Oxidative stress-mediated CTL susceptibility in reservoir clones despite limited antigen expression

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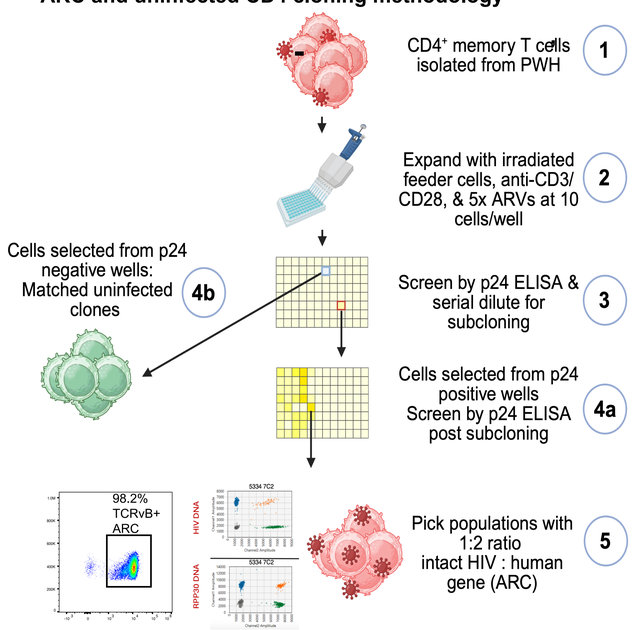
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INTRODUCTION and METHODS

The HIV reservoir is made up of a predominantly latent, clonally enriched, yet highly scarce population of CD4+ T-cells (1-100/1x10^6 CD4s), making molecular and functional studies of these cells challenging. Here, we leverage a technique to isolate and propagate **authentic reservoir clones (ARCs)** from PLWH to functionally characterize potential properties contributing to their persistence.

a

ARC and uninfected CD4 cloning methodology



b

Molecular and phenotypic characterization (5' CITE-seq + flow cytometry)

***Ability to study pure monoclonal ARCs in tandem with synchronous uninfected clones**

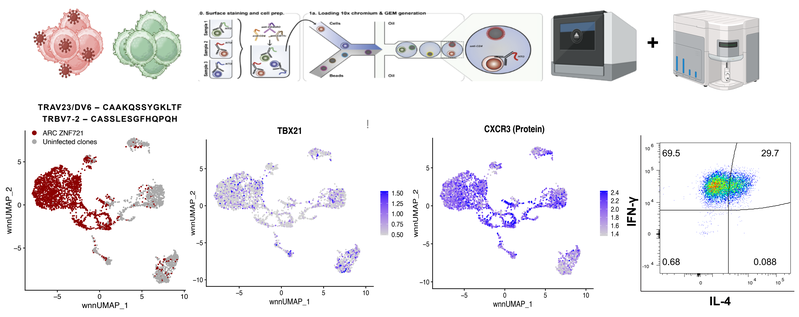
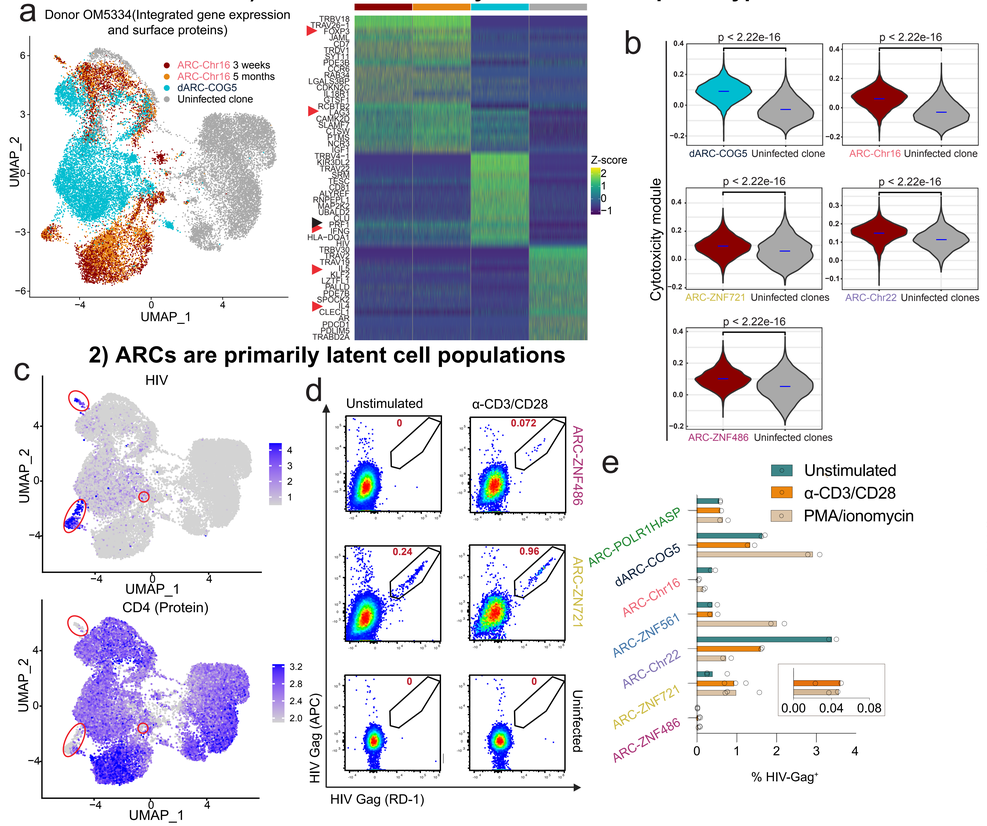


Figure 1. ARC and uninfected clone cloning methodology and characterization assays. **a)** Cell culture methodology to isolate ARCs using a 10 cell/well propagation method with HIV-Gag ELISA screening followed by iterative rounds of limiting dilution sub-cloning and further ELISA screens. ARCs are then confirmed by IPDA and TCRvB staining when applicable. **b)** Representative example of pipeline to confirm monoclonality in ARCs and characterize their molecular/immunophenotypic profiles compared to synchronous uninfected clones (or other ARCs). Showing data from OM5334 donor and ARC-ZN721.

RESULTS

1) ARCs have stable cytotoxic immunophenotypes



2) ARCs are primarily latent cell populations

3) ARCs can have a degree of CTL susceptibility in prolongued co-cultures

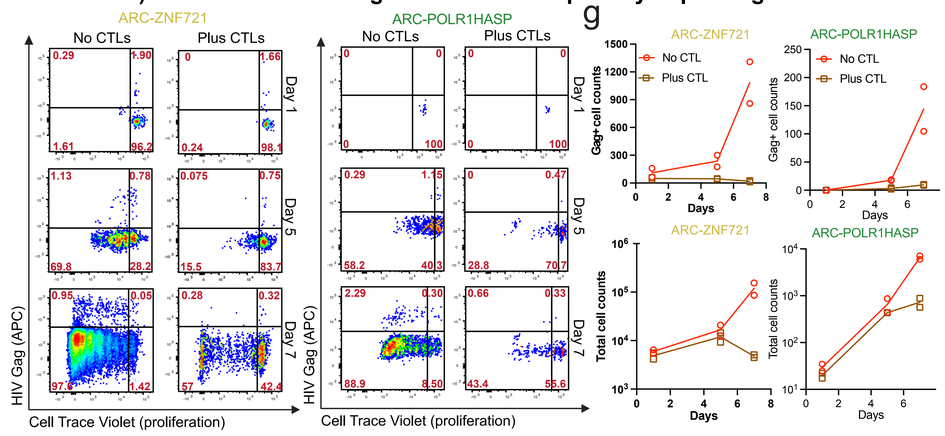
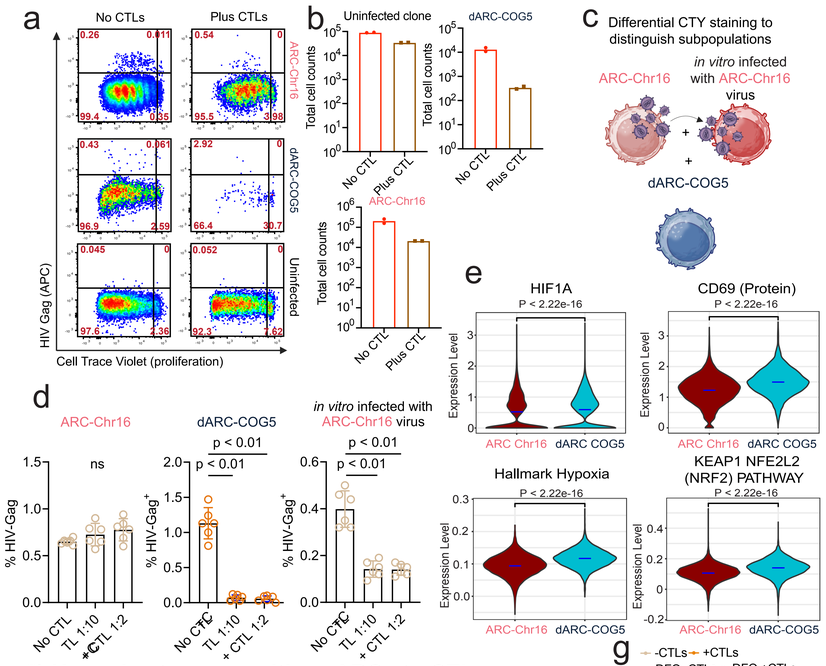


Figure 2. ARCs display a stable cytotoxic immunophenotype and are highly latent but have a degree of susceptibility to cytotoxic T-lymphocytes (CTL). **a)** Representative 5' CITE-seq UMAP clustering (left) of two ARCs (one at multiple timepoints post-isolation) and an uninfected clone and top DEGs between them (right) . **b)** Cytotoxicity module scoring on ARCs anduninfected clones. Measures of HIV-expression by CITE-seq **c)** and flow cytometry **d-e)** including LRA treatment. Measures of HIV expression and proliferation in two ARCs in the prescence or absence of an autologous HIV-specific CTL clone ('slowcure' assay) showing representative plots **f)** and summary data **g)**.

4) CTL-resistance demonstrated by an ARC is linked low oxidative stress



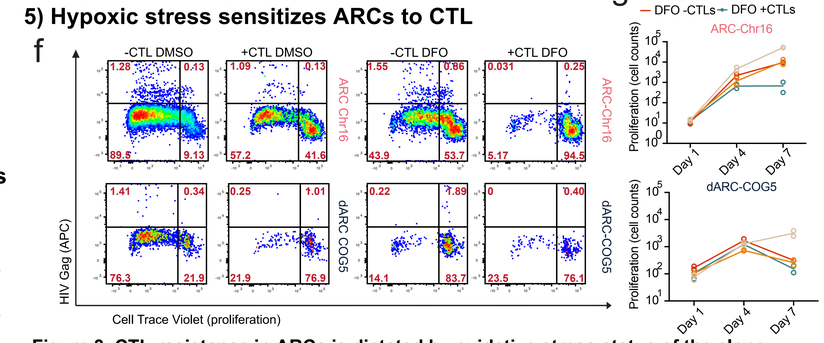


Figure 3. CTL-resistance in ARCs is dictated by oxidative stress status of the clone. **a-b)** Slowcure assays on CTL-resistant (ARC-Chr16) and a CTL susceptible (dARC-COG5)ARC and an uninfected clone. Schematic **c)** and summary data **d)** of competitive killing assays with ARC-Chr16, dARC COG5 and total CD4 T-cells infected with ARC-Chr16 virus. **e)** Expression of CTL-susceptibility factors by CITE-seq of ARC-Chr16 and dARC COG5. Slowcure assays on ARC-Chr16 and dARC-COG5 using the FDA-approved hypoxia inducer DFO to sensitize ARCS to CTL showing representative flow plots **f)** and summary data **g)**.

CONCLUSIONS

Authentic reservoir clones (ARCs) are monoclonal populations of cells with enriched cytotoxic immunophenotypes and are able to maintain their foundational ex vivo profiles long term through in vitro propagation.

ARCs are highly latent, with only small fractions of the clones productively expressing HIV at any given time, but can still be eroded by constant, strict CTL pressure.

CTL-resistance in ARCs is dictated by their metabolic/oxidative stress status and they can be sensitized to elimination through treatment with deferoxamine.

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