HIV-1 Infection of Trophoblasts Is Independent of gp120/CD4 Interactions but Relies on Heparan Sulfate Proteoglycans

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Mother-to-child transmission of human immunodeficiency virus type 1 (HIV-1) is the leading cause of HIV infection in infants. Direct infection of trophoblasts—cells forming the placental barrier—may cause this transmission. Entry of HIV-1 into trophoblasts is unusual for this retrovirus, because it is associated with endocytosis. However, given that trophoblasts express no or few receptors/coreceptors required for virus internalization, the mechanism underlying this event remains ambiguous. In the present study, we show that HIV-1 entry and infection of polarized trophoblasts are independent not only of CD4 but also of envelope (Env) glycoproteins gp120 and gp41. Virus internalization, cytoplasmic release, reverse transcription, integration, and HIV-1 gene expression occurred with both fusion-incompetent and Env-deficient viruses. Importantly, fusion-independent infection was observed when we used viruses produced in a natural cellular reservoir (i.e., primary human cells). Finally, HIV-1 requires heparan sulfate proteoglycans for uptake in trophoblasts. Together, our findings illustrate that HIV-1 utilizes an unusual pathway for entering human polarized trophoblasts.

More than 2 million children are living with HIV-1 worldwide, and 90% of these infections resulted from transmission from the mother [1] occurring in utero, at birth, or via breast-feeding [2]. HIV-1 has been detected in newborns at delivery [3–5] and in aborted fetuses [6–9]. Importantly, women are becoming infected at a higher frequency than men [10], and access to antiretroviral treatment is limited in developing countries [11]. Hence, definition of the mechanism(s) underlying vertical transmission is urgently needed.

For in utero HIV-1 transmission to occur, the virus must cross the selective placental barrier, which is composed of a double layer of polarized epithelial-type cells: cytotrophoblasts and syncytiotrophoblasts. The postulated models include cell-to-cell transfer, direct infection of the trophoblasts, and transcytosis of viral particles across trophoblasts (reviewed in [12]). The mechanism underlying infection with cell-free virus remains largely unknown. HIV-1 has been shown to productively infect trophoblasts both in vitro [13–16] and in vivo [5, 17–19]. However, they exhibit a much lower susceptibility to productive HIV-1 infection than do CD4+ T cells [14, 15, 20].

HIV-1 entry in CD4+ T cells is mediated by the virus-encoded envelope (Env) glycoproteins gp120 and gp41. These proteins interact with the primary cellular receptor CD4 and its coreceptors (e.g., CXCR4 or CCR5), which leads to the formation of a fusion pore in the host cell membrane (reviewed in [21]). Contrary to this, we have established that, on contact with human tro-
phoblasts, HIV-1 is rapidly and massively endocytosed and vi-
rions are predominantly trapped within the endosomal com-
partments [22]. It is noteworthy that this internalization event
is crucial for HIV-1 infection to proceed in trophoblasts, given
that drugs that inhibit the function of endosomes block virus
infection [22, 23].

Although HIV-1 infection in trophoblasts relies on endo-
cyosis, the initial events of the virus life cycle in this cell type
are ill defined. For instance, the expression of CD4 remains
very low to absent in primary trophoblasts, whereas the ex-
pression of coreceptors may vary during the course of preg-
nancy [15, 24, 25]. Some previous studies have addressed
the contribution of CD4, CXCR4, and CCR5 in HIV-1 infection
of trophoblasts, and the published reports are contradictory,
either involving gp120/CD4/coreceptor interactions [20, 26] or
not [16, 25, 27]. Hence, the role played by CD4 and coreceptors
in HIV-1 entry into trophoblastic cells is still unclear, but it is
reasonable to assume that CD4, CXCR4, and CCR5 are unlikely
to play a significant role, because they are expressed at such
low levels if present. In agreement with this, several groups
have shown recently that CD4 is not required for HIV-1 in-
fec tion in trophoblasts [27–30]. In this context, we developed
2 hypotheses. First, other attachment receptors must be in-
volved to compensate for the low level of CD4/CXCR4/CCR5
in these cells. Second, if HIV-1 infection of trophoblasts is
independent of the known receptor/coreceptors for entry, the
virus may not rely on the Env glycoproteins for such an event.
The central objective of the present work was to test the validity
of these postulates.

MATERIALS AND METHODS

Cells. Human trophoblastic cell lines (JAR and JEG-3), em-
byronic kidney 293T cells, the leukemic T cell line Jurkat (clone
E6.1), and peripheral blood mononuclear cells (PBMCs) from
healthy donors were maintained as described elsewhere [14].

Preparation of virus stocks. The NL4-3 (X4), JR-CSF (R5),
NL4-3-Luc E’R’, HXB2-env (X4), Ada-M-env (R5), and
HCMV-G molecular constructs used have been described
elsewhere [23]. The NL4-3-Luc E’R’ expression vector was
used to generate Env-deficient reporter viruses (NL4-3ΔEnv).
pHCMV-G codes for the broad-host-range vesicular stomatitis
virus envelope glycoprotein G (VSV-G) [31]. The fusion-com-
petent pSM-wild type (WT) and fusion-incompetent pSM-
570R Env expression vectors were derived from the HXB-2
backbone [32]. Viruses were produced by calcium phosphate
transfection of 293T cells, as described elsewhere [14]. Viruses
loaded with the β-lactamase (βlaM)–Vpr fusion protein were
produced by cotransfection of 293T cells with pCMV-βlaM-
Vpr (provided by W. C. Greene, Gladstone Institute of Virology
and Immunology, San Francisco, CA), together with some of
the expression vectors described above. Virus preparations were
also generated through acute infection of PBMCs with NL4-3
or JR-CSF.

Infection assays. Cells were incubated at 37°C for 24 h with
NL4-3ΔEnv particles or recombinant luciferase-encoding vi-
ruses pseudotyped with SM-WT or SM-570R (1 × 10^5 target
cells with 0–500 ng of p24 or, in some experiments, with 250
ng of p24 together with 10 μg/mL zidovudine [ZDV]). Virus
gene expression was induced with tumor necrosis factor (TNF)–
α (10 ng/mL), as described elsewhere [14]. JAR cells were incu-
bated for 30 min at 4°C with soluble CD4 (scCD4; 10 μg/mL),
an isotype-matched irrelevant control antibody (10 μg/mL
IgG2a), or a blocking anti-CD4 antibody (10 μg/mL SIM2),
after which Ada-M or HXB-2 pseudotyped reporter viruses
(250 ng of p24) were added. Jurkat cells were exposed to the
control antibody or SIM2 for 30 min at 37°C before inoculation
with pseudotyped viruses (100–150 ng of p24). T-20 (Roche
Bioscience) was added at various doses (0, 0.2, 1.0, and 5.0 μg/
ML) at 4°C to JAR cells concomitantly with Ada-M or HXB-
2 pseudotyped reporter viruses (250 ng of p24). Jurkat cells
were treated with 1 μg/mL T-20 before exposure to HXB-2
pseudotypes or NL4-3 viruses (100–150 ng of p24). Luciferase
activity was measured in lysed cells. For Jurkat cells infected
with NL4-3, cell supernatants were harvested and frozen at
−20°C until they were assayed using a homemade p24 test [33].

Virus absorption and internalization assays. Cells were
exposed to pseudotyped SM-WT, pseudotyped SM-570R, NL4-
3, or NL4-3ΔEnv (250 ng of p24 for 1 × 10^6 cells) for 0–4 h
at 4°C (to test virus absorption) or 37°C (to test virus inter-
nalization). To test the kinetics of virus absorption, the cells
were washed 3 times with ice-cold PBS. As for the kinetics
of virus internalization, the cells were acid treated (0.5 mol/L NaCl
and 1% acetic acid) for 1 min at 4°C and washed 3 times with
ice-cold PBS to remove all uninternalized viral particles. Cells
were lysed in ice-cold lysis buffer, as described elsewhere [23].
Heparin sodium (0, 50, 250, and 500 IU/mL; Leo-Pharma) was
added at 4°C together with NL4-3ΔEnv, Ada-M, or HXB-2
pseudotypes (250 ng of p24). JAR cells were pretreated with 0,
2, 20, 200, and 400 μU/mL heparinase (Sigma-Aldrich) for 1.5
h at 37°C.

βlaM-Vpr–mediated cleavage of CCF2/AM. The βlaM-
Vpr assay was based on a technique established elsewhere [34,
35]. Briefly, polarized JAR cells were exposed to pseudotyped
SM-570R or NL4-3ΔEnv viruses that were all loaded with
βlaM-Vpr fusion protein (400 ng of p24 for 1 × 10^6 cells) for
2 h at 37°C. The virus-cell mixture was incubated with CCF2/
AM dye (Aurora Bioscience) for 1 h at room temperature, and
the βlaM reaction was maintained for 24 h at room temperature
in CO2-independent medium supplemented with 10% fetal calf
serum (FCS) and 2.5 mmol/L probenecid (Sigma-Aldrich).
Figure 1. HIV-1 infection of polarized trophoblasts independent of CD4. A, Polarized JAR cells exposed to Ada-M or HXB-2 pseudotyped viruses for 1 h at 37°C in the presence or absence of soluble CD4 (sCD4; 10 μg/mL). The cells were then washed extensively and lysed. Viral entry was measured by evaluating the amount of p24 in each cell lysate. Data shown are means ± SDs of triplicate samples and are representative of 3 independent experiments. In some experiments, polarized JAR cells were first pretreated with PBS (vehicle), the control antibody, or SIM.2 and then exposed to Ada-M (B) or HXB-2 (C) pseudotyped reporter viruses for 24 h at 37°C. For Jurkat cells, the cells were pretreated with PBS (vehicle), an isotype-matched irrelevant antibody (control antibody/IgG2a) or SIM.2 before exposure to reporter viruses pseudotyped with SM–wild-type (WT) Env (D) for 24 h at 37°C. The cells were then either left unstimulated or stimulated for 24 h with tumor necrosis factor (TNF)-α (10 ng/mL). Viral infection was monitored by measuring luciferase activity in each cell lysate. Values from the luminometer are expressed as relative light units. Data are the fold increase ± SD of treated cells over mock-infected cells without stimulation and are representative of 3 independent experiments.

Both the emission spectra of CCF2/AM (520 nm) and its cleaved product (447 nm), as well as the degree of βαM-mediated cleavage, were assessed as described elsewhere [36].

Cell fractionation assays. Polarized JAR cells were exposed to pseudotyped (i.e., SM-WT, SM-570R, and VSV-G), NL4-3, or NL4-3ΔEnv viruses (250 ng of p24 for 1 × 10^6 cells) for 4 h at 37°C. The cellular membranes were disrupted, and the 2 distinct fractions (i.e., cytosolic and endosomal) were isolated as described elsewhere [37].

Real-time polymerase chain reaction (RT-PCR). Polarized JAR cells (1 × 10^6) were either left untouched or were exposed to pseudotyped (i.e., SM-WT, SM-570R, and VSV-G) or NL4-3ΔEnv viruses (250 ng of p24) (a ratio of 1 × 10^6 cells to 100 ng of p24 was used for Jurkat) for 48 h at 37°C. For the experiment with the fusion inhibitor T-20, the drug was added (1.0 μg/mL) at 4°C to polarized JAR cells (1 × 10^6) concomitantly with NL4-3 or JR-CSF viruses produced in PBMCs (110 ng of p24). Total cellular DNA was isolated using the DNeasy Tissue Kit as recommended by the manufacturer (QIAGEN). To quantify the amount of integrated viral DNA, we used the RT-PCR approach described by Suzuki et al. [38].

RESULTS

HIV-1 entry and infection of human trophoblasts, independent of CD4 and gp120/gp41. Given that HIV-1 infection of trophoblasts relies heavily on the cellular endocytic machinery [22], maintaining cell polarity when studying such process is of prime importance. If the purification of primary human trophoblasts results in the loss of cell polarity [39], the human
Figure 2. No inhibition of HIV-1 infection of polarized JAR cells by fusion inhibitor T-20. Polarized JAR cells were exposed to Ada-M (A) or HXB-2 (B) pseudotyped reporter viruses in the presence of the indicated concentrations of T-20 for 24 h at 37°C. In some experiments, Jurkat cells were exposed to wild-type NL4-3 viruses (C) in the absence or presence of 1.0 μg/mL T-20 for 24 h at 37°C. Cells were then either left untreated or stimulated for 24 h with tumor necrosis factor (TNF-α) (10 ng/mL) together with a second dose of T-20. For cells infected with reporter viruses, luciferase activity was monitored in each cell lysate. Values from the luminometer are expressed as relative light units (RLU). For Jurkat cells infected with fully competent viruses (i.e., NL4-3), infection was evaluated by estimating the amount of p24 in each cell lysate. Data are means ± SDs of quadruplicate samples and are representative of 3 independent experiments.
Viral internalization in polarized trophoblasts independent of gp120/gp41. Polarized JAR cells were exposed to SM–wild-type (WT) pseudotyped (A), NL4-3 (B), SM-570R pseudotyped (C), or Env-deficient (NL4-3ΔEnv) (D) virus for the indicated times either at 4°C (to estimate absorption) or 37°C (to estimate internalization). The cells were then treated as described in Materials and Methods. Viral absorption and internalization were measured by evaluating the amount of p24 in each cell lysate. Data are means ± SDs of triplicate samples and are representative of 3 independent experiments.

Figure 3.

both found to infect JAR cells (figure 4B and 4C). Again, virus infection was markedly reduced by ZDV, illustrating true viral infection. Experiments performed with another choriocarcinoma cell line (i.e., JEG-3) confirmed that virus infection in trophoblastic cells can be achieved in a gp120/gp41-independent manner (figure 4D). However, no LTR-driven luciferase activity could be detected in the absence or presence of TNF-α when Jurkat cells were inoculated with NL4-3ΔEnv virions in escalating doses (figure 4E).

Env-mutated and Env-deficient HIV-1 accessing the cytoplasm of polarized JAR cells. The distribution of HIV-1 within vesicular and cytosolic compartments was assessed in polarized JAR cells because productive HIV-1 infection has been reported to result from the release of p24 within the cytosol [41]. VSV-G pseudotypes were used as positive controls for the assay because, although VSV-G pseudotypes enter cells through endocytosis, there is a rapid shift from the endocytic machinery to the cytoplasm [42]. VSV-G pseudotypes were indeed predominantly located within the cytosolic fraction of JAR cells (figure 5A). Next, we found that 37% and 58% of the total internalized p24 content were located in the cytoplasm of JAR cells on infection with SM-WT pseudotyped and NL4-3 viruses, respectively. Importantly, fusion-incompetent and Env-deficient viruses were found to access the cytoplasm of polarized trophoblastic cells with efficiencies comparable to that of WT virus.

An alternative strategy was used to confirm the capacity of HIV-1 to gain access to the cytoplasm through a gp120/gp41-independent process. The fluorescence resonance energy transfer–based HIV-1 fusion assay exploits the incorporation of βlaM-Vpr chimeric proteins inside progeny viruses and their subsequent release into the cytoplasm of target cells as a marker of fusion. Infection with SM-570R and NL4-3ΔEnv viruses resulted in βlaM-mediated cleavages ranging from 4.60% to 19.33% and 7.98% to 11.70%, respectively (table 1). It is important to specify that, although HIV-1 is internalized primarily by endocytosis in trophoblasts [22], CCF2-AM is cytoplasmic and does not reach these compartments [34]. Therefore, in trophoblasts, βlaM-mediated cleavage of CCF2-AM occurs when HIV-1 is gaining access to the cytoplasm after escaping the endosomal compartments.

Fusion-incompetent and Env-deficient viruses integrating within the genome of polarized trophoblasts. The presence of integrated viral DNA was next assessed in polarized trophoblastic cells after infection with fusion-incompetent and Env-deficient viruses by use of a RT-PCR test described elsewhere [38]. As expected, we found that the number of integrated viral DNA copies obtained on infection with VSV-G
Figure 4. Viral infection in polarized trophoblasts independent of gp120/gp41. Polarized JAR cells were exposed to increasing concentrations of pseudotyped SM–wild-type (WT) (A), pseudotyped SM-570R (B), or Env-deficient reporter (NL4-3ΔEnv) (C) virus in the absence or presence of zidovudine (ZDV; 10 μmol/L) for 24 h at 37°C. JEG-3 cells were exposed to pseudotyped Ada-M, pseudotyped HXB-2, NL4-3ΔEnv, pseudotyped SM-WT, or pseudotyped SM-570R reporter viruses (D). In some experiments, Jurkat cells were exposed for 24 h at 37°C to SM-WT pseudotyped (500 ng of p24), SM-570R pseudotyped (500 ng of p24), or NL4-3ΔEnv (1, 10, 50, and 150 ng of p24) (E) viruses. The cells were then stimulated for 24 h with tumor necrosis factor (TNF–α) (10 ng/mL). Luciferase activity was monitored in each cell lysate. Values from the luminometer are expressed as relative light units (RLU). Data are means ± SDs of quadruplicate samples and are representative of 3 independent experiments.

Pseudotypes was much higher than when WT HIV-1 was used (figure 5B). High quantities of integrated HIV-1 DNA copies were detected in trophoblasts on infection with SM-WT, in agreement with the notion of true HIV-1 infection in these cells. Importantly, the number of viral DNA copies obtained on infection with SM-570R and NL4-3ΔEnv viruses was comparable to the number observed when WT NL4-3 particles were used. We next tested viral integration using Jurkat cells as con-
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Figure 5. Comparable cytosolic release of p24 and integration in the genome of polarized trophoblasts among wild-type (WT), fusion-incompetent, and Env-deficient viruses. Polarized JAR cells were exposed to pseudotyped SM-WT, pseudotyped SM-570R, NL4-3, pseudotyped Env-deficient (NL4-3ΔEnv), or pseudotyped virus envelope glycoprotein G (VSV-G) viruses for 4 h at 37°C (A). The cells were then washed, trypsinized, and resuspended in a swelling buffer. The cells were next disrupted by Dounce homogenization, and p24 levels were evaluated in each cellular fraction (i.e., cytosolic and vesicular). Data are representative of 3 independent experiments and are expressed as means ± SDs of the ratio of p24 present in the cytosol or vesicles over the sum of p24 present in the 2 fractions. In some experiments, polarized JAR cells were either left untreated (mock) or exposed to pseudotyped VSV-G, pseudotyped SM-WT, pseudotyped SM-570R, or NL4-3ΔEnv viruses for 48 h at 37°C (B). In other experiments, polarized JAR cells were either left uninfected (mock) or exposed to fully competent viruses (i.e., NL4-3 or JR-CSF) that were produced in peripheral blood mononuclear cells (PBMCs) for 48 h at 37°C (C). Virus infection was performed either in the presence (+) or absence (−) of the fusion inhibitor T-20. Total cellular DNA was isolated, and real-time polymerase chain reaction assays were conducted to quantify reverse-transcribed and integrated HIV-1–specific DNA. Data are means ± SDs of quadruplicate samples and are representative of 3 independent experiments.

trols. Although integrated provirus was observed on infection of Jurkat cells with SM-WT, none was detected on infection with SM-570R or NL4-3ΔEnv viruses (data not shown).

The experiments conducted so far were done using viral preparations produced in human embryonic kidney 293T cells. Is the Env-independent virus infection of trophoblasts related to unique fusogenic properties derived from 293T cells (virus-producer cells) that would be in turn be acquired by HIV-1 on budding, or could this phenomenon be also seen when using viruses produced in other physiological cell types? Fully competent R5 (i.e., JR-FL) and X4 (i.e., NL4-3) viruses were harvested after infection of PBMCs and were used to infect trophoblasts in the presence or absence of T-20 before assessment of the amount of integrated viral DNA copies. We found that
T-20 had no impact on the number of integrated viral DNA copies on infection of trophoblasts with HIV-1 produced in PBMCs (figure 5C). In a control experiment, integration of NL4-3 particles in Jurkat cells was reduced by 75% after treatment with T-20 (data not shown).

**HIV-1 internalization in polarized trophoblasts, mediated at least partly through heparan sulfate proteoglycans (HSPGs).**

HSPGs have been suggested to be putative HIV-1 attachment/entry receptors on the basis of the demonstration that heparin treatment, which removes all cell-surface heparan sulfate chains, diminishes HIV-1 infection of HeLa-CD4 epithelial cells and monocyte-derived macrophages [43, 44]. Heparin strongly decreased entry of the 2 HIV-1 strains tested (figure 6A and 6C). To verify whether the effect induced by heparin was indeed related to receptor inhibition and not due to a steric hindrance phenomenon related to heparin, JAR cells were treated with heparinase to remove heparan sulfate from the cell surface before the cells were exposed to Ada-M or HXB-2 pseudotypes. A decrease in virus internalization was observed after treatment with heparinase (figure 6B and 6D). Moreover, internalization of NL4-3ΔEnv viruses was also diminished after pretreatment either with heparin (figure 6D) or heparinase (figure 6F). To define whether this diminution in viral internalization translated into reduced viral infection, infection assays were conducted in JAR cells pretreated with heparin. This treatment induced a 50% reduction in HIV-1 infection (figure 6G).

**DISCUSSION**

The present article addresses key aspects of the HIV-1 replicative cycle in polarized human trophoblasts. We used different technical strategies to demonstrate that the process of virus infection proceeds through a gp120/CD4-independent mechanism: (1) a blocking anti-CD4 antibody and sCD4, (2) the fusion inhibitor T-20, (3) fusion-incompetent viruses, and (4) viral particles lacking both gp120 and gp41 Env glycoproteins. Given that the cytoplasmic delivery of viral material, reversion transcription (controlled by ZDV), and viral DNA integration are potent markers of true HIV-1 infection in CD4+ T cells and considering that these various events are observed in polarized trophoblasts after exposure to Env-deficient viruses at levels comparable to WT virions, we conclude that the process of HIV-1 internalization leading to the infection of polarized trophoblastic cells is independent of the normal interactions between CD4/coreceptors and gp120. Importantly, integration was seen in the presence of T-20 when fully competent viruses produced in natural cellular reservoirs, such as PBMCs, were used. This fact brings great credibility to the biological relevance of our findings. It is of interest to note that gp120-independent infection of CD4+ epithelial cells and CD4+ T cells by HIV-1 has been documented previously [45]. Moreover, although our experiments were performed with cell-free viruses, it has been previously proposed that HIV-1 infection of trophoblastic cells occurs mainly via cell-to-cell contact [27, 46]. The explanation for this discrepancy is presently unknown but could be related to differences in experimental methodologies, such as the use of polarized trophoblastic cells in the present work.

We also provide evidence that HSPGs play an important role in mediating Env-independent internalization of HIV-1 within trophoblasts. Heparan sulfate within HSPGs is linear, polysulfated, and, thereby, highly negatively charged glycosaminoglycan polysaccharides, and its binding to a wide variety of ligands is largely dependent on ionic interactions [47]. This notion fully agrees with the observation that, although HIV-1 internalization is extremely rapid, binding of HIV-1 to trophoblasts is weak [14]. In support of the idea that HSPGs may be involved in HIV-1 trophoblast infection in vivo, syndecan-1 is expressed throughout pregnancy by syncytiotrophoblasts, whereas syndecan-2, syndecan-4, and glypican-1 are expressed by villous and extravillous cytotrophoblasts as well as by syncytiotrophoblasts [48]. Neither heparin nor heparinase completely blocked viral uptake in trophoblasts, which suggests that other types of receptors may act concomitantly for HIV-1 internalization in trophoblasts. It has been shown that transcytosis of HIV-1 across the trophoblastic cell line BeWo can be blocked by antibodies to galactosyl ceramide [13]. In addition, several putative alternate HIV-1 receptors, which are recognized to act as such on other cell types, are present on the surface of trophoblasts (including C-type lectins, as the mannose receptor). It has also been hypothesized that vertical transmission of HIV-1 may be favored by the presence of dendritic cell–specific intercellular adhesion molecule 3–grabbing nonintegrin (DC-SIGN)/DC-SIGN–related–expressing cells within the placental environment [49]. However, this property would apply to transmission via microbreaches, because these attachment receptors are expressed in maternal macrophages, placental capillaries, and Hofbauer cells but not in trophoblasts [49].

We have previously shown that endosome inhibitors abolish HIV-1 infection of trophoblastic cells [22, 23], which implies that the process of virus infection is endocytic in nature. On
Figure 6. HIV-1 internalization in polarized trophoblasts reduced by heparin and heparinase treatment. Polarized JAR cells were exposed to pseudotyped Ada-M (A), pseudotyped HXB-2 (B), or Env-deficient (NL4-3ΔEnv) (E) viruses for 1 h at 37°C in the absence or presence of increasing concentrations of heparin. In some experiments, polarized JAR cells were first pretreated with increasing doses of heparinase before exposure to pseudotyped Ada-M (C), pseudotyped HXB-2 (D), or NL4-3ΔEnv (F) viruses for 1 h at 37°C. Cells were finally washed and lysed, and HIV-1 internalization was assessed by evaluating the amount of p24 in each cell lysate. In some experiments, JAR cell were pretreated with heparin (+) or not pretreated (−) and exposed to NL4-3ΔEnv reporter viruses and then stimulated with tumor necrosis factor (TNF-α) (10 ng/mL) (G). Luciferase activity was monitored in each cell lysate. Values from the luminometer are expressed as the ratio of TNF-α-stimulated cells to unstimulated cells. Data are means ± SDs of quadruplicate samples and are representative of 3 independent experiments.
the basis of our present data, we conclude that HIV-1 endo-
somal escape occurs in trophoblastic cells through a mechanism
that is independent of the viral Env glycoproteins. The precise
mechanism through which HIV-1 can reach the cytoplasm and
integrate within the host chromatin of polarized trophoblasts
is still undefined. However, it is well documented that HIV-1
carries a lipid membrane and acquires a wide array of host cell
proteins upon budding (reviewed in [50]). HIV-1 may have
evolved to exploit the endosomal hydrosoles to escape into the
cytoplasm. However, this hypothesis is not supported by some
recent data [23]. Alternatively, HIV-1 might undergo fusion
within the endosomes via interactions between virus-anchored
host proteins and endosomal proteins within trophoblasts. In-
terestingly, it was recently shown that the nucleocapsid of VSV
is exported into the cytoplasm after the back-fusion of internal
vesicles with the limiting membrane of late endosomes [51].
Moreover, it is recognized that HIV-1 infection from the en-
dosomes is possible in macrophages, lymphocytic cells, and
HeLa cells when viral particles are spared from degradation
[52, 53]. Finally, images of HIV-1 particles internalized in en-
docytic vesicles and undergoing fusion with endosomal mem-
brane have been observed in macrophages and trophoblastic
cells [16, 54].

In summary, the data presented in this study further support
the idea that HIV-1 infection of trophoblasts is fundamentally
different from what is known for CD4+ T cells. Our model
proposes that the initial interactions between HIV-1 and po-
larized trophoblasts occur partly through a HSPG-mediated
absorption mechanism. This process leads to a rapid and mas-
see endocytosis of virions. Infection ensues in the absence of
the viral Env glycoproteins gp120 and gp41 through endosomal
escape and access to the cytoplasm. Collectively, these data
provide key information about the early events associated with
the HIV-1 life cycle in polarized human trophoblasts.

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