Methamphetamine Use Disorder in PWH on ART is Associated with Elevated NLRP3 Inflammasome Activation

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BACKGROUND

Methamphetamine (MA) use disorder among people with HIV (PWH) is linked to lower antiretroviral therapy (ART) adherence, increased viral resistance, and poorer clinical outcomes[1]. We recently demonstrated that MA exposure during ART is associated with increased HIV transcription of the viral reservoir. *In vitro* and animal studies have shown that MA leads to dysregulated immune activation and inflammation[2,3]. Here, using a multiomic approach, we identified distinct immune signaling pathways and HIV RNA transcript levels that varied by MA exposure from lymphoid tissues in PWH on ART.

METHODS

Ten ART-suppressed PWH with MA use disorder were evaluated longitudinally across six visits (**Fig. 1, Table 1**). At two visits, blood samples were paired with inguinal lymph node samples collected via fine-needle aspiration. MA levels were measured in plasma, serum, and urine. HIV transcription (RT-ddPCR)[4] and protein markers of immune phenotype and function were measured (flow cytometry) from PBMCs. Single cell gene expression was analyzed from lymph node samples. Linear mixed-effect models were used to associate MA concentrations to measures of HIV transcription and to host immune responses.

Figure 1. Cohort design and sample collection

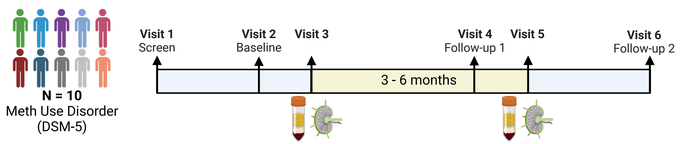


Table 1. Participants characteristics

Characteristics	PWH MA+ (N = 10)
Age (years)	54.5 (46 - 63)
Sex at birth (% male)	9 (90%)
Race/Ethnicity (self-reported)	
African-American (%)	0 (0%)
Asian (%)	1 (10%)
White/Hispanic/Latino (%)	1 (10%)
White/European American (%)	8 (80%)
Nadir CD4+ T cell count (cells/mm^3)	385.4 (7 - 759)
Baseline CD4+ T cell count (cells/mm^3)	587.7 (201 - 937)
Baseline CD4:CD8 ratio	0.94 (0.33 - 1.87)
Duration of ART Suppression (years)	5.125 (1 - 10)
Baseline Viral Load (copies/mL)	
Not detected	4 (40%)
Detected < 30	6 (60%)

Mean (min. – max.)

HIV transcription in MA use disorder is associated with NLRP3 inflammasome activation in myeloid cells and upregulation of metabolic and inflammatory pathways in lymph nodes of PWH

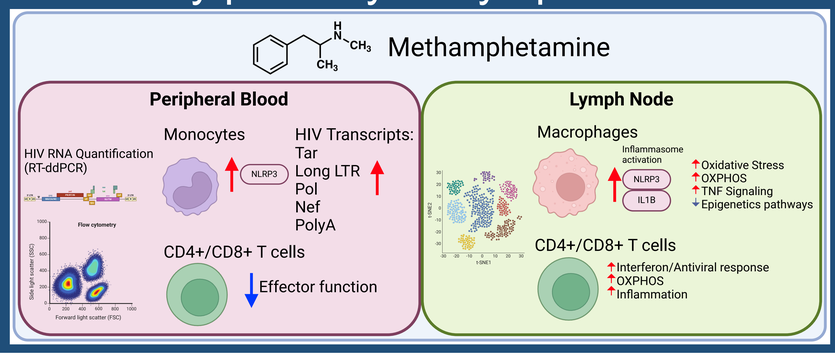
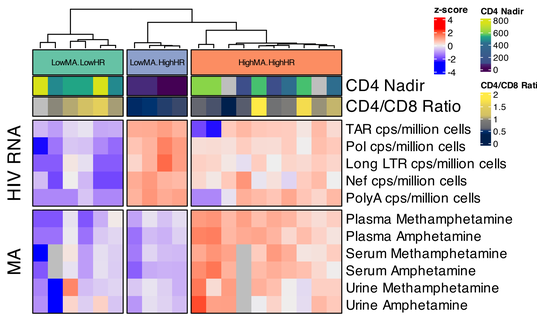
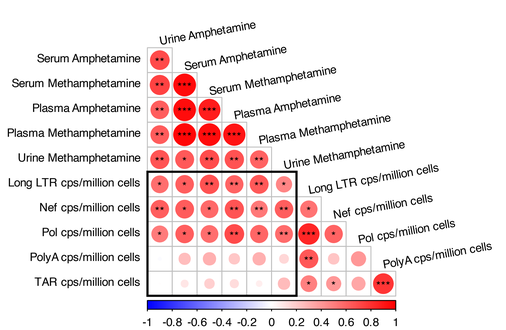


Figure 2. Hierarchical clustering of MA concentrations and HIV RNA transcript[4] levels in blood identified 3 distinct clusters of participants



We identified 3 groups of participants: i) Low MA and Low HIV RNA (LowMA.LowHR); ii) low MA and high HIV RNA (LowMA.HighHR); and iii) high MA and high HIV RNA (HighMA.HighHR). Notably, the LowMA.HighHR group was found to have lower nadir CD4+ T cell count and CD4/CD8 ratio compared to the other groups suggesting non-MA-induced mechanisms associated with higher HIV RNA transcription[4].

Figure 3. PWH on ART with higher MA exposure had increased levels of HIV reservoir transcripts in peripheral blood



Spearman correlation matrix of participants in the LowMA.LowHR vs. HighMA.HighHR groups. *p < 0.05, **p < 0.01, ***p < 0.001.

Figure 4. Individuals with higher MA concentrations and higher HIV transcription had increased expression of NLRP3 inflammasome associated markers (e.g., IL-1β) from peripheral myeloid and CD4+ T cell subsets and decreased expression of markers of effector function (GZMB and IFNG) from CD4+ and CD8+ T cells

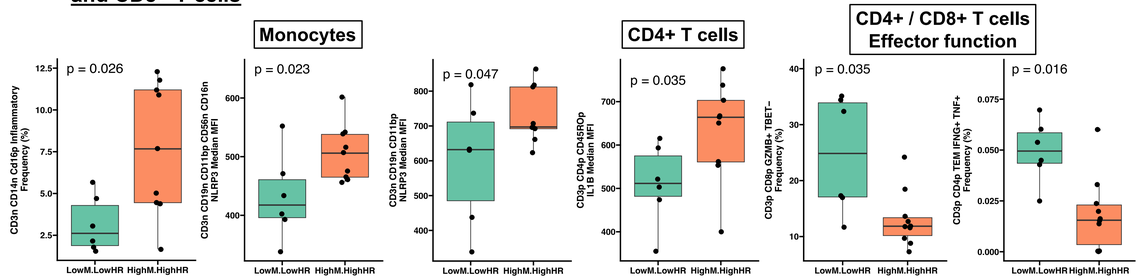
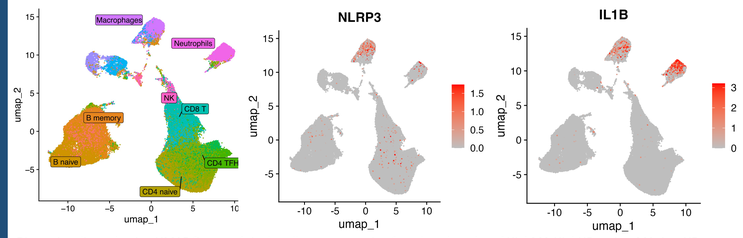


Figure 5. *NLRP3* and *IL1B* genes were highly expressed in macrophages and neutrophils from lymph nodes (LN).



Dimensionality reduction UMAP figures of single cell gene expression from lymph nodes of HighMA.HighHR vs. LowMA. LowHR group.

Figure 6. Individuals with high MA concentrations and high HIV transcription had increased oxidative stress and TNF signaling and decreased epigenetic modification signaling from LN macrophages

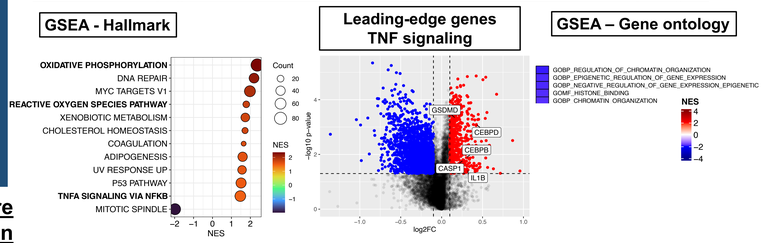
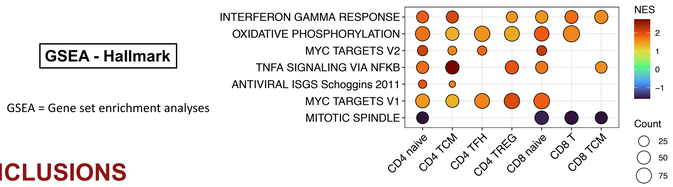


Figure 7. Individuals with high MA concentrations and high HIV transcription had increased antiviral (interferon), oxidative stress, and TNF signaling from LN CD4+ and CD8+ T cells



GSEA = Gene set enrichment analyses

CONCLUSIONS

- ART suppressed PWH who use MA and have higher concentrations of MA exhibit increased HIV reservoir transcription in the peripheral blood.
- Individuals with high MA and HIV transcription demonstrate increased NLRP3 inflammasome activation and decreased CD4+ and CD8+ T cells effector function in the periphery.
- Higher MA exposure and HIV reservoir transcription was also associated with increased *NLRP3* and *IL1B* expression, with upregulation of oxidative stress and inflammatory pathways and downregulation of epigenetic pathways, from lymph node macrophages while CD4+ and CD8+ T cell subsets from lymph nodes demonstrated upregulation of genes associated with antiviral (interferon) responses.
- To our knowledge, these are the first human data to demonstrate a mechanistic link between MA exposure, HIV reservoir transcription, and NLRP3 inflammasome activation in PWH on ART.

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