AN INTESTINAL TISSUE MODEL FOR TARGETED STRATEGIES DIRECTED TO TISSUE RESERVOIRS

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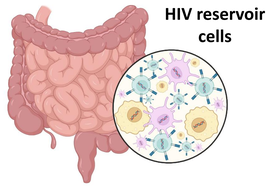
BACKGROUND

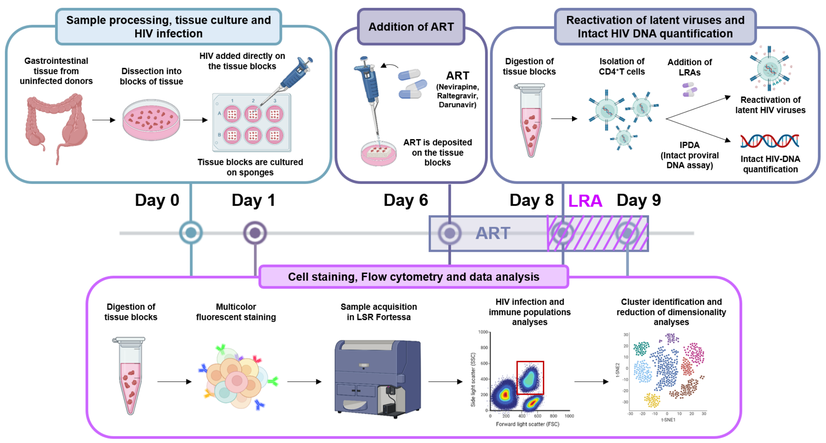
The gastrointestinal (GI) tract constitutes one of the main anatomical sites in which HIV establishes persistence, mainly during acute infection and when ART is initiated. However, sample availability from people living with HIV limits its study.

We have developed an intestinal tissue model to:

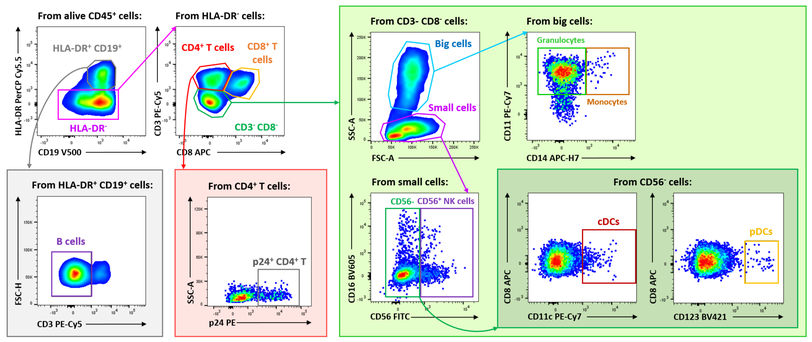
- Evaluate the impact of HIV infection on immune cell populations.
- Identify HIV-reservoir cells.
- Study the action of different Latency Reversal Agents (LRAs) in different key CD4⁺ T subsets.



EXPERIMENTAL DESIGN

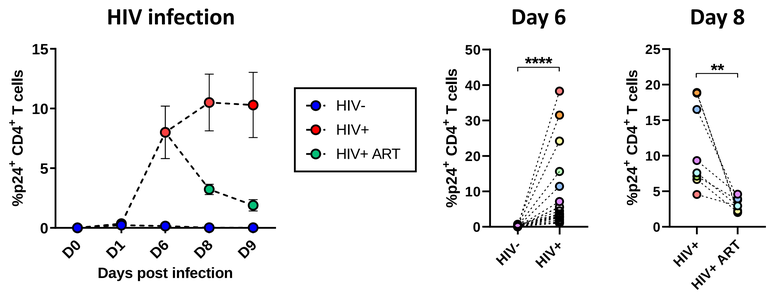


Gating strategy of immune populations present in the intestinal tissue

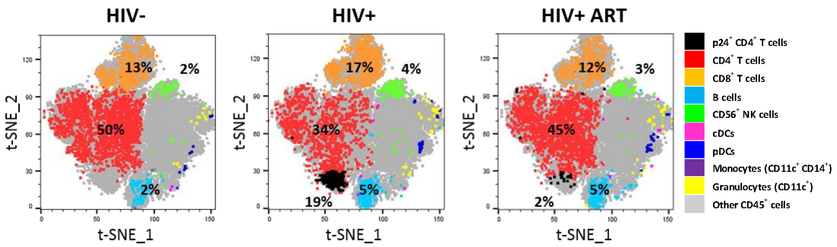


RESULTS AND CONCLUSIONS

Human intestinal model of HIV persistent infection

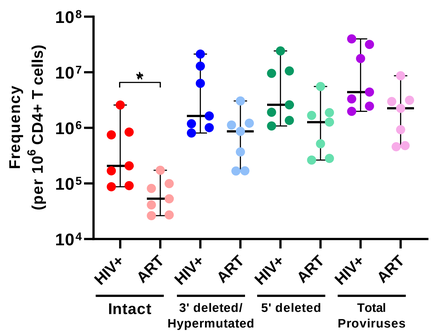


The optimal model consisted in 9 days of HIV_{BAL} infection of unstimulated tissue blocks and the introduction of ART from day 6. At day 6, we could observe a potent HIV infection (mean=8.01%, p<0.0001) that was reduced when ART was administered for at least two days (from 11.2 to 3.1% p=0.0078).



T-distributed Stochastic Neighbour Embedding (t-SNE) representations displaying the major cell clusters present in live CD45⁺ gate, based on FSC and SSC, of a representative human intestinal tissue uninfected, HIV-infected and HIV-infected and ART-treated at day 8. HIV caused a significant decline in CD4⁺ T cells (p=0.03), an increase of NK cells (p=0.06) and an expansion of B cells (p= 0.03).

ART reduces intact HIV-DNA in CD4⁺ T cells from the intestinal tissue

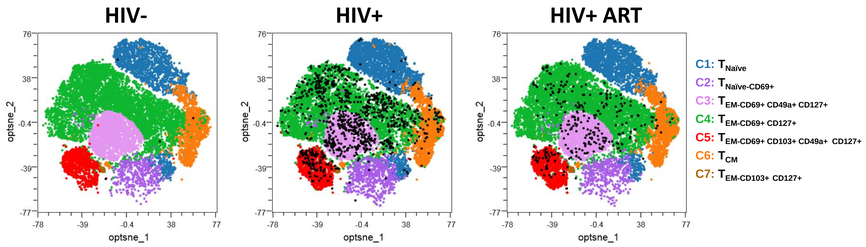


Absolute frequencies of intact and defective proviruses present in isolated CD4⁺ T cells from human intestinal tissue HIV-infected and HIV-infected and ART-treated at day 8. Total proviruses were determined as the sum of intact, 5' defective, and 3' defective proviruses. Horizontal lines indicate median values.

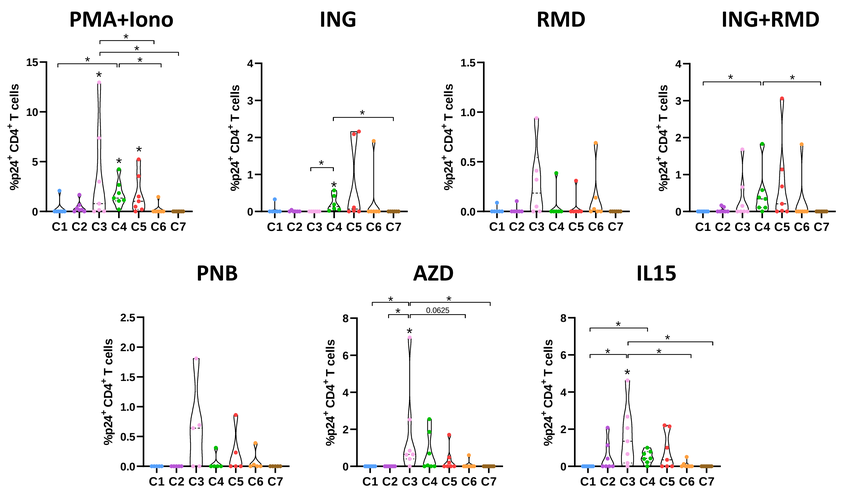
Two days of ART significantly reduced the number of CD4⁺ T cells harbouring intact HIV proviruses (p=0.0156).

Evaluation of HIV-reactivation on HIV-infected CD4⁺ T subsets

Identification of 7 subpopulations of CD4⁺ T cells present in the intestinal tissue at day 8 using the algorithm FlowSOM. Clustering analysis was performed based on the expression of the markers: CD45RO, CCR7, CD69, CD103, CD127 and CD49a.



The effect of different LRAs, including PMA+Ionomycin, Ingenol (ING), Romidepsin (RMD), AZD5582 (AZD), Panobinostat (PNB), IL-15, and the combination of ING+RMD, was evaluated in the 7 subsets of CD4⁺ T cells.



Addition of LRAs on day 8 revealed the presence of viral reservoir cells in most tested CD4⁺ T subsets, which presented differential response to the LRAs assessed.

CONCLUSIONS

- This tissue model recapitulates major attributes of HIV infection, including changes in key immune populations throughout productive and treated infection.
- Most memory CD4⁺ T subsets constituted HIV reservoirs after ART, however current LRAs only impacted specific populations.

Overall, we present a novel tissue model that might provide significant insights into new strategies specifically designed to target tissue reservoirs.

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