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Short Communication: Preferential Killing of HIV Latently Infected CD4$^+$ T Cells by MALT1 Inhibitor

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Abstract

We report that the addition of an host paracaspase MALT1 inhibitor, MI-2, to HIV latently infected ACH-2, Jurkat E4, and J-LAT cells accelerated cell death in the presence of cell stimuli or the protein kinase C agonist, bryostatin 1. MI-2-mediated cell death correlated with the induction of the cellular RNase MCPIP1 and requires the presence of viral component(s). Altogether, the combination of MI-2 and bryostatin 1 displays selective killing of HIV latently infected CD4$^+$ T cells.

Human immunodeficiency virus 1 (HIV-1) persists even under highly active antiretroviral therapy because of a reservoir of long-lived, quiescent, memory CD4$^+$ T cells, which constitute a fraction of resting CD4$^+$ T cells (rCD4$^+$ T).[1–4] These cells harbor chromosomally integrated HIV-1 proviruses that are transcriptionally silent and immunologically unrecognizable, and hence constitute the majority of the latently infected reservoir. The ''Shock and kill'' strategy envisions that proviral reactivation can be achieved by the addition of small molecules called latency reversing agents (LRAs), which activate HIV-1 transcription in the latent HIV reservoir. Under this theory, a complete (or near-complete) reactivation of HIV-1 will induce *de novo* virion synthesis, viral cytopathic effect, immune clearance, and the ultimate death of the latent reservoir. Unfortunately, this approach faces serious challenges revealed by many recent findings, including the heterogeneous reservoirs of HIV-1 latency,[5] insufficiency for LRAs alone to reactive patient-derived cells,[6,7] a very small proportion of replication competent provirus that can be reactivated by any given LRA,[8] and the fact that even when virus activation is achieved, the immune system often fails to clear the infected cells.[9]

We have previously reported that a cellular RNase monocyte chemotactic protein-induced protein 1 (MCPIP1) restricts HIV-1 infection in resting CD4$^+$ T cells.[10] Interestingly, MCPIP1 is rapidly degraded in activated primary T cells.[10] We[11] and others[12] subsequently demonstrated that MCPIP1 was cleaved in activated human and mouse CD4$^+$ T cells by the mucosa-associated lymphoid-tissue lymphoma-translocation gene 1 (MALT1), a paracaspase whose activity is critically important for activation of T and B lymphocytes.[13,14] MALT1 cleaves MCPIP1 at the C-terminal side of an arginine residue of the PEST sequence found in its substrates, including Bcl10, CYLD, and A20.[15] Of note, MCPIP1 knockout mice displayed hyperactivation of CD4$^+$ T cells, including memory CD4$^+$ T cells.[12,16]

Based on these findings, we postulated that blocking MALT1-dependant MCPIP1 cleavage in activated CD4$^+$ T cells may restore MCPIP1 levels and confer resistance to HIV-1. Among several reported MALT1 inhibitors, MI-2 was shown to selectively bind to and inhibit the cleavage activity of MATL1.[17] MI-2 contains a reactive chloromethyl amide and covalently binds to and irreversibly blocks MALT1 cleavage activity (Fig. 1A, B).[17] To examine the effect of MI-2 on MALT1-mediated MCPIP1 cleavage, we treated Jurkat T cells with MI-2 and found that MCPIP1 is rapidly upregulated on addition of MI-2 (Fig. 1C). Interestingly, the protein levels of another two MALT1 substrates, A20 and CYLD, either modestly changed or did not change at all following MI-2 treatment.

Next, we sought to test whether MI-2 treatment confers resistance to HIV in reactivated HIV latently infected cells. We chose to work with three cell line-based HIV latency models, including the J-Lat Tat-GFP (A2 clone) and J-Lat full length 9.2 clone from Verdin's laboratory,[18] the Jurkat E4 clone from Karn's group,[19] and the ACH-2 clone,[20] which all harbor a latent HIV provirus in various forms. To our surprise, although MI-2 is nontoxic to animals, it induced massive cell death in cell line-based HIV latency models when cell activation signals were supplied (Supplementary Fig. S1; Supplementary Data are available online at www.liebertpub.com/aid). Measured by an ATP-based metabolic luminescent assay,

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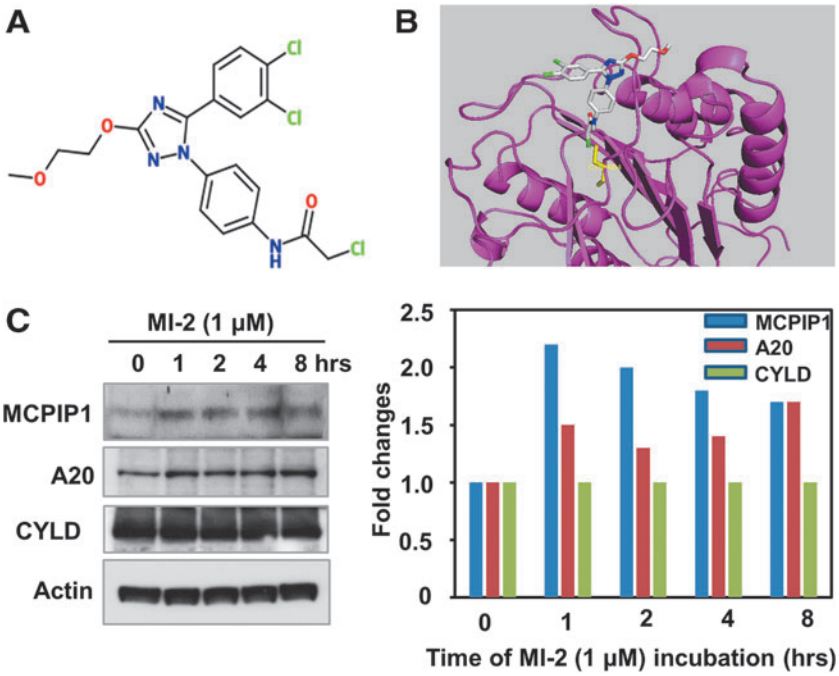

FIG. 1. MI-2 induces MCPIP1 expression in Jurkat T cells. **(A)** Chemical structure of MI-2. **(B)** MI-2 binds to the catalytic pocket of MALT1, which is shown in *magenta* with C464 in *yellow*. MI-2 (in stick model) is shown with carbons in *gray*, oxygens in *red*, nitrogens in *blue*, and chlorines in *green*. **(C)** 1×10^6 Jurkat T cells were treated with MI-2 (1 μM) for indicated periods of time. The cells were harvested and the whole cell lysates were subjected to analysis by Western blot with MCPIP1 (GeneTex GTX110807), A20 and CYLD antibodies (*left panel*). Actin was probed as loading control. *Right panel*: quantification of the blot by ImageJ.

the half-maximum toxicity (CC_{50}) of MI-2 on J-Lat Tat-GFP (A2 clone) ranges between 1 and 2 μM (Fig. 2). Addition of phorbol 12-myristate 13-acetate (PMA) or anti-CD3/anti-CD28 antibody combo, however, accelerated MI-2-mediated cell death by shifting the curve to the left (CC_{50} ~100–200 nM). To examine whether a specific LRA will have similar effect, bryostatin 1, a potent modulator of protein kinase C,[21] was added to the cells. As shown in Figure 3A–D, bryostatin 1 significantly enhanced the MI-2-mediated cytotoxicity in all three latency models by an order of magnitude. Consequently, the production of infectious HIV from the bryostatin 1-treated ACH-2 cells was reduced by 4 logs (Fig. 3E). At 10 nM, bryostatin 1 alone strongly activated the proviral transcription without causing any cell death (Supplementary Fig. S2).

Finally, we determined whether MI-2 in combination with bryostatin 1 selectively targets reservoir with lethal effect. As shown in Figure 4A and B, MI-2 in combination with bryostatin 1 does not accelerate cell death of uninfected Jurkat T nor CEM cells, suggesting that the accelerated cell death achieved in the HIV latently infected cell lines involves viral components. The question is whether the observed cell death is exclusively dependent on MCPIP1 induction. Previously we have shown that elevated MCPIP1 enhanced stress-induced apoptosis in RAW264.7 cells.[22] We speculate that HIV latently infected $CD4^+$ T cells are more prone to MCPIP1-mediated cell death on reactivation. Since MCPIP1 is an RNase and shown by others to directly bind viral RNA,[23] newly transcribed HIV mRNA species in the reactivated cells may trigger MCPIP1-mediated cell death. In other words, elevated MCPIP1 on MI-2 treatment detects HIV mRNA species and causes cell death. If true, such a mechanism may only require the transcriptional reactivation

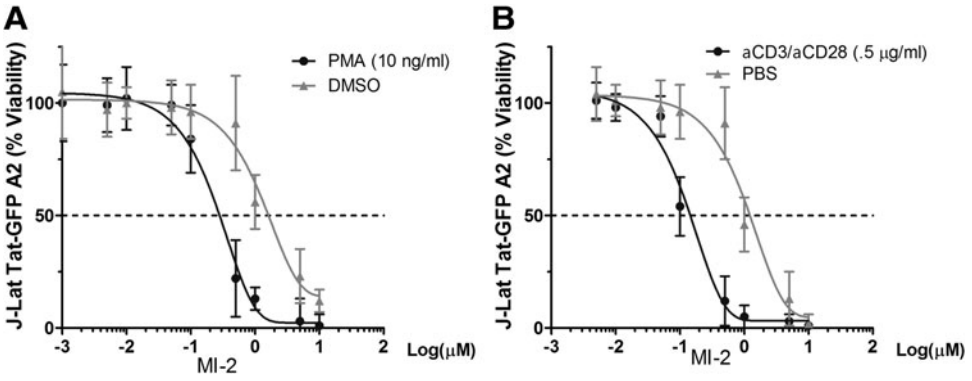

FIG. 2. Cell activation increases MI-2-mediated cell death in HIV latently infected cells. **(A)** J-Lat Tat-GFP A2 cells were seeded at 0.25×10^6/ml and were treated with dimethyl sulfoxide (DMSO) or stimulated with phorbol 12-myristate 13-acetate (PMA; 10 ng/ml) and increasing concentrations of MI-2. **(B)** Anti-CD3/anti-CD28 antibody combo was added to stimulate cells. $n = 3$, error bars represent standard deviations.

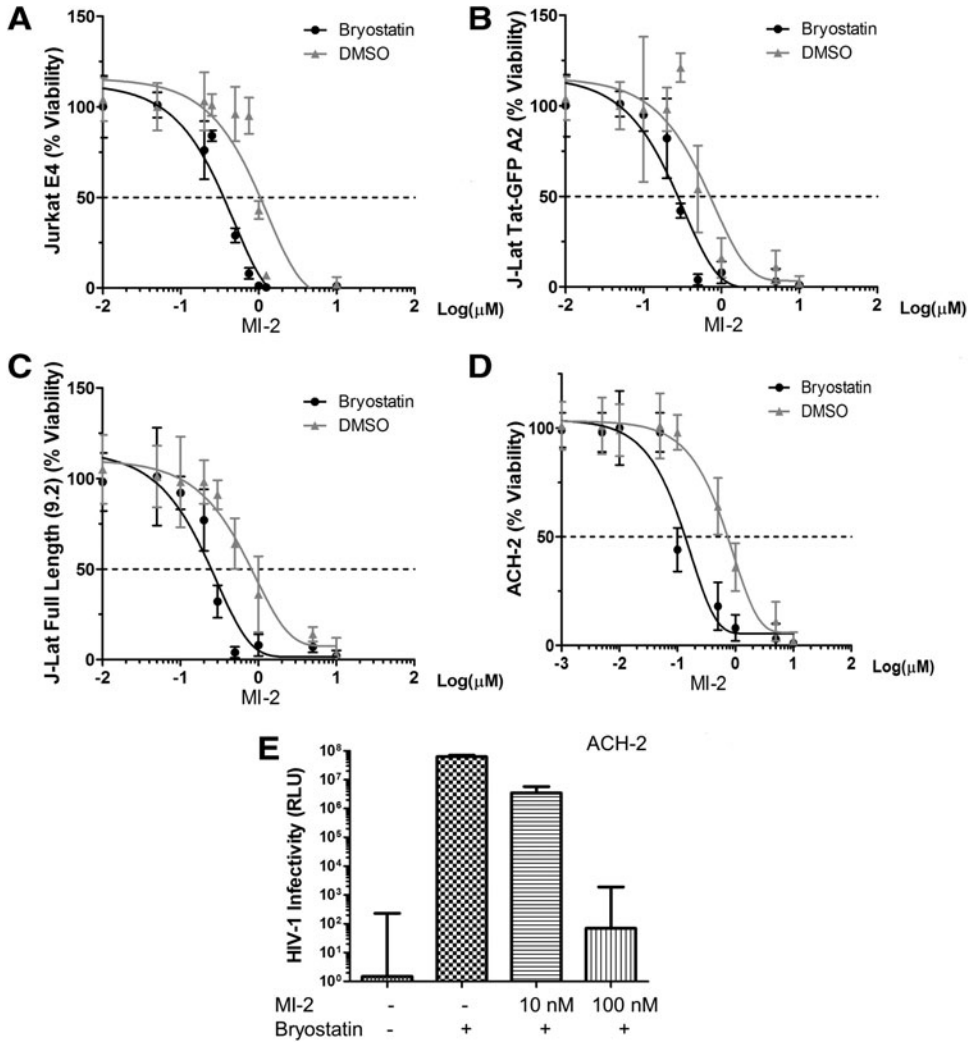

FIG. 3. Bryostatin 1 increases MI-2-mediated cell death in HIV latently infected cells. **(A)** Jurkat E4 clone was seeded at 0.25×10^6/ml and was treated with DMSO or stimulated with bryostatin 1 (10 nM) and increasing concentrations of MI-2. **(B)** Bryostatin 1 (10 nM) was added to stimulate J-Lat Tat-GFP A2 cells and increasing concentrations of MI-2. **(C)** Bryostatin 1 (10 nM) was added to stimulate J-Lat Full length 9.2 cells and increasing concentrations of MI-2. **(D)** Bryostatin 1 increases MI-2-mediated cell death in HIV latently infected ACH-2 cells. cART (Lamivudine, Emitricitabine, Indinavir, and Tenofovir, 10 μM of each) was added to prevent spread and the contribution of unintegrated viral species. **(E)** HIV produced from MI-2-treated ACH-2 cells was titered on TZM-bl reporter cells. Equal number of ACH-2 cells were treated as indicated for 2 days. Cells were then pelleted down and then resuspended in fresh medium to ensure no carryover of compounds. After 24 h, supernatants were collected and titered on TZM-bl cells.

of proviruses, which can be easily achieved by LRAs. Ongoing experiments are underway to test this possibility.

Collectively, our data suggest that the MALT1 inhibitor in combination with LRA represents a novel approach to kill HIV latently infected T cells. The therapeutic index (TI, ~ 10) of MI-2 warrants in-depth follow-up analysis. Interestingly, two studies of MALT1 inhibitors revealed that MALT1 cleavage activities can also be achieved through reversible binding of MALT1 by one MI-2 analog, MI-2A3,[17] and another small molecule named mepazine.[24] These compounds may offer wider therapeutic window because reversible inhibition of MALT1 is expected to cause less death to bystander cells. Further investigations of these compounds using primary latency model and *ex vivo* HIV-1 latency model will confirm the validity of this novel approach.

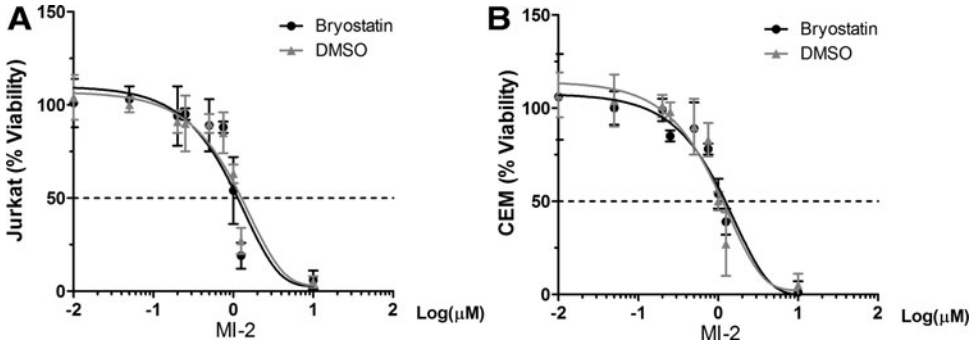

FIG. 4. Bryostatin 1 does not increase MI-2-mediated cell death in uninfected Jurkat T **(A)** or CEM T cells **(B)**. Cells were seeded at 0.25×10^6/ml and were treated with DMSO or stimulated with Bryostatin 1 (10 nM) and increasing concentrations of MI-2.

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Author Disclosure Statement

No competing financial interests exist.

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