SUPPLEMENTAL MATERIAL

MATERIALS AND METHODS

Cell culture. TZM-bl cells, obtained through the NIH AIDS Reagent Program (NIH-ARP, catalog number 8129), and HEK293T cell line [American Type Culture Collection (ATCC), CRL-3216] were maintained in DMEM with 10% FBS as described (1). Hut/CCR5 cell line was maintained in RPMI1640 with 10% FBS (2). Hut/CCR5 cell line is derived from Hut78 $CD4^+$ T-cell line with high level CCR5 expression, supporting HIV-1 infection and replication (2). All cell lines used in this study were maintained at 37°C, 5% CO_2 and tested negative for mycoplasma contamination using a universal mycoplasma detection kit (ATCC 30-1012K).

HIV-1 production. HIV-1 containing S534P mutation (M1, M3 and M4) are not able to initiate virus-cell fusion (1). To achieve single-cycle infection, WT Env or VSV-G was used to pseudotype these viruses for efficient cell entry. To generate M1/Env, M3/Env and M4/Env viruses, HEK293T cells were co-transfected with M1, M3 or M4 proviral DNA plasmids and pIIIenv, a plasmid expressing WT Env of HIV-1_{NL4-3}, using polyethylenimine as described (1). For HIV-1 production of M1/VSV-G, M3/VSV-G and M4/VSV-G, HEK293T cells were co-transfected with the mutant proviral DNA plasmids and pMD2.G that expresses VSV-G protein. pMD2.G was a gift from Didier Trono (Addgene plasmid number 12259). Culture medium was changed at 6 h post-transfection, and cell supernatants were collected at 48 h post-transfection as described (1).

HIV-1 infection and passage in cells. Hut/CCR5 cells were infected with WT replication-competent HIV-1_{NL4-3}, single-cycle WT Env- or VSV-G-pseudotyped mutant viruses as described

(1, 3). At 2 h post-infection, cells were washed 3 times with phosphate buffered saline (PBS) to remove extra-cellular free viruses. Fresh media were added every 2-3 days. For cells infected with WT or Env-pseudotyped mutant viruses, cell supernatants were collected at 7 dpi. For cells infected with VSV-G-pseudotyped viruses, since syncytia appear later than WT HIV-1, culture media were collected at 13 dpi. Fresh Hut/CCR5 cells were added to the culture at 11 dpi to facilitate virus recovery.

HIV-1 p24 quantification and kinetics of HIV-1 infection in Hut/CCR5 cells. HIV-1 p24 levels in replication-competent viral stocks were quantified by an enzyme-linked immunosorbent assay using anti-p24-coated plates (AIDS and Cancer Virus Program, NCI-Frederick, MD) as described (4). HIV-1_{NL4-3} PR-mutant M1, M3, or M4 pseudotyped either with WT Env or VSV-G were used to infect 4×10^5 Hut/CCR5 cells with equal amounts of viruses (400 ng p24) for 2 h. After infection cells were washed three times with PBS and then cultured in complete medium for the indicated times. WT HIV-1_{NL4-3} was used as a positive control in the infection assay. The supernatants containing HIV-1 were collected every two days from 3 to 15 dpi for measuring viral infectivity. To maintain the viability of WT HIV-1_{NL4-3} infected cells, 1×10^6 fresh Hut/CCR5 cells were also supplied to the infected cells. To quantify viral infectivity, viruses equivalent to 0.2 ng p24 were used to infect 5×10^4 TZM-bl cells in 24-well plates for 2 h and then washed out. The infected TZM-bl cells were cultured for 2 days and lysed for the luciferase assay as described (1). Cellular protein concentrations were quantified using a bicinchoninic acid assay (Pierce) and all luciferase results were normalized based on total protein input.

Cloning and sequencing of HIV-1 *env* gene. The HIV-1 PR mutants (M1, M3 and M4) have been reported in our recent study (1). To amplify the *env* gene of infectious viruses recovered from WT Env- or VSV-G-pseudotyped mutant viruses, Hut/CCR5 cells were infected with the recovered viruses for 3 days, the cells were collected and cellular DNAs were extracted with Blood & Tissue Kit (QIAGEN, catalog number 69504). After PCR amplification using high-fidelity DNA polymerase (Invitrogen, catalog number 12532-016), the full-length *env* cDNA were inserted into a pRK vector as described (5). After transformation into Stbl2 competent cells, colonies were randomly picked and cultured for plasmid DNA extraction. After digestion with *Sal*I and *Not*I endonucleases (New England BioLabs) to confirm the *env* cDNA insertion, 6 clones from each recovered virus were selected for Sanger DNA sequencing.

SUPPLEMENTAL REFERENCES

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