Histone Deacetylase (HDAC) and Histone Acetyltransferase (HAT) Inhibitors Have Opposing Effects on Cellular Susceptibility to HIV Infection

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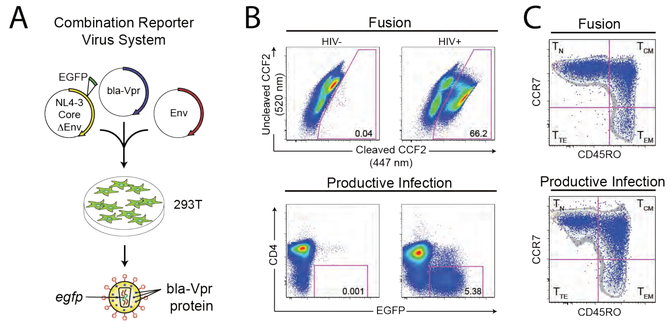
Introduction

- The latent reservoir remains a major obstacle for HIV eradication strategies, as this population can persist for long periods of time.
- The HIV "shock-and-kill" strategy has been proposed involving use of histone deacetylase (HDAC) inhibitors such as vorinostat (SAHA) to reactive proviral transcription while under cART, allowing the immune system to eliminate reactivated cells while antiretroviral drugs prevent reinfection.
- Here we demonstrate a novel effect of HDAC inhibitors to augment HIV productive infection, independent of their ability to reactivate latent provirus.
- Conversely, our findings led us to hypothesize that Histone Acetyltransferase (HAT) inhibitors may act beneficially to reduce productive infection by HIV-1, demonstrating that alterations in protein acetylation can significantly modulate cellular susceptibility to HIV.

Figure 1: HIV Combination Reporter Virus System



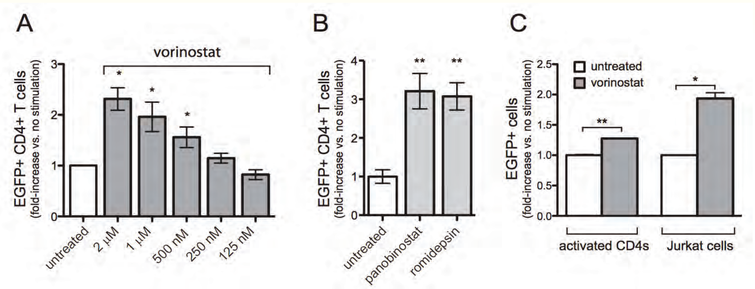
(A) 293T cells are co-transfected with plasmids encoding an egfp-containing HIV core (pNL4-3-deltaEnv-EGFP), β-lactamase-Vpr, and either HIV env JOTO.TA1.2247 (X4-Tropic) or REJO.D12.1972 (R5-Tropic). (B) Viral fusion is detected by flow cytometry as measured by β-lam-Vpr mediated cleavage of CCF2 fluorescent dye. Productive infection is measured by LTR-drvien EGFP expression coupled with Nef-mediated downregulation of surface CD4 receptor. C. CD4+ T cell subsets undergoing fusion and productive infection can be identified within the context of this system. (Shown in blue)

Methods

- CD4+ T cells were treated with HDAC or HAT inhibitors and spinoculated with combination reporter viruses at 1200 g for two hours. Cells were incubated with either 2 μM vorinostat, 2 μM tubacin, 200 nM panobinostat, 200 nM romidepsin, 10 μM garcinol, or 10 μM curcumin 4 hours prior to addition of virus.
- 2-LTR circle and integration products were amplified in parallel qPCR reactions using HIV specific primers and multiplexed. Samples were pooled and sequenced using the Ion Torrent PGM.
- For replication competent experiments, Primary CD4 T cells were stimulated with 100 IU/ml IL-2, infected with X4-tropic replication-competent virus, and spinoculated. Cells and supernatants were harvested at days 1, 3, 5, 7, 9, 11, and 13 postinfection. Productive infection was measured by accumulation of EGFP. Viral concentration in supernatants were quantified by p24 ELISA and normalized to cell viability.

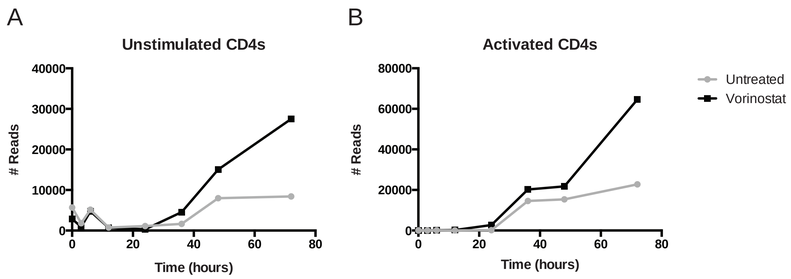
Results

Figure 2. The HDAC inhibitor vorinostat increases susceptibility of primary CD4+ T cells to productive infection by HIV-1



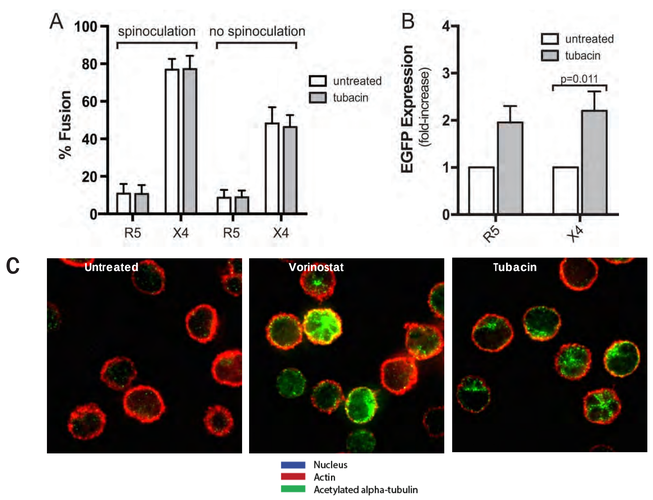
(A) 4 hour pre-treatment with vorinostat enhanced productive infection of X4-tropic HIV-1 in a dose-dependent manner. (B) Vorinostat mediated effects occur independent of co-receptor tropism. (C) Productive infection of T cells was enhanced by 4 hour pre-treatment with additional pan-HDAC (Panobinostat, Romidepsin) and HDAC6 specific (Tubacin) inhibitors.

Figure 3. Vorinostat increases efficiency of nuclear import



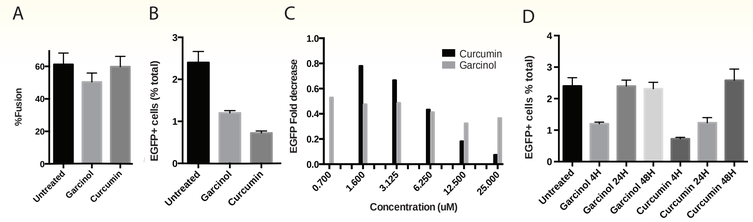
Treatment with vorinostat significantly increased the number of 2-LTR circle sequence reads by 72 hours postinfection. Primary unstimulated (A) and polarized, activated (B) CD4 T cells were infected with X4-tropic reporter viruses and spinoculated. At various time points following infection, the cells were harvested and DNA was extracted for qPCR of 2-LTR circles, followed by deep sequencing to confirm specificity.

Figure 4. Specific inhibition of HDAC6 by Tubacin recapitulates effects of broad spectrum HDAC inhibitors



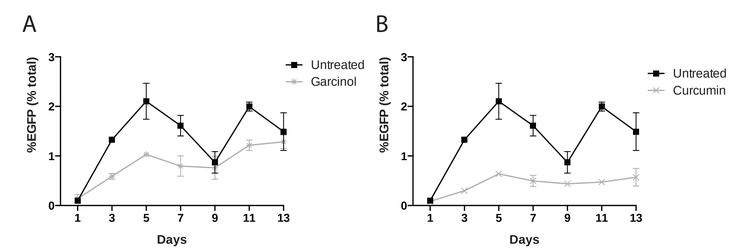
(A,B) Pre-treatment of primary CD4+ T cells with tubacin significantly increased EGFP production in R5- and X4- tropic reporter viruses. (C) Vorinostat and tubacin upregulated acetylated microtubule networks in primary unstimulated CD4+ T cells. Images acquired with Deltavision Core imaging system at 100x magnification.

Figure 5. HAT inhibitors garcinol and curcumin decrease susceptibility of primary CD4+ T cells to productive infection by HIV-1



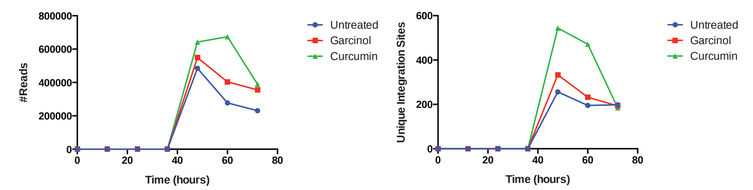
(A) 4 hour pre-treatment with HAT inhibitors garcinol and curcumin does not significantly affect viral fusion. However, (B) both inhibitors decrease productive infection of X4 tropic HIV, albeit curcumin to a greater extent. These effects occur in both (C) dose and (D) time dependent manners.

Figure 6. HAT inhibitors decrease productive infection of HIV in a replication competent spreading infection



A. Pre-treatment of primary CD4+ T cells with (A) garcinol and (B) curcumin decreased EGFP production of replication competent X4 tropic virus. Most notably, treatment with curcumin significantly prevented spreading infection of HIV.

Figure 7. Treatment with garcinol and curcumin increases HIV integration and number of unique integration sites



Pre-treatment of primary CD4+ T cells with HAT inhibitors increases both (A) number of HIV integration sequence reads and (B) overlapping consensus regions of DNA, or "contigs". Reactions normalized by ng input DNA. Sequencing was performed following nested PCR of MseI and BglII digested DNA fragments containing multiplexed adaptors.

Conclusions

- Treatment with vorinostat significantly increases the vulnerability of uninfected cells to HIV-1 by enhancing both kinetics and efficiency of post-entry viral events. The increased susceptibility observed with tubacin pre-treatment suggest a previously unknown cytoplasmic mechanism of HDAC inhibitors promoting viral infection by induction of stable acetylated microtubule networks.
- Conversely, treatment with HAT inhibitors garcinol and curcumin significantly decrease productive infection, demonstrating that the axis of acetylation can directly modulate susceptibility to HIV infection. Additional work will need to be done to dissect the mechanism of these inhibitors and determine where in the viral life cycle they act to decrease productive infection.

Funded by 5T32GM008056-31, NIH-1R01HD077886-01, amfAR-108257-51-RGRL and the Cleveland Foundation. Special thanks to CROI Young Investigator Award program.