PREDOMINANCE OF NON-T FOLLICULAR HELPER CELL SUBSETS IN THE LYMPH NODE VIRAL RESERVOIR DURING ART

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Background

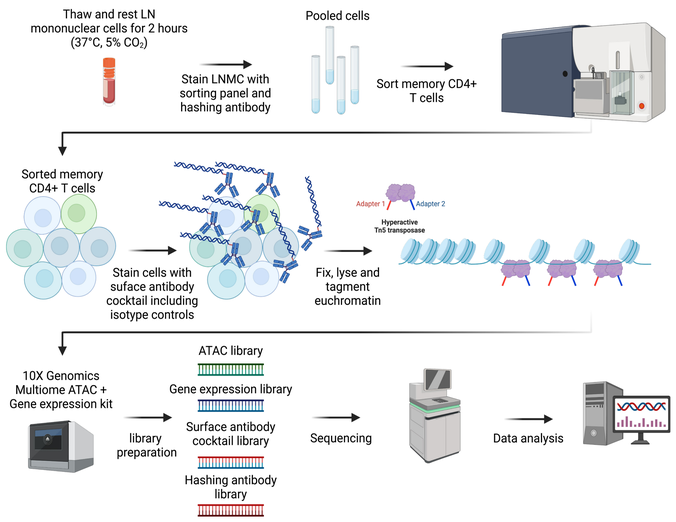
HIV-1 cure strategies require a comprehensive understanding of cellular phenotype of HIV tissue reservoirs for effective and selective targeting. While recent data have demonstrated the highly heterogeneous nature of peripheral blood CD4+ T cells subsets comprising the HIV reservoir of antiretroviral therapy (ART) treated people with HIV (PWH), CD4+ T follicular helper (Tfh) cells are generally considered as the predominant cellular reservoir of virus within lymphoid tissue. However, there are limited data supporting this during ART. Here, we re-examined this notion in lymph nodes of viremic and ART-treated PWH using unbiased multiomic analysis.

Aim

Identify the landscape of cellular phenotypes in the HIV tissue reservoir using single-cell resolution and without any ex vivo manipulation.

Methods

scDOGMAseq[1] profiling of the lymphoid tissue HIV reservoir from viremic and ART-treated PWH



[1] DOGMAseq protocol from Mimitou et al., *Nature Biotechnology* 2021
Graphics made with BioRender

Sample and Donor information

HIV-

Participant ID	ART duration (days)	pVL (copies/ml)
U287*	-	Undetectable
U294*	-	Undetectable
U190	-	Undetectable
U191	-	Undetectable

ART

Participant ID	ART duration (days)	pVL (copies/ml)
A002	476	<40
A024*	784	Undetectable
A170	770	Undetectable
A213	7326	Undetectable
A262	1197	Undetectable
A304	1035	Undetectable
A025*	489	Undetectable
A303	355	Undetectable
A127	749	<40
A256	1027	<40

Viremic

Participant ID	ART duration (days)	pVL (copies/ml)
C028	-	25,951
C087	-	1,771,593
C098	-	1,019,989
C102	-	1,692,571
C107	-	6,326,452
C111	-	552,134
C229	-	3,882,624
C242	-	715,509
C282	-	3,087,470
C101*	-	1,935,095
C235*	-	259,708
C250*	-	1,067,647

* Inguinal lymph node. All others are cervical lymph nodes (LN).

People with HIV and without HIV were originally recruited by the Centro de Investigación en Enfermedades Infecciosas at the Instituto Nacional de Enfermedades Respiratorias (CIENI-INER) in Mexico City, Mexico. All donors provided informed consent for LN tissue donation in compliance with protocols set forth by the Ethics Committee and the Ethics in Research Committee of the INER (study number: B03-16) and the Institutional Review Board at the University of Pennsylvania.

Extensive diversity of CD4+ T cells in lymph nodes

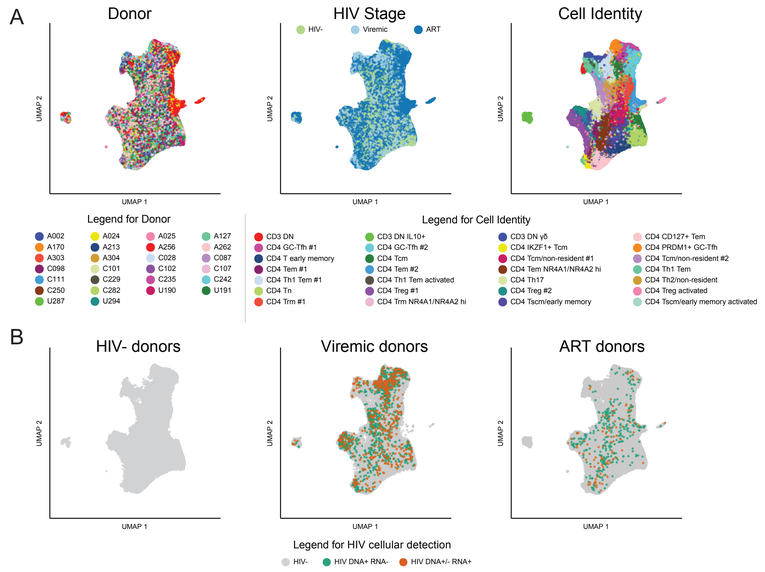


Figure 1: UMAP representations of the scDOGMAseq dataset colored by various metadata and features. The UMAP representation (same for all sub-figures) was built from a weighted nearest-neighbors graph of ATAC and RNA modalities. A) Each subplot features cells colored by donor, HIV stage (HIV-, viremic, or ART-treated), and cellular identity as manually annotated (subplots from left to right, respectively). B) *HIV cellular detection* - cells in UMAP coordinate space colored by detection of HIV DNA and/or RNA separated by HIV stage.

Discordant reservoir composition between viremic and ART-treated PWH

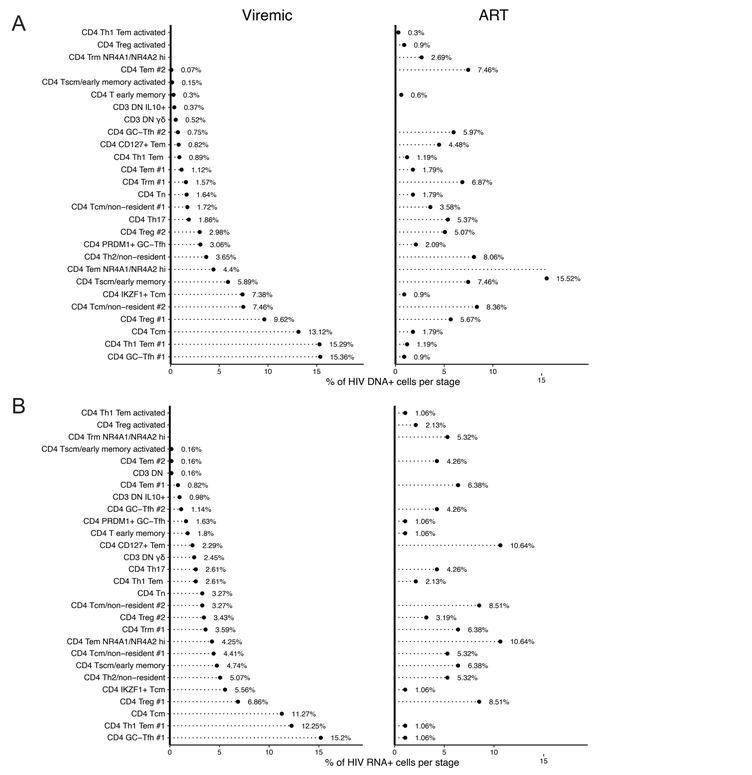


Figure 2: (A) Percentage of HIV DNA (irrespective of HIV RNA) cells found by cluster separated by donor and disease state (viremia versus ART-treated); x axis represents the percent of HIV DNA+ cells that were found in each annotated cluster. (B) Percentage of HIV DNA+/- RNA+ cells found by cluster separated by donor and disease state (viremia versus ART-treated); x axis represents the percent of HIV RNA+ cells that were found in each annotated cluster.

Extent of reservoir complexity beyond Tfh phenotypes

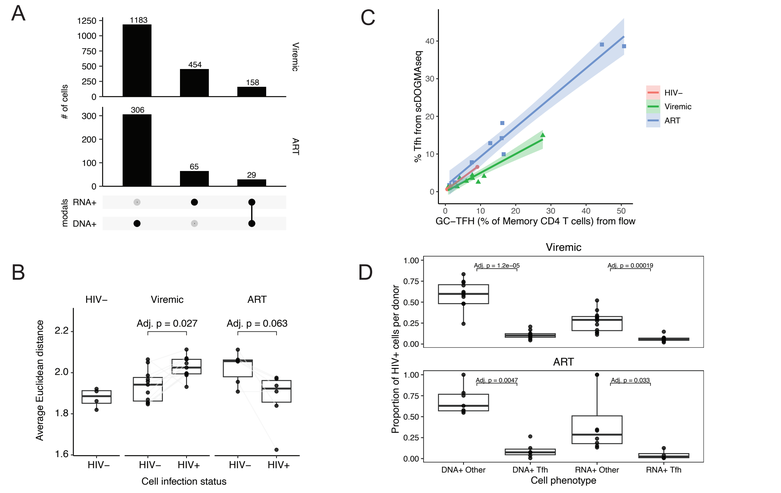


Figure 3: (A) Distribution of infected cells aggregated by infection status (no HIV+ cells were detected in people without HIV) and how they were marked as infected (detection by DNA via the ATAC modality, RNA modality, or both modalities. (B) Heterogeneity of HIV+ cells versus HIV- cells in multidimensional space (Harmony corrected latent semantic indexing of DNA and of RNA modalities). HIV- cells were sampled based on the donor's size of HIV+ cells and bootstrapped 100 times for average distance calculation. Paired Wilcoxon test with Holm correction. (C) Correlation of GC-Tfh detection from flow cytometry and from scDOGMAseq. All linear correlations have Adjusted R values greater than 0.9 with p-values < 0.05. (D) Proportion of HIV+ cells with a Tfh phenotype or a non-Tfh (Other) phenotype from viremic or ART-treated donors. Comparisons are done in the HIV DNA+ or the HIV RNA+ cells with Wilcoxon test and Holm correction.

Conclusions

- High resolution mapping of more than 500k CD4+ T cells from lymph nodes of people without HIV, people with viremic HIV, and people with treated HIV documents the extensive diversity of CD4+ T cells in tissue.
- Transcriptionally inactive and active HIV-1 infected CD4+ T cells from the lymphoid tissue are predominantly in non-Tfh cell subsets in the HIV tissue reservoir during ART.
- HIV+ cells are instead more concentrated in resting/recently activated cells that have not been previously described.
- Stark phenotypic differences exists between HIV+ cells of chronic and ART-treated PWH.

- Heterogeneity of HIV+ cells in the lymph node presents a challenge to cure strategies.
- Sole targeting of follicular HIV+ cells would miss a large portion of the lymph node reservoir especially during ART.

Future Directions

- Additional analyses are underway to understand the differential regulation and expression patterns of the latent (HIV DNA+ RNA-) versus transcriptionally active reservoir.
- Understanding the longitudinal characteristics of the peripheral and tissue reservoir over time on ART.
- Ongoing work is underway to better understand reservoir dynamics upon ART treatment in both peripheral blood and tissue via a cross-sectional study cohort.

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