Universitätsklinikum Essen

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Metabolic Shut Down of CD4 T Cells Activity Induced by HAART

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Background & Aim

Metabolism plays a pivotal role in a cell's ability to maintain their viability and fulfil their effector functions. It has been shown that cells in chronically HIV-infected individuals become exhausted and undergo a progressive loss of hierarchical functions, but the changes in their cellular metabolism remain unclear. In this study we evaluate the impact of HIV infection and individual HAART regimens on two major metabolic pathways – oxidative phosphorylation and glycolysis as well as on cellular function.

Methods

Different cell types were isolated from PBMC of HIV-infected treatment-naïve and treated individuals as well as from healthy donors. Cells were stimulated in the presence of different ART regimens and their metabolic profiles were analysed by the extracellular flux analyser Seahorse XFp. We used multicolour flow cytometry to study the function and phenotype of PBMC of each individual and determined changes in ROS production as well as mtDNA content by qPCR.

Results

NK cells, B cells, CD4 and CD8 T cells from HIV infected treatment-naïve individuals displayed significantly reduced basal and maximal respiration compared to healthy controls. The metabolic capacity strongly correlated with the expression of the inhibitory receptor PD-1 ($p<0.0001$) and immune activation level (defined as HLA-DR+ CD38+ expression; $p<0.0001$). Interestingly, while long-term HAART treatment robustly restored the bioenergetic profile of NK cells, B cells and CD8 T cells, it had a negative effect on CD4 T cells, particularly in Dolutegravir (DLG) containing regimens.

We therefore assessed the impact of individual antiretrovirals on CD4 T cell metabolism. Strikingly, the integrase inhibitors (INSTI) Elvitegravir (EVG) and DLG, but not Raltegravir (RAL), shut down the basal and maximal respiration of CD4 T cells. This significantly altered the functional profiles of the cells by driving them from a balanced polyfunctional response to a TNFα-dominated 'stress' immune response. Analysis of mitochondrial ROS and mtDNA quantities revealed increased mitochondrial toxicity, but not general cytotoxicity, in the presence of these drugs.

I) Immune cells from HIV⁺ treatment-naïve individuals display reduced metabolic activity

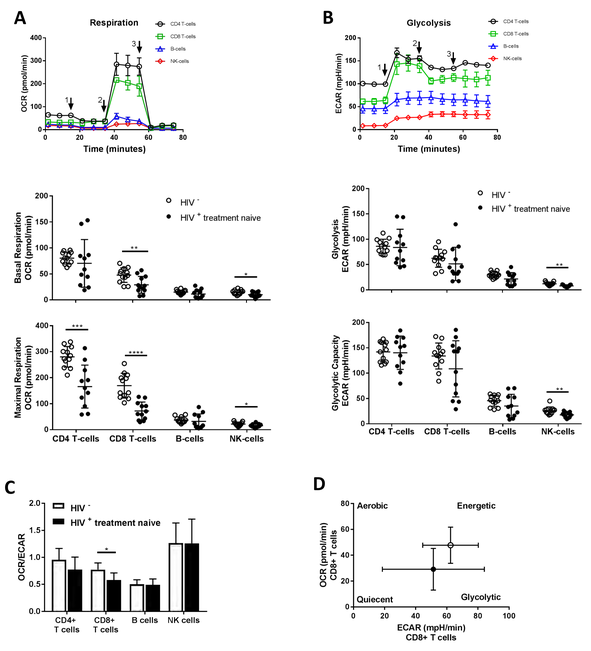


Figure 1: (A) Representative plot of OCR and comparison of basal and maximal mitochondrial respiration between HIV treatment-naïve individuals and healthy controls. (B) Representative plot of ECAR. Glycolysis and glycolytic capacity. (C) Differences in OCR/ECAR ratio. (D) Schematic graph of energetic profiles of investigated groups. Data of two groups: HIV⁻ (n=12), HIV⁺ treatment-naïve (n=11) are shown as mean ± SD. * indicates p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 by Student´s t-test.

II) Metabolic capacity correlates with expression of inhibitory and activation markers

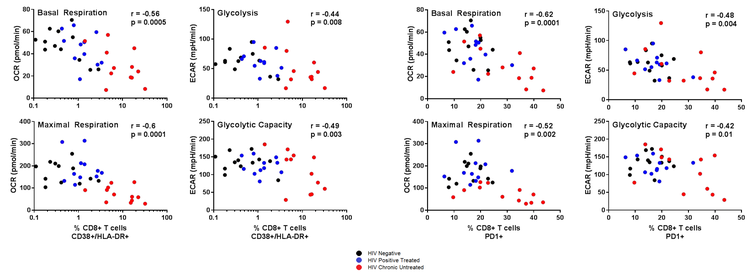


Figure 2: Negative correlation found between activation (characterized as expression of CD38⁺/HLA-DR⁺) and metabolism as well as between exhaustion (characterized as expression PD-1⁺) and metabolic parameters. Data of three groups: HIV⁻ (n=12), HIV⁺ treatment-naïve (n=11) and HIV⁺ HAART treated (n=12) individuals.

III) HAART has a negative impact on oxidative metabolism of CD4⁺ T cells

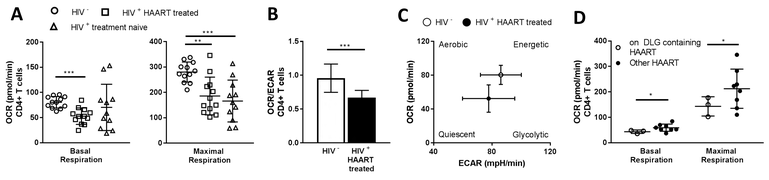
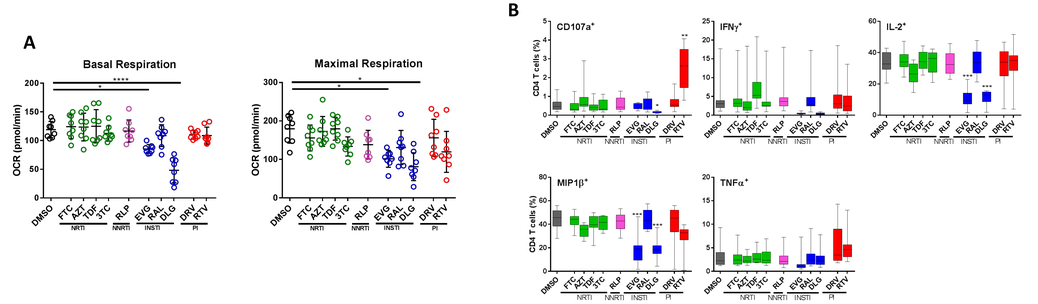


Figure 3: (A) Basal and maximal respiration of HIV⁻ and HIV⁺ HAART treated individuals showing a significant decrease in OCR upon 3 day stimulation with anti CD3/CD28 beads. (B) HIV⁺ HAART treated individuals display lower OCR/ECAR ratio and (C) are less energetic compared to HIV⁻ controls. (D) Decreased oxidative metabolism of HIV⁺ individuals on HAART containing DLG compared to others. Data of all groups HIV⁻ (n=12), HIV⁺ HAART treated (n=12), HIV⁺ treatment-naive (n=11) are shown as mean ± SD. * indicates p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 by One-Way ANOVA.

IV) HAART containing DLG or EVG influences metabolism and functionality of CD4⁺ T cells



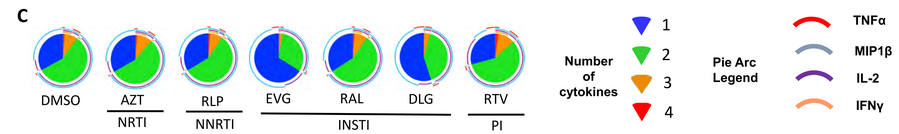


Figure 4: Basal (A) and maximal respiration (B) of CD4 T cells exposed to different ART regimens for 3 days showing significant decrease in OCR in cells treated with DLG and EVG. (C) Decreased secretion of cytokines: CD107a, IFNγ, IL-2, MIP1β and TNFα upon 3 day stimulation with SEB in presence of different ART regimens. (D) Switch from polyfunctional response to SEB stimulation to TNFα dominating monorespose. Data are representative of n=8 HIV⁻ individuals and shown as mean ± SD. * indicates p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 by One-Way ANOVA.

V) Increased mtROS production and mtDNA content

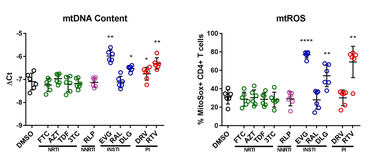


Figure 5: CD4 T cells exposed to different ART regimens for 3 days showing increased production of mtROS when exposed to DLG, EVG and RTV. (B) Increased mtDNA content of CD4 T cells upon incubation with DLG and EVG for 3 days. Data are representative of n=6 HIV⁻

Conclusion

Taken together, our data demonstrate a substantial disruption in the metabolic activity of lymphocytes during chronic HIV infection that is restored through antiretroviral therapy. However, two INSTI, DLG and EVG, diminish the metabolic activity in CD4 T cells, leading to a switch in functionality and impairment of overall function.

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