INCREASED HIV TARGET CELLS IN FGT DURING FOLLICULAR PHASE OF HIV SERONEGATIVE WOMEN

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Background: Ovarian hormones are known to modulate the immune system in the female genital tract (FGT). We sought to define the impact of menstrual cycle phase on the mucosal microenvironment and HIV target cell availability.

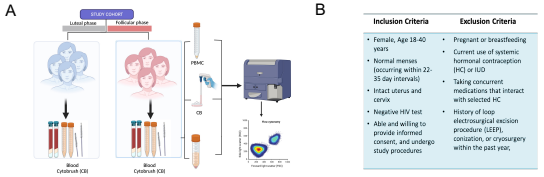
Methods: Here, we characterized the distribution, phenotype and function of CD4 T cells with special emphasis on HIV target cells (CCR5+ and α4β7+) and tissue resident memory (TRM) CD4 T cells in FGT of cycling women from endocervical cytobrush (CB) and compared with peripheral blood. We isolated cells from blood and CB of 91 healthy women and performed multi-color flow cytometry to characterize the various subsets of CD4 T cells between two study visits, timed to coincide with the luteal and follicular phases based on the participant's last menstrual period. We assessed association of cellular marker by visit, stratified by specimen type (blood and CB).

Results: In the blood and the FGT, mean levels of several cell types were higher in the follicular compared to the luteal phase including: CCR5 (p=0.041) and CCR5+α4β7+ double positive cells (p=0.04) were higher in follicular compared to luteal phase. We also found significantly greater expression of activation marker in the FGT (HLADR, p=0.041) in the CB follicular phase specimens compared to luteal phase. Similarly, we found increased expression of TRMs with significantly higher expression of CD103+ (p=0.033), CD69+(p=0.029) and CD69+CD103+ (p=0.044) in the follicular CB samples compared to luteal phase. This CD69+CD103+ population expresses significantly higher levels of HIV target cells CCR5 but not α4β7 in the follicular phase (p=0.006) compared to the luteal phase. Interestingly, although there is higher activation found in the follicular phase, the regulatory T cells were significantly increased in the follicular phase (Fox-P3: p<0.016).

Conclusion: We found evidence of enrichment of HIV target cells with higher activation and tissue resident and regulatory profile in the follicular phase, suggesting that CCR5+ α4β7+ TRM cells could be more susceptible to HIV infection during this phase of menstrual cycle.

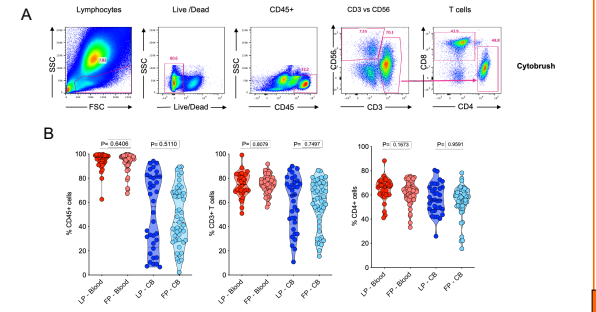
Note: Based on the hormonal data we stratified the samples into luteal and follicular phase and analyzed the data post CROI abstract submission. Since we only have few hormonal data, we would like compare those data for the current presentation.

Study Design & Methods



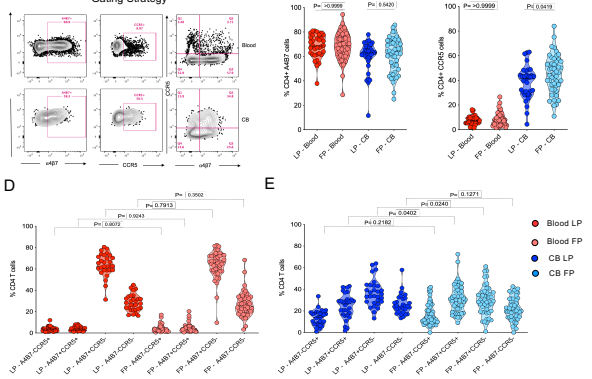
Participant Characteristic	Number of Participants (%) or median (N=49)
Age	29 years (average) rs29
Ethnicity	
African American	20 (40.8%)
White/Caucasian	16 (32.6%)
Multi-racial	7 (14.2%)
Asian	3 (6.1%)
Not specified	3 (6.1%)
Self-reported sexually transmitted infection in the last 6 months	6.1%
Visit Characteristic	
Visits during luteal/follicular phase	35/49 in numbers
Symptomatic genital infection	0%
Asymptomatic sexually transmitted infection	
Gonorrhea	-
Chlamydia	-
HSV ½ DNA	-
Trichomonas	6.1%
Self-reported vaginal sex within 1 week of study visit	69.3%

Gating Strategy for Identifying T cell subsets in Cytobrush



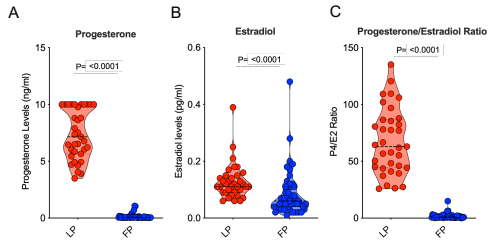
(A) Representative flow plots showing gating strategy for T cells in cytobrush (B). Violin plots showing the frequency of CD45+ (Leukocytes), CD3+ T cells & CD4 T cells in Blood & Cytobrush between luteal and follicular phase

HIV target cells CCR5 single & CCR5+α4β7+ double positive cells are significantly higher in follicular phase (Cytobrush)



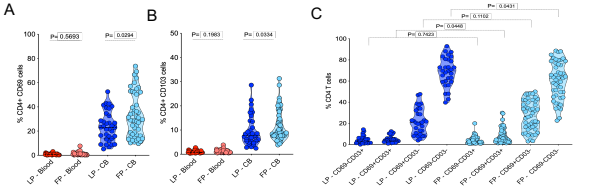
(A) Representative flow plots showing the expression of CCR5 and α4β7 in blood and cytobrush. (B). α4β7 expression in Blood & CB. (C). CCR5 expression in Blood & CB. (D). α4β7 vs CCR5 expression in Blood & cytobrush

Progesterone and Estradiol levels are higher in luteal phase



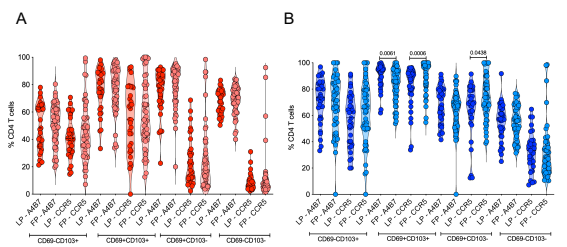
(A) Progesterone levels comparing luteal and follicular phase. (B) Estradiol levels comparing luteal and follicular phase. C). Progesterone/Estradiol ratio comparing luteal and follicular phase.

Tissue Resident Memory cells (TRM) cells significantly higher in follicular phase (Cytobrush)



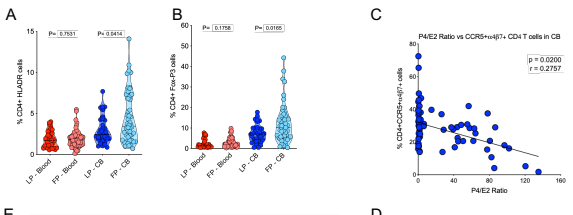
A) Violin plot shows the frequency of CD69 single positive CD4 T cells between blood and cytobrush. (B). Violin plots show the frequency of CD103 single positive CD4 T cells. (C). Violin plots shows the frequency of single and double positive TRM subsets comparing luteal and follicular phase of mensural cycle.

CCR5 expression was higher in TRMs during follicular phase (Cytobrush)



(A) Violin plot shows the expression of CCR5 and α4β7 on blood CD4 T cells which express CD69 and CD103 and tissue resident CD4 T cells in cytobrush. Between the CD69 and CD103, CD69+ cells express high levels of CCR5 in cytobrush

Progesterone levels influences the CCR5+α4β7+ target cells and TRM cells in the cytobrush



CD4 T cell subsets	CD4 Markers in Blood comparing Luteal vs Follicular phase	CD4 Subsets in Cytobrush comparing Luteal vs Follicular phase based on hormonal level
CD4+CCR5+ (Target cell)	No change	Significant (p=0.042)
CD4+α4β7+ (Target cell)	No change	No significant (p=0.6194)
CD4+CCR5+α4β7+ (Target cell)	No change	Significant (p=0.040)
CD4+HLADR+	No change	Significant (p=0.041)
CD4+CD38+	No change	No significant (p=0.9440)
CD4+CD69	No change	Significant (p=0.04)
CD4+CD103	No change	Significant (p=0.0330)
CD4+CD69+CD103+	No change	Significant (p=0.05)
CD4+Fox-P3+	No change	significant (p=0.0165)
CD4+Ki67+	No change	No significant (p=0.5510)

(A) Violin plot shows the expression of HLADR, (B) Fox-P3+ CD4 T cells.(C). Correlation of target CD4 T cells with P4/E2 ratio. (D). Correlation of Tissue Resident CD4 T cells with P4/E2 ratio. Progesterone/Estradiol ratio was compared both in luteal and follicular phase. Spearman correlation was used for analysis. (E). Cumulative data for displaying the statistical analysis for CD4 T cell subsets in the current study. Mann Whitney test was used for comparing two phases.

Conclusions

- **Enrichment of target cells during follicular phase of mensural cycle in Cytobrush**
- **Enrichment of Tissue resident CD4 T cells are higher in follicular phase.**
- **TRMs express higher CCR5 levels during follicular phase of mensural cycle in Cytobrush.**
- **Activation and regulatory CD4 T cells are higher in Cytobrush during follicular phase**
- **P4/E2 ratio influence the levels of target cells and tissue resident CD4 T cells in the Cytobrush**

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