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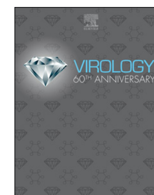

Associations between HIV-1 DNA copy number, proviral transcriptional activity, and plasma viremia in individuals off or on suppressive antiretroviral therapy

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ABSTRACT

The relationships between HIV-1 DNA copy number, proviral transcriptional activity, and residual plasma viremia in individuals off and on ART are not well defined. To address this, we performed a cross-sectional study of 12 viremic donors and 23 ART-treated virologically suppressed (plasma HIV-1 RNA < 20 copies/ml) donors. We report a strong association between HIV-1 DNA copy number and HIV-1 transcriptional activity in blood that persists on suppressive ART, but not between transcriptional activity and the levels of persistent viremia on ART. The latter finding contrasts with that in viremic donors and suggests that most HIV transcription in donors on suppressive ART does not result in virion production. This uncoupling of proviral transcription and viremia warrants closer investigation.

1. Introduction

Although HIV-1 replication is suppressed during combination antiretroviral therapy (ART), replication-competent HIV-1 proviruses persist in latently-infected CD4$^+$ T-cells (CD4), and their remarkably long half-life of 44 months prevents a “sterilizing” cure from ART alone (Finzi et al., 1997; Siliciano et al., 2003). In addition, various forms of cell-associated (CA) HIV-1 RNA continue to be synthesized during ART (Chun et al., 2003; Fischer et al., 2000; Lassen et al., 2004), and ultrasensitive PCR assays can detect HIV-1 virions in the plasma (Maldarelli et al., 2007; Palmer et al., 2008), indicating that HIV-1 proviral transcription and small amounts of virion production persist on ART.

With the growing interest in controlling or eliminating HIV-1 reservoirs, plasma HIV-1 RNA, along with total HIV-1 DNA and unspliced RNA, have been used as virologic markers of HIV-1 persistence to assess the therapeutic effect of new pharmacological and immunological approaches to achieve a functional cure of HIV-1 (Archin et al., 2014; Elliot et al., 2014; Rasmussen et al., 2014). Molecular markers offer reliable and inexpensive ways to monitor changes in the number and transcriptional activity of HIV-1-infected cells. This approach is potentially valuable because the gold standard of measuring the HIV-1 latent reservoir – the quantitative viral outgrowth assay (QVOA) – is expensive and time consuming, and other approaches to improving or replacing QVOA still require *ex vivo* cell culture (Laird et al., 2013; Procopio et al., 2015). Molecular markers of HIV-1 have also been evaluated extensively, and a number of observational studies have characterized the dynamics of their decay as a result of ART (Besson et al., 2014; Furtado et al., 1999; Koelsch et al., 2008; Palmer et al., 2008). Although some researchers have questioned the value of molecular markers because they do not correlate with the latent reservoir measured by QVOA (Eriksson et al., 2013), others have suggested that HIV-1 DNA and RNA could provide important information on the time to virological relapse after the interruption of ART (Li et al., 2016; Pasternak et al., 2009; Sneller et al., 2017; Williams et al., 2014).

HIV-1 DNA, unspliced RNA and plasma viral RNA represent three distinct stages in the lifecycle of HIV-1 replication, i.e., infection of CD4$^+$ T-cells, transcription of proviruses and production of virions, respectively. It is therefore not surprising that these molecular markers are correlated during untreated viremia (Furtado et al., 1999). However, it is still unclear how suppressive ART alters these correlations. To explore their relationships on ART, we conducted a cross-sectional study of molecular markers in virologically suppressed donors on stable ART and compared them to viremic donors off ART. Although previous studies have explored associations between HIV-1 DNA and CA HIV-1 RNA (Malatinkova et al., 2015; Procopio et al., 2015), HIV-1 DNA and

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Table 1
Baseline characteristics.

		Total N = 35	GROUP Viremic N = 12	Suppressed N = 23
Age	Min, Max	24, 66	24, 58	26, 66
	Median (Q1, Q3)	50 (44, 54)	49 (35, 53)	51 (45, 57)
Sex	M	28 (80%)	11 (92%)	17 (74%)
	F	7 (20%)	1 (8%)	6 (26%)
Race	African American	22 (63%)	10 (83%)	12 (52%)
	Caucasian	12 (34%)	1 (8%)	11 (48%)
	Hispanic	1 (3%)	1 (8%)	0 (0%)
Nadir CD4 cell count (cells/mm^3)[a]	N			21
	Min, Max			23, 578
	Median (Q1, Q3)			227 (89, 314)
ART Duration at time of screening (years)	< 4			9 (39%)
	> 4			11 (48%)
	missing			3 (13%)
Pre-ART HIV-1 RNA level (log$_{10}$ (copies/ml)[a]	N			20
	Min, Max			3.7, 6.7
	Median (Q1, Q3)			5.0 (4.3, 5.1)
Pre-ART HIV-1 RNA level (copies/ml)[a]	≤ 10 K			2 (9%)
	10–100 K			11 (48%)
	≥ 100 K			7 (30%)
	missing			3 (13%)
Screening CD4 cell count (cells/mm^3)	N	35	12	23
	Min, Max	17, 1667	17, 1053	386, 1667
	Median (Q1, Q3)	628 (392, 835)	240 (102, 810)	642 (486, 835)
Screening CD4 cell count (%)	N	33	11	22
	Min, Max	3, 55	3, 45	24, 55
	Median (Q1, Q3)	32 (25, 40)	17 (8, 31)	35 (30, 43)

[a] Data not available for the Viremic group.

plasma viremia (Chun et al., 2011; Mexas et al., 2012), or CA HIV-1 RNA and plasma viremia (Procopio et al., 2015), the current study examines each of these correlations within the same set of samples; and importantly, compares viremic patients to those suppressed on ART.

2. Materials and methods

2.1. Clinical specimens

The study was approved by the University of Pittsburgh Institutional Review Board. Study participants were recruited from the University of Pittsburgh AIDS Center for Treatment and provided written informed consent. Two groups of participants were enrolled: (1) viremic donors with plasma HIV-1 RNA > 1000 copies/ml (Roche COBAS AmpliPrep/COBAS TaqMan, v2.0) who were not currently receiving ART (some participants had a history of ART exposure); and (2) virologically suppressed donors who had been on stable suppressive ART for at least 6 months with plasma HIV-1 RNA < 20 copies/ml (Roche). In a subset of donors, samples were collected at two timepoints (5 of 12 viremic donors and 19 of 23 virologically suppressed donors).

Sample specimens were collected through either large-volume phlebotomy (100–180 ml) or leukapheresis, and then processed to peripheral blood mononuclear cells (PBMC) by Ficoll-Paque density gradient centrifugation (Sigma-Aldrich, USA) within 4 h of collection. The cells were cryopreserved in 5–10 million aliquots and stored in liquid nitrogen before analysis. Plasma was harvested from whole blood by double centrifugation (400 g × 10 min, followed by 1350 g × 15 min) and stored at −80 °C before analysis.

2.2. Isolation and quantification of HIV-1 DNA and unspliced HIV-1 RNA

Total HIV-1 DNA and unspliced HIV-1 RNA levels in PBMC were quantified as reported (Hong et al., 2016b). Briefly, total nucleic acid (TNA) was isolated from 2.5 million PBMCs using Proteinase K/Guanidinium HCl/Guanidinium Thiocyanate lysis and isopropanol precipitation. TNA was split and one half used for HIV-1 DNA quantitation in triplicate by qPCR and one half treated with Dnase and then used for CA-HIV-1 RNA quantitation in triplicate by RT-qPCR. The target for both assays is the 3' end of *pol*. The limit of detection for both HIV-1 DNA and RNA was one copy per reaction, as determined by limiting dilution analysis of DNA and RNA standards. Nucleic acid input of 700 (DNA) or 300 (RNA) ng was used for each qPCR (DNA) or RT followed by qPCR (RNA) reaction. The number of cell equivalents was estimated by qPCR targeting CCR5 according to a published protocol (Malnati et al., 2008), and was used to normalize HIV-1 DNA and unspliced RNA per million cells. The results were further normalized, as appropriate, by the percentage of CD4$^+$ T-cells, i.e., HIV-1 DNA copies/million CD4$^+$ T-cells was calculated by dividing HIV-1 DNA copies/million cells by CD4$^+$ T-cell percent. For those donors from which samples were obtained at two timepoints (as above), the mean values from duplicate assays are reported.

2.3. Quantification of plasma HIV-1 RNA

Plasma HIV-1 RNA was quantified with single copy sensitivity (single copy assay; SCA) using qRT-PCR targeting a highly conserved region in *pol* (Cillo et al., 2014). The limit of detection for HIV-1 RNA was one copy per reaction as determined by limiting dilution analysis of HIV-1 transcripts. For those donors from which samples were obtained at two timepoints (as above), the mean values from duplicate assays are reported.

2.4. Flow cytometric analysis

CD4$^+$ T-cell percentage in PBMC was determined by flow cytometry either by clinical assay or in-house. Briefly, cells were fixed using BD Cytofix buffer and analyzed for surface expression of T-cell markers using CD3-V450, CD4-APC-H7 and CD8-PE using an LSRII cytometer with FACSDiva software (BD Biosciences). All antibodies were obtained from BD Biosciences.

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