HIV Persists in Colon and Blood CCR6+CD4+ T-Cells During Viral Suppressive ART


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The mastaba, House of eternity
Christo & Jeanne Claude

Fromentin R et al Plos Pathogens 2016
Mucosal Immunity and HIV Infection

HIV infection characterized by:

- Rapid and massive depletion of CD4+ T-cells from the gut-associated lymphoid tissues (GALT)
- Viral replication in memory CCR5+ T-cells from the GALT
- However, GALT CD4 T cells contribute for a large part to HIV reservoir

Ponte, Routy et al. Ebiomedicine 2016
The body’s second brain: Enteric nervous system > $100 \times 10^6$ neurons

Gut = Largest surface area ($400 \text{m}^2$) to maximize digestion/absorption

Large surface in contact with the external environment: Microbiota & byproducts
Chemokine Receptor and Gut Homing of T-Cells

- The chemokine receptor CCR6 is a gut-homing molecule mediating migration of the cells into Peyer’s Patches where its ligand CCL20 is located.
- CCR6 identifies memory CD4+ T-cells with Th17 (CCR4+CCR6+) and Th1Th17 (CXCR3+CCR6+) polarization profiles.
- CCR6+ T cells express more CCR5 and post-entry HIV-permissiveness factors than their negative counterpart.
- Retinoic Acid (RA) is a vitamin A metabolite involved in gut-homing through the induction of CCR9 & α4β7.

Gosselin/Monteiro et al., J Immunol, 2010
Montiriro et al. J immunol 2011
Preferential HIV-DNA Integration in Memory CCR6+ T-Cells of Viremic Untreated HIV-Infected Patients

Gosselin/Monteiro et al., J Immunol, 2010

Rapid CCR6 T cell depletion in blood
Not restored by ART

RI: Recent infection, CI: Chronic infection

p=0.03
p=0.0001
ATRA Enhances HIV Permissiveness in Memory CCR6+ T-Cells in vitro

Monteiro et al., J Immunol, 2011

ATRA, all trans retinoic acid
Hypothesis and objectives

• **Hypothesis:** CCR6 is a marker for CD4+ T-cells that preferentially harbor replication-competent in ART-treated subjects

• **Objectives:**
  – To investigate the contribution of colon and blood CCR6+CD4+ T-cells to HIV persistence during ART
  – To assess the ability of ATRA to reactivate HIV reservoirs in CCR6+CD4+ T-cells
Clinical Parameters of Chronically Infected HIV-positive on ART

Table I. Clinical parameters of CI on ART subjects

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<th>Patient ID#</th>
<th>CD4 counts*</th>
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<th>Time since infection&amp;</th>
<th>ART regimen</th>
<th>Time of aviremia§</th>
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</table>

#, plasma viral load in all patients <40 HIV-RNA copies/ml; *, cells/µl; &, months
Reduced Frequency of CCR6+CD4+ T-Cells in ART treated vs. Uninfected Individuals

Leukapheresis → PBMC → Phenotyping

CD4+ T-cells

CD4+CD45RA-

CD4+CD45RA-

EM/TM CCR6+
CM CCR6+

EM/TM CCR6-
CM CCR6-

CM CCR6-
Man-Whitney p<0.0001

CM CCR6+
Man-Whitney p=0.0078

EM/TM CCR6-
Man-Whitney, not significant

EM/TM CCR6+
Man-Whitney p=0.0001

Gosselin/Wiche Salinas et al. submitted
CCR6+ vs. CCR6- CM Subsets Harbor Superior Levels of Integrated HIV DNA in ART treated Individuals

PBMC
MACS purified CD4+ T-Cells
FACS purified Memory Subsets
Integrated HIV PCR

HIV-DNA integration in CCR6+/CCR6- subsets

HIV+ on ART (n=5)
Friedman p=0.0031
Contribution to HIV reservoir

ART-treated subjects (n=5)
Friedman p=0.033
Relative contribution to HIV reservoir

Gosselin/Wiche Salinas et al. submitted
Free-Cell Isolation from Colon Biopsies

• Challenges to overcome:
  – Small amount of material
    • Adaptation of the techniques
  – Surface marker loss due to enzymatic digestion
    • Use of Liberase DL
    • Specific antibody clones for epitopes that are less affected
    • O/N Resting to restore the expression
    • Work on fresh samples
  – Contamination by gut flora
    • Use of specific antibiotics (piperacillin/tazobactam)
Free-Cell Isolation from Colon Biopsies

- Technique:
  - Liberase DL Enzymatic Digestion
  - 3-4 Cycles of Mechanical Disruption using a Blunt-Ended Needle
  - Free-Cell isolation and washing
  - O/N Resting
  - Staining for Flow Cytometry
  - Cell Sorting
  - Total HIV PCR

N = 30 biopsies

Gosselin/Wiche Salinas et al. submitted
The Frequency of Memory CCR6+ T-Cells Is Enriched in the Colon of HIV+ on ART Individuals

A

All viable cells
Lineage- cells
CD3+ T-cells
CD4+CD45RA-

CD326
CD3
CD45RA
CD3

Frequency memory CCR6+ T-cells
Paired t-Test p=0.0013

PBMC
Colon

Sample ID
B20 B21 B22 B23 B24 B25 B26 B27 B28 B29 B30 B31 B32 EC1

(% of CD3+CD4+CD45RA-)

CCR6+ cells
Blood and Colon CCR6+ versus CCR6- T-Cells Are Preferentially Infected During ART

**Blood**

Gag HIV-DNA in blood CD4+ T-cells

*Paired t-Test = 0.045*

**Colon**

Gag HIV-DNA in colon CD4+ T-cells

*Paired t-Test = 0.003*

Gosselin/Wiche Salinas et al. submitted
**Preferential Reactivation of HIV-1 Reservoirs in Blood CCR6+ T-Cells and enhanced by ATRA**

### Day 0
- MACS/FACS cell isolation
- TCR triggering ± ATRA (2x10^6 cells/well)

### Day 3
- Harvest media
- Add IL-2 ± ATRA
- HIV-p24 ELISA

### Day 6
- Harvest media
- Add IL-2 ± ATRA
- HIV-p24 ELISA

### Day 9
- Harvest media and cells
- HIV-p34 ELISA
- HIV-p24 ICS

**HIV reactivation: Day 9**
- Friedman p=0.0004

- **intra cellular staining for p24**
  - TEM/TM: ART#19; Day 9

- **HIV reactivation: Day 9**
  - Friedman p=0.001

**Total memory (CD45RA-); CM (CD45RA-CCR7+); EM/TM (CD45RA-CCR7-)**

Gosselin/Wiche Salinas et al. submitted
In HIV-infected individuals receiving viral suppressive ART:

- CCR6 is a marker for memory TH17 CD4+ T-cells enriched in for replication-competent HIV-DNA in both colon and blood

- Blood CCR6+ vs CCR6- CM harbor superior levels of integrated HIV-DNA contributing the most to the pool of infected CM

- HIV reactivation was mainly detected in CCR6+ T-cells

- ATRA promotes HIV latency reversal in a TCR-dependent manner, suggesting an important contribution of the intestinal environment to viral replication and/or reactivation

HIV eradication strategies should target viral persistence in CCR6+CD4+ T-cells from various anatomic sites
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