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cGAS induced type I IFN responses in dendritic cells from HIV elite controllers.

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Introduction

Cell-intrinsic HIV-1 immune recognition by IFI16 in CD4 T cells can cause pyroptosis, CD4 T cell loss, and increased immune activation. However, such cell-intrinsic immune responses in alternative cell subsets are thought to contribute to protective immune activity against HIV-1. Indeed, our previous studies indicated an accumulation of viral reverse transcripts and a rapid and sustained cell-intrinsic type I IFN response in primary conventional dendritic cells (cDC) from Elite controllers (EC) upon HIV-1 infection, which was functionally relevant for inducing and supporting effective HIV-1-specific CD8 T cell responses. However, molecular mechanisms accounting for such efficient cell-intrinsic immunity against HIV in cDCs from these patients remain unknown .

Hypothesis

Potent type I IFN responses in human primary dendritic cells from HIV elite controllers are dependent on effective induction of cGAS and are associated with inflammatory functional maturation profiles.

Materials and methods

Ex vivo infection of human peripheral blood cDCs.

▪ PBMC from EC, untreated chronic progressors (CP), and HIV-1 negative subjects were *ex vivo* infected with HIV-1-VSV-G-GFP in the presence of 5ug/ml polybrene. After 24, 48h post infection cDCs were isolated and expression of putative cytoplasmic DNA sensors was analyzed by RT-PCR.

Isolation of cDC and qantitative RT-PCR analysis of DNA sensors.

▪ Total RNA was obtained from BDCA1+ cDCs purified by MACS (purity > 95%) from HIVneg, CP, and EC cultured during 24 or 48h in the presence of media or HIV-1. cDNA was subsequently synthesized and transcriptional levels of IFI16, cGAS, STING and IFNβ were quantified using individual qPCR assays or TaqMan MicroFluidic cards.

Single cell-level RNAseq analysis of innate responses against HIV-1 in cDC from an Elite Controller.

▪PBMCs from an elite controller were cultured in the presence of media or HIV-1 for 48h and cDCs were sorted at the single cell level for RNAseq analysis performed by John Trobetta and Dr. Alex Shalek. Biostatistical analysis of the transcriptional patterns observed in single cDCs cultured in the presence of media or HIV-1 was performed by Dr. Zhengyu Ouyang.

SiRNA-mediated knock down of cGAS expression in primary cDCs.

▪Primary cDCs were isolated from HIVnegative PBMC as described and nucleofected with control scramble (Sc) or cGAS-specific (cGAS) siRNAs. At 24h post-nucleofection, SC and cGAS-siRNA treated cDCs were infected with a VSVG-pseudotyped HIV-1 virus and transcriptional levels of cGAS and type I IFNs were assessed by qPCR at 24h p.i.

Results

▪Expression of the DNA sensor cGAS was more efficiently induced at early time points after exposure to HIV-1 (24h) in cDCs from EC, in contrast to HIV-infected CP or healthy individuals (**Fig1**, left panel). In the other hand, the downstream effector of cGAS STING and the alternative sensor IFI16 were similarly upregulated in all study cohorts at 24h p.i, but more significantly induced at later time points post infection in elite controllers (48h) (**Fig.1**; lower panel). Induction of type I IFN secretion in cDC depended on recognition of viral reverse transcripts by cGAS (**Fig.6**), but was largely unaffected by IFI16 or other known antimicrobial DNA sensors (data not shown).

Figure 1

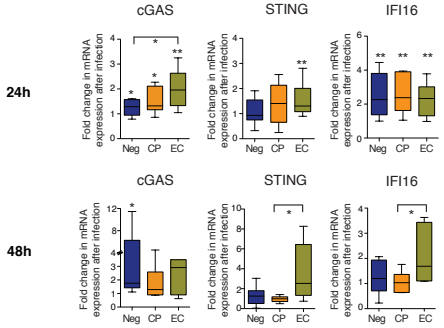


Figure 1. Induction of cytosolic DNA sensors in cDCs from EC. Fold change in cGAS, STING and IFI16 mRNA expression levels in indicated study cohorts at 24 (upper panels) and 48 (lower panels) hours after ex-vivo infection with HIV-1. Induction of mRNA expression in comparison to baseline levels was tested for statistical significance using the Wilcoxon matched-pairs signed-rank tests. Significant differences between distinct cohorts were calculated using the Mann Whitney test. No correction for multiple comparisons was applied.

▪ Characterization of innate immune responses against HIV-1 in cDCs from an elite controller at the single-cell level by RNAseq revealed the existence of distinct subpopulations of cells responding to HIV-1 defined by principle component analysis, named as HIV-A, HIV-B and HIV-C (**Fig. 2**). While transcriptional patterns of HIV-C DCs overlapped with control media-treated cells (**Fig.2**), HIV-A and HIV-B were characterized by specific signatures (37 and 208 specific genes for A and B, respectively) (**Fig.3**) distinct from untreated cells that suggested different activation pathways or status in response to HIV-1.

Figure 2

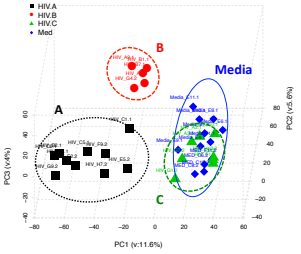


Figure 2. Identification of differential transcriptional patterns on single-DCs exposed to HIV-1. Principle component analysis of single DCs cultured for 48h in the presence of Media (Med) or HIV (HIV). Graph represents tridimensional distribution of single DCs in PC1, PC2 and PC3 based on the expression of 13798 total detectable genes. Three patterns of HIV-treated DCs (HIV-A, HIV-B, HIV-C) are observed.

Figure 3

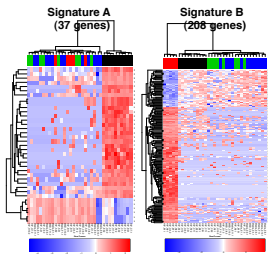


Figure 3. Gene signatures of A and B HIV-exposed DC population. Heatmaps reflecting unique gene expression signatures (High/Low) in HIV-A (37genes; left panel) and HIV-B (208 genes; right panel) single-DC populations are shown. Gene signatures were defined by maximum/minimum levels of a given gene that were smaller/larger in a group compared to all other samples with at most 3 exceptions..

Figure 4

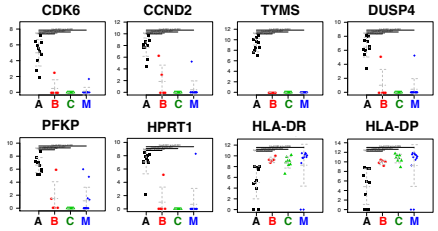


Figure 4. HIV-A DC signature is characterized by genes involved in cell cycle and metabolism. Plots showing expression levels (TPM= Transcripts per million) of CCND2, CDK6, TYMS, DUSP4, PFKP, HPRT1, HLA-DR, HLA-DP generated by RNAseq for single DCs treated with Media (M, blue) or HIV (A,black; B,red; C, green), included in the specific signature of HIV-A. DCs treated with media Statistically significant differences among different DC populations were calculated using a t student and a Wilcoxon rank-sum test (shown on the upper area of graphs).

▪ HIV-A cDC signature was characterized by downregulation of MHC-class II genes and the induction of genes involved in cell-cycle such as CCND2 and CDK6 and regulation of metabolism such as the enzymes TYMS (thymidylate synthetase), DUSP4(phosphatase), PFKP (Phosphofructokinase), HPRT1 (Phosphoribosyl transferase 1), suggesting responses more associated with cellular stress than efficient maturation (Fig.4). In contrast, HIV-B cDC signature was characterized by a very significant upregulation of the IFN-induced genes IFITM3, IFIT3 and IFI30, the chemokines CXCL9, CXCL10 (IP-10), CXCL16, and the activation marker CD38 (Fig.5), indicating efficient maturation of cDCs in response to HIV-1. Such functional responses seemed to be associated with a very efficient induction of cGAS in HIV-B DCs (Fig.5). Importantly, si-RNA-mediated knock down of cGAS in primary cDCs dramatically prevented the induction of IFNb in response to HIV-1, suggesting that this sensor is required to effective innate immune responses against HIV-1 in these cells.

Figure 5

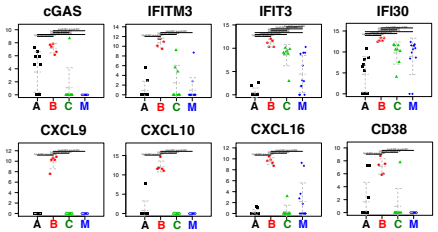


Figure 5. HIV-B DC signature is characterized by high expression of cGAS, ISGs and functional maturation of DCs. Plots showing expression levels (TPM= Transcripts per million) of cGAS, IFITM3, IFIT3, IFI30, CXCL9, CXCL10, CXCL16 and CD38 generated by RNAseq for singles DC treated with Media (M, blue) or HIV (A,black; B,red; C, green), included in the specific signature of HIV-B. Statistically significant differences among DC populations were calculated using a t student and a Wilcoxon rank-sum test (shown on the upper area of graphs).

Figure 6

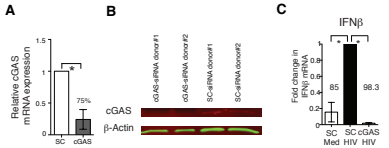


Figure 6. Si-RNA mediated silencing of cGAS prevents type I IFN responses in cDCs exposed to HIV-1. Efficacy of siRNA-mediated knowdown of cGAS expression in primary cDC. Data indicate mRNA (left panel) and protein (right panel) expression levels of cGAS in cDCs nucleofected with scramble control (SC) or cGAS-specific siRNAs by qPCR (A) and western blot (B), respectibely. (C) Normalized IFNβ expression in primary cDCs nucleofected with scrambled (SC) or cGAS-specific siRNAs, followed by infection with HIV-1. Data from n=4 experiments are shown. Differences in type I IFN responses among untreated or SC- or cGAS-nucleofected cDCs were tested for statistical significance using a Kruskal-Wallis test with post-hoc Dunn's test * p<0.05; ** p<0.01.

Conclusions

▪ Cell-intrinsic immune recognition of HIV-1 by cGAS in cDC induces type I IFN responses that initiate complex changes in gene expression profiles leading to functional maturation that can enhance antiviral immunity in elite controllers. These data suggest that cell-intrinsic immune recognition of HIV in CD4 T cells and in cDC can have distinct, and partially opposing functions in HIV-1 disease pathogenesis.