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

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Abstract

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, prior 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 (naïve, central memory (CM), 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 naïve CD4+ ($P=0.03$), 33% more EM CD4+ ($P=0.0002$) and 37% fewer CM CD8+ ($P=0.02$) T-cells. Among HIV-positives we observed only one of these three relationships: higher

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percentage of EM CD4+ among HCV viremic women. Further, the association with EM CD4+ among HIV-positives was limited to individuals with diminished immune status (total CD4+ count 500 cells/ μ L), 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.

Keywords

hepatitis C virus; HIV; T-cell; phenotype; activation; differentiation

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 following 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 non-specific immune activation in individuals with chronic HCV infection has been hypothesized to contribute to the development of extra-hepatic 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 three studies reported lower percentages of naïve CD4+ T-cells, and one study also reported a lower percentage of naïve CD8+ T-cells in HCV-viremic individuals as compared to HCV-seronegative controls.¹⁰⁻¹² The data from these studies were not consistent, though, with regard to 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 to naïve 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 (e.g., CD38 and HLA-DR) in HCV-viremic individuals compared to 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 one 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 prior 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 Lastly, while the association of HCV viremia with T-cell differentiation and activation in HIV-positive individuals has been studied,^{10;15-19;21;29} no prior 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 six US sites during three 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 semi-annual basis, interviews are conducted, a physical exam 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 semi-annual visit to obtain demographic, behavioral and clinical information. This includes past and current injection drug use, alcohol use, cigarette smoking, and adherence to prescribed antiretroviral therapy regimens. The definition of highly active antiretroviral therapy (HAART) in WIHS was guided by the DHHS/Kaiser Panel [DHHS/Kaiser 2008] guidelines and is defined as: the reported use of three or more antiretroviral medications, one of which has to be a PI, an NNRTI, one of the NRTIs abacavir or tenofovir, an integrase inhibitor (e.g., raltegravir), or an entry inhibitor (e.g., Maraviroc or enfuvirtide). A clinical exam 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 semi-annual 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 (e.g., the total percentage of CD4+ T-cells) are similar whether measured in fresh or frozen specimens.^{32;33}

On the other hand, the data are conflicting as to whether more detailed phenotyping of T-cells by FACS, including the percentage of naïve 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 of cryopreservation, and cell viability is a concern. For these reasons we measured T-cell phenotypes in fresh unfrozen blood samples in the current study.

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

Upon arrival at Rush University, peripheral blood samples were diluted in PBS without Ca^{++} and Mg^{++} and processed over Lymphocyte Separation Media (Mediatech). The interface containing PBMCs was collected, washed in PBS w/o Ca^{++} and Mg^{++} and stained for flow cytometric evaluation. PBMC were stained with Fixable Aqua Dead Cell stain (Invitrogen), washed and stained with fluorochrome conjugated antibodies against the following cell surface markers: CD3, CD8, CD25, CD38, CD45RA, CCR7, HLA-DR (BD Biosciences), and CD4 (Invitrogen). 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 $\text{CD3}^{+}/\text{CD4}^{+}$ or $\text{CD3}^{+}/\text{CD8}^{+}$ T-cells, including: activated (CD38^{+} and HLA-DR^{+}), naïve (CD45RA^{+} (RA^{+}) and CCR7^{+} (R7^{+}), central memory ($\text{RA}^{-}/\text{R7}^{+}$), effector memory ($\text{RA}^{-}/\text{R7}^{-}$) and terminally differentiated effector ($\text{RA}^{+}/\text{R7}^{-}$) T cell markers. T-regulatory cells ($\text{CD3}^{+}/\text{CD4}^{+}/\text{CD25}^{+}/\text{FoxP3}^{+}$) 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 $600\text{--}5.0 \times 10^5$ IU/ml, as previously described,²⁹ or the COBAS Taqman assay, which has a linear range of $10\text{--}2.0 \times 10^8$ IU/ml (both from Roche Diagnostics, Branchburg, NJ). Follow-up HCV antibody testing was conducted in 1998-99 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 (PCR) assays in HIV-infected women using assays that had lower levels of detection of 80 copies/mL and total CD4^{+} and total CD8^{+} T-cell counts (cells/ μL) 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 while 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 semi-annual WIHS visit. If a woman reported usage of an HCV medication at any time in the preceding six 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 following their most recent HCV RNA test and we considered the dataset 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 naïve, central memory (CM), effector memory (EM), or terminally differentiated effector (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 log₁₀ transformed prior to 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 three 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, >500), HIV RNA viral load (undetectable, lower limit of detection (LLD)-4,000 copies/mm³, 4,001-20,000 copies/mm³, 20,001-100,000 copies/mm³, >100,000 copies/mm³), HAART use (yes/no) and percent self-reported adherence with the prescribed HAART regimen (no HAART, <75% adherence, 75%-95%, >95%). Lastly, 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 two different binary thresholds to define high versus low total CD4+ count; i.e., using a total CD4+ count of 500 or 350 CD4+ T-cells/μL.

RESULTS

Selected Characteristics of the Study Population

The dataset 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 non-viremic 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, interquartile range (IQR) 97.1-99.0.

HIV-Negative Women

Table 2 and Supplementary Table 1 show the relationship between HCV viremia in HIV-negative women and the percentage of naïve, central memory (CM), effector memory (EM), terminally differentiated effector (TE) and activated CD4+ and CD8+ T-cells and Tregs. In both unadjusted analyses (Table 2 and Figure 1) and in analyses adjusted for age, race/ethnicity, smoking and BMI (Table 2), HCV viremic women had lower percentages of naïve CD4+ cells (25% lower in adjusted analyses vs. the mean percentage (28.8%) in HCV non-vireemics; $P=0.03$) and higher percentages of EM CD4+ cells (33% higher in adjusted analyses vs. the mean percentage (38.7%) in HCV non-vireemics; $P=0.0002$) 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 non-vireemics; $P=0.02$). Excluding the three women who reported recent IDU had no impact on the findings (data not shown).

HIV-Positive Women

Table 3 and Supplementary Table 2 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 Figure 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 non-vireemics; $P=0.01$) while Tregs were 17% higher (vs. the mean percentage (5.2%) in HCV non-vireemics; $P=0.02$) 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 <500 or <350 CD4+ T-cells/ μ L), 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 <500 CD4+ cells/ μ L (14% higher EM CD4+ cells vs. the mean percentage (45.7%) in HCV non-vireemics; $P=0.03$) or as <350 CD4+ cells/ μ L (18% higher EM CD4+ cells vs. the mean percentage (49.2%) in HCV non-vireemics; $P=0.01$). 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/ μ L). 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+ 500 cells/ μ L ($P=0.03 \rightarrow P=0.09$) and with percentage of activated CD4+ cells among women with total CD4+ 350 cells/ μ L ($P=0.05 \rightarrow P=0.15$).

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 naïve 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 one of these three relationships, namely, a higher EM CD4+ percentage in those with HCV viremia. Further, this relationship was limited to the subset of HIV-positives with diminished immune status (e.g., total CD4+ count 350 T-cells/ μ L). 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 naïve CD4+ T-cells in HIV-negative women with vs. without HCV viremia is consistent with data reported in all three prior studies that examined this relationship.¹⁰⁻¹² However, the current paper 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 naïve CD4+ T-cell percentages.

Furthermore, the low percentage of naïve 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. While not all associations of HCV viremia with naïve, CM and EM CD4+ and CD8+ T cell phenotypes were statistically significant, the pattern of these associations was consistent with this interpretation; i.e., 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 naïve and CM T-cells would be expected to result in a reduced capacity to mount T-cell 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 (HBV)

vaccination.³⁸ However, this hypothesis assumes that it is in fact individuals with naïve T-cell lymphopenia that respond less well to HBV vaccination, which would need to be verified.

Non-specific (generalized) accelerated T-cell differentiation in HCV infection might also play a role in extra-hepatic conditions that occur at higher rates in HCV-positives, such as cardiovascular disease (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 While 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

We had hypothesized that associations of HCV viremia in HIV-positives with high total CD4+ T-cell 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+, even though 31 and 41 women with HCV viremia (out of a total of 63 among HIV-positives) were among those with >500 and >350 CD4+ T-cells/ μ L, 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 HIV patients on stable HAART,¹⁸ though it was not observed by us in our prior 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 two prior studies.^{10;17} However, the current study was much larger than these prior 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 prior WIHS studies in the pre-HAART era^{15;29} and in the Scandinavian HAART users.¹⁸ While a marginal association ($P=0.06$) of HCV viremia with CD8+ activation was observed in the current 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 prior study¹⁸ vs. CD38+ and HLA-DR+ in the current investigation) or from population (e.g., sex or racial) differences.

Several limitations to the current 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. While all women who were categorized as HCV viremic had at least two positive HCV RNA tests during follow-up, it is possible that a small fraction of these women cleared HCV spontaneously following their most recent HCV RNA test, conducted either in 1997-2000 or in 2006-2007. Furthermore, while 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. Injection drug use (IDU)

declined dramatically among WIHS women following 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 (i.e., 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. Lastly, 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 extra-hepatic disease among HCV viremic individuals.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Scatter plots and box plots of T-cell phenotypes by HCV viremia status for HIV-negative (Panels A and B) and HIV-positive (Panels 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)). Further, the upper and lower vertical bars indicate the range of the data within $1.5 \times \text{IQR}$; and points marked with asterisks and circles represent outliers ($1.5\text{-}3.0 \times \text{IQR}$) and far outliers ($>3.0 \times \text{IQR}$), respectively.

Table 1

Selected characteristics among HIV-negative and HIV-positive women by HCV RNA status

	HIV- (N = 158)			HIV+ (N=464)		
	HCV- (N = 140) ^a	HCV+ (N = 18)	P ^b	HCV- (N=401) ^a	HCV+ (N=63)	P ^b
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
1994-95	55 (39)	11 (61)		186 (46)	51 (81)	
2001-02	58 (41)	7 (39)		144 (36)	12 (19)	
2011-	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 (%) ^c			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 months, 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 (%)			d			0.08
>500	d	d		196 (50)	31 (49)	
350-500	d	d		108 (28)	11 (17)	
<350	d	d		87 (22)	21 (33)	
Nadir CD4+ T cell count, N (%)			d			0.64
>500	d	d		28 (7)	3 (5)	
350-500	d	d		49 (12)	6 (10)	
<350	d	d		324 (81)	54 (86)	

	HIV- (N = 158)			HIV+ (N=464)		
	HCV- (N = 140) ^a	HCV+ (N = 18)	P ^b	HCV- (N=401) ^a	HCV+ (N=63)	P ^b
HIV RNA viral load, N (%) undetectable	<i>d</i>	<i>d</i>	<i>d</i>	218 (56)	31 (49)	0.64
LLD-4,000 copies/mm ³	<i>d</i>	<i>d</i>		118 (30)	23 (37)	
4,001-20,000 copies/mm ³	<i>d</i>	<i>d</i>		18 (5)	4 (6)	
20,001-100,000 copies/mm ³	<i>d</i>	<i>d</i>		28 (7)	3 (5)	
>100,000 copies/mm ³	<i>d</i>	<i>d</i>		9 (2)	2 (3)	
HAART use past 6 months, N (%)			<i>d</i>			0.73
No	<i>d</i>	<i>d</i>		39 (10)	7 (11)	
Yes	<i>d</i>	<i>d</i>		362 (90)	56 (89)	
HAART compliance, N (%) ^e			<i>d</i>			0.94
>95%	<i>d</i>	<i>d</i>		302 (84)	47 (85)	
75-95%	<i>d</i>	<i>d</i>		45 (12)	6 (11)	
<75%	<i>d</i>	<i>d</i>		14 (4)	2 (4)	

IQR, interquartile range; IDU, injection drug use; LLD, lower limit of detection; HAART, highly active antiretroviral therapy

^aThe HIV-/HCV- group includes 137 HCVAb- and 3 HCV Ab+/HCV RNA- women while the HIV+/HCV- group includes 364 HCVAb- and 37 HCV Ab+/HCV RNA- women.

^bP-values are from Pearson's chi-square tests, Fisher's exact tests or t-tests (for continuous variables)

^cAlcohol use was defined as: light (<3 drinks/week), moderate (3-13 drinks/week), heavy (>13 drinks/week)

^dNot applicable

^eTwo women who reported HAART use in the past 6 months had missing HAART compliance data

Table 2

Associations between HCV viremia and T cell phenotypes, HIV-negative women

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

^a Adjusted for age (continuous term), race/ethnicity (non-Hispanic Black, Hispanic, non-Hispanic White/other), cigarette smoking (current, former/never) and body mass index (BMI - thin/normal, overweight, obese).

^b Ten women did not have complete covariate information and were not included in adjusted models

^c T cell phenotype abbreviations: effector memory (EM), central memory (CM) and terminally differentiated effector (TE)

^d T-cell phenotype distributions that were not normally distributed, including percentage of CD4+ activated, CD8+ CM, and CD8+ activated T-cells were log₁₀ transformed prior to analysis

Table 3

Associations between HCV viremia and T cell phenotypes, HIV-positive women

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

^a Adjusted for age (continuous term), race/ethnicity (non-Hispanic Black, Hispanic, non-Hispanic White/other), cigarette smoking (current, former/never) and body mass index (BMI – thin/normal, overweight, obese), current and nadir CD4+ T cell counts (<350, 350-500, >500), HIV viral load (undetectable, lower limit of detection (LLD)-4,000 copies/mm³, 4,001-20,000 copies/mm³, 20,001-100,000 copies/mm³, >100,000 copies/mm³), highly active antiretroviral therapy (HAART) use (yes/no) and percent compliance to a prescribed HAART regimen (no HAART, <75%, 75%-95%, >95%)

^b Twenty seven women did not have complete covariate information and were not included in adjusted models

^c T cell phenotype abbreviations: effector memory (EM), central memory (CM) and terminally differentiated effector (TE)

^d T-cell phenotype distributions that were not normally distributed, including percentage of CD4+ activated, CD8+ CM, and CD8+ activated T-cells were log₁₀ transformed prior to analysis

Table 4

Analysis of associations between HCV viremia and T-cell phenotypes stratified by CD4+ T-cell count, HIV-positive women

T-cell phenotype ^{a b}	Cut-point of 500 CD4+ T-cells/ μ L			Cut-point of 350 CD4+ T-cells/ μ L		
	>500 cells	500 cells	>350 cells	350 cells		
	β (95% CI) ^c (n = 219) ^d	P	β (95% CI) ^c (n = 218) ^d	P	β (95% CI) ^c (n = 334) ^d	P
CD4+ naïve	-2.2 (-7.8, 3.4)	0.44	-3.9 (-9.5, 1.6)	0.17	0.4 (-4.2, 5.1)	0.86
CD4+ CM	-0.04 (-2.3, 2.2)	0.97	-1.3 (-3.5, 1.0)	0.28	-0.1 (-2.0, 1.9)	0.96
CD4+ EM	3.2 (-2.7, 9.0)	0.28	6.4 (0.6, 12.2)	0.03	0.5 (-4.3, 5.4)	0.83
CD4+ TE	-1.0 (-3.3, 1.3)	0.41	-1.2 (-3.5, 1.1)	0.31	-0.9 (-2.9, 1.0)	0.35
CD4+ activated	0.1 (-0.02, 0.2)	0.13	0.2 (0.1, 0.3)	0.002	0.1 (0.0, 0.1)	0.17
CD4+ Treg	0.2 (-0.9, 1.3)	0.71	2.0 (1.0, 3.1)	0.0002	0.4 (-0.5, 1.3)	0.33
CD8+ naïve	0.9 (-4.4, 6.3)	0.73	-1.4 (-6.7, 3.9)	0.60	1.9 (-2.6, 6.5)	0.41
CD8+ CM	0.02 (-0.1, 0.1)	0.71	0.02 (-0.1, 0.1)	0.74	0.02 (-0.1, 0.1)	0.77
CD8+ EM	0.04 (-5.8, 5.8)	0.99	0.1 (-5.7, 5.8)	0.98	-0.8 (-5.7, 4.1)	0.76
CD8+ TE	-1.3 (-6.7, 4.1)	0.63	1.2 (-4.1, 6.6)	0.65	-1.4 (-6.0, 3.2)	0.56
CD8+ activated	0.1 (0.0, 0.2)	0.17	0.1 (0.0, 0.2)	0.08	0.1 (0.0, 0.2)	0.19

^a T-cell phenotype abbreviations: effector memory (EM), central memory (CM) and terminally differentiated effector (TE)

^b T-cell phenotype distributions that were not normally distributed, including percentage of CD4+ activated, CD8+ CM, and CD8+ activated T-cells were log10 transformed prior to analysis

^c Adjusted for age (continuous term), race/ethnicity (non-Hispanic Black, Hispanic, non-Hispanic White/other), cigarette smoking (current, former/never) and body mass index (BMI – thin/normal, overweight, obese), nadir CD4+ T cell counts (<350, 350-500, >500), HIV viral load (undetectable, lower limit of detection (LLD)-4,000 copies/mm³, 4,001-20,000 copies/mm³, 20,001-100,000 copies/mm³, >100,000 copies/mm³), highly active antiretroviral therapy (HAART) use (yes/no) and percent adherence with a prescribed HAART regimen (no HAART, <75% adherence, 75%-95%, >95%)

^d 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=41 HCV RNA+); >350 cells (n=21 HCV RNA+)