Sustained HIV Release By Persisting CD4+ T Cells During Latency Disruption

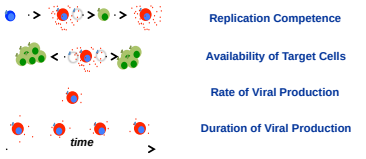
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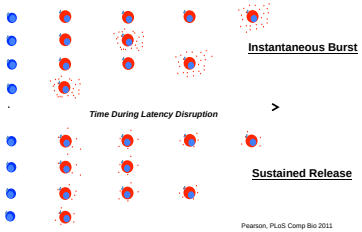
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Background.
The Ability of HIV To Establish Infection Depends On:



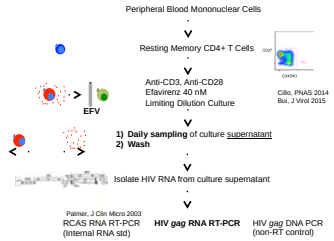
Models of Virus Production:



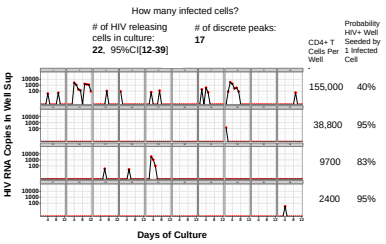
Key Questions Addressed:

1. What is the duration and rate of HIV release for CD4+ T cells during latency disruption?
2. How much total HIV is released?
3. How much cell-to-cell variability is there for these parameters?

Method.
Detecting HIV Release Dynamics:

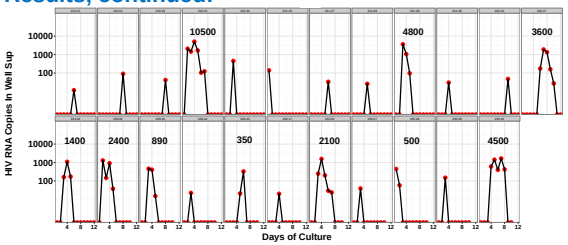


Results:



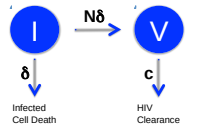
HIV RNA released into well culture supernatant versus time for one donor. HIV RNA copies in well calculated from copies in RT-PCR reaction, dilution factors, and retroviral RNA recovery. Estimate for the number of HIV releasing CD4+ T cells in culture determined by the fraction of positive wells at a given cell concentration using the method by Hu and Smyth (ELDA R Package, J Immunol Methods, 2009).

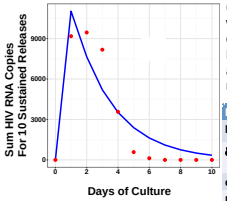
Results, continued:



Sustained release accounts for >95% of HIV RNA detected in wells at limiting dilution. The 23 positive wells from 4 donors each have a greater than 77% chance of having been seeded by a single virus releasing cell. The total detected HIV RNA copies for the sustained release wells are noted directly above the peaks.

	10 Multi-day Peaks	13 Single-day Peaks
Total HIV RNA	31082 (97%)	1108 (3%)
HIV RNA /LD well	3110	85
Median time to +	3d (1-6d)	4d (1-9d)





CD4+ T cells undergoing latency disruption at limiting dilution release 1500 HIV RNA copies /day /seeded infected cell. The sum of HIV RNA copies on each day for each of the 10 sustained release wells above is represented by the red points, with day 1 and subsequent days normalized to the first day of virus production. The blue line shows best fit of the data using a model of infected cells **I** producing **V** amount of virus (Pearson, PLoS Comp Bio 2011). Model includes a daily virus washout, as done experimentally. The HIV RNA decay constant **c** was determined by detecting the decay of released HIV RNA placed in culture with stimulated CD4+ T cells from an uninfected donor in the presence of efavirenz, to inhibit new rounds of infection.

Parameter	Description	Value
N	Total released HIV RNA	**3900** HIV RNA copies /cell
δ	Infected cell death constant	**0.39** /day
c	HIV RNA decay	**0.22** /day (by experiment)
Nδ	Release rate	**1500** HIV RNA copies /day /cell

Conclusions:

1. **>95% of detected HIV occurred as a sustained release for an average total release of 3900 HIV RNA copies per infected cell.**
2. **Average release rate of 1500 HIV RNA copies per seeded infected cell per day for a duration of 2-6 days.**
3. **Total detected HIV RNA varied from 350-10500 copies with time to first positive ranging from 1 to 6 days.**

Implications and Possibilities:

1. **Advances understanding and quantification of the HIV reservoir.**
2. **Time to virus eradication** in treatment interruption models depends on *infected cell frequency* and *half-life*, but also ***virus release*** and ***cell-to-cell variability in release***. Conway PLoS Comp Bio 2011, Hill PNAS 2014, Rouzine PNAS 2014
3. **Sustained release opens the possibility that spreading infection can be eliminated by rapid immune recognition of an infected cell at the very beginning of the release process.**

