New HIV-1 Cell-Cell Transmission Assay Identifies Cell Type Dependency of PG9 And P16 Neutralization

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BACKGROUND

HIV-1 efficiently replicates in vivo by direct transmission from infected to uninfected CD4+ T cells, which is highly resistant to broadly neutralizing antibodies (bnAbs). But an accurate and sensitive measurement of HIV-1 transmission between cells remains challenging. Here we developed new ultrasensitive HIV-1 cell-to-cell transmission based on replication-activated nanoluciferase gene and evaluated infectivity and neutralizing activity of bnAbs in primary and immortalized T cells.

METHODS

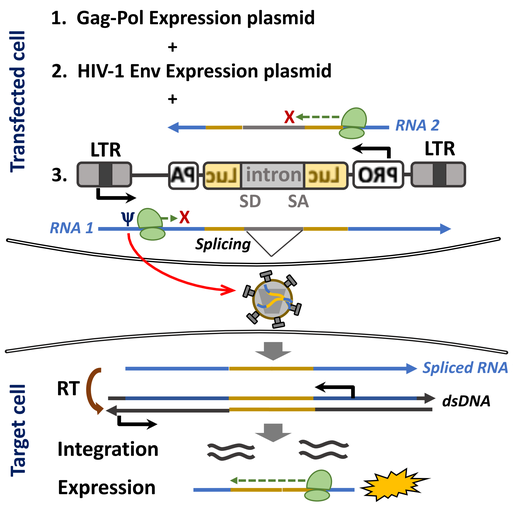


Figure 1. A concept of intron-regulated reporter vectors. The reversion of expression cassette and interruption with intron blocks the translation of reporter protein from RNA 1 and RNA2 in producer cells. Reverse transcription in target cells flips the cassette and activate reporter expression

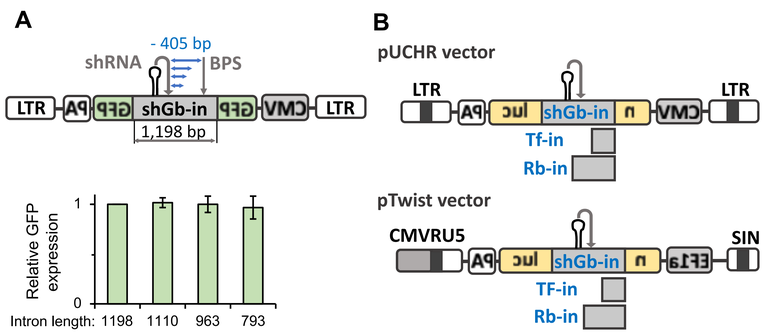


Figure 2. A. Shortening of inactivating intron. **B.** Generation of lentiviral in-Nluc reporter vectors with three different introns from ***γ***-globin with anti-intronic shRNA (**shGb**), mouse TNF-β (**Tf**), and ribozyme (**Rb**)

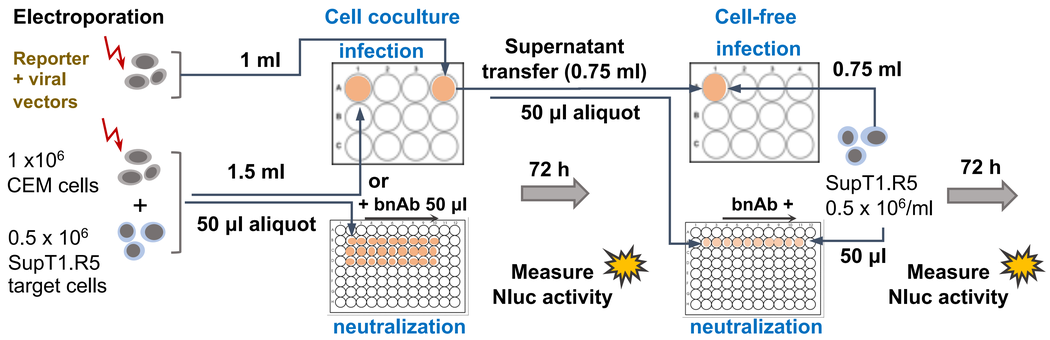


Figure 3. Experimental pipeline for measuring both HIV-1 cell-to-cell transmission and free virus infection between T cells

RESULTS

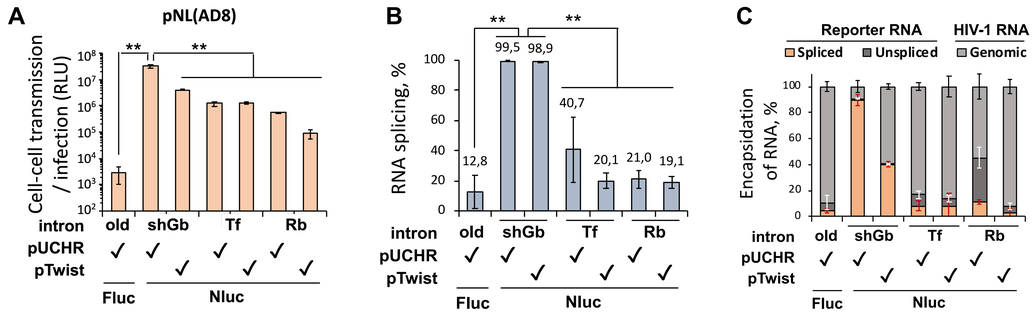


Figure 4. pUCHR-in-Nluc vector with shGb intron displays the best sensitivity to detect NL4(AD8) transmission (**A**), the highest level of splicing of the reporter RNA encapsidated into pseudoviral particles (**B**) and NL4(AD8) virions (**C**).

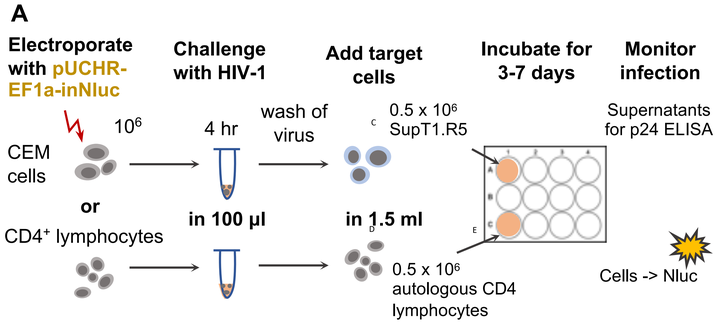


Figure 5. A. Flowchart of experiment for detection of HIV-1 replication using in-Nluc vector transfection followed by virus infection. HIV-1 spreading was monitored in parallel by Nluc expression (**B**) or Gag release in supernatant (**C**).

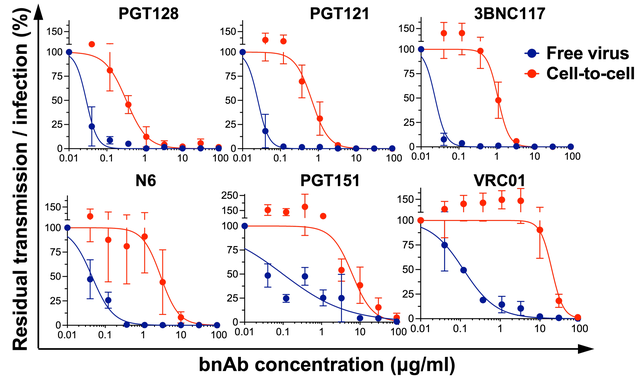


Figure 6. Neutralization of HIV-1 AD8 Env in cell-to-cell and free virus infection. Cell-to-cell transmission was at least 10-fold less sensitive to all tested bnAbs than free virus infection.

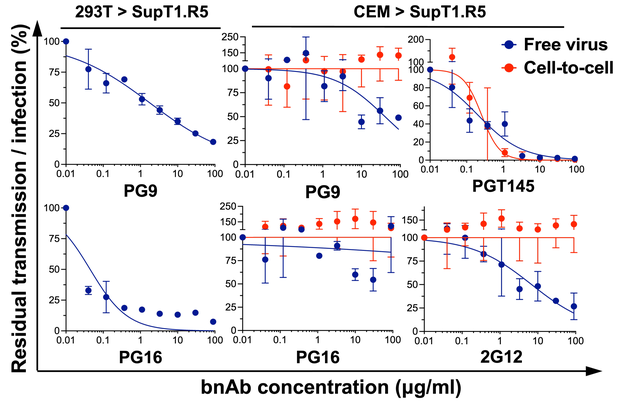


Figure 7. Neutralization of HIV-1 AD8 Env with V1/V2-targeting bnAbs. HIV-1 is sensitive to PG9 and PG16 bnAbs when generated in 293T cells but resistant if produced in CEM T cells.

CONCLUSIONS

- the new ultrasensitive assay can measure HIV-1 cell-cell transmission in T cells and accurately detect bnAb neutralization efficiency
- the type of transmission and cell origin contribute to HIV-1 sensitivity to bnAbs

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REFERENCE Dmitriy Mazurov et al. Ultrasensitive quantification of HIV-1 cell-to-cell transmission in primary human CD4+ T cells measures viral sensitivity to broadly neutralizing antibodies. mBio, 2023 Dec 8:e0242823. doi: 10.1128/mbio.02428-23. *Figures 1-6 were adapted from this paper.*