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.

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

E Saba[1,2], J-C Grivel[1], C Vanpouille[1], B Brichacek[1], W Fitzgerald[1], L Margolis[1] and A Lisco[1]

[1]Section of Intercellular Interactions, Program in Physical Biology, Eunice Kennedy-Shriver National Institute of Child and Human Development, National Institutes of Health, Bethesda, Maryland, USA. [2]San Raffaele Vita-Salute University, Dibit, AIDS Immunopathogenesis Unit, Milan, Italy. Correspondence: L Margolis (margolil@mail.nih.gov) or A Lisco (liscoa@mail.nih.gov)

"***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

cells, macrophages, T-lymphocytes, or other cells and that such virus could be rescued from cervico-vaginal MCs (with phenotype and biological features yet to be defined) under appropriate experimental conditions. Moreover, quantitative rather than qualitative data on HIV-1_{R5}/HIV-1_{X4} replication in MC co-cultures with indicator cells, as well as in cervico-vaginal tissues themselves, should be presented by Ariën *et al.* to conclude that HIV-1_{R5} and HIV-1_{X4} are transferred by MCs *equally* efficiently.

Recent reports[3,4] demonstrate that HIV-1 mucosal transmission requires a local expansion of a small founder population of infected cells in the cervico-vaginal mucosa in order to establish a systemic infection. Thus, a robust viral production in the genital tract rather than the simple retention and transfer of infectious particles appears to be critical for HIV-1 transmission. Therefore, in contrast to Ariën *et al.*, we believe that our results indicate that one of the HIV-1_{X4} gatekeeping mechanisms exist in the cervico-vaginal mucosa, preventing HIV-1_{X4} from replicating as efficiently as HIV-1_{R5}.

Nevertheless, we agree with Ariën *et al.* that other mechanisms beyond the simple difference in coreceptor expression in cervico-vaginal cells are underlying gatekeeping: As we stated in our paper and elsewhere[2,5] and demonstrated for cervico-vaginal mucosa, there is no sole and exclusive impenetrable gatekeeper but rather multiple imperfect gatekeepers at different anatomical sites that collectively prevent HIV-1_{X4} transmission via different routes.[5] Future studies should identify the nature and decipher the molecular mechanisms of these gatekeeping barriers to develop new HIV-1 preventive strategies.

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