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

**Background**

1. **The major barrier to an HIV-1 cure is the latent reservoir**, which refers to stably suppressed HIV-infected donor cells that can lead to clonal expansion of proviruses rather than their elimination.
2. **The most effective latency reversing agents are also potent T-cell activators**.1,2
3. **Although rapid cell death is a common feature of activated HIV-infected T cells**, recent studies show that virus-producing cells can persist and expand in vivo.3,4
4. **The present study explores whether activation of CD4+ T-cells from chronically suppressed HIV-infected donors can lead to clonal expansion of proviruses rather than their elimination.**

**Methods**

- Experiments were performed in five chronically-suppressed HIV-1 infected individuals.
- Total CD4+ T-cells (tCD4) were isolated from large volume blood draw and stimulated with PMA/IONOMycin (50/100 ng/ml) between days 0-7 and days 21-28.
- Experiments were also performed with peripheral blood mononuclear cells (PBMC) in donors 2 and 5.
- Replication-competence of virions produced from total CD4+ T-cells in donor 2 was determined by the viral outgrowth assay.
- HIV-1 RNA in cell-free supernatants was quantified by qRT-PCR using the Amplicor COBAS TaqMan assay.
- HIV-1 cell-associated DNA (CAD) was quantified by in-house qPCR.
- Single Genome Sequencing (SGS) was performed to characterize proviruses and virion RNA.

**Results**

Table 1. Donor Clinical Characteristics.

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**Conclusions**

- **SGS reveals complex proviral dynamics after cell activation.**
- Many proviruses do not produce virus, consistent with the high frequency of defective proviral genomes.6
- A subset of proviruses show no virion production following repeat stimulation, suggesting death of cells containing that proviruses.
- New proviruses can be expressed with repeat stimulation, consistent with previous studies.6
- A subset of proviruses are expressed with both stimulations.
- Non-producing proviruses can proliferate.
- Double-producing proviruses can persist and proliferate, including those that are replication-competent.
- **Reversal of HIV-1 latency by CD4+ T cell activation results in multiple outcomes of proviruses, 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.

**Acknowledgements**

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