CD4+ T-cells expressing negative checkpoint receptors are associated with decreased mitochondrial oxidative phosphorylation in chronic HIV

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Background

Despite virally suppressive antiretroviral therapy (ART) regimens, chronic HIV is associated with increased expression of multiple negative checkpoint receptors (NCRs) on CD4+ and CD8+ T-cells that has been associated with T-cell dysfunction, immune activation and HIV viral persistence. The underlying mechanisms are multifactorial. However, the role of cellular immunometabolism in HIV remains under investigated. We assessed the relationship between T-cell immune exhaustion and cellular bioenergetics as determined by mitochondrial oxidative phosphorylation (OXPHOS) in peripheral blood mononuclear cells (PBMCs) in a well characterized cohort of chronic HIV-infected individuals on ART.

Methods

From 2009 to 2012, the Hawaii Aging with HIV cohort enrolled patients with documented HIV infection, age≥40 years old, and on stable ART ≥3 months. OXPHOS complex I (CI, NADH dehydrogenase) and complex IV (CIV, cytochrome *c* oxidase) protein levels in PBMCs were quantified using immunoassays, as previously described.[1] Monocyte subsets and markers of T-cell activation, senescence, and exhaustion were measured on PBMC by flow cytometry.[2] Plasma inflammatory mediators were quantified using a multiplex assay. HIV-uninfected group (N=44) of similar age, gender, and ethnicity had available OXPHOS levels.

Results

Of 149 HIV+ patients, median age was 51 years, current CD4 count 505 cells/uL, and nadir CD4 count 150 cells/uL. Majority (88.4%) were male and had undetectable plasma HIV RNA<50 copies/ml (83.7%). Of the older NRTIs, 7.5% were on zidovudine, 1.9% were on didanosine. No patients were on integrase inhibitors. Current didanosine use was associated with significantly lower median PBMC CI (23.5 vs 66.5, p=0.03) and CIV (21.9 vs 49.5, p=0.05) protein levels.

Among HIV+ patients, lower CI protein levels correlated with lower CD4 count (r = 0.19, p=0.02), CD4% (r = 0.18, p=0.02), and CD4/CD8 ratio (r = 0.18, p=0.03). Higher percentages of NCR-bearing T-cells correlated with lower OXPHOS levels (Table).

Spearman correlation between negative checkpoint receptors in T-lymphocytes and PBMC mitochondrial OXPHOS

Negative checkpoint receptors (%)	Complex I (optical density (OD)/µg of protein×10³)	Complex IV (OD/µg of protein×10³)
TIM3+ CD8 T-cells	-0.21 (p=0.18)	-0.30 (p=0.05)
TIGIT+ TIM3+ CD8 T- cells	-0.16 (p=0.31)	-0.32 (p=0.04)
TIGIT+ CD4 T- cells	-0.33 (p=0.03)	-0.35 (p=0.02)
PD1+ TIGIT+ CD4 T-cells	-0.35 (p=0.03)	-0.36 (p=0.02)
PD1+ TIM3+ CD4 T-cells	-0.31 (p=0.05)	-0.29 (p=0.06)
TIGIT+ TIM3+ CD4 T-cells	-0.40 (p=0.009)	-0.42 (p=0.006)

Presented are Spearman's rho (r) and p-values.

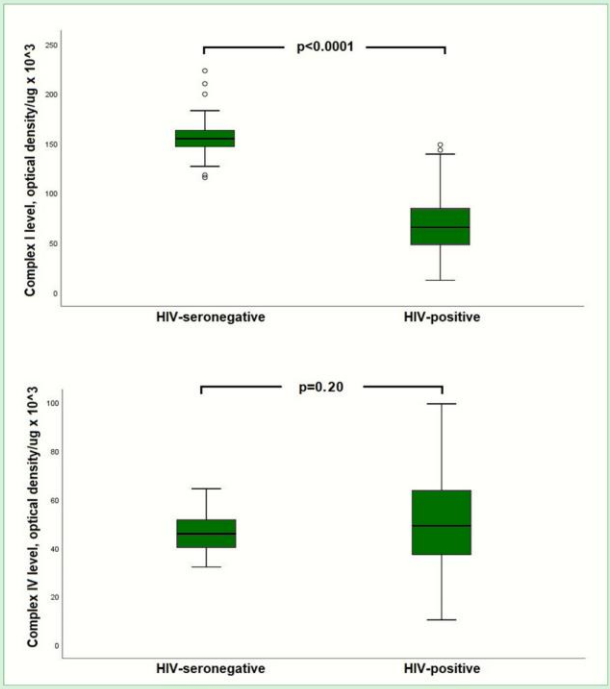

Figure 1. Comparison of PBMC OXPHOS protein levels between HIV+ and seronegative group.

Linear regression analyses of immunologic parameters associated with PBMC OXPHOS protein levels

	Multivariable*	
	Complex I	Complex IV
CD4 percent	0.21 (p=0.015)	0.19 (p=0.026)
CD4/CD8 ratio	0.23 (p=0.008)	0.20 (p=0.022)
MCP-1	-0.24 (p=0.008)	-0.17 (p=0.059)
MPO	-0.25 (p=0.005)	-0.31 (p<0.001)
SAA	-0.42 (p<0.001)	-0.37 (p<0.001)
SAP	-0.42 (p<0.001)	-0.45 (p<0.001)
sVCAM	-0.25 (p=0.006)	-0.33 (p<0.001)
Intermediate ($CD14^{++}CD16^{+}$) monocyte %	-0.26 (p=0.002)	-0.23 (p=0.007)
TIGIT+ CD4 T- cell (%)	-0.35 (p=0.039)	-0.38 (p=0.022)
PD1+ TIGIT+ CD4 T-cells (%)	-0.35 (p=0.037)	-0.41 (p=0.016)
PD1+ TIM3+ CD4 T-cell (%)	-0.31 (p=0.084)	-0.37 (p=0.040)
TIGIT+TIM3+ CD4 T-cell (%)	-0.40 (p=0.018)	-0.49 (p=0.004)

* Presented are β-coefficient and p-values. Separate multivariable linear regression analyses were performed for each immunologic parameter, adjusted for age, use of zidovudine or didanosine, and HIV RNA (detectable vs undetectable). Plasma cytokines were log-transformed. NCR-bearing CD8 T-cells were *not* significant on multivariable regression.

Conclusions

In our cohort of older HIV patients on stable ART, mitochondrial CI protein levels were significantly decreased compared to HIV-uninfected persons. Lower OXPHOS levels in PBMCs correlated with disease severity, as assessed by CD4% and CD4/CD8 ratio. Lower PBMC OXPHOS levels correlated with higher soluble plasma markers of inflammation (MCP-1, MPO, SAA, SAP, and sVCAM), and higher percentages of the pro-inflammatory intermediate monocyte subset. It has been well documented that HIV proteins exert direct toxicity to the mitochondria.[3] Inflammatory mediators are known to induce mitochondrial dysfunction, increasing reactive oxygen species (ROS), leading to a vicious cycle of mitochondrial damage and inflammation.[4]

Decreased CI and CIV protein levels in PBMCs were strongly associated with increased frequency of TIGIT+TIM3+ CD4 T-cells. We have previously reported that TIGIT expression on CD4 T-cells correlates with total HIV DNA and residual immune activation despite suppressive ART.[2] The expression of TIM-3 on CD4 T-cells prior to ART predicted the time to viral rebound after treatment interruption.[5] Our findings suggest that immunometabolism may play a role in HIV persistence. Further studies are needed to investigate the relationship between CD4 T-cell exhaustion, immunometabolism, and HIV persistence.

References:
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