Background

- HIV RNA shedding from the genital tract (GT) during antiretroviral therapy (ART) that suppresses the median plasma HIV RNA to <30 copies/mL (c/mL) is defined as discordant (DSC) shedding.
- DSC has been reported in up to 50% of persons. It is hypothesized to be due to HIV replication due to poor drug penetration.

Hypothesis

- DSC shedding is caused by production of HIV viruses from clonally expanded cells without full cycles of viral replication, primarily in persons with larger proviral reservoirs.

Specific Aims

- Determine if DSC shedding is associated with increasing HIV diversity from Maltese Common Ancestor (MICA), which would suggest HIV replication.
- Determine if DSC shedding is associated with larger HIV reservoirs (higher HIV RNA in PBMC) and clonal expansion of infected cells.

Methods

- Longitudinal study of HIV-infected ARV-naive men and women initiating ART in Lima, Peru
- Genital and blood samples were collected every 3 months for 24 months to assess DSC shedding.
- Cervical vaginal lavage (CVL) collected with 10 mL 1xPBS, lower limit of quantification: 50 c/mL.
- Seminal plasma (SP): 10 mL 0.2xPBS.
- Sexual Tissue Flow™ (sTFF): 100 mL 1,25xPBS.
- Plasma HIV RNA and DNA quantified by real-time PCR.
- Single genome amplification (SGA) and phylogenetic analysis
- Single genome amplification (SGA) of env (235-1073 nt) and pol (1234-1650 nt) was performed. DNAase treatment was used prior to RNA SGA.
- Maximum likelihood (ML) trees and pairwise distances generated in DIVER using PhyML.
- Evaluation of viral replication
- Genomic diversity of DSC env sequences
- Genetic distance of DSC SGA vs. pre-ART sequences from the MICA
- Antiretroviral (ARV) drug resistance in patient from the Lima database.

Statistical Analysis

- Subsets grouped by detection vs. no detection of 31 episode of DSC genital rectal shedding
- Pre-ART continuous variables (CD4 cell count, age, plasma HIV RNA, log10 concentrations, HIV DNA PBMC concentrations) across genders were evaluated with the Whitney-Mann test.
- Spearman's correlation was used to evaluate the relationship of HIV RNA and DNA with several demographic-age (SingelPhd, Per, La Jolla, CA) and ART treatment
- All tests were two-sided with a p-value <0.05 considered significant.

Results

- 126 ART-naive Peruvians were enrolled; 89 completed all study visits with 82/89 (92%) having sustained ART suppression.
- DSC shedding was observed in 23/49 (38%) subjects at 36/72/3 (5%) visits
- Median HIV RNA in DSC specimens were: CVL: 620 c/mL (IQR: 53-1890 c/mL); SP:350 c/mL (IQR: 207-5950 c/mL); and RS: 5,880 c/mL (IQR: 1,758-14,484 c/mL).
- Pre-ART subjects with vs. without DSC shedding had higher HIV DNA in PBMC and a younger median HIV RNA in CVL (Figure 1).
- After DTNase treatment, HIV sequences only amplified from 2/38 (5%) DSC specimens.
- Phenotypic analysis of DSC shedding SP and CVL env RNA sequences consistent with production of viruses from a proliferating cell (Figure 2).
- No drug resistance was detected in pol sequences.
- The reproducibility of HIV RNA detection from CVL at time of DSC shedding was 95% through In-house and Abbott assays (Table 1).
- DSC suppression, HIV RNA and DNA in CVL were highly correlated (Figure 3) (SP and RS samples were depleted by attempted SGA, thus DNA was not quantified).

Summary and Conclusions

- Greater pre-ART HIV RNA and DNA concentrations in subjects with vs. without DSC shedding, suggesting a greater viral burden (Figure 1).
- Time-ordered evolution and accumulation of drug resistance, typical of HIV replication, was not observed in the two subjects with successful SGA of DSC shedding.
- "Diagnosis" of DSC shedding can be falsely (+) due to amplification of HIV DNA (Figure 3) and variation in detection of HIV RNA near the limit of detection (Table 1) from genital samples during ART suppression.
- Multiple monotypic HIV env RNA and DNA sequences, without evidence of divergence, suggests production of viruses from a clonally expanded cell.

References


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