Cocaine and Peripheral Serotonin Drive Impaired T-cell Function and HIV Persistence

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BACKGROUND

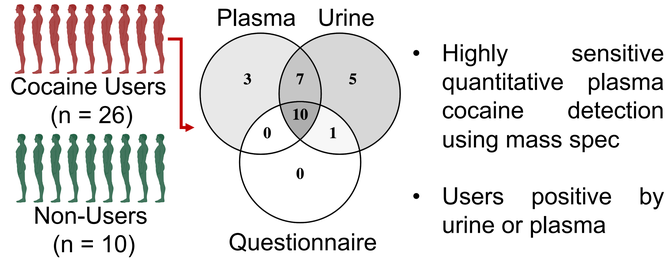
- Substance abusers are prevalent amongst HIV-infected individuals (In the US, 18-20% of HIV+ are IDU).
- Chronic cocaine users show lower reduction in HIV RNA levels following HAART.

We hypothesize that pro-inflammatory responses downstream of cocaine use drive HIV persistence

METHODS

Non Users **Cocaine Users**

STUDY COHORT AND EXPERIMENTAL PLAN



- Highly sensitive quantitative plasma cocaine detection using mass spec
- Users positive by urine or plasma

Ex vivo –Omic assessment between two groups

- 23 plasma cytokines (Mesoscale Discovery U-Plex)
- Plasma metabolites/neurotransmitters (Semi-quantitative Liquid Chromatography mass spectrometry)
- Plasma cocaine metabolites (quantitative mass spectrometry)
- 28 color flow cytometry: Innate, T cell function
- Whole blood RNA-sequencing
- Inducible HIV in CD4 T cells using Tat-Inducible Limiting Dilution Assay (TILDA)
- -Omic integration: Mechanistic multiomic models that are associated with CD4 T cell function and HIV persistence
- Validation: Impact of key metabolites/neurotransmitters on T cell activation/exhaustion in vitro
- Comparable CD4 counts, CD4:CD8 T cell ratio and TILDA and other demographics between users and non-users

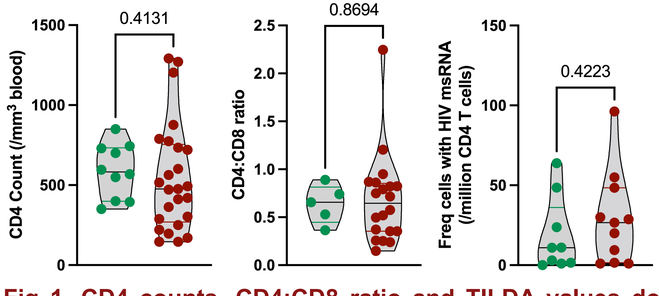


Fig 1. CD4 counts, CD4:CD8 ratio and TILDA values do not differentiate the 2 groups. Mann-Whitney U-test.

RESULTS

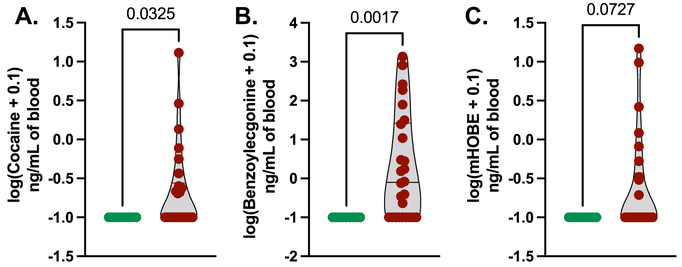
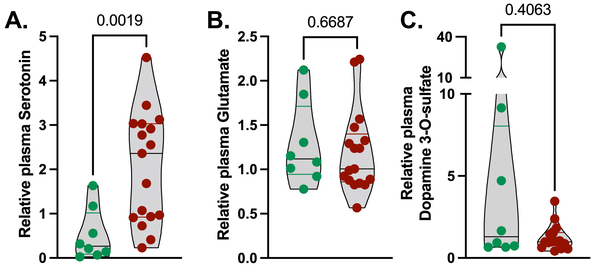


Fig 2. Significant increase in plasma cocaine metabolites in users. Absolute quantification of plasma cocaine (A), benzoylecgonine (B) and mHOBE (C) using mass spectrometry shows that chronic cocaine users have upto a 4-log dynamic range of cocaine metabolites in whole blood. Mann-Whitney U test.

Fig 3. Plasma Serotonin is significantly increased in chronic cocaine users. Levels of peripheral neurotransmitters measured using LC/MS showed that relative levels of plasma serotonin (A) were higher in users vs non-users, while glutamate (B) and dopamine (C) levels remained comparable .



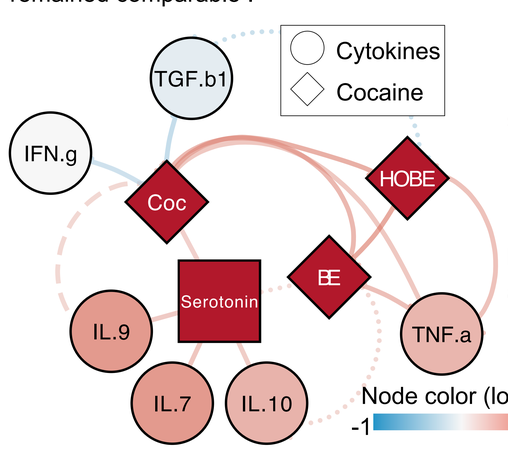
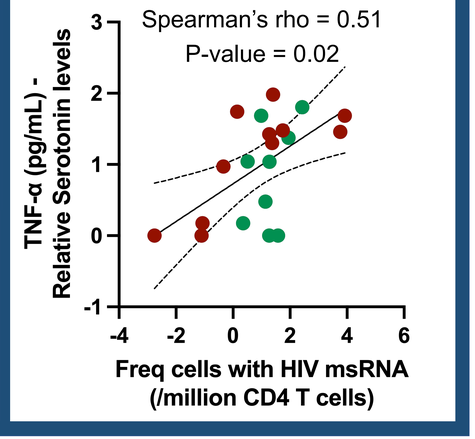


Fig 4. Plasma cocaine metabolites and plasma serotonin levels are associated with increased pro-inflammatory milieu. Correlation network showing the interaction between plasma drug levels, peripheral neurotransmitters and cytokines. These data highlight that levels of cocaine and serotonin are higher in the users and are associated with increased plasma TNF-α, IL-7, IL-9 and reduced levels of anti-inflammatory cytokine TGF-β. Solid edges: p<0.05. Dotted edges: p<0.1

Feature selection determines that TNF-α and Serotonin levels in plasma drives levels of inducible HIV.

LASSO based feature selection – using plasma features that are associated with cocaine levels – was used to define the least number of variables that are the best determinants of inducible HIV. Linear modeling where equal weights of TNF-α were subtracted from Serotonin was found to strongly correlate with inducible HIV.



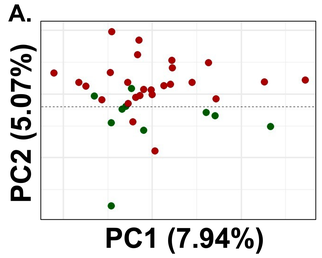


Fig 5. Cocaine users have distinct blood transcriptome. PC analyses shows distinct clustering of cocaine users in blood (A) where interferon, inflammatory responses, and cell proliferation/activation pathways were upregulated with TNF-α (B).

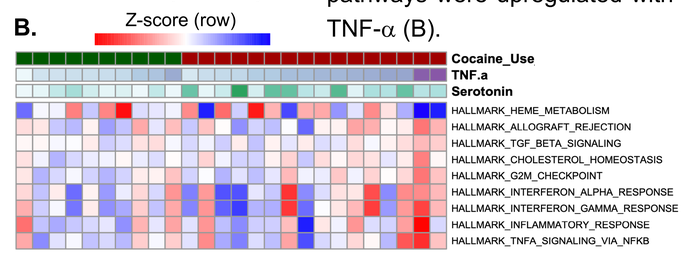
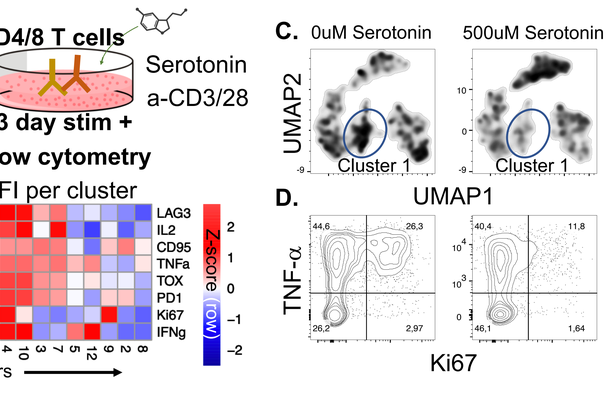


Fig 6. Serotonin limits T cell activation in vitro. (A) In vitro experimental design. (B) MFI heatmap of unbiased CD4 T cells phenograph clusters. (C) Significant decline in effector cluster 1 shown in UMAP projection of Serotonin stimulated CD4 T cells. (D) Lower Ki67 and TNF-α in Serotonin stimulation



CONCLUSION AND NEXT STEPS

- Plasma cocaine levels associated with peripheral Serotonin and pro-inflammatory milieu (higher TNF-α) drives HIV persistence
- Higher levels of TNF-α are linked to a pro-inflammatory transcriptome and can be subdued with Serotonin in vitro
- Further studies required to assess the role of cocaine induced peripheral serotonin on HIV latency

ACKNOWLEDGEMENTS

Funding support from the following grants:
5R01DA042712-05, 5R01DA043263-05
5R61DA047022-03

NIH National Institute on Drug Abuse