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A Small Fraction of Proviruses Express Unspliced HIV RNA in Vivo

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BACKGROUND:

The vast majority of proviruses that persist on ART are defective. Of the minority that are intact (~2%), the fractions that are latent or transcriptionally active are not known. To address this question, we determined the fraction of proviruses that express HIV RNA in vivo in cell populations carrying either intact or defective proviruses.

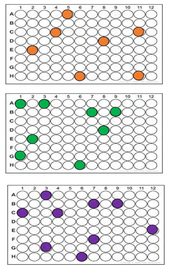
METHODS:

PBMCs were obtained from Patient #1 reported in Maldarelli, et al. This donor had multiple clones of cells that contain intact or defective proviruses. Intact proviruses that could be activated to produce replication-competent virus were identified using a limiting dilution VOA. Proviral expression was determined by single genome pro-pol sequencing of HIV DNA and RNA from multiple aliquots of PBMC diluted to an endpoint.

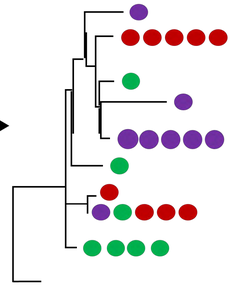
1)Collect PBMCs

2)Aliquot to ~2-10 HIV expressing cells per vial

3)Sequence single HIV CAR molecules



4)Analyze phylogenetically



Results:

Figure 1a. Replication competent clones: Three Wildtype (OG-1, AMBI-1, and OG-2) clones were found to be replication competent by the viral outgrowth assay (VOA). Each clone had a small fraction of cells that expressed unspliced HIV RNA.

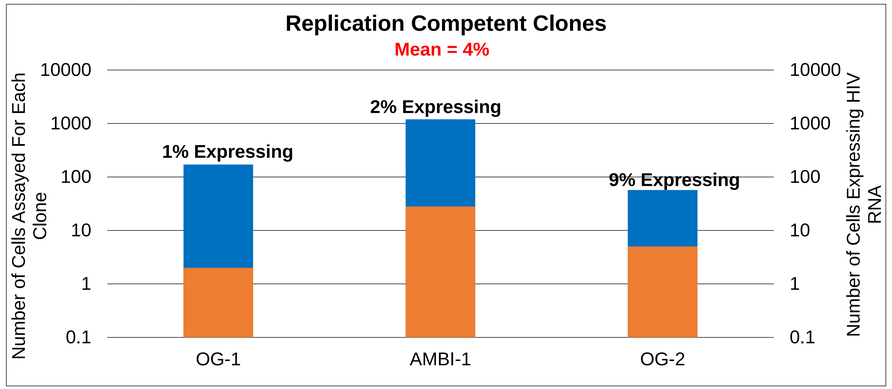


Figure 1b. Defective clones: Defective clones contained stop codons. The fraction of expressing cells in defective clones in not different from clones with replication-competent proviruses.

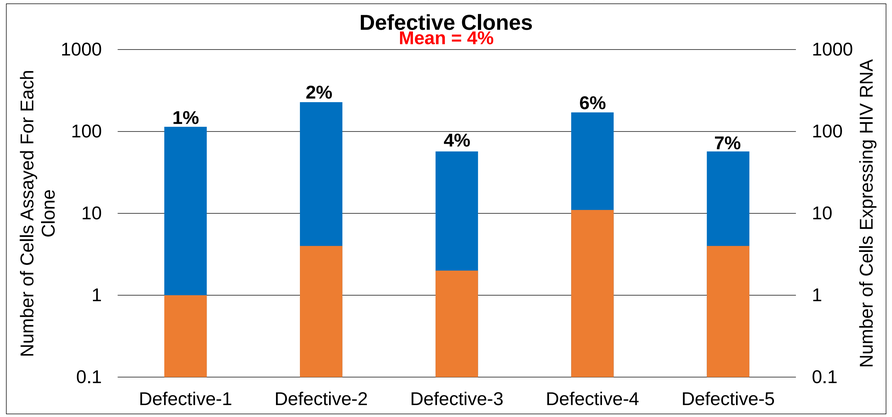
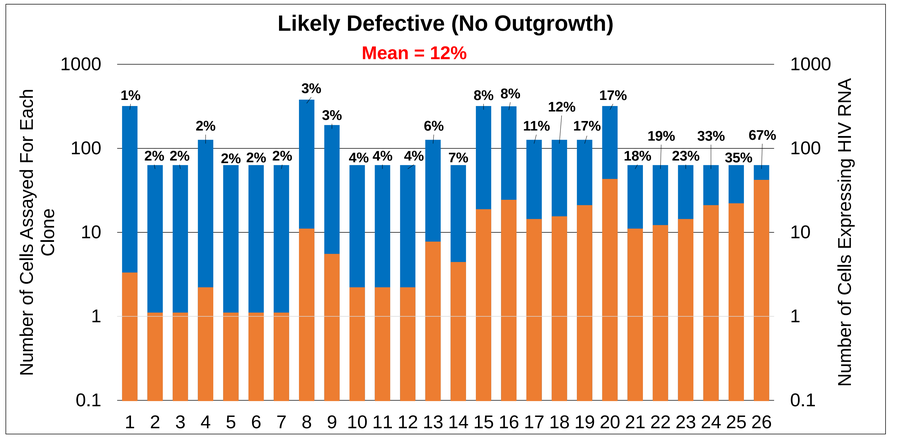


Figure 1b. Likely Defective (No Outgrowth) clones: Likely Defective clones did not contain obvious stop codons but no outgrowth in the VOA was observed. The fraction of cells within each clones that expressed HIV RNA in vivo ranged from 1% to 67%.



Conclusions:

The large majority (>80%) of infected cells that persist on ART are either latent or incapable of HIV RNA expression. A small fraction of proviruses within infected clones expressed unspliced HIV RNA, but this fraction was not significantly different between clones carrying intact proviruses from clones containing obviously defective proviruses, indicating that HIV RNA expression appears similarly detrimental (or non-detrimental) for infected cells regardless of whether the provirus they carry is intact.

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