The Journal of Experimental Medicine

DC-SIGN–mediated Infectious Synapse Formation Enhances X4 HIV-1 Transmission from Dendritic Cells to T Cells

Jean-François Arrighi,[1] Marjorie Pion,[1] Eduardo Garcia,[1] Jean-Michel Escola,[1] Yvette van Kooyk,[2] Teunis B. Geijtenbeek,[2] and Vincent Piguet[1]

[1]*Department of Dermatology and Venereology, University Hospital of Geneva, 1211 Geneva, Switzerland*
[2]*Department of Molecular Cell Biology and Immunology, VUMC, 1081 BT Amsterdam, Netherlands*

Abstract

Dendritic cells (DCs) are essential for the early events of human immunodeficiency virus (HIV) infection. Model systems of HIV sexual transmission have shown that DCs expressing the DC-specific C-type lectin DC-SIGN capture and internalize HIV at mucosal surfaces and efficiently transfer HIV to $CD4^+$ T cells in lymph nodes, where viral replication occurs. Upon DC–T cell clustering, internalized HIV accumulates on the DC side at the contact zone (infectious synapse), between DCs and T cells, whereas HIV receptors and coreceptors are enriched on the T cell side. Viral concentration at the infectious synapse may explain, at least in part, why DC transmission of HIV to T cells is so efficient.

Here, we have investigated the role of DC-SIGN on primary DCs in X4 HIV-1 capture and transmission using small interfering RNA–expressing lentiviral vectors to specifically knockdown DC-SIGN. We demonstrate that DC-$SIGN^-$ DCs internalize X4 HIV-1 as well as DC-$SIGN^+$ DCs, although binding of virions is reduced. Strikingly, DC-SIGN knockdown in DCs selectively impairs infectious synapse formation between DCs and resting $CD4^+$ T cells, but does not prevent the formation of DC–T cells conjugates.

Our results demonstrate that DC-SIGN is required downstream from viral capture for the formation of the infectious synapse between DCs and T cells. These findings provide a novel explanation for the role of DC-SIGN in the transfer and enhancement of HIV infection from DCs to T cells, a crucial step for HIV transmission and pathogenesis.

Key words: HIV/SIV • virological synapse • RNA interference • lentiviral vectors • trans infection

Introduction

The major mode of HIV propagation worldwide is through sexual transmission. Model systems of HIV sexual transmission have shown that small amounts of virus cross mucosal surfaces and are captured by DCs to reach replication-competent sites in lymphoid tissue (for review see references 1–4). Direct infection of specific DC subsets by HIV is well demonstrated in vitro, at least with large amounts of virus. However, HIV/SIV-infected DCs are rarely detected in vivo (3). HIV can take an alternative route. DCs capture and store HIV in an intracellular compartment in the absence of viral replication and subsequent present infectious virions to T cells (5–7). Whether sexual transmission of HIV is mediated by direct infection of DCs and/or via the capture pathway remains unresolved (reference 8 and for review see references 1, 3, 4, 9)

In addition to classical HIV receptors and coreceptors (CD4, CCR5, and CXCR4), DCs express a type II transmembrane protein with an external C-type lectin domain, DC-SIGN (CD209), that captures and transmits HIV to T cells (10–12). DC-SIGN is expressed on the surface of several subsets of immature and, to a lesser extent, mature DCs (13, 14). The natural ligands for DC-SIGN are ICAM-3 and ICAM-2, molecules that contribute to transient DC–T cell attachment (13) and to transmigration of DCs across the vascular endothelium (15), respectively. Some DC subsets, such as Langerhans cells, do not express DC-SIGN, but they may use additional molecules such as Langerin, another

J.-F. Arrighi and M. Pion contributed equally to this work.

Address correspondence to Vincent Piguet, Dept. of Dermatology and Venereology, University Hospital of Geneva, 4-752, 24 Rue Micheli-du-Crest, 1211 Geneva, Switzerland. Phone: 4-1-22-372-94-65; Fax: 4-1-22-372-94-70; email: vincent.piguet@medecine.unige.ch

Abbreviations used in this paper: MOI, multiplicity of infection; si, small interfering.

 J. Exp. Med. © The Rockefeller University Press • 0022-1007/2004/11/1279/10 $8.00
Volume 200, Number 10, November 15, 2004 1279–1288
http://www.jem.org/cgi/doi/10.1084/jem.20041356

C-type lectin, to bind and transmit HIV to T cells (14, 16). Currently, DC-SIGN is the only known C-type lectin that enhances transmission from DCs to T cells (10, 17).

DC-SIGN and its homologue L-SIGN (CD209L/DC-SIGNR) function as attachment factors for HIV-1, HIV-2, and SIV (11, 18, 19). In fact, DC-SIGN binds gp120 with high affinity (20).

Interestingly, DC-SIGN–associated virus remains infectious for prolonged periods of time in the absence of replication within DCs (10, 21, 22). Although professional APCs such as DCs are rich in degradative compartments that are important in antigen processing, and some degradation of HIV-1 occurs, DCs appear unable to completely digest this virus (8, 17, 23). The mechanisms that mediate the prolonged survival or the increase in infectivity of virions captured by DCs via DC-SIGN are unclear at present, but may involve trafficking of HIV through a nonlysosomal endosomal compartment that has yet to be fully defined (17, 24, 25). These data are supported by results demonstrating that trypsin treatment of HIV-exposed DCs does not decrease the efficiency of DC-mediated virus transmission to T cells (26).

After encountering $CD4^+$ T cells, virions internalized by DCs relocalize to sites of contact with T cells at the infectious synapse (references 8, 27 and for review see reference 28). Infectious synapse formation is likely to be initiated by a normal cellular process in which DCs form transient contacts with T cells without the requirement for antigen specificity. Subsequently, T cells perform a "scanning" to enable recognition of any cognate peptide specificities presented by the APC (29, 30). In DC–T cell conjugates, HIV-1 virions internalized by DCs concentrate at the contact surface with the T cell, whereas the HIV-1 receptors CD4 and CCR5 appear to be partially enriched on the T cell at the site of contact with the DC (27). Viral receptor recruitment and virus focusing at this synapse may explain, at least in part, why DC transmission of HIV to T cells is such an efficient process (5, 10, 31). However, mechanisms of DC–T cell infectious synapse formation remain elusive.

To analyze the role of DC-SIGN in infectious synapse formation, we disrupted the expression of this C-type lectin with lentiviral vectors (LV-si-SIGN) stably expressing small interfering (si)RNA targeting DC-SIGN. DCs knocked down for DC-SIGN enabled us to define the sequential role of this receptor in HIV attachment (X4 HIV-1), internalization, and transfer of viral infectivity from DCs to $CD4^+$ T cells through an infectious synapse. Although DC-SIGN contributes significantly to X4 HIV-1 binding on DCs at 4°C, viral capture and internalization by DCs at 37°C after 2 h is similar in presence or absence of DC-SIGN. Importantly, DC-SIGN knockdown DCs are selectively impaired for the formation of infectious synapses between DCs pulsed with X4 HIV-1 and uninfected $CD4^+$ resting T cells. Nevertheless, DC-$SIGN^-$ DCs retain their capacity to form conjugates with resting $CD4^+$ T cells. As a consequence, transfer of X4 HIV-1 infection by DC-$SIGN^-$ DCs to target cells is severely impaired (downstream of viral capture). Together, these results identify the C-type lectin DC-SIGN as an important component of the infectious synapse that facilitates transfer of X4 HIV-1 infection from DCs to T cells.

Materials and Methods

Vectors Construction and siRNA. Lentivectors and siRNA sequences used in this work have been described previously (32). LV-si-SIGN11 is an efficient siRNA-expressing lentivector enabling stable DC-SIGN and L-SIGN knockdown (32). LV-si-SIGN8 is an siRNA-expressing lentivector enabling stable specific DC-SIGN (and not L-SIGN) knockdown (32). LV-si-SIGN26 is an inefficient siRNA-expressing lentivector (32)

Production of Lentiviral Vectors. The 293T and HeLa cell lines were cultured in Dulbecco's modified Eagle's medium supplemented with 10% FCS. All recombinant lentiviral vectors were produced by transient transfection of 293T cells according to standard protocols (33) as described previously (32). Vector titers were determined by transduction and flow cytometry analysis of GFP expression in HeLa cells. Titers ranged between 2×10^7 and 5×10^8 HeLa transducing units per milliliter.

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 described previously (34). DC progenitors were transduced and analyzed on a FACSCalibur cell analyzer (Becton Dickinson) as reported previously (35) with some modifications (32). After 3–6 wk of primary culture, DC progenitors were transduced with LV-si-SIGN lentivectors at an MOI of 20 and were induced to differentiate into immature DCs for 6 d with 50 ng/ml GM-CSF and IL-4 or into mature DCs by further addition of LPS for the last 2 d. DCs were harvested at day 6, analyzed by flow cytometry and used in subsequent assays. Additional technical details have been described previously (32).

Flow Cytometric Analysis and Monoclonal Antibodies. Flow cytometric analysis was performed as described previously (36). Cells were analyzed on a FACScalibur cell analyzer (Becton Dickinson). Data were analyzed using WINMDI software by J. Trotter (The Scripps Institute, La Jolla, CA). All antibodies have been described previously (32).

Cellular Sorting. Immature or mature DCs were sorted on a FACSVantage SE cell sorter (Becton Dickinson) as GFP^+ DC-$SIGN^-$ cells (LV-si-SIGN11) using PE-labeled anti–DC-SIGN mAb or GFP^+ cells (empty vector). Cells were systematically reanalyzed after sorting using allophycocyanin-labeled anti–DC-SIGN mAb.

Viral Stocks. Viral stocks were generated by transfection of 293T cells with calcium-phosphate coprecipitated proviral plasmid pR9, which encodes for a full length HIV-1 X4 strain (37), or pR9 IN-HA, which encodes for a full length infectious HIV-1 X4 strain provirus bearing an HA-tagged integrase (a gift from F. Bushmann, Salk Intitute, La Jolla, CA). Infectious titer of viral stocks was evaluated by limiting dilution on $CD4^+$ HeLa P4-2 cells containing β-*gal* gene under the control of the HIV promoter, expressed as infectious units per milliliter, which gives us values of MOI. Alternatively, titer values were determined by measuring HIV-1 $p24^{gag}$ using an ELISA kit (Beckman Coulter). GFP-labeled HIV-1 X4 strain (HIV W-xxF-GFP) has been described previously (38).

Viral Binding and Capture Assays Sorted transduced DCs or Raji cell lines (8×10^4 cells/well) were incubated with HIV-1

X4 (100 ng of $p24^{gag}$ or approximately an MOI of 1) in 80 μl of total volume for 2 h at 4°C. Viral binding assays were performed with stably siRNA-expressing Raji cell lines described previously (32) or with DCs transduced with empty vector or LV-si-SIGN11. Untransduced DCs were also incubated in the presence of 1 mg/ml mannan for 30 min at 37°C before virus exposure for 2 h at 4°C. Cells were vigorously washed seven times with cold PBS + 1% HSA interspersed with centrifugations to remove unadsorbed virus, and finally lysed with 50 μl of PBS containing 1% Triton X-100. The $p24^{gag}$ content of the lysate was determined by ELISA. Results were expressed as percentage of $p24^{gag}$ binding in control cells (Raji-LV-DC-SIGN or untransduced DCs).

For viral capture assays at 37°C, transduced DCs (2.5×10^5 cells/well) were incubated with HIV-1 X4 (MOI 1) in 80 μl of total volume for 2 h at 37°C. Simultaneous labeling of surface DC-SIGN and intracellular $p24^{gag}$ was performed using allophycocyanin-coupled anti–DC-SIGN mAb, and subsequent intracellular staining of HIV-1 $p24^{gag}$ was performed using Cytofix-Cytoperm (BD Biosciences) and PE-coupled anti-$p24^{gag}$ mAb (clone KC57 RD1; Beckman Coulter). Cells were washed, fixed in 1% paraformaldehyde and analyzed using a FACScalibur.

Analysis of DC-SIGN–mediated Transfer of HIV Infection to Target Cells. The ability of Raji transfectants and DCs to transfer virus particles to target cells was determined by coculturing HIV-pulsed cells with $CD4^+$ HeLa P4-2 cells in 24-well plates in a single round assay. In brief, virus-pulsed Raji or DCs were washed extensively to remove unadsorbed virus, and 1,000 Raji or DCs were cocultured with $CD4^+$ HeLa P4-2 cells. Viral transfer was determined by measuring the number of $CD4^+$ HeLa P4-2 infected cells. Results were expressed as percentage of each condition compared with control cells (Raji-LV-DC-SIGN or empty vector-transduced DCs).

Immunofluorescence Microscopy, Infectious Synapse Assay, and DC–T Cell Clusters Formation Assays. Highly purified resting $CD4^+$ T cells were prepared as described previously (39), resulting in a population of resting $CD4^+$ T cells with a degree of purity superior to 95% as determined by postpurification FACS analysis. For infectious synapse assays, 3×10^5 $CD4^+$ T cells were left to adhere on poly-L-lysine-treated glass coverslips for 2 h at 37°C. Mature sorted DCs (10^5 cells) were pulsed with HIV IN-HA for 2 h at 37°C (MOI = 5). DCs were washed twice and left to adhere at 37°C on coverslips for 10 or 30 min to allow contact with previously seeded T cells. Cells were fixed by a 20-min incubation in 3% paraformaldehyde at room temperature, further permeabilized with 0.05% saponin, and washed several times with PBS containing 10% FCS and human IgG (20 μg/condition). Cells were stained with primary mouse anti–HA-11 mAb (dilution of 1:1,000; Covance-Babco) and secondary donkey anti–mouse coupled to rhodamine (Jackson ImmunoResearch Laboratories) (dilution of 1:500). Nuclei were stained with DAPI (Molecular Probes). Samples were analyzed on an Axiovert 200 fluorescence microscope (Carl Zeiss MicroImaging, Inc.) equipped with a cooled charge–coupled device camera as described previously (40). We measured an infectious synapse as a DC–T cell conjugate where the majority of HIV is focused at the zone of contact with the $CD4^+$ T cells (>75% of HIV), which can be readily determined by immunofluorescence microscopy. We imaged and quantified in each experiment ~60–70 DC–T cell conjugates for the 10-min time point and 170–180 DC–T cell conjugates for the 30-min time point (see Fig. 6 B).

For HIV and DC-SIGN colocalization in the infectious synapse, mDCs (10^5 cells) were loaded with a GFP-labeled HIV-1 X4 for 2 h (MOI = 10) and left to adhere on coverslips for 30 min at 37°C to allow contact with previously seeded T cells as described before. Cells were fixed, permeabilized, and stained with primary anti–DC-SIGN mAb (dilution of 1:5) (clone 120507; R&D Systems) and secondary donkey anti–mouse coupled to rhodamine. Mounted slides were examined on an Axiovert 200 fluorescence microscope (Carl Zeiss MicroImaging, Inc.). Confocal laser scanning microscopy was performed with a Nipkow QLC100 module (Visitron Systems).

DC–T cell conjugate formation was quantified by counting DC–T cell conjugates on the whole surface of coverslips prepared for the infectious synapse assay. Alternatively, DC–T cell cluster formation was measured by flow cytometric analysis. For this purpose, empty vector- or LV-si-SIGN11-transduced DCs were labeled with anti–CD1a-APC and incubated over time with $CD4^+$ T cells labeled with anti–CD3-PE or with the fluorescent dye PKH26 (Sigma-Aldrich). Flow cytometric analysis of DC–T cell cluster formation was performed by determining the percentage of PKH^+ cells within each GFP^+CD1a^+ cellular population using a FACScalibur cell analyzer. This method allowed us to measure triple positive ($PKH^+GFP^+CD1a^+$) DC–T cell conjugates.

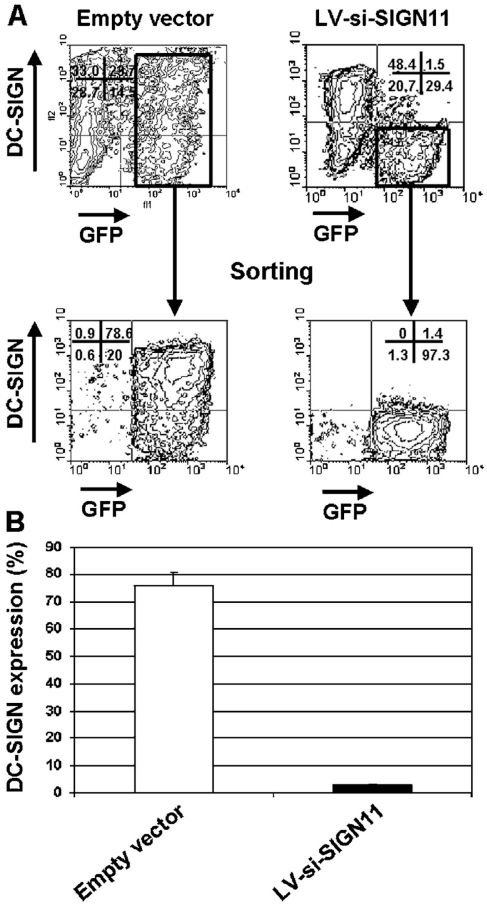

Figure 1. Generation of DCs knocked down for DC-SIGN expression. (A) DC progenitors were transduced with the indicated lentiviral vectors and differentiated into mature DCs. GFP^+ DCs were sorted for empty vector-transduced cells (left), whereas GFP^+ DC-$SIGN^-$ cells were sorted for LV-si-SIGN11 (right). Cell percentages corresponding to each quadrant of two-dimensional plots are shown. One representative experiment out of eight is shown. (B) Percentage of DC-SIGN expression is shown for each sorted GFP^+ cellular population. Means ± SEM of eight independent experiments is shown.

Results

Transduction of DC Progenitors with siRNA-expressing Lentiviral Vectors Allows Generation and Sorting of DC-SIGN⁻ DCs. Recently, we reported an experimental system that allows us to generate human DCs stably knockdown for DC-SIGN expression (32). DC progenitors are amplified in vitro from cord blood CD34⁺ cells with Flt3-ligand, stem cell factor, and thrombopoietin. Subsequently, they are transduced with siRNA-expressing lentiviral vectors targeting DC-SIGN expression. Transduced DC progenitors are induced to differentiate either into immature DCs for 6 d with GM-CSF and IL-4, or into mature DCs by addition of LPS for the last 2 d. We have identified an siRNA-expressing lentiviral vector (LV-si-SIGN11) that very potently inhibits DC-SIGN expression (32). Sorting of DCs with surface DC-SIGN knockdown is facilitated by GFP expression of transduced cells (Fig. 1). As shown in Fig. 1 A, GFP⁺ cells are selected in the case of empty vector-transduced DCs, whereas gating is performed on GFP⁺ DC-SIGN⁻ cells for LV-si-SIGN11–transduced DCs (top right). Flow cytometric analysis of sorted cellular populations for GFP and DC-SIGN expression is shown in Fig. 1 A (bottom). DC-SIGN expression was 75.7 ± 4.9% in empty vector-transduced DCs with a mean fluorescence intensity between 10^2 and 10^3 and 2.6 ± 0.6% in LV-si-SIGN11–transduced DCs with a mean fluorescence intensity close to background values (mean ± SEM, $n = 8$; Fig. 1 B). In conclusion, this experimental system allows us the generation and selection of primary DCs that are negative for DC-SIGN expression.

DC-SIGN Contributes to HIV Binding on DCs, But Not to HIV Capture and Internalization. To evaluate the contribution of DC-SIGN for HIV attachment on DCs, we performed binding assays at 4°C using an X4 viral strain and measured virion binding to DCs by $p24^{gag}$ ELISA. Attachment of HIV on DC-SIGN⁻ DCs at 4°C was reduced by ~40%, whereas the carbohydrate mannan decreased HIV binding to DCs by 60% (Fig. 2 A). Viral attachment to DC-SIGN knockdown Raji transfectants was severely decreased by 80% (Fig. 2 A).

Next, we examined whether DC-SIGN was required for viral capture and internalization by DCs after 2 h at 37°C (Fig. 2 B). For this purpose, we used a well-characterized FACS-based assay measuring viral capture through detection of intracellular $p24^{gag}$. Strikingly, after 2 h of internalization of HIV at 37°C (MOI = 1), we measured similar levels of viral capture in DC-SIGN⁺ and DC-SIGN⁻ DCs. Although there was a small decrease of viral capture for DC-SIGN⁻ DCs, it did not reach statistical significance when compared with DCs transduced with an empty vector (mean ± SD: 77 ± 12% vs. 84 ± 5%, respectively; Fig. 2 C). To strengthen these findings, we trypsinized DCs after viral capture before FACS analysis to remove surface-bound virus. We observed identical HIV internalization in DC-SIGN⁺ and DC-SIGN⁻ DCs after trypsin treatment (unpublished data).

In conclusion, although DC-SIGN promotes binding of X4 HIV-1 to DCs at 4°C, it is not required for viral capture and internalization by DCs at 37°C over time.

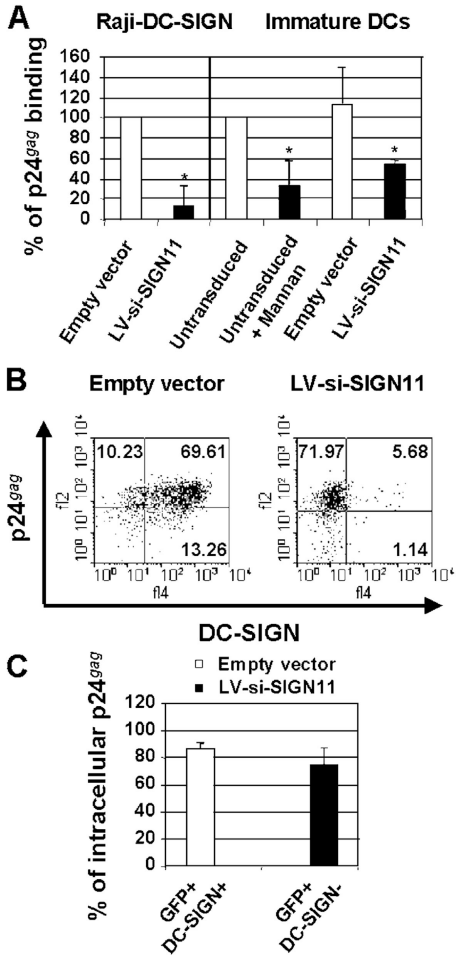

Figure 2. Analysis of HIV binding and capture by DC-SIGN⁻ DCs or DC-SIGN⁺ DCs. (A) DC-SIGN increases binding of X4 HIV-1 to Raji–DC-SIGN transfectants or immature DCs. Cells were pulsed with HIV (100 ng of $p24^{gag}$) for 2 h at 4°C. Cell-bound virus was determined by a $p24^{gag}$ ELISA. Results are expressed as percentage of $p24^{gag}$ binding compared with control cells (Raji-LV–DC-SIGN or untransduced DCs). Mean ± SD of three independent experiments is shown. *, Statistically significant differences (Student's *t* test, $P < 0.05$). (B) DC-SIGN⁻ DCs (LV-siSIGN 11) capture X4 HIV-1 as efficiently as DC-SIGN⁺ DCs. DCs were incubated with X4 HIV-1 at an MOI of 1 for 2 h at 37°C. Cells were immunostained for surface DC-SIGN and labeled with antibodies against intracellular HIV $p24^{gag}$. The percentage of double positive cells for HIV-$p24^{gag}$ and DC-SIGN was determined on GFP⁺ cells. One representative experiment out of three is presented. (C) Quantification of HIV internalization by DC-SIGN⁺ and DC-SIGN⁻ DCs. Results are expressed as the percentage of $p24^{gag}$ staining in GFP⁺ DC-SIGN⁺ cells for empty vector-transduced DCs, or in GFP⁺ DC-SIGN⁻ cells for LV-si-SIGN11-transduced DCs. Mean ± SD of three independent experiments is shown. No statistical significant difference was observed between DC-SIGN⁺ DCs and DC-SIGN⁻ DCs (LV-siSIGN 11) (Student's *t* test, $P > 0.05$).

DC-SIGN Increases Transfer of HIV Infectivity from DCs to Target Cells in Trans. To investigate whether DC-SIGN facilitates the transmission of X4 HIV-1 to target cells in trans, we incubated Raji cells expressing DC-SIGN (LV–DC-SIGN) or DC-SIGN⁻ Raji cells (LV-si-SIGN8 and 11) with HIV for 2 h (MOI = 1) at 37°C and measured transmission to CD4⁺ HeLa P4-2 cells in a single round infection assay. LV-si-SIGN8 encodes a DC-SIGN–specific

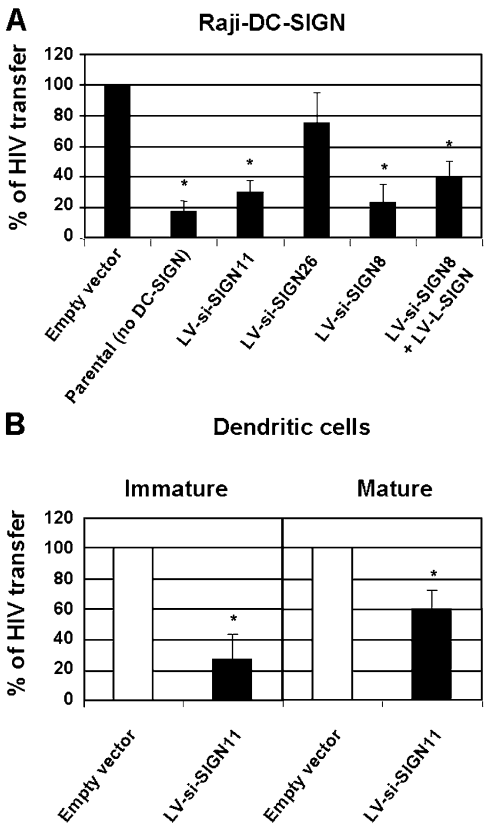

Figure 3. DC-SIGN facilitates transfer of HIV infectivity to target cells in trans. (A and B) DC-SIGN–mediated transfer of HIV to CD4$^+$ HeLa-P4-2 cells. Raji transfectants expressing DC-SIGN (A) or immature and mature DCs (B) were transduced with siRNA-expressing lentiviral vectors. DCs or Raji transfectants expressing DC-SIGN were subsequently sorted into GFP$^+$ DC-SIGN$^-$ (LV-si-SIGN8 and 11) and GFP$^+$ DC-SIGN$^+$ (empty vector, LV-si-SIGN26). GFP$^+$ DC-SIGN$^-$ cells (LV-si-SIGN8) were transduced with L-SIGN (LV-L-SIGN) and are GFP$^+$ DC-SIGN$^-$ L-SIGN$^+$ cells. Cells were incubated with X4 HIV-1 at an MOI of 1 at 37°C for 2 h. Infected cells were loaded onto target CD4$^+$ HeLa-P4-2 cells, and transfer of HIV infectivity was scored in a single round infection assay. Results are expressed as percentage of the number of infected CD4$^+$ HeLa-P4-2 cells compared with control cells (Raji–DC-SIGN [A] or empty vector-transduced DCs [B]). Mean ± SD of three independent experiments is shown. *, Statistically significant differences (Student's *t* test, $P < 0.05$).

siRNA, whereas LV-si-SIGN11 downregulates both DC-SIGN and L-SIGN. LV-si-SIGN26 encodes an inefficient siRNA (32). DC-SIGN$^+$ Raij cells were very efficient in transferring HIV to target cells, whereas the DC-SIGN–knocked down Raji cells with either LV-si-SIGN8 or 11 were inefficient to transfer HIV infectivity to target cells (~70% inhibition; Fig. 3 A). Importantly, this effect did not result from nonspecific inhibition because it was rescued by expressing the DC-SIGN homologue L-SIGN in the DC-SIGN–knocked down Raji cells (condition LV-si-SIGN8 + LV-L-SIGN; Fig. 3 A). The rescue with L-SIGN was partial and correlates with the lower transfer HIV infectivity mediated by L-SIGN compared with DC-SIGN (41).

Subsequently, we analyzed whether DC-SIGN enhanced transfer of HIV infection from primary human DCs to CD4$^+$ HeLa P4-2 cells (Fig. 3 B). Immature DC-SIGN$^-$ DCs showed a decreased capacity to transfer infectious virus to Hela CD4$^+$ cells (75% inhibition) compared with control DCs (Fig. 3 B). In mature DCs, HIV transfer to target cells was decreased by ~40% (Fig. 3 B). It is conceivable that the remaining infectivity transmitted by mature DC-SIGN$^-$ DCs is mediated by additional lectins that are yet to be identified. Strengthening these findings, we recently reported that suppressing DC-SIGN from DCs abrogates their capacity to transmit HIV-1 infectivity to activated primary CD4$^+$ T cells in trans (32).

Together, these results confirm the role of DC-SIGN as a crucial HIV receptor that mediates HIV-1 transmission from DCs to target cells, including activated T cells. Furthermore, we also demonstrate that DC-SIGN enhances viral transmission in trans downstream from viral capture because viral capture is equal in DC-SIGN$^-$ DCs and in DC-SIGN$^+$ DCs in this system.

DC-SIGN Is Dispensable for the Formation of DC–CD4$^+$ T Cell Clusters. As DC-SIGN is known to interact with ICAM-3 on resting CD4$^+$ T cells, we analyzed whether disruption of DC-SIGN would prevent formation of clusters between DCs and resting CD4$^+$ T cells. A loss of recruitment of T cells by DC-SIGN$^-$ DCs could provide an obvious explanation for the decrease in viral transfer from DC-SIGN$^-$ DCs to T cells.

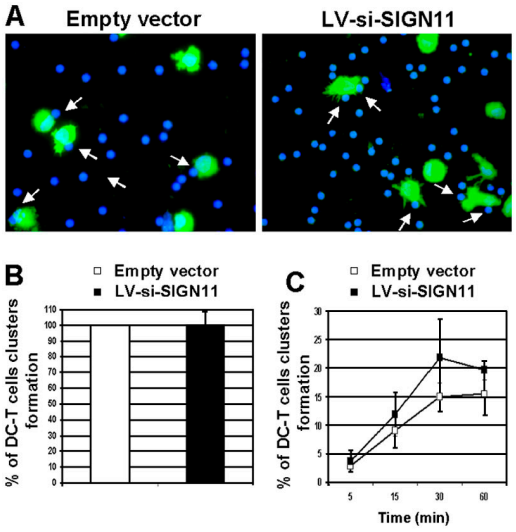

Figure 4. DC-SIGN is not required for DC–T cell cluster formation. (A) Sorted mature DCs (GFP$^+$) were incubated with highly purified resting CD4$^+$ T cells for 30 min at 37°C, allowing DC–T cell cluster formation. Cell nuclei were stained using DAPI (blue). Arrows denote typical DC–T cell conjugates. Representative results of three independent experiments are shown. (B) The number of immunological synapses was determined in each experimental condition by counting on microscope slides. The percentage of DC–T cell clusters formed by LV-si-SIGN11-transduced DCs is shown compared with control DCs (empty vector). Mean ± SD of three independent experiments is shown. (C) Kinetics analysis of DC–T cell cluster formation was performed by flow cytometric analysis. Empty vector- or LV-si-SIGN11-transduced DCs were incubated over time with highly purified resting CD4$^+$ T cells. DC-SIGN$^-$ DCs (LV-siSIGN 11) form DC–T cells conjugates as efficiently as DC-SIGN$^+$ DCs. Mean ± SD of three independent experiments is shown.

First, we measured immunological conjugate formation between DCs and highly purified resting $CD4^+$ T cells by immunofluorescence analysis. DCs were incubated with $CD4^+$ T cells for 30 min. Cell size and DAPI staining allowed immediate identification of DC–T cell conjugates (Fig. 4 A). Quantification of the DC–T cell conjugates demonstrates that DC-SIGN knockdown DCs were able to form conjugates with T cells as efficiently as untransduced DCs (Fig. 4, A and B).

To further evaluate whether DC-SIGN was required for the formation of DC-T cells conjugates, we performed time course experiments using a flow cytometric-based assay that allowed us to detect DC–resting $CD4^+$ T cell conjugates. DCs were stained with anti-CD1a mAb, and $CD4^+$ T cells were loaded with a fluorescent dye (PKH26) or stained with anti-CD3 mAb (Fig. 4 C). Both T cells staining methods gave identical results (unpublished data). DC–T cell conjugates were detected as early as 5 or 15 min, and the maximal number of conjugates was detected at 30 min. We did not observe any requirement for DC-SIGN in the formation of DC–resting $CD4^+$ T cell conjugates.

DC-SIGN Promotes Infectious Synapse Formation between DCs and $CD4^+$ T Cells. DC-SIGN knockdown DCs form conjugates efficiently with resting $CD4^+$ T cells. For that reason, we investigated whether the abrogated transfer of HIV infection from DC-SIGN knockdown DCs to T cells (32) could be explained by a role of DC-SIGN in DC–T cell infectious synapse formation. We developed the following infectious synapse assay: DCs pulsed with HIV-1, containing an HA-tagged integrase (X4 strain), were incubated with highly purified resting $CD4^+$ T cells for various times before fixation and analysis by immunofluorescence microscopy.

We first analyzed whether DC-SIGN was present in the infectious synapse between mature DCs and resting $CD4^+$ T cells. DC-SIGN was expressed at high levels in both immature and mature DCs as we reported recently (32). DC-SIGN could readily be detected at the infectious synapse by conventional fluorescence microscopy (Fig. 5). Using confocal microscopy, we confirmed that DC-SIGN was present in the infectious synapse (Fig. 5).

Due to the presence of DC-SIGN in the infectious synapse, next we examined whether DC-SIGN was promoting the formation of an infectious synapse between DCs pulsed with X4 HIV and resting $CD4^+$ T cells. Infectious synapses defined by viral focusing at the DC–T cell contact site appeared after 10 min of incubation (Fig. 6 B). A larger proportion of DC–T cell clusters was detected after 30 min, with 60–70% of mature DCs showing viral focusing at the zone of contact with resting $CD4^+$ T cells. When immature DCs were used, only 15–20% of infectious synapses were formed in DC–T cell clusters and fewer DC–T cells conjugates were formed between immature DCs and $CD4^+$ T cells (unpublished data), in agreement with previously published results (8, 27). For this reason, we performed all our infectious synapse experiments using mature DCs pulsed with HIV. Occasionally, we also observed that HIV virions were relocalized from intracellular endocytic compartments to a DC surface distinct from a zone of contact with the T cell (unpublished data). This was possibly due to an early dissociation between DCs and T cells, and these events were excluded from subsequent analysis. We also observed by microscopy that HIV infection was transferred from mature DCs to resting $CD4^+$ T cells because viral staining was detected on single T cells as well as on T cells in DC–T cell conjugates in areas that were distinct from the infectious synapse as observed previously (8). The amount of viral transfer from DCs to resting $CD4^+$ T cells was difficult to quantify by microscopy; therefore, we measured infectious synapses in DC–T cell conjugates.

Transduction of DCs with empty lentiviral vectors did not affect the formation of infectious synapses, with ~70% of mature DC–T cell clusters displaying infectious synapses (Fig. 6 A, a and b). In contrast, when we used an siRNA lentiviral vector targeting DC-SIGN expression (LV-si-SIGN11), infectious synapse formation in DC-$SIGN^-$ DC–T cell clusters was decreased (Fig. 6 A, c and d).

Interestingly, in DC-$SIGN^-$ DCs, HIV remained concentrated in an intracellular endocytic compartment, even in the presence of T cells, instead of focusing at the infectious synapse (Fig. 6 A, c and d). This viral pattern was very similar to DC-$SIGN^+$ DCs that had not encountered T cells (unpublished data). In fact, HIV relocalizes more efficiently from an intracellular endocytic compartment to the DC surface upon encountering a T cells in DC-

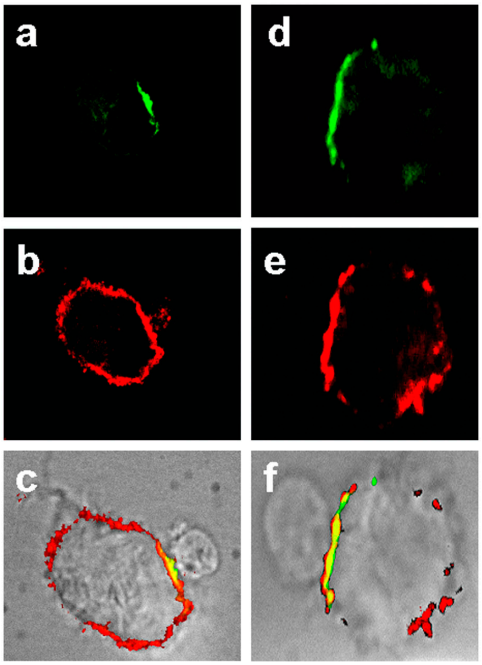

Figure 5. DC-SIGN is present in the infectious synapse between DCs and $CD4^+$ T cells. Mature DC-$SIGN^+$ DCs were loaded with HIV-GFP for 2 h at 37°C and incubated with highly purified resting $CD4^+$ T cells for 30 min at 37°C, allowing infectious synapse formation. Two representative examples are shown (a–c and d–f). DC-SIGN was readily detected at the infectious synapse by confocal microscopy, appearing sometimes enriched (f), but not consistently (c). (green) HIV-GFP; (red) DC-SIGN.

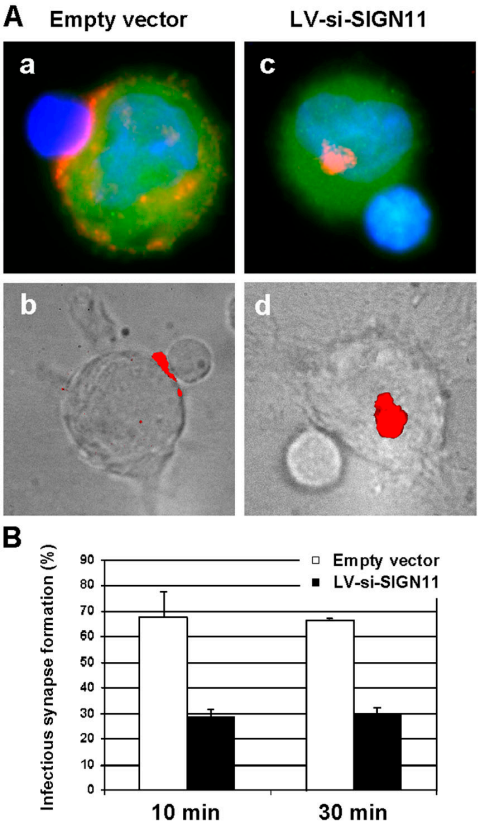

Figure 6. DC-SIGN promotes infectious synapse formation between DCs and CD4$^+$ T cells. (A) Transduced mature DCs were sorted into GFP$^+$ DC-SIGN$^-$ (LV-si-SIGN11) or GFP$^+$ DC-SIGN$^+$ (empty vector) cells. Sorted mature DCs were loaded with HIV IN-HA for 2 h at 37°C, and incubated with highly purified resting CD4$^+$ T cells for 30 min at 37°C, allowing infectious synapse formation. Representative examples of infectious synapses obtained between DCs transduced with control lentiviral vectors and CD4$^+$ resting T cells are shown (a and b). DC-SIGN$^-$ DCs (transduced with LV-si-SIGN11) are unable to redistribute internalized HIV from intracellular pools to the infectious synapse (c and d). (a and c) Immunofluorescence microscopy. (b and d) Confocal analysis. (green) GFP-expressing DCs; (red) HIV; (blue) DAPI (nuclei of both DCs and T cells). (B) Kinetics of infectious synapse formation. Quantification over time of infectious synapse formation in DC–T cell immunological conjugates was performed in DC-SIGN$^+$ DCs (transduced with empty vector) and in DC-SIGN$^-$ DCs (transduced with LV-si-SIGN11). Mean ± SD of three independent experiments is shown.

SIGN$^+$ DCs (Fig. 6 A, a and b) than in DC-SIGN$^-$ DCs (Fig. 6 A, c and d).

To evaluate if DC-SIGN requirement for infectious synapse formation was rapid, we performed a time course experiment to measure and quantify infectious synapse formation over time. Clearly, the inhibition of HIV transfer to the infectious synapse was independent of the time used because 60% inhibition was observed at both 10 and 30 min (Fig. 6 B). Quantitative results at earlier time points could not be obtained because the number of infectious synapses formed within that time was too low for analysis. Nevertheless, this kinetics experiment indicates that DC-SIGN requirement for infectious synapse formation is rapid and sustained over time.

Discussion

In this paper, we have demonstrated a novel role for DC-SIGN downstream from viral capture. This C-type lectin promotes the formation of an infectious synapse between DCs pulsed with X4 HIV-1 and resting CD4$^+$ T cells, thereby allowing optimal transfer of HIV infection from DCs to T cells.

Not only do our results show that DC-SIGN increases HIV binding on the DCs as reported in several other studies (7, 8, 10, 11, 17, 32, 42) but, more importantly, our knockdown system demonstrates that internalization pathways in addition to DC-SIGN participate in viral capture by DCs. These findings are also in agreement with recent results obtained in an ex vivo model of human cervical tissue explants (7). Interestingly, binding to HIV-gp120 to DCs appears more dependent on DC-SIGN than full viral particles (10, 32), suggesting that molecules incorporated in virions other than HIV gp-120 may enhance binding of HIV to DCs through a DC-SIGN–independent process (43, 44). Other DC subtypes, such as Langerhans cells, do not express DC-SIGN and may bind HIV-1 gp120 through additional molecules such as the Langerin and/or mannose receptor (14, 16, 45, 46), but whether these molecules allow for efficient transfer of HIV infection in trans remains to be established.

Notably, X4 HIV-1 capture and internalization after 2 h at 37°C by DC-SIGN knockdown DCs was identical to DC-SIGN$^+$ DCs. Our results were obtained with a high viral input, and we cannot exclude that at a very low viral input, DC-SIGN would increase viral capture, especially if the contact between HIV and DCs was over a short period of time. Nevertheless, our observation indicates that viral capture occurs efficiently through DC-SIGN–independent internalization pathways. Although we cannot formally demonstrate that HIV is stored in the same intracellular compartment in the presence or absence of DC-SIGN, the amount of virus captured as well as the morphology of HIV storage compartment in DC-SIGN$^+$ DCs and DC-SIGN$^-$ DCs at immunofluorescence resolution appear very similar (unpublished data). DC-SIGN–independent viral internalization may be receptor dependent or receptor independent. In macrophages, for instance, HIV internalization is mediated by macropinocytosis in a receptor-independent manner (47).

Although DC-SIGN does not appear to promote in viral capture and internalization in our system, this receptor clearly increases the transfer and enhancement of HIV infection to target cells in trans, especially to CD4$^+$ T cells (32). To evaluate the function of DC-SIGN in the recruitment of resting CD4$^+$ T cells, possibly via ICAM-3–DC-SIGN interactions, we measured the capacity of DC-SIGN$^-$ DCs to form conjugates with resting CD4$^+$ T cells. We could demonstrate that DC-SIGN is dispensable for DC–resting CD4$^+$ T cell conjugate formation. In contrast, we recently showed that DC-SIGN$^-$ DCs did not bind efficiently to ICAM-3–coated beads (32), suggesting that other interactions in addition to ICAM-3–DC-SIGN in-

teractions promote binding of DCs to resting $CD4^+$ T cells.

It has been known for many years that cell-to-cell transmission is the major mode of transfer of HIV infectivity from DCs to T cells (5, 31). The recent description of an infectious synapse between DCs pulsed with HIV and T cells may provide an explanation for DC–T cell retroviral transmission (8, 27). We have identified in this paper the first molecule on the DC side of the infectious synapse that promotes infectious synapse formation, the C-type lectin DC-SIGN. This unexpected function of DC-SIGN provides a potential explanation for the high efficiency with which DCs transfer and enhance X4 HIV-1 infectivity to T cells in trans. Whether DC-SIGN has a similar function in the transfer of HIV using CCR5 from DCs to T cells remains to be established.

Although our results provide evidence that DC-SIGN increases infectious synapse formation, the precise function of DC-SIGN in this process remains to be determined. We have shown that DC-SIGN is present in the infectious synapse (Fig. 5). It is conceivable that DC-SIGN recruits internalized HIV from its storage compartment and rapidly redirects virions to the plasma membrane, explaining that HIV focuses at the infectious synapse. Alternatively, when DC–T cell clusters form, DC-SIGN might mediate a signal enabling stored virus to reach contact zones with T cells. The rapid kinetics of HIV transfer to infectious synapses (~5–10 min), the absence of colocalization of internalized HIV with DC-SIGN (reference 8 and unpublished data), and the presence of DC-SIGN in infectious synapses (27) argues in favor of a signaling model. Indeed, DC-SIGN might act as a sensor on the surface of DCs. Upon contact with T cells, DC-SIGN could rapidly send reverse signals to the DCs that allow internalized antigens to be presented at the DC surface. Previous studies already demonstrated that C-type lectins (DC-SIGN and Dectin-1) could modulate intracellular signals mediated by Toll receptors (48, 49).

Although DC-SIGN clearly favors infectious synapse formation, additional components of this machinery remain to be identified. Whether reorganization of cytoskeleton is required as shown for HTLV-1 and HIV-1 spread between T lymphocytes is still unsolved (references 50, 51 and for review see reference 28).

In conclusion, our paper provides a new insight into the mechanisms of transfer of infectious virus from DCs to T cells via an infectious synapse, a step that is critical for HIV transmission and pathogenesis.

We thank D. Trono for helpful discussions during the preparation of this paper, F. Leuba for excellent technical help, and D. Wohlwend for technical assistance with FACS analysis and cell sorting.

This work was supported by the Geneva Cancer League and Swiss National Science Foundation grant no. 3345-67200.01 (to V. Piguet). V. Piguet is the recipient of a Professor SNF position (PP00A–68785).

The authors have no conflicting financial interests.

Submitted: 6 July 2004
Accepted: 17 September 2004

References

1. Pope, M., and A.T. Haase. 2003. Transmission, acute HIV-1 infection and the quest for strategies to prevent infection. *Nat. Med.* 9:847–852.
2. Shattock, R.J., and J.P. Moore. 2003. Inhibiting sexual transmission of HIV-1 infection. *Nat. Rev. Microbiol.* 1:25–34.
3. Steinman, R.M., A. Granelli-Piperno, M. Pope, C. Trumpfheller, R. Ignatius, G. Arrode, P. Racz, and K. Tenner-Racz. 2003. The interaction of immunodeficiency viruses with dendritic cells. *Curr. Top. Microbiol. Immunol.* 276:1–30.
4. Piguet, V., and A. Blauvelt. 2002. Essential roles for dendritic cells in the pathogenesis and potential treatment of HIV disease. *J. Invest. Dermatol.* 119:365–369.
5. Pope, M., M.G. Betjes, N. Romani, H. Hirmand, P.U. Cameron, L. Hoffman, S. Gezelter, G. Schuler, and R.M. Steinman. 1994. Conjugates of dendritic cells and memory T lymphocytes from skin facilitate productive infection with HIV-1. *Cell.* 78:389–398.
6. Blauvelt, A., H. Asada, M.W. Saville, V. Klaus-Kovtun, D.J. Altman, R. Yarchoan, and S.I. Katz. 1997. Productive infection of dendritic cells by HIV-1 and their ability to capture virus are mediated through separate pathways. *J. Clin. Invest.* 100:2043–2053.
7. Hu, Q., I. Frank, V. Williams, J.J. Santos, P. Watts, G.E. Griffin, J.P. Moore, M. Pope, and R.J. Shattock. 2004. Blockade of attachment and fusion receptors inhibits HIV-1 infection of human cervical tissue. *J. Exp. Med.* 199:1065–1075.
8. Turville, S.G., J.J. Santos, I. Frank, P.U. Cameron, J. Wilkinson, M. Miranda-Saksena, J. Dable, H. Stossel, N. Romani, M. Piatak Jr., et al. 2004. Immunodeficiency virus uptake, turnover, and 2-phase transfer in human dendritic cells. *Blood.* 103:2170–2179.
9. Geijtenbeek, T.B., and Y. van Kooyk. 2003. DC-SIGN: a novel HIV receptor on DCs that mediates HIV-1 transmission. *Curr. Top. Microbiol. Immunol.* 276:31–54.
10. Geijtenbeek, T.B., D.S. Kwon, R. Torensma, S.J. van Vliet, G.C. van Duijnhoven, J. Middel, I.L. Cornelissen, H.S. Nottet, V.N. KewalRamani, D.R. Littman, C.G. Figdor, and Y. van Kooyk. 2000. DC-SIGN, a dendritic cell-specific HIV-1-binding protein that enhances trans-infection of T cells. *Cell.* 100:587–597.
11. Pohlmann, S., G.J. Leslie, T.G. Edwards, T. Macfarlan, J.D. Reeves, K. Hiebenthal-Millow, F. Kirchhoff, F. Baribaud, and R.W. Doms. 2001. DC-SIGN interactions with human immunodeficiency virus: virus binding and transfer are dissociable functions. *J. Virol.* 75:10523–10526.
12. Geijtenbeek, T.B., A. Engering, and Y. Van Kooyk. 2002. DC-SIGN, a C-type lectin on dendritic cells that unveils many aspects of dendritic cell biology. *J. Leukoc. Biol.* 71: 921–931.
13. Geijtenbeek, T.B., R. Torensma, S.J. van Vliet, G.C. van Duijnhoven, G.J. Adema, Y. van Kooyk, and C.G. Figdor. 2000. Identification of DC-SIGN, a novel dendritic cell-specific ICAM-3 receptor that supports primary immune responses. *Cell.* 100:575–585.
14. Turville, S.G., P.U. Cameron, A. Handley, G. Lin, S. Pohlmann, R.W. Doms, and A.L. Cunningham. 2002. Diversity of receptors binding HIV on dendritic cell subsets. *Nat. Immunol.* 3:975–983.
15. Geijtenbeek, T.B., D.J. Krooshoop, D.A. Bleijs, S.J. van Vliet, G.C. van Duijnhoven, V. Grabovsky, R. Alon, C.G. Figdor, and Y. van Kooyk. 2000. DC-SIGN-ICAM-2 inter-

action mediates dendritic cell trafficking. *Nat. Immunol.* 1:353–357.
16. Turville, S., J. Wilkinson, P. Cameron, J. Dable, and A.L. Cunningham. 2003. The role of dendritic cell C-type lectin receptors in HIV pathogenesis. *J. Leukoc. Biol.* 74:710–718.
17. Kwon, D.S., G. Gregorio, N. Bitton, W.A. Hendrickson, and D.R. Littman. 2002. DC-SIGN-mediated internalization of HIV is required for trans-enhancement of T cell infection. *Immunity.* 16:135–144.
18. Pohlmann, S., F. Baribaud, and R.W. Doms. 2001. DC-SIGN and DC-SIGNR: helping hands for HIV. *Trends Immunol.* 22:643–646.
19. Bashirova, A.A., T.B. Geijtenbeek, G.C. van Duijnhoven, S.J. van Vliet, J.B. Eilering, M.P. Martin, L. Wu, T.D. Martin, N. Viebig, P.A. Knolle, et al. 2001. A dendritic cell–specific intercellular adhesion molecule 3-grabbing nonintegrin (DC-SIGN)–related protein is highly expressed on human liver sinusoidal endothelial cells and promotes HIV-1 infection. *J. Exp. Med.* 193:671–678.
20. Curtis, B.M., S. Scharnowske, and A.J. Watson. 1992. Sequence and expression of a membrane-associated C-type lectin that exhibits CD4-independent binding of human immunodeficiency virus envelope glycoprotein gp120. *Proc. Natl. Acad. Sci. USA.* 89:8356–8360.
21. Smith, B.A., S. Gartner, Y. Liu, A.S. Perelson, N.I. Stilianakis, B.F. Keele, T.M. Kerkering, A. Ferreira-Gonzalez, A.K. Szakal, J.G. Tew, and G.F. Burton. 2001. Persistence of infectious HIV on follicular dendritic cells. *J. Immunol.* 166: 690–696.
22. Trumpfheller, C., C.G. Park, J. Finke, R.M. Steinman, and A. Granelli-Piperno. 2003. Cell type-dependent retention and transmission of HIV-1 by DC-SIGN. *Int. Immunol.* 15: 289–298.
23. Moris, A., C. Nobile, F. Buseyne, F. Porrot, J.P. Abastado, and O. Schwartz. 2004. DC-SIGN promotes exogenous MHC-I-restricted HIV-1 antigen presentation. *Blood.* 103: 2648–2654.
24. Sol-Foulon, N., A. Moris, C. Nobile, C. Boccaccio, A. Engering, J.P. Abastado, J.M. Heard, Y. van Kooyk, and O. Schwartz. 2002. HIV-1 Nef-induced upregulation of DC-SIGN in dendritic cells promotes lymphocyte clustering and viral spread. *Immunity.* 16:145–155.
25. Engering, A., T.B. Geijtenbeek, S.J. van Vliet, M. Wijers, E. van Liempt, N. Demaurex, A. Lanzavecchia, J. Fransen, C.G. Figdor, V. Piguet, and Y. van Kooyk. 2002. The dendritic cell-specific adhesion receptor DC-SIGN internalizes antigen for presentation to T cells. *J. Immunol.* 168:2118–2126.
26. Ayehunie, S., R.W. Groves, A.M. Bruzzese, R.M. Ruprecht, T.S. Kupper, and E. Langhoff. 1995. Acutely infected Langerhans cells are more efficient than T cells in disseminating HIV type 1 to activated T cells following a short cell-cell contact. *AIDS Res. Hum. Retroviruses.* 11:877–884.
27. McDonald, D., L. Wu, S.M. Bohks, V.N. KewalRamani, D. Unutmaz, and T.J. Hope. 2003. Recruitment of HIV and its receptors to dendritic cell-T cell junctions. *Science.* 300: 1295–1297.
28. Piguet, V., and Q.J. Sattentau. 2004. Dangerous liaisons at the virological synapse. *J. Clin. Invest.* 114:605–610.
29. Revy, P., M. Sospedra, B. Barbour, and A. Trautmann. 2001. Functional antigen-independent synapses formed between T cells and dendritic cells. *Nat. Immunol.* 2:925–931.
30. Mempel, T.R., S.E. Henrickson, and U.H. Von Andrian. 2004. T-cell priming by dendritic cells in lymph nodes occurs in three distinct phases. *Nature.* 427:154–159.
31. Cameron, P.U., P.S. Freudenthal, J.M. Barker, S. Gezelter, K. Inaba, and R.M. Steinman. 1992. Dendritic cells exposed to human immunodeficiency virus type-1 transmit a vigorous cytopathic infection to CD4+ T cells. *Science.* 257:383–387.
32. Arrighi, J.F., M. Pion, M. Wiznerowicz, T.B. Geijtenbeek, E. Garcia, S. Abraham, F. Leuba, D. Dutoit, O. Ducrey-Rundquist, Y. van Kooyk, et al. 2004. Lentiviral-mediated RNA interference of DC-SIGN expression abrogates HIV transmission from dendritic cells to T cells. *J. Virol.* 78: 10848–10855.
33. Zufferey, R., D. Nagy, R.J. Mandel, L. Naldini, and D. Trono. 1997. Multiply attenuated lentiviral vector achieves efficient gene delivery in vivo. *Nat. Biotechnol.* 15:871–875.
34. Arrighi, J.F., C. Hauser, B. Chapuis, R.H. Zubler, and V. Kindler. 1999. Long-term culture of human CD34(+) progenitors with FLT3-ligand, thrombopoietin, and stem cell factor induces extensive amplification of a CD34(−)CD14(−) and a CD34(−)CD14(+) dendritic cell precursor. *Blood.* 93: 2244–2252.
35. Salmon, P., J.F. Arrighi, V. Piguet, B. Chapuis, R.H. Zubler, D. Trono, and V. Kindler. 2001. Transduction of CD34+ cells with lentiviral vectors enables the production of large quantities of transgene-expressing immature and mature dendritic cells. *J. Gene Med.* 3:311–320.
36. Arrighi, J.F., C. Soulas, C. Hauser, S. Saeland, B. Chapuis, R.H. Zubler, and V. Kindler. 2003. TNF-alpha induces the generation of Langerin/(CD207)+ immature Langerhans-type dendritic cells from both CD14-CD1a and CD14+CD1a− precursors derived from CD34+ cord blood cells. *Eur. J. Immunol.* 33:2053–2063.
37. Gallay, P., T. Hope, D. Chin, and D. Trono. 1997. HIV-1 infection of nondividing cells through the recognition of integrase by the importin/karyopherin pathway. *Proc. Natl. Acad. Sci. USA.* 94:9825–9830.
38. Turelli, P., V. Doucas, E. Craig, B. Mangeat, N. Klages, R. Evans, G. Kalpana, and D. Trono. 2001. Cytoplasmic recruitment of INI1 and PML on incoming HIV preintegration complexes: interference with early steps of viral replication. *Mol. Cell.* 7:1245–1254.
39. Ducrey-Rundquist, O., M. Guyader, and D. Trono. 2002. Modalities of interleukin-7-induced human immunodeficiency virus permissiveness in quiescent T lymphocytes. *J. Virol.* 76:9103–9111.
40. Piguet, V., L. Wan, C. Borel, A. Mangasarian, N. Demaurex, G. Thomas, and D. Trono. 2000. HIV-1 Nef protein binds to the cellular protein PACS-1 to downregulate class I major histocompatibility complexes. *Nat. Cell Biol.* 2:163–167.
41. Pohlmann, S., E.J. Soilleux, F. Baribaud, G.J. Leslie, L.S. Morris, J. Trowsdale, B. Lee, N. Coleman, and R.W. Doms. 2001. DC-SIGNR, a DC-SIGN homologue expressed in endothelial cells, binds to human and simian immunodeficiency viruses and activates infection in trans. *Proc. Natl. Acad. Sci. USA.* 98:2670–2675.
42. Baribaud, F., S. Pohlmann, G. Leslie, F. Mortari, and R.W. Doms. 2002. Quantitative expression and virus transmission analysis of DC-SIGN on monocyte-derived dendritic cells. *J. Virol.* 76:9135–9142.
43. Bobardt, M.D., A.C. Saphire, H.C. Hung, X. Yu, B. Van der Schueren, Z. Zhang, G. David, and P.A. Gallay. 2003. Syndecan captures, protects, and transmits HIV to T lymphocytes. *Immunity.* 18:27–39.
44. Gummuluru, S., V.N. KewalRamani, and M. Emerman.

2002. Dendritic cell-mediated viral transfer to T cells is required for human immunodeficiency virus type 1 persistence in the face of rapid cell turnover. *J. Virol.* 76:10692–10701.
45. Nguyen, D.G., and J.E. Hildreth. 2003. Involvement of macrophage mannose receptor in the binding and transmission of HIV by macrophages. *Eur. J. Immunol.* 33:483–493.
46. Kawamura, T., F.O. Gulden, M. Sugaya, D.T. McNamara, D.L. Borris, M.M. Lederman, J.M. Orenstein, P.A. Zimmerman, and A. Blauvelt. 2003. R5 HIV productively infects Langerhans cells, and infection levels are regulated by compound CCR5 polymorphisms. *Proc. Natl. Acad. Sci. USA.* 100:8401–8406.
47. Marechal, V., M.C. Prevost, C. Petit, E. Perret, J.M. Heard, and O. Schwartz. 2001. Human immunodeficiency virus type 1 entry into macrophages mediated by macropinocytosis. *J. Virol.* 75:11166–11177.
48. Geijtenbeek, T.B., S.J. Van Vliet, E.A. Koppel, M. Sanchez-Hernandez, C.M. Vandenbroucke-Grauls, B. Appelmelk, and Y. Van Kooyk. 2003. Mycobacteria target DC-SIGN to suppress dendritic cell function. *J. Exp. Med.* 197:7–17.
49. Gantner, B.N., R.M. Simmons, S.J. Canavera, S. Akira, and D.M. Underhill. 2003. Collaborative induction of inflammatory responses by dectin-1 and Toll-like receptor 2. *J. Exp. Med.* 197:1107–1117.
50. Jolly, C., K. Kashefi, M. Hollinshead, and Q.J. Sattentau. 2004. HIV-1 cell to cell transfer across an Env-induced, actin-dependent synapse. *J. Exp. Med.* 199:283–293.
51. Igakura, T., J.C. Stinchcombe, P.K. Goon, G.P. Taylor, J.N. Weber, G.M. Griffiths, Y. Tanaka, M. Osame, and C.R. Bangham. 2003. Spread of HTLV-I between lymphocytes by virus-induced polarization of the cytoskeleton. *Science.* 299: 1713–1716.