Reversing HIV-1 Latency by Targeting RasGRP1-dependent Biogenesis of P-TEFb

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BACKGROUND: The deliberate reactivation of latent HIV-1 to enable clearance of persisting latently infected memory T cells – the Shock and Kill strategy – has had limited success because efficient, non-toxic latency-reversing agents (LRAs) remain to be discovered. We are taking a direct approach to LRA development based on the principle that proviral reactivation is tightly coupled to the posttranscriptional biogenesis of P-TEFb, a cellular transcription elongation factor whose expression is highly restricted in resting memory T cells. Recently, we reported that naturally occurring diacylglycerol (DAG)-mimicking protein kinase C (PKC) agonists stimulate the posttranscriptional expression of the cyclin T1 (CycT1) subunit of P-TEFb to reactivate latent HIV-1 primarily via the RasGRP1-Ras-Raf-MEK-MAPK ERK1/2 signaling pathway rather than through PKC enzymes. Here we describe synthetic agonists that preferentially bind RasGRP1 over PKC and can safely activate P-TEFb to reverse HIV-1 latency in primary CD4$^+$ T cells.

METHODS: Synthetic DAG indololactones, with known differential affinities for PKC and RasGRP, were tested for their ability to stimulate P-TEFb expression in healthy donor-derived memory CD4$^+$ T cells. Combinatorial LRA studies were performed in a primary CD4$^+$ T-cell latency model to examine the effectiveness of DAG indololactones at synergizing with LRAs that target proviral transcription initiation.

RESULTS: Three of the 4 DAG indololactones we tested were more effective at inducing CycT1 and active P-TEFb than the T-cell activation markers CD69 and CD25. The DAG indololactone 2A-127, which preferentially binds RasGRP1 over PKC-α more than 60-fold, synergized with the HDACi SAHA in reactivating latent HIV-1 in primary T cells. DAG indololactones and other DAG-mimicking agonists stimulate CycT1 protein synthesis by activating mTORC1 kinase through ERK1/2 MAPK signaling.

CONCLUSIONS: DAG indololactones can stimulate the posttranscriptional expression of P-TEFb in memory T cells at concentrations below the threshold needed to induce T-cell activation through a RasGRP1-mediated ERK1/2-mTORC1-S6K-rpS6 pathway. These agents can synergize with HDAC inhibitors, thereby bolstering the hypothesis that a two-pronged strategy that targets P-TEFb biogenesis and stimulates RNA polymerase II recruitment to the HIV-1 promoter is needed to reverse HIV-1 latency efficiently.

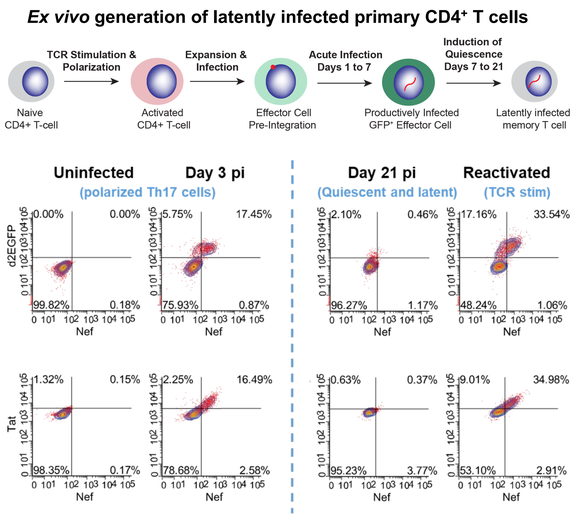


Figure 1. *(Top)* Scheme for generating latently infected polarized CD4$^+$ Th17 cells from healthy donor-derived naïve CD4$^+$ T cells. *(Bottom)* Immunofluorescence co-staining for Tat and Nef to monitor proviral gene expression by flow cytometry in acutely infected, latently infected and reactivated primary CD4$^+$ Th17 cells.

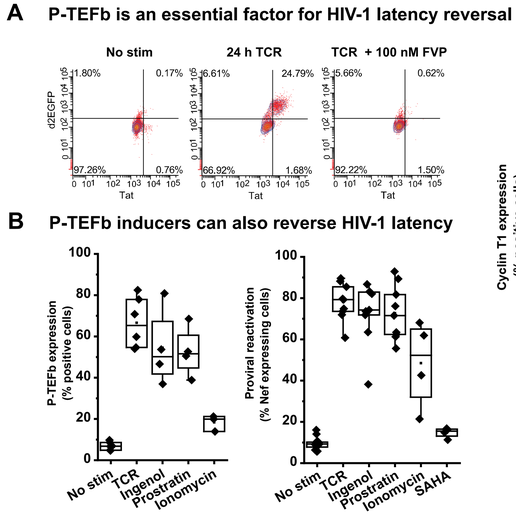


Figure 2. *(A)* An inhibitor of P-TEFb kinase, flavopiridol (FVP) abrogates the TCR-induced reactivation of proviral HIV-1 in primary Th17 cells. *(B)* Measurement of transcriptionally active P-TEFb *(Left)* and latent HIV-1 reactivation *(Right)* by immunofluorescence flow cytometry following 24 h treatment with various stimuli.

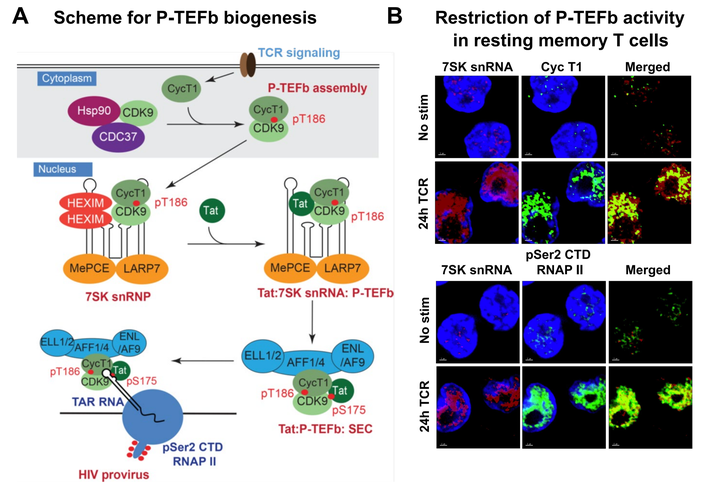


Figure 3. *(A)* Proposed scheme for the biogenesis of P-TEFb in memory T cells and its exchange from 7SK snRNP to assemble the super elongation complex (SEC) on proviral HIV. *(B)* Subcellular expression of the Cyclin T1 subunit of P-TEFb *(Top)* and the P-TEFb-modified pSer2 CTD RNAP II *(Bottom)* before and after T-cell receptor (TCR) activation. Images were captured at 100X using a deconvolution microscope. Scale bars: 1 μm.

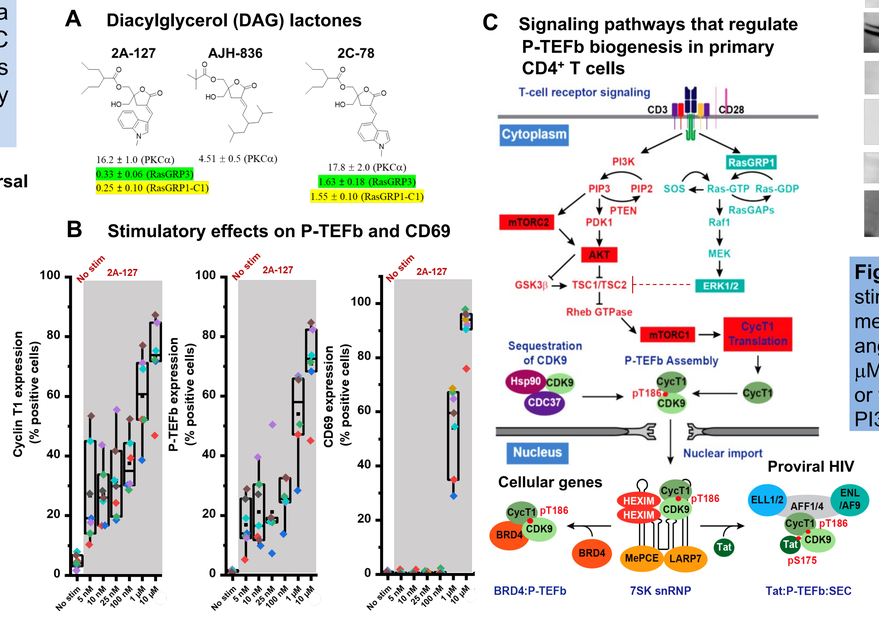


Figure 4. *(A)* Structures and *in vitro* binding affinities of the DAG lactones tested in the current study. The two DAG indololactones 2A-127 and 2C-78 exhibit preferential binding to RasGRP over PKC. *(B)* 2A-127 stimulates P-TEFb expression at concentrations below the threshold needed to induce the T-cell activation marker CD69. Memory CD4$^+$ T cells from two different donors (male and female) were challenged with increasing concentrations of 2A-127 for 24 h prior to being subjected to immunofluorescence flow cytometry to examine the expression of Cyclin T1, transcriptionally active P-TEFb (dual expression of Cyclin T1 and pSer175 CDK9) and CD69. Data plotted on the graphs are color-coded based on experiment. (C) Signaling mechanisms that regulate the biogenesis and nuclear induction of P-TEFb in memory CD4$^+$ T cells.

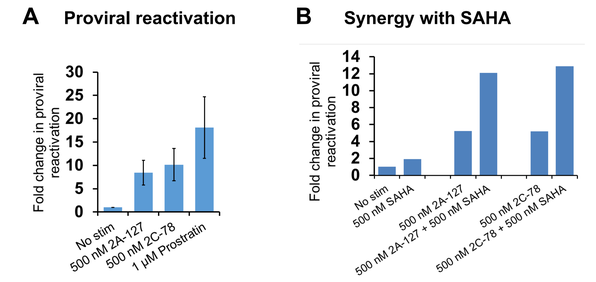


Figure 5. DAG indololactones synergize with the HDACi SAHA to reactivate latent HIV-1 in an *ex vivo* primary cell model. *(A)* Individual 24-h treatments with 2A-127, 2C-78 or the PKC agonist prostratin at the indicated concentrations. Results shown are an average of three separate experiments. Error bars indicate SEM. *(B)* Combined treatments with 500 nM of 2A-127 or 2C-78 with 500 nM SAHA provides a synergy in latent proviral reactivation. In both A and B proviral reactivation was assessed by immunofluorescence flow cytometry as the dual inducible expression of Tat and Nef.

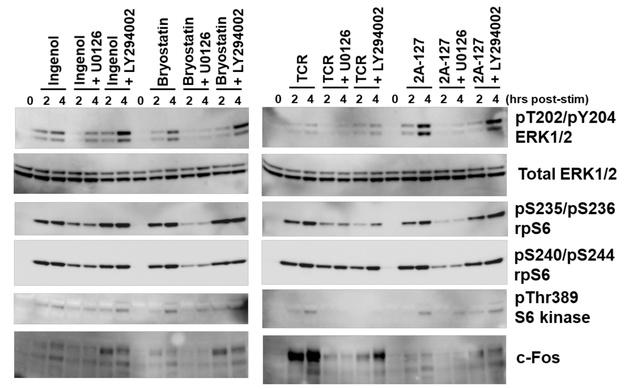


Figure 6. DAG-mimicking C1 domain agonists, including 2A-127, stimulate mTORC1 activity through ERK1/2. Healthy donor-derived memory CD4$^+$ T cells were challenged with 50 nM ingenol-3-angelate or 10 nM bryostatin 1 *(Left)* or the DAG-lactone 2A-127 at 1 μM or stimulated through the TCR *(Right)* for the times indicated with or without pre-treatment with the MEK inhibitor U0126 (10 μM) or the PI3K inhibitor LY294002 (5 μM).

A two-pronged strategy that induces P-TEFb expression with a RasGRP1-selective C1 domain agonist and stimulates HIV-1 promoter activity with a HDAC inhibitor could be an effective 'Shock' approach for reversing HIV-1 latency in memory CD4$^+$ T cells.

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