Lentivirus-Mediated RNA Interference of DC-SIGN Expression Inhibits Human Immunodeficiency Virus Transmission from Dendritic Cells to T Cells

Jean-François Arrighi,1 Marjorie Pion,1 Maciej Wiznerowicz,2 Teunis B. Geijtenbeek,3 Eduardo García,1 Shahnaz Abraham,2 Florence Leuba,1 Valérie Dutoit,1 Odile Ducray-Rundquist,2 Yvette van Kooyk,3 Didier Trono,2 and Vincent Piguet1*

Department of Dermatology and Venereology, University Hospital of Geneva,1 and Department of Genetics and Microbiology, CMU, Faculty of Medicine, University of Geneva,2 Geneva, Switzerland, and Department of Molecular Cell Biology and Immunology, VUMC, Amsterdam, The Netherlands3

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In the early events of human immunodeficiency virus type 1 (HIV-1) infection, immature dendritic cells (DCs) expressing the DC-specific intercellular adhesion molecule 3-grabbing nonintegrin (DC-SIGN) receptor capture small amounts of HIV-1 on mucosal surfaces and spread viral infection to CD4+ T cells in lymph nodes (22, 34, 45). RNA interference has emerged as a powerful tool to gain insight into gene function. For this purpose, lentiviral vectors that express short hairpin RNA (shRNA) for the delivery of small interfering RNA (siRNA) into mammalian cells represent a powerful tool to achieve stable gene silencing. In order to interfere with DC-SIGN function, we developed shRNA-expressing lentiviral vectors capable of conditionally suppressing DC-SIGN expression. Selectivity of inhibition of human DC-SIGN and L-SIGN and chimpanzee and rhesus macaque DC-SIGN function, we developed shRNA-expressing lentiviral vectors capable of conditionally suppressing DC-SIGN expression. Selectivity of inhibition of human DC-SIGN and L-SIGN and chimpanzee and rhesus macaque DC-SIGN was obtained by using distinct siRNAs. Suppression of DC-SIGN expression inhibited the attachment of the gp120 envelope glycoprotein of HIV-1 to DC-SIGN transfectants, as well as transfer of HIV-1 to target cells in trans. Furthermore, siRNA-expressing lentiviral vectors were capable of efficiently suppressing DC-SIGN expression in primary human DCs. DC-SIGN-negative DCs were unable to enhance transfer of HIV-1 infectivity to T cells in trans, demonstrating an essential role for the DC-SIGN receptor in transferring infectious viral particles from DCs to T cells. The present system should have broad applications for studying the function of DC-SIGN in the pathogenesis of HIV as well as other pathogens also recognized by this receptor.

MATERIALS AND METHODS

Vector construction and siRNAs. The pSUPER, pSUPER-si-GFP (si-GFP), pLV-Th, and pLV-TR-KRAB-dsRed (LV-KRAB) constructs were described previously (9, 54). Oligonucleotides encoding shRNAs directed against human DC-SIGN mRNA were designed according to the method of Elbashir et al. (17) and purchased from MWG Biotech (Ebersberg, Germany) (see Table 1 for

*Corresponding author. Mailing address: Department of Dermatology and Venereology, HUG, 4-752, 24 Rue Micheli-du-Crest, 1211 Geneva, Switzerland. Phone: (4122) 372.94.65. Fax: (4122) 372.94.70. E-mail: vincent.piguet@medecine.unige.ch.
FIG. 1. Design and screening of siRNAs targeting human DC-SIGN mRNA sequence. (A) Design of siRNAs. Six different siRNAs (siGFP to siSIgN26) targeting DC-SIGN were designed to target distinct parts of the DC-SIGN mRNA sequence. siRNA11 targeted tandem repeats (R1 to R8) present in exon III. TM, transmembrane domain. (B) Screening of siRNA-expressing vectors. Subconfluent 293T cells were cotransfected with pSUPER control plasmid, si-GFP, or si-SIGN constructs along with human DC-SIGN and pEGFP-N1 expression plasmids. The mean fluorescence intensity of DC-SIGN and GFP expression was measured in the GFP-positive and the DC-SIGN-positive cell populations, respectively. Histograms show the percentages of inhibition relative to that under the pSUPER control experimental condition. Means ± standard errors of results from four independent experiments are shown.

siRNA sequences). These oligonucleotides were annealed and ligated into pSUPER downstream of the H1 promoter, giving rise to pSUPER-si-DC-SIGN constructs (referred to hereafter as si-SIGN constructs). Both strands of si-SIGN constructs were sequencing using T3 and T7 primers. The H1 promoter cassette in pLV-TH was replaced by the H1-siRNA cassette excised from si-SIGN constructs, giving rise to LV-si-DC-SIGN lentiviral vectors. Human DC-SIGN (23) and L-SIGN (6) genes were cloned into lentiviral vectors pLOX (LV-DC-SIGN) and pWPXL (LV-L-SIGN), respectively, replacing the green fluorescent protein (GFP) marker gene. Chimeranese and rhymes macaque DC-SIGN expression plasmids were described previously (20).

siRNA screening test. Subconfluent 293T cells were cotransfected with 5 μg of pSUPER control plasmid or si-SIGN constructs and 0.5 μg of human DC-SIGN and pEGFP-N1 (Clontech, Palo Alto, Calif.) expression plasmids by calcium phosphate precipitation. Medium was changed after 16 h, and 24 h later, flow cytometric analysis of GFP and DC-SIGN expression using phycoerythrin (PE)-labeled anti-human DC-SIGN monoclonal antibody (MAb) was performed. To test for the specificity of si-SIGN constructs, the same assay was performed with the human DC-SIGN expression plasmid replaced by a plasmid expressing L-SIGN (PE-labeled anti-human L-SIGN MAb) or chimeranese or rhymes macaque DC-SIGN (PE-labeled anti-human DC-SIGN MAb).

Production of lentiviral vectors. All recombinant lentiviruses were produced by transient transfection of 293T cells according to standard protocols (58). Briefly, subconfluent 293T cells were cotransfected with 20 μg of the plasmid vector, 15 μg of pCMV-deltaR8.91, and 5 μg of pMD2.G-VSVG by calcium phosphate precipitation. Vector titers were determined by transfection and flow cytometric analysis of GFP expression in HeLa cells. Titers ranged between 2 × 10^7 and 5 × 10^5 HeLa-transducing U per ml.

Viral stocks. Viral stocks were generated by transfection of 293T cells with calcium phosphate-coprecipitated proviral plasmid pR9 which carries a full-length HIV-1 X4 strain (19). Infectious titers of viral stocks were evaluated by limiting dilution on HeLa P4 cells (11) and expressed as infectious units per milliliter. The titer values were also determined by measuring HIV-1 p24Ab by using an enzyme-linked immunosorbent assay (ELISA) kit (Beckman Coulter, Parc, France).

Generation of cell lines stably expressing siRNAs. Raji B cells were cultured as described elsewhere (55). Various Raji cell lines stably expressing siRNAs were generated by transduction with LV-DC-SIGN, LV-si-SIGN8, LV-si-SIGN11, LV-si-SIGN26, LV-L-SIGN, or empty vector. The HeLa P4-R5 cell lines stably expressing siRNAs were generated by transduction with LV-DC-SIGN, LV-si-SIGN8, LV-si-SIGN11, or LV-si-SIGN26. Stable knockdown or expression of the genes of interest by the different cell lines was determined by flow cytometric analysis.

Preparation and transduction of DC progenitors. Cord blood samples were obtained according to institutional guidelines of the ethical committee. CD34+ cells were purified and cultured as previously described (4). DC progenitors were transduced and analyzed on a FACSCalibur cell analyzer (Becton Dickinson, Mountain View, Calif.) as reported in reference 41 with the following modifications. After 3 to 6 weeks of primary culture, DC progenitors were transduced with LV-si-SIGN lentiviral vectors at a multiplicity of infection (MOI) of 20 for 16 h in the presence of hematopoietic growth factors. Cells were then extensively washed, and 2 × 10^6 transduced DC progenitors per ml of Iscove’s modified Dulbecco medium Glutamax 1 supplemented with 10% fetal calf serum and antibiotics (Life Technologies, Basel, Switzerland) were induced to differentiate into immature DCs for 6 days with a solution containing 50 ng/ml each granulocyte-macrophage colony-stimulating factor (GM-CSF) and interleukin-4 (IL-4; Strathmann Biotec, Hamburg, Germany) in the presence of 50 μM 2-mercaptoethanol (Sigma, Buchs, Switzerland) or into mature DCs by further addition of 20 ng of lipopolysaccharide (LPS; Escherichia coli strain 055:B5; Difco, Detroit, Mich.) for the last 2 days. DCs were harvested at day 6, analyzed by flow cytometry, and used in subsequent assays.

Cellular sorting. Immature DCs were sorted on a FACSIncoming SE cell sorter (Becton Dickinson) as GFP positive-DC-SIGN negative (LV-si-SIGN11) or GFP positive-DC-SIGN negative (empty vector). Cells were systematically reanalyzed after sorting. Viability and correctness of surface phenotype were excellent (>95%) up to 24 h postsorting. Sorted cells were counted and used for viral assays as described below.

Analysis of DC-SIGN-mediated transfer of HIV-1 infection to target cells. In order to measure DC-SIGN-mediated trans-enhancement of HIV-1 infection of T cells, aliquots of 5 × 10^5 Raji transfectants or sorted DCs were incubated for 2 h with HIV-R9 virus at 37°C at a MOI of 0.001, extensively washed, and directly cocultured with activated peripheral blood leukocytes (PBLs). Viral production in the coculture supernatants was monitored by reverse transcriptase assay.

Flow cytometric analysis and MAbs. Flow cytometric analysis was performed as described previously (5). Cells were analyzed on a FACSCalibur cell analyzer (Becton Dickinson). Data were analyzed using WINMDI software by J. Trotter at Scripps Institute (La Jolla, Calif.).

Analysis of DC-SIGN expression by the different cell lines was determined by the FACSCalibur flow cytometer. The percentage of GFP-positive cells within the total population was used to calculate the percentage of cells expressing DC-SIGN. The percentage of cells expressing DC-SIGN was determined using an enzyme-linked immunosorbent assay (ELISA) kit (Beckman Coulter, Paris, France).
TABLE 1. Human DC-SIGN cDNA target sequences for siRNAs

<table>
<thead>
<tr>
<th>siRNA</th>
<th>Exon</th>
<th>Sequence (5′ to 3′)</th>
</tr>
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<tbody>
<tr>
<td>siRNA2</td>
<td>I</td>
<td>AAAGGCTGAGAGGCCCCTGGAT</td>
</tr>
<tr>
<td>siRNA3</td>
<td>II</td>
<td>AACCTCTCTCTCTACGGCCTT</td>
</tr>
<tr>
<td>siRNA8</td>
<td>III</td>
<td>AGAGGGCAATCTACAGAGACC</td>
</tr>
<tr>
<td>siRNA11</td>
<td>IV</td>
<td>AAGGGTCCGCTGGGCTGGCCCTT</td>
</tr>
<tr>
<td>siRNA26</td>
<td>VI</td>
<td>AACTGGCCACGATCCATCACC</td>
</tr>
<tr>
<td>siRNA35</td>
<td>VA</td>
<td>AAAAGTGCGGGAGAATTATAGG</td>
</tr>
</tbody>
</table>

* Six different siRNAs directed against human DC-SIGN were designed to correspond to distinct parts of the DC-SIGN mRNA sequence. Target sequences of the DC-SIGN cDNA and corresponding exons are shown.

RESULTS

siRNA-expressing vectors suppress DC-SIGN expression. In order to silence human DC-SIGN expression, we followed the design rules of Elbashir et al. (17) and found 41 different siRNAs potentially targeting the DC-SIGN gene. Among them, six siRNAs corresponding to distinct parts of the DC-SIGN cDNA sequence were chosen (Fig. 1A; siRNA sequences are indicated in Table 1). siRNAs were cloned into pSUPER downstream of the H1 promoter, giving rise to siSIGN constructs.

The ability of each siRNA to inhibit DC-SIGN expression was then tested by cotransfection of 293T cells with pSUPER control plasmid, pSUPER-si-GFP, or si-SIGN constructs along with human DC-SIGN and pEGFP-N1 expression plasmids. This knockdown screening test allowed the identification of four siRNAs effective at inhibiting DC-SIGN expression as assessed by flow cytometric analysis (Fig. 1B). si-SIGN11 was the most effective (97% inhibition) while not affecting GFP expression. si-SIGN11 titration showed dose-dependent inhibition of DC-SIGN expression (data not shown). The same assay was used to test the siRNAs against human L-SIGN, rhesus macaque DC-SIGN, and chimpanzee DC-SIGN. Interestingly, some siRNAs could inhibit human L-SIGN as well as primate DC-SIGN expression despite the presence of mismatches between siRNA and specific mRNA sequences (Table 2). Again, si-SIGN11 was the most effective at inhibiting human L-SIGN and primate DC-SIGN. Interestingly, only si-SIGN8 specifically inhibited human DC-SIGN.

Lentivirus-mediated delivery of siRNA suppresses DC-SIGN expression in cell lines. siRNAs were then cloned into lentiviral vectors for stable expression in cell lines (54). In order to evaluate the effectiveness of siRNA-expressing lentiviral vectors (referred to hereafter as LV-si-SIGN) at inhibiting DC-SIGN expression, various Raji cell lines stably expressing siRNAs were generated (Fig. 2A). Transduction with LV-si-SIGN8 or LV-si-SIGN11 along with LV-DC-SIGN induced suppression of DC-SIGN expression. Results of flow cytometric analysis of DC-SIGN expression on Raji stable transfectants correlated with the results obtained using the knockdown screening test described above (Fig. 1B and 2A). LV-si-SIGN8 did not inhibit L-SIGN expression, confirming the specificity of the vector for DC-SIGN downregulation (Fig. 2A and Table 2). It is of note that stable DC-SIGN knockdown could be observed over 6 months (data not shown).

To ensure that DC-SIGN downregulation occurred through RNAi and not translational repression through a micro-RNA mechanism, levels of mRNA encoding DC-SIGN in different Raji cell lines stably expressing siRNAs were measured by RT-PCR (Fig. 2B). Very low levels of DC-SIGN mRNA were found in Raji cell lines transduced with LV-si-SIGN8 or LV-si-SIGN11 compared to those in control cell lines, whereas levels comparable to those in the DC-SIGN-expressing cell line were found in cell lines transduced with LV-si-SIGN26.

In addition, we studied the effect of DC-SIGN-specific siRNAs on the expression of the CD4 receptor and the CXCR4 coreceptor involved in HIV binding. For this purpose, various HeLa P4-R5 cell lines stably expressing siRNAs were

TABLE 2. siRNA specificity

<table>
<thead>
<tr>
<th>Construct</th>
<th>Human DC-SIGN</th>
<th>Human L-SIGN</th>
<th>Chimpanzee DC-SIGN</th>
<th>Rhesus macaque DC-SIGN</th>
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<tbody>
<tr>
<td>si-SIGN2</td>
<td>+ (0)</td>
<td>− (0)</td>
<td>+ (0)</td>
<td>− (0)</td>
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<tr>
<td>si-SIGN3</td>
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<td>+ (1)</td>
<td>+ (0)</td>
<td>+ (0)</td>
</tr>
<tr>
<td>si-SIGN8</td>
<td>+ (0)</td>
<td>− (1)</td>
<td>− (1)</td>
<td>− (2)</td>
</tr>
<tr>
<td>si-SIGN11b</td>
<td>+ + (0/0/0/0/0/0)</td>
<td>+ + (2/2/2/2/2)</td>
<td>+ + (0/0/0/0/0)</td>
<td>+ + (1/0/0/2/5)</td>
</tr>
<tr>
<td>si-SIGN26</td>
<td>− (0)</td>
<td>− (1)</td>
<td>− (1)</td>
<td>− (0)</td>
</tr>
<tr>
<td>si-SIGN35</td>
<td>− (0)</td>
<td>− (1)</td>
<td>− (1)</td>
<td>− (1)</td>
</tr>
</tbody>
</table>

* Subconfluent 293T cells were cotransfected with pSUPER control plasmid, si-GFP, or si-SIGN constructs along with human DC-SIGN and pEGFP-N1 expression plasmids. Flow cytometric analysis of DC-SIGN expression was performed. The mean fluorescence intensity of DC-SIGN expression was measured in the GFP-positive cell population. To test for the specificity of si-SIGN constructs, the same assay was performed with the human DC-SIGN expression plasmid replaced by plasmids expressing L-SIGN or chimpanzee or rhesus macaque DC-SIGN. Percentages of inhibition relative to that under the pSUPER control experimental condition were then calculated. + +, strong inhibition; +, inhibition; −, no inhibition. The numbers within brackets correspond to mismatches between siRNAs and specific mRNA sequences. Ø, no significant homology.

b si-SIGN11 targets six repeated sequences with 100% homology on the human DC-SIGN mRNA.
generated. Whereas transduction with LV-si-SIGN11 induced significant suppression of DC-SIGN expression, CD4 and CXCR4 expression levels were not affected (data not shown). Identical results were obtained using LV-si-SIGN8 (data not shown).

**Suppression of DC-SIGN expression by drug-inducible siRNAs.** Various HeLa cell lines were generated by transduction with the indicated lentiviral vectors. While both LV-si-SIGN8 and LV-si-SIGN11 suppressed DC-SIGN expression, only LV-si-SIGN11 suppressed L-SIGN expression. One month posttransduction, flow cytometric analysis of GFP, DC-SIGN, and L-SIGN expression was performed. The percentages of cells positive for the indicated cell surface markers are shown. (B) Effect of siRNAs on DC-SIGN mRNA. Total RNA extracted from Raji transfectants was subjected to RT-PCR to assess levels of mRNA corresponding to DC-SIGN and cyclophilin. Both LV-si-SIGN8 and LV-si-SIGN11 downregulated DC-SIGN mRNA expression but not cyclophilin mRNA expression.

**Fig. 2.** Suppression of DC-SIGN expression in cell lines through lentivirus-mediated delivery of siRNAs. (A) Generation of cell lines stably expressing siRNAs. Stable Raji transfectants were generated by transduction with the indicated lentiviral vectors. While both LV-si-SIGN8 and LV-si-SIGN11 suppressed DC-SIGN expression, only LV-si-SIGN11 suppressed L-SIGN expression. One month posttransduction, flow cytometric analysis of GFP, DC-SIGN, and L-SIGN expression was performed. The percentages of cells positive for the indicated cell surface markers are shown. (B) Effect of siRNAs on DC-SIGN mRNA. Total RNA extracted from Raji transfectants was subjected to RT-PCR to assess levels of mRNA corresponding to DC-SIGN and cyclophilin. Both LV-si-SIGN8 and LV-si-SIGN11 downregulated DC-SIGN mRNA expression but not cyclophilin mRNA expression.

**Fig. 3.** Conditional suppression of DC-SIGN expression was evaluated in HeLa cell lines transduced with LV-DC-SIGN, LV-si-SIGN11, and LV-KRAB in the presence (+) or absence (−) of doxycycline (DOX). One week posttransduction, flow cytometric analysis of GFP and DC-SIGN expression was performed. Cell percentages corresponding to each quadrant of two-dimensional plots are shown.

**Transduction of DC progenitors with siRNA-expressing lentiviral vectors allows the generation of DC-SIGN-negative DCs.** We next evaluated the potential of siRNA-expressing lentiviral vectors to suppress DC-SIGN expression at the surface of DCs. For this purpose, we transduced DC progenitors amplified in vitro from cord blood CD34+ cells with Flt3 ligand, stem cell factor, and thrombopoietin (4, 41). Transduced DC progenitors were then induced to differentiate into immature DCs for 6 days with GM-CSF and IL-4. Flow cytometric analysis of DCs revealed that LV-si-SIGN11 completely suppressed DC-SIGN expression compared to that in control cells or cells transduced with the inefficient LV-si-SIGN26 (Fig. 4A). LV-si-SIGN8 was less potent in inhibiting DC-SIGN expression, although the majority of the cells showed receptor knockdown. CD1a expression levels were comparable between GFP-positive and GFP-negative DCs (data not shown). A sim-
ilar phenotype for DC-SIGN was obtained using LPS as a maturation factor. Upregulation of HLA-DR and CD83 as well as downregulation of macrophage mannose receptor (CD206) was always observed upon maturation with LPS (Fig. 4B). In addition, transduction with lentiviral vectors did not modulate the expression of HLA-ABC, CD4, or CXCR4 (data not shown). Finally, no IFN-α could be detected in the supernatants of day-6 transduced immature DCs (data not shown).

DC-SIGN knockdown impedes HIV-1 gp120 adhesion to target cells. To test the ability of LV-si-SIGN to inhibit HIV-1 gp120 attachment to Raji transfectants (Fig. 5A) or DC-SIGN-negative DCs (Fig. 5B), we used a well-established fluorescent-bead adhesion assay (23, 25). Beads coated with HIV-1 gp120, M. tuberculosis cell wall component ManLAM, or ICAM-3 were incubated with cells stably expressing siRNAs. HIV-1 gp120, ManLAM, and ICAM-3 binding to cells transduced with LV-si-SIGN11 was severely reduced (Fig. 5). The levels of inhibition obtained using this assay were consistently reproducible with LV-si-SIGN11, but mannan or blocking antibodies showed more intra-assay variation (data not shown).

DC-SIGN knockdown severely reduces trans-enhancement of HIV-1 infection of T cells. Finally, we investigated whether DC-SIGN-negative DCs were capable of trans-enhancing HIV-1 infectivity in activated PBLs. Similar results were obtained using primary human DCs and Raji DC-SIGN transfectants (Fig. 6). While sorted DCs transduced with a control lentiviral vector could enhance the transfer of small amounts of virus HIV-R9 (MOI of 0.001) to activated PBLs, the same amount of virus in medium alone or added to DC-SIGN-negative DCs could not be transferred to activated PBLs (Fig.
lectin is not required for DC ontogeny, and this finding is compatible with the main role of DC-SIGN as a pathogen recognition receptor. Indeed, DC-SIGN plays a major role in the capture of HIV and M. tuberculosis on some DC subtypes (34, 35). However, other DC subtypes, such as Langerhans cells, do not express DC-SIGN and may mediate transfer of HIV through additional molecules or may require productive infection to transfer HIV to T cells (30, 37, 50, 51). In addition, the versatility of lentiviral vectors should enable the knockdown of DC-SIGN in a large number of targets, including transgenic animals. Indeed, advances in RNAi technology have provided a rapid loss-of-function method for assessing gene function in mammalian cells (9, 16). Moreover, highly stable and functional systems for silencing of gene expression in animal models are available (31, 40, 44, 53). In particular, a recent report describes a lentivirus-based system to functionally silence genes in transgenic mice by RNAi (40). Therefore, the generation of transgenic animals which would help to elucidate DC-SIGN function in HIV or simian immunodeficiency virus (SIV) pathogenesis is currently possible.

Furthermore, DC-SIGN can bind nonviral pathogens such as Leishmania mexicana (12), Schistosoma mansoni (52), M. tuberculosis (26, 47), Helicobacter pylori (52), and Candida albicans (10), as well as viral pathogens including Ebola virus (2), SIV-1, SIV-2 (38), HIV-2 (38), cytomegalovirus (27), hepatitis C virus (33, 39), and severe acute respiratory syndrome coronavirus (57). Therefore, our system based on lentivirus-mediated delivery of drug-inducible siRNAs targeting DC-SIGN will allow for new insights on this pathogen recognition receptor.

Another important application of our system is to reassess DC-SIGN functions. Most results described so far on the role of DC-SIGN relied on the use of blocking antibodies and mannan (22, 49). While these blocking agents are valuable, significant variations may be observed between results of studies using these tools because the block depends on the affinity of DC-SIGN for its ligand compared to that for the inhibitor used. The system developed here is nonequivocal because it is not affected by the binding properties of inhibitors that are more or less specific on diverse cell types. Lentiviral vectors conditionally expressing shRNAs targeting DC-SIGN could also be used in monocyte-derived DCs. However, we expect a less-efficient downregulation of DC-SIGN, because monocytes are more refractory to transduction than CD34+ cell-derived DC precursors (35; J.-F. Arrighi and V. Piguet, unpublished results). Furthermore, DC-SIGN is expressed at very high levels when monocytes are incubated in GM-CSF and IL-4 for 24 to 48 h (23), potentially limiting the effect of lentivirus-mediated RNAi of this receptor in this system.

Using lentiviral vector-mediated RNAi, we could demonstrate the unique role of DC-SIGN in enhancing transfer of HIV infectivity from DCs to T lymphocytes in trans. Finally, knockdown of DC-SIGN on primary DCs enables us to study unequivocally the function of this receptor in early events of HIV infection as well as its role in the pathogenesis of other viruses recognized by DC-SIGN.

**DISCUSSION**

Here we describe a novel system that enables potent suppression of DC-SIGN expression in a conditional manner. In order to disturb DC-SIGN expression, we developed lentiviral vectors conditionally expressing shRNAs targeting DC-SIGN. These tools allowed for the efficient suppression of DC-SIGN expression in various cell lines and in primary human DCs. By silencing DC-SIGN expression, we could inhibit the attachment of the gp120 envelope glycoprotein of HIV-1 to DCs negative for DC-SIGN as well as the transfer of HIV infection from DCs to T cells in trans.

Knockdown of DC-SIGN in DC precursors derived from CD34+ hematopoietic stem cells also revealed that this C-type lectin is not required for DC ontogeny, and this finding is compatible with the main role of DC-SIGN as a pathogen recognition receptor. Indeed, DC-SIGN plays a major role in the capture of HIV and M. tuberculosis on some DC subtypes (34, 35). However, other DC subtypes, such as Langerhans cells, do not express DC-SIGN and may mediate transfer of HIV through additional molecules or may require productive infection to transfer HIV to T cells (30, 37, 50, 51). In addition, the versatility of lentiviral vectors should enable the knockdown of DC-SIGN in a large number of targets, including transgenic animals. Indeed, advances in RNAi technology have provided a rapid loss-of-function method for assessing gene function in mammalian cells (9, 16). Moreover, highly stable and functional systems for silencing of gene expression in animal models are available (31, 40, 44, 53). In particular, a recent report describes a lentivirus-based system to functionally silence genes in transgenic mice by RNAi (40). Therefore, the generation of transgenic animals which would help to elucidate DC-SIGN function in HIV or simian immunodeficiency virus (SIV) pathogenesis is currently possible.

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