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The Inducible HIV-1 Reservoir is Predicted by Combinations of pre- and on-ART Parameters

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Abstract

Background: Antiretroviral therapy (ART) suppresses HIV-1 replication but an inducible viral reservoir persists in resting CD4+ T (rCD4) cells. We have found that the amount of inducible HIV-1 RNA following *ex vivo* activation of purified rCD4 cells varies >1000-fold across donors. Here, we sought to identify the reasons for this wide variation in inducible HIV-1.
Methods: We studied 10 consecutive HIV-1 infected participants on suppressive ART with HIV-1 RNA in plasma <50 cps/mL for ≥1.5 years (median: 11 years). Plasma and PBMC were collected and rCD4 cells ($CD3^+CD4^+CD25^-CD69^-HLA\text{-}DR^-$) were purified by negative selection. rCD4 cells were treated for 7 days with anti-CD3/CD28-coated beads in media containing efavirenz. The amount of virus released into the culture supernatants was quantified by Roche AmpliPrep/COBAS TaqMan. We evaluated 9 continuous and 7 categorical variables for relationships with the amount of inducible HIV-1 RNA. Correlations were evaluated by Spearman's correlation and differences in levels of inducible virus between categorical variables were evaluated by Wilcoxon rank sum test or one-way ANOVA, as appropriate. A principal component analysis (PCA) and regression analysis (PCRA) were performed using the 9 continuous variables to identify qualities associated with the amount of inducible HIV-1.
Results: Inducible HIV-1 RNA was not significantly different between categorical variables. Amongst the continuous variables, only cellular HIV-1 RNA was weakly correlated with inducible HIV-1 (ρ=0.67,p=0.04). Given the poor predictive power of individual variables, we next performed a PCA to determine the proportion of the variance that was explained by linear combinations of the 9 continuous variables. The first 3 dimensions of the PCA explained 80% of the variance in inducible virus. Inducible HIV-1 was positively associated with pre-ART plasma HIV-1 RNA, low-level plasma viremia, and cellular HIV-1 RNA and DNA in PBMC; and inversely associated with CD4 counts at nadir, as well as CD4 counts on suppressive ART.
Conclusions: 80% of the inter-patient variation in the amount of inducible HIV-1 from rCD4 cells was explained by 3 principal components that included pre-ART plasma HIV-1 RNA, low-level plasma viremia on ART, cell-associated HIV-1 DNA and RNA levels, and CD4 counts at nadir and on suppressive ART. These findings support a model in which no single variable predicts the size of the latent inducible reservoir, but combinations of immunologic and virologic measures explain much of the observed variance in inducible HIV-1.

Introduction

- **Predictors of the infectious virus reservoir have been proposed[1-2], but a simple surrogate has been elusive**
- **We have observed >1000-fold variation in virion-associated HIV-1 RNA following activation of resting CD4+ T cells**

Objectives

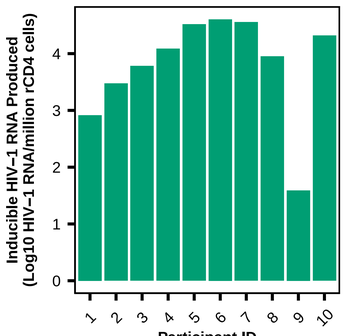
- **Evaluate categorical and continuous variables for relationships with the size of the inducible reservoir**
- **Determine the contribution of pre- and post-ART variables on the size of the inducible reservoir**
- **Assess whether combinations of variables can explain the variation in levels of inducible HIV-1**

Materials and Methods

Study Population and Entry Criteria: HIV-1 positive participants on suppressive ART for ≥1.5 years from the Pittsburgh AIDS Center for Treatment (PACT) at the University of Pittsburgh Division of Infectious Diseases were selected as volunteer blood donors. Historical clinical data (e.g. pre-ART CD4, nadir CD4, pre-ART viral load, etc.) were obtained from these participants. This study was conducted with the approval of the University of Pittsburgh IRB.
Isolation and culture of rCD4 cells: Plasma was first separated from blood and stored for downstream single copy assay (SCA) analysis as previously described[3]. 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 as previously described[4], and were cultured for 7 days in the presence of anti-CD3/CD28 activation beads. The supernatant was removed, and HIV-1 RNA levels were quantified by Roche TaqMan 2.0.
Molecular assays of viral persistence: Low-level viremia was quantified as previously described using two-step qRT-PCR iSCA[3]. 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[4].
Statistical Analyses: Wilcoxon rank sum tests or one-way ANOVA tests (where appropriate) were used to evaluate differences in the size of the inducible reservoir for categorical variables. Spearman's correlation was used to evaluate correlations between the inducible reservoir and continuous variables. A principal component analysis[5] was used to evaluate whether linear combinations of variables could explain the variance in inducible virus production. Principal component regression analysis was used to evaluate the utility of the principal components in quantitatively predicting the size of the inducible reservoir. All statistical analyses were performed using R[6].

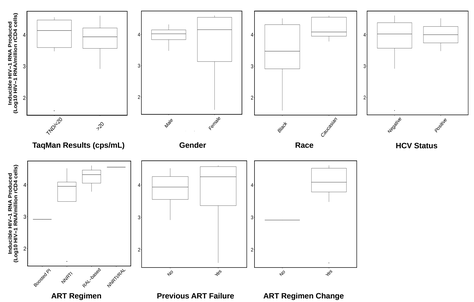
Results

Figure 1. Inter-participant variation in levels of inducible HIV-1 RNA following T cell activation



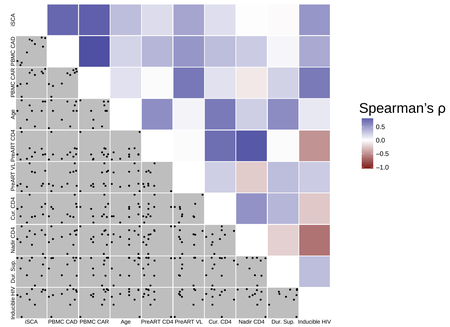
The variation in HIV-1 RNA produced per million rCD4 cells across a panel of 10 consecutive donors was >1000-fold (median: 10,560 copies/10^6 rCD4 cells; range: 39 to 33,113 copies/10^6 rCD4 cells).

Figure 2. Differences in levels of inducible virus production was not readily explained by categorical variables



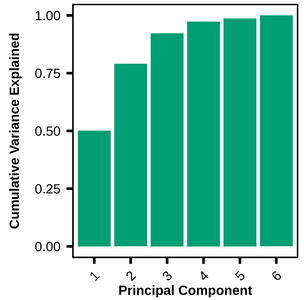
A total of 7 categorical variables were evaluated for relationships with inducible virus production. There were no differences between levels of inducible HIV-1 produced across any set of categorical variables including TaqMan positivity, gender, race, HCV serostatus, current ART regimen, previous ART failure or whether ART was changed.

Figure 3. Relationships between continuous variables and the size of the inducible reservoir



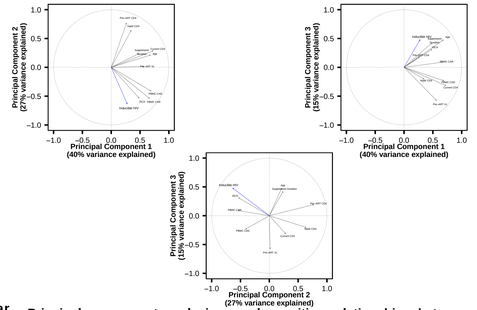
Correlative inter-relationships between the 9 continuous variables evaluated and their relationship to inducible HIV-1 following T cell activation. Cellular HIV-1 RNA was the only variable that correlated significantly with inducible virus production (ρ=0.67, p=0.04).

Figure 4. Overall variance in the dataset explained by principal component analysis



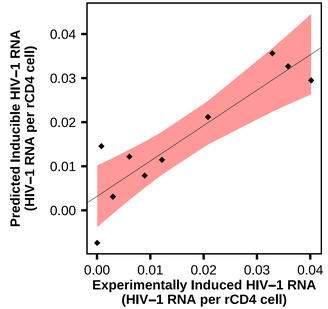
Principal component analysis was used to determine if linear combinations of the 9 continuous variables could explain the variance in the data. We found that 3 principal components explained >80% of the variance in the data.

Figure 5. Relationships between the 9 continuous variables, the principal components, and the inducible reservoir



Principal component analysis reveals positive relationships between iSCA, cellular HIV-1 RNA and DNA and the inducible reservoir, and inverse relationships between current CD4 count and nadir CD4 count and the size of the inducible reservoir.

Figure 6. Levels of inducible virus predicted by principal component regression correlates with observed virus production



Using principal component regression analysis (PCRA) based on the first 3 principal components and including iSCA, cellular HIV-1 RNA and DNA, current CD4 count, nadir CD4 count and pre-ART viral load as explanatory variables, we found that predicted virus production and experimentally observed virus production were correlated (ρ=0.79, p=0.0098).

Conclusions

- **The amount of HIV-1 RNA produced by rCD4 cells following activation is highly variable between donors.**
- **Unspliced cellular HIV-1 RNA from PBMC was weakly correlated with inducible virus production.**
- **PCA revealed that residual viremia, cellular HIV-1 DNA and RNA, and pre-ART HIV-1 RNA were positively associated with inducible virus; whereas, nadir CD4 and current CD4 were inversely associated.**
- **Predicted levels of inducible HIV-1 RNA from PCRA correlated strongly with measured levels of inducible HIV-1 RNA from resting CD4 cells.**

References

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