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Viral Factors Associated with Cytokine Expression During HCV/HIV Co-Infection

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Abstract

Co-infection with human immunodeficiency virus (HIV) is associated with reduced hepatitis C virus (HCV) treatment response and accelerated HCV disease. Cytokines, as mediators of immune responses, inflammation, and fibrogenesis, may underlie important differences in HCV pathogenesis during HIV co-infection. We previously found that serum interleukin-8 (IL-8) and tumor necrosis factor- α (TNF- α) increased after HCV therapy with interferon (IFN) in HCV/HIV co-infected patients; however, cytokine levels were not predictive of HCV therapeutic response. Here, we examined viral factors associated with expression of IL-8, TNF- α , and transforming growth factor- β 1 (TGF- β 1) in uninfected, HCV mono-infected, HIV mono-infected, and HCV/HIV co-infected persons. HIV co-infection was associated with decreased IL-8 detection but not TNF- α detection. A significant interaction effect demonstrated that HIV infection was associated with elevated TGF- β 1 in HCV-positive individuals but not in HCV-negative individuals. The induction of a sustained profibrotic signal, such as TGF- β 1, by HIV may cause accelerated liver fibrosis during HCV/HIV co-infection and may hinder the host's ability to mount an effective HCV-specific immune response. Further studies are warranted to identify noninvasive markers of liver disease for the clinical management of HCV disease, particularly when liver biopsies have not been performed or are contraindicated.

IN THE UNITED STATES, an estimated 150,000–300,000 persons are infected with both hepatitis C virus (HCV) and human immunodeficiency virus (HIV).¹ HIV co-infection is associated

with reduced HCV treatment response rates and has an adverse impact on liver fibrosis, HCV RNA levels, and HCV disease progression.² The essential roles played by cytokines in mediating immune responses, inflammation, and fibrogenesis suggest that this complex signaling network could influence HCV persistence and the virologic response to therapeutic interventions.

Cytokine dysregulation is common among HIV-positive persons³ and may further contribute to ineffective immune responses against HCV. We, therefore, hypothesized that certain cytokines, such as interleukin-8 (IL-8), tumor necrosis factor- α (TNF- α), and transforming growth factor- β 1 (TGF- β 1) would be expressed differentially in HCV/HIV co-infected persons compared with HCV mono-infected persons because of their essential roles in hepatic inflammation and fibrogenesis. IL-8 is a proinflammatory cytokine/chemokine that is elevated in the serum of persons with chronic HCV compared with uninfected controls.⁴ IL-8 is also associated with inflammation,⁵ severe hepatitis activity,⁶ and decreased susceptibility to interferon (IFN).^{4,7} TNF- α is an important regulator of IL-8 expression and is associated with liver inflammation.⁸ TGF- β 1 is a major regulator of liver fibrogenesis and may contribute to the development of hepatocellular carcinoma.^{9,10} Furthermore, several studies have found an association between serum levels of IL-8, TNF- α , or TGF- β 1 and virologic response to HCV therapy,^{4,11–13} although these findings were not confirmed in HCV/HIV co-infected persons initiating HCV therapy.¹⁴ The objectives of the current study were (1) to examine the effects of HIV and HCV infection status on serum IL-8, TNF- α , and TGF- β 1 levels and (2) to identify virologic variables associated with cytokine expression in HCV-positive people.

Subjects with available sera were pooled from several sources, including pretreatment samples of HCV/HIV co-infected participants ($n = 106$) enrolled in two AIDS Clinical Trial Group (ACTG) treatment trials, ACTG 383¹⁵ ($n = 19$) and ACTG 5071¹⁶ ($n = 65$), as well as participants in observational cohorts from the Massachusetts General Hospital (MGH, $n = 22$). As comparison groups, 68 HCV mono-infected (HCV antibody positive and HCV RNA positive), 48 HIV mono-infected (HIV antibody positive), and 20 uninfected persons (HCV antibody negative and HIV antibody negative) were selected from observational cohorts at MGH based on sample availability.

Demographic and virologic characteristics are presented in Table 1. Nineteen of 70 (27%) MGH participants with available data and 43 of 65 (66%) ACTG 5071 subjects had undetectable HIV RNA levels; all HIV RNA levels were detectable in ACTG 383 subjects. Nine MGH patients and 3 ACTG 383 subjects had undetectable HCV RNA levels at the time of sample collection; HCV RNA levels were detectable in all ACTG 5071 subjects. The four infection groups differed in gender, with a higher proportion of males in the HIV mono-infection group ($p = 0.0008$). The median age of the uninfected controls was lower than for the other three groups ($p < 0.0001$). The proportion of individuals with genotype 1 in the HCV/HIV co-infection group was higher than that in the HCV mono-infection group ($p = 0.02$). No ACTG 5071 participants had prior treatment with IFN or ribavirin, although 86% had a history or current use of anti-retroviral therapy (ART). Per the enrollment criteria, no ACTG 383 participants had treatment with IFN for at least 3 months prior to study entry, although 42% had a history of ART. There was a history of HCV and HIV

treatment in 50% and 59%, respectively, among the HCV/HIV co-infected participants enrolled at MGH. Median HCV and HIV RNA levels were 5.46 log₁₀ IU/mL and 2.60 log₁₀ copies/mL, respectively.

IL-8 detection rates were significantly different among the four infection groups: 30% in uninfected controls, 15% in HIV mono-infected persons, 70% in HCV/HIV co-infected persons, and 91% in HCV mono-infected persons (Fig. 1A). The difference in IL-8 detection rates was not statistically significant between the uninfected controls and the HIV mono-infection groups (Table 2); however, other pairwise differences were highly significant. Serum detection rates for the proinflammatory cytokine TNF- α were low for all infection groups (Fig. 1B): 0% in uninfected controls, 19% in HCV mono-infected persons, 28% in HCV/HIV co-infected persons, and 29% in HIV mono-infected persons. None of the differences in TNF- α detection rates among the mono-infected and co-infected groups was statistically significant (Table 2). Because of the variable patient characteristics, logistic regression models were fit using age, gender, HIV status, and HCV status as potential predictors of IL-8 or TNF- α detection among all 242 participants. For IL-8, HIV infection (odds ratio point estimate [OR] 0.27, 95% confidence interval [CI] 0.13–0.57, $p = 0.005$) and HCV infection (OR 16.64, CI 7.89–35.11, $p < 0.0001$) were statistically significant. Age and gender were not significant in the multivariate model. Thus, according to our model, HCV infection is positively associated with IL-8 detection, whereas HIV infection is negatively associated with IL-8 detection. For TNF- α , HIV infection was associated with increased TNF- α detection (OR 2.31, CI 1.16–4.58, $p = 0.017$). Age, gender, and HCV infection were not associated with TNF- α detection in the multivariate model.

A major objective of our study was to identify virologic factors associated with cytokine expression among HCV-positive persons, 68 with HCV mono-infection and 106 with HCV/HIV co-infection in the current study (Table 3). In a subanalysis restricted to these HCV-positive persons, HIV co-infection was found to be statistically significant in the univariate analysis ($p < 0.0001$). HCV treatment history, HCV genotype, and gender were not significantly associated with IL-8 detection rate in the univariate analysis, although HCV RNA level ($p = 0.11$) and age ($p = 0.10$) were marginally significant. We then considered age, HIV co-infection, HCV RNA level, and HCV treatment in a multivariate model to account for potential confounding. Gender and HCV genotype were excluded from the multivariate model because they were not significant in the univariate analysis, and there was no evidence of potential confounding with other covariates. In the multivariate model, both HIV co-infection and HCV RNA level were statistically significant. HIV co-infection was associated with decreased IL-8 detection (OR 0.23, CI 0.10–0.54, $p = 0.0003$), whereas high HCV RNA levels were associated with increased IL-8 detection (OR 10.66, CI 3.54–32.12, $p = 0.0004$). For TNF- α , HCV genotype 1 was marginally associated with a higher TNF- α detection rate (28% for genotype 1 vs. 15% for non-1, $p = 0.08$). However, when restricting the analysis to only HCV-positive individuals, HIV status, a factor associated with increased TNF- α detection rate in the infection status analysis, was no longer statistically significant (data not shown). Age, gender, HCV RNA level, and HCV treatment history were also not significantly associated with TNF- α detection.

Serum levels of the fibrogenic cytokine TGF- β 1 were detectable in all participants (Fig. 1C). Mean TGF- β 1 levels (expressed as log₁₀ pg/mL) were 4.49 in HCV/HIV co-infected persons, 4.34 in HCV mono-infected persons, 4.52 in HIV mono-infected persons, and 4.70 in uninfected controls. The differences among the four infection groups were statistically significant for each pairwise comparison, except for the comparison between the HIV mono-infection and HCV/HIV co-infection groups (Table 2). Linear regression models were fit using viral infection status, age, and gender as potential predictors of mean TGF- α 1 levels among all 242 participants. After adjusting for HIV and HCV infection status, gender and age were not significantly associated with TGF- β 1 levels. The interaction effect between HIV and HCV infection status was found to be statistically significant ($p < 0.0001$). Specifically, the model demonstrated that HIV infection was associated with elevated TGF- α 1 in HCV-positive individuals but not in HCV-negative individuals. However, as HCV RNA levels are elevated in HCV/HIV co-infected persons, the role of HIV infection was explored further in HCV-positive persons, adjusting for the effects of HCV RNA levels. In a sub-analysis restricted to the HCV-positive subjects (Table 4), HIV co-infection was significantly associated with higher TGF- β 1 levels compared with HCV mono-infection (mean = 4.49 vs. 4.34 log₁₀ pg/mL, $p = 0.0003$). Furthermore, HCV treatment was associated with lower TGF- α 1 levels compared with individuals never treated for HCV (mean = 4.35 vs. 4.46 log₁₀ pg/mL, $p = 0.033$). High HCV RNA levels (>6 log₁₀ IU/mL) were also associated with a significant increase in TGF- β 1 levels compared with those with low HCV RNA levels (mean = 4.53 vs. 4.37 log₁₀ pg/mL, $p = 0.0003$). In the multivariate regression model adjusted for potential confounding by age, HIV co-infection, HCV RNA level, and HCV treatment history, HCV RNA level remained marginally significant ($p = 0.067$), although HIV co-infection and HCV treatment history were no longer significant after controlling for HCV RNA level.

Correlations among serum cytokine levels were also analyzed using Spearman rank tests. Among all 242 participants, IL-8 and TNF- α levels were positively correlated ($r = 0.125$, $p = 0.05$). However, the statistically significant association between IL-8 and TNF- α levels among HCV mono-infected persons ($r = 0.401$, $p = 0.0007$) appeared to drive this relationship, as IL-8 and TNF- α levels were not correlated when the analysis was restricted to the HCV/HIV co-infection group ($r = 0.068$, $p = 0.49$). TGF- β 1 levels were not correlated with either IL-8 or TNF- α levels. However, TGF- β 1 levels were marginally associated with IL-8 levels in the HCV/HIV co-infection group ($r = 0.184$, $p = 0.06$) but not in the HCV mono-infection group or uninfected controls. No correlation was found between TNF- α and TGF- β 1 in any infection group.

In a previous study, we examined whether changes in serum cytokine levels were associated with HCV virologic, biochemical, or histologic response to therapy.¹⁴ Although IL-8 and TNF- α levels were elevated after HCV therapy, baseline cytokine levels were not predictive of treatment response among HCV/HIV co-infected individuals. This is in contrast to previous data from HCV mono-infected persons, suggesting that serum levels of IL-8, TNF- α , or TGF- β 1 could serve as predictors of the virologic response to HCV therapy.^{4,11–13} Whereas previous studies found that increased IL-8 levels were associated with nonresponse to HCV treatment,^{4,7,17} the present study suggests that excess production of IL-8 above that

present during HCV mono-infection does not occur frequently in the context of HIV co-infection. Our data also confirmed a significant positive correlation between serum IL-8 and TNF- α levels. However, this correlation was present only in the HCV mono-infection group, suggesting that HIV may perturb the regulatory pathways that exist between these two cytokines. We also found that HIV infection was associated with increased TGF- α 1 levels among HCV-positive persons but not among HCV-negative persons. Thus, the higher HCV RNA levels commonly described during HCV/HIV co-infection may further contribute to liver fibrosis by induction of TGF- α 1 expression.

Several limitations to our study should be noted. First, because a convenience sampling approach was used to increase the sample size and to capture subjects with different viral infection status, caution should be taken when interpreting these data. We did, however, carefully consider the cohort characteristics by multivariate modeling that accounted for age, treatment history, and viral load; nonetheless, other important variables may exist that were not included here. Second, liver biopsy material was not available for the vast majority of people studied. Thus, we could not directly assess intrahepatic expression of these and other cytokines. Third, the limited volume of available serum required us to focus on those cytokines most likely to be involved in HCV disease pathogenesis, although other cytokines may also be involved in mediating interactions between HIV and HCV. Finally, although we did find relatively high TGF- β 1 levels in healthy controls with no underlying liver disease or immunodeficiency, platelets also release TGF- β 1. Thus, elevated TGF- β 1 levels among uninfected controls may be the result of higher platelet counts¹⁸ or non-white race/ethnicity.¹⁴

Several studies have suggested that serum cytokines may be important markers of liver inflammation and fibrosis.^{8,19–21} The primary objective of this study was to identify potential virologic determinants of cytokine expression, as intrahepatic disease markers are not routinely assessed in HIV mono-infected individuals or healthy controls or for all HCV-infected individuals. Collectively, such studies are likely to provide new insights into pathogenesis during HCV/HIV co-infection and may identify noninvasive markers of hepatic inflammation and fibrosis among HCV/HIV co-infected persons. For instance, the induction of a sustained profibrotic signal, such as TGF- β 1, by HIV may cause accelerated liver fibrosis during HCV/HIV co-infection and may hinder the host's ability to mount an effective HCV-specific immune response. Further studies are warranted to test these hypotheses and to identify noninvasive markers of liver disease for the clinical management of HCV disease, particularly when liver biopsies have not been performed or are contraindicated.

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FIG. 1.

IL-8 (**A**), TNF- α (**B**), and TGF- β 1 (**C**) levels by viral infection status. Serum levels of IL-8, TNF- α , and TGF- β 1 were quantified using standard enzyme-linked immunoassays and normalized to a standard curve. The lower limit of detection (LLD) for each assay was 3.5 pg/mL for IL-8, 1.6 pg/mL for TNF- α , and 7.0 pg/mL for TGF- α 1. Sample measurements below the LLD were assigned a value of 0. Values are expressed as log₁₀ transformed values (pg/mL). Lines in **C** denote mean TGF- β 1 levels.

Table 1

Study Population Characteristics^a

<i>Infection status</i> (total = 242)	<i>Uninfected</i> (n = 20)	<i>HIV</i> <i>mono-infection</i> (n = 48)	<i>HCV</i> <i>mono-infection</i> (n = 68)	<i>HCV/HIV</i> <i>co-infection</i> (n = 106)
Gender (n = 217) *				
Male	10 (50%)	21 (87%)	40 (59%)	84 (80%)
Female	10 (50%)	3 (13%)	28 (41%)	21 (20%)
Age (n = 213) ** (median years)	28.5	40.00	49.00	43.00
HCV genotype (n = 164) ***				
Genotype 1	—	—	41 (61%)	76 (78%)
Non-1	—	—	26 (39%)	21 (22%)
History of HCV treatment (n = 242)	—	—	35%	10%
HCV RNA (n = 168) (median log ₁₀ IU/mL)	—	—	5.07	6.07
HIV RNA (n = 136) (median log ₁₀ copies/mL)	—	3.34	—	1.80
History of HIV treatment (n = 242)	—	4%	—	73%

^aPlasma HCV RNA levels were quantified using the Roche Amplicor Monitor kit (Nutley, NJ), with a lower limit of detection (LLD) of 600 IU/mL. Plasma HIV viral loads were quantified using the Roche Amplicor Monitor kit, with an LLD of 50–500 copies/mL. HCV genotypes were determined using the LiPA assay. HCV RNA levels were converted to IU/mL prior to analysis using a conversion factor of 2.7 copies/IU according to the manufacturer's instructions. Associations between dichotomous or categorical variables were examined using Fisher's exact tests, and comparisons involving continuous variables were evaluated using the Kruska-Wallis tests. Numbers in parentheses in the first column denote the number of subjects with available data for analysis.

* $p = 0.008$ for the comparison across all groups.

** $p < 0.0001$ for the comparison across all groups.

*** $p = 0.02$ for the comparison between two groups.

Table 2Cytokine Comparisons by Infection Status^a

		<i>IL-8</i>		<i>TNF-α</i>		<i>TGF-β1</i>	
	<i>Infection status</i>	<i>Detection rate</i>	<i>p value</i>	<i>Detection rate</i>	<i>p value</i>	<i>Mean (log₁₀)</i>	<i>p value</i>
Co-infection vs. mono-infection	HCV ⁺ HIV ⁺	74 (70%)	<0.0001	30 (28%)	NS ^b	4.49	NS
	HCV ⁻ HIV ⁺	7 (15%)		14 (29%)		4.52	
	HCV ⁺ HIV ⁺	74 (70%)	β 0.0009	30 (28%)	NS	4.49	β 0.0003
	HCV ⁺ HIV ⁻	62 (91%)		13 (19%)		4.34	
Uninfected vs. mono-infection	HCV ⁻ HIV ⁻	6 (30%)	NS	0 (0%)	0.007	4.70	0.0007
	HCV ⁻ HIV ⁺	7 (15%)		14 (29%)		4.52	
	HCV ⁻ HIV ⁻	6 (30%)	<0.0001	0 (0%)	0.030	4.70	<0.0001
	HCV ⁺ HIV ⁻	62 (91%)		13 (19%)		4.34	

^aDue to the high number of samples below the assay LLD, IL-8 and TNF- α levels were dichotomized as detectable or undetectable in all analyses. All TGF- β 1 levels were detectable and thus analyzed as continuous data. Results were compared by Pearson's chi-square test for IL-8 or TNF- α and the *t*-test for TGF- β 1.

^bNS, not significant ($p > 0.15$).

Table 3

IL-8 LOGISTIC REGRESSION ANALYSIS AMONG HCV-POSITIVE PERSONS^a

	n	IL-8 detection	p value	
			Univariate	Multivariate
Age, years			0.10	NS ^b
45	82	60 (73%)		
>45	91	76 (84%)		
Gender			NS	^c
Male	124	94 (76%)		
Female	49	42 (86%)		
HIV co-infection			<0.0001	0.0003
Yes	106	74 (70%)		
No	68	62 (91%)		
HCV RNA			0.11	0.0004
>6 log ₁₀ IU/mL	61	53 (87%)		
6 log ₁₀ IU/mL	107	82 (77%)		
HCV genotype			NS	^c
Genotype 1	117	97 (83%)		
Non-1	47	37 (79%)		
HCV treatment			NS	NS
Ever	35	30 (86%)		
Not reported	139	106 (76%)		

^aThis subanalysis included 68 HCV mono-infected and 106 HCV/HIV co-infected individuals. Logistic regression models were used for the dichotomized variables (Table 3), and linear regression models were used for the log₁₀ transformed variables (Table 4). Wald confidence intervals were used for the odds ratios in the logistic regression models. Age, gender, HIV status, HCV RNA, HCV genotype, and HCV treatment history were considered as covariates in the regression analyses among HCV-positive subjects. Multivariate models examined the joint effects of the variables of interest on cytokine levels after adjusting for potential confounding. Because of the likely associations among HIV co-infection, HCV RNA level, and HCV treatment history, we adjusted for these variables in multivariate models, even when they were not statistically significant in univariate analyses.

^bNS, not significant ($p > 0.15$).

^cThese variables were not entered in the multivariate model.

Table 4TGF- β 1 LINEAR REGRESSION ANALYSIS AMONG HCV-POSITIVE PERSONS^a

	n	Mean TGF- β 1 (log ₁₀ transformed)	p value	
			Univariate	Multivariate
Age, years			NS	NS ^b
45	82	4.45		
>45	91	4.42		
Gender			NS	^c
Male	124	4.44		
Female	49	4.42		
HIV co-infection			0.0003	0.130
Yes	106	4.49		
No	68	4.34		
HCV RNA			0.0003	0.067
>6 log ₁₀ IU/mL	61	4.53		
6 log ₁₀ IU/mL	107	4.37		
HCV genotype			NS	^c
Genotype 1	117	4.44		
Non-1	47	4.38		
HCV treatment			0.0330	NS
Ever	35	4.35		
Not reported	139	4.46		

^a See Table 3 for statistical analysis.^b NS, not significant.^c These variables were not included in the multivariate model.