

## **HCV stimulates HIV immune activation in HIV/HCV co-infected subjects on HAART**

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## Abstract

**Background:** HIV viremia is associated with chronic systemic immune activation, which is the strongest predictor of disease progression. Lowering HIV load through a HAART regimen medically and cognitively improves HIV mono-infected and HIV/HCV co-infected individuals. We have characterized unique gene expression profiles in monocytes from HIV and HIV/HCV co-infected subjects. Monocytes from subjects with HIV viremia are highly activated while subjects with low viral load are similar to non-infected controls. We hypothesize that HCV co-infection increases immune activation above HIV infection alone. We used the HIV expression profiles to determine the activation state of monocytes from HIV/HCV co-infected individuals who had undetectable viral loads.

**Methods:** A chronic immune activation (CIA) index for HIV subjects was generated using CD14<sup>+</sup> monocytes from 44 subjects (14 undetectable HIV viral load and 30 HIV<sup>+</sup> with detectable viral loads). Analysis of monocytes gene expression by high-density microarrays identified 148 genes as significantly ( $p < 0.05$ ) up- or down-regulated at >2 fold with Student's t test and corrected for multiple comparisons (Benjamini and Hochberg). Of these 148 genes, 19 genes with >3 fold up-regulation were included in the CIA index. Next, CD14<sup>+</sup> monocytes from 12 HIV/HCV co-infected subjects on HAART with undetectable HIV viral loads were evaluated for expression of the 19 genes in the CIA index.

**Results:** Linear regression using only HIV+ subjects was performed using genes in the CIA index and log transformed viral load ( $R^2 = 0.7157$ ,  $p < 0.0001$ ). Higher HIV viral loads were associated with increased monocyte activation. While all co-infected subjects (HIV undetectable) had a monocyte activation profile greater than that suggested by their HIV viral load alone, two subjects had highly activated monocytes with an HIV calculated viral load (with associated high immune activation index) approaching 20,000 RNA copies/mL, a range that may place the subject at added health risks if chronic activation is sustained.

**Conclusions:** Based on immune activation as represented in the CIA index, individuals co-infected with HIV/HCV are at greater risk for disease progression compared to HIV mono-infected individuals despite undetectable HIV viral loads. This elevated immune activation may place these individuals at increased risk for HIV and HCV disease complications.

## Methods

### **Subjects**

We recruited and consented 44 HIV seropositive subjects and 12 seronegative controls. HIV seropositive subjects were subdivided into 2 groups based on a clinical definition of viral load status with 22 subjects with low viral load (LVL, <10,000 RNA copies/ml) and 22 subjects with HVL (>10,000 RNA copies/ml). We also recruited and consented 14 HCV mono-infected subjects (HCV loads mean of  $1.5 \times 10^6$  IU/ml) and 12 HIV/HCV co-infected subjects (HIV undetectable viral load [ $<50$  copies/ml] and HCV load mean of  $3.7 \times 10^6$  IU/ml). **All HCV mono-infected and HIV/HCV co-infected subjects had detectable HCV viral loads, were not on HCV treatment nor abusing alcohol or drugs at the time of blood draw.**

### **Monocyte and RNA isolation and gene expression arrays**

CD14<sup>+</sup> monocytes were isolated from whole blood and processed as previously described (5). Peripheral blood mononuclear cells (PBMCs) were enriched by density gradient centrifugation of cell preparation tubes (CPT). CD14<sup>+</sup> monocytes were isolated using magnetic beads conjugated with anti-CD14 antibody (Miltenyi Biotech). Total RNA was isolated from monocytes using the RNeasy Micro Kit (Qiagen). The integrity of RNA was evaluated on the Agilent Bioanalyzer 2100 using an RNA 6000 Pico LabChip (Agilent Technologies). Complementary RNA (cRNA) was synthesized and labeled with biotin using iExpress iAmplify kit (GE Healthcaare). Ten

micrograms of cRNA were then hybridized to Codelink Whole Human Genome Bioarrays. The slide was scanned and the image was analyzed using Codelink Expression Analysis software (GE Healthcare). Microarray data were normalized with loess normalization using R and Bioconductor package. Determination of differential gene expression significance and multiple testing correction / false discovery rate adjustments were performed using GeneSpring GX 7.3 software package (Agilent). Gene ontology analysis was conducted using the GeneSpring GX software package.

## Introduction

It is estimated that approximately 25-30% of all HIV positive individuals in the United States are also co-infected with the hepatitis C virus (HCV). HIV-associated dementia (HAD) is a well-described life-threatening complication of uncontrolled HIV while HCV mono-infection is associated with mild neurocognitive impairment (1,2,3). However, HIV/HCV co-infection increases risk for HIV disease progression and ultimately neurocognitive impairment. Chronic immune activation is a driver of HIV disease progression and recent data suggest that it may also be responsible for disease progression in HIV/HCV co-infected subjects (4). Therefore, monocyte activation could be an important indicator of future cognitive impairment and disease progression, and may be more predictive than HIV viral loads.

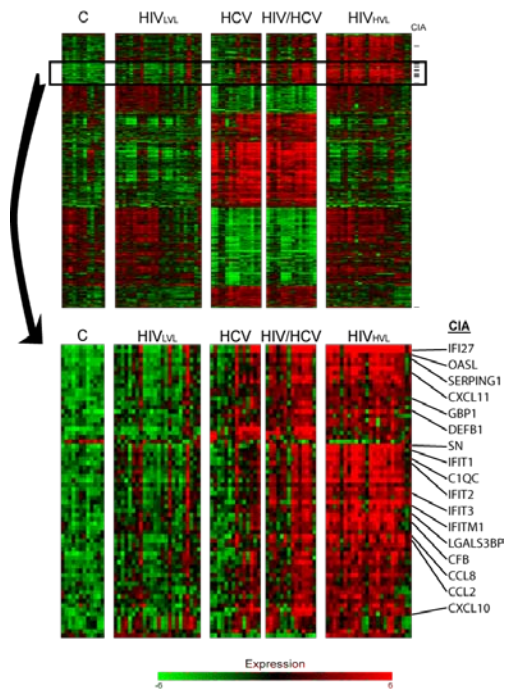
We developed gene expression profiles to characterize the impact of HIV/HCV co-infection on monocyte activation. Our results suggest that HIV/HCV co-infection affects the monocytes in a unique manner, different than either virus alone and may attenuate the beneficial neurological effects of highly active antiretroviral therapy (HAART) on HIV/HCV co-infected individuals.

## Results

### 1) HIV and HCV induce different monocyte expression profiles

Global gene expression analysis using high-density cDNA microarrays was performed on cells isolated from 44 HIV-seropositive subjects and 12 HIV seronegative controls. HIV<sub>LVL</sub> (n=22), HCV (n=14), HIV/HCV (n=12) and HIV<sub>HVL</sub> (n=22). The impact of HCV on monocyte gene expression is different from that caused by HIV. In **Figure 1** (top panel), heat map illustrating monocyte gene expression for healthy controls, HIV and/or HCV- infected subjects. The top portion of the heat map indicates similar gene expression patterns in controls and subjects with HIV<sub>LVL</sub>. In contrast, dramatic gene induction was observed in the subjects with HIV<sub>HVL</sub>. In the middle part of the heat map, the impact of HCV infection is obvious with gene expression significantly elevated in both HCV mono-infected and HIV/HCV co-infected subjects compared to controls but not in the HIV-infected subjects. Genes in the boxed area are expanded in the bottom panel (Fig. 1). Elevated genes in the HIV<sub>HVL</sub> are similarly elevated in the HIV/HCV co-infected subjects and at a diminished level in the HCV mono-infected subjects. HIV loads in the HIV/HCV co-infected were **undetectable** and yet this group has an elevated profile compared to control, HIV<sub>LVL</sub> or HCV mono-infected subjects.

These results suggest that HCV is amplifying the HIV effect on the immune system.

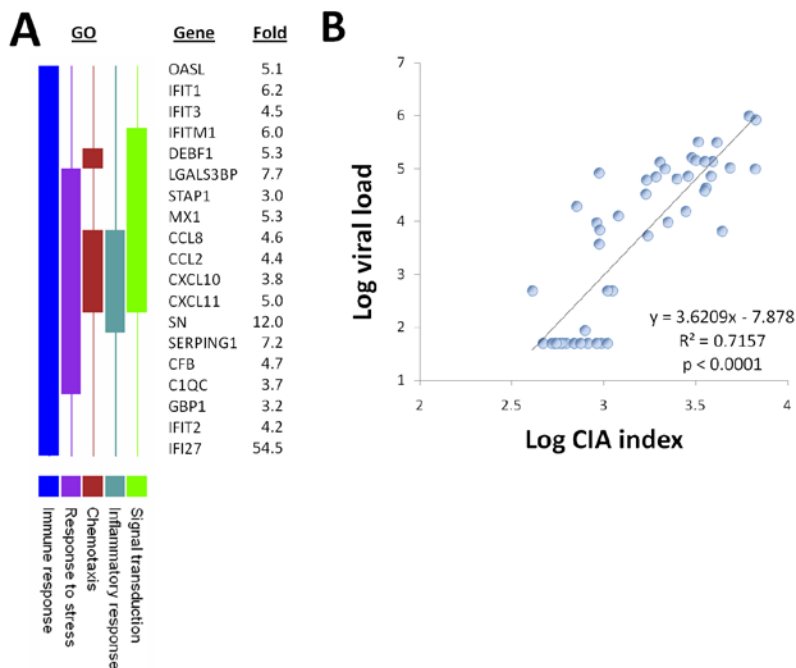


**Figure 1. Heat maps depicting gene expression in monocytes isolated from control and infected subjects. Top panel:** Genes that are differentially expressed between controls and the disease conditions (grouped as columns): HIV low viral load (HIV<sub>LVL</sub>), HCV (mono-infected), HIV/HCV co-infected and HIV high viral load (HIV<sub>HVL</sub>). **Bottom panel:** Expanded area identifying DE genes common to both HIV<sub>HVL</sub> and HIV/HCV co-infected subjects. Genes selected for the monocyte chronic immune activation (CIA) index are designated at the right margin.

**2) Monocyte activation is defined by the chronic immune activation index (CIA)**

We developed a chronic immune activation (CIA) index based on a limited number of genes elevated in subjects with HIV. The index is comprised of 19 genes related to immune activation that are up-regulated 3 to 54-fold in HIV<sub>HVL</sub> subjects (Fig. 2A). Subclassification of gene function includes signal transduction, inflammatory response and chemotaxis. To generate a CIA value for each subject, the mean intensities of all 19 genes were taken for each sample and calculated

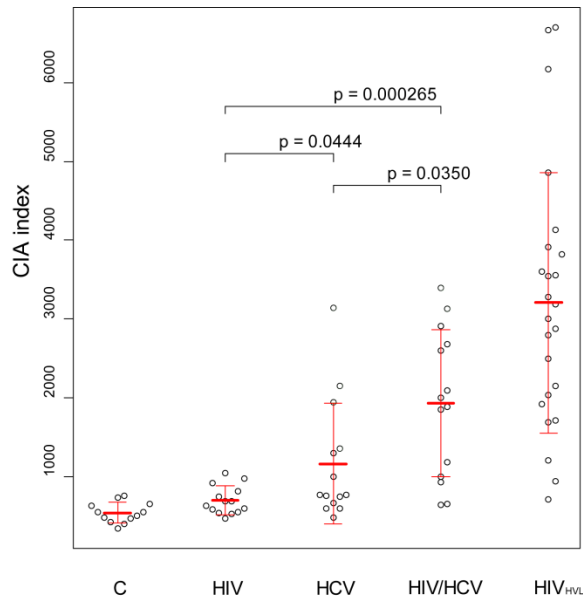
as follows: 
$$CIA = \frac{\sum_{i=1}^n Intensity_i}{n}$$
, where *Intensity* equals corrected and normalized signal intensity for each gene, while *n* represents the 19 selected genes. For each subject, the CIA value and corresponding viral load were plotted and linear regression analysis showed a Pearson's  $R^2 = 0.7157$  with  $P < 0.0001$  (Fig. 2B). Correlation between CIA and log viral load indicates that the CIA index can be used to define monocyte activation in HIV-1-infected subjects.



**Figure 2. Chronic immune activation index (CIA).** **(A)** List of genes, fold change and associated Gene Ontology (GO) categories for genes in the CIA index. CIA genes with  $\geq 3$  fold elevated expression in HIV<sub>HVL</sub> vs control. **(B)** Correlation of CIA index and viral load for subjects with HIV infection. Data are shown with regression line, correlation coefficient and significance.

### **3) Monocyte activation state differentiates HIV mono-infected from HIV/HCV co-infected subjects.**

Monocyte CIA values were calculated for healthy controls (n=12), HIV mono-infected (n=14; <50 HIV RNA copies/ml), HCV mono-infected (n=14), HIV/HCV co-infected (n=12; <50 HIV RNA copies/ml), and HIV<sub>HVL</sub> (n=22; >10,000 HIV RNA copies/ml) (Fig. 3). All subjects were age-matched. This analysis illustrates that subjects with HIV who are controlled on HAART (<50 HIV RNA copies/ml) have an insignificant rise in monocyte activation compared to healthy controls. By comparison, mono-infected HCV individuals exhibit an increase in monocyte activation that is further exacerbated by co-infection with HIV. This occurred in the absence of a detectable HIV viral load. These results demonstrate that co-infection with HCV amplifies the HIV monocyte activation profile and suggests increased risk of disease progression.



**Figure 3. HIV/HCV co-infection increases monocyte activation above either HIV or HCV mono-infection.** Monocyte chronic immune activation (CIA) was calculated for healthy donors (C), subjects with HIV (with undetectable viral load), HCV (mono-infected), HIV/HCV (co-infected with undetectable HIV viral load) and HIV<sub>HVL</sub> (mono-infected with high viral load). Statistical analysis was performed with Student's t test.

Based on the CIA value for each HIV/HCV co-infected individual, we generated a theoretical or computed HIV viral load (Table) using the highly significant correlation between CIA and HIV viral load (Fig. 2B). These results indicate a range of monocyte activation in co-infected individuals, with low activation in subjects 1 – 3, which was similar to HIV subjects with undetectable loads. In contrast, subjects 10 – 12 exhibited viral loads calculated to be in excess of 30,000 HIV RNA

copies/ml. Monocyte activation may prove to be a useful metric in determining who is at greatest risk for disease progression.

**Table. HIV viral load, CIA and computed HIV viral load**

Subjects	HIV VL	CIA	Computed HIV VL
HIV	< 50	699 ± 181	349 ± 336
<b>HIV/HCV</b>			
01	<50	638	189
02	<50	658	213
03	<50	929	740
04	<50	1,003	977
05	<50	1,187	1,793
06	<50	1,856	9,069
07	<50	1,886	9,606
08	<50	2,007	12,030
09	<50	2,094	14,030
10	<50	2,602	30,783
11	<50	2,684	34,458
12	402	3,395	80,708

HIV VL= HIV viral load (RNA copies/ml)

CIA = monocyte chronic immune activation index

Computed HIV VL = computed viral load based on CIA value

HIV = mean ± S.D. for CIA and computed HIV VL (n = 14 subjects)

## Discussion

Returning the immune system to normal homeostasis is an important goal that would likely benefit both HIV and/or HCV-infected subjects. Antiviral therapy will not be sufficient to consistently suppress HIV or clear HCV in the near future and therefore making the immune system as “normal” as possible may be helpful in living with these viruses. Risk of disease progression is presently measured

principally by HIV viral load alone but surprisingly we find that immune activation is a more accurate predictor of HIV disease progression and risk for cognitive impairment than is HIV viral load. A different but significant pattern of activation was found in HIV/HCV co-infected individuals, which was higher than predicted based exclusively on HIV viral load. This implies that in some HIV+ individuals, HIV co-infection with HCV substantially increases immune activation which may accelerate immune dysfunction and cognitive impairment.

## Summary

- Monocytes respond differently to HCV and HIV infection
- CIA index is a metric for HIV-induced monocyte activation which correlates with viral load
- HCV co-infection elevates monocyte activation (CIA) without increased viral load
- HIV/HCV co-infection increases monocyte activation and therefore the computed viral load

## References

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