**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 conundrites cell (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, uncoated chrosoegene (CP), and HIV−1 negative subjects were ex vivo infected with HIV−1-SVGS-GFP in the presence of 5 μg/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 quantitative RT-PCR analysis of DNA sensors.**

- Total RNA was obtained from BMDs−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 TNFa 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 and cDCs were sorted at the single cell level for RNAseq analysis performed by John Trubett and Dr. Alex Sheik. Biostatistical analysis of the transcriptional patterns observed in single cDCs cultured in the presence of media or HIV−1 was performed by Dr. Zhongyuan Ouyang.

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

- Primary cDCs were isolated from HIV−negative 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 VSV−pseudotyped HIV−1 virus and transcriptional levels of cGAS and type I IFNs were assessed by qPCR at 24h pi.

**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 (Fig 1, left panel). In the other hand, the downstream effector of cGAS STING and the alternate sensor IFI16 were similarly upregulated in all study cohorts at 24h pi, but more significantly induced at later time points post infection in elite controllers (48h pi) (Fig 1, lower panel). Induction of type I IFN secretion in cDCs dependent on recognition of viral reverse transcripts by cGAS (Fig 6), but was largely unaffected by IFI16 or other known antiviral microbial sensors (data not shown).

**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.