ORIGINAL

IXI triplet residues within the Env-V3 tip can influence the replication potential of R5-tropic HIV-1

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Abstract : The HIV-1 envelope glycoprotein (Env)-gp120 plays a critical role in viral entry by binding to the receptor CD4 and coreceptors CCR5/CXCR4. The V3 loop in CCR5-tropic HIV-1 Env-gp120 is a key domain responsible for interacting with CCR5. Based on virus mutation and adaptation studies, our structural modeling predicted that the IXI triplet residues within the V3 loop of Env-gp120 interact with hydrophobic cores in CCR5, potentially modulating viral infectivity. In this study, we investigated the functional significance of the IXI triplet in HIV-1 replication. Substitutions at the central "X" position of the IXI triplet significantly affected viral replication in both macaque and human cell lines without altering Env expression. Combinatorial mutations with the V3 tip residue G310R showed either enhancing or suppressive effects on replication in macaque cells, highlighting a complex interplay between the IXI triplet and V3 tip. Furthermore, the impact of these mutations varied between different HIV-1 strains, suggesting that some strain-specific sequence/structural context(s) is critical for the observed biological effect. Taken together, these findings indicate that the IXI triplet can be a novel motif influencing HIV-1 replication and underscore the importance of V3 loop variability in coreceptor interaction and viral growth potential. J. Med. Invest. 73 : 80-87, February, 2026

Keywords **:** *HIV-1, Envelope, V3, replication*

INTRODUCTION

HIV-1 Envelope glycoprotein (Env) protruding outside from the virion functions at the virus-receptor binding and viral entry into host cells, and is the sole target for neutralizing antibodies (1-5). HIV-1 Env has a high ability to mutate, and thus its functional and structural changes by mutations strongly affect virus replication and survival competencies in various environments. HIV-1 Env is synthesized as an Env-gp160 precursor in the ER and forms a trimer. Upon transport to the Golgi apparatus, trimerized Env-gp160 is cleaved by furin and/or furin-like proteases to produce Env-gp120/gp41. The resultant trimer of the hetero dimer of Env-gp120/gp41 is transported to the plasma membrane and incorporated into virions (1-5).

HIV-1 Env-gp120 plays a role in binding to receptor CD4 and coreceptor CCR5 and/or CXCR4. Based on sequence variation, Env-gp120 is divided into constant (C1 to C5) and variable (V1 to V5) regions (1-5). Of these, the V3 loop region is a key domain involved in the interaction with coreceptors. Alterations in V3 loop sequences have been linked to changes in coreceptor usage (6-10), which classifies HIV-1 strains into CCR5 (R5)-, CXCR4 (X4)-, and dual-tropic (R5/X4) viruses. R5-tropic HIV-1 is typically associated with viral transmission, while X4- and dual-tropic viruses tend to emerge during later stages of disease progression. In infected individuals, a coreceptor switch from R5 to X4 can occur, and this phenomenon can be related to rapid progression to AIDS (11-13). Frequencies of coreceptor switch seem to be different among HIV-1 subtypes, suggesting that the difference in V3 sequences and structures affects coreceptor usage (14-18).

HIV-1 coreceptor CCR5 is one of the G-protein-coupled receptors with seven transmembrane spanning domains (2, 19-21). A truncated protein CCR5Δ32 with a 32 bp deletion is not able to function as an HIV-1 coreceptor due to its lack of expression on the cell surface. People homozygous for the CCR5Δ32 allele are largely protected against HIV-1 infection (22-24), suggesting the importance of CCR5 in viral transmission/infection. The expression level on the cell surface of CCR5 can influence its conformation and also the HIV-1 entry and pathogenesis (2, 19, 20). For valid HIV-1 infection control, it is crucial to understand how HIV-1 Env-gp120 interacts with CCR5, and to identify mutations in these molecules that alter their interaction and thereby influence viral replication.

Through our series of mutation/adaptation studies on HIV-1, we have reported the mutations in the V3 loop that intervene the interaction between Env-gp120 and CD4/CCR5, affecting the replication ability of R5-tropic HIV-1 NL-DT562 (562) (25-27). In addition to the experimental virological data, our *in silico* structural modeling predicted that the ITI triplet residues (305 to 307 residues in 562) within the V3 loop can contribute to the interaction between CD4, Env-gp120, and CCR5 (25). In this study, we aimed to biologically evaluate the functional role of the ITI triplet within the Env-gp120 V3 loop in HIV-1 replication.

MATERIALS AND METHODS

Clones

HIV-1 proviral clones 562, 562EX, Y3 (GenBank : LC816726),

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and B3AD8 used in this study have been reported previously (25-28). Env mutant clones were generated by site-directed mutagenesis as described previously (25-28).

Cells

Monolayer cell line, HEK293T (ATCC CRL-1573) (29) was cultured and maintained in Eagle's minimal essential medium containing 10% heat-inactivated fetal bovine serum. Macaque HSC-F (30) and human MT4/CCR5 (26) lymphocyte cell lines were cultured and maintained in RPMI 1640 medium containing 10% heat-inactivated fetal bovine serum (for MT4/CCR5 cells, 200 μg/mL of hygromycin B [Sigma-Aldrich] was added).

Multicycle replication assay

Virus samples were prepared from HEK293T cells transfected with proviral clones by the calcium-phosphate coprecipitation method as described previously (31, 32). Virus amounts were determined by virion-associated reverse transcriptase (RT) assay as previously described (32, 33) or by the HIV-1 p24 antigen enzyme-linked immunosorbent assay kit (ZeptoMetrix Corporation). To examine viral growth potential, equal amounts of virus samples were inoculated into HSC-F cells. For MT4/CCR5, viruses were spin-infected into the cells (34). Infected HSC-F cells were cultured in the presence of IL-2 (Bio-Rad). After infection, culture supernatants were periodically collected, and virus replication was monitored by RT assays (32, 33) or real-time RT-PCR using virion-associated viral genomes as described previously (35). The assays were repeated using independently prepared virus stocks and cell cultures. The reproducibility of the assays was determined by comparing the growth kinetics between parental clones as standards and the samples tested. In the repeated experiments, the viral growth potential was judged by the peak day of virus production, and if the viral growth kinetics were similar, by the virion production level on the peak day.

Env expression in cells and on virions

HEK293T cells were transfected with proviral clones by Lipofectamine 2000 (Invitrogen). On day 2 post-transfection, virions released into the culture supernatants were collected by ultracentrifugation. The cells and collected virions were lysed for Western blotting analysis as previously described (36, 37). Total protein amounts of transfected cells and Gag-p24 amounts of collected virions were quantified by the DC protein assay kit (Bio-Rad) and by the HIV-1 p24 antigen enzyme-linked immunosorbent assay kit, respectively. The equal amounts of proteins (1 ug) and Gag-p24 (0.5 ng and 10 ng for Gag-p24 and Env analyses, respectively) were resolved by SDS-PAGE. Western blotting analyses were performed using anti-HIV-1 gp120 (ab21179, abcam), anti-HIV-1 Gag-p24 (183-H12-5C) (catalog no. 3537 ; NIH Research and References Reagent Program), and anti-β-actin clone AC-15 (Sigma-Aldrich Co.) antibodies as previously described (36).

RESULTS

In silico structural analysis predicted that the ITI triplet residues within the V3 loop of HIV-1 562 Env-gp120 can be involved in the interaction with CCR5

HIV-1 coreceptor CCR5 consists of the N-terminal segment, three extracellular loops, three intracellular loops, and cytoplasmic C-terminal tail. The bundle of seven transmembrane-spanning segments forms chemokine recognition site 2 (CRS2) (2, 19-21). The N-terminal segment makes contact to the Env-gp120 bridging sheet, which is formed upon CD4 binding (38, 39), and the V3 loop is inserted into the CRS2 (21, 40). As we reported previously (25), our structural modeling of human CD4/CCR5 and HIV-1 562 Env was performed using a recent cryo-electron microscopy (cryo-EM) structure of the CD4-full-length gp120-unliganded CCR5 complex (21) as a template. Then, to investigate the interaction of the V3 loop and CCR5, molecular dynamics simulations were carried out under the condition that CCR5 portions in the homology modeling of the CD4-full-length gp120-unliganded CCR5 complex were embedded in a lipid bilayer (25). Notably, our model predicted that the ITI triplet residues can interact with two hydrophobic cores in CCR5 and thus can function as a structural element that modulates the interaction between the V3 loop and CCR5 (Fig. 1). We hypothesized that local conformational changes in the V3 loop region can influence the binding affinity of Env-gp120 to CD4 and CCR5, thereby affecting viral infectivity (25).

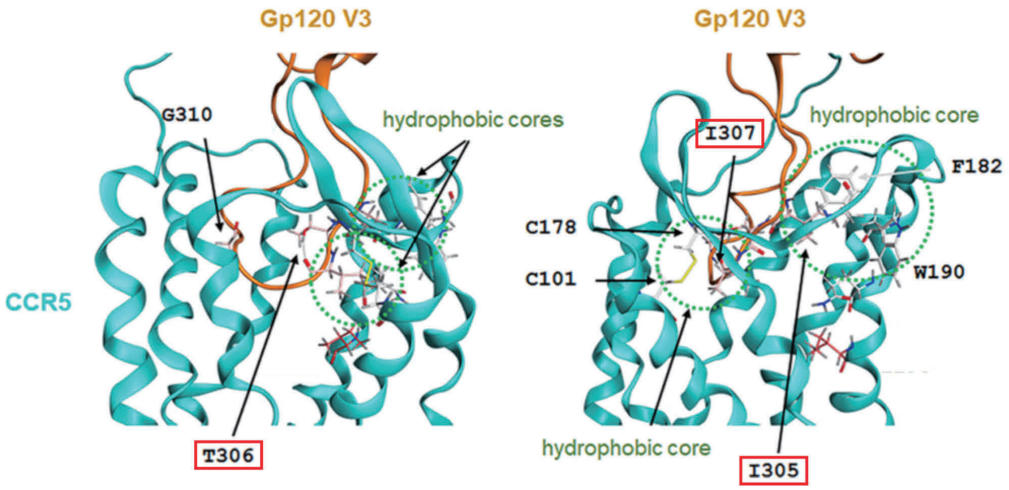

Figure 1. Structural model of the interaction between HIV-1 Env-gp120 V3 loop and human CCR5. Adapted from Koma *et al*., J Virol 95 : e02177-20, 2021, with permission from the American Society for Microbiology. The IXI triplet residues in HIV-1 (562) Env-gp120 V3 loop are highlighted with red boxes. Two hydrophobic cores are presented as green dotted circles. The CRS2 pocket mentioned in the Results section is located within the transmembrane domain of the CCR5 receptor and is surrounded by its hydrophobic cores. The interactions between the IXI triplet residues and hydrophobic cores involve the connection between : V3 I305 and CCR5 F182/W190 ; V3 I307 and CCR5 C178/C101. Views from two arbitrary angles are shown.

The T site within the ITI triplet has different amino acid residues among R5-tropic HIV-1 strains

Since the differences in HIV-1 subtypes and V3 loop sequences affect the interaction between Env and CD4/CCR5 (6-10, 14-18), we compared V3 loop sequences from three distinct R5-tropic HIV-1 clones that we have generated (Fig. 2) (28). Clones 562 and B3AD8 carry the envelope genes from HIV-1 subtype B SF162 and AD8 clones, respectively, whereas clone Y3 harbors a clinical isolate subtype CRF_08BC envelope gene (27, 28, 41, 42). We noticed that in the V3 loop sequences, amino acid residues at the T site within the ITI triplet in 562 Env differ among the three clones analyzed, that is, B3AD8 and Y3 carry H and R, respectively. The ITI triplet residues are located immediately upstream of a conserved V3 tip (308GPG310, 562 numbering), which is deeply inserted into the CRS2 pocket (Figs. 1 and 2) (21). We postulated that as predicted by our structural model (Fig. 1), alterations in the T site residue can change the local structure of V3 tip, turning into varying viral growth potential.

Mutations at the T site within ITI triplet and a G310R mutation differently affect HIV-1 562 replication ability in macaque and human cell lines

To examine the effect of amino acid variations at the T site within ITI triplet on HIV-1 replication, we newly generated 562 mutant clones carrying IHI, IRI, and IGI of the triplet residues. We chose these amino acids because polar amino acids H and R are found in B3AD8 and Y3 Env (Fig. 2) and because G is a nonpolar amino acid with different amino acid property from T, H, and R. Besides, we have shown that G310R mutation within the V3 tip of 562 Env-gp120 increases viral growth potential specifically in macaque cells, not in human cells, via the enhancement of binding to macaque CD4 and CCR5 (25). Concerning a potential association between the ITI triplet and V3 tip, we were also interested in whether the combinations of T site mutations and G310R affect viral replication. To determine their combinatorial effects, both macaque-tropic HIV-1 (562) and human-tropic HIV-1 (562EX) clones were used for the analysis. While 562 and 562EX clones have the same envelope genes, the partial *gag* and the full-length *vif* sequences are distinct from each other, enabling efficient viral replication depending on their cell tropisms (25, 27).

Viruses were prepared for multicycle replication assays by transfecting HEK293T cells with proviral clones and were infected into macaque HSC-F and human MT4/CCR5 cell lines. As shown in Fig. 3, the alteration of amino acid residues at the T site within ITI triplet varied viral replication ability in both macaque and human cell lines. In HSC-F cells, IHI, IRI, and IGI mutant clones exhibited enhanced growth potential compared to a parental 562 clone carrying the ITI triplet residues (Fig. 3A). Consistent with our previous result (25), the G310R mutation at the V3 tip increased growth potential of 562 in macaque cells. The combination of IHI and G310R mutations improved replication ability than either IHI or G310R alone, whereas viral replication was attenuated by the combination of IRI or IGI with G310R to a degree lower than 562 (Fig. 3A). In MT4/CCR5 cells, while the IRI mutant clone grew similarly to a parental 562EX clone, IHI and IGI mutations enhanced replication ability compared to 562EX (Fig. 3B). Consistent with our previous data (25), the G310R mutation completely abolished viral growth potential, and viral replication was not resilient in combination with either IHI, IRI, or IGI mutation (Fig. 3B).

In summary, amino acid alterations at the T site varied viral replication potential (Fig. 3 and Table 1). Notably, the combination of ITI mutations and G310R increased and decreased viral replication ability compared to each ITI mutation and G310R alone in macaque cells (Fig. 3 and Table 1), suggesting that there may be a mutually affected interaction between the ITI triplet and V3 tip. These results showed that virus growth potential is evidently influenced by amino acid residues at the T site within ITI triplet as we hypothesized (Fig. 1), and thus we named this triplet as the IXI triplet residues hereafter.

Table 1. Viral growth of various mutant clones in macaque HSC-F and human MT4/CCR5 cell lines

562/562EX	Growth in macaque cells[1]	Growth in human cells[1]
ITI (WT)	+	+
IRI	+++	+ or ++
IHI	++	+++
IGI	++++	+++
WT + G310R	+++	−
IRI + G310R	± or −	−
IHI + G310R	+++	−
IGI + G310R	± or +	−

[1]The results in Fig. 3 were qualitatively summarized. Growth abilities of viral clones relative to WT (562 or 562EX, +) were shown as follows : ++, higher virion production level on the peak day ; +++, peak day earlier by 3 to 6 days ; ++++, peak day earlier by 9 days ; ±, lower virion production level on the peak day ; −, undetectable virion production.

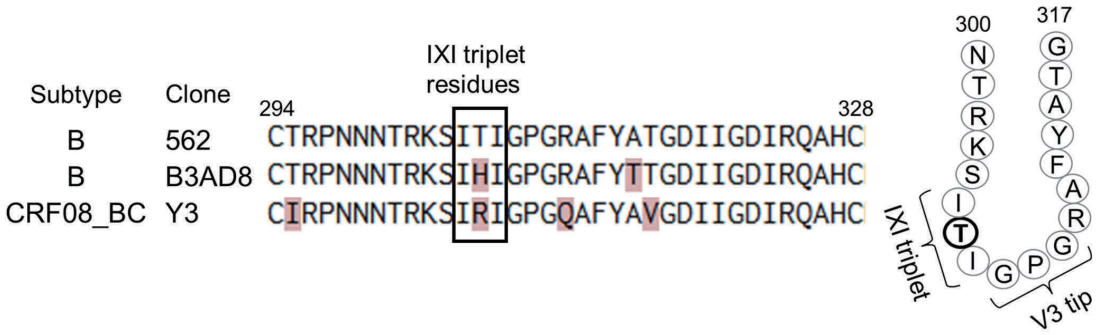

Figure 2. The alignment of V3 loops sequences from three distinct HIV-1 clones.
HIV-1 subtypes and clone names are presented. The IXI triplet residues are highlighted with a black box. The shaded boxes indicate different amino acid residues from those of clone 562. The sequence alignment was done using Benchling [Biology Software]. (2022). Retrieved from https://benchling.com. Organization of the partial V3 region is presented along with the highlight of the IXI triplet and V3 tip of 562 Env-gp120. The numbers indicate amino acid positions of HIV-1 562 Env.

Mutations at the X site within IXI triplet enhance or reduce the replication ability of R5-tropic HIV-1 562EX

Viral replication ability of 562 carrying the ITI triplet was changed by H and R substitutions at the T site from distinct HIV-1 Env sequences (Fig. 3). To investigate whether amino acid properties can be related to the variation in virus growth potential, we analyzed the Env-gp120 V3 loop sequences of HIV-1 subtype B based on the HIV-1 sequence database (http://www.hiv.lanl.gov) (Fig. 4). The X site within IXI triplet was one of the highest variable residues in the V3 sequences analyzed, and several amino acids including P, N, and S existed at a certain frequency in addition to H and R (Fig. 4).

To further examine the effect of X amino acid residues,

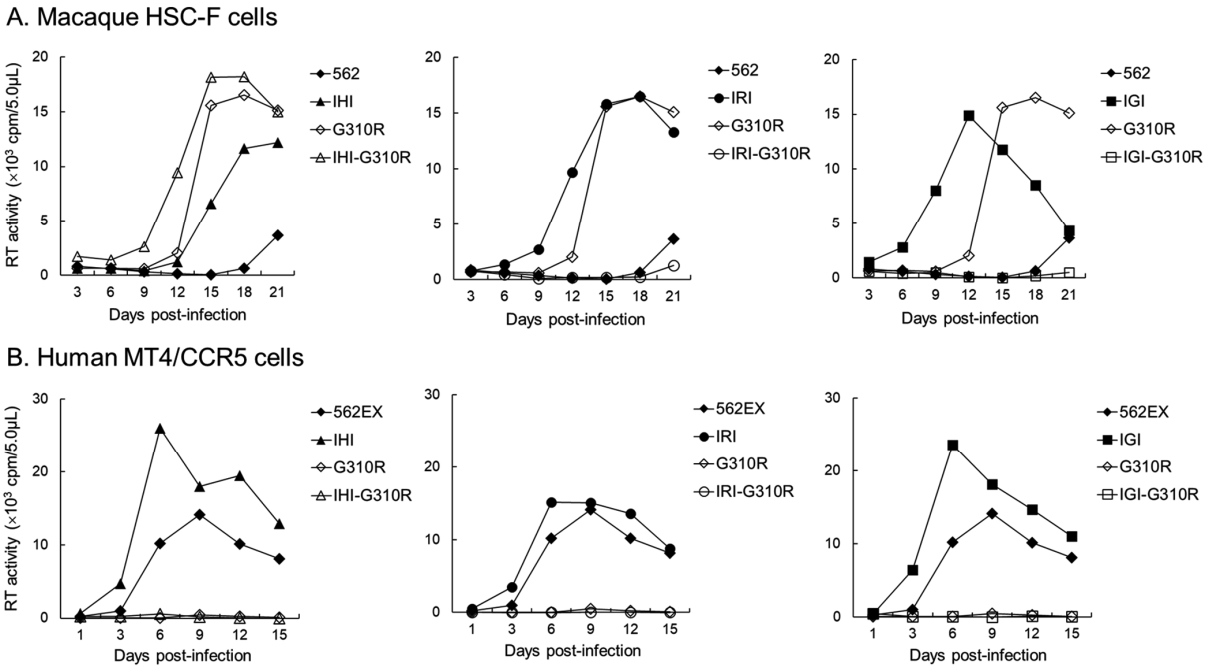

Figure 3. Effect of mutations within the IXI triplet (IHI, IRI, and IGI) and V3 tip of Env-gp120 (G310R) on HIV-1 growth in macaque (A) and human (B) cell lines. Viruses were prepared from HEK293T cells transfected with the indicated clones. The equal amounts of viruses (5×10^5 and 2×10^5 RT units for macaque HSC-F and human MT4/CCR5 cells, respectively) were inoculated into HSC-F (2×10^5) or were spin-infected into MT4/CCR5 (1×10^5) cells. Virus replication was monitored using RT activity in the culture supernatants. For easy comparison, viral replication kinetics of WT 562 and 562EX as well as their G310R mutants are presented in each panel of A and B, respectively. The viral growth potentials are assessed by the peak day of virus production, and if the viral growth kinetics are similar, by the production level on the peak day. The infection experiment was repeated with similar results.

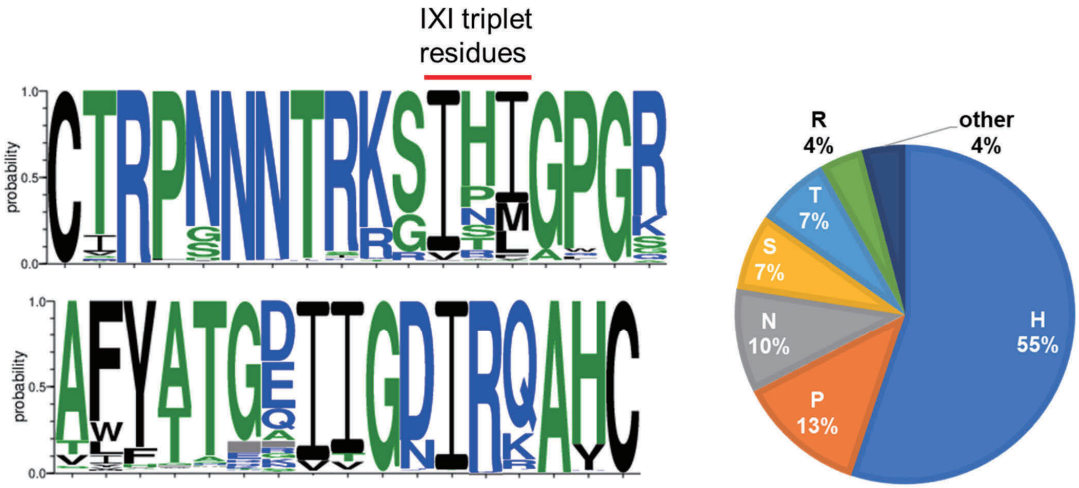

Figure 4. Sequence alignment of the V3 loop among HIV-1 subtype B viruses. Sequences (2334 sequences corresponding to amino acid positions 294 to 328 of HIV-1 562) of HIV-1 subtype B and amino acid logo were obtained from the HIV-1 sequence database (Los Alamos National Laboratory, http://www.hiv.lanl.gov/) on November 16th, 2022. Gaps in the logo were manually removed. The IXI triplet residues are indicated. The pie chart on the right shows the frequencies of amino acid residues at the X site within the IXI triplet.

especially the difference in amino acid properties, on virus replication potential, we newly generated IXI triplet mutant clones, IPI, ISI, IDI, and IFI. Reasons we selected these amino acids were as follows : 1) P and S were found in the V3 sequences analyzed (Fig. 4), and 2) D and F display quite different amino acid properties from other amino acids tested (H, R, T, and G) (Table 2). Growth kinetics of X site mutant clones (IRI, IHI, IGI, IPI, ISI, IDI, and IFI) were compared with that of a parental 562EX clone in human MT4/CCR5 cells (Fig. 5). Consistent with our data (Fig. 3B), while IRI clones exhibited similar growth kinetics to 562EX clone, IHI and IGI mutations enhanced viral growth potential compared to 562EX carrying the ITI triplet (Fig. 5A). All newly generated clones IPI, ISI, IDI, and IFI grew poorly relative to 562EX, though the extent of reduction was different among mutations (Fig. 5A).

To determine if variation in viral replication ability is due to

Table 2. Amino acid properties of analyzed residues at the X site of IXI triplet and their effects on viral growth of 562EX and Y3 in a human cell line MT4/CCR5

Amino acid		Size	Chemical property	Side chain	562EX[1]	Y3[1]
Threonine	T	Medium-small	Neutral hydrophilic	Uncharged polar	WT	ND
Histidine	H	Large	Basic	Basic (positive charge)	+	+
Glycine	G	Small	Aliphatic	Nonpolar	+	−
Arginine	R	Medium-large	Basic	Basic (positive charge)	± or +	WT
Proline	P	Medium-small	Imine	Nonpolar	−	ND
Serine	S	Small	Neutral hydrophilic	Uncharged polar	−	+
Aspartic acid	D	Medium-small	Acidic	Acidic (negative charge)	−	ND
Phenylalanine	F	Large	Aromatic	Nonpolar	−	±

[1]Viral growth potential : +, better than WT ; ±, similar to WT ; −, poorer than WT ; ND, not determined.

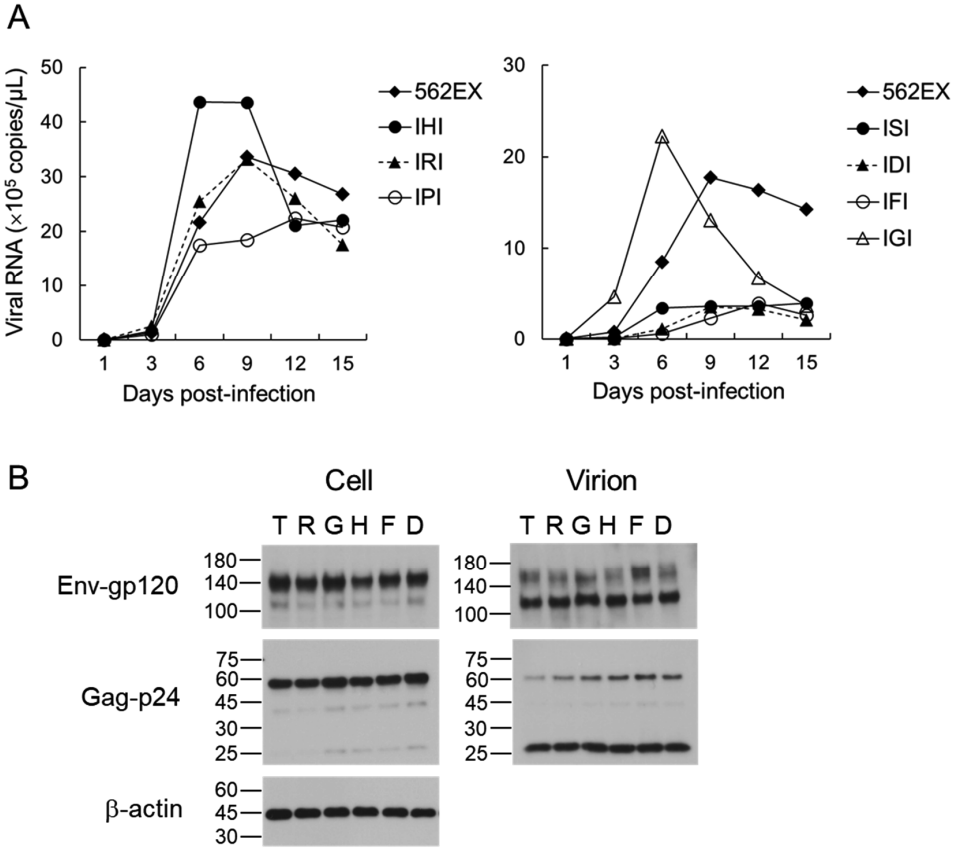

Figure 5. Effect of mutations at the X site within the IXI triplet on virological phenotypes of HIV-1 562. (A) Growth kinetics. Viruses were prepared from HEK293T cells transfected with proviral clones indicated, and equal amounts (2×10^5 RT units) were spin-infected into MT4/CCR5 (1×10^5) cells. Virus replication was monitored by real-time RT-PCR using virion-associated viral genomes released into the culture supernatants. The infection experiment was repeated with similar results. (B) Env expression in cells and on virions. HEK293T cells were transfected with IXI mutants of HIV-1 562 Env-gp120. On day 2 post-transfection, virions released into the culture supernatants were collected by ultracentrifugation. The virion- and cell-samples were lysed and subjected to Western blotting analysis using anti-gp120, anti-Gag-p24, and anti-β-actin antibodies. The letters T, R, G, H, F, and D indicate the amino acid residues at the X site within ITI triplet.

the effect of IXI mutations on Env expression, mutant clones exhibiting distinct viral growth kinetics were analyzed for Env expression in cells and on virions. Mutant clones used for analysis included a parent clone (562EX, ITI) and those with similar growth (IRI), increased growth (IHI and IGI), and decreased growth (IDI and IFI) potentials relative to the 562EX. Proviral clones were transfected into HEK293T cells, and the cells and the virions released into the culture supernatants were monitored for Env expression. Expression levels and patterns of Env in mutant clones tested were similar to those of 562EX both in cells and on virions (Fig. 5B), indicating no appreciable effects of the IXI mutations on Env expression. Taken together, although specific amino acid properties were not found that can explain the variation in viral replication ability, these results showed that viral growth potential varies depending on substituted amino acid residues at the X site within IXI triplet of HIV-1 562EX Env-gp120.

Amino acid residues at the X site within IXI triplet differently affect virus growth potential between distinct R5-tropic HIV-1 562EX and Y3

We identified amino acid residues that increase (IHI and IGI) and decrease (IFI, ISI, IDI, and IPI) viral replication in 562EX (Fig. 5). To examine whether the X site mutations also affect viral growth potential of a different HIV-1 clone, we used R5-tropic HIV-1 Y3 because of its distinct nature from 562EX (see the subtype and Env sequence in Fig. 2). Mutations that effectively varied 562EX viral replication potential were introduced into a parental Y3 clone carrying IRI, and the resultant IHI, IGI, IFI, and ISI mutant clones were used for replication assay (Fig. 6 and Table 2). Like 562EX, the Y3-IHI mutation enhanced growth potential relative to a parental Y3 clone. The Y3-IFI clone grew similarly to the Y3 clone. In contrast to 562EX, IGI and ISI mutant clones exhibited slower and faster growth kinetics, respectively, than that of the Y3 clone. This result suggested that the growth potential of R5-tropic HIV-1 can be affected by mutations at the X site within IXI triplet, whereas the effect of substituted amino acid residues on virus replication can be different among HIV-1 strains.

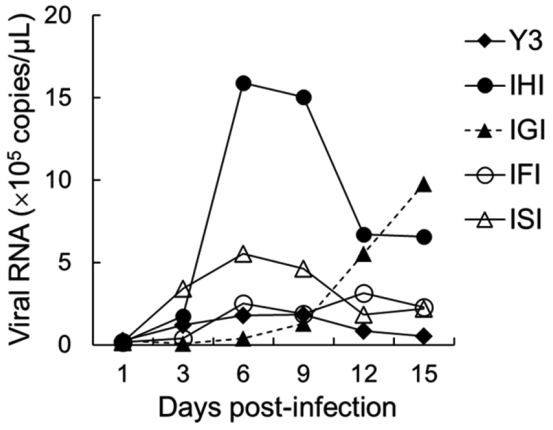

Figure 6. Effect of mutations at the X site within the IXI triplet on the replication potential of HIV-1 Y3. Viruses were prepared from HEK293T cells transfected with proviral clones indicated, and equal amounts (50 ng of Gag-p24) were spin-infected into MT4/CCR5 (1×10^5) cells. Virus replication was monitored by real-time RT-PCR using virion-associated viral genomes released into the culture supernatants. The infection experiment was repeated with similar results.

DISCUSSION

We have previously reported an *in silico* structural model of human CD4-R5-tropic 562 Env-gp120-human CCR5 complex based on a cryo-EM structure (21) to explore the effect of mutations in the 562 Env-gp120 V3 on its interaction with CCR5 (Fig. 1) (25). Together with virological analysis, our structural model predicted that the ITI triplet residues within the V3 loop can be involved in the interaction of Env-gp120 with CD4 and CCR5, and thereby can influence viral replication ability (25). The investigation of the V3 loop sequences in this study showed that amino acid residues at the T site within ITI triplet of 562/562EX are relatively variable in the HIV-1 population (Figs. 2 and 4). Based on the alignment of various V3 sequences and the properties of amino acids they encode, we newly generated a series of mutant clones and virologically characterized them. Although no relationship was noted between the changes in viral replication ability and the properties of the substituted amino acid residues, we clearly demonstrated that the T site mutations vary the growth potential of 562/562EX depending on altered residues without any effects on Env expression (Figs. 3 and 5), and thus we named the triplet residues as IXI. In fact, the X site mutations within IXI triplet in Y3, which has a distinct Env from 562EX, also increased or decreased viral replication ability (Figs. 2 and 6), though the mutational effects on viral growth potential was different between 562EX and Y3 (Table 2). These results showed that the IXI triplet residues within the Env-gp120 V3 loop can be a novel motif that modulates the replication ability of R5-tropic HIV-1.

As for the IXI triplet at positions 305-307 (562 numbering), I305, I307, and F313 have been reported to form a hydrophobic patch that can contribute to the Env-gp120 trimer association (43, 44). As expected for this role of I residues in the Env-gp120, analysis of V3 loop consensus sequences from 391 R5-tropic HIV-1 subtype B Env showed that two I sites within the IXI triplet displayed low entropy but high entropy for the X site (16), implying higher variability in amino acid residues at the X site than two I sites. Since mutations at the X site influenced the sensitivity against certain neutralizing antibodies targeting the V3, it has been proposed that this amino acid variability at the X site may be responsible for defining the structure of the V3 tip (16). Our data would support this possible interaction between the X site and the V3 tip, because the combination of different IXI mutations and the V3 tip G310R differently affected the 562-virus growth potential in macaque cells (Fig. 3A). We have also proposed from our structural model that changes in the interaction between the ITI triplet and the V3 tip may regulate the interplay in the ternary CD4-Env-gp120-CCR5 complex (25). Intriguingly, it has been reported that amino acid residues at the X site have covarying pair residues within the V3 loop, e.g., the X site and residues at position 304 and 311 (562 numbering) (16, 45). Such covarying pair residues might change the effect of the IXI triplet residues on viral growth potential. In fact, the effect of the X site mutations on viral growth was different between 562EX and Y3, which have different amino acid residues at position 311 (R and Q, respectively) (Figs. 5 and 6).

It has been suggested that the V3 loop region can electrostatically modulate the global structure of Env-gp120 outer domain and can contribute to Env structural stability through its interaction with gp120 core (46-48). We have previously reported that the G310R mutation within the V3 loop can affect the binding energies for both CD4 and CCR5, as calculated by *in silico* modeling (25). Thus, mutations within the V3 loop in Env-gp120 can change not only the V3 loop conformation itself but also overall Env structure and stability. These multiple influences of the V3 loop on Env structure/function may explain that there was no

correlation between viral replication ability and amino acid properties at the X site within the IXI triplet in this study, that is, the X site residue is not necessarily the sole determinant of interaction with CCR5. Mutations within the IXI triplet may vary viral replication potential by directly affecting CCR5 binding or by modulating both CD4 and/or CCR5 binding through changing the V3 loop conformation, the interaction among residues within the V3 loop described above, and/or the Env structure. Further studies are needed to elucidate the mechanism by which amino acid residues at the X site alter the V3 tip structure and the interaction in the ternary CD4-Env-gp120-CCR5 complex, and thereby viral growth potential.

The difference in the V3 loop sequences among the HIV-1 population suggests their structural variability. HIV-1 coreceptor CCR5 has various ligands and adopts various oligomerization statuses and conformations (19, 20). Structural flexibilities in both the V3 loop and CCR5 allow accommodation of the V3 loop into the CRS2 pocket in CCR5 even when the V3 loop sequence is mutated. Various mutations in the Env-gp120 can affect the interplay with receptor CD4 and coreceptors CCR5/CXCR4. In order to control HIV-1 infection/replication, it is pivotal to comprehensively understand the functional and structural interactions of Env-gp120–receptor/coreceptors and specifically clarify the Env mutations that could be associated with the interactions.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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