UNEQUAL DISTRIBUTION OF INTACT HIV PROVIRUSES IN CELLS EXPRESSING PD-1 AND/OR CTLA-4

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Katie Fisher[1,2], Timothy E. Schlub[3], Zoe Boyer[1,2], Thomas A. Rasmussen[4], Ajantha Rhodes[4], Rebecca Hoh[5], Frederick M. Hecht[5], Steven G. Deeks[5], Sharon R. Lewin[4,6,7], Sarah Palmer[1,2]

[1.] Centre for Virus Research, The Westmead Institute of Medical Research, The University of Sydney, Sydney, NSW, 2145, Australia ; [2.] Sydney Medical School, Westmead Clinical School, Faculty of Medicine and Health, The University of Sydney, Sydney, NSW, 2006, Australia; [3.] Sydney School of Public Health, Faculty of Medicine and Health, The University of Sydney, Sydney, NSW, 2006, Australia; [4.] Department of Infectious Diseases, The University of Melbourne at The Doherty Institute for Infection and Immunity, Melbourne, VIC, Australia; [5.] Division of HIV, Infectious Diseases and Global Medicine, Department of Medicine, Zuckerberg San Francisco General Hospital, University of California San Francisco, California, 94110, United States of America; [6.] Department of Infectious Diseases, Alfred Hospital and Monash University, Melbourne, VIC, Australia; [7.] Victorian Infectious Diseases Service, Royal Melbourne Hospital at The Doherty Institute for Infection and Immunity, Melbourne, VIC, Australia

BACKGROUND

HIV-1 persists in CD4 T-cells of people with HIV (PWH) on antiretroviral therapy (ART). Determining cell surface markers that enrich for genetically-intact HIV-1 genomes is vital in developing curative strategies. The immune checkpoint markers programmed cell death protein-1 (PD-1) and cytotoxic T-lymphocyte associated protein-4 (CTLA-4) have been identified as potential markers for enrichment of HIV-1 provirus.

PARTICIPANTS AND METHODS

We obtained peripheral blood (PB) from 16 PWH under effective ART. Memory CD4 T-cells were FACS-sorted into 4 populations: PD-1$^-$CTLA-4$^-$ (double negative, DN), PD-1$^+$CTLA-4$^-$ (PD-1$^+$), PD-1$^-$CTLA-4$^+$ (CTLA-4$^+$) and PD-1$^+$CTLA-4$^+$ (double positive, DP). Using the full-length individual proviral sequencing assay, we identified genetically-intact proviruses, and proviruses with intact open reading frames (ORFs) for the HIV-1 proteins *p24* and *nef.*

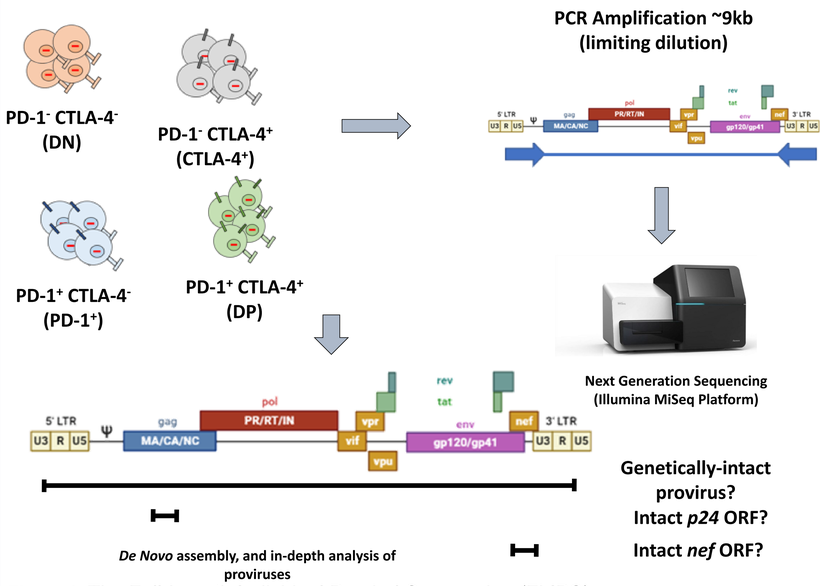


Figure 1. The Full-Length Individual Proviral Sequencing (FLIPS) assay (Hiener et al. 2017) was used to obtain near full-length HIV-1 proviruses and classify these as either genetically intact or defective.

INTACT HIV-1 INFECTION FREQUENCY

Genetically-intact proviruses were less frequent in CD4$^+$ T cells expressing CTLA-4. However, many of the comparisons of HIV-1 proviruses with specific characteristics between CD4$^+$ T cells expressing PD-1 and/or CTLA-4 were affected by strong participant-specific differences, including comparisons of intact infection frequencies.

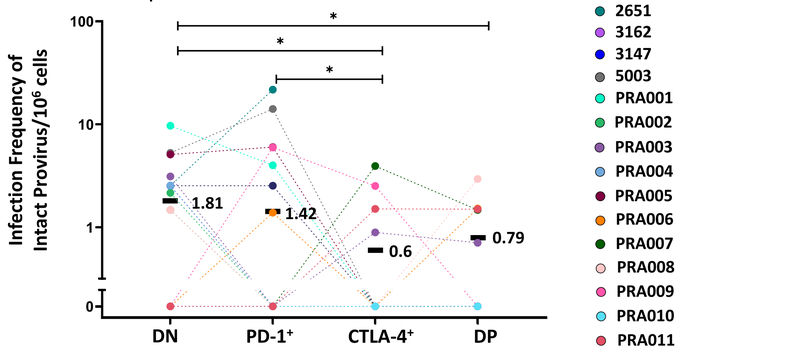


Figure 2. Genetically-intact HIV-1 proviruses are less frequent in CD4$^+$ T cells expressing CTLA-4. Infection frequency per 10^6 cells of genetically-intact HIV-1 proviruses was higher in DN and PD-1$^+$ cells compared to CTLA-4$^+$ and DP cells, with evidence for PD-1$^+$>CTLA-4$^+$ (p=0.04), DN>CTLA-4$^+$ (p=0.01) and DN>DP cells (p=0.03). The difference in intact infection frequency between DN and PD-1$^+$ cells was highly variable across participants (p=0.009), with some participants exhibiting a higher intact infection frequency in DN cells, while others exhibited a higher intact infection frequency in PD-1$^+$ cells. Data is the estimated infection frequency per 10^6 cells for each subset.

INFECTION FREQUENCY OF PROVIRUSES WITH AN INTACT *p24* ORF

The lack of enrichment of genetically-intact proviruses in PD-1$^+$ compared to PD-1$^-$ cells does not agree with previous work investigating the presence of proviruses that can express p24 protein (Pardons et al., 2019). We investigated whether this was due to an enrichment of proviruses with an intact *p24* ORF in PD-1$^+$ cells, which may be able to express p24 protein despite being genetically-defective.

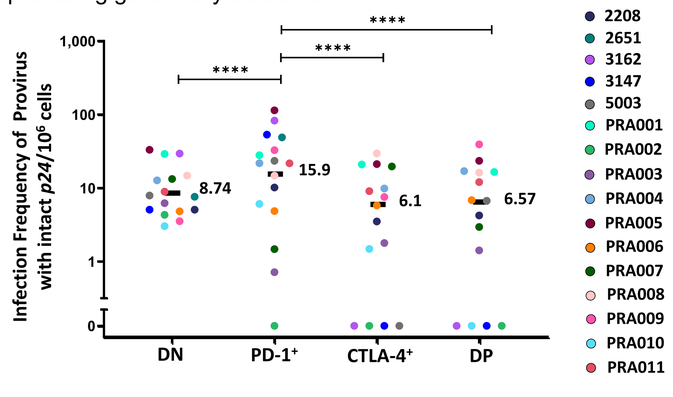


Figure 3. Proviruses that can express p24 protein are enriched in PD-1$^+$ cells. The infection frequency per 10^6 cells of proviruses with an intact *p24* ORF was found to be highest in PD-1$^+$ cells, followed by DN, DP and CTLA-4$^+$ cells, with evidence for PD-1$^+$>DN, PD-1$^+$>CTLA-4$^+$ and PD-1$^+$>DP (all p<0.00001). These differences were also participant-dependent (all p<0.001). Data is the estimated infection frequency per 10^6 cells for each subset.

CONTRIBUTION OF CELLULAR PROLIFERATION TO HIV-1 PERSISTENCE IN CD4$^+$ T CELLS EXPRESSING PD-1 AND/OR CTLA-4

The contribution of cellular proliferation to the persistence of HIV-1 proviruses during effective ART is well-known. To investigate the contribution of cellular proliferation to HIV-1 persistence in CD4$^+$ T cells expressing PD-1 and/or CTLA-4, we compared the proportion of sequences within each subset that were part of an expansion of identical sequences (EIS; groups of two or more identical sequences).

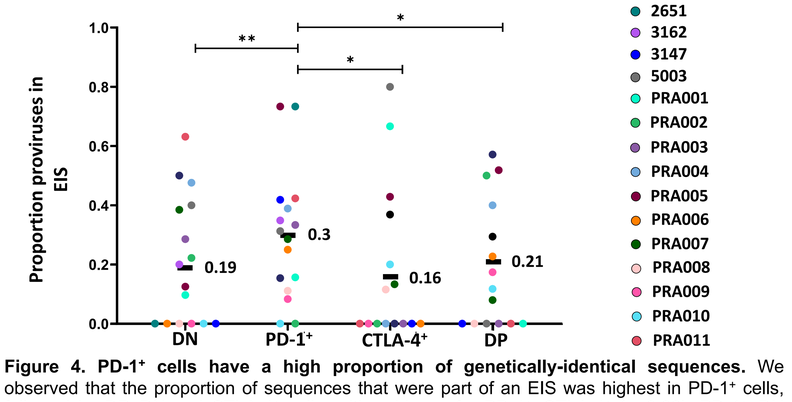


Figure 4. PD-1$^+$ cells have a high proportion of genetically-identical sequences. We observed that the proportion of sequences that were part of an EIS was highest in PD-1$^+$ cells, followed by DP, DN and CTLA-4$^+$ cells, with evidence for PD-1$^+$>CTLA-4$^+$ (p=0.02), PD-1$^+$>DN (p=0.001) and PD-1$^+$>DP (p=0.05). The differences in the proportion of sequences that were part of an EIS between all cell subsets were affected by participant-specific variability (p<0.03 for all). Data is the estimated infection frequency per 10^6 cells for each subset.

INFECTION FREQUENCY OF PROVIRUSES WITH AN INTACT *nef* ORF

The HIV protein Nef has been shown to have important functions in immune escape by downregulating expression of surface MHC-I, thereby allowing an HIV-1-infected cell to escape detection by HIV-1-specific CD8+ T cells and contributing to HIV-1 persistence (Duette et al, 2022). We investigated whether CD4$^+$ T cells expressing PD-1 and/or CTLA-4 are enriched for proviruses with an intact *nef* ORF, which may enhance HIV-1 persistence in these cells.

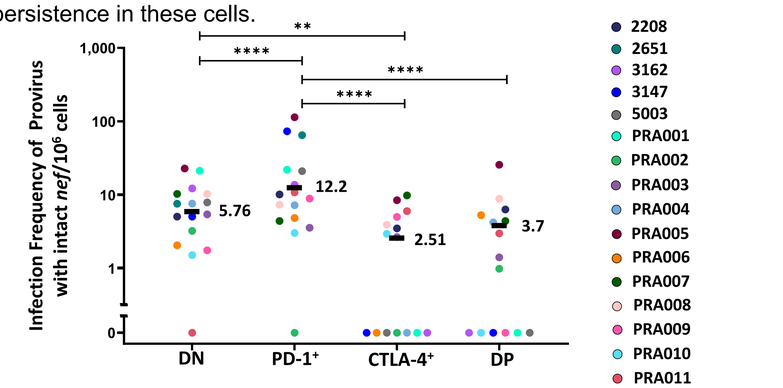


Figure 5. Proviruses with genetically-intact *nef* are enriched in PD-1$^+$ cells and less frequent in CTLA-4$^+$ cells. The infection frequency per 10^6 cells of proviruses with an intact *nef* ORF was found to be lower in CTLA-4$^+$ and DP cells compared to DN and PD-1$^+$ cells, with evidence for PD-1$^+$>CTLA-4$^+$, PD-1$^+$>DP and PD-1$^+$>DN (all p<0.00001), and DN>CTLA-4$^+$ (p=0.002). These differences all varied across participants (p≤0.02 for all). Data is the estimated infection frequency per 10^6 cells for each subset.

CONCLUSIONS

- PD-1$^+$ cells in the peripheral blood have high levels of proviruses with intact *nef* ORFs and that genetically-identical to another sequence, indicating that these cells have characteristics that contribute to HIV-1 persistence.
- PD-1$^+$ cells also have high levels of proviruses with intact *p24* ORFs, which can affect studies of HIV-1 persistence during ART
- CTLA-4 expression is a marker for HIV-1 proviruses that are more likely to be genetically-defective.
- We observed significant participant variation in the enrichment of HIV-1 proviruses with intact genomes or specific intact ORFs across these memory CD4$^+$ T cell subsets.
- Consideration of additional cellular markers will likely be needed to consistently identify cells harbouring potentially replication-competent HIV-1.

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Author contact information: katie.fisher@sydney.edu.au

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