1. Infection requires CD4 protein on the surface of the cell as receptor. Therefore, it can only infect CD4+ (“helper”) T cells and a few others.
2. Almost all infected cells die within a day or two after infection.
3. Infected CD4 cells make enough virus particles to infect about the same number of new cells (10^7-10^9).
4. Therefore, the infection in an individual persists by constant, repeated cycles of infection and cell death (about 1 a day).
5. These properties are also found in the benign SIV-monkey infections, but in humans there is a slow loss of total CD4 cells, leading eventually to failure of the immune system.

1. After early primary infection, HIV gives lifelong persistent infection leading to AIDS after about 10 years (on average).
2. Persistence is due to constant replication of the virus and killing of 10^7-10^9 infected CD4+ T cells at about 1 cycle per day.
3. Smaller fractions of “latently infected” cells that live much longer after infection are probably unimportant for the natural history of the infection, but very important for failing treatment.
4. Constant replication day after day, year after year, leads to extensive genetic variation.
   • Antigenic escape.
   • Drug resistance.
   • Variation in coreceptor usage.
5. The system remains in an extraordinarily robust quasi steady state for thousands of replication cycles before progressing to disease.
6. We still don’t know how HIV causes AIDS.
**Question:** What is happening to the viremia at “undetectable” levels? Ongoing low-level replication or release from latently infected cells?

**Hypothesis:** More potent therapeutic regimens will give a lower level of viremia at maximal suppression.
Single-Copy Assay (SCA)

- Real-time RT-PCR assay to measure viral RNA levels down to a single copy in 3 ml
- 50 to 75 times more sensitive than commercial assays
- Can monitor previously "undetectable" viremia
- This assay is being used to define the origin of persistent viremia

Pailler et al. JCM 2003

Question: Is the difference in potency in the two arms of the trial reflected in the level of viremia?

Conclusion: Distribution of persistent viremia among patients is independent of PI or NNRTI Regimen. Implies that virus comes from cells infected before therapy.
**Accumulation and Loss of HIV-Infected Cells**

- **Early infection**
  - Productive infection
  - "Dead" infection

- **Chronic infection**
  - Potent regimen
  - Less potent regimen

- **Chronic infection: Suppressive Therapy**
  - Potent regimen
  - Less potent regimen

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**How Long Can Detectable Viremia Persist on Therapy?**

- The Abbott 720 trial:
  - 21 patients on suppressive LPV/r-based therapy
  - No viremia > 50 copies RNA/ ml for more than 7 years
  - ca 18 samples each analyzed by SCA

**Conclusion:** Viremia persists for more than 7 years. Longitudinal analysis reveals an additional third and fourth phase of viral decay
Probing the Mechanism of Chronic Viremia Using Antiretroviral Intensification

Intensification with Raltegravir (ACTG 5244)

- Randomized cross-over trial of RAL intensification in patients with HIV RNA <50 c/mL on currently recommended ART
- Primary objective: To compare HIV RNA level by SCA averaged between wks 10/12 in subjects who add RAL to subjects who do not add RAL to their background regimen

No Reduction in Low-Level Residual Viremia after Raltegravir Intensification
Conclusions

1. No effect of integrase on persistence was detected in either the appropriate patient group or in any individual patient in either study.

2. This result is independent of study site, treatment regimen (4 different), or antiretroviral agent (4 different).

3. The viral population with persistent replication as the source of persistent low-level viremia.

4. Thus, all indications are that persistent, low-level viremia comes from cells infected prior to the start of therapy.

5. The source of these cells remains to be determined, but the prime suspects are latency infected CD4+T cells.

Genetic Diversity of HIV-1 Populations in Infected Individuals

Within-Host Virus Evolution
Multiple Alignment File

P6, protease, RT

Env – V1V2, V3

gag-pro-pol

Sequence individual genomes

Multiple Alignment File

Palmer et. al. J. Clin Micro Jan 2005

Antiretroviral Therapy and Chronic Infection

Chronic infection is characterized by relatively stable levels of viremia comprising highly diverse virus populations for long periods of time.

- How does the virus population evolve under these conditions?

Therapy leads to profound reduction in HIV-1 RNA levels relative to on-therapy steady state viremia

- How does the genetic structure of the virus population change with time?

HIV-1 Populations Evolve within Patients Over Years of Infection

consensus
2000
2004
2007
What is the Impact of Antiretroviral Therapy (ART) on HIV-1 Genetic Diversity in Plasma?

- Does HIV-1 diversity decline as the virus load is reduced on ART?
- What is the impact of long-term ART on the diversity and structure of HIV-1 populations?
- What is the genetic resemblance of rebound virus populations compared to pretreatment virus?

No Obvious Change in Population Structure for up to 1 year on ART

No Obvious Change in Population after One Year on ART in 9/10 Subjects
What is the Impact of Antiretroviral Therapy (ART) on HIV-1 Genetic Diversity in Plasma?

- Does HIV-1 diversity decline as the viral load is reduced on ART?

- What is the impact of long-term ART on the diversity and structure of HIV-1 populations?
  - Emergence of minor viruses?

- What is the genetic relatedness of rebound virus populations compared to pre-therapy virus?
No Significant Change in HIV Diversity or Divergence of Rebound Viremia

Little Divergence in Rebound Viremia from Pre-therapy After Long-term ART

Suggested Model
**Conclusions:**
Can we Cure HIV Infection?

- HIV diversity does not decline following initiation of ART
  -indicating that low-level and long-lived cells are infected with variants from this population.

- A restricted group of HIV-1 variants (identical sequences) emerges after years of suppressive ART
  -suggests that these are active in a few chronically-infected cells or expansion of one or more chronically-infected cells.

- Rebound viremia after long-term therapy has little divergence from pre-treatment virus populations
  -suggesting that these cells were infected before therapy is the source for viral rebound.

- Long-lived cells must be further characterized and targeted to eradicate HIV-1 infection
  -suggesting that these cells may be the source for ongoing infection.

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**Elite Controllers**

- 0.5% of HIV-1 infected population spontaneous HIV-1 RNA <50 copies/ml

- Known as HIV-1 controllers/elite controllers/elite suppressors

- Low level viremia <50 copies/ml by standard assay

- Co-cultivation with PBMC yields replication competent virus

- Plasma viruses contain no gross genetic defects and replicate well in culture

- Superinfecting virus replicates to high levels
  -Ongoing replication in vivo?

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**Study Subjects**

- 21 HIV-1 controllers – Danish HIV-1 database

- 25 non controllers from an NIH cohort

- Min. 3 HIV-1 RNA <50 copies/ml within 1 year + no therapy

- Median duration of infection 11 years (IQR 7-18)

- Total of 257 plasma samples – median of 14 plasma samples available per patient

- HLA available in 16 individuals – 6/16 (38%) B*5701/27 positive
**Viremia by SCA**

Minimum median: 0.3 copies/ml  
Maximum median: 9.8 copies/ml

**Ongoing Replication?**

- SGS from 1-5 ml of plasma
- Amplification success in ~1/3 of samples
- A total of 337 single genome sequences of p6-rt and env
- SGS data on ≥2 time points in a total of 15 patients

**Time Dependent Clustering**

Rooted ML trees
Conclusions

- Evidence of evolution:
  - Increasing root-to-tip distances of rooted maximum likelihood trees
  - 3-4 fold lower rate of replication compared to non-controllers
- Suggests replication and adaptation is specific cellular immune responses but not humoral immune response in HIV-1 controllers
- Suggests that, unlike patients on ART, the virus undergoes full cycles of replication in HIV-1 controllers
- Most likely reflects an unusual host-virus relationship in which there is a CTL response against one or a few virus epitopes from which the virus cannot easily escape at great cost to its replicative fitness
- Despite their superficial similarities to patients on therapy, elite controllers are not good models for patients with drug-suppressed viremia

Acknowledgements

- NCI - Frederick HIV Drug Resistance Program
- University of Pittsburgh - J. Mellors

Acknowledgements

- NIAID/CCMD Clinic
  - C. Lane
  - J. Mican
  - R. Davey
  - M. Polis
  - J. Kovacs
  - R. Dewar
  - C. Rehm
  - J. Metcalf
Acknowledgements

Abbott Laboratories
- M. King
- G. Hanna
- S. Brun
- D. Kempf

Abbott Laboratories

NCI - Frederick HIV Drug Resistance Program

The ACTG 5244 Team
- R. Gupta
- J. Eron
- D. Margolis
- Many others

NCI - Frederick HIV Drug Resistance Program

We acknowledge with gratitude the participation of patients in these studies.