Single-cell multi-omic profiling of tissue-specific immune and non-immune cells across the gut in ART-suppressed people living with HIV

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Despite effective antiretroviral therapy (ART), people living with HIV (PLWH) experience persistent immune activation and inflammation, which may contribute to intestinal immune dysregulation and impaired gut barrier function. The cellular organization of distinct intestinal compartments in ART-suppressed PLWH remains incompletely characterized. We used single-cell multi-omic profiling to investigate tissue-specific immune and epithelial cell states across the gut.

Gut biopsies from six ART-suppressed PLWH stratified by soluble CD14 (sCD14) levels (n=4 low, n=2 high) were collected from the ileum, colon and rectum. CD45+ and CD45− fractions were isolated prior to sequencing. Using the BD Rhapsody platform, we performed single-cell RNA sequencing and 43-plex AbSeq profiling to jointly characterize transcriptional and protein-level cellular states across immune and non-immune compartments.

Single-cell profiling identified distinct tissue-associated immune populations across sites, including a cycling, germinal center-like B-cell population enriched in the rectum, and a cytotoxic γδ T-cell population, defined transcriptionally and positive for the activation marker CD69 at the protein (ADT) level, present at higher frequencies in colon and ileum. Activated CD4+ T cells, defined at the RNA level, showed PD1 positivity at the protein, with the highest frequency in the rectum. Despite lower non-immune recovery, epithelial populations were resolved into colonocytes, enterocytes and goblet cells; notably, the patient with the highest sCD14 level was the only one showing a markedly expanded non-immune fraction. In the non-immune compartment, pathway analysis revealed a shift from interferon/TNF-driven inflammatory signatures (low sCD14) to metabolic signatures, including oxidative phosphorylation (high sCD14); in the immune compartment, a shift from strong proliferative/metabolic signatures (low sCD14) to weaker complement- and TNF-associated signatures (high sCD14).

Our single-cell multi-omic analysis reveals tissue-specificity of the intestinal immune compartment in ART-suppressed PLWH, including cycling, germinal center-like B-cell and cytotoxic γδ T-cell populations, alongside evidence that systemic sCD14 levels may relate to gut epithelial-immune balance.