PRESENTATION OF COGNATE ANTIGENS BY DENDRITIC CELLS CAUSES STOCHASTIC HIV EXPRESSION

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Background

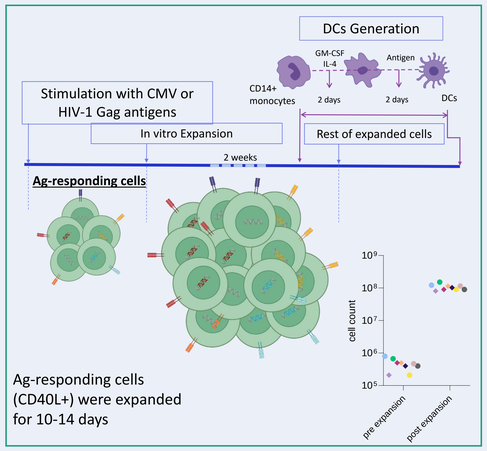
- Encounter with cognate antigens (Ag) is a major cause of proliferation and persistence of HIV-infected CD4+ T cells.
- Understanding to which extent physiological T cell activation elicits latency reversal is key for cure efforts.

Does encounter with cognate antigen lead to latency reversal?

How do we compare HIV reactivation mediated by Ag recognition versus global T cell activators in interpretable way?

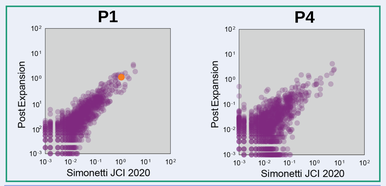
1 Methods

Due to low frequency of HIV-infected Ag-reactive cells, expansion is needed to study Ag-mediated proviral reactivation.

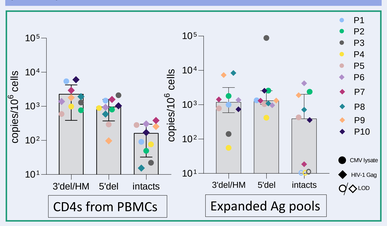


Ag-responding cells (CD40L+) were expanded for 10-14 days

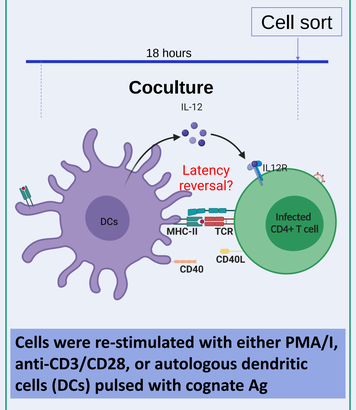
Expanded pools preserved their TCR repertoire



The frequency and composition of proviruses in the expanded pools were highly variable across participants

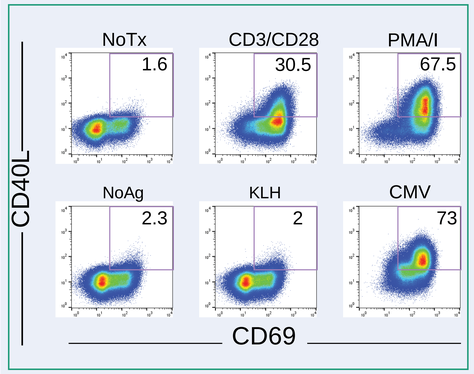


2 Results

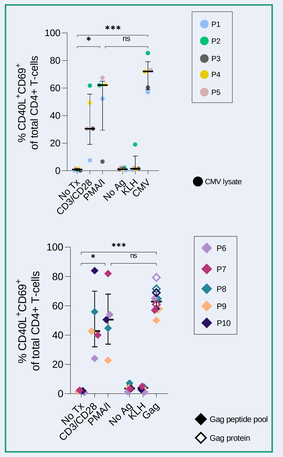


Cells were re-stimulated with either PMA/I, anti-CD3/CD28, or autologous dendritic cells (DCs) pulsed with cognate Ag

Encounter with cognate antigen induced upregulation of CD69 and CD40L

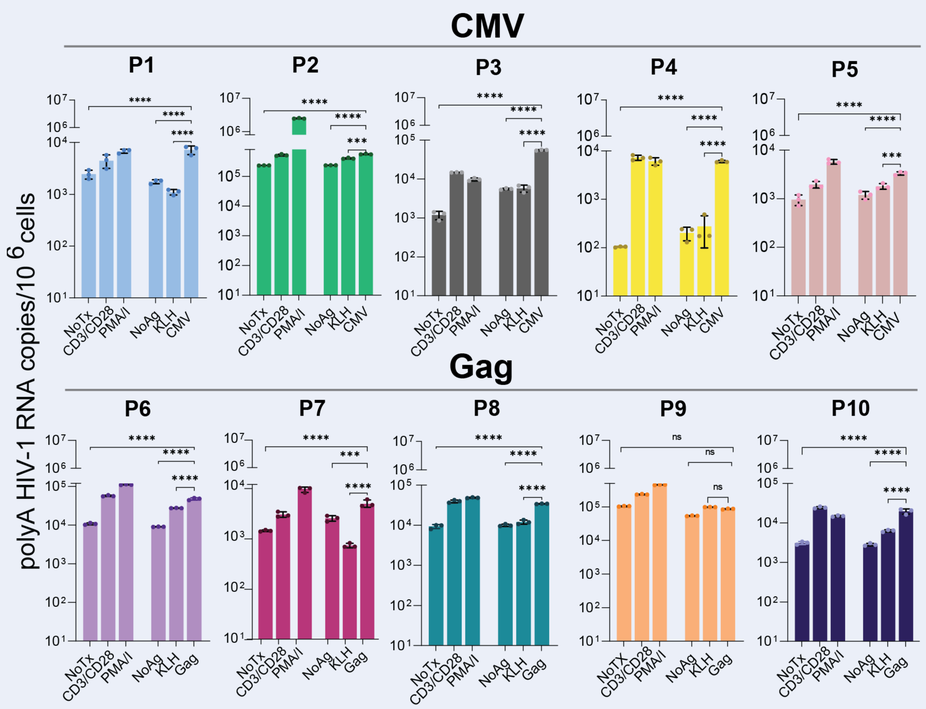


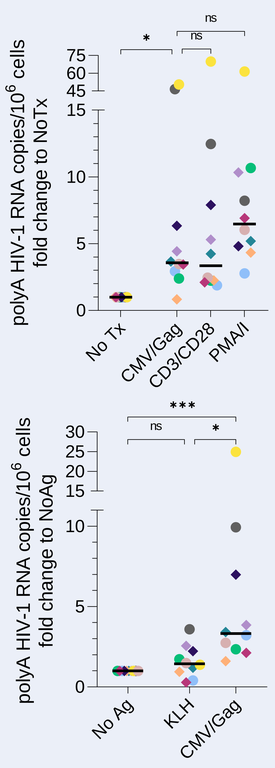
CD40L as a marker of TCR engagement is upregulated upon encounter with cognate Ag



3 Results

Encounter with cognate antigen induced increased HIV expression

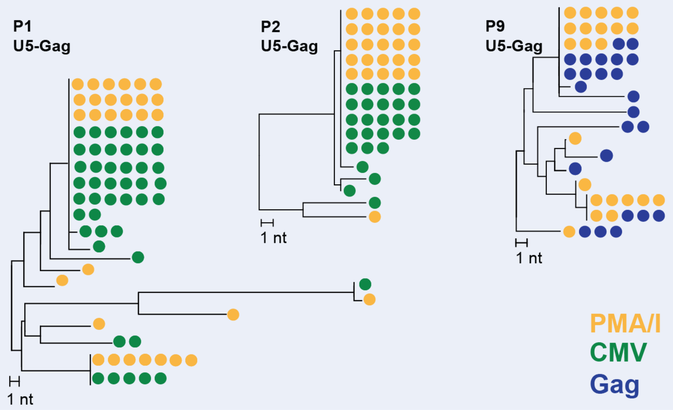




Bulk cell-associated HIV RNA measurements upon restimulation with either PMA/I, anti-CD3/CD28, or autologous DCs pulsed with either cognate Ag (CMV/Gag) or irrelevant Ag (KLH)

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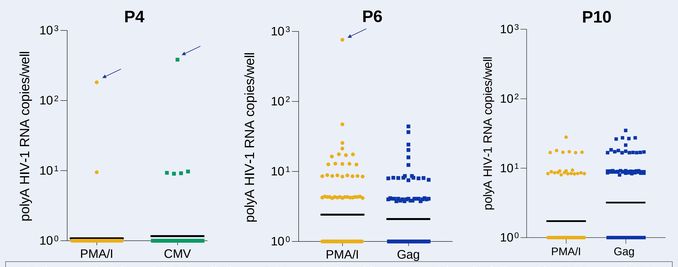
RNA sequences are present both in Ag and PMA/I stimulation



Cell-associated RNA was isolated from CD4+ T cells treated with PMA/I or autologous DCs pulsed with cognate Ag and used for cDNA synthesis. cDNA was then diluted at end point for single genome sequencing of the U5-gag region.

5

Measuring HIV RNA at a single-cell-resolution elicits the presence of rare high RNA-producing cells



Cells were distributed in 96-well plates. RNA was isolated by magnetic beads, followed by DNAse and HIV poly-A tail-anchored cDNA synthesis.

Conclusions

- We have developed an approach to study Ag-mediated proviral reactivation and compare it to global T cell activators in interpretable way .
- Cognate Ag induced increased HIV expression, but this increase showed inter-participant variability.
- We observed quantitative and qualitative differences in HIV transcriptional profiles when CD4+ T cells encountered their cognate Ag compared to strong T cell activators.
- RNA sequence analysis showed similar breadth of proviruses induced across different conditions.

References

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