EXPRESSION OF UNSPLICED HIV-1 RNA INDUCES INFLAMMASOME ACTIVATION IN MYELOID CELLS

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BACKGROUND: Despite the success of combination antiretroviral therapy, persistent viral RNA expression in tissue reservoirs such as macrophages and CNS-resident microglia and associated chronic inflammation remain a major barrier to HIV-1 cure strategies. We have previously reported that cytoplasmic expression of unspliced intron-containing viral RNA (icRNA) leads to innate immune activation in human macrophages and microglia, though the icRNA sensing mechanism has remained unclear (Akiyama, H., *et al. Nat Commun* (2018); Akiyama, H., *et al. J Virol* (2020). In this study, we provide evidence of icRNA-dependent, MAVS-mediated sensing and inflammasome activation in both macrophages and microglia.

METHODS: Monocyte-derived macrophages (MDM) derived from purified human CD14$^+$ PBMCs, human microglia cell line (CHME3), and iPSC-derived human microglia were infected with replication-competent (R5-tropic) or reporter viruses expressing icRNA accessing distinct nucleocytoplasmic RNA export pathways. Infections were performed in the presence or absence of small molecule inhibitors targeting virus replication cycle, or upon knockdown of innate immune sensing pathways. Viral RNA expression and inflammasome activation were determined by qRT-PCR, IL-1β ELISA, immunoblotting, and Caspase-1 activation (FLICA) assay.

RESULTS: Infection of MDMs and microglia with WT HIV-1, but not M10/Rev-mutant HIV-1 (that lacks cytoplasmic icRNA export) induced IL-1β secretion. Interestingly, MPMV constitutive transport element (CTE)-mediated rescue of icRNA export in Rev/M10 HIV-1 also primed NLRP3 expression and triggered secretion of IL-1β. IL-1β production was independent of viral icRNA sequence, viral protein (Gag-Pol, Vif, Vpr, Vpu, Nef and Env) expression, and was attenuated upon pre-treatment with HIV-1 RT (Efavirenz), integrase (Raltegravir), transcription (Spironolactone) or icRNA export (KPT335) inhibitors, or treatment with caspase-1 inhibitors (z-VAD, VX-765). Additionally, knockdown of MAVS, but not STING or abrogation of endosomal TLR nucleic acid sensing pathways, significantly attenuated IL-1β production, confirming that cytosolic HIV-1 icRNA sensing is required for inflammasome activation in macrophages and microglia.

CONCLUSIONS: Collectively, our findings suggest that provirus establishment and persistent cytoplasmic expression of HIV-1icRNA alone, even in the absence of viral protein expression, is sufficient to activate inflammasomes and might contribute to chronic innate immune activation in tissue-resident macrophages.

Establishment of HIV-1 infection and *de-novo* viral RNA expression in MDMs triggers caspase-dependent IL-1β secretion

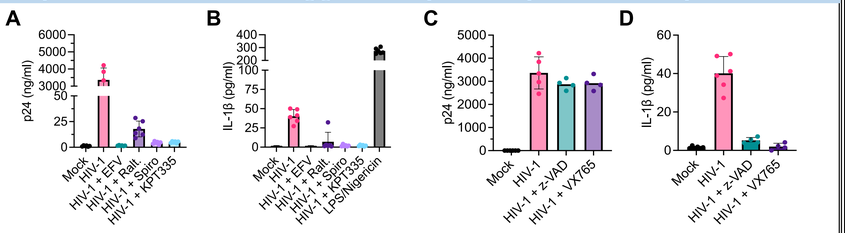


Figure 1. (**A** and **C**) MDMs (from at least 4 independent donors) were infected with R5-tropic HIV-1/YU2 (MOI=1, + SIV_{mac} Vpx VLPs) in the absence or presence of 1μM Efavirenz (EFV, RT inhibitor), 30μM Raltegravir (RAL, Integrase inhibitor), 1μM Spironolactone (Spiro, transcription inhibitor), 0.1μM KPT335 (*Rev/CRM1* inhibitor), z-VAD (pan-caspase inhibitor?) or VX765 (Caspase1 and -4 inhibitor). Infection establishment and viral spread was determined by quantifying p24^{gag} in supernatants at day 3 post-infection (3 dpi) by an ELISA (mean±SEM). (**B** and **D**) Quantitation of IL-1β secretion at 3 dpi by an ELISA.

Nuclear export of HIV-1 icRNA is required for inflammasome activation and IL-1β secretion in HIV-infected MDMs

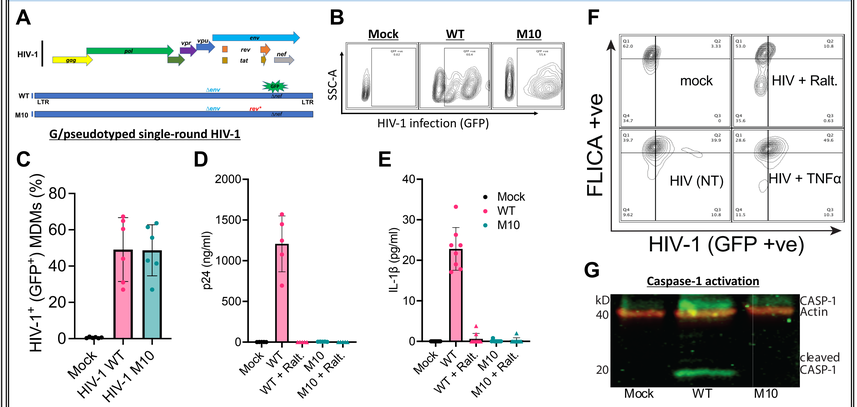


Figure 2. (**A**) Schematic diagram of HIV-1 "WT" (functional *Rev-CRM1 export*) and *Rev M10 mutant* (*rev**), lacking *Rev-CRM1* export. (**B** and **C**) Representative GFP expression and % infection of MDMs with single-round HIV-1 (LaiΔenvGFP, MOI=1, + SIV_{mac} Vpx VLPs) via flow cytometry. Cells were pre-treated with either DMSO or 30μM Raltegravir. (**D** and **E**) p24^{gag} production and IL1-β secretion in culture supernatants measured by ELISA at 3 dpi in the presence or absence of RAL. (**F**) Fluorescent-activated Caspase-1 in HIV-1 infected cells, determined by flow cytometry. (**G**) Caspase-1 activation and cleavage by immunoblotting. Data from at least 6 independent replicates/donors (mean±SEM).

Inflammasome activation in HIV-infected CHME3 microglia requires *de-novo* icRNA expression and nuclear export

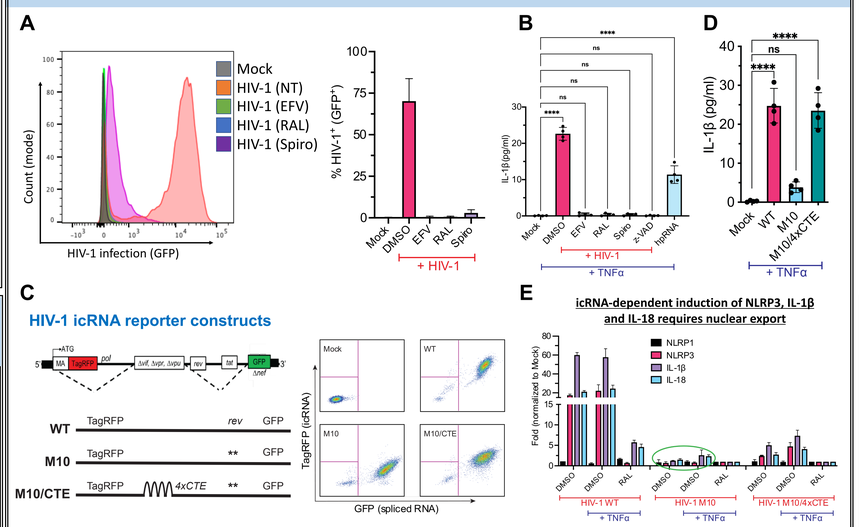


Figure 3. (**A**) Left: representative infection of human microglia cell line (CHME3) with single-round HIV-1 (LaiΔenvGFP, MOI=2) via flow cytometry. Cells were pre-treated with either DMSO or 1μM EFV, 30μM RAL, or 1μM Spironolactone. Right: % infection based on GFP expression. (**B**) quantitation of IL-1β secreted in culture supernatants at 3 dpi by ELISA. (**C**) Left: schematic diagram of HIV-1- based TagRFP icRNA reporter viruses; WT, *Rev mutant M10 (**), or Rev-mutant* expressing MPMV constitutive transport element (4xCTE). Right: flow cytometry profiles of CHME3 microglia infected with reporter viruses. (**D**) IL-1β from CHME3 culture supernatants infected with WT or mutant TagRFP reporter constructs in the presence or absence of RAL, at 3 dpi by ELISA. (**E**) mRNA expression of key inflammasome components in HIV-1/TagRFP infected CHME3 microglia at 3 dpi by qRT-PCR. Data from 4 independent replicates (mean±SEM).

HIV-1 infection of iPSC-derived microglia and icRNA expression triggers IL-1β secretion

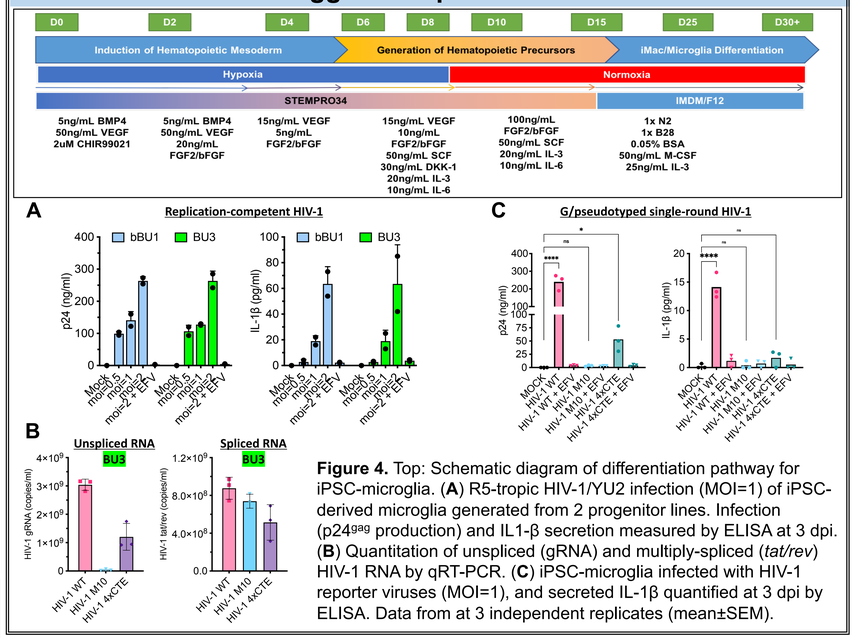


Figure 4. Top: Schematic diagram of differentiation pathway for iPSC-microglia. (**A**) R5-tropic HIV-1/YU2 infection (MOI=1) of iPSC-derived microglia generated from 2 progenitor lines. Infection (p24^{gag} production) and IL1-β secretion measured by ELISA at 3 dpi. (**B**) Quantitation of unspliced (gRNA) and multiply-spliced (*tat/rev*) HIV-1 RNA by qRT-PCR. (**C**) iPSC-microglia infected with HIV-1 reporter viruses (MOI=1), and secreted IL-1β quantified at 3 dpi by ELISA. Data from at 3 independent replicates (mean±SEM).

Inflammasome activation in HIV-infected CHME3 microglia requires cytosolic sensing of viral icRNA via MAVS

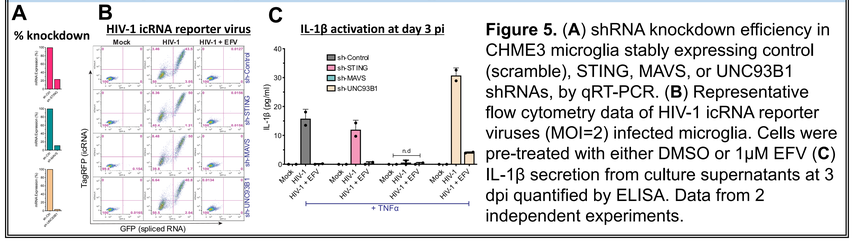


Figure 5. (**A**) shRNA knockdown efficiency in CHME3 microglia stably expressing control (scramble), STING, MAVS, or UNC93B1 shRNAs, by qRT-PCR. (**B**) Representative flow cytometry data of HIV-1 icRNA reporter viruses (MOI=2) infected microglia. Cells were pre-treated with either DMSO or 1μM EFV (**C**) IL-1β secretion from culture supernatants at 3 dpi quantified by ELISA. Data from 2 independent experiments.

Conclusions

- ❑ Inflammasome activation in macrophages and microglia requires *de-novo* expression and nuclear export of HIV-1 icRNA
- ❑ Expression of HIV-1 icRNA is sufficient to prime inflammasome expression and caspase dependent IL-1β secretion in macrophages and microglia
- ❑ Cytosolic sensing of icRNA and inflammasome activation in CHME3 microglia is mediated by MAVS
- ❑ Targeting icRNA expression and nuclear export in long-lived tissue-resident macrophages is a potential strategy to combat HIV-associated chronic inflammation

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