Glutaminolysis Alters CD4+ T-Cell Immunometabolism And Inhibits HIV Transcription

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BACKGROUND

- We recently reported (Giron et al., *Nature Comm.* 2021) that the plasma levels of the metabolite glutamic acid predict a delayed time-to-viral-rebound and a higher probability of achieving a post-treatment controller phenotype after antiretroviral therapy (ART) interruption (Fig. 1).
- Glutamic acid metabolism (**glutaminolysis**) can fuel the tricarboxylic acid (TCA) cycle through its conversion to α-ketoglutarate (α-KG), which modulates the cellular immunometabolic and epigenetic landscapes.
- We aimed to investigate the potential direct impact of glutamic acid and α-KG on latent HIV transcription in vitro and ex vivo.

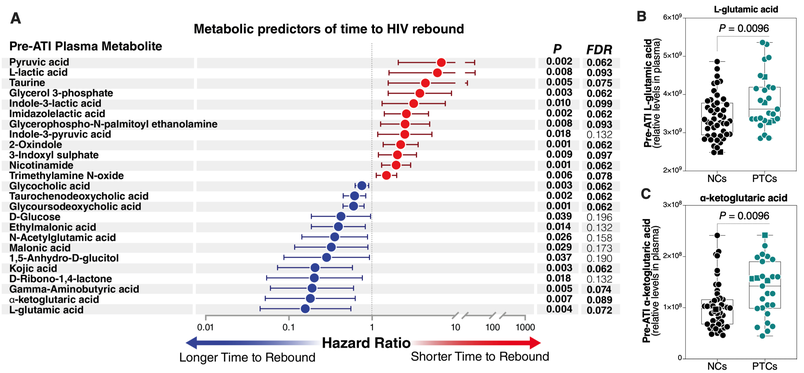


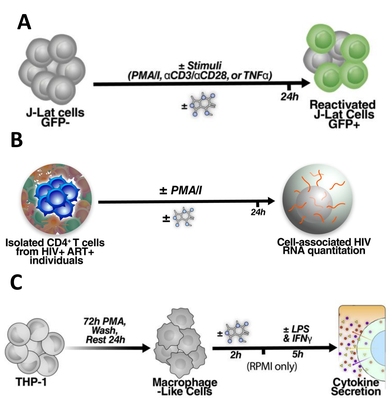
Figure 1. Pre-ATI plasma metabolic biomarkers associate with time-to-viral-rebound and with PTC phenotype. (A) Metabolites that associate with a longer (blue) or shorter (red) time-to-viral rebound during ATI (Cox proportional-hazards model). False Discovery Rate (FDR) was calculated using Benjamini-Hochberg correction. (B-C) Levels of L-glutamic acid and α-KG in plasma distinguish PTCs from NCs.

METHODS

A. JLat 5A8 cells were stimulated with appropriate stimuli in the presence or absence of L-glutamic acid or α-KG. MFI of HIV-regulated green fluorescent protein (GFP) expression was measured by flow cytometry.

B. Primary CD4⁺ T cells were isolated from HIV+ART+ individuals and stimulated with PMA/I in the presence of L-Glutamic acid. Cell-associated HIV RNA quantification was performed using qPCR and the effects of glutamic acid on the transcriptome of primary CD4+ T cells were evaluated by RNA-seq

C. THP-1 cells were differentiated into macrophage-like cells. Cells were treated with L-glutamic treated before LPS/IFNγ stimulation and cytokine secretion was measured.



RESULTS

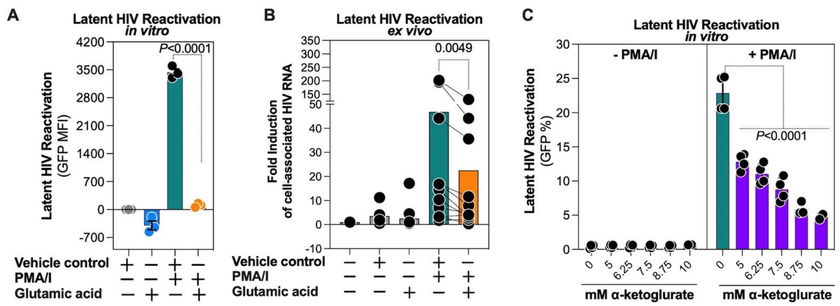


Figure 3. L-Glutamic acid modulates HIV reactivation. In vitro (A) (n = 3 independent experiments) or ex vivo (B) treatment with L-glutamic acid inhibited the ability of PMA/I to reactivate HIV. (C) Treatment with α-KG also inhibited the ability of PMA/I to reactivate HIV in a dose-dependent manner.

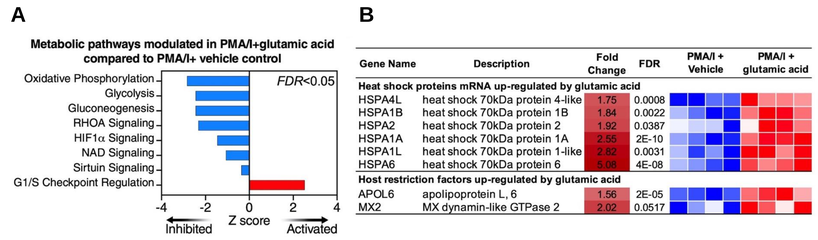


Figure 4. L-Glutamic shifts cell metabolism. (A) Transcriptomic analyses revealed that, during PMA/I stimulation, glutamic acid inhibited several metabolic pathways that induce HIV transcription and replication including, oxidative phosphorylation, glycolysis, HIF1α signaling, NAD signaling, and Sirtuin signaling. (B) In contrast, glutamic acid induced the mRNA expression of several proteins that suppress HIV transcription and replication, including HSP70 and the anti-HIV host restriction factors APOL6 and MX2 (FDR<0.05; Blue= lower expression, red= higher expression.

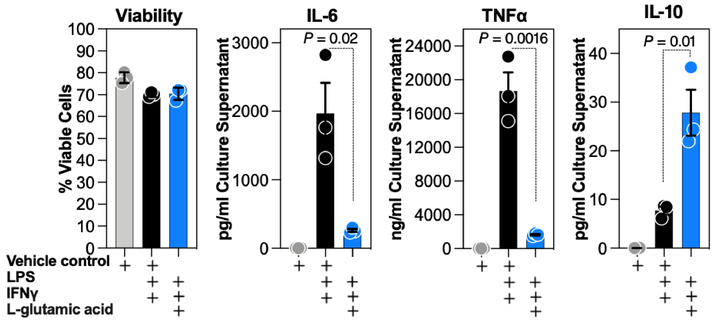
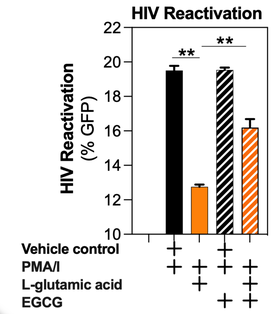
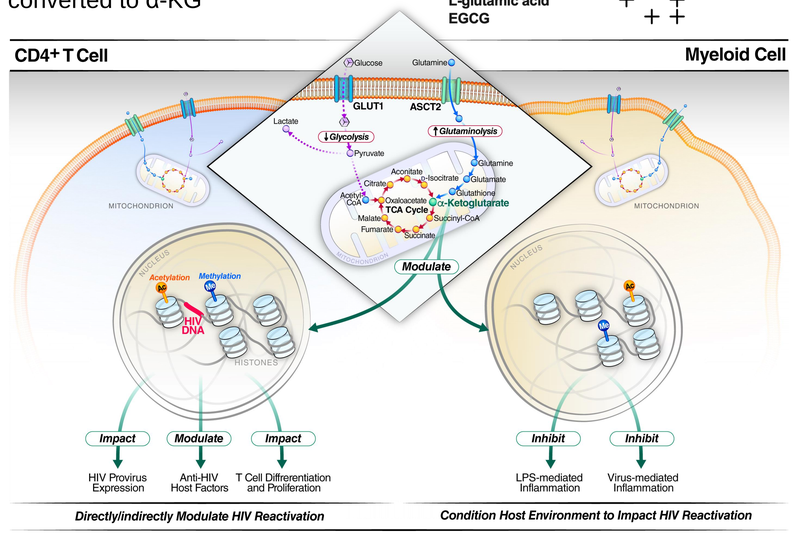


Figure. 5. L-glutamic acid inhibits meyloid inflammation. Differentiated THP-1 cells were treated with L-glutamic acid before LPS/IFNγ stimulation. L-glutamic acid inhibited LPS/IFNγ-mediated secretion of pro-inflammatory cytokines but increased the secretion of anti-inflammatory cytokines.

Figure 5. Treatment with EGCG (an inhibitor of the conversion of L-glutamic acid to α-KG) partially reversed the L-glutamic-acid-mediated inhibition of PMA/I-mediated induction of HIV reactivation. This suggested that L-glutamic acid inhibits HIV transcription, at least partly, by being converted to α-KG





CONCLUSIONS

- Beyond the utility as biomarkers, we confirmed plausible mechanistic links between L-glutamic acid, α-ketoglutarate, and HIV latency reactivation.
- Glutaminolysis, a metabolic pathway previously associated with post-treatment control of HIV, may shift the immunometabolic status of CD4+ T cells to inhibit HIV transcription and replication.
- Future investigations are needed to understand the mechanistic underlings of how glutaminolysis may regulates HIV control during ART and post-ART cessation. This understanding may allow the development of novel immunometabolic approaches to recapitulate the post-treatment controller phenotype.

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