

Role of L-selectin CD62L in HIV-1 Attachment to Endothelial Cells and *Trans* infection of CD4+ T Lymphocytes



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ABSTRACT

Background: Cell-to-cell transmission is an efficient way for retroviruses to infect CD4+ T cells. For example, HIV-1 exploits immune cell communications such as that between dendritic cells (DCs) or macrophages and CD4+ T cells to achieve a more efficient viral spread. Endothelial cells also interact with CD4+ T cells during their passage into the blood. However, the involvement of this type of interaction in HIV-1 propagation remains poorly defined. HIV-1 incorporates a variety of host-derived proteins, including the L-selectin CD62L, during its budding process. CD62L participates in the immune response by allowing rolling of leukocytes during the process of transmigration. Interestingly, endothelial cells express various ligands of CD62L. Considering the natural role of L-selectin and its presence on HIV-1 particles, we postulated that CD62L-carrying virions might be more efficiently transferred from endothelial cells to CD4+ T cells than viruses lacking this adhesion molecule.

Methods: Human umbilical vein endothelial cells (HUVECs) and CD4+ T cells were isolated from different healthy donors. Isogenic virions either lacking or bearing host CD62L were generated by the calcium phosphate co-precipitation method in 293T cells. The extent of virus binding was assessed by measuring the p24 content, whereas virus transfer between endothelial and CD4+ T cells was evaluated by measuring p24 in cell-free supernatants at various days after the co-culture.

Results: We found that viruses bearing CD62L bind with more efficiency to HUVECs than viruses lacking this host protein. Moreover, infection *in trans* was also increased when using CD62L-bearing viruses. Interestingly, the impact of CD62L on both viral binding and transfer was abolished upon treatment of virions with a blocking antibody.

Conclusion: Altogether, these results suggest that CD62L enhances attachment of HIV-1 particles to endothelial cells, a process resulting in a more efficient HIV-1 transfer toward CD4+ T lymphocytes. Additional work is needed to shed light on the CD62 ligand(s) responsible for the observed phenomenon and also to define whether other step(s) in the virus life cycle can be affected upon acquisition of CD62L. These observations confirm that host-derived proteins located on the exterior of HIV-1 can modulate the biology of this retrovirus.

HYPOTHESIS

HIV-1 incorporates many host-derived proteins (e.g. ICAM-1, LFA-1 and HLA-DR), including the L-selectin CD62L, during its budding process. Endothelial cells express various ligands of CD62L such as E-selectin, MadCAM-1, PCLP and sgp200. The majority of these ligands are expressed on HEV (high endothelial venules) in secondary lymphoid organs, where naive T cells diapedesis take places. Considering the role of L-selectin and its presence onto HIV-1 particles, we postulate that CD62L, when present on the virion may initiate viral binding on endothelial cells and facilitate HIV-1 transmission to CD4+ T lymphocytes.

RESULTS

Infectious NL4-3-based virus co-transfected with CD62L plasmid in 293T cells bearing CD62L

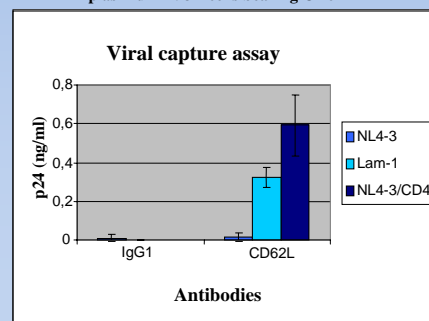


Figure 1. Fully infectious NL4-3-based viruses were generated by calcium phosphate cotransfection in 293T cells. These viruses were devoid of CD62L while Lam-1 viruses expressed this protein. Viruses produced in CD4+ T cells were used as positive control.

Both NL4-3 and Lam-1 viruses had the same infectivity in the LuSIV cell line

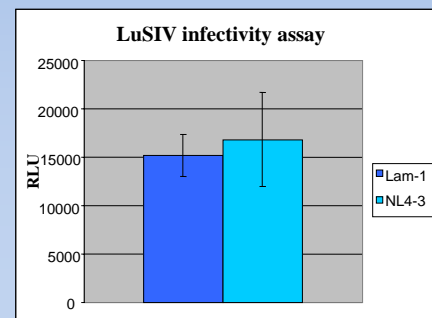


Figure 2. NL4-3 and Lam-1 viruses were used to infect LuSIV cells. After 48 hours, cells were lysed and luciferase assay was performed

Viruses bearing CD62L were transferred more rapidly to CD4+ T cells than virus devoid of this selectin

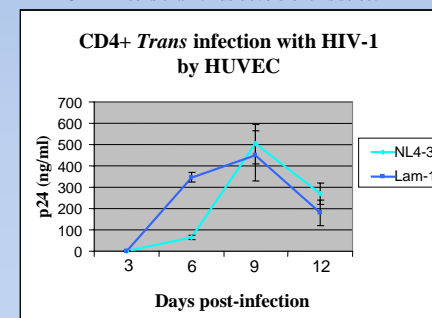


Figure 5. After binding assays, HUVECs were washed twice and incubated 5 minutes with CD4+ T lymphocytes. T cells were then washed and grown for 9 days. HIV-1 transfer between endothelial cells and lymphocytes was evaluated by measuring the p24 content in supernatant harvested 3, 6 and 9 days after transfer.

NL4-3 and Lam-1 viruses had the same infectivity in the Jurkat cell line

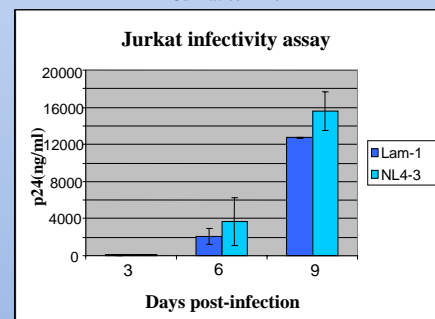


Figure 3. Both viruses were used to infect Jurkat cells. After 3, 6 and 9 days post-infection, supernatant was harvested and p24 ELISA was performed to quantify the virus

Viruses bearing CD62L (Lam-1) bind more easily on HUVECs than viruses lacking this protein (NL4-3)

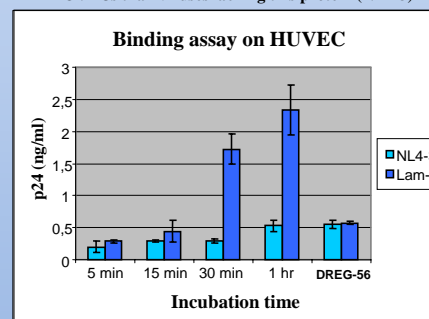


Figure 4. Viruses bearing or lacking CD62L were used to perform a binding assay on HUVECs. Viruses were incubated for the indicated time periods with HUVECs, and the antibody DREG-56 was used to block the interaction between CD62L and its ligands on HUVECs. Virus binding assays were measured by p24 ELISA after cell lysis.

CONCLUSION

Taken together, these results suggest that CD62L play an important role in the attachment of HIV-1 particles on HUVEC, a phenomenon linked to a better HIV-1 transfer toward CD4+ T lymphocytes. Additional work is needed to shed light on the CD62L ligand(s) responsible for the observed phenomenon and also to define whether other step(s) in the virus life cycle (entry, infection) can be affected by the presence of CD62L. Further binding assays will be performed with other endothelial cells like human BMVECs (brain microvascular endothelial cells) which express more ligands of CD62L than HUVECs.

Finally, these observations confirm that host-derived proteins located on the exterior of HIV-1 can modulate the biology of this retrovirus.

ACKNOWLEDGMENTS

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