# Susceptibility of Cervical Explant Tissue of Transgender Men to HIV-1 Infection

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## BACKGROUND

- Administration of testosterone is an integral component of masculinizing hormonal therapy for individuals seeking gender affirming care.
- Testosterone immunomodulates immune cells through androgen receptors (ARs) resulting in altered immune responses.
- Systemically, testosterone administration upregulates TIGIT in circulatory CD4+ T cells, promotes expansion of T-regulatory cells, and anti-inflammatory macrophages while also dampening IFN-1 and upregulating TNFα and IFNγ secretion in pDCs.
- *Given the broad range of inflammatory effects of testosterone on immune cells, it is likely that similar immunological effects will also manifest at sites of mucosal HIV-1 transmission such as the cervix, and thus impact transgender men (TGM) receiving testosterone therapy.*
- New informative mucosal HIV-1 infection models are needed to better understand the biological risk factors for HIV-1 transmission to TGM compared to cisgender women (CIS).
- Here we applied a research strategy using an innovative *ex vivo* cervical explant tissue (CET) model, illustrated below, to assess the immune environment in cervical mucosa from cisgender women (CIS) and the testosterone-induced immune recalibration on HIV target cells, cytokine signaling, and cell-specific transcriptomic states of the cervical mucosa from **TGM.**

## METHODS

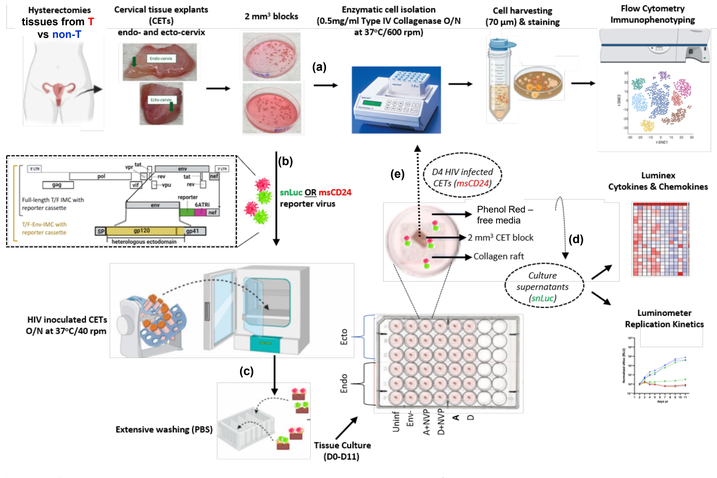



**Figure 1. Research strategy used to understand the impact of testosterone therapy on the cervico-mucosal microenvironment**. Remnant cervical tissues from **CIS** and **TGM** undergoing medically indicated hysterectomies, were separated into endo- and ecto-cervix for subsequent sectioning into 2 mm³ CET blocks that were either **(a)** collagenase digested for cell isolation and immunophenotyping or **(b)** inoculated (in a non-polarized manner and without mitogenic stimulation) with 2.5E+05 TZMbl IU of full-length HIV-1 transmitted/founder (TF) infectious molecular clones (T/F-IMC) engineered to express snLuc or msCD24 reporter genes. **(c)** Initial virus inoculum was extensively washed off the CET blocks after the O/N inoculation followed by tissue culture of individual CET blocks up to 11 days. Infection was analyzed using **(d)** supernatants of each individual CET block (snLuc-T/F-IMC infection) from all donors to assess replication kinetics (D2-11) and cytokine signaling (D4) OR (**e**) characterized using a 26-color flow cytometry panel (msCD24-T/F-IMC infection): CD45, CD14, CD11c, CD3, TCR-αβ, CD45RA, CD68, CD206, CD163, CD4, CD8, CCR5, CXCR4, p24, msCD24, CD209, CD207, CD169, α4β7, CD38, HLA-DR, CD80/86, CCR7, IFNγ, IL-10 and the live-dead marker. In preliminary work, flow cytometry was done in parallel to single cell RNA sequencing of pre-sorted CD45+ immune cells from CIS and TGM with and without HIV-1.

Cervical tissue explants from transgender men display increased frequency of HIV-1 target CD4+CCR5+α4β7+ T cells and macrophages, transcriptional and inflammatory remodeling consistent with heightened mucosal HIV-1 permissiveness, and enhanced HIV-1 replication kinetics

## RESULTS

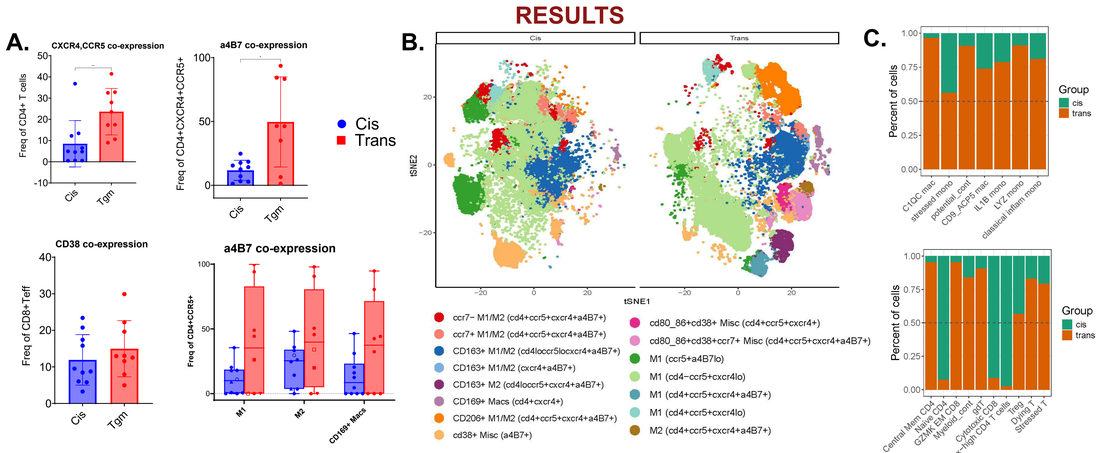



**Figure 2. Differential expression of HIV-1 susceptibility & activation markers and, transcriptomic profiles of cervical immune cells isolated from cervical tissue obtained from transgender men and cisgender women. U**sing a 28-parameter flow cytometry panel, immune cells were identified and characterized using **(A)** traditional gating strategies and (B) automated clustering using FlowSOM into CCR5+CXCR4+CD4+ T cells including co-expression of a4B7, CD38+ effector CD8+ T cells and CD4+CCR5+a4B7+ proinflammatory (HLA-DR+CD80/86+), anti-inflammatory (CD206+CD163+) and CD169+ macrophage subsets. Box-and-whisker plots display median frequencies, interquartile ranges, and full distributions; two-tailed Mann–Whitney U tests were used for statistical comparisons. These data suggest hormone-linked mucosal HIV-1 susceptibility and activation phenotypes. (C) Differential transcriptomic states in pre-sorted CD45+ Immune cells from transgender men and cisgender women.

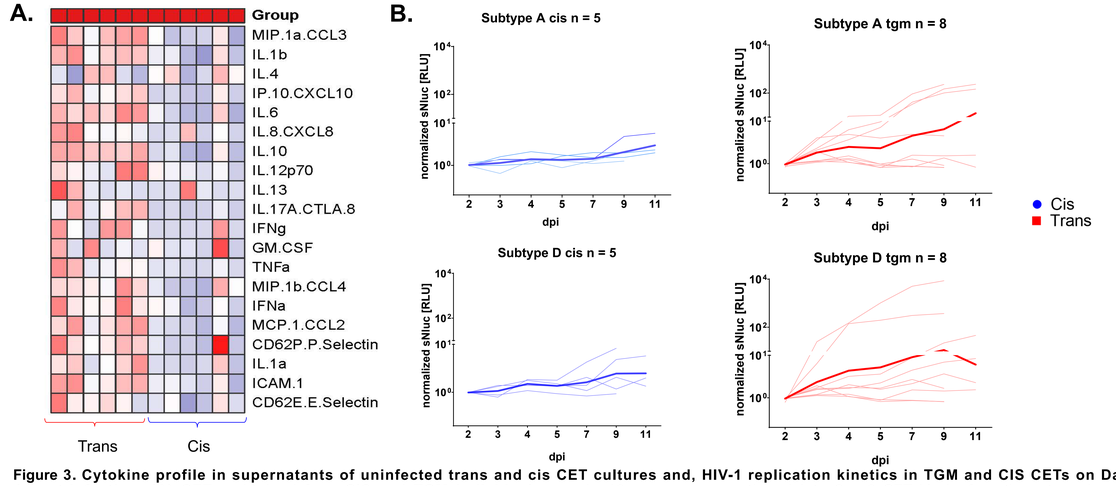



**Figure 3. Cytokine profile in supernatants of uninfected trans and cis CET cultures and, HIV-1 replication kinetics in TGM and CIS CETs on Day 4 post-infection. (A)** CETs obtained from CIS and **TGM individuals**, aged >18 years were sectioned into 2mm3 CET blocks from the ectocervix and endocervix with and without snLuc T/F IMCs and left in culture for 4 days. Culture supernatants were collected form non-HIV-1 CETs and assessed for cytokines/chemokines using 20-plex Luminex. Each column is representative of each TGM (**left**) or CIS donor (**right**) showing ecto- and endo-cervix adjacently. Expression values are scaled by row and visualized using the z-score. **(B)** Without mitogenic stimulation, 2mm3 CET blocks were inoculated (in a non-polarized manner) with 2.5E+05 TZMbl IU of HIV-1 clade A (**A.R880F** or **A.191845**) or D (**D.191859**) full length snLuc-T/F-IMC in the presence or absence of RT inhibitor, nevirapine (NVP). Initial virus inoculum was extensively washed off the CET blocks after 18 hours, followed by tissue culture of the CET blocks + NVP over 11days. Infection was analyzed via snLuc Relative Light unit (RLU) luminescence activity in supernatants of each individual CET block from all donors. Graphed data represent geo mean RLU values from the individual blocks of each donor taken at each time point (thin lines), and the overall geomean derived from these values (bold line) .

## CONCLUSIONS

- We demonstrate that cervical tissue explants from trans-men exhibit increased frequencies of HIV target cells, including $CD4^+CCR5^+\alpha4\beta7^+$ T cells and macrophages co-expressing CD4, CCR5, and α4β7, compared to cis-women
- Using our innovative CET model of *ex-vivo* HIV-1 infection, and full-length transmitted founder IMCs engineered for reporter gene expression, we found these alterations to coincide with enhanced HIV-1 replication kinetics *ex vivo.*
- Preliminary findings from scRNA-seq of pre-sorted $CD45^+$ cells reveal depletion of naïve $CD4^+$ T cells , accumulation of $GZMK^+CD8^+$ T cells, loss of canonical cytotoxic T lymphocytes, and expansion of pro-inflammatory myeloid clusters in CET derived cells from transgender men compared to those from cisgender women.
- Multiplex cytokine profiling further demonstrates a mixed pro- and anti-inflammatory secretory milieu in transgender CET culture supernatants.
- Together, these findings suggest **a transcriptionally and functionally distinct cervical immune landscape associated with heightened HIV-1 permissiveness in transgender men on gender affirming hormone therapy.**
- SIGNIFICANCE: By characterizing effects on the mucosal immune environment at the primary site of HIV-1 entry and transmission, this study contributes biological insights needed to better inform tailored HIV prevention strategies, including PrEP optimization and hormone-informed risk assessment in transgender populations.

## ACKNOWLEDGEMENTS:

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**LAY SUMMARY**: We found more HIV-1 susceptible immune cells in cervical tissue samples from transgender men compared to cisgender women, and the virus (HIV-1) was able to more readily infect these samples in lab studies. This suggests important biological differences that may influence HIV-1 risk and prevention in transgender communities.