The Role of Migratory Dendritic Cells in Establishing HIV Dissemination

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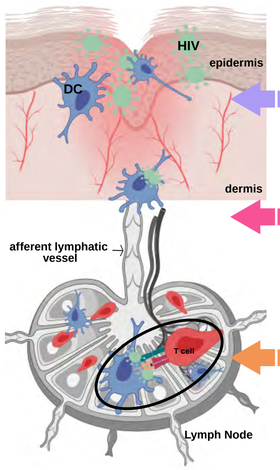
Summary

HIV-1 dissemination from the genital mucosal tract to the lymphoid organs is the first critical step towards systemic infection. HIV-1 can disseminate either as free-virus, or it can be transported to lymphoid tissues by migratory cells. Our previous studies strongly argued that the trafficking of cell-associated HIV-1 from the genital mucosa to lymphoid organs played a dominant role in viral spread early after sexual transmission in humanized mice. Here, we further extend these observations by addressing the role of migratory dendritic cells (DCs) in the capture, retention, and transfer of HIV-1 to susceptible CD4$^+$ T cells through *trans*-infection; a route of viral transmission that occurs through cell- to-cell contact. Using a three-dimensional (3D) model that recapitulates stromal networks of lymph nodes, we demonstrated that DCs retain their ability to migrate into lymph nodes following virus capture, and are able to *trans*-infect T cells by forming stable contacts, through env:CD4, and ICAM-1:LFA-1 interactions. This study successfully unravels the molecular mechanism that allows DC:T cells *trans*-infection to occur, and also confirms that DC trafficking can be an earliest target to block local transmission and limit HIV spread.

Global Question

To what extent do migratory DCs contribute to HIV acquisition and HIV dissemination during mucosal transmission?

Aims



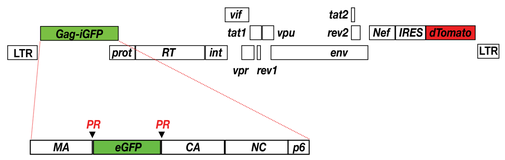
1) To investigate the capture and retention dynamics between DCs and HIV-1.

2) To determine whether HIV-captured DCs have the capacity to migrate towards the lymph node.

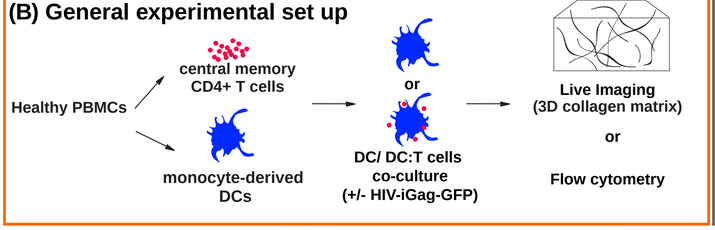
3) To investigate the molecular mechanisms that determine the contact dynamic between DC:T during *trans*-infection.

Experimental Approach

(A) Structure of the HIV-GAG-iGFP/dTOMATO

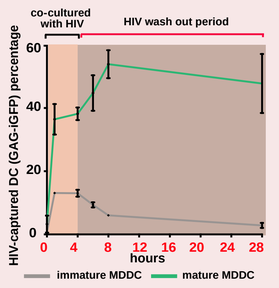


(B) General experimental set up



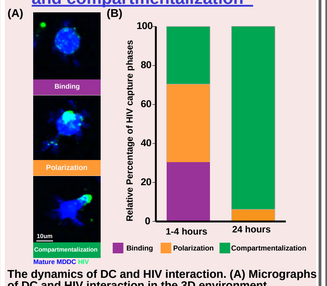
(1) Mature DCs can capture and retain HIV through compartmentalization over 24 hours

Mature DCs can capture and retain HIV more efficiently than immature DCs



Comparison of HIV capture percentage between immature and mature DCs.

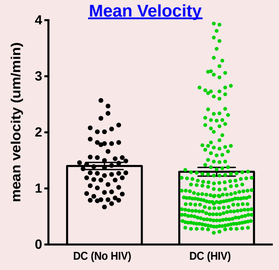
Mature DCs retain HIV via binding, polarization and compartmentalization



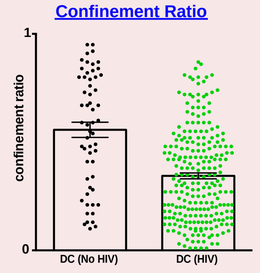
The dynamics of DC and HIV interaction. (A) Micrographs of DC and HIV interaction in the 3D environment, characterized by 3 phases: (1) binding, (2) polarization, (3) compartmentalization (B) Relative proportion of DC:HIV dynamics phases at 1-4 hrs and 24 hrs.

(2) HIV-captured DCs preserve their migratory ability in the 3D environment

Mean Velocity

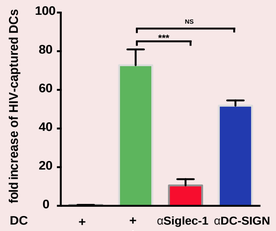


Confinement Ratio



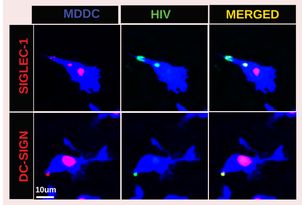
(3) Siglec-1, and to a lesser extent DC-SIGN, mediates HIV binding and compartmentalization

Blocking Siglec-1 receptor decreases HIV capture



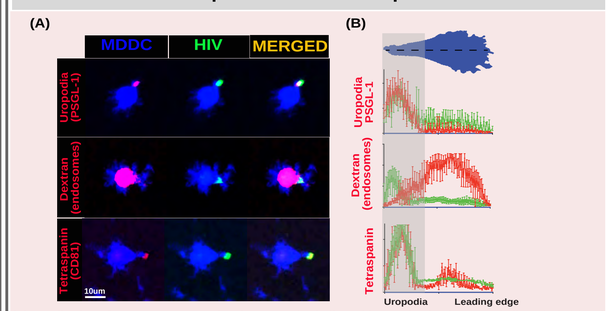
Difference in HIV capture capacity between untreated and treated DCs (+/- HIV).

DC retains HIV in a Siglec-1 receptor rich compartment



Co-localization of HIV (green) with Siglec-1/DC-SIGN receptors (red) in a migratory DC.

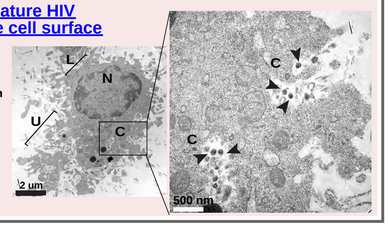
(4) Migratory DCs store HIV in a CD81 tetraspanin-rich compartment at the uropodia



(A) Localization of HIV compartments in migratory DCs, indicated by P-selectin glycoprotein ligand-1(PSGL-1) /dextran/ CD81 tetraspanin. (B) Fluorescence distribution of HIV (green line) and PSGL-1/dextran/ CD81 tetraspanin (red line) in migratory DCs. Mean fluorescent line profile through the center of DCs are shown.

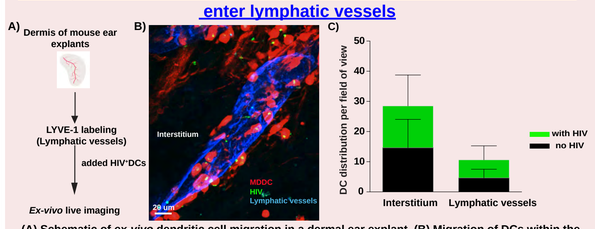
Uropodia retains mature HIV and are exposed to the cell surface

Transmission electron micrograph of HIV-captured migratory DCs. (N: nucleus. C:HIV compartment, L: leading edge, U: uropodia arrowhead: mature HIV)



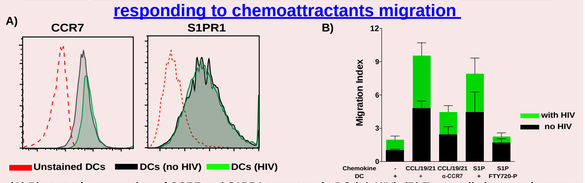
(5) HIV-captured DCs have the capacity to respond towards lymph node-homing chemokines CCL19/21 and S1P

HIV-captured DCs are able to migrate within the interstitial space and enter lymphatic vessels



(A) Schematic of *ex-vivo* dendritic cell migration in a dermal ear explant. (B) Migration of DCs within the dermis of ear explants. (C) Quantification of the number of DCs (+/-) within interstitium and lymphatic vessels calculated by morphometric analysis.

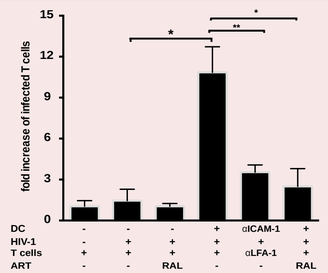
Blocking CCR7 and S1PR1 receptors prevents HIV-captured DCs responding to chemoattractants migration



(A) Phenotypic expression of CCR7 and S1PR1 receptors in DC (+/- HIV). (B) Transwell chemotaxis assays to assess the chemotactic properties of DCs (+/- HIV; treated/untreated) toward medium alone or medium supplemented with CCL19/21 or S1P.

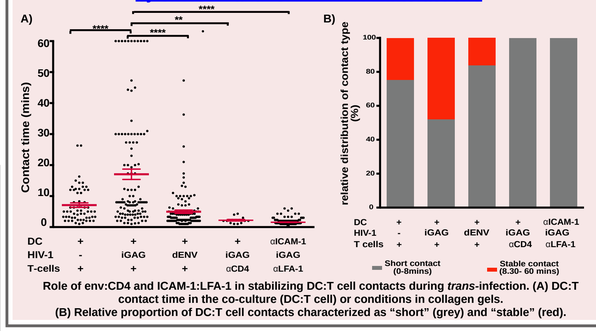
(6) Dynamic 3D characterization of HIV$^+$ DCs and T cells contacts that facilitate *trans*-infection

ICAM-1:LFA-1 blockade decreases DC:T cells *trans*-infection



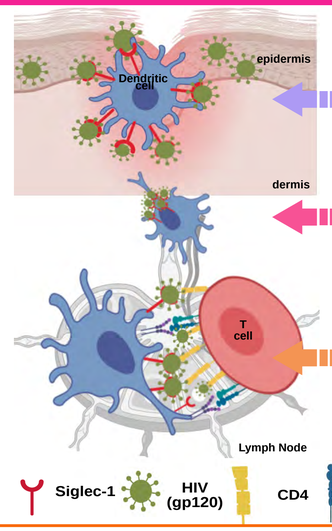
HIV dissemination through dynamic DC:T cell interactions in collagen. Number of fold increase in infected T cells, measured by dTOMATO expression, after the indicated co-culture (DC:T cell) or virus alone (cell-free) or conditions in collagen gels. Ral = Raltegravir.

DC:T cells *trans*-infection requires stable contact formation, mediated by env:CD4 and ICAM-1:LFA-1 interactions



Role of env:CD4 and ICAM-1:LFA-1 in stabilizing DC:T cell contacts during *trans*-infection. (A) DC:T contact time in the co-culture (DC:T cell) or conditions in collagen gels.
(B) Relative proportion of DC:T cell contacts characterized as "short" (grey) and "stable" (red).

Proposed DC:T cells *trans*-infection mechanism model



1) DCs bind to the HIV via Siglec-1 receptor.

2) DCs compartmentalize HIV, and migrate towards the lymph node.

3) Env:CD4 and ICAM-1:LFA-1 interactions stabilize DC:T cells contact to allow *trans*-infection.

Conclusions

1. Visual characterization of dynamic DC:T cell contact behaviors in a 3D matrix reveals that HIV alters the nature of these interactions.
2. DCs can readily compartmentalize HIV and migrate towards lymph node-homing chemokines to engage target CD4$^+$ T cells.
3. Siglec-1:HIV binding near the cell surface of migratory DCs function to restrain and facilitate stable contacts with motile CD4$^+$ T cells. Stability is further enhanced by ICAM-1:LFA-1 contacts to mediate *trans*-infection.

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