Application of an HIV Entry Assay to Identify HIV Target Cells in the Female Genital Tract

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Abstract

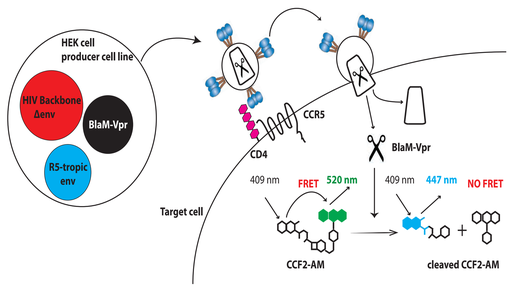
Background: The HIV pandemic disproportionately affects women, with the majority of new infections occurring through receptive vaginal sex. The cellular targets of HIV infection in the female genital tract (FGT) are poorly defined; however, possible correlates of HIV susceptibility include the HIV co-receptor - CCR5, early activation marker - CD69, and the mucosal homing integrin - $\alpha4\beta7$. Measuring actual infection in cervical cells in a biologically relevant *ex vivo* HIV infection assay has been difficult due to the low cell yield of cervical cytobrush sampling or cervical biopsies. Here, we validate a biologically-relevant HIV entry assay to rapidly quantify HIV entry of an early sexually transmitted CCR5-tropic HIV pseudovirus in unstimulated cervical mononuclear cells (CMCs) obtained using minimally-invasive cervical cytobrush sampling.

Methodology: Peripheral blood mononuclear cells (PBMCs) and CMCs were obtained from HIV-negative female participants in Nairobi, Kenya. Cells were incubated with R5-tropic HIV pseudovirus incorporating β-lactamase-vpr (BlaM-Vpr) in its capsid, which cleaves a fluorescence reporter dye upon cytosolic entry. HIV entry in various T cell subsets was analyzed 12h post-infection by multi-parameter flow cytometry based on expression of cell surface receptors relevant to mucosal homing and/or HIV acquisition such as CCR5, CD69, and $\alpha4\beta7$.

Results: HIV entry in CMCs was specific to CD4 T cells, and blocked by CCR5 inhibitor-maraviroc but not by CXCR4 inhibitor-AMD3100. Cervical CD4 T cells demonstrated 2.8 fold greater HIV entry compared to blood from matched participants. HIV entry in CD4 T cells correlated between the two compartments and with CCR5 expression but not CD4 mean fluorescent intensity (MFI). Cervical CD69+ and $\alpha4\beta7$+ CD4 T cells had 1.9 and 1.6 fold higher HIV entry than CD69- and $\alpha4\beta7$- CD4 T cells (p=0.001 and p=0.02) respectively, possibly due a 2-fold increase in CCR5 expression (p<0.0001 and p=0.0006).

Conclusion: We apply a rapid and sensitive assay to quantify HIV entry in cervical CD4 T cells and demonstrate that activated and $\alpha4\beta7$+ CD4 T cells are preferential targets of CCR5-dependent HIV entry. The assay has potential for field studies as an intermediate endpoint to assess the impact of clinical parameters on the risk of HIV acquisition.

Overview: BlaM-Vpr HIV entry assay



Results

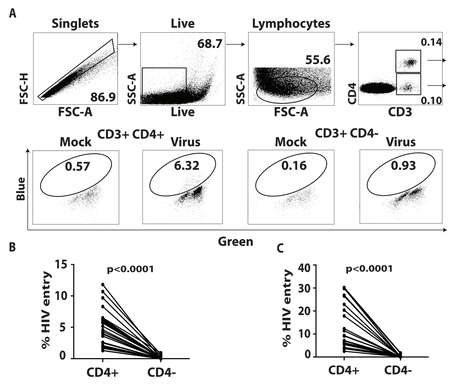


Figure 1. HIV entry in CD4+ and CD4- T cells in PBMCs and CMCs incubated *ex vivo* with R5-tropic BlaM-Vpr pseudovirus.
(A) Gating on (from left to right) singlets, live cells, lymphocytes, and CD4+ and CD4- T cells in cervical specimens. Bottom rows show representative viral entry or blue versus green signal in CD4+ and CD4- T cells in mock- and virus-treated samples. For clarity, 80000 events are shown. Overall % HIV entry into CD4+ and CD4- T cells in PBMCs (B) and CMCs (C). N=20 and 24 for CMCs and PBMCs respectively.

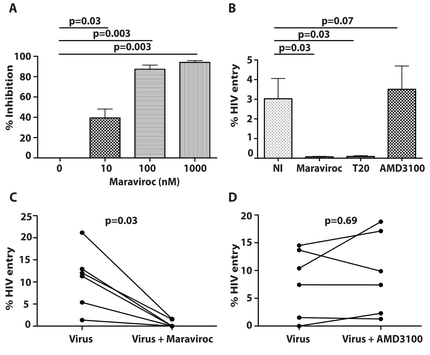


Figure 2. Effect of various HIV-entry inhibitors on cytosolic entry of CCR5-tropic BlaM-Vpr pseudovirus in CMCs and PBMCs. PBMCs were titrated with 0-1000nM maraviroc (A) and treated with 1μM maraviroc, 1μM fuzeon, or 250nM AMD3100 prior to addition of BlaM-Vpr pseudovirus (B). CMCs were split into three parts and treated with mock, virus, or virus plus 1μM maraviroc (C) or 250nM AMD3100 (D). All inhibitors were incubated with samples for 30 min at room temperature prior to addition of virus. NI=No inhibitor. N=6 for all panels.

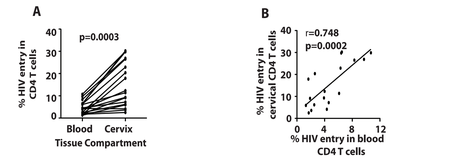


Figure 3. Relationship between blood and cervical CD4 T cell susceptibility to HIV entry. Comparison (A) and correlation (B) of HIV entry in CD4 T cells between PBMCs and CMCs from 18 matched samples. Median values are shown and statistical comparisons are done by Wilcoxon rank-pairs signed rank test. B=Blood, C=Cervix.

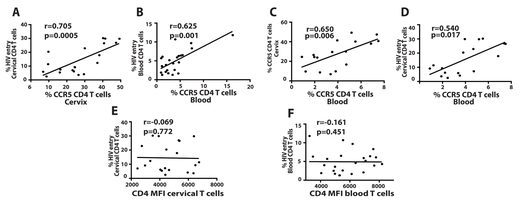


Figure 4. Relationship between CCR5 and CD4 expression and HIV entry. Correlation between % CCR5 CD4 T cells and HIV entry in CMCs (A) and PBMCs (B). Correlation of % CCR5 CD4 T cells in blood with % CCR5 CD4 T cells (C) and % HIV entry (D) in CMCs. Association between CD4 MFI and HIV entry in cervical (E) and blood (F) T cells. All correlations were performed by Spearman correlation. N=20 for CMCs and 24 for PBMCs.

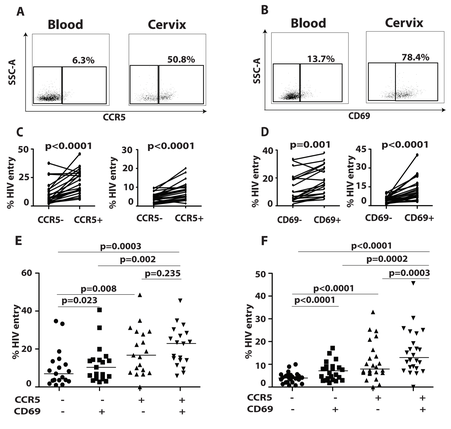


Figure 5. Preferential HIV entry in CCR5+ and CD69+ CD4 T cells. Gating strategy for CCR5+ and CCR5- CD4 T cells (A) and CD69+ and CD69- CD4 T cells (B) in blood (left) and cervical (right) compartments. Comparison of HIV entry between CCR5+ and CCR5- CD4 T cells and CD69+ and CD69- CD4 T cells in 20 cervical (C) and 24 blood (D) specimens. Comparison of HIV entry between CCR5-CD69-, CCR5-CD69+, CCR5+CD69- and CCR5+CD69+ CD4 T cells in 19 cervical (E) and 24 blood specimens (F). Graphs show median values and statistical comparisons are done by Wilcoxon rank-pairs signed rank test.

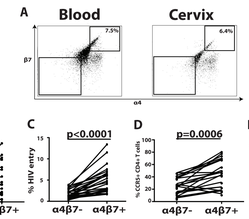


Figure 6. Comparison of HIV entry between $\alpha4\beta7$- and $\alpha4\beta7$+ CD4 T cells. Gating strategy for $\alpha4\beta7$+ and $\alpha4\beta7$- CD4 T cells in blood (left) and cervical (right) compartments (A). Comparison of HIV entry and % CCR5 expression in $\alpha4\beta7$+ and $\alpha4\beta7$- CD4 T cells subsets from cervical (B and D) and blood (C and E) specimens. N=20 for cervical and 24 for blood specimens.

Take Home Points

- The R5-tropic BlaM-Vpr HIV pseudovirus assay allows rapid and robust quantification of HIV entry into cervical CD4 T cells
- HIV entry into cervical T cells is CD4- and CCR5-dependent; however, it correlates with CCR5 expression but not CD4 MFI
- Cervical CD4 T cells are more susceptible to HIV entry than blood
- CD69+ and $\alpha4\beta7$+ CD4 T cells are preferential targets of HIV entry in cervix and blood, possibly due increased CCR5 expression
- CD4 T cell susceptibility in cervix correlates with CCR5 expression by and HIV entry into blood CD4 T cells

Acknowledgements

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