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Short communication

HIV endocytosis after dendritic cell to T cell viral transfer leads to productive virus infection

Imma Clotet-Codina[a,1], Berta Bosch[a,1], Jordi Senserrich[a], María Teresa Fernández-Figueras[b], Ruth Peña[a], Ester Ballana[a], Margarita Bofill[a,c], Bonaventura Clotet[a], José A. Esté[a,*]

[a] Retrovirology Laboratory IrsiCaixa, Hospital Universitari Germans Trias i Pujol, Universitat Autònoma de Barcelona, 08916 Badalona, Spain
[b] Department of Pathology, Hospital Universitari Germans Trias i Pujol, Universitat Autònoma de Barcelona, 08916 Badalona, Spain
[c] Institució Catalana de Recerca i Estudis Avançats, Spain

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ABSTRACT

Contacts between HIV-producing T cells and primary CD4+ T cells may induce the uptake of HIV by target cells that are endocytosed into trypsin-resistant compartments. We have now compared the mechanism of virus transmission from T cell-to-T cell versus infected dendritic cells (DCs)-to-T cell. In cocultures of HIV-1-infected DCs with primary CD4+ T cells, virus transmission to the target cells was resistant to trypsin treatment and could only be prevented by the anti-SUgp120 antibody IgGb12 but not by TAK-779, C34 or AZT. Importantly, upon stimulation of purified HIV-1-loaded CD4+ T cells with PHA/IL-2, cells became productively infected as measured by intracellular CAp24 staining and antigen determination in the cell supernatant. These results suggest that the viral endocytic transfer may represent a escape mechanism in the presence of drugs targeting HIV-1 entry or the host immune system.

HIV-1 entry is a validated target for antiretroviral therapy (Esté and Telenti, 2007). Agents targeting the CD4 receptor, HIV coreceptors, and TMgp41-dependent fusion effectively block HIV replication, HIV envelope-induced cell death, syncytium formation and cell-to-cell virus transmission (Berger et al., 1999; Esté, 2003; Moncunill et al., 2008a). However, we and others have shown that T cell-to-T cell virus transfer may occur in the presence of HIV-1 entry inhibitors targeting virus coreceptors or TMgp41-dependent fusion (Blanco et al., 2004; Bosch et al., 2005; Chen et al., 2007; Ruggiero et al., 2008). In the presence of HIV entry inhibitors, cell-to-cell virus transfer may be mediated by a clathrin-dependent endocytic process that also depends on the interaction between the viral envelope SUgp120 and the cellular CD4 receptor (Bosch et al., 2005, 2008; Ruggiero et al., 2008). Endocytosed virus may often lead to poor or no infectivity and degradation. However, internalized HIV-1 particles may not reach Lamp1 positive compartments, suggesting that during HIV-1 transfer by cell–cell contacts, virions can be taken up by endocytosis but may not be degraded in lysosomes (Bosch et al., 2008).

Dendritic cells (DCs) can be productively infected by HIV-1 (*cis*-infection) (Burleigh et al., 2006) or, alternatively, DCs can mediate HIV-1 transmission to target T cells (*trans*-infection) (Cameron et al., 1992; Wiley and Gummuluru, 2006). That is, transfer of HIV-1 to CD4+ T cells may be composed of two different mechanisms, the first resulting from the uptake of virus particles by DCs in a short period of time, the second requiring DC infection and occurring after a few days (Nobile et al., 2005; Turville et al., 2004). Here, we show that cell-to-cell HIV-1 transmission from infected DCs to primary CD4+ T lymphocytes may occur through a trypsin-resistant endocytosis of viral particles by T cells similar to that observed between infected and uninfected T cells. Importantly, endocytosis of viral particles by T cells may lead to productive infection upon T cell stimulation.

To evaluate virus transfer form DCs to T cells, DCs were differentiated from isolated peripheral blood monocytes cultured in RPMI 1640 (Invitrogen, Madrid, Spain) with 10% FCS, 1000 U/ml GM-CSF (Peprotech, London, UK) and 1000 U/ml interleukin-4 (IL-4) (Peprotech, London, UK) as described before (Bosch et al., 2006). DCs were identified following cell surface markers (CD14+, HLA-DR, CD83 and CD86) as previously described (de Arquer et al., 2007; Engering et al., 2002; Shannon et al., 2005; Wang et al., 2007). Immature DCs (iDCs) were positive for the CD4 receptor but expressed low levels of both HIV-1 coreceptors (CXCR4 and CCR5) (data not shown). To generate productively infected DCs, iDCs were acutely infected with the R5 tropic HIV-1_{BaL} strain. Percentage of positive CD14, DC-SIGN, CXCR4 and CD4 DCs decreased when infected. After 16 days,

* Corresponding author at: Fundació irsiCaixa, Laboratori de Retrivirologia, Hospital Universitari Germans Trias i Pujol, Crta. del Canyet s/n, 08916 Badalona, Spain. Tel.: +34 934656374; fax: +34 934653968.
E-mail address: jaeste@irsicaixa.es (J.A. Esté).
[1] These authors contributed equally to the study (dual first authorship).

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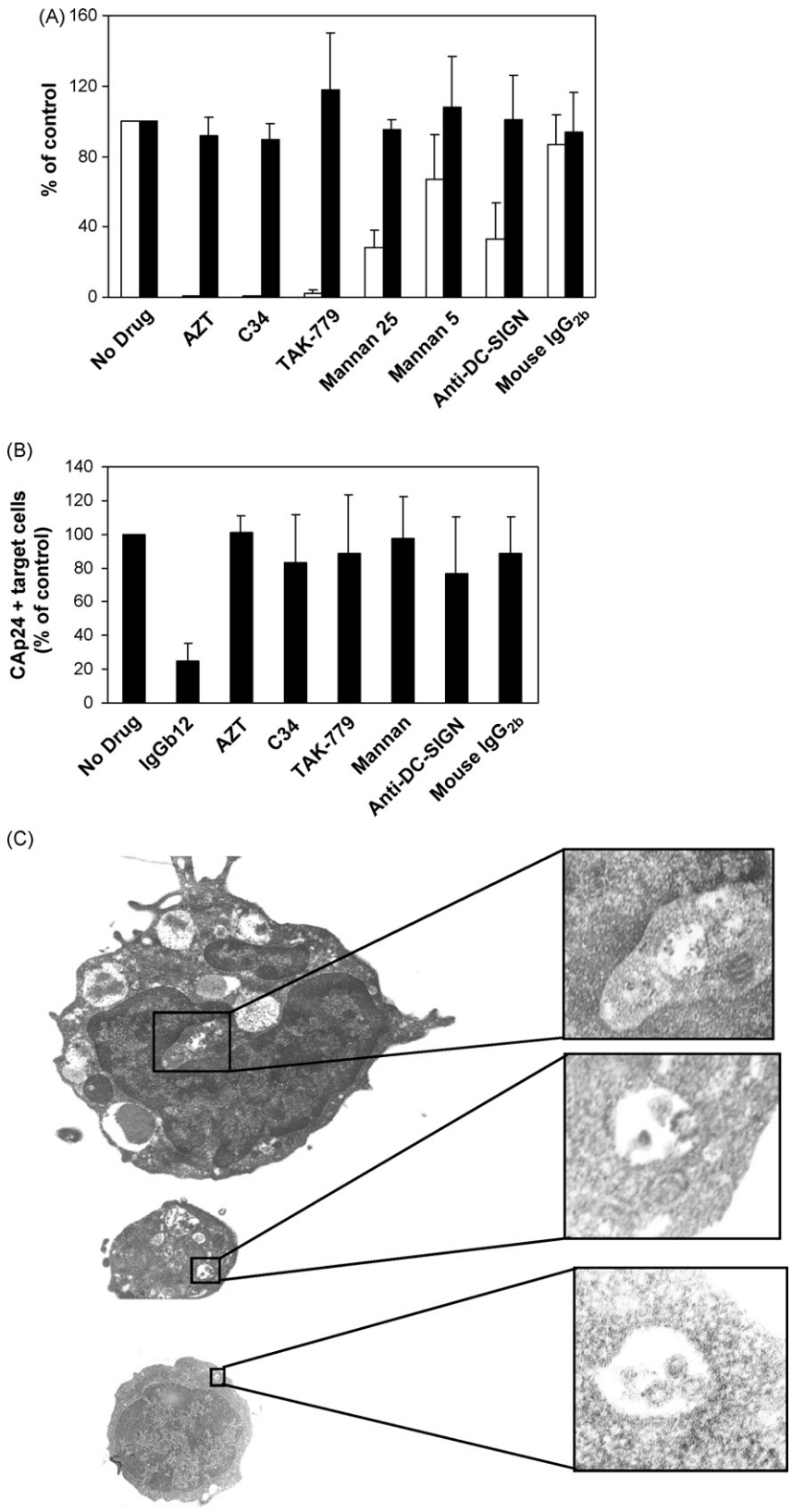

Fig. 1. Inhibition of HIV-1 replication and transfer of HIV by dendritic cells to CD4+ T lymphocytes. (A) Infection of DCs with HIV-1_{BaL} was performed in the presence or the absence of the following drugs and antibodies: AZT at 0.5 μg/ml, C34 at 2 μg/ml, TAK-779 at 0.5 μg/ml, mannan at 25 and 5 μg/ml, the anti-DC-SIGN mAb at 20 μg/ml and the isotype control mouse IgG2b at 20 μg/ml. HIV-1 replication was evaluated at day 9 post-infection by CAp24 antigen production (white bars). Results are represented relative to the HIV-1_{BaL}-infected but not treated (no drug, 100%) control. Cell viability was measured in drug- or antibody-treated uninfected DCs cultures by flow cytometry (black bars). Results are represented relative to the untreated (no drug, 100%) control. Data represent the mean ± S.D. (standard deviation) of three independent experiments. (B) DCs were infected with HIV-1_{BaL} for 16 days prior to overnight coculture with unstimulated primary CD4+ T lymphocytes in the presence or absence of the following drugs and antibodies that were present during the entire coculture: IgGb12 mAb at 10 μg/ml, AZT at 1 μg/ml, C34 at 5 μg/ml, TAK-779 at 5 μg/ml, mannan at 100 μg/ml, the anti-DC-SIGN mAb at 20 μg/ml and the isotype control mouse IgG2b at 20 μg/ml. To assess HIV-1_{BaL} antigen transfer, after overnight cocultures cells were treated with trypsin,

a maximum amount of CAp24 antigen in supernatant was observed which correlated with the maximum of intracellular CAp24 antigen staining (37% CAp24+ DCs). The total amount of intracellular CAp24 antigen could be the result of two different contributions: newly synthesized viral particles and virus present in endosomes that had been internalized by DCs. Therefore, 16-day cultured, infected or uninfected DCs were used for subsequent experiments. When analyzing the expression of CD83 and CD86 in the CAp24+ population (defined as infected DCs), 62% and 77% of CAp24+ cells were positive for CD83 and CD86, respectively, confirming that HIV-1 infection induced DC maturation as measured by CD83 and CD86 expression (Fantuzzi et al., 2004) (data not shown), although we cannot exclude the possibility that this increase in the amount of cells positive for CD83 and CD86 could be partially due to the death of uninfected DCs which conserve a CD83 and CD86 low phenotype.

As expected, the antiretroviral drugs AZT, C34 and TAK-779 completely blocked HIV-1_{BaL} replication in DCs (Fig. 1A). Compared to the positive control, mannan and the anti-DC-SIGN mAb (Jameson et al., 2002) inhibited HIV-1_{BaL} replication by up to $72 \pm 10\%$ and $67 \pm 20\%$, respectively, whereas viral replication was not affected by treatment with an isotype control mouse IgG2b. None of the agents were cytotoxic at the concentrations tested (Fig. 1A). Our results confirm the capacity of iDCs to become productively infected with HIV-1 and also show that the infection of iDC occurs through a mechanism in which manose-binding C-type lectin and DC-SIGN may be involved (Arrighi et al., 2004; Burleigh et al., 2006; Geijtenbeek et al., 2000; Nobile et al., 2005).

Then, we coculture HIV-infected DCs with freshly isolated, unstimulated PBMC (to minimize productive virus replication in target cells), as shown before for infected and uninfected T cells cocultures (Blanco et al., 2004; Bosch et al., 2005), in the absence or presence of known anti-HIV agents. Infected DCs were able to transfer CAp24 antigens into 10–35% of target cells depending on the cell donor used after overnight cocultures of DCs and T cells. Viral transmission was not affected by DC-SIGN inhibitors (mannan or the anti-DC-SIGN mAb), AZT, C34 or TAK-779 (Fig. 1B). Conversely, the mAb IgGb12, which blocks the SUgp120–CD4 interaction, was able to inhibit virus transmission to the target cell up to $75 \pm 11\%$ (Fig. 1B), in agreement with (Frankel et al., 1998). Electron microscopy (following the procedure described by Blanco et al. (2004)) of overnight DC-T cell cocultures confirmed the transfer of HIV-1 to T cells (DCs and T cells were clearly identified by size and heterochromatin structure). Endosomes containing viral particles were observed in infected DCs as previously shown (Izquierdo-Useros et al., 2007) but also in target T cells. Endosomes containing viral particles were also found in T cells in the presence of the CCR5 antagonist TAK-779 (Fig. 1C).

From overnight cocultures between unstimulated CD4+ T lymphocytes and productively HIV-1_{BaL}-infected DCs, the CD4+ T cell population was purified by negative selection achieving a purity of $98 \pm 1\%$ (Fig. 2A). Cells were trypsinized to remove extracellular bound virions and stimulated with PHA and IL-2. Intracellular CAp24 staining of T lymphocytes showed up to 10% of positive cells. CAp24 antigen could be detected in the supernatant of purified T cells (1.25×10^6 pg/ml) and the mean fluorescence intensity in T cells increased (Fig. 2B) suggesting the initiation of virus replication upon T cell stimulation. The contribution of contaminating HIV-infected DCs ($2 \pm 1\%$) in the total amount of CAp24 detected in the supernatant of purified T cells after 6 days of culture with PHA/IL-2 was evaluated using CD4+ T cells isolated from IgGb12 treated DC-T cell cocultures, where virus transfer was mostly blocked (Fig. 1B). After 6 days of culture, the production of CAp24 by this T cell population was 14% compared to the untreated (no drug) control (Fig. 2C). This value would correspond to the maximum contribution, if any, of contaminating DCs in the total amount of CAp24 released by the control (untreated) cells. Taken together, these results suggest that the virus transferred to CD4+ T cells during DC–T cell coculture could establish a productive infection after PHA/IL-2 activation of recipient T cells.

Supernatant from isolated CD4+ T lymphocytes could infect U87.CD4/CCR5 cells as measured by antigen detection (Fig. 2D) and syncytium formation in U87.CD4/CCR5 cells stained with 1 μM Hoechst 33324 (Moncunill et al., 2008b) (Fig. 2E).

Thus, we demonstrate that similar to T cell-to-T cell cocultures, virus transmission may occur between infected DCs and CD4+ T cells and, most importantly, that an apparently passive transfer of virus particles from infected DCs to resting CD4+ T cells may lead to productive infection upon stimulation of the CD4+ T cells. We confirm that HIV infection leads to DC maturation (as seen by cell surface marker expression) and efficient virus transmission to target cells independently of C-type lectins (Wang et al., 2007). Apparent coexpression of the mature DC phenotype with intracellular CAp24 antigen suggests that maturation of DCs could be a requirement for virus replication or, what could be more likely, that HIV-1 can induce a certain degree of maturation in DCs (Harman et al., 2006; Smed-Sörensen et al., 2005). Thus, HIV-1 may sequester the natural function of immature DCs (the capture and processing of viral antigen) to generate the appropriate conditions, that is DC maturation, to initiate a productive infection. Virus attached to DC-SIGN promotes such productive infection. Indeed, DC-SIGN plays a role in virus capture and infection of DCs. Both mannan and a mAb targeting DC-SIGN inhibited HIV-1_{BaL} replication in DCs, confirming its role in HIV-1 *cis*-infection. However, as observed in T cell–T cell cocultures (Blanco et al., 2004; Bosch et al., 2005; Chen et al., 2007; Wang et al., 2007) once DCs were productively infected, virus transmission to CD4+ T cells was only blocked by an agent targeting the SUgp120–CD4 interaction but not by the CCR5 antagonist TAK-779, a TMgp41 inhibitor C34, or the RT inhibitor AZT. Detection of viral antigen was resistant to trypsin and concomitant to the detection of endosomes containing viral particles in the target T cells.

Viral antigen in T cells must come from the infected DCs as supported by several evidences: (1) short time cocultures (overnight) do not allow for productive infection to occur, (2) cocultures were performed with non-stimulated CD4+ T cells, in which virus replication is minimized, (3) virus transfer to T cells occurred also in the presence of HIV entry inhibitors targeting CCR5, TMgp41-dependent fusion or AZT and finally, (4) virus detection in T cells was trypsin-resistant, suggesting virus internalization through an endocytic route. Nevertheless, upon T cell activation, HIV-1-loaded isolated T cells, became infected and produced virus that was secreted into the cell culture medium.

Recently, Hübner et al. (Hubner et al., 2009) use 3D microscopy to confirm previous demonstrations of endocytic HIV cell-to-cell transfer through virological synapses and transmission through endocytic routes that may serve as viral reservoirs. We and others have shown that virus-cell fusion and endocytosis may coexist in the same cell as the later is corecepetor independent and often leads to lysis of virus particles or regurgitation of HIV to the extra-

stained for intracellular CAp24 antigen and analyzed by flow cytometry. For each condition, 5000 events corresponding to unstimulated CD4+ T lymphocytes, identified by CMFDA (5-chloromethyl-fluorescein diacetate) staining and morphological parameters, were analyzed. Data are represented relative to the non-treated control and are the mean ± S.D. of five experiments. (C) HIV-1_{BaL}-infected DCs (top) were cocultured with CD4+ T cells in the absence (middle) or presence of TAK-779 (bottom) and analyzed by electron microscopy. Virus particles could be detected in effector and target cells in intracellular trypsin-resistant vesicles.

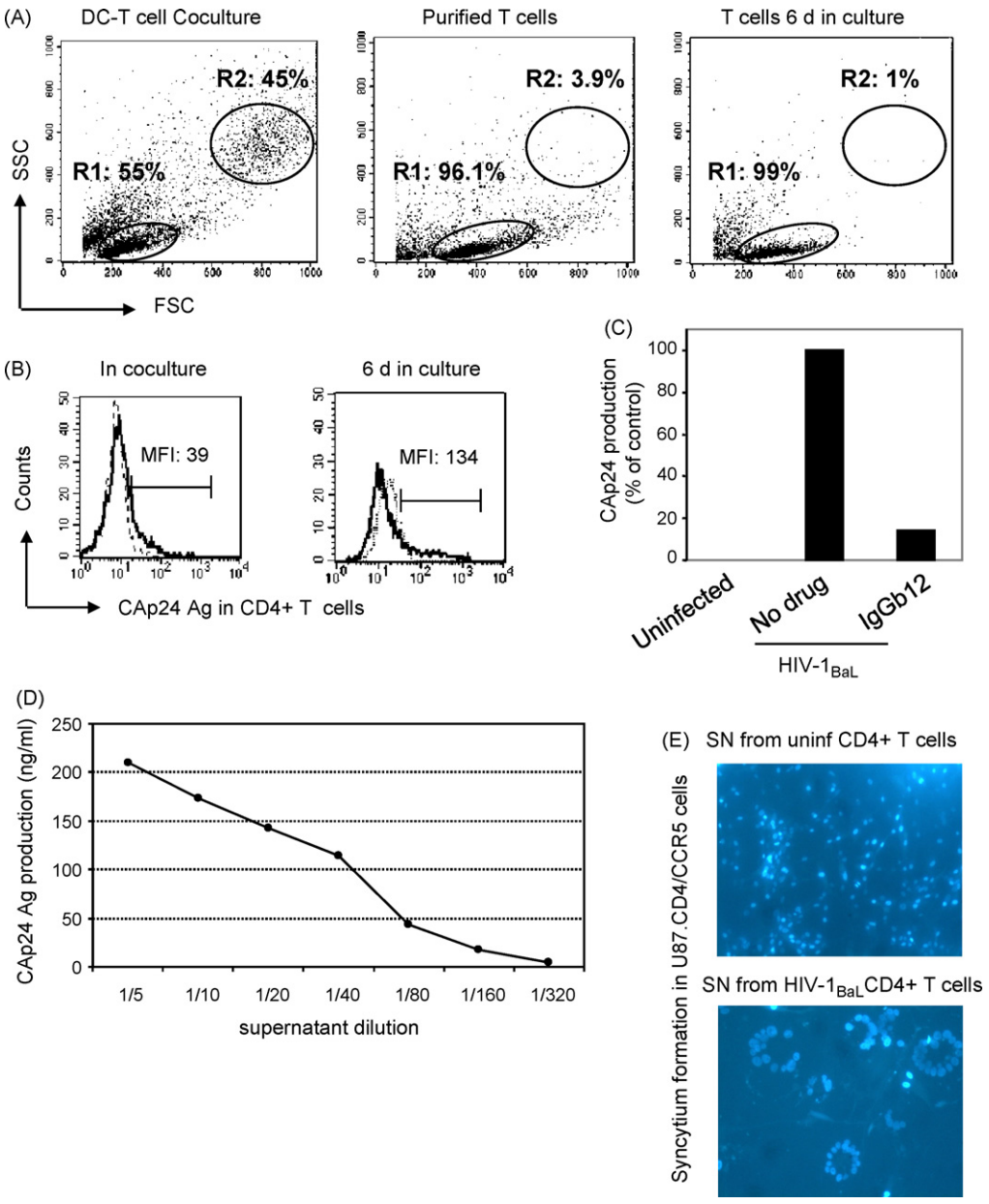

Fig. 2. Transfer to CD4+ T lymphocytes leads to T cell infection and viral production. (A) Purified CD4+ T lymphocytes from 15-h cocultures with 16-day HIV-1_{BaL}-infected DCs were stimulated and cultured for 6 days. Dot plots show the percentage of T lymphocytes (R1) and DCs (R2) in coculture (left), purified T cells (middle) or after stimulation and 6 days in culture (right). (B) Mean fluorescence intensity (MFI) of the intracellular CAp24 antigen expression in T lymphocytes from overnight cocultures (left) with uninfected DCs (dashed line) or HIV-1_{BaL}-infected DCs (black line), or cultivated for 6 days after isolation from cocultures (right) as measured by flow cytometry. (C) CAp24 antigen produced by CD4 T cells purified from cocultures with uninfected DCs (uninfected) or HIV-1_{BaL}-infected DCs in the absence (no drug) or the presence of mAb IgGb12 (IgGb12). (D) Supernatant from stimulated purified CD4+ T lymphocytes after 6 days in culture was recovered and used to infect U87.CD4/CCR5 cells (24-h inoculation). The amount of CAp24 antigen produced 5 days after infection with 2-fold dilutions of the supernatant is expressed in ng/ml. (E) Infectivity of released viral particles was confirmed by Hoechst 33324 staining of syncytium formation in U87.CD4/CCR5 cells. Briefly, U87.CD4/CCR5 cells challenged for 5 days with the supernatant from stimulated purified CD4+ T lymphocytes were stained with 1 μM Hoechst 33324. Cultures were visualized in a Nikon Eclipse TE-200 fluorescence microscope and syncytia with more than four nuclei were scored as positive.

cellular compartment. The results of Hübner et al. and ours maybe in apparent contrast to previous confocal and electron microscopy analysis, which concluded that there was no overlap between HIV-1 antigen and early endosomes and found no evidence of virus particles within endosomal structures (Jolly et al., 2004; Martin and Sattentau, 2009). In conclusion, our results suggest that infected DCs passively transfer HIV-1 particles to CD4+ T cells in the presence of HIV-1 entry inhibitors targeting coreceptors or TMgp41. In conditions in which HIV-1 attachment, coreceptor engagement and fusion are limited or hampered by anti-HIV agents or neutralizing antibodies (Chen et al., 2007), virus endocytosis could result in an escape mechanism leading to productive infection.

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