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 - α4β7. Measuring actual infection in cervical cells is a biologically relevant way to examine 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 pseudovirions in unmutilated cervical monocytic 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 pseudovirions 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 HIV infection such as CCR5, CD40, and α4β7.

Results: HIV entry in CMCs was specific to CD4 T cells, and blocked by CCR5 inhibition whereas not by CXCR4 inhibitor AMD3100. Cervical CD4 T cells demonstrated 2.8 fold greater HIV entry compared to blood matched participants. HIV entry in CMCs correlated between the two compartments and with CCR5 expression but not CD4 mean fluorescent intensity (MFI). Cervical CD69+ and CD69- CD4 T cells had 1.3 and 1.6 fold higher HIV entry than CD69- and CD69+ T cells (p<0.01) and p<0.002 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 CD4+ CD69 T cells in both blood and cervical compartments 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

Figure 1. HIV entry in CD4- and CD4+ T cells in PBMCs and PBMCs incubated in vitro 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 HIV infection such as CCR5, CD40, and α4β7.

Figure 2. Effect of various HIV entry inhibitors on cytosolic entry of CCR5-tropic BlaM-Vpr pseudoviruses in CMCs and PBMCs. PBMCs were treated with 0.1000 μl mAbs (A) and treated with 1.1000 μl mAbs, 1.1000 μl BlaM-Vpr, and 0.1000 μl CD40+ T cell line in PBMCs (B). All inhibitors were added in culture for 3 h at room temperature prior to addition of virus. N=4 inhibitor and 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.

Figure 4. Relationship between blood and cervical CD4 T cell susceptibility to HIV entry: Comparison (A) and correlation (B) of % CCR5 CD4 T cells in blood with % CCR5 CD4 T cells in cervical (E) and blood (D) specimens. N=20 for cervical and 24 for blood specimens.

Figure 5. Preferential HIV entry in CCR5+ and CD69+ CD4 T cells. Gating strategy for CCR5+ and CD69+ CD4 T cells (A) and CD69- and CCR5+ CD4 T cells (B and C). Percentage of HIV entry was calculated (D) for CCR5+ and CD69+ CD4 T cells in 20 cervical (E) 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.

Take Home Points

• The 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 CD69+ 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.

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