Few liver-infiltrating cells express CXCR3 in HIV/HCV patients commencing ART

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ABSTRACT

Co-infections with HCV and HIV accelerate the progression of both conditions and hamper effective treatment. Here we describe expression of CXCR3 on liver infiltrating cells and peripheral T-cells from co-infected patients commencing ART in Indonesia. CXCR3 was expressed by small numbers of intrahepatic inflammatory cells, mostly in the portal areas. Numbers did not change on ART and were markedly lower than numbers of CD4$^+$ and CD8$^+$ cells in the liver. The data suggest that CXCR3 may contribute to liver infiltration but demonstrates a dynamic situation, changing as the immune system recovers on ART.

TEXT

Hepatitis C (HCV) / human immunodeficiency virus (HIV) co-infection is common in intravenous drug users as the viruses share a route of transmission. Co-infection accelerates hepatic cirrhosis and liver failure\textsuperscript{1}, so it is plausible that the mechanisms driving hepatic inflammation and fibrosis may be differ from those invoked by HCV alone, and may change in HIV patients beginning antiretroviral therapy (ART).

CXCR3 has been identified as the chemokine receptor most clearly expressed in cirrhotic liver and mechanisms implicating this receptor in hepatic inflammation and fibrosis have been proposed\textsuperscript{2}. Of the CXCR3 reactive chemokines, circulating CXCL10 levels correlated most clearly with fibrosis\textsuperscript{3,4}. Elevated expression of CXCR3 has been also demonstrated on resting peripheral CD8$^+$ T-cells from HIV/HCV infected patients stable on ART\textsuperscript{5}. CXCL10 levels in plasma and polymorphisms in the encoding gene also affected fibrosis and a response to therapy.
in HCV/HIV co-infection\textsuperscript{6,7}. However CXCL10 levels decline on ART\textsuperscript{8}, so the impact of this cytokine on HCV hepatitis may change.

Understanding the effect of ART on liver histopathology in asymptomatic HCV co-infected patients requires liver biopsies collected before and after ART in well-characterized cohorts. Here we describe further studies of a prospective cohort\textsuperscript{9}, in which we obtained sequential liver biopsies at baseline and after 48 weeks to monitor the infiltration of cells expressing CD4 and CD8\textsuperscript{10}. Here histopathological changes in the liver were correlated with expression of CXCR3 on circulating and intrahepatic cells and levels of CXCL10 in plasma.

We recruited HIV/HCV co-infected patients as they began ART in the HIV/AIDS clinic of Cipto Mangunkusumo Hospital, Jakarta in 2008-9. Patient demographics have been described\textsuperscript{9,10}. Inclusion criteria were HCV seropositivity, age 17–50 years, no previous ART or therapy for HCV disease and <200 CD4\textsuperscript{+} T-cells/µL blood. Exclusion criteria were hepatitis B virus seropositivity, clinically apparent liver cirrhosis, contraindications for liver biopsy, kidney or heart failure, pregnancy or alcohol excess. Liver biopsies were performed at baseline (48 patients) and week 48 (34 patients). Plasma HIV RNA and HCV RNA levels were measured using a Cobas Amplicore Monitor (Roche Molecular Systems; Pleasanton, CA). Lower limits of detection were 400 copies HIV RNA/mL and 200 copies HCV RNA/mL. The study was approved by the Ethics Committee for Research on Human Subjects, University of Indonesia. Informed consent was obtained for all procedures.

Biopsies used modified Menghini needles, fixed with formalin and stained to evaluate necroinflammation (score 0–18) and fibrosis (score 0–6) using Ishak’s scale\textsuperscript{10}. Expression of CXCR3 was assessed using sections stained with anti-mouse anti-CXCR3 antibody (2Ar1; Abcam; Cambridge, MA), followed by the Starr Trek Universal HRP Detection System (Biocare;
The Hague, Netherlands) and diaminobenzidine tetrahydrochloride substrate. Specimens were counterstained with haematoxylin. Seventeen samples (9 baseline and 8 from 48-weeks) were excluded as insufficient tissue remained. CXCR3 expression on infiltrating cells was counted over 3-5 portal areas and the average number of cells per field was determined. Positive controls were human lymphoid tissue and negative controls were liver biopsies stained without primary antibodies. Hepatic expression of CXCL10 was evaluated in representative samples using a rabbit polyclonal antibody (Abcam).

Plasma levels of CXCL10 were measured by Cytometric Bead Array (BD Biosciences; SanJose, CA). Chemokine receptor expression was assessed using peripheral blood mononuclear cells (PBMC) cryopreserved in liquid nitrogen. Expression of CXCR3 on monocytes and CD4+ T-cells was assessed using anti-CD14 PE-Cy7 (BioLegend; San Diego, CA), anti-CD4 FITC and anti-CXCR3 APC. Expression of CXCR3 on CD8+ T-cells was assessed using anti-CD3 PerCP-Cy5.5, anti-CD8 FITC and anti-CXCR3 APC (BD Biosciences). 100 000 events per sample were analyzed on a FACS Canto cytometer and analyzed with FlowJo v7.6 (TreeStar; Ashland, OR). Mononuclear cells were gated by forward and side light scatter11.

Data were compared using non-parametric Mann-Whitney tests. Statistical significance was defined as p < 0.05. Correlations were assessed using Spearman statistics.

Numbers of intrahepatic CD4+ and CD8+ cells and the necroinflammatory and fibrosis scores have been reported10. Here we show that CXCR3 was expressed by small numbers of inflammatory cells before ART [1.4 (0.67-1.7) cells per field] (Table 1), with most positive cells being in the portal areas (Supplementary Figure 1a and b). The two cases with a few positive cells in the
lobular area had the largest numbers of positive cells in the portal area. In these cases, some CXCR3+ cells resembled endothelial cells. All CXCR3+ cells were morphologically distinct from hepatocytes. Numbers did not change on ART and were markedly lower than numbers of cells expressing CD4 or CD8. Before ART, numbers of CXCR3+ cells in the liver correlated directly with numbers of CD4+ cells (r=0.5, p=0.01), but not significantly with CD8+ cells (r=0.15, p=0.36). There was no correlation between numbers of CXCR3+ cells and necroinflammatory scores (r=0.08, p=0.62) or fibrosis (r=-0.02, p=0.87), but all scores were low to moderate (ie: none had severe HCV disease). There was also no correlation between plasma HCV RNA and CXCR3+ cells in the liver before and after ART (r=0.02, p=0.86 and r=0.16, p=0.42). Expression of CXCL10 was sought but yielded only weak diffuse staining that could not be quantified. We also detected CXCR3+ infiltrating cells in liver biopsies from HCV mono-infected patients with relatively severe disease, but had insufficient samples for a quantitative study (Supplementary Figure 1c).

At Week 48, the number of CD4+ cells in the liver had risen whilst numbers of CD8+ cells declined10, paralleling changes seen in the blood11. Correlations between numbers of CD4+ and CD8+ cells and cells expressing CXCR3 in the liver were not significant, with a trend towards inverse relationships (r = -0.33, p=0.16 and r = -0.20, p=0.42; resp.).

PBMC cryopreserved at Week 36 were used to assess expression of CXCR3 on circulating cells as this may influence which cells are in the liver at week 48. CXCR3 was expressed on 16(9-30)% of CD4+ T-cells, 24(17-30)% of CD8+ T-cells and 0.5(0.3-1.1)% of CD14+ cells (monocytes) in peripheral blood at Week 36. Numbers of CXCR3+ cells in the liver at 48 weeks correlated inversely with the proportion of circulating CD4+ (r= -0.44, p=0.045) and CD8+ (r= -
0.40, p=0.07) T-cells expressing CXCR3 at Week 36, but did not associate with the small numbers of monocytes expressing CXCR3 (r=0.025, p=0.91).

Plasma levels of CXCL10 were 1426(1105-2406) pg/ml at baseline, 464(246-922) pg/ml at 36 weeks and 691(356-1064) pg/ml at 48 weeks when expressed as median (interquartile range). Hence levels declined between Weeks 0 and 36 (Mann Whitney, p<0.0001), but stabilized by Week 48 (p=0.34). There was no correlation between levels of CXCL10 at Week 0 and any population assessed in the liver at that time (r = -0.05-0.14, p=0.47-0.79). Numbers of CXCR3+ cells in the liver at Week 48 correlated with plasma CXCL10 at Week 36 (r = 0.45, p=0.03), suggesting a role for CXCL10 in T-cell infiltration into the liver. However CXCL10 levels at Week 48 did not show this correlation (r=0.02, p=0.90).

Overall in this well-characterized cohort of co-infected patients, intrahepatic CXCR3+ cells exist in low numbers in the portal areas without significant changes over the first year on ART. A portal distribution is consistent with recent arrival from peripheral blood, as demonstrated previously in cases of chronic hepatic inflammation. Here it was rare to find positive cells in lobular regions. This may reflect the distribution of CXCR3-binding chemokines in the liver as differential expression of CXCL9, CXCL10 and CXCL11 in portal and lobular areas co-localized with CXCR3 expression in advanced HCV mono-infections.

We noted a weak positive correlation between numbers of CXCR3+ cells and CD4+ or CD8+ cells in the liver at baseline, which switched to weak negative correlations at Week 48. This suggests a change in the role of CXCR3 and is consistent with the marked decline in levels of CXCL10 levels in plasma over time on ART.
CXCR3 expression was demonstrated on circulating CD4$^+$ and CD8$^+$ T-cells at levels similar to those reported previously, with slightly lower expression in HIV/HCV patients compared with HCV mono-infection$^{12}$. Here there was also a negative correlation between numbers of CXCR3$^+$ cells in the liver and CXCR3 expression on circulating CD4$^+$ T-cells at Week 48. This could reflect migration of CXCR3$^+$ T-cells from the blood to the liver or may arise randomly if CXCR3 was irrelevant to liver infiltration. Detailed longitudinal studies are needed to determine how the chemokine is relevant to liver infiltration at specific times on ART.

**ACKNOWLEDGEMENTS**

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**REFERENCES**


Supplementary Figure 1.

Images are from representative HIV/HCV co-infected patients assessed before ART (Panel A) and after 48 weeks on ART (Panel B). Portal areas of the liver show moderate inflammation with 1 to 2 mononuclear inflammatory cells positive for CXCR3 in brown color (arrows). Panel C is from a HCV mono-infected patient displaying more severe portal inflammation with some mononuclear inflammatory cells positive for CXCR3. (magnification 400x).

209x50mm (300 x 300 DPI)
Table 1. Intrahepatic inflammation and CXCR3 expression.

<table>
<thead>
<tr>
<th></th>
<th>Week 0 (n=39)</th>
<th>Week 0 (n=26)</th>
<th>Week 48 (n=26)</th>
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</thead>
<tbody>
<tr>
<td><strong>Systemic markers</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD4(^+) T cells/µL</td>
<td>28 (7-65)</td>
<td>35 (8-56)</td>
<td>122 (85-148)</td>
</tr>
<tr>
<td>Plasma HIV RNA, log(_{10})</td>
<td>5.3 (4.9-5.5)</td>
<td>5.3 (4.9-5.5)</td>
<td>&gt;2.7 in 3 patients</td>
</tr>
<tr>
<td>Plasma HCV RNA, log(_{10})</td>
<td>5.6 (3.0-5.9)</td>
<td>5.7 (4.8-6.0)</td>
<td>5.6 (5.6-6.0)</td>
</tr>
<tr>
<td><strong>Histopathological markers</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Necroinflammatory score (0-18)</td>
<td>5 (4-7)</td>
<td>5 (4-6)</td>
<td>4 (3-6)</td>
</tr>
<tr>
<td>Fibrosis score (0-6)</td>
<td>1 (1-1)</td>
<td>1 (1-2)</td>
<td>1 (1-2)</td>
</tr>
<tr>
<td>Portal CD4(^+) lymphocytes(^a)</td>
<td>29 (24-32)</td>
<td>28 (24-31)</td>
<td>55 (45-70)</td>
</tr>
<tr>
<td>Portal CD8(^+) lymphocytes(^a)</td>
<td>46 (40-55)</td>
<td>44 (38-53)</td>
<td>33 (30-42)</td>
</tr>
<tr>
<td>Portal CXCR3(^+) lymphocytes(^a)</td>
<td>1.4 (0.67-1.7)</td>
<td>1.3 (0.57-1.7)</td>
<td>1.0 (0.65-2.0)</td>
</tr>
</tbody>
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\(^a\) For each individual, average numbers of cells per field were determined by examination of 3-5 fields in the portal region. Data are presented as median (interquartile range) reflecting individual variation.
Supplementary Figure 1.

Images are from representative HIV/HCV co-infected patients assessed before ART (Panel A) and after 48 weeks on ART (Panel B). Portal areas of the liver show moderate inflammation with 1 to 2 mononuclear inflammatory cells positive for CXCR3 in brown color (arrows). Panel C is from a HCV mono-infected patient displaying more severe portal inflammation with some mononuclear inflammatory cells positive for CXCR3. (magnification 400x).