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Research Paper

The Effect of Latency Reversal Agents on Primary CD8 + T Cells: Implications for Shock and Kill Strategies for Human Immunodeficiency Virus Eradication

Victoria E. Walker-Sperling [a], Christopher W. Pohlmeyer [a], Patrick M. Tarwater [b], Joel N. Blankson [a,*]

[a] Center for AIDS Research, Department of Medicine, Johns Hopkins University School of Medicine, USA
[b] Division of Biostatistics and Epidemiology, Paul L. Foster School of Medicine, El Paso, TX, USA

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ABSTRACT

Shock and kill strategies involving the use of small molecules to induce viral transcription in resting CD4 + T cells (shock) followed by immune mediated clearance of the reactivated cells (kill), have been proposed as a method of eliminating latently infected CD4 + T cells. The combination of the histone deacetylase (HDAC) inhibitor romidepsin and protein kinase C (PKC) agonist bryostatin-1 is very effective at reversing latency in vitro. However, we found that primary HIV-1 specific CD8 + T cells were not able to eliminate autologous resting CD4 + T cells that had been reactivated with these drugs. We tested the hypothesis that the drugs affected primary CD8 + T cell function and found that both agents had inhibitory effects on the suppressive capacity of HIV-specific CD8 + T cells from patients who control viral replication without antiretroviral therapy (elite suppressors/controllers). The inhibitory effect was additive and multi-factorial in nature. These inhibitory effects were not seen with prostratin, another PKC agonist, either alone or in combination with JQ1, a bromodomain-containing protein 4 inhibitor. Our results suggest that because of their adverse effects on primary CD8 + T cells, some LRAs may cause immune-suppression and therefore should be used with caution in shock and kill strategies.

1. Introduction

Latently infected CD4 + T cells are the major barrier to HIV-1 cure efforts. The cells contain integrated proviruses that are transcriptionally silent and thus able to evade detection and clearance by the immune system. The shock-and-kill cure strategy seeks to first reactivate these latent viruses without causing global T cell activation followed by clearance of the reactivated cells by the immune system (reviewed in Siliciano and Siliciano, 2013; Archin and Margolis, 2014). Latency reactivating agents (LRAs) are drugs that induce HIV-1 transcription. Notable drug classes include PKC agonists and HDAC inhibitors (HDACi), which have been very effective in inducing HIV-1 transcription in cell lines (Contreras et al., 2009; Xing et al., 2011; Li et al., 2013; DeChristopher et al., 2012). Unfortunately, in vitro experiments with primary resting CD4 T cells from patients on suppressive antiretroviral therapy (ART) regimens suggest that most individual LRAs are unable to induce substantive amounts of HIV-1 transcription with the notable exception of PKC agonists bryostatin-1-1 (Bullen et al., 2014) and ingenol (Spivak et al., 2015). However, LRA combinations in the same system are capable of inducing significant HIV-1 transcription (Laird et al., 2015; Jiang et al., 2015; Darcis et al., 2015).

The other half of the cure strategy deals with killing newly reactivated infected CD4 + T cells. Recent experiments suggest that reactivation from latency is not enough to induce cell death (Shan et al., 2012), and therefore there may be a need for immune mediated eradication. Expanded CD8 + T cell lines were able to clear reactivated latently infected resting CD4 + T cells following exposure to the HDAC inhibitor, vorinostat (Sung et al., 2015). However primary CD8 + T cells from patients on suppressive ART regimens that were pre-stimulated with overlapping Gag peptides were unable to consistently reduce the amount of HIV-1 mRNA induced from autologous resting CD4 + T cells that were activated with PMA and ionomycin (Walker-Sperling et al., 2015).

The combination of romidepsin and bryostatin-1 has been shown to be one of the best inducers of latent HIV-1 in primary CD4 + T cells (Laird et al., 2015). However, bryostatin-1 has been showed to be involved in the modulation of NFkB and NFAT (Williams et al., 2004) and romidepsin is known to affect the function of NK cells and CD8 + T cells (Kelly-Sell et al., 2012; Jones et al., 2014). Other HDAC inhibitors have furthermore been known to induce Treg cells in vitro (Akimova et al., 2010; Tao et al., 2007). The immunomodulatory activity of the two drug classes thought to be most promising in cure efforts therefore

* Corresponding author at: Department of Medicine, Johns Hopkins University School of Medicine, 855 N. Wolfe Street, Rangos 552, Baltimore, MD 21205, USA.
E-mail address: jblanks@jhmi.edu (J.N. Blankson).

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needs to be further studied in the context of CD8 + T cell elimination of reactivated latently infected CD4 + T cells.

In this study, we sought to determine the ability of HIV-specific CD8 + T cells from patients with progressive HIV-1 disease on ART (chronic progressors) to kill HIV-infected CD4 + T cells after treatment with LRAs. To elucidate the contribution of the drug treatments the HIV-specific response, suppression of infection was examined with elite suppressor CD8 + T cells that had been pre-treated with different LRAs, including an HDAC inhibitor, a bromodomain-containing protein 4 inhibitor, and multiple PKC agonists. Finally, we examined the mechanisms that may have contributed to the effects of drug treatment on CD8 + T cell function. Our results have implications for the HIV-1 cure agenda.

2. Methods

2.1. Donor blood samples

HIV-1 positive and HIV-1 negative blood samples were obtained from donors with written, informed consent and handled according to a Johns Hopkins University IRB approved protocol. The chronic progressors studied were HIV-1 positive individuals who were started on suppressive ART therapy during chronic infection and have a viral load of <20 copies of HIV RNA/mL. Elite suppressors are patients who have maintained undetectable viral loads without antiretroviral therapy. The clinical characteristics of the patients are summarized in Table 1.

2.2. Primary cell isolations

PBMCs were obtained from whole blood via Ficoll-Paque PLUS gradient centrifugation (GE Healthcare Life Sciences). PBMCs underwent negative selection for CD4 + T cells using the MACS system (CD4 Isolation Kit, Miltenyi Biotech). Resting CD4 + T cells were further isolated from the bulk population by depleting CD25 +, CD69 +, and HLA-DR + cells (CD25 microbeads, CD69 Isolation Kit, and HLA-DR microbeads; Miltenyi Biotech). When applicable, CD8 + T cells were obtained via positive selection from PBMCs (CD8 microbeads, Miltenyi Biotech) prior to any negative selection performed in experiments described below.

2.3. Latency reactivation ex vivo and autologous suppression

Resting CD4 + T cells isolated from fresh blood samples from ART-suppressed individuals as described above ("Primary Cell Isolation") were plated in 12-well plates with 5×10^6 (DeChristopher et al., 2012) cells per replicate in non-stimulating media (RPMI 1640 + Glutamax, 10% FBS) and treated alone for six hours with the combination of bryostatin-1 (B, 10 nM; Sigma Aldrich) and romidepsin (R, 40 nM; Selleck Chemicals) in the presence of efavirenz (EFV, 10 μM) and raltegravir (RAL, 4 μM) to prevent new infection and more closely mimic in vivo conditions. CD8 + T cells were isolated at this time as described above from PBMCs that had been previously stimulated for seven days in the presence of 100 U IL-2/mL and overlapping consensus Gag and Nef peptides (10 μg/mL; AIDS Reagent Database). At the conclusion of the six hours, the pre-stimulated CD8 + T cells were co-cultured with the resting CD4 + T cells at a 1:1 effector:target ratio and concentration of 5×10^6 (DeChristopher et al., 2012) cells/mL for another 18 h in the presence of B/R, EFV, and RAL at the same concentration as the initial treatment. The CD8 + T cells were not washed prior to co-culture with resting CD4 + T cells. In a second set of experiments, the CD8 + and CD4 + T cells were co-cultured together for 24 h in the presence of B/R, EFV, and RAL. For each replicate, at the conclusion of the full 24 h, supernatant samples and the full 5×10^6 (DeChristopher et al., 2012) cell samples were harvested and placed in TRIzol LS and TRIzol (Life Technologies), respectively, for the isolation of supernatant and cell-associated RNA.

2.4. Isolation and quantification of cell-associated and supernatant HIV-1 mRNA

Cell-associated and supernatant RNA were isolated, and the HIV-1 mRNA present in those samples was then quantified as previously described (Bullen et al., 2014; Laird et al., 2015; Walker-Sperling et al., 2015). Supernatant RNA samples were measured on a Roche LightCycler 480 Real-Time PCR thermocycler with TaqMan Fast Advanced Mastermix (Applied Biosystems) run as per manufacturer's instructions and the following primers: Forward (5′ → 3′) CAGATGCTGC ATATAAGCAGCTG (9501–9523), Reverse (5′ → 3′) TTTTTTTTTTTTTTT TTTTTTTGAAGCAC (9629-poly A). The probe used is as follows: (5′ → 3′) FAM-CCTGTACTGGGTCTCTCTGG-MGB (9531–9550) (all nucleotide coordinates relative to HXB2 consensus sequence). The molecular standard curves used for the quantification were generated using serial dilutions of a TOPO plasmid containing the final 352 nucleotides of the HIV-1 genomic RNA with the addition of 30 deoxyadenosines on the 5′ end to mimic the poly-A tail.

2.5. Latency reactivation agents' effects on autologous suppression of ex vivo infection

Autologous bulk CD8 + and CD4 + T cells were freshly isolated from PBMCs from elite suppressors as described above ("Primary Cell Isolation") for a modified version of a previously described HIV suppression assay (Buckheit et al., 2012). CD8 + T cells were treated for six hours in non-stimulating media (RPMI 1640 + Glutamax, 10% FBS) with either nothing, DMSO, romidepsin (40 nM), JQ1 (1 μM; Sigma Aldrich),

Table 1
Clinical characteristics of the chronic progressors studied.

Subject	Current CD4 + T cell count	Nadir CD4 + T cell count	Time on suppressive regimen	Current regiment	HLA-A	HLA-B
CP8	424	18	8 years	3TC, RAL EFV	1, 68	57, 58
CP9	991	190	8 years	TDF, FTC, DRV/c	34, 68	58, 81
CP11	1032	177	8 years	TDF, FTC, DRV/r	2, 11	25, 57
CP 14	646	12	4 years	DRV/r, DTG		
CP 16	921	203	5 years	3TC, ABC, DTG	29, 20	42, 81
CP25	584	NA	4 years	TDF, FTC, RAL	3, 30	8, 42
ES 3	1149	NA	NA	NA	25, 68	51, 57
ES 6	601	NA	NA	NA	23	15, 57
ES 9	798	NA	NA	NA	2, 30	27, 57
ES 22	1033	NA	NA	NA	30, 31	15, 57
ES 24	1742	NA	NA	NA	24, 30	7, 57
ES 31	1236	NA	NA	NA	3	27, 58

3TC: lamivudine, ABC: abacavir, FTC: emtricitabine, TDF: tenofovir, DTG: dolutegravir, EFV: efavirenz, RAL: raltegravir, DRV/c: cobicistat boosted darunavir, DRV/r: ritonavir boosted darunavir.
NA: Not applicable.