Note

High levels of chronic immune activation in the T cell compartment of HCV/HIV-1 co-infected patients on HAART is reverted by IFNα and ribavirin treatment

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Chronic immune activation is a driver of Human Immunodeficiency virus 1 (HIV-1) disease progression. Here, we describe that subjects with chronic hepatitis C virus (HCV)/HIV-1 co-infection display sharply elevated immune activation as determined by CD38 expression in T cells. This occurs despite effective anti-retroviral therapy, in both CD8 and CD4 T cells, and is more pronounced than in the appropriate mono-infected control groups. Interestingly, suppression of HCV by pegylated interferon-α and ribavirin treatment reduces activation. High HCV loads and elevated levels of chronic immune activation may contribute to the fast rates of viral disease progression observed in HCV/HIV-1 co-infected patients.
Hepatitis C virus (HCV) establishes chronic infection in a majority of infected humans. 15-30% of patients with Human Immunodeficiency Virus 1 (HIV-1) infection are also infected with HCV (23, 26), and this patient group experience an augmented rate of HCV disease progression, with increased liver-related morbidity, and higher HCV loads (2, 5, 6, 29). T cell responses to HCV are generally poor in chronic infection and this deficiency is exacerbated in HCV/HIV-1 co-infection. This is likely due in part to HIV-mediated loss of CD4 helper T cells, which in turn impairs the function of HCV-specific CD8 T cells (15, 17). Spontaneous clearance of chronic HCV infection is rare in mono-infection, and probably even more so in HIV-1 co-infected subjects although a few such cases have been described (7, 10, 30). Interestingly, some studies suggest that HCV/HIV-1 co-infection is associated with an increased rate of HIV-1 disease progression (14, 22, 24).

HCV can be successfully treated with pegylated IFNα (peg-IFNα)+ribavirin. A sustained virological response, with eradication of the virus, is obtained in only 50-80% of patients with HCV mono-infection, and rates are generally lower in HCV/HIV-1 co-infected subjects (19). Several viral and host factors influence peg-IFNα+ribavirin treatment outcome, where the HCV genotype, viral burden, age and gender are among the most important. Cellular sensitivity to IFNα, and the ability to increase expression of IFNα-regulated genes are important for the response to treatment (20, 25, 27). Because of the key role of chronic immune activation in driving HIV-1 disease ((8, 11, 16) and reviewed in (9)), and the interplay between the two viruses in the co-infected host, we were interested in analyzing the levels of T cell immune activation in patients with chronic HCV on the background of an HIV-1 co-infection effectively controlled by highly active antiretroviral therapy (HAART).

The study subjects were from the Viral Dynamics and Immunology in HCV/HIV-1 Co-infection (DICO) study including patients from the Karolinska University Hospital in Stockholm, Sweden (13), as well as the Hvidovre Hospital and Rigshospitalet in...
Copenhagen, Denmark, and Aalborg and Aarhus university hospitals (Table 1). 14 HCV/HIV-1 co-infected subjects who had not received prior HCV therapy were sampled at baseline, and 11 of these were further sampled during and after treatment with peg-IFNα-2a (Pegasys, Roche AB, Sweden) and ribavirin (Copegus, Roche AB, Sweden). Co-infected subjects were on stable HAART for at least one year, and had no active intravenous drug use for at least six months before the start of the study. Nine HIV-1 mono-infected subjects on stable HAART and 14 chronically HCV mono-infected subjects were included. The 21 uninfected control subjects were healthy volunteers donating blood at the Stockholm Blood bank. All study subjects were of Caucasian white ethnic origin. The study protocols were approved by local institutional reviews boards as well as the Swedish National Medical Products Agency and the Danish Medicinal Agency, and the Danish Data Protection Agency. Peripheral blood mononuclear cells were isolated from heparinized whole blood samples by Lymphoprep gradient centrifugation (Axis-Shield, Oslo, Norway).

To address the role of immune activation in HCV/HIV-1 co-infection we analyzed CD38 expression in the CD4 and CD8 T cell compartments in subjects chronically co-infected with HCV and HIV-1, in comparison to mono-infected controls and healthy blood donors (Fig. 1A) (Table 1). Anti-CD3 PE-Cy7, anti-CD4 Pacific Blue, anti-CD8 PerCP, and anti-CD38 APC were from BD Biosciences (San Diego, CA, USA). Multicolor flow cytometry data was acquired on a CyAn ADP instrument (Beckman Coulter Inc, Fullerton, CA, USA), and analyzed using FlowJo software (Tree Star, OR, USA) (12). These patient groups allowed the investigation of T cells in patients with uncontrolled HCV infection on the background of a drug-controlled HIV-1 infection. We observed considerably elevated CD38 expression in both CD8 and CD4 T cells in HCV/HIV-1 co-infected subjects in comparison to mono-infected subjects and healthy controls (Fig. 1B and C, respectively). In CD4 T cells, CD38 expression was significantly elevated also in HCV mono-infected subjects (Fig. 1C).
There was a direct correlation between CD38 expression in CD4 T cells and CD8 T cells, indicating a coordinated elevation of immune activation levels in both T cell subsets (Fig. 1D). CD38 expression in CD8 and CD4 T cells tended to be more pronounced in HCV genotype 1 infection as compared to genotype 2 or 3 infection (Fig. 1E and F). Together, these data indicate that HCV/HIV-1 co-infection is associated with high levels of chronic activation of both CD8 and CD4 T cell compartments despite effective control of HIV-1 replication by HAART.

11 out of the HCV/HIV-1 co-infected subjects started a 48-week HCV treatment with peg-IFNα+ribavirin and were sampled at weeks 0, 4, 12, and 72 (24 weeks post-treatment). Plasma HCV RNA level was measured using the COBAS TaqMan HCV test with a limit of detection of 15 IU/ml (Roche Diagnostics, Branchburg, NJ, USA). Baseline comparison between HCV mono-infected and HCV/HIV-1 co-infected subjects revealed significantly higher HCV loads in co-infected patients (Fig. 2A), confirming previous observations (2, 5, 6, 29). Upon initiation of peg-IFNα+ribavirin treatment, viral load was rapidly reduced (Fig. 2A). At week 12, HCV RNA was undetectable in a majority of patients.

We assessed T cell activation by means of CD38 expression in T cells during treatment, and observed a significant reduction in the frequency of activated CD8 and CD4 T cells (Fig. 2B and C). It is interesting to note that the initial response to treatment was different in the two T cell compartments. Activation levels at week 4 increased slightly in the CD8 T cell compartment, whereas the CD4 T cells displayed reduced CD38 expression already at this time point. Thus, immune activation as assessed by CD38 expression decreased in response to IFNα+ribavirin treatment, and CD4 and CD8 subsets differed in the initial response to treatment.

HCV/HIV-1 co-infection presents a formidable challenge to the human immune system. The present study indicates that HCV/HIV-1 co-infected patients have high levels of
CD8 and CD4 T cell immune activation, as indicated by CD38 expression, despite effective
HAART-mediated suppression of HIV. This was associated with high HCV loads in the co-
infected patients and the CD38 expression declined when HCV replication was suppressed by
IFNα+ribavirin treatment. This may suggest that the high levels of immune activation was
driven by high HCV viral loads. It is well known that reversible T cell lymphopenia is a side
effect of IFNα+ribavirin treatment. CD8 and CD4 T cell counts rebounded after treatment
(Fig. 2D and E), whereas the percentage of CD38+ T cells did not. However, it remains
possible that the reduced frequency of CD38+ T cells upon IFNα+ribavirin treatment could be
due to direct effects of treatment on T cells, which may cause them to enter tissues other than
the blood or go into apoptosis. Further studies are needed to address these distinct but not
mutually exclusive possibilities.

It is not entirely clear how immune activation drives HIV disease progression,
and what is the primary driver of immune activation. One hypothesis is that destruction of the
immune system in the gut in acute HIV-1 infection leads to leakage of microbial products into
circulation, promoting activation via innate pattern recognition (4). Another possibility has
been suggested by studies of the sooty mangabey monkey, the natural host of SIVsm. This
virus is non-pathogenic in this monkey, but much more pathogenic in the rhesus macaque.
This was recently linked to differences in immune activation and levels of type I IFN
expression triggered by the virus (21), where high levels of IFN were associated with
pathogenic immune activation. To what extent these findings can be extended to human HIV
infection remains undetermined. Our finding that IFNα+ribavirin treatment reduces immune
activation may seem to contradict a role for type I IFNs in pathogenic immune activation.
However, it is important to remember that only one species of type I IFN, IFNα-2a, is used
for treatment. It remains possible that the precise species of IFN, the dose, and mode of
delivery might influence the effects on immune activation.
One might speculate that the direct recognition by TLRs of viral RNA is likely to be the basis of immune activation in HCV infection. The damage to the immune system inflicted by HIV-1 leads to impaired immune control of HCV with higher HCV loads, which may in turn feed the observed elevation in immune activation. Interestingly, however, it was recently noted that microbial translocation into the circulation can be more severe in HCV co-infected subjects than in HIV-1 mono-infected subjects, and this could be one mechanism behind accelerated HCV disease progression in HIV-1 infected patients (1). Whereas we observe that CD38 expression in T cells decrease when HCV replication is suppressed, the levels of CD38 remain higher than in normal donors. It is clearly possible that excessive microbial translocation or impaired clearance of microbe-derived products from circulation contributes to this pattern.

HCV liver disease has become a leading cause of death in HCV/HIV-1 co-infected patients who have access to HAART, and HCV disease progresses faster in this group of patients (2, 5, 6, 29). Some studies suggest that this is also true for HIV-1 disease (14, 22, 24), although this is not observed in all cohorts (28). The high levels of immune activation may contribute to rapid rates of viral disease progression, by impairing the ability of the immune system to respond. Interestingly, we recently observed that a subset of CD8 T cells expressing the Fc-receptor CD16 is numerically elevated in chronically HCV infected subjects, and that these cells mediate NK cell-like cytolytic function (3). Expression of CD16 correlated with that of CD38, suggesting that development of NK-like activity in T cells may be part of the immunopathogenesis of HCV disease. Also of note, Kovacs et al. reported high levels of CD38 expression in CD8 T cells HCV/HIV-1 co-infected women who were not on HAART (18). We observe here that high levels of activation do not require active HIV-1 replication, and activation levels are even higher in CD4 T cells. Taken together, the results of
the present study suggest that chronic immune activation is a component in the pathogenesis of HCV infection, and might be particularly important in the context of HIV-1 co-infection.
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FIGURE LEGENDS

FIG. 1. Elevated CD38 levels in CD4 and CD8 T cells in HCV/HIV-1 co-infected subjects despite effective HAART. Representative dotplots of CD38 staining in CD8 T cells (A). CD38 expression in CD8 T cells in co-infected patients (n = 14) in comparison to HCV (n = 11) and HIV-1 (n = 9) mono-infected subjects as well as to healthy donors (n = 21) (B). CD38 expression in CD4 T cells in the four study groups (C). Spearman rank correlation between the CD38 expression in CD4 and CD8 T cells in co-infected subjects (D). CD38 expression in CD8 T cells (E), and CD4 T cells (F) in co-infected patients with HCV genotype 1 (Gt1) (n = 10) infection, HCV genotype 2 or 3 (Gt2/3) infection (n = 4), and healthy donors (n = 21). Mean and standard error are shown. Statistical analysis performed was one-way ANOVA on ranks followed by Dunn’s multiple comparison test, where * indicates P<0.05, ** indicates P<0.01 and *** indicates P<0.001. All statistical analyses were performed using GraphPad Prism software 5.0 (GraphPad Software Inc., San Diego, CA).

FIG. 2. Reduction of T cell activation upon IFNα and ribavirin treatment. HCV/HIV-1 co-infected subjects (n = 14) had significantly higher HCV RNA levels (log10 IU/mL) at baseline (BL) in comparison to HCV mono-infected subjects (n = 11), ** P<0.01 as determined by the Mann-Whitney rank sum test. Viral load decreased upon treatment (T) with peg-IFNα and ribavirin (A). CD38 expression in CD8 (B) and CD4 (C) T cells in co-infected subjects (n = 11), measured at BL, during treatment (T) with peg-IFNα and ribavirin, and 24 weeks post treatment (PT) corresponding to week 72 after treatment start. CD8 (D) and CD4 (E) T cell counts in co-infected subjects, measured at BL, during T with peg-IFNα and ribavirin, and 24 weeks PT. Statistical analysis in B-E was performed using the repeated measures one-way ANOVA on ranks followed by Dunn’s multiple comparison test where * indicates P<0.05, ** indicates P<0.01, and *** indicates P<0.001. All statistical analyses
were performed using GraphPad Prism software 5.0 (GraphPad Software Inc., San Diego, CA).
TABLE 1. Patient characteristics at baseline.

<table>
<thead>
<tr>
<th></th>
<th>HCV/HIV-1 co-infected</th>
<th>HCV mono-infected</th>
<th>HIV mono-infected</th>
<th>Healthy controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subjects, n</td>
<td>14</td>
<td>11</td>
<td>9</td>
<td>21</td>
</tr>
<tr>
<td>Sex, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>3 (21%)</td>
<td>5 (50%)</td>
<td>2 (22%)</td>
<td>9 (43%)</td>
</tr>
<tr>
<td>Male</td>
<td>11 (79%)</td>
<td>6 (50%)</td>
<td>7 (78%)</td>
<td>12 (57%)</td>
</tr>
<tr>
<td>Age (years) median (range)</td>
<td>43 (21-53)</td>
<td>52 (43-59)</td>
<td>46 (29-67)</td>
<td>45 (32-59)</td>
</tr>
<tr>
<td>CD4 Absolute median cells/µL (range)</td>
<td>499 (250-1370)</td>
<td>729 (200-1746)</td>
<td>605 (370-720)</td>
<td>845 (441-1491)</td>
</tr>
<tr>
<td>CD4 % median (range)</td>
<td>27 (12-45)</td>
<td>44 (38-57)</td>
<td>30 (21-41)</td>
<td>46 (31-67)</td>
</tr>
<tr>
<td>CD8 Absolute median cells/µL (range)</td>
<td>910 (530-2440)</td>
<td>666 (352-754)</td>
<td>770 (520-1770)</td>
<td>443 (195-776)</td>
</tr>
<tr>
<td>CD8 % median (range)</td>
<td>50 (24-70)</td>
<td>26 (15-39)</td>
<td>46 (24-64)</td>
<td>NA</td>
</tr>
<tr>
<td>HIV ART, n (%)</td>
<td>14 (100%)</td>
<td>NA</td>
<td>9 (100%)</td>
<td>NA</td>
</tr>
<tr>
<td>HIV load median copies/mL (range)</td>
<td>&lt;50 (0.068-20.0x10⁶)</td>
<td>NA</td>
<td>&lt;50 (0.023-3.65x10⁶)</td>
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<td>HCV genotype, n (%)</td>
<td></td>
<td></td>
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<tr>
<td>1</td>
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<td>2</td>
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<td>4 (38%)</td>
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<tr>
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<td>2 (14%)</td>
<td>5 (46%)</td>
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<tr>
<td>Serum HCV RNA median IU/mL (range)</td>
<td>3.64x10⁶ (0.068-20.0x10⁶)</td>
<td>0.57x10⁶ (0.023-3.65x10⁶)</td>
<td>NA</td>
<td>NA</td>
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NA indicates not applicable