Transcriptional States of B cells Producing Broadly Neutralizing Antibodies that target HIV-1 Envelope

Tyler Evangelous[4], Madison Berry[1], Todd Bradley, PhD[2], Isabela Pedroza-Pacheco, PhD[3], Ipsita Mohanty[1], Bhavna Hora, PhD[1], Yousef Abuahmad[1], Anthony M. Moody, MD[1], Derek W. Cain, PhD[1], Persephone Borrow, PhD[3], Kevin Wiehe, PhD[1], Barton F. Haynes, MD[1], and Wilton B. Williams, PhD[1]

[1]Duke University, Durham, NC, USA; [2]Children's Mercy Hospital, Kansas City, MO, USA; [3]University of Oxford, Oxford, UK., [4]Brody School of Medicine, Greenville, NC.

Tyler Evangelous
Brody School of Medicine
East Carolina University
Greenville, North Carolina 27858
evangeloust22@students.ecu.edu

INTRODUCTION

- Broadly neutralizing antibodies (bnAbs) target the HIV-1 envelope (env) on multiple different HIV strains and are attractive targets for HIV-1 vaccines (Figure 1).

- Previous studies have shown that HIV-1 bnAb induction is disfavored by host immunity, given their genetic properties and auto-/polyreactive nature.[1,2,3,4]

- The transcriptional programs of HIV-1 Env bnAb B cells may provide insights into the nature of these B cells and inform future vaccine strategies to harness them.

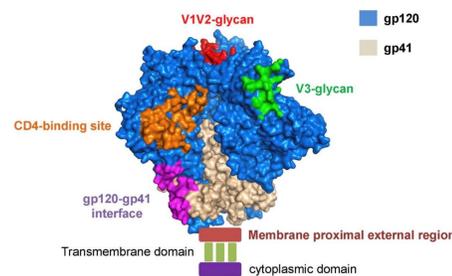### Figure 1: Structure of HIV-1 env protein

Figure 1: Structure of HIV-1 env protein (gp120 and gp41) showing frequent bnAb binding sites, which include V1V2-glycan, V3-glyan, CD4-binding site, gp12-gp41 interface, and Membrane proximal external region (MPER).

PURPOSE OF STUDY

To understand the nature and properties of HIV-1 envelope-reactive bnAb B cells using a high-resolution assay that provides paired VDJ and transcriptome of single cells

MATERIALS & METHODS

- Negative B cell enrichment from peripheral blood cells (PBMCs) of 4 HIV-1 infected individuals from whom we previously isolated and characterized HIV-1 Env bnAbs of different specificities, and 3 seronegative controls.
  - CH505: CH103 and CH235—CD4-bindng site bnAbs.
  - CH848: DH270—V3 glycan bnAb.
  - CH219: CH31—CD4bs bnAb.
  - CH457: CH27—CD4bs bnAb.

- High-throughput single cell encapsulation and library preparation (10X Genomics).

- Next generation sequencing (NGS) with Illumina instruments.[8]

- Processing of raw sequencing reads for gene expression (GEX) and VDJ data using cellranger (10X genomics).[8]

- BCR immunogenetics and clonality inferred using the Cloanalyst software program.

- Gene expression profiling was done using Seurat[5] followed by reduction of the dimensionality of single cell transcriptomes to two dimensions by Uniform Manifold Approximation and Projection (UMAP). Cells were clustered using a graph-based approach to create a single-cell map of cell clusters.

RESULTS

Figure 2: UMAP projections of the B cell clusters from HIV+ and HIV- individuals.
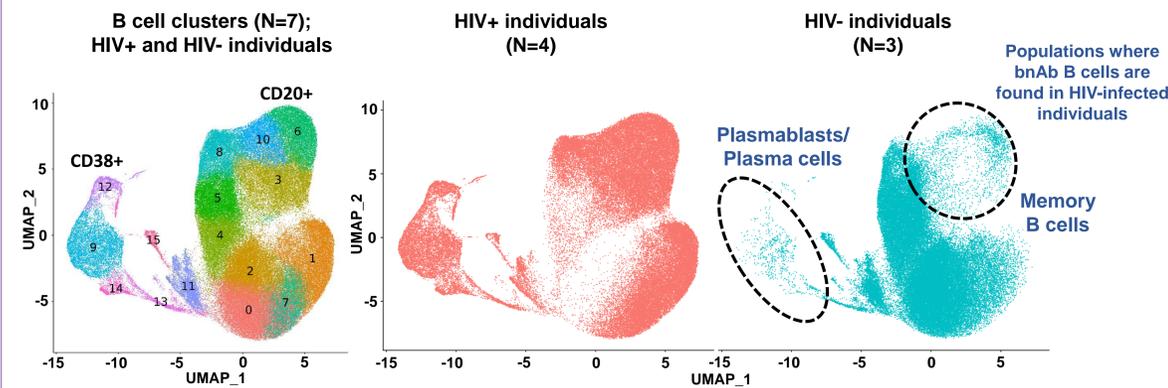

Figure 2: UMAP projections of the 16 unique B cell clusters from HIV+ and HIV- individuals. On the left, B cells from HIV+ and HIV- B cell clusters can be grouped into 2 main populations, CD38+ (left) and CD20+ (right). Shown in the middle (red, HIV+ individuals) and right (teal, HIV- individuals) are the B cells separated by infection status. The two B cell populations are plasmablasts/plasma cells (left) and memory B cells (right). **HIV-1 Env-reactive B cells demonstrated unique transcriptional profiles compared to those from HIV-1 seronegative individuals.**

Figure 3: Identification of bnAb B cells and the transcriptional overlap of bnAb clusters
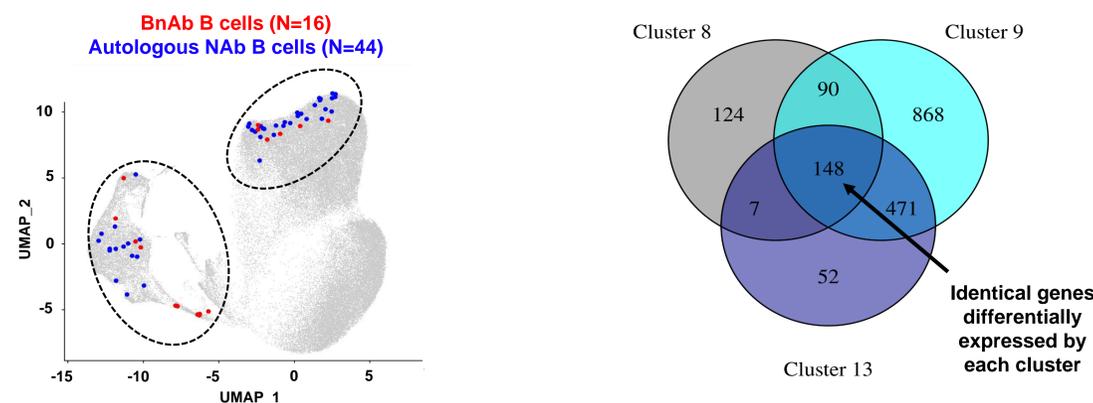

Figure 3: (Left) Previously isolated and characterized antibodies can be recovered in single-cell data. Antibody sequences from bnAbs (red, N=16) and Nabs (blue, N=44) are paired with single cell data and shown overlayed on a UMAP projection with the HIV+ B cells. These B cells are in regions underpopulated in HIV- individuals. BnAb B cells and Nab B cells cluster together. **(Right)** BnAb B cells are most frequent in 3 clusters: Clusters 8, 9 and 13. To understand the transcriptional programs unique to those clusters, we examined the overlap of differentially expressed genes in those clusters to compared to all other B cell clusters. There were 148 shared differentially expressed genes between clusters 8, 9 and 13. **Pathway analysis revealed candidate genes (n=148) enriched in B cell clusters with bnAb B cells.**

Figure 4: Pathway analysis of shared differentially expressed genes in bnAb clusters
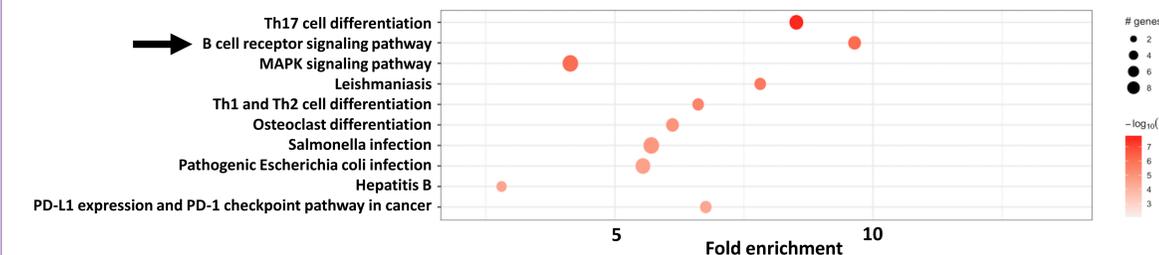

Figure 4: Transcriptional pathway analysis shows upregulation of the B cell receptor signaling pathway. Annotated transcriptional pathways are shown on the Y axis. The size of the circle is related to the number of genes found in the pathway and the darker the color of the circle is related to a smaller P value. **Differential expression of genes in the B cell receptor signaling pathway were investigated to determine transcriptional signatures of bnAb B cells.**

Table 1: Genes involved in the B cell receptor signaling pathway

Gene	Mean Adjusted P value	Mean LogFC
CD22	4.16E-112	-0.7562872
CD72	6.82E-100	-0.6441664
PTPN6 (SHP1)	7.54E-168	-0.1959335

Table 1: Genes in the B cell clusters with the highest number of bnAb B cells involved in the B cell receptor signaling pathway are downregulated and implicate loss of regulation. Genes in the table are shown with the mean adjusted P value and mean log fold change (LogFC). **Down-regulation of CD22 and SHP-1 may lead to hyper-responsiveness, thus making such B cells prone to constitutive signaling (or self antigen mediated) that may lead to anergy, and possibly loss of responsiveness to Env stimulation.** [7,8,9]

Table 2: Summary of the cohort and number of B cells studied to define transcriptional states of bnAb B cells elicited by HIV-1 infection

Cohort	Number of individuals	Total B cells
HIV-1 infected (bnAbers)**	4	83,216*
HIV-1 naïve controls	3	75,707*
COVID-19 dataset[6]	620	251,377

Table 2: Summary of the HIV+ and HIV- cohort used in this study, and for the future COVID-19 dataset that will be integrated with ours to better inform B cell dysfunction in the context of HIV-1 infection. Extensive literature search for other single cell datasets in the context of both acute and chronic infection yielded a publicly available single-cell COVID-19 atlas of over 3 million cells – 251,377 B cells. **From our HIV+ and HIV- dataset, we recovered 158,923 B cells with paired BCR information. The B cells from the COVID-19 single cell atlas will be integrated with our HIV-1 dataset to better understand B cell dysfunction.**
*Antigen-unbiased B cells, VH + VL pairs, **BnAbers: CH505, CH848, CH0219 and CH0457.

CONCLUSION

- HIV-1 Env bnAbs and autologous NAbs are produced by B cells within transcriptionally-distinct B cell subsets that are expanded in HIV-infected individuals.

- The transcriptional co-clustering of bnAbs and autologous NAbs suggests that bnAbs may not require the expression of specialized transcriptional programs in order to achieve their neutralization breadth.

- The transcriptional profile of B cell clusters with the highest number of bnAb B cells implicate loss of B cell regulation that may lead to anergy.

- These data raise the hypothesis that appropriate immunogens may be needed to rescue anergic B cell cells and select improbable mutations to achieve bnAb status.

FUTURE STUDIES

Future studies seek to integrate our HIV+ single-cell dataset with other infections, including COVID-19, to better understand if B cell dysfunction is unique to HIV-1 infection, and/or may be manipulated during HIV-1 vaccination.

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