DISTINCT HIV RESERVOIR MEASURES CORRELATE WITH INTACT AND DEFECTIVE PRO-VIRAL DNA**

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Abstract

Background: A major priority for HIV cure strategies remains how best to measure persistence of HIV despite suppressive antiretroviral therapy (ART) in chronic HIV infection. Several assays have been developed to measure the HIV reservoir. We assessed the association between six distinct HIV measures on ART (intact and defective pro-viral DNA, total HIV DNA, integrated HIV DNA, integrated HIV Gag and Pol, and inducible RNA or p24).

Methods: Peripheral blood mononuclear cells (PBMC) from 64 HIV+ subjects chronically suppressed on ART at <50 HIV-1 copies/ml were assessed for a) intact and 3' or 5' deleted and/or hypermutated HIV DNA by IPDA (Accelevir), b) total HIV DNA by multiplex qPCR, c) integrated HIV DNA by Alu-gag PCR, d) integrated HIV Gag and Pol by droplet digital PCR (ddPCR) following pulsed-field gel electrophoresis (PFGE), and e) latency re-activation *in vitro* measured by both cell-associated tat/rev induced limiting dilution assay (TILDA), and by HIV p24 single molecule array (SIMOA). Spearman tests were used to test relationships between HIV measures.

Results: Total qPCR HIV DNA, integrated Alu-gag PCR or PFGE/ddPCR HIV DNA, as well as inducible HIV RNA or p24 were positively associated with each other (e.g. Alu-gag PCR HIV DNA and inducible HIV RNA: p=0.0319, spearman's rho=0.5211). IPDA-intact HIV DNA was positively associated with total qPCR HIV DNA (p=0.0158, rho=0.4298) and integrated HIV DNA (i.e. Alu-Gag PCR (p=0.0048, rho=0.3779; PFGE/ddPCR HIV Gag: p=0.0387, rho=0.5376; PFGE/ddPCR HIV Pol: p=0.0343, rho=0.5484). Finally, IPDA-3' defective HIV DNA was positively associated with integrated PFGE/ddPCR HIV Pol (p=0.0418, rho=0.5308) and with inducible HIV RNA (p=0.0431, rho=0.5278). IPDA-5' defective HIV DNA was positively associated with total qPCR HIV DNA (p=0.0008, rho=0.571), and integrated HIV DNA (i.e. Alu-Gag PCR: p=0.0039, rho=0.3866; PFGE/ddPCR HIV Gag: p=0.0004, rho=0.7929; PFGE/ddPCR HIV Pol: p=0.0272, rho=0.5679).

Conclusion: In steady-state ART suppression, our data support (1) an association of total or -integrated HIV with IPDA-intact or -defective HIV, and (2) an association of inducible HIV with IPDA-defective HIV.

Introduction

The establishment of reservoir **(1-3)** creates the greatest challenge in HIV eradication. Development of eradication strategies is dependent both by the understanding of the nature of the reservoir and by the development of rapid and scalable assay that are able to characterize it accurately **(4)**. A great number of assays have been used to identify the reservoir. Yet, many questions remain about the ability of current techniques to characterize the reservoir accurately. It is crucial for eradication and cure strategies to use assays able to detect infected cells and distinguish intact from defective proviruses **(5-8)**. Recently, the intact proviral DNA assay (IPDA) was developed to measure the HIV-1 reservoir that separately quantifies intact and defective proviruses **(6)**. In a cohort of ART-suppressed chronically HIV-1-infected subjects we performed a correlation analysis of IPDA with five distinct HIV measures that are traditionally used for characterization of the latent HIV reservoir.

Subjects, Materials & Methods

Study participants

A total of 64 HIV+ individuals participated in the study. All participants were chronically suppressed on antiretroviral therapy (ART) at <50 HIV-1 copies/ml for ≥1 year and had ≥450 CD4+ T cells/mm^3 at the time of samples collection.

Methods

Intact proviral DNA assay (IPDA)

Assessed in CD4+ T cells isolated from leukapheresis-derived cells by IPDA (Accelevir). Results were expressed as count of intact or copies of 3' or 5' deleted and/or hypermutated HIV pro-viruses per 10^6 CD4+ T cells.

Total HIV DNA assay

Performed on CD4+ T cells isolated from cryopreserved leukapheresis-derived cells by multiplex qPCR (HIV-1 Total DNA Quantitative Kit, Ultrabio Technologies, Bothell, WA). Results were expressed as copies of total HIV DNA per 10^6 CD4+ T cells.

Integration assay

a) Performed on CD4+ T cells isolated from cryopreserved whole blood-derived PBMC by Alu-gag–PCR. Results were expressed as copies of integrated HIV DNA per 10^6 CD4+ T cells.

b) Performed on cryopreserved leukapheresis-derived cells enriched for CD4+ T cells using pulse-field gel electrophoresis (PFGE) and droplet digital PCR (ddPCR). Results were expressed as copies of integrated HIV DNA per 10^6 CD4+ T cells.

In vitro latency re-activation

Assessed in CD4+ T cells isolated from leukapheresis-derived cells following stimulation for 24hrs with PMA/ionomycin or DMSO (control) by:

a) Tat/rev induced limiting dilution assay (TILDA)

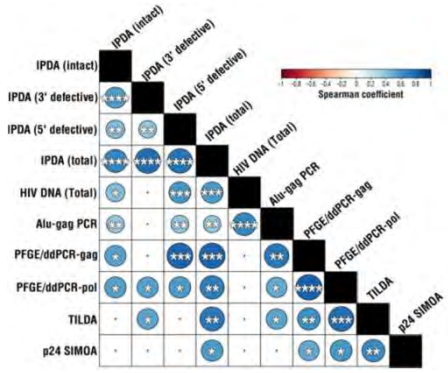
b) HIV p24 Single Molecule Array (SIMOA).

Induced measures for TILDA and p24 SIMOA were used following subtraction of the constitutive measures [i.e. (PMA and ionomycin) – DMSO).

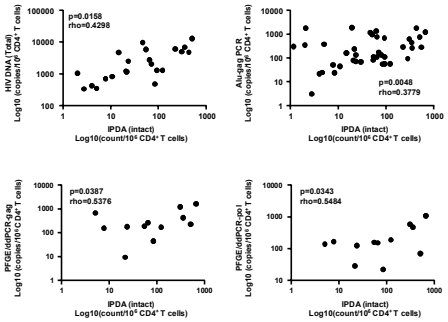
Statistical analysis

Data were described as medians, 25th and 75th percentiles. Relations between HIV measures were assessed by Spearman's Rank correlation test. P-values <0.05 were considered statistically significant. All statistics were performed with JMP Pro15 (SAS Institute, Cary, NC).

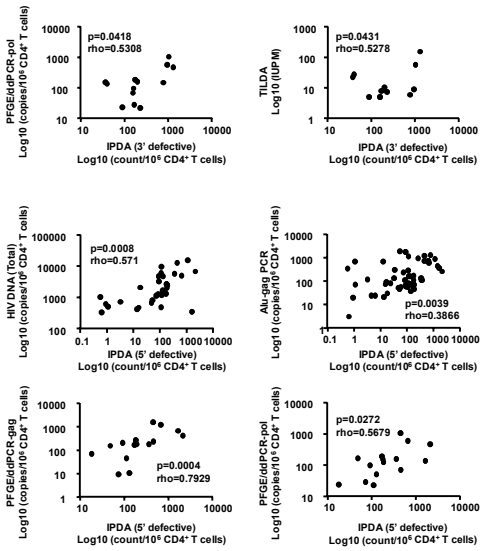
1 Associations between six distinct HIV measures of HIV DNA and induced HIV RNA and p24



2 Positive association of intact HIV proviruses with total and integrated HIV DNA



3 Positive association of defective HIV proviruses with total, integrated and induced HIV RNA



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****UPDATE NOTICE** The poster was updated from the original abstract submission to include data from a larger cohort and additional assays.

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Conclusions

- Positive association of non-IPDA-total and -integrated HIV DNA measures with IPDA-total, -intact or -defective proviruses
- Positive association of inducible HIV measures with IPDA-total or -defective proviruses.
- Positive association between non-IPDA HIV measures
- Positive association between IPDA HIV measures

Interpretation

Data support the added value of combining measures from IPDA or integrated provirus assays with measures from inducible HIV assays in order to evaluate intact or integrated proviruses with the potential for antigen expression, of relevance to eradication and potential for immune stimulation, respectively

Future Directions

- Validation of data in a larger cohort and in samples from tissues.
- Validation of IPDA results by full genome sequencing
- Exploration of the impact of correlations in cure-directed studies testing target interventions.

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