Impact of CD4 mimetics-resistant mutations on susceptibilities to anti-Env nMAbs

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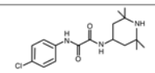
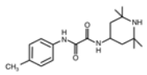
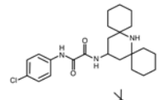
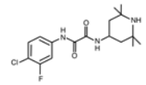
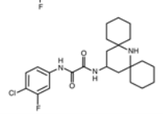
Background

CD4 mimic small compounds (CD4MCs), NBD-556 and its analogues inhibit the gp120-CD4 interaction (TABLE 1) [1-3] and can also induce conformational changes in the gp120 architecture thereby exposing masked epitopes of neutralizing antibodies on the Env protein [2,3]. In this study, we induced the CD4MCs resistant variants using primary isolate to elucidate the interactions between gp120 and CD4MCs, and also investigated the phenotypic change in the CD4MCs resistant isolates against other entry inhibitor and anti-Env neutralizing monoclonal antibodies (nMAbs).

Methodology

To induce resistant variants against five CD4MCs (NBD-556, YYA-021, HAR-171, JRC-II-191 and HAR-431) and sCD4 in vitro, we exposed PM1 cells to the bulked and cloned primary KP-5P virus (subtype B, R5) (FIG. 1). We constructed infectious clones with CD4MC-resistant mutation following in vitro selection. The susceptibility of mutated Env/NL4-3 infectious clones to a CCR5 inhibitor (maraviroc, MVC), 3D6 (anti-CD4bs) and 4E9C (anti-CD4i epitope) were evaluated using single-round assay in TZM-bl cells.

TABLE 1. Anti-HIV activity and cytotoxicity of CD4MCs[a]

Name	Structure	IC_{50} (μM)[b]	CC_{50} (μM)[c]
NBD-566		0.53	71
YYA-021		11	>300
HAR-171		0.35	122
JRC-II-191		0.34	91
HAR-431		0.14	10

[a]All data are the mean values for at least three independent experiments. [b]The IC_{50} values of the WST-8 assay are based on the inhibition of HIV-1 induced cytopathogenicity in PM1/CCR5 cells. [c]The CC_{50} values are based on the reduction of the viability of mock-infected PM1/CCR5 cells.



FIG. 1. The gp120 sequences of the infectious clone KP-5P as compared with the sequence of HIV-1 consensus B. The single-letter amino acid code is used. The HXB_2 reference sequence is given at the top, and each amino acid is numbered relative to the HXB_2 reference sequence. Dots indicate amino acid sequence identity and dashes indicate deletions. Val255, Thr375, and Met426 substitutions are denoted by black rectangular boxes. Potential N-glycosylation sequons (PNGS) are denoted by gray rectangular boxes.

Results

V255M was most frequent mutation in six of ten CD4MC resistant viruses, while T375I mutation was most frequent in three of ten CD4MC resistant viruses (FIG. 3 and 7). M426I was observed as a major clone in only YYA-021 resistant virus using the bulked virus (FIG. 3). To investigate a relationship between the resistant mutations in gp120 and sensitivity to CD4MCs, we constructed Env-mutant clones carrying a single mutation within the KP-5P gp160 backbone, and determined the sensitivity of each clone to CD4MCs. The three mutations, V255, T375 and M426 were associated with high level of resistance to all CD4MCs tested (FIG. 5). Furthermore, we examined susceptibilities of the mutated clones to CCR5 inhibitor MVC, CD4bs nMAb 3D6 and CD4i nMAb 4E9C. In this assay in TZM-bl cells, there was no substantial difference between the wild type and CD4MCs resistant clones in sensitivity of MVC. The clones containing V255M or M426I substitutions became relatively resistant to 4E9C compared to the wild type clone, whereas the clone with T375I showed low sensitivity to both 3D6 and 4E9C nMAbs. This finding suggests that the T375I mutation can negatively affect the neutralizing activity of CD4bs and CD4i nMAbs for inducing the conformational change in the Env more than V255I and M426I mutations.

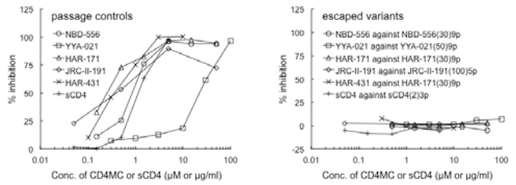


FIG. 2. Inhibitory activities of CD4MCs and sCD4 toward infection of the bulked KP-5P escape variants from CD4MCs and sCD4. PM1/CCR5 cells ($2x10^5$) were exposed to $200TCID_{50}$ of passage controls CD4MC-ecaped variants and then cultured in the presence of various concentrations of CD4MCs. The IC_{50} values were determined using the WST-8 assay on day 7 of culture.

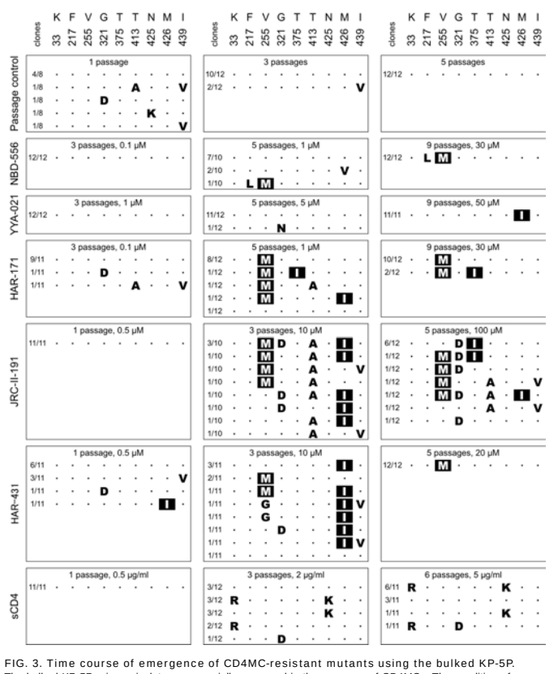


FIG. 3. Time course of emergence of CD4MC-resistant mutants using the bulked KP-5P. The bulked KP-5P primary isolates were serially passaged in the presence of CD4MCs. The condition of passage experiments are referred to as "9 passages, 30 μM", where "9 passages" stands for passage number, and "30 μM" is the concentration of CD4MC in the selection culture. V255M, T375I, and M426I substitutions are denoted by black rectangular boxes. Dots denote sequence identity. The numbers on the left show the numbers of clones with the listed sequence among the total number of clones tested.

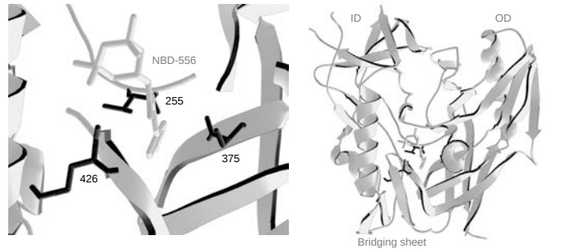


FIG. 4. The locations of the mutations induced by CD4MC in the Phe43 cavity of gp120 core. The crystal structures of NBD-556 in complex with HIV-1 gp120 (C1086) was retrieved from the PDB (3TGS) [4]. The side chains of the mutated residues (255, 375, and 426) that appeared during *in vitro* selection are shown in black.

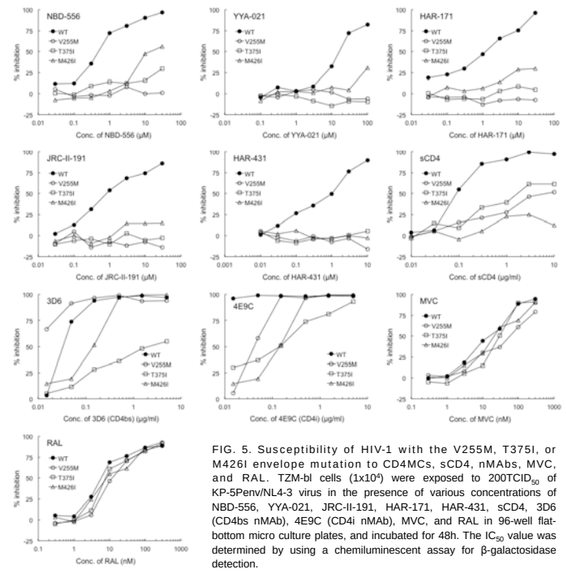


FIG. 5. Susceptibility of HIV-1 with the V255M, T375I, or M426I envelope mutation to CD4MCs, sCD4, nMAbs, MVC, and RAL. TZM-bl cells ($1x10^4$) were exposed to $200TCID_{50}$ of KP-5Penv/NL4-3 virus in the presence of various concentrations of NBD-556, YYA-021, JRC-II-191, HAR-171, HAR-431, sCD4, 3D6 (CD4bs nMAb), 4E9C (CD4i nMAb), MVC, and RAL in 96-well flat-bottom micro culture plates, and incubated for 48h. The IC_{50} value was determined by using a chemiluminescent assay for β-galactosidase detection.

TABLE 2. Susceptibility of HIV-1 with the V255M, T375I, or M426I envelope mutation to CD4MCs, sCD4, nMAbs, MVC, and RAL.

	compound	IC_{50}[a] (fold resistance)[b] WT	V255M	T375I	M426I
CD4MCs	NBD-556 (μM)	0.48 (1)	>30 (>63)	>30 (>63)	14 (29)
	YYA-021 (μM)	16 (1)	>100 (>6.3)	>100 (>6.3)	>100 (>6.3)
	JRC-II-191 (μM)	0.82 (1)	>30 (>37)	>30 (>37)	>30 (>37)
	HAR-171 (μM)	1.2 (1)	>30 (>25)	>30 (>25)	>30 (>25)
	HAR-431 (μM)	0.90 (1)	>30 (>33)	>30 (>33)	>30 (>33)
	sCD4 (μg/ml)	0.13 (1)	>10 (77)	2.6 (20)	>10 (77)
NAbs	Maraviroc (nM)	14 (1)	58 (4.1)	29 (2)	21 (1.5)
	3D6 (CD4bs, μg/ml)	0.033 (1)	<0.015 (<0.45)	2.2 (67)	0.022 (0.67)
	4E9C (CD4i, μg/ml)	<0.015 (1)	0.042 (>2.8)	0.11 (>7.3)	0.14 (>9.3)
	Raltegravir (nM)	7.7 (1)	14 (1.8)	8.0 (1)	10 (1.3)

(a) TZM-bl cells ($1x10^4$) were exposed to $200TCID_{50}$ of KP-5Penv/NL4-3 virus in the presence of various concentrations of NBD-556, YYA-021, JRC-II-191, and HAR-171, HAR-431, sCD4, 3D6 (CD4bs Nab), 4E9C (CD4i Nab), MVC, and RAL in 96-well flat-bottom micro culture plates, and incubated for 48h. The IC_{50} value was determined by using a chemiluminescent assay for β-galactosidase detection. (b) Numbers in parentheses represent fold changes of IC_{50}s against each virus compared to IC_{50} against the wild type. The fold change values is color coded as follows: green = < 5.0 fold; tangerine = 5.0-20 fold; red = >20 fold.

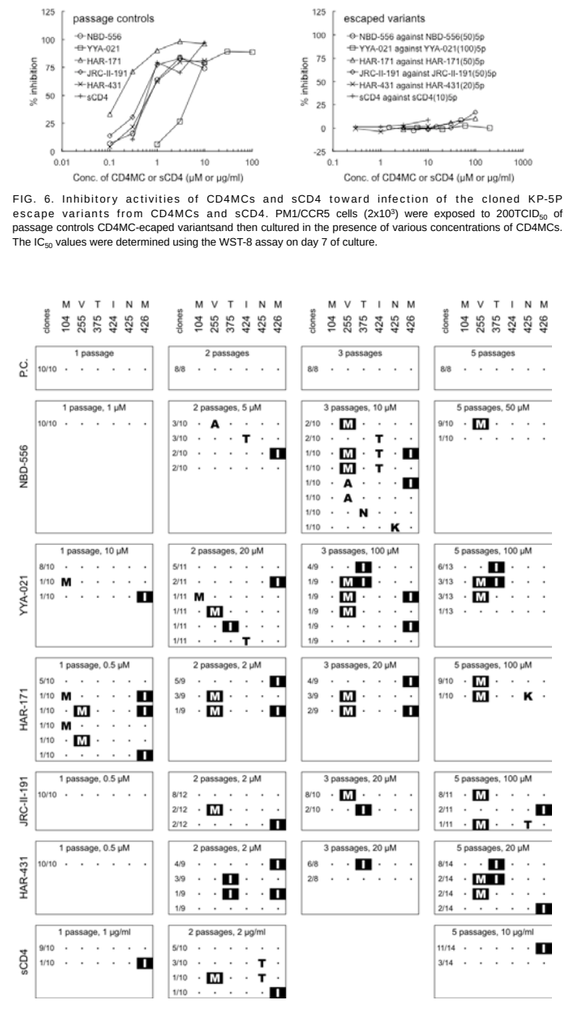


FIG. 6. Inhibitory activities of CD4MCs and sCD4 toward infection of the cloned KP-5P escape variants from CD4MCs and sCD4. PM1/CCR5 cells ($2x10^5$) were exposed to $200TCID_{50}$ of passage controls CD4MC-ecaped variantsand then cultured in the presence of various concentrations of CD4MCs. The IC_{50} values were determined using the WST-8 assay on day 7 of culture.

FIG. 7. Time course of emergence of CD4MC-resistant mutants using the cloned KP-5P. The cloned KP-5Penv/NL4-3 viruses were serially passaged in the presence of CD4MCs. The condition of passage experiments are referred to as "5 passages, 50 μM", where "5 passages" stands for passage number, and "50 μM" is the concentration of CD4MC in the selection culture. V255M, T375I, and M426I substitutions are denoted by black rectangular boxes. Dots denote sequence identity. The numbers on the left show the numbers of clones with the listed sequence among the total number of clones tested.

Conclusions

V255M, T375I and M426I, which are located inside of the Phe43-cavity are key mutations for acquiring highly resistant ability to CD4MCs. The clones containing V255M or M426I substitutions became relatively resistant to the 4E9C compared to the wild type clone, whereas the clone with T375I showed low sensitivity to both 3D6 and 4E9C nMAbs. T375I may contribute to the closing of neutralizing antibody epitopes for CD4i and CD4bs nMAbs.

References

1. Zhao Q, Ma L, N. Neamati N, *et al.*, *Virology*, 339, 213–25, 2005.
2. Yoshimura K, Harada S, Tamamura H, Matsushita S, *et al.*, *J. Viol.* 84, 7558–68, 2010.
3. Narumi T, Yoshimura K, Harada S, Matsushita S, Tamamura H, *et al.*, *Bioorg. Med. Chem.*, 19, 6735-42, 2011.
4. Kwon YD, Finzi A, Sodroski JG, Kwong PD, *et al.*, *Proc. Natl. Acad. Sci. USA*, 109, 5663-8, 2012.