National Institute of Allergy and Infectious Diseases

Utilizing Defective HIV-1 Provirus as a Novel “Barcode” for Studying T-Cell Dynamics

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Vinie Kouamou[1], Mindy Smith[1], Brad T. Sherman[2], Catherine A. Rehm[1], Weizhong Chang[2], H. Clifford Lane[1], Hiromi Imamichi[1]

[1]National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD, United States, [2]Frederick National Laboratory for Cancer Research, Frederick, MD, United States.

BACKGROUND

- Achieving an understanding of T-cell dynamics and distribution of proviral DNA within T cell subsets is of critical importance in achieving a better understanding of the relationships between effector memory, central memory and naïve CD4 cell subsets.
- Current techniques, such as labeling and tracking, and BrdU incorporation, used to measure cell half-life are unable to distinguish between signal reduction due to cell decay and cell division over time.
- Defective HIV-1 proviruses once integrated into the host genome of a single cell in people with HIV (PWH) can persist for years and faithfully duplicate during somatic cell division (**‘the signal is preserved**’). In this study, we investigated the relationships between T cell subsets utilizing the natural “barcoding” provided by replication-incompetent defective HIV-1 proviruses.

METHODS

- Cryopreserved cells were obtained from 2013 to 2023 from a 54-year-old white male with HIV-1 infection. During this time, his plasma HIV-RNA levels remained <20-40 copies/mL with transient viral blips below 454 copies/mL, and his median CD4 count was 651 cells/µL (range: 434-756 cells/µL).
- CD4 cell subsets were isolated by flow cytometry sorting with a combination of CD45RO and CD27 antibody staining to identify naïve (NA, CD27+CD45RO-), central memory (CM, CD27+CD45RO+) and effector memory (EM, CD27-CD45RO+) subsets.
- HIV-1 proviruses in CD4 cell subsets and unsorted PBMCs were characterized by 5'LTR-to-3'LTR PCR single-genome amplification and direct amplicon sequencing.

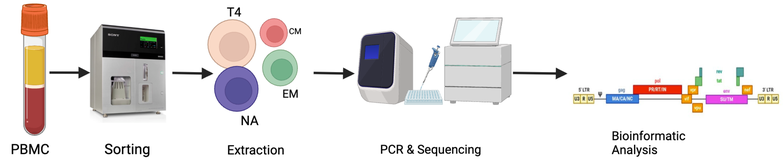
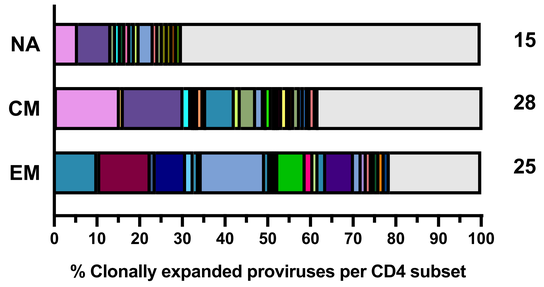


Figure 1: Flowchart of the study method

RESULTS



Sequencing of 1,174 HIV-1 proviruses in the three CD4 cell subsets and PBMCs identified 78 expanded defective HIV-1 provirus clones, each harboring distinct defective provirus barcodes.

Figure 2: Distribution of the clones per CD4 subset

23 of the 78 clones persisted for ≥3 yrs (median: 6 yrs, range: 3-10 yrs). The highest number of provirus-barcoded clones was found in the CM pool (n=28) followed by the EM (n=25) and NA (n=15) pools, corresponding to 61%, 79%, 30% of the total provirus sequences detected in CM, EM and NA cells, respectively

One of the dominant clones (the pink-colored sections of the bars in the figure is a 6 kb HIV-1 provirus with hypermutations. It was first detected in CM cells and 3 yrs later in NA cells (clones 1 and 4 in Figure 3). In addition, clonal conversions from EM to CM (2 provirus-barcoded clones) and reversions from CM to NA (5 provirus-barcoded clones) were observed.

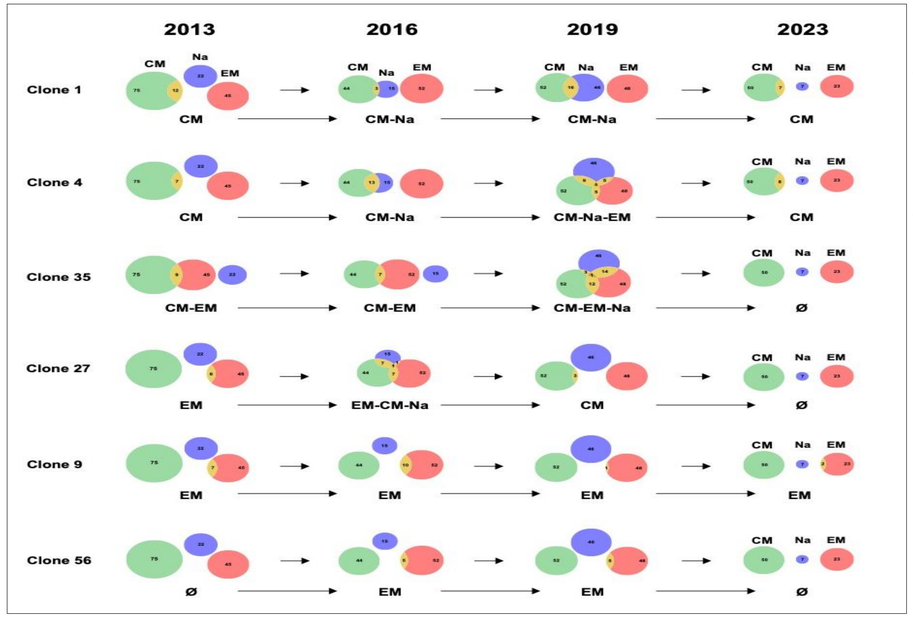
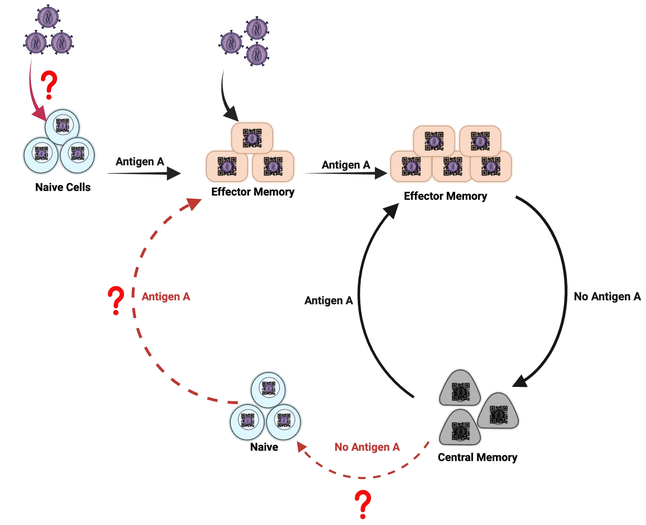


Figure 3: Fate of the T cell using defective HIV-1 provirus

CONCLUSIONS



Clonal conversion (EM-CM) and reversion (CM-NA) were observed

- The strategy of using expanded defective HIV-1 provirus clones as a novel “barcode” enables high-resolution insights into T-cell dynamics and demonstrates the durability of clonal populations in each subset.
- In contrast to the current dogma of effector memory populations being short-lived, these data demonstrated that they are able to persist in vivo for several years or more.

ACKNOWLEDGEMENTS

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