poster board # 273

LOSS OF CERVICAL GAMMA DELTA 1 T CELLS IN HIV INFECTED WOMEN

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Background

Background: Human gamma delta (GD) T cells are essential components of the adaptive and innate immune system and play a well-documented role in epithelial barrier protection and tumor surveillance. Two subsets of GD T cells, defined by the use of either the V delta 2 (D2) or V delta 1 (D1) TCR, predominate. The majority of the circulating GD T cells bear V D2 TCR while the majority of the tissue-associated GD T cells are D1.

We hypothesized that, being the major component of intraepithelial mucosal compartment, endocervical GD T cells may have a decisive role in HIV infection and could help to control HIV replication.

Aim

To evaluate endocervical intraepithelial gamma delta (GD) T cells of women from Miami participating in the **Women's Interagency HIV Infection Study (WIHS),** the largest longitudinal cohort of women with HIV and at risk for HIV infection in the United States.

Material and Methods

Institutional Review Board (University of Miami Miller School of Medicine) approval was obtained prior to recruitment and any assessment or study related procedures.

Study activities took place at **University of Miami HIV Research Unit in collaboration with the Miami Women and HIV Interagency Study (WIHS**) and the **Miami Center for AIDS Research (CFAR).** HIV infected and HIV uninfected at risk pre-menopausal women were recruited (Table 1).

Participants underwent vaginal examination with collection of endocervical cytobrush samplles and peripheral blood. Cervical samples were collected by inserting Cytobrush® Plus GT cell collector into the endocervix. The cytobrush was processed and the extracted cells were resuspend in 1 ml of IMDM and counting was performed using a Beckman Coulter automated cell counter (The Vi-CELL Series Cell Viability Analyzer) with the trypan blue dye exclusion method (total viable cell concentration were reported). Frequency and phenotype of CD3+ T lymphocytes and GD T cells were determined in cervical cytobrush samples and peripheral blood by multicolor flow cytometry.

Table 1. Characteristics of Study Participants

Characteristics	Participants n=37	
N	HIV - =18; HIV +=19	
Age	HIV+ =35.66 (SD=6.46)	
	HIV- =33.31 (SD=6.26)	NS
On ART	14	
CD4 count (cells/ul)	N=16	
(only HIV+)	Mean absolute=1030 (SD=606)	
Plasma HIV load (copies/ul)	N=15	
(only HIV+)	Detectable VL=7	
	VL=1.7 $\log_{10}$ (SD=2.1)	
Days since last menses	HIV+ =16 (SD=7.8)	
	HIV- =19 (SD=7.3)	NS
Cervical cell yield	HIV- = 700,000 (300,000-1.800,000)	
	HIV+ = 700,000 (300,000-1.7,000)	NS
Cervical CD45 yield	HIV+ =25,000 (4,000-90,000)	
	HIV- =10,000 (2,000-170,000)	NS
Cervical CD3 yield	HIV+ = 20,000 (200- 500,000)	
	HIV- =16,100 (100-90,000)	NS
Cervical CD4:CD8 ratio	HIV+ = 2.3:1	
	HIV- = 0.18:1	

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Results

I) Endocervical gamma delta T cells

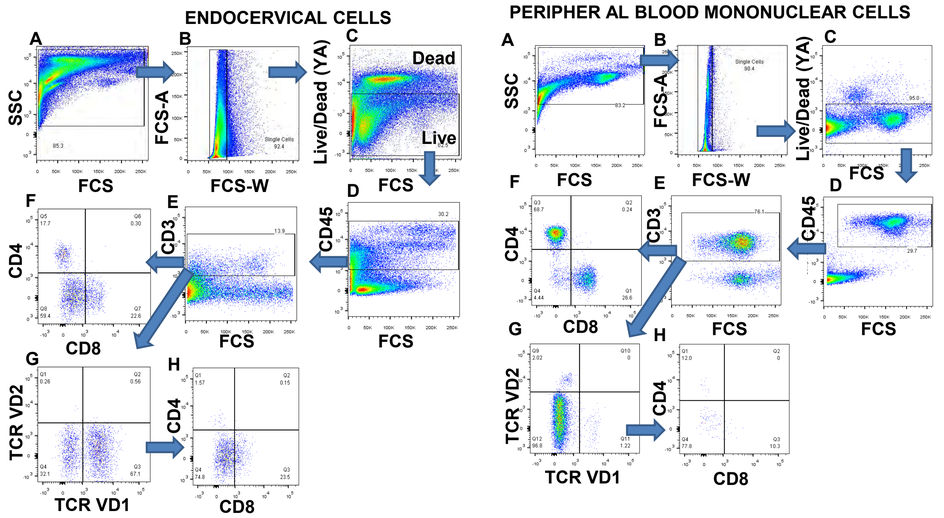


Figure 1. Representative flow cytometry gating strategy for enumeration of gamma delta (GD) T cell subpopulation in endocervical mucosa and peripheral blood . Identification of endocervical T cell populations by following gating strategy: A) First, the large gate is set on Forward/Side scatter of acquired endocervical cytobrush cells B) FCS-A versus FSC-W plot for the exclusion of cell doublets C) Viability is defined by exclusion of dead cells using LIVE/DEAD Fixable Yellow Amine Dead Cell Stain. Viable cells are therefore identified by gating on the viability dye-negative population D) live cells expressing CD45 E) gated CD45+ cells expressing CD3 F) gated CD3+ cells analyzed for CD4 and CD8 expression G) and for TCRVD1 and TCRVD2 H) gated TCRVD1+ cells expressing CD4 and CD8.

II) Peripheral blood gamma delta T cells

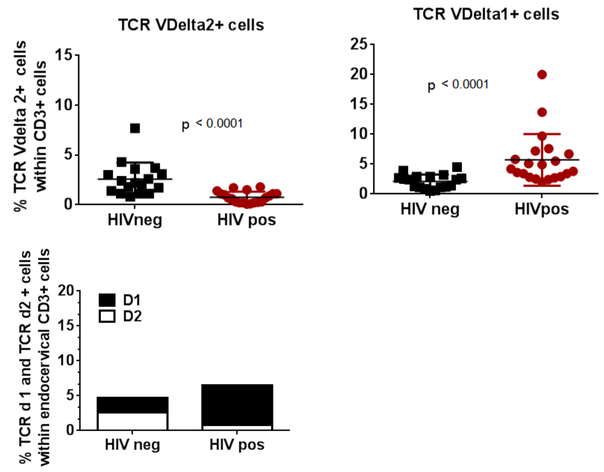


Figure 2. Comparison of percentages of peripheral blood D1 and D2 T cells in HIV infected and uninfected women. PBMC samples from HIV infected and uninfected women were analyzed by flow cytometry A) frequency of TCR V delta2+ cells (D2) and TCR V delta1+ (D1) (B) within endocervical CD3+ cells C) combined data from A and B

III) Endocerviocal gamma delta T cells

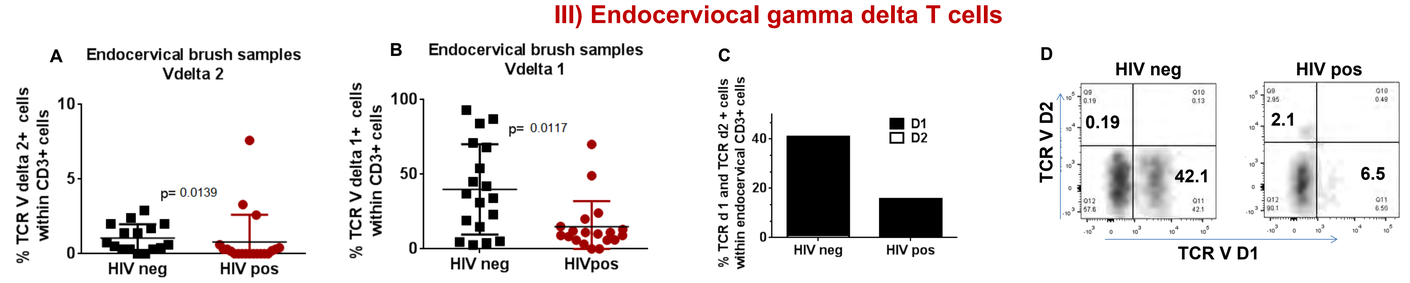


Figure 3. Comparison of percentages of endocervical D1 and D2 T cells in HIV infected and uninfected women. Endocervical brush samples form HIV infected and uninfected women were analyzed by flow cytometry A) frequency of TCR V delta2+ (D2) cells and TCR V delta1+ (D1) (B) within endocervical CD3+ cells C) combined data from A and B D) representative flow cytometry data for HIV negative and HIV positive endocervical CD3+ cells showing expression of TCR VD2 and TCR VD1.

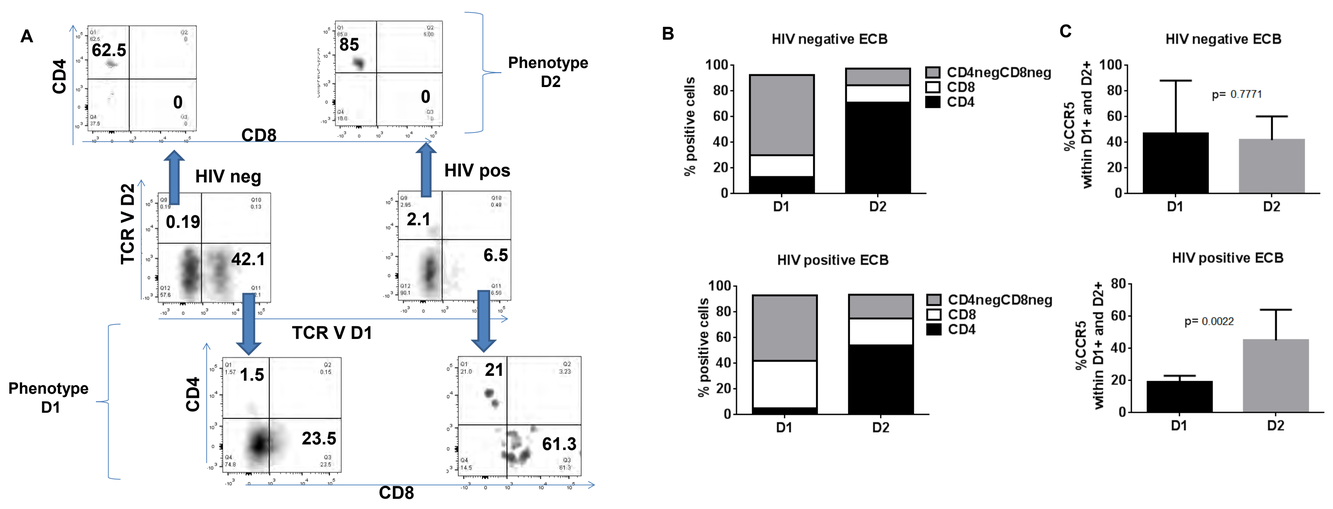


Figure 4. Phenotypic characteristics of endocervical D1 T cells in HIV infected and uninfected women. Endocervical TCR D1+ (D1) and TCR D2+ (D2) cells from HIV negative and HIV positive women were analyzed for expression of surface molecules: CD4, CD8 and CCR5. **A)** Representative flow cytometry data of endocervical CD3+ cells (middle panels) form HIV infected and uninfected women expressing TCR V D2 or TCR V D1. Expression of CD4 and CD8 on TCR V D2 gated cells (upper panels) and TCR VD1 gated cells (lower panels). Numbers in the quadrants are showing percent of positive cells. B) Percent of CD4, CD8 and double negative (CD4-CD8-) cells within the D1 and D2 cells from HIV negative (upper panel) and HIV positive (lower panel) women. C) Percent of CCR5 positive cells within D1 and D2 cells from HIV negative (upper panel) and HIV positive (lower panel).

Conclusions

- ❑ **We report for the first time, the unique GD1 T cells are predominant T cells subset within endocervical cells in women with HIV and at risk for HIV**
- ❑ **By eliminating cervical GD1 cells, HIV cripples an important antiviral effector subset and removes a normal control over inflammation resulting in HIV persistence and progressing disease**
- ❑ **We propose that evaluation of GD T cells responses could be used as a novel marker of mucosal vulnerability in women with HIV infection or at risk for HIV**