Small Molecule Inhibition of Intrinsic Stress Pathways Substantially Reduces HIV Transcription upon T Cell Activation

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Background

Advances in the treatment of HIV-1 infection have dramatically reduced the death rate from AIDS in the United States since 1996, when protease inhibitor–based combination therapy regimens came into widespread use. Although antiretroviral therapy (ART) is effective in suppressing HIV-1 replication by blocking various steps in the viral life cycle, it is not curative due to the existence of a latent viral reservoir in resting memory CD4⁺ T cells. The LR may be established when HIV-1 infects fully or partially activated CD4⁺ T cells that then return to a resting memory state as part of the normal physiological process after encountering cognate antigens. Thus, latent proviruses are found predominantly in various subsets of resting memory cells but not in naïve cells. Studies in model systems have identified many latency reversing agents (LRAs), but few are efficacious in cells isolated from HIV-1 infected patients. Dose-response studies across multiple chemical classes of LRAs in model cells and patient cells have led to our hypothesis that generalized cell stress may play a role in initiating HIV transcription. Here, we study the effect of antioxidants and stress pathway inhibitors on HIV transcription in latently infected cells from HIV-positive patients.

Methods

Resting T cells were isolated from HIV-1 infected individuals with suppression of viremia on cART. Cells were treated for 24 hours with the protein kinase C (PKC) agonist bryostatin and the synthetic Nrf2 agonist HBB2 to mimic downstream T cell activation signaling and cell stress, respectively. Treatment was done in the presence or absence of the reactive oxygen species (ROS) scavenger N-acetylcysteine (NAc) and the HSF-1 inhibitor KRIB11. Intracellular HIV mRNA was measured by RT-qPCR.

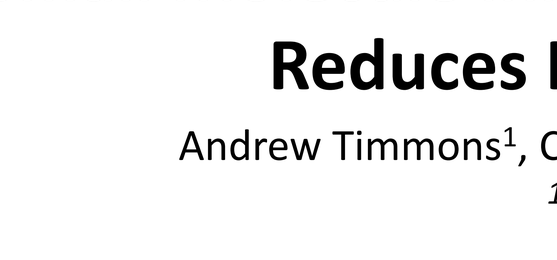


Figure 1 – HIV mRNA was detected by qPCR using a primer/probe set that anneals to the PolyA tail and the 3' distal region of the genome. These primers and probes allow for detection of HIV mRNA regardless of splicing variant, and excludes readthrough transcripts.

Results

In model systems HIV reactivation only occurs at concentrations close to toxic concentrations. However, this pattern is not observed in cells taken from HIV infected patients.

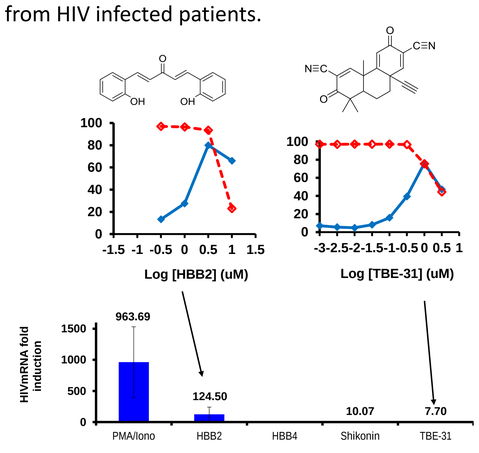


Figure 2 – TOP: Dose response curves of Bis(2)-Hydroxybenzylidine Acetone (HBB2) and tricyclic bis(cyano enone) 31 in BCL2 transduced model cells. Red indicates viability of cells, and blue represents percent HIV expression relative to maximal T cell activation. BOTTOM: HIV mRNA fold induction over DMSO in primary patient cells at maximally effective concentration in model cells.

Results

A combinatorial screen in primary cells reveals interesting synergy with HBB2. The finding that a cell stress inducing molecule increases the expression of HIV mRNA is novel.

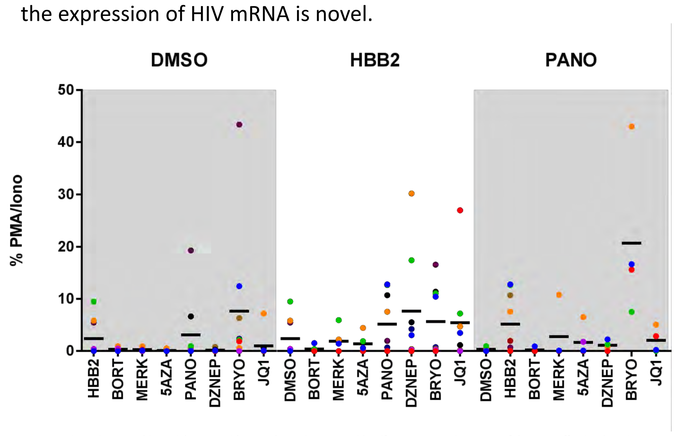
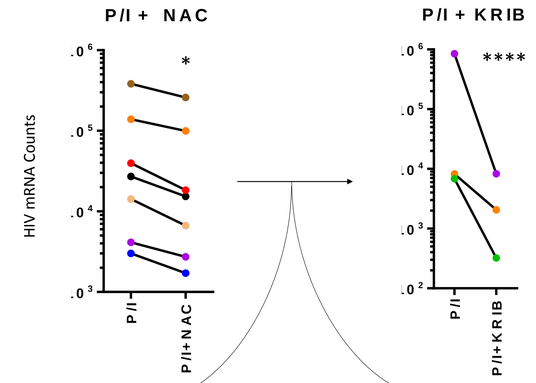
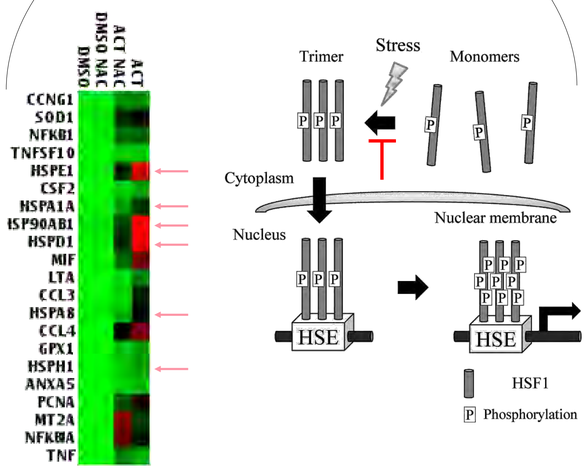


Figure 3 – A combinatorial screen with several putative LRAs to identify synergy. HBB2 was one of the most effective drugs in combination. This is observed with several different classes of LRAs. HIV mRNA was measured by qPCR as described previously.

Increasing oxidative stress appears to have a positive effect on HIV transcription. Reducing oxidative stress with N-acetylcysteine (an ROS scavenger) reduces HIV expression but does not reduce T cell activation





Gene expression assays were performed on cells treated with NAC in the presence and absence of T cell activating stimulus. In cells treated with NAC, there appeared to be a blunted expression of multiple heat shock proteins and transcription factors. As a follow-up experiment the heat shock inhibitor KRIBB11 was used in lieu of NAC, resulting in a much more significant negative effect on the expression of HIV.

Results

In contrast to the inhibition of heat stress using KRIBB11, induction of heat shock via an increase in temperature did not seem to have any effect on the kinetics of HIV expression from P/I treated cells. It did, however, have an effect on the kinetics of virion release from cells exposed to CD3/CD28 costimulation.

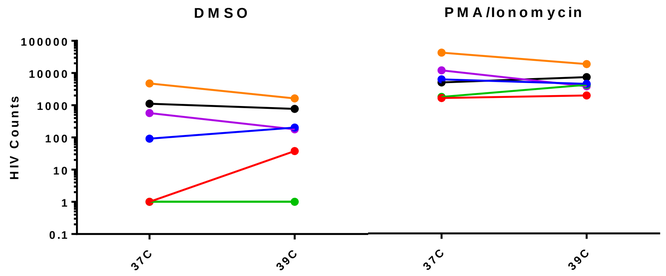


Figure 4 – Increasing temperature does not increase the amount of HIV expressed intracellularly in cells treated with PMA/Ionomycin. T cells were activated with PMA/Ionomycin for 24 hours and then lysed in Trizol solution. Total RNA was isolated from the lysate, and HIV mRNA was specifically amplified using the VQA assay as described previously. In response to

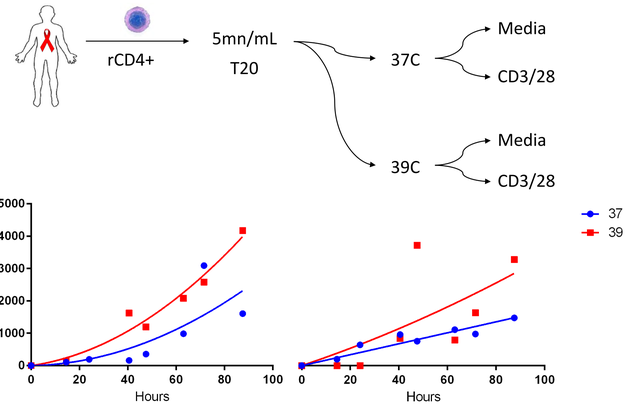


Figure 5 – Increasing temperature increases the amount of HIV released into the supernatant in cells treated with CD3/CD28. Primarily resting CD4+ T cells were isolated from an infected donor and were stimulated with CD3/CD28 dynabeads. Stimulation took place at either 37C or 39C in the presence of T20, which blocks all nascent infection. Supernatant was measured every 12 hours and assessed for levels of HIV mRNA in the supernatant.

Conclusions

We assessed whether intrinsic cell stress pathways play an important role in the reactivation of HIV from its latent reservoir in resting CD4+ T cells. We hypothesize that cell stress itself can act as a latency reversing agent and that cell stress is a contributing component to HIV reactivation. Inhibitors of the oxidative and heat shock stress responses substantially reduce the amount of HIV expressed in activated T cells by approximately 55 and 80%, respectively. In addition, inducing heat stress by raising the incubation temperatures of stimulated cells increased expression and the production of virions in CD3/CD28 stimulated cells, and not in PMA/Ionomycin stimulated cells. These data seem to suggest a fundamental difference between the activation of HIV with PMA/Ionomycin and CD3/CD28 costimulation.

Acknowledgements

We thank Dr. Korin Bullen for providing data from model cell experiments. We also thank Dr. Paul Talalay for advice and reagents used for model cell experiments. Dr. Gregory Laird provided experimental advice and assistance. This work was funded by the Howard Hughes Medical Institute and the National Institutes of health.