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Effects of HCV Treatment on Cytokine Expression During HCV/HIV Coinfection

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Abstract

There is growing evidence that cytokine expression is linked to hepatitis C virus (HCV) pathogenesis and treatment response rates among HCV-monoinfected persons. However, because of the profound effects of human immunodeficiency virus (HIV) coinfection on HCV, it is not clear if these observations are also true for HCV/HIV-coinfected individuals. Serum expression of the proinflammatory cytokines interleukin-8 (IL-8) and tumor necrosis factor- α (TNF- α) and the fibrogenic cytokine transforming growth factor- β 1 (TGF- β 1) were measured in HCV/HIV-coinfected persons at baseline and at week 24 of HCV therapy. Higher levels of IL-8 and TGF- β were demonstrated among nonwhite subjects at baseline. Increases in TNF- α and IL-8 expression were found at week 24 of HCV therapy, suggesting that enhanced proinflammatory cytokine production may occur during HCV treatment. However, cytokine levels were not predictive of HCV virologic, biochemical, or histologic response. Although previous studies conducted among HCV-monoinfected individuals have suggested that cytokine levels could predict the virologic response to therapy, no such associations were observed among HCV/HIV-coinfected persons, suggesting that they may respond differently to treatment than do their HCV-monoinfected counterparts.

INTRODUCTION

In the United States, an estimated 150,000–300,000 persons are infected with both hepatitis C virus (HCV) and human immunodeficiency virus (HIV). The critical roles that cytokines

play in immune responses, inflammation, and fibro-genesis suggest that this complex network of molecules may contribute to viral persistence in chronic hepatitis C infection as well as regulate the response to therapeutic interventions. Mounting evidence suggests that the cytokine environment is altered during HCV mono-infection.^{1,2} For instance, interleukin-2 (IL-2) and interferon- γ (IFN- γ), are upregulated in chronic HCV patients compared with uninfected controls, and levels of these cytokines are correlated with histologic fibrosis,³ whereas IL-2, IFN- γ , and IL-8 are associated with inflammatory activity.⁴ There is growing evidence that cytokine expression is also linked to HCV treatment response, with lower baseline levels generally correlated with virologic response.⁴⁻¹³ Thus, certain cytokines may be useful as easily measured, predictive markers of treatment response.

We, therefore, quantified serum expression of IL-8, tumor necrosis factor- α (TNF- α), and transforming growth factor- β 1 (TGF- β 1) among HCV/HIV-coinfected persons enrolled in an HCV treatment trial to explore (1) the demographic and clinical variables associated with baseline levels and changes in cytokine expression after HCV treatment initiation and (2) the effects of HCV therapy on cytokine expression.

MATERIALS AND METHODS

Study design

Study subjects were 52 individuals enrolled in the U.S. Adult AIDS Clinical Trial Group (ACTG) study A5071 between December 2000 and April 2001 who had baseline and week 24 sera available for cytokine quantification. A5071 was a prospective, randomized, phase II/III trial evaluating the safety and efficacy of pegylated IFN plus ribavirin (PegIFN-RBV) vs. IFN plus RBV (IFN-RBV) in HCV/HIV-coinfected individuals with stable HIV disease who had not previously been treated with IFN. The study was described in detail in the original publication.¹⁴ Serum IL-8, TNF- α and TGF- β levels were assessed prior to treatment (baseline) and after 24 weeks of PegIFN-RBV or IFN-RBV. As measures of HCV treatment response, HCV virologic response (undetectable HCV RNA) and histologic response (2 point drop in total hepatic activity index [HAI] from baseline) were assessed at week 24. Biochemical response was defined as normalization of serum alanine aminotransferase (ALT) levels in persons with ALT greater than the upper limit of normal (ULN) at entry. All study patients were treated for 24 weeks, but only virologic responders and histologic responders completed 48 weeks of treatment. Plasma HCV RNA levels were quantified using the Roche Amplicor Monitor kit (Roche Diagnostic Systems, Inc., Branchburg, NJ), with a lower limit of detection (LLD) of 60 IU/mL for the primary study end point at week 24. Plasma HIV viral loads were quantified using the Roche Amplicor Monitor kit, with an LLD of 50 copies/mL. HCV genotypes were determined using the LiPA assay (Innogenetics, Gent, Belgium).

Immunoassays

Whole blood was separated by centrifugation at 1500 rpm for 15 min, and serum aliquots were frozen at -80°C until use. Serum levels of IL-8, TNF- α , and TGF- β 1 were quantified using standard enzyme-linked immunoassays (ELISAs) according to the manufacturer's

instructions (R&D Systems, Minneapolis, MN). The 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- β 1. Sample measurements less than the LLD were assigned a value of 0.

Statistical analysis

Associations between dichotomous or categorical variables were assessed using Fisher's exact tests, and McNemar's exact tests were conducted on changes over time. Comparisons between two groups involving continuous data were evaluated using Wilcoxon rank-sum tests, whereas changes over time were assessed by signed rank tests. Correlations were evaluated using Spearman rank tests. For baseline IL-8 analysis, log-normal regression models that accounted for left-censored observations were used to evaluate joint effects in multivariate models, and likelihood ratio tests were used to assess the significance of covariate effects. All p values were two-sided, and only p values < 0.05 were considered statistically significant without adjustments for multiple testing.

RESULTS

Study population characteristics

Baseline characteristics of the 52 participants prior to initiation of HCV therapy are shown in Table 1. No statistically significant differences were found in baseline characteristics between the study subjects and the remaining 81 A5071 participants, except for the HIV RNA detection rate. Whereas 29% of the current study participants had detectable HIV RNA at baseline, 47% of the remaining A5071 participants had detectable HIV RNA ($p = 0.05$). Consistent with the parent A5071 study results, PegIFN-RBV was found to be superior to IFN-RBV in reducing HCV RNA in our study sample ($p = 0.0006$) but their effects were similar on HAI and ALT changes. Similarly, abnormal ALT levels were reduced after treatment ($p = 0.0002$), although there was no change in HAI.

Cytokine levels at baseline and associations with clinical variables

At baseline, 86% of the participants had detectable serum IL-8, and 22% had detectable TNF- α (Fig. 1). TGF- β 1 was detectable for all participants. Baseline medians (ranges) were 1.52 (0–3.52), below the LLD (0–2.07), and 4.58 (4.10–4.81) \log_{10} pg/mL for IL-8, TNF- α and TGF- β 1, respectively. We next explored associations between baseline serum cytokine expression and baseline demographics and clinical variables of interest. HIV RNA detection, HCV genotype, HCV RNA, and HAI were not significantly associated with IL-8 levels. Non-white race/ethnicity ($p = 0.026$) and higher fibrosis score ($p = 0.0044$) were associated with increased IL-8, and decreased CD4 cell count ($p = 0.061$) and age ($p = 0.070$) were marginally associated with baseline IL-8 in univariate analyses. Their joint effects were assessed in a multivariate log-normal regression model. Race/ethnicity ($p = 0.025$) and baseline fibrosis score ($p = 0.026$) remained significant; however, CD4 count and age were no longer significant after adjusting for race/ethnicity and fibrosis score (Table 2). For TNF- α , there was a marginal negative correlation with HCV RNA ($p = 0.09$); however, no other associations were found to be statistically significant. For TGF- β 1, there was an association between increased TGF- β 1 and nonwhite race/ethnicity ($p = 0.002$), although no other significant associations were found.

Changes in cytokine levels after 24 weeks of HCV treatment

As no differences in cytokine levels were detected between PegIFN-RBV and IFN-RBV, they were grouped together for all subsequent analyses. After 24 weeks of HCV treatment, IL-8 detection increased from 86% at baseline to 94%, whereas TNF- α detection increased from 22% to 63%. Median IL-8 level increased marginally from 1.52 log₁₀ pg/mL at baseline to 1.68 log₁₀ pg/mL (range 0.00–2.68) at week 24 ($p = 0.08$). Median TNF- α level increased significantly from undetectable to 0.46 log₁₀ pg/mL (range 0.00–2.16, $p = 0.007$). TGF- β 1 was detectable in all samples at both times, and median TGF- β 1 remained steady at 4.58 log₁₀ pg/mL over 24 weeks of HCV treatment ($p = 0.19$).

Association analyses

No significant associations were found between week 24 changes from baseline ($_{0 \rightarrow 24}$) in IL-8 or TGF- β 1 levels and $_{0 \rightarrow 24}$ in HIV RNA, CD4 cell count, HCV RNA, HAI, or fibrosis score. No significant associations were observed between IL-8, TNF- α , or TGF- β 1 $_{0 \rightarrow 24}$ and HCV treatment, race/ethnicity, HCV genotype, HCV virologic response, histologic response, or biochemical response. The associations between baseline IL-8, TNF- α , or TGF- β 1 levels and week 24 HCV virologic, histologic, or biochemical response were also not significant. Furthermore, baseline levels of the three cytokines, as well as changes in their levels from baseline, were not significantly correlated with each other.

DISCUSSION

Several previous studies have examined cytokine production in response to therapy during HCV mono-infection.^{12,13,15,16} These data suggest that serum levels of cytokines, such as IL-8, TNF- α , and TGF- β 1, might be important predictors of the virologic response to HCV therapy. In the present analysis, we measured cytokine levels to determine if cytokine expression was also associated with HCV virologic, histologic, or biochemical response among HCV/HIV-coinfected persons.

We found a statistically significant association between race/ethnicity and IL-8 expression, with higher IL-8 levels among nonwhite participants. Fibrosis score was also positively correlated with IL-8 at baseline. In multivariate analysis, race/ethnicity and fibrosis score remained significantly associated with IL-8 expression. Nonwhite race/ethnicity was also associated with significantly higher levels of TGF- α 1 but not TNF- β , suggesting that differences in cytokine expression among diverse ethnic groups could have important implications for HCV.

A5071 subjects showed a significant reduction in HCV RNA after HCV treatment; thus, we evaluated if virologic response was mediated by proinflammatory or fibrogenic cytokines, such as IL-8, TNF- α and TGF- β 1. Whereas detection rates for IL-8 and TNF- α increased after initiation of HCV therapy, median TGF- β 1 levels were stable over the 24-week treatment period. Importantly, the associations between baseline (or changes at week 24 from baseline) IL-8, TNF- α , or TGF- β 1 levels and week 24 HCV virologic, histologic, or biochemical response were not significant. The study sample size was sufficient to detect a correlation magnitude of ~ 0.4 between cytokine expression and HCV RNA changes after

HCV treatment with 83% power. Therefore, either the statistical power of the measured cytokine levels to predict virologic response is lower than what is allowed with this sample size (16 responders and 36 nonresponder), or despite the increased proinflammatory cytokine expression demonstrated in the current study, these cytokines are not critical mediators of HCV treatment response in HCV/HIV-coinfected persons.

Several limitations to our study should be considered. First, coinfecting persons were selected from a larger clinical trial based on sample availability; thus, negative findings may reflect limited power to detect small differences in cytokine expression. Although our study participants had lower HIV RNA detection compared with the remaining A5071 subjects, they were otherwise representative of the A5071 study population. Second, studies using paired serum and liver biopsies are lacking from the HCV literature, making it difficult to determine empirically whether peripheral cytokine levels accurately reflect intrahepatic cytokine expression. As liver biopsies were performed only on study participants who did not achieve HCV virologic response, biopsy material was not available for the majority of A5071 study participants. Therefore, we sought to evaluate serum cytokines as noninvasive markers of treatment response.^{3,5,17–19} Finally, it is likely that multiple cytokines, particularly endogenous IFNs, are also involved in mediating interactions between HIV and HCV but were not evaluated in the current study. Although a direct comparison between HCV-monoinfected and HCV/HIV-coinfected persons was not considered in the original A5071 study design, the current substudy demonstrates the inability of cytokine levels to predict virologic response to therapy in HCV/HIV-coinfected persons, as has been documented previously in HCV-monoinfected individuals.

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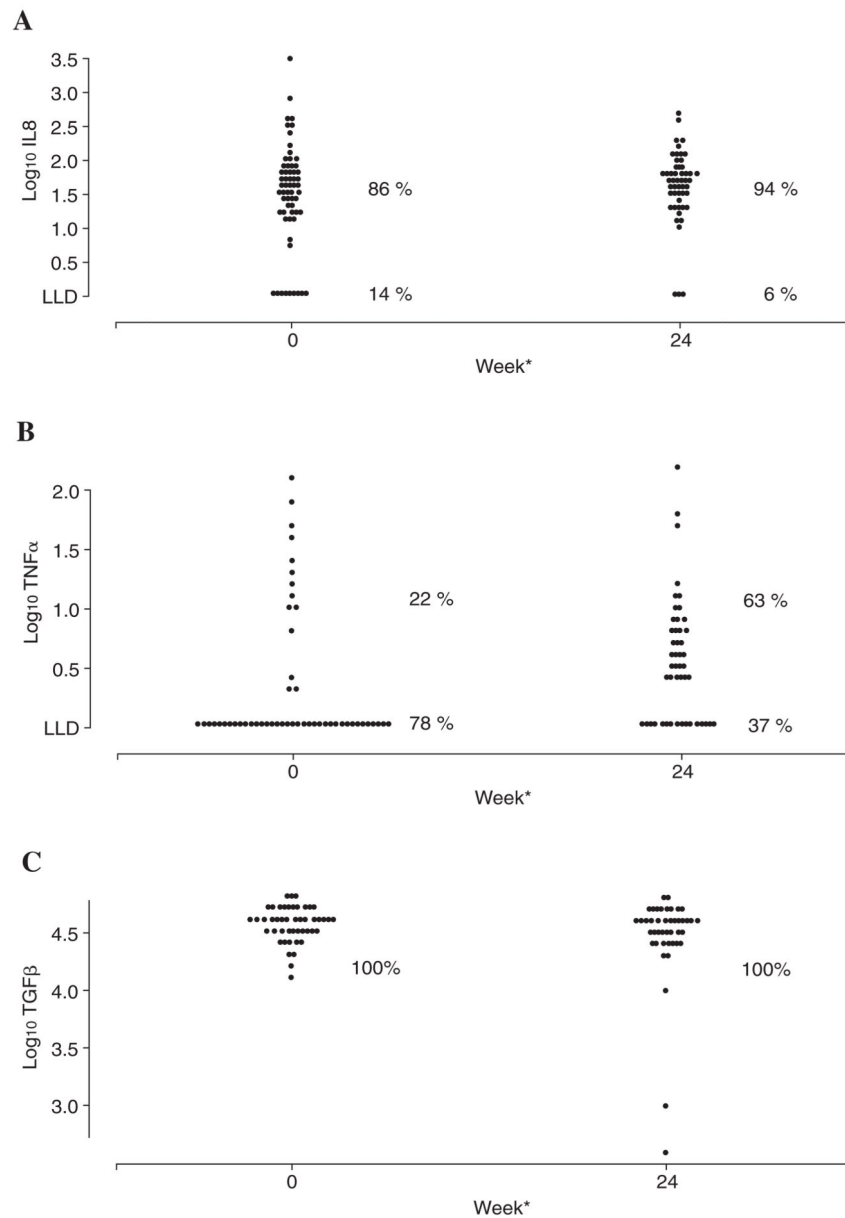


FIG. 1. (A) IL-8, (B) TNF- α , and (C) TGF- β 1 levels at baseline and at 24 weeks of HCV treatment. Values are expressed as \log_{10} transformed values (pg/mL). LLD on (A) and (B) denotes the lower level of detection for each assay. (A) $*p = 0.082$ comparing the median change between weeks 0 and 24. (B) $*p = 0.007$ comparing the median change between weeks 0 and 24. (C) $*p = 0.19$ comparing the median change between weeks 0 and 24.

Table 1

Baseline Population Characteristics

Characteristic	Total (n = 52)
Male gender	42 (81) ^a
Race/ethnicity	
White	23 (44)
Black	20 (38)
Hispanic	6 (12)
Other	3 (6)
Age (years)	
Median (range)	45 (27–60)
HCV genotype 1	41 (79)
HCV therapy	
IFN-RBV	27 (52)
PegIFN-RBV	25 (48)
HIV therapy	
Naive/not reported	6 (12)
NRTI ^b	8 (15)
NRTI + NNRTI (or PI)	27 (42)
NRTI + NNRTI + PI	11 (21)
CD4 count (cells/mL)	
Median (range)	445 (131–1240)
HCV RNA (log ₁₀ IU/mL)	
Median (range)	6.31 (4.35–6.63)
HIV RNA (log ₁₀ copies/mL)	
Detectable	15 (29)
Hepatic activity index	
Median (range)	5 (2–10)
Fibrosis score	
Median (range)	2 (0–6)
Abnormal ALT	36 (69)

^aData are number of participants (%) unless otherwise noted.

^bNRTI, nucleoside reverse transcriptase inhibitor; NNRTI, nonnucleoside reverse transcriptase inhibitor; PI, protease inhibitor.

Table 2

Log-Normal Regression Analysis of Baseline IL-8

Characteristic	Univariate <i>p</i> value	Multivariate <i>p</i> value
Race/ethnicity (nonwhite)	0.026	0.025
Baseline HIV RNA (detectable vs. undetectable)	0.82	<i>a</i>
HCV genotype (1 vs. non-1)	0.32	<i>a</i>
Baseline ALT (≤ ULN vs. > ULN)	0.12	<i>a</i>
Baseline CD4	0.061	0.360
Baseline HCV RNA	0.53	<i>a</i>
Baseline hepatic activity index	0.25	<i>a</i>
Baseline fibrosis score	0.0044	0.026
Age	0.070	0.460

^aNot entered in the multivariate model ($p > 0.10$).