Immune gene spatial profiling of the HIV lymph node reservoir

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1. Background

- HIV is currently incurable owed to persistence within tissue-resident CD4⁺ T cell reservoirs, even during effective highly active anti-retroviral therapy (HAART).
- Single cell transcriptomics of HIV-infected, lymph node resident CD4⁺ and CD8⁺ cells suggests HIV modulates the germinal center immune microenvironment for the survival of viral reservoirs[1,2], but without spatial context within the lymph node.
- Means to image HIV within infected lymph nodes using immunohistochemistry (IHC) and RNA scope were recently developed at the Ndhlovu Lab, by which HIV Gag (p24) antigen correlated with HIV RNA signal within lymph node germinal centers[3].

Coupling transcriptomics with HIV p24 detection in lymph nodes, **this study aims to explore HIV modulation of the germinal center immune microenvironment in direct spatial context.**

Objectives:

1. **Study immune gene expression in HIV+ lymph nodes displaying p24 heterogeneity.**
2. **Compare immune gene expression between HIV+ and HIV- subjects.**

3.1 HIV infection modulates germinal center immune genes

Within HIV+ lymph nodes, p24-negative germinal centers form an immune gene expression cluster separate to germinal centers with varying p24 signal.

- Uninfected germinal centers display a uniform pattern of immune gene expression.
- HIV-infected (p24 low-high) regions form multiple sub-clusters: **infection causes transcriptional heterogeneity.**

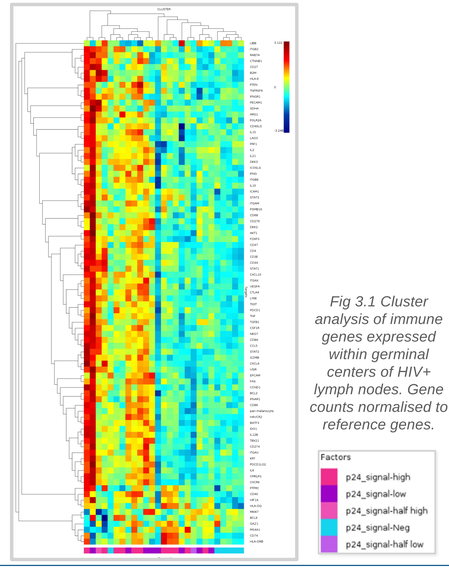

Fig 3.1 Cluster analysis of immune genes expressed within germinal centers of HIV+ lymph nodes. Gene counts normalised to reference genes.

3.2 Lymph node reservoirs display immune activation

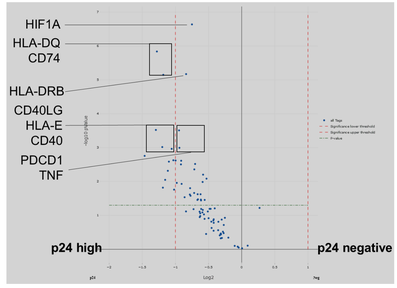

Fig 3.2A Volcano plot of Linear Mixed Model test, plotted for each gene by Log2 change between p24 high and p24 negative GCs in HIV positive LN (x axis) against statistical significance (y axis).

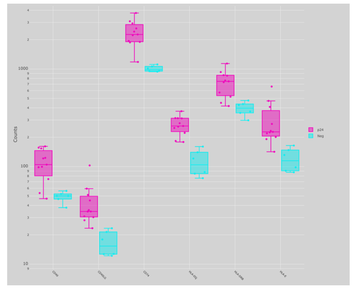

Fig 3.2B Box plots of genes significantly upregulated in p24-high GCs compared to p24-negative GCs of HIV-positive lymph nodes according to linear mixed modelling. Each dot represents a ROI. Counts normalised to reference genes.

Germinal centers in which high p24 signal was detected, significantly upregulated HLA and MHCII markers, as well as CD40 and CD40 Ligand compared to uninfected germinal centers.

3.3 Immune gene silencing in HIV-infected subjects

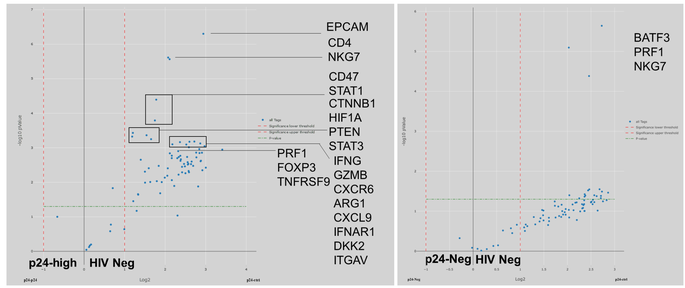

Fig 3.3 Volcano plot of Linear Mixed Model test, plotted for each gene by Log2 change for p24 high (left) or p24 negative GCs (right) in HIV positive LN compared to healthy controls (HIV neg) (x axis) against statistical significance (y axis). Fold change based on gene counts normalised by scaling to nuclei count.

Lymph nodes of healthy controls displayed unique transcriptional patterns to HIV-infected subjects.

- majority of immune genes were upregulated in healthy controls, suggesting downmodulation by HIV.
- Cytolytic response genes, NKG7, STAT1, STAT3, GZMB and PRF1 were significantly downregulated in HIV+ germinal centers
- Uninfected germinal centers of HIV positive lymph nodes are still transcriptionally distinct from HIV negative lymph nodes.

2. Study Design

Immune spatial profiling was carried out by performing GeoMx Digital Spatial profiling and Ncounter readout for HIV+ lymph nodes (n=7) alongside HIV- control lymph nodes (n=2).

- Lymph nodes were probed with the Immune Pathways Panel for Ncounter RNA readout with added custom spike ins and stained for germinal centers (CD20) and nuclei (Syto13) (Fig. 1a).
- HIV p24 IHC was performed for serial sections (Fig1b) and overlayed in GeoMx DSP software to select HIV infected and uninfected germinal centers for probe collection (Fig. 2).
- Probes were counted on the NCounter platform and analysed using GeoMx DSP software.

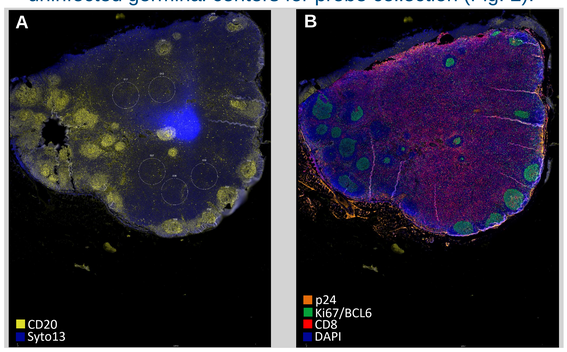

Fig. 1 Matching serial sections of human lymph nodes. A) 20X scan imaged on the GeoMx DSP. B) Serial section stained for HIV Gag (p24) to serve as an overlay for region of interest selection.

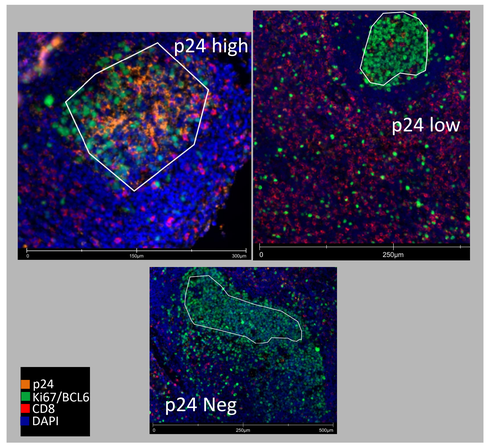

Fig. 2 Regions of interest for collection of immune gene RNA probes. IHC staining of a serial section of each lymph node was used to distinguish between HIV infected (p24) or HIV uninfected germinal centers.

4. Conclusions

1. Lymph node germinal center HIV reservoirs, identified by p24 signal, produce unique immune gene transcriptional profiles.
2. Immunoregulatory pathways may be manipulated by HIV infection to aid persistence of tissue-resident HIV reservoirs, inhibiting HIV cure.
3. Markers of immune gene activation in p24-rich germinal centers are expected following chronic activation by HIV, observed in other studies.
4. Future work includes expansion of spatial profiling using whole human atlas assays via NGS readout as well as single-cell RNA-seq for lymph node cells and PBMCs matched to serial sections to identify complete regulatory pathways for HIV cure targets.

5. Acknowledgments

- Funding from the Bill and Melinda Gates foundation
- Postdoctoral fellowship under Sub-Saharan African Network for TB/HIV Research Excellence (SANTHE)
- Clinical staff and participants of the Lymph Node Study and FRESH
- Ndhlovu Lab members

6. References

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