Multiple Origins of Virus Persistence in HIV Controllers

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The Cascade

Number and percentage of HIV-infected persons engaged in selected stages of the continuum of HIV care in the United States

Even with drugs that are 100% effective at suppressing virus we are unable to achieve this in 100% of infected people

This is one reason we need a cure
How To Cure HIV-Infected People

• Multiple mechanisms account for HIV persistence

• The unifying theme is to find and diminish the size of the HIV reservoir
  – Reduce seeding of latent pool with early/more ART
  – Reverse latency (shock and kill)
  – Increase HIV-specific immune function (vaccines)
  – Reduce immune activation
  – Gene therapy targeting the virus and the host
  – Allogeneic stem cell transplantation

Combination therapy may be necessary
Hematopoietic transplantation with cells resistant to infection

- Doing well off ARV therapy > 6 years
- No replication competent HIV
- No PBMC DNA, intermittent very low plasma HIV RNA and rectal DNA
- Waning HIV antibodies and no HIV-specific T cells
- Normal levels T cell activation

Even though this procedure works, it is highly unlikely that it will ever translate into an accessible approach
Boston Patients: Virus Recrudescence

Hematopoietic transplantation with cells susceptible to infection

Despite 1000 – 10,000 fold reductions in reservoir size, virus rebounded

Modeling: latent reservoir will have to be depleted > $10^5$ fold (Hill, PNAS ‘14)
Patient A: Where Did the Virus Come From?

A single virus accounts for recrudescence

Plasma RNA 5 days after HIV rebound

HIV DNA pre-transplant

Consensus B

Heinrich 2015
The HIV Reservoir

• How big is the HIV reservoir?

• Where does HIV reside?

• What is its transcriptional state?
  – can we see it?

• Where is the HIV reservoir that matters?
  – will it cause damage off cART?

Developing treatments that target HIV-infected cells is a part of the path towards a cure
How Big Is The Reservoir That Matters?

Total HIV DNA

Replication competent HIV

Defective HIV DNA (88%)
Intact and inducible (12%)
Replicates in vitro (0.1%)

Size of relevant reservoir is unknown: between red and yellow

Ho, Cell, 2013; Cillo, PNAS 2014; Erikkson Plos Path 2013
Size of reservoir correlates with and/or is enriched in cells:

- proliferating (Ki67)\textsuperscript{1,2}
- activated (CD38, HLA-DR, CCR5, CD4/CD8 ratio) \textsuperscript{1-6}
- migrate to tissue (CXCR3) \textsuperscript{7}
- exhausted (PD-1, LAG-3, TIGIT) \textsuperscript{1,8}
- stem memory T cells\textsuperscript{10}
- central and transitional memory T cells on cART\textsuperscript{1,10,11}
- GC-T follicular helper cells – major source of replicating virus in SIV controllers, higher frequency in HIV viremics\textsuperscript{12,17}

- effector memory T cells low frequency on cART
- sustained clonal expansion of infected T cells contributes to HIV persistence on cART\textsuperscript{13-16}

\textsuperscript{1}Chomont, Nat Med '09, \textsuperscript{2}Murray JV '14, \textsuperscript{3}Hatano JID '12, \textsuperscript{4}Cockerham PLoS ONE '14, Hatano JID '14, \textsuperscript{5}Wang JID '14, \textsuperscript{6}Riddler (unpublished), \textsuperscript{7}Lewin (unpublished), \textsuperscript{8}Frometin (PLoS Path '16), \textsuperscript{9}Chun JCI '05, \textsuperscript{10}Buzon Nat Med '14, \textsuperscript{11}Linqvist JCI '12, \textsuperscript{12}Perreau JEM '13, \textsuperscript{13}Josefsson PNAS '13, \textsuperscript{14}Wagner Science '14, \textsuperscript{15}Malderelli Science '14, \textsuperscript{16}Cohn Cell '15, \textsuperscript{17}Fukazawa Nat Med '15
Three Histories of HIV-infected Cells In Vivo

Distinct mechanisms may reflect different reservoirs
Understanding Persistence By Sequencing

Each process may show specific HIV sequence profile.
### HIV Controllers

<table>
<thead>
<tr>
<th>Donor ID</th>
<th>Clinical Group</th>
<th>CD4 count (cells/µL)</th>
<th>Plasma HIV RNA (copies/mL)</th>
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Controllers may have a lower bar to eradication

Boritz et al. *Cell* 2016
Quantify and sequence virus in plasma and CD4 T cell subsets.
Infection Burden of CD4 T Cell Subsets

EM and TM comprise most infected cells in blood

Low and equal transcription
Clonal Sequencing of HIV in Cell Subsets and Plasma

Non-controller
VRC200-016
VL 22,240 copies/mL

Controller
SCOPE 1214
VL 45 copies/mL

Diverse
Not compartmentalized

Plasma diverse, cell-associated repetitive
Highly compartmentalized

Non-controller

Controller

Plasma
Naive
CM
EM
Controller HIV sequences less diverse than non-controller in *all* blood CD4 T cell subsets, but *not* in plasma virus.
Reduced TCR diversity due to expanded T cell clones
Are clusters of identical virus expanded infected T cell clones?
Reduced TCR diversity due to expanded T cell clones
Are clusters of identical virus expanded infected T cell clones?
Compare virus sequence from samples 3 years apart:

- Evolution of plasma virus
- No evolution of virus in clusters of identical sequence
HIV strains in blood cells are evolutionarily close to MRCA (they are old)

Strains occurring in most cells (i.e. most expanded clones) are most remote from plasma

Repeated HIV sequences in controller blood CD4 T cells have attributes of old expanded cellular clones
Virus persistence is not explained by replicative bursts

(2) Viability of Virus Sequence

Sort subsets within CM, TM and EM:
- 2 large clusters of identical virus (80%)
- Cluster 1 is profoundly hypermutated
- Cluster 2 contains fatal stop codon
• Deep sequencing of integration sites to identify expanded clones
• Match integration sites to clonal env sequences

Large clusters derive from expanded clones
Sequences from expanded clones account for majority of HIV
Causes and Consequences of Control

- Each sequence defined as repeat/singlet and whether hypermutated or not
- Non-controllers dominated by non-hypermutant singlets: ongoing replication
- Most sequences in controllers are repeats and sometimes hypermutated
- Absolute levels of hypermutant and repeated sequences are lower in controllers

Hypermutants, expanded mutated clones and repeats are not cause of virus control, they are the result of virus control
HIV in blood is predominantly contained within the EM and TM CD4 T cell subsets

HIV strains in blood CD4 T cells are distant from plasma virus and show low diversity

Large clusters of identical HIV sequences are detected especially within the EM subset

Large clusters of identical HIV sequences have the genetic attributes of expanded clones and not of replicative bursts
If large cell-associated clusters of virus are distant from plasma virus and often defective then what is the origin of diverse virions in plasma?

Can expanded infected T cells produce virus?
In Vitro Stimulation of CD4 T Cell Subsets

Stimulation #1: Virions that match plasma virus and virions that match a clonal expansion — but it’s non-infectious

Stimulation #2: Virions that match same non-infectious clonal expansion and virions that match another clonal expansion — appears viable
History of Unique Induced Viruses

Type of virus sequence
- Cell repeat, >10^2 copies/mL
- Cell repeat 10^-10 copies/mL
- Cell repeat 1-10 copies/mL
- Cell repeat 10^1-1 copies/mL
- Cell singlet
- Induced virion, no cell match

Induced virion CD4 T cell subset
- CM
- TM
- EM

**S1270, VL <40 copies/mL**
- Cell repeat, >10^2 copies/mL
- Cell repeat 10^-10 copies/mL
- Cell repeat 1-10 copies/mL
- Cell repeat 10^1-1 copies/mL
- Cell singlet
- Induced virion, no cell match

**S1495, VL 525 copies/mL**
- Cell repeat, >10^2 copies/mL
- Cell repeat 10^-10 copies/mL
- Cell repeat 1-10 copies/mL
- Cell repeat 10^1-1 copies/mL
- Cell singlet
- Induced virion, no cell match

**V912, VL 442 copies/mL**
- Cell repeat, >10^2 copies/mL
- Cell repeat 10^-10 copies/mL
- Cell repeat 1-10 copies/mL
- Cell repeat 10^1-1 copies/mL
- Cell singlet
- Induced virion, no cell match

**S1349, VL <40 copies/mL**
- Cell repeat, >10^2 copies/mL
- Cell repeat 10^-10 copies/mL
- Cell repeat 1-10 copies/mL
- Cell repeat 10^1-1 copies/mL
- Cell singlet
- Induced virion, no cell match

**S1788, VL 41 copies/mL**
- Cell repeat, >10^2 copies/mL
- Cell repeat 10^-10 copies/mL
- Cell repeat 1-10 copies/mL
- Cell repeat 10^1-1 copies/mL
- Cell singlet
- Induced virion, no cell match
History of Unique Induced Viruses

- S1270, VL <40 copies/mL
- S1495, VL 525 copies/mL
- V912, VL 442 copies/mL
- S1349, VL <40 copies/mL
- S1495, VL 525 copies/mL

**Type of virus sequence**
- **Induced virion**
- **Cell repeat, >10^2 copies/mL**
- **Cell repeat 10^1-1 copies/mL**
- **Cell single**

**Induced virion CD4 T cell subset**
- CM
- TM
- EM

**History of Unique Induced Viruses**
- S1270, VL <40 copies/mL
- S1495, VL 525 copies/mL
- S1349, VL <40 copies/mL
- V912, VL 442 copies/mL
- S1495, VL 525 copies/mL
Small population of unique inducible viruses from cells that appear to have been infected recently — where?

**History of Unique Induced Viruses**

**Type of virus sequence**
- Cell repeat, >$10^2$ copies/mL
- Cell repeat 10-10$^2$ copies/mL
- Cell repeat 1-10 copies/mL
- Cell singlet
- Induced virion, no cell match

**Induced virion CD4 T cell subset**
- CM
- TM
- EM
When a Cell is Infected with a Dead Virus

- Differentiation
- Proliferation
- Trafficking

Tissue

Blood
Sequencing Informs Pathogenesis

- Tissue
- Blood
- Trafficking
- Differentiation
- Proliferation
Can we find a *cellular* match in lymph node for *virion* sequences in plasma?
High LN HIV DNA and RNA levels especially in Tfh cells
Infected cells in blood and LN differ qualitatively
Some HIV-infected LN EM cells might arise by differentiation of HIV-infected Tfh, rather than by direct infection of EM-like cells. EM-like cells can leave the lymph node and enter circulation.
Genetic Attributes of Virus in LN CD4 T Cells

- LN virus in *all* T cell subsets has attributes of actively replicating virus:
  - proximity to plasma virus
  - abundant transcript

- Blood and LN in controllers are distinct reservoirs
Genetic Attributes of Virus in LN CD4 T Cells

$T_{FH}$ virus is largest actively replicating pool

Virus in other LN subsets is closely related to $T_{FH}$ virus
• Cells from some expanded clones of HIV-infected CD4 T cells can produce virions on restimulation

• Most HIV-infected CD4 T cells in LN have a follicular (GC Tfh and non-GC Tfh) phenotype

• There is a substantial contribution of virus from other LN CD4 T cell subsets

• HIV strains in all LN CD4 T cell subsets are closely related to plasma virus and appear to be actively replicating
Significance

• Higher levels of cell-associated HIV DNA do not equate with most relevant HIV reservoir

• Nature of different subsets of infected cells affects the maintenance and nature of that virus reservoir

• Three processes govern HIV reservoir dynamics:
  1) Long term maintenance of old strains in expanding blood T cell clones that persist even though they may produce virus
  2) Distinct upstream process of active replication predominantly, but not exclusively, in LN follicles
  3) Small population of circulating cells that inducibly produce HIV strains that resemble active LN reservoir

• Three different target populations for cure strategies
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