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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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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 *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 *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 ±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

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FIGURE LEGENDS

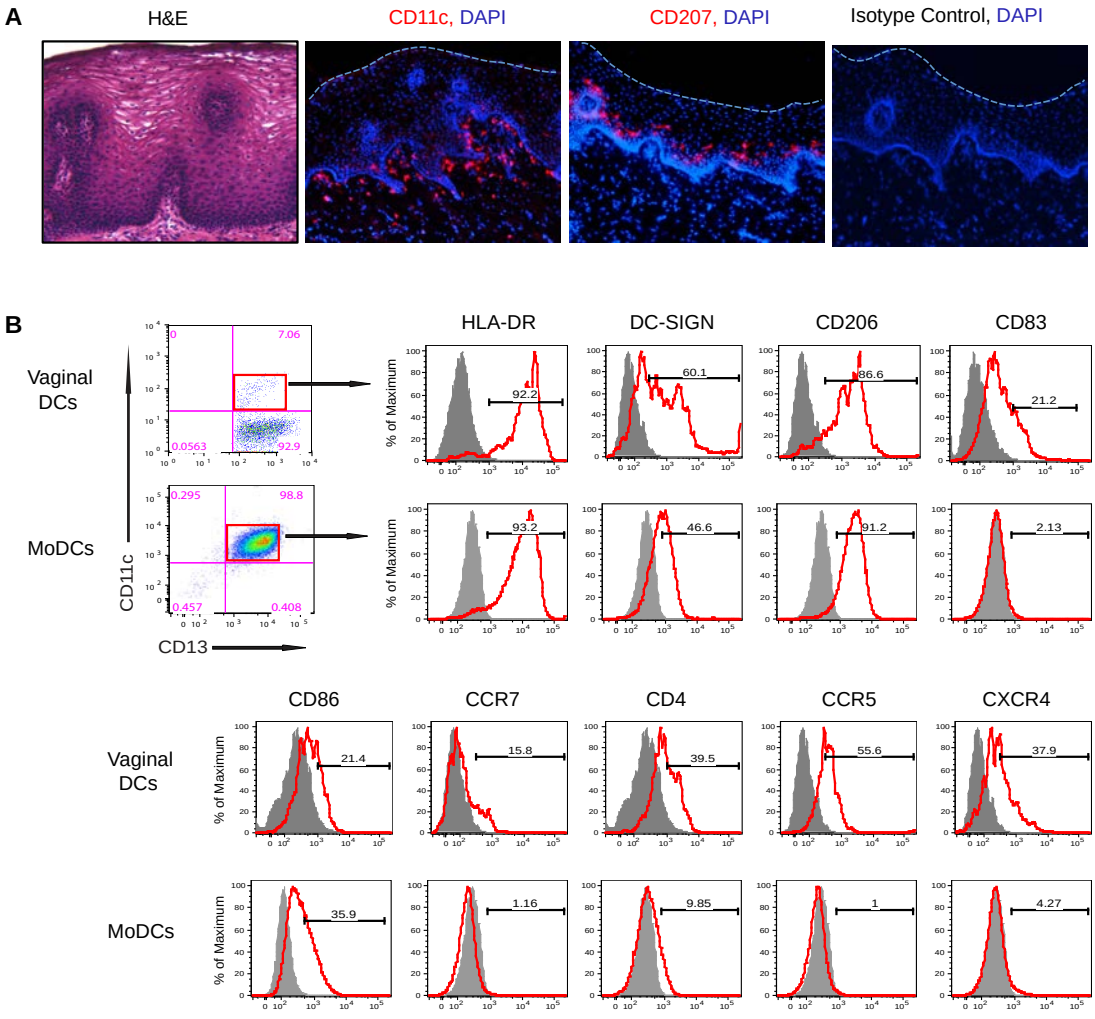
Figure 1. Tissue localization and phenotype of human vaginal DCs. **A.** Vaginal DCs ($CD11c^+$) and Langerhans cells ($CD207^+$) display distinct localization in intact vaginal mucosa. **B.** $CD13^+CD11c^+$ vaginal DCs display myeloid features and differ from control $CD13^+CD11c^+$ monocyte-derived 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 37^0C. 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 R5 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 % $HIV\text{-}1^+$ $CD13^+CD11c^+$ 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



C

	Vaginal DCs		MoDCs		*P*-Value
	Mean[a]	SD[b]	Mean	SD	
$CD4^+$	36.6	15.1	4.3	4.1	0.0095
$CCR5^+$	41.5	20.8	0.6	0.5	0.0095
$CXCR4^+$	31.4	12.4	1.6	1.4	0.0357
$CD4^+CCR5^+$	19.7	9.3	0.2	0.1	0.0095
$CD4^+CXCR4^+$	18.5	9.6	1.1	0.5	0.0357

[a] Mean percentage of positive cells (n = 4)

[b] SD: Standard deviation

Fig. 2

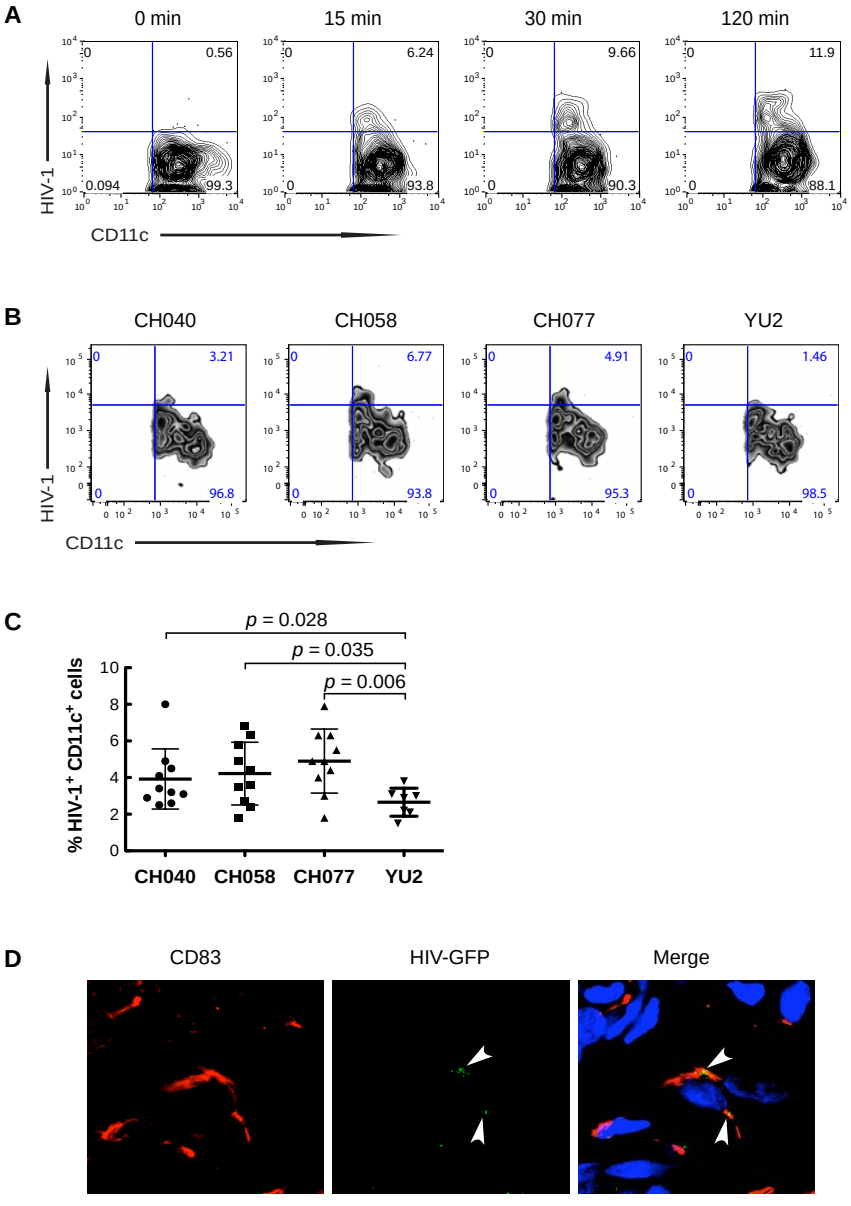


Fig. 3

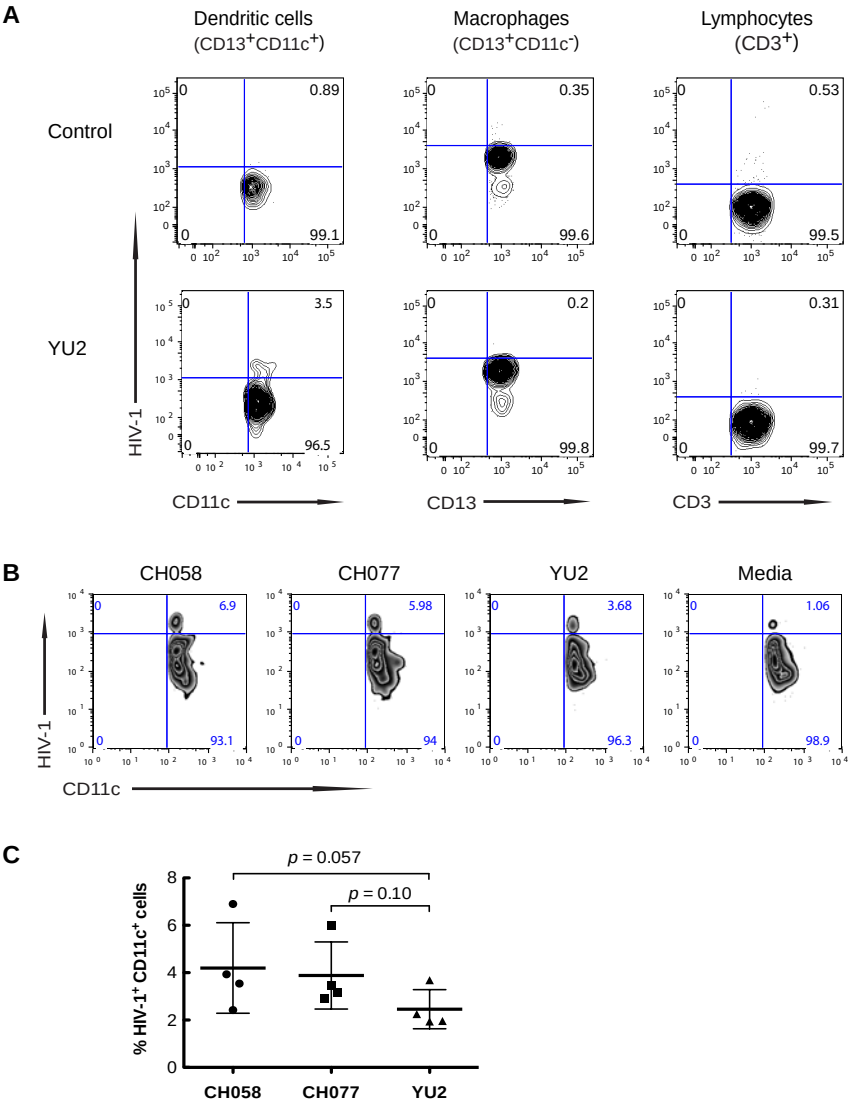


Fig. 4