# Disruption of intestinal germinal centers during HIV infection

325

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## BACKGROUND

Aim of the study was to investigate **how HIV affects humoral responses in secondary lymphoid tissue.**

- Humoral responses are a pillar of host protection against pathogens, including viruses
- HIV induces humoral responses that are not protective and unable to control viral replication
- Humoral responses occur mostly in secondary lymphoid tissue and involve the formation of the Germinal Center (GC) and interaction between T (specifically T follicular Helper cells, TFH) and B cells.
- HIV induces massive depletion of CD4+ T cells in secondary lymphoid organs, likely impacting humoral responses

## METHODS

- Prospective enrollment of people with viremic HIV infection (PWVH, n=18), people with suppressed HIV infection (PWSH, n=46) and HIV non-infected controls (NC, n=80) undergoing colonoscopy
- Gut-associated tissue was mechanically and enzymatically digested to prepare cell suspension for further downstream analysis:
  - Single cell RNA sequencing (scRNAseq)
  - Multiparameter flow cytometry
- Immunohistochemistry (IHC) staining was done on formalin-fixed, paraffine-preserved tissue.
- Analysis of scRNA data including data normalization and batch effect correction was performed via the Seurat package
- Comparisons across groups were performed using Mann-Whitney U test or Kruskal-Wallis test with Dunn's correction for multiple comparisons. Median and interquartile are shown in summary data plots. Correlation was obtained using Spearman's r correlation coefficient

## RESULTS

- After initial clustering of scRNAseq data, B cells and Plasma Cells were subclustered and 5 B cell clusters were identified in the colon and 6 in the ileum (Figure 1 A).
- Lower fraction of overall B cells was observed in both colon and ileum during viremic HIV infection (Figure 1B)

**HIV infection is associated with a profound loss of intestinal Germinal Center B cells and may represent a major source of perturbation of humoral immunity at these sites of viral replication**

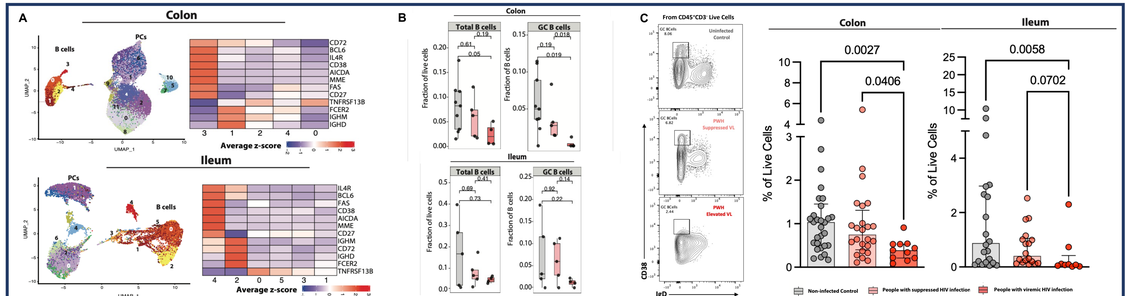


**Figure. 1: (A)** Uniform Manifold Approximation and Projection (UMAP) showing, colon-derived (top) and ileum-derived (bottom) B cell and plasma cells clusters (re-clustered from main clusters) scRNA seq. Heatmap showing the average normalized log expression z-score of canonical B cell markers across different colonic-derived (top) and ileum-derived (bottom) B cell sub-clusters. **(B)** scRNA seq data demonstrating the frequency of B cell subclusters derived from the colon (top) and ileum (bottom) across the three groups (HIV- controls (HIV NC), n=9 for colon and n=5 for ileum; people with suppressed HIV (PWSH), n=5 for colon and ileum, both; people with viremic HIV (PWVH) n=4 for colon and ileum, both). **(C)** Representative gating strategy for flow cytometric identification of germinal center B cells (left) and summary plots quantifying colonic and ileal germinal center B cells (HIV- controls (HIV NC) n=51; People with suppressed HIV infection (PWSH) n=35; People with viremic HIV infection (PWVH) (n=16).

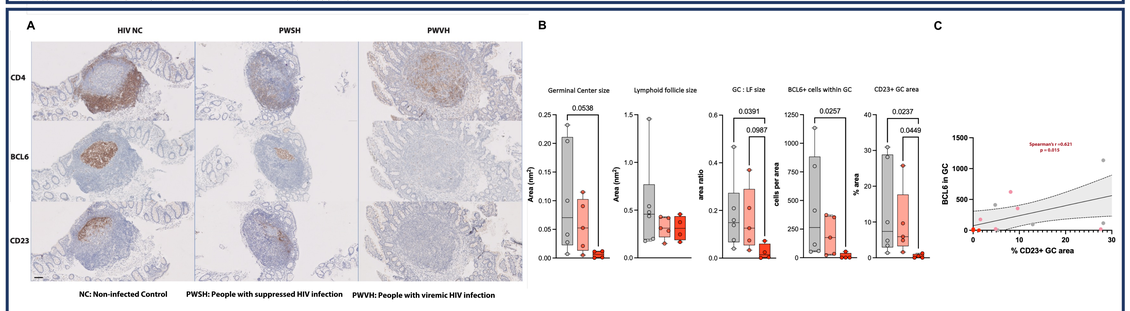


**Figure. 2: (A)** Representative Immunohistochemistry (IHC) images of intestinal lymphoid follicles for each of the three groups showing staining for CD4 (top panels), BCL6 (middle panels) and CD23 (bottom panels). Scale bar represents 100µm. **(B)** Summary data for IHC (HIV NC n=6; PWSH, n=5; PWVH, n=4), quantifying Germinal Center (GC) size, lymphoid follicle size, GC size, the ratio between GC and lymphoid follicle size, the number of BCL6+ cells in each GC and the number of BCL6+ cells in each GC and the % area positive for CD23 staining within each GC. **(C)** Correlation analysis between the area with positive CD23 staining and the number of BCL6+cells within each GC.

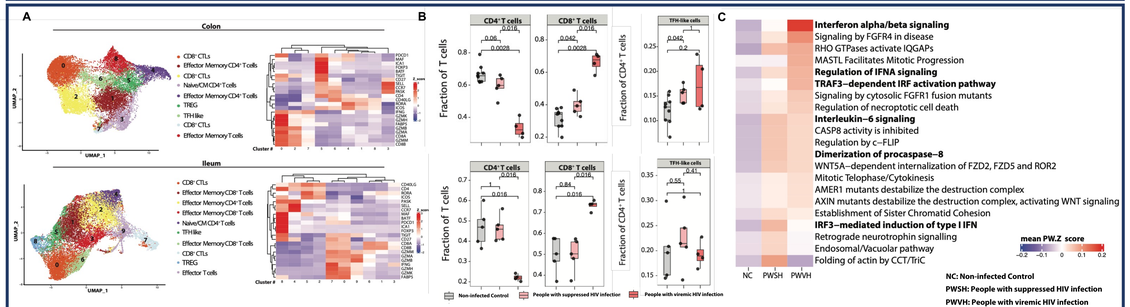


**Figure. 3: (A)** Uniform Manifold Approximation and Projection (UMAP) showing, colon-derived (top) and ileum-derived (bottom) T cells clusters (re-clustered from main clusters) scRNA seq. Heatmap showing the average normalized log expression z-score of canonical T cell markers across different colonic-derived (top) and ileum-derived (bottom) B cell sub-clusters. **(B)** scRNA seq data demonstrating the frequency of T cell subclusters derived from the colon (top) and ileum (bottom) across the three groups (HIV- controls (HIV NC), n=9 for colon and n=5 for ileum; people with suppressed HIV (PWSH), n=5 for colon and ileum, both; people with viremic HIV (PWVH) n=4 for colon and ileum, both). **(C)** Heatmap representing the mean z-score enrichment for selected pathways within the ileum-derived TFH-like T cell subcluster.

## RESULTS

- **GC B cells were almost absent in the gut-associated lymphoid tissue during viremic HIV infection, in both colon and ileum (Figure 1B).**
- Flow cytometry analysis confirmed significant reduction of GC B cells in both colon and ileum during HIV viremic infection (Figure 1C).
- IHC staining showed a **reduced size of GC during viremic HIV infection**, despite overall similar lymphoid follicle size **(Figure 2 A, B).**
- **BCL6+ cells in the GCs** (putative GC B cells) **were significantly reduced during viremic HIV infection (Figure 2 A, B).**
- The **CD23+ area** (putative follicular dendritic cells) within each GC **was significantly lower during viremic HIV infection** and correlated with the number of BCL6+ cells within the same GC (Figure 2 B and C).
- After initial clustering of scRNAseq data, T cells were subclustered and 9 T cell clusters were identified in the colon and 10 in the ileum (Figure 3 A).
- Lower fraction of overall CD4+ T cells and higher fractions of overall CD8 T cells were observed in both colon and ileum Frequency of TFH cells was not different across groups in the ileum and slightly increased during viremic HIV infection in the colon (Figure 3B).
- Pathway analysis revealed an increase in interferon signaling and response, inflammatory and apoptotic signaling pathways during viremic HIV infection (Figure 3C).

## CONCLUSIONS

- **Disruption of GCs and depletion of GC B cells** in gut-associated secondary lymphoid tissue might help explain **the lack of protective immunity during HIV infection** and might have **implications for the persistence of HIV in these tissue sites**

## ADDITIONAL KEY INFORMATION

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