CROI 2015 SEATTLE, WASHINGTON February 23-26, 2015

285

The Alarmin HMGB1 is crucial for the acquisition of pDC Effector Functions

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BACKGROUND

Plasmacytoid dendritic cells (pDCs) are the main producers of interferon-alpha (IFN-α), a powerful innate antiviral cytokine. IFN-α production by pDCs is promoted by their cross-talk with NK cells, which triggers NK cell-cytotoxicity through the up-regulation of the death ligand TRAIL. HIV-1 directly activates pDCs by inducing their maturation and their production of IFN-α. HIV-1 is also involved in the differentiation of pDCs into TRAIL-expressing killer cells. The impact of NK cells on IFN-α-production and TRAIL-expression by pDCs in the context of HIV-1 infection is unknown.

OBJECTIVE

Given the essential role of both pDCs and NK cells in the control of HIV-1 infection, the objective of this study was to determine the impact of NK-pDC cross-talk on the reciprocal triggering of antiviral activities.

METHODS

pDCs and NK cells were negatively sorted from PBMC of healthy donors. NK cells were kept either unstimulated (rNK) or activated with PMA/ionomycin for 2h (aNK). pDCs were either uninfected or infected with R5-HIV-1 BAL (pDC_{HIV}) at various concentrations and cocultured with NK cells at different ratios for 24h. The fate of both cell types was studied using multiparametric flow cytometry combined to Multianalyte Profiling technology and Gene array approach.

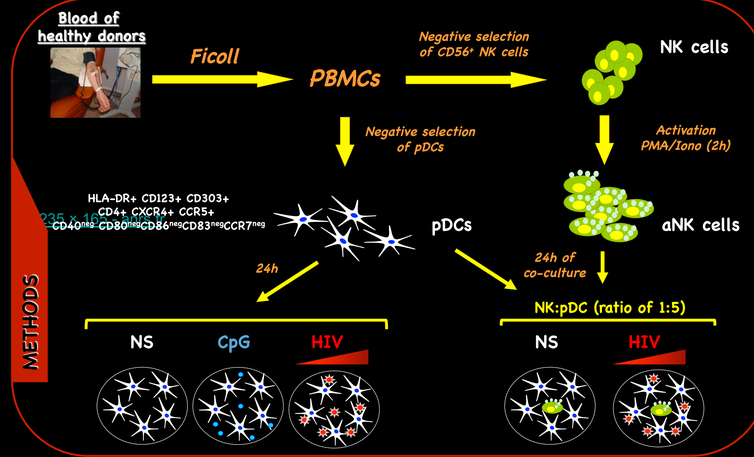
RESULTS

HIV-1-infection of primary pDCs induces the expression of maturation markers (HLA-DR, CD80, CD83, CD86) and the homing receptor CCR7. It also induces the emergence of TRAIL-expressing IFN-α-producing pDCs in a dose-dependent manner. At low concentrations of HIV-1, the crosstalk of pDC_{HIV} with aNK cells significantly increased the production of IFN-α, the proinflammatory mediators IL-12, TNF-α and IFN-γ and the β-chemokines MIP-1α and MIP-1β. It also triggered TRAIL expression on aNK cells. At high concentrations of HIV-1, aNK cells did not significantly modulate the production of these cytokines and chemokines. Interestingly, the alarmin HMGB1 released by pDCs upon HIV-1 infection, plays an essential role during NK-pDC cross-talk, and its inhibition by Glycyrrhizin, N-ethyl pyruvate or anti-HMGB1 Abs prevented pDCs maturation, the release of IFN-α and β-chemokines, and the emergence of TRAIL-expressing aNK cells and pDCs.

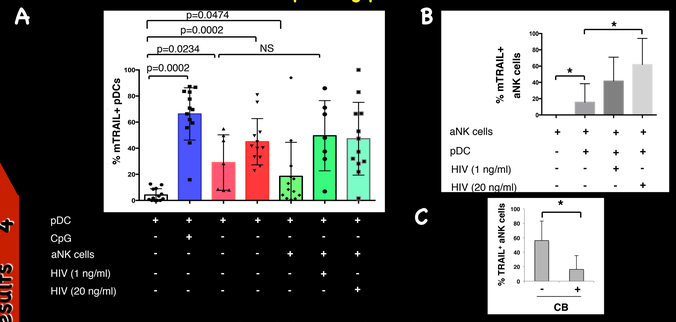
CONCLUSION

Our findings highlight the crucial role of NK-pDC_{HIV} cross-talk in the acquisition of effector antiviral functions by pDCs and NK cells and reveal the essential role of HMGB1 during this cross-talk.

METHODS



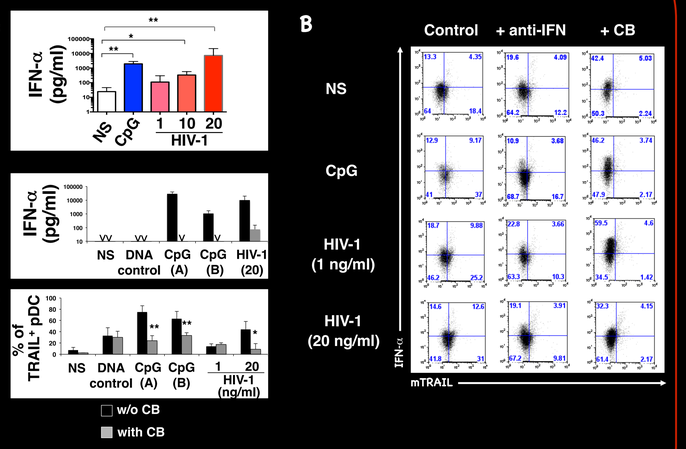
Impact of HIV-1 concentration on the emergence of TRAIL-expressing pDCs and NK cells



Results 4

pDCs were infected with low (1 ng/ml) or high (20 ng/ml) concentrations of HIV-1 in the presence or absence of aNK cells. The expression of TRAIL was monitored at the surface of pDCs (**A**) or aNK cells (**B** and **C**). Clobenproprit (CB) was used in co-cultures to prevent the release of IFN-α (**C**). HIV-1 did not induce the expression of TRAIL at the surface pf NK cells (data not shown). In co-cultures, only high levels of HIV-1 significantly increased the expression of TRAIL at the surface of aNK cells.

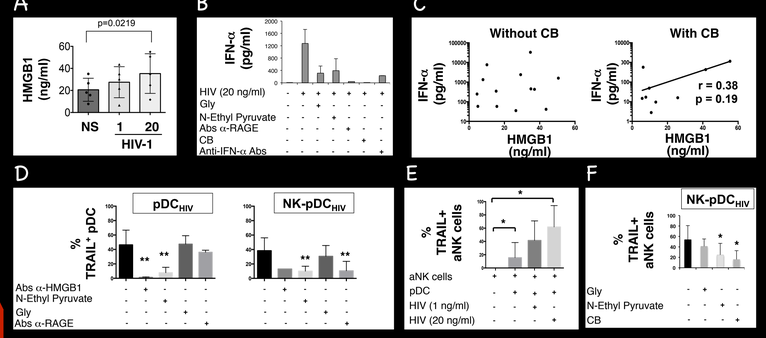
Impact of HIV-1 concentration on the emergence of TRAIL-expressing pDCs. Influence of IFN-α



Results 2

HIV-1 induces the release of IFN-α by pDCs in a dose-dependent manner **(A)**. IFN-a was neutralized either by the addition of specific antibodies (+ anti-IFN) or Clobenpropit (CB), a chemical molecule that inhibits specifically the release of IFN-α **(B and C)**. Neutralization of IFN-α significantly prevented the expression of mTRAIL at the surface of pDCs **(C and D)**.

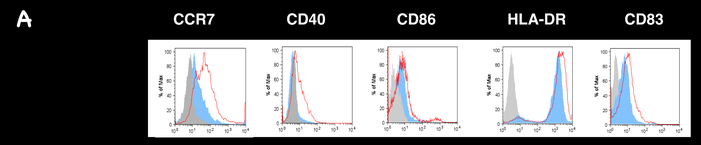
Released during NK-pDC cross-talk, the alarmin HMGB1 is essential to the release of IFN-a by pDCs and the induction of cytoxic TRAIL+ pDCs and NK cells



Results 5

Quantification by ELISA of the release of HMGB1 **(A)** and IFN-α **(B)** in supernatants of NK-pDC co-cultures. HMGB1 was neutralized by the addition of Glycyrrhizin (Gly) or N-ethyl pyruvate and IFN-α by Clobenpropit (CB) or anti-IFN-α Abs. RAGE, the major HMGB1 receptor expressed on pDCs, was blocked with specific antibodies. Correlation was found between HMGB1 and IFN-α quantified in the supernatants of cells cultivated in the presence of CB **(C)**. Inhibition of the interaction between HMGB1 and RAGE by anti-RAGE Abs prevented the expression of TRAIL at the surface of HIV-infected pDCs when the cells were cultivated alone or in the presence of aNK cells **(D)**. The culture of aNK cells with pDCs increased significantly the expression of TRAIL at their surface, this expression increased dramatically when pDCs were infected **(E)**. The expression of TRAIL on aNK cells was not modulated directly by the virus (data not shown). the presence of HMGB1 (Gly and N-ethyl pyruvate) and IFN-α (CB) inhibitors prevented the expression of TRAIL on aNK cells **(F)**.

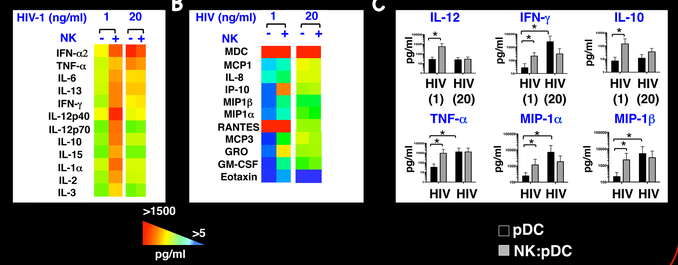
HIV-1 induces the maturation of pDCs in a dose-dependent manner



Results 1

CpG (A) or HIV-1 (B) increases on pDCs the expression of all studied maturation markers following 24h exposure.

Low HIV-1 concentrations do not induce the release of cytokine/chemokines by pDCs unless aNK cells are added. In contrast, aNK cells have no impact at high virus concentrations



Cytokines/chemokines were quantified by Luminex.

Results 3

Conclusion

Altogether, our results show that:

- low concentrations of HIV-1 (as found early after HIV-1 mucosal transmission) require the cross-talk of pDCs with NK cells for pDCs activation and Th1 microenvironment.

However, this Th1 response may occur too early at this stage of HIV-1 infection and may be inefficient to limit HIV-1 mucosal transmission.

- high concentrations of HIV-1 (as found following local expansion of the virus in mucosal tissues) are able to activate the secretion by pDCs of anti-viral cytokines/chemokines and the cytotoxic functions of both NK cells and pDCs. The alarmin HMGB1 is involved in this process.

However, the functional activation of cytotoxic cells may occur too late to control mucosal dissemination of the virus.

