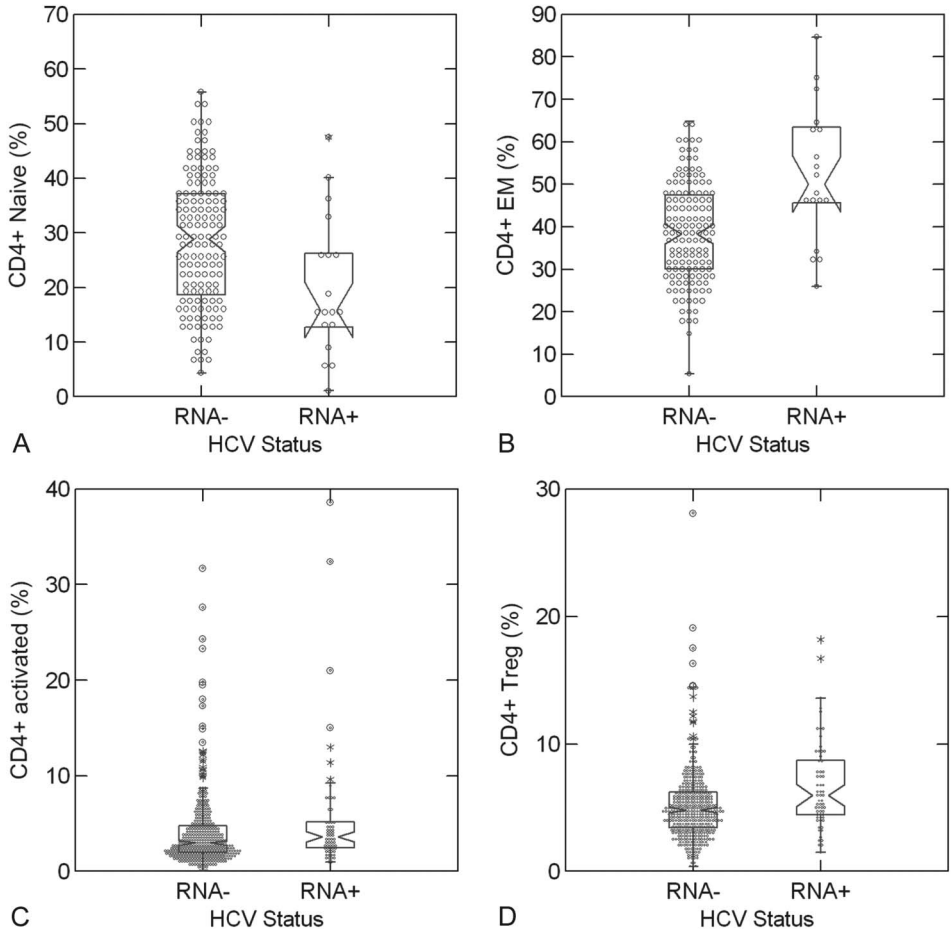

FIGURE 1. Scatter plots and box plots of T-cell phenotypes by HCV viremia status for HIV-negative (A and B) and HIV-positive (C and D) women. Shown are those T-cell phenotypes with significant associations in both unadjusted and adjusted analyses. Box plots are interpreted as follows: the waist is the median; diagonal lines indicate 95% confidence intervals about the median; lower and upper horizontal lines indicate 25th and 75th percentiles of the distribution [interquartile range (IQR)]. Furthermore, the upper and lower vertical bars indicate the range of the data within 1.5 × IQR; and points marked with asterisks and circles represent outliers (1.5–3.0 × IQR) and far outliers (>3.0 × IQR), respectively.

and with percentage of activated $CD4^+$ cells among women with total $CD4^+$ ≤350 cells per microliter ($P = 0.05$ to $P = 0.15$).

DISCUSSION

The relationship of HCV viremia with T-cell differentiation and activation phenotypes varied greatly by HIV status in this study. In HIV-negative women, we found that HCV viremia was significantly associated with lower percentage of naive $CD4^+$ and higher percentage EM $CD4^+$ T cells, as well as a lower percentage of CM $CD8^+$ T cells. In contrast, among HIV positives, we observed only 1 of these 3 relationships, namely, a higher EM $CD4^+$ percentage in those with HCV viremia. Furthermore, this relationship was limited to the subset of HIV positives with diminished immune status (eg, total $CD4^+$ count ≤350 T cells/μL). In the women with diminished immune status, we additionally observed associations of HCV viremia with higher percentages of activated $CD4^+$ T cells and Tregs, whereas among HIV-positive women with high $CD4^+$ count, we found no significant associations between HCV viremia and T-cell phenotype percentages. Thus, the findings not only differed by HIV-serostatus but also by $CD4^+$ count among HIV-positive women.

The low percentage of naive $CD4^+$ T cells in HIV-negative women with versus without HCV viremia is consistent with data reported in all 3 previous studies that examined this relationship.[10–12] However, this article is the first to involve a comparison group from the same low-income population with high rates of smoking and alcohol use as the HCV viremic individuals, instead of healthy controls from the general population. These factors can affect T-cell differentiation and activation and, therefore, the current data clarify the relationship between HCV viremia and low-naive $CD4^+$ T-cell percentages.

Furthermore, the low percentage of naive $CD4^+$ cells in HCV viremic HIV-negative women, in combination with the low percentage of CM $CD8^+$ T cells (another early differentiation phenotype) but high percentage of EM $CD4^+$ (a late differentiation phenotype), suggests that T-cell differentiation may be accelerated in the setting of chronic HCV infection. Although not all associations of HCV viremia with naive, CM and EM $CD4^+$ and $CD8^+$ T-cell phenotypes were statistically significant, the pattern of these associations was consistent with this interpretation; that is, accelerated T-cell differentiation in HCV-viremic individuals (Table 2). The lack of statistical significance may relate to the small number of women with HCV viremia among the HIV negatives.

If correct, this conclusion has important implications. Specifically, the reduction in naive and CM T cells would be expected to result in a reduced capacity to mount T-cell

TABLE 3. Associations Between HCV Viremia and T-Cell Phenotypes, HIV-Positive Women

	Unadjusted		Adjusted*†	
T-Cell Phenotype‡§	β (95% CI) (n = 464)	*P*	β (95% CI) (n = 437)	*P*
$CD4^+$ naive	−3.9 (−7.9 to 0.1)	0.05	−2.0 (−6.0 to 1.9)	0.31
$CD4^+$ CM	−0.9 (−2.4 to 0.7)	0.28	−0.5 (−2.1 to 1.1)	0.55
$CD4^+$ EM	6.0 (1.8 to 10.2)	0.01	3.5 (−0.5 to 7.6)	0.09
$CD4^+$ TE	−1.2 (−2.9 to 0.4)	0.13	−1.0 (−2.7 to 0.6)	0.23
$CD4^+$ activated	0.1 (0.0 to 0.2)	0.02	0.1 (0.0 to 0.2)	0.007
$CD4^+$ Treg	1.5 (0.8 to 2.3)	0.0002	0.9 (0.1 to 1.6)	0.02
$CD8^+$ naive	−1.9 (−5.6 to 1.8)	0.32	0.2 (−3.6 to 4.0)	0.92
$CD8^+$ CM	0.1 (0.0 to 0.1)	0.28	0.02 (−0.1 to 0.1)	0.69
$CD8^+$ EM	1.0 (−3.0 to 5.1)	0.62	−0.6 (−4.7 to 3.5)	0.77
$CD8^+$ TE	0.5 (−3.2 to 4.1)	0.80	0.2 (−3.7 to 4.1)	0.91
$CD8^+$ activated	0.1 (0.0 to 0.2)	0.31	0.1 (0.0 to 0.2)	0.06

*Adjusted for age (continuous term), race/ethnicity (non-Hispanic black, Hispanic, non-Hispanic white/other), cigarette smoking (current, former/never) and body mass index (thin/normal, overweight, obese), current and nadir $CD4^+$ T-cell counts (<350, 350–500, >500), HIV viral load (undetectable, LLD—4000, 4001–20,000, 20,001–100,000, and >100,000 copies/mm^3), HAART use (yes/no), and percent compliance to a prescribed HAART regimen (no HAART, <75%, 75%–95%, and >95%).

†Twenty-seven women did not have complete covariate information and were not included in adjusted models.

‡T-cell phenotype abbreviations: EM, CM, and TE.

§T-cell phenotype distributions that were not normally distributed, including percentage of $CD4^+$ activated, $CD8^+$ CM, and $CD8^+$ activated T cells were $\log_{10}$ transformed before analysis.

LLD, lower limit of detection.

responses against novel and previously encountered antigens, including vaccine antigens. This could explain in part why HCV positives have lower response rates to hepatitis B virus vaccination.[38] However, this hypothesis assumes that it is in fact individuals with naive T-cell lymphopenia that respond less well to hepatitis B virus vaccination, which would need to be verified.

Nonspecific (generalized) accelerated T-cell differentiation in HCV infection might also play a role in extrahepatic conditions that occur at higher rates in HCV positives, such as CVD. For example, a high percentage of EM $CD4^+$ T cells was associated with higher levels of carotid atherosclerosis and with carotid artery disease in a recent study.[39] Although these biologic relationships are not completely characterized, it is clear that understanding the origins of generalized immune differentiation and inflammation in CVD patients is a clinically relevant topic.[40]

TABLE 4. Analysis of Associations Between HCV Viremia and T-Cell Phenotypes Stratified by $CD4^+$ T-Cell Count, HIV-Positive Women

	Cut-point of 500 $CD4^+$ T Cells/μL				Cut-point of 350 $CD4^+$ T Cells/μL			
	>500 Cells		≤500 Cells		>350 Cells		≤350 Cells	
T-Cell Phenotype*†	β (95% CI)‡ (n = 219)§	*P*	β (95% CI)‡ (n = 218)§	*P*	β (95% CI)‡ (n = 334)§	*P*	β (95% CI)‡ (n = 103)§	*P*
$CD4^+$ naive	−2.2 (−7.8 to 3.4)	0.44	−3.9 (−9.5 to 1.6)	0.17	0.4 (−4.2 to 5.1)	0.86	−6.6 (−13.6 to 0.3)	0.06
$CD4^+$ CM	−0.04 (−2.3 to 2.2)	0.97	−1.3 (−3.5 to 1.0)	0.28	−0.1 (−2.0 to 1.9)	0.96	−1.5 (−4.4 to 1.4)	0.32
$CD4^+$ EM	3.2 (−2.7 to 9.0)	0.28	6.4 (0.6 to 12.2)	0.03	0.5 (−4.3 to 5.4)	0.83	9.1 (1.8 to 16.3)	0.01
$CD4^+$ TE	−1.0 (−3.3 to 1.3)	0.41	−1.2 (−3.5 to 1.1)	0.31	−0.9 (−2.9 to 1.0)	0.35	−1.0 (−3.9 to 2.0)	0.52
$CD4^+$ activated	0.1 (−0.02 to 0.2)	0.13	0.2 (0.1 to 0.3)	0.002	0.1 (0.0 to 0.1)	0.17	0.1 (0.0 to 0.3)	0.05
$CD4^+$ Treg	0.2 (−0.9 to 1.3)	0.71	2.0 (1.0 to 3.1)	0.0002	0.4 (−0.5 to 1.3)	0.33	1.6 (0.3 to 3.0)	0.02
$CD8^+$ naive	0.9 (−4.4 to 6.3)	0.73	−1.4 (−6.7 to 3.9)	0.60	1.9 (−2.6 to 6.5)	0.41	−2.7 (−9.5 to 4.1)	0.43
$CD8^+$ CM	0.02 (−0.1 to 0.1)	0.71	0.02 (−0.1 to 0.1)	0.74	0.02 (−0.1 to 0.1)	0.77	0.02 (−0.1 to 0.2)	0.76
$CD8^+$ EM	0.04 (−5.8 to 5.8)	0.99	0.1 (−5.7 to 5.8)	0.98	−0.8 (−5.7 to 4.1)	0.76	−0.3 (−7.6 to 7.0)	0.94
$CD8^+$ TE	−1.3 (−6.7 to 4.1)	0.63	1.2 (−4.1 to 6.6)	0.65	−1.4 (−6.0 to 3.2)	0.56	3.0 (−3.9 to 9.9)	0.39
$CD8^+$ activated	0.1 (0.0 to 0.2)	0.17	0.1 (0.0 to 0.2)	0.08	0.1 (0.0 to 0.2)	0.19	0.1 (−0.1 to 0.2)	0.56

*T-cell phenotype abbreviations: EM, CM, and TE.

†T-cell phenotype distributions that were not normally distributed, including percentage of $CD4^+$ activated, $CD8^+$ CM, and $CD8^+$ activated T cells were $\log_{10}$ transformed before analysis.

‡Adjusted for age (continuous term), race/ethnicity (non-Hispanic black, Hispanic, non-Hispanic white/other), cigarette smoking (current, former/never) and body mass index (thin/normal, overweight, obese), nadir $CD4^+$ T-cell counts (<350, 350–500, and >500), HIV viral load (undetectable, LLD—4000, 4001–20,000, 20,001–100,000, and >100,000 copies/mm^3), HAART use (yes/no), and percent adherence with a prescribed HAART regimen (no HAART, <75% adherence, 75%–95%, >95%).

§The number of women who were HCV RNA+ in strata of $CD4^+$ count are as follows: >500 cells (n = 31 HCV RNA+); ≤500 cells (n = 31 HCV RNA+); >350 cells (n = 41 HCV RNA+); ≤350 cells (n = 21 HCV RNA+).

LLD, lower limit of detection.

We had hypothesized that associations of HCV viremia in HIV positives with high total $CD4^+$ T cell count but not those with low total $CD4^+$ count would be similar to associations in HIV negatives, but this was not the case. Instead, the associations of T-cell phenotypes with HCV viremia was limited to HIV positives with low $CD4^+$, although 31 and 41 women with HCV viremia (of a total of 63 among HIV positives) were among those with >500 and >350 $CD4^+$ T cells per microliter, respectively. Specifically, among HIV positives with diminished immune status, we observed a significant association of HCV viremia with high percentage of activated $CD4^+$ T cells. A similar association was seen in a study of Scandinavian patients with HIV on stable HAART,[18] although it was not observed by us in our previous studies in the WIHS cohort in the pre-HAART era.[15,29] We also observed an association of HCV viremia with high Treg percentage in HIV positives with diminished immune status, a finding that is inconsistent with data from 2 previous studies.[10,17] However, this study was much larger than these previous studies, and as mentioned above, ours was the first to study differences in associations by total $CD4^+$ count.

We were also surprised by the absence of associations with $CD8^+$ T-cell phenotypes in HIV positives because a significant association of HCV viremia with $CD8^+$ T-cell activation was observed in our previous WIHS studies in the pre-HAART era[15,29] and in the Scandinavian HAART users.[18] Although a marginal association ($P = 0.06$) of HCV viremia with $CD8^+$ activation was observed in this study of HIV positives, it is possible that HAART use attenuates the relationship between HCV viremia and $CD8^+$ T-cell activation. The conflicting findings between the current investigation and the Scandinavian study could relate to differences in the definition of T-cell activation ($CD38^+$ in the previous study[18] vs. $CD38^+$ and HLA-DR+ in the current investigation) or from population (eg, sex or racial) differences.

Several limitations to this study must be considered in the interpretation of these data. First, HCV viremia status was not ascertained at the same WIHS visit at which we measured T-cell phenotypes. Although all women who were categorized as HCV viremic had at least 2 positive HCV RNA tests during follow-up, it is possible that a small fraction of these women cleared HCV spontaneously after their most recent HCV RNA test, conducted either in 1997–2000 or in 2006–2007. Furthermore, although some WIHS women who were HCV seronegative at enrollment were tested for HCV antibody again a few years later, HCV antibody testing is not conducted at each WIHS visit. IDU declined dramatically among WIHS women after enrollment, but even if some women acquired new HCV infections after enrollment, or if a few women classified as having chronic HCV were actually HCV RNA negative at the T-cell phenotype testing visit, the results would still be biased toward rather than away from the null (ie, increasing the likelihood of false-negative but not false-positive findings). Second, liver function biomarkers had not been measured on all women at the time of analysis, and we were therefore unable to examine T-cell phenotypes in relation to liver disease. Finally, the current data are cross-sectional, and prospective investigations are therefore needed to verify that HCV infection is indeed antecedent to changes in T-cell phenotype percentages.

In conclusion, the data suggest that HCV viremia is associated with changes in the general distribution of T-cell phenotypes in circulation. The changes involved, however, differ by HIV status and by total $CD4^+$ T-cell count in HIV positives. If correct, further efforts to define the T-cell changes that are not specific for HCV may help inform efforts to reduce extrahepatic disease among HCV-viremic individuals.

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