Studying Dual Role of Glycosylation in Resistance to Broadly Neutralizing Antibodies In Vitro

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Background

The envelope (Env) protein of HIV evades neutralization by antibodies in part by varying glycosylation patterns on Env's surface. One of these key glycosylation sites is at amino acid position 276 in the CD4 binding site. Canonically, a mutation at this site causing the removal of the glycan confers sensitivity to CD4 binding site bNAb, VRC01. In recent viral escape experiments, we conversely determined increased resistance to VRC01 with the removal of this glycan depending on the HIV Env in question.

Methods

The glycan mutation was observed after *ex vivo* CD4+ T cells were infected with NL4.3 expressing a subtype AC Env 246.F3, and the virus replicated in the presence of increasing concentrations of VRC01. Mutations were determined using single genome sequencing (SGS) and tested for bNab resistance in the Tzm-Bl single round neutralization assay. To test the effect of removing glycan 276 in different virus subtypes, the N276D mutation was introduced into each Env of the global panel (DeCamp et al.). Mutant and wildtype plasmids tested for bNAb sensitivity in the pseudovirus neuralization assay. IC50s were compared between wildtype and mutant to calculate fold changes. Because of documented 3-fold variation in the assay, changes >3 were deemed to be more resistant and <0.333 to be more sensitive.

Results

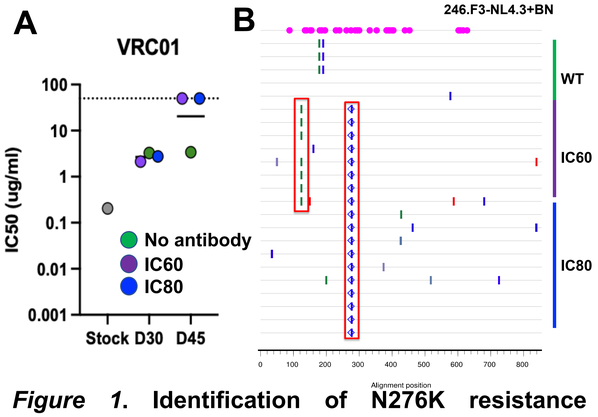

Figure 1. **Identification of N276K resistance mutation.** (**A**) Outgrowth viral supernatant from cells infected with 246.F3 and replicated in the presence or absence of VRC01 was tested for sensitivity to VRC01. IC50s are graphed. Dotted line indicates the highest bNAb concentration tested (50 µg/mL). Solid lines denote geometric mean. (**B**) SGS was performed to generate *env* sequences. Alignments were analyzed using the LANL Highlighter tool. Fuchsia dots represent glycosylation sites. Color ticks indicate an amino acid difference from stock virus. Diamonds indicate a lost glycan. Red boxes denote fixed mutations.

Results

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A

Mutant IC50			Antibody															
			VRC01		VRC07-523		3BNC117		VRC13		N6		10E8v4-V5R		PGDM1400		10-1074	
			CD4 binding site		CD4 binding site		CD4 binding site		CD4 binding site		CD4 binding site		gp41		V2 Apex		V3 Glycan supersite	
	Virus	*Subtype*	WT	N276D	WT	N276D	WT	N276D	WT	N276D	WT	N276D	WT	N276D	WT	N276D	WT	N276D
Virus	CNE55	01_AE	0.4	0.05	0.1	0.027	0.104	13.48	0.386	2.24	0.34	0.05	0.01	0.05	1.19	0.01	0.03	50.00
	CNE8	01_AE	0.4	0.204	0.3	0.051	0.367	0.91	0.259	0.82	0.12	0.05	0.04	0.24	0.01	0.01	50.00	50.00
	CH119	07_BC	0.3	1.85	0.1	0.195	0.227	50.00	0.121	3.23	0.05	0.19	0.03	0.36	50.00	0.93	0.02	0.18
	BJOX00200	07_BC	2.0	26.5	0.1	25.1	2.460	50.00	0.992	27.78	0.22	0.29	0.19	0.49	0.21	50.00	0.07	0.11
	398.F1	A1	50.0	0.06	41.6	0.05	50.000	0.38	50.000	0.38	0.98	0.08	0.14	0.75	50.00	33.14	0.10	0.05
	246.F3	A1C	0.2	4.81	0.0	1.21	16.883	50.00	5.375	50.00	0.04	0.69	0.01	2.17	0.01	0.24	50.00	50.00
	TRO.11	B	0.5	11.8	0.2	0.173	0.412	0.107	4.595	1.77	0.23	1.88	0.00	0.25	0.00	3.75	0.08	0.18
	X2278	B	0.2	0.03	0.1	0.041	0.110	0.04	0.126	0.15	0.10	0.00	0.07	0.13	0.01	25.12	0.01	0.07
	25710	C	0.5	0.222	0.0	0.060	0.036	50.00	0.014	2.07	0.21	0.02	0.01	0.13	0.01	0.02	50.00	0.10
	CE0217	C	2.1	0.06	0.2	0.038	0.338	0.07	1.093	0.31	0.46	0.05	0.13	0.91	0.01	0.13	50.00	0.05
	CE1176	C	4.3	16.4	0.6	0.091	0.451	0.06	1.850	1.57	0.78	0.06	0.03	0.05	0.28	25.02	0.05	0.04
	X1632	G	0.1	0.07	0.0	0.007	0.010	1.13	0.024	2.73	0.04	0.04	0.01	0.05	0.02	25.22	0.01	50.00

mAb concentrations µg/ml:
<0.100
0.100-1.00
1.00-10.0
10.0-50.0
>50.0

B

IC50 Fold Change		*Antibody*							
Virus	*Subtype*	VRC01	VRC07-523	3BNC117	VRC13	N6	10E8v4-V5R	PGDM1400	10-1074
398.F1	A1	0.217	0.58	1.69	3.2	1.49	22.7	0.663	1.96
246.F3	A1C	11.4	3.89	136	193	5.9	51	34.9	1.00
CNE55	01_AE	0.090	0.609	380	165.8	0.248	8.58	1.60	1.00
CNE8	01_AE	0.099	0.205	2.68	0.75	0.105	1.93	1.33	1.00
TRO.11	B	33.5	1.57	1.02	4.58	5.54	31.5	3.14	5.83
X2278	B	0.181	3.68	4.40	6.15	0.095	20.9	1322	7.67
CH119	07_BC	0.918	1.39	20.33	3.25	0.87	1.87	4.33	2.51
BJOX02000	07_BC	0.530	0.605	1.00	0.556	0.300	3.44	1.00	1.2
CE1176	C	0.24	0.155	0.139	0.851	0.074	1.81	125	0.80
25710	C	0.434	0.326	121	0.450	0.089	129	8.00	1.32
CE0217	C	0.248	0.475	0.650	2.48	0.52	12.75	18.6	4.38
X1632	G	0.302	0.333	0.067	0.508	1.05	3.29	2910	1.00

Fold-Change
<0.333
0.334-2.99
>3

Figure 2. **Effect of removing N276 glycan within global panel Envs. (A)** Pseudoviruses of each Env containing an N276D mutation were tested along with wildtype Envs for sensitivity a panel of 8 bNAbs targeting 4 sites on the virus. IC50s are shown and color-coded according to legend. (**B**) Fold change between mutant and wildype IC50 was calculated and variations greater than 3-fold are colored according to the legend. We observe that loss of the glycan can confer resistance or increased sensitivity to various CD4bs bNAbs depending on the Env.

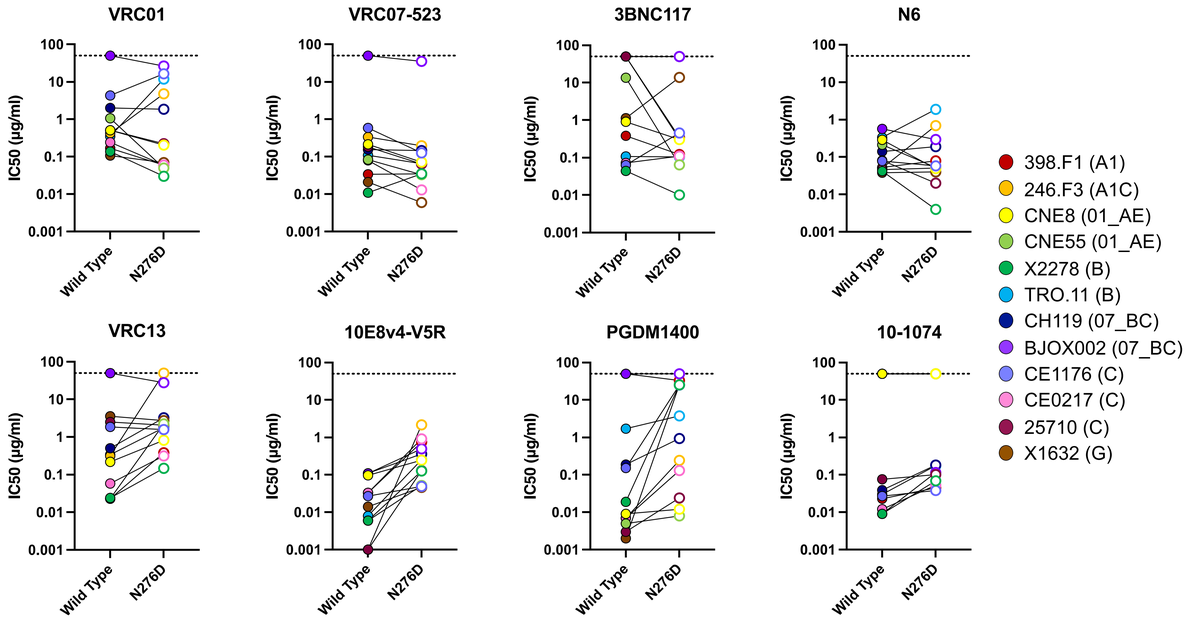

Figure 3. **Change in IC50s of N276D mutants versus wild type envelopes of deCamp et al. global panel**. IC50 values shown in figure 2A are shown here graphed by antibody to show differential role of glycan removal in multiple CD4 binding site antibodies.

Conclusions

Previous research has shown that the removal of the 276 glycan confers increased sensitivity or no change in IC50 against bNAb VRC01 (Lynch et al.). Preliminary experiments demonstrated that the removal of the N276 glycan conferred complete resistance of subtype AC Env 246.F3 to VRC01. In these experiments, we showed that the N276D mutation in the CD4 binding site, resulting in the removal of the glycan at that position, implicated a dual role for glycosylation in sensitivity to VRC01 class bNAbs. We showed that when this mutation was conferred in the envelopes of the deCamp et al global panel, there was a range of fold changes in IC50s against VRC01 class antibodies, inducing both increased sensitivity and increased resistance depending on the envelope. This shows that there is a dual role of glycosylation at position 276 in bNAb neutralization at the CD4 binding site. In future experiments, we intend to determine the structural impacts of the removal of this glycan to more deeply understand the changes in neutralization profiles against not only VRC01 class antibodies, but also the off target bNAbs also tested in these experiments.

References

- deCamp A, et al. (2014) Global panel of HIV-1 env reference strains for standardized assessments of vaccine-elicited neutralizing antibodies. *Journal of Virology* 88(5):2489–2507.
- Lynch RM, et al. (2015) HIV-1 fitness cost associated with escape from the VRC01 class of CD4 binding site neutralizing antibodies. *Journal of Virology* 89(8):4201–4213.

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DC CFAR District of Columbia Center for AIDS Research