EXPRESSION OF UNSPLICED HIV-1 RNA INDUCES NLRP1 INFLAMMASOME ACTIVATION IN MYELOID CELLS

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BACKGROUND: Despite the success of combination antiretroviral therapy (ART), chronic HIV-1 infection in long-lived tissue-resident cells such as macrophages and CNS-resident microglia and associated chronic inflammation remain a major barrier to successful HIV-1 management strategies. Inflammasome activation is a critical checkpoint in innate immune responses and HIV-1 has been proposed to activate multiple inflammasome sensors, including NLRP3 (by multiple mechanisms) and CARD8 (by HIV-1 protease). Recent work in our laboratory has shown that expression of HIV-1 intron-containing RNAs (icRNA) is sufficient to activate innate immune responses. As ART does not affect viral RNA expression in long-lived infected cells, persistent expression of HIV-1 RNAs thus presents a novel mechanism for inflammasome activation and chronic inflammation, observed in people with HIV (PWH).

METHODS: Primary human monocyte-derived macrophages (MDMs, differentiated from CD14+ monocytes) or TNF-stimulated phorbol 12-myristate 13-acetate (PMA)-differentiated THP-1 macrophages were infected with HIV-1. Supernatants from infected cells were collected and assayed by ELISA for IL-1β. Transfected siRNAs were used to knock down putative sensors in MDMs, while lentiviral constructs were used to overexpress putative sensors in THP-1 macrophages.

RESULTS: We found that inflammasome activation in MDMs was dependent on the nuclear export of HIV-1 icRNAs, similar to the activation of type I interferon. Surprisingly, unlike induction of type I IFN responses, MAVS-signaling was not required for inflammasome activation and IL-1β secretion. IL-1β secretion was dependent on caspase-1 and the inflammasome sensor NLRP1, but unlike HIV-mediated activation of CARD8 inflammasome, not dependent on viral protease or enzymatic activity. Furthermore, knockdown of NLRP1 did not affect the activation of type I IFN responses in MDMs, suggesting cytosolic icRNA expression engages divergent innate immune sensing pathways. Finally, HIV-1 infection also induced inflammasome activation and IL-1β secretion in NLRP1-overexpressing THP1/PMA macrophages, confirming that NLRP1 senses HIV-1 icRNA expression.

CONCLUSIONS: Persistent expression of HIV-1 icRNAs in long-lived infected cells may contribute to persistent immune activation in PWH via NLRP1 activation. Future studies in our laboratory will address the mechanism of HIV-mediated NLRP1 activation.

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

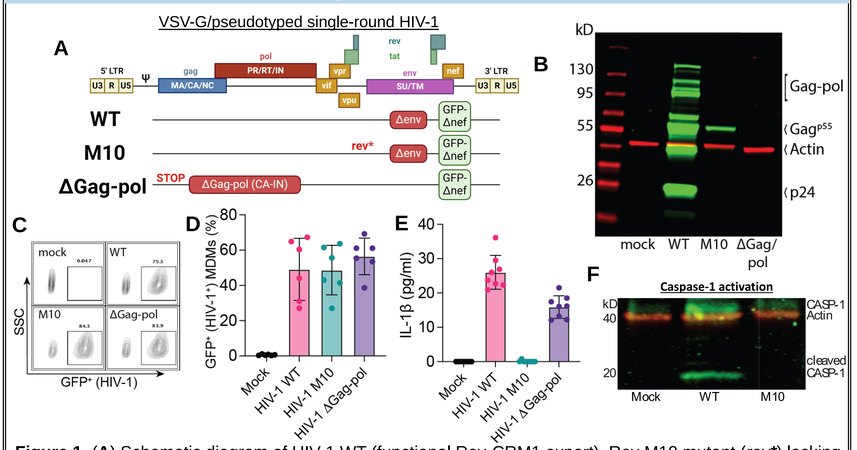


Figure 1. (**A**) Schematic diagram of HIV-1 WT (functional Rev-CRM1 export), Rev M10 mutant (*rev**) lacking Rev-CRM1 export ,and ΔGag-pol mutant lacking Gag/Gag-pol expression. (**B**) Western blot of 293T cells transfected with WT and mutant viral plasmids. (**C** and **D**) Representative GFP expression and % infection of MDMs with single-round HIV-1 viruses (LAI isolate, MOI 1, + SIV_{mac} Vpx VLPs) via flow cytometry. (**E**) IL-1β secretion in culture supernatants measured by ELISA at 3 dpi. (**F**) Caspase-1 cleavage by immunoblotting. Data from at least 6 independent replicates/donors (mean±SEM).

NLRP1 and CASP1 control inflammasome activation in HIV-infected MDMs

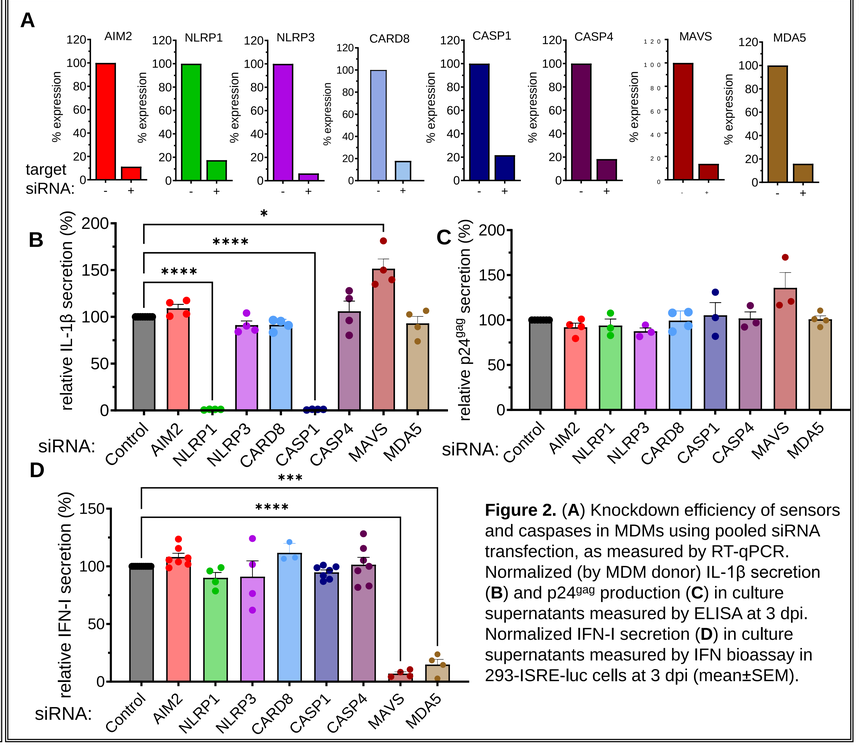


Figure 2. (**A**) Knockdown efficiency of sensors and caspases in MDMs using pooled siRNA transfection, as measured by RT-qPCR. Normalized (by MDM donor) IL-1β secretion (**B**) and $p24^{gag}$ production (**C**) in culture supernatants measured by ELISA at 3 dpi. Normalized IFN-I secretion (**D**) in culture supernatants measured by IFN bioassay in 293-ISRE-luc cells at 3 dpi (mean±SEM).

NLRP1 overexpression in THP-1/PMA macrophages enables sensing of HIV-1 icRNA and inflammasome activation in an HIV-protease-independent manner

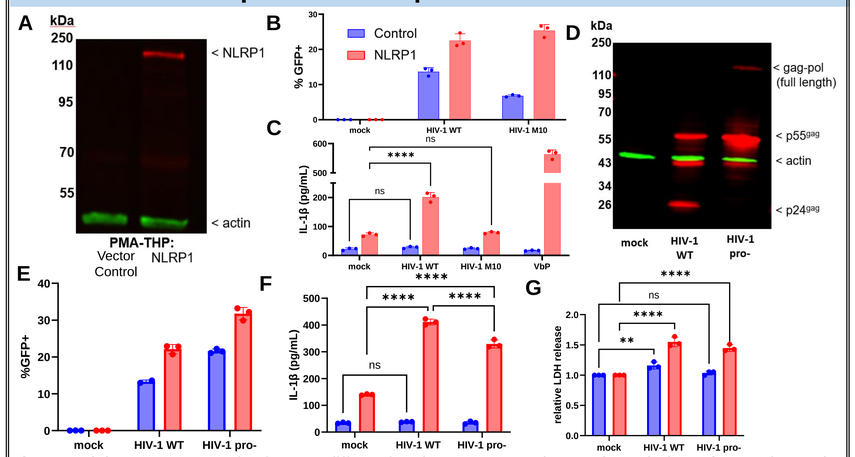
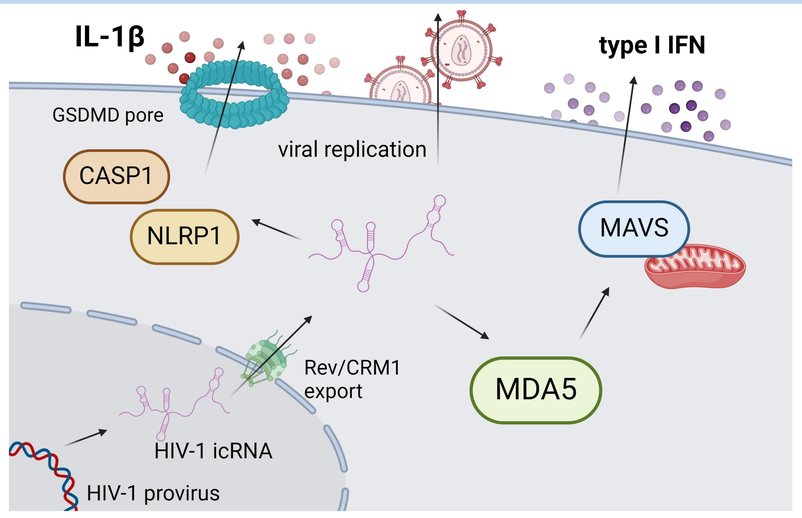


Figure 3. (**A**) NLRP1 expression in PMA-differentiated THP-1 macrophages. (**B** and **C**) IL-1β secretion and infection (GFP) efficiency measured at 3 dpi in THP macrophages infected with single-round HIV-1 and stimulated with 10 ng/ml TNFα. VbP = NLRP1/CARD8 activator, 100 μM treatment for 24h. (**D**) Western blot of 293T cell lysates transfected with single-round HIV-1 WT plasmid or protease-inactive (mutant PR residues 25, 49, 50) HIV-1 plasmid. (**E**, **F**, and **G**) IL-1β secretion (ELISA), GFP positivity (flow cytometry), and LDH release (colorimetric assay) of cell supernatants harvested at 3 dpi of THP macrophages infected with HIV-1 WT or protease mutant single-round virus.

Conclusions



Rev-dependent export of HIV-1 icRNAs induces both NLRP1 inflammasome activation and MDA5/MAVS-dependent type I IFN secretion.

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