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Alterations in the balance of Th1 cells to Th17 and Th22 cells in HIV-1/HCV co-infection is associated with immune activation, microbial translocation and liver fibrosis

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Background

- HIV/HCV: more rapid & frequent progression to liver fibrosis\(^1\)

- Mechanisms driving liver fibrosis likely multiple & complex

- Increased microbial translocation may drive liver fibrosis:
  - promoting systemic immune activation\(^2\)-\(^4\)
  - LPS signalling via TLR4 in the liver\(^5\)

- Th17\(^6\) & Th22\(^7\) cells:
  - Integral to maintaining the immune integrity of the gut mucosa
  - Depletion in HIV may lead to increased microbial translocation

- CD4 T cell subsets have interdependent relationships
  - Ratios more important than proportions in determining immune control
  - Th17 & Th1 cells have reciprocal relationship

Hypothesis

Alterations in Th1, Th17 and Th22 cells in HIV-1 infection are associated with microbial translocation and immune activation and the rapid development of fibrotic liver disease in HIV-1/HCV co-infection.
Methods (1)

- Cross-sectional study
- Groups:
  1. HC
  2. HCV
  3. HIV ART
  4. HIV NAÏVE
  5. HCV HIV ART
  6. HCV HIV NAÏVE

- Blood samples:
  - 6 colour flow cytometry to determine CD4 T cell subsets frequencies:
    1. Th1 (CXCR3+CCR5+)
    2. Treg (CD25+CD127lo)
    3. Th17 (CCR4+CCR6+CCR10-)
    4. Th22 (CCR4+CCR6+CCR10+)
Methods (3)

• Blood samples (continued):
  – ELISAs on serum
    • Neopterin (marker of immune activation)
    • LBP (markers of microbial translocation)

• FibroScan

• Statistical analysis: (Graphpad Prism version 5.0)
  – Categorical variables: Chi-squared analysis / Fisher’s exact test
  – Continuous variables: Kuskal Wallis test with Dunn’s post test
    (to correct for multiple comparisons)
  – Correlations: Spearman’s rank correlation coefficient
    \( r = +/- 0.5000 \) taken as significant
## Baseline demographic and clinical data

<table>
<thead>
<tr>
<th></th>
<th>TOTAL (n=101)</th>
<th>HC (n=16)</th>
<th>HCV (n=21)</th>
<th>HIV ART (n=16)</th>
<th>HIV NAÏVE (n=20)</th>
<th>HCV HIV ART (n=18)</th>
<th>HCV HIV NAÏVE (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>AGE in years</strong></td>
<td></td>
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<tr>
<td>Median (IQR)</td>
<td>40.5 (12.35)</td>
<td>52.4 (8.39)</td>
<td>47.7 (11.44)</td>
<td>40.3 (8.09)</td>
<td>44.9 (6.39)</td>
<td>43.7 (7.81)</td>
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<tr>
<td><strong>GENDER</strong></td>
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<tr>
<td>Male: n (%)</td>
<td>15 (94)</td>
<td>16 (76)</td>
<td>16 (100)</td>
<td>16 (80)</td>
<td>17 (94)</td>
<td>9 (90)</td>
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<tr>
<td><strong>ETHNICITY</strong></td>
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<td>White: n (%)</td>
<td>15 (94)</td>
<td>13 (62)</td>
<td>13 (81)</td>
<td>16 (80)</td>
<td>14 (77)</td>
<td>7 (70)</td>
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<tr>
<td>Hispanic: n (%)</td>
<td>0 (0)</td>
<td>3 (14)</td>
<td>2 (13)</td>
<td>1 (5)</td>
<td>3 (17)</td>
<td>3 (30)</td>
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<tr>
<td>Black: n (%)</td>
<td>0 (0)</td>
<td>1 (5)</td>
<td>1 (6)</td>
<td>2 (10)</td>
<td>2 (10)</td>
<td>0 (0)</td>
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<tr>
<td><strong>CD4 %: Median</strong></td>
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<tr>
<td>(IQR)</td>
<td>48.2 (42.1-52.1)</td>
<td>45.1 (35.4-56.1)</td>
<td>37.7 (29.2-42.5)</td>
<td>15.7 (13.5-20.7)</td>
<td>35.2 (13.5-20.7)</td>
<td>27.8 (17.4-32.5)</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>cells/µl: Median</td>
<td>777 (561-950)</td>
<td>781 (501-1081)</td>
<td>619 (542-880)</td>
<td>283 (169-338)</td>
<td>646 (519-919)</td>
<td>434 (369-709)</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>(IQR)</td>
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<tr>
<td><strong>CD4:CD8</strong></td>
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<tr>
<td>Median (IQR)</td>
<td>1.72 (1.51-2.75)</td>
<td>1.99 (1.14-3.19)</td>
<td>0.96 (0.64-1.33)</td>
<td>0.25 (0.19-0.39)</td>
<td>0.80 (0.57-1.22)</td>
<td>0.46 (0.36-0.74)</td>
<td>P &lt; 0.001</td>
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<tr>
<td><strong>Years since HIV diagnosis</strong></td>
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<tr>
<td>Median (IQR)</td>
<td>NA</td>
<td>NA</td>
<td>12.7 (8.3-16.2)</td>
<td>5.1 (2.8-9.0)</td>
<td>9.6 (7.4-15.5)</td>
<td>4.9 (2.8-9.4)</td>
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<tr>
<td><strong>Years on ART</strong></td>
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<tr>
<td>Median (IQR)</td>
<td>NA</td>
<td>NA</td>
<td>8.0 (3.2-11.3)</td>
<td>NA</td>
<td>9.6 (3.7-11.6)</td>
<td>NA</td>
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<tr>
<td><strong>Years since HCV diagnosis</strong></td>
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<tr>
<td>Median (IQR)</td>
<td>NA</td>
<td>11.0 (7.5-16.0)</td>
<td>NA</td>
<td>NA</td>
<td>8.0 (5.0-10.3)</td>
<td>4.5 (2.4-6.0)</td>
<td>P &lt; 0.001</td>
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<tr>
<td><strong>HCV Genotype</strong></td>
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<tr>
<td>1: n (%)</td>
<td>NA</td>
<td>15 (71)</td>
<td>0 (0)</td>
<td>NA</td>
<td>NA</td>
<td>17 (94)</td>
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<tr>
<td>2: n (%)</td>
<td>NA</td>
<td>0 (0)</td>
<td>1 (5)</td>
<td>NA</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td></td>
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<tr>
<td>3: n (%)</td>
<td>NA</td>
<td>5 (24)</td>
<td>NA</td>
<td>NA</td>
<td>1 (6)</td>
<td>1 (10)</td>
<td></td>
</tr>
<tr>
<td>4: n (%)</td>
<td>NA</td>
<td>8 (80)</td>
<td>1 (10)</td>
<td>1 (10)</td>
<td>0 (0)</td>
<td>0 (0)</td>
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</tr>
</tbody>
</table>

*P < 0.01, P < 0.001*
Frequency of Th17 & Th22 cells

- No difference in Th17 or Th22 cells in HCV mono-infection
- Trend to reduced Th17 cells in HIV mono-infection
  - Confirmed with functional tests: IL-21+CD4 T cells
- Th22 cells depleted in HIV mono-infection
  - Confirmed with functional tests: IL-22+CD4 T cells
Frequency of Th17 & Th22 cells

- Th17 and Th22 cells depleted in HIV/HCV co-infection
  - Confirmed with functional tests: IL-21+CD4 T cells & IL-22+CD4 T cells
Frequency of Treg & Th1 cells

- No difference between groups in Treg cells
- Th1 cells increased in HCV
- Th1 cells unchanged in HIV
Frequency of Treg & Th1 cells

- Marked depletion of Th1 cells in HIV/HCV co-infection
  - Striking compared to HCV mono-infection
  - Deletion compared to HIV mono-infection
Shifts in CD4 T cell subsets

- HIV/HCV co-infection reduced Th1:Th22 & Th1:Th17 cell ratio
Correlations: immune activation & CD4 T cell subsets

HCV mono-infection

- Th17: $r = 0.4339$

- Th22: $r = 0.4776$

- Th1: $r = 0.4236$

HIV mono-infection

- $r = 0.5737$

- Th22: $r = 0.5316$

- Th1: $r = 0.1167$
Correlations:
immune activation & CD4 T cell subsets

HCV mono-infection
Th17: \( r = -0.4339 \)
Th22: \( r = -0.4776 \)
Th1: \( r = -0.4236 \)

HIV mono-infection
Th17: \( r = -0.5737 \)
Th22: \( r = -0.5316 \)
Th1: \( r = -0.1167 \)

HIV/HCV co-infection
Th17: \( r = 0.1515 \)
Th22: \( r = 0.1459 \)
Correlations:
microbial translocation & CD4 T cell subsets

HCV mono-infection

r 0.3829

HIV mono-infection

r -0.5029

Th17

Th22

r -0.3800

Th1

r -0.3717
Correlations: microbial translocation & CD4 T cell subsets

HCV mono-infection

Th17

HIV mono-infection

r -0.5029

HIV/HCV co-infection

r -0.0303

HCV mono-infection

Th22

r 0.3829

HIV mono-infection

r -0.3800

r -0.0973

HIV/HCV co-infection

r -0.5000

Th1

r -0.3717

r -0.1509
Correlations: immune activation & CD4 T cell shifts

HCV mono-infection

- Th1:Th17: $r = -0.1544$

HIV mono-infection

- Th1:Th17: $r = 0.5170$

HCV mono-infection

- Th1:Th22: $r = -0.0542$

HIV mono-infection

- Th1:Th22: $r = 0.4380$
Correlations: immune activation & CD4 T cell shifts

**HCV mono-infection**
- $\text{Th1:Th17} \quad r = -0.1544$

**HIV mono-infection**
- $\text{Th1:Th22} \quad r = -0.0542$
- $\text{Th1:Th17} \quad r = 0.5170$

**HIV/HCV co-infection**
- $\text{Th1:Th22} \quad r = 0.4380$
- $\text{Th1:Th17} \quad r = -0.5515$
- $\text{Th1:Th22} \quad r = -0.4788$
Correlations: microbial translocation & CD4 T cell shifts

HCV mono-infection

Th1:Th17 vs. LBP: $r = -0.6311$

HIV mono-infection

Th1:Th17 vs. LBP: $r = 0.4688$

Th1:Th22 vs. LBP: $r = -0.5604$

HIV/HCV co-infection

Th1:Th22 vs. LBP: $r = 0.4399$
Correlations:
microbial translocation & CD4 T cell shifts

HCV mono-infection

HIV mono-infection

HIV/HCV co-infection

Th1:Th17

Th1:Th22

Correlations: microbial translocation & CD4 T cell shifts
Correlations:
Liver stiffness

In HCV & HIV groups immune activation was positively associated with liver stiffness
In HCV & HIV groups immune activation was positively associated with liver stiffness.

Liver stiffness (Kpa)

- Th1
  - HIV/HCV co-infection: $r = -0.6239$
  - In HCV & HIV groups immune activation was positively associated with liver stiffness.

- Th1:Th17
  - HIV/HCV co-infection: $r = -0.6991$

- Th1:Th22
  - HIV/HCV co-infection: $r = -0.6869$
Conclusions

**HCV mono-infection**
- preferential expansion of Th1 cells
- Shifts towards Th1 cells associated with reduced levels of microbial translocation

**HIV mono-infection**
- preferential depletion of Th17 and Th22 cells
- Depleted Th17 cells associated with increased levels of immune activation and microbial translocation

**HIV/HCV co-infection**
- depletion of Th1, Th17 & Th22 cells
  - AND
- Shifts away from Th1 cells towards Th17 and Th22 cells
- Shifts away from Th1 cells associated with increased levels of microbial translocation, immune activation and liver stiffness.

In HIV mono-infection a preferential reduction in Th17 cells may lead to increased levels of microbial translocation & immune activation.

In HIV/HCV co-infection an additional lack of Th1 cell expansion with alterations in the balance of Th1 to Th17 cells may contribute toward development of liver fibrosis through secretion of pro-inflammatory cytokines.
Limitations

• Cross-sectional – causality cannot be determined

• Accuracy of markers used for microbial translocation

• Liver fibrosis assessment: use of transient elastography rather than biopsy

• Lack of paired gut or liver samples
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SSAT  
British HIV Association

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British HIV Association
BHIVA

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16–19 April 2013

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