Phenotypic Characterization of Lymphocytes in HCV/HIV Co-infected Patients

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Abstract

While hepatitis C virus (HCV)-specific immune responses are attenuated in HCV/HIV co-infected patients compared to those infected with HCV alone, the reasons for this remain unclear. In this study, the proportions of regulatory, naïve, and memory T cells, along with chemokine receptor expression, were measured in co-infected and mono-infected patients to determine if there is an alteration in the phenotypic profile of lymphocytes in these patients. HCV/HIV co-infected patients had increased proportions of $CD4^+$ naïve cells and decreased proportions of $CD4^+$ effector cells when compared to HCV mono-infected patients. The proportions of $CD4^+$ Tregs and $CD4^+$ $CXCR3^+$ T cells were also significantly lower in co-infected patients. A decrease in $CD4^+$ Tregs and subsequent loss of immunosuppressive function may contribute to the accelerated progression to liver disease in co-infected individuals. Dysregulation of immune responses following reduction in the proportions of $CD4^+$ $CXCR3^+$ Th-1 cells may contribute to the reduced functional capacity of HCV-specific immune responses in co-infected patients. The findings of this study provide new information on the T-cell immunophenotype in HCV/HIV co-infected patients when compared to those infected with HCV alone, and may provide insight into why cell-mediated immune responses are diminished during HCV infection.

Introduction

Clearance of hepatitis C (HCV) is associated with vigorous multi-specific $CD4^+$ and $CD8^+$ T-cell responses, whereas individuals who develop chronic infection tend to have weak, narrowly focused responses (13,25,44). Studies in chimpanzees have shown that depletion of $CD4^+$ or $CD8^+$ cells prevents HCV viral clearance (22,39). These observations suggest that there is an association between viral clearance and specific cell-mediated immunity. In HCV/HIV co-infected patients, HCV-specific T-cell responses are weaker and less frequent compared to HCV mono-infected patients (10,14), suggesting that the immunosuppression resulting from HIV infection compromises immune responses to HCV. However, several studies have shown that total CD4 counts are not associated with reduced T-cell responses (10,21); it is therefore possible that a qualitative defect in $CD4^+$ and/or $CD8^+$ cells, resulting from HIV infection, could be responsible for the diminished cellular immune responses to HCV in HCV/HIV co-infected patients.

Regulatory T cells (Tregs) are a subset of $CD4^+$ T cells responsible for controlling the responses of other T cells, and protection from self-reactivity by suppressing the proliferation of effector T cells (40,41). Recent studies have shown that regulatory T cell activity is increased in patients with chronic HCV infection compared to those who clear infection (7,9,34); these studies have also shown that Tregs contribute to the dysfunction of HCV-specific $CD8^+$ T cells by suppressing proliferation and IFN-γ production, suggesting that Tregs contribute to HCV persistence. There is currently limited information available on regulatory T cells in HCV/HIV co-infected patients.

Naïve and memory T cells can be distinguished based on the expression of two cell surface molecules: CD45 and the

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chemokine receptor CCR7 (28,35). CCR7 is a chemokine receptor, expressed by various subsets of immune cells, whose main function is concerned with the homing of dendritic cells and T cells to the lymph nodes (18,19). It has been widely adopted as a marker for distinguishing between naïve and memory T cells; cells expressing the CD45RA isoform and CCR7 are classified as naïve cells (CD45RA$^+$ CCR7$^+$); central memory cells (T$_{CM}$) have a CD45RA$^-$ CCR7$^+$ phenotype, and effector memory cells are classified as those negative for both CD45RA and CCR7 (CD45RA$^-$ CCR7$^-$) (35). In HCV-infected patients, CD8$^+$ T$_{CM}$ cells residing in the periphery are capable of differentiating into effector memory cells, which are recruited to the liver. CD8$^+$ effector cells in the liver were found to have reduced functional efficiency, as evidenced by reduced IFN-γ production (1,29). Clearly, memory cells play an important role in controlling HCV infection, as *in vitro* depletion of CD8$^+$ memory cells has been shown to result in viral persistence, and depletion of CD4$^+$ memory cells is associated with the emergence of CTL escape mutants (22,39). Currently there are no detailed reports comparing central and effector memory T-cell populations in HCV/HIV co-infected and HCV mono-infected patients.

Chemokines and their receptors play an important role in the pathogenesis of HCV infection. Th-1 cells preferentially express the chemokine receptors CXCR3 and CCR5 (24,36). CXCR3 binds to the 10-kDa IFN-γ-inducible protein (IP-10), whereas CCR5 binds to macrophage inflammatory protein-1α (MIP-1α), macrophage inflammatory protein-1β (MIP-1β), and RANTES. These Th-1 associated chemokine/receptor pairs appear to predominate in HCV infection (5,38). Chemokines and their receptors also play an important role in the pathogenesis of HIV. Two chemokine receptors, CCR5 and CXCR4, are important in facilitating cellular entry of HIV. CCR5 is the major co-receptor for macrophage-tropic strains of HIV (3), whereas CXCR4 facilitates entry of T-tropic HIV strains (17). The role of chemokine receptors such as CXCR3 and CCR5 in HCV/HIV co-infection has not been well characterized.

The aim of this study was to perform a phenotypic characterization of T lymphocytes in HCV/HIV co-infected and HCV mono-infected patients to determine if there is an alteration in the CD4$^+$ and/or CD8$^+$ T-cell phenotype profile, which could help explain the diminished cellular immune responses to HCV in HCV/HIV co-infected patients.

Materials and Methods

Study participants

Four patient groups were included in this study: HCV/HIV co-infected ($n = 11$), HCV mono-infected ($n = 15$), HIV mono-infected ($n = 9$), and healthy controls ($n = 8$). For all participants, HCV infection was defined by positive serum HCV antibodies and detectable viral RNA; HIV infection was defined by seropositivity for HIV. Healthy control subjects were negative for both HCV and HIV antibodies. All HCV patients were infected with genotype 1. Seven of eleven HIV/HCV co-infected and seven of nine HIV mono-infected patients were receiving antiretroviral treatment. None of the anti-HCV-positive patients had received interferon therapy at the time of the study. All studies were conducted with the approval of the ethical review board of each institution.

Virological, biochemical, and histopathology tests

Anti-HCV antibodies were tested using an enzyme-linked immunosorbent assay (ELISA; Ortho Diagnostic Systems, Amersham, UK) and confirmed by a recombinant immunoblot assay (RIBA; Chiron Corporation, Emeryville, CA). The presence of HCV RNA in serum was determined by reverse transcription-polymerase chain reaction (RT-PCR) (11). Antibodies to HIV were tested by ELISA (Abbott Laboratories, Chicago, IL) and confirmed by an immunoblot assay (INNOLiPA; Innogenetics, Ghent, Belgium). HCV and HIV viral loads were measured using branched DNA-based VERSANT™ assays (Bayer Diagnostics, Tarrytown, NY). Alanine aminotransferase (ALT) (44) and aspartate aminotransferase (AST) (31) levels were measured using commercial spectrophotometric kits (BioMerieux, France, and Roche Diagnostics, Mannheim, Germany). Liver fibrosis scores were evaluated according to the guidelines of Ishak *et al*. (23).

Flow cytometric analysis

Frozen PBMCs were thawed and stained with various combinations of the following fluorochrome-conjugated antibodies: CD3-FITC, CD3-PE, CD4-PerCP, CD8-PerCP, CD45RA-FITC, CD25-PE, CXCR3-PE (BD Pharmingen, San Diego, CA), CCR5-APC, and CCR7-APC (R&D Systems, Oxford, U.K.). Intracellular staining for Foxp3 was performed using an APC anti-human Foxp3 staining set (eBioscience, San Diego, CA). Four-color flow cytometric analysis was performed on a FACS Calibur flow cytometer using CellQuest software (BD Biosciences, Oxford, UK). At least 100,000 events from the lymphocyte gate were acquired for memory and regulatory T-cell analysis, and at least 30,000 events from the lymphocyte gate were collected for CXCR3 and CCR5 analysis. Isotype-matched control antibodies were used to monitor background fluorescence and to set quadrant gates. For all analyses, cells were first gated on CD3$^+$ lymphocytes. For naïve/memory cell analysis, cells were further gated on CD4$^+$ or CD8$^+$ cells, and dot plots were created based on CD45RA versus CCR7. For regulatory T-cell analysis, cells were further gated on CD25$^+$ cells and dot plots were formed based on CD4 versus Foxp3. For chemokine receptor analysis, dot plots displaying CD4 or CD8 versus CCR5 or CXCR3 were formed based on the CD3$^+$ lymphocyte gate.

Measurement of serum chemokine levels

Serum IP-10, MIP-1α, MIP-1β, and RANTES levels were measured using a multiplex human chemokine Linco-plex kit (Linco Research Inc., St. Charles, MO) as described previously (33). Multi-analyte profiling was performed on a Luminex-100 system and XY Platform (Luminex Corporation, Austin, TX).

Statistical analyses

All statistical analyses were performed using Statview software 5.0.1 (SAS Institute, Cary, NC). Patient cohorts were compared using the Mann-Whitney U test; Fisher's exact test was used for categorical data. Correlations between parameters were estimated using Spearman's correlation coefficient (r). p values <0.05 were considered statistically significant.

Results

Study population characteristics

Four groups of patients were recruited into this study; HCV/HIV co-infected, HCV mono-infected, HIV mono-infected, and healthy controls. The proportions of regulatory CD4$^+$ T cells, naïve and memory cells, and chemokine receptor surface expression were measured by flow cytometry in these four groups in an attempt to determine if differences exist in the phenotypic profile of lymphocytes in HCV/HIV co-infected patients. The demographics and laboratory parameters of the three infected patient groups are summarized in Table 1. There were no significant differences between the three infected groups and healthy controls with regard to age and gender.

Regulatory CD4$^+$ T cells

Regulatory CD4$^+$ T cells (Tregs) were defined as CD4$^+$ T cells positive for both CD25 and Foxp3. Fig. 1A shows representative dot plots obtained from each of the four study groups. All three infected groups had decreased proportions of CD4$^+$ Tregs compared to healthy controls ($p < 0.05$) (Fig. 1B). There was no significant difference between the proportion of CD4$^+$ Tregs in HCV and HIV mono-infected patients. HCV/HIV co-infected individuals had a lower percentage of CD4$^+$ Tregs compared with both the HCV and HIV mono-infected groups; statistical significance was only observed with the HCV mono-infected group ($p = 0.0007$). A decrease in CD4$^+$ Treg proportions in the co-infected group suggests that the immunosuppressive function of Tregs may be reduced in these patients. We have previously shown that serum IP-10 levels are increased in HCV/HIV co-infected patients compared to those infected with HCV alone (33). The elevated IP-10 levels observed in HCV/HIV co-infected patients is further evidence of an impaired immunosuppressive function. IP-10 levels were compared with the proportions of CD4$^+$ Tregs to determine if an increase in IP-10 levels was associated with a decrease in CD4$^+$ Tregs;

no correlation was found. Similarly, no association was found between decreased CD4$^+$ Tregs and increased HCV viral load, ALT levels, or liver fibrosis. A negative correlation between CD4$^+$ Tregs and AST levels was observed, but this was not statistically significant ($r = -0.533$, $p = 0.131$).

Naïve and memory cells

According to the classification of Sallusto *et al.* (35), the following subsets of naïve and memory cells were measured: naïve cells (CD45RA$^+$ CCR7$^+$), central memory cells (T$_{CM}$, CD45RA$^-$ CCR7$^+$), and effector memory cells (T$_{EM}$, CD45RA$^-$ CCR7$^-$). A further subset of CD8 lymphocytes, terminally differentiated effector memory cells (T$_{EMRA}$, CD45RA$^+$ CCR7$^-$) were also measured. Representative dot plots are displayed in Fig. 2A.

All three infected groups had a lower proportion of naïve CD4$^+$ cells compared to healthy controls; however, this was only significant in the HCV mono-infected group ($p = 0.0006$) (Fig. 2B). HCV/HIV co-infected patients had a significantly increased proportion of naïve CD4$^+$ cells compared to HCV mono-infected patients ($p = 0.04$). There was no significant differences in the proportion of CD4$^+$ naïve cells between HIV mono-infected patients and either of the HCV-infected groups. The distribution of CD4$^+$ central memory cells was similar in all four study groups. All three infected groups had an increased proportion of CD4$^+$ effector memory cells compared to healthy controls; however, this was only significant in the HCV mono-infected group ($p = 0.02$). HCV/HIV co-infected patients had a significantly decreased proportion of CD4$^+$ T$_{EM}$ cells compared to HCV mono-infected patients ($p = 0.01$). There was no significant difference in the proportion of CD4$^+$ T$_{EM}$ cells between HIV mono-infected patients and either of the HCV-infected groups.

HCV viral loads were compared with naïve and effector memory CD4$^+$ T-cell proportions. In the HCV mono-infected group, no association was found between HCV viral load and naïve or effector memory CD4$^+$ T cells. In the HCV/

TABLE 1. SUMMARY OF PATIENT CHARACTERISTICS

	HCV/HIV (n = 11)	HCV only (n = 15)	HIV only (n = 9)
Age, median (range), years	49 (26–61)	31 (24–53)	35 (27–61)
Male/female	9/2	7/8	9/0
HCV genotype 1a/1b	11/0	9/6	NA
HCV RNA, median (range), copies $\times 10^6$/mL	7.02 (0.7–24.3)	8 (0.03–21.4)	NA
ALT, median (range), IU/L	53 (21–149)	62 (16–282)	28 (17–64)
AST, median (range), IU/L	68 (33–122)	60 (26–212)	27 (19–47)
CD4$^+$, median (range), cells/μL	386 (190–730)	NT	351 (193–729)
HIV RNA, median (range), copies/mL	52 (<50-46,385)	NA	<50 (<50–120,528)
Fibrosis score[a]			NT
0–1	0	2	
2	3	5	
3	1	2	
4	2	3	
≥5	2	2	

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; NT, not tested; NA, not applicable.
[a]Fibrosis scores were not available for 3 HCV/HIV co-infected patients and 1 HCV mono-infected patient.
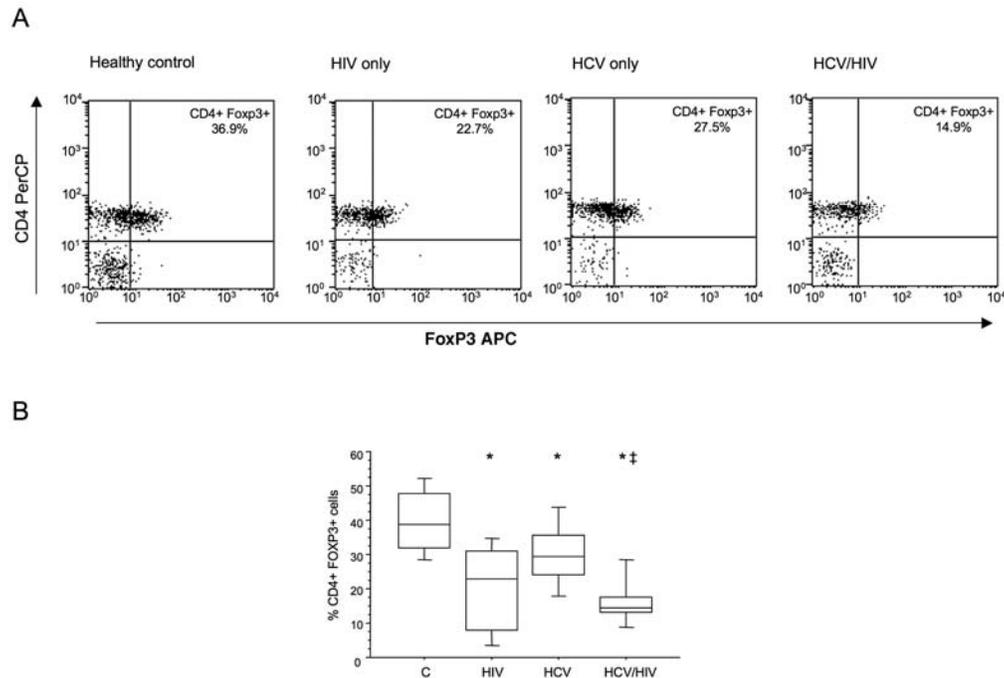
A

B

FIG. 1. (**A**) Representative dot plots obtained from the measurement of CD4$^+$ T-regulatory cells by flow cytometry. The cells were first gated on CD3$^+$ lymphocytes. CD3$^+$ T cells were further gated on CD25$^+$ cells, and dot plots were created showing CD4 versus Foxp3. Percentage values show the percentage of CD4$^+$ Foxp3$^+$ T cells. (**B**) Measurement of CD4$^+$ T-regulatory cells in healthy controls (C) (n = 8), HIV mono-infected (HIV) (n = 9), HCV mono-infected (HCV) (n = 15), and HCV/HIV co-infected patients (HCV/HIV) (n = 11). Results are illustrated by box plots, each box displays the median and interquartile ranges. Groups were compared using the Mann-Whitney U test. *Denotes statistical significance versus healthy controls; ‡denotes statistical significance versus HCV mono-infected.

HIV co-infected group, there was no correlation between HCV viral load and the proportion of naïve cells. However, an increase in viral load was associated with a decrease in effector memory CD4$^+$ T cells (r = −0.833, p = 0.02). There was no correlation between liver enzyme levels or liver fibrosis score and CD4$^+$ naïve/memory cells in any of the study groups. Similarly, no association was found between CD4 count and CD4$^+$ naïve /memory cells in either of the HIV-infected groups.

All three infected groups had decreased proportions of CD8$^+$ naïve cells compared to healthy controls (Fig. 2C) (p < 0.05). No differences were observed in the distribution of CD8$^+$ naïve cells between the infected groups. The proportions of CD8$^+$ central memory cells in the four study groups were comparable. An increase in the frequency of effector memory cells was observed in the three infected groups compared to healthy controls (p0.05); no difference in the distribution of these cells between the infected groups was observed. The frequency of T$_{EMRA}$ cells was similar in all four groups. HCV viral loads were correlated with naïve and effector memory CD8$^+$ T cells. No association was found between HCV viral load and naïve or effector memory CD8$^+$ T cells in either of the HCV-infected groups, nor was there any association between liver enzyme levels or liver fibrosis score and CD8$^+$ naïve/memory cells. CD4$^+$ cell counts were compared with the proportions of naïve and effector memory cells in the HCV/HIV co-infected group and no association was found.

CXCR3 and CCR5 expression

CXCR3 and CCR5 were measured on CD4$^+$ and CD8$^+$ T cells in the four study groups. Fig. 3A shows representative dot plots obtained from the measurement of CXCR3 expression in each of the four study groups. CXCR3 expression on CD4$^+$ cells was increased in all three infected groups when compared to healthy controls; this was significant only in the HCV mono-infected group (p = 0.003) (Fig. 3B). There was no significant difference in the proportion of CD4$^+$ CXCR3$^+$ T cells between HIV mono-infected patients and either of the HCV-infected groups. A significant decrease in CD4$^+$ CXCR3$^+$ T cells was observed in the HCV/HIV co-infected group when compared to HCV mono-infected patients (p = 0.02). Both HIV and HCV mono-infected groups had similar proportions of CD8$^+$ CXCR3$^+$ T cells when compared to healthy controls; HCV/HIV co-infected patients had a decreased proportion of CD8$^+$ CXCR3$^+$ T cells when compared to the other three study groups, and this was statistically significant for the HCV mono-infected group (p = 0.02) (Fig. 3C).

No correlation was found between CXCR3 expression and viral load, liver enzyme levels, or liver fibrosis score in either of the HCV-infected groups. In HCV/HIV co-infected patients an increase in CD4 count was associated with an increase in CXCR3 expression on CD4$^+$ and CD8$^+$ T cells (r = >0.5, p < 0.05). Since CXCR3 is the chemokine receptor for IP-10, CXCR3 expression was correlated with IP-10 levels (data not shown). In HCV/HIV co-infected patients, a de-
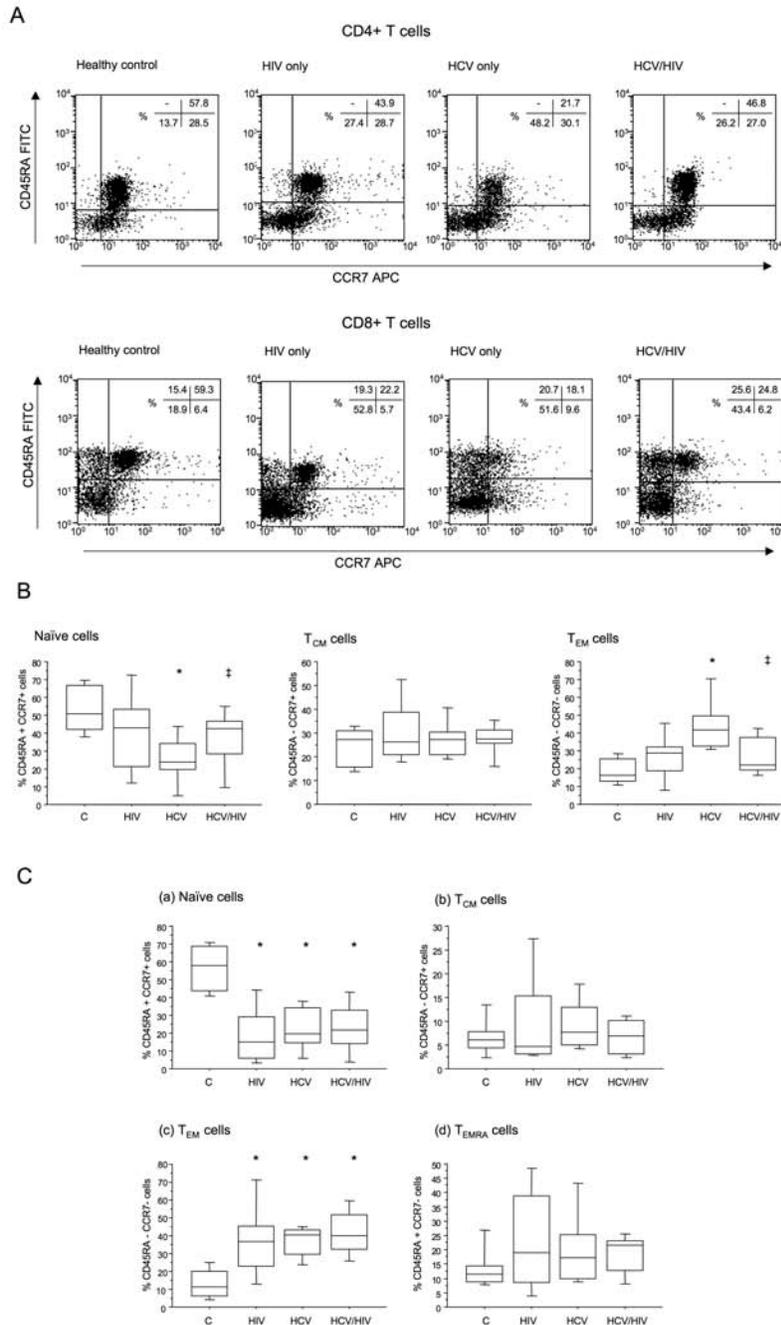
FIG. 2. (**A**) Representative dot plots obtained from the measurement of naïve cells, central memory cells, and effector memory cells by flow cytometry. Cells were first gated on CD3$^+$ lymphocytes. CD3$^+$ T cells were then gated on CD4$^+$ or CD8$^+$ cells, and dot plots were formed based on CD45RA versus CCR7. Naïve cells were classified as CD45RA$^+$ CCR7$^+$ cells; central memory cells were classified as CD45RA$^-$ CCR7$^+$ cells, and effector memory cells were defined as CD45RA$^-$ CCR7$^-$ cells. CD8$^+$ terminally differentiated effector cells were classified as CD45RA$^+$ CCR7$^-$ cells. Percentage values for each of these populations are displayed in the upper right quadrant of each box plot. (**B**) Measurement of CD4$^+$ and (**C**) CD8$^+$ naïve T cells, T_{CM} cells (central memory), T_{EM} cells (effector memory), and T_{EMRA} cells (terminally differentiated effector memory, CD8$^+$ only) in healthy controls (**C**) (n = 8), HIV mono-infected (HIV) (n = 9), HCV mono-infected (HCV) (n = 15), and HCV/HIV co-infected patients (HCV/HIV) (n = 11). Results are illustrated by box plots, and each box displays the median and interquartile ranges. Groups were compared using the Mann-Whitney U test. *Denotes statistical significance versus healthy controls; ‡denotes statistical significance versus HCV mono-infected.

crease in CXCR3 expression on CD4$^+$ and CD8$^+$ T cells was associated with an increase in IP-10 levels (r = −0.767, p = 0.03 and r = −0.709, p = 0.03, respectively). This association was not observed in the HCV mono-infected group.

The levels of CD4$^+$ CCR5$^+$ T cells were similar in all four study groups (Table 2). HIV mono-infected patients had lower proportions of CD8$^+$ CCR5$^+$ T cells compared to healthy controls and HCV/HIV co-infected patients (p <
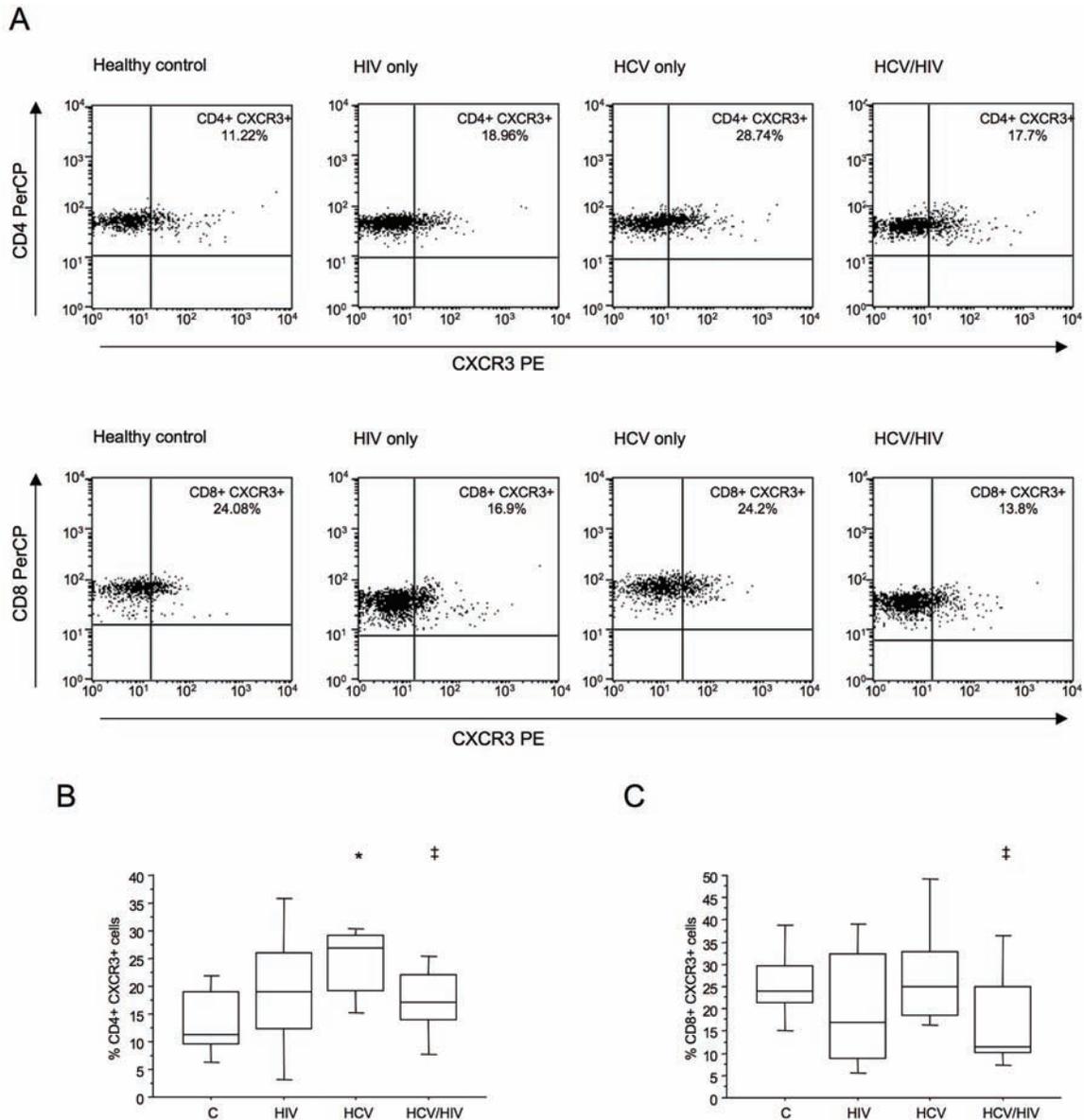
FIG. 3. (**A**) Representative dot plots obtained from the measurement of CXCR3 expression on CD4$^+$ T cells and CD8$^+$ T cells by flow cytometry. For measurement of T lymphocytes, cells were first gated on CD3$^+$ lymphocytes. Dot plots displaying CD4 or CD8 versus CXCR3 were constructed based on the CD3 lymphocyte gate. (**B**) CXCR3 expression on CD4$^+$ and (**C**) CD8$^+$ T cells in healthy controls (C) (n = 8), HIV mono-infected (HIV) (n = 9), HCV mono-infected (HCV) (n = 15), and HCV/HIV co-infected patients (HCV/HIV) (n = 11). Results are illustrated by box plots, and each box displays the median and interquartile ranges. Groups were compared using the Mann-Whitney U test. *Denotes statistical significance versus healthy controls; ‡denotes statistical significance versus HCV mono-infected.

0.05) (Table 2). No significant differences were observed between any of the other groups. No correlation was found between CCR5 expression and viral load, liver enzyme levels, or liver fibrosis score in either of the HCV-infected groups. Similarly, there was no association between CD4 count and CCR5 expression in the co-infected group. Since CCR5 is the chemokine receptor for MIP-1α, MIP-1β, and RANTES, the concentration of these chemokines was compared with CCR5 expression on CD4$^+$ T cells, CD8$^+$ T cells, and B cells in HCV mono-infected and HCV/HIV co-infected patients (data not shown). No statistically significant correlations were observed in either of the HCV-infected groups.

Discussion

The efficacy of HCV-specific immune responses are reduced in HCV/HIV co-infected patients (10,14), but the reasons for this have not been fully clarified. In this study, the proportions of regulatory CD4$^+$ T cells, naïve and memory cells, and chemokine surface expression on lymphocytes were measured by flow cytometry in HCV/HIV co-infected and mono-infected patients in an attempt to determine if an alteration in the phenotypic profile of lymphocytes could be implicated in the diminished HCV-specific immune responses observed in HCV/HIV co-infected patients.

TABLE 2. CCR5 EXPRESSION ON CD4$^+$ AND CD8$^+$ T CELLS

	Healthy control group	HIV mono-infected	HCV mono-infected	HCV/HIV co-infected
CD4$^+$ CCR5$^+$	2.5 (1.0–7.7)	7.1 (0.9–17.5)	3.9 (1.1–11.8)	4.8 (1.8–16.8)
CD8$^+$ CCR5$^+$	9.6 (5.0–26.0)	2.5 (1.4–10.3)[a]	5.0 (0.9–16.6)	10.52 (1.9–16.1)[b]

[a]Denotes statistical significance versus healthy controls.
[b]Denotes statistical significance versus HIV mono-infected.
Values depict median (range) percentages of either CD4$^+$ CCR5$^+$ or CD8$^+$ CCR5$^+$ T cells. Groups were compared using the Mann-Whitney U test.

To date, there has only been one documented report looking at the proportion of circulating CD4$^+$ Tregs during HCV/HIV co-infection (42). This study examined the effect of IL-2 immunotherapy on the frequency and phenotype of Tregs in HCV/HIV co-infected patients. However, no HCV or HIV mono-infected groups were included, making our study the first to compare circulating CD4$^+$ Tregs between HCV/HIV co-infected and mono-infected patients. The role of CD4$^+$ Tregs during HCV infection has not yet been fully clarified, with some studies demonstrating an increase in CD4$^+$ Tregs compared to healthy controls and patients with resolved infection (7,9), while others have shown no difference in the proportion of Tregs between persistent and resolved infection (27). Many previous studies did not account for possible variation due to genotype differences among their HCV-infected subjects. Furthermore, Tregs were often simply classified as CD4$^+$ CD25$^+$ cells, with no additional markers such as Foxp3 for regulatory activity taken into consideration. This makes comparisons difficult, but could explain the differences in results obtained in the present study, in which we observe that HCV-infected patients had decreased proportions of circulating CD4$^+$ Tregs compared to healthy controls. An upregulation of CD4$^+$ Tregs has been demonstrated in the liver during HCV infection (43), possibly in an effort to attenuate the heightened hepatic immune response observed during HCV infection, thus depleting the proportions of CD4$^+$ Tregs in the periphery.

Both HIV-infected groups also had decreased proportions of CD4$^+$ Tregs compared to healthy controls. CD4$^+$ Tregs were further depleted in HCV/HIV co-infected patients compared to the HCV mono-infected group. Previous studies have also shown depletion of CD4$^+$ Tregs during HIV infection (4,15) and in other viral infections such as HTLV-1 and herpes virus (20,45). In HIV-infected patients, this decrease in CD4$^+$ Tregs was associated with a decrease in total CD4 cell numbers (15), but no correlation was found between CD4$^+$ Tregs and CD4 counts in this study. Tregs have been shown to be present at significantly higher proportions in sites of HIV replication, such as the lymphoid tissues and gastrointestinal mucosa, compared to the periphery (4,16). One hypothesis is that the recruitment of Tregs to lymphoid tissues decreases their proportions in the periphery, making them unavailable for recruitment to the liver, thereby preventing them from controlling the localized cellular immune response, and perhaps contributing to liver damage in co-infected patients. In addition, the majority of HIV-infected patients were receiving HAART, which has been to shown to restore CD4$^+$ cells and CD4$^+$ Tregs (4,6), so it is possible that the restoration of CD4 counts as a result of HAART could be masking the true *in vivo* effect that co-infection with HIV has on CD4$^+$ Tregs.

Information on the proportions of naïve and effector memory cells in the circulation and at sites of infection, during HCV and HIV infection, could provide information on the diminished immune responses observed in both of these infections. In the present study, the peripheral naïve and memory CD4$^+$ T-cell pool in HCV mono-infected patients was predominantly composed of effector memory cells, whereas in both HIV-infected groups, naïve cells were the predominant phenotype. It is possible that HIV is directly responsible for the depletion of effector CD4$^+$ cells, and naïve CD4$^+$ cells are increased as a result of HAART. There are very few reports in the literature looking at naïve and central/effector memory T-cell subsets during HCV/HIV co-infection. It is difficult to compare our study with previous studies (2,30,37), as ours is the first that compares naïve and central/effector memory T cells in HCV/HIV co-infected and HCV mono-infected patients. In addition, these studies used different criteria for defining naïve and memory cells than those used in our study, making comparisons inappropriate. Our data demonstrate that naïve CD4$^+$ cells were increased and effector cells correspondingly decreased in HCV/HIV co-infected patients when compared to those infected with HCV alone. Since CD4$^+$ T cells play an important role in the activation of cytotoxic CD8$^+$ T cells, this lack of CD4$^+$ effector cells in HCV/HIV co-infected patients could contribute to the dysfunctional effector CD8$^+$ T-cell responses observed in the progression of HIV infection (26,31), and could also contribute to the reduced efficacy of immune responses to HCV in co-infected patients when compared to those infected with HCV alone.

The chemokine receptor CXCR3 is predominantly expressed on activated Th-1 T cells and plays an important role in recruiting activated T cells to sites of infection (24,36). There are currently no documented reports measuring CXCR3 expression on T cells during HCV/HIV co-infection. Our study reports, for the first time, the comparison of CXCR3 expression on T cells in HCV/HIV co-infected and HCV mono-infected patients. CXCR3 expression on CD4$^+$ and CD8$^+$ T cells was decreased in HCV/HIV co-infected patients when compared to those infected with HCV only. Decreased CXCR3 expression on CD8$^+$ cells has also been demonstrated during advanced stages of HIV infection (8). Clearance of HCV infection is associated with the development of HCV-specific Th-1 responses and, since CXCR3 is expressed predominantly on Th-1 cells, the decreased proportions of CD4$^+$ CXCR3$^+$ T cells suggests there may be a depletion of Th-1 cells in co-infected patients. In addition, a lack of CXCR3 expression could impair recruitment of Th-1 cells to the liver and therefore contribute to the defective HCV-specific immunity in co-infected patients.

The decrease in CXCR3 expression on CD4$^+$ and CD8$^+$ T cells was associated with an increase in serum IP-10 levels. It is possible that due to decreased proportions of CXCR3$^+$ T cells, IP-10 is upregulated in an attempt to recruit more CXCR3$^+$ T cells to the liver. On the other hand, "chemorepulsion" may be occurring, in which cells migrate away from an area of high chemokine concentration (12,32). Therefore, in HCV/HIV co-infected patients, the elevated IP-10 concentration could disrupt the chemokine sensing gradient, and as a result CXCR3$^+$ T cells migrate away from the site of infection, resulting in downregulation of CXCR3 expression. We have also shown that the proportions of effector memory T cells are also decreased in HCV/HIV co-infected patients compared to those infected with HCV only. Since CXCR3 is expressed on a large proportion of effector cells and plays a role in recruiting effector cells to sites of infection, it would be interesting in future studies to measure the CXCR3 expression levels on effector cells to determine if it is this population of cells that are depleted during HCV/HIV co-infection.

Taken together the results of this study have generated some novel and interesting data regarding the T-cell immune repertoire during HCV/HIV co-infection. However, one primary caveat is that the majority of HIV-infected patients were in receipt of HAART and therefore had low HIV viral loads; due to the immune restoration capabilities of HAART, the true effects of the immunosuppressive nature of HIV may not have been fully appreciated in this study or similar studies. Ideally, future studies should be performed using HAART-naïve patients, but establishing such a cohort with sufficient patient numbers could prove a challenge and have significant ethical implications. It should also be noted that since HIV is an infection that can sequester lymphocytes in the lymph nodes and other sites of HIV replication, this could have an impact on the assessment of lymphocyte subsets in the periphery. However, given that there were no significant differences between the HCV and HIV mono-infected groups throughout any of the analyses, co-infection with HIV and HCV can be proposed as being the causative factor for differences observed in the T-cell phenotype profiles.

Conclusion

The findings of this study have provided novel information on the composition of the T-cell immune repertoire in HCV/HIV co-infected patients compared to those infected with HCV alone. It is possible that the differences observed in the T-cell phenotype of co-infected patients may play a role in the diminished cell-mediated immune responses observed during HCV infection. Our results are also strengthened by the fact that unlike previous studies, all of our HCV-infected patients were infected with the "difficult to treat" genotype 1. Although we were unable to assay specific cellular immune responses in this study, our data raise a number of important questions with regard to HCV/HIV co-infected patient immune function that warrant examination in the future. Together these findings could have important implications for understanding the pathogenesis of disease in HCV/HIV co-infected patients.

Acknowledgments

Disclosure Statement

No competing financial interests exist.

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