New developments in understanding the latent reservoir for HIV

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Disclosures: None
A stable latent reservoir for HIV

Chun et al, Nat Med 1995
Reactivation of latent HIV

Naive

Memory

Long half-life of memory cells

Proliferation driven by cytokines, Ag, or integration site related effects

Chun et al, Nat Med 1995
An assay for latently infected cells

180-200 ml blood

Purified resting CD4⁺ T cells

PHA + irradiated allogeneic PBMC

5×10^6

10^6

2×10^5

4×10^4

8×10^3

1.6×10^2

Negative control

1/1,000,000

d2: add CD4⁺ lymphoblasts from HIV-donors

d7: add CD4⁺ lymphoblasts from HIV-donors

Chun et al., Nature, 1997
Finzi et al., Science, 1997
Slow decay of latently infected CD4+ T cells

- Half-life: 44 months
- Time to eradication: > 73.4 years

Frequency (per 10^6 cells)

Time on ART (years)

Finzi et al., Nature Med., 1999
Challenges for imaging

• Low frequency of latently infected cells

• Low or absent viral gene expression in latently infected cells (<100 copies HIV-1 RNA/10^6 cells) which increases 100 fold on T cell activation

• Latently infected cells are widely distributed in lymphoid organs and other sites and are constantly recirculating
Residual viremia

Plasma HIV RNA (copies/ml)

Time on ART (days/years)

- Sensitive to current regimen
- Archival
- Non-evolving

Hermankova et al, JAMA, 2001
Kieffer et al, J Infect Dis, 2004
Nettles et al, JAMA, 2005
Effect of intensification on residual viremia

Plasma HIV RNA (copies/ml)

- Start Therapy
- Limit of Detection (50 copies/ml)
- 1 copy/ml

Time on ART (days/years)

Add 4th drug

Dinozo et al., PNAS, 2009
Effect of intensification

Mean Plasma HIV RNA (copies/ml)

ATV/r
LPV/r
EFV

Phase of study

ART
Intensification
ART

Plasma virus

Resting CD4+ T cells
Activated CD4+ T cells
Monocytes
PBMC

Time post entry (d) → 0 100 300 400 600

HXB2
SF2
JRFL
Rates of reactivation of cells in the latent reservoir

- Level of residual viremia can be used to estimate rate of reactivation of cells from the reservoir. Hill et al (PNAS 2014) estimate 57 cells/d
  - Other estimates
    - 0.17 cells/d based on time to rebound in clinical trials (Pinkevych et al, PLoS Pathogens 2016)
    - 0.33-0.70 cells/d based on rebound of bar-coded SIV variants (Fennessey et al, PLoS Pathogens 2017)
- Even with high estimates, cells exiting the reservoir are rare and likely to be widely distributed, making imaging difficult
Rates of reactivation of cells from the reservoir

- How many target molecules/cell are required for detection?
- How many infected cells/unit volume of tissue are required for detection?

Rothenberger et al, PNAS, 2015
In half of patients studied, residual viremia is dominated by a small number of clones. These sequences do not show evidence of sequence evolution. These sequences appear to represent clonal expansion of individual infected cells.
Does reservoir stability require replenishment by new infection events?

Start Therapy

Limit of Detection (50 copies/ml)
Is it possible for ART to block all new infection events?

\[
f_a/f_u = (D/IC_{50})^m
\]

- \(f_a\) = fn affected
- \(f_u\) = fn unaffected
- \(D\) = dose
- \(m\) = slope parameter (Hill coefficient)

Shen et al, Nat Med 2008
Shen et al, Sci Trans Med 2011
Sampah et al, PNAS 2011
Jilek et al, Nat Med 2012
Rosenbloom et al, Nat Med 2012
Rabi et al, JCI 2012
Linear-Log Dose Response Curve

Infection (% of control) vs Concentration/IC$_{50}$

$C_{min}$ and $C_{max}$

$m=1$, $m=1.5$, $m=2$, $m=3$, $m=5$

References:
- Shen et al, Nat Med 2008
- Shen et al, Sci Trans Med 2011
- Sampah et al, PNAS 2011
- Jilek et al, Nat Med 2012
- Rosenbloom et al, Nat Med 2012
- Rabi et al, JCI 2012
Log-Log Dose Response Curve

Infection (% of control) vs Concentration/IC₅₀

IC₅₀

C_{min} C_{max}

Shen et al, Nat Med 2008
Shen et al, Sci Trans Med 2011
Sampah et al, PNAS 2011
Jilek et al, Nat Med 2012
Rosenbloom et al, Nat Med 2012
Rabi et al, JCI 2012
Slope parameter for different classes of antiretroviral drugs

Shen et al, Nat Med 2008
Jilek et al, Nat Med 2012
Rosenbloom et al, Nat Med 2012
Laskey et al, JCI Insight 2016
Log-Log Dose Response Curve

Infection (% of control) vs Concentration/IC$_{50}$

- $C_{min}$ and $C_{max}$
- IC$_{50}$
- $m=1$, $m=1.5$, $m=2$, $m=3$, $m=5$
- NNRTI, PI
- Shen et al, Nat Med 2008
- Shen et al, Sci Trans Med 2011
- Sampah et al, PNAS 2011
- Jilek et al, Nat Med 2012
- Rosenbloom et al, Nat Med 2012
- Rabi et al, JCI 2012
Some classes of antiretroviral drugs are remarkably effective.
The best PIs can cause a 10 log (10,000,000,000 fold) reduction in a single round of infection!
The InSTIs have lower slope values but work well in combination with other drugs.
ART works well enough to stop viral replication completely – but does not target latent HIV.
Chronic Hepatitis C infection

- Continuous, high level viremia
- Rapid viral evolution
- Drug resistance with suboptimal treatment

Feld et al., NEJM, 2015
Inhibition of HCV replication by direct acting antiviral drugs

- HCV antivirals also have cooperative dose response curves and synergies that produce very high IIP
- HCV infection is readily curable
- HCV has no latent form

Koizumi et al., PNAS 2017
Persistent HIV-1 replication maintains the tissue reservoir during therapy

Ramon Lorenzo-Redondo, Helen R. Fryer, Trevor Bedford, Eun-Young Kim, John Archer, Sergei L. Kosakovsky Pond, Yoon-Seok Chung, Sudhir Penugonda, Jeffrey G. Chipman, Courtney V. Fletcher, Timothy W. Schacker, Michael H. Malim, Andrew Rambaut, Ashley T. Haase, Angela R. McLean & Steven M. Wolinsky

Time on ART (months)

Labile infected cells populations dominate early

Blankson et al, JID 1999
Rosenbloom et al., submitted
Early sampling can create the appearance of clocklike evolution

- Imaging studies of the latent reservoir should only be done after >6-8 months of suppressive ART when labile populations of infected cells have decayed.

Rosenbloom et al., submitted
Design of molecular imaging strategies requires understanding that nature of viral sequences in tissues

- Viral outgrowth
- Total HIV DNA
- Integrated HIV DNA
- Total HIV DNA
- 2 LTR circles
- Residual viremia

<table>
<thead>
<tr>
<th>Assay</th>
<th>Viral outgrowth</th>
<th>Total HIV DNA</th>
<th>Integrated HIV DNA</th>
<th>Total HIV DNA</th>
<th>2 LTR circles</th>
<th>Residual viremia</th>
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<tbody>
<tr>
<td>Cell/tissue</td>
<td>Resting CD4</td>
<td>PBMC</td>
<td>Resting CD4</td>
<td>PBMC</td>
<td>Rectal CD4</td>
<td>PBMC</td>
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<tr>
<td>Cohort</td>
<td>Chronic</td>
<td>Acute</td>
<td>Chronic</td>
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<tr>
<td>Plasma</td>
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</tbody>
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- Plasmas HIV RNA (copies/ml)
- rho = 0.19, p = 0.31
- rho = 0.07, p = 0.71

Eriksson et al, PLOS Pathogens, 2013
Non-induced proviruses

Resting CD4+ T cells

PHA + irradiated allogeneic PBMC

Are they inducible?

5x10^6 10^6 2x10^5 4x10^4 8x10^3 1.6x10^2 Negative control

full length, single genome analysis

d2: add CD4+ lymphoblasts from HIV-donors

d7: add CD4+ lymphoblasts from HIV-donors

p24 Ag

Ho et al, Cell, 2013
Landscape of HIV proviruses
Landscape of HIV proviruses

**Key:**
- Intact
- Hypermutated
- Hypermutated and deleted
- Packaging signal deletion
- Very large internal deletion
- Deletion at 3’ end of genome
- Deletion at 5’ end of genome

**ART during chronic infection**
- Intact: 36%
- Hypermutated and deleted: 21%
- Deletion at 5’ end of genome: 8%
- Deletion at 3’ end of genome: 7%
- Packaging signal deletion: 8%
- Very large internal deletion: 2%
- Missed by subgenomic PCR: 2%

**ART during acute infection**
- Intact: 22%
- Hypermutated and deleted: 26%
- Deletion at 5’ end of genome: 5%
- Deletion at 3’ end of genome: 18%
- Packaging signal deletion: 19%
- Very large internal deletion: 8%
- Missed by subgenomic PCR: 2%

**Single round of infection**
- Intact: 59%
- Hypermutated and deleted: 5%
- Deletion at 5’ end of genome: 5%
- Deletion at 3’ end of genome: 27%
- Packaging signal deletion: 4%

- Arise during (-) strand synthesis
- Not in plasma virus
- Missed by subgenomic PCR

QVOA, intact, and total proviruses

- Are they replication-competent?
- Can they be induced *in vivo*?

Ho et al Cell, 2013
Reconstructing intact non-induced proviruses

Ho et al, Cell, 2013
Replication capacity of intact non-induced proviruses

Ho et al, Cell, 2013
Can intact non-induced proviruses be induced?

Time (days)

PHA

Ho et al Cell, 2013
Hosmane et al, JEM 2017
Repetitive stimulation induces additional proviruses

Ho et al Cell, 2013
Hosmane et al, JEM 2017
QVOA, intact, and total proviruses

- Each round of stimulation induces additional proviruses
- A single round of maximal T cell activation does not induce all latent proviruses
- The number of intact proviruses provides a much more accurate upper limit on reservoir size than standard DNA PCR assays
- Imaging of cells in “deep latency” may be particularly challenging

Ho et al Cell, 2013
Bruner et al, Nat Med, 2016
Hosmane et al. JEM 2017
Expanded clones with major defects

Bruner et al, Nat Med 2016
Clonal expansion detected by integration site analysis

Maldarelli et al, Science, 2014

Wagner et al, Science, 2014
Proliferation of infected cells

• Antigen drives T cell proliferation but also induces viral gene expression. Productively infected cells die quickly.

• Cytokines like IL-7 can drive homeostatic proliferation of memory T cells, possible expanding the reservoir, but may also reverse latency.
Latently infected cells can proliferate *in vitro* without producing virus.

Expanded cellular clone
PPC

Independent isolates of replication-competent HIV with identical sequence

Hosmane et al, JEM 2017
Hypotheses to explain identical isolates

- Dominant virus population
- Point mutation
- Clonal expansion
Intrapatient genetic distances between isolates

Intrapatient genetic distances

Independent isolates with identical sequence

Closely related isolates

Subject #
P01
P02
P05
P06
P09
P10
P11
P12

Intrapatient genetic distance (relative to patient maximum)

Number of occurrences

Hosmane et al, JEM 2017
Expanded cellular clones account for the majority of the reservoir

Hosmane et al, JEM 2017
Slow decay of latently infected CD4⁺ T cells

Half-life: 44 months
Time to eradication: > 73.4 years

Finzi et al., Nature Med., 1999
Thanks

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