Differential restoration of HIV-specific CD8+ T cells by aminoacid and lipid metabolism modulation

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Background

Priming of CD8+T cells with dendritic cells (DC) is a promising approach to reduce viral reservoirs in people with HIV (PWH). However, HIV-specific CD8+T responses in PWH are affected by PD-1, TIGIT and TIM3 expression in these cells, mechanisms underlying these associations remain unclear and could be crucial to improve immunotherapies against HIV-1 (**Figure 1**). Here, we studied transcriptional, metabolic and functional characteristics of memory CD8+T cells differentially expressing these checkpoint receptors and identified new modulators potentially improving HIV-1 Gag-specific responses in PWH.

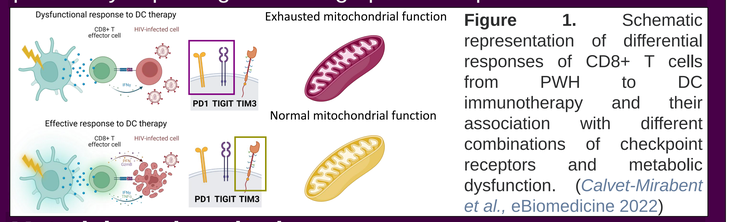


Figure 1. Schematic representation of differential responses of CD8+ T cells from PWH to DC immunotherapy and their association with different combinations of checkpoint receptors and metabolic dysfunction. (*Calvet-Mirabent et al.*, eBiomedicine 2022)

Materials and methods

CD8+T cells were co-cultured with monocyte-derived DC activated with STING and TLR3 agonists and PD-1-TIGIT-TIM3- (TN), PD-1+TIGIT+TIM3- (PD-1/TIGITDP) and PD-1-TIGIT-TIM3+ (TIM3SP) memory CD8+T cells were sorted for RNA-seq analysis (n=4). Relevant transcriptional metabolic and functional pathways were identified by IPA. Selected differentially expressed genes (DEG) were validated by FACS (n=13) (**Figure 2A**). Mitochondrial mass and ROS content was assessed using MitroTracker and MitoSOX probes, respectively (**Figure 2B**). Dependence of OXPHOS on lipid metabolism was tested in CD8+T cell subsets by SeaHorse. Effect of lipid (LTC4) and aminoacid (Ornithine) metabolites in the polarization of HIV-1-specific CD8+T cells (n=9) was evaluated by analysis of IFNγ, TNFα and CD107a (**Figure 2C**) and by elimination of autologous p24+CD4+T cells (n=8) (**Figure 2D**).

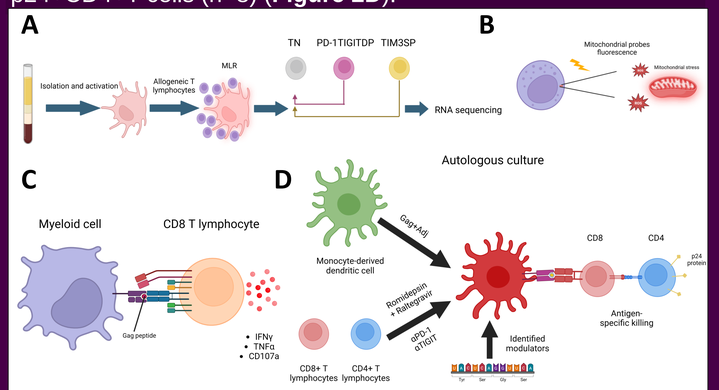


Figure 2. Schematic representation of methodology used in the study. (A): Protocol used to induce and sort different populations of CD8+ T memory lymphocytes with specific patterns of checkpoint receptors after culture with allogeneic DC. (B): Use of mitochondrial fluorescent probes to define mitochondrial mass and stress. (C): Analysis of cytokine profiles of HIV-1-specific T cell in response to HIV-1 Gag peptide stimulation. (D): Evaluation of killing capacity of HIV-specific CD8+ T cells.

Stimulation of lipid and aminoacid metabolism may represent new targets to restore exhausted TIGIT+ HIV-specific CD8+ T cells function in PWH

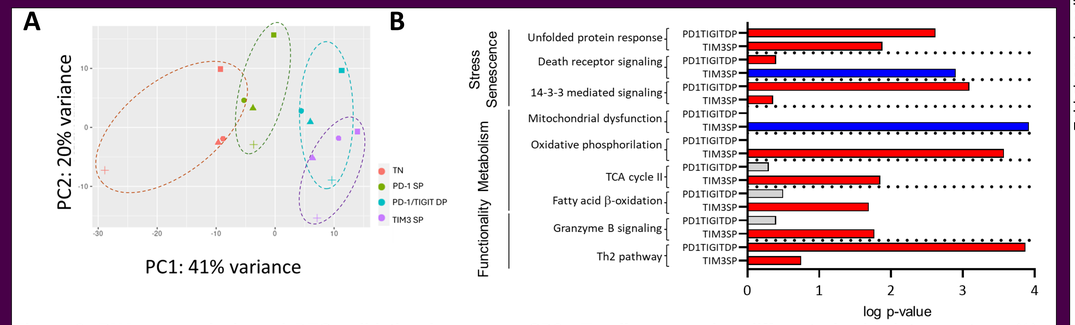


Figure 3. RNA-seq analysis and DEG validation in memory CD8+ T cells expressing different checkpoint receptors. (A): Principal component analysis (PCA) of transcriptional patterns of sorted PD1-/TIGIT-/TIM3- (TN), PD1+/TIGIT+/TIM3- (PD1/TIGITDP), PD1-/TIGIT-/TIM3+ (TIM3SP) CD8+ T cell memory cell subsets induced in the presence of allogeneic DC from n=4 donors. (B): IPA analysis of predicted pathways associated to metabolism for DEG TN, PD-1/TIGITDP, and TIM3SP. Upregulated and downregulated pathways are represented in red and blue, respectively. Level of significance is defined by a –log2 p values.

Results

1671 DEG identified in TIM3SP cells were associated with upregulation of modulators of OXPHOS, TCA cycle, and amino acid/lipid metabolism. In contrast, 866 DEG from PD-1/TIGITDPCD8+ T lymphocytes associated with immune function and mitochondrial dysfunction (**Figure 3A, B**). PD-1/TIGITDP CD8+T cells exhibited higher (p=0.002) ROS accumulation than TIM3SP from PWH (**Figure 4A**). In addition, TIM3SP CD8+T cells from PWH contained higher Arginase2+ (p=0.0002) and MGST2+ (p=0.0156) cells compared to TN cells (**Figure 4B**). Consistently, maximal mitochondrial-dependent respiration in TIM3SP, but not in PD1/TIGITDP CD8+T cells was dependent on fatty acid β-oxidation (p=0.0156) (**Figure 4C-D**).

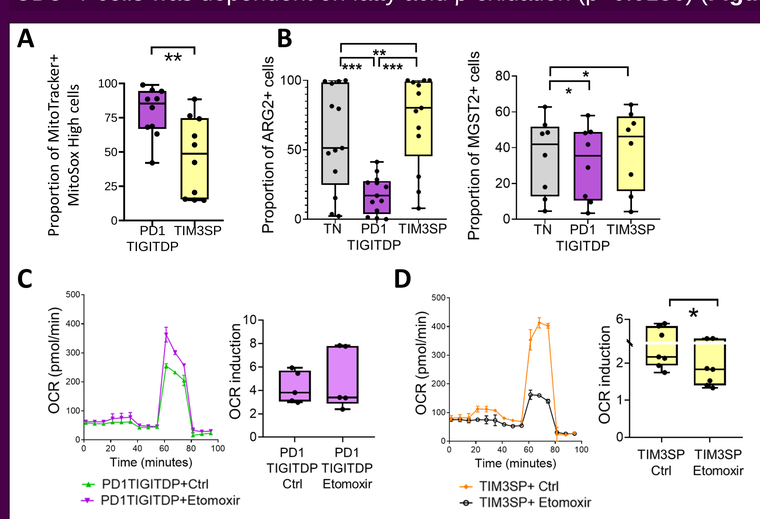


Figure 4. Validation of the expression and functionality of the pathways identified by RNA-seq. (A): Proportions of MitoTracker+ MitoSox High CD8+ T cells and (B): proportions of ARG2+ (left) and MGST2+ (right) CD8+ T cells in TN, PD1/TIGITDP and TIM3SP populations from PWH. (C): SeaHorse analysis of oxygen consumption rate (OCR) and fold change of induction of respiration by FCCP of TIM3SP or PD1/TIGITDP (D) CD8+ T in the absence or presence of fatty acids β-oxidation inhibitor etomoxir at 60 min timepoint. Statistical significance was calculated using two-tailed Wilcoxon test (*p<0.05, **p<0.01, ***p<0.001).

Notably, an increase of HIV Gag-specific IFNγ+CD107a+CD8+T cells was observed after Ornithine (p=0.0034) and LTC4 (p=0.0371) modulation. TNFα expression was also induced in the presence of Ornithine (p=0.0049) (**Figure 5A**). Finally, modulation of CD8+T cells with DC, TIGIT/PD1 blockade and Ornithine consistently and significantly reduced proportions of autologous HIV-1 infected cells *in vitro* (p=0.0156) (**Figure 5B**).

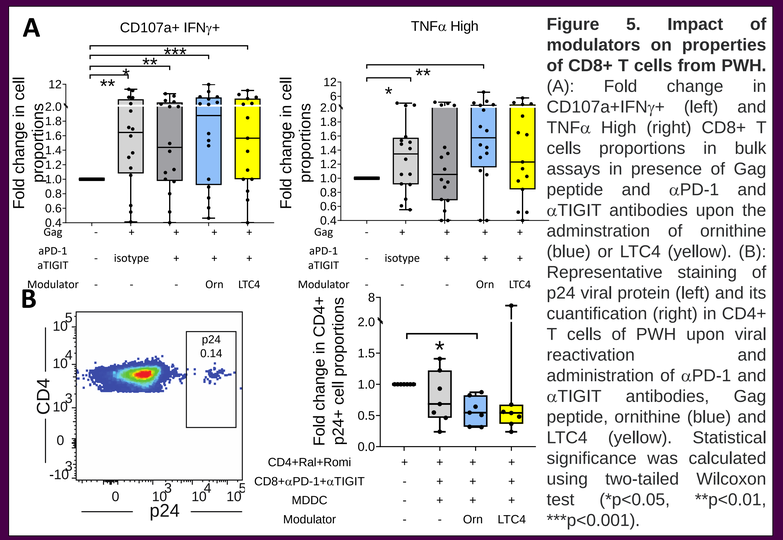
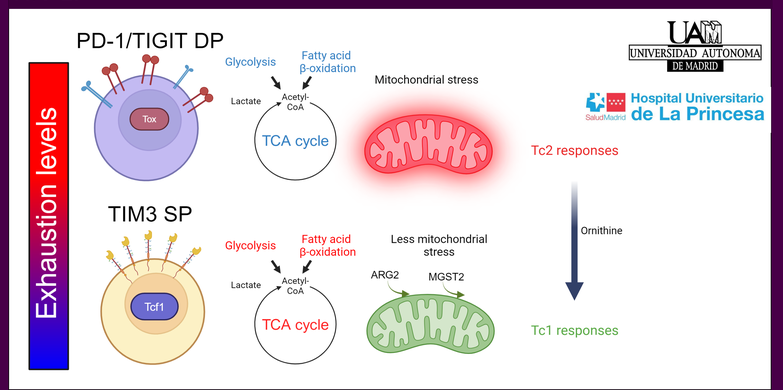


Figure 5. Impact of modulators on properties of CD8+ T cells from PWH. (A): Fold change in CD107a+IFNγ+ (left) and TNFα High (right) CD8+ T cells proportions in bulk assays in presence of Gag peptide and αPD-1 and αTIGIT antibodies upon the adminstration of ornithine (blue) or LTC4 (yellow). (B): Representative staining of p24 viral protein (left) and its cuantification (right) in CD4+ T cells of PWH upon viral reactivation and administration of αPD-1 and αTIGIT antibodies, Gag peptide, ornithine (blue) and LTC4 (yellow). Statistical significance was calculated using two-tailed Wilcoxon test (*p<0.05, **p<0.01, ***p<0.001).

Conclusion

TIM3SP cells represent a less exhausted, metabolically plastic population with different cytotoxic profiles compared to exhausted PD-1/TIGITDP cells. We have shown that stimulation of aminoacid and lipid metabolism of PD1/TIGITDP T cells from PWH potentiated the induction of functional Tc1-like IFNγ TNFα+ responses against HIV-1. Thus, our data may be relevant to improve immunotherapies against HIV-1.



ACKNOWLEDGMENTS

This study was supported by the Agencia Estatal de Investigación (PID2021-127899OB-I00; CNS2023-144841; RYC2018-024374-I)

and CIBER of Infectious diseases (CIBERINFEC), ISCIII (CB21/13/00107)

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