288

HIV INFECTIONS IN NOVEL CD1a CELLS FROM HUMAN VAGINAL MUCOSA

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Background

Transfer across mucosal surfaces, such as the vaginal mucosa, account for the majority of HIV-1 infections in the world. The identity and susceptibility of potential target cells in the vaginal mucosa (VM) remain unclear.

METHODS

Skin and vaginal cells were obtained from women undergoing elective reduction mammoplasties and vaginal repairs respectively. Discontinuous density gradients were used to isolate epithelial and subepithelial cells, and anti-human CD1a magnetic beads were used to further purify the epithelial cells. Isolated cells were characterized by using flow cytometry, electron-microscopy (EM) and Western blotting. Ability of different CCR5 (R5) or CXCR4-using (x4) HIV-1 to fuse, integrate, and replicate in these cells was examined by using the BlaM-Vpr fusion assay, Alu-PCR integration and growth curves respectively.

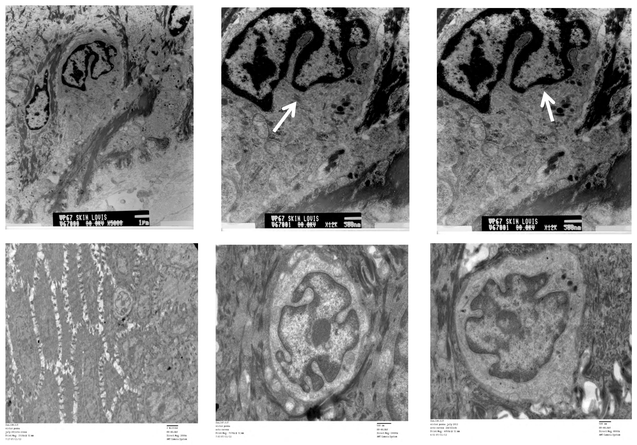
RESULTS

Similar to skin derived Langerhans cells (LCs), vaginal epithelial cells, termed CD1a+ VDCs , either contained low numbers or no Birbeck Granules, a hallmark of all LCs. HIV-1 Yu2 (R5) but not NL4-3 (X4) replicated in CD1a+ VDCs. The CD1a+ VDCs expressed the CXCR4 receptor, and NL4-3 both fused and integrated in the CD1a+ VDCs. Vaginal subepithelial tissue resident lymphocytes were CD69 and displayed a memory phenotype. Both YU2 and NL4-3 replicated in vaginal lymphocyte cultures. Interestingly, both NL4-3 and YU2 replicated in CD1a+ VDCs exposed to virus, washed and co-incubated with vaginal subepithelial lymphocytes 3 days post exposure.

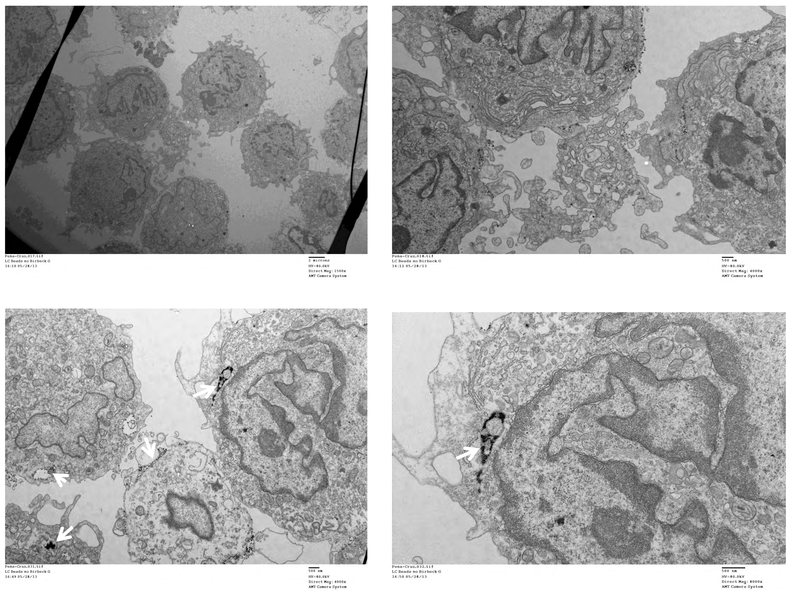
CONCLUSIONS

CD1a+ VDCs differentially support replication of CCR5- but not CXCR4-using HIV-1. These unique CD1a+ VDCs are located at the portal of virus entry and are only susceptible to types of HIV-1 strains that initiate most infections, which suggests that CD1a+ VDCs are likely the earliest target cells for HIV-1 in the vaginal mucosa.

Electron Microscopy:
Skin LCs with Birbeck Granules (BG) and VM with no BG.



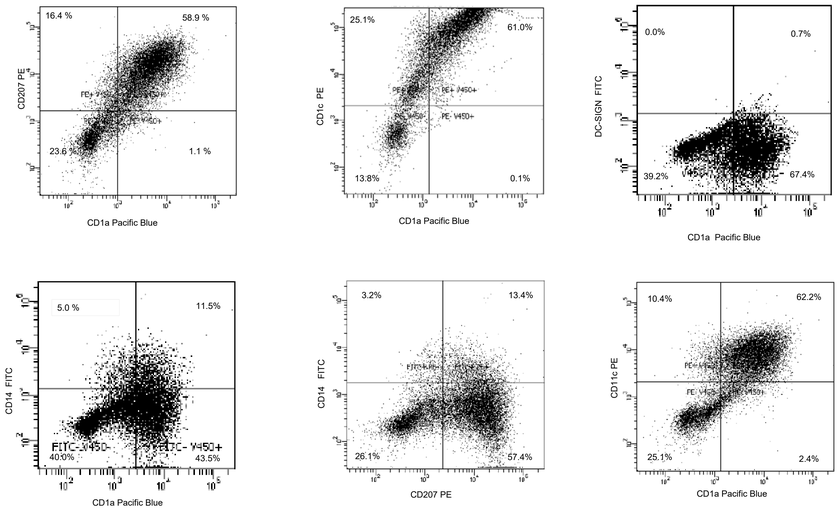
Extraction of CD1a cells: epidermis and epithelium are incubated with Dispase and trypsin. Cell suspension is Centrifuged through a density gradient then, CD1a beads.

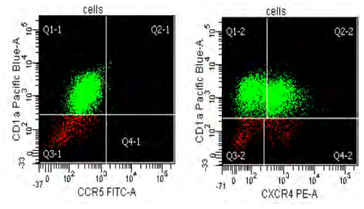


Arrows= anti- CD1a beads

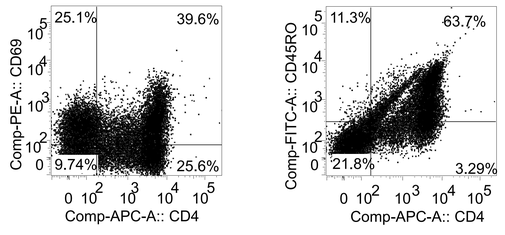
No BG

Facs analysis:Vaginal CD1a cells

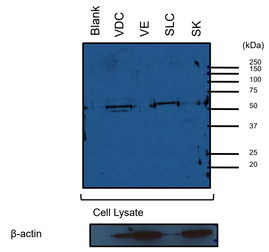




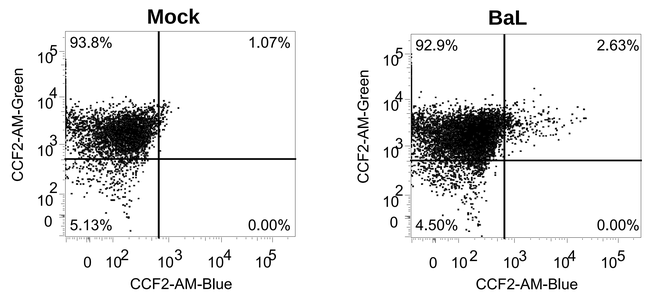
Vaginal Subepithelial T-cells

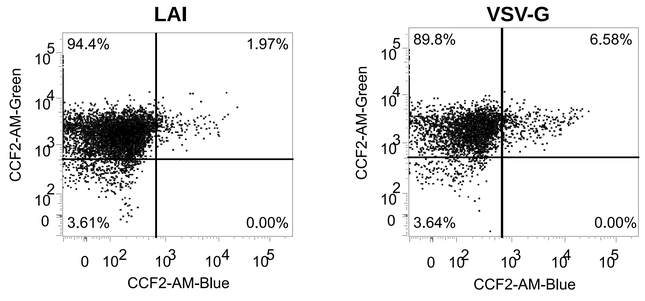


Western Blot for BG in SLC and CD1a+ VDCs

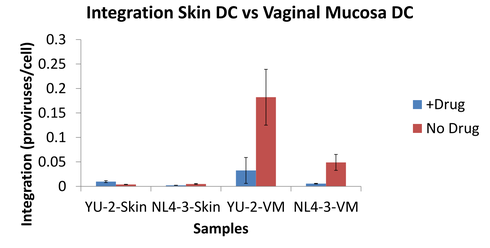


Virus Fusion





Virus Integration



Virus Growth

