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Antigen-specific immune responses and liver histology in HIV and hepatitis C coinfection

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Abstract

Objective—To test the hypothesis that antigen-specific interferon (IFN) γ responses are correlated with milder liver disease in subjects coinfecting with HIV-1 and hepatitis C virus (HCV).

Design—Cellular immune responses were studied in a cohort with HIV/HCV coinfection (n = 107) who underwent liver biopsy.

Methods—We measured HCV-specific and recall responses in peripheral blood mononuclear cells using IFN γ and interleukin (IL)-10 ELISpots, and correlated these immune responses with liver histology. The relationship of immunologic, virologic and clinical variables to inflammation and fibrosis was modeled using recursive partitioning.

Results—There were significant negative correlations between inflammatory scores and IFN γ production in response to the HCV proteins core, NS5 and summed HCV responses. Lower fibrosis scores were also correlated with higher IFN γ production in response to NS5 and summed HCV proteins. Higher IFN γ production in response to *Candida* was significantly associated with lower inflammatory and fibrosis scores. In multivariable models, factors associated with severe fibrosis were lower IFN γ responses to *Candida* and summed HCV proteins. Factors associated with severe inflammation were detectable HIV viral load and lower HCV viral load, while predictors of mild inflammation included undetectable HIV viral load and higher IFN γ response to *Candida*.

Conclusions—In this cohort of subjects coinfecting with HIV and HCV, antigen-specific IFN γ responses are correlated with milder inflammation and fibrosis. Immunological responses best predicted severity of fibrosis, while clinical variables and recall antigen responses best predicted severity of inflammation.

Keywords

Hepatitis C virus; liver histology; cellular immunity; interferon gamma; fibrosis; interleukin 10

Introduction

The majority of individuals who develop hepatitis C virus (HCV) infection fail to spontaneously clear the virus and are at risk for progressive liver inflammation and fibrosis. While cellular immune responses are clearly important for spontaneous recovery from acute infection, the role of these CD4 or CD8 T cells in chronic infection is less clear [1,2]. The prevailing hypothesis of liver injury in HCV is that CD4 and CD8 T-cell responses mediate HCV-related liver damage while engaged in an ineffectual effort to clear the chronic infection [3]. However, previously published studies are conflicting regarding whether type-1-like immune responses found in peripheral blood mononuclear cells (PBMC) are protective against liver damage [4,5] or are associated with more advanced liver disease [6]. We have recently reported that development of a vigorous CD4 type 1 response very early in hepatitis C infection (within 6 months) appears to protect against more rapid liver fibrosis in the setting of *Schistosomiasis mansoni* coinfection [7]. However, HCV-specific cellular immune responses are difficult to examine in chronic HCV infection because they are of low magnitude in PBMC [4,8–14].

Persons with chronic human immunodeficiency syndrome (HIV) and HCV coinfection, particularly those with CD4 cell counts $< 200 \times 10^6/l$, have a higher rate of fibrosis development and are more likely to progress to severe liver disease [15]. Coinfected persons have variable degrees of immunosuppression that may provide insight into the relationship between cellular immune functions and the degree of liver damage as assessed by liver biopsy. Studies of persons with HIV and chronic HCV coinfection have also demonstrated low frequency HCV-specific responses in PBMC. One study found that one out of 19 persons with HIV/HCV coinfection had interferon (IFN) γ secretion to HCV core protein and three out of 19 had IFN γ secretion to NS3 [16]. However, no prior studies of HIV/HCV coinfection have correlated immune responses with liver histology [16–18].

In this study, we examined both HCV-specific and recall antigen responses in PBMC of persons with HIV/HCV coinfection entering an anti-HCV treatment trial. As expected, this coinfecting cohort had very low frequency HCV-specific immune responses using sensitive ELISpot assays, but the large size of the cohort allowed us to examine relationships between antigen-specific immune responses and liver inflammation and fibrosis. We also used recursive partitioning to identify relationships between various clinical factors that may affect HCV-related disease progression and cellular immune responses in order to assess the relative role of immune responses in histological outcome.

Materials and methods

Subjects

One-hundred and thirty-three eligible subjects were enrolled in the Adult AIDS Clinical Trial Group protocol A5071, a randomized, open-label multicenter trial comparing pegylated IFN α -2a plus ribavirin with standard IFN α -2a three times a week plus ribavirin [19]. HIV-infected subjects 18 years or older were eligible for trial participation if they had chronic HCV infection confirmed by HCV RNA detection and had not been previously treated with IFN α -containing regimens. A liver biopsy demonstrating abnormal histology consistent with chronic hepatitis C was required within 48 weeks of study entry, although median time from entry liver biopsy to study enrollment was 33 days. Study eligibility required normal or elevated serum alanine aminotransferase (ALT) levels and negative tests for hepatitis B surface antigen. Liver biopsies were blindly scored by a central hepatopathologist using the modified Histology Activity Index (HAI) [20]. The current report analyzed all subjects who had adequate PBMC for analysis at study entry ($n = 107$). A5071, including the assays performed for this report, was reviewed and approved by the Ethics Committees of the Beth Israel Deaconess Medical Center and all participating sites. All subjects participating in the study provided written informed consent before any study related procedures. The protocol and all study procedures were conducted in conformity with the ethical guidelines of the Declaration of Helsinki. Methodology was established with HIV negative subjects recruited from Beth Israel Deaconess Medical Center and Boston Medical Center, Boston, MA after review and approval by the Ethics Committees of both sites, and these subjects provided written informed consent.

Recombinant HCV proteins

The recombinant HCV proteins used were derived from HCV genotype 1b and included core protein (amino acids 1–115), and nonstructural (NS) proteins NS3 (amino acids 1007–1534), and NS5 (amino acids 2622–2868) (Mikrogen, Munich, Germany).

ELISpot assay

PBMC were isolated by Ficoll-Hypaque (Amersham Biosciences, Piscataway, New Jersey, USA) density-gradient centrifugation and cryopreserved. Ninety-six-well polyvinylidene difluoride plates (Millipore Corporation, Bedford, Massachusetts, USA) were coated under sterile conditions with 100 μ l primary monoclonal antibody [mAb; anti-IFN γ , anti-interleukin (IL)-10; Endogen, Woburn, Massachusetts, USA] at a concentration of 10 μ g/ml in bicarbonate buffer and incubated overnight at 4°C. Excess antibody was removed and wells were blocked with complete media [RPMI 1640 (GIBCO, Grand Island, New York, USA) supplemented with 10 mM HEPES, penicillin (100 IU/ml), streptomycin (100 μ g/ml), and 10% heat inactivated human AB serum (RPMI-AB; Biowhittaker, Walkersville, Maryland, USA)] for 1 h. PBMC (2.5×10^5) were cultured for 48 h in the presence of the recombinant HCV protein core, and nonstructural proteins NS3 and NS5 at 1 μ g/ml. Positive control wells consisted of phytohemagglutinin (PHA, 5 μ g/ml, Sigma, St. Louis, Missouri, USA) and *Candida* cellular antigen (20 μ g/ml, Greer Labs, Lenoir, North Carolina, USA). Negative control wells were media alone and buffer (Mikrogen). After 48 h, plates were washed four times with phosphate buffered saline (PBS) and four times with PBS containing

0.05% Tween20 (PBS-T). Fifty microliters of biotin-conjugated secondary mAb (anti-IFN γ or anti-IL-10, Endogen) were added to each well at a concentration of 0.2 μ g/ml and incubated for 2 h at room temperature. After the plates were rinsed with PBS-T, 100 μ g/ml streptavidin-horseradish peroxidase (1 : 6000, Endogen) were added for 90 min. Plates were washed with PBS-T and 100 μ l substrate [3',9' aminoethyl-carbazole (Sigma) in dimethyl formamide and sodium acetate buffer (0.05 M, pH 5.0) and 0.3% H₂O₂] was added and incubated until the appearance of brown spots in the wells, then rinsed with tap water. The numbers of spots per well were scored using a dissection microscope. Averaged numbers of spot forming cells (SFC) in control wells were subtracted from antigen-stimulated wells to correct for spontaneous cytokine production. Non-specific responses were low in this assay. Background (buffer control) average numbers of SFC were zero in 66% of the cohort for IFN γ and 74% for IL-10. In nine healthy control subjects, one subject had one IFN γ SFC to each of NS3 and NS5; a second control subject had one IFN γ SFC to core and one IL-10 SFC to NS5. All other control subjects had zero IFN γ or IL-10 SFC to all HCV antigens, and all had IFN γ *Candida* responses (data not shown). Persons performing the ELISpot assays were blinded to all cohort data except unique patient identifiers and dates of sample collection.

Statistical methods

Data were analyzed in SAS 6.12 (SAS Institute, Carey, North Carolina, USA) using nonparametric tests including the Kruskal–Wallis test and Spearman rank correlation. All tests were two-tailed and evaluated at a significance level of 0.05. No adjustment for multiple comparisons was made in these analyses. Recursive partitioning using CART (Classification and Regression Trees) in Splus V3.4 (MathSoft Inc., Insightful Corporation, Seattle, Washington, USA) was performed to identify subgroups highly associated with the severity of liver histology [21]. HCV-specific responses were considered as responses against a single protein as well as summed for responses against core, NS3 and NS5. Clinical variables included sex, race and HCV genotype, and entry age, injecting drug use, CD4 cell count, antiretroviral regimen [protease inhibitor (PI) versus non-PI], and HIV and HCV viral loads. Immune response data were included as continuous variables, and CART selected 'cut points' associated with histologic outcome. These have been characterized as 'high' and 'low', and the actual value may be specific to this dataset. Numerous models were generated, and the most parsimonious with high sensitivity and specificity for histology are presented. Extensive cross-validation and 'pruning' was not performed in order to identify small subgroups with unique characteristics for visual inspection.

Results

Subject characteristics

Table 1 shows demographics characteristics for the 107 subjects in whom cellular immune responses were studied. There were no significant differences between the 107 subjects studied and the 133 eligible subjects (data not shown). Subjects with HCV genotype 1 had similar demographics to that of the whole cohort (Table 1). Of note, only 12/107 (11%) of subjects were markedly immunocompromised with CD4 cell counts < 200 $\times 10^6$ /l and 88% of the cohort was on some form of antiretroviral therapy. HAI scores for inflammation

(components A–D summed) and fibrosis (component E) in pretreatment liver biopsies were dichotomized into ‘mild’ and ‘severe’ based on median values for the cohort. Mild inflammation, represented by scores on the HAI index A–D of 1–5, was seen in 59 subjects (55%) and severe inflammation, represented by scores of > 5 was seen in 48 subjects (45%). Likewise, mild fibrosis, represented by scores of HAI-E 0–2 was noted in 62 subjects (58%) and severe fibrosis with scores of HAI-E 3–6 in 45 subjects (42%).

Cellular immune responses

As expected based on previous literature, the frequency of HCV-specific immune responses in the peripheral blood was low [8,10–12]. Although at least half of the subjects had an undetectable response, the large size of the cohort did allow analysis of the observed responses and histology. As seen in Table 2, subjects with mild inflammation had significantly higher IFN γ secretion to NS5 and *Candida* compared to subjects with severe inflammation. Subjects with mild fibrosis scores had significantly higher secretion of IFN γ to NS3, to the sum total of HCV-specific responses (‘summed HCV’; numbers of SFC to core, NS3 and NS5 added together) and to *Candida*. In contrast, PBMC responses with IL-10 secretion in response to HCV proteins did not distinguish the degree of fibrosis or inflammation although there was higher IL-10 secretion to *Candida* in subjects with mild fibrosis compared to those with severe fibrosis. IFN γ responses to *Candida*, a nonspecific indicator of immune function, were detected in over 75% of the subjects, with a median of 24 SFC/10⁶ PBMC. In comparison, a separate analysis of 41 persons with HCV mono-infection demonstrated responses in 88% of subjects with a median of 69 IFN γ SFC/10⁶ PBMC to *Candida* (data not shown). Thus, consistent with the relatively high median CD4 cell count, this cohort did not have a significant degree of immune dysfunction and the results were similar to those seen in HCV mono-infected subjects.

Table 3 details the Spearman rank correlation coefficients between HCV-specific and *Candida* responses and liver inflammation and fibrosis scores. Milder inflammatory scores correlated with the IFN γ response to HCV core ($r = -0.21$; $P = 0.03$), NS5 ($r = -0.33$; $P < 0.001$), summed HCV ($r = -0.32$; $P < 0.001$), and *Candida* ($r = -0.35$; $P < 0.001$). In addition, milder fibrosis scores correlated with responses to NS5 ($r = -0.23$; $P = 0.02$), summed HCV ($r = -0.22$; $P = 0.02$) and *Candida* ($r = -0.37$; $P < 0.001$). There was a weaker correlation between inflammation and IL-10 secretion to summed HCV ($r = -0.20$; $P = 0.05$).

The antigens used in the ELISpot assay are derived from HCV genotype 1. In order to determine whether the above results were specific for genotype 1 HCV, analyses in all subjects were compared with those in genotype 1 subjects ($n = 85$, 79%) as shown in Table 3. Despite the smaller numbers of subjects with genotype 1, milder inflammatory scores again correlated with IFN γ secretion to NS5, to summed HCV, and to *Candida*; and with IL-10 secretion to NS5 ($r = -0.24$; $P = 0.03$), to summed HCV ($r = -0.22$; $P = 0.05$). There was also an inverse correlation between IFN γ secretion to NS5 and *Candida* and fibrosis scores. There was no significant correlation between IL-10 and fibrosis scores in genotype 1 subjects.

The ability to mount a detectable immune response was not simply a function of the degree of immunosuppression as measured by CD4 cell count, as there were no significant correlations in this cohort between any immune responses studied and absolute CD4 cell counts at trial entry (data not shown).

Multivariable modeling of clinical and immune parameters with outcome of liver histology

It is likely that many of the immune parameters are interrelated in an individual subject, and may be linked to various clinical parameters, so recursive partitioning was used to better understand their predictive value for liver histology relative to clinical parameters. With this statistical technique, the value that best describes a group is chosen by the model, without an *a priori* definition of what constitutes a ‘negative’ or ‘positive’ response, as is typically done when responses are compared to those observed in control subjects. The relative contributions made by immune responses in predicting severe fibrosis (HAI E score > 2) and severe inflammation (summed HAI A–D components > 5) were evaluated in models that also included clinical variables. The immune variables included were IFN γ and IL-10 secretion to core, NS3, NS5, summed HCV, and *Candida*. The clinical variables were age, race, sex, injecting drug use status, HCV genotype, HCV viral load, HIV viral load, and CD4 cell count. Figure 1 demonstrates a classification and regression tree (CART) of variables associated with severe fibrosis. Overall, 42% of all subjects had severe fibrosis. Subjects were most likely to have severe fibrosis if they had poor IFN γ secretion to *Candida* (defined in this dataset as < 22 SFC/10⁶ PBMC) and poor secretion of IFN γ to summed HCV proteins (defined as < 1 SFC/10⁶ PBMC). Of those subjects in our cohort with poor responses to both *Candida* and summed HCV proteins, 71% had severe fibrosis, as compared to 42% of the overall group. Subjects were more likely to have mild fibrosis if they had a stronger IFN γ response to *Candida* (defined as > 22 SFC/10⁶ PBMC) and no detectable IFN γ response to core, where only 15% had severe fibrosis. Interestingly, in this model, variables such as age, HIV or HCV viral load and CD4 cell count did not predict fibrosis. The overall model had a sensitivity of 0.56 and specificity of 0.84.

A CART model for inflammation is shown in Fig. 2. Overall, 45% of the subjects had severe inflammation. In contrast to the model predicting severe fibrosis, factors related to HIV viral control and effects on CD4 cell counts were more predictive of severity of inflammation (Fig. 2). Those with undetectable HIV viral loads and strong IFN γ immune responses to *Candida* (defined as > 22 SFC/10⁶ PBMC) had a low likelihood of having severe inflammation (15% versus 45% in the cohort as a whole). A higher CD4 cell count (> 648 $\times$ 10⁶/l) in those with lower IFN γ immune responses to *Candida* was also associated with milder inflammation, where only 12% had severe inflammation compared to 59% of those with lower CD4 cell counts. Subjects with detectable HIV viral loads (> 50 copies/ml) and lower HCV viral loads (defined as log₁₀ HCV RNA < 6.25) had a higher likelihood of having severe inflammation (84%). Here, HCV-specific immune responses did not contribute to the overall model of inflammation. The overall model had a sensitivity of 0.81 and specificity of 0.71.

Discussion

This study found that antigen-specific, IFN γ immune responses are associated with milder inflammation and milder fibrosis in a relatively immunocompetent cohort of subjects with HIV/HCV coinfection. Mouse models of liver injury demonstrate that excessive type 1 immune responses (including IFN γ and IL-12) are associated with inflammation, but not fibrosis [22,23]. In contrast, type 2 responses (including IL-4, IL-5 and IL-13) are associated with an enhancement in hepatic fibrosis. However, few studies have examined relationships between liver histology and patterns of cytokine production in humans. Rosen *et al.* found in an HCV monoinfected cohort that HCV-specific Th1 responses, particularly against non-structural HCV proteins, were associated with milder histology [4]. A prospective study of HCV-specific immune responses early after acute infection showed that in those patients who developed chronic HCV infection, those with strong IFN γ responses also had a slower progression of fibrosis, while subjects who had increased IL-4 production were more likely to have rapid fibrosis progression [7]. It is unclear in these cohorts whether insufficient type 1 responses or excessive type 2 responses lead to enhanced fibrosis progression [7].

One strength of this study is its size. Peripheral HCV-specific responses occur in low frequency in most cohorts of persons with chronic HCV infection, regardless of coinfection status [4,10–12,14,16–18]. Despite the low frequency of responses in the peripheral blood, the size of this cohort combined with a sensitive ELISpot assay allowed determination of possible associations between antigen-specific IFN γ responses and outcomes of liver inflammation and fibrosis. There were limited but statistically significant differences in the univariate comparisons of immune responses with liver histology when dichotomized into ‘mild’ and ‘severe’ (Table 2). Notably, the two most significantly different antigen-specific immune responses (IFN γ responses against summed HCV and *Candida*) were also selected by CART (which also dichotomized the outcome of histology) as predictors of liver fibrosis. Thus, different approaches to analysis of the large database selected the same immunologic parameters as being associated with milder liver disease. Spearman rank correlations used the data more fully in univariate analysis since the ordinal histology scores were ranked instead of split into two groups (Table 3). Using this different statistical approach, multiple significant correlations were noted between antigen-specific IFN γ secretion and mild liver histology. This cross-sectional study cannot make any conclusions about causality and rates of progression, but these findings add to the studies that describe an association between IFN γ and milder liver histology [4,7].

In addition, previous literature has relied upon arbitrary values to determine whether or not an immune response is present or absent. For example, many laboratories use the mean plus several standard deviations of values obtained in normal controls as a cutoff value in ELISpot, with responses above this cutoff considered to be significant. Our study did not select arbitrary defined cutoff values as ‘true’ responses, but rather looked for associations between immune responses and clinical outcomes. CART use recursive partitioning to select variables that best split a cohort into those with and without an outcome of interest [24]. In this study, variables, such as numbers of SFC secreted in response to a specific antigen, were split at the value that best distinguished mild versus severe histology scores. Because

over half the cohort had no detectable HCV-specific responses, selecting a value of '> 1 SFC' indicated that subjects with one or more SFC secreting a cytokine had a different outcome than those who had no detectable secretion. CART is also useful in this setting to examine relative relationships between multiple clinical parameters and immune responses, which has not been done in previous studies. CART was used to examine relationships between these immune responses and the extensive clinical data collected during the clinical trial (such as age, sex, race, CD4 cell count) with respect to outcomes of liver fibrosis and inflammation. The cutoffs identified by CART in our analysis should not be seen as definitive points, but rather as a framework for future prospective studies examining the relationship of immune responses with rates of fibrosis progression.

There were no associations between CD4 cell counts and any immune responses on univariate analysis, indicating that ability to mount cellular immune responses is not purely a function of the degree of immunosuppression, but may be related to a qualitative defect. Of note, there were too few subjects (11%) with severe immunosuppression, so the association of immune responses and severe fibrosis in those with CD4 cell counts $< 200 \times 10^6/l$ could not be evaluated [25]. We did not have nadir CD4 cell counts available for this cohort, nor information on whether subjects were 'long-term nonprogressors' with respect to their HIV, which has been shown to increase the frequency of HCV-specific responses in coinfecting persons in other cohorts [17]. Data on duration of HCV infection for this cohort was also not available, so immune responses could not be correlated with rate of progression of liver fibrosis.

Overall, higher levels of IFN γ secretion to recall and HCV proteins were associated with less severe inflammation and less fibrosis on univariate analyses and using recursive partitioning. In contrast, IL-10 showed minimal associations with severity of liver disease. In conclusion, both clinical and immune parameters have a role in predicting liver histology in subjects with HIV/HCV coinfection. Increased IFN γ HCV-specific and recall immune responses are associated with milder liver inflammation and fibrosis. These findings may have implications for future research in immunomodulatory therapy for HCV infection.

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Fig. 1. CART for predicting the outcome of severe fibrosis

Forty-two percent of the overall cohort had severe fibrosis (HAI E scores > 2). The CART shows predictors that identified subgroups with higher or lower frequencies of severe fibrosis. All percentages indicate classified subjects with severe fibrosis compared to the total subjects in that classified subgroup.

Fig. 2. CART for predicting the outcome of severe inflammation

Forty-five percent of the overall cohort had severe inflammation (HAI A–D scores > 5). The CART shows predictors that identified subgroups with higher or lower frequencies of severe inflammation. All percentages indicate classified subjects with severe inflammation compared to the total subjects in that classified subgroup.

Table 1

Baseline demographic characteristics.

Characteristic	All subjects (<i>n</i> = 107)	Genotype 1(<i>n</i> = 85)
Male	83%	81%
Race		
White, non-Hispanic	50%	44%
Black, non-Hispanic	34%	38%
Other	17%	19%
Age in years (median)	45	45
13–39	21%	19%
40–49	57%	59%
> 50	22%	22%
Previous IV drug use	64%	66%
Median CD4 cell count ($\times 10^6/l$)	476	490
100–299	20%	19%
300–500	35%	32%
> 500	45%	49%
Antiretroviral regimen		
None	12%	13%
Any NRTI	83%	81%
Any protease inhibitor	54%	55%
HIV RNA load undetectable (< 50 copies/ml)	60%	59%
Log ₁₀ (HCV RNA) IU/ml (median)	6.28	6.30

Table 2

Differences in immune responses between subjects with mild and severe liver histology.

Antigen	Mild inflammation [median (5%, 95% range)]	Severe inflammation [median (5%, 95% range)]	<i>P</i> ^a	Mild fibrosis [median, (5%, 95% range)]	Severe fibrosis [median (5%, 95% range)]	<i>P</i>
IFN- γ -Core	0 (0, 12)	0 (0, 1)	0.22	0 (0, 8)	0 (0, 3)	0.48
IFN- γ -NS3	0 (0, 23)	0 (0, 11)	0.94	0 (0, 23)	0 (0, 8)	0.03
IFN- γ -NS5	0 (0, 20)	0 (0, 4)	0.05	0 (0, 13)	0 (0, 4)	0.08
IFN- γ -Sum ²	1 (0, 24)	0 (0, 17)	0.12	3 (0, 25)	0 (0, 12)	0.01
IFN- γ - <i>Candida</i>	53 (0, 415)	12 (0, 189)	0.005	73 (0, 415)	7 (0, 152)	<0.001
IL10-Core	0 (0, 7)	0 (0, 12)	0.43	0 (0, 23)	0 (0, 1)	0.74
IL10-NS3	0 (0, 45)	0 (0, 23)	0.31	0 (0, 63)	0 (0, 23)	0.18
IL10-NS5	0 (0, 53)	0 (0, 6)	0.06	0 (0, 53)	0 (0, 5)	0.68
IL10-Sum ²	0 (0, 77)	0 (0, 75)	0.13	0 (0, 77)	0 (0, 33)	0.17
IL10- <i>Candida</i>	3 (0, 456)	0 (0, 241)	0.21	4 (0, 456)	0 (0, 241)	0.04

^a Values calculated using the nonparametric Kruskal Wallis test.

^b Sum of responses (spot forming cells; SFC) to HCV proteins Core, NS3 and NS5, IFN, Interferon; IL, interleukin.

Table 3

Spearman correlation coefficients of immune responses with liver histology.

Antigen	Inflammation		Fibrosis	
	All subjects	Genotype 1	All subjects	Genotype 1
IFN γ -Core	-0.21 [*]	-0.18	-0.02	0.12
IFN γ -NS3	-0.16	-0.15	-0.16	-0.08
IFN γ -NS5	-0.33 ^{***}	-0.30 ^{**}	-0.23 [*]	-0.22 [*]
IFN γ Sum1	-0.32 ^{***}	-0.31 ^{**}	-0.22 [*]	-0.14
IFN γ -Candida	-0.35 ^{***}	-0.36 ^{***}	-0.37 ^{***}	-0.33 ^{**}
IL10-Core	-0.004	-0.01	-0.02	-0.001
IL10-NS3	-0.17	-0.19	-0.13	-0.11
IL10-NS5	-0.18	-0.24 [*]	-0.07	-0.10
IL10 Sum1	-0.20 [*]	-0.23 [*]	-0.14	-0.12
IL10-Candida	-0.03	-0.02	-0.19	-0.18

^a Sum of responses to HCV proteins Core, NS3 and NS5.^{*} 0.01 < *P* ≤ 0.05;.^{**} 0.001 < *P* ≤ 0.01;.^{***} *P* ≤ 0.001. IFN, Interferon; IL, interleukin.