Impaired Antigen Presentation and TGF-β Signaling Fosters Th17 Differentiation in PLWH under ART

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BACKGROUND

Innate Lymphoid Cells (ILCs), the innate counterpart to adaptive T helper cells, are divided into ILC1s, ILC2s and ILC3s, distinguished by their canonical transcription factors T-bet, GATA3, and RORγt, respectively. Compelling evidence from different groups (Sonnenberg, Littman, or Rudensky) indicates that **innate RORγt+ cells from the lower gut, including ILC3s** and other antigen presenting cells, play a **critical role** in promoting **immune tolerance** by **presenting microbiota antigens to naïve T cells**, thereby **maintaining the Treg/Th17 balance**. This balance essential for gut homeostasis, when disrupted, contributes to inflammatory bowel disease. **By altering ILC3 to T cells interactions** in the **gut, HIV** could **affect Treg differentiation, Treg/Th17 balance and reservoir seeding in the gut**.

METHODS

Single-cell RNA sequencing was performed on gut tissues from four compartments (duodenum, ileum, colon, rectum) of people living with HIV (PLWH) on ART (n=7, CIENI, Mexico) and people living without HIV (PLWoH) (n=9, from CIENI, the RID-HIV consortium, and the Gut Reference Survey). Using Seurat (v5.0) in R, batch effects were corrected, and cells were processed and annotated through a combination of automated and manual methods. Differential gene expression analysis was conducted on functional and differentiation pathways in ILC3s and Tregs, respectively.

ADDITIONAL INFORMATION

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RESULTS

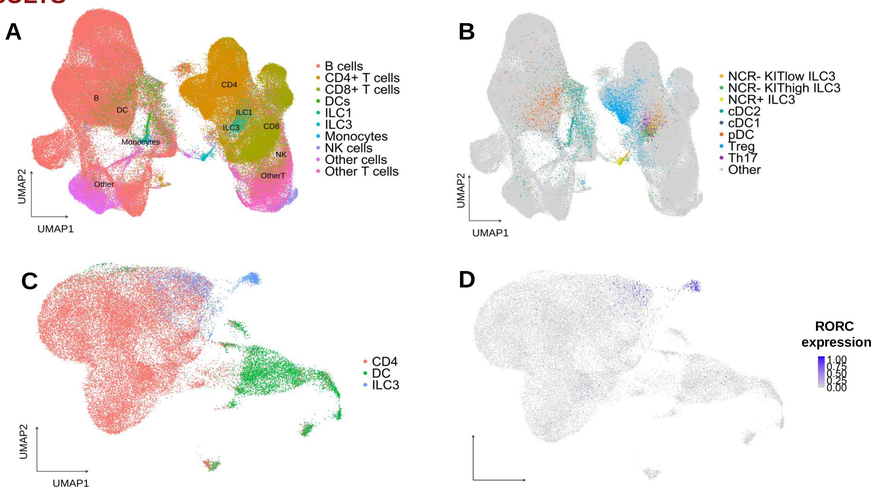


Figure 1.
A: UMAP projection of all cells across all gut compartments. **B**: Three subsets of ILC3s were identified: NCR- KITHigh, NCR- KITLow, and NCR+ ILC3s. **C**: CD4, DC and ILC3 were selected and re-clustered. **D**: RORC expression co-localized with ILC3s, and to a lesser extend with CD4 T cells, but not with DCs, confirming ILC3s as the main innate RORγt+ population in our dataset.

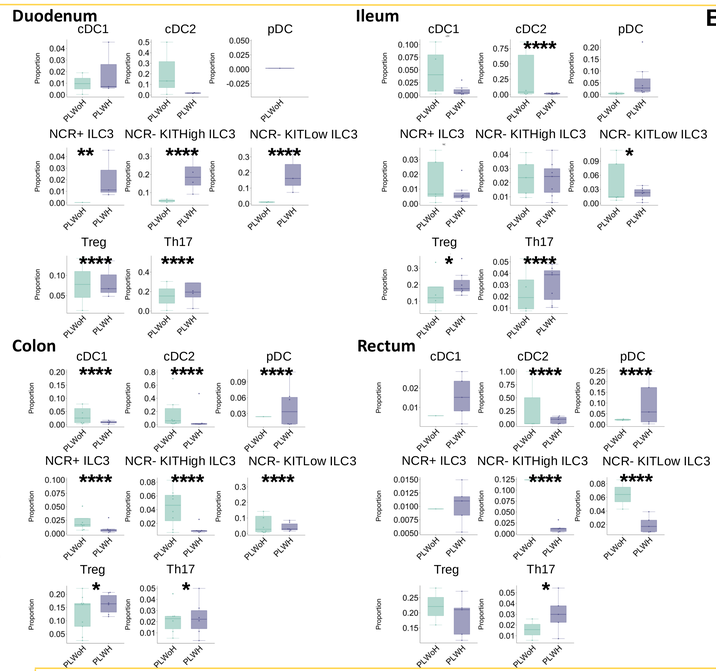


Figure 2.
A: Cell frequencies per compartment are shown for PLWH and PLWoH. Across all compartments from the lower gut, PLWH showed a significant decrease in ILC3 subsets frequencies. Th17 cell frequencies increased in all compartments, while Treg frequencies increased only in the duodenum and colon, to a lesser extent (Chi² test).
B: Treg/Th17 ratio was lower in PLWH compared to PLWoH in all compartments. This suggests a Treg/Th17 imbalance in the gut of PLWH that could be explained by an impairment of ILC3-T cell interactions.

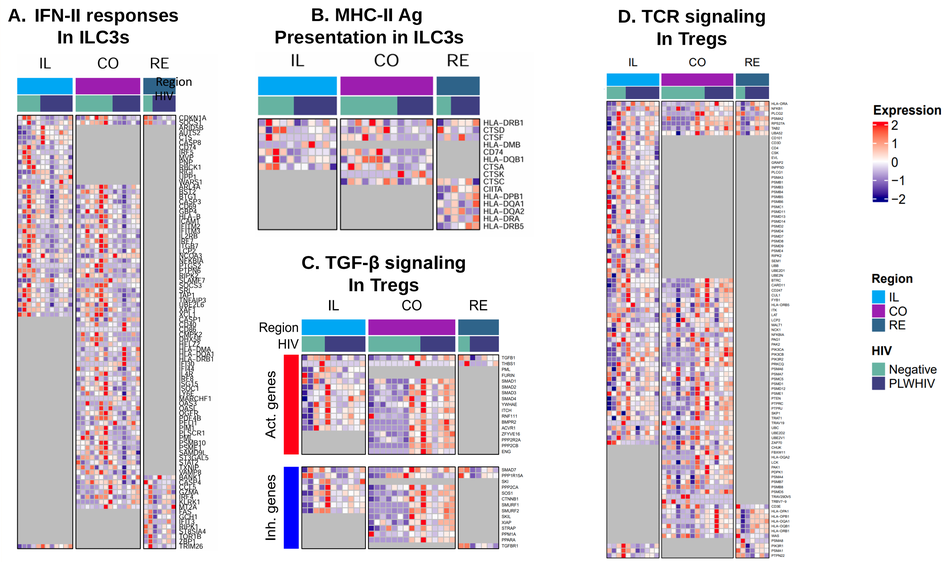
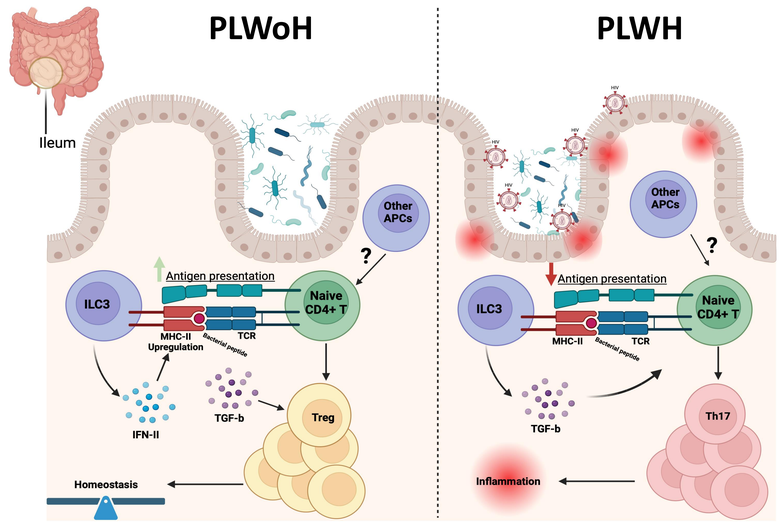


Figure 3. Heatmaps of genes significantly up/downregulated in PLWH, related to IFN-II responses and MHC-II Ag presentation pathways in ILC3s, and related to TGF-β signaling pathway in Tregs. **A**: IFN-II responses were decreased in ILC3s of PLWH, in all compartments. **B**: Genes related to MHC-II antigen presentation such as CD74, cathepsins and HLA-DR/DQ were decreased in ileal and colonic ILC3s from PLWH. **C**: Both activating (act.) and inhibitory (inh.) genes involved in TGF-β signaling were upregulated in Tregs of PLWH. **D:** TCR signaling was respectively down and upregulated in the ileum and the colon of PLWH. These results reveal an impaired MHC-II antigen presentation machinery in ILC3s, and disrupted TGF-β signaling in Tregs, essential for their differentiation.

MODEL



We provide evidence **for impairment of antigen presentation in ILC3s and TCR and TGF-β signaling in Tregs**, all required for Tregs differentiation. **This contributes to the Treg/Th17 imbalance and promotes HIV reservoir seeding in the gut of PLWH despite ART**, as **Th17 are highly susceptible to infection**. Ongoing studies on gut-derived ILC3s from newly diagnosed PLWH aim to assess early ILC3-T cell dynamics and ART's impact on immune restoration.