T Memory Stem Cells: A Long-term Reservoir for HIV-1

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A well accepted mechanism of HIV persistence is the stable infection of resting long-lived memory cells.

They decay very slowly with an average half-live of 44 months (Silicano et al; Nat Med 2003).

Major cellular reservoirs for HIV (Chomont et al; Nat Med 2009):
- Central Memory cells (CM)
- Transitional Memory cells (TM)

However, some evidences suggest the presence of other cellular reservoirs (Bailey et al; 2006 J Virol).
Identification of T Stem Cells Memory (T_{SCM})

- T_{SCM} represent a recently-discovered subpopulation of T cells that persist for extremely long periods of time.

A human memory T cell subset with stem cell-like properties

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- Phenotypic markers are available to identify this new subset (CD45RA, CCR7, CD62L, CD28, CD27, CD127, CD122, CD95)
Due to their extremely long half-live and self-renewal capacity, $T_{SCM}$ might be a key reservoir for HIV, contributing to HIV persistence and representing a significant barrier for HIV eradication.
Aims

• To determine the proportion of $T_{SCM}$ cells in different patient cohorts

• To ascertain if $T_{SCM}$ cells are an in-vivo reservoir for HIV

• To evaluate the in-vitro and in-vivo susceptibility of $T_{SCM}$ cells to HIV infection

• To determine the contribution of $T_{SCM}$ cells to the pool of HIV infected cells
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Materials & Methods

• Flow cytometry of $T_{SCM}$ and CD4 subsets (NA, CM, EM and TD) from:

  - HAART-treated (n=20) with 10 years <50 copies/ml
  - EC (n=18) with at least 8 years <50 copies/ml
  - HIV negatives (n=9)
Gating Strategy

CD4

CD45RA

CD62L

CD95

International AIDS Society
Stronger Together
No differences in the proportion of $T_{SCM}$ between cohorts
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• Total HIV-1 DNA quantification by ddPCR and qPCR of $T_{SCM}$ and CD4 subsets (NA, CM, EM and TD)
Droplet Digital PCR (ddPCR)

HIV-1 DNA

<table>
<thead>
<tr>
<th>C-</th>
<th>EM</th>
<th>CM</th>
<th>TD</th>
<th>$T_{SCM}$</th>
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</thead>
<tbody>
<tr>
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<td>A05</td>
<td>C05</td>
<td>D05</td>
<td>E05</td>
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CCR5

<table>
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<tr>
<th>C-</th>
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<tr>
<td>D06</td>
<td>D09</td>
<td>F09</td>
<td>G09</td>
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</tbody>
</table>

Event Number

FAM Amplitude
$T_{SCM}$ cells are an in-vivo reservoir for HIV-1.
Highest per cell levels of HIV within the $T_{SCM}$ cells
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Materials & Methods

• Ex-vivo infection of PBMC from HIV-negative without ex-vivo stimulation (n=8)

  GFP R5, GFP VSV-G
  MOI = 0.01

  PBMCs
  4 days

• Determination of GFP+ cells by flow cytometry
Ex-vivo infection

CD4 0.50%

TD 0.56%

EM 0.35%

CM 0.56%

NA 0.43%

SCM 2.84%
$T_{SCM}$ have higher ex-vivo infection levels compared to CD4 subsets.
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Materials & Methods

• Sorting of $T_{SCM}$ and CD4 subsets from untreated HIV infected individuals with high viral load (n=3)

• Quantification of cell-associated HIV RNA of each CD4 subset by qPCR
$T_{SCM}$ are susceptible to in-vivo HIV-1 infection

HIV-1 RNA

<table>
<thead>
<tr>
<th></th>
<th>SC M</th>
<th>NA</th>
<th>CM</th>
<th>EM</th>
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<td>n=3</td>
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</tbody>
</table>

HIV RNA/ug RNA

- $10^{-5}$
- $10^{-4}$
- $10^{-3}$
- $10^{-2}$
- $10^{-1}$
- $10^{0}$
- $10^{1}$
- $10^{2}$
- $10^{3}$
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Materials & Methods

• Longitudinal sorting of $T_{SCM}$ and CD4 subsets from HAART-treated (n=8)
  - earliest time point: months after infection
  - latest time point: after 10 years of suppressive HAART

• Total HIV-1 DNA quantification of each subset by qPCR

• Calculation of the percentage of contribution of each subset to the pool of HIV infected CD4+ T cells
Constant levels of HIV-1 DNA within the $T_{SCM}$ over time

![Graph showing constant levels of HIV-1 DNA over time in different intervals.](image-url)
Increased contribution over time of $T_{SCM}$ to the HIV-1 infected pool

Short-term HAART

- % Frequency CD4
  - EM: 42%
  - CM: 17%
  - NA: 19%
  - SC: 1%
  - TD: 1%

- % Contribution
  - EM: 14%
  - CM: 33%
  - NA: 13%
  - SC: 16%
  - TD: 24%

Long-term HAART

- % Frequency CD4
  - EM: 40%
  - CM: 14%
  - NA: 21%
  - SC: 1%
  - TD: 1%

- % Contribution
  - EM: 24%
  - CM: 24%
  - NA: 16%
  - SC: 35%
  - TD: 1%

n=8
Conclusions

• In HAART-treated and EC subjects, high per-cell levels of HIV-1 DNA were observed in CD4 T_{SCM} exceeding corresponding levels in central-memory or effector-memory CD4 T cells.
• In ex-vivo assays, T_{SCM} were more susceptible to HIV-1 infection than memory or naïve CD4 T cells.
• In in-vivo assays, T_{SCM} contained viral RNA, indicating T_{SCM} are infected in vivo.
• Longitudinal investigations of HIV-1 DNA over 10 years demonstrated an increasing contribution of T_{SCM} to the total viral reservoir, while the contribution of more mature effector-memory and terminally-differentiated CD4 T cells tended to decline.

Tscm serve as a long-lasting reservoir for HIV-1 that importantly contributes to viral persistence. Targeting this specific cell compartment by immunological or pharmacological interventions may contribute to reducing viral persistence in vivo.
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