DONOR-DERIVED ENV MUTATION SCANNING REVEALS ANAB AND BNAB VULNERABILITIES

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BACKGROUND

Rapid viral evolution during untreated HIV-1 infection results in escape from autologous neutralizing antibodies (aNAbs). Escape often occurs within variable regions of the env gene, and at known epitopes of broadly neutralizing antibodies (bNAbs). Following ART initiation, viral evolution ceases, but aNAb activity persists against some viral variants in the latent reservoir. We hypothesized that identifying common escape mutations to aNAb will offer insight into the efficacy of passive and active immunization strategies.

METHODS

We implemented site-directed mutagenesis[1] (SDM) to alter a specific amino acid through the introduction of a mutation as laid out in **Figure 1A**. Substitutions were introduced via PCR and plasmids were transformed in E Coli. Mutation fidelity was verified by Sanger sequencing. Plasmids were then transfected into HEK 293T cells with an NL4-3Δ *env* backbone. Pseudovirus (PsV) was then produced and titered over Tzm-bl cells (Fig. 2). Neutralization assays were performed using wild-type (parent) and mutated PsV. PsV were incubated with either autologous, purified IgG (aNAb) from donor plasma or a broadly neutralizing antibody (bNAb). **Figure 1B** demonstrates the overall experimental protocol, with the primary readout being luminescence following culture with IgG and PsV.

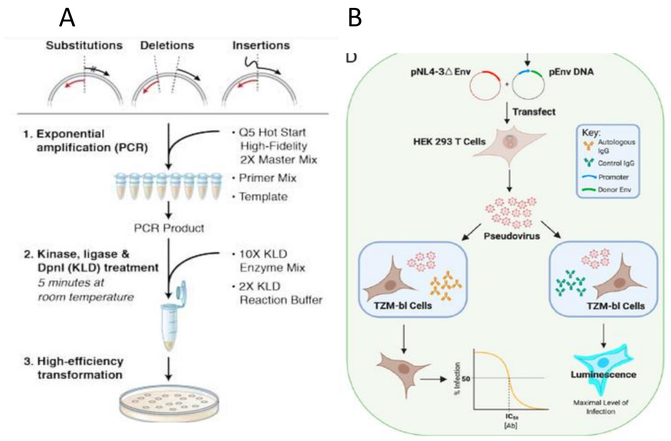


Figure 1. Experimental Methods (A) Site-directed mutagenesis[1] (B) Pseudovirus production and Tzm-bl based neutralization assay

Site-specific mutation of patient-derived envelope impacts bNAb and aNAb potency. This investigation provides insight into how mechanisms of aNAb escape are archived in the latent reservoir and has implications for immunotherapy-based approaches for an HIV cure.

RESULTS

Specific amino acid signatures are associated with resistance to aNAbs

A

PTID 12 analyses

Patient	Isolate	FASTA Seq name	Auto. IC50 ug/mL	289	291	379	386	411	332	340	343	388	455
12	UD.B42A	012.ST.B08B.39	2.3	K	P	R	O	E	K	N	R	T	T
12	ST.B28	012.ST.B28X.01	7	K	P	R	O	E	K	N	R	T	T
12	ST.B12	012.ST.B12X.02	20.4	O	S	G	O	G	O	D	R	T	I
12	PP.B35	012.PP.B35X.01	33	O	S	G	N	A	O	N	K	A	T
12	AB.B14	012.AB.B14X.01	33.1	O	S	G	N	E	N	N	K	A	T
12	AB.B11	012.AB.B11X.01	37.2	O	S	G	N	A	O	N	K	A	T
12	UD.A06B	012.UD.A68B.01	39.7	K	P	R	D	V	I	D	R	T	I
12	UD.A05	012.UD.B41B.04	46	O	S	G	N	E	O	N	K	A	T
12	AB.A06	012.AB.A06X.01	49.4	O	S	G	N	E	O	N	K	A	T
12	ST.B17	012.ST.B17X.01	59.9	O	S	G	N	E	O	D	R	T	I
12	ST.B13	012.ST.B13X.04	83.3	K	P	R	D	V	K	D	R	T	I
12	ST.A08	012.ST.A08X.01	120.7	K	P	R	D	G	N	D	R	T	I
12	UD.B37B	012.PP.A01A.05	171.2	O	S	G	N	E	O	N	K	A	T
12	AB.A07	012.AB.A07X.01	182.6	O	S	G	N	E	O	N	K	A	T
12	UD.B29A	012.UD.B29A.01	377	K	P	R	D	V	K	D	R	T	I

Red=Associated with Control
Blue=Associated with Ab resistance
O = glycan

B

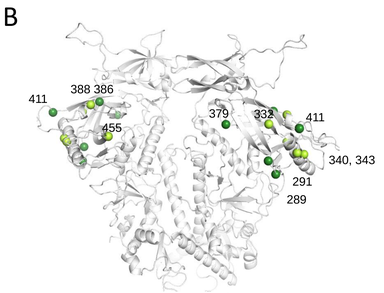


Figure 2. (A) Participant 12 isolates from extracted p24 positive outgrowth wells in a previously published study[2] (B) Model of trimeric HIV-1 envelope annotated with relevant positions

Neutralization data were collected from a previous study investigating the role of aNAbs in the suppression of outgrowth *ex vivo*[2]. Sequence data paired with neutralization data were used in a genetic signatures analysis[3]. **Figure 2A** shows the output of this analysis revealing relevant positions that are associated with antibody resistance in this analysis. **Figure 2B** represents those amino acid positions on a 3D model of the HIV envelope trimer. These amino acids are found mainly on the outer face of gp120 and some positions are suspected to be N-Linked glycosylated.

Mutation at N332 confers phenotypic change in model viral isolates

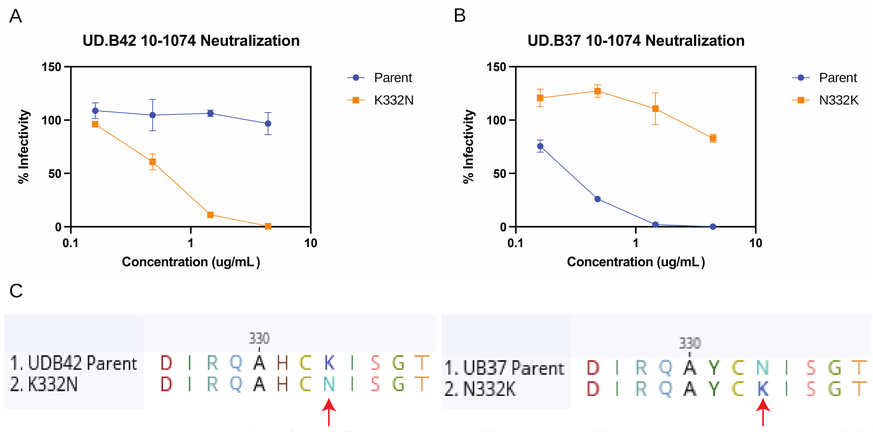


Figure 3. 10-1074 bNAb dose-response in Tzm-bl neutralization assay with (A) UDB42 and (B) UDB37 position 332 mutations. (C) Example of the amino acid mutation at position 332 in both UDB42 and UDB37 isolates.

Figure 3A shows that when a glycan is introduced at position 332, the bNAb 10-1074 can neutralize UDB42. **Figure 3B** shows that when a glycan is removed at position 332, 10-1074 is no longer able to neutralize UDB37. Figure 3C points to position 332 having the specified amino acid change. These results provide evidence that mutations induced through SDM can alter the phenotype of the viral isolates studied.

Phenotypic change induced in presence of aNAbs

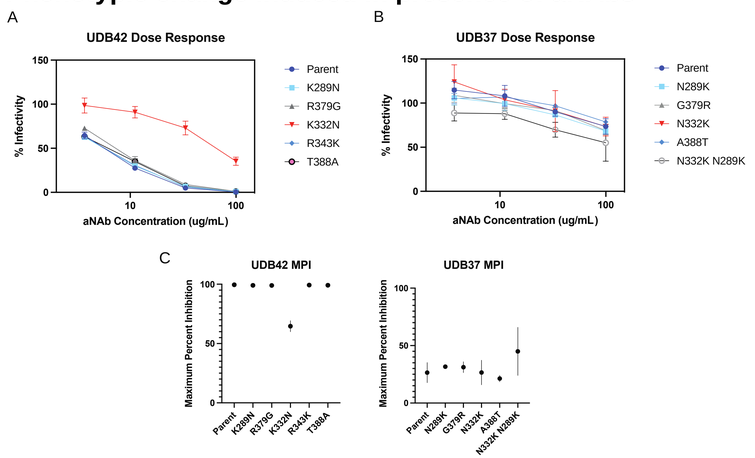


Figure 4. aNAb dose-response in Tzm-bl neutralization assay with mutations at specific sites in (A) UDB42 and (B) UDB37. (C) Maximum percent inhibition (MPI) at 100 µg/mL for parent and mutant viral isolates.

In the above experiments, aNAbs were isolated from Participant 12 plasma. Single amino acid changes were investigated for their potential to mediate phenotypic change in model viral isolates. Most specific site mutations had no phenotypic change when compared to the parent envelopes. At the key position N332 in **Figure 4A**, the introduction of a glycan confers resistance to aNAbs in the UDB42 envelope. However, in **Figure 4B**, we demonstrate no phenotypic change when compared to the parent UDB37 in any single amino acid change. Moreover, removal of two glycans at positions 332 and 289 in isolate UDB37 induced no phenotypic change. **Figure 4C**, highlights the lack of detectable phenotype changes in many of the mutated envs using maximum percent inhibition. A stark decrease in MPI though is observed when a glycan is added at position 332 in env UDB42. These results suggest that a neutralizing epitope in UDB42 may be occluded by the addition of a glycan at position 332.

CONCLUSIONS

- We have a robust process to introduce mutations in patient-derived envelope.
- Mutation at a known bNAb epitope induced a phenotypic change in bNAb potency
- The addition of a glycan at position 332 in a sensitive viral isolate, UDB42, confers resistance by aNAb, but removal of a glycan at position 332 and 289 in a resistant viral isolate, UDB37, does not sensitize to aNAb.
- Future studies using this approach will be key to understand how aNAb escape is archived in the reservoir, and what therapeutic strategies can be used to target aNAb resistant envelopes

ADDITIONAL KEY INFORMATION

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Additional Resources
1. https://www.neb.com/products/e0554-q5-site-directed-mutagenesis-kit#Product%20Information
2. Bertagnolli, L. N., et al., (2020). Autologous IgG antibodies block outgrowth of a substantial but variable fraction of viruses in the latent reservoir for HIV-1. PNAS, 117(50)
3. https://www.hiv.lanl.gov/content/sequence/GENETICSIGNATURES/gs.html