Abstract no # 589

Enhanced Neutralization of HIV-1 with Fusion Inhibitor Resistant Mutations

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Introduction

The membrane fusion is an important step to successful viral infection which has been recognized as a therapeutic target for HIV/AIDS. Effect of mutations resistant to Enfuvirtide on neutralization efficiency and envelope structure has been studied before (reported previously). Several novel fusion inhibitors, such as C34, SC34 and SC34EK, have been developed and shown higher genetic barrier for resistance induction than Enfuvirtide.

Aim and objective of the study

The objective of this project is to investigate the sensitivities of novel fusion inhibitor-resistant HIV-1 mutants to several broad and conventional neutralizing antibodies.

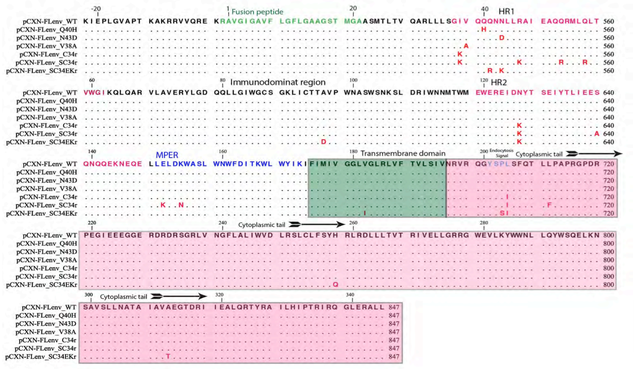


Fig1. Distribution of fusion inhibitor resistant mutations in functional domains of gp41

Materials and methods

Fusion inhibitors: Enfuvirtide (T20), C34, SC34, SC34EK.

Resistant Mutants: Fusion inhibitor resistant envelope expression plasmids were prepared by site directed mutagenesis in the gp41 using HIV-1 JR-FL$_{WT}$ *env* gene. All mutants confer resistance to fusion inhibitors with respect to JR-FL wild type.

Pseudovirus preparation: 293T cells were co-transfected with plasmids to express Env and pSG3ΔEnv.

Neutralization Assay: Neutralization phenotype of resistant mutations were investigated using TZM-bl cell and monoclonal antibodies (MAbs) against CD4 binding site (CD4bs), V3, CD4-induced site (CD4i), gp41 membrane proximal external region (MPER) and gp41.

Flow cytometry: Binding of the antibodies with each resistant mutant HIV-1 Env expressed on 293T cells were analyzed by flow cytometry. Env binding with each antibody was normalized by measuring mean fluorescence by per molecule of 2G12.

Results

Fusion inhibitor resistant mutants showed reduced infectivity.

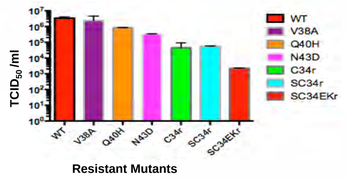


Fig2. All mutants showed reduced infectivity compared to that of wild type indicating effect of mutations in fitness defect.

Enhancement of neutralization sensitivity by gp41 mutations:
T-20 resistant mutant N43D showed enhanced neutralization by 4E10 (3.5×), 10E8 (12×) and also by anti-V3 antibodies, 1C10 (3.1x) and KD247 (4.3x). SC34EK-resistant virus was significantly sensitive to anti-MPER antibodies (4E10; 22.8x, 2F5; 20.6x, 10E8; 12.4x). MAb 49G2 and 42F9, which fail to neutralize the wild type JR-FL pseudovirus, neutralized C34 and SC34 resistant mutants. Anti-V3 MAbs neutralized C34, SC34 and SC34EK resistant mutants with 3 to 10 fold lower IC_{50} values than that of wild type. 4E10 and 2F5 failed to neutralize SC34 resistant mutants probably due to mutations in MPER region (Fig3 and Table1).

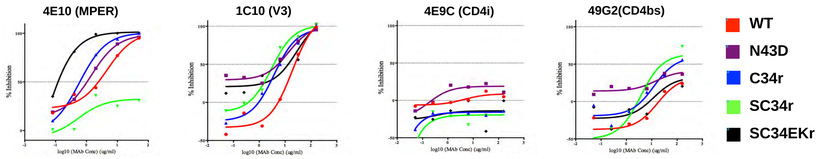


Fig3: Neutralization curves showing neutralization of fusion inhibitor resistant mutants by MAbs against major epitopes on HIV-1 envelope glycoprotein. Experiments were repeated three times and representative of each experiment has been shown.

	gp41	gp41 MPER			CD4bs					V3			CD4i		
gp41 mutants	2E8S34	4E10	2F5	10E8	b12	VRC01	49G2	82D5	42F9	1C10	KD247	16G6	916B2	4E9C	17b
V38A	>100	NA	NA	0.366	0.03	0.05	>200	>100	>150	6.752	7.599	>100	>100	>50	>50
Q40H	>100	NA	NA	0.260	0.01	0.03	>200	>100	>150	5.876	7.494	>100	>100	>50	>50
N43D	>100	0.66	0.43	0.042	0.02	0.04	>200	>100	>150	4.556	1.922	>100	>100	>50	>50
C34r	>100	0.58	0.41	0.294	0.01	0.02	33.4	>100	27.505	2.770	2.068	13.44	>100	>50	>50
SC34r	>100	>50	>50	0.070	0.03	0.05	22.3	>100	21.311	2.290	1.345	7.13	>100	>50	>50
SC34EKr	>100	0.101	0.041	0.041	0.02	0.07	77	>100	>150	2.580	1.288	13.2	>100	>50	>50
JR-FL WT	**>100**	**2.30**	**0.84**	**0.506**	**0.011**	**0.098**	**100**	**>100**	**>150**	**14.314**	**8.317**	**74.15**	**>100**	**>100**	>50

gp41 mutants	2E8S34	4E10	2F5	10E8	b12	VRC01	49G2	82D5	42F9	1C10	KD247	16G6	916B2	4E9C	17b
V38A	NA	NA	NA	1.4	0.4	1.9	NA	NA	NA	2.1	1.1	NA	NA	NA	NA
Q40H	NA	NA	NA	1.9	0.8	2.8	NA	NA	NA	2.4	1.1	NA	NA	NA	NA
N43D	NA	3.5	1.9	12.0	0.5	2.3	NA	NA	NA	3.1	4.3	NA	NA	NA	NA
C34r	NA	4.0	2.0	1.7	0.8	4.0	3.0	NA	5.45	5.2	4.0	5.5	NA	NA	NA
SC34r	NA	NA	NA	7.2	0.4	1.9	4.5	NA	7.04	6.3	6.2	10.4	NA	NA	NA
SC34EKr	NA	22.8	20.6	12.4	0.6	1.4	1.3	NA	NA	5.5	6.5	5.6	NA	NA	NA

Table1. Neutralization sensitivity (IC_{50} µg/ml) of fusion inhibitor-resistant mutants to various MAbs (top section). Fold change of IC_{50} (WT/mutant) of fusion inhibitor resistant mutants to various MAbs (Bottom section). (Highlight: red >10, yellow > 3, Orange - Neutralization phenotype appeared in mutants against weekly neutralized antibodies)

Antibody binding analysis by FACS:

	V3 loop MAb			CD4 binding site MAb		
Mutants	KD247	16G6	1C10	49G2	42F9	VRC01
V38A	0.9	1.0	0.9	1.0	1.0	1.03
Q40H	1.0	1.0	0.9	0.9	1.2	1.10
N43D	0.9	0.9	0.8	0.9	1.1	1.00
C34r	1.7	1.9	3.0	2.7	3.3	1.52
SC34r	1.3	1.6	2.1	2.2	2.3	1.43
SC34Ekr	1.5	1.8	1.3	2.2	3.1	1.54
175P	5.4	7.4	13.9	5.3	5.5	1.29

Fold Change Color: 1.0-1.5 | 1.5-2.0 | 2.0-2.5 | 2.5-3.0 | >3.0

Table2. Antibody binding with cell surface expressed envelope showed C34r, SC34r and SC34EKr resistant mutants have increased binding. Overall week correlation was observed between neutralizing and binding activities (r = 0.383, P = 0.0213).

Effects of each C34r mutants: JRFL mutant envelope bearing single C34r associated mutants (I37K, N126K, L204I) were prepared by site-directed-mutagenesis. Effect on viral infectivity and binding with 1C10 (anti-V3), 42F9 (anti-CD4bs) and VRC01 (anti-CD4bs broad neutralizing antibody) was analyzed (Fig4 and Fig5).

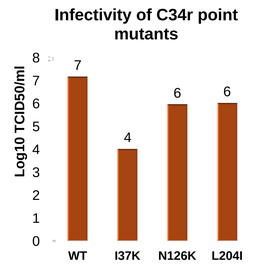


Fig4. C34r single mutants showed lower infectivity compared to WT. I37K mutant has almost 3 log reduction in infectivity.

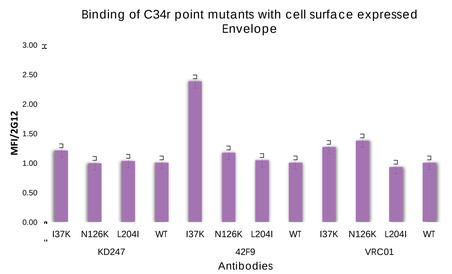


Fig5. I37K showed about 2.5 fold increased binding with CD4bs conventional MAb 42F9. On the other hand N126K and L204I dose not have any significant difference in binding.

Conclusion

- Here we report, the enhancement in the neutralization sensitivity of novel fusion inhibitors (C34, SC34, SC34EK) resistant mutants against gp41-MPER, CD4bs and V3 epitopes.
- All anti-V3 antibodies tested showed increased sensitivities against mutant resistant to C34, SC34 and SC34EK resistant mutants. This data suggest that changes in gp41 influence V3 epitope on gp120.
- Weak correlation was observed between neutralizing and binding activities.
- Non-neutralizing MAb (42F9) turned out neutralizing against fusion inhibitor resistant mutants (C34r, SC34r).
- Next generation fusion inhibitors and MAbs could be a potential combination for future regimen of combined antiretroviral therapy.

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