HIV Reservoir Dynamics: Implications for HIV Latency Establishment and Reversal

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• Persistence of HIV infection under ART is due to a reservoir of latently infected cells that remain indefinitely despite full therapeutic suppression of virus replication.

• HIV latency is triggered by several mechanisms that lead to the silencing of virus expression including epigenetic DNA modification through methylation and histone deacetylation, limited availability of critical transcription factors and inefficient elongation of the nascent viral transcripts.

• **Understanding the mechanisms responsible for the establishment, maintenance, and reversal of the HIV reservoir under ART is essential to develop novel approaches for HIV eradication.**
Eradication strategies to target HIV in these subsets will need to address the heterogeneity of this complex population that harbors the latent reservoir.
HIV Reservoir

Mechanisms of persistence

Reservoir Quantification

Evaluating Therapeutic Interventions

In vitro models

CURE HIV
EMPLEO A COMBINACIÓN DE EX VIVO Y IN VITRO APPROCHESES TO STUDY MECHANISMS OF HIV LATENCY

<table>
<thead>
<tr>
<th>SCOPE N = 20</th>
<th>Florida N = 17</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (Yrs)</td>
<td>Infected (Yrs)</td>
</tr>
<tr>
<td>56 (48-63)</td>
<td>19 (14-23)</td>
</tr>
<tr>
<td>CD4 nadir (cells/mm³)</td>
<td>Infected (Yrs)</td>
</tr>
<tr>
<td>78 (22-220)</td>
<td>221 (27-316)</td>
</tr>
<tr>
<td>CD4 count (cells/mm³)</td>
<td>Infected (Yrs)</td>
</tr>
<tr>
<td>525 (304-701)</td>
<td>387 (285-715)</td>
</tr>
<tr>
<td>CD4:CD8 ratio</td>
<td>Infected (Yrs)</td>
</tr>
<tr>
<td>0.5 (0.32-0.77)</td>
<td>0.66 (0.57-1.0)</td>
</tr>
<tr>
<td>Viral Load</td>
<td>Infected (Yrs)</td>
</tr>
<tr>
<td>Undetectable</td>
<td>16 (12-23)</td>
</tr>
<tr>
<td>Yrs Infected</td>
<td>Infected (Yrs)</td>
</tr>
<tr>
<td>6.2 (4.2-8.9)</td>
<td>4.6 (3.0-6.3)</td>
</tr>
</tbody>
</table>

LARA: latency and reversal assay
**T_{EM}** SUBSET HAS HIGHEST FREQUENCY OF INTEGRATED HIV DNA IN VIRALLY SUPPRESSED COHORT

Kulpa et al 2018 submitted
**Tem subset shows highest frequency of the inducible reservoir in virally suppressed cohort**

![Graph showing frequency of cells in each memory CD4+ subset and frequency of integrated HIV DNA/million CD4+ T cells.](image)

**TCM** and **TTM** show significantly lower frequency compared to **TEM**.

**HIV msRNA**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Frequency (%</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unstimulated</td>
<td>TCM</td>
<td>25%</td>
</tr>
<tr>
<td></td>
<td>TTM</td>
<td>33%</td>
</tr>
<tr>
<td></td>
<td>TEM</td>
<td>32%</td>
</tr>
<tr>
<td>PMA + iono</td>
<td>TCM</td>
<td>22%</td>
</tr>
<tr>
<td></td>
<td>TTM</td>
<td>26%</td>
</tr>
<tr>
<td></td>
<td>TEM</td>
<td>52%</td>
</tr>
</tbody>
</table>

*Kulpa et al 2018 submitted*

**Tem** have high efficiency of latency reversal. What about **Tcm**?
Transcriptional profiling demonstrates relationship between acquisition of effector function and latency reversal.

Kulpa et al. 2018 submitted
LRA treatment induces gene expression patterns of $T_{EM}$ in $T_{CM}$ subset from virally suppressed cohort.
Modulation of epigenetic modifications correlate with inducible reservoir measure in $T_{CM}$ and $T_{EM}$ subsets

What is the efficacy of different classes of Latency Reversing Agents in different memory CD4+ T cell subsets?

Kulpa et al 2018 submitted
LARA: *In vitro* model of HIV latency to study mechanisms of latency establishment and reversal

Schematic of LARA *in vitro* model of HIV latency

- Buffy coat from HIV-negative donor
- Enrich memory CD4+ T cells
- Infect HIV clone 89.6
- Add IL-2 + saquinavir
- Add TGFβ + IL-7 + H-80 conditioned medium + saquinavir + efavirenz + raltegravir
- Day 3/6/LRA

Memory subset distribution:

<table>
<thead>
<tr>
<th>Day</th>
<th>T_CM</th>
<th>T_TM</th>
<th>T_EM</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>16%</td>
<td>40%</td>
<td>41%</td>
</tr>
<tr>
<td>6</td>
<td>13%</td>
<td>39%</td>
<td>42%</td>
</tr>
<tr>
<td>13</td>
<td>7%</td>
<td>64%</td>
<td>20%</td>
</tr>
</tbody>
</table>

Contribution of each subset to the pool of HIV-infected cells (%)

Kulpa et al 2018 submitted
LATENCY REVERSAL: CD4+ MEMORY SUBSETS SHOW DIFFERENTIAL RESPONSES TO LATENCY REVERSING AGENTS

Kulpa et al 2018 submitted
Together with our ex vivo data support the acquisition of an effector phenotype correlates with HIV expression and latency reversal in quiescent subsets like $T_{CM}$ and LARA model is effective tool for studying mechanisms of HIV latency

Kulpa et al 2018 submitted
LARA model can also be employed to examine factors that contribute to establishment of HIV latency and persistence.

**Examine cytokines and cellular interactions that mediate HIV latency**
The role of TGF-β in HIV latency establishment:
Increasing concentrations of TGF-β during LARA latency culture results in an increased frequency of latently infected cells.

TGF-β promotes survival of infected cells

Increasing concentrations of TGF-β during latency culture demonstrate stable viability with decreased overall cell #

However, increasing concentrations of TGF-β show increased retention of HIV infected cells.
CD8 T cells control of HIV infection include both cytolytic and non-cytolytic mechanisms.
Preliminary experiments focus on characterizing the role of CD8+ T cells in the establishment of HIV latency in CD4+ cells.
Perform experiments with CD4:CD8 T cell co-cultures using *in vitro* platform with HIV naïve donors

**establishment of HIV infection**

- **Day 0**
  - Enrich memory CD4+ T cells
  - Buffy coat from HIV negative donor
  - Enrich total CD8+ T cells

- **Day 3**
  - Infect HIV clone 89.6

- **Day 6**
  - CD4 alone
  - CD4:CD8 1:1
  - IL-2 + saquinavir
  - CD4:CD8 1:5
  - CD8 alone

- **Activate 3 days**
  - αCD3/CD28+IL-2
Quantify impact on HIV expression through flow cytometry (CD4-GAG+) & qPCR (frequency of cells carrying integrated HIV DNA)

**establishment of HIV latency**

- **day 0**
  - Buffy coat from HIV negative donor
  - Enrich memory CD4+ T cells
  - Enrich total CD8+ T cells

- **rest**

- **day 3**
  - Infected HIV clone 89.6

- **CD4 alone**
- **CD4:CD8 1:1**
- **CD4:CD8 1:5**
- **CD8 alone**

**CD4:CD8 1:1**

- **activate 3 days αCD3/CD28+IL-2**

**CD4:CD8 1:5**

**CD8 alone**

**frequency of CD4-Gag+ expression cells by flow cytometry in memory CD4 T cells & each memory subset**

**frequency of cells carrying integrated HIV DNA cells by qPCR**
Co-culture of HIV-infected memory CD4+ T cells with activated total CD8+ cells shows a significant reduction in HIV-GAG+ cells
Co-culture of HIV-infected memory CD4+ T cells with activated total CD8+ cells shows a significant reduction in HIV-GAG+ cells
Memory CD4+ T cell subsets all show similar decreased frequency of HIV-GAG+ cells in the presence of CD8+ T cells.
However, there is no significant reduction in the frequency of HIV-infected CD4+ T cells

These data suggest CD8 T cells can play a role in the establishment of HIV latency that we are currently investigating further...
Conclusions

• We have demonstrated memory CD4+ T cell subsets constitute a dynamic reservoir of latently HIV-infected cells
• The T_{EM} subset shows the highest frequency of inducible reservoir in a cohort of virally suppressed HIV-infected individuals
• T_{CM} subset differentiation to effector phenotype is correlated with latency reversal
• LARA in vitro model supports these conclusions and offers platform for more in depth mechanistic studies
• Expanded our studies to characterize contexts that are important for the establishment and maintenance of HIV latency to aid in the development of therapeutic intervention approaches.
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