Viral escape from Broadly Neutralizing Antibodies in non-subtype B viruses.

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Background

Broadly neutralizing antibodies (bNAbs) provide a useful tool for HIV cure strategies because of their ability to target conserved regions on the envelope (Env) protein in the context of both virions and infected cells. Multiple clinical trials infusing both mono- and combination bNAb therapies into people living with HIV demonstrated transient viral suppression after infusion. The major obstacle to more effective treatment, however, continues to be the ease of viral escape. In order to understand escape, we are determining common escape pathways from bNAbs to better inform future combination trials

Methods

Viral stocks were isolated from HIV-infected PBMC obtained from the U.S. Military HIV Research Program (MHRP). Briefly, limiting dilutions of donor derived PBMC were cultured in the presence of uninfected *ex vivo* target CD4⁺ T cells for 7 days in a 96-well plate. Cultures were then tested for p24 and dilutions that fell within a Poisson distribution were expanded in a T-25 flask and harvested every 48 hours for 6 days. Virus sequences were characterized via single genome sequencing and input into the LANL RIP program to determine viral subtype. Neutralization profiles were determined against 8 bNAbs to be used in downstream *in vitro* resistance assays. Resistance assays were performed by infecting *ex vivo* CD4⁺ cells with chosen donor-derived viral stocks in the presence of varying susceptible bNAb concentrations. Cultures were maintained for 56 days with suboptimal concentrations of bNAbs that were slowly increased to induce escape. Replication kinetics as well as genotypic and phenotypic measures of bNAb resistance were measured throughout as described in figure 3.

Results

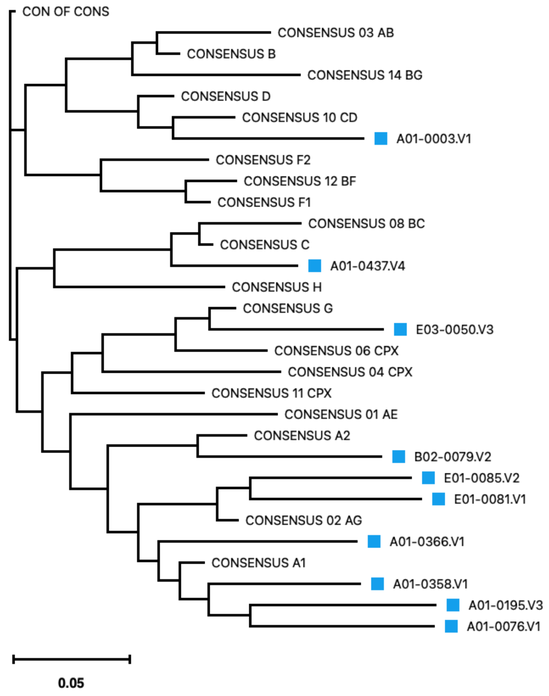


***Figure 1*. Phylogenetic tree of isolated MHRP viral stocks.** Consensus sequences of isolated MHRP viral stocks were first MUSCLE aligned in Geneious with LANL 2004 subtype consensus alignment. A maximum-likelihood tree was calculated in MEGA and rooted on the Con of con. All viruses grouped with the same viral subtype consensus sequence as determined by the LANL RIP program. Isolated viruses are denoted by a blue square.

Results

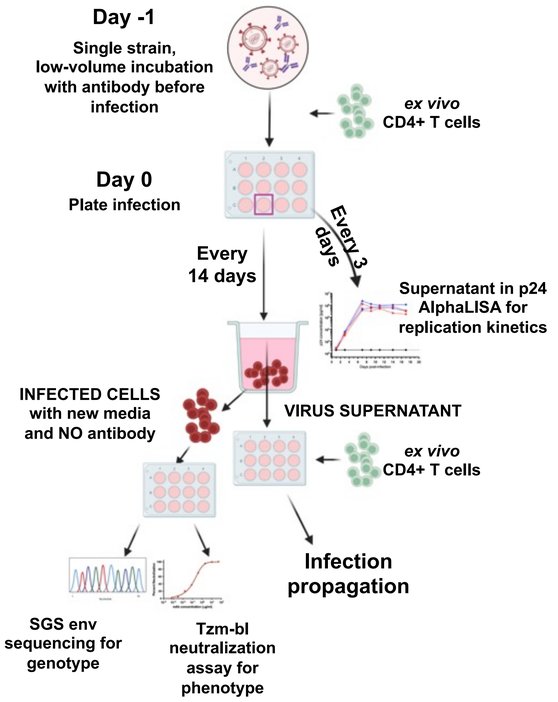


***Figure 3*. Schematic of *in vitro* viral resistance assay.** Virus and bNAb pairing are incubated for 30min. before a high MOI standing infection of pre-stimulated CD4 enriched *ex vivo* T cells. Infections are plated and monitored for replication kinetics by measuring p24 every 3 days. Every 14 days, supernatant is used to infect new CD4 T cells for infection propagation. New media without any bNAb is added to infected cells for virus to grow for 24 hours. This cell free/antibody free virus is then measured for resistance phenotypically and genotypically.

Results

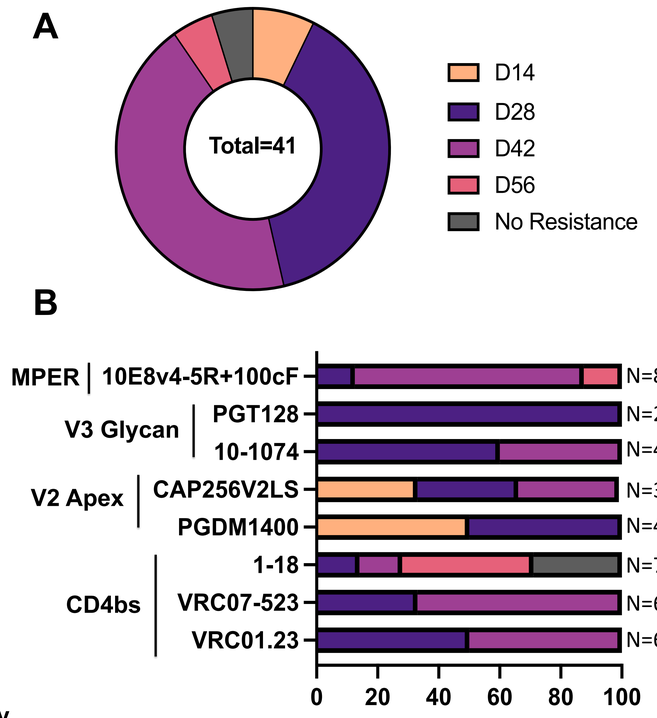


***Figure 4*. Time to resistance of MHRP viral panel in escape assay.** Overall time to resistance of antibody-virus pairings (A) shows most viruses generate resistance between D28 and D42. When broken down by bNAb (B), V2 apex antibodies were escaped from at earlier timepoints, on average, with CD4bs and optimized bNAbs taking more time for the virus to develop resistance. Of note is that CD4bs bNAb 1-18 was the only antibody in which certain viruses were unable to escape.

Results

		VRC07-0523		VRC01.23		1-18		PGDM1400		CAP256V2LS		10-1074		PGT128		10E8v4-5R+100cF	
Virus	**Subtype**	IC50	IC80	IC50	IC80	IC50	IC80	IC50	IC80	IC50	IC80	IC50	IC80	IC50	IC80	IC50	IC80
A01-0358.V1	A1	4.16	9.90	0.457	1.26	0.150	0.450	0.137	0.452	50.0	50.0	50.0	50.0	0.273	3.14	0.116	7.59
E03-0050.V3	G	5.74	14.9	0.949	1.96	0.928	4.76	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	3.93	12.9
A01-0366.V2	A1D	0.153	1.19	0.084	0.168	0.471	1.79	50.0	50.0	0.00001	0.015	50.0	50.0	2.57	5.72	0.688	*50.0*
B02-0079.V2	A2	3.23	6.29	0.392	0.431	0.293	0.674	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	8.30	39.7
A01-0195.V3	A1	0.137	0.444	0.059	0.221	50.0	50.0	7.67	50.0	0.001	3.38	0.113	0.341	2.17	50.0	3.94	16.8
E01-0085.V2	CRF02_AG	50.0	50.0	8.99	29.5	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	3.48	37.3
E01-0081.V1	CRF02_AG	3.12	7.45	2.06	4.69	1.57	4.31	0.785	14.2	50.0	50.0	0.315	0.765	50.0	50.0	3.35	36.0
A01-0003.V1	D	50.0	50.0	1.83	3.96	8.11	35.3	0.100	0.395	0.189	7.90	50.0	50.0	50.0	50.0	50.0	50.0
A01-0437.V4	C	0.420	1.04	0.265	0.656	1.23	3.74	0.119	15.5	0.00001	0.015	0.035	0.086	0.200	0.904	0.363	1.46
A01-0076.V2	A1,D	2.34	12.6	0.308	3.53	0.594	5.09	50.0	50.0	0.0001	1.68	0.121	1.22	50.0	50.0	2.10	37.2
	Breadth	80%	80%	100%	100%	80%	80%	50%	40%	50%	50%	40%	40%	40%	30%	90%	80%
	Potency	1.22	3.85	0.546	1.48	0.806	3.05	0.397	2.51	0.0003	0.399	0.111	0.407	0.743	2.53	1.65	16.2

***Figure 2*. Neutralization profiles of isolated MHRP viral stocks.** Isolated viral stocks were tested for sensitivity against a panel of 8 bNAbs targeting 4 sites on the virus. IC50s are shown and color-coded according to legend. Breadth was calculated by determining the percentage of bNAbs that the viral strain was susceptible to (IC50<50 ug/mL). Potency is the geometric mean of all IC50 values for a given strain.

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

Future Directions

Future experiments will expand this study to begin determining viral escape from combination bNAb . This reproducible *in vitro* bNAb escape assay will lead to a deeper understanding of viral escape, to better inform the design of highly effective bNAb cocktails targeting multiple conserved sites.

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