# Defective response to Toll-like receptor 3 and 4 ligands by activated monocytes in chronic hepatitis C virus infection

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## SUMMARY

Toll-like receptors (TLR) have a critical role in innate immunity against pathogens. We investigated the cytokine response to TLR stimulation in peripheral blood cells of subjects infected with hepatitis C virus (HCV) and / or human immunodeficiency virus (HIV) in the Women Interagency HIV Study (WIHS) cohort. Interleukin (IL)-6 in response to TLR3 and TLR4 ligands such as polyinosinic-polycytidylic acid and lipopolysaccharide was significantly compromised in HCV-infected women. High spontaneous secretion of IL-6 suggested pre-existing cell activation as a factor mediating reduced responses to TLR3 and TLR4 stimulation. To a lesser extent, tumour necrosis factor-α and IL-1β responses to TLR stimulation were also compromised. Monocytes, but not B cells or NK cells, were identified as the cell population spontaneously secreting cytokines and also as the cells responding to TLR stimulation. These results highlight a functional defect in antigen-presenting cells of women with HCV infection or co-infection. In women with existing HIV co-infection, decreased cytokine function of antigen-presenting cells suggests another mechanism contributing to immune dysfunction in addition to the HIV-associated CD4 defect.

### Keywords

cytokines; hepatitis; hepatitis C virus; human immunodeficiency virus; inflammation; Toll-like receptors

## INTRODUCTION

Infection with hepatitis C virus (HCV) often results in viral persistence and chronically infected individuals have an increased rate of liver cirrhosis and hepatocellular carcinoma. Th1-type immune responses and cytotoxic T lymphocyte activity appear associated with viral clearance [1–3]. Accordingly, T-cell responses to HCV proteins are readily detected during acute infection, but both CD4 and CD8 functions become significantly impaired in the chronic phase [reviewed in 4]. The scarcity of HCV-specific memory T-cell responses during chronic infection has been proposed to be the result of interference by HCV proteins which bypasses immune mechanisms protective to the host.

In human immunodeficiency virus (HIV)-infected individuals, HCV co-infection appears linked to increased morbidity and mortality [5–7]. We and others have previously reported more extensive T helper cell dysfunction in patients with HIV / HCV co-infection compared

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with individuals with either HIV or HCV mono-infection [8,9]. Although reduced CD4 cell counts in HIV-infected individuals have a significant impact on immune function, emerging investigations suggest that additional levels of dysfunction linked to HCV may also exist. In some studies, dendritic cells (DC) generated *in vitro* from peripheral blood of HCV-infected individuals appear impaired in their capacity for antigen presentation [10–12]. Although other studies found reduced percentages of myeloid and plasmacytoid DC, their capacity for IFN-α secretion and allogeneic antigen presentation was comparable with uninfected controls [13,14]. Thus, the potential effect of HCV infection on antigen presentation remains inconclusive. Emerging data [13,14] as well as our unpublished observations suggest that the T-cell impairment in patients with HCV mono-infection is specific for HCV, while memory responses to unrelated pathogens like CMV remain robust.

Several studies have demonstrated that HCV could infect and establish a reservoir in peripheral blood. Cells of the monocytic lineage appear permissive for HCV replication [15–18]. The effect of such extrahepatic infection remains unclear, although initial studies reported secretion of tumour necrosis factor (TNF)-α and interleukin (IL)-8 by *in vitro* infected monocytes [19] and high IL-8 levels in the serum of infected patients [20].

Toll-like receptors (TLR), primary components of innate immunity, play a critical role in defense against pathogens [reviewed in 21]. Both TLR3 [reviewed in 22] and TLR4 [23–26] have a recognized role in antiviral immunity. In this study, we investigated the *ex vivo* capacity of peripheral blood mononuclear cells (PBMC) from women with HCV mono-infection or co-infected with HIV to respond to TLR 3 and 4 ligands. The results highlight a state of functional activation associated with HCV infection reflected by a reduced response to TLR ligands and high spontaneous secretion of the pro-inflammatory cytokine IL-6 and to a lesser extent also TNF-α and IL-1β. Monocytes were found to be the only cell type secreting spontaneously pro-inflammatory cytokines as shown in depletion experiments and intracellular staining. The aberrant secretion of pro-inflammatory cytokines by monocytes in HCV infection does not appear to be driven directly by HCV viraemia.

## METHODS

### Subjects

This was a cross-sectional study nested within the Women's Interagency HIV Study [WIHS, 27]. Women with confirmed HCV serological status enrolled in the Southern California WIHS were evaluated during a scheduled visit between March 2003 and December 2004. HCV serostatus was established at enrollment, 2–10 years prior to initiation of this study. None of the HCV+ women were receiving anti-HCV therapy. Cytokine responses to TLR stimulation were investigated in 43 women, of whom 14 were co-infected (HIV+ / HCV+), 16 were HIV+ only and 13 were HCV+ only. Healthy individuals ($n = 20$) confirmed to be seronegative for HIV and HCV were used as a control group. Written consent was obtained from all subjects, and the Institutional Review Board (IRB) of the University of Southern California approved the study.

### HIV and HCV viral loads

Plasma HIV-RNA levels at the time of cytokine testing were determined using the Nucleic Acid Sequence Based Amplification assay (NucliSens; bioMerieux, Inc., Durham, NC, USA) lower detection limit 25 copies / mL. HCV-RNA was detected by real-time PCR assay using the Cobas Amplicor HCV Monitor 2.0 (Roche Diagnostics®, Basel, Switzerland), with a dynamic range of 10–200 000 000 IU / mL.

### PBMC stimulation

Fresh PBMC were cultured in serum-free medium (X-vivo 20; BioWhittaker, Walkersville, MD, USA) at $2 \times 10^5$ cells / well for 24 h in 96-well round-bottom plates. Supernatants from cultures with medium only were used to evaluate spontaneously secreted cytokines. Parallel cultures received TLR3 and TLR4 stimulants [polyinosinic-polycytidylic acid (pIC), 200 μg /mL or lipopolysaccharide (LPS), 20 ng /mL respectively, both from Sigma, St. Louis, MO, USA]. These concentrations of pIC and LPS were established as optimal in preliminary experiments. Supernatants were recovered at 16 h of culture and stored frozen until batch analysis.

### Cytometric bead array assay for IL-1β, IL-6, IL-10, IL-12 and TNF-α

The cytometric bead array (CBA) assay was performed using undiluted tissue culture supernatants. For samples with high levels of cytokines, supernatants were retested at 1:5 dilution as described by the supplier (BD Biosciences, San Jose, CA, USA). For this assay, soluble cytokines were captured on microparticles and then measured using a fluorescence-based detection system and flow cytometry analysis as described previously [28,29]. A series of 10 dilutions using cytokine standards were run in each assay for generation of standard curves. Samples were analysed in a FACSCalibur flow cytometer (Becton Dickinson, Franklin Lakes, NJ, USA) using the BD CBA Analysis Software, (Becton Dickinson). Spontaneous secretion of each cytokine was defined as the mean $\pm$ 3 SD of background release in PBMC from healthy controls ($n = 20$). The lower limits for IL-1β, IL-6, IL-10, IL-12 and TNF-α using this criterion were 12, 73, 3, 2.5 and 24 pg /mL respectively.

### Depletion of monocytes, B cells and NK cells

Specific cell populations were removed by immunomagnetic separation. Briefly, PBMC were incubated for 30 min at 4 °C with mouse monoclonal antibodies specific for CD14, CD19 or CD56 for removal of monocytes, B cells or NK cells respectively. Following washing, anti-mouse Ig-coated magnetic beads (Dynabeads; Dynal, Oslo, Norway) were added at a 10:1 ratio. Bound cells were retained on a magnetic field. The preparations after two-rounds of depletion contained <2% of the target population as monitored by flow cytometry. Depleted preparations were recounted and stimulated with TLR ligands or left in medium only for spontaneous release.

### IL-6 and TNF-α detection by intracellular staining

Peripheral blood mononuclear cells were stimulated for 5 h with TLR ligands or without stimuli for direct *ex vivo* detection of cytokines. Frozen PBMC were used for the detection of TNF-α. For analysis of both cytokines, Brefeldin A (1 μM; BD PharMingen, San Jose, CA, USA) was added during the last 4 h of incubation. Cells were stained with APC-labelled anti-CD14, FITC-labelled anti-CD19 monoclonal antibody (MAb) and CyC5-labelled anti-CD56. After fixation / permeabilization, cells were incubated with PE-labelled anti-human MAb to IL-6 or TNF-α, or an isotype control in wash / permeabilization buffer according to the manufacturer's instructions (all reagents from BD PharMingen). Stained preparations were analysed on a FACSCalibur flow cytometer (Becton Dickinson). Fifty thousand eventswere scored for each sample.

### Statistical analyses

Student's *t*-test was used for the comparison of continuous variables between two groups. Analysis of variance (ANOVA) was used for comparison of continuous variables between three or more groups. Further comparisons between any two groups within these multiple groups were conducted using Scheffe's method. Proportions between categorical groups

were compared using chi-squared test or Fisher's exact test. Wilcoxon Rank Sum test was used for the comparison of IL-6 ratios. Two-tailed $P$-value <0.05 was considered statistically significant.

## RESULTS

### Characteristics of the study populations

In general, the study groups were similar except that a history of injection drug use was more common in the 27 women with HCV infection (92% in the 13 mono-infected and 86% in the 14 co-infected) compared with the 16 women with HIV mono-infection (0%, $P <$ 0.0001, Table 1).As expected, CD4 counts were significantly lower in women with HIV infection, either as mono-infection (mean 493 cells / μL) or as co-infection (448 cells / μL), compared with women with HCV mono-infection (1045 cells / μL, $P < 0.0001$).

### High spontaneous cytokine secretion

Compared with healthy controls, HCV (54%) and HIV / HCV positive women (64%) had high levels of spontaneous IL-6 release ($P < 0.05$, Fig. 1a). Spontaneous secretion of IL-6 in women with HIV mono-infection was not statistically different from the value observed in healthy controls (34.1 and 8.1 pg /mL respectively). Spontaneous TNF-α was significantly higher among HIV / HCV-positive women (Fig. 1b, 16 pg / mL) compared with both HIV mono-infected (3.3 pg / mL) and controls (3.2 pg / mL, $P < 0.05$ for both comparisons). Compared with controls, levels of spontaneous IL-1β were also significantly enhanced in HCV-positive women (Fig. 1c, 7.8 and 1.4 pg /mL respectively, $P < 0.05$). However, IL-6 was the most abundantly spontaneously secreted cytokine among HIV / HCV-positive women, with overall levels about 10-fold higher than TNF-α and 50-fold higher than IL-1β. Spontaneous secretion of IL-10 was observed at low levels in only four women, but overall there were no differences between the groups. No spontaneous secretion was observed for IL-12.

### Compromised IL-6 responses to TLR stimulation

Contrasting the spontaneous secretion, IL-6 response to TLR3 (pIC) stimulation was significantly reduced in HCV-infected women (Table 2). Moreover, most HCV-mono-infected and HIV-co-infected women presented both high spontaneous cytokine secretion and low TLR-mediated cytokine responses. Similar results were obtained in cultures stimulated with the TLR4 ligand LPS, indicating that aberrant pro-inflammatory cytokine secretion in these patients comprises both TLR3 and TLR4 responses. Overall, only one HCV-positive patient (4%) had responses comparable with healthy controls for all cytokines.

### Other cytokine responses to TLR stimulation

Levels of IL-1β and TNF-α in response to TLR3 (pIC) or TLR4 (LPS) stimulation were also reduced among women with HCV infection, either as mono-infection or in HIV co-infection (Table 2). Despite the overall trend, IL-1β among HCV-infected women (mono- or co-infected) was significantly reduced only compared with HIV+ women (but not healthy controls) for both TLR3 and TLR4 stimulation. A similar pattern was observed for TNF-α, except that the response to TLR4 ligand among co-infected women was not statistically different from all other groups. Levels of the regulatory cytokine IL-10 were likewise reduced among women with HCV infection compared with women with HIV mono-infection but not different from healthy controls, although IL-10 values were overall lower than those of IL-6, IL-1β and TNF-α. IL-12 was seldom detected and there were no differences between the groups (data not shown).

### Monocytes involved in spontaneous secretion of pro-inflammatory cytokines

Following depletion of monocytes / macrophages (CD14+ cells), B cells (CD19+) or NK cells (CD56+), only depletion with anti-CD14 antibody resulted in nearly abolished secretion of pro-inflammatory cytokines both for spontaneous and TLR-mediated secretion (shown for IL-6, TNF-α and IL-1β in Fig. 2a,b). This observation was further confirmed by intracellular staining for IL-6 and TNF-α (Fig. 2c). Flow cytometry data shown are PBMC from a woman with HIV / HCV co-infection indicating a positive IL-6 signal in 32.8% of CD14+ cells (4.4% for the corresponding isotype control, Fig. 2c upper row). No IL-6 signal was detected in CD19+ or CD56+ cells. Staining for TNF-α indicated a positive signal in 23.1% of CD14+ cells compared to 4.5% in the corresponding isotype control (Fig. 2c, lower row). No TNF-α signal was detected in CD19+ (panels to the left) or CD56+ cells. This pattern of cytokine secretion by CD14+ cells but not by CD19+ or CD56+ cells was identical in a total of six patients in independent experiments.

## DISCUSSION

This study detected a reduced IL-6 response to TLR ligands in patients with HCV infection irrespective of HIV status. High spontaneous secretion of IL-6 was observed among HCV-infected women. Noteworthy, levels of spontaneous secretion in co-infected women were similar to the levels observed in women with HCV mono-infection, suggesting that the cytokine dysregulation is linked to HCV infection. Although the impaired TLR-mediated IL-6 response was more noticeable in patients with high spontaneous secretion, absolute levels of stimulated IL-6 secretion were overall reduced in HCV infection.

Toll-like receptors, primary components of innate immunity, play a critical role in defence against pathogens [reviewed in 21]. Specifically, TLR3 has a well-recognized role in antiviral immunity [reviewed in 22]. Also TLR4 has been implicated before in responses to viral infections [23,24]. In humans, respiratory syncytial virus [25] and coxsackie virus B4 [26] stimulate IL-6 secretion via TLR4 signalling. Moreover, the HCV NS5A protein has been recently reported to activate TLR4 but not TLR3 in human B cells [30]. A recent microarray analysis indicated compromised expression of mRNA for TLRs in HCV patients with poor treatment response [31]. However, this defect appears to involve primarily the receptor for single-stranded RNA TLR7 and only marginally other TLRs.

Our depletion studies demonstrate that cells expressing the monocytic marker CD14 mediated the spontaneous secretion of IL-1β, IL-6 and TNF-α, as well as the TLR-mediated cytokine secretion. Cells of the monocyte / macrophage lineage are characteristically very potent producers of cytokines and have a relevant role in amplification and modulation of innate responses by their release of cytokines. Although abnormal function of B cells and NK cells have been associated with HCV infection [reviewed in 32], our depletion experiments found no evidence of contribution by NK cells or B cells in either spontaneous or induced secretion of the cytokines investigated. Intracellular staining further confirmed the sole involvement of monocytes / macrophages in this response. Compromised IFN-α and IL-6 responses to a combination of TLR ligands in plasmacytoid but not myeloid DC from HCV-infected subjects was reported recently [33]. Although we did not investigate specifically DC, removal of monocytes / macrophages from PBMC was sufficient to nearly ablate spontaneous secretion of IL-6, TNF-α and IL-1β.

Several reasons may account for monocyte activation *ex vivo* and the resulting spontaneous cytokine secretion. Monocytes may be directly infected by HCV and several studies have detected negative strand HCV RNA in monocytes indicating HCV replication, albeit to low efficiency [15–18]. Infection of monocytes by HCV may account for a defective cytokine response by the infected cells. It is also plausible that non-infected monocytes are affected

by infected ones in a bystander fashion because of release of viral proteins. For example, HCV core and NS3 proteins were reported to induce secretion of TNF-α and IL-10 by normal monocytes *in vitro* [11]. Our study detected significantly higher levels of spontaneous TNF-α secretion in a substantial number of co-infected women, but differing from Dolganiuc's observations, no evidence of spontaneous IL-10 secretion was found in our experimental system. Recently, secretion of TNF-α and IL-8 was detected in cultures containing HCV-infected monocytes [19]. Yet, the question whether the cytokine defect was mediated in a direct or a bystander fashion remains to be investigated. In other studies, HCV core and NS3 proteins mediated NFkB and inflammatory activation *in vitro* by using the TLR-2 pathway [34]. Such a mechanism involving key elements of intracellular activation is in agreement with our observation of enhanced spontaneous cytokine secretion but impaired TLR-mediated cytokine response. In contrast, recent studies characterized two additional mechanisms of interference with innate immunity involving disruption of retinoic acid-inducible gene I (RIG-I) and cleavage of the TLR3 adaptor protein TRIF, both effects mediated by HCV NS3 / 4A [35,36]. In both cases, the end result is inhibition of interferon regulatory factor 3 (IRF-3) and NFkB in cells expressing NS3 / 4A. The apparently contradictory observations of our *ex vivo* studies with these *in vitro* investigations may be explained by a dual model of viral interference, where infected cells are subjected to disruption of RIG-I and TRIF signalling, but bystander uninfected cells are activated by circulating viral products.

Monocyte / macrophage activation *ex vivo* could also be a consequence of tissue injury, for example in liver. High serum levels of IL-6 in chronic HCV infection have been reported [37–39], as well as IL-6 mRNA in liver tissue of infected patients [40]. Elevated circulating levels of IL-10, IL-12 and TNF-α have been reported previously [39,41]. However, to our knowledge, a direct contribution to IL-6 production by monocytes / macrophages in peripheral blood of HCV-infected patients has not been investigated. Interestingly, a recent study indicated that IL-6 secretion could be induced by the HCV core protein [42]. Although the possibility that IL-6 being stimulated by endogenous core protein seems plausible in our patients, the question requires further investigation because a direct correlation between spontaneous secretion of IL-6 and levels of viraemia could not be established in the current groups.

Earlier studies by our group and others provided evidence of lymphocyte activation in peripheral blood of patients with HIV [43–45] or HIV / HCV co-infection [46]. It seems plausible that spontaneous secretion of cytokines by monocytes contributes to the activation of lymphocytes observed *ex vivo* and further studies are needed to characterize this possible relationship. It also seems likely that activated monocytes may function inefficiently as mediators of inflammatory responses during antigen presentation and this may play a role in the reduced cell-mediated responses reported previously among HIV / HCV co-infected patients [8,9]. Because of the immunodeficiency linked to reduced CD4 numbers in HIV infection, such a defect becomes evident in HIV / HCV co-infected individuals but not in those with HCV mono-infection that have normal CD4 numbers. Further and expanded investigations are needed to clarify the role of IL-6 and other inflammatory cytokines in disorders associated with chronic HCV infection, both as mono-infection and in co-infection with HIV.

## Abbreviations

**CBA**       cytometric bead array

**DC**        dendritic cells

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|---|---|
| **HCV** | hepatitis C virus |
| **HIV** | human immunodeficiency virus |
| **LPS** | lipopolysaccharide |
| **MAb** | monoclonal antibody |
| **PBMC** | peripheral blood mononuclear cells |
| **pIC** | polyinosinic-polycytidylic acid |
| **TLR** | Toll-like receptors |
| **TNF** | tumour necrosis factor |
| **WIHS** | Women Interagency HIV Study |

## Acknowledgments

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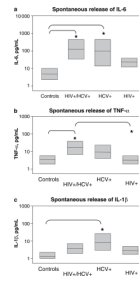
**Fig. 1.**
Altered cytokine response in hepatitis C virus infection. Spontaneous release of (a) interleukin (IL)-6, (b) tumour necrosis factor (TNF)-α and (c) IL-1β. Peripheral blood mononuclear cells from healthy controls or infected patients were cultured in medium only. Supernatants recovered at 16 h of culture were tested for released cytokines. Data shown represent total values of secreted IL-6, TNF-α and IL-1β in pg /mL. Group values that were significantly different (*$P < 0.05$) are indicated in each plot.
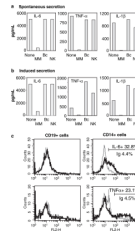
**Fig. 2.**
Monocytes secrete cytokines both spontaneously and in response to Toll-like receptor stimulation. (a) Untreated peripheral blood mononuclear cells (PBMC) (None) or PBMC depleted from monocytes (CD14+), B cells (CD19+) or NK cells (CD56+) by immune-magnetic separation were cultured in medium alone. (b) PBMC untreated or population-depleted were stimulated with lipopolysaccharide for 16 h. Secreted cytokines in the supernatants were evaluated by cytometric bead array assay. Data shown [from a patient with hepatitis C virus (HCV) mono-infection] is representative of identical results from four separate patients. (c) Intracellular staining for interleukin (IL)-6 (upper row) in B cells (CD19+) and monocytes (CD14+) from a patient with human immunodeficiency virus/HCV co-infection. Staining in NK cells (CD56+) was also negative (data not shown). The grey line depicts the flow cytometric profile with an isotype control antibody (4.4% of CD14+ cells) and the black line indicates IL-6 staining (32.8% of the CD14+ population). Staining for tumour necrosis factor (TNF)-α was positive in 23.1% of CD14+ cells (4.5% for the isotype control). No TNF-α signal was detected in CD56+ (NK) cells (data not shown).

**Table 1**

Demographic and Clinical characteristics of the study populations

| Characteristics | Group | HCV+/HIV+ | HIV+ | HCV+ | P-value |
|---|---|---|---|---|---|
| n | | 14 | 16 | 13 | |
| Injection drug use | Ever | 12 (86) | 0 (0) | 12 (92) | <0.0001 |
| HAART-last 6 months | Yes | 8 (57) | 13 (81) | | NS |
| Length of HAART[†] (years) | | 3.6 | 4.1 | | NS |
| CD4 (cells/μL)[†] | | 448 | 493 | 1045[*] | <0.0001 |
| Nadir CD4[†] | | 239 | 250 | 788[*] | <0.0001 |
| ALT (U/L)[†] | | 57 | 30 | 52 | NS |
| HIV (Log10 RNA copies/mL)[†] | | 1.97 | 1.49 | | NS |
| HCV (Log10 IU/mL)[†] | | 4.78 | | 4.16 | NS |

Data are no. (%) of women, unless otherwise indicated.

HCV, hepatitis C virus; HIV, human immunodeficiency virus; NS, not significant.

[*] Significantly higher than the other two groups.

[†] Group mean.

**Table 2**

Analysis of cytokine secretion in response to TLR stimulation by infection status

| Stimuli | Cytokines | n | Infection | Geometric Mean | 95% CI | P-value* HIV | HCV | Control |
|---|---|---|---|---|---|---|---|---|
| pIC | IL-1β | 14 | HCV/HIV | 130.8 | 48.2–354.8 | < 0.05 | NS | NS |
| | | 10 | HIV | 1739.9 | 962.8–3144.3 | | <0.05 | NS |
| | | 13 | HCV | 222.6 | 89.9–551.2 | | | NS |
| | | 20 | Control | 442.6 | 230.9–848.2 | | | |
| | IL-6 | 14 | HCV/HIV | 6046.7 | 3529.9–10358.0 | NS | NS | <0.05 |
| | | 10 | HIV | 12367.0 | 11574.4–13214.0 | | NS | NS |
| | | 13 | HCV | 6184.4 | 4709.7–8120.9 | | | <0.05 |
| | | 20 | Control | 13041.3 | 10719.2–15866.5 | | | |
| | TNF-α | 14 | HCV/HIV | 814.3 | 359.7–1843.1 | <0.05 | NS | NS |
| | | 10 | HIV | 3187.9 | 2415.5–4207.3 | | <0.05 | NS |
| | | 13 | HCV | 610.4 | 311.2–1196.9 | | | NS |
| | | 20 | Control | 1783.1 | 1329.2–2391.9 | | | |
| | IL-10 | 14 | HCV/HIV | 61.5 | 21.4–176.1 | <0.05 | NS | NS |
| | | 10 | HIV | 535.8 | 346.0–829.7 | | <0.05 | NS |
| | | 13 | HCV | 64.7 | 20.1–208.4 | | | NS |
| | | 20 | Control | 141.3 | 70.8–282.0 | | | |
| LPS | IL-1β | 14 | HCV/HIV | 349.4 | 128.4–951.2 | <0.05 | NS | NS |
| | | 16 | HIV | 1970.5 | 1327.6–2924.9 | | <0.05 | NS |
| | | 13 | HCV | 355.0 | 142.9–882.1 | | | NS |
| | | 20 | Control | 664.9 | 363.9–1214.9 | | | |
| | IL-6 | 14 | HCV/HIV | 7956.2 | 5898.6–10731.7 | NS | NS | NS |
| | | 16 | HIV | 10836.6 | 9900.4–11861.4 | | <0.05 | NS |
| | | 13 | HCV | 5531.1 | 3529.2–8668.5 | | | <0.05 |
| | | 20 | Control | 10719.4 | 7888.8–14565.6 | | | |
| | TNF-α | 14 | HCV/HIV | 1385.2 | 628.7–3051.7 | NS | NS | NS |
| | | 16 | HIV | 1990.3 | 1194.7–3315.9 | | <0.05 | NS |
| | | 13 | HCV | 488.1 | 210.3–1133.0 | | | NS |

| Stimuli | Cytokines | n | Infection | Geometric Mean | 95% CI | P-value* HIV | HCV | Control |
|---|---|---|---|---|---|---|---|---|
| | | 20 | Control | 1671.2 | 1079,8–2586.5 | | | |
| | IL-10 | 14 | HCV/HIV | 79.4 | 26.1–241.2 | <0.05 | NS | NS |
| | | 10 | HIV | 515.7 | 358.8–741.3 | | <0.05 | <0.05 |
| | | 13 | HCV | 52.3 | 16.6–165.0 | | | NS |
| | | 20 | Control | 86.8 | 39.9–188.8 | | | |

pIC, polyinosinic-polycytidylic acid; LPS, lipopolysaccharide; IL, interleukin; TNF, tumour necrosis factor; HCV, hepatitis C virus; HIV, human immunodeficiency virus; NS, not significant.

*Statistical significance when comparing the infection group in a row with the infection group in a column.