Poster # 280

Evaluating the Link between Mitochondrial State and HIV-specific CD8+ T Cell Exhaustion

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INTRODUCTION

- In the setting of chronic, untreated HIV infection, HIV-specific CD8+ T cells become exhausted in that they demonstrate poor proliferative and effector capacity.[1,2,3]
- Reversing or preventing exhaustion is an important HIV cure target; however, the regulation of exhaustion in HIV-specific CD8+ T cells is poorly understood.[3]
- Exhausted antigen-specific CD8+ T cells in other settings (e.g., HBV[4,5] and some cancers[6]) have a distinct mitochondrial state marked by: increased mitochondrial mass (MM), increased cellular reactive oxygen species (ROS) content, and decreased mitochondrial membrane potential (MMP).[4,5,6]
- Little is known about how mitochondrial state relates to the exhaustion of HIV-specific CD8+ T cells.[7,8]

RESEARCH QUESTION

How do the mitochondrial features* of HIV-specific CD8+ T cells relate to their functional capacity and level of exhaustion?

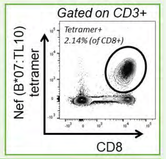
**as defined by mitochondrial mass (MM), reactive oxygen species (ROS) content, and mitochondrial membrane potential (MMP)*

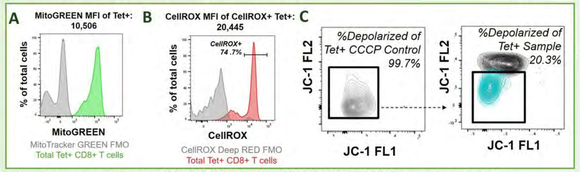
METHODS

1. Screening of HIV-infected individuals in the UCSF-based SCOPE cohort for MHC Class I tetramer responses (HLA-*A02, *03, *24; B*07, *27-restricted; specific for Gag, Pol, Nef). Characteristics of individuals included in study are summarized below:

Clinical Values (Median)	HIV+ Viremic (VIR) n=8	HIV+ ART-suppressed (ART) n=11	HIV+ Controller (CTRL) n=8	Uninfected (UI) n=10
Age (years)	40.5	57	52.5	54.5
% Male	75.0%	90.9%	75.0%	100%
CD4 count (cells/mm^3)	452	513	733	-
CD4 nadir (cells/mm^3)	381	147	437	-
HIV VL (copies/ml)	20,861	all <75 *(on ART median=12y)*	all <40	-

2. Phenotyping of tetramer+ (tet+) HIV-specific CD8+ T cells via flow cytometry using the following markers: Live/dead, CD3, CD4, CD8, tetramer, CCR7, CD45RA, CD127, PD-1.
3. Proliferative capacity after 6 day *in vitro* peptide stimulation assessed via CellTrace Violet (CTV) staining.
4. Mitochondrial characteristics evaluated via fluorescent mitochondrial dyes.





Median fluorescence intensity (MFI) of ***(A)*** *MitoTracker GREEN (MitoGREEN) for mitochondrial mass,* ***(B)*** *%CellROX+ for ROS Content and* ***(C)*** *frequency of cells with depolarized mitochondria via JC-1 staining for mitochondrial membrane potential (high %depolarized= low MMP).*

Fig. 1: Bulk effector and HIV-specific (tet+) CD8+ T cells display a hyperactivated mitochondrial state that does not vary significantly according to HIV clinical group

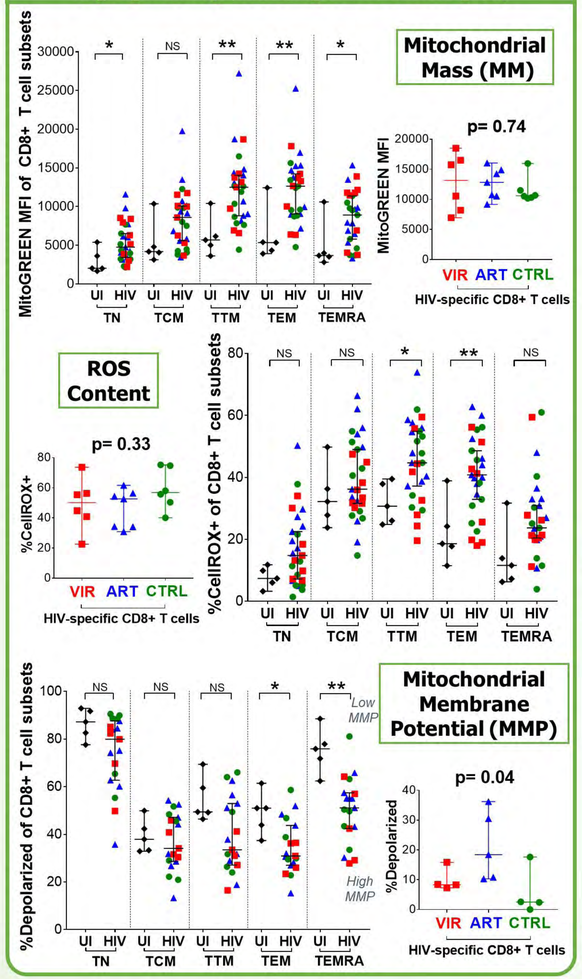
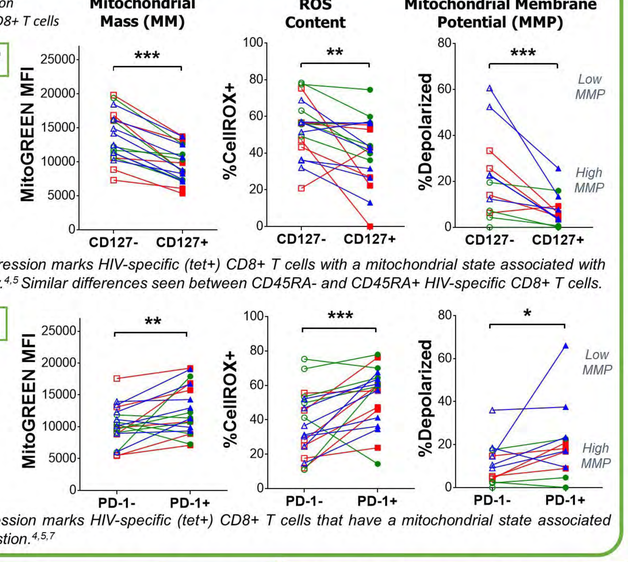


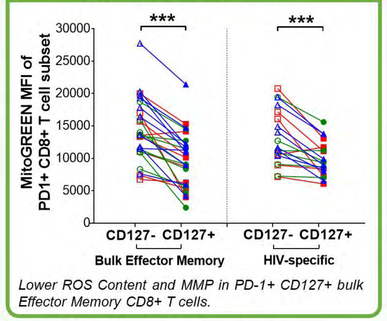
Fig. 2: Expression of CD127 or PD-1 identifies HIV-specific (tet+) CD8+ T cells with distinct mitochondrial states



CD127 expression marks HIV-specific (tet+) CD8+ T cells with a mitochondrial state associated with functionality.[4,5] Similar differences seen between CD45RA- and CD45RA+ HIV-specific CD8+ T cells.

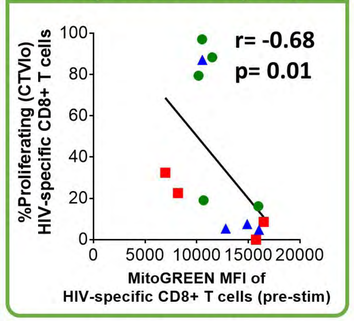
PD-1 expression marks HIV-specific (tet+) CD8+ T cells that have a mitochondrial state associated with exhaustion.[4,5,7]

Fig. 3: PD-1+ cells co-expressing CD127 have lower MM



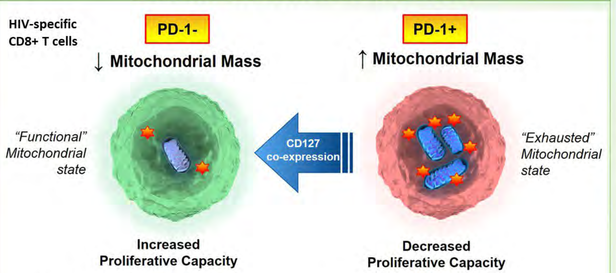
Lower ROS Content and MMP in PD-1+ CD127+ bulk Effector Memory CD8+ T cells.

Fig. 4: MM negatively correlates with proliferative capacity



Legend: ◆ Uninfected ■ Viremic ▲ ART-Suppressed ● Controller *p < 0.05, **p < 0.01, ***p < 0.001

The exhaustion of HIV-specific CD8+ T cells is related to their mitochondrial state



CONCLUSIONS

- Bulk effector CD8+ T cells from HIV-infected individuals display a more hyperactivated mitochondrial state as compared to those from HIV-uninfected individuals.
- PD-1+ HIV-specific CD8+ T cells have a mitochondrial state that is associated in other infectious models with exhaustion and metabolic stress, which may be attenuated by CD127 co-expression.
- HIV-specific CD8+ T cells with lower MM demonstrate enhanced proliferative capacity.
- These findings highlight a potential role of the mitochondrial state in HIV-specific CD8+ T cell exhaustion.
- Metabolic pathways that influence CD8+ T cell functionality could potentially be targeted to overcome HIV-specific CD8+ T cell exhaustion.

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ACKNOWLEDGEMENTS

We would like to thank all the study participants. CD Deguit is supported by the Philippine Department of Science and Technology-Science Education Institute. RL Rutishauser is supported by a NIH T32 training grant AI060530. These studies and the SCOPE cohort are also supported by a CFAR Mentored Scientist Award to RL Rutishauser, additional NIH funding (5U19A1096109-05, 5K24AI069994, R01HD074511, AI110271), the UCSF/Gladstone Institute of Virology and Immunology CFAR (P30 AI027763), the Delaney AIDS Research Enterprise (DARE AI096109), and the amfAR Institute for HIV Cure Research (amfAR grant number 10901).

This research was done as a Master's Thesis for CD Deguit under the guidance of the following mentors: Rachel L. Rutishauser (Research Mentor, UCSF), Joseph M. McCune and Peter W. Hunt (Primary Investigators, UCSF) and Paul Mark B. Medina (Thesis Adviser, UP Manila).