Reservoir-harboring T-cell clones have a cytotoxic immunophenotype that may affect HIV inducibility

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Alberto Herrera[1,2], Isabella Ferreira[1], Tan Thinh Huynh[1], Emily Stone[1], Noemi Linden[1,2], Cintia Bittar[3], Marina Caskey[3], Michel C. Nussenzweig[3]
R. Brad Jones[1,2]

[1] Infectious Disease Division, Weill Cornell Medicine, New York, NY, USA, [2] Immunology and Microbial Pathogenesis Program, Weill Cornell Graduate School of Medical Sciences, New York, NY, USA, [3] Laboratory of Molecular Immunology, The Rockefeller University, New York, NY 10065, USA .

INTRODUCTION

- The HIV reservoir is enriched for clonal CD4+ T-cells harboring intact proviruses after years of antiretroviral therapy (ART).
- In this study, we characterize the immunophenotypes of '**authentic reservoir-harboring clones**' **(ARCs)** isolated and propagated from people living with HIV (PLWH) and aim to understand the contribution of these profiles to persistence.

METHODS

- Individual ARCs from PLWH on ART (n=4) were isolated by limiting dilution of memory CD4+ T-cells and propagated with irradiated feeders and anti-CD3/CD28 stimulation in media containing ARVs and bNAbs.
- Wells were screened for the presence of ARCs by Gag-p24 ELISA and infected cell purity was validated by IPDA and single-cell TCR-sequencing.
- Transcriptional, surface protein profiles and HIV-expression of ARCs were characterized by ECCITE-seq (single cell multiomics of gene-expression, surface proteins and V(D)J reconstruction) .
- Latency reversal activity and granzyme release from ARCs were assessed using bryostatin or PMA/Ionomycin, followed by intracellular flow cytometry analysis.

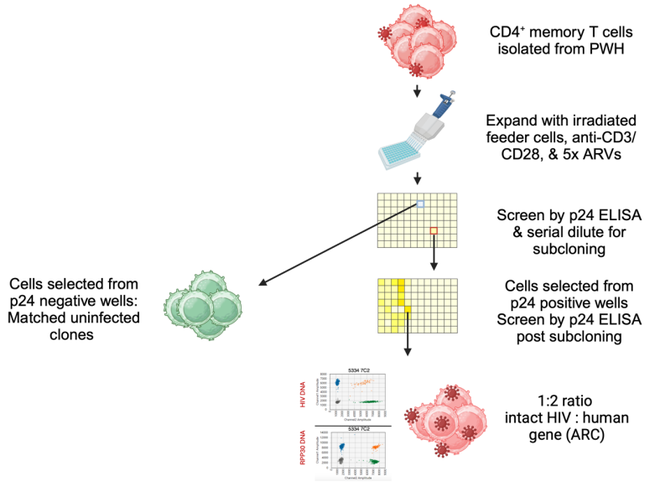


Figure 1. Schematic for the isolation and propagation of ARCs and matched uninfected clones.

RESULTS

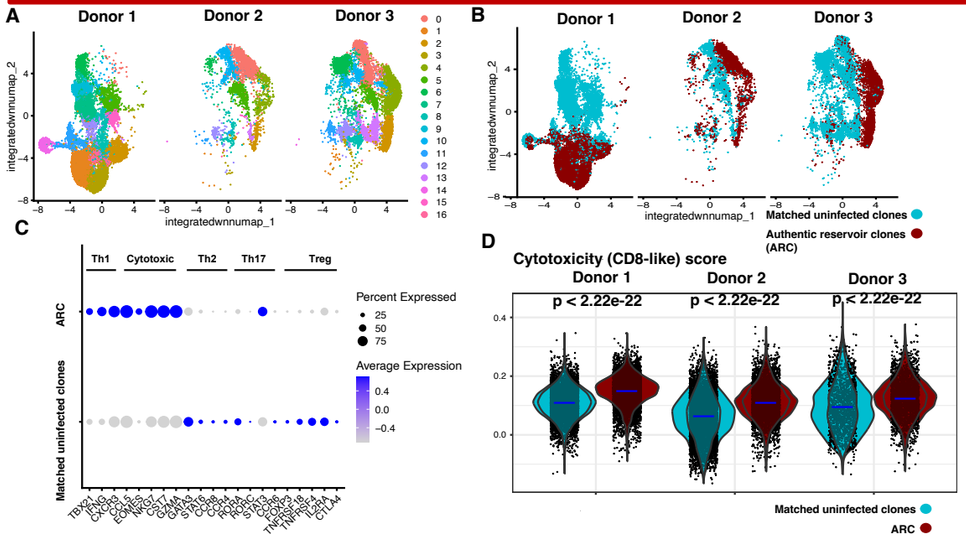


Figure 2. Authentic reservoir clones (ARCs) are enriched for cytotoxic Th1 profiles compared to autologous uninfected clones. A,B, Harmonized UMAP plots integrating gene expression and surface protein (155 surface proteins) expression profiles from ARCs (n=3) and autologous uninfected clones (n=10) from 3 PLWH showing unbiased clustering (A) and colored by clone infection status (B). **C**, Dot plot showing the percentage of cells expressing and the average expression of Th-subset defining transcripts and selected cytotoxic genes in ARCs vs uninfected clones. **D**, Violin plots showing transcriptional score of a CD8 cytotoxic module pulled from profiles defining T-cell functional states (Szabo *et al* 2019).

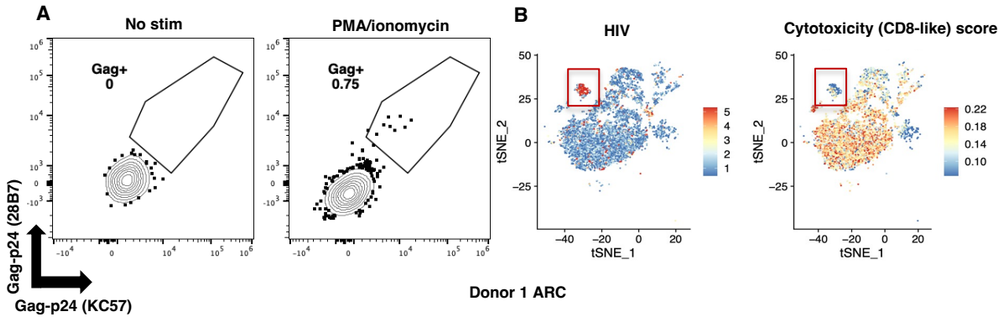


Figure 3. While mostly latent, a small sub-population of cells from the same ARC exhibits high expression of HIV and displays downregulation of cytotoxic programs. A, Representative flow cytometry plots of Gag-p24 protein expression with and without latency reversal activation using PMA/ionomycin stimulation for 24 hours on cells from donor 1 ARC . **B**, HIV transcript expression (left) and cytotoxic CD8-like module score (right) on cells from single-cell multiomics analysis subset on donor 1 ARC.

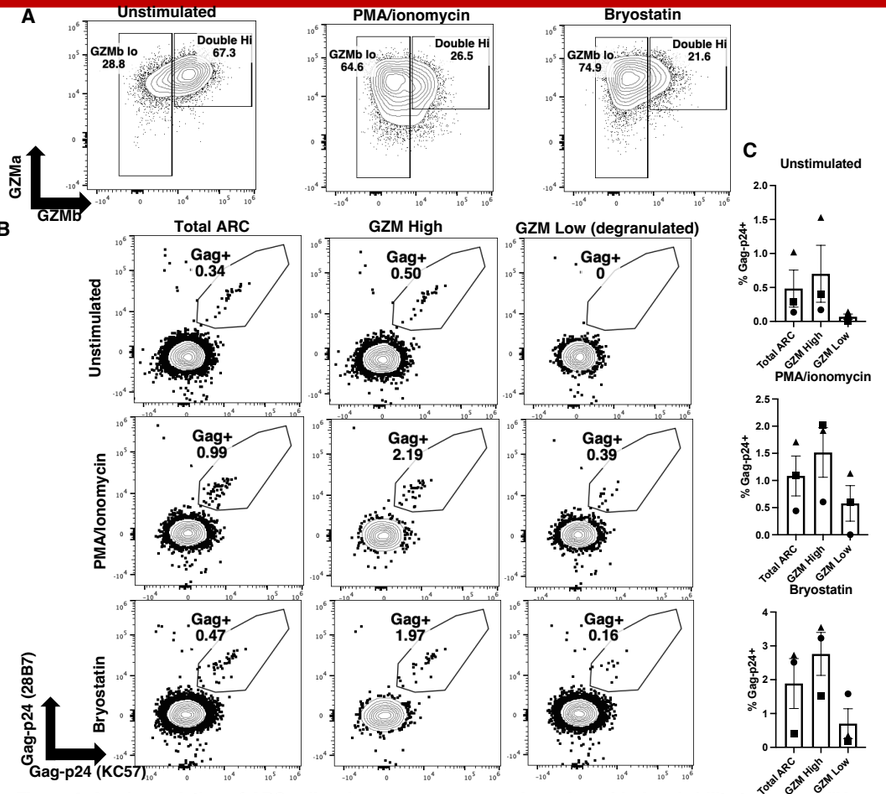


Figure 4. A subpopulation of ARC cells releases granzymes and may have hindered ability to induce HIV expression after reactivation with latency reversal agents . A,B Representative flow cytometry plots showing granzyme loss (release) (A) and Gag-p24 expression (B) in ARC cells after activation with latency reversal agents (PMA/ionomycin and bryostatin) for 24 hours. **C**, Summary plots of data from B across ARC cells from 3 donors.

CONCLUSIONS AND FUTURE DIRECTIONS

- ARCs isolated and propagated from the blood of ART suppressed PLWH are enriched for TNF-a producing Th1 cells with cytotoxic profiles.
- ARCs display a mostly latent phenotype but some cells within the same ARC can transcribe high levels of HIV and produce HIV antigens.
- ARC cells with low retention of granzyme after reactivation are depleted for cells expressing HIV antigens.
- Future work will assess mechanisms by which cytotoxic profiles are enforced in ARCs and dissect the features of this phenotype that may affect HIV inducibility.

ACKNOWLEDGEMENTS

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