STINGING CD4+ T CELLS TO RENDER THEM REFRACTORY TO HIV INFECTION

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BACKGROUND

HIV rapidly infects CD4+ T cells where it can establish latent infection. Identifying immune mechanisms which can render CD4+ T cells refractory to infection represents a critical step in limiting infection and viral spread during acute infection. Immune cells have multiple mechanisms of detecting viral infection and one of these is through nucleic acid sensing in the cytosol. It has recently been shown that STING signaling downstream of cGAS activation by cytosolic DNA is essential for detecting and combating HIV infection; though this has been studied mainly in the context of myeloid cells. However, it is known that STING is expressed and has functional signaling in T cells. In this study, we identify that the STING signaling pathway functions in CD4+ T cells and can be activated to mediate a cellular state refractory to HIV infection. ***We hypothesize that activation of STING prior to or immediately following HIV exposure renders CD4+ T cells refractory to infection, preventing viral spread and dissemination, while augmenting normal antiviral immunity to promote global viral clearance.***

METHODS

- Purified memory CD4+ T cells were stimulated with 1 μM diABZI for 24 hrs. Cell pellets were obtained and LC-MS global abundance proteomics was performed. Proteins significantly induced or reduced (FDR<0.05) were identified and pathway enrichment analysis performed using ClueGO.
- Purified total CD4+ T cells were stimulated with the known STING agonist 1 μM cGAMP for 18 hrs and Type I IFNs and CXCL10 quantified in the supernatant using the U-Plex platform from MesoScale Discovery.
- Using purified memory CD4+ T cells from healthy human donors, we stimulated cells with titrating doses (0.1 μM-50 μM) of the STING agonist diABZI for 18 hours followed by infection with HIV 89.6. IFNa was used a positive control for reduced HIV infection. p24 levels were quantified by flow cytometry (FCM) after 72 hours of infection, in the presence of 5 uM Saquinavir (HIV protease inhibitor that prevents viral budding. Using CRISPR mediated knockout, we next identified the critical role for STING sensing of HIV in limiting infection in purified CD4+ T cells. Control (CTL), STING KO, and NLRX1 KO (a known negative regulator of STING signaling) cells were infected and p24 quantified, as described before.

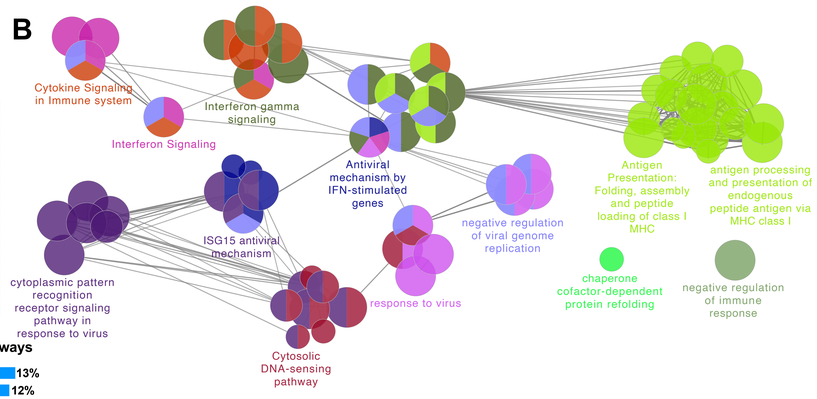
Activation of ***STING signaling in CD4+ T cells*** induces antiviral proteins which render cells ***refractory to HIV infection***

RESULTS

A

Top 50 induced proteins by diABZI							
1	IFIT2	14	IFI44	27	TRANK1	39	HGF
2	IFIT3	15	CMPK2	28	TRAFD1	40	STAT2
3	IFIT1	16	OAS1	29	APOL2	41	SAMD9L
4	OASL	17	ISG20	30	ZBP1	42	DDX58
5	HERC5	18	HLA-C	31	CCR7	43	HLA-A
6	CD69	19	DHX58	32	GBP4	44	IRF9
7	IFIH1	20	PARP14	33	KPNA2	45	ZC3HAV1
8	MX1	21	HELZ2	34	XAF1	46	LGALS3BP
9	ISG15	22	TFRC	35	MX2	47	NUB1
10	GBP5	23	POTEJ	36	HLA-C	48	PARP9
11	CAP1	24	AFAP1	37	DTX3L	49	SIPA1L1
12	RTP4	25	RNF149	38	OAS2	50	FND3CA
13	MT2A	26	GBP7				

B



C

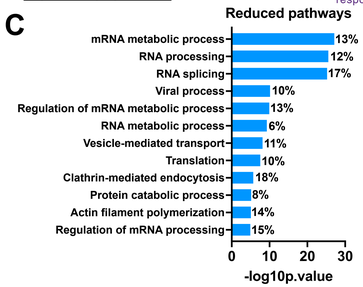


Figure 1. STING potently increases expression of antiviral and innate immune proteins in CD4+ T cells. Purified human memory CD4+ T cells were treated with the STING agonist diABZI for 24 hours and then global abundance proteomics performed by LC-MS. (A) The top 50 induced proteins (fdr <0.05) include antiviral (IFITs, OASs, MXs, ISGs), innate immune (IRF9, DDX58) and MHC Class I (HLA-A, HLA-C) proteins. Key antiviral/immune proteins are bolded in **red**. (B-C) Pathway enrichment analysis using ClueGO reveals, as expected, that induced proteins map to key pathways of antiviral and innate immunity. Pathways involved in RNA processing, protein translation, cytoskeleton, and vesicle trafficking are enriched for suppressed proteins. % enriched proteins are noted next to pathways.

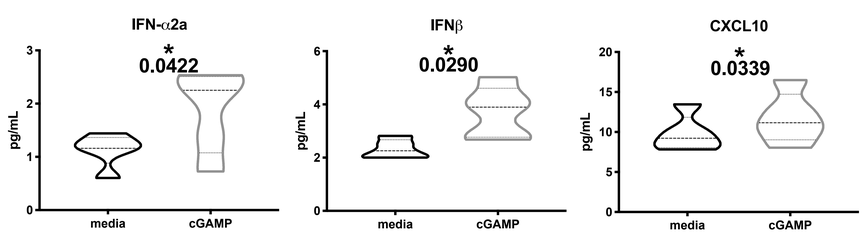


Figure 2 STING signaling induces Type I IFNs Purified CD4+ T cells (n=5) were treated with cGAMP. Expression of Type I IFNs (IFNα, IFNβ) and the ISG CXCL10 were quantified in the supernatants 24 hours post-stimulation. Non-parametric, paired t-test was used for statistical analysis.

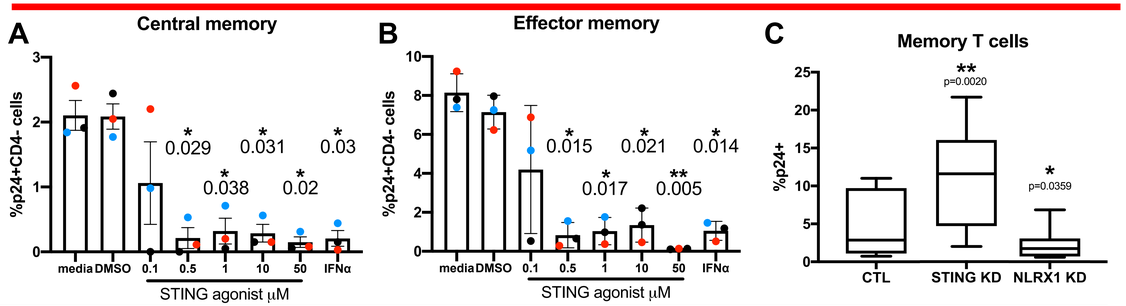
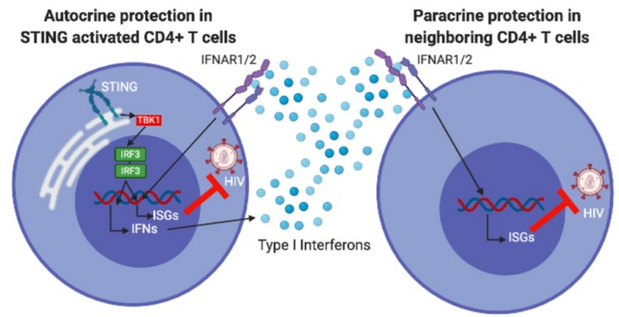


Figure 3. STING signaling in primary memory CD4+ T cells controls HIV infection in vitro. (A-B) Purified CD4+ T cells were treated with the STING agonist diAZBI for 16 hrs and then infected with HIV for 72 hrs. Significantly reduced p24+ cells were observed in central and effector memory cells. **(C)** Purified memory CD4+ T cells were nucleofected with CRISPR RNPs against STING, NLRX1 (a known negative regulator of STING signaling) or non-targeting control and then infected with HIV and p24 levels quantified. Statistics: paired t-test.

CONCLUSIONS



Together, our data clearly demonstrate that STING signaling is functional in CD4+ T cells and induces a state of antiviral immunity through upregulation of antiviral proteins and production of Type I IFNs. This provides mechanistic insight into the STING pathway in CD4+ T cells as a means to mediate refractoriness to HIV infection. Thus, this pathway lands a “1-2” punch of heightening antiviral immunity while suppressing host machinery needed for viral replication. The prospect of treating with a STING agonist as part of post-exposure therapeutics represents a novel and impactful means of helping stop HIV infection in its tracks while restoring the normal antiviral immune response needed to control the remaining virus. In the future, we will use Multi-omics analysis to better understand the critical role of STING signaling in preventing viral infection in humans. Through use of NHP models, we will be able to directly interrogate how activation of this cascade prior to or concurrent with infection impedes viral dissemination and reservoir seeding while restoring normal antiviral and cytotoxic responses in vivo.

ADDITIONAL KEY INFORMATION

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