IFI16 controls PVR and Gal-9 expression and its targeting improves immunotherapy against HIV-1.

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BACKGROUND

Stimulation of HIV-1 specific CD8+ T cells with Monocyte-derived dendritic-cells (MDDC) in an attractive immunotherapy strategy against HIV-1. However, chronic inflammation in People with HIV (PWH) has been linked to low CD4/CD8 T cell ratios, activation of the inflammasome and may affect the expression of checkpoint ligands in monocytes (Mo), limiting efficacy of MDDCs. Here, we studied the impact of specific innate inflammasome sensors modulating checkpoint ligands in Mo and MDDC from PWH and their ability to activate HIV-1 specific T cells.

METHODS

PBMC of a total of n= 29 PWH on ART characterized by low (<0.8; LR; n=17) and normal (>1; NR; n=12) CD4/CD8 T cell ratios were recruited for the study. CD14+ Mo were isolated from PBMC and exposed to LPS, Flagllin, Poly I:C, CL097 or gag peptides for 16h. Expression of CD40, PDL1, PVR and Galectin-9 was assessed by FACS. IL1β was analyzed in culture supernatants by ELISA. Inflammasome sensor mRNA expression was analyzed by qPCR. IFI16 silencing in Mo and MDDC from PWH was performed by specific siRNA and compared to cells treated with scramble controls. Finally, Caspase (Z-VAD-FMK), NLRP3 (MCC950) and AIM2/IFI16 (ODN-TTAGGG) inhibitors were used to address modulation of checkpoint ligands in MDDC from PWH and their ability to activate polyfunctional HIV-1 Gag-specific CD8+ T cells.

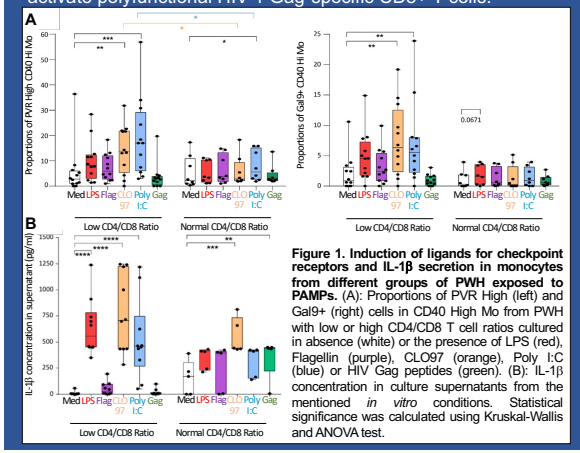


Figure 1. Induction of ligands for checkpoint receptors and IL-1β secretion in monocytes from different groups of PWH exposed to PAMPs. (A): Proportions of PVR High (left) and Gal9+ (right) cells in CD40 High Mo from PWH with low or high CD4/CD8 T cell ratios cultured in absence (white) or the presence of LPS (red), Flagellin (purple), CLO97 (orange), Poly I:C (blue) or HIV Gag peptides (green). (B): IL-1β concentration in culture supernatants from the mentioned *in vitro* conditions. Statistical significance was calculated using Kruskal-Wallis and ANOVA test.

Higher induction of PVR and Gal9 expression in Mo/DC from PWH with low CD4/CD8 T cell ratios is dependent on the IFI16 inflammasome

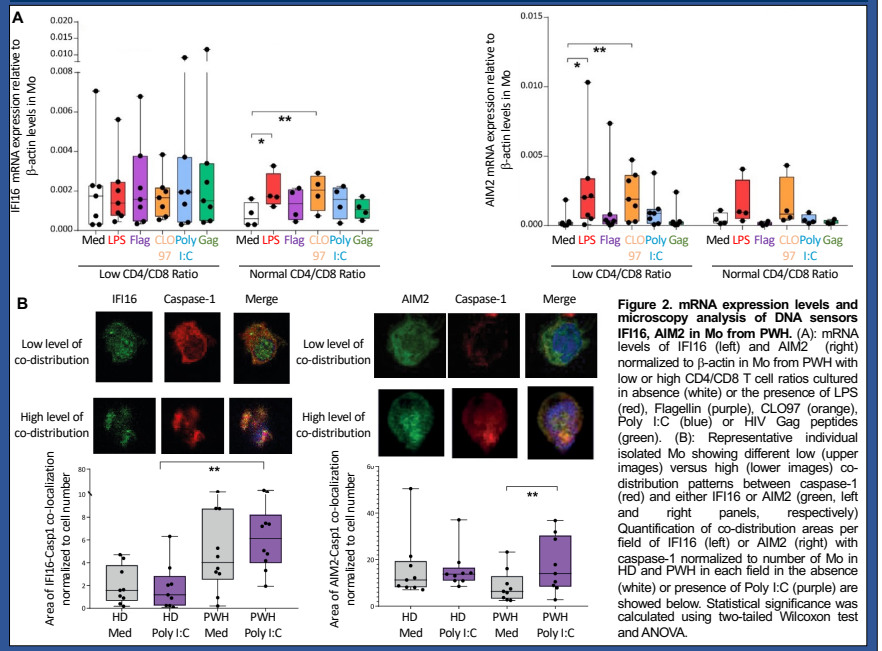


Figure 2. mRNA expression levels and microscopy analysis of DNA sensors IFI16, AIM2 in Mo from PWH. (A): mRNA levels of IFI16 (left) and AIM2 (right) normalized to β-actin in Mo from PWH with low or high CD4/CD8 T cell ratios cultured in absence (white) or the presence of LPS (red), Flagellin (purple), CLO97 (orange), Poly I:C (blue) or HIV Gag peptides (green). (B): Representative individual isolated Mo showing different low (upper images) versus high (lower images) co-distribution patterns between caspase-1 (red) and either IFI16 or AIM2 (green, left and right panels, respectively) Quantification of co-distribution areas per field of IFI16 (left) or AIM2 (right) with caspase-1 normalized to number of Mo in HD and PWH in each field in the absence (white) or presence of Poly I:C (purple) are showed below. Statistical significance was calculated using two-tailed Wilcoxon test and ANOVA.

RESULTS

Expression of PVR (p=0.0002) and Galectin-9 (p=0.0078) was significantly increased in CD40hi Mo from LR PWH, compared to those with NR PWH after CL097 stimulation (**Figure 1A**). Also, a higher increase in IL1β secretion upon LPS and CL097 (p<0.0001) was observed in Mo from LR PWH, indicating inflammasome overactivation (**Figure 1B**). DNA sensing inflammasome sensors such as AIM2 and IFI16, were specifically either induced in response to CL097 (AIM2; p=0.0214) or basally upregulated (IFI16) in the Mo from LR PWH group (**Figure 2A**). Upon the stimulation with Poly I:C, IFI16 and caspase-1 were significantly more co-localized in Mo from PWH compared to HIV- donors (HD) (**Figure 2B, left**). On the other hand, higher colocalization of AIM2 and caspase-1 was also observed in Mo from PWH upon Poly I:C stimulation compared to media control condition, but levels were similar to those observed in HD (Figure 2B, right). IFI16 siRNA-mediated Knock down in Mo from LR PWH led to a significant reduction of PVR (p=0.0156) and Galectin-9 (p=0.0391) expression after CL097 or STING agonist stimulation. Inhibition of caspases (p=0.0195) and AIM2/IFI16 (p=0.0039 prevented upregulation of PVR in MDDC from PWH. Finally, inhibition of casases or IFI16/AIM2 by potentiated IFNγ+ TNFα+ CD107a+ HIV-1-specific CD8+ T cell responses in vitro.

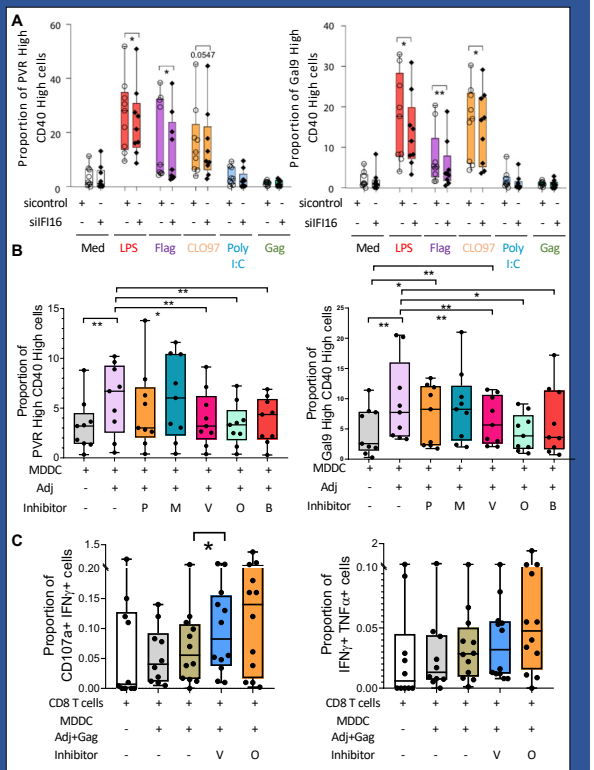


Figure 3. Dependence of expression of checkpoint ligands on IFI16 in activated monocytes from different groups of PWH and functional impact of inflammasome inhibition in ability of MDDC to activate effective HIV-1 specific CD8+ T cell responses. (A): Proportions of CD40 High PVR High (left) and CD40 High Gal9 High Mo (right) upon treatment with siRNA specific for IFI16 cultured in absence (white) or the presence of LPS (red), Flagellin (purple), CLO97 (orange), Poly I:C (blue) or HIV Gag peptides (green). (B): Proportions of PVR High CD40 High MDDCs in control condition (white) or activated with Poly I:C and 2'3'-c'diAM(PS)2 (Adj) (pink) and treated with inhibitors: Parthenolide (P, orange), MCC950 (turquoise), Z-VAD-FMK (ruby), ODN-A151 (mint) or BX795 (red). (C): Proportions of CD107a+ IFNγ+ (left) and IFNγ+TNFα+ CD8+ T cells (right) in preseence of MDDC activated with Gag peptides and adjuvants and tretated with V or O. Statistical significance was calculated using two-tailed Wilcoxon test and ANOVA.

CONCLUSIONS

IFI16 inflammasome regulates expression of ligands for checkpoint receptors in Mo and MDDC from PWH and its targeting may be useful to enhance immunotherapies against HIV-1.

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