Identification of Innate Lymphoid Cell Subsets in the Human Female Genital Tract that Respond to HIV

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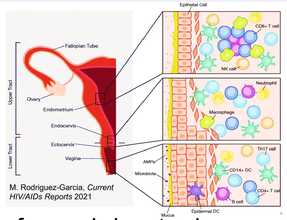
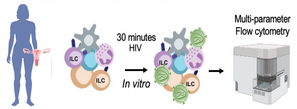
BACKGROUND



Women acquire HIV mainly through sexual contact. Epithelial barriers and innate immune cells protect against HIV infection[1], as we have described for genital neutrophils[2,3]. Identification of novel innate immune mechanisms that reinforce mucosal protection against HIV infection in the female genital tract (FGT) will enable strategies to prevent HIV acquisition. Innate lymphoid cells (ILCs) are tissue-resident cells specialized in cytokine secretion that provide mucosal protection against infections in mice. However, human ILCs are poorly characterized and their potential role in preventing HIV acquisition in the female genital mucosa is unknown.

METHODS



Human hysterectomies (n=35) were enzymatically digested to generate mixed-cell suspensions from endometrium, endocervix, and ectocervix. ILC phenotype and function was assessed with high-dimensional spectral flow cytometry for surface and intracellular molecules (22-parameters). To define ILC-mediated anti-HIV responses, genital mixed-cell suspensions were stimulated with HIV for 30 min in vitro, and cytokine content (IL-22 and IFNγ) and degranulation (CD107a) analyzed by flow cytometry.

Identification of ILC subsets that produce high levels of IL-22 and IFN-γ and degranulate at homeostasis

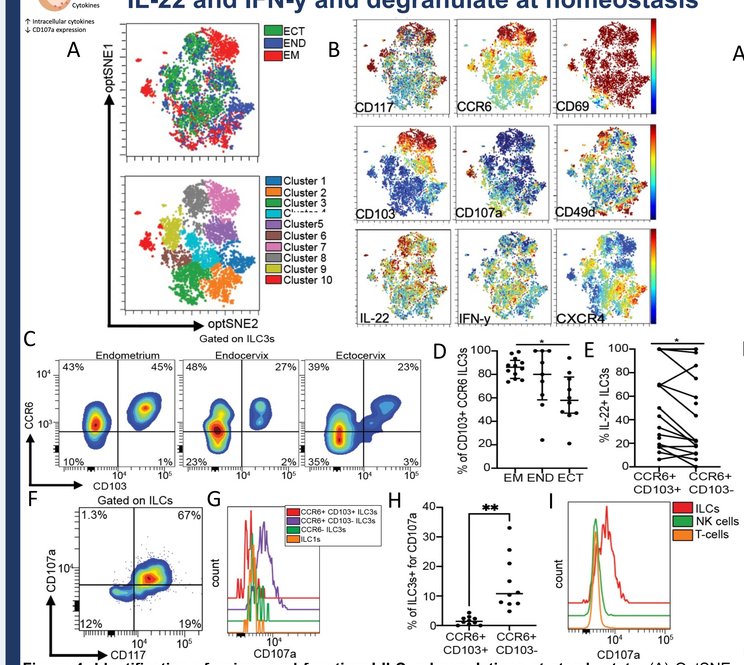


Figure 4: Identification of unique and functional ILC subpopulations at steady-state. (A) OptSNE plot of genital ILCs generated with 3 patients and 3 matching tissues via unsupervised clustering, overlayed tissue of origin (top) and FlowSOM clustering (bottom). (B) Scaled expression of markers. (C) Contour plot of CCR6 and CD103 expression on genital ILC3s from one matched patient. (D) Quantification of percent of CD103+CCR6+ ILC3s across tissues. (E) Percent of IL-22+CCR6+ ILC3s in CD103+ and CD103- populations. (F) Contour plot of CD107a and CD117 expression, gates were set with FMO. (G) Histogram of CD107a expression in ILC subsets (H) % of CD107a+ cells on ILC3 subsets. (I) Histogram of CD107a expression in genital ILCs, NK cells (CD56 bright), and T cells (CD3+)

CCR6+CD103+ ILC3s and CD103+ ILC1s respond to HIV with degranulation and release of IL-22 and IFN-γ

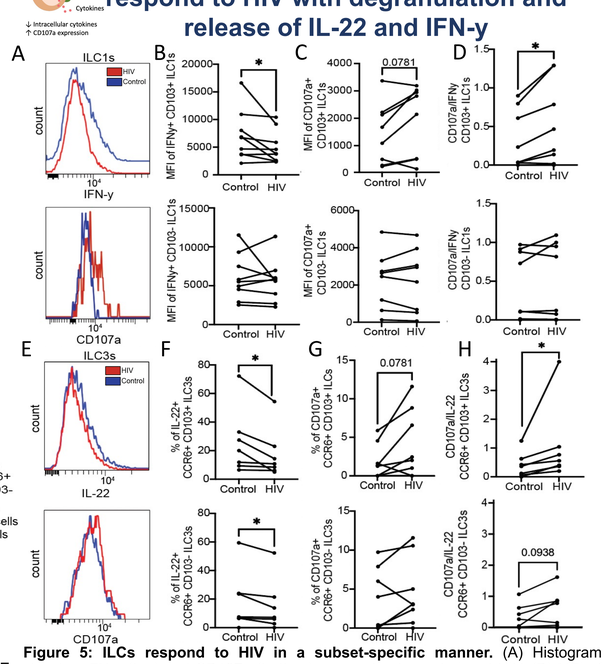
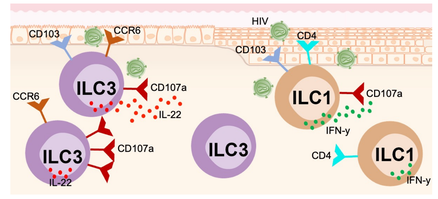


Figure 5: ILCs respond to HIV in a subset-specific manner. (A) Histogram showing IFN-γ (top) and CD107a (bottom) expression in control and HIV. (B) MFI of IFN-γ expression in control and HIV in ILC1 subsets (top CD103+, bottom CD103-). (C) MFI of CD107a in CD103+ (top) and CD103- (bottom) ILC1s. (D) Response ratio of CD107a expression divided by IFN-γ content in control and HIV. (E) Histogram showing IL-22 (top) and CD107a (bottom) expression in control and HIV. (F) % of IL-22+ILC3s in control and HIV in (top CCR6+CD103+, bottom CCR6+CD103-). (G) % of CD107a+ cells in CCR6+CD103+ (top) and CCR6+CD103- (bottom) ILC3s. (H) Response ratio of CD107a expression divided by IL-22 content in control and HIV.

CONCLUSIONS



- ILCs reside in the FGT in a compartment-specific manner (ILC1s dominating in the lower tract and ILC3s more predominant in the endometrium).
- Genital ILCs produce antiviral cytokines under steady-state.
- We identified two ILC3 subpopulations active during homeostasis: CCR6+CD103+ (highest IL-22) and CCR6+CD103- (highest degranulation).
- CD103+ ILC1s and CCR6+CD103- ILC3s release IFN-γ and IL-22 respectively in response to HIV stimulation, suggesting a role in the maintenance of an antiviral environment and the ability to mount an antiviral response.
- Our investigation suggests ILCs could be a novel mechanism for protection against HIV in the FGT.

REFERENCES & FUNDING

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RESULTS

Identification of compartment-specific distribution of ILCs in the female genital tract.

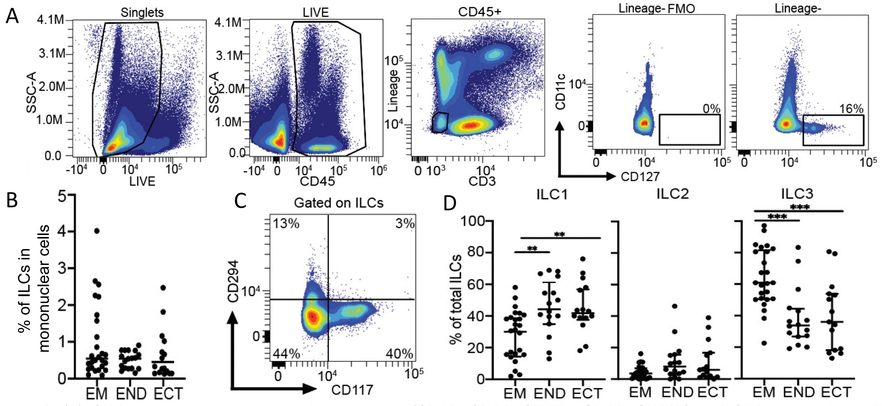

Figure 1. (A) CD45+ live singlets are gated on lineage- (CD19, CD14, CD11b, CD16, CD66b) and CD3- populations. The lineage- population is gated on CD11c- and CD127+ expression, gate is set with an FMO. (B) Quantification of ILC numbers in three different tissues (endometrium, endocervix, and ectocervix) per total mononuclear cells (n= 70). (C) Contour plot of ILC subsets. ILC1s are CD294- and CD117-, ILC2s are CD294+, and ILC3s are CD117+. (D) Quantification of the ILC1, ILC2, and ILC3 percentage of total ILCs in the three regions (EM=endometrium; END= endocervix; ECT= ectocervix)

Genital ILCs produce IFN-γ and IL-22 under homeostatic conditions

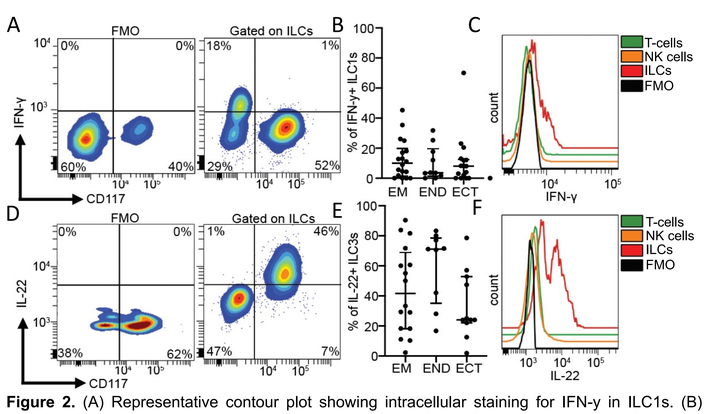

Figure 2. (A) Representative contour plot showing intracellular staining for IFN-γ in ILC1s. (B) Quantification percentage of intracellular IFN-γ+ ILC1s. (C) Histograms comparing intracellular IFN-γ content in ILCs (lineage-, CD127+), T-cells (CD3+), and NK cells (CD56+ high). (D) Contour plot showing intracellular staining for IL-22 in ILC3s. (E) Percentage of IL-22+ ILC3s. (F) Histograms comparing intracellular IL-22 content in ILCs, T-cells, and NK cells.

Genital ILCs express HIV receptors

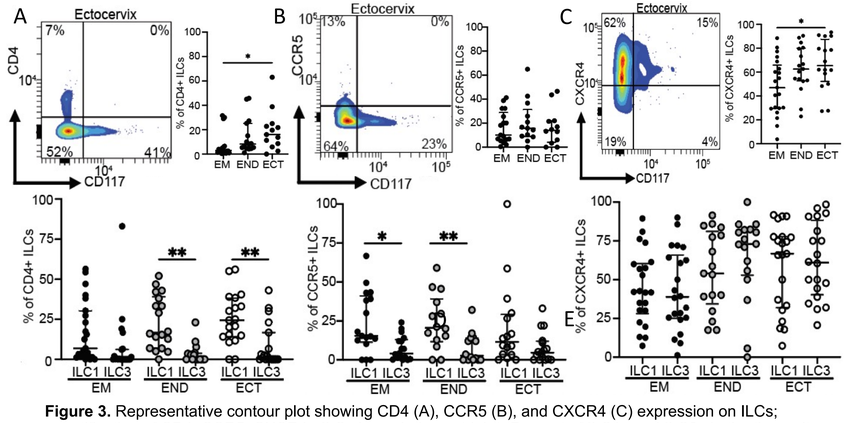

Figure 3. Representative contour plot showing CD4 (A), CCR5 (B), and CXCR4 (C) expression on ILCs; quantification of CD4, CCR5, CXCR4+ ILCs in each tissue (right). Percent of ILC1s and ILC3s in the three sites that express CD4, CCR5, and CXCR4 (bottom)