ORIGINAL

Amino acid alterations in Gag that confer the ability to grow in simian cells on HIV-1 are located at a narrow CA region

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Abstract : We previously generated a prototype monkey-tropic human immunodeficiency virus type 1 (HIV-1) designated NL-DT5R. This viral clone has a small region of simian immunodeficiency virus (SIV) within Gag capsid (CA) protein and also SIV Vif protein, but displays a poor growth phenotype in simian cells. To improve the growth potential of NL-DT5R, we have constructed a series of its *gag* variant viruses. Out of fourteen viral clones generated, five were infectious for simian HSC-F cells, and two of the infectious variants grew similarly with NL-DT5R. Taking their genome structures into consideration, our data here clearly show that a narrow CA region within the Gag protein, i.e., the domain around cyclophilin A (CypA)-binding loop, is critical for the growth ability of HIV-1 in simian cells. J. Med. Invest. 56 : 21-25, February, 2009

Keywords **:** *HIV-1, Gag, CA, CypA, TRIM5α*

INTRODUCTION

The narrow host range of human immunodeficiency virus type 1 (HIV-1) has been a major obstacle for establishing the animal model system for studies of viral replication and pathogenesis *in vivo* (1). HIV-1 infects and causes disease only in humans. To conquer this difficulty, we have recently generated a monkey-tropic HIV-1 designated NL-DT5R (1-3). Its genome contains a *gag* sequence encoding simian immunodeficiency virus from rhesus monkeys (SIVmac) capsid (CA) element, corresponding to the HIV-1 cyclophilin A (CypA)-binding loop, and the entire SIVmac *vif* gene (2). However, by subsequent studies, NL-DT5R was found to grow in simian cells more poorly both *in vitro* and *in vivo* than a standard SIVmac designated SIVmac 239, which induces the AIDS in monkeys and is widely used for model studies of HIV-1/AIDS. As a result of its biological property, NL-DT5R was unable to induce AIDS in the animals (3). Extensive attempts to improve the growth ability of NL-DT5R in simian cells through modification of Gag-CypA region and Vif by recombinant DNA techniques have been so far unsuccessful (4, 5). Another research group has also reported that a monkey cell-tropic HIV-1 designated stHIV-1 is successfully generated by genetic manipulation and virus adaptation in cells (6). It was suggested that three amino acid mutations in *gag* might be important for the ability of HIV-1 to grow in simian cells (6).

In this study, to potentiate growth capability in simian cells of a prototype monkey-tropic HIV-1 clone, various SIVmac sequences and mutations were introduced into the *gag* of NL-DT5R, and the resultant recombinants/mutants were monitored for

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their growth properties in simian HSC-F cells. We show here clearly that any viruses without the SIVmac CypA region do not grow in HSC-F cells, and demonstrate the importance of CypA region for the species tropism of HIV-1.

MATERIALS AND METHODS

Cells

A human monolayer cell line 293T (7) was maintained in Eagles's minimal essential medium containing 10% heat-inactivated fetal bovine serum. A simian lymphocytic cell line HSC-F (8) was maintained in RPMI-1640 medium containing 10% heat-inactivated fetal bovine serum.

Transfection

Sub-confluent 293T cells in 90 mm dishes were transfected with 20 μg of proviral clones by the calcium-phosphate co-precipitation method, and on day 2 post-transfection, cell-free virus samples for infection experiments were prepared as previously described (9).

Infection

HSC-F cells ($1\text{-}10\times10^6$) were infected with an equal amount of viruses [$1\text{-}10\times10^6$ reverse transcriptase (RT) units] prepared from transfected 293T cells, and monitored for RT production at intervals as previously described (9).

RT assay

Virus production in transfected 293T cells and viral growth property in infected HSC-F cells were determined by monitoring RT activity of the culture supernatants. RT assay using ^{32}P-dTTP has been previously described (10).

DNA constructs

Full-length infectious molecular clones of HIV-1 and SIVmac, designated pNL4-3 (9) and pMA239 (11), respectively, have been previously described. A monkey-tropic infectious DNA clone of HIV-1 designated NL-DT5R has been previously described (2). An infectious clone designated NL-DT5R/4-3 is a derivative of NL-DT5R carrying CA of NL4-3, and grows more poorly than NL-DT5R in simian HSC-F cells (4). Various *gag* recombinants and mutants of NL-DT5R were constructed by the QuikChange site-directed mutagenesisi kit (Stratagene, La Jolla, CA, USA) as previously described (12).

RESULTS AND DISCUSSION

Our previous results have indicated that modifications in *gag* (encoding CypA-binding loop) and *vif* genes of NL-DT5R do not improve its growth ability in simian cells (4, 5). We, therefore, constructed new *gag* recombinants between NL-DT5R and MA239 of SIVmac to obtain HIV-1 type viruses which would be more replication-competent than NL-DT5R in simian cells. We previously reported that recombinants between HIV-1 NL4-3 and SIVmac MA239 within five regions of CA-p2 are viable and infectious for human M8166 cells (12). Based on these results, recombinants in this study were carefully designed as shown in Fig. 1 and Table 1.

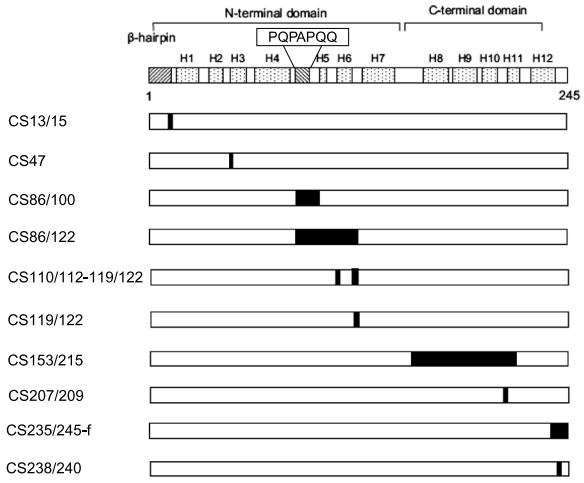

Figure 1 Structure of Gag CA-p2 region of various recombinant clones derived from a monkey-tropic HIV-1 NL-DT5R. Sequences of SIVmac239 are indicated by black areas (for details, see Table 1). Structural domains of HIV-1 Gag CA-p2 (19, 20) are shown at the top. Amino acid sequence PQPAPQQ represents the region of SIVmac239 corresponding to the CypA-binding loop of HIV-1 CA (PVHAGPIAP). H, α-helix.

To examine growth potentials in simian cells of new viral clones (10 clones in Fig. 1 and Table 1), they were transfected into 293T cells, and cell-free virus samples were prepared on day 2 post-transfection. Viruses obtained were then inoculated into HSC-F cells, and viral growth was monitored by RT assay. Representative results are shown in Fig. 2 and all the data are summarized in Table 1. As shown in Fig. 2, NL-DT5R readily established a spreading infection albeit grew more poorly than SIVmac239. The peak infection day for SIVmac239 and NL-DT 5R were 6 and 18, respectively. In contrast to NL-DT5R, eight of ten recombinants (Fig. 1 and Table 1) were not infectious at all for HSC-F cells (Fig. 2

Table 1. Amino acid sequences of SIVmac inserted into CA-p2 region of NL-DT5R, a derivative of HIV-1 NL4-3

Recombinants	Amino acid sequences of MA239 inserted[1]	Growth in HSC-F cells
CS13/15	(13) PLS (15)	(-)
CS47	(47) T (47)	(+)
CS86/100	(86) PQPAPQQGQLREPSGSD (100)	(+)
CS86/122	(86) PQPAPQQGQLREPSGSDIAGTTSSVDEQIQWMYRQQNPI (122)	(-)
CS110/112-119/122	(110) EQI (112)	(-)
	(119) YRQQN (122)	
CS119/122	(119) YRQQN (122)	(-)
CS153/215	(153) QGPKEPFQSYVDRFYKSLRAEQTDAA	(-)
	VKNWMTQTLLIQNANPDCKLVLKGLGVNPTL	
	EEMLTA (215)	
CS207/209	(207) PTL (209)	(-)
CS235/245-f	(235) LKEALAPVPIPFAA (245)	(-)
CS238/240	(238) APV (240)	(-)

[1]The first and last amino acid numbers of HIV-1 NL4-3 CA-p2 replaced with sequences of SIVmac MA239 are indicated in parentheses. GenBank accession numbers for pNL4-3 and pMA239 are AF324493 and M33262, respectively.

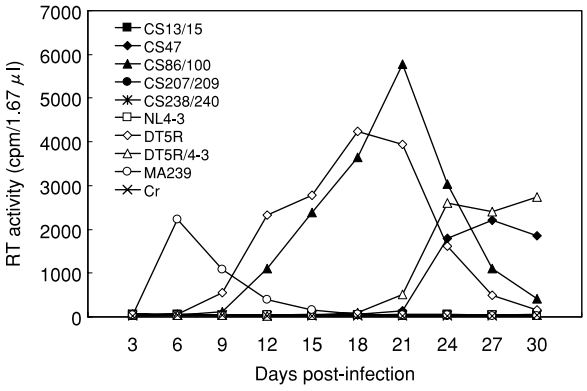

Figure 2 Growth kinetics in HSC-F cells of Gag CA-p2 recombinants. Input cell-free viral samples were prepared from 293T cells transfected with the clones indicated, and an equivalent RT units were inoculated into HSC-F cells. Viral growth was monitored at intervals by RT activity in the culture supernatants. As a negative control (Cr), pUC19 was used.

and Table 1). Even the two infectious recombinants (CS47 and CS86/100) did not grow better than NL-DT5R. In particular, CS47, which actually is a point mutant of NL-DT5R, grew much more poorly than NL-DT5R similarly with DT5R/4-3 carrying the HIV-1 type CA (4).

Recently, Hatziioannou, *et al.* reported that their monkey cell-tropic HIV-1 designated stHIV-1 differs from the parental HIV-1 clone in only minor ways other than the genetically engineered CA and Vif substitutions (6). They found three coding mutations in *gag* (K110I, A209V and P371L) and six silent mutations in *gag* and *pol* (6). Based on this report, we introduced the three nonsynonymous mutations into NL-DT5R to have viral clones that would grow better than NL-DT5R in simian cells (Fig. 3A).

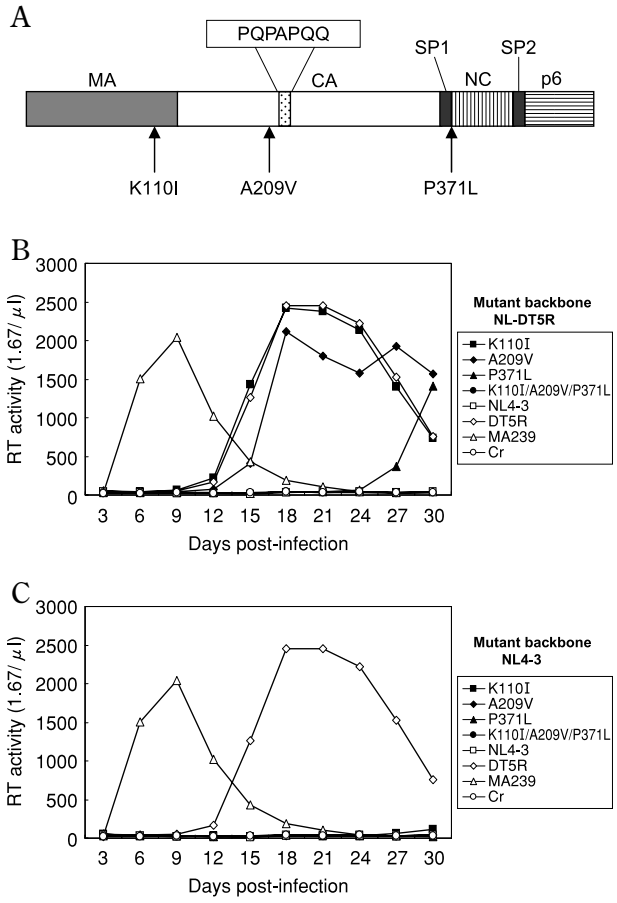

Figure 3 Growth kinetics in HSC-F cells of Gag mutants. (A) Locations of mutations in Gag. Mutations at the indicated positions are introduced into NL-DT5R and NL4-3 (mutants in panels B and C, respectively). Amino acid sequence PQPAPQQ represents the region of SIVmac239 corresponding to the CypA-binding loop of HIV-1 CA (PVHAGPIAP). MA, Gag matrix protein ; NC, Gag nucleocapsid protein ; SP1 and SP2, Gag spacer proteins 1 (p2) and 2 (p1), respectively. (B) and (C) Growth kinetics of various mutants clones in HSC-F cells were determined as described in the legend to Fig. 2. Results obtained in one experiment are shown separately in (B) and (C) for clarity.

Proviral clones containing each and all the three mutations were constructed for this purpose. Cell-free virus samples derived from these clones were prepared as described above, and inoculated into HSC-F cells. As shown in Fig. 3B, while control viruses SIVmac239 and NL-DT5R grew in a consistent manner, two (A209V and P371L) out of the four mutants displayed retarded growth phenotype. Although K110I grew similarly well with NL-DT5R, the triple mutant K110I/A209V/P371L did not at all. To know whether the three mutations can confer the ability to grow in simian cells on HIV-1 type CA, four mutant clones in the context of NL4-3 were constructed as above and monitored for their growth potentials in HSC-F cells. As clearly seen Fig. 3C, the growth of the four mutants were undetectable or negligible during the observation period, indicating the reported coding mutations in *gag* (6) are biologically inactive or meaningless in simian cells.

In this study, we have constructed ten new recombinants between the NL-DT5R clone of monkey-tropic HIV-1 and the standard pathogenic clone SIVmac239, and four new *gag* mutants of NL-DT5R in anticipation of improved growth potentials in simian cells. In addition to the changes relative to HIV-1 (Gag-CypA region and Vif) which the prototype monkey-tropic virus NL-DT5R carries, the new viral clones contain alterations in scattered regions of Gag-CA and in the other parts of Gag. Our results here clearly showed that region(s) around the CypA-binding loop of HIV-1 is critical for viral growth ability in simian cells. Cellular TRIM5α is well known to interact with this region and restrict retroviral replication (13-18). In this regard, it is interesting to note that NL-DT5R does not overcome the barrier imposed by TRIM5α (4). Virological studies to evade the TRIM5α restriction are in progress in our laboratory.

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