DC-SIGN–Mediated Transfer of HIV-1 Is Compromised by the Ability of Leishmania infantum to Exploit DC-SIGN as a Ligand

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DC-SIGN (dendritic cell–specific intercellular adhesion molecule 3–grabbing nonintegron) binds human immunodeficiency virus type 1 (HIV-1) and facilitates transfer of virus to permissive cells. Leishmania parasites also exploit DC-SIGN as a receptor. Here, we report that transfer of HIV-1 to target cells is markedly reduced when DC-SIGN+ cells are preincubated with Leishmania amastigotes before pulsing with virions. Moreover, binding of HIV-1 to DC-SIGN+ cells is diminished by the presence of Leishmania amastigotes. Our findings provide novel insight into the complex interactions between HIV-1 and Leishmania parasites. The ability of both HIV-1 and Leishmania parasites to bind to the same cell-surface constituent to gain entry into dendritic cells might have an impact on the immunological and pathological events associated with HIV-1 infection.

Dendritic cells (DCs), which are essential antigen-presenting cells found at body surfaces and in lymphoid tissues, appear to play a determinant role in transmission of HIV-1. This process is facilitated by virtue of DCs being the initial target cell type of HIV-1 and of their unique capacity to migrate from virus entry sites in the peripheral blood to T cell–rich areas in lymphoid tissues. Intimate contact between DCs and T cells has been shown to promote transmission of HIV-1 by locally concentrating virus, viral receptors, and coreceptors, as well as certain adhesion molecules. Previous studies have provided evidence that, in addition to CD4 and chemokine receptors CCR5 and CXCR4, DC-SIGN (DC-specific intercellular adhesion molecule 3–grabbing nonintegron; also called “CD209”) is largely responsible for the capture of HIV-1 and the amplification of the infection in the DC–T cell milieu [1]. In contrast to CD4, which is the primary cellular receptor, DC-SIGN functions not as a classical HIV-1 entry receptor but rather as a transreceptor that binds to HIV-1 and transfers the virus very efficiently to neighboring permissive target cells [1]. Recently, it has been discovered that DC-SIGN is also exploited by protozoan parasites of the genus Leishmania, which are opportunistic pathogens in HIV-1 infection, to gain entry into DCs [2]. Leishmania/HIV-1 coinfection is considered to be a serious threat in countries where both pathogens are widespread. This parasite has been shown not only to up-regulate HIV-1 transcription and virus production [3] but also to down-regulate HIV-1–mediated giant cell formation [4], which suggests that there is a complex relationship between the 2 microorganisms. Leishmania pifanoi and Leishmania infantum amastigotes can infect DCs, because these parasites can efficiently bind to DC-SIGN [2]. It has been proposed that DCs will carry the bound parasites during their migration from the initial infection sites to the most proximal draining lymph nodes, an event contributing to the establishment of a parasite reservoir.

Despite the reported propensity of both HIV-1 and Leishmania parasites to utilize DC-SIGN as a portal of entry into DCs, the consequences of these findings for HIV-1 capture and transfer have not been previously investigated. By use of both DC-SIGN transfectants and primary human immature DCs, we demonstrate here that ligation of L. infantum amastigotes to DC-SIGN leads to reduced retention and transfer of HIV-1.

**Methods.** Virions were produced by transient calcium phosphate transfection of human 293T cells with the R5-tropic HIV-1 infectious molecular clone JR-CEF, and virus stocks were normalized for virion content, as described elsewhere [5]. Stationary L. infantum promastigotes were transferred into MAA/20 medium that consisted of modified medium 199 (Gibco BRL) with Hank’s salts, supplemented with 0.5% soybean trypsin-casein (Pasteur Diagnostics), 15 mmol/L d-glucose, 5 mmol/L L-glutamine, 4 mmol/L NaHCO3, 0.023 mmol/L bovine hemin, 25 mmol/L HEPES (pH, 6.5), and 20% fetal bovine serum, to differentiate...
Figure 1. Significant reduction of HIV-1 transfer upon preincubation with *Leishmania infantum* amastigotes. B-THP-1/DC-SIGN cells (A and B) and primary human immature monocyte-derived dendritic cells (iMDDCs) (C and D) were preincubated for 1 h at 37°C in the absence or presence of *L. infantum* amastigotes ("L. i.") at the indicated parasite-cell ratios. Next, cells were pulsed with R5-tropic virions (i.e., JR-CSF at 25 ng of p24/1 × 10^6 target cells) for 2 h at 37°C. The cell/parasite/virus mixture was next cocultured with either TZM-bl indicator cells (A and C) or phytohemagglutinin-activated CD4+ T cells (B and D). Virus replication was monitored by assessing luciferase activity (in relative light units [RLU]) in TZM-bl indicator cells at 2 days after coculture and p24 levels in CD4+ T cells at 6 days after coculture. Data are from triplicate samples and are representative of 3 independent experiments. Statistically significant differences were obtained when samples infected with HIV-1 only were compared with samples infected with HIV-1 and *L. infantum* at a 10:1 parasite-cell ratio (A, P = .001; B, P = .019; C, P = .012; and D, P = .025).

Flow cytometry analysis was performed to test the binding of *L. infantum* amastigotes to B-THP-1/DC-SIGN cells. Briefly, cells were either left untreated or exposed to *L. infantum* amastigotes for 1 h at 37°C to reach the saturation state and were subsequently washed with PBS to eliminate unbound parasites. Cells were then stained with a fluorescein isothiocyanate–labeled anti–DC-SIGN antibody (anti–human CD209; clone eB-h209 [eBioscience]).

The effect of *L. infantum* on DC-SIGN–dependent capture and transfer of HIV-1 was measured by first pulsing DC-SIGN+ cells—that is, B-THP-1/DC-SIGN cells (1 × 10^6)—and iMDDCs (3.3 × 10^5)—either with HIV-1 alone or with *L. infantum* before HIV-1. DC-SIGN+ cells either were not preincubated or were preincubated with *L. infantum* amastigotes (10 or 15 parasites/target cell) for 1 h at 37°C, and free parasites were washed out with PBS. Cells were then briefly exposed to JR-CSF virus (25 ng of p24/1 × 10^6 cells) for 2 h at 37°C. Next, cells were extensively washed with PBS to remove untrapped pathogens and were cocultured with either TZM-bl indicator cells.
Leishmania-dependent decrease of HIV-1 transfer, as a result of competition for DC-SIGN binding, B-THP-1 and B-THP-1/DC-SIGN cells were first incubated for 1 h at 37°C in the absence or presence of *L. infantum* amastigotes ("*L. i.*") at the indicated parasite-cell ratios. Next, cells were pulsed for 1 h at 37°C with R5-tropic viruses (i.e., JR-CSF at 25 ng of p24/1 × 10^6 target cells). The levels of cell-associated virus were quantified by measuring the p24 content. Data are from triplicate samples and are representative of 3 independent experiments. A statistically significant difference (P = .017) was obtained when samples infected with HIV-1 only were compared with samples infected with HIV-1 and *L. infantum* at a 10:1 parasite-cell ratio.

(1.5 × 10^6; plated 24 h before coculture) or PHA-activated CD4+ T cells (1 × 10^5). Virus replication was evaluated by measuring reporter gene activity by use of a luciferase assay at 2 days after coculture and measuring p24 production at 6 days after coculture. In the HIV-1–binding experiment, B-THP-1/DC-SIGN cells (1 × 10^6) were first exposed to *L. infantum* amastigotes for 1 h at 37°C. Unbound parasites were washed out with PBS. Next, JR-CSF virus (25 ng of p24/1 × 10^6 cells) was added, and the parasite/HIV-1/cell mixture was incubated for another 2 h at 37°C. After extensive washes with PBS, the cell pellets were lysed, and the amounts of cell-associated virus were quantified by estimating p24 content. Results are expressed as the mean ± SD values of samples tested in triplicate. Statistical significance (P < .05) of differences between groups was determined by analysis of variance.

**Results.** Exposure of B-THP-1/DC-SIGN cells to *L. infantum* amastigotes led to a dose-dependent diminution of DC-SIGN staining. For example, DC-SIGN expression was reduced by 65% ± 6.6% and 31% ± 8.4% when parasite-cell ratios of 10:1 and 5:1 were used, respectively (data not shown). This suggests that *L. infantum* amastigotes bind to DC-SIGN on the surface of B-THP-1/DC-SIGN cells. Given the crucial role played by the transmembrane lectin DC-SIGN in HIV-1 capture and transfer, we next examined whether the interaction between DC-SIGN and *L. infantum* could affect the ability of DC-SIGN+ cells to transfer HIV-1 in trans to target cells. The capacity of B-THP-1/DC-SIGN cells to capture and transfer HIV-1 to TZM-bl indicator cells (figure 1A) and primary human CD4+ T cells (figure 1B) was severely reduced upon preincubation with *L. infantum* amastigotes. A similar phenomenon was observed when primary human iMDDCs were used in combination with TZM-bl indicator cells (figure 1C) and CD4+ T cells (figure 1D). The efficiency of HIV-1 transfer by B-THP-1/DC-SIGN cells is an order of magnitude higher than that of iMDDCs. Consequently, surface expression of DC-SIGN was measured on both DC-SIGN transfectants and iMDDCs, because this factor may have an impact on virus transfer. Comparative flow cytometry analyses indicated that B-THP-1/DC-SIGN cells do indeed express higher surface levels of DC-SIGN than do iMDDCs (data not shown). It should be noted that the efficiency of HIV-1 transfer was similarly decreased after simultaneous incubation of cells with both *L. infantum* amastigotes and HIV-1 (data not shown). Altogether, these results support the notion that HIV-1 dissemination is negatively modulated upon binding of *L. infantum* amastigotes to DC-SIGN+ cells.

In an attempt to shed light on the precise mechanism through which the parasite can influence virus transmission, we next investigated the ability of *L. infantum* to affect DC-SIGN–mediated capture of HIV-1 particles. Attachment of progeny virus to the surface of DC-SIGN+ cells was not affected by the presence of the parasites (figure 2), which provides indirect evidence that attachment of *L. infantum* to such cells requires the presence of DC-SIGN. On the contrary, virus binding to B-THP-1/DC-SIGN cells was significantly reduced by *L. infantum*, in a dose-dependent manner.

**Discussion.** There is now compelling evidence that DC-SIGN is involved in the binding and internalization of *Leishmania* parasites in both the promastigote and amastigote infective stages [2, 9]. Thus, our results suggesting that *L. infantum* amastigotes interact with DC-SIGN are perfectly in line with the notion that DC-SIGN plays a role in pathogen–DC interactions through its affinity for natural surface glycans found on the surface of this human pathogen. The present study was focused on *L. infantum*, because infection with this *Leishmania* species is the third most frequent parasitic disease in HIV-1–infected individuals, after *Toxoplasma gondii* and *Cryptosporidium parvum* infection. Previous studies have investigated possible *Leishmania*/DC-SIGN interactions through the use of fluorescence-labeled parasites in combination with blocking agents such as anti–DC-SIGN antibodies and soluble mannan [2, 9], whereas we used a technical strategy that relied on incubation of DC-SIGN+ cells with *Leishmania* amastigotes, followed by staining with anti–DC-SIGN antibody. This procedure provided direct useful information on both the intensity of DC-SIGN cell-surface expression and the percentage of DC-SIGN binding sites that were bound specifically by *Leishmania* parasites. Moreover, this method allowed us to demonstrate that the *L. infantum*/DC-SIGN association is strong, since it resisted extensive and vigorous washes. Our
virus transfer experiments further revealed that HIV-1 transfer to target cells, such as primary CD4+ T cells, was significantly reduced when DC-SIGN+ cells, including primary human iMDDCs, were first exposed to the parasites before pulsing with HIV-1. The diminished capacity of DC-SIGN+ cells to transfer HIV-1 was linked with a reduction in virus capture. These findings are reminiscent of previous observations of antibodies directed against DC-SIGN that have been shown to inhibit the uptake of both human pathogens by DCs [2, 9, 10]. It is important to emphasize that DCs were exposed to Leishmania parasites for a short period, therefore suggesting that the reduced HIV-1 transfer is most likely attributable to an effect whereby cell-surface DC-SIGN is occupied by L. infantum and is no longer accessible to HIV-1. It can also be proposed that DC-SIGN is internalized upon L. infantum binding. Additional studies are warranted to shed light on this issue.

At first sight, the demonstration that Leishmania parasites in the amastigote form impair HIV-1 transfer from DCs to target cells seems to constitute a paradox, since virus load in HIV-1/Leishmania–coinfected patients is higher than that in HIV-1–infected control individuals without coinfection. However, some explanations can be proposed for this apparent contradiction. For example, although DC-SIGN plays an important role in the process of HIV-1 infection in trans, it must be stressed that DC-SIGN affects virus transfer only from cells that are either not susceptible or weakly susceptible to productive HIV-1 infection. It is now well established that HIV-1 entry into target cells occurs mainly through the interaction of the viral envelope protein gp120 with cell-surface receptor CD4 and chemokine coreceptors. Thus, the reported augmentation of HIV-1 load in patients who are also infected with Leishmania parasites may be the consequence of a parasite-mediated increase in CCR5 expression [11], an effect that may predominate over the impact on the DC-SIGN trans-infection pathway. Interestingly, patients with active visceral leishmaniasis showed increased surface expression of CXCR4 on circulating CD4+ T cells, compared with that in healthy donors, a process that could render T lymphocytes more susceptible to HIV-1 infection [12].

To the best of our knowledge, there are no published studies that have included quantitative analyses of L. infantum amastigotes in a statistically significant number of HIV-1–infected patients. Therefore, it is still unknown whether parasites can be found at levels sufficiently high to possibly interfere with transmission of HIV-1. However, in studies of limited numbers of individuals, it has been shown that HIV-1–infected individuals carry many more amastigotes in their peripheral circulation than do their HIV-1–negative counterparts [13, 14]. Moreover, data from in vitro studies indicate that HIV-1 enhances the intracellular growth of Leishmania parasites [15]. Therefore, it can be proposed that our findings bear some physiological relevance in the setting of coinfected patients.

Altogether, our results highlight the very complex nature of possible interactions between Leishmania parasites and HIV-1 in dually infected patients. To the best of our knowledge, this is the first study to propose that the demonstrated competition between HIV-1 and L. infantum amastigotes for DC-SIGN binding might impact the immunological and pathological events associated with HIV-1 infection. This observation certainly expands our knowledge of the relationship between these 2 human pathogens. Since anti–DC-SIGN antibodies can block the uptake of both human pathogens, novel therapeutic strategies could be designed that use, for example, soluble receptors for blocking interactions between DCs and these 2 microorganisms.

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References

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