Vaginal Myeloid Dendritic Cells Transmit Founder HIV-1

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Running title: Vaginal DCs transmit HIV-1 founder virus

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Text: 1,230 words
Abstract: 72 words
ABSTRACT

We report that primary human vaginal dendritic cells (DCs) display a myeloid phenotype and express CD4, CCR5 and CXCR4. Vaginal CD13⁺CD11c⁺ DCs rapidly and efficiently bound transmitted/founder (T/F) CCR5-tropic (R5) viruses, transported them through explanted vaginal mucosa, and transmitted them in trans to vaginal and blood lymphocytes. Vaginal myeloid DCs may play a key role in capturing and disseminating T/F R5 HIV-1 in vivo and are candidate “gatekeeper” cells in HIV-1 transmission.

Key words: vaginal dendritic cells, transmitted/founder virus, vaginal mucosa, trans-infection, explant
Dendritic cells (DCs) play a critical role in HIV-1 transmission (1-12). We recently reported that DCs in human intestinal mucosa rapidly captured HIV-1, transported the virus through the intestinal lamina propria, and transmitted it \textit{in trans} to peripheral blood and intestinal lymphocytes, implicating intestinal DCs in HIV-1 entry through gut mucosa (13). In contrast, surprisingly little is known about the role of human vaginal DCs in heterosexual HIV-1 transmission. Using primary human vaginal cells and explanted vaginal mucosa, we show that vaginal myeloid DCs efficiently capture, transport and transmit transmitter/founder (T/F) viruses \textit{in trans} to vaginal lamina propria and systemic lymphocytes, suggesting that vaginal DCs are candidate “gatekeeper” cells in heterosexual HIV-1 transmission.

**Human vaginal lamina propria contains myeloid DCs that express HIV-1 receptors.** In normal vaginal mucosa from healthy women not receiving hormone therapy, CD11c$^+$ DCs were located predominantly in the basilar lamina propria and infrequently in the epithelium, whereas CD207$^+$ (langerin)$^+$ Langerhans cells were present predominantly in the lower region of the epithelium and not in the lamina propria (Fig. 1A). Among isolated vaginal mononuclear cells (MNLs) (12, 14) that expressed myeloid marker CD13, 9.1 $\pm$3.0% (n=11) expressed DC marker CD11c, indicating a myeloid DC population. Most vaginal CD13$^+$CD11c$^+$ DCs expressed HLA-DR, and a substantial proportion expressed DC-SIGN, mannose receptor CD206, maturation marker CD83, co-stimulatory molecule CD86, and lymph node homing receptor CCR7 (Fig. 1B). Importantly, vaginal DCs also expressed CD4 (36.6%), CCR5 (41.5%) and CXCR4 (31.4%), and a substantial proportion co-expressed CD4 plus CCR5 (19.7%) and CD4 plus CXCR4 (18.5%) (Fig. 1B,C). Thus, vaginal CD13$^+$CD11c$^+$ DCs express receptors that bind HIV-1 (12, 15-18). Except for DC-SIGN, the percentage of vaginal DCs that express these receptors was significantly different from that of control monocyte-derived DCs (MoDCs) (n=4, data not shown), underscoring the importance of using primary vaginal DCs in vaginal transmission studies.
**Vaginal myeloid DCs efficiently capture transmitted/founder (T/F) R5 virus.** In cultures of freshly isolated human vaginal MNLs inoculated with YU2 Env-pseudotyped GFP-Gag virus-like particles (VLPs) (19) at an MOI=10, VLPs were detected in 6.2% of CD13⁺CD11c⁺ DCs at 15 min, increasing to 11.9% of the DCs at 2 h post-inoculation by flow cytometry gating on the CD13⁺ cells for CD11c and GFP (Fig. 2A). Next, we determined the ability of isolated vaginal DCs to capture T/F viruses. To more closely mimic natural transmission, we used a pre-determined optimal MOI=0.1 (from MOIs=0.01-1.0) for infectious T/F viruses. Among vaginal MNLs isolated from a representative donor, 3.21% to 6.77% of the CD11c⁺ DCs bound T/F viruses and 1.46% bound control YU2 at an optimal MOI=0.1 at 2 h (Fig. 2B). The T/F viruses CH040, CH058 and CH077, which were used in these experiments, are subtype B viruses and were cloned from Fiebig stage II/III donors (20, 21). Vaginal CD11c⁺ DCs from 10 different donors captured T/F viruses CH040, CH058 and CH077 1.5-, 1.6- and 1.8-fold, respectively, more efficiently compared with the capture of YU2 (p=0.028, 0.035 and 0.006, respectively) (Fig. 2C), indicating that vaginal DCs efficiently captured T/F viruses. Interestingly, the magnitude of vaginal DC capture of T/F viruses is similar to the magnitude recently reported by Parrish and colleagues (22) for MoDC capture of T/F viruses. Finally, we investigated whether DCs in vaginal mucosa using tissue explants (13, 14, 23) could take up virus in situ. HIV-1-GFP VLPs inoculated onto explants were detected in cells expressing DC marker CD83 in vaginal mucosa 30 min post-inoculation (Fig. 2D). Thus, both isolated and tissue vaginal DCs capture HIV-1.

**Vaginal myeloid DCs capture and transport HIV-1 through vaginal mucosa.** In contrast to non-migrating Langerhans cells (24), mucosal DCs migrate to draining lymph nodes to present antigen to naive T cells (25). Therefore, we determined whether vaginal DCs could capture HIV-1 inoculated onto freshly constructed, leak-proof explants of vaginal mucosa, as we have previously described (14, 23), and transport the virus through the mucosa. YU2 or media were inoculated onto vaginal explants, and 2 h later the cells that had migrated through the tissue into the lower chamber were collected and
analyzed for HIV-1 by flow cytometry gating on CD13^+CD11c^+, CD13^+CD11c^- and CD3^+ for DCs, macrophages and lymphocytes, respectively, and using KC57-FITC to detect intracellular virus. Among the cells in the lower chamber, only CD13^+CD11c^+ cells, and neither CD13^+CD11c^- macrophages nor CD3^+ lymphocytes, contained HIV-1 (Fig. 3A). The migrating CD13^+CD11c^+ cells also expressed HLA-DR, DC-SIGN, CD206, CD83, CD86 and CCR7 (data not shown). Thus, the cells that capture and transport HIV-1 through vaginal mucosa within the first 2 h after inoculation are myeloid DCs. We next inoculated the apical surface of explants with T/F viruses CH058 and CH077 and 2 h later detected both viruses in CD13^+CD11c^- DCs in the lower chamber (Fig. 3B). In four separate vaginal tissues, DCs captured and transported T/F virus CH058 1.7-fold and CH077 1.6-fold more efficiently than chronic virus YU2 (Fig. 3C).

**Vaginal myeloid DCs efficiently transmit T/F HIV-1 in trans to lymphocytes.** Having shown that mDCs are the only lamina propria cells that capture and transport T/F HIV-1 through explanted vaginal mucosa (Fig. 3), we next explored whether the DCs that migrated through the mucosa could transmit infectious virus to target mononuclear cells. YU2 HIV-1 was applied to the apical surface of vaginal explants, and 2 h later cells that had migrated into the lower chamber were collected and co-cultured with PHA-stimulated heterologous peripheral blood lymphocytes (PBLs) or autologous vaginal lamina propria MNLs for up to 4 days, after which the cells were analyzed for HIV-1 replication by flow cytometry, using KC57-FITC intracellular staining of CD3^+ T cells and p24 ELISA. Cells that migrated into the lower chamber of explants inoculated with YU2, but not supernatant or cells from media-inoculated explants, infected both blood (Fig. 4A) and vaginal T cells (Fig. 4C) in trans, resulting in progressive p24 production in the cultures of migrated DCs plus either PBLs (Fig. 4B) or mucosal MNLs (Fig. 4D). Virus replication occurred exclusively in the PBLs and MNLs, since ≥95% mucosal DCs die within 24 of culture (data not shown). Thus, infectious R5 virus inoculated onto explanted vaginal tissue was taken up by vaginal DCs, transported through the mucosa...
and trans-infected systemic and mucosal mononuclear target cells. We performed the same trans-
infection assay with T/F virus or chronic R5 virus. Due to the small size of each vaginal specimen,
only a limited number of explants could be established, allowing evaluation of only two T/F viruses
and one control virus per donor tissue. CD11c+ DCs in vaginal mucosa from four separate donors
transmitted T/F viruses CH058 2.0-fold and CH077 1.9-fold compared with the chronic virus YU2
(n=4, p=0.029) to blood lymphocytes (Fig. 4E,F). Thus, vaginal DCs efficiently captured and
transported T/F virus through the mucosa and trans-infected lymphocytes.

Here we used primary human vaginal cells and tissues to elucidate the role of vaginal DCs in
HIV-1 transmission biology. Vaginal DCs captured HIV-1 several-fold more efficiently than MoDCs,
possibly due to the higher expression level of receptors that bind HIV-1, and trans-infected
lymphocytes (2, 15-18). Vaginal myeloid DCs also more efficiently captured T/F viruses and more
efficiently transported T/F viruses through vaginal mucosa than control YU2 virus. Further, vaginal
myeloid DCs transmitted T/F viruses to lymphocytes with significantly higher efficiency compared
with YU2. Together, these findings suggest that vaginal DCs play a key role in HIV-1 genital
transmission and could contribute to the selection of founder virus in heterosexual HIV-1 transmission.

Acknowledgments

This work was supported by National Institutes of Health grants AI093151 and AI106395 (RS),
AI083127, RR-20136 and DK064400 (Mucosal HIV and Immunobiology Center) (PDS); UAB Center
for AIDS Research (CFAR) and Comprehensive Cancer Center Pilot Grant Program (RS);
Immunology, Autoimmunity and Transplantation Strategic Planning (IAT) and amfAR, the Foundation
for AIDS Research 108015-49-RGRL (PDS); and the Research Service of the Veterans Administration
(PDS and JCK).
REFERENCES


**FIGURE LEGENDS**

**Figure 1.** Tissue localization and phenotype of human vaginal DCs.  
A. Vaginal DCs (CD11c+) and Langerhans cells (CD207+) display distinct localization in intact vaginal mucosa.  
B. CD13+CD11c+ vaginal DCs display myeloid features and differ from control CD13+CD11c+ monocyte-derived DCs (MoDCs) in the levels of receptor expression maturation and activation. Profiles are from a representative donor (n=4).  
C. Expression of HIV-1 receptor and co-receptor on vaginal DCs and MoDCs. MNLs were isolated from hysterectomized vaginal tissue from otherwise healthy women not receiving hormone therapy, stained with fluorescence-conjugated antibodies to CD11c and the indicated markers, and analyzed by flow cytometry by gating on the CD13+CD11c+ population.

**Figure 2.** Vaginal myeloid DCs efficiently take up T/F R5 virus.  
A. DCs among isolated vaginal MNLs capture HIV-1. Cultures of isolated vaginal MNLs were inoculated with YU2 envelope pseudotyped GFP-Gag virus-like particles (VLPs) and incubated at 37°C. At 0, 15, 30 and 120 min post-inoculation, cells were harvested and analyzed by flow cytometry gating on CD13+ cells for CD11c and GFP.  
B,C. Vaginal myeloid DCs efficiently capture T/F viruses. Vaginal MNLs from a representative donor (B) and 7-10 donors (C) were incubated for 2 h with T/F viruses CH040, CH058 and CH077 or control virus YU2 at MOI=0.1 and then analyzed by flow cytometry for CD13+CD11c+ cells containing HIV-1, gating on CD13+ myeloid cells positive for KC57-FITC intracellular staining. Values in C are % HIV-1+ CD13+CD11c+ cells among vaginal MNLs from individual donors; bars correspond to mean ±SD; p values were calculated by the Mann-Whitney test.  
D. Vaginal myeloid DCs capture HIV-1 in explanted mucosa. YU2-GFP VLPs were inoculated onto the apical surface of explanted vaginal mucosa, and 30 min post-inoculation explants were harvested, sectioned, stained and analyzed by confocal microscopy for CD83+ DCs containing GFP-tagged VLPs.
Figure 3. Vaginal myeloid DCs capture and transport T/F HIV-1 through vaginal mucosa. A. Vaginal myeloid DCs, not macrophages or lymphocytes, capture and transport HIV-1 through vaginal mucosa. YU2 was inoculated onto the apical surface of explanted vaginal mucosa, and, after 2 h incubation, the cells that had migrated into the lower chamber were harvested and analyzed by flow cytometry using antibodies CD11c-APC, CD13-APC, CD3-PE to identify CD13⁺CD11c⁺ DCs, CD13⁺CD11c⁻ macrophages and CD3⁺ lymphocytes, respectively, and KC57-FITC to detect intracellular virus. B,C. Vaginal myeloid DCs capture and transport T/F R5 virus CH058 1.7-fold and CH077 1.6-fold more efficiently compared with control virus YU2 (n=4, p=0.057 and 0.10, Mann-Whitney test). Profiles in B were from a representative donor; values in C are from 4 donors with mean ±SD.

Figure 4. Vaginal myeloid DCs that capture and transport T/F HIV-1 through vaginal mucosa efficiently transmit T/F viruses to lymphocytes. A-D. Vaginal myeloid DCs transmit HIV-1 to peripheral blood lymphocytes (PBLs) and vaginal lymphocytes. YU2 was inoculated onto the apical surface of explanted vaginal mucosa, and 2 h later the cells in the lower chamber were harvested and co-cultured with PHA-stimulated PBLs or vaginal lamina propria MNLs. After 4 days incubation, cells were analyzed by flow cytometry for HIV-1 replication using KC57-FITC intracellular staining and gating on CD3⁺ T cells (A,C). YU2 replication also was determined by p24 ELISA on days 2 and 4 (B,D). E,F. Vaginal DCs transmitted T/F virus CH058 2.0-fold and CH077 1.96-fold more efficiently than control virus YU2 (n=4; p=0.029 and 0.029, Mann-Whitney test). Assay was performed with explanted vaginal mucosa as in A,B with the indicated viruses. Profiles in E are from a representative donor. Values in F are from 4 donors; with bars correspond to mean ±SD.
Fig. 1

A

H&E

CD11c, DAPI

CD207, DAPI

Isotype Control, DAPI

B

Vaginal DCs

MoDCs

CD11c

CD13

CD86

CCR7

CD4

CCR5

CXCR4

MoDCs

CD86

CCR7

CD4

CCR5

CXCR4

C

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<th>( P )-Value</th>
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<tr>
<td>CCR5</td>
<td>41.5 ± 20.8</td>
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<td>CXCR4</td>
<td>31.4 ± 12.4</td>
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<td>CD4(^+)CCR5(^+)</td>
<td>19.7 ± 9.3</td>
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<td>CD4(^+)CXCR4(^+)</td>
<td>18.5 ± 9.6</td>
<td>1.1 ± 0.5</td>
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\(^{a}\)Mean percentage of positive cells (\( n = 4 \))

\(^{b}\)SD: Standard deviation
Fig. 2

A. CD11c

B. CH040, CH058, CH077, YU2

C. % HIV-1+ CD11c+ cells

D. CD83, HIV-GFP, Merge
Fig. 3

A

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<tr>
<td>Dendritic cells (CD13⁺CD11c⁺)</td>
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<td>Macrophages (CD13⁺CD11c⁻)</td>
<td>0.35</td>
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<td>Lymphocytes (CD3⁺)</td>
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B

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<tr>
<td>CD11c</td>
<td>CD11c</td>
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<tr>
<td>p = 0.057</td>
<td>p = 0.10</td>
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C

% HIV-1⁺CD11c⁺ cells

CH058 | CH077 | YU2

p = 0.057 | p = 0.10
Fig. 4

A Control

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<tr>
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<th>Migrated cells + PBLs</th>
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<td>CD3</td>
<td>98.3</td>
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B 2 4

Days Post-infection

C Control

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<th>Supernatant + vMNLs</th>
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<td>CD3</td>
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D 2 4

Days Post-infection

E CH058 CH077 YU2 Media

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<td>HIV-1&lt;FL1-A&gt;</td>
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<td>CD3</td>
<td>98.6</td>
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F 0.0 0.5 1.0 1.5

% HIV-1+ lymphocytes

p = 0.0286

p = 0.0286