Extracellular Acyl-CoA-Binding Protein prevents autophagy and T-cell function in people with HIV

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BACKGROUND

T-cell responses require large amounts of energy to produce cytokines and cytolytic molecules. T-cells primarily on glycolysis, oxidative phosphorylation (OxPhos) and autophagy to generate energy. In people with HIV (PWH), we have demonstrated the critical role of autophagy in efficient T-cell responses. Autophagy and glutaminolysis enable antigen-specific CD4 T-cells to produce IL-21, which stimulates lipophagy and enhances the production of perforin and granzyme B by antigen-specific CD8 T-cells (*Loucif, Autophagy 2021*). Moreover, autophagy levels are elevated in T-cells of HIV elite controllers (ECs) - a rare subset of PWH capable of controlling HIV replication without antiretroviral therapy (ART) - compared to progressors.

Factors associated with impaired autophagy and OxPhos induction in T-cells of PWH on ART remain unknown.

Autophagy is in part regulated by the acyl-CoA-binding protein (ACBP) which has a dual function: **Intracellular ACBP promotes autophagy** by shuttling activated Acyl-CoA bound lipids to cellular organelles including the mitochondria. However, **secreted extracellular ACBP inhibits autophagy** and increases appetite.

Herein, we assessed whether extracellular ACBP influences autophagy and T-cell function in PWH.

METHODS

- ACBP and cytokines levels were quantified in plasma by ELISA and multiplexes assays.
- Intracellular levels of cytokines, ACBP and autophagy molecules were assessed by flow cytometry. Proliferation was assessed by flow cytometry measuring CFSE dilution.
- PBMCs were stimulated with anti-CD3 antibodies, PMA+ionomycin or HIV peptides (Gag, Pol, Env, Nef). Recombinant ACBP (recACBP) was added at specified concentrations for 16-18h. Brefeldin A was included for intracellular cytokine labelling.
- Metabolites were quantified in serum samples by GC-MS.

DEMOGRAPHICS

		Elite controllers	HIV ART+	Controls without HIV
Number of participants		37	55	31
Age		45.5 (25-72)	54 (26-74)	52 (23-75.5)
Sex	Female	9 (24%)	5 (9%)	8 (26%)
	Male	28 (86%)	50 (91%)	23 (74%)
Viral load (copies/mL)		<50 (<20-<50)	<50 (<20-<50)	NA
Infection duration (years)		6 (0.7-27)	17.2 (2-33)	NA
ART duration (years)		NA	13.7 (0.2-25.4)	NA
CD4 count (cells/µL)		640 (290-1200)	546 (1.4-1462)	827 (281-1173)
CD8 count (cells/µL)		649 (211-1460)	719 (2.46-1475)	391 (188-1245)
CD4/CD8		1.02 (0.34-3.06)	0.7 (0.2-2.1)	2.1 (0.38-3.98)

Circulating ACBP levels are elevated in PWH and are associated with inflammation. Extracellular ACBP inhibits autophagy induction and impairs T-cell responses, highlighting the need for ACBP inhibition strategies to enhance immune function towards an HIV cure in PWH.

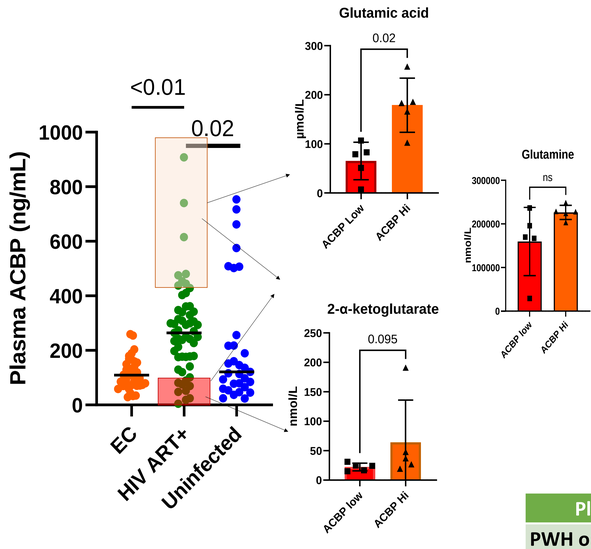


Figure 1: Plasma ACBP levels were higher in ART-treated PWH and associated with alterations in circulating metabolites.
Left: Plasma ACBP levels. Kruskal-Wallis' test with Dunn's post test. Multivariable analyses showed that age, sex and weight did not influence these differences.
Right: Serum metabolites from 5 participants with high (orange) or low (red) ACBP levels. Mann Whitney's test.

Table 2: In ART-treated PWH, plasma ACBP levels correlated positively with plasma inflammatory markers but correlated inversely with IL21 levels. Multivariable analysis indicated independence of these correlations with age and sex. Spearman's test.

Plasma levels of ACBP vs. other markers			
PWH on ART	Factor	Slope	P-value
Growth factor	G-CSF	**0.48**	**0.002**
	GM-CSF	**0.44**	**0.006**
Homeostatic factors	IL-7	**0.48**	**0.002**
	IL-15	**0.4**	**0.012**
Inflammation	IFN-α2	**0.37**	**0.02**
	IFN-γ	**0.4**	**0.01**
	IL-1β	**0.43**	**0.007**
	sCD40L	**0.67**	**0.003**
Immune function	**IL-21**	**-0.54**	**<0.001**

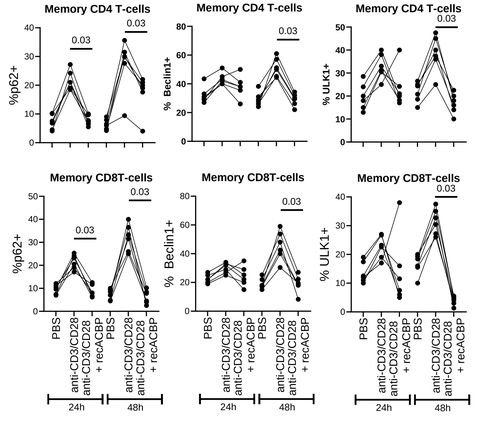
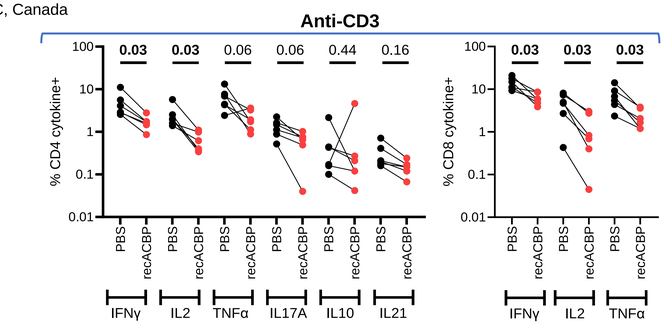


Figure 2: Extracellular ACBP prevented autophagy induction upon T-cells activation. PBMCs from healthy donors were stimulated or not for 24 and 48h with anti CD3/CD28 antibodies and levels of autophagy markers p62, Beclin-1 and ULK1 were assessed by flow cytometry in memory CD4 (top panels) and CD8 (bottom panels) T-cells. Wilcoxon's tests.



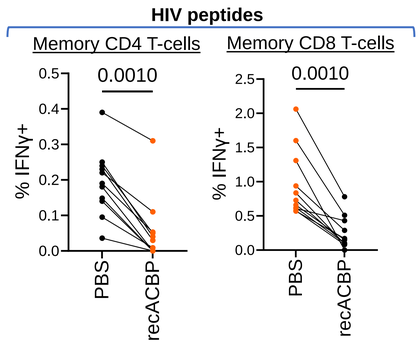


Figure 3: Extracellular ACBP suppressed polyclonal and HIV-specific T-cell responses. RecACBP (10µg/mL) was added to PBMCs from ART-treated donors and stimulated with anti-CD3 (1µg/mL) or HIV peptides Intracellular cytokine production was assessed in memory CD4 and CD8 T-cells by flow cytometry. Wilcoxon's tests. Similar results for TNF-a and IL-2 production as well as CFSE dilution (data not shown)

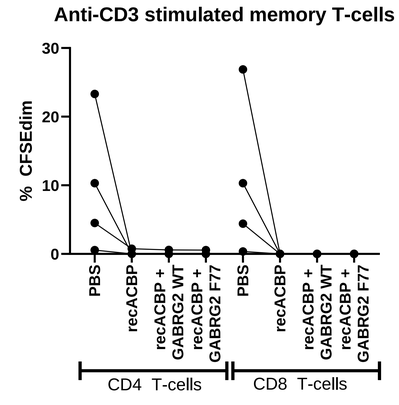


Figure 4: Extracellular ACBP inhibited T-cell activation independently of the GABA-receptor. In mice, Anagnostopoulos *et al.* (*Cell Death Dis 2024*) demonstrated that ACBP inhibits autophagy in the liver through the $GABA_A$ Receptor. Addition of $GABA_AR$ peptides (WT mutated (F77)) blocked the effect of recACBP in mice. These $GABA_AR$ peptides did not prevent the inhibitory effect of ACBP on CD4 or CD8 T-cells following anti-CD3 stimulation.

CONCLUSIONS

↗ plasma ACBP levels → Altered serum metabolite composition.
↗ plasma ACBP levels → ↗ inflammation but ↘ IL-21 plasma levels
RecACBP → prevents autophagy induction and cytokine secretion by CD4 and CD8 T-cells following anti-CD3 or HIV-peptide stimulation.
➔ Targeting extracellular ACBP with blocking antibodies could enhance autophagy and promote more effective immune responses in PWH.

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