Selective Cell-Free or Cell-to-Cell HIV-1 Infection by gp41 Cytoplasmic Tail Mutants

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Abstract

The gp41 transmembrane subunit of the HIV-1 envelope (Env) has a cytoplasmic tail (CT) ~150 amino acids (aa) long. The CT has several functions during the viral life cycle, including the i) endocytosis of cell-surface Env ii) packaging Env into viral particles and iii) controlling the fusogenic potential of Env. Full truncation of the gp41 CT (ΔCT144) does not directly impair viral fusion and can generate infectious virus in a cell-type dependent manner. Smaller deletions result in non-infectious virus particles, although Env is still expressed and packaged. Only the full truncation, ΔCT144, has been tested in the context of cell-to-cell infection. We therefore set out to systematically examine which domains of the CT are required for cell-to-cell infectivity in comparison to those needed for cell-free infection.

Infectivity Assays

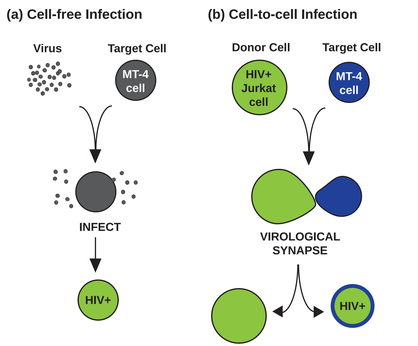


Figure 3. Flow cytometry based assays used to measure HIV-1 infection. (a) In the cell-free infectivity assay, MT-4 target cells are incubated with NL-GI virus particles to allow infection. (b) In the cell-to-cell infectivity assay, Jurkat cells are nucleofected with NL-GI proviral DNA 24 h prior to the assay. Donor Jurkat cells and MT-4 target cells are labeled with Cell Proliferation dyes and co-cultured to allow infection. In single-round infection assays, cell culture media is replaced with media containing 10 μM AZT 18 h after co-culture. 40 h after initial infection, GFP expression levels are detected by flow cytometry, indicating HIV-1 infection.

HIV-1 gp41 CT Mutants

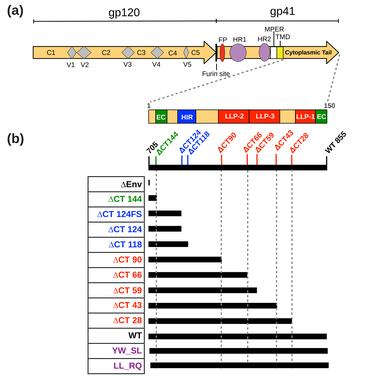


Figure 1. Domains in the gp41 cytoplasmic tail (CT). (a) Schematic of the HIV-1 Env protein showing major structural and functional domains in gp120 and gp41. EC, endocytosis signal; HIR, highly immunogenic region; LLP, lentiviral lytic peptide. Adapted from Checkly et al., 2011. (b) gp41 CT mutants constructed by overlap PCR. Adapted from Jiang & Aiken, 2007.

HIV-1 Envelope Expression

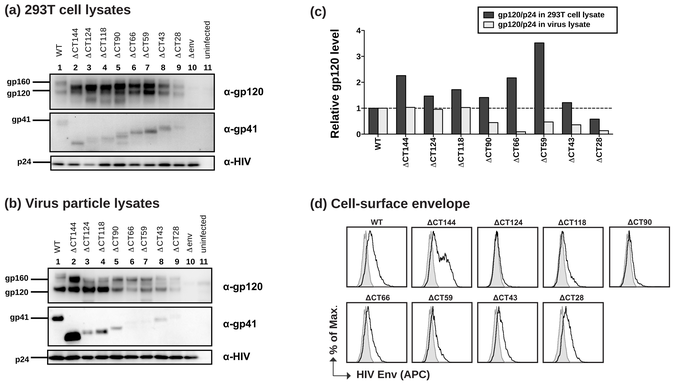


Figure 2. HIV-1 envelope expression by gp41 CT truncation mutants. (a & b) Western blot of (a) 293T cell lysates or (b) virus particle lysates of WT NL-GI or gp41 CT mutants. (c) Quantification of gp120 levels expressed in 293T lysates and virus particle lysates. Env expression was calculated as a ratio of gp120/p24 and expressed relative to WT. (d) Cell surface HIV-1 Env on Jurkat cells expressing WT NL-GI or gp41 CT mutants (black line) compared to Δenv (filled grey). Env levels were detected on cells expressing high levels of HIV-1 infection with pooled polyclonal HIV-IG antibody.

LLP-3 Point Mutants

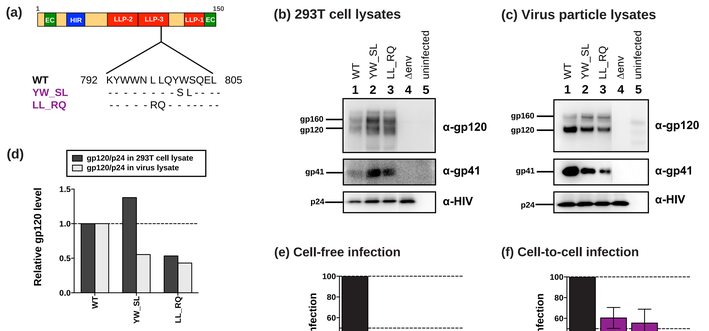


Figure 6. HIV-1 envelope expression and single round infectivity of gp41 LLP-3 mutants YW_SL and LL_RQ in MT-4 cells. (a) Amino acid sequence alignment of gp41 LLP-3 mutants in relation to the full-length, wild type (WT) NL4-3 gp41 CT. Amino acid identity (-) and substitutions are shown. (b & c) Western blot of (b) 293T cell lysates or (c) virus particle lysates of WT NL-GI or gp41 LLP-3 mutants. (d) Quantification of gp120 levels expressed in 293T lysates and virus particle lysates. (e & f) Infection levels of MT-4 cells after (e) cell-free or (f) cell-to-cell infection by gp41 LLP-3 mutants, expressed as a percentage of WT NL-GI. Error bars represent the SEMs from the means of at least three independent experiments.

Single & multi-round infectivity assays: Cell-free vs. Cell-associated inocula

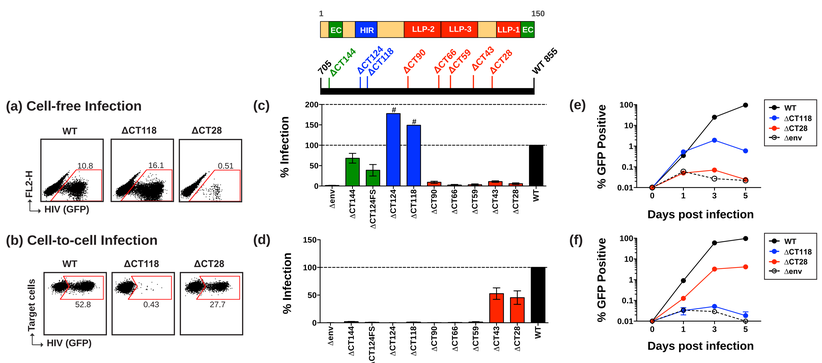


Figure 4. Infectivity of gp41 CT truncation mutants in MT-4 cells. (a & b) Representative flow cytometry plots of (a) cell-free or (b) cell-to-cell infection of MT-4 cells by WT, ΔCT118 or ΔCT28 NL-GI. (c & d) Single round infection levels after (c) cell-free or (d) cell-to-cell infection, expressed as a percentage of WT NL-GI. Error bars represent the SEMs from the means of at least three experiments. # indicates a single experiment performed in duplicate. (e & f) Multi-round infection of MT-4 cells initiated at Day 0 with (e) cell-free or (f) cell-associated WT, ΔCT118, ΔCT28 or Δenv NL-GI.

Cell-to-cell Transfer and Fusion assays

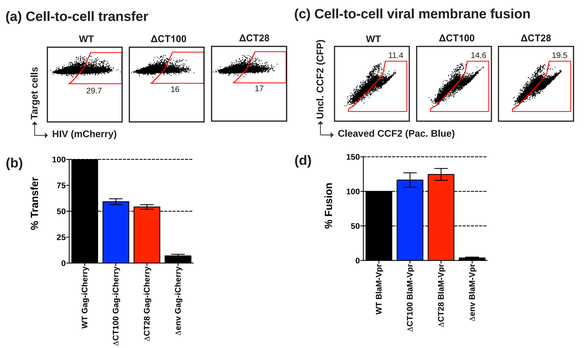


Figure 5. Cell-to-cell transfer and cell-to-cell viral membrane fusion of gp41 CT mutants into primary CD4+ T cells. (a) Representative flow cytometry plots and (b) cell-to-cell transfer levels of WT, ΔCT100, ΔCT28 or Δenv Gag-iCherry into primary CD4+ T cells. (c) Representative flow cytometry plots and (d) cell-to-cell viral membrane fusion levels of WT, ΔCT100, ΔCT28 or Δenv NL4-3 and BlaM-Vpr into primary CD4+ T cells. Error bars represent the SEMs from the means of two experiments performed in duplicate using CD4+ T cells from three blood donors.

Conclusions

- Several gp41 CT mutants have striking selective deficiencies in cell-free but not cell-to-cell infectivity, or vice versa.
- Functional properties of Env required for cell-free and cell-to-cell infection are genetically distinct.
- Infectivity differences may reflect a different intrinsic regulatory influence of the CT on cell-associated vs. particle-associated Env, or differential interaction with host or viral proteins.

Future studies will:

- Define the block in cell-to-cell infection of large truncation muants.
- Use these mutants to determine the contributions of one mode of infection in isolation.

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