Host Gene SLC35A2 Restricts CXCR4-Tropic HIV-1 Replication at the Stage of Viral Fusion

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BACKGROUND

Despite the diversity of HIV-1 variants in a chronically infected individual, during transmission only a few variants seed new infections. These founder viruses predominantly utilize the CCR5 co-receptor to facilitate viral entry into CD4+ T cells over the CXCR4 co-receptor. The host factors that contribute to this transmission bottleneck are not well understood. We have recently identified a host gene involved in glycosylation, SLC35A2, that restricts CXCR4-tropic (X4) viruses and enhances CCR5-tropic (R5) virus replication, which recapitulates the selection pattern observed in natural transmission.

SLC35A2 has opposing effects on viral fusion and infection of R5- vs X4-tropic viruses in primary CD4+ T cells that mirror selection observed during transmission, suggesting this host gene could have a critical role in driving the HIV-1 transmission bottleneck.

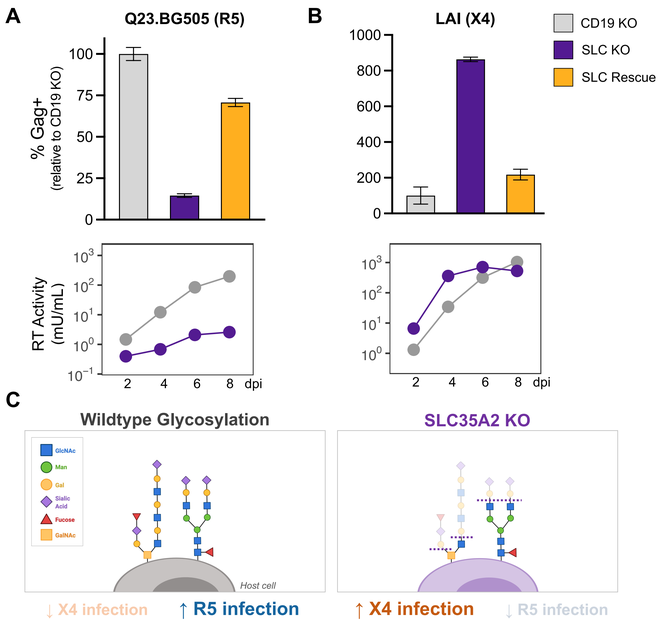
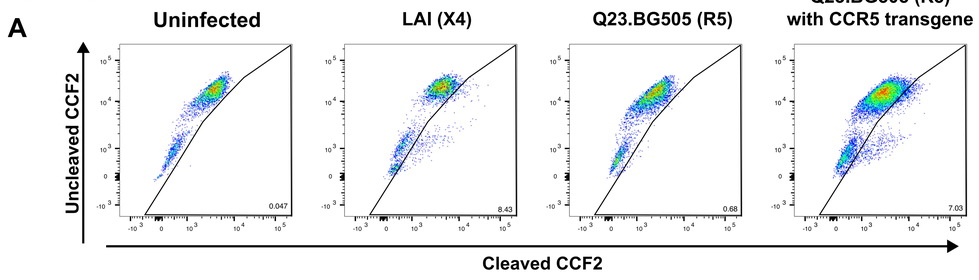


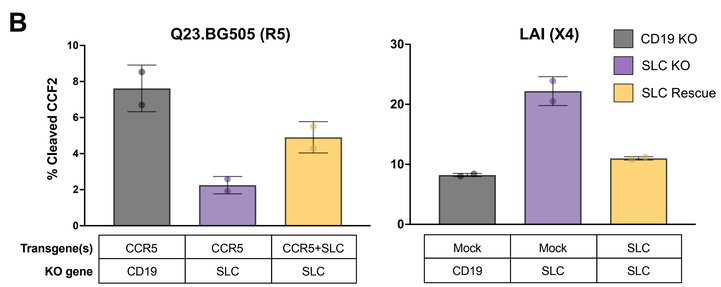
Figure 1. Effect of SLC35A2 knockout (SLC KO) in primary CD4+ T cells on HIV-1 infection. Infection of CD4+ T cells with R5 virus Q23.BG505 (A) or X4 virus LAI (B) demonstrates opposing effects of SLC KO on infection. These effects are rescued with SLC35A2 complementation. Percent Gag+ cells 3dpi is shown in the top graphs and RT activity over time post infection is shown below. (C) Working model of the tropic-specific effect of SLC35A2 KO on HIV-1 infection due to dysfunctional glycosylation in the SLC KO cells. Modified from Itell, et al., Nat Microbiol. 2024 Nov.

METHODS

SLC35A2 was inactivated by CRISPR-Cas9 in primary CD4+ T cells (SLC knockout, KO) and several early steps of viral replication were evaluated: (1) virus-cell fusion by the BlaM-Vpr fusion assay (Cavrois, et al., 2014); (2) binding of HIV-1 Gag-iGFP to cells; (3) production of viral reverse transcription (RT) intermediates measured by qPCR (Mbisa, et al., 2009). Unpaired t tests were used to determine significance of effects in SLC KO compared to control cells and p-values are shown.

RESULTS





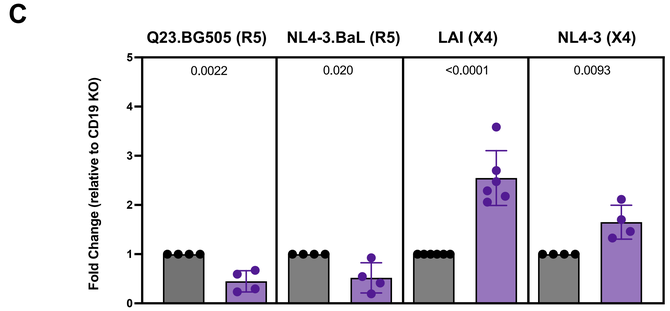


Figure 2. SLC53A2 KO in primary CD4+ T cells reduces viral fusion of R5 viruses and increases fusion of X4 viruses. (A) Gating strategy for detecting viral fusion with the BlaM-Vpr assay. To increase detection of fusion with R5 viruses, CCR5 was expressed as a transgene. (B) Fusion of Q23.BG505 (R5) and LAI (X4) BlaM-Vpr viruses, measured by detection of cleaved CCF2 substrate, in CD4+ T cells from a representative donor with technical duplicates. (C) Aggregate fusion data from four viruses (R5: Q32.BG505, NL4-3.BaL and X4: LAI, NL4-3). The assay was performed on CD4+ T cells from at least four unique donors across two to three independent experiments.

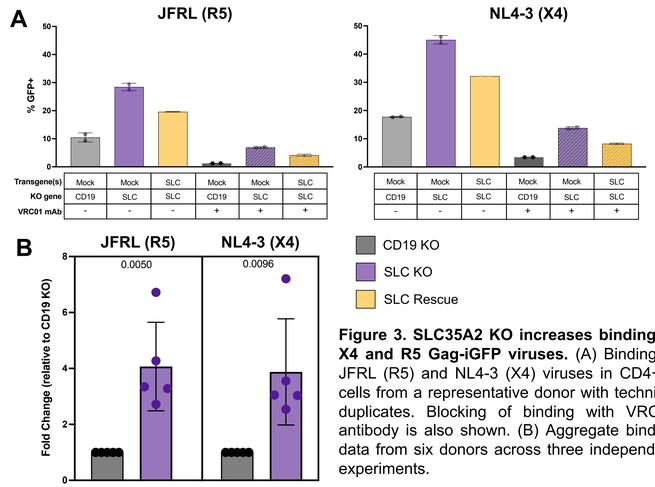


Figure 3. SLC35A2 KO increases binding of X4 and R5 Gag-iGFP viruses. (A) Binding of JFRL (R5) and NL4-3 (X4) viruses in CD4+ T cells from a representative donor with technical duplicates. Blocking of binding with VRC01 antibody is also shown. (B) Aggregate binding data from six donors across three independent experiments.

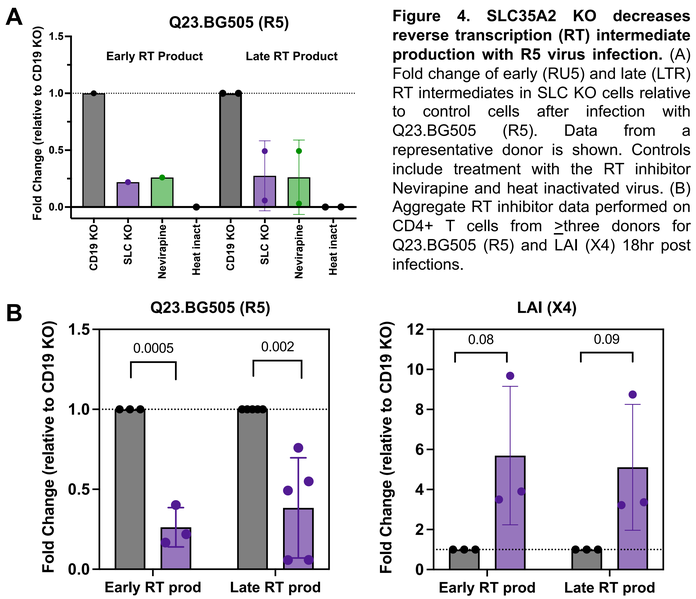


Figure 4. SLC35A2 KO decreases reverse transcription (RT) intermediate production with R5 virus infection. (A) Fold change of early (RU5) and late (LTR) RT intermediates in SLC KO cells relative to control cells after infection with Q23.BG505 (R5). Data from a representative donor is shown. Controls include treatment with the RT inhibitor Nevirapine and heat inactivated virus. (B) Aggregate RT inhibitor data performed on CD4+ T cells from ≥three donors for Q23.BG505 (R5) and LAI (X4) 18hr post infections.

CONCLUSIONS

- SLC35A2 reduces binding of both X4- *and* R5-tropic HIV-1.
- SLC35A2 has opposing effects on viral fusion that are consistent with its effects on infection: it reduces X4 viral fusion and enriches R5 viral fusion.
- Similarly, when SLC35A2 is expressed, the production of R5 viral RT intermediates is increased and X4 RT intermediates are reduced.
- These results suggest SLC35A2 is an X4-specific restriction factor that inhibits viral fusion and conversely enhances R5 fusion. Given SLC35A2 impacts glycan structure, we hypothesize its effects are through changes in glycosylation of the co-receptors.

References

Itell, et al., Nat Microbiol. 2024.
Cavrois, et al., Bio Protoc. 2014.
Mbisa, et al., Methods Mol Bio. 2009.

Acknowledgements

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