Majority of Infected Cells in Peripheral Blood of PWH on ART Transcribe Cell-Associated HIV *gag* RNA

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Background

Although previous studies have detected HIV-1 RNA in ART-treated people with HIV (PWH), the prevailing dogma is that this "residual transcription" represents a rare exception rather than the rule and the reservoir is thought to be generally silent. For the most part, this stems from the absence of sufficiently accurate and sensitive assays to enumerate the reservoir cells that transcribe HIV-1 RNA.

Study design and results

We developed **AREA** (Active Reservoir Evaluation Assay) – a novel sensitive and precise assay based on limiting dilution-multiplex seminested RT-qPCR that measures frequencies of cells transcribing HIV-1 cell-associated *gag*, *env*, and multiply spliced (MS, *tat*/*rev*/*nef*) RNA. We applied this assay to evaluate the active reservoir in peripheral blood of 7 untreated (UT), 11 ART-treated, and 3 elite controller (EC) PWH without ex vivo cellular stimulation. The highest frequencies of *gag* RNA+, *env* RNA+, and MS RNA+ cells were measured in UT, intermediate in ART-treated, and the lowest in EC. Surprisingly, in ART-treated PWH, majority (median, 66.8%) of HIV-1 *gag* DNA+ cells transcribed *gag* RNA. However, only a median of 1.7% of these *gag* RNA+ cells transcribed MS RNA, due to post-transcriptional latency blocks and/or proviral genetic defects that prevented HIV-1 splicing. In comparison, in UT PWH, 47.0% of HIV-1 *gag* DNA-positive cells transcribed *gag* RNA, but of the latter, 10.0% transcribed MS RNA. Only a median of 8.4% of *env* DNA+ cells transcribed *env* RNA in ART-treated PWH, however in UT PWH this percentage was 91.3%. The median *env* RNA+ / *gag* RNA+ cell frequency ratio was 0.33 in ART-treated and 1.23 in UT PWH, with the corresponding median *env* DNA / *gag* DNA ratios of 1.08 and 1.17, respectively. This suggests the existence of a latency block at the distal elongation stage of HIV-1 transcription in ART-treated PWH.

Conclusions

AREA revealed a large, previously underestimated, HIV-1 active reservoir in ART-treated PWH, questioning transcriptional latency as the main mechanism of HIV-1 persistence on ART. However, the low percentages of MS RNA+ cells in all groups implies that with or without ART, most gag RNA+ cells are still not productively infected. Our results argue for the shift of HIV-1 latency reversal strategies from stimulating HIV-1 transcription initiation and proximal elongation to the downstream steps such as distal elongation and splicing of HIV-1 RNA. AREA can serve as a robust tool for the measurement of HIV-1 reservoir in the curative interventions.

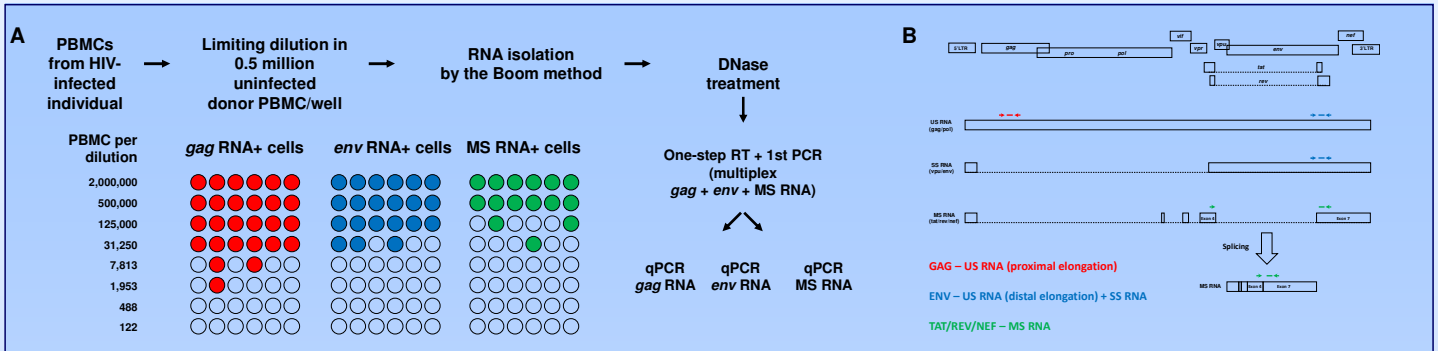


Figure 1. (**A**) Overview of AREA. Unstimulated or pre-stimulated PBMCs from HIV-infected individuals are distributed in limiting dilution (typically 6-8 dilutions X 6-8 aliquots/dilution). Total cellular RNA is isolated from each well, treated with DNase, and subjected to a multiplex one-step RT-PCR using primers specific for *gag*, *env*, and multiply spliced (MS) HIV-1 RNA. Aliquots from this RT-PCR reaction are used as templates for separate seminested quantitative PCRs for *gag*, *env*, or MS RNA. Frequencies of cells with *gag*, *env*, or MS RNA are determined using the maximum likelihood method. AREA corrects for a number of factors that influence the output: (i) RT-PCR inhibition by cellular RNA, (ii) primer/probe-template mismatches (participant-matched primers and probes are used), (iii) RNA loss during isolation. (**B**) Locations of primers and probes used in the assay. The *gag* primers detect a region in the middle of the *gag* ORF on the unspliced (US) RNA. The *env* primers target the 3' end (GP41 region) on the US and singly spliced RNA. The MS primers target the exon-exon junctions of the MS RNA and do not detect US RNA.

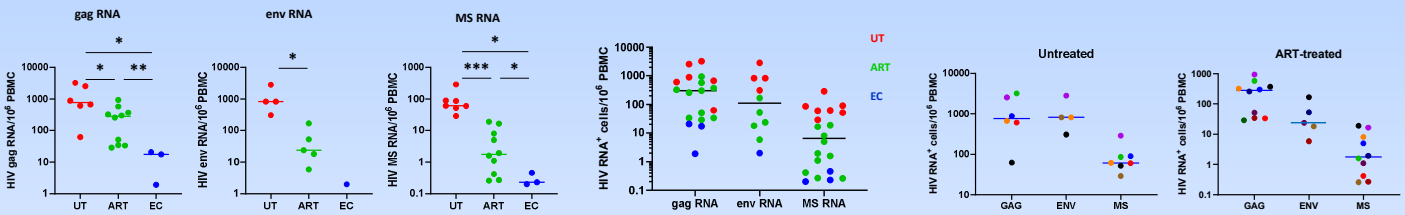


Figure 2. Frequencies of *gag*+. *env*+, and MS RNA+ cells in the study participants. UT, untreated PWH (red). ART, ART-treated PWH (green). EC, elite controllers (blue). Mann-Whitney tests were used to calculate statistical significance.

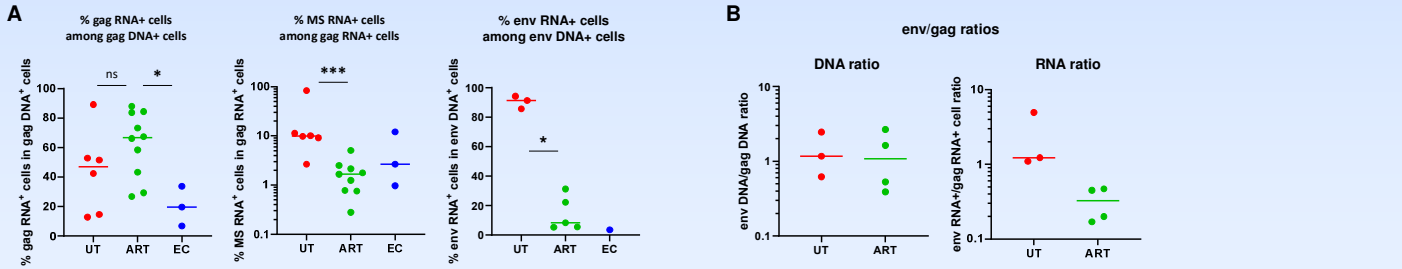


Figure 3. (**A**) Percentages of *gag* RNA+ cells among *gag* DNA+ cells, of MS RNA+ cells among *gag* RNA+ cells, and of *env* RNA+ cells among *env* DNA+ cells. (**B**) *env* DNA / *gag* DNA and *env* RNA+ / *gag* RNA+ cell ratios. Mann-Whitney tests were used to calculate statistical significance.

PLAIN LANGUAGE SUMMARY

Our findings indicate that HIV-1 persistence on ART is not equivalent to transcriptional silence as most reservoir cells produce viral RNA even in the absence of *ex vivo* stimulation. Therefore, we argue for the shift of HIV-1 latency reversal strategies from stimulating HIV-1 transcription initiation to stimulating the downstream steps of viral gene expression.

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