# Differences in CD4+ T-cell Immune Activation in HIV, Hepatitis C (HCV), and HIV/HCV Coinfection are Influenced by HIV and HCV Infection Status


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## Revised Abstract


**Background:** HIV/HCV-coinfected patients have accelerated liver disease compared to HCV-monoinfection. In poorly-controlled HIV with chronic hepatitis C (CHC), immune activation has been primarily attributed to CD8 T-cell subsets. In well-controlled HIV, data comparing inflammatory cytokines and immune activation between HIV/HCV-coinfection with CHC to HIV/HCV-seropositive patients with cleared-HCV are underreported.
**Methods:** Fifty-nine age (median, 53 years) and sex (male=43/female=16)-matched patients were stratified: A) HIV-monoinfection (N=15), B) HCV-monoinfection with CHC (N=15), C) HIV/HCV-coinfection with CHC (N=14), and D) HIV/HCV-seropositive with cleared-HCV (N=15). All HIV+ patients had undetectable HIV viremia, and current CD4 counts in strata A and C were matched to CD4 at estimated timepoint of HCV clearance in strata D (median, CD4=420 cells/ml**).** Risk factors were recorded and serum collected for CD4 and CD8 immune activation (CD38/HLADR) and immune senescence (CD28) markers via flow cytometry, and luminex-multiplex cytokine assay. Immune and inflammatory markers were analyzed across strata using pair-wise t-tests; additional correlations of immune markers and cytokines were examined by multivariate analysis.
**Results:** CD38+HLADR+ expression on CD4+ T-cells was significantly increased in HIV/HCV-coinfection with CHC versus HCV-monoinfection and HIV/HCV-seropositive with cleared-HCV. Total CD4+HLADR+ expression was significantly increased in HIV/HCV-coinfection with CHC versus HIV-monoinfection and HIV/HCV-seropositive with cleared-HCV. Total CD4+CD38+ and CD4+CD38+HLADR- expression was significantly higher in HIV/HCV-seropositive with cleared-HCV than HCV-monoinfection and significantly higher in HIV-monoinfection than HCV-moninfection (Figure 1). Other potential predictors of CD4+ activation included elevated transaminases and other habits (Table 2). IL10 production was significantly lower in HIV-monoinfection versus HIV/HCV-coinfection with CHC (p=0.0002). No other differences in CD8+ immune activation markers, CD28, or cytokines across strata were detected.
**Conclusions:** In contrast to previously reported increased CD8 activation in poorly-controlled HIV, there were no differences in CD8 activation in our populations with undetectable HIV viremia. CD4 immune activation with HLADR+ expression in HIV/HCV-coinfection with well-controlled HIV may arise from chronic HCV viremia. Conversely, CD38+ expression in CD4 cells may be driven by underlying HIV infection in both HIV-monoinfection and HIV/HCV-coinfection.


## Introduction

- Given shared modes of transmission, hepatitis C coinfection is common among HIV-positive patients
- Compared to patients with HCV monoinfection, coinfected patients have more rapid progression of liver disease
- Inflammatory cytokine responses and immune activation likely play a pivotal role in the immunopathology of HIV/HCV coinfection and liver fibrosis
- In poorly-controlled HIV with chronic hepatitis C (CHC), immune activation has been primarily attributed to CD8 T-cell subsets (Kovacs, et al)
- Patterns of immune activation in HIV/HCV-coinfected patients with well-controlled HIV have not been fully defined.

## Materials and Methods

- Location: Ruth M. Rothstein CORE Center, Chicago, IL, USA
- Study Period: June, 2011 to December, 2011
- Study Population:
  - 59 patients were stratified as follows:
    - A) HIV-monoinfection (N=15)
    - B) HCV-monoinfection with CHC (N=15)
    - C) HIV/HCV-coinfection with CHC (N=14)
    - D) HIV/HCV-seropositive with cleared-HCV (N=15)
  - Patients were matched for: age, sex, and current CD4 counts in strata A and C were matched to CD4 at estimated time of HCV clearance in strata D (median CD4 = 420 cells/ml).
- Data collected:
  - HIV and HCV risk factors
  - Expression of markers of CD4 and CD8 immune activation (CD38/HLADR) and immune senescence (CD28) (assessed via flow cytometry)
  - Cytokine production: IL-6, IL-8, IL-10, IL-12p70, IL-17A, IP-10, MIP-1α, IFN-γ, and TNF-α (assessed via luminex-multiplex cytokine assay)
  - Transient elastography (TE)-measured liver fibrosis scores
- Statistical analysis:
  - Immune and inflammatory markers compared across strata using Tukey multiple comparison test adjusted by variables in table 2
  - Multivariate analyses were built for variables with an alpha significance <0.05 in univariate analysis
  - All analyses were performed using SAS Version 9.2 (Cary, NC)

## Results

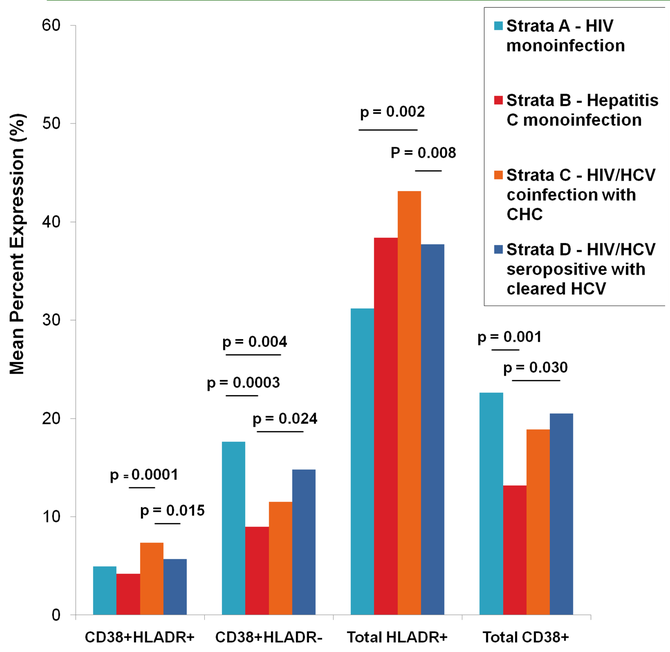
### Table 1. Baseline Characteristics

| Variable | Strata A (N=15) | Strata B (N=15) | Strata C (N=14) | Strata D (N=15) | *P* |
|---|---|---|---|---|---|
| **Demographics** | | | | | |
| Sex (no.) | | | | | 1.00 |
| Female | 4 | 4 | 4 | 4 | |
| Male | 11 | 11 | 10 | 11 | |
| Age, years | 53 (51-56) | 54 (51-56) | 54 (52-56) | 53 (42-66) | 0.95 |
| Race/Ethnicity (no.) | | | | | 0.21 |
| Black/non-Hispanic | 11 | 12 | 9 | 13 | |
| White/non-Hispanic | 4 | 2 | 4 | 0 | |
| White/Hispanic | 0 | 1 | 1 | 2 | |
| **Laboratory values** | | | | | |
| ALT | 18 (11-82) | 43 (19-78) | 23 (14-88) | 22 (10-77) | 0.0009 |
| AST | 19 (14-52) | 37 (23-84) | 30 (18-83) | 24 (18-55) | 0.0002 |
| Albumin | 4.2 (3.8-5.1) | 4.0 (3.4-4.7) | 4.3 (2.7-4.7) | 4.2 (3.8-5.0) | 0.29 |
| hs-CRP | 4.0 (0.5-11.3) | 1.2 (0.2-36.9) | 0.7 (0.2-27.2) | 3.5 (0.2-65.3) | 0.08 |
| **Risk factors (no.)** | | | | | |
| Sexual preference | | | | | 0.01 |
| MSM | 6 | 1 | 1 | 1 | |
| Heterosexual | 7 | 14 | 11 | 14 | |
| Bisexual | 2 | 0 | 2 | 0 | |
| Alcohol use | | | | | 0.32 |
| > once a week | 4 | 1 | 2 | 0 | |
| < weekly, but > monthly | 2 | 1 | 2 | 1 | |
| Monthly or less | 9 | 13 | 10 | 14 | |
| Injection drug use ever | | | | | <0.0001 |
| Yes | 1 | 9 | 13 | 9 | |
| No | 14 | 6 | 1 | 6 | |
| TE-derived fibrosis score* | 4.4 (3.0-11.6) | 7.9 (4.7-29.9) | 7.6 (3.2-22.3) | 5.9 (3.0-9.1) | 0.001 |
| Current CD4 count | 495 (296-956) | - | 457 (299-651) | 668 (295-1117) | 0.01 |
| Nadir CD4 count | 168 (22-415) | - | 225 (13-428) | 302 (124-918) | 0.08 |
| Duration of ARVs, years | 9 (1-25) | - | 11 (3-15) | 6 (0-20) | 0.59 |
| Estimated duration of HIV, years | 12 (2-25) | - | 17 (5-31) | 19 (1-28) | 0.69 |
| Estimated duration of HCV, years | - | 24 (15-40) | 30 (12-38) | 26 (3-37) | 0.23 |

Values are expressed as median (range) unless otherwise noted
Abbreviations: ARV, antiretroviral; hsCRP, high sensitivity C-reactive protein; MSM, men who have sex with men; TE, transient elastography.
*TE-derived fibrosis scores correlate with Metavir fibrosis staging system as follows: <7.1 = stage 0-1, 7.1-9.4 = stage 2, 9.5-12.4 = stage 3, and ≥ 12.5 = stage 4.

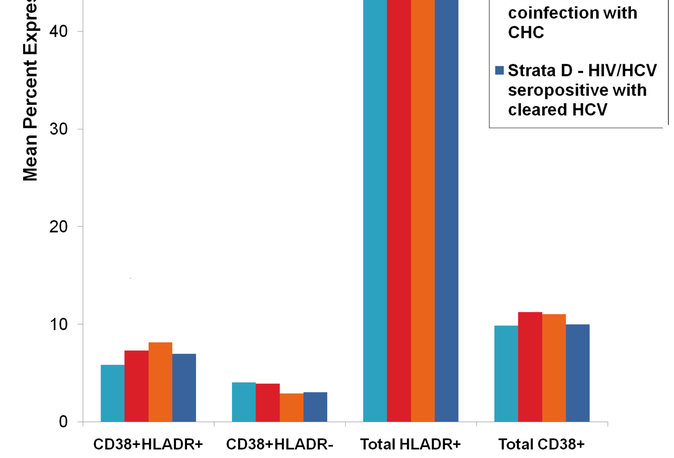
**Cytokine Results:**
- In univariate analysis IP-10, TNF-α, and IL-10 production were significantly lower in HIV-monoinfection compared to HIV/HCV-coinfection with CHC (p = 0.001, 0.05, and 0.01 respectively)
- In multivariate analysis, only IL-10 production was significantly lower in HIV-monoinfection versus HIV/HCV-coinfection with CHC (p=0.0002).

### Figure 1: Comparison of CD4 T-cell Activation Expression by HIV and Hepatitis C Strata via Multivariate Analysis




*Statistically significant differences between strata ($p < 0.05$) denoted with line

### Figure 2: Comparison of CD8 T-cell Activation Expression by HIV and Hepatitis C Strata via Univariate Analysis




*No statistically significant differences between strata found

### Table 2. Multivariate Analysis of Significant Associations with CD4 T-cell Markers of Immune Activation

| Predictor Variable | CD38+HLADR+ | CD38+HLADR- | Total CD38+ | Total HLADR+ |
|---|---|---|---|---|
| **Fibrosis Stage** | | | | |
| Stage 0-1 | Referent | Referent | Referent | Referent |
| Stage 2 | -4.88 (-6.3 – -3.38)* | -5.60 (-10.11 – -1.09)* | -10.49 (-15.90 – -5.08)* | -13.40 (-21.55 – -5.25)* |
| Stage 3 | -2.90 (-4.42 – -1.37)* | -6.37 (-10.94 – -1.81)* | -9.27 (-14.74 – -3.81)* | -3.77 (-12.00 – 4.47) |
| Stage 4 | -2.49 (-4.37 – -0.61)* | -1.75 (-7.39 – 3.89) | -4.24 (-11.00 – 2.52) | -5.79 (-15.96 – 4.39) |
| ALT | 0.14 (0.08 – 0.19)* | 0.20 (0.03 – 0.36)* | 0.33 (0.13 – 0.53)* | 0.21 (-0.09 –0.51) |
| AST | -0.06 (-0.12 – -0.00)* | -0.06 (-0.23 – 0.12) | -0.12 (-0.33 – 0.09) | -0.14 (-0.46 – 0.17) |
| hsCRP | 0.04 (0.00 – 0.09) | -0.05 (-0.15 – 0.08) | -0.01 (-0.16 – 0.15) | 0.14 (-0.09 – 0.37) |
| D dimer | 1.07 (-0.04 – 2.18) | 0.16 (-3.17 – 3.49) | 1.23 (-2.77 – 5.23) | -0.25 (-6.27 – 5.77) |
| Total cholesterol | -0.00 (-0.02 – 0.01) | -0.09 (-0.13 – -0.04)* | -0.09 (-0.14 – -0.04)* | 0.13 (0.06 – 0.21)* |
| History of injection drug use (IDU) | 0.35 (-0.78 – 1.48) | 4.50 (1.11 – 7.88)* | 4.84 (0.79 – 8.89)* | -7.66 (-13.77 – -1.55)* |
| Coffee (> 2 cups/day vs. none) | -2.16 (-4.18 – -0.14)* | -5.27 (-11.32 – 0.79) | -7.43 (-14.69 – -0.17)* | -1.76 (-12.70 – 9.18) |
| Alcohol (≤ monthly vs. > weekly intake) | 1.68 (0.00 – 3.36)* | 0.97 (-4.05 – 5.99) | 2.65 (-3.36 – 8.67) | 6.48 (-2.58 – 15.55) |
| **Ethnicity** | | | | |
| White/non-Hispanic vs. Black/non-Hispanic | 0.24 (-1.33 – 1.81) | 4.35 (-0.36 – 9.05) | 4.59 (-1.05 – 10.22) | -5.84 (-14.33 – 2.65) |
| White/Hispanic vs. Black/non-Hispanic | -0.91 (-2.97 – 1.15) | -2.99 (-9.16 – 3.17) | -3.90 (-11.29 – 3.49) | -1.62 (-12.75 – 9.51) |

Values determined by Maximum Likelihood Estimates using Generalized Linear Model. Reported as estimates of mean difference (95% CI). For categorical variables, the estimate is mean difference between two compared groups; for continuous variables, the estimate is mean difference for one unit increment of independent variable
Abbreviations: hs-CRP, high sensitivity C-reactive protein; TE, transient elastography.
Strata was also included in multivariate model, though not reported here.
*P < 0.05

## Study Limitations

- Small sample size as a pilot study
- Subjects with a sustained virologic response (SVR) following HCV treatment and subjects with spontaneously cleared HCV infection were analyzed as a singular strata

## Conclusions

- This well-controlled study highlights patterns of CD4 immune activation in HIV/HCV-coinfection, as manifested by HLADR+ and CD38+ expression:
  - HLADR+ expression in HIV/HCV-coinfection with well-controlled HIV appears to arise from chronic HCV viremia
  - CD38+ expression is likely driven by underlying HIV infection
- No differences in CD8 activation were detected between our strata of subjects with well-controlled HIV infection
  - Contrasts with findings in earlier studies (Kovacs, et al) with generally poorly-controlled HIV in which there was increased CD8 activation among coinfected patients
- Production of the anti-inflammatory cytokine IL-10 was significantly lower in HIV-monoinfection versus HIV/HCV-coinfection with CHC (p=0.0002)
- There were no consistent correlations between immune activation and TE-determined liver fibrosis stage inferred by HIV and HCV infection status
  - Patients with TE-determined mild to moderate liver fibrosis appear to have overall lower levels of CD4 immune activation markers
  - Significantly higher TE-determined liver stiffness values were measured in HCV monoinfection and HIV/HCV coinfection with CHC, in which the highest levels of CD4 HLADR+ expression were recorded, though in HCV monoinfection the lowest levels of CD38+ expression were observed
- Increased CD4+ immune activation in patients with longstanding HIV/HCV coinfection with CHC may contribute to amplified inflammatory responses, such as increased IL-10 production, that may lead to deleterious immune exhaustion, having an impact extending beyond known accelerated liver fibrosis
  - Successful clearance of HCV may abrogate these immune activation and inflammatory responses
- Additional studies exploring the implications of immune activation in the setting of HCV treatment with newly licensed direct-acting antivirals (DAAs), as well as DAAs and therapeutic HCV vaccines in development, are needed

## References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