TCR SEQUENCING REVEALS CLONAL EXPANSIONS OF INDUCIBLE RESERVOIRS IN SPECIFIC SUBSETS

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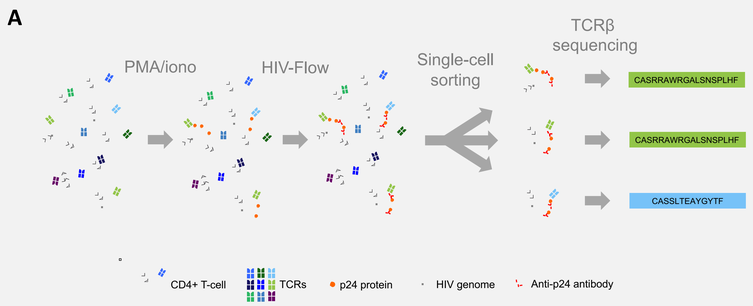
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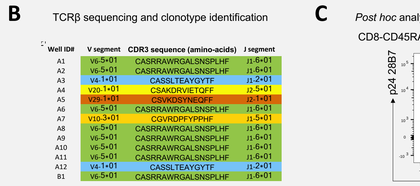
Introduction

Clonal expansions occur in the persistent HIV reservoir as demonstrated by the duplication of HIV genes and/or integration sites reported in several studies. However, these approaches do not permit to phenotypically analyze these expanded clones of infected cells nor the inducibility of the proviruses.
We took advantage of the uniqueness of the T-cell receptor (TCR) expressed by a given T-cell clone to track HIV-infected cells.

Methods

Blood samples from 8 individuals on suppressive ART for at least 2 years were collected longitudinally. Clonotype characterization of HIV-infected cells was determined by combining **index single-cell sorting of HIV-infected cells** by HIV-Flow **and TCR sequencing** (Figure 1A). TCR clonotypes were retrieved from TCR sequences using the IMGT database (example for participant #1, 1st sample in Figure 1B) and their memory phenotype was assessed *post hoc* (Figure 1C).
A subset of p24- cells was analyzed to determine TCR diversity in the CD4+ T-cell compartment.





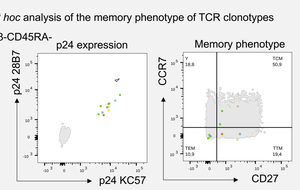
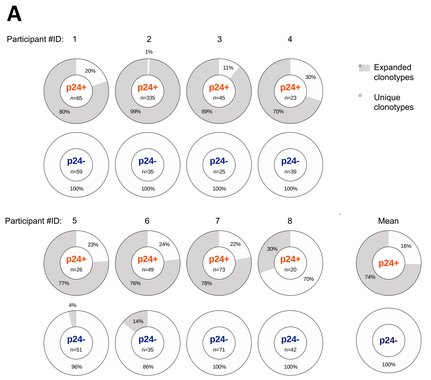


Figure 1. Strategy for analyzing the TCR repertoire of single-sorted HIV-infected cells.
A: Overview of methods; **B:** Example of TCR clonotypes identification for participant #1 (1st sample) after single-cell sorting of p24+ cells; **C:** Dot plots of the memory phenotypes of the TCR clonotypes.

Results

The majority of the translation-competent reservoir is clonally expanded

We obtained the **TCRβ sequences from 636 p24+** and 357 p24- single-sorted cells, among those, we identified 98 and 353 different TCR clonotypes, respectively. **Clonal expansions** were detected in the pool of p24+ cells from **all participants** (Figure 2) and accounted for the majority of reservoir cells (mean, 74%). We observed a median of 2 independent clonal expansions per sample (range, 1-5 expanded clonotype per sample), each expansion being detected in a median of 5 p24+ cells (range, 2-187).



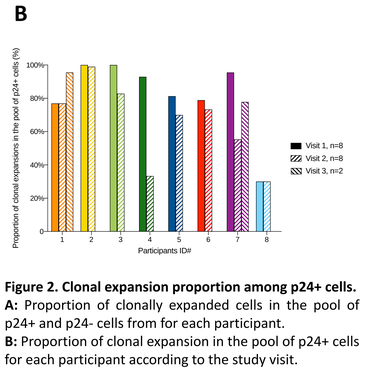


Figure 2. Clonal expansion proportion among p24+ cells.
A: Proportion of clonally expanded cells in the pool of p24+ and p24- cells from for each participant.
B: Proportion of clonal expansion in the pool of p24+ cells for each participant according to the study visit.

Expanded clonotypes in p24+ cells often persist on ART

Over time on ART, clonally-expanded p24+ cells could whether be further detected, indicating persistent clonal expansion, or not, indicating transient clonal expansion.
Expanded infected clonotypes persisted on ART in 7/8 individuals (except participant #5).
However, the dynamic of the HIV reservoir on ART greatly varied between individuals, with some participants showing a stable repertoire, whereas others displayed emergence of new clonotypes over time (Figure 3).

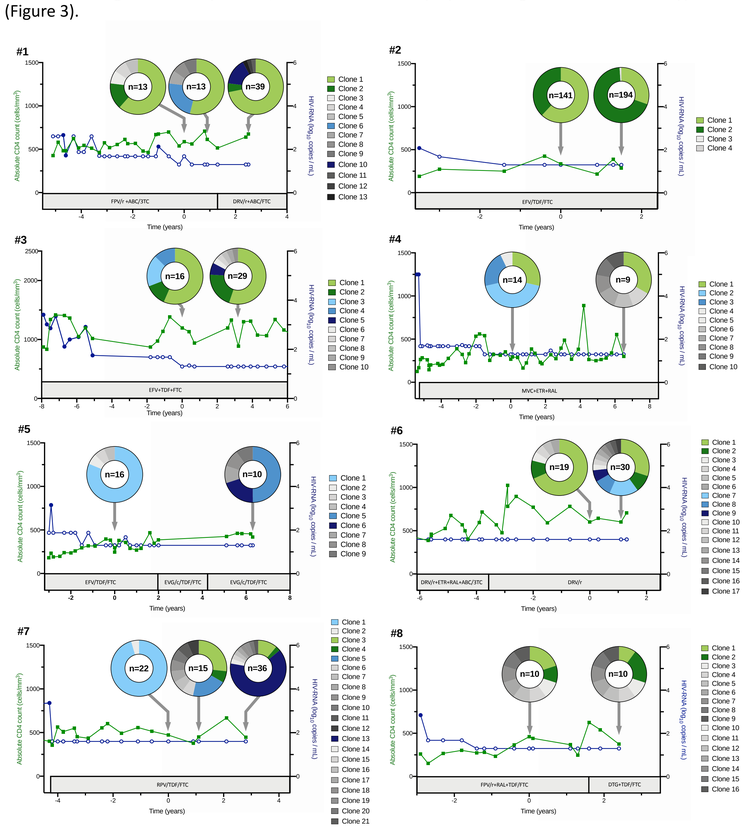


Figure 3. Clonal expansion proportion among p24+ cells.
Proportion of TCRβ clones among the pool of p24+ cells among the different study participants (ranked #1 to #8) and according to the study.

The TCR repertoire of p24+ cells is biased when compared to p24- cells

There was **a bias** in the selection of V and J segments in p24+ cells (Figure 4A) when compared to p24- cells (Figure 4B). This bias was mainly introduced by clonal expansions in the HIV reservoir.

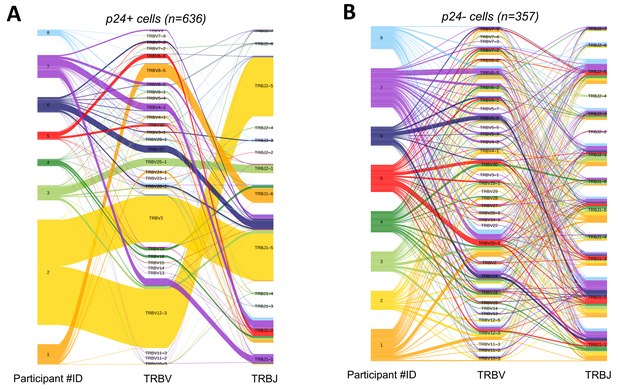
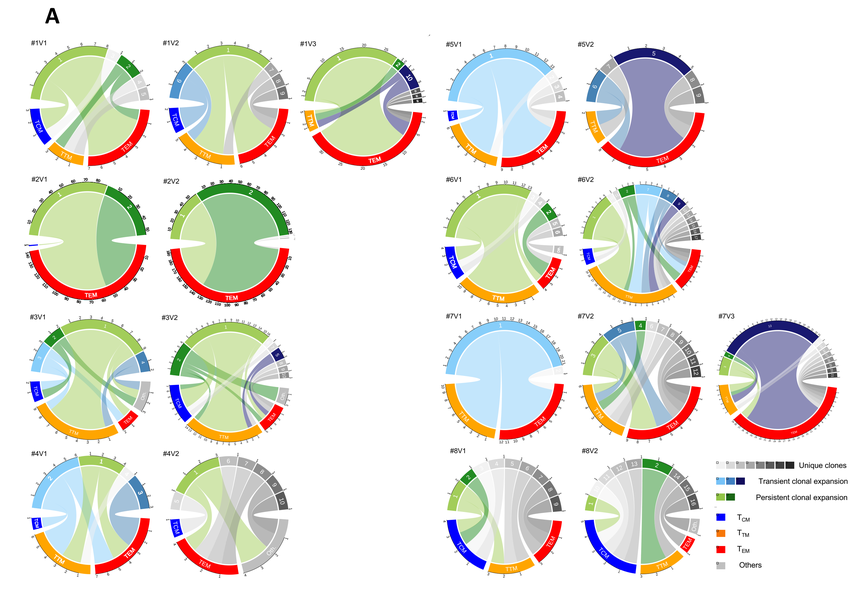


Figure 4. V and J segment association in the pool of p24+ and p24- cells.
Association of V and J segment (links) for both p24+ **(A)** and p24- cells **(B)**, each color represent a participant.

Clonally-expanded p24+ cells display multiple memory phenotypes

Expanded p24+ clonotypes systematically displayed at least two different memory phenotypes (Figure 5A). Expanded infected clones were overrepresented in the most differentiated cells (i.e. transitional, T_{TM}; and effector memory, T_{EM}). Nonetheless, these expanded clones were also identified within the central memory (T_{CM}) compartment from the majority of the participants, albeit at lower frequencies (Figure 5B).
Importantly, the memory phenotype of these expanded reservoir cells was maintained over time on ART



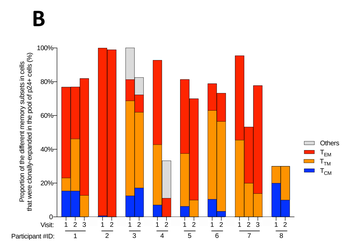


Figure 5. Expanded p24+ clonotypes display multiple memory phenotypes.
A: Distribution of TCRβ clones from p24+ among memory subsets each study participant (ranked #1 to #8) and according to the study visit (V1 to V2 or V3). This circular representation shows the link between a specific clone (top half of the circle) and its memory subset(s) (bottom half of the circle). The circular axis represents the number of isolated and sequenced p24+ cells in each clone/subset.
The clone color code is defined as follows: expanded clones persisting over time in green; expanded clones detected at a single visit in blue; and unique clonotypes in grey. The memory subset color code is defined as follows: T_{CM} in blue; T_{TM} in orange; T_{EM} in red; and others in grey.
B: Proportion of the different memory phenotypes among cells that were clonally-expanded cells in the pool of p24+ cells for all participants and all study visits.

Conclusions

We developed a method to obtain TCR sequences from single HIV-infected cells in individuals on ART.
Using this approach, we demonstrate that:
(1) clonal expansions highly contribute to the persistence of the translation-competent HIV reservoir, (2) clonally-expanded cells display multiple memory phenotypes, and (3) the dynamic of the repertoire of the HIV reservoir varies between individuals.
Our results suggest that:

→ A**ntigen stimulation is a major driver of the HIV reservoir dynamics** during ART.

→ Infected T cell **clonotypes displaying a differentiated phenotype are the progeny of infected central memory cells** undergoing clonal expansion during ART (Figure 6).

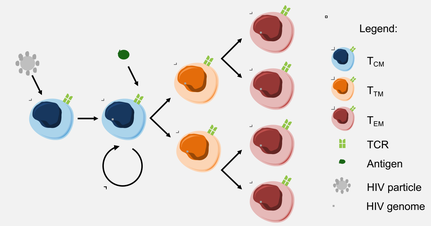


Figure 6. T_{CM} cells represent a long-lived source of reservoir cells
T_{TM} and T_{EM} cells represent the main contributors to the pool of infected cells, but are the progeny of infected T_{CM} cells that have long survival and self-renewal capacities, and can expand upon antigen stimulation and replenish the pool of infected cells.

Acknowledgements

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