SMAC Mimetic-APG Reactivates HIV and Reduces Viral Reservoirs in Humanized-mice

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BACKGROUND

Antiretroviral therapy (ART) suppresses HIV replication but does not eliminate viral reservoirs (VRs). VRs have elevated levels of inhibitors of apoptosis proteins (IAPs), prolonging their survival. PKC agonists efficiently reactivate VRs but are unable to reduce the size of VRs. As well, these agents activate the canonical NF-κB pathway and in so doing, elicit generalized activation and inflammation. Consequently, alternative approach(es), which can reactivate HIV and eliminate infected cells without triggering systemic activation, are needed. In this context, small molecules known as mimetics of the second mitochondrial activator of caspases (SMAC mimetics, SM), designed originally for cancer therapeutics, have garnered increasing attention because of their ability to specifically activate the non-canonical NF-κB pathway. Indeed, the SM AZD-5582 has been shown to reactivate HIV in resting CD4⁺T cells although its effect on other cell subsets was not known (Nixon et al., 2020 Nature). In this study, we assessed whether novel bivalent SM-APG has the ability to reverse HIV latency and affect VRs in vivo. It was also of interest to determine if SM-APG has any effect on other immune cell subsets.

METHODS

We first studied the effect of SM-APG on CD4⁺ T cell models of HIV latency and on HIV-CRMZ dual reporter virus-infected primary CD4⁺ T cells (E2-Crimson under the HIV LTR and ZS-Green under the EF1α promoter). Thereafter, SM-APG was assessed in virally suppressed, HIV-infected humanized BLT mice (hu-mice) for its ability to induce HIV reactivation and modulates VRs.

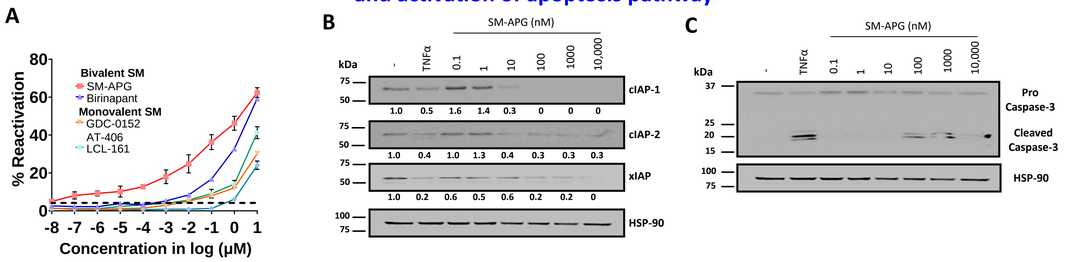
Acknowledgements

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Take-home message: Novel bivalent SM-APG efficiently reactivates HIV in CD4⁺ T cell models of latency. In virally suppressed, humanized BLT mice, SM-APG induces viral reactivation, reduces frequency of cells carrying integrated viral genomes and decreases the extent of viral rebound.

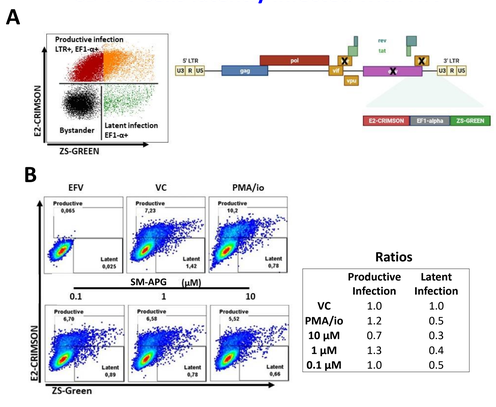
RESULTS

Fig. 1: SMAC mimetics reactivate HIV in latently infected model cells and lead to degradation of IAPs and activation of apoptosis pathway



SM-APG was most efficient at reactivating HIV in 2D10 cells among all the SM tested (Panel A). Degradation of IAPs (Panel B) and cleavage of pro caspase-3 to active caspase-3 (Panel C) were observed in 2D10 cells treated with SM-APG. Additional, not shown data: SM-APG specifically activates the non-canonical pathway and that upon treatment with SM-APG, reactivated 2D10 cells expressed high levels of caspase-3 compared to unreactivated cells.

Fig. 2: SM-APG reduces the frequency of primary CD4⁺ T cells latently infected with HIV



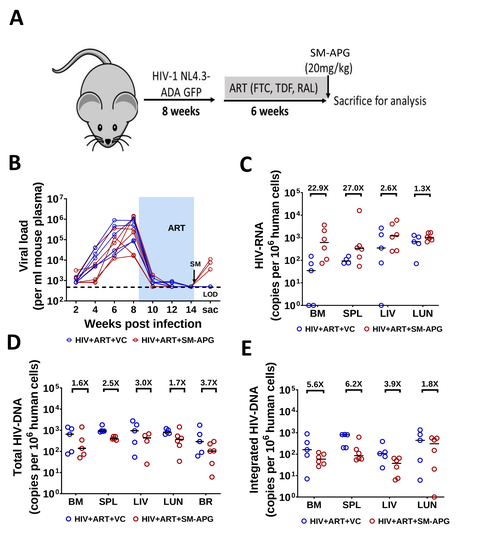
Ratios	Productive Infection	Latent Infection
VC	1.0	1.0
PMA/io	1.2	0.5
10 μM	0.7	0.3
1 μM	1.3	0.4
0.1 μM	1.0	0.5

Primary CD4⁺T cell populations were infected with the HI.fate dual-fluorescent virus and analyzed by flow cytometry. Productively and latently infected cell populations were as identified (Panel A). Treatment of infected cells with SM-APG led to a decrease in the frequency of both populations although the latent cell subset was more affected (Panel B).

Author contact information

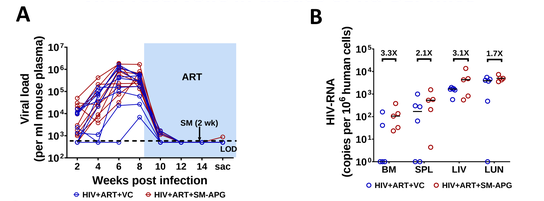
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Fig. 3: A single dose of SM-APG induces HIV RNA expression and modestly reduces viral reservoirs in tissues of virally suppressed hu-BLT mice



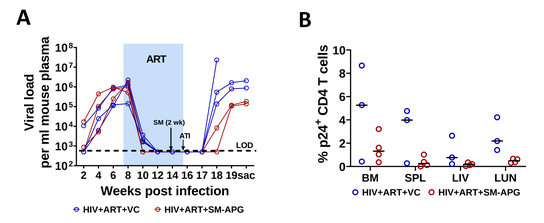
In virally suppressed animals (Panel A), a single dose, 48-hour treatment of SM-APG was sufficient to reactivate HIV allowing for detection of viremia (Panel B). We observed a meaningful increase in cell-associated HIV RNA levels in tissues (Panel C) and a significant decrease in levels of viral genome-harbouring cells (Panels D and E). Additional, not shown data: SM-APG treatment did not affect cell viability in uninfected mice. Neither was T cell activation status in uninfected or infected animals.

Fig. 4: Multiple dose treatment of SM-APG decreases viral reservoirs in tissues of hu-BLT mice



Sequential administration of SM-APG in the presence of uninterrupted ART did not increase viremia (Panel A) but enhanced detection of cell-associated HIV RNA in bone marrow, spleen, liver and lung tissues (Panel B). SM-APG treatment was correlated with a reduction in the frequency of viral genome-habouring cells in tissues including the brain (Panel C). Additional, not shown data: SM-APG decreased levels of Th17, Tregs and dendritic cells.

Fig. 5: Multiple dose treatment of SM-APG lowers the magnitude of viral rebound in hu-mice



Viral rebound upon ART interruption was significantly reduced (by 10 to 100-fold) in mice treated with SM-APG compared to those receiving the vehicle control (Panel A). The decrease in viral rebound was correlated with a lower level of virus-expressing CD4⁺ T cells (Panel B).

CONCLUSIONS

- Novel bivalent SM-APG efficiently reactivates HIV in CD4⁺ T cell models of latency. By increasing the level of active caspase-3 in infected cells, SM-APG has the potential to reduce VRs.
- A single dose, 48-hour treatment of SM-APG can reverse latency in humanized mice thus, increasing plasma viral load and enhancing HIV RNA in tissues.
- Sequential treatment of SM-APG in the presence of uninterrupted ART leads to a decrease in the frequency of infected cells and maintains viral suppression.
- The level of viral rebound is lowered in SM-APG-treated mice.