Early Initiation of ART Preserves Mucosal Th17 Cells and Reverses HIV-related Immune Activation

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Mucosal Th17 cells are instrumental for the maintenance of the gut epithelial barrier, are depleted early (<6 months) during HIV infection and are only partially restored under long-term ART
- Kim CJ, J Immunol 2013

Loss of mucosal Th17 cells contributes to microbial translocation and sets the stage for ongoing immune activation

The timing of mucosal Th17 depletion during early acute infection and the impact of early initiation of ART are not well understood
Mucosal Sub-study Design (RV254)

Screened NAT/EIA
52,767 samples; 89 acute HIV infections identified

3 days
75 enrolled
in the main protocol

Optional procedure
Sigmoid Biopsy

2 days
Optional ART

Sigmoid Biopsies and PBMC collected in Fiebig I to III

Time of Diagnosis
37 participants

6 months post ART
27 participants

24 months post ART
16 participants
## Cohort Characteristics

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Acute HIV-infected (n=37)</th>
<th>ART-naïve chronically HIV-infected (n=5)</th>
<th>HIV-uninfected (n=9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age [years]</td>
<td>29</td>
<td>24</td>
<td>31</td>
</tr>
<tr>
<td>MSM [%]</td>
<td>83</td>
<td>100</td>
<td>80</td>
</tr>
<tr>
<td>Fiebig Stage, n</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>13</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>II</td>
<td>3</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>III</td>
<td>21</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Median time of infection [days]</td>
<td>14.5</td>
<td>298</td>
<td>NA</td>
</tr>
<tr>
<td>Median plasma HIV RNA ([\log_{10} \text{ copies/ml}])</td>
<td>5.4</td>
<td>4.9</td>
<td>NA</td>
</tr>
<tr>
<td>Median sigmoid colon HIV RNA ([\log_{10} \text{ copies/mg tissue}])</td>
<td>2.4</td>
<td>ND</td>
<td>NA</td>
</tr>
<tr>
<td>Median CD4 count ([\text{cell/mm}^3])</td>
<td>437</td>
<td>515</td>
<td>NA</td>
</tr>
<tr>
<td>CRF01_AE [%]</td>
<td>74</td>
<td>ND</td>
<td>NA</td>
</tr>
</tbody>
</table>
Flow Cytometry Methodology

- 18 to 23 biopsy pieces (approx. Φ 2mm) were collected from the sigmoid colon and processing was started within 30 min of sample collection.

- Isolation of MMC by ≤3 rounds of Collagenase II digestion at 20 min each in the presence of Benzonase.

<table>
<thead>
<tr>
<th>Cell Yield [x10^6/gr tissue]*</th>
<th>w/o Benzonase (n=18)</th>
<th>with Benzonase (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median</td>
<td>43.0</td>
<td>62.5</td>
</tr>
<tr>
<td>Min</td>
<td>14.9</td>
<td>29.5</td>
</tr>
<tr>
<td>Max</td>
<td>50.4</td>
<td>74.8</td>
</tr>
</tbody>
</table>

*HIV-uninfected subjects
Flow Cytometry Methodology – cont’d

- For all functional assays isolated MMC were subject to Percoll gradient purification (implemented later during the study)

- Cells were rested ON after Collagenase II Isolation/Percoll and subsequently stimulated for 5 hours with PMA/Ionomycin or for 6 hours with antigens

- MMC used for phenotyping were stained directly after Collagenase II digestion
In situ Hybridization and Immunohistochemistry Methodology (Jake Estes, Claire Deleage)

1 to 5 biopsy pieces (approx. Φ 2mm) were embedded in paraffin and subjected to *in situ* hybridization, immunohistochemistry and quantitative image analysis

- HIV-1 CRF01_AE specific riboprobes were generated for ISH (targeting Gag, Pol, Vif/Vpr/Vpu/Tat/Rev, Env, Nef
- For Immunohistochemistry samples were stained using anti-CD4, anti-CD163 and anti-CD86 antibodies
- Quantitative image analysis was performed
At Time of
Acute HIV Infection Diagnosis
CD4+ T cells in the LP significantly decreased in FI/II but not the number and frequency of bulk CD4+ T cells.
In Fiebig I and II HIV RNA+ cells were found in LP and LA while in Fiebig III HIV RNA+ cells were restricted to LA.
Gating Strategy – Th17 cells

Fiebig I

IL-17

IL-22

IL-17/IL22

IL-17/IL22 (unstim)
Frequencies and absolute number of mucosal Th17 cells remain intact during Fiebig I/II and correlate inversely with colonic viral load.
Mucosal Th17 cell function (IL-2, IL-22, IFN\(\gamma\)) remains intact during Fiebig I/II
Mucosal immune activation occurs by Fiebig I/II

<table>
<thead>
<tr>
<th>HIV RNA viral load</th>
<th>%CD8 DR/CD38</th>
<th>%CD4 DR/CD38</th>
</tr>
</thead>
<tbody>
<tr>
<td>colonic</td>
<td>r=0.38</td>
<td>r=0.21</td>
</tr>
<tr>
<td>plasma</td>
<td>p=0.02</td>
<td>p=0.19</td>
</tr>
<tr>
<td>sCD14</td>
<td>r=0.38</td>
<td>r=0.15</td>
</tr>
<tr>
<td></td>
<td>p&lt;0.001</td>
<td>p=0.32</td>
</tr>
<tr>
<td></td>
<td>p=0.01</td>
<td>p=0.48</td>
</tr>
</tbody>
</table>
Loss of mucosal Th17 cells inversely correlates with plasma levels of inflammation markers.

- CRP [pg/ml] vs. %CD4 Th17: $r = -0.42$, $p = 0.03$
- IP-10 [pg/ml] vs. %CD4 Th17: $r = -0.71$, $p < 0.001$
- HA [ng/ml] vs. %CD4 Th17: $r = 0.53$, $p = 0.004$
- TNF$\alpha$ [pg/ml] vs. %CD4 Th17: $r = -0.49$, $p = 0.03$
After Antiretroviral Therapy
Initiation of ART at Fiebig I/II preserves mucosal Th17 cell frequencies

\[\% CD4^{IL17}\]

- Fiebig I/II
- Fiebig III
- HIV-
- CHI

\(p=0.05\)
\(p=0.04\)
Initiation of ART at Fiebig I/II preserves mucosal Th17 cell function (IL-2, IL-22, IFNγ)
Initiation of ART during Fiebig I/II prevents increase of mucosal T\textsubscript{reg}
Initiation of ART at Fiebig I/II fully reverses mucosal CD8+ T cell immune activation
Initiation of ART at Fiebig I/II prevents mucosal CD4+ T cell immune activation
Summary

- CD4+ T cell loss during FI/II mainly occurs on the LP and to a lesser extent in the LA.
- Initiation of ART at Fiebig I/II prevents loss of mucosal Th17 cells and Th17 cell multifunctionality.
- Initiation of ART in Fiebig III does not restore the initial loss of multifunctional mucosal Th17 cells, despite partial recovery of Th17 cell numbers.
- Even though significant local and systemic immune activation is observed in Fiebig I/II, if treatment is initiated, it is fully reversed after 6 and 24 months of ART.
- Data argue for early and aggressive treatment intervention.
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Systemic immune activation occurs by Fiebig I/II

- HIV RNA viral load
  - Colonic: r=0.41, p=0.01
  - Plasma: r=0.27, p=0.09
  - sCD14: r=0.0006, p=0.97

%CD8 HLA-DR/CD38

- HIV- (n=9): p=0.001
- F1 (n=13): p=0.02
- FIII (n=21): p<0.001
- CHI (n=5): p<0.001
Initiation of ART at Fiebig I/II fully reverses systemic immune activation