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Using EMPIRIC saturation mutagenesis to identify novel Env trimers for vaccines

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Background

Protective HIV-1 vaccines may need to induce antibodies that neutralize diverse HIV-1. A major target for cross-reacting neutralizing antibodies (nabs) is the CD4 binding site (CD4bs) on the envelope glycoprotein trimer (Env). There is an urgent need to identify novel Env trimers where the CD4bs is optimally displayed without exposing immunogenic sites that induce non-neutralizing antibodies or strain specific nabs.

We used a novel saturation mutagenesis approach, EMPIRIC (Exceedingly Meticulous and Parallel Investigation of Randomized Individual Codons) to investigate effects of the CD4 binding loop region on trimer structure and to identify Env mutants with enhanced exposure of the CD4bs. This region of Env contains (1) CD4 contact sites, (2) residues that modulate trimer opening and (3) sites that influence macrophage-tropism. Here, we describe Env mutants that carry a more exposed CD4bs yet differ in the exposure of the V3 loop at the trimer apex

Materials and Methods

We used EMPIRIC saturation mutagenesis to create two plasmid libraries containing all possible mutations for aa 361-370 and 371-380. Libraries were cloned into LN40 env, an R5 non-macrophage-tropic Env cloned from an AIDS patient lymph node, and incorporated in a replication competent pNL4.3 chimera.



Figure 1: The CD4 binding loop in LN40 Env. Red stars, CD4 contact residues; blue circles, residues lining the Phe43 cavity.

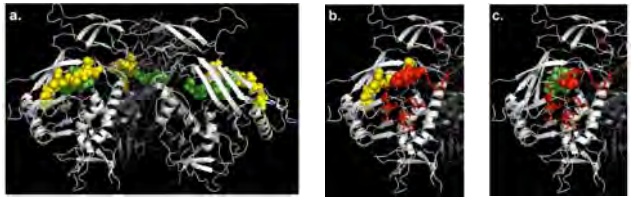


Figure 2: Structure of the HIV-1 Env trimer (4NCO). a) The yellow and green spheres are residues form 361-370 and 371-380, respectively. b) and c) In red, we represent the CD4 binding loop. V1-V2 and V3 loops are shown in grey at the apex of the trimer.



Figure 3: LN40/NL4.3 libraries were transfected into 293T cells and progeny virus rescued. This virus was used to infect PBMCs and the replication fitness of each mutant estimated by deep sequencing progeny virus.

Env mutants that conferred wt replication or higher were investigated for their properties via Env+ pseudoviruses using Env mabs via neutralization and a trimer binding assay.

Acknowledgements

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Results

The effect of specific Env mutations on Env structure and neutralization sensitivity, structure and function

We studied 33 mutations that were selected at wt levels or higher in PBMCs. We evaluated changes in Env structure by testing the sensitivity of Env+ pseudovirions neutralization by sCD4 and mabs including b6, 447-52D and 17b. Results of 13 mutants are shown in Table 1. Several substitutions enhanced exposure of CD4bs and V3 loop.

LN40 Env wt and mutants			IC50s (μg/ml) sCD4	447-52D (V3 crown)	b6 (CD4bs)	17b (CD4i)
LN40 wt			>50	40	>50	>50
373	R373K	b	>50	35.2	>50	nt
	R373M	w	>50	>50	>50	nt
	R373Q	b	44.2	25.2	>50	nt
	R373E	b	23.9	2.4	>50	>50
	R373N	b	43.9	12.9	>50	>50
375	S375H	b	15.3	>50	>50	nt
	S375T	b	38.2	>50	>50	nt
	S375F	b	7.6	>50	>50	nt
	S375W	b	8.7	>50	>50	>50
	S375Y	b	5.9	>50	>50	>50
377	N377V	b	19.0	1.3	>50	>50
380	G380A	w	32.0	<0.2	33.5	>50
	G380P	b	21.7	<0.2	15.3	>50

Table 1: The effect of LN40 mutations identified by EMPIRIC on Env structure and neutralization. We used direct mutagenesis to introduce each mutation into an LN40 Env+ expression vector, prepared Env+ pseudoviruses and tested neutralization with sCD4 and different mAbs. In this table, we represent the IC50 of mAb for each Env mutant.

>100 fold
<4<100 fold
>2-<4 fold

b: beneficial mutation
w: wt-like mutation
nt: not tested

The effect of substitutions of residues 375, 377 and 380 on diverse Envs

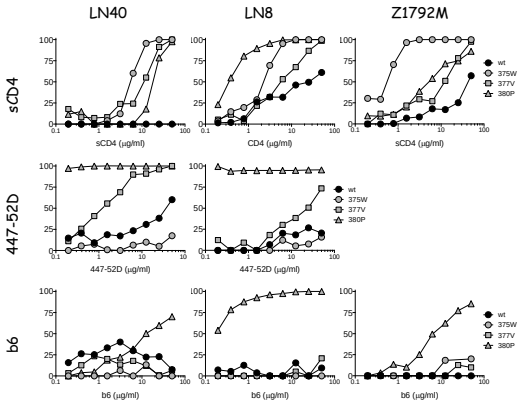


Figure 4: Substitutions at residues 375, 377 and 380 confer similar effects on clade B LN40, LN8 and clade C Z1792M Envs. Neutralization profiles for sCD4, 447-52D and b6 are shown. Each substitution had similar effects on the three different Envs. consistently increasing sensitivity to sCD4. However, only 380P conferred sensitivity to mab b6. 377V and 380P exposed the V3 loop crown (447-52D epitope), while 375W didn't. Of note, Z1792M does not carry the 447-52D epitope.

Env substitutions 375W, 377V, and 380P modulated the PG9 epitope at trimer apex

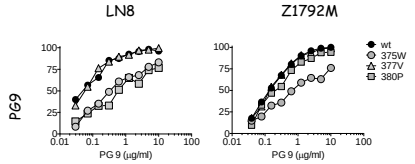


Figure 5: Env substitutions proximal to the Phe-43 cavity, reduced sensitivity to V2q, mabs, PG9, consistent with a more open trimer association domain(TAD) and trimer.

375W and 380P enhance macrophage infection

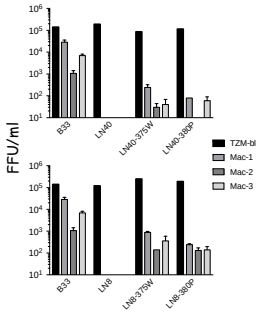


Figure 6: Macrophage infection mediated by LN40 and LN8 Env mutants 375W and 380P. Macrophage infectivity was measured using monocyte derived macrophages from three independent donors. 375W and 380P mutants (but not wt) Envs conferred infection of macrophages by Env+ pseudoviruses. However, infectivity was substantially lower than that of a pseudovirus carrying B33 Env, an R5 mac-tropic Env.

375W and 380P both increase exposure of the CD4bs (Table 1) and both enhanced macrophage infectivity (Fig 6). However, the effect was modest indicating that additional changes are need for full macrophage infection.

Summary

Env region	Substitution	Specificity	Affected Env region: CD4bs	V3 loop[1]	TAD V2q epitope[2]	2G12 glycans
Phe-43 cavity	375W	cross clade	more exposed	still hidden	reduced	shifted
CD4 binding loop, C-terminal flank	377V	cross clade	more exposed	exposed	reduced	shifted
	380P	cross clade	more exposed	highly exposed	reduced	shifted

1. Not tested on Z1792M.; 2. Not tested on LN40.

Our data indicate that the residues identified play an important role in enhancing CD4 binding and/or regulating trimer opening

Conclusion

We identified specific residues in the CD4 binding loop that expose the CD4bs, yet differ in their effects on the trimer apex and V3 loop exposure in clade B and C Envs. Env mutants that enhance the exposure of the CD4bs yet maintain a closed trimer structure with the V3 loop occluded may be desired immunogens for inclusion in vaccines aimed at eliciting CD4bs nabs.