Stable total HIV-1 DNA levels prior and post ART interruption in chronic HIV

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Abstract

Background: The persistence of the HIV reservoir during antiretroviral therapy (ART) is a barrier to HIV eradication. The impact of viremic episodes on cell-associated HIV DNA or RNA in the context of ART-mediated suppression remains unknown.

Methods: Peripheral blood mononuclear cells (PBMC) from 23 ART-suppressed, chronically HIV-1-infected subjects were evaluated at initiation of treatment interruption (TI, baseline), during HIV viremia soon after TI, and following ART-mediated HIV viral load (VL) re-suppression for: a) T cell activation [CD38, HLA-DR, CD28, CD95 and TNFRII expression on $CD4^+$ or $CD8^+$ T cells] by flow cytometry, and b) viral measures including: i) cell-associated HIV DNA [total HIV DNA (pol copies) and episomal 2-long terminal repeat (2-LTR) circles] by droplet digital PCR (ddPCR), and ii) cell-associated HIV unspliced (gag), multi spliced RNA (tat/rev) and poly-A tailed transcripts (PolyA), by reverse transcriptase-ddPCR. Differences between time points were tested using Wilcoxon Signed-Rank or paired t-tests. Correlations were assessed using Spearman tests. All statistics used JMP Pro11.

Results: The median HIV VL during TI [median time=4 weeks, interquartile range (IQR)=4-9] was 72900 copies/ml (IQR=32558-127193). When compared to pre-TI levels, TI resulted in a decrease in $CD4^+$ T cells/mm^3 ($p<0.0001$), increase in T cell activation [e.g. $CD8^+CD38^+$ percent $p=0.0005$], and increase in cell-associated HIV DNA (pol $p<0.0001$, 2-LTR $p=0.0362$) and RNA (gag $p<0.0001$, tat/rev $p<0.0001$, PolyA $p<0.0001$). Upon resumption of ART, HIV re-suppression occurred after a median of 16 weeks (IQR=12-22) and resulted in restoration of $CD4^+$ T cells/mm^3, reduction of T cell activation, and return of total HIV DNA and cell-associated HIV RNA to pre-TI levels. However, levels of activated (e.g. $HLA\text{-}DR^+$) $CD4^+$ T cells and 2-LTR circles remained higher than pre-TI levels ($p=0.0171$ and $p=0.0323$, respectively) even after viral re-suppression. Pre-TI mean fluorescent intensity of CD38 on $CD4^+HLA\text{-}DR^+$ T cells was positively associated with total HIV DNA levels observed during TI ($p=0.034$, Rho=0.4437) and after viral re-suppression ($p=0.0289$, Rho=0.4658).

Conclusions: ART-mediated HIV VL re-suppression restored total HIV DNA to pre-TI levels, but retained higher 2-LTR levels. In addition, pre-TI T cell activation levels were positively correlated with total HIV DNA levels observed during TI and following viral re-suppression.

Introduction

Antiretroviral therapy (ART) reduces HIV viremia to undetectable levels, yet the persistence of ART resistant reservoirs[1,2] despite undetectable plasma HIV viral load (VL) is a major barrier to HIV eradication. As a result, there is a clear need to identify strategies that could maximize reservoir clearance. In ART-suppressed chronically HIV-1-infected subjects, we investigated whether a viremic episode after treatment interruption (TI) could alter cell-associated viral measures when comparing time-points with suppressive ART before and after TI.

Hypothesis

A viremic episode as a result of TI would result in an increase of cell-associated HIV load in spite of a subsequent ART resumption and HIV VL re-suppression.

Subjects, Materials & Methods

Study subjects

A total of 23 ART-suppressed, chronically HIV-1 infected subjects were evaluated at baseline of TI while on ART, at HIV viremia during TI, and following ART-mediated HIV VL re-suppression. Study subject characteristics are shown in Section 1 .

Methods

Cryo-preserved PBMC were evaluated for: a) T cell activation [frequency (%) and mean fluorescence intensity (MFI) of CD38, HLA-DR, CD28, CD95 and TNFRII expression on $CD3^+$, $CD4^+$, $CD8^+$ T cells] by flow cytometry[3,4], and b) viral measures including: i) cell-associated HIV DNA [total HIV DNA (pol copies) and episomal 2-long terminal repeat (2-LTR) circles] by droplet digital PCR (ddPCR)[5], and ii) cell-associated HIV unspliced (gag), multi spliced RNA (tat/rev) and poly-A tailed transcripts (PolyA), by reverse transcriptase-ddPCR.

Statistical analysis

Immune phenotypes were described as medians, 25^{th} and 75^{th} percentiles. Differences between time points were compared by Wilcoxon Signed-Rank or paired t-tests depending on data distribution. Correlations between T cell activation and viral measures were assessed using Spearman tests based on data distribution. P-values <0.05 were considered statistically significant. All statistics were performed with JMP Pro11 (SAS Institute, Cary, NC). Results are shown in Sections 2-4.

1 Patient Characteristics

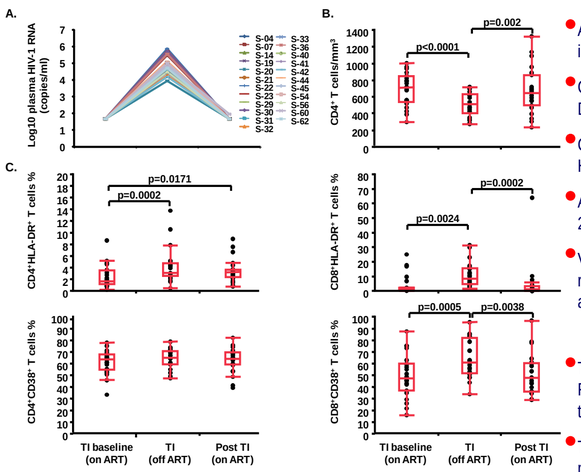
Subject ID	Age	Sex	Racial group	Number of previous TIs* (duration in weeks)	Study TI* duration (weeks)	HIV VL** at study TI* start (on ART***) (copies/ml)	HIV VL** at study TI* (off ART***) (copies/ml)	HIV VL** post study TI* (on ART***) (copies/ml)	Post study TI* duration until HIV VL** re-suppression (weeks)
S-04	38	Male	White	1 (2)	4	<50	72900	<50	16
S-07	59	Male	White	1 (2)	4	<50	317984	<50	16
S-14	29	Male	White	0	16	<50	55430	<50	29
S-19	39	Male	Black	3 (2, 4, 6 each)	12	<50	28981	<50	17
S-20	23	Male	Black	0	28	<50	8970	<50	7
S-21	39	Male	White	0	9	<50	37372	<50	15
S-22	49	Male	White	1 (2)	4	<50	750000	<50	22
S-23	47	Male	White	1 (2)	4	<50	556372	<50	20
S-29	45	Male	Black	0	4	<50	71012	<50	26
S-30	45	Male	White	2 (2, 4 each)	4	<50	517473	<50	27
S-31	45	Male	White	3 (2, 4, 6 each)	8	<50	32558	<50	12
S-32	41	Male	White	0	9	<50	18034	<50	13
S-33	49	Female	Black	0	4	<50	73066	<50	17
S-36	40	Male	White	0	5	<50	363774	<50	13
S-40	55	Male	White	0	5	<50	94489	<50	30
S-41	54	Male	White	3 (2, 4, 6 each)	8	<50	124747	<50	8
S-42	41	Male	White	1 (2)	4	<50	16083	<50	12
S-44	43	Male	White	1 (2)	4	<50	127193	<50	16
S-45	39	Male	White	1 (2)	4	<50	67248	<50	12
S-54	42	Male	White	1 (2)	4	<50	116243	<50	22
S-56	41	Male	White	1 (2)	4	<50	31377	<50	17
S-60	50	Male	White	0	5	<50	95361	95	7
S-62	48	Male	Black	0	9	<50	50715	<50	15

*TI: treatment interruption

**HIV VL: Plasma HIV-1 RNA measured at specified time point (i.e. on ART: prior or post study TI; off ART: at the end of study TI duration)

***ART: Antiretroviral therapy

2 Changes in HIV VL, $CD4^+$ T cell count and T cell activation during TI and Following ART-mediated HIV VL Re-suppression



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Conclusions

- As expected, viremia during TI (median duration=4 weeks, IQR=4-9) results in an increase in T cell activation and decrease in $CD4^+$ T cell count.
- $CD4^+$ T cell activation at baseline of TI associates positively with cell-associated HIV DNA during TI and following HIV VL re-suppression.
- $CD4^+$ and $CD8^+$ T cell activation during TI associate positively with cell-associated HIV DNA and RNA during TI.
- ART resumption and HIV VL re-suppression after a median of 16 weeks (IQR=12-22) do not reverse elevated $CD4^+HLA\text{-}DR^+$ following TI.
- Viremia during TI increases all measures of cell-associated HIV DNA and RNA. ART resumption and HIV VL re-suppression result in restoration of initial levels of cell-associated HIV DNA and RNA measures with the exception of 2-LTRs.

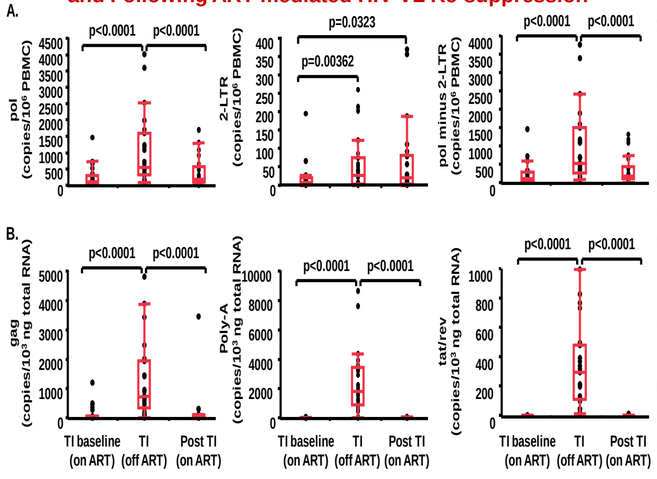
Interpretation

- There is no evidence of detectable change in cell-associated total HIV DNA and RNA upon ART-mediated HIV VL re-suppression after TI in subjects who initiated treatment during chronic infection.
- The persistence of the TI-mediated increase in 2-LTR levels in spite of ART-mediated HIV VL re-suppression suggests a delay in the decay kinetics for this measure (or retention of low level viral replication) in the presence of retained $CD4^+$ T cell activation.

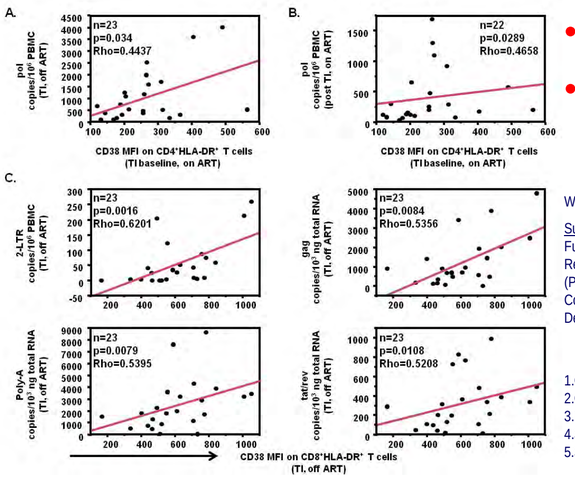
Future Direction

- Measurement of cell-associated integrated HIV DNA, to corroborate the cell-associated total HIV DNA measurements.
- Assessment of the effect of a longer period of ART-mediated HIV VL re-suppression on cell-associated HIV load, to determine whether the persistence of TI-mediated increase in 2-LTR levels reflects a delay in the decay kinetics for this measure.

3 Changes in cell-associated HIV DNA and RNA during TI and Following ART-mediated HIV VL Re-suppression



4 Association between T cell Activation and cell-associated HIV DNA and RNA



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