

Short
CommunicationVirus-associated host CD62L increases
attachment of human immunodeficiency virus type 1
to endothelial cells and enhances *trans* infection of
CD4⁺ T lymphocytes

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Previous studies have identified several host-derived cell-surface proteins incorporated within emerging human immunodeficiency virus type 1 (HIV-1) particles. Some of these molecules play a role in different steps of the virus life cycle and are often advantageous for the virus. We report here that the leukocyte L-selectin (also called CD62L) remains functional when inserted within the envelope of HIV-1. Indeed, we demonstrate that adsorption of virions to endothelial cells is enhanced upon acquisition of host-derived CD62L. The more important binding of CD62L-bearing HIV-1 particles resulted in a more efficient virus transmission to CD4⁺ T lymphocytes. Capture and eventual transfer of such CD62L-bearing virions by the endothelium could play a role in the pathogenesis of HIV-1 infection.

The classical way for human immunodeficiency virus type 1 (HIV-1) to infect CD4⁺ T cells involves interactions between gp120 and a complex made of cell-surface CD4 and an appropriate chemokine co-receptor (i.e. CXCR4 or CCR5), followed by fusion with the plasma membrane (Clapham & McKnight, 2001; Pierson & Doms, 2003; Piguet & Sattentau, 2004). However, it is now well established that initial interactions between the viral particle and the cell surface are far more complex than initially thought, and they are notably modulated by a variety of interactions occurring between gp120 and different cell-surface molecules. Such interactions include those between gp120 and dendritic cell (DC)-specific intercellular adhesion molecule 3 (ICAM-3)-grabbing nonintegrin (DC-SIGN); L-SIGN, another C-type lectin found in liver lymph nodes; heparan sulphate proteoglycans (HSPG); and langerin or mannose receptor (Bashirova *et al.*, 2001; Bobardt *et al.*, 2003; Cantin *et al.*, 2005; Dakappagari *et al.*, 2006; Geijtenbeek *et al.*, 2000; Turville *et al.*, 2002, 2003). Moreover, it is now accepted that the HIV-1 attachment step can also be influenced by interactions between virus-anchored host-cell membrane components and their natural ligands once expressed on target cells (Kolegraff *et al.*, 2006; Ott, 1997, 2002). Amongst the different incorporated host proteins, ICAM-1, leukocyte function-associated antigen-1 (LFA-1), HLA-DR, CD28 and CD152 have been shown to play a key role in the initial events in the HIV-1 life cycle (Cantin *et al.*, 1997; Castilletti *et al.*, 1995; Fortin *et al.*, 1997; Giguère *et al.*, 2005; Paquette *et al.*, 1998). Thus, the enhancement of viral attachment through these additional interactions may modulate the *cis* and/or *trans* infection with HIV-1.

Trans infection is defined as an initial capture of virions by carrier cells, which are not or weakly susceptible to productive infection, followed by an efficient virus transfer to more permissive target cells (Geijtenbeek *et al.*, 2000; Piguet & Sattentau, 2004). Several studies have demonstrated that DCs, macrophages, B lymphocytes, epithelial and endothelial cells can serve as HIV-1 carriers (Abbate *et al.*, 1999; Arias *et al.*, 2003; Bounou *et al.*, 2004; De Milito, 2004; Turville *et al.*, 2004). Following HIV-1 binding, some carrier cells can internalize virions, whereas others can retain them under an intact form on their plasma membrane. In both cases, a close contact between these ‘Trojan horse’ cells and CD4⁺ T cells is needed to transmit HIV-1.

Endothelial cells possess the capacity to capture circulating T cells as part of the process of diapedesis. CD62L and LFA-1 and their respective ligands on endothelial cells, i.e. E-selectin and ICAM-1, all participate in the slowing down and adhesion of leukocytes, the first steps in lymphocyte homing. Also called L-selectin, CD62L is a C-type lectin largely distributed on B and T cells, monocytes and neutrophils. CD62L supports leukocyte tethering and rolling along vascular endothelium by interacting with carbohydrates presented by various specific endothelial cell ligands, including CD34, podocalyxin-like protein and E-selectin (McEver & Cummings, 1997; Rosen, 2004; Sasseti *et al.*, 1998). These are widely expressed on vascular endothelium but mainly remain in a non-functional state. The majority of functional ligands are expressed on high endothelial venules (HEVs), which are located in lymphoid organs such as lymph nodes. To become functional, ligands

require sialylation, fucosylation and sulphation of their mucin-like domain (Pablos *et al.*, 2005). Ligands expressed on vascular endothelium other than HEVs become functional in response to pro-inflammatory cytokines (Pablos *et al.*, 2005). Nonetheless, it has been reported that CD62L can also interact with the cutaneous lymphocyte antigen (CLA) found on vascular endothelium other than HEVs in the absence of cytokines (Tu *et al.*, 1999).

Interestingly enough, it has been shown that CD62L is incorporated in HIV-1 produced in primary human CD4⁺ T lymphocytes (Bastiani *et al.*, 1997). However, whether this molecule plays a role when present on virions remains to be determined. Considering the natural role played by CD62L in the rolling step and given that this cell-membrane constituent is incorporated within HIV-1, we hypothesized that endothelial cells could efficiently capture CD62L-carrying virions and then transfer such trapped virus to CD4⁺ T cells.

In an attempt to investigate these two interconnected possibilities, we first produced isogenic viruses either lacking (NL4-3 and NL4-3Balenv) or bearing (NL4-3/CD62L and NL4-3Balenv/CD62L) host-derived CD62L molecules by the calcium phosphate co-precipitation method in 293T cells as described previously (Fortin *et al.*, 1997). We used the full-length infectious molecular clone of HIV-1 pNL4-3 or pNL4-3Balenv and pLam-1, a cDNA encoding human CD62L (kindly provided by T. F. Tedder, Duke University Medical Center, Durham, NC, USA) (Tedder *et al.*, 1989). In pNL4-3Balenv, the env gene of the X4-tropic NL4-3 strain has been replaced with that of R5-using Bal strain. Virus preparations were normalized for virion content using an in-house enzymic assay specific for the viral capsid protein p24 (Bounou *et al.*, 2002). It should be noted that 293T cells were transfected with a quantity of the CD62L expression vector leading to a surface-expression level of CD62L similar to what is seen in primary human CD4⁺ T cells (data not shown). The presence of CD62L on the exterior of HIV-1 particles was verified using a previously described virus capture test (Martin & Tremblay, 2004).

As illustrated in Fig. 1(a), CD62L-bearing NL4-3 particles were captured by magnetic beads coated with an anti-CD62L (Lam1-116), thus confirming that host-derived CD62L is efficiently acquired by newly formed HIV-1. In this set of experiments, antibodies specific for CD45 and class I were used as negative and positive controls, respectively. Similar observations were made when using NL4-3Balenv (data not shown). The physiological relevance of such findings was addressed by comparing CD62L incorporation in viruses produced in transfected 293T cells and primary human CD4⁺ T cells, and we found that the level of virus-associated host CD62L is comparable for the two virus preparations tested (Fig. 1b). Next, to assess whether host CD62L can promote binding of virions to endothelial cells, human umbilical vein endothelial cells (HUVECs) were used to capture the virus stocks studied.

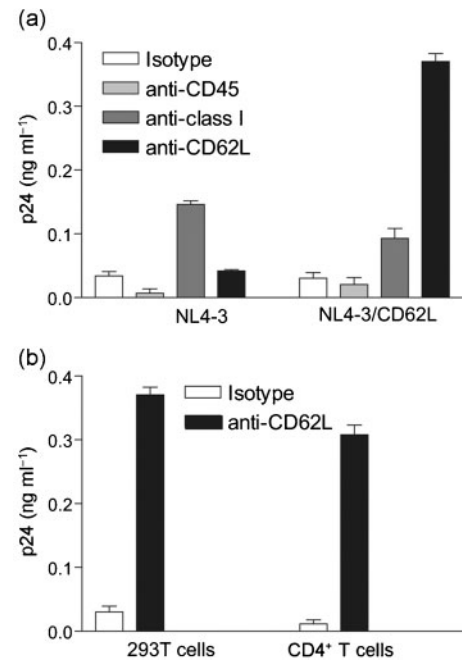


Fig. 1. CD62L is incorporated in HIV-1. (a) Viruses produced in 293T cells were captured with magnetic beads coated with the antibodies shown. (b) Viruses produced in 293T cells or primary human CD4⁺ T cells were captured with magnetic beads coated with an anti-CD62L or a control isotype-matched antibody. Results shown are the means \pm SD of triplicate samples and are representative of five separate experiments.

For the virus-binding assay, HUVECs (1×10^5) were exposed to similar concentrations of each virus preparation (standardized in terms of p24 content, 10 ng p24 was used) for the indicated times at 37 °C. After several washes, unbound viruses were removed and the cell-virus mixture was treated with 200 μ l lysis buffer (HEPES 20 mM, NaCl 150 mM and Triton 0.5%), and the p24 content was evaluated by ELISA. Data shown in Fig. 2 demonstrate that CD62L-bearing X4 (a) and R5 (b) virions bind more efficiently to HUVECs than viruses lacking host-derived CD62L. Furthermore, there was a time-dependent increase in the level of CD62L-bearing viruses bound to the surface of HUVECs, whereas attachment of virions lacking host-derived CD62L remained almost constant over time. Interestingly, although the capture of the virus stocks tested was lower with primary CD4⁺ T cells compared to HUVECs, there was no difference in the binding efficiency between virions either lacking or bearing CD62L when capture was performed with CD4⁺ T lymphocytes (data not shown). The participation of endothelial cells in HIV-1 transfer has already been reported by Bobardt *et al.* (2003). Given that virus-associated host CD62L leads to a more important attachment of HIV-1 onto HUVECs, we hypothesized that this might also affect the process of HUVEC-mediated virus infection of CD4⁺ T lymphocytes *in trans*. To address this issue, HUVECs were exposed to

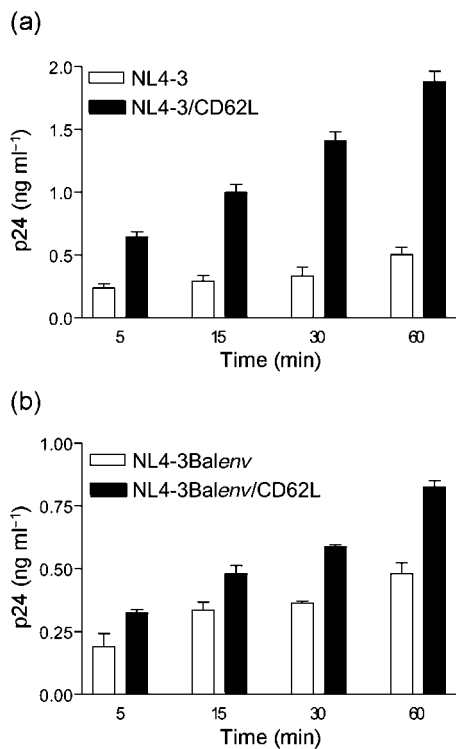


Fig. 2. Attachment of HIV-1 to HUVECs is increased following CD62L incorporation. HUVECs were first exposed to NL4-3 (a) and NL4-3Balenv (b) either lacking or bearing host-derived CD62L for the indicated time periods, washed extensively and subjected to a p24 test. The data shown represent the means \pm SD of triplicate samples and are representative of five independent experiments performed with samples from different donors.

the virus stocks tested, washed extensively and co-cultured for 15, 30 and 60 min with purified CD4⁺ T cells (1×10^5). Next, CD4⁺ T lymphocytes were removed from adherent HUVECs and washed with PBS to eliminate uninternalized virions. Finally, cells were grown for 9 days in complete RPMI medium supplemented with recombinant human interleukin 2. Virus transfer between endothelial cells and CD4⁺ T cells was evaluated by measuring the p24 content in cell-free supernatants harvested at days 3, 6 and 9 after transfer. Data depicted in Fig. 3(a) demonstrate that the HUVEC-mediated transfer to CD4⁺ T lymphocytes is more efficient with CD62L-bearing viruses at the earliest time points tested (i.e. 15 and 30 min) but not at the latest time point tested (i.e. 60 min). This suggests that the insertion of host-derived CD62L within HIV-1 will accelerate the kinetics of virus transfer from HUVECs to more susceptible target cells. We next tested whether part of the observed enhancement of HUVEC-mediated *trans* infection of CD4⁺ T cells could be due to an increased infectivity of CD62L-bearing viruses. We thus evaluated the susceptibility of CD4⁺ T lymphocytes to infection with viruses either lacking or bearing host-derived CD62L. In

brief, activated CD4⁺ T cells (1×10^5) were exposed to similar concentrations of the virus stocks studied (10 ng p24), and at days 3, 6 and 9 post-infection, 100 μ l cell-free supernatant was harvested, replaced with fresh medium, and frozen at -20°C until assayed for p24 content. Although T lymphocytes have been demonstrated to express P-selectin glycoprotein ligand-1 (PSGL-1) (McEver & Cummings, 1997), a ligand of CD62L, CD62L-bearing viruses were not more infectious for CD4⁺ T cells than isogenic virions lacking this host cell-surface component (Fig. 3b). It should be stated that similar observations were made when CD4⁺ T cells were exposed to lower concentrations of the virus stocks tested (i.e. 1, 2 and 5 ng p24) (data not shown).

Trans infection is a phenomenon widely used by different viruses like Ebola and human cytomegalovirus (Alvarez *et al.*, 2002; Halary *et al.*, 2002; Herrmann & Lagrange, 2005), as well as by bacteria (*Mycobacterium tuberculosis*) (Herrmann & Lagrange, 2005) and parasites (*Leishmania*) (Zhao *et al.*, 2005) to increase their infection efficiency. HIV-1 also infects CD4⁺ T cells in a *trans* infection mode via, for example, DCs and this type of infection is much more efficient than the 'classical' way by which free virions come into contact with receptors and co-receptors expressed on permissive target cells. The results displayed in this work indicate that transmission of viruses bearing CD62L is more efficient than transfer of viruses lacking host CD62L when using a co-culture system made of HUVECs and CD4⁺ T lymphocytes. Based on this information, it can thus be postulated that CD62L-bearing virions will more firmly attach to the endothelium surface than viruses lacking this host cell-membrane component. This might translate under *in vivo* conditions to a superior transfer of HIV-1 particles to CD4⁺ T cells, which are naturally rolling onto the surface of endothelial cells during the process of lymphoid homing to lymphoid organs.

Enveloped viruses such as HIV-1 acquire parts of the host cell-membrane and consequently host-cell-derived molecules as integral parts of their mature envelopes while emerging from infected cells (reviewed by Cantin *et al.*, 2005). It has been demonstrated in various studies that these host-derived constituents display multiple effects on the virus life cycle (Cantin *et al.*, 2005). Of high relevance to the present study, it has been shown that some HIV-1-associated host proteins and more particularly cell-adhesion molecules can participate in the attachment step and result in an increased adsorption onto and infection of target cells bearing the appropriate counter-receptors. The metalloproteinase-dependent L-selectin, also called CD62L, can be added to the list of virus-anchored host cell-surface components that can modulate the complex interactions between HIV-1 and the target cell surface. More precisely, we obtain evidence indicating that adsorption of HIV-1 particles to the surface of endothelial cells (i.e. HUVECs) is increased upon incorporation of host CD62L and this leads to a more potent *trans* infection of CD4⁺ T cells.

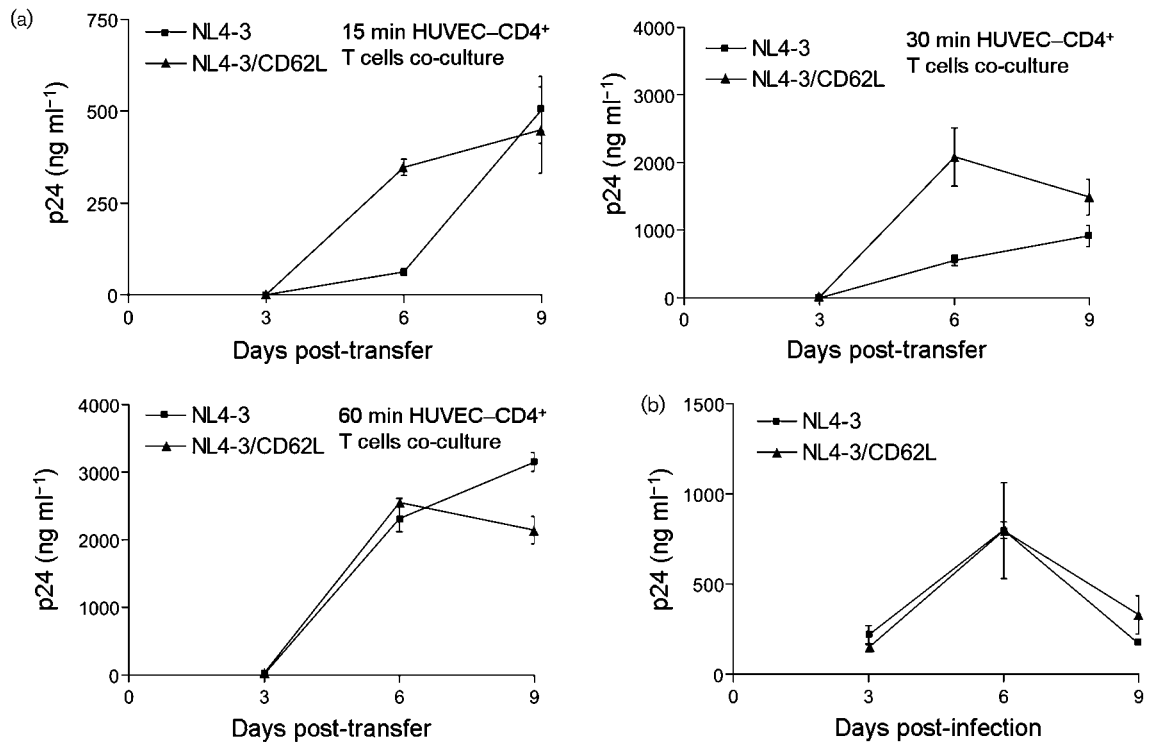


Fig. 3. HUVEC-mediated transfer of HIV-1 to CD4⁺ T cells is promoted when using CD62L-bearing viruses. (a) HUVECs were first exposed to the virus stocks tested, washed and co-cultured for 15, 30 and 60 min with CD4⁺ T cells. Next, CD4⁺ T cells were removed, put in culture and the p24 content was estimated at the indicated time periods. (b) Purified CD4⁺ T cells were exposed to the virus stocks tested and virus production was assessed at the indicated time points. The data shown represent the means \pm SD of triplicate samples and are representative of three independent experiments.

Interestingly, a wide array of functional ligands of CD62L are expressed on HEVs in lymphoid organs where T cells migrate and HIV-1 can thus be constantly in contact with these permissive cells. Moreover, proinflammatory cytokines are known to be secreted at high levels during HIV-1 infection (Decrion *et al.*, 2005). Since some CD62L ligands become functional in the presence of proinflammatory cytokines, a greater number of viruses might consequently bind to the vascular endothelium. So, the well-described inflammatory state seen in HIV-1-infected patients might possibly favour the spread of infection by allowing a better endothelium-mediated *trans* infection of CD4⁺ T cells. We are now trying to identify which of the different ligands expressed on HUVECs can associate with virus-anchored host CD62L. For example, it is known that heparan sulphate chains of syndecans bind to CD62L (Norgard-Sumnicht & Varki, 1995). Another possible counter-receptor is CLA, an essential carbohydrate component of vascular endothelium L-selectin ligands (Tu *et al.*, 1999). However, we cannot exclude the possibility that some other, yet to be defined, CD62L ligands might be responsible for the observed phenomenon.

In conclusion, it can be proposed that the vascular endothelium, which is estimated to cover a surface of at least 600 m², can modulate HIV-1 pathogenesis by

favouring a more efficient virus transfer to susceptible target cells such as CD4⁺ T lymphocytes. However, it is also possible that endothelial cells can remove virus from the peripheral circulation, thereby sequestering it from susceptible target cells. Additional studies are warranted to define the precise contribution of endothelial cells in the progression of HIV-1-associated diseases.

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