A Novel Non-invasive Approach to Sample Female Genital Tract Immune Cell Populations

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Background

- T cells in the human female genital tract (FGT) are key mediators of susceptibility to and protection from infection, including HIV and other sexually transmitted infections.
- There is a critical need for increased understanding of the distribution and activation of T cell populations in the FGT.
- Current sampling methods require a healthcare provider and are expensive, limiting the ability to study these populations longitudinally.
- Menstrual discs are a disposable menstrual product that collects cervicovaginal fluid (secretions from the cervix and fluid pooled in the vaginal fornices).

Develop a non-invasive approach to sample the FGT for immune cells

Approach

- 5 healthy, adult, reproductive-aged participants were enrolled.
- Each participant donated CVF 3 days in a row, 7 to 11 days from LMP.
- Blood was also drawn and processed for PBMCs.

Membranal disc processing

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Objectives

- Develop a non-invasive approach to sample the FGT for immune cells.
- Use menstrual discs to sample CVF.
- Track each participant's menstrual cycle.
- Blood was also drawn and processed for PBMCs.

Immune Cell Recovery from CVF

- After gating out Tregs (CD25+FoxP3+), conventional CD4+ T cells were further gated by CCR7 and CD45RA to define EMRA, EMRA, and TCM populations for PBMC (left) and CVC (right) samples.
- CD8+ T cells were also gated by CCR7 and CD45RA to determine memory subtypes, as well as by CD69 and CD103 to determine tissue residency (CD69+ CD103+).
- Frequency of conventional CVC CD4+ memory subtypes across samples.
- Frequency of CVC CD8+ memory subtypes across samples.

Reproducibility of T Cell Population Structure

- Participants were sampled across three (CVC) and one (PBMC) day. Results for samples with greater than 100 cells in the parent population were compared to the PBMC frequency using a Wilcoxon signed rank test. Star indicates p ≤ 0.05.
- Summary plots of frequencies of activation and suppression markers between paired PBMC and CVC memory T cell populations. (A) Concordance of paired PBMC and CVC memory T cell populations.
- Heat map characterizing the median expression of CVC T cell markers across individuals and samples. (B) Heat map characterizing the median expression of T cell markers in each identified cluster. (C) Dimensional reduction of all markers relevant to T cells performed and is represented as a UMAP including all CD3+ T cells across samples. (D) Proportions of cell clusters comparing each sample, arranged by participant and visit.

Conclusions

- We have established a low cost, self-applied method that will facilitate longitudinal studies and reduce the need for participants to interface with a clinical setting.
- We can assess mucosal vaccine responses with this method.