DC-specific ICAM-3-grabbing nonintegrin mediates internalization of HIV-1 into human podocytes


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Mikulak J, Teichberg S, Arora S, Kumar D, Yadav A, Salhan D, Pullagura S, Mathieson PW, Saleem MA, Singhal PC. DC-specific ICAM-3-grabbing nonintegrin mediates internalization of HIV-1 into human podocytes. Am J Physiol Renal Physiol 299:F664–F673, 2010. First published July 14, 2010; doi:10.1152/ajprenal.00629.2009.—Human immunodeficiency virus (HIV)-1 has been demonstrated to contribute to the pathogenesis of HIV-associated nephropathy. In renal biopsy studies, podocytes have been reported to be infected by HIV-1. However, the mechanism involved in HIV-1 internalization into podocytes is not clear. In the present study, we evaluated the occurrence of HIV-1 internalization into conditionally immortalized human podocytes and the mechanism involved. Human podocytes rapidly internalized R5 and X4 HIV-1 primary strains via an endocytosis-dependent pathway, without establishing a productive infection. The HIV-1 internalization was dendritic cell-specific ICAM-3-grabbing nonintegrin (DC-SIGN) receptor mediated. The role of DC-SIGN was confirmed by using specific blocking antibodies and transfection with small interfering (si) RNA/DC-SIGN. Since podocyte HIV-1 trafficking was not altered by pH-modulating agents, it appeared that HIV-1 routing occurred through nonacid vesicular compartments. Interestingly, transfection of podocytes with neither siRNA/caveolin-1 nor siRNA/clathrin heavy chain inhibited podocyte viral accumulation. Thus it appears that clathrin-coated vesicles and caveosomes may not be contributing to HIV-1-associated membrane traffic.

DC-SIGN; DEC 205; HIV-1 receptor; HIV-associated nephropathy

Visceral epithelial cells (podocytes) are highly specialized and differentiated cells which support the glomerular capillaries, synthesis of glomerular basement membrane, and regulation of glomerular permselectivity (31). The study of podocytes in culture had been controversial because of its terminally differentiated phenotype. However, this problem was taken care of by the development of a constitutively immortalized human podocyte cell line by transfection with the temperature-sensitive simian virus 40 (SV40)-T gene (50). These cells exhibit a regular, cobblestone-like polygonal phenotype and express podocyte-specific O-acetylated ganglioside, WT-1 protein, nephrin, and CD2AP, providing a suitable phenotype for studying pathways involved in human renal disorders including HIV-associated nephropathy (HIVAN).

Many studies confirmed the central role of podocytes in the pathogenesis of HIVAN (29, 66, 67), which undergo characteristic phenotypic alteration-dysregulated phenotype, re-entry of the cell cycle, and proliferation (2). Podocyte dysfunction appears to be the result of the combination of the direct effect of viral gene product expression (66, 68), the specific host, and genetic factors (26, 28, 30, 44). However, much of our understanding of the effect of HIV-1 on podocytes is related to the rodent models, which allow only the study of the postentry phase of the HIV-1 life cycle; therefore, little is known about the interaction between HIV-1 and human podocytes in general, and the mechanism involved in podocyte HIV-1 entry in particular.

In general, HIV-1 enters susceptible cells by fusion of its envelope with the plasma membrane after binding to the CD4 molecule (11) and interaction with the chemokine coreceptors CCR5 or CXCR4 (54). Nevertheless, HIV-1 entry into CD4-negative human cells has been widely reported (1, 5, 15, 57, 59). In the majority of instances, HIV-1 entry into CD4-negative cells has been reported to occur through the endocytic pathways (35, 38, 62, 63) and at times, resulted in the productive infection (13, 63).

Dendritic cell-specific ICAM-3-grabbing nonintegrin (DC-SIGN) is an endocytic receptor, which belongs to the C-type lectin family. This large group of proteins [such as the macrophage mannose receptor (MMR) and DEC-205] are specialized in the recognition of carbohydrate structures present on cellular and viral proteins and are implicated in several biological processes, including cell adhesion and antigen presentation (17, 53). The role of C-type lectins in HIV-1 pathogenesis has been highlighted by studies demonstrating their capability to bind HIV-1 in a CD4-independent manner (10, 23, 41, 58, 65), which may further increase viral transmission to permissive cells (19). DC-SIGN is highly expressed in dendritic cells (56, 60) and was originally cloned for its ability to bind and internalize heavily glycosylated HIV-1 gp120 protein (10). Interestingly, the expression of DC-SIGN has also been demonstrated in other cells, including macrophages (53) and platelets (9).

In the present study, we have shown that human podocytes express the DC-SIGN receptor, which efficiently captures HIV-1. Specific antibody-blocking studies inhibited entry of the virus in podocytes transfected with siRNA/DC-SIGN as well as podocyte transfer of HIV-1 to T cells confirmed the functionality of DC-SIGN in these cells. The interaction between HIV-1 and human podocytes resulted in the activation of podocyte HIV-1 trafficking and degradation. Our results provide insights into the role of the attachment factors on human podocytes involved in HIV-1 internalization that appears to involve the DC-SIGN-dependent endocytic pathway.
MATERIALS AND METHODS

Reagents. Chloroquine (CLQ), ammonium chloride (NH₄Cl), bafilomycin A1, and aldrithiol-2 (AT-2) were purchased from Sigma-Aldrich (St. Louis, MO). T-20 (Roche, Indianapolis, IN) was obtained from NIH AIDS Research and Reference Reagent Program, (cat. no. 9845). Clarithrin heavy chain (HC) siRNA (h), caveolin-1 siRNA (h), clarithrin HC (TD.1) mouse monoclonal antibody, and caveolin-1 (N20) rabbit polyclonal antibody were obtained from Santa Cruz Biotechnology (Santa Cruz, CA).

Cells and viruses. Immortalized human podocytes were developed from primary human podocytes by transfection with the temperature-sensitive SV40-T gene and were characterized by their expression of WT-1 and nephrin (50). Human peripheral blood mononuclear cells (PBMCs) were prepared from peripheral blood mononuclear cells of HIV-1-negative donors as described previously (23). PBMCs were isolated from blood obtained from the New York Blood Bank (unidentified donors). Monocytes were obtained from PBMCs that adhered to plastic plates and cultured for 6 days in medium containing 100 U/ml GM-CSF (Sigma-Aldrich) and 10 ng/ml IL-4 (PeproTech, Rocky Hill, NY) to generate immature dendritic cells (IDCs). Several strains of HIV-1 were used, including R5 HIV-1_92US660 and X4 HIV-1_92HT999. Viral inclusions were adjusted according to reverse transcriptase (RT) activity to 20 × 10⁴ cpm/ml. In cocultivation studies, latex particles activated with phophomagglutinin (PhA; 5 µg/ml, Sigma) were added to podocytes in a ratio of 2:1 in a medium supplemented with 20 U/ml IL-2 (Roche). In blocking studies, cells were pretreated with specific antibodies: anti-DEC-205 (MG38; Serotec, Raleigh, NC); anti-CXCR4 (NIH AIDS Research and Reference Reagent Program, cat. no. 4083); anti-MMR (15-2, Santa Cruz Biotechnology); anti-DC-SIGN (ab13487, Abcam, Cambridge, MA); or a matching isotype control (Serotec) for 30 min before HIV-1 treatment. For inactivation with AT-2, a viral stock solution was treated with 1 mM AT-2 in DMSO or DMSO alone for 1 h at 37°C. Treatment agents were removed by ultrafiltration with a centricrifugal filter device with a YM-100 MW membrane (Millipore, Bedford, MA). A control virus preparation was processed in parallel with inactivated samples. Human podocytes were transfected with specific siRNA against DC-SIGN (Invitrogen), clarithrin HC (Santa Cruz Biotechnology), caveolin-1 (Santa Cruz Biotechnology), or control siRNA at concentration of 40 nM, using as a transfection reagent Lipofectamine 2000 (Invitrogen). After 6 h of transfection, the cells were grown for another 48 h and then used for Western blot analysis or treatment with HIV-1. 293T cells were transfected with pol gene Primer Sequence

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer Sequence</th>
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<tr>
<td>CD4</td>
<td>5’-GGGAGCTGTTGTTGAGAGC3’</td>
</tr>
<tr>
<td>CCR5</td>
<td>5’-GACCATTGAGGAGAAGGCT-3’</td>
</tr>
<tr>
<td>CXCR4</td>
<td>5’-AAATATTGAGGAGGAGGCT-3’</td>
</tr>
<tr>
<td>DC-SIGN</td>
<td>5’-CCCAAGATTGCTGATGAGGCT-3’</td>
</tr>
<tr>
<td>L-SIGN</td>
<td>5’-GGGTAGAGAGGATGAGGCT-3’</td>
</tr>
<tr>
<td>DE-205</td>
<td>5’-GGGTAGAGAGGATGAGGCT-3’</td>
</tr>
<tr>
<td>MMR</td>
<td>5’-GGGAGCTGTTGTTGAGAGC3’</td>
</tr>
<tr>
<td>GAPDH</td>
<td>5’-GGGAGCTGTTGTTGAGAGC3’</td>
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Flow cytometric analysis. Podocytes were washed with PBS and blocked with PBS containing 20% human serum. After washing, cells were stained with specific antibodies (anti-DEC-205-FITC (MG38; eBiosciences, San Diego, CA); anti-DC-SIGN-FITC (DCN46; BD Pharmingen, San Jose, CA); and anti-CXCR4 (C-20; Santa Cruz Biotechnology) and corresponding isotype antibody (BD Pharmingen, San Diego, CA), were stained with specific antibodies [anti-DEC-205-FITC (MG38; eBiosciences, San Diego, CA); anti-DC-SIGN-FITC (DCN46; BD Pharmingen, San Jose, CA); and anti-CXCR4 (C-20; Santa Cruz Biotechnology) and corresponding isotype antibody (BD Pharmingen)]. After washing, cells were stained with appropriate secondary FITC-conjugated antibody (BD Pharmingen) and then fixed with 4% buffered formalin. Staining was analyzed with FACs Calibur (Becton Dickinson, San Jose, CA) using Cell Quest software.

DC-SIGN, dendritic cell-specific ICAM-3-grabbing nonintegrin; MMR, macrophage mannose receptor.
Cell viability in the presence of used drug concentrations was determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT; Sigma) assay as described previously (39).

Statistical analysis. Student’s t-test was used to calculate P values.

RESULTS

HIV-1 entry into human podocytes. Human podocytes were exposed to primary strains of HIV-1 with different coreceptor usage, R5 HIV-192US660 and X4 HIV-192HT599. There was no difference in viability of cells treated under control and experimental conditions (data not shown). As shown in Fig. 1A, human podocytes exposed to either R5 or X4 viral strains were positive for the specific HIV-1 strong-stop DNA (LTR RU5), the HIV-1 early reverse transcript. The adequacy of washing was tested on HIV-1-nonpermissive 293T cells, transfected with either control or DC-SIGN plasmid. Both control (mock) and cells expressing control-plasmid were negative for LTR RU5, whereas, 293T cells expressing DC-SIGN were positive for LTR RU5 (Fig. 1B). The time course effect on podocyte HIV-1-LTR RU5 content was studied by incubating human podocytes with the primary HIV-192US660 for variable time periods. HIV-1 strong-stop DNA was detected in podocytes within 30 min, whereas, podocyte viral load peaked at 2 h and decreased by 24 h (Fig. 1C).

Intracellular viral localization by EM studies. To confirm HIV-1 internalization, EM studies were carried out. Podocytes showed the presence of intracellular viral-like particles in the vacuoles located in close proximity to endoplasmic reticulum (ER) (Fig. 2).

Noninfectious entry of HIV-1 into human podocytes. Since we did not detect the HIV-1 late reverse transcripts (pol, gag) (Fig. 1C) and because the nascent synthesis of HIV-1 strong-stop DNA has been reported to occur also within a viral particle (64), we wanted to ascertain whether the presence of LTR RU5 was the result of reverse transcription that occurred in the host cell or whether it was a part of the incorporated viral particle. In these studies, HIV-1 was inactivated with AT-2, which eliminates infectivity but preserves conformational and functional integrity of viral surface protein (49). AT-2-treated virions bound comparably to native virions (to target cells) but showed arrest of the progression of viral life cycle. Since there was no difference in strong-stop DNA content between AT-2-HIV-1 vs. native-HIV-1-treated podocytes, it appeared that the proviral DNA was part of the virions (Fig. 3).

Because cell-free HIV-1 transcytosis has been proposed as a potential mechanism for viral crossing to several tissue-specific cells (3, 4, 21, 33), we studied whether the cell-free HIV-1 could cross human podocytes and escape (without the establishment of productive infection). After treatment with HIV-1 (2 h), podocytes were trypsinized, washed, and seeded onto Transwells (0.4-μm membrane separating podocytes from primary human activated PBMCs). During 14 days of cocultivation, no HIV-1 p24 antigen was detected (data not shown). Similarly, the cell-free supernatants harvested at 24 and 48 h (after the cells were exposed to HIV-1 and washed)
were not able to establish the productive infection in activated PBMCs (data not shown). These findings indicate that the internalization of captured HIV-1 by human podocytes results in the intracellular hijacking and degradation of viral particles.

Expression of HIV-1 receptors in human podocytes. The primers used for gene amplification of HIV-1 receptors in CIHPs are described in Table 1. We did not find any detectable levels of gene expression for CD4 and CCR5 receptors (Table 2). Consistent with the published data (25), we found very low CXCR4 gene expression. Moreover, podocytes showed mRNA expression of C-type lectins, including DEC-205 and DC-SIGN, and no expression of MMR. Data on podocytes and PBMCs (RT-PCR) are shown in Fig. 4A. Percentages of human podocytes showing surface expression of different receptors were as follows: DEC-205 (25%), DC-SIGN (3%), and CXCR4 (0%) (Fig. 4B), whereas Western blot analysis showed relatively high expression of DC-SIGN protein, very low expression of DEC-205 protein, and no expression of CXCR4 (Fig. 4C). Because other homologs of DC-SIGN protein, l-SIGN (also called DC-SIGNR) (53), have been reported to be able to bind HIV-1 (48), we also verified its expression by real-time PCR; however, as shown Table 2, we did not detect the expression of l-SIGN transcripts in CIHPs.

DC-SIGN-dependent endocytosis of HIV-1 into human podocytes. To rule out podocyte HIV-1 entry through fusion (conventional HIV-1 receptors), we evaluated the effect of the T-20, a known HIV-1 fusion inhibitor, on podocyte viral entry. Although T-20 completely inhibited the infection of primary R5 and X4 HIV-1 strains in T cells, it had no effect on the internalization of the same primary R5 and X4 HIV-1 strains in podocytes (data not shown). In addition, pretreatment of podocytes with an anti-DC-SIGN antibody markedly decreased the effect of yeast mannan on podocyte HIV-1 entry. As shown in Fig. 6, there was complete inhibition of HIV-1 entry in podocytes in the media containing 0.45 M sucrose. Because C-type lectins are bound to the high-mannose-containing carbohydrates (16), we evaluated the effect of yeast mannan on podocyte HIV-1 entry. As shown in Fig. 6, mannan (5 mg/ml) blocked the internalization of virus by 80%. These findings supported the role of mannose in podocyte HIV-1 entry.

To identify the role of the specific lectin involved in podocyte HIV-1 entry, we carried out blocking studies. Podocytes were pretreated with specific antibodies against DEC-205 and DC-SIGN before incubation with HIV-1. As shown in Fig. 5A, preincubation with an anti-DC-SIGN antibody markedly de-

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Table 2. Analysis of expression of HIV-1 receptors by real-time PCR

<table>
<thead>
<tr>
<th>Receptor</th>
<th>PBMCs</th>
<th>Podocytes</th>
<th>IDCs</th>
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<tbody>
<tr>
<td>CD4</td>
<td>34.550 ± 452</td>
<td>00 ± 00</td>
<td>*</td>
</tr>
<tr>
<td>CXCR4</td>
<td>1.028.0 ± 120.2</td>
<td>2.0 ± 0.7</td>
<td>*</td>
</tr>
<tr>
<td>CCR5</td>
<td>40.0 ± 5.8</td>
<td>0.0 ± 0.0</td>
<td>*</td>
</tr>
<tr>
<td>DEC-205</td>
<td>45.0 ± 2.3</td>
<td>10.0 ± 2.5</td>
<td>*</td>
</tr>
<tr>
<td>MMR</td>
<td>2.5 ± 0.9</td>
<td>0.0 ± 0.0</td>
<td>*</td>
</tr>
<tr>
<td>DC-SIGN</td>
<td>*</td>
<td>8.0 ± 2.9</td>
<td>1,905.0 ± 145.7</td>
</tr>
</tbody>
</table>

Values are means ± SD and represent relative gene expression (2 ΔΔct), normalized to GAPDH, of 2 independent experiments. Total RNA, extracted from podocytes, activated peripheral blood mononuclear cells (PBMCs), and immature dendritic cells (IDCs), was subjected to real-time RT-PCR amplification using primers (Table 1) for classic human immunodeficiency virus (HIV)-1 receptors, including CD4, CCR5, CXCR4, and C-type lectin receptors, including DEC-205, MMR, and DC-SIGN. * , Not done.
creased HIV-1 entry in human podocytes. In contrast, no inhibition of podocyte HIV-1 entry was observed with pretreatment with anti-DEC-205 or a correspondent isotype. The effect of the anti-DC-SIGN antibody was dose dependent (with the saturation of inhibition at a concentration of 10 μg/ml) (Fig. 5B). In addition, transfection with specific siRNA/DC-SIGN significantly inhibited the entry of virus in human podocytes compared with the control siRNA (Fig. 7).

Human podocytes express DEC-205/DCL-1 fusion mRNA transcripts. Since anti-DEC-205 did not inhibit HIV-1 entry into podocytes, we hypothesized that the podocytes may not be expressing classic (wild-type) DEC-205 receptors. Recently, a novel DEC-205/DCL-1 fusion protein has been demonstrated in a Hodgkin’s lymphoma cell line and in human dendritic cells (6, 27). The DEC-205/DCL-1 fusion protein is a splice variant of DEC-205 and a novel C-type lectin, DCL-1, that carries the extracellular domains of DEC-205 and a transmembrane and cytoplasmic domain derived from DCL-1 (Fig. 8B). To detect mRNA for DEC-205 (between CTLD10 and the cytoplasmic domain) and DCL-1 (CTLD and the cytoplasmic domain), we have used specific primers for the cytoplasmic tail of this receptor compared with the wild-type of the DCL-1 receptor and DEC-205/DCL-1 fusion transcripts (Fig. 8A). Upon sequence analysis of the DEC-205/DCL-1 fusion protein and wild-type DCL-1, using primers shown in Fig. 8C, the transcripts were found to correspond to the DEC-205/DCL-1 fusion protein variant (Ref. AY184222) and DCL-1 (Ref. AY314007).

DC-SIGN is a functional receptor for HIV-1 entry into human podocytes. It has been reported that in dendritic cells, DC-SIGN not only captures HIV-1 but also contributes to cytoplasmic viral sequestration before viral transfer to T cells (18, 32). Moreover, stably DC-SIGN-transfected cells were not only capable of retaining HIV-1 for 3–6 days in culture but were also able to transmit HIV-1 to target cells (T cells) (18, 32). To determine whether the DC-SIGN receptor expressed in human podocytes is a functional receptor for viral sequestration and/or viral transmission to the target cells, we cocultivated HIV-1-pulsed podocytes with activated PBMCs. HIV-1 could establish productive infection in lymphocytes for up to 6 days (Fig. 9), indicating that DC-SIGN expressed on human podocytes has the potential for viral sequestration. Since we could detect the HIV-1 strong-stop DNA (by PCR) only up to 72 h, it appeared that DC-SIGN could sequester only a minimal (undetectable) number of infectious viral particles in podocytes for a longer time.
HIV-1 trafficking in human podocytes. To gain insight into viral traffic, we evaluated the effect of vesicular pH-modulating agents on podocyte HIV-1 strong-stop DNA content. Cells were pretreated with CLQ and NH₄Cl, the agents known to block vesicle acidification as well as the endo-lysosomal degradation system. CLQ and NH₄Cl did not alter podocyte HIV-1 accumulation (Fig. 10A). To confirm this further, cells were pretreated with bafilomycin A followed by evaluation for podocyte viral accumulation. As shown in Fig. 10B, bafilomycin A did not modulate podocyte viral accumulation. These findings indicate that podocyte viral trafficking might have occurred through pH-neutral vesicles. Since clathrin-coated vesicles and caveosomes also contribute to the endocytic pathway (34, 47), we studied the role of clathrin-coated vesicles and caveosomes on HIV-1 membrane traffic in podocytes. Podocytes were transfected with the specific siRNA/clathrin HC or siRNA/caveolin-1 or control siRNA, followed by viral entry protocol. As shown in Fig. 11A, cells transfected with siRNA/caveolin-1 showed diminished expression of caveolin compared with cells transfected with control siRNA and cells transfected with siRNA/clathrin HC.
Similarly, cells transfected with siRNA/clathrin HC displayed diminished expression of clathrin compared with control and cells transfected with siRNA/caveolin-1 (Fig. 11B). Cells transfected with either siRNA/caveolin-1 or siRNA/clathrin HC did not modulate viral accumulation in podocytes (Fig. 11C).

**DISCUSSION**

Human podocytes rapidly internalized R5 and X4 HIV-1 primary strains via an endocytosis-dependent pathway. Human podocytes expressed DC-SIGN receptors, which mediated capture of R5 and X4 HIV-1 primary strains. Since the internalized virus did not undergo reverse transcription and the amount of viral components decreased with time, it appears that HIV-1 particles in human podocytes are directed for degradation. Interestingly, a small fraction of the captured replication-competent viral particles survived inside the podocytes and could establish viral replication after transmission to target cells. These results are consistent with previously published studies showing that DC-SIGN not only captured and internalized HIV-1 particles but also enhanced trans-infection to T cells (18, 32).

We have previously reported that DEC-205 mediated HIV-1 entry in renal tubular cells (23, 39). Interestingly, podocytes also expressed DEC-205 receptors. Therefore, we expected a potential role of DEC-205 in podocyte HIV-1 entry. However, the blocking study with the specific anti-DEC-205 antibody did not support this notion. In addition, pH-modulating agents (contrary to renal tubular cells) did not show any effect on virus trafficking in podocytes. These findings indicated that pH-dependent endocytic compartments involved in renal tubular cell HIV-1 trafficking are not involved in podocyte HIV-1 trafficking. Further investigation revealed podocyte-expressed DEC-205/DCL-1 fusion protein instead of wild-type DEC-205 receptors. This hybrid protein carries the outer domain of DEC-205 and transmembrane and cytoplasmic domain of DCL-1 (6). These findings provided an explanation for non-functional DEC-205. At present, the role of DCL-1 and the functional significance of DEC-205/DCL-1 fusion protein are not known. However, expression of this protein in dendritic cells was associated with the abrogation of endocytic activity (6). We propose that podocyte expression of DEC-205/DCL-1 may be contributing to the lack of endocytic HIV-1 entry in podocytes.

We have recently demonstrated that HIV-1 entry into human podocytes was inhibited by disruption of specific membrane microdomains by cholesterol depletion (39). However, we did not evaluate any selective pathway in these studies. In dendritic cells, a significant portion of DC-SIGN resides in cellular membrane microdomains (7). Moreover, HIV-1 binding and

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**Fig. 8.** Detection of DEC-205/DCL-1 fusion mRNA transcripts in human podocytes. A: primers specific for DEC-205, DCL-1, and DEC-205/DCL-1 fusion were used to detect mRNA transcripts in cDNA from human podocytes. One representative experiment of 2 is shown. B: schematic representation of protein structures encoded by detected mRNA transcripts. C: primers used for amplification and sequencing of DEC-205/DCL-1 fusion protein and DCL-1 protein: CP7 (DCL-1), 5'-AAGGAATAGTGGTGAAAACGAGAATCAG-3'; CP8 (DEC-205), 5'-GAAGATTATTTGTCGAGCTCTATCGT-3'; CP9 (DCL-1), 5'-GGAAGTGGTGAAAACGAGAATCAG-3'.

**Fig. 9.** DC-SIGN in human podocytes mediates transmission of virus to the target cells. Podocytes were pulsed with HIV-12US660. Before incubation, viral stock was treated with 200 U/ml of RNase-free DNase. After 4 h of incubation with HIV-1, cells were trypsinized, washed, and cultured for an additional 3 and 6 days. Afterward, cells were washed extensively and activated T cells were added (ratio, 1:2). Viral replication was measured in lymphocyte supernatants (collected at different time points after cocultivation) by p24 assay. Values are means ± SD of 2 independent experiments. Mock pulsing served as a negative control.
uptake in IDCs have been reported to occur through a cholesterol-sensitive pathway (20). Many viruses, including HIV-1, target lipid rafts during both cellular entry and viral assembly (14, 37, 55). Recently, new data on the postendocytic trafficking of raft markers have provided insights into postendocytic trafficking. Interestingly, one of the better known lipid raft-mediated trafficking pathways discovered for SV40 involved pH-neutral vesicles (46).

The phagocytic pathway of the DC-SIGN receptor remains controversial. DC-SIGN binding has been shown to many pathogens (8, 51, 56, 64); however, several studies indicate that the internalization pathway of DC-SIGN in dendritic cells is dependent on the type of ligand. The antigen Lewis X and mycobacterial ManLAM are efficiently captured and internalized through DC-SIGN and are targeted to lysosomes (19). On the other hand, DC-SIGN-mediated HIV-1 entry was routed to nonlysosomal compartments and it was associated with sustained virulence in dendritic cells (32). Similarly, the hepatitis C virus entered via DC-SIGN/α-SIGN escape lysosomal degradation (36). Our study in human podocytes suggest the involvement of the neutral pH-compartment in the trafficking pathway of HIV-1; however, further studies are required to delineate the exact route.

In the present study, HIV-1 could establish productive infection in lymphocytes for up to 6 days, indicating that DC-SIGN expressed on human podocytes has the potential for viral sequestration. Since we could detect the HIV-1 strong-stop DNA (by PCR) only up to 72 h, it appeared that DC-SIGN could sequester only a minimal (undetectable) number of infectious viral particles in podocytes for a longer time. An alternative possibility can be considered that a smaller number of viral particles might have persisted by being attached to the cellular surface in the trypsin-resistant compartments. However, EM studies did not show any presence of HIV-1 particles on the surface of the human podocytes. Moreover, EM studies showed the presence of HIV-1 particles inside the cells. Thus it appears that there may be two possible sources for viral transfer to the immune cells.

We conclude that human podocytes have the capability to capture and internalize HIV-1 through a pH-independent endocytic pathway, which utilizes DC-SIGN receptors.

**GRANTS**

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the authors.
REFERENCES


