Immune Modulation With Rapamycin as a Potential Strategy for HIV-1 Eradication

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BACKGROUND

Despite effective combination antiretroviral therapy (cART), HIV-1 persists in a long-lived latent reservoir in resting memory CD4+ T cells. These latently infected cells represent a major barrier to eradication. A prominent paradigm for HIV-1 cure involves the reactivation of viral transcription in latently infected CD4+ T cells by small molecules. Agents that elicit global T cell activation have been shown to be effective in HIV-1 latency reversal, but cannot be used in a clinical setting due to severe adverse effects associated with this strategy. Due to this toxicity, agents eliciting any level of T cell activation have been avoided in the "shock and kill" approach to eradication, significantly limiting the repertoire of compounds that can be used. The adverse response associated with immune activation is commonly attributed to massive cytokine release by T cells. However, this toxicity may be avoided using concurrent immunosuppressant treatment. The goal of this study was to identify immunomodulatory compounds that inhibit cytokine release and proliferation during a T cell activation approach, without affecting HIV-1 gene expression.

METHODS & RESULTS

Methods: Resting CD4+ T cells were isolated from HIV-1 infected individuals on effective cART treatment. Cells were treated for 24 hours with αCD3/αCD28 stimulation only, stimulation plus rapamycin or cyclosporin, or a no treatment control. To measure reactivation of latent HIV 1, cellular RNA was isolated, reverse transcribed, and qPCR was performed to specifically identify polyadenylated HIV-1 transcripts. Supernatant cytokine release and cell proliferation were assayed by flow cytometry.

Results: We found that rapamycin, an mTOR inhibitor, did not inhibit HIV-1 transcriptional response to αCD3/αCD28 stimulation at concentrations up to 5ug/ml. At these same concentrations, rapamycin was effective at inhibiting pro-inflammatory cytokine release and cell proliferation in response to this stimulation. The calcineurin inhibitor cyclosporin has been previously described to inhibit HIV-1 expression in a cell line (1), consistent with our results that cyclosporin abrogated both cytokine release and HIV-1 expression induced by αCD3/αCD28 treatment.

RESULTS: Rapamycin does not inhibit expression of HIV-1 by αCD3/αCD28 stimulation

To determine whether the immunosuppressant rapamycin would affect transcription of HIV-1 elicited by global T cell activation, we purified rCD4s from HIV-1 infected individuals on suppressive ART and treated 5 million cells each with vehicle alone, αCD3/αCD28 alone, or αCD3/αCD28 plus rapamycin (5 ug/ml) or cyclosporin (500 nM) for 24 hours. Following treatment RNA was isolated from lysed cells and intracellular HIV-1 mRNA was measured using previously described primers and probes specific to HIV-1 polyadenylated transcripts (2).

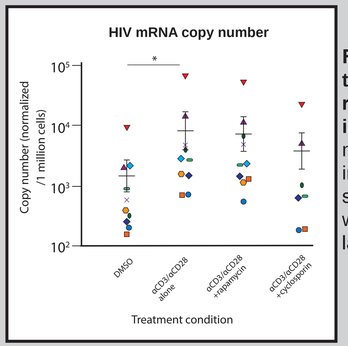


Figure 1: RT-PCR results for intracellular HIV-1 mRNA in rCD4s isolated from infected individuals. αCD3/αCD28 treatment alone induced a significant increase in HIV-1 mRNA transcripts (p = 0.048). Co-treatment with rapamycin did not downregulate this effect.

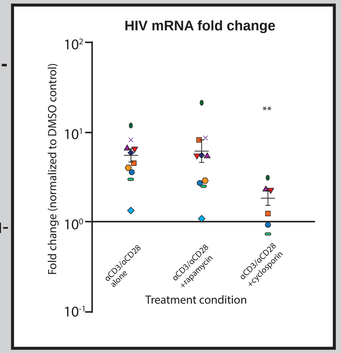


Figure 2: RT-PCR results shown as fold change. There was no significant difference in fold induction by αCD3/αCD28 with or without rapamycin cotreatment. However, in cells treated with αCD3/αCD28 and cyclosporin, HIV-1 mRNA was measured to be significantly lower than that induced by αCD3/αCD28 antibody treatment alone (p = 0.003).

RESULTS: Effects of immunosuppressant treatment on in vitro predictors of clinical toxicity

In order to be clinically relevant, rapamycin must not only allow for HIV-1 expression, but also inhibit toxicity associated with T cell activation. A primary mediator of unacceptable clinical toxicity is pro-inflammatory cytokine release. Therefore, we measured supernatant concentration of IL-2, TNF, and IFNγ.

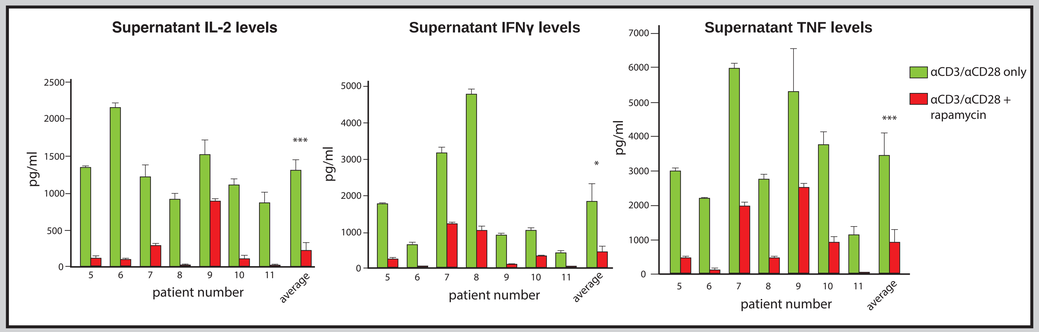


Figure 3: Effects of rapamycin treatment on pro-inflammatory cytokine release. Co-treatment with rapamycin significantly inhibited pro-inflammatory cytokine release caused by T cell activation, including IL-2 (p = 0.0009), IFNγ (p = 0.019), and TNF (p = 0.0002). Both DMSO only and αCD3/αCD28 plus cyclosporin elicited little to no cytokine release, and so are not represented.

RESULTS: Toxicity continued

Another in vitro predictor of in vivo immune toxicity is cellular proliferation. We measured the effect of each treatment condition on proliferation and cell death using healthy donor PBMC. 5 million cells per condition were treated with DMSO alone, αCD3/αCD28 antibodies, or αCD3/αCD28 with rapamycin or cyclosporin.

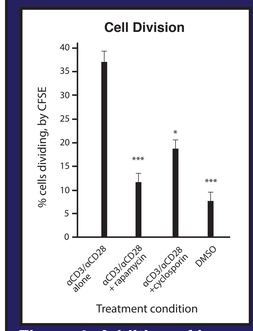


Figure 4: Addition of immunosuppressants decreases cellular proliferation in PBMC. αCD3/αCD28 antibody stimulation caused significant cell division when compared with DMSO alone (p = .0001). Cell division was significantly decreased by addition of rapamycin (p = .0002) or cyclosporin (p = .01).

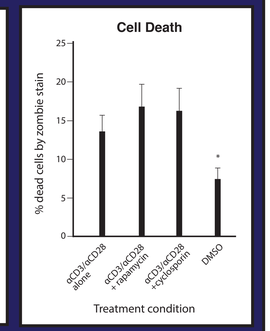


Figure 5: Analysis of cellular death by Zombie Aqua in healthy donor PBMC. All conditions induced significantly higher levels of cellular death than the DMSO only control (DMSO only compared with αCD3/αCD28 only, p = 0.018).

CONCLUSIONS

Rapamycin downregulates toxic effects of T cell activation without affecting expression of HIV-1. These findings suggest that latency reversing agents that induce some level of T cell activation may be safely and effectively used in the presence of immunomodulators such as rapamycin.

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