Viral Reservoirs, Latency, and Mechanisms of HIV Persistence

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Viral dynamics in patients on antiretroviral drugs

Plasma HIV-1 RNA (copies/ml)

Time on HAART (days)

0 100 200 300

0.001 0.01 0.1 1 10 100 1000 10000 100000

Start antiretroviral drugs

Eradication in 2-3 years

Below limit of detection (50 copies/ml)
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**Physiology of resting and activated CD4+ T cells**

- Infection of activated and resting CD4+ T cells
- Establishment and maintenance of a latent reservoir

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**NFκB sites in the HIV LTR**

U3 R U5

- Modulatory region
- Enhancer
- Core

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**Establishment and maintenance of a latent reservoir**

- Ag†††
- HIV
- Naive Memory

- Generation of latently infected cells
- Reactivation of latently infected cells

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Frequency of latently infected CD4+ T cells as a function of time on HAART

- Time on HAART (years)
- Frequency (per 10^6 resting CD4+ cells)

\[ t_{1/2} = 3.7 \text{ years} \]
\[ 73.4 \text{ years} \]

Finzi et al., Nat. Med., 1999

Residual viremia and blips in patients on HAART

- Time on HAART (days)
- Plasma HIV-1 RNA (copies/ml)

\[ \text{Limit of detection (50 copies/ml)} \]

Start HAART

J. Siliciano et al., Nat. Med., 2003

Frequency of latently infected CD4+ T cells as a function of time on HAART

- Time on HAART (years)
- Frequency (per 10^6 resting CD4+ cells)

\[ t_{1/2} = 6 \text{ months} \]
\[ 6-10 \text{ years} \]

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Dynamics of the latent pool

Persistence of archival wild type and drug resistant variants in the latent reservoir

HIV evolution

Darwin: “Survival of the fittest”
HIV: “Survival of all major forms, active replication of the viral variants that are the fittest under current conditions”
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Assay for latent HIV in viremic patients

Activated with PHA+ irradiated allogeneic PBMC in presence of RT and integrase inhibitors

Analyze virions released by limiting dilution RT-qPCR

Archived nevirapine resistance

- 30% of reservoir viruses are highly resistant to entire class of NNRTIs
- Archived NNRTI resistance seen in > 40% of mothers who received sdNVP
- NNRTIs are the key components of the only practical HAART regimens for developing countries

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Residual viremia reflects release from reservoirs. Residual viremia and blips in patients on HAART support this hypothesis: HAART stops all replication.

Residual viremia in patients on HAART:
- Viruses in the plasma should resemble viruses in the reservoir.
- There should be continuous ongoing replication.

Analysis of residual viremia in patients on HAART:
- Plasma HIV-1 RNA levels decrease over time on therapy.
- Resting CD4+ T cells are also analyzed.

RT codons associated with resistance to:
- AZT
- 3TC
- EFV

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Residual viremia and blips in patients on HAART

Start HAART

Plasma HIV-1 RNA (copies/ml)

Limit of detection for genotyping

Limit of detection (50 copies/ml)

New set point on HAART

Is resistance present? Should Rx be changed?

Blips are not related to fluctuations in drug levels

Analysis of blips with frequent sampling

Patient 113

Nettles et al., JAMA, 2005

No new mutations during blips

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Blips do not represent clinically significant elevations in viremia

- A single predominant plasma sequence represents 72.1% of all plasma sequences
- The same exact patient-specific sequence was isolated 161 times in multiple independent blood samples taken over a 3 year period
- This sequence was profoundly underrepresented among resting and activated CD4+ T cells in blood

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Why hasn’t this been seen before?

- No other group has examined plasma virus in patients with < 50 copies/ml HIV RNA
- Precautions must be taken to avoid PCR resampling which gives the false impression of a dominant sequence; Hence, all analysis done by limiting dilution PCR
- Precautions must be taken to avoid PCR errors which give the false impression of evolutionary diversity; Hence, limiting dilution PCR, direct sequencing, and error detection algorithms

The predominant plasma sequence represents a predominant plasma clone.
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Characteristics of the PPC
- PPCs seen in half of the patients studied; Others have less abundant plasma unique clones
- These clones are released into the plasma over months to years without evolution; Cannot be readily explained by continuous replication – would expect mutations to arise, at least at neutral sites
- No evidence of unique patterns of drug resistance or immune escape
- Highly underrepresented in circulating CD4+ T cells

Hypothesis on the origin of the PPCs
- A rare infection event leads to stable infection of a progenitor cell in the monocyte-macrophage lineage
- Through cell division, the integrated viral genome is copied without error and distributed into many progeny cells derived from a single progenitor cell
- These cells are distributed into the tissues as macrophages and continue to produce virus for their normal lifespan
- The existence of a 2nd major source of residual viremia in patients on HAART greatly complicates HIV eradication, particularly if renewable progenitor cell is involved

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The latent reservoir is not replenished by ongoing viral replication in patients on HAART.

Conclusions

• Eradication of HIV infection cannot be achieved with antiretroviral therapy alone because of a small pool of latently infected resting CD4+ T cells.
• The latent reservoir stores drug resistant viruses that arise in the setting of suboptimal therapy, thereby permanently limiting treatment options.
• HAART largely stops ongoing replication; the low level viremia that continues despite HAART does not require the development of new drug resistance mutations and may largely reflect release from stable reservoirs.
• There can be viremia without viral evolution, and therefore long control of viral replication is possible.
• There appears to be a second major source of low level viremia in at least some patients; this second reservoir greatly complicates the problem of eradication.