Single-Cell Profiling of HIV-1 Infection and Response to Early ART in an Ex Vivo Human Tonsil Model

B-03

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BACKGROUND

- Lymphoid tissues (lymph node, spleen, tonsil) are key sites where HIV can replicate or persist at low levels despite ART.
- Tonsils are rich in T cell niches that are different than peripheral blood, but their respective contribution to productive HIV infection are relatively less well understood.
- Which CD4⁺ T cell states in the tonsil support productive HIV-1 replication, and how are these states affected by early ART?

METHODS

- Palatine tonsil resections from healthy donors (n=10) were sectioned into 2×2 mm tissue blocks and cultured for 24 hours on foam rafts at the air–liquid interface.
- Tissue blocks were infected with CXCR4-tropic HIV-1 NL43-mCherry (10 ng p24) and cultured at 37C for eight days.
- Combination ART (tenofovir 5 µM, emtricitabine 70 nM, raltegravir 20 nM) was added with media changes: D2 and D5.
- Free supernatant cells were analyzed by flow cytometry for viability, mCherry infection, and immune cell markers.
- Single-cell RNA sequencing (10X Chromium 3’ GEX) was performed on D8 and reads mapped to a custom reference[1].

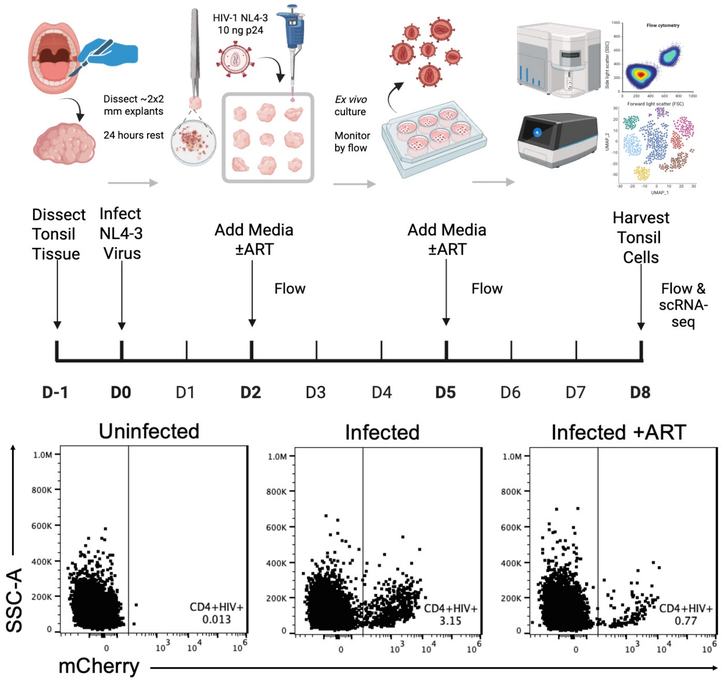


Figure 1. Experimental timeline and fluorescent reporter readout of productive HIV infection in tonsil explants. Top: Schematic of the ex vivo tonsil explant model. Tonsil tissue is dissected into ~2×2 mm explants (D−1), infected with HIV-1 NL4-3 (10 ng p24 per block) on D0, and cultured on collagen rafts with media ± ART added on D2 and D5. Explants are monitored by flow cytometry during culture and tonsil cells are harvested on D8 for terminal flow and single-cell RNA-seq library preparation. **Bottom:** Flow dot plots from uninfected, infected, and infected +ART conditions at D8 timepoint from one representative donor. Gated on single, live, CD45+CD3+CD8- .

Single-cell profiling of ex vivo human tonsil explants shows that productive HIV-1 infection is abundant in specific CD4 T-cell states and follows a viral-burden spectrum that early ART effectively reduces but does not fully eliminate.

RESULTS

- **Tonsil explants support robust infection.** In untreated tonsil explants across ten donors, mCherry+ reporter signal increased over eight days, whereas combination ART markedly reduced productive infection without majorly altering overall T cell viability or CD4/CD8 composition. A small pool of HIV-infected cells persist in ART.
- **HIV RNA burden tracks along a metabolic gradient.** Ordering cells by HIV RNA bin (0 = unexposed, uninfected; 1 = exposed, uninfected; 2-7, lowest to highest HIV state) reveals a continuum from low to high transcript levels associated with progressive induction of respiratory chain gene programs. ART treatment blunts this induction.
- **Infection is abundant in a handful of T-cell states.** Among all HIV RNA⁺ T cells (n ≈ 3,000), five subsets dominate: CD4 Tfh (20%), CD4 Trm (17%), CD4 T naive (12%), CD8 gdTem (11%), and CD4 Tph (10%) cells.

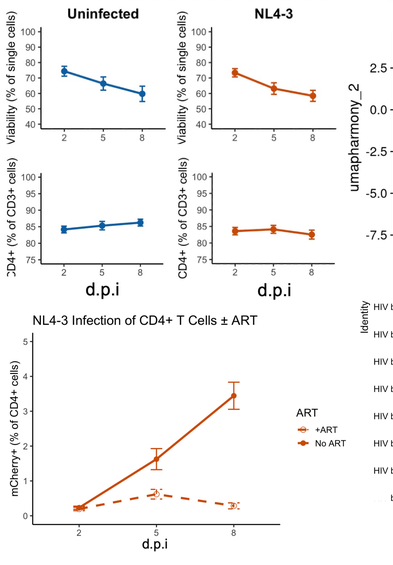


Figure 2. Viability, CD4 compartment, and productive infection in *ex vivo* human tonsil explant cultures. Top: Viability of total single cells over time (% live singlets by) for **uninfected (left, blue)** and NL4-3–**infected (right, red)** cultures. **Middle:** Frequency of CD4⁺ cells within the CD3⁺ T cell gate over time, showing preserved CD4 representation during culture. **Bottom:** Frequency of productively infected cells (mCherry⁺ among CD4⁺ T cells) over time in NL4-3–infected cultures maintained with or without antiretroviral drugs (ART). Lines and error bars indicate mean ± SEM across ten donors (n=10).

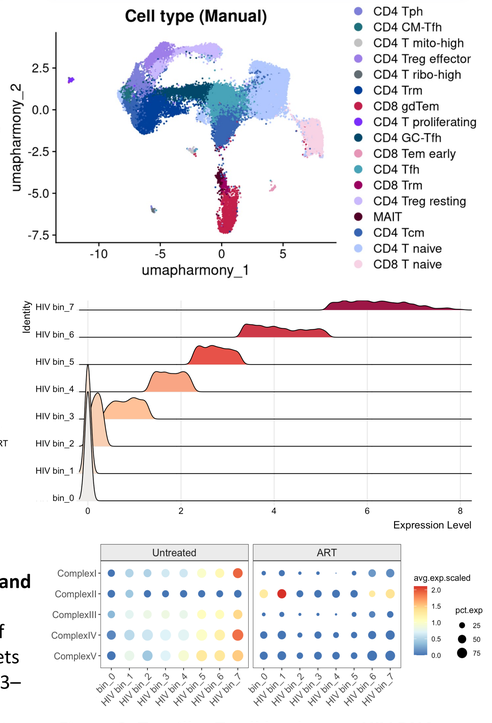


Figure 3. Tonsillar T cell landscape, HIV RNA burden, and metabolic state. Top: UMAP of T cells (n= 42,998, 4 donors) colored by subset. Manual annotation assisted by Human Tonsil Atlas[2]. **Middle:** Ridge plots showing the distribution of HIV-1 NL-CI transcript levels across ordered HIV RNA bins (0 = unexposed, uninfected; 1 = exposed, uninfected; 2-7, lowest to highest HIV state). **Bottom:** Dot plot of ETC (Complex I–V) gene signatures across HIV bins.

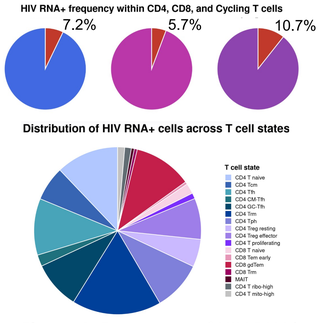


Figure 4. Frequency of HIV RNA⁺ cells by T cell lineage or subset. Within-lineage frequency of HIV RNA⁺ cells (≥ 2 UMI) among CD4 T cells, CD8 T cells, and cycling T cells. Pies show the proportion of HIV RNA⁺ (colored wedge) vs HIV RNA⁻ cells (background wedge).

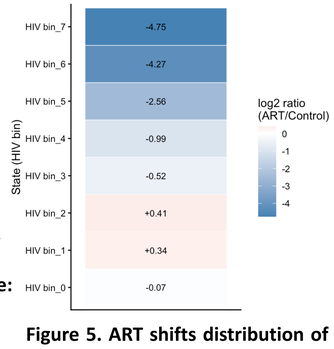


Figure 5. ART shifts distribution of HIV states. Heatmap shows the $\log_2$ ratio of cell frequency in ART-treated versus untreated cultures.

CONCLUSIONS

- **HIV RNA⁺ cells are abundant in a subset of CD4 T-cell states** (including CD4 Trm, Tfh/GC-Tfh, Tph, and effector Treg), whereas other states contribute relatively few infected cells.
- **Early ART markedly limits, but does not completely prevent, the persistence of infected CD4+ T cells.** Delayed combination ART started two days after infection reduces the frequency of CD4+ T cells in the high HIV RNA bins. Yet, a pool of low HIV RNA bin cells remain with ART, and their mechanism is unclear.
- **Higher viral RNA burden is associated with metabolic activation**. Increasing viral transcript levels track with stronger respiratory chain and mitochondrial gene signatures, pointing to the metabolic state of the cell as a potential marker—and possible modulator—of active and latent infection.

FUTURE DIRECTIONS

- Expand studies to CCR5-tropic HIV-1 JRFL-mCherry virus.
- *In situ* imaging and sequencing of HIV-infected tissues.
- Apply Seahorse metabolism assay to HIV-infected cells.

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REFERNCES

1.Freeman TL, Zhao C, Schrode N, Fortune T, Shroff S, Tweel B, et al. HIV-1 activates oxidative phosphorylation in infected CD4 T cells in a human tonsil explant model. Front Immunol. 2023;14:1172938.
2. .Massoni-Badosa R, Aguilar-Fernández S, Nieto JC, Soler-Vila P, Elosua-Bayes M, Marchese D, et al. An atlas of cells in the human tonsil. Immunity. 2024;57(2):379–99.e18.

PLAIN LANGUAGE SUMMARY

We dissected apart small pieces of donated tonsil tissue in the laboratory, then infected them with HIV, and treated them with a combination of anti-HIV medicines. We found that only certain kinds of immune cells in the tonsil make most of the virus, and that early treatment lowers the number of highly infected cells while still leaving some infected cells behind.