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## **HIV Infection Induces Rapid Activation of HAND-Associated Inflammatory Signatures in Microglia**

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HIV-associated neurocognitive disorders (HAND) continue to be a significant burden to people living with HIV-1 (PLWH) despite antiretroviral therapy (ART). Microglia within the central nervous system (CNS) are targeted by HIV-1 early and persists during chronic infection. Microglia play a central role in promoting neuroinflammation and HAND symptoms seen in PLWH. Exploring the transcriptional changes in microglia following HIV-1 infection may reveal mechanisms underlying the development of HAND.

iPSC-derived microglia (iMG) were infected with HIV-1 BaL and samples on days 1, 2, 4, 6 and 8 post-infection were collected for bulk RNA-sequencing. Chromatin accessibility by ATAC-seq was performed on infected samples from days 1 and 4 post-infection. HIV-infected monocyte-derived macrophages (MDMs) were sampled in parallel as a comparator in our analyses.

The integration of RNA-seq-derived differentially expressed genes (DEGs) with ATAC-seq-identified differentially accessible regions (DARs) uncovered a subset of genes demonstrating simultaneous changes in transcript levels and promoter accessibility. Inflammatory pathways were triggered early following infection of microglia, in a pattern that differed from MDMs. Genes identified as both DARs and DEGs were significantly enriched in key biological pathways, including TNF $\alpha$  signaling via NF $\kappa$ B, IL6/JAK/STAT3 signaling, interferon-alpha responses, and apoptosis. DiffTF analysis identified NF $\kappa$ B family members as key regulators of the responses, with significant contributions from AP-1 family members FOS, JUNB, and additionally the interferon regulatory factor (IRF) transcription factors IRF1, IRF8, IRF 7 and IRF9.

In summary, HIV infection of microglia triggered the rapid onset of inflammatory profiles similar to those observed in studies of patients diagnosed with HAND.