Barriers that limit cell-free HIV-1 entry into macrophages are overcome after cell-cell fusion with infected T cells.

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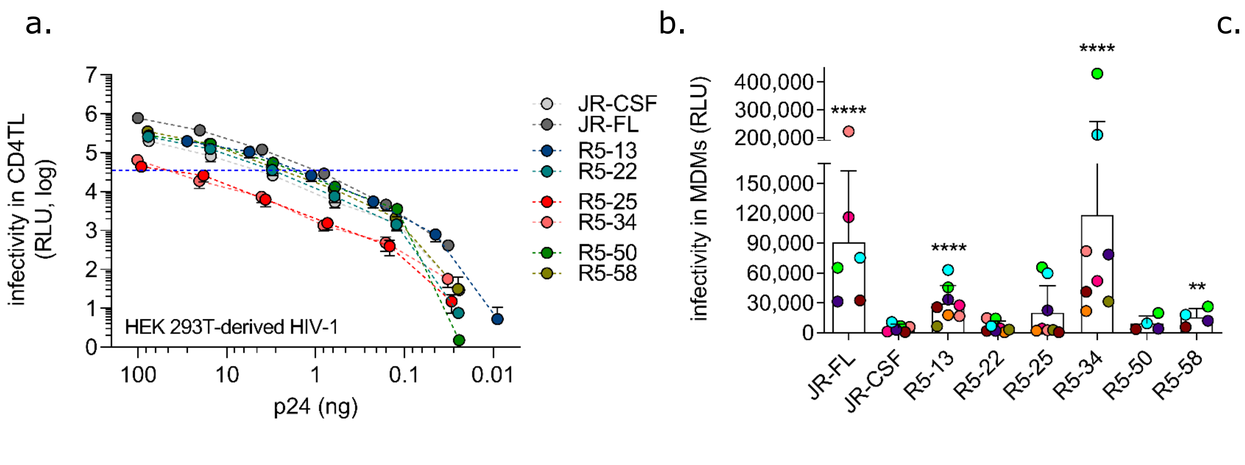
✔ Macrophages (MΦ) contribute to dissemination and persistence of HIV-1. In persons living with HIV-1, infected MΦ are found in a wide range of tissues. Paradoxically, cellular tropism assays indicated that HIV-1 isolates are T-tropic and only rarely M-tropic, most often due to inefficient viral entry into MΦ. However, these assays, because they use cell-free viral particles, might not reflect all modes of MΦ infection in vivo, in particular through cell-to-cell viral transfer. Here, we investigated whether virus isolates, previously characterized as non-M-tropic viruses in cell-free infection assays, could efficiently infect MΦ through cell-cell fusion with infected CD4+ T cells.

✔ We investigated the capacity of CCR5- and/or CXCR4-using Env-pseudotyped viruses and infectious molecular clones to infect MΦ by a cell-free mode or through viral transfer from infected CD4+ Jurkat T cells. Envs representative of the different stages of HIV-1 infection were used, including transmitted/founder Envs known to be non-M-tropic.

✔ Single-round infection and virus-cell fusion assays showed that most of these viruses in the form of cell-free particles are inefficient to enter MΦ, whereas they can effectively infect primary CD4+ T cells (**Fig. 1**). In contrast, **all viruses were efficiently transferred to MΦ after Env-dependent cell-cell fusion of MΦ with infected CD4+ T cells**, ultimately leading in most cases to productively infected multinucleated giant cells (MGCs) (**Fig. 2**). Results also showed that MΦ infection through cell-cell fusion with infected T cells overcome barriers that normally restrict entry of cell-free viruses into MΦ. Indeed, **formation of infected T cell/MΦ conjugates enhanced interactions between Env and CD4 and CCR5** (**Fig. 3** and **Fig. 4**), rendering viral entry into MΦ less dependent on expression levels of those receptors.

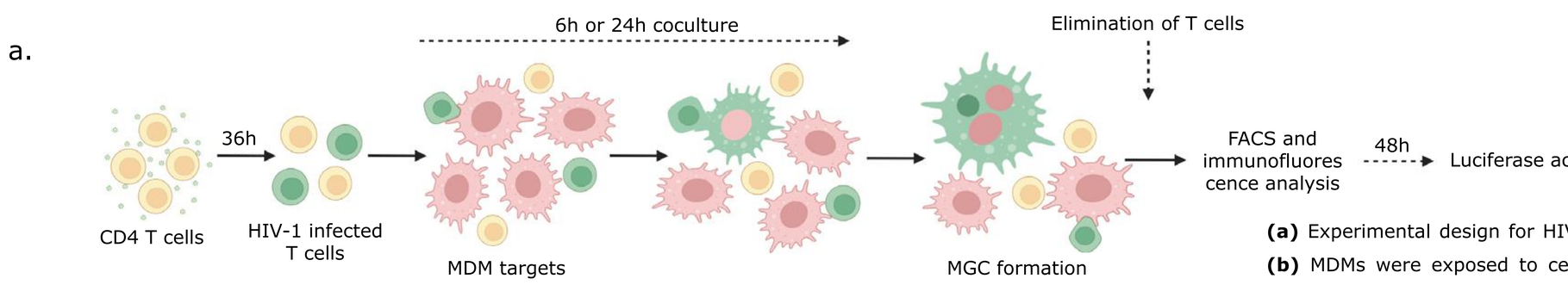
✔ These data suggest that M-tropism of HIV-1 is more widespread than initially thought based on cellular tropism assays. They also suggest that MΦ infection may be facilitated in CD4+ T cell rich tissues. These data renew our understanding of the role of MΦ in HIV-1 transmission and pathogenesis and the formation of tissue reservoirs.

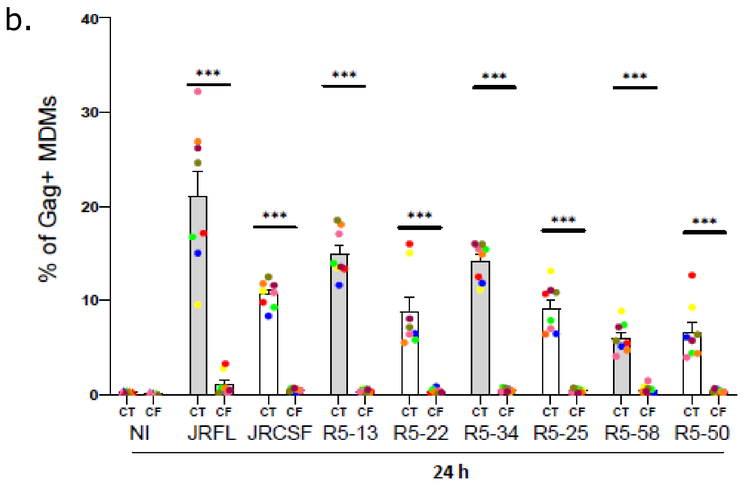
I- Cell-free T-tropic HIV-1 particles enter and infect MDMs at low efficiency

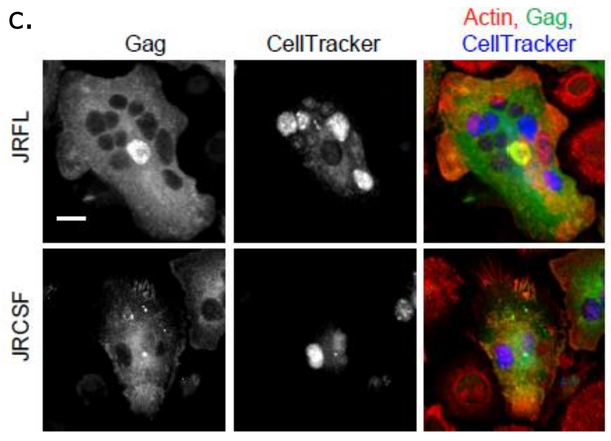


(a) The infectivity of cell-free luciferase reporter viruses pseudotyped with primary R5 Envs was determined on primary CD4+ T lymphocytes (CD4TL) as a function of the Gag p24 content. Similar amounts of infectious viruses (dotted line) were then used to infect monocyte-derived-macrophages (MDMs) **(b)** Only two viruses consistently infected MDMs, R5-13 and R5-34, similarly as the M-tropic strain JR-FL. All others did not or only marginally, similarly as the non-M-tropic strain JR-CSF. The differences in dot colors represent MDMs from different donors. **(c)** R5 and X4 T-tropic viruses are impaired in their ability to enter MDMs, as assessed by the BLam-vpr/CCF2 virus-cell fusion assay. Thus non-M-tropic viruses are unable to infect MΦ due to a blockage at the viral entry step.

II- Cell-to-cell transfer to macrophages of non-M-tropic HIV-1 through cell-cell fusion with infected T cells



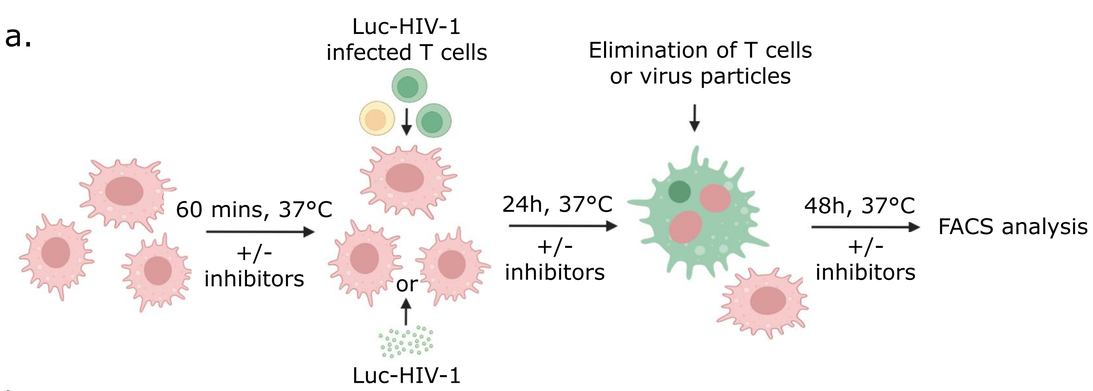


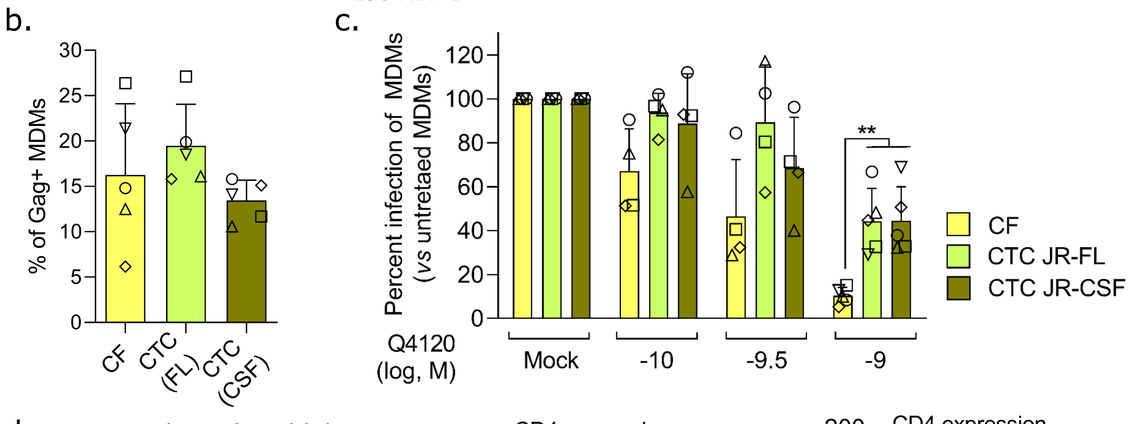


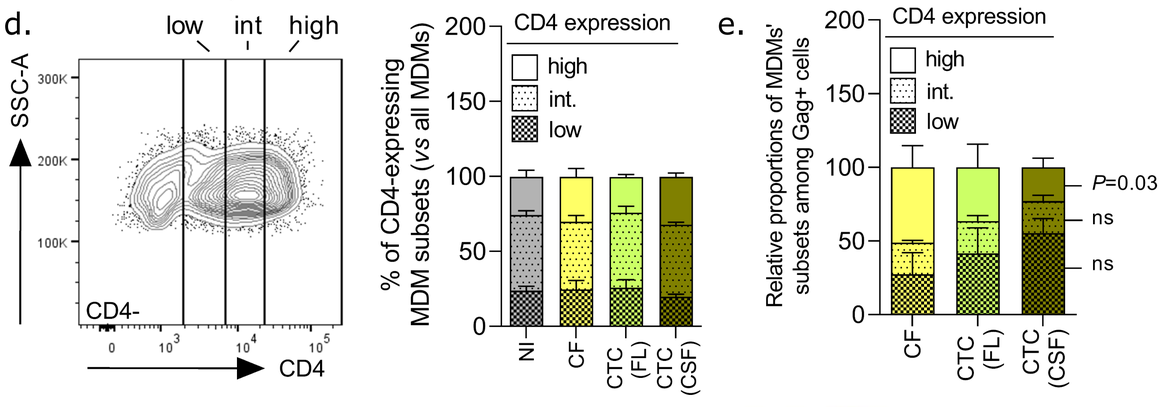
(a) Experimental design for HIV-1 cell-to-cell transfer from infected CD4+ T cells to MDMs. **(b)** MDMs were exposed to cell-free (CF) virus-containing supernatants of infected Jurkat cells or cocultured (CT) with Jurkat cells infected with M- (grey bars) or non-M-tropic (white bars) viruses. Viral transfer to MDMs revealed by the presence of Gag+ MDMs was quantified by flow cytometry. Results show that **all R5 viruses, including non-M-tropic viruses, are transferred to MDMs from infected T cells**. In contrast, no Gag + MDMs were detected when using supernatants from infected Jurkat cells. Similar results were obtained with the X4 viruses shown in Fig. 1C.

(c) Jurkat cells infected with M- or non-M-tropic Env-pseudotyped viruses were prelabeled with CellTracker and co-cultured for 6h with MDMs. MDMs were then stained with anti-Gag, phalloidin (actin) and DRAQ5 (nuclei), and analyzed by confocal microscopy (scale bar, 10 µm). Results show that fusion between infected Jurkat cells and MDMs ultimately leads to the formation of Gag-expressing MGCs. Representative results for the M- and non-M tropic Envs JRFL and JRCSF are shown.

III- Increased efficiency of CCR5 and CD4 use in HIV-1 transfer between infected T cells and MDMs

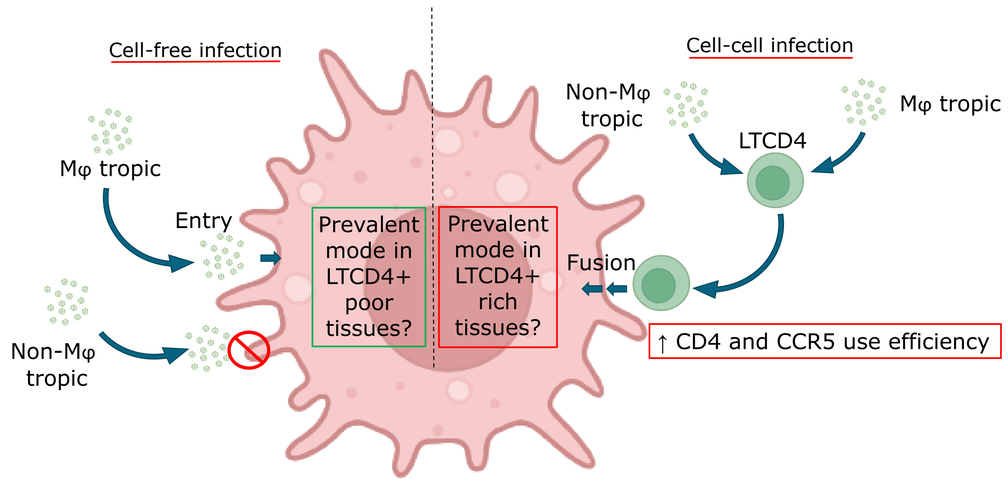






(a) Experimental scheme of experiments. **(b)** The experiments analyzing CD4 (here) or CCR5 (not shown) dependence for MΦ infection were performed under conditions of similar proportions of Gag+ MDMs exposed to cell-free (CF) JR-FL or Jurkat cells infected with JR-FL (FL) or JR-CSF (CSF) viruses (CTC). **(c)** Dose-dependent inhibition by Q4120 of MDM infection by CF JRFL or through CTC viral transfer. MDM infection through CTC transfer is more resistant to Q4120 than infection with CF viruses. **(d-e)** The proportion of infected MDMs by both modes of infection was then analyzed as a function of CD4 cell-surface expression level. MDMs expressing low, intermediate or high levels of CD4 were selected **(d)** an then compared for their permissiveness to infection by CF viruses or through CTC transfer of JRFL or JRCSF **(e)**. Results show an enrichment in high-CD4 expressing cells among Gag+ MDMs, which was more pronounced in infection experiments with CF viruses than through CTC viral transfer. Considered altogether, these data indicate that MΦ infection through CTC transfer is less dependent on CD4 expression level, compared to the cell-free mode, suggesting greater efficiency in CD4 usage. Similar experimental setup led to the same conclusions regarding CCR5 usage.

IV- Proposed model



We propose that the mode of MΦ infection varies according to the CD4TL content in tissues. While the cell-free mode would be prevalent in CD4TL-depeleted tissues (e.g. the brain), viral transfer from infected CD4TL would become prevalent in CD4TL-rich tissues (lymph nodes, mucosa).

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