HIV INFECTION OF TFH: INTERROGATING THE SAMHD1 CONTRIBUTION THROUGH HIV-2

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BACKGROUND

SAMHD1 is a host restriction factor with recognized impact in HIV reservoir establishment and suggested to be involved in follicular helper T cell (Tfh) biology. We explore here the ability of HIV-2 to counteract SAMHD1 due to the expression of Vpx, to further investigate the interplay of SAMHD1 and HIV in Tfh.

METHODS

We sort-purified two distinct subsets of CD4+ T-cells from tonsillar tissue (n=6) known to feature distinct levels of SAMHD1, namely: Tfh, defined as CXCR5^{bright}ICOS^{bright}PD-1^{bright}, and quiescent triple negative (TN), defined as CXCR5^{neg}ICOS^{neg}PD-1^{neg}, using FACS Aria (BD). Both Tfh and TN subsets were infected with HIV-2 and HIV-1 primary isolates with either CXCR4 or CCR5 co-receptor tropism. In parallel, CD4+ naïve T-cells were magnetically sorted from buffy-coats and differentiated into Tfh using a standardized protocol of TCR stimulation and cytokine exposure (TGF-β, IL-12, IL-6, IL-1β) upon infection with HIV-2 or HIV-1 dual-tropic virus. Cell associated total viral DNA and gag mRNA levels were quantified by qPCR, as well as SAMHD1 and Bcl6 expression. Cell phenotype was assessed utilizing high dimensional spectral flow cytometry (Cytek, Aurora), and the data was visualized utilizing the dimension reduction technique UMAP.

ADDITIONAL KEY INFORMATION

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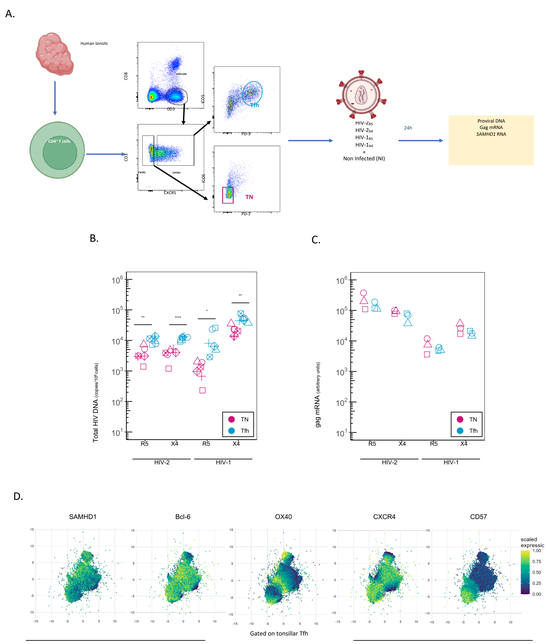
Funding: This work was supported by the following grants: PTDC/MED-IMU/30474/2017 - cofunded by FEDER LISBOA-01-0145-FEDER-030474, through POR Lisboa 2020 - Programa Operacional Regional de Lisboa, do PORTUGAL 2020 and Fundação para a Ciência e a Tecnologia; and Gilead Genesis to AES; GAPIC Program from FMUL to AP; CMC, AVA, ACT, and AGS received fellowships co-funded by FCT and FEDER, and GBF by Janssen-Cilag Farmacêutica.

Acknowledgments: We thank the ENT of Hospital de Santa Maria/Centro Hospitalar Lisboa Norte/Lisboa Portugal, the Instituto Português do Sangue e Transplantação (IPST), the Flow Cytometry Unit and Biosafety Level 3 facilities at Instituto de Medicina Molecular JLA and all donors for their collaboration.

These first data using primary isolates show that **HIV-2**, despite harboring **Vpx**, has ***no significant advantage to infect CD4 T cells, regardless of their SAMHD1 levels, as well as to modulate Tfh differentiation***, with implications for HIV-2 benign course.

RESULTS I



Sort-purified Tfh (CXCR5^{bright}ICOS^{bright}PD-1^{bright}) and quiescent triple negative (TN, CXCR5^{neg}ICOS^{neg}PD-1^{neg}) from human tonsils, with clearly distinct SAMHD1 levels, were infected with HIV-2 or HIV-1 primary isolates, using either CCR5 or CXCR4 (A).

We found that, despite higher SAMHD1 expression, TN featured comparable levels of total viral DNA and gag mRNA upon HIV-2 and HIV-1 infections (B-C).

Nevertheless, total viral DNA levels were higher in Tfh, irrespective of viral strain (B).

SAMHD1 protein expression in Tfh (CD3+CD4+CXCR5^{bright}ICOS^{bright}PD-1^{bright}) from human tonsils (ex-vivo, n=9) was heterogeneous and did not correlate with other markers of Tfh differentiation (D).

CONCLUSIONS

HIV-2, despite harboring Vpx, did not feature a significant advantage to infect tonsillar CD4 T cells, irrespectively of their SAMHD1 levels, as well as to modulate in-vitro TFH differentiation. These first data using HIV-2 primary isolates prompt the investigation of additional paths in the establishment of Tfh reservoirs, and their implications for the benign course of HIV-2.

RESULTS II

We asked whether HIV-2 infection biased Tfh generation from circulating naïve CD4 T cells (A).

Levels of cell-associated total viral DNA were similar 72h post-infection with dual-tropic HIV-2 and HIV-1 primary isolates (B).

The frequency of the generated Tfh (C), and their detailed phenotype (D-E) were remarkably similar in all conditions.

