Candida-Responsive Cells Contain Intact HIV-1 DNA Despite Limited Clonality in Blood

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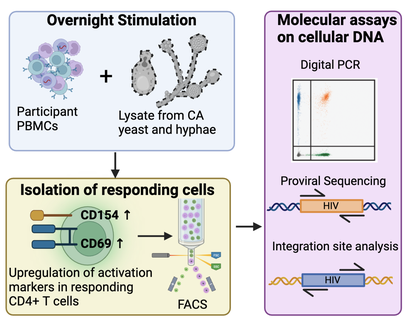
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BACKGROUND

- The persistence of a stable reservoir of HIV-1 infected CD4⁺ T cells during antiretroviral therapy (ART) remains the central barrier to a cure.
- Antigen-driven proliferation of infected cells is a major driver of reservoir persistence, yet the role of repeated antigen stimulation from commensal microbes is poorly characterized.[1]
- *Candida albicans* (CA), a ubiquitous commensal fungus, elicits Th17-polarized responses, which remain underexplored compared to Th1/CTL-polarized populations.[2-4]
- We **hypothesized** that CA antigens contribute to reservoir persistence in blood by driving the proliferation of HIV-infected CD4⁺ T cells.

METHODS

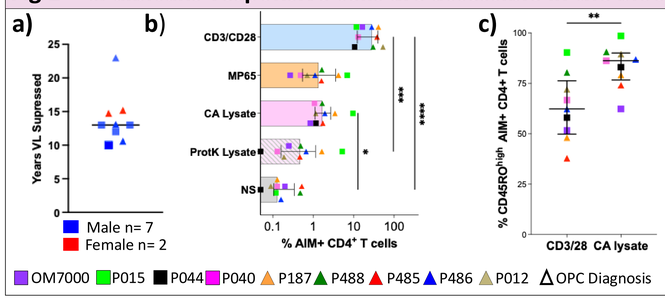
- CD8 depleted PBMCs from nine long term ART-suppressed people living with HIV (PLWH) were stimulated with anti-CD3/CD28 beads, the immunodominant CA antigen MP65, CA protein lysate, proteinase K digested CA lysate (Prot K), or not stimulated (NS)
- Responsive (AIM+) CD4⁺ T cells were isolated based on activation-induced markers (AIM: CD69, CD154, CD137), along with CA non-responsive cells (NR).
- Genomic DNA from sorted cells was analyzed using intact proviral DNA assay (IPDA) to quantify intact and defective proviruses, proviral sequencing and integration site analysis to assess proviral clonality, and TCR-β sequencing to evaluate clonal distribution.
- In a participant with an expanded clone, near full length (NFL) proviral sequencing and ligation mediated PCR integration site analysis (LM-PCR/ISA) was performed.



C. albicans-responsive T cells contribute to reservoir persistence despite lower clonal expansion in peripheral blood.

RESULTS

Fig 1 – *C. albicans*-responsive cells are accessible in the blood of PLWH

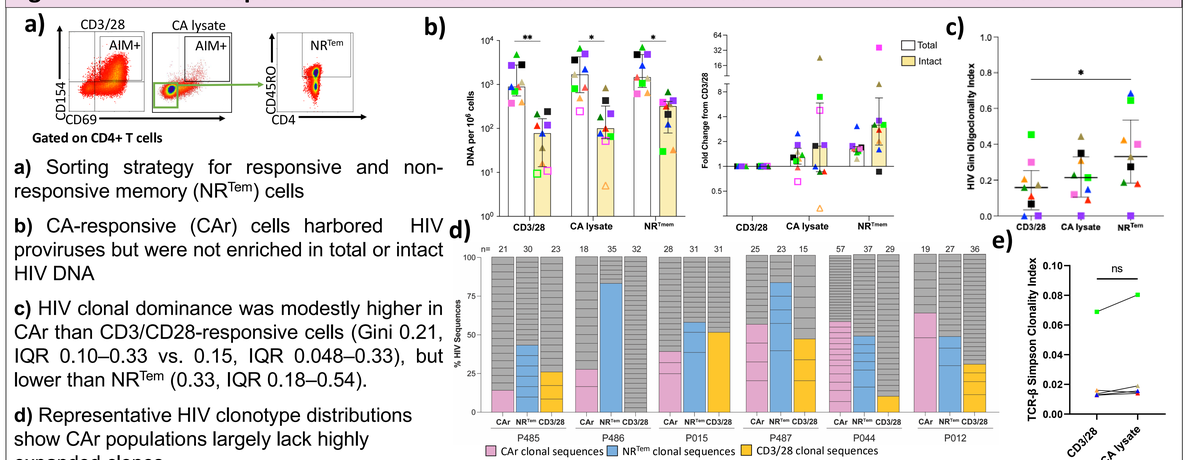


a) Nine long-term virally suppressed participants (median 13 years; IQR 11.3–14.97); 5/9 with prior oropharyngeal candidiasis (OPC) diagnosis.

b) Median 1.7% (IQR 1.1–2.7) of blood CD4⁺ T cells respond to CA lysate; responses drop to 0.5% (IQR 0.2–1.2) after Proteinase K digestion. The immunodominant peptide MP65 elicits a comparable frequency (1.4%, IQR 0.5–3.6).

c) CA-responsive CD4⁺ T cells are predominantly memory (CD45RO^high), consistent with specific activation of memory cells.

Fig 2 – *C. albicans*-responsive CD4⁺ T cells harbor defective and intact HIV DNA but lack clonal enrichment



a) Sorting strategy for responsive and non-responsive memory (NR^Tem) cells

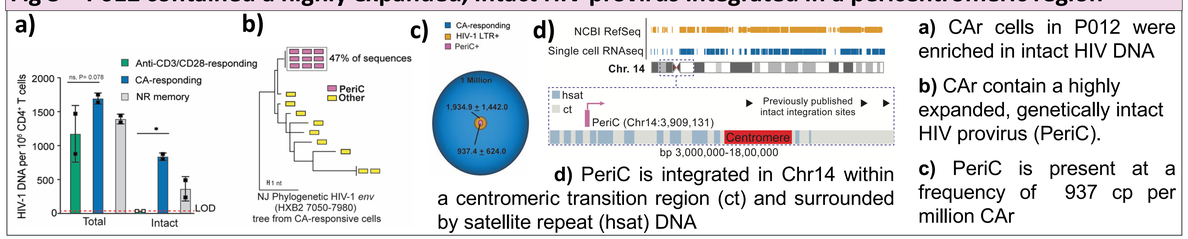
b) CA-responsive (CAr) cells harbored HIV proviruses but were not enriched in total or intact HIV DNA

c) HIV clonal dominance was modestly higher in CAr than CD3/CD28-responsive cells (Gini 0.21, IQR 0.10–0.33 vs. 0.15, IQR 0.048–0.33), but lower than NR^Tem (0.33, IQR 0.18–0.54).

d) Representative HIV clonotype distributions show CAr populations largely lack highly expanded clones.

e) Overall, circulating CAr cells are not characterized by clonal dominance relative to non-specifically stimulated cells.

Fig 3 – P012 contained a highly expanded, intact HIV provirus integrated in a pericentromeric region



a) CAr cells in P012 were enriched in intact HIV DNA

b) CAr contain a highly expanded, genetically intact HIV provirus (PeriC).

c) PeriC is present at a frequency of 937 cp per million CAr

d) PeriC is integrated in Chr14 within a centromeric transition region (ct) and surrounded by satellite repeat (hsat) DNA

CONCLUSIONS

Although *C. albicans* colonizes mucosal and skin surfaces[3], CA-responsive CD4⁺ T cells are detectable in peripheral blood using CA lysate stimulation. Responses were protein antigen-dependent, as demonstrated by reduced cell activation upon proteinase K treatment. The responding cells were also predominantly CD45RO^high, consistent with a memory phenotype.

Circulating CAr cells harbor HIV-1 proviruses but are not enriched for highly expanded clones, distinguishing them from typical virus-responsive Th1/CTL populations.[5] The limited TCR-β clonality suggests this is an intrinsic feature of antifungal responses, likely reflecting Th17-skewed immunity and the tissue-localization of antigen exposure[3-4]. As Th17 cells are concentrated at mucosal sites, studying fungal-responsive cells within these compartments will be essential to defining their role in HIV reservoir persistence.

Although clonality was generally low, one participant (P012) exhibited a highly expanded clone with a provirus integrated in a centromeric transition region of the p arm of acrocentric Chr 14, indicating that clonal expansion can occur but is not a dominant feature of circulating CAr cells in PWLH.

Taken together, our results show that HIV persists in less characterized T cell populations responsive to commensal pathogens.

Sources:

1. **Simonetti et al, 2021**
2. **Christophe et al, 2021** PMID: 33232448
3. **M. Swidergall, 2022.** PMID: 35778599
4. **Bacher et al, 2019** PMID: 30799037
5. **Collora et al, 2022** PMID: 35320704

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PLAIN LANGUAGE SUMMARY

Blood immune cells responding to a common fungi can carry hidden HIV, even though they are not the highly expanded cell types usually linked to viral persistence. This suggests that everyday immune responses to microbes that inhabit our body may play an underappreciated role in helping HIV infected cells survive during treatment.

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