Multiple Rounds of Activation Induce Replication-Competent HIV-1 from Resting Memory CD4+ T Cells in the Latent Reservoir

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INTRODUCTION

HIV-1 establishes latency in resting memory CD4+ cells, which are the major barrier to eradication of the disease. The quantitative viral outgrowth assay (Q-VOA) is a valuable culture assay which can detect individual latently infected cells. No other assay has given a higher frequency of cells containing replication-competent virus. In this assay, resting CD4+ T cells are isolated from ART-suppressed patients and are subjected to a single round of maximum activation with phytohemagglutinin (PHA) and allogeneic, irradiated PBMCs from uninfected donors. We have recently shown that the size of the latent reservoir (LR) may be significantly larger than has been previously measured with the Q-VOA. A significant fraction of non-induced proviruses from p24-negative wells of the Q-VOA are intact. We hypothesized that multiple rounds of maximum T cell activation might induce additional outgrowth of these intact, non-induced proviruses.

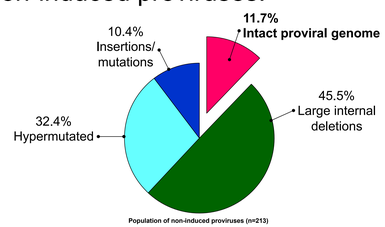
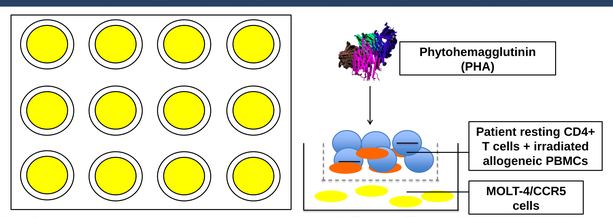


Image from Ho, et al., *Cell*. 2013.

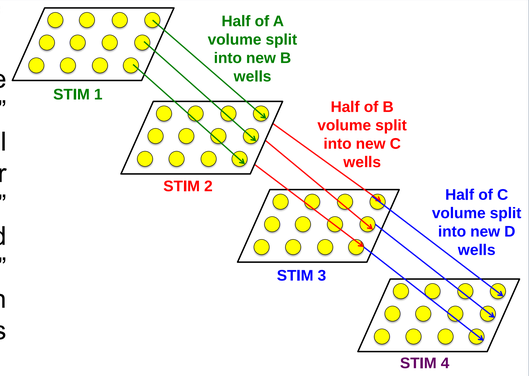
MATERIALS AND METHODS

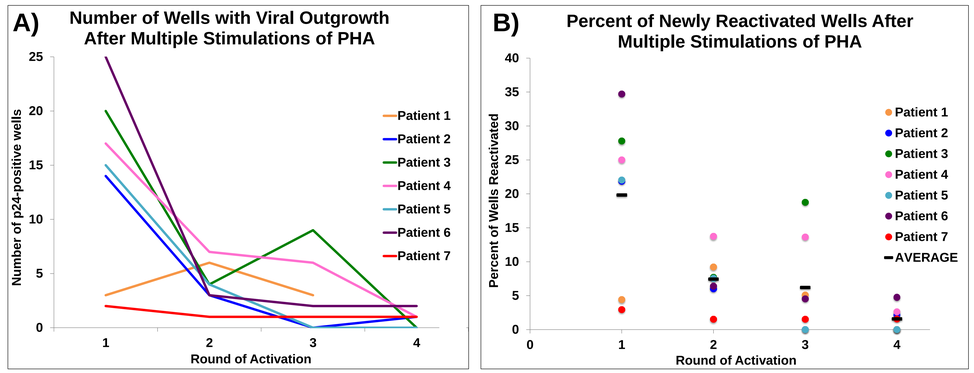


200,000 patient resting memory CD4+ T cells were plated per well in 12-well transwell plates that contained a 0.4 micrometer membrane pore separating upper and lower chambers. The upper chamber contained patient resting CD4+ T cells combined with irradiated healthy donor allogeneic PBMCs. PHA was added at a final concentration of 0.5 µg/mL per well and removed 24 hours later. Each experiment involved 64-72 replicate wells.

RESULTS

Figure 1. Scheme for Multiple Rounds of Maximum T Cell Activation.
After 8 days of culture with PHA, half of the volume from both chambers in each "A" transwell was split into a new "B" transwell for a second round of PHA stimulation. After another 8 days, half the volume from the "B" transwell was split again into a third replicate "C" transwell and further into a "D" transwell after 8 more days. Viral outgrowth was measured by p24 ELISA 21 days following each respective PHA stimulation.





C)

	STIM 1	STIM 2	STIM 3	STIM 4	Total Wells Stimulated	STIM 1 IUPM	Final IUPM	Started ART
Patient 1 (M)	3	6	3	N/A	12/68	.225	0.97	1996
Patient 2 (F)	14	3	0	1	18/64	1.234	1.651	2005
Patient 3 (F)	20	4	9	0	33/72	1.627	2.938	2004
Patient 4 (F)	17	7	6	1	31/68	1.438	3.042	2003
Patient 5 (F)	15	4	0	0	19/68	1.246	1.638	2003
Patient 6 (M)	25	3	2	2	32/72	2.132	2.938	2003
Patient 7 (M)	2	1	1	1	5/68	0.149	.381	1999

Figure 2. Multiple rounds of PHA induce additional replication-competent viruses.
Resting CD4+ T cells from 7 ART-suppressed patients were analyzed. A p24 ELISA was used 21 days following each PHA addition to determine additional viral outgrowth. (**A**) Raw number of p24-positive wells after each round of activation, (**B**) Percentage of newly reactivated p24-positive wells following each PHA stimulation. Black bar is the average from all seven patients at each stimulation. (**C**) Summary of results seen in (**A**) and (**B**).

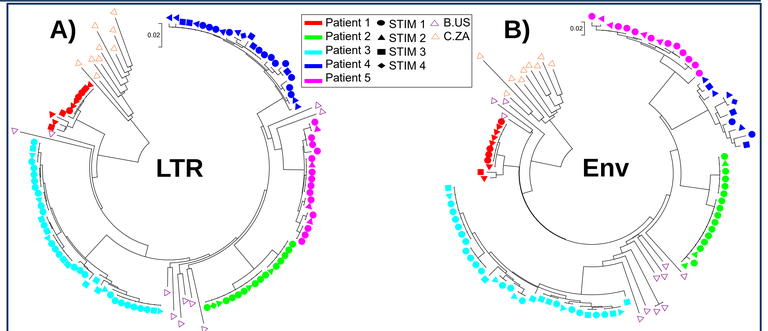


Figure 3. Phylogenetic analysis of LTR and Env from replication-competent viruses after multiple rounds of activation.
A Neighbor-Joining tree was generated for phylogenetic analysis of all viruses collected from Patients 1-5. RNA in the supernatant of p24-positive wells was collected, converted to cDNA, and sequenced. In **(A)** a 500-bp region of LTR, including the U3 and part of R, was sequenced in addition to **(B),** a 500-bp region of Env that included the V3 and V4 variable loops.

CONCLUSIONS

Multiple rounds of maximum T cell activation induced the outgrowth of additional replication-competent viruses from resting CD4+ T cells in ART-suppressed patients. Phylogenetic analysis of LTR and Env from all replication-competent viruses showed no correlation of sequence with rounds of stimulation. These results indicate the possibility of stochastic reactivation of latently infected resting memory CD4+ T cells *in vivo*.

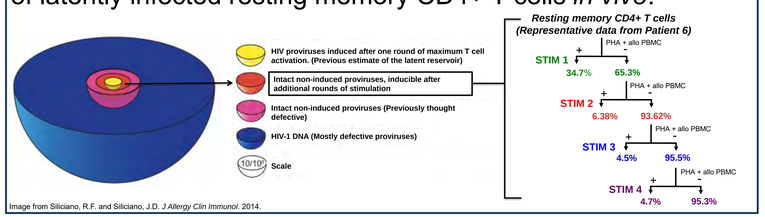


Image from Siliciano, R.F. and Siliciano, J.D. *J Allergy Clin Immunol*. 2014.

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