The "gatekeeper" hypothesis challenged in a human cervico-vaginal tissue model for HIV-1 transmission

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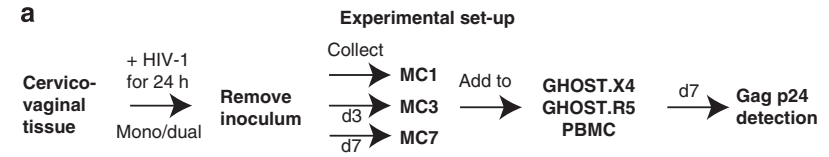
To the Editor: In an article published in a recent issue of *Mucosal Immunology*, Saba *et al.*[1] describe new insights in the early events of human immunodeficiency virus (HIV)-1 infection and investigate the "gatekeeping" mechanism that selects between R5 and X4 tropic HIV-1. Using the R5 BaL and X4 LAI labstrains, the authors conclude that productive infection of cervico-vaginal T lymphocytes with R5 virus is much more efficient compared with X4 HIV-1. Although informative, the replication kinetics and biological properties of labstrains are not representative for HIV-1 circulating in humans.

Earlier work from our laboratory showed that paired R5 and X4 biological clones isolated from the quasispecies of HIV+ individuals replicated equally well in activated T cells, whereas X4 tropic clones replicated to higher levels in cocultures of dendritic cells (DCs) and CD4+ T lymphocytes.[2,3] We found that this may be related to changes in coreceptor expression upon T-lymphocyte stimulation by DCs.[2]

We have now pursued this work and performed dual infection/competition experiments with equal infectious dose of R5 and X4 biological clones isolated



b Monoinfections with R5 and X4 biological clones

		GHOST.R5 Migratory cells			GHOST.X4 Migratory cells			PBMC Migratory cells		
VI	CR	d1	d3	d7	d1	d3	d7	d1	d3	d7
820-1	R5	+	+	+	–	–	–	+	+	+
820-4	X4	–	–	–	+	+	+	+	+	+
761-1	R5	+	+	+	–	–	–	+	+	+
761-4	X4	–	–	–	+/–	+	+	+	+	+
943-3	R5	+	+	+	–	–	–	+	+	+
943-4	R5	+	+	+	–	–	–	+	+	+
943-1	X4	–	–	–	+	+	+	+	+	+
943-2	X4	–	–	–	+	+/–	–	+	+	+

c Dual infections with R5 and X4 biological clones

		GHOST.R5 Migratory cells			GHOST.X4 Migratory cells			PBMC Migratory cells		
R5	X4	d1	d3	d7	d1	d3	d7	d1	d3	d7
820-1	820-4	+	+	+/–	+	+	+	+	+	+
761-1	761-4	+	+	+	+	+	+/–	+	+	+
943-3	943-1	+	+	+/–	+	+	+	+	+	+
943-3	943-2	+	+	+	+/–	–	–	+	+	+
943-4	943-1	+	+	+	+	+/–	+	+	+	+
943-4	943-2	+	+	+	+	+/–	–	+	+	+

\+ High-level replication
+/– Low-level replication
– No replication

Figure 1 (**a**) Experimental set-up: cervico-vaginal tissue was obtained from three premenopausal women undergoing hysterectomy, and sliced in blocks of approximately 3×3×3 mm. Cervico-vaginal tissue was exposed to 500 TCID50 (50% tissue culture infective dose) of either X4, R5, or an equal-infectious-dose mixture of X4 and R5 human immunodeficiency virus (HIV)-1, in 24-well tissue culture plates. Virus titres were determined on phytohemagglutinin (PHA)/interleukin (IL)-2-activated peripheral blood mononuclear cells (PBMCs) from an HIV-negative blood donor. Eight biological clones from three patient isolates were used (VI820—CRF02_AG, VI761—subtype D, and VI943—subtype B).[2,3] Migratory cells (MCs) were collected 24 h post-infection and washed 5× with RPMI medium containing 10% fetal calf serum. This MC fraction was termed MC1. At the same time, each tissue explant was extensively washed (5×) to remove the inoculum. Cervico-vaginal explants were further maintained in cell culture medium for 7 days and additional MC fractions were collected on days 3 and 7 post-infection. Equal numbers of MC1, MC3, and MC7 were incubated with GHOST.X4, GHOST.R5, and PHA/IL-2-stimulated PBMCs. Virus replication was measured in culture supernatant at day 7 of co-incubation, using a Gag p24 antigen detection enzyme-linked immunosorbent assay (ELISA). (**b**) Control infections with single viruses (monoinfections) showed that MC1, MC3, and MC7 from cervico-vaginal tissue are able to transfer both X4 and R5 HIV-1 to PHA/IL-2-activated PBMCs, whereas productive infection of X4 and R5 viruses was only detected in GHOST.X4 and GHOST.R5 cells, respectively. This observation confirmed the coreceptor tropism of the different biological HIV-1 clones. (**c**) Dual infections/competitions with equal-infectious-dose mixtures of X4 and R5 clones revealed that both biotypes are able to infect cervico-vaginal tissue. (+) High-level replication: optical density (OD) >1.0 in ELISA, (+/–) low-level replication: OD >0.2 and <1.0, (–) no replication: OD <0.2. A typical background control in our Gag p24 ELISA generates OD values <0.1. VI, virus isolate; CR, coreceptor.

from HIV+ patients in cervico-vaginal tissue obtained from premenopausal women undergoing hysterectomy. The viral inoculum was removed 24 h post-infection and migratory cells (MCs)[4] were harvested at 1, 3, and 7 days post-infection. Subsequently, equal amounts of MCs were added to GHOST.X4 and GHOST.R5 cell lines and to phytohemagglutinin (PHA)/interleukin-2-activated peripheral blood mononuclear cells (PBMCs) (**Figure 1a**). Gag p24 levels were measured 7 days after onset of coculture. MCs appeared to efficiently disseminate both R5 and X4 variants to activated PBMC both in mono- and dual infections (**Figure 1b** and **c**). As expected, MCs carrying R5 HIV-1 or X4 HIV-1 were able to disseminate virus almost exclusively to GHOST.R5 or GHOST.X4 cells, respectively. Interestingly, dual infections/competitions with R5 and X4 biological clones from the same patient showed that both clones are transferred efficiently from MCs to GHOST.R5, GHOST.X4, and PBMCs. This observation was performed with eight biological clones from three different HIV+ patients in three independent tissue donors.

In contrast to the results published by Saba *et al.*,[1] showing that the X4 LAI virus does not replicate in cervico-vaginal tissue, our findings suggest that both R5- and X4-tropic HIV-1 can infect MCs in cervico-vaginal tissue and that both can efficiently be transferred onto other HIV target cells (e.g., T cells).

Seminal work by Hu *et al.*[4] showed that the MC fraction from cervico-vaginal tissue roughly consists of $CD3^-HLA\text{-}DR^+$ (i.e., DC-SIGN+ DC) and $CD3^+HLA\text{-}DR^-$ cells (i.e., T cells) that emigrate out of the explant in two phases. While the number of emigrating lymphocytes increased over time, the majority of DC migrated quickly within the first 24 h and carried most of the infectious HIV. It is well established that DC can transfer HIV to T cells bo th in *trans* and in *cis*. We have collected MC fractions at 1, 3, and 7 days post-inoculation and found consistently that both R5 and X4 viruses were efficiently transferred to PHA-activated T lymphocytes. Although it is still unclear how long DCs can retain HIV in an infectious state in the absence of productive infection, this process is supposed to last somewhere between hours and 2–3 days.[5] Although our observation with MC from days 1 and 3 may result from virus retained by DC and transfer to PHA-activated T lymphocytes, the results with day 7 MC strongly suggest that productive infection of DC and/or T lymphocytes within the MC also occurs. This observation is in line with recent evidence showing focal infected founder populations of cells (DCs and T lymphocytes) in the endocervix.[6]

Altogether, our observations suggest that other mechanisms than coreceptor expression at the cervico-vaginal site are underlying the "gatekeeping" mechanism upon sexual HIV transmission.

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DISCLOSURE

The authors declare no conflict of interest.

REFERENCES

1. Saba, E. *et al.* HIV-1 sexual transmission: early events of HIV-1 infection of human cervico-vaginal tissue in an optimized *ex vivo* model. *Mucosal. Immunol.* **3,** 280–290 (2010).
2. Ariën, K.K., Gali, Y., El-Abdellati, A., Heyndrickx, L., Janssens, W. & Vanham, G. Replicative fitness of CCR5-using and CXCR4-using human immunodeficiency virus type 1 biological clones. *Virology* **347,** 65–74 (2006).
3. Vanham, G. *et al.* Dendritic cells, exposed to primary, mixed phenotype HIV-1 isolates preferentially, but not exclusively, replicate CCR5-using clones. *AIDS* **14,** 1874–1876 (2000).
4. Hu, Q. *et al.* Blockade of attachment and fusion receptors inhibits HIV-1 infection of human cervical tissue. *J. Exp. Med.* **199,** 1065–1075 (2004).
5. Turville, S.G. *et al.* Immunodeficiency virus uptake, turnover, and 2-phase transfer in human dendritic cells. *Blood* **103,** 2170–2179 (2004).
6. Li, Q. *et al.* Glycerol monolaurate prevents mucosal SIV transmission. *Nature* **458,** 1034–1038 (2009).

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Response to "The "gatekeeper" hypothesis challenged in a human cervico-vaginal tissue model for HIV-1 transmission"

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"***Eppur si esiste*** **(And yet it exists)"**

To the Editor: The exclusive ability of HIV-1_{R5} (not HIV-1_{X4}) to transmit infection *in vivo* suggests the existence of "gatekeeping" mechanisms that select one HIV variant over the other. In response to our paper,[1] which showed that human cervico-vaginal tissue *ex vivo* preferentially supports the productive infection of HIV-1_{R5} rather than that of HIV-1_{X4}, Ariën *et al.*[2] report that cervico-vaginal migratory cells (MCs) are able to transfer equally both HIV-1_{R5} and HIV-1_{X4} to CD4-CCR5- and CD4-CXR4-transfected osteosarcoma cell lines and to activated peripheral blood mononuclear cells. Ariën *et al.* concluded that, "in contrast" with our results, their "findings suggest that both HIV-1_{R5} and HIV-1_{X4} can infect MCs and be transferred onto other HIV-1 target cells".

We think that the results of our two groups are complementary rather than contradictory: the fact that HIV-1_{R5} replicates more efficiently than HIV-1_{X4} in cervico-vaginal tissue does not contradict the evidence that both infectious HIV-1_{X4} and HIV-1_{R5} virions can be retained by inoculated tissue in or on dendritic