CARD8 Activation in Myeloid Cells as a Driver of Inflammation in HIV Infection

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BACKGROUND

Myeloid cells play an essential role in HIV persistence and chronic inflammation. Inflammasome activation in response to “danger-associated stimuli” can lead to pyroptotic cell death and release of inflammatory cytokines such as IL-1ß and IL-18.

HIV-1 protease has been shown to activate the CARD8 inflammasome in CD4 T cells, which undergo pyroptosis but do not produce IL-1ß and IL-18.

We hypothesize that CARD8 can be activated in myeloid cells upon exposure to HIV, and this can in turn drive inflammation.

METHODS

Monocyte-derived macrophages (MDMs) were cocultured with HIV-infected autologous CD4 T cells or free viral particles. In selected experiments, CARD8 or CASP-1 knockout (KO) cells were used for coculture. Supernatants were collected at 8 hours for IL-1ß and IL-18 ELISA. P values were calculated using t test or ANOVA test.

RESULTS

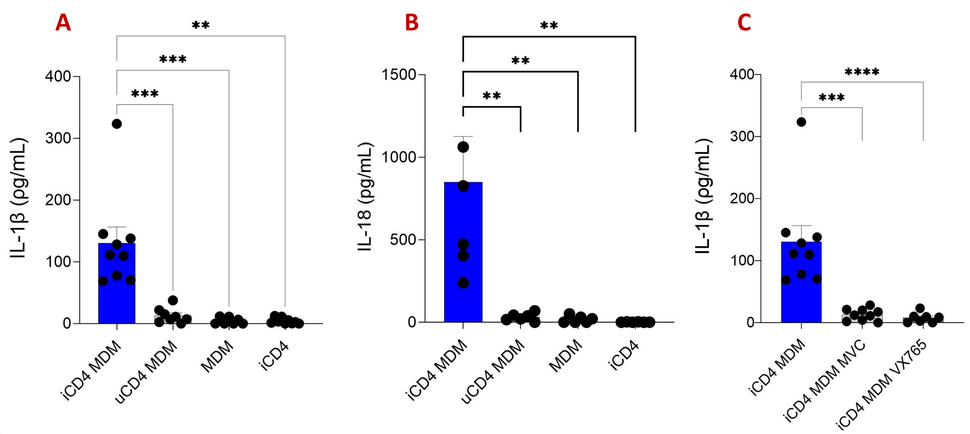


Figure 2.
Coculture of MDMs with autologous CD4 T cells infected with CCR5-tropic HIV increased release of IL-1ß **(A)** at 8 hours compared to uninfected cocultures (n=9, 111 vs 9 pg/mL), MDMs alone (0 pg/mL) and infected CD4 T cells alone (2.6 pg/mL) p<0.0001. Similar results were obtained for IL-18 **(B).**

IL-1ß release was prevented by pre-incubation with the caspase-1 inhibitor VX-765 (8.6 pg/mL) or the CCR5-inhibitor maraviroc (12 pg/ml) **(C).**

CARD8 activation upon coculture with HIV-infected CD4 T cells triggers the release of inflammatory cytokines in myeloid cells, potentially contributing to persistent inflammation in HIV infection

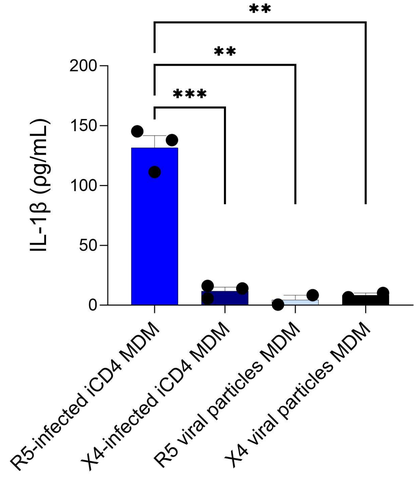


Figure 1. Coculture of MDMs with autologous CD4 T cells infected with CCR5-tropic HIV isolates resulted in increased release of IL-1ß at 8 hours compared to CXCR4-tropic HIV-infected CD4s (138 vs 14 pg/mL) as well as MDM exposed to either R5-tropic or X4-tropic viral particles (8 and 4 pg/mL, respectively), p=0.009. Similar increases were observed for IL-18 (not shown).

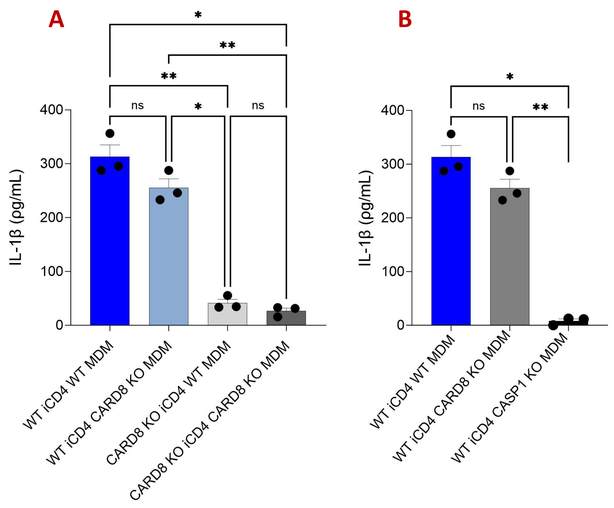
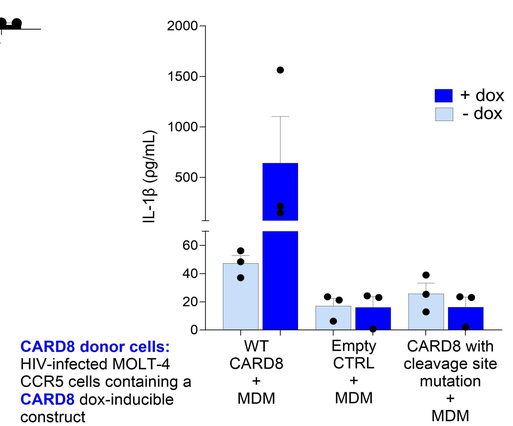


Figure 4. MDM coculture with HIV-infected MOLT-4/CCR5 cells carrying a doxycycline-inducible WT CARD8 construct resulted in higher IL-1ß release (215 pg/mL) compared to cells carrying an empty vector (23 pg/mL) or a cleavage-site mutated CARD8 construct (23 pg/mL).

Figure 3. MDM coculture with infected CARD8 KO CD4 T cells resulted in significantly lower IL-1ß release compared to Cas9 CD4 controls (n=3, 35 vs. 295 pg/mL, p=0.008) **(A).** CARD8 KO in MDMs did not affect cytokine release. However, caspase-1 KO in MDMs abolished cytokine release (7 pg/mL, p=0.003) **(B).**



CARD8 donor cells: HIV-infected MOLT-4 CCR5 cells containing a **CARD8** dox-inducible construct

CONCLUSIONS

Activation of the CARD8 inflammasome upon coculture with infected CD4 T cells results in release of inflammatory cytokines from autologous myeloid cells. This is abolished by CARD8 knockout as well as by the use of drugs that either prevent HIV entry in myeloid cells or block the downstream signaling through caspase-1. Our findings offer novel clues into HIV pathogenesis by providing a mechanism for myeloid cell activation during untreated infection. Since some HIV proviruses remain translationally active even during effective antiretroviral therapy, it is possible that HIV proteins can continue to fuel inflammation by activating CARD8 in myeloid cells even when new rounds of infection are prevented. Therefore, it is important to understand whether CARD8 inhibition could decrease chronic inflammation in people living with HIV.

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