Assessing Macrophage Inflammation and Expression of Neurotransmitter Machinery in Patients with Comorbid HIV and Depression

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Introduction

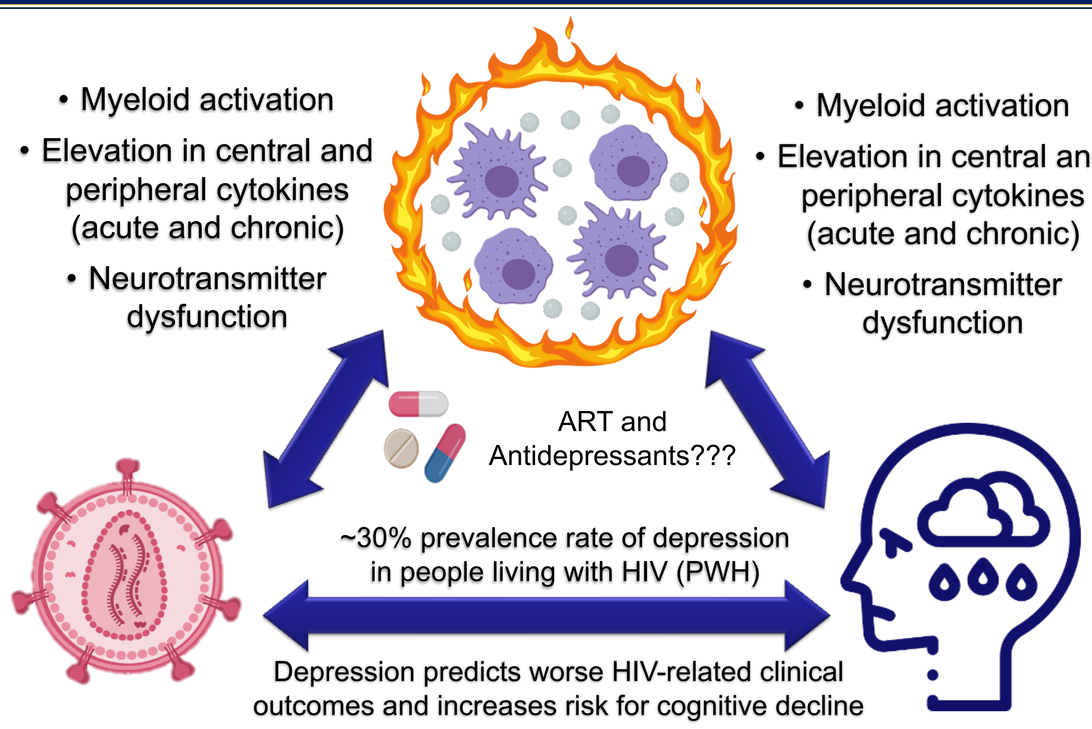
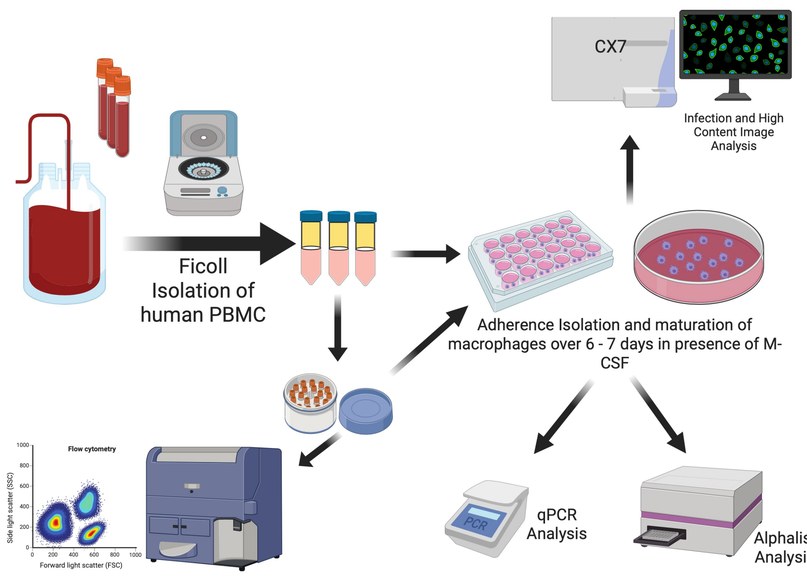


Figure 1: Interactive Impact of Depression, HIV, and Inflammation on Myeloid Cells. Adapted from Rakshasa-Loots et al., 2022. Created with Biorender.

Methods

Figure 2: Experimental Workflow of Processing Fresh and Frozen Monocyte-derived Macrophages. Created with Biorender.



Ficoll-Pacque gradient centrifugation separated the peripheral blood mononuclear cells (PBMC) from de-identified blood from the New York Blood Center or virally suppressed HIV+ individuals with/without depression from the Drexel Comprehensive NeuroHIV Center (CNHC). Depression cutoffs were determined PHQ-9. Patient samples were frozen down after isolation and banked through the CNHC until ready to use.

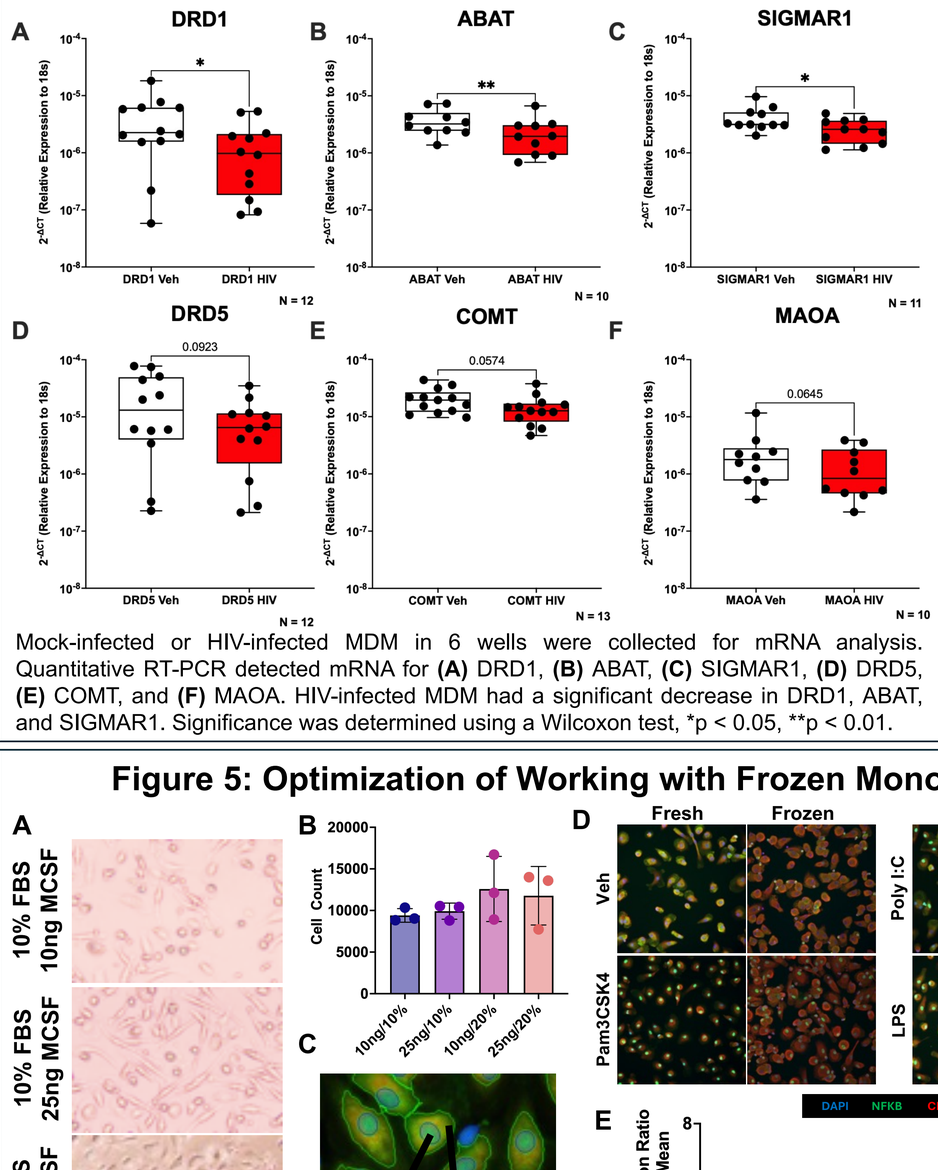
Fresh or thawed PBMC were matured into monocyte-derived macrophages (MDM) using adherence isolation. Cells were cultured for 6-7 days in media containing macrophage colony stimulating factor (M-CSF).

Fresh healthy MDM were mock-infected or infected with 1/ng/mL HIV for 7 days and analyzed for gene expression by qPCR (**Figure 3**).

PBMC characterization of CNHC samples was analyzed by flow cytometry (**Figure 4**). Conditions were optimized for frozen MDM (**Figure 5**) and treated with vehicle, PAMCSK4 (TLR2 Agonist), Poly i:c (TLR3 Agonist), and LPS (TLR4 Agonist) for 1 hour to assess Nf-κb nuclear translocation) and 24 hours to assess cytokine release (**Figure 6**).

Results

Figure 3: Expression of Genes Involved in Neurotransmitter Signaling, Synthesis, and Degradation in HIV-Infected Human Macrophages



Mock-infected or HIV-infected MDM in 6 wells were collected for mRNA analysis. Quantitative RT-PCR detected mRNA for **(A)** DRD1, **(B)** ABAT, **(C)** SIGMAR1, **(D)** DRD5, **(E)** COMT, and **(F)** MAOA. HIV-infected MDM had a significant decrease in DRD1, ABAT, and SIGMAR1. Significance was determined using a Wilcoxon test, *p < 0.05, **p < 0.01.

A

HIV+ No Depression (N = 11)		HIV+ Depression (N = 19)	
Variable	Statistic	Variable	Statistic
Age (years)[a]	51.5 (6.1) [41-58]	Age (years)[a]	50.1 (7.9) [34-60]
Gender (% men)	50%	Gender (% men)	33.3%
Ethnicity		Ethnicity	
Black/African American	100%	Black/African American	100%
HCV status (% +)	0%	HCV status (% +)	0%
Current CD4[a]	750.6 (226.1) [495 - 1169]	Current CD4[a]	799.9 (327.7) [185 - 1332][b]
ART Status (% on)	100%	ART Status (% on)	100%
PHQ-9[a]	2.2 (1.5) [0-4]	PHQ-9[a]	11.3 (5.8) [5-23]

[a] Mean (standard deviation) [range]
[b] Statistic based on 16/19 donors

B

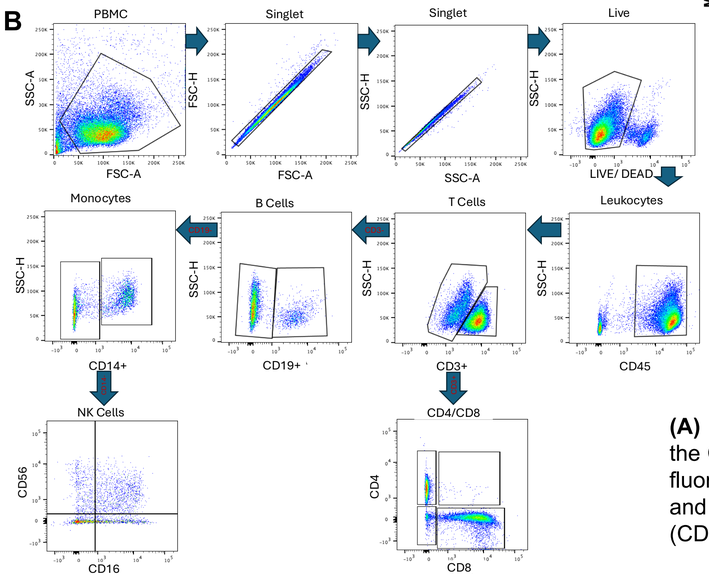
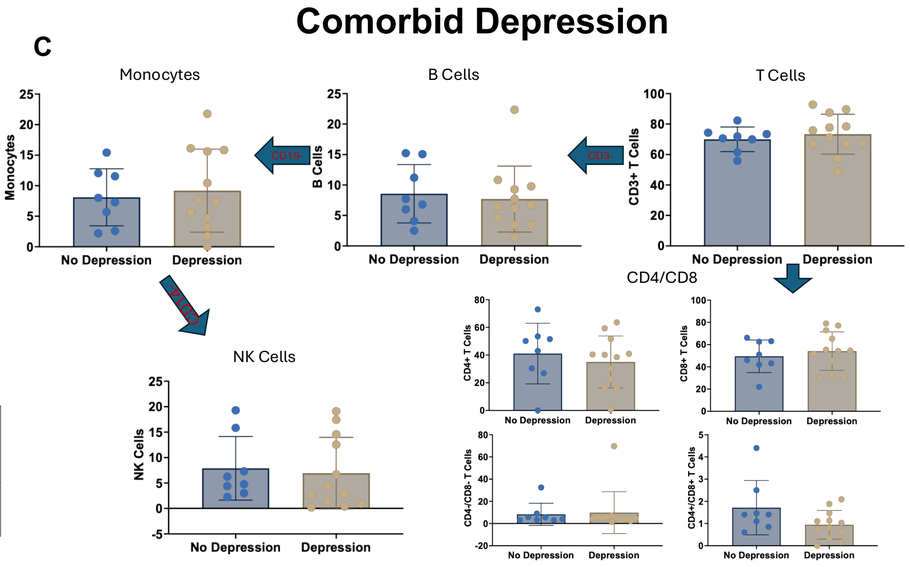
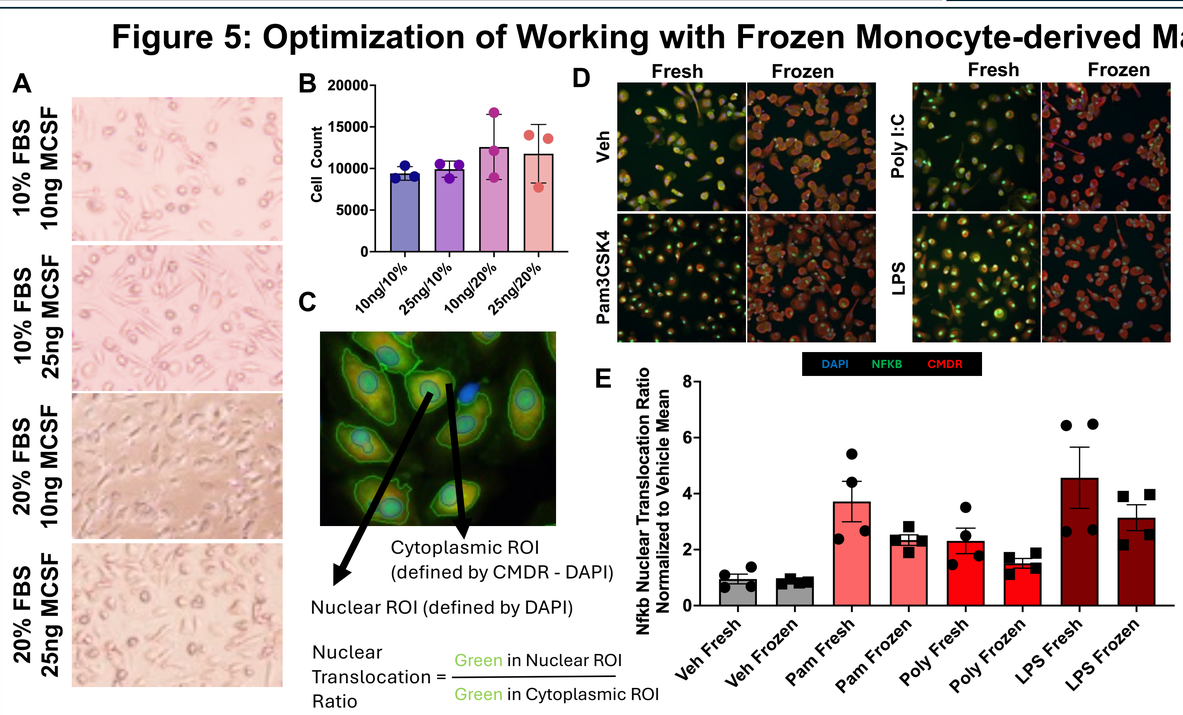


Figure 4: PBMC Characterization of HIV-infected Virally Suppressed Patients with Comorbid Depression



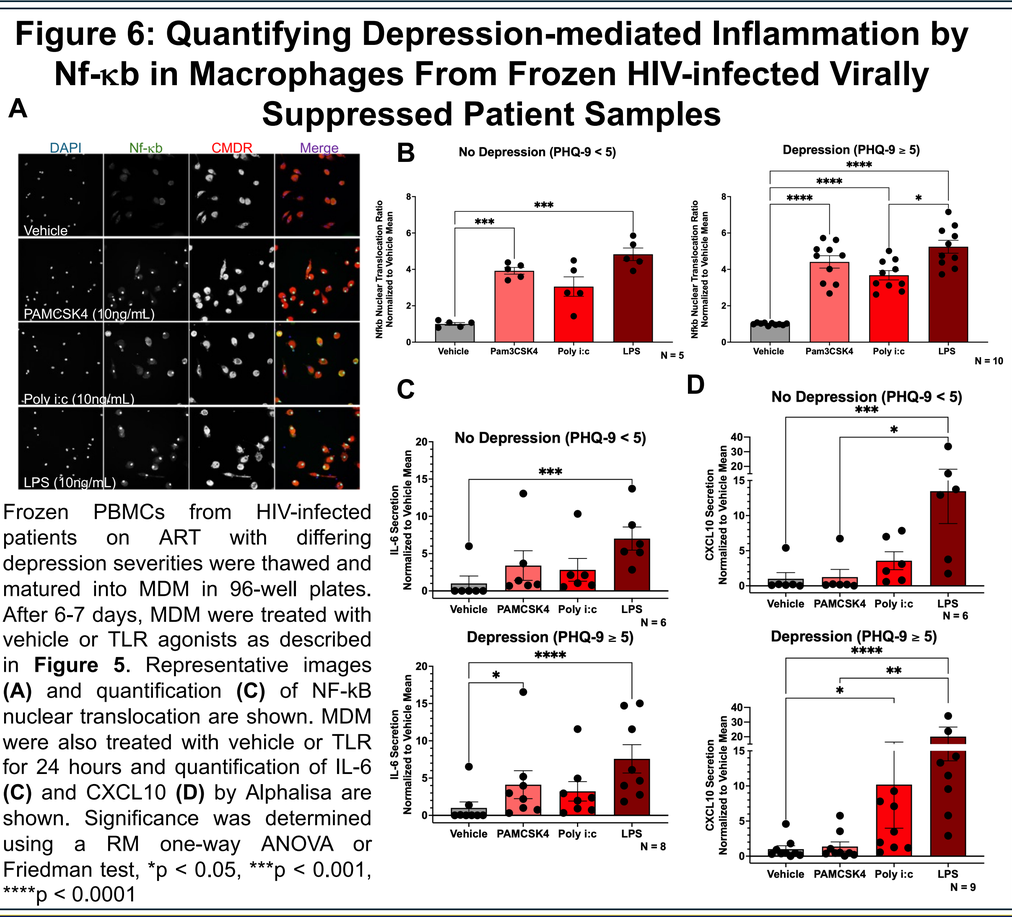
(A) Table 1 summarizes demographic, viral, and depression data of the donors used from the CNHC cohort. **(B)** A representative flow gating strategy is shown. We utilized a panel of fluorescently conjugated antibodies against extracellular markers that are subtype specific and includes T-cells (CD3+, CD4+, CD8+), NK cells (CD3-/CD56+/CD16+/-), Monocytes (CD14+), and B-cells (CD19+). **(C)** Quantification of each indicated PBMC subtype.

Figure 5: Optimization of Working with Frozen Monocyte-derived Macrophages



MDM were cultured in 10% or 20% FBS with 10 or 25ng M-CSF in 24-well plates **(A)**. MDM were also either media-fed or washed at days *in vitro* (DIV) 3. Analyses including cell counts **(B)** determined 10% FBS and 10ng M-CSF with a DIV 3 wash was the optimal condition. Fresh and frozen MDM were treated with vehicle or the TLR agonists PAMCSK4, Poly i:c, or LPS for 1 hour. Description of analysis of Nf-κb nuclear translocation is shown in **C**. Representative images **(D)** and quantification **(E)** of NF-kB nuclear translocation are shown, with no statistical difference between fresh and frozen responses. Significance was determined using a paired t test, *p < 0.05.

Figure 6: Quantifying Depression-mediated Inflammation by Nf-κb in Macrophages From Frozen HIV-infected Virally Suppressed Patient Samples



Frozen PBMCs from HIV-infected patients on ART with differing depression severities were thawed and matured into MDM in 96-well plates. After 6-7 days, MDM were treated with vehicle or TLR agonists as described in **Figure 5**. Representative images **(A)** and quantification **(C)** of NF-kB nuclear translocation are shown. MDM were also treated with vehicle or TLR for 24 hours and quantification of IL-6 **(C)** and CXCL10 **(D)** by Alphalisa are shown. Significance was determined using a RM one-way ANOVA or Friedman test, *p < 0.05, ***p < 0.001, ****p < 0.0001

Discussion and Future Directions

- Primary human monocyte-derived macrophages (MDM) express mRNA related to neurotransmitter signaling pathways that significantly change with HIV infection. These studies will guide targets to measure in frozen clinical samples.
- Optimization of frozen MDM demonstrated that 10% FBS and 10ng M-CSF with a DIV 3 wash was the most ideal to move forward analyzing patient-derived samples.
- We can assess PBMC characteristics by flow cytometry as well as Nf-κB nuclear translocation and cytokine release by high-content assays for frozen clinical cohort samples from people living with HIV and depression
- A detailed understanding of depression-mediated changes in inflammation and neurotransmitter signaling will be critical for identifying efficacious therapies to mitigate treatment-resistant depression, suboptimal ART efficacy, and persistent cognitive symptoms in people with HIV.

Acknowledgments

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Dr. Stephanie Matt's Faculty Profile