Effect of Primary CD8+ T Cells on Romidepsin and Bryostatin-Treated CD4+ T Cells

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Introduction

Latently infected CD4+ T cells represent a major barrier to HIV-1 cure efforts. The current "shock and kill" strategy relies upon the use of latency reactivating agents (LRAs) to selectively induce production of HIV-1 mRNA and proteins in resting CD4+ T cells to make them visible to the adaptive immune system. Previous work has shown that treating primary latently infected cells with combinations of LRAs is more effective than single drug treatments. One such combination is bryostatin-1 and romidepsin, the latter of which has further been shown to induce reactivation in vivo. However, romidepsin and other HDAC inhibitors have been associated with impaired CTL responses. Thus, we sought to examine the stimulated, primary effector CD8+ T cell response of cART-treated HIV+ individuals to latently-infected CD4+ T cells cultured with the LRAs bryostatin and romidepsin (B/R).

Methodology

Resting, CD4 T cells from 6 cART-treated HIV-positive chronic progressors were treated with the combination B/R to reactivate latently infected cells, and primary CD8 T cells that had been stimulated with overlapping Gag and Nef peptides and IL-2 for seven days were added after 6 hours. Cell-associated and supernatant HIV mRNA from 24 hours post-stimulation was measured via qPCR to determine the efficacy of the CD8 effector response. A modified, FACS-based HIV-1 suppression assay using CD4+ and drug-treated CD8+ T cells from elite suppressors was used to determine the effect of LRAs on HIV-specific CD8+ T cell responses, and HIV-negative donor PBMCs were treated with LRAs to determine non-specific effects.

Results

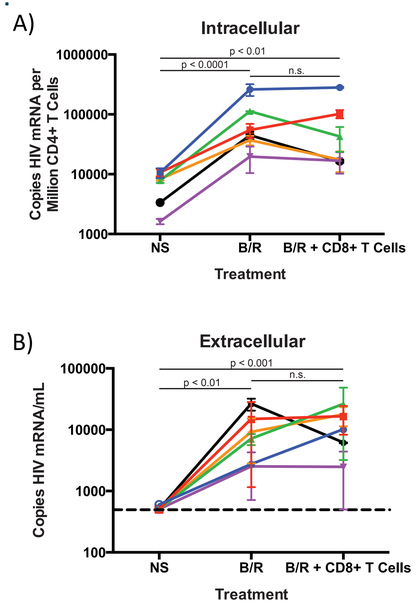


Figure 1. Latently-infected resting CD4+ T cells treated with bryostatin-1 and romidepsin upregulate HIV-1 mRNA production that is not inhibited by Gag and Nef-stimulated CD8+ T cells. Resting CD4+ T cells from HIV positive donors were treated with bryostatin (10 nM) and romidepsin (40 nM) for 6 hours before addition of autologous CD8+ T cells stimulated with overlapping Gag and Nef peptides for 7 days. After a further 18 hour incubation with drugs, RNA isolated via Trizol extraction from culture supernatant and cell lysates was quantified with qPCR specific for HIV-1 mRNA. Each individual (indicated by a colored line) had up to three replicates of 5 x 10^6 CD4+ T cells cultured per condition. A) Average cell-associated HIV-1 mRNA. B) Average supernatant HIV-1 mRNA.

● Detectable
○ Undetectable
---- Limit of Detection

Results

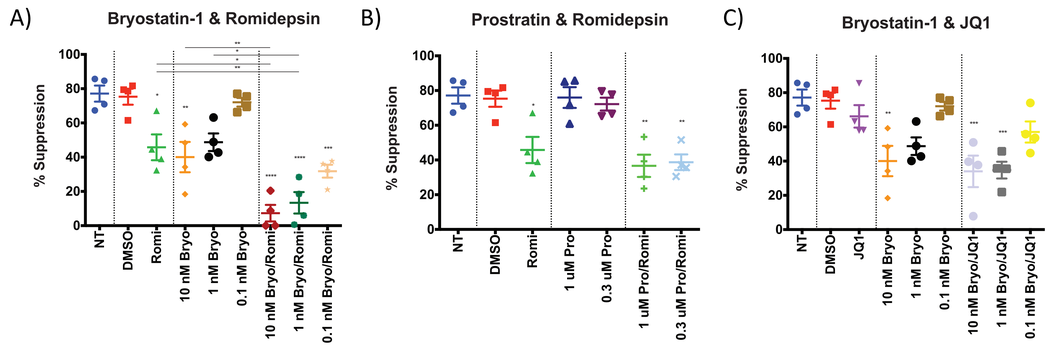


Figure 2. CD8+ T cell-mediated suppression of HIV-infected CD4+ T cells is inhibited by treatment with romidepsin and bryostatin-1. CD8+ T cells from elite suppressors were treated with vehicle (DMSO), bryostatin (10, 1, or 0.1 nM), prostratin (1 or 0.3 uM), romidepsin (40 nM), or JQ1 (1 uM) alone or in combination for 6 hours before washing and addition to autologous CD4+ T cells infected with pseudovirus $HIV_{NL4\text{-}3\Delta env\text{-}gfp}$. Percent suppression was calculated as follows: 100% x (1 – [% GFP of CD4s cultured with CD8+ T cells] / [% GFP of CD4s cultured with no effectors]). A) Effect of bryostatin and romidepsin treatment of CD8 T cells. B) Effect of prostratin and romidepsin treatment. C) Effect of bryostatin and JQ1 treatment. * p < 0.05, ** p < 0.01, *** p < 0.001, **** p < 0.0001

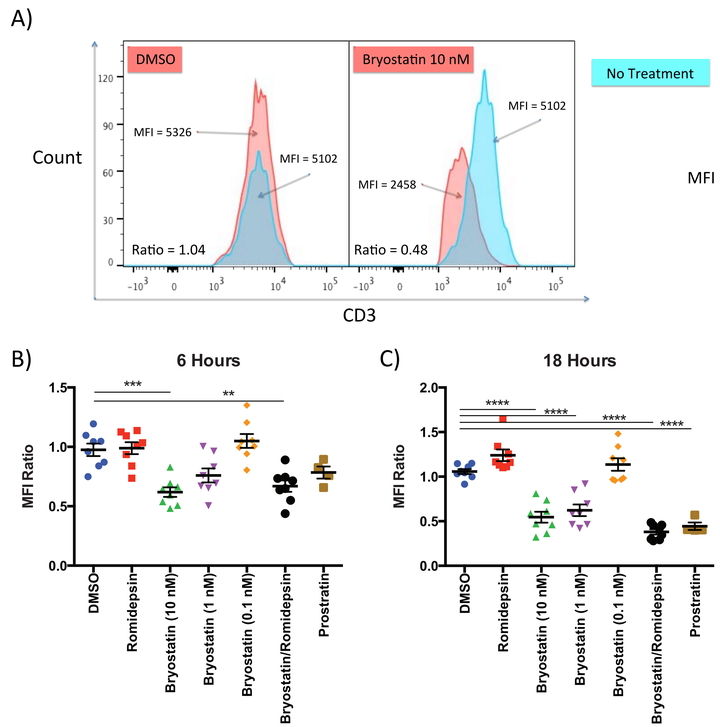


Figure 3. Treatment of CD8+ T cells with bryostatin-1 alone and the combination of bryostatin-1 and romidepsin decreases CD3 expression. CD8+ T cells from HIV-negative donors were treated with vehicle (DMSO), romidepsin (40 nM), bryostatin (10, 1, or 0.1 nM), or prostratin (0.3 uM) for 6 hours or 18 hours. Expression of CD3 was determined via FACS analysis of mean fluorescence intensity. A) Diagram with for MFI ratio calculation and representative plots from 6 hours of treatment. B) 6 hours. C) 18 hours. * p < 0.05, ** p < 0.01, *** p < 0.001, **** p < 0.0001

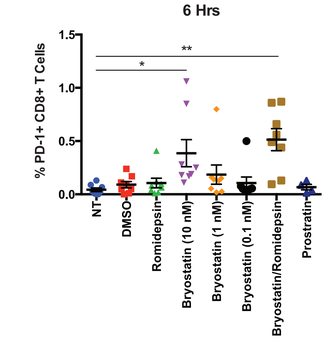


Figure 4. Treatment of CD8+ T cells with bryostatin-1 alone and the combination of bryostatin-1 and romidepsin significantly increases PD-1 expression after six hours. CD8+ T cells from HIV-negative donors were treated with vehicle (DMSO), romidepsin (40 nM), bryostatin (10, 1, or 0.1 nM), romidepsin (40 nM) and bryostatin (10 nM), or prostratin (0.3 uM) for six hours. PD-1 expression was determined via FACS analysis. * p < 0.05, ** p < 0.01

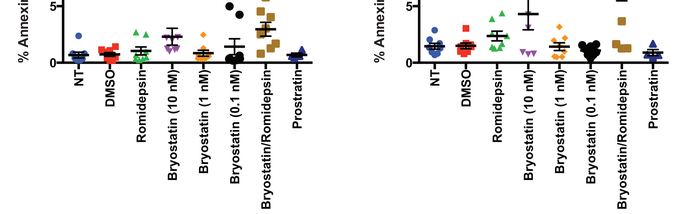


Figure 5. Treatment of CD8+ T cells with the combination of bryostatin-1 and romidepsin increases annexin V expression. CD8+ T cells from HIV-negative donors were treated with vehicle (DMSO), romidepsin (40 nM), bryostatin (10, 1, or 0.1 nM), romidepsin (40 nM) and bryostatin (10 nM), or prostratin (0.3 uM) for A) 6 hours or B) 18 hours. Annexin V expression was determined via FACS analysis. * p < 0.05, ** p < 0.01

Conclusions

While cell-associated and supernatant HIV-1 mRNA is produced in latently-infected, resting CD4+ T cells in response to bryostatin-1 and romidepsin treatment, autologous stimulated CD8+ T cells from chronic progressors are incapable of inhibiting production of either. The HIV-specific CD8+ T cell response of elite suppressors was also significantly negatively effected by bryostatin-1 and romidepsin treatment. The mechanism behind the impairment of CD8+ T cell function may due to downregulation of CD3, increased cell death, increased PD-1 expression, or a combination of the three. Potential LRA therapies should therefore be examined for their broad effects on adaptive immunity before use in the context of an HIV-1 cure.