Reduced Metabolic Flux Protects HIV-Expressing CD4+ T-cells from CTL-Mediated Elimination

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INTRODUCTION

- HIV latency is a driver of persistence; however, cells expressing HIV RNA and proteins can still be detected after years of antiretroviral therapy (ART) and appear to drive ongoing stimulation of HIV-specific cytotoxic T lymphocytes (CTL).
- We have identified the existence of a rare subset of HIV-expressing CD4+ T-cells **resistant to CTL-mediated** elimination (**survivors**) using a primary in vitro infection model (Figure 1).
- In this study, **we characterize these resistant, surviving cells and use cells from people living with HIV (PLWH) to evaluate mechanisms of resistance.**

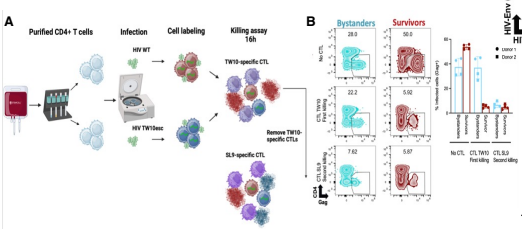


Figure 1. Existence of a rare subpopulation of HIV-expressing CD4+ T-cells resistant to CTL attack.

METHODS

- CD4+ T-cells from HLA-B57⁺ donors were infected with either HIV$_{JRSCF}$ or HIV$_{JRSCF_TW10esc}$ containing a single escape mutation in the Gag-TW10 epitope.
- CD4+ T-cells infected with these strains were dyed with different labeling dyes, mixed and co-cultured with a TW10 epitope-specific CTL clone.
- **Surviving WT** and **Bystander TW10esc** cells were sorted based on HIV–Env surface expression and profiled by ECCITE-seq.
- Cellular reactive oxygen species (ROS) levels were measured via flow cytometry using DCFH-DA.
- Bioenergetic capacity and dependency at the single-cell level were measured by SCENITH profiling.
- HIV-infected CD4+ T-cells were pre-treated with the FDA-approved compound DFO in additional killing assays.
- EX-vivo CD4+ T-cells from PLWH were sorted based on levels of ROS detection and HIV DNA was quantified by IPDA.

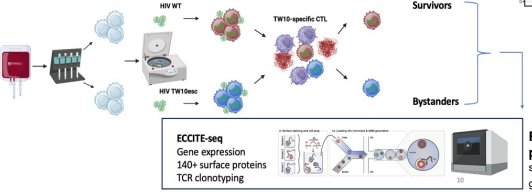


Figure 2. Sorting schematic for surviving and bystander infected CD4+ T cells after CTL targeting.

RESULTS

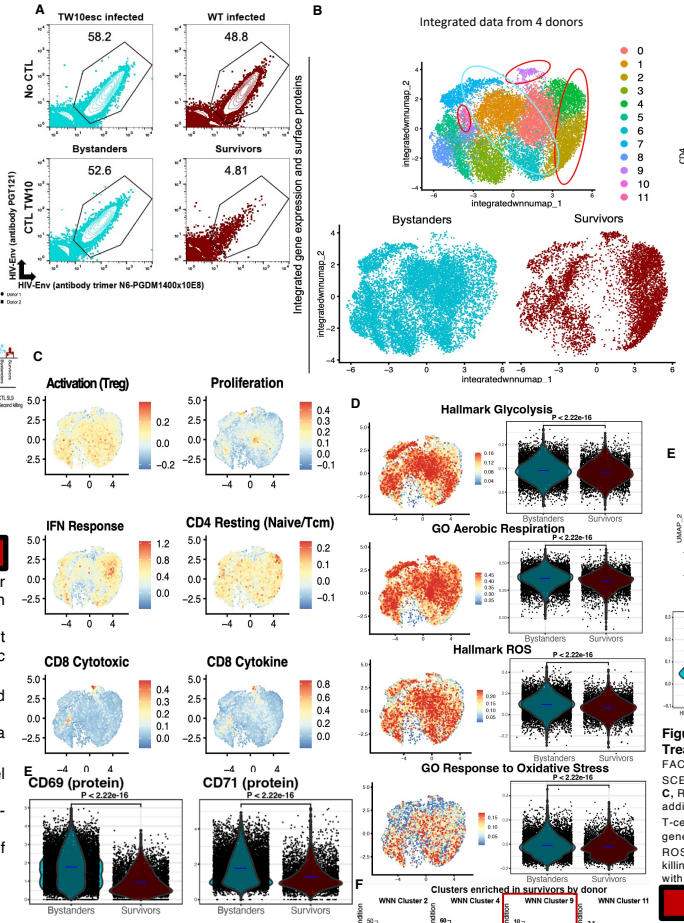


Figure 3. HIV-infected, CTL-resistant CD4+ T-cells have unique transcriptional and surface protein profiles, denoting low levels of activation and oxidative stress. A, Representative flow cytometry plots showing gating of surviving and bystander HIV-expressing cells sorted after a killing assay. **B**, Unbiased integrated gene and surface protein expression clustering of surviving and bystander cells from 4 donors. **C,** Module scoring of activation and resting T-cell gene sets from Szabo *et al.*, 2019. **D,** Module scoring of metabolism gene sets. **E,** Surface protein expression of activation markers. **F,** Summary plots showing the enrichment of specific clusters amongst survivor cells, highlighting consistent enrichment of cytotoxic cluster 9 across all donors.

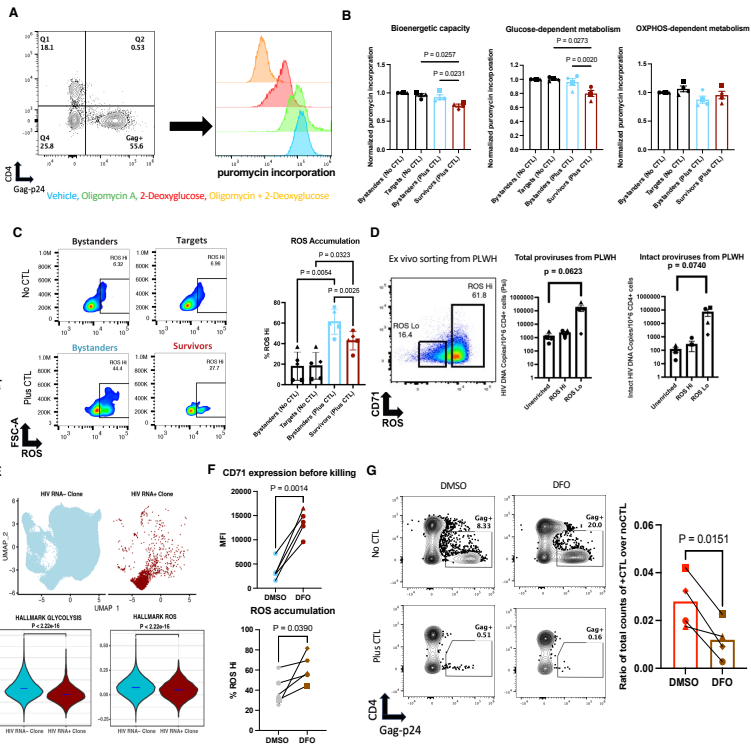


Figure 4. Diminished levels of metabolic flux and ROS accumulation in CTL-resistant HIV-expressing survivors; Treatment with FDA-approved hypoxia-inducer DFO sensitizes infected cells for improved elimination. A, Representative FACS plots showing the effect of metabolic inhibitors on translation capacity (puromycin incorporation) in HIV-expressing cells during SCENITH profiling. **B**, Quantification of translation capacity in bystander and target cells without and with addition of CTLs for killing assays. **C,** Representative FACS plots (left) of 4 independent experiments showing ROS accumulation in bystander and target cells without and with addition of CTLs for killing assays and quantification (right). **D,** IPDA results from unenriched, and FACS sorted ROS Hi and ROS Lo CD4+ T-cells from PLWH showing number of cells per million T-cells harboring total and intact HIV proviruses. **E**, Module scoring of metabolism gene-sets on HIV-RNA+ and RNA- T -cell clones from PLWH from Collora *et al.*, 2022. **F**, Summary plots of CD71 expression (top) and ROS accumulation (bottom) after treatment with the FDA-approved iron-chelator and hypoxia-inducing compound DFO or vehicle before killing. **G,** Representative FACS plots of Gag-p24 expression (left) from 4 independent experiments and surviving cells after killing assays with and without pre-treatment with DFO.

CONCLUSIONS

- Surviving, CTL-resistant, HIV-expressing CD4+ T-cell targets are enriched amongst cells with cytotoxic profiles and with low levels of activation, metabolism processes and oxidative stress.
- In PLWH samples, CD4+ T-cells with low ROS levels are enriched for cells harboring HIV proviruses.
- Infected cells can be sensitized to upregulate ROS and the transferrin receptor (CD71), key components of ROS-mediated cell death and ferroptosis, and become vulnerable to elimination by CTLs.

ACKNOWLEDGEMENTS

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