# HIV drives CARD8 Inflammasome Activation and Proinflammatory Cytokine Release by Myeloid Cells

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## BACKGROUND

Inflammasome activation in response to "danger-associated stimuli" including HIV can lead to the release of inflammatory cytokines such as IL-1ß and IL-18 as well as pyroptotic cell death. The HIV-1 protease can activate the CARD8 inflammasome in CD4 T cells, resulting in T-cell loss during untreated HIV infection. Since myeloid cells are equipped to mount an inflammatory response upon inflammasome activation, we hypothesize that CARD8 activation in these cells might be driving IL-18 production upon HIV infection.

## METHODS

We tested our hypothesis using an in vitro, ex vivo, and in vivo model. In vitro, we cocultured monocyte-derived macrophages (MDMs) with HIV-infected autologous CD4 T cells (CD4s) or HIV viral particles. We used either CARD8 knockout (KO) or Cas9 control cells. Ex vivo, we co-cultured MDMs with autologous CD4s from people living with HIV (PLWH) in which latent HIV-1 was reactivated by CD3/CD28 antibodies. For in vivo experiments, humanized mice were engrafted with either Cas9 control or CARD8 KO CD34+ cells and then infected with HIV at week 9. Plasma or supernatant levels of IL-18 and IL-1ß were measured by ELISA. P values were calculated using t test or ANOVA test.

## RESULTS

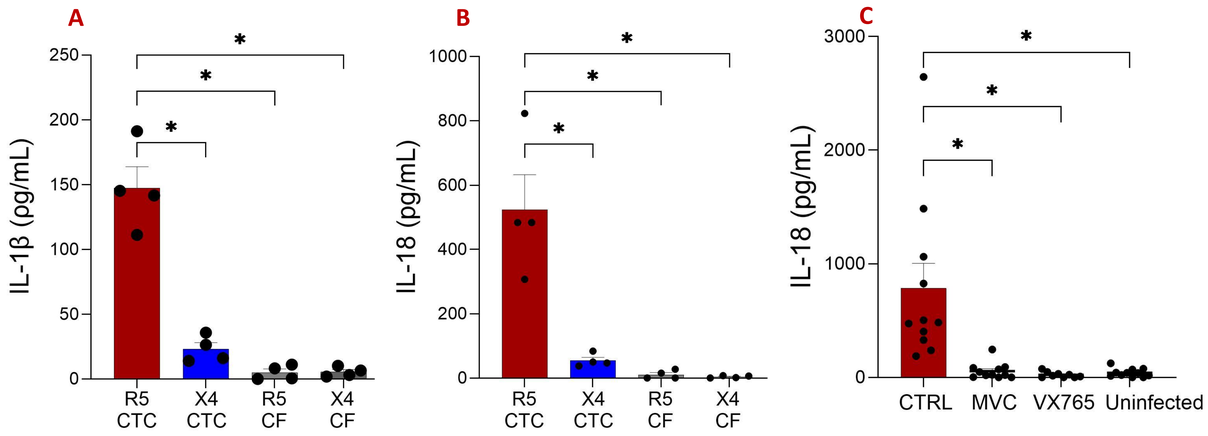



**Figure 1.** Coculture of MDMs with autologous CD4 T cells infected with CCR5-tropic HIV (cell-to-cell, CTC) resulted in increased release of IL-1ß **(A)** at 24 hours compared to CXCR4-tropic infected CD4s as well as MDM exposed to either R5-tropic or X4-tropic viral particles (cell-free, CF). Similar increases were observed for IL-18 **(B)**. IL-18 release was blocked by pre-incubation with the caspase-1 inhibitor VX-765 or the CCR5-inhibitor maraviroc **(C).** N=4

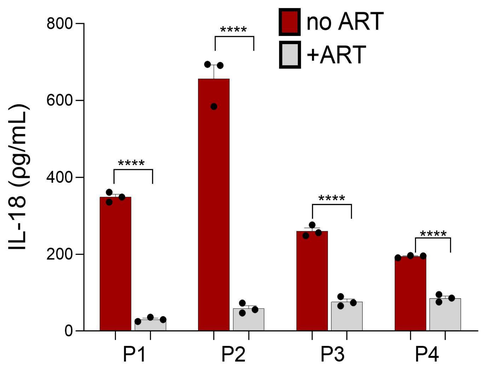



**Figure 2.** CD4 T cells from PLWH triggered IL-18 release when they were cultured without ART to allow for viral reactivation (n=4) and then added to autologous MDMs. Cytokine release was prevented when CD4s were cultured with ART.

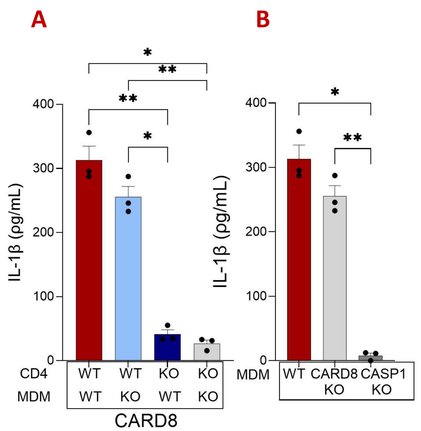



**Figure 3.** Coculture of CARD8 KO CD4 T cells with MDMs resulted in significantly lower IL-1ß release compared to Cas9 CD4 controls, n=3 **(A)**. CARD8 KO in MDMs did not affect cytokine release. However, caspase-1 KO in MDMs prevented IL-1ß release, n=3 **(B)**.

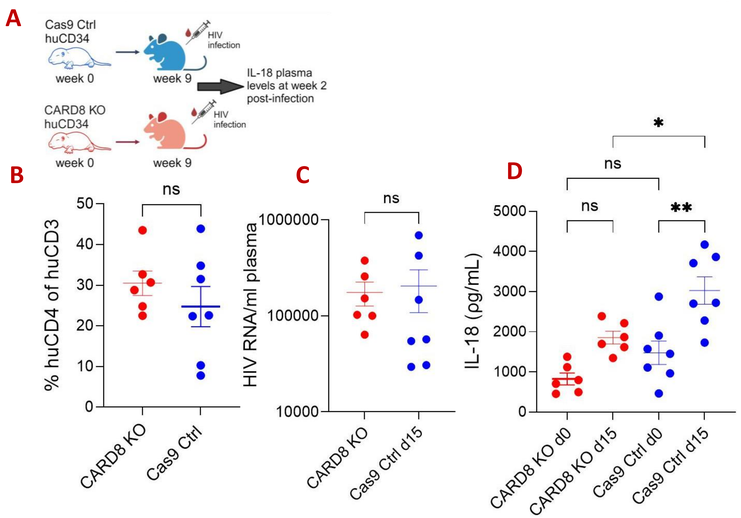



**Figure 4.** Humanized mice were engrafted with either CARD8 KO (n=6) or Cas9 control (n=7) CD34+ cells and infected with HIV at week 9 **(A)**. CARD8 KO did not affect CD4 development **(B)** or HIV viral loads **(C)**. CARD8 KO and Cas9 control mice had similar IL-18 levels at baseline **(D)**. However, CARD8 KO mice had a blunted inflammatory response to HIV compared to controls. While IL-18 levels trended up after infection in both groups, this increase was significant only in the Cas9 control group **(D)**.

**CARD8 activation upon coculture with HIV-infected CD4 T cells triggers the release of inflammatory cytokines in myeloid cells in vitro, ex vivo, and in vivo, which could in turn contribute to persistent inflammation in HIV infection**

## CONCLUSIONS

CARD8 inflammasome activation drives the release of inflammatory cytokines from autologous myeloid cells in vitro and ex vivo. Moreover, CARD8 KO decreases IL-18 surge during untreated HIV infection in humanized mice. These findings offer novel insights into HIV pathogenesis since they provide a mechanism for myeloid cell activation during acute infection. Since inflammation appears to persist even during effective antiretroviral therapy, understanding the contribution of CARD8 activation in PLWH could offer new strategies to decrease chronic inflammation.

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