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HIV-Induced Type I Interferons Promote Viral Latency in Infected Macrophages

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Background

The existence of latent HIV reservoirs, consisting of both T cells and myeloid cells, represents the major obstacle to viral cure. There remain crucial gaps in our understanding of the molecular mechanisms that lead to latent infection in myeloid cells. We hypothesize that HIV-induced type I IFN signaling promotes a state of transcriptional latency in HIV-1 infected macrophages.

Methods

We examined HIV-1 replication kinetics and the effects of type I IFN signaling on HIV-1 replication in an in vitro monocyte-derived macrophage (MDM) model. Transcription factor recruitment to the 5' LTR was evaluated using chromatin immunoprecipitation (ChIP). Chromatin structure at the promoter was assessed by restriction enzyme accessibility. Single cell RNA sequencing (scRNA-Seq) was utilized to determine changes in gene expression in infected macrophages. The contribution of innate immune receptors to latency was determined using shRNA.

Results

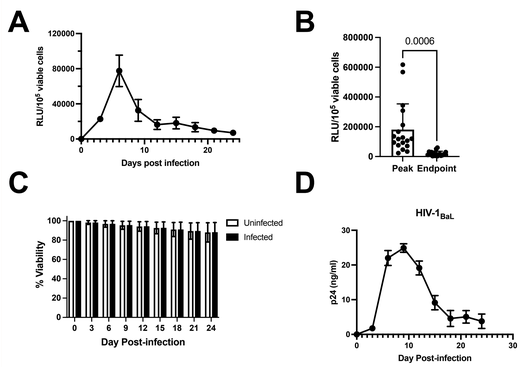


Figure 1. MDMs enter a low-level persistent/latently infected state over time in culture. (A-C) MDMs were infected with a single round replication-competent HIV-1 reporter virus that encodes secreted nanoluciferase. Luciferase activity over time for a single donor (A), comparison of peak versus endpoint viral replication for 18 donors (B) and cellular viability for 18 donors (C) are shown. (D) MDMS were infected with replication competent HIV-1_{BaL}. One representative donor is shown.

Results

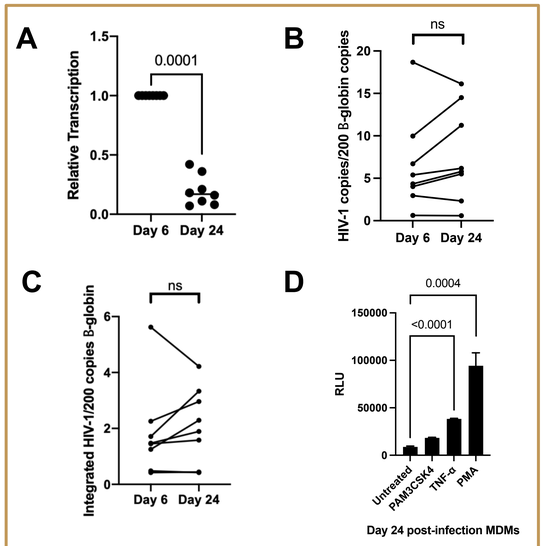


Figure 2. HIV-1 latency is characterized by reversible transcriptional latency. MDMs were infected with single round replication-competent HIV-1 and cultured for up to 24 days. HIV-1 transcription (A), total cellular HIV-1 DNA (B), and integrated proviral DNA (C) were measured at days 6 and 24 post infection. (D) Treatment of MDMs at day 24 post infection with latency reversing agents.

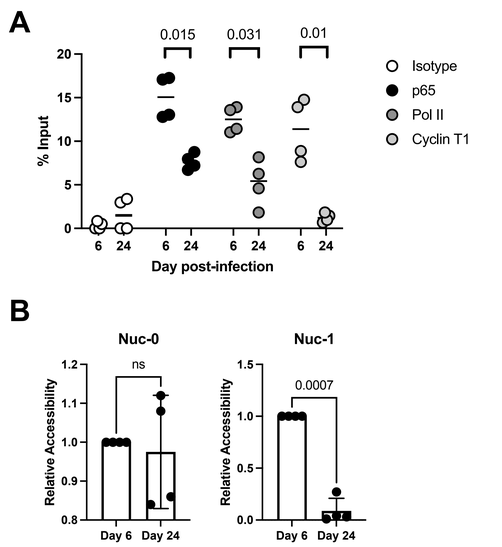
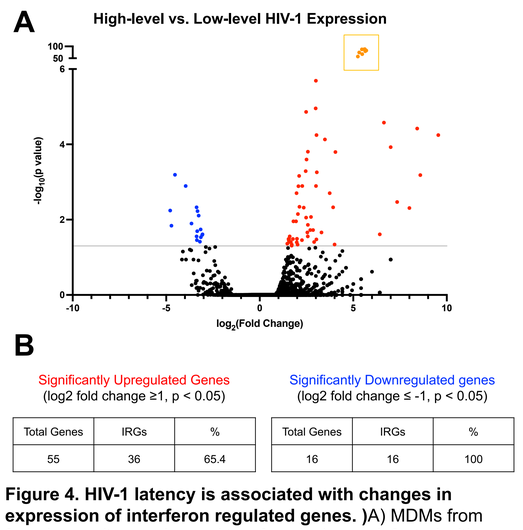


Figure 3. Latent HIV-1 infection in MDMs is associated with changes in transcription factor recruitment and chromatin structure at the 5' LTR. MDMs were infected with HIV-1 and cultured for up to 24 days. Transcription factor association with the HIV-1 5' LTR was assessed by ChIP (A) and chromatin structure was analyzed by restriction enzyme accessibility in purified nuclei (B).

Results



Total Genes	IRGs	%
55	36	65.4

Total Genes	IRGs	%
16	16	100

Figure 4. HIV-1 latency is associated with changes in expression of interferon regulated genes.)A) MDMs from four donors (two males and two females) were infected with HIV-1. At day seven post infection, the MDMs were harvested and submitted for scRNASeq. A volcano plot comparing MDMs with high levels of viral transcripts and those with low levels of viral transcripts are shown. (B) Data from MDMs with high levels of viral transcripts and low levels of viral transcripts were analyzed using the Interferome database to assess for enrichment for IRGs. Orange – viral genes.

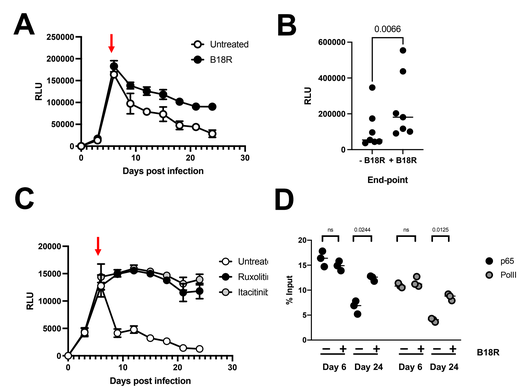


Figure 5. HIV-1 latency is induced by type I IFN signaling. (A-B) MDMs were infected HIV-1 and cultured for up to 24 days in the absence or presence of the soluble type I IFN inhibitor, B18R (100 ng/mL), starting at day six post infection. Data from one representative donor (A) and composite endpoint data from multiple donors (B) are shown. (C) MDMs were infected with HIV-1 and cultured for up to 24 days in the absence or presence of ruxolitinib (10 μM), itacitinib (10 μM), or vehicle control starting at day six post infection. Data from one representative donor are shown. (D) MDMs were infected with HIV-1 and cultured for up to 24 days in the absence or presence of B18R (100 ng/mL), starting at day three post infection. Cells were harvested at day 6 or 24 post infection and transcription factor association with the HIV-1 5' LTR was assessed by ChIP.

Results

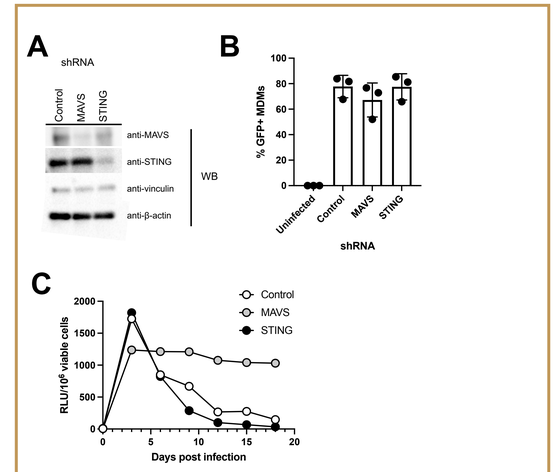


Figure 6. MAVS signaling is required for the establishment of HIV-1 latency in MDMs. (A-C) MAVS and STING expression were knocked down using retrovirally-encoded shRNAs. (A) Expression was measured by western blot. Data from one representative donor are shown. (B) MDMs were infected HIV-1 encoding GFP, cultured for three days, and analyzed by flow cytometry. (C) MDMs were infected with HIV-1 expressing nanoluciferase and cultured for 18 days. Data from one representative donor are shown.

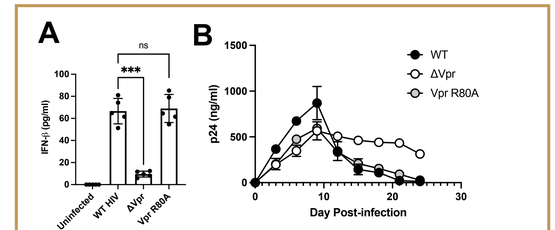


Figure 7. HIV-1 accessory protein Vpr contributes to the establishment of latency. MDMs were infected with wild-type HIV-1_{BaL}, HIV-1_{BaL} ΔVpr, or HIV-1_{BaL} with Vpr R80A. (A) IFN-β production was measured by ELISA at day three post infection. (B). Viral replication was measured by p24 ELISA. Data from one representative donor are shown.

Conclusions

1. Macrophages from subset of donors enter a state reminiscent of viral latency.
2. The transition to latency is associated with decreased NF-kB recruitment to the LTR.
3. Type I IFN signaling induces HIV-1 latency by blocking NF-kB and RNA polymerase II recruitment to the LTR.
4. Latency requires MAVS signaling.
5. HIV-1 Vpr contributes to the induction of viral latency in macrophages.