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Reversal of HIV-1 latency by activation of patient-derived CD4+T-cells results in clonal expansion and sustained production of infectious virus from a subset of cells

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Background: The "kick-and-kill" strategy, consisting of latency reversal followed by death of cells with activated proviruses, has been proposed as a means of eliminating the HIV-1 reservoir. However, the most effective latency reversing agents are also potent T-cell activators (Cillo, PNAS 2014). Recent studies show that virus producing cells can persist and expand in vivo (Maldarelli, Science 2014). We hypothesized that activation of patient-derived CD4+ T-cells can lead to clonal expansion of proviruses rather than their elimination.

Methods: To study the effects of latency reversal by CD4+ T-cell activation on virus production and cell survival, we established an ex vivo cell culture system involving stimulation of patient-derived CD4+ T cells with PMA/ionomycin (day 1-7), followed by rest (day 7-21), and then restimulation (day 21-28) in the presence of raltegravir and efavirenz to block virus spread. Cell-associated HIV-1 DNA and virion RNA in the supernatant were quantified by qPCR at weekly intervals. Single genome sequencing (SGS) was performed to characterize proviruses and virion RNA. Replication-competence of virions produced was determined by co-culture with CD8-depleted blasts from HIV negative donors.

Results: Experiments were performed with purified CD4+ T-cells from 5 consecutive donors who had been suppressed on ART for 2 or more years (median = 13.4 years). In all experiments, HIV-1 RNA levels in supernatant increased following initial stimulation, decreased during the rest period, and increased again with restimulation. Cell-associated HIV-1 DNA levels did not show a consistent pattern of change. SGS revealed several different outcomes of cells containing specific proviruses: 1) virus production following the first but not the second stimulation; 2) virus production only following the second stimulation; 3) virus production following both stimulations; 4) no virus production with either stimulation, 5) proviral expansion without virus production; and importantly 6) proviral expansion with virus production, including replication-competent virus.

Conclusions: These results indicate that reversal of HIV-1 latency by CD4+ T cell activation results in multiple outcomes of proviral-containing cells including clonal expansion of proviruses that can produce infectious virions. These findings underscore the complexity of eliminating HIV reservoirs and the need for strategies to kill virus-producing cells before they can proliferate.