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**Epigenetic regulation of a persistent HIV reservoir in human brain myeloid cells despite durable ART**

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Long-lived and self-renewing myeloid cells may harbor persistent, replication-competent HIV and serve as stable reservoirs for the virus. In this study, we isolated human brain myeloid cells (BrMCs) from several regions using rapid research autopsy donations from people with HIV (PWH) who were under suppressive antiretroviral therapy (ART) in the "Last Gift" program. We isolated CD3+ CNS T cells and CD11b+ BrMCs using a careful sequential protocol. We were able to isolate up to  $1 \times 10^6$  viable BrMCs per gram of brain tissue from PWH, with up to 95% of these BrMCs being TMEM119+ microglia. The levels of total and integrated HIV DNA were similar in BrMCs isolated from two ART-suppressed and two ART-interrupted PWH (ART stopped < 3 weeks before death). We found that HIV largely remained silent in BrMCs, in which the latent virus could be induced by epigenetic regulators (SAHA and CM272), but not the NF- $\kappa$ B agonist PEP005. Viral outgrowth was detected after induction and co-culture of BrMCs with PBMC PHA blasts. We were able to recover HIV in induced BrMCs from one ART-suppressed donor, which was Macrophage-tropic R5 virus and can productively re-infect both BrMCs and PBMCs isolated from HIV-negative donors. Conversely, CNS T cells yielded no viral outgrowth, although we obtained 10-1000x fewer T cells than BrMCs from the same donors. Our data demonstrate the first evidence of replication-competent HIV in isolated BrMCs, most probably microglia, from virally suppressed PWH, which is largely controlled by epigenetic regulations.