Role of PD-1+/Tfh Cells in HIV-1 persistence

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Anti-Retroviral Therapy (ART) Reduces HIV Viremia to Undetectable Levels

- Initiation of ART
- Limit of detection
- Cessation of therapy
Why/where does HIV persist in infected individuals?

Two main phenomena:

Residual HIV replication

- CD4 T cells?
- Macrophages?
- DCs?

- Size: estimated $10^6$ cells
- Half life of memory CD4 T cells: 44 months
- Estimated time for eradication: ~70 years under full virus suppression by ART
- Not susceptible to ART
- Not susceptible to the immune system

Latent HIV reservoir

- $T_{CM}/T_{TM}$ CD4 T cells

- Covert cellular reservoir
- Privileged anatomical compartment
- Resistant to HIV cytopathic effect
- Minimal virus spreading
- Replenishment of the latent cellular reservoir
“Shock and Kill” strategy to target HIV-infected CD4 T cells

Latently infected CD4 T cell

Trigger viral reactivation

‘Shock’

Latently infected cell is reactivated

HIV RNA

HIV proteins

HIV virus particles

Immune system

‘Kill’

Dying infected cell

Uninfected cell

ART
To identify marker(s) to specifically target HIV-1 infected cells using immunotherapy

- Latently infected CD4 T cell
- HIV genome
- Trigger viral reactivation
- ‘Shock’
- Latently infected cell is reactivated
- HIV RNA
- HIV proteins
- HIV virus particles
- Dying infected cell
- Immune system
- ‘Kill’
- Uninfected cell
- ART

HIV infected CD4 T cell
- Identification of a specific marker of HIV infected cells
- Targeted by toxin conjugated antibody
- Destruction of HIV infected cell
- Toxin
- G E S I D A 2017
In Blood, CM and TM are the major HIV-1 reservoirs

Integrated HIV-1 DNA in Sorted CD4 T cell subsets in Blood of treated HIV-1 infected patients

Chomont et al., Nature Medicine 2009
CD32a is a marker of CD4 T cells harboring replication competent virus

Descours et al., Nature 2017
Lymphoid organs are the primary anatomical compartments for HIV replication and spreading
In germinal centers a new CD4 T-cell subset was discovered and named follicular helper T (Tfh) cells.
Tfh-cell phenotype and functions

Nutt et al., Nature Immunology 2011
Tfh and CXCR5-PD-1+ CD4 T-cell populations support active HIV replication and production in viremic HIV-1 infected individuals

Adapted from Perreau et al., J Exp Med 2013
Hypothesis of the study

- Tfh cells isolated from lymph nodes of aviremic HIV-infected treated individuals are enriched in cells containing inducible replication competent virus and the major source persistent HIV-1 transcription.
## Cohort

<table>
<thead>
<tr>
<th>Subject ID</th>
<th>Age</th>
<th>Sex</th>
<th>Duration of HIV infection (years)</th>
<th>CD4 Cell Count at enrollment (cells/ul)</th>
<th>Viral Load at Enrollment (copies/ml)</th>
<th>Time on HAART (years)</th>
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</table>
Sorting of memory CD4 T-cells from blood and lymph node based on CXCR5 and/or PD-1 expression

Selection of blood CD4 T-cell populations

Selection of LN CD4 T-cell populations
T follicular helper T cells represent about 65% of total LN memory PD-1+ CD4 T cells
Virus Outgrowth Assay (VOA) – Determination of Replication Competent HIV-1

Allogeneic CD8-depleted PBMCs from HIV negative subject

Limiting dilution format

Patients

Limiting dilution format

Sorted Blood/LN memory (CD45RA-) CD4 T-cell populations

HIV-1 infected CD4 T cells

Allogeneic CD8 depleted PBMCs

HIV 1

Readout: 1. HIV-1 RNA (RT PCR)
2. P24 (ECL)

Day 0

Day 5

Day 14

1.10^5

2.10^4

4.10^3
Replication Competent HIV-1 in Blood and LN PD-1 Negative and PD-1+/Tfh Cell Populations in Long-Term ART Treated Aviremic HIV-1 Patients – Cumulative Data

Day 14

HIV-1 RNA

HIV-1 P24

CXCR5
PD-1

Blood
LN

Banga et al., NMED 2016
Levels of HIV Replication in LN CXCR5\(^+\) and PD-1\(^+\)/Tfh Cell Populations of Long-Term ART Treated Aviremic Patients Negatively Correlate with the Duration of Treatment

**Lymph Node**

**HIV-1 RNA**

- DN: \( r = -0.5342; P = 0.071 \)
- CXCR5: \( r = -0.8142; P = 0.0018 \)
- PD1: \( r = -0.9015; P < 0.0001 \)

**P24**

- DN: \( r = -0.3708; P = 0.1480 \)
- CXCR5: \( r = -0.7173; P = 0.0062 \)
- PD1: \( r = -0.8498; P = 0.0007 \)

Banga et al., NMED 2016
What are the frequencies of cells containing replication competent and infectious virus in blood and LN compartments?

Mean frequency of cells containing replication competent (RUPM) and infectious virus (IUPM) per million cells determined using Extreme Limiting Dilution Assay.
LN PD-1+ CD4 T cells are enriched with replication competent and infectious virus

Mean frequency of cells containing replication competent (RUPM) and infectious virus (IUPM) per million cells determined using Extreme Limiting Dilution Assay

Banga et al, NMED 2016
LN PD-1+ CD4 T cells represent the major source of replication competent and infectious virus.

Banga et al., NMED 2016
Conclusions

- LN PD-1+/Tfh Cells are enriched in inducible replication competent and infectious HIV in treated aviremic HIV-infected subjects

- Are LN PD-1+/Tfh a major source for active virus transcription?
Increased Levels of HIV Cell Associated RNA in LN PD-1+/Tfh Cell Populations of Long-Term ART Treated Aviremic Patients

Cell-associated RNA

Active transcription occurs preferentially within PD-1+/Tfh cells likely because of their greater state of activation
Persistent Levels of HIV Cell Associated RNA in LN PD-1⁺/Tfh Cell Populations of Long-Term (up to 12 years) ART Treated Aviremic Patients

Cell-associated RNA

Persistent HIV-1 transcription in PD-1⁺/Tfh cells may help to explain why virus rebound is consistently observed after ART interruption.
Size and Number of GCs and Number of PD-1+ cells within GCs progressively decreased upon prolonged ART.

- Long-term ART was associated with a progressive shift from an activation to a quiescent state of the LN tissue.
IC molecule expression on blood and LN CD4 T-cell populations

Treated aviremic HIV-infected individual #137

Gated on LN CD3^+CD4^+CD45RA^- cells

CXCR5^+PD-1^- CD4 T cells
CXCR5^+PD-1^- CD4 T cells
CXCR5^+PD-1^+ CD4 T cells
CXCR5^+PD-1^+ CD4 T cells
CXCR5^hiPD-1^hi (TFH) CD4 T cells
IC molecule expression on blood and LN CD4 T-cell populations

TIGIT and PD-1 are the 2 main IC molecules expressed on blood and LN CD4 T-cell populations
IC-ligand expression on blood and LN cell populations

**BLOOD**

- **B cell populations**
  - Naive
  - Gated on Non-naive B cells
  - Gated on Non-GC

- **Monocytes and DCs**
  - Monocytes
  - cDCs

**LYMPH NODE**

- **B cell populations**
  - Naive
  - Gated on Non-naive B cells
  - Gated on Non-GC

- **DCs**
  - CD1c^hiHLA-DR^hi
IC-ligand expression on blood and LN cell populations

CD155 (TIGIT-ligand) and PD-L1/L2 are expressed on blood and LN myeloid cell populations but not on B cell populations
Impact of IC/IC-L interaction on HIV production from blood and LN memory CD4 T cells

- Anti-CD3/anti-CD28
- Emtricitabine
- Anti-CD3/anti-CD28
- Recombinant IC-L
- Day 5 HIV-1 RNA (RT PCR)
- Purified resting memory CD4 T cells
- Latently HIV-1 infected CD4 T cells
- HIV 1
Impact of IC/IC-L interaction on HIV production from blood and LN memory CD4 T cells

HIV treated individual

**BLOOD**
Gated on blood CD3+CD4+CD45RA- cells

**LYMPH NODE**
Gated on LN CD3+CD4+CD45RA- cells

<table>
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<tr>
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<th>PD-1</th>
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<td>BLOOD</td>
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<td>Lymph Node</td>
<td>32.3</td>
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</table>
Impact of IC/IC-L interaction on HIV production from blood and LN memory CD4 T cells

- Recombinant IC-L significantly reduce HIV-1 RNA production from blood and LN memory CD4 T cells.

![Graph showing levels of HIV RNA in blood and lymph node with IC-L interaction](image)

The graph illustrates the levels of HIV RNA (copies/ml) in blood and lymph node samples under different conditions of IC-L interaction. The + and - symbols represent the presence or absence of specific molecules (PDL1, PDL2, CD155, α-CD3/α-CD28) in the reaction.

* Sign indicates a significant reduction in HIV RNA levels.
Do PD-1-expressing and PD-L1 expressing cells colocalize?
Do PD-1-expressing and PD-L1 expressing cells colocalize?

Untreated viremic HIV-infected individual
Do PD-1-expressing and PD-L1 expressing cells colocalize?

Treated HIV-infected individual
ART treatment initiation induces substantial changes in IC/IC-L expression.

**Graph 1:**
- **X-axis:** Healthy, Viremic, Treated
- **Y-axis:** Number of PD-1$^{\text{high}}$ cell/mm$^2$
- **Legend:**
  - Black circles: Healthy
  - Gray squares: Viremic
  - Red circles: Treated
- **Annotations:**
  - *: Statistical significance

**Graph 2:**
- **X-axis:** extra, GC
- **Y-axis:** Proportion of PD-L1$^+$ area
- **Legend:**
  - Black circles: Healthy
  - Gray squares: Viremic
  - Red circles: Treated
- **Annotations:**
  - *: Statistical significance

![Graph 1](https://example.com/graph1.png)
![Graph 2](https://example.com/graph2.png)
ART treatment initiation induces substantial changes in IC/IC-L tissue distribution

- Suggesting that IC/IC-L interactions might be altered in GC areas of cART treated HIV-1 infected individuals
Evaluation of anti-PD-1 Mabs efficiency to reactivate HIV-1 from latency

Autologous irradiated CD8-depleted PBMCs

Unexposed/isotype control (Negative Controls)

Anti-CD3/anti-CD28 (positive control)

Anti-PD-1

14 days

Day 14 HIV-1 RNA (RT PCR)

Purified resting memory CD4

Latently HIV-1 infected CD4 T cells

Allogeneic CD8-depleted PBMCs

HIV 1

Adapted from Banga et al., JVI 2015
The fraction of the provirus induced by anti-PD-1 MAbs corresponded to about 21% of the one induced by anti-CD3/anti-CD28 MAbs.
Conclusions

- LN PD-1+/Tfh Cells are enriched in inducible replication competent and infectious HIV in treated aviremic HIV-infected subjects
- LN PD-1+/Tfh cells serve as the major source for active and persistent virus transcription after long-term ART, associated with a progressive shift from an activation to a quiescent state of the LN tissue and an altered IC-ligand distribution
- LN PD-1+/Tfh cells may represent a major obstacle for HIV functional cure/eradication
- These results provide the scientific rationale for the development of therapeutic interventions targeting PD-1+ cells
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