Mapping the Spatial Landscape of Lymph Nodes with Undetectable Versus Active HIV Replication

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BACKGROUND

- Despite its effectiveness in suppressing active HIV replication, antiretroviral therapy (ART) does not eliminate the long-lived pool of HIV reservoir cells.
- Lymphoid tissues are a major reservoir even in the context of ART, but most studies on latent reservoirs focus on peripheral blood.
- The complex interplay between immune cells and the mechanisms that maintain viral persistence in lymphoid tissue remain incompletely understood.
- In this study, we used a spatial proteomic method to map immune microenvironments in HIV infected tissue with different levels of viremia.

METHODS

- Lymph node (LN) tissue from 6 people with HIV (PWH; n=3 undetectable, n=3 viremic) were obtained from the Pathology Anatomy Unit from Hospital Universitario La Princesa (Madrid, Spain). LNs from people without HIV (PWOH) were included as negative controls (n=5).
- We used MIBI-TOF (Multiplexed Ion Beam Imaging by Time of Flight), to simultaneously image 40 proteins in these tissues.

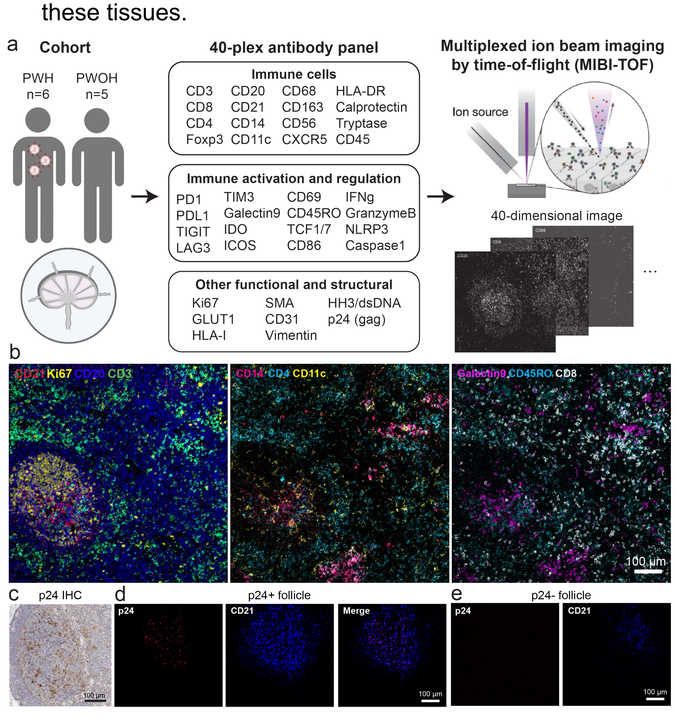


Figure 1. (a) Study design. (b) Representative MIBI-TOF overlays of a single field-of-view (800 μm x 800 μm), where the colors correspond to protein expression. (c) Representative chromogenic immunohistochemistry stain of the p24 antibody in LN. (d) Representative example of p24+ LN. (e) Representative example of p24- LN.

PROFILING THE IMMUNE LANDSCAPE IN LYMPH NODES

- We profiled over 1.8 million cells and characterized the spatial relationships between 20 cell types in their native tissue context.
- We enumerated 1079 different features for each image, including various spatial features such as cellular microenvironments (MEs).
- MEs dominated by CD8+ T cells expressed various activation and inflammasome markers, whereas MEs dominated by T follicular helper cells expressed exhaustion markers.

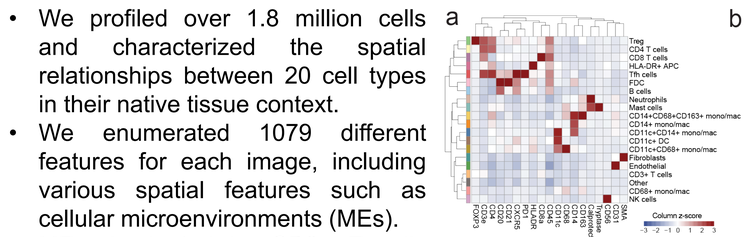


Figure 2. (a) Heatmap of cell phenotypes identified in the images. (b) Representative MIBI-TOF overlay and corresponding cell phenotype map, colored according to the color bar in (a).

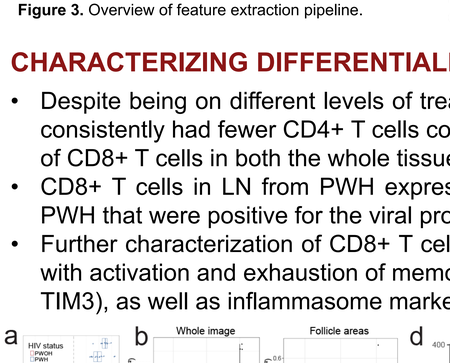


Figure 3. Overview of feature extraction pipeline.

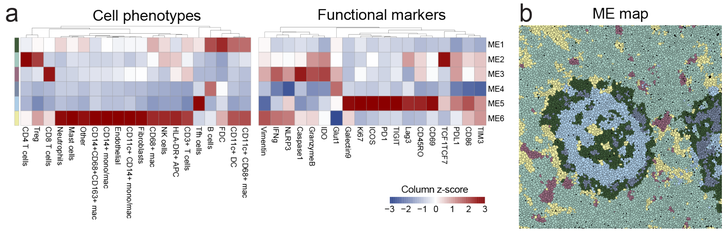


Figure 4. Cellular microenvironments (MEs) were identified by enumerating the number of each cell phenotype within a 50-pixel radius around each cell, then using k-means clustering to group cells using these neighborhoods. (a) Heatmaps of the number of each cell phenotype assigned to each ME and the expression of functional markers in each ME. (b) Same field-of-view as shown in Fig. 2b, colored according to cell microenvironment where colors correspond to the color bar in (a).

CHARACTERIZING DIFFERENTIALLY EXPRESSED CD8+ T CELLS

- Despite being on different levels of treatment and harboring different levels of viremia, LNs from PWH consistently had fewer CD4+ T cells compared to PWOH (p= 0.0006), while there were higher numbers of CD8+ T cells in both the whole tissue (p=0.024) and specifically within follicles (p=0.021).
- CD8+ T cells in LN from PWH expressed more Granzyme B, and were further enriched in LN from PWH that were positive for the viral protein p24 (p=0.011).
- Further characterization of CD8+ T cells in p24+ regions revealed higher levels of markers associated with activation and exhaustion of memory and effector CD8+ T cells (CD45RO, ICOS, TIGIT, Galectin9, TIM3), as well as inflammasome markers Caspase1 (p=0.03) and NLRP3 (p=0.3, FC=1.90).

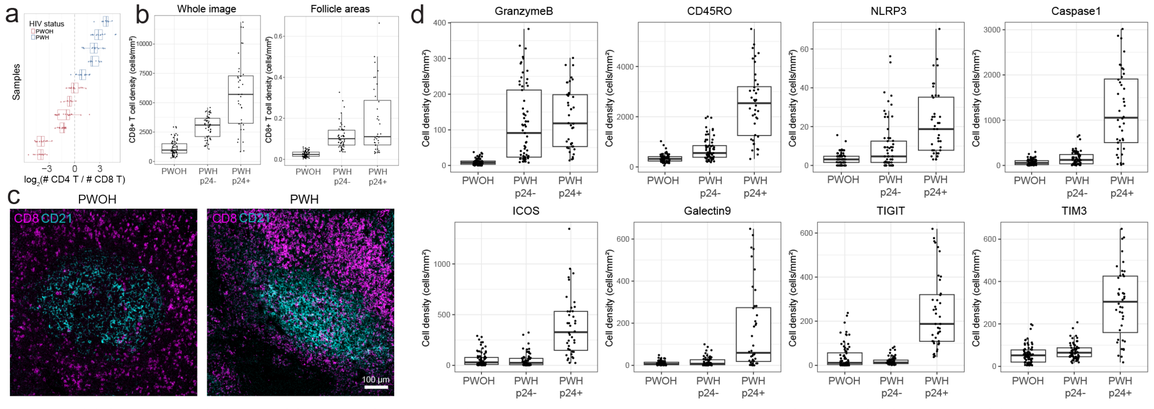


Figure 5. (a) Comparison of the ratio of CD4+ and CD8+ T cells for all samples. Each dot is one imaged area of the LNs. (b) Comparison of CD8+ T cell density across conditions, in both the whole imaged area (left) and within follicle regions (right). "PWH p24+" indicates samples from LNs that showed positive staining for p24. (c) Representative MIBI-TOF overlays showing CD8+ T cell infiltration into follicles. (d) Characterization of functional marker positivity in CD8+ T cells across the different groups. Each dot is one imaged area of the LNs.

DIFFERENTIAL SPATIAL RELATIONSHIPS

- Using QUICHE, a graph-based method designed to discover cellular niches that are differentially enriched between groups, we found that LNs from PWH were significantly enriched for CD8+ T cells near antigen-presenting cells (CD11c+, HLADR+), fibroblasts (SMA+), and endothelial cells (CD31+), while LNs from PWOH were enriched for CD4+ T cell niches.

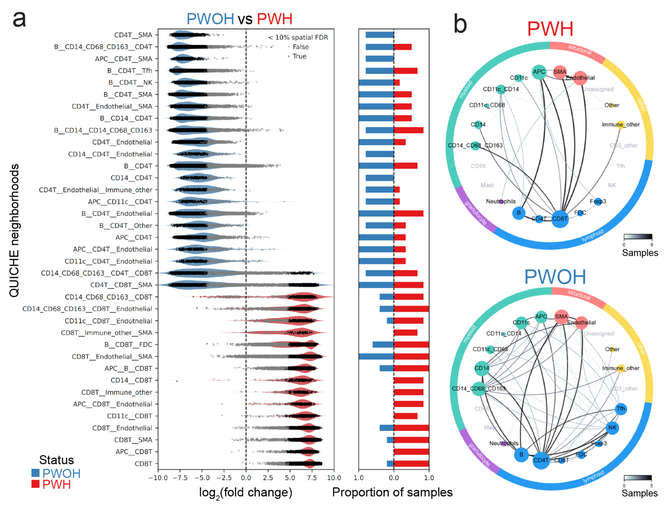


Figure 6. (a) Violin plots show the top differentially-enriched niche neighborhoods within PWOH (blue) or PWH (red) LN samples. (b) Niche networks for differentially-enriched neighborhoods in PWH (top) or PWOH (bottom). Nodes represent cell types and edge weights correspond to the number of unique samples with the corresponding interaction. Node size is proportional to connectedness, as measured by eigenvector centrality.

CONCLUSIONS

- Using spatial proteomics to map the immune landscape in LNs from PWH and PWOH, we identified novel markers characterizing CD8+ T cell subsets enriched in PWH with different levels of viremia, implying that active viral replication confers long-term changes on the immune landscape in LNs.
- Collectively, these data provide new insights into complex cell networks associated with viral replication at an important tissue reservoir site, which could be relevant for future HIV cure strategies.

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