A three-step heterologous prime-boost immunization schedule results in development of cross-neutralizing VRC01-class antibodies

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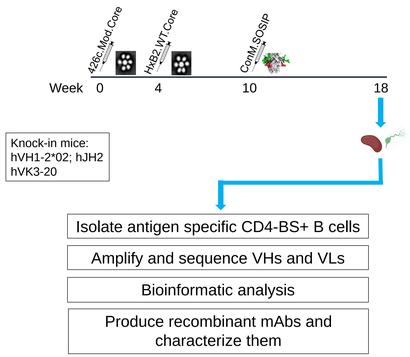
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BACKGROUND

HIV continues to be a major global public health issue with ~39.9 million people living with it at the end of 2023 (WHO). An effective vaccine is therefore needed, that can elicit diverse immune responses including broadly neutralizing antibodies (bnAbs). One such class of bnAbs that recognize the CD4-binding site on the HIV-1 Env are the VRC01-class of antibodies. They are derived from the pairing of VH1-2*02 antibody heavy chains with 5-amino acid long CDRL3 expressing light chains and have been isolated from several people living with HIV-1. They not only prevent infection of humanized mice by HIV-1 and of non-human primates by S(H)IV, but also prevented HIV-1 acquisition from susceptible viruses in two phase 3 clinical trials (HVTN 703/704). Hence, VRC01-class antibody elicitation is expected to be essential for an effective HIV-1 vaccine. Here, for the first time, we report on 'prime-boost' immunization regimens that lead to accelerated development of VRC01-class antibodies capable of neutralizing diverse heterologous, tier 2 HIV-1 viruses.

METHODS

Figure 1: Study design. Transgenic mice *(engineered by Fred Alt lab)* were immunized* at indicated time points with adjuvanted immunogens, bled every two weeks during the course of the study, and sacked for blood, spleen, and lymph node tissues, at week 18. Downstream analysis was carried out as shown in the schematic below.



RESULTS

Figure 2: VRC01-class antibodies isolated at the end of immunization display a wide range of Env reactivities, including against fully glycosylated Env trimers using Biolayer Interferometry (BLI) assay. The maximum signal obtained in the assay against each Env is plotted for the individual mAbs. Mature (blue) and germline (black) VRC01 bnAbs are included as internal controls.

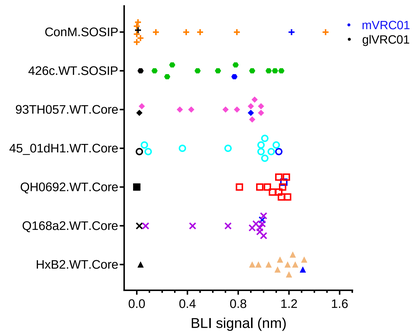


Figure 3: VRC01-class antibodies isolated at the end of the immunization display cross-neutralizing activities against tier 2 viruses. Shown values represent IC50 neutralization in µg/mL. Bold/shaded values indicate neutralizing activity. % neutralization on right indicates the percent of viruses neutralized, where purple is >20% neutralization and yellow is <20% neutralization of the total no. of viruses.

	ID50 (µg/mL)																
	426c	25710-2.43	Ce1176_A3	Ce703010217_B6	X1632_S2_B10	CNE55	246-F3_C10_2	BJOX002000.03.2	CH119.10	TRO.11	H703_0472_030s	H703_2805_080Es	H704_0128_220_RE_pb001_s	H704_0855_080_EsN	H704_1180_070EsN	H704_1535_030sN	% (n=16)
mAb	Clade C	Clade C	Clade C	Clade C	Clade G	CRF01_AE	Clade AC	CRF07_BC	CRF07_BC	Clade B	Clade C	Clade C	Clade F1/B	Clade B	Clade A1/B	Clade B	
G3R3-1	**1.48**	>50	>50	>50	>50	**3.21**	>50	>50	>50	>50	**0.76**	>50	**0.09**	**0.90**	>50	**0.94**	37.5
G3R3-2	**2.08**	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	**0.29**	>50	>50	**27.12**	18.8
G3R3-4	**18.83**	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	6.3
G3R3-7	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	**42.76**	6.3
G3R3-8	**1.03**	>50	>50	**2.60**	>50	>50	>50	>50	>50	>50	>50	**1.06**	**25.97**	>50	>50	>50	25.0
G3R3-9	**10.54**	**11.71**	>50	**7.97**	>50	>50	>50	>50	>50	**15.46**	>50	>50	**0.21**	>50	>50	>50	31.3
G3R3-10	**2.10**	**3.19**	>50	**5.10**	>50	>50	>50	>50	>50	>50	**13.97**	**0.46**	**3.40**	>50	>50	>50	37.5
CH01-31	2.23	0.28	0.07	0.09	0.11	0.15	0.15	*nd*	2.00	0.46	0.04	>25.0	0.39	0.22	0.06	0.12	
mVRC01	1.27	0.22	*nd*	*nd*	0.09	0.20	*nd*	36.63	0.62	0.24	0.06	>5.0	0.18	0.07	0.25	0.14	
gVRC01	>50	>50	*nd*	*nd*	>50	>50	*nd*	>50	>50	>50	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*	

Figure 4: G3R3-1, G3R3-9, and G3R3-10 exhibit great breadth. A summary of neutralizing activity with a larger panel (119 total) of tier 2 viruses for a select few VRC01-class antibodies isolated at the end of immunization is shown below.

VRC01 mAb	No. of viruses neutralized	%	No. of clades neutralized	%
G3R3-1	25/119	21	10/15	67
G3R3-9	29/119	24	10/15	67
G3R3-10	27/119	23	13/15	87

Figure 5: Comparison of number of amino acid changes in the HC and LC of paired sequences isolated post final immunization. Each symbol represents a paired sequence.

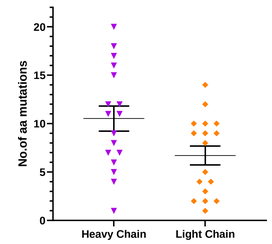
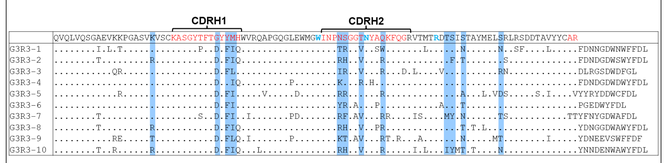


Figure 6: Comparison of type of amino acid changes in the HC of paired sequences isolated post final immunization. Highlighted residues are mutations found in mature, human, VRC01-class bnAbs.



CONCLUSIONS

- We isolate cross-neutralizing VRC01-class antibodies, after 3 immunizations.
- These mAbs neutralize up to 38% of heterologous tier 2 viruses including major HIV-1 clades.
- Our 'Prime-Boost' immunization schema leads to selection of AAs found in VRC01-class bnAbs.
- Overall, **VRC01-class antibodies with tier 2 cross-neutralizing activities are developed after only 3 immunizations.**

FUTURE DIRECTIONS

We are utilizing different approaches to further increase the breadth of the elicited cross-neutralizing VRC01 antibodies, including additional immunizations following the ConM.SOSIP immunization.

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