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Cell type-dependent retention and transmission of HIV-1 by DC-SIGN

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Abstract

DC-SIGN (CD209) is a C-type lectin expressed by several groups of dendritic cells (DC), including those derived from blood monocytes and DC found beneath genital epithelium. DC-SIGN binds the envelope glycoprotein of HIV-1 and facilitates transmission of infectious virus to permissive CD4+ T cells. We have compared the capacity of DC-SIGN in different cell types to bind, retain and transmit infectious HIV-1 to T cells. The analyzed cells included monocyte-derived DC, and three different DC-SIGN-expressing transfectants termed THP, 293 and HOS. Our results show that DC-SIGN transfectants were able to bind HIV-1 virions comparably to DC. However, only the THP monocytic cell line shared with DC the capacity to retain for several days virus that was infectious for T cells. In both THP-DC-SIGN transfectants and DC, but not in 293 cells, HIV-1 was localized to intracellular compartments that did not double label for endosomal and lysosomal markers or for DC-SIGN itself. Virus remained detectable in these compartments for at least 2 days. Anti-DC-SIGN antibodies blocked the binding and transmission of HIV-1 in DC-SIGN transfectants, as monitored by PCR for HIV LTR/gag and p24 ELISA. However anti-DC-SIGN antibodies did not block virus binding and transmission to T cells as well in DC as in THP-DC-SIGN transfectants. Thus, the function of DC-SIGN in HIV-1 transmission depends on its cellular context, since only DC and the THP monocyte cell line, but not 293 and HOS, are able to use DC-SIGN to retain HIV-1 in a highly infectious state for several days.

Introduction

Dendritic cells (DC) are antigen-presenting cells found at body surfaces and in lymphoid tissues (1). DC can migrate from peripheral tissues and blood to lymphoid organs where they interact with T cells. Therefore DC are critically positioned to capture HIV-1 and spread the infection to CD4+ T cells. The entry of HIV-1 into cells requires the interaction of viral envelope protein, gp120, with two cell surface receptors. CD4 binds HIV-1, while the chemokine receptors CCR5 and CXCR4 serve as co-receptors for entry of R5 and X4 viruses respectively (2). HIV-1 entry into target cells can be influenced by several additional factors. Heparin sulfate and mannose receptors may facilitate attachment of virions to the cell surface (3–5), and co-receptors may need to be clustered (6) and/or be located in lipid rafts (7).

DC-SIGN (DC-Specific ICAM-3 Grabbing Non-integrin; CD209) is a C-type lectin that specifically recognizes glycan ligands (8). DC-SIGN is expressed primarily on DC, such as those derived from blood monocytes as well as DC located beneath genital surfaces and within lymphoid tissues (9). DC-SIGN is already known to have several functions. It can participate in the interactions between DC and T cells by binding ICAM-3 (10). DC-SIGN also can bind ICAM-2 on endothelial cells and potentially regulate the transmigration of DC from blood into tissues (11). Non-viral pathogens like *Leishmania* amastigotes (12) as well as other viral pathogens like Ebola (13,14), SIV-1, SIV-2 (15), HIV-2 (15) and HCV (16) can bind to DC-SIGN. Importantly, DC-SIGN binds the gp120 envelope glycoprotein of HIV-1 (15,17,18) and facilitates the transmission of HIV-1 from DC-SIGN transfectants to target CD4+ T cells (15,18,19). Originally it was shown that DC-SIGN could function independently from CD4 and CCR5 to transmit virus to permissive T cells (18,19). More recently it was also noted that *cis* expression of DC-SIGN in transfectants permitted more efficient entry of HIV-1 and SIV via CD4 and co-receptor (20). DC-SIGN contains putative tyrosine and dileucine motifs in its cytosolic domain (21), and can act as

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an endocytic receptor facilitating processing and presentation of antigens (22). The endocytic function of DC-SIGN plays an important role in its capacity to transmit HIV-1 to T cells (19).

Although it is known that DC-SIGN can enhance HIV-1 infection of T cells, it was of interest to explore the cellular environment required for the capacity of DC-SIGN to retain and transmit infectious HIV-1 over the course of several days. Here we have compared the capacity of DC-SIGN in different cellular contexts to bind and retain infectious virus for 2–6 days in culture, and then transmit HIV-1 to T cells. Three different types of transfectants were able to bind HIV-1, but only the THP monocytic cell line shared with DC the capacity to internalize and retain HIV-1 in an infectious state for presentation to T cells. Our finding that DC-SIGN retains infectious HIV-1 in select cell types may help to characterize the function of this lectin in disseminating HIV-1 and other pathogens.

Methods

Plasmid preparation

Full-length DC-SIGN was PCR amplified from a human thymic cDNA library. The primers used for PCR amplification were: 5-hDC-SIGN (5′-GACATGAGTGACTCCAAGGAACCAAGACTGCAGCA-3′, with an *Eco*RI site), 3-hDC-SIGN (5′-CTACGCAGGAGGGGGGTTTGGGGTGGCAG-3′, with *Bam*HI and *Not*I sites). The sequence of full-length DC-SIGN was confirmed to be identical with the published sequences from Curtis *et al*. (17) and Geijtenbeek *et al*. (18). DC-SIGN was cloned into the mammalian expression vector pN1-eGFP (Clontech, Palo Alto, CA) to which green fluorescence protein has been deleted, but which still retains the neomycin (G418) selection marker.

DC

To prepare DC from precursors in blood, $CD14^+$ monocytes were positively selected from peripheral blood mononuclear cells (PBMC; from buffy coat or venapuncture specimens) using CD14 magnetic beads as suggested by the vendor (Miltenyi Biotec, Auburn, CA). $CD14^+$ cells (1.5×10^6) were plated in six-well dishes in 3 ml RPMI 1640 medium supplemented with 5% human serum, recombinant granulocyte macrophage colony stimulating factor (100 IU/ml, Leukine; Immunex, Seattle, WA) and recombinant human IL-4 (20 ng/ml; R & D Systems, Minneapolis, MN). The cells were fed on days 2 and 4 with the same concentration of the cytokines. At day 6, most of the non-adherent cells were immature DC with a $CD14^-$, $HLA\text{-}DR^+$, $CD3^-$ and $CD83^-$ phenotype. Mature $CD83^+$ DC then were induced by adding inflammatory cytokines (IL1-β, tumor necrosis factor-α and IL-6 at 10 ng/ml, and prostaglandin E_2 at 1 μg/ml).

Transfectants

293 (human embryonic kidney) and HOS (human osteosarcoma) cells, obtained from the AIDS Research and Reference Reagent Program, expressing DC-SIGN were generated by stable transfection using the Lipofectamine agent, as suggested by the vendor (Gibco/BRL, Gaithersburg, MD). Geneticin-resistant clones were expanded and analyzed by flow cytometry using anti-DC-SIGN antibodies. Similarly, HOS cells expressing CD4 and CCR5 (obtained from the AIDS Research and Reference Reagent Program) were transfected with DC-SIGN plasmid. HOS cells were maintained in DMEM medium (Gibco/BRL) supplemented with 10% FCS, 1 μg/ml puromycin and 1 mg/ml geneticin. THP, a monocytic cell line, and THP-DC-SIGN transfectants were kindly provided by Dr Dan Littman (New York University). MAGI, a HeLa cell clone expressing human CD4, CCR5 and HIV-LTR-β-gal developed by Michael Emerman's laboratory, was obtained from the NIH AIDS Research Reagent Program.

Quantitation of HIV-1 receptors

Primary DC and DC-SIGN transfectants were stained with the anti-DC-SIGN mAb 507 and 612 (R & D Systems), anti-CD4–phycoerythrin (PE) (clone Leu-3a; Becton Dickinson, San Jose, CA), and anti-CCR5 (clone 2D7; PharMingen, San Diego, CA). Cells were incubated for 45 min at 4°C with each antibody diluted to the optimal concentration for immunostaining. After washing with cold PBS supplemented with 2% FCS, the cells were incubated with PE-conjugated mAb (Biosource, Camarillo, CA) for 30 min at 4°C, washed a further 3 times and analyzed on a FACScan with CellQuest software (Becton Dickinson). To quantify expression of HIV-1 receptors, we used a quantitative FACS assay (QFACS) with microbeads containing known amounts of anti-Ig molecules (Sigma, St Louis, MO).

HIV-1 infection of cells

Cells were infected with the Ba-L isolate, grown in mitogen-stimulated PBMC and tittered in MAGI cells to determine infectious units (IU). In general, 10^5 cells were pulsed with graded doses of HIV-1 Ba-L starting with 300 pg, equivalent to 3000 IU or 0.03 m.o.i. for 2 h at 37°C. In the experiments in which the effect of antibodies were studied, the indicated antibodies were added for 30 min at room temperature before virus addition and kept during the infection period. The cells were washed 4 times and 5×10^4 virus-pulsed cells co-cultured with phytohemagglutinin-activated PBMC (1×10^5) immediately after the 2-h HIV-1 pulse or after 2–6 days of culture. To avoid overgrowth, the HIV-1-pulsed transfected cells were exposed to ionizing irradiation (1500 rad). Culture supernatants were collected at different time points and the p24 antigen released into supernatants measured by ELISA. For PCR analysis, infected cultures were collected at the indicated time points and proviral DNA was amplified for the detection of early (RU/5) and late reverse transcripts (LTR/gag) as previously described (23). HIV-1 copy numbers in 50,000 cells per lane were estimated by comparison with graded doses of ACH-2 cells, which contain one copy of proviral DNA per cell. HLA-DQ sequences were amplified as a control for DNA input. For detection of p24 at the single-cell level, infected cultures were fixed with 4% paraformaldehyde in PBS for 30 min on ice. Cells were permeabilized for 15 min in 0.1% saponin, and double stained with p24–FITC (Coulter, Miami, FL) and CD3–PE (clone SK7; BD PharMingen, San Diego, CA).

HIV-1 binding assays

To analyze the binding of HIV-1 Ba-L to cells, the cells were preincubated with or without antibodies for 20 min at room

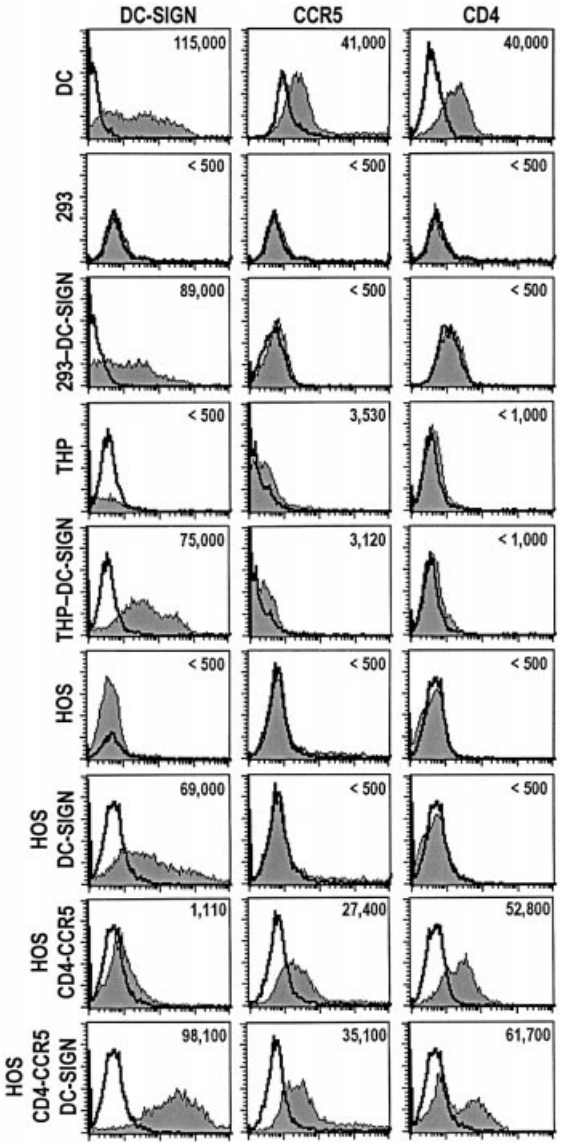

Fig. 1. Expression of HIV-1 receptors on DC and DC-SIGN transfectants. The number on each panel represents antibody-binding sites quantified as described in Methods. The histograms reflect binding of the specific antibody (gray) and isotype-matched control antibody (white).

temperature before the addition of HIV-1 (300 pg). Cells were extensively washed after 2 h; part of the cells were then lysed in 0.5 % Triton, and the rest were cultured for 2 days and then lysed. Cell lysates were assayed for p24 by ELISA (Coulter).

Confocal microscopy

DC-SIGN-expressing cells were infected on ice or at 37°C with Aldritiol-2 (AT-2) (24)-inactivated HIV-1 particles at a high dose, 100 ng/10^5 cells (a kind gift of J. Lifson), and washed. The cells were examined after 2 h and 2 days of culture. For microscopy, the cells were attached to printed slides (Carlson Scientific, Peotone, IL) coated with polylysine (Sigma) and left to adhere at 37 or 4°C for 30 min in serum-free medium. The cells were washed and fixed for 30 min in 4% paraformaldehyde in PBS. Fixed cells were permeabilized in 0.05% saponin for 15 min, and double stained for HIV-1 gag (mAb KC57 FITC–IgG1; Coulter) and anti-DC-SIGN (clone 507; IgG2b) for 1 h at room temperature followed by goat anti-IgG2b Alexa 546 at a dilution of 1:300 (Molecular Probes, Eugene, OR). We also stained cells with combinations of anti-gag and anti-caveolin (Santa Cruz Biotechnology, Santa Cruz, CA), anti-EEA1 (Santa Cruz Biotechnology), and anti-LAMP–biotin (PharMingen). Nuclei were stained with DAPI. Fluorescence was examined with a model AX70 Olympus laser scanning microscope.

Results

Quantitation of DC-SIGN expression on DC and transfectants

To further understand the involvement of DC-SIGN in HIV-1 infection, we established several cell lines stably expressing DC-SIGN. Quantitation of DC-SIGN and other receptors for HIV-1 (CD4, CCR5) on each cell type was assessed using Ig-coated fluorescent beads. As expected from prior reports, immature DC expressed the two co-receptors for HIV-1, CD4 and CCR5, and high levels of DC-SIGN (Fig. 1). In contrast, the 293 and THP cell lines were negative for all three HIV-1-binding molecules (Fig. 1). Upon transfection with DC-SIGN, the cell lines expressed the protein. The expression of DC-SIGN on the transfectants was stable over the course of multiple experiments and the different cell lines expressed comparable average levels of DC-SIGN per cell (Fig. 1).

Interaction of HIV-1 with DC-SIGN-expressing cells

To measure the binding of HIV-1 to DC-SIGN-expressing cells, cells were incubated with relatively low doses of virus (3000 IU corresponding to 300 pg of p24/10^5 cells) for 2 h, washed and lysed in 0.5% Triton. Cell lysates were assessed for virus binding by quantifying HIV-1 gag (p24) by ELISA. The added virions bound to cells expressing DC-SIGN efficiently (Fig. 2; 4–10 pg of bound p24) and the extent of binding depended on the input dose of virus (not shown). Background binding to cells expressing vector alone was low (Fig. 2). Two different commercially available DC-SIGN antibodies (507 reacting with DC-SIGN and 612 reacting with DC-SIGN/DC-SIGNR) and the AZND1 antibody (18) were used to inhibit HIV-1 binding. The inhibition was at least 50% on 293-DC-SIGN cells and >95% on THP-DC-SIGN transfectants (Fig. 2A). Therefore, the transfectants used DC-SIGN to bind HIV-1 and, in the case of THP cells, mAb to DC-SIGN almost totally blocked binding.

Next, binding of HIV-1 was assessed in immature DC and DC matured with inflammatory cytokines. Both types of DC express the receptors for HIV-1, CD4, CCR5 and DC-SIGN, with higher expression of the receptors on the immature cells (not shown). The level of CD4 and CCR5 expression varied in immature DC from different donors at least 10-fold, but there was much less variation with respect to DC-SIGN. HIV-1 bound to DC comparably to DC-SIGN transfectants analyzed in parallel, but the anti-DC-SIGN antibodies only slightly decreased HIV-1 binding to DC; in contrast the THP-DC-SIGN transfectants that were almost totally blocked (*cf.* Fig. 2A with B). T blasts, which were negative for DC-SIGN

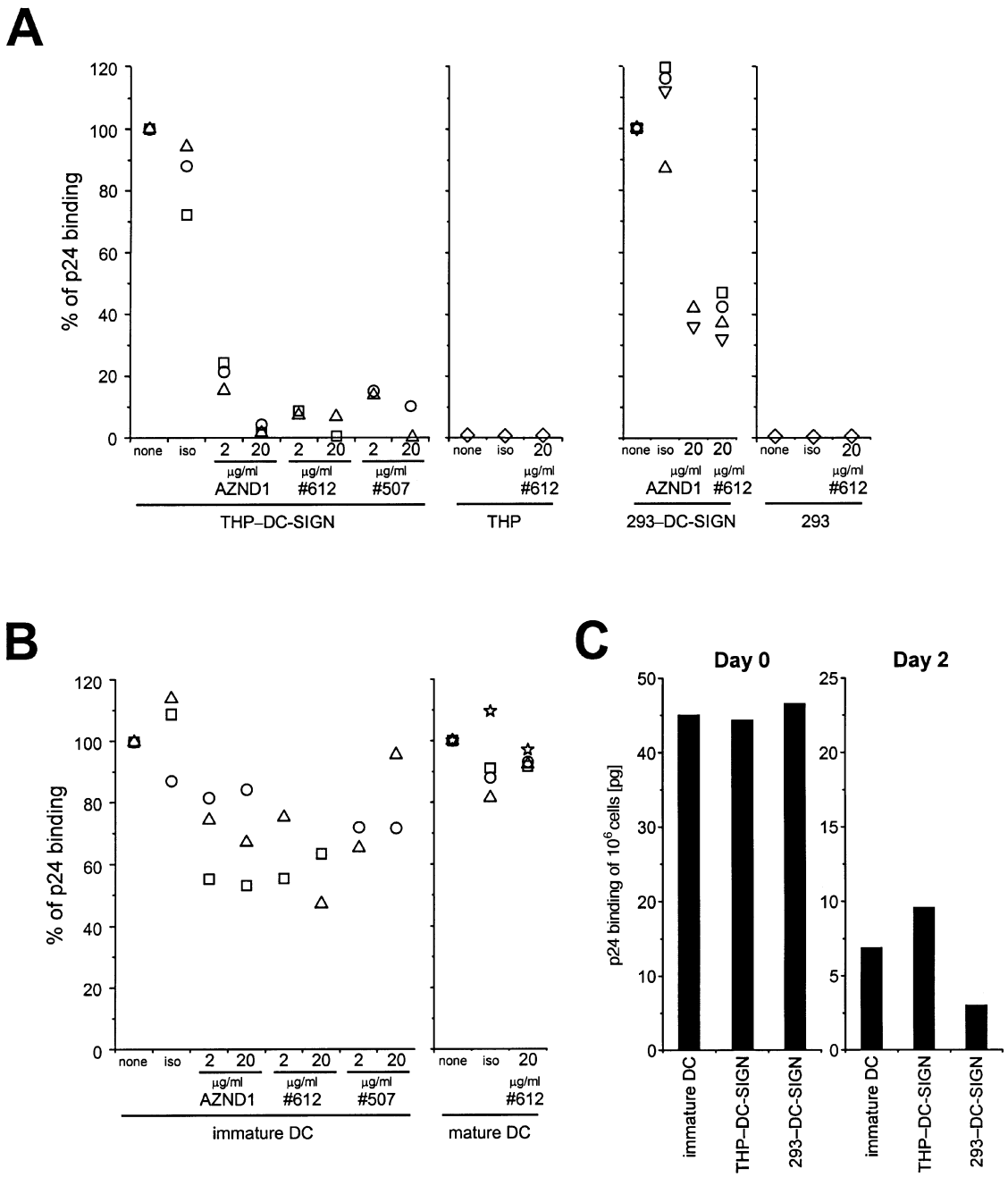

Fig. 2. Binding of HIV-1 to DC and DC-SIGN transfectants. Cells (10^5) were incubated with anti-DC-SIGN antibodies or isotype controls at a concentration of 15 µg/ml for 20 min at room temperature and then infected with HIV-1 (300 pg of p24 that is equivalent to 3000 IU) for 2 h at 37°C. Some cells were washed and lysed in 0.5% Triton X-100 immediately, while others were lysed after 2 days. The amount of p24 antigen in the cell lysates was quantified by ELISA. Each symbol corresponds to an individual experiment. (A) HIV-1 captured by DC-SIGN transfectants after 2 h. (B) HIV-1 captured by immature and mature DC after 2 h. (C) Comparison of HIV-1 bound to cells after a 2-h capture (day 0) and retained by cells after 2 days (day 2).

expression, were at least 4- to 5-fold less active in binding HIV-1 than DC-SIGN transfectants or DC and the binding to T blasts was not sensitive to anti-DC-SIGN antibodies (not shown).

We then assessed retention of the captured virus in culture for 2 days after infection. The amount of virus retained by DC and THP-DC-SIGN cells at this time point was 10 times less compared to the 2-h pulse (Fig. 2C). Nevertheless, we could readily quantify virus by p24 ELISA (Fig. 2C). It is worth noting that although the level of p24 retained by the cells after 2 days was reduced, the retained virus, as seen below, was still sufficient to set up a robust infection in T cells.

Thus, cells differ in their capacity to retain HIV-1 and also DC differ from DC-SIGN transfectants in that anti-DC-SIGN antibodies do not efficiently block HIV-1 binding.

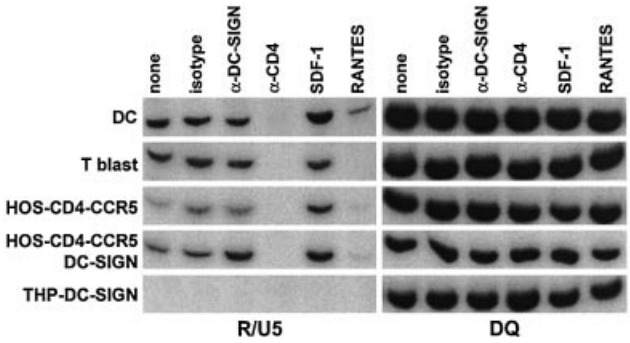

Fig. 3. Effect of HIV-1 receptor inhibitors on proviral DNA synthesis in DC and DC-SIGN transfectants. The indicated cells were pretreated or not for 20 min with anti-DC-SIGN antibodies 507 and 612 at 7.5 μg/ml each, anti-CD4 Leu3a and RPTA4 (PharMingen) at 7.5 μg/ml each or SDF1 and RANTES (R & D Systems) at 100 nM. The cells were infected with 3000 IU of HIV-1. After 5 h, cells were collected, washed and the equivalent of 5×10^4 assessed for RU/5 sequences by PCR indicative of early reverse transcripts. DQ sequences were amplified as a control for DNA input (23).

Effect of anti-CD4, RANTES and anti-DC-SIGN antibodies on direct infection of DC, T blasts and DC-SIGN transfectants

We monitored infection and entry in cells at the level of early reverse transcripts of proviral DNA. The anti-CD4 antibodies and RANTES blocked the infection in DC and T blasts, whereas anti-DC-SIGN antibodies did not (Fig. 3). However, as expected from their lack of CD4 and CCR5 (Fig. 1), THP-DC-SIGN cells were not susceptible to infection. To assess the role of DC-SIGN in transfectants that express CD4 and CCR5, we made stable DC-SIGN transfectants in HOS cells previously transfected with CD4 and CCR5. As seen in Fig. 1, DC-SIGN is highly expressed in these transfectants. Virus entry was blocked by anti-CD4 antibodies and RANTES, but not by anti-DC-SIGN antibodies, in HOS-CD4-CCR5 or HOS-CD4-CCR5-DC-SIGN transfectants (Fig. 3). Thus, CD4 and CCR5, but not DC-SIGN, serve as receptors for virus entry into both HOS cells and DC. We next investigated transmission of HIV-1 from DC-SIGN-expressing cells to activated T cells, since this may proceed independently of infection.

DC-SIGN-expressing cell lines differ in their capacity to retain infectious HIV-1 for transmission to T cells

We compared the DC-SIGN transfectants to DC for their capacity to retain infectious HIV-1, using cells that were exposed to virus for 2 h at 37°C, washed and then added to T cells immediately or after 3–6 days in culture. The latter approach was to test whether the virus retained by the DC-SIGN-expressing cells was infectious for subsequent transmission to T cells. As seen in Fig. 4A (left panel), if activated T cells were added immediately after infection, both 293-DC-SIGN and THP-DC-SIGN cells could transmit HIV-1, much like DC. However, 293-DC-SIGN cells were unable to retain the virus in an infectious state for 3 days (Fig. 4A, middle panel). The lack of T cell infection was also evident even when activated T cells were added 24 h after infection of 293-DC-SIGN cells (not shown). HOS-DC-SIGN cells acted similarly to the 293 transfectants, being able to transfer virus to T cells only if tested immediately and not if cultured 1–3 days prior to addition of the T cells (Fig. 4B). In contrast, infected THP-DC-SIGN cells and DC could still transmit virus to activated T cells, even when the T cells were added 3 days later (Fig. 4A, middle panel). If T blasts were added after 6 days, only DC but not THP-DC-SIGN cells could transmit HIV-1 (Fig. 4A, right panel). Therefore, the DC and THP-DC-SIGN transfectants, but not 293 or HOS transfectants, could retain HIV-1 in a form that is infectious for T cells for at least 3 days, whereas DC could transmit infection to T cells even after 6 days.

Effect of anti-DC-SIGN antibodies on transmission of HIV-1 from DC-SIGN transfectants and DC to T cells

Several anti-DC-SIGN antibodies were also tested for their capacity to block HIV-1 transmission from HIV-1-infected cells to T cells. Two different assays were utilized with cells that were preincubated with anti-DC-SIGN antibodies, exposed to virus for 2 h and after extensive washes co-cultured with activated T cells. First, to detect early infection in T cells, LTR/gag sequences were analyzed by PCR after 36 h of co-culture. Anti-DC-SIGN antibodies completely inhibited virus transfer from THP-DC-SIGN cells as visualized by PCR (Fig. 5A), confirming previous results (14,19). In contrast, DC-SIGN antibodies did not completely block the transfer of HIV-1 from either immature or mature DC (not shown) to T cells (Fig. 5A), even when the two antibodies were used at 20 μg/ml. As seen in Fig. 5(A), the AZND1 antibody inhibited more strongly than the 612 and 507 antibodies (or the combination of 507 and 612; data not shown). Second, HIV-1 transfer over longer culture periods was assessed by measuring p24 in the supernatants by ELISA. With this assay we compared DC-SIGN infected cells that were co-cultured with activated T cells added at day 0 or after 2 days of infection (Fig. 5B). The results were identical, indicating that in THP-DC-SIGN transfectants, HIV-1 transmission is completely blocked by anti-DC-SIGN antibodies (Fig. 5A and B, lower panels). In DC, anti-DC-SIGN antibodies did not block HIV-1 transfer completely, but in some experiments with higher doses of AZND1 antibody the inhibition was 80–90% (Fig. 5B, upper panels). Thus, as noted in the capture assay, in DC there may be an alternative DC-SIGN-independent pathway whereby DC can bind and retain HIV-1 for transmission to T cells.

Co-expression of DC-SIGN with CD4 and CCR5 enhances viral infection and transmission in HOS cells

Previous work has shown that DC-SIGN either does not mediate virus entry (18) or that *cis* expression of DC-SIGN allows for more efficient entry of HIV-1 (20). The HOS transfectants provided an opportunity to evaluate the role of DC-SIGN in cells that also expressed the CD4 and CCR5 receptors for viral entry, like DC. For this, cells were infected with different virus titers and then proviral DNA was amplified. We did observe a 3- to 5-fold increase in infection of the CD4- and CCR5-expressing HOS cells when DC-SIGN was also expressed, but only when the cells were infected with limiting doses of HIV-1 (Fig. 6A). More impressive differences in quantitative terms were seen during culture with T cells. When the HOS-CD4-CCR5 cells were tested for HIV-1 transmission to T cells (immediately after infection), the expression of DC-SIGN markedly enhanced the production of p24 in the HOS-T

cell co-culture. This was evident with a quantitative single-cell staining of p24 in the T blasts by FACS (Fig. 6B). For the latter, HOS transfectants were infected with HIV-1 and co-cultured with activated T blasts for 6 days. $CD3^+$ cells were gated and enumerated for staining to newly produced intracellular p24 antigen. Infection of the T blasts was increased at least 10-fold

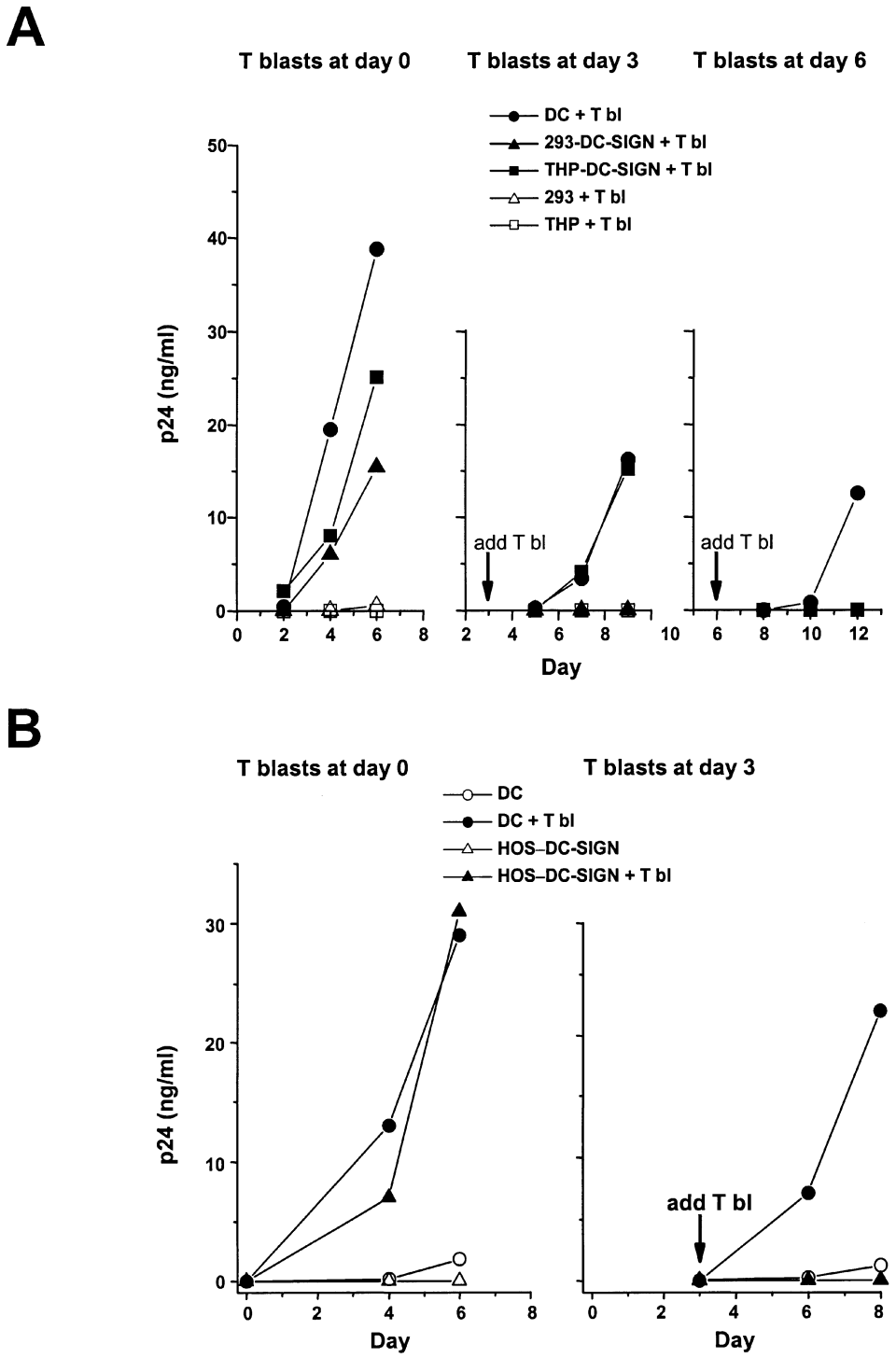

Fig. 4. Transmission of HIV-1 to T blasts by immature DC and DC-SIGN transfectants. Cells (10^5) were infected with 300 pg of p24 (equivalent to 3000 IU) for 2 h at 37°C. Cells were washed and 5×10^4 infected cells were incubated immediately with 10^5 T blasts (day 0). In parallel, other aliquots of virus-pulsed cells were cultured for 3 or 6 days prior to addition of T blasts (day 3 or day 6). p24 was measured in the supernatants by ELISA at the indicated time points. (A) Comparison of DC with 293-DC-SIGN and THP-DC-SIGN transfectants. (B) Comparison of DC with HOS-DC-SIGN.

by the presence of DC-SIGN in the HOS cells transfectants. Therefore, DC-SIGN is able, in CD4- and CCR5-bearing HOS cells, to enhance infection and transmission to T blasts at low virus titers.

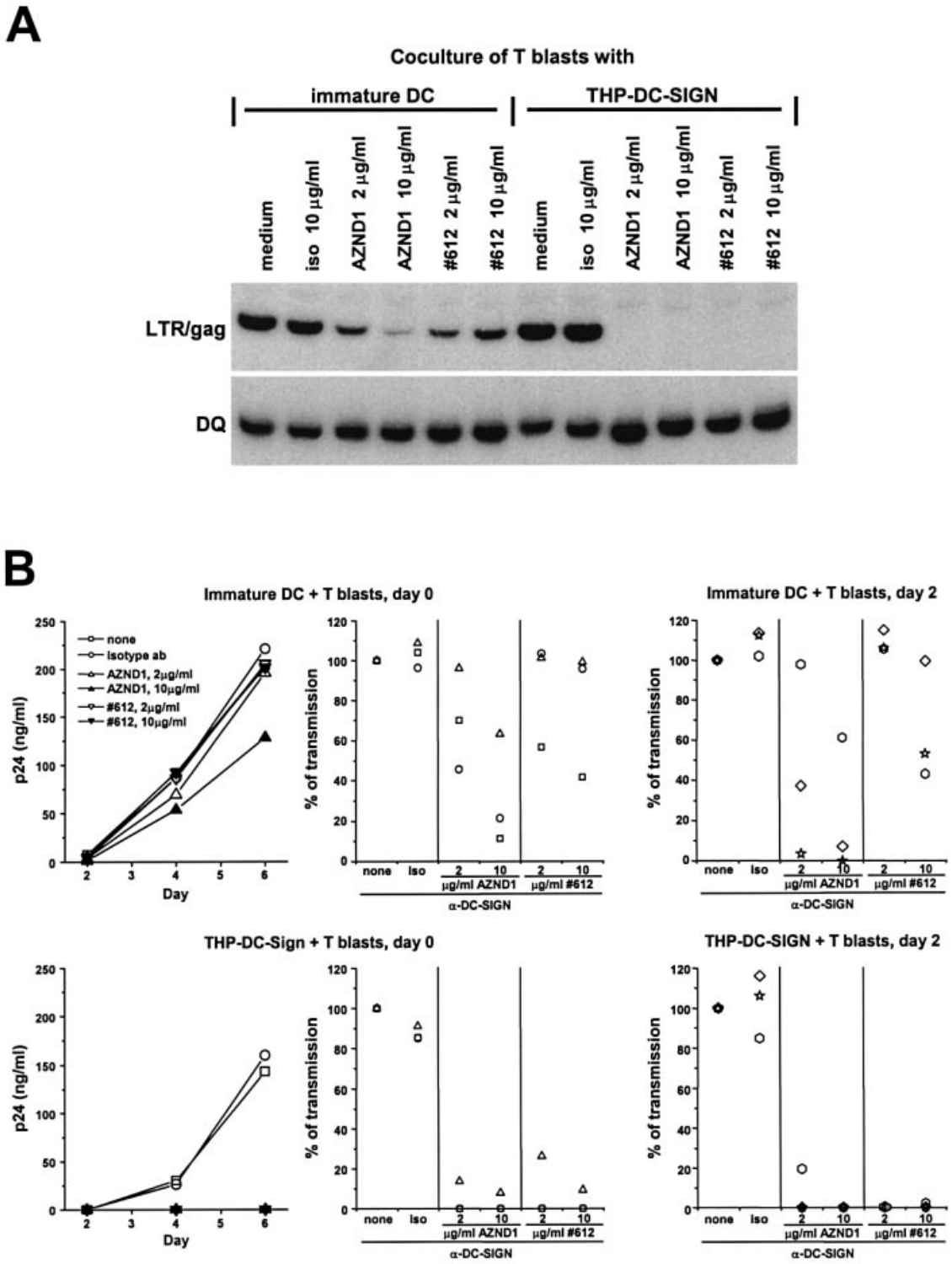

Fig. 5. Effect of anti-DC-SIGN antibodies on HIV-1 transmission. Cells were incubated with the indicated doses of anti-DC-SIGN antibodies or isotype controls at a concentration of 10 μg/ml for 20 min at room temperature and then infected with HIV-1 for 2 h at 37°C. Cells were washed 4 times and 5×10^4 infected cells co-cultured with 10^5 T blasts. (A) Detection of full-length proviral DNA (LTR/gag) after 36 h of co-culture. DQ sequences were amplified for control of DNA input (23). (B) p24 secreted in the culture media as detected by ELISA. Each symbol in the middle and right panel corresponds to an individual experiment.

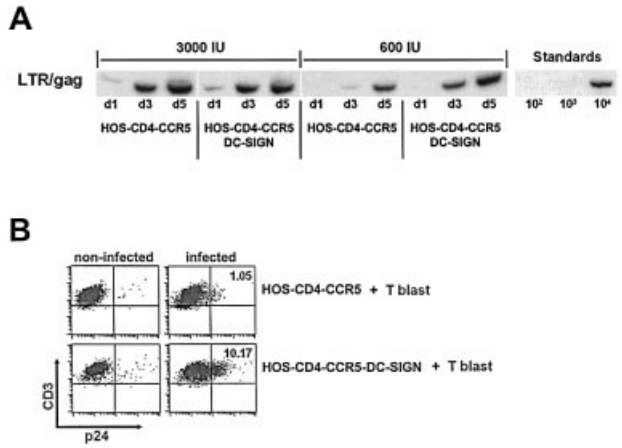

Fig. 6. Expression of DC-SIGN on CD4 and CCR5 HOS transfectants increases infection and transmission to T blasts. HOS cells (10^5) were infected with the indicated viral doses of HIV-1 for 2 h at 37°C. After extensive washes, cells were studied directly (A) or incubated with activated T cells (B). (A) Formation of LTR/gag reverse transcripts after 1, 3 or 5 days of culture in HOS cells that express CD4 and CCR5 in the absence or presence of DC-SIGN, at two different doses of HIV-1. Graded doses of ACH-2 cell lysates were amplified in parallel for quantitation of proviral DNA in samples. (B) FACS for p24 expression. HOS cells were infected with HIV-1 and, after washing, cells were co-cultured with activated T cells. After 6 days, cells were collected, double stained for p24 and CD3, and the gated CD3 cells were analyzed for p24.

Visualization of p24-containing compartments in HIV-1-infected cells

To investigate the fate of bound and internalized virus in DC and THP-DC-SIGN transfectants as a function of time, cells were infected with high titers of AT-2-inactivated HIV-1 (24) for 2 h on ice or at 37°C and then washed to remove unbound virus. The infected cells were attached to slides, and double stained for p24 and specific markers of cellular compartments immediately after the 2-h pulse with HIV-1 or after culture for 2 days. If cells were infected on ice, virus localized predominantly at the surface, with some co-localization of p24 with DC-SIGN (yellow labeling) in the transfected cells, consistent with a function of DC-SIGN in virus capture in the transfectants (Fig. 7). However, in DC, there was little overlap between the DC-SIGN and anti-p24 labeling, suggesting alternative methods of virus binding than DC-SIGN (Fig. 7). When cells were infected at 37°C for 2 h, p24 staining was detected within intracellular granules, but there was no double labeling with antibodies for several endocytic compartments. The intracellular $p24^+$ granules lacked EEA1 for early endosomes, caveolin for caveolae or LAMP for late endosomes and lysosomes (not shown); furthermore, there was no double labeling for DC-SIGN and p24 (Fig. 7). When the infected cells were studied after 2 days in culture, p24 persisted within granules, often in the perinuclear region (Fig. 7), but again without evident co-localization with DC-SIGN. However, virus persistence was only observed in DC and THP-DC-SIGN cells, and not in 293-DC-SIGN (Fig. 7). This indicates that in DC and THP-transfectants, HIV-1 can be sequestered and retained in vacuoles that are distinct from many known endocytic compartments, and that the virus may have dissociated from DC-SIGN.

Discussion

The most interesting feature of these studies is that retention and transmission of HIV-1 by DC-SIGN depend on the cellular environment. Although 293-DC-SIGN and HOS-DC-SIGN bind virus and transmit infectivity to activated T cells, this was achieved only if T cells were added immediately after the virus pulse. THP-DC-SIGN kept the virus in an infectious state for 2–3 days, whereas only DC transmitted infectious virus after 6 days. This indicates that monocyte-derived cells retain virions and competence to infect target T cells after a prolonged period from primary infection. Some cell-restricted mechanisms, present in THP-1 and DC, but not in 293 and HOS, seem to be required for DC-SIGN to exhibit its striking capacity to retain HIV-1 in an infectious state for T cells. It has been shown by others that following interaction of HIV with DC-SIGN, the virus is rapidly internalized in THP-DC-SIGN (19). DC, like THP-DC-SIGN cells, internalize HIV-1 and, by confocal microscopy, virus could be visualized inside cells 2 days after infection. However, the viral particles could not be detected by microscopy in 293-DC-SIGN and HOS-DC-SIGN after 2 days of infection. The intracellular $p24^+$ compartments did not double label with antibodies to early endosomes, caveolae or late endosomes/lysosomes. At the time points studied, the internalized virions also were not co-localized with DC-SIGN, suggesting dissociation or weak interaction with DC-SIGN. Thus, DC and the THP line have specialized compartments that can protect viruses from degradation. The function of these compartments in transmission of HIV-1 to T cells may be critical, especially in the case of cells that have similarity to the THP cells and DC used in this study.

A high amount of DC-SIGN is expressed by DC [Fig. 1 and (14,18)]. We speculate that, in addition to the sequestration and transmission of infectious HIV-1, an additional function of DC-SIGN is to concentrate and tether low amounts of virus to the cell surface, thus facilitating the interaction with CD4 and CCR5. Alternatively virions bound to DC-SIGN could undergo conformational changes that increase binding affinity for CD4 and CCR5. These situations may occur and recapitulate an *in vivo* infection. Our hypotheses are supported by the results showing that $CD4^+$ and $CCR5^+$ cells transfected with DC-SIGN are infected more efficiently at very low virus titers.

Transmission to T cells was greatly enhanced using DC-SIGN transfected versus non-transfected cell lines and transmission in the former was blocked often almost completely by inclusion of anti-DC-SIGN antibodies. However, in the monocyte-derived DC that we studied, the antibodies did not completely block transmission. This was evident with three different sources of anti-DC-SIGN antibodies that recognize the carbohydrate recognition domain (CDR) of DC-SIGN (9,18), although inhibition was more pronounced with the AZND1 antibody. In DC, virus transmission seems to depend on several factors such as direct infection with HIV-1 (25–28), capture of virus by DC-SIGN (18,19) or by other transmitting receptors homologous to DC-SIGN. Macaque DC express very low levels of DC-SIGN, but are still able to transmit virus to T cells (29), and in a recent study it was suggested that factors other than DC-SIGN play a role in the ability of DC to transmit HIV-1 (14). Interestingly enough, other DC subsets such as

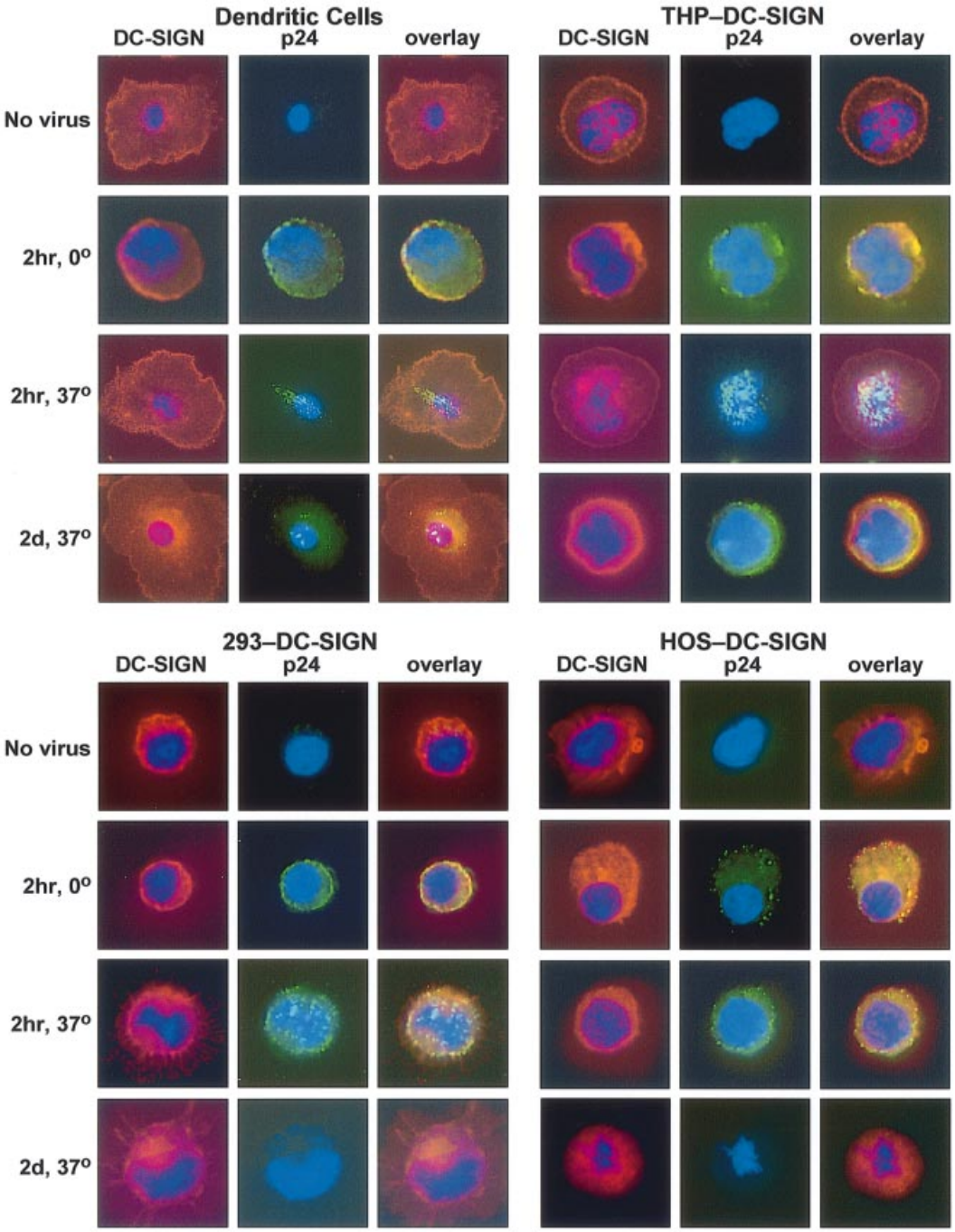

Fig. 7. Localization of HIV-1 by confocal microscopy in DC-SIGN-expressing cells. Cells were infected with AT-2 virus (100 ng of p24 for 10^5 cells) at 37°C or on ice for 2 h and then washed. Some cells were analyzed immediately, while others were examined after 2 days of culture. The cells were stained for p24 (green), DC-SIGN (red) and nuclei (blue).

Langerhans cells and plasmacytoid cells express HIV-1 co-receptors, and little or no DC-SIGN, but can be infected and transmit HIV-1 (30,31). In contrast, a subset of DC-SIGN$^+$ DC in human blood was shown to transmit HIV-1 to T cells (32).

Others have shown that the role of DC-SIGN in the capture of infectious agents extends beyond HIV-1 to other pathogens like *Leishmania* (12), SIV (15), Ebola (13) and HCV (16). It will be of interest to determine if the function of DC-SIGN in supporting infection with these other agents also in some way depends upon the presence of this C-type lectin in a DC environment.

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Abbreviations

AT-2	Aldritol-2
DC	dendritic cell
PBMC	peripheral blood mononuclear cell
PE	phycoerythrin

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