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Biomarkers of the Latent Reservoir of HIV-1 in Patients on Suppressive Antiretroviral Therapy

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Abstract

Background: Simple biomarkers of the inducible HIV reservoir have not been identified. We investigated whether the frequency of infected cells in blood and their transcriptional activity is related to the inducible HIV reservoir from resting CD4+ T cells in persons on virologically-suppressive antiretroviral therapy (ART).
Methods: Total blood mononuclear cells (PBMC) were isolated from leukapheresis product obtained from volunteers on suppressive ART for ≥1 year. Cellular unspliced HIV-1 RNA (CA-RNA) and proviral HIV-1 DNA (CA-DNA) in uncultured PBMC were quantified by qPCR assays targeting 3' integrase. Resting CD4+ T (rCD4) cells were isolated from PBMC by negative selection, and were then activated with PMA/ionomycin in the presence of 300 nM efavirenz. On day 6 of culture, supernatants were collected from replicate wells, centrifuged (500 x g for 5 min), and stored at -80°C. HIV-1 RNA in supernatants (surrogate for virion production) was quantified by COBAS Roche AmpliPrep/TaqMan v2.0. Correlations between variables were assessed using Spearman's correlation, with a two-side type I error rate of 5%.
Results: A total of 21 donors were evaluated. Levels of HIV-1 RNA produced after 6 days of treatment with PMA/ionomycin varied >1000 fold among the donors; the median was 4447 copies/mL of culture supernatant, ranging from of 38 to 42756 copies/mL. The median level of CA-RNA was 37 (range: <1 to 355) copies per 10^6 PBMC, and the median level of CA-DNA was 283 (range: 7 to 2972) copies per 10^6 PBMC. CA HIV-1 RNA and DNA levels were strongly correlated with each other (rho=0.80,p<0.001), and both were strongly correlated with inducible virion production (rho=0.76, p<0.001 for CA-RNA; rho=0.75, p<0.001 for CA-DNA).
Conclusions: Inducible virion production from resting CD4+ T cells *ex vivo* is strongly associated with the number of HIV-infected cells and the basal level of HIV-1 RNA transcription in isolated PBMC. These data indicate that simple, high-throughput and relatively inexpensive measures of cellular HIV-1 RNA and DNA can be used to estimate the size of the inducible reservoir in patients on suppressive ART. These approaches could also be useful in assessing the effectiveness of interventions targeting the latent HIV reservoir.

Introduction

- **Biomarkers of the latent reservoir in patients on suppressive ART are critically needed to evaluate *in vivo* therapeutics**
- **Predictors of the infectious reservoir[1-2] and time to rebound[3-4] have been proposed, but simple surrogates has been elusive**

Objectives

- **Quantify molecular biomarkers of HIV-1 persistence in samples obtained from peripheral blood**
- **Investigate relationships between molecular biomarkers and culture based spontanoues/inducible virion production**
- **Evaluate relationship between variables**

Materials and Methods

Study Population and Entry Criteria: Consecutive HIV-1 positive participants on suppressive ART for ≥1 year from Quest Clinical Research were selected as volunteers to undergo simultaneous plasma- and leuka-pheresis. Participants were anonymized prior to distribution of samples.
Isolation and culture of rCD4 cells: Plasma obtain from plasmapheresis was spun at 500xg for 10 minutes, and the supernatant was removed and spun a second time at 1350xg for 15 minutes and was then stored for downstream large volume integrase single copy assay (iSCA) analysis as previously described[5]. Peripheral blood mononuclear cells (PBMC) were separated by Ficoll-hypaque density gradient centrifugation. PBMC were cryopreserved for qRT-PCR quantification of cellular HIV-1 DNA and RNA[4]. rCD4 cells were then isolated by negative selection using a depletion cocktail (Stemcell Technologies). PBMC and rCD4 cells were cultured for 7 days with 50 ng/mL phorbol 12-myristate 13-acetate (PMA) and 500 ng/mL ionomycin or medium alone in the presence of 100 nM efavirenz/100 nM elvitegravir. After 7 days, supernatants were removed, spun at 500xg for 10 mins, and HIV-1 RNA levels were quantified by Roche COBAS AmpliPrep/TaqMan v2.0.
Molecular assays of viral persistence: Low-level viremia was quantified as previously described using two-step qRT-PCR iSCA[5]. Cellular HIV-1 RNA was quantified from PBMC by two-step qRT-PCR, and cellular HIV-1 DNA was quantified by qPCR as previously described[6].
Statistical Analyses: Spearman's correlation was used to evaluate relationships between levels of virion production from cultures and molecular measures of HIV-1 persistence. A generalized linear model was constructed to evaluate predictors of the inducible reservoir. All figures were generated in R using ggplot2, and all statistical analyses were performed using R.

Results

Table 1. Study participant characteristics

Participant	Age	CD4 count (cells/mm3)	CD4%	Mega-iSCA (cps/mL)	CA HIV-DNA (cps/10^6 PBMC)	CA HIV-RNA (cps/10^6 PBMC)	ART Regimen
1	69	742	41.2	1.1	523	74	EFV/TDF/FTC/RAL
2	54	653	36.3	<0.05	172	82	Unknown
3	57	587	36.7	1.2	245	40	3TC/ABC/DTG
4	55	762	23.8	1.7	192	3	DRV/r/3TC/ABC
5	51	692	17.3	6	2972	355	DRV/r/TDF/FTC/ RAL/MVC
6	54	607	26.4	2.3	283	37	EFV/TDF/FTC
7	61	1022	29.2	0.81	1061	92	Unknown
8	58	632	23.4	11.4	636	177	ETV/MVC
9	50	1345	26.9	6.34	80	35	DRV/LPV/r/3TC/ABC/ ZDV/DTG
10	54	590	26.8	<0.74	611	53	Unknown
11	52	422	28.1	<0.46	78	4	ATV/r/TDF/FTC/DTG
12	53	958	38.3	ND	504	90	ETV/TDF/FTC/RAL/MVC
13	52	832	39.6	2.8	211	21	ATV/LPV/r/3TC/ABC
14	64	725	48.3	0.67	453	70	Unknown
15	64	538	33.6	<0.087	31	15	LPV/r/TDF/3TC/ABC/ ZDV
16	39	556	39.7	ND	160	28	EFV/TDF/FTC
17	56	882	42	ND	7	<1	TDF/FTC/RAL/ BMS663068
18	54	699	36.8	ND	366	13	ATV/TDF/3TC
19	57	780	39	ND	381	39	Unknown
20	47	796	36.2	ND	289	11	DRV/r/DTG
21	62	783	43.5	ND	91	2	TDF/FTC/RAL
Median (IQR)	54 (52-60)	725 (599-814)	36 (27-40)	1.2 (0.18-3.6)	283 (126-514)	37 (12-78)	N/A

ND = Not done; N/A = Not applicable

Figure 1. Correlogram showing relationships between the 11 variables studied

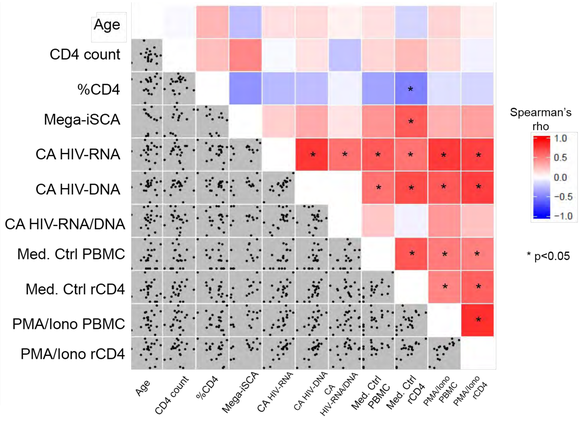
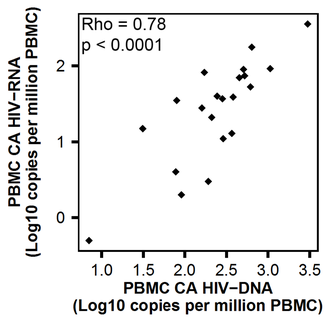
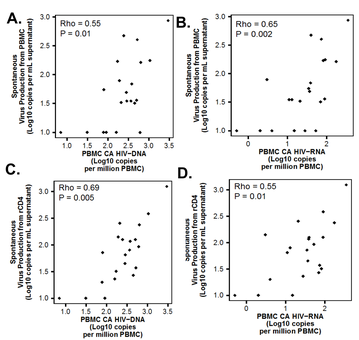


Figure 2. Cellular HIV-1 RNA and proviral DNA in peripheral blood mononuclear cells are positively correlated



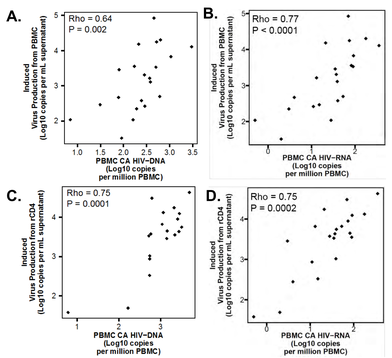
Levels of cellular HIV-1 RNA (CA HIV-RNA) and proviral HIV-1 DNA (CA HIV-DNA) in PBMC are strongly correlated in a sample of 21 donors on suppressive ART, indicating that the frequency of infected cells is associated with the transcriptional activity of the infected cells.

Figure 3. Relationships between cellular HIV-1 DNA and RNA and levels of virions spontaneously released from cultured cells



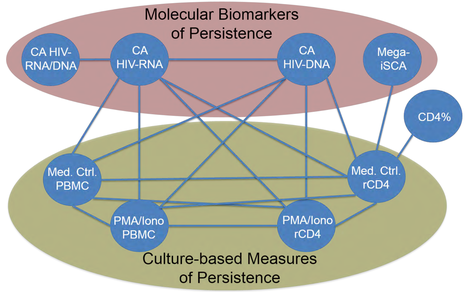
Spontaneous virus production from unstimulated PBMC (A. and B.) and rCD4 cells (C. and D.) in culture is related to both the frequency of HIV-1 infected cells (CA HIV-DNA), and the levels of HIV-1 RNA transcription (CA HIV-RNA) within PBMC.

Figure 4. Relationships between cellular HIV-1 DNA and RNA and levels of virions produced from PMA/Ionomycin treated cells



Inducible virus production from PBMC (A. and B.) and rCD4 cells (C. and D.) treated with PMA/Ionomycin in culture is related to both the frequency of HIV-1 infected cells (CA HIV-DNA), and the levels of HIV-1 RNA transcription (CA HIV-RNA) within PBMC.

Figure 5. Interaction network between molecular biomarkers of persistence and culture-based measures of persistence



Interaction network showing variables as nodes, and statistically significant Spearman's correlations as connections between the nodes. Connections between molecular biomarkers of persistence and culture-based measures of persistence are clearly evident.

Conclusions

- **Cellular HIV-1 RNA and proviral DNA in PBMC are strongly correlated**
- **Spontaneous virion production from PBMC and rCD4 cells is positively associated with levels of cellular HIV-1 RNA and DNA in PBMC**
- **Cellular HIV-1 RNA and proviral DNA in PBMC are strongly correlated with inducible virion production from PBMC and rCD4 cells**
- **Molecular biomarkers of persistence, and culture-based measures of virion production are interrelated**
- **Simple measures of the frequency of infected cells and their transcriptional activity are related to levels of the spontaneous and inducible virion production**

References

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