Potential Cross-Reactive HIV-1 Neutralization in Fusion Peptide-Primed SHIV-Infected Macaques

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BACKGROUND

An effective antibody-based HIV-1 vaccine will require the consistent induction of potent cross-reactive HIV-1-neutralizing responses. Natural HIV-1 infection can generate broad and potent neutralizing responses but does so in only a small fraction of those infected. Nonetheless, such responses and their constituent broadly neutralizing antibodies can serve as guides for HIV-1 vaccine design.

Vaccination with HIV-1 antigens can induce consistent responses in preclinical animal models. Epitope-focusing approaches have elicited consistent, but low titer, cross-clade neutralizing responses against multiple sites of vulnerability including the fusion peptide site[1-3]. However, consistent, broad, and potent neutralizing responses against HIV-1 have not been achieved by vaccination.

METHODS

To demonstrate feasibility towards the consistent, broad, and potent neutralizing responses, we combined vaccination targeting the fusion peptide-site of vulnerability with infection by simian-human immunodeficiency virus (SHIV)[4]. Such responses – especially if they could be consistently induced against a specific target site and deconvoluted into constituent monoclonal antibodies – could provide valuable guides for how the immune system can produce consistent, broad, and potent neutralizing responses against HIV-1.

Main Findings

SHIV-infection boosts neutralizing plasma responses to ~100 ID50 with 45-77% breadth on a 208-strain panel

A

Sample ID	Pre-SHIV (week70) BG505.N611Q ID_{50} or IC_{50}	Neut reduction with FP8v1
DJ85	2554	41.8%
GPZ6	940	14.1%
CR5N	326	8.6%
8L2H	784	5.4%
A23E	47	6.0%
TRNM	1116	62.8%
HERH	1075	10.3%
RD6A	1144	5.0%
JC69	46	4.5%
S3ZB	177	7.5%
VRC34.01	0.019	100.0%
VRC01	0.0840	4.9%

B

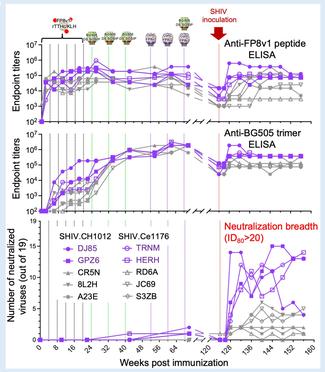
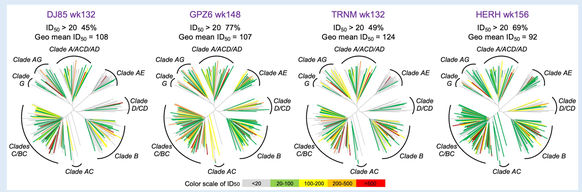


Figure 2. SHIV-infection boosting yields broadly neutralizing plasma responses in vaccinated macaques in eight weeks

A. Fusion peptide-competition assays indicate plasma neutralizing activity against BG505.N611Q, a fusion-peptide sensitive mutant, to be mediated by fusion peptide-competed response in 4 of 10 macaques.
B. Longitudinal plasma ELISA endpoint titers and ID80-neutralization breadth.

A



B

Site of vulnerability	CD4-binding site			Subunit interface	V2 apex	V3 glycan	MPER		Subunit interface	Fusion peptide		
Template antibody	VRC01-like	b12-like	HJ16-like	8ANC195-like	PG9-like	PGT128-like	2F5-like	10E8-like	35O22-like	PGT151-like	Residual score*	Median signal#
Macaque plasma DJ85 wk132	0.05	0.02	0.05		0.07	0.09		0.12		0.60	-0.48	0.05
GPZ6 wk148	0.02		0.11	0.06	0.02	0.06		0.16		0.57	-0.11	0.04
TRNM wk132	0.05		0.05		0.08	0.03		0.05	0.04	0.70	-0.67	0.05
HERH wk156	0.06	0.09	0.14		0.09	0.05		0.08		0.50	-0.14	0.07

Prevalence values >=0.25 are highlighted with a dashed box.
* Residual score: indication of the presence of a potentially unknown specificity.
# Median signal: median of the NFP delineation scores for the ten specificities from the reference antibody set.

Figure 3. SHIV-infection boost neutralizing plasma responses to ~100 ID50 with 45-77% breadth in top four macaques, with neutralization signature indicating fusion peptide-directed responses

A. Dendrograms of ID_{50} titers for DJ85 wk132, GPZ6 wk148, TRNM wk132 and HERH wk156 plasmas on a 208-strain panel.
B. Neutralization fingerprint analysis on 129 strains reveals fusion peptide-directed responses to predominate.

Cryo-EM revealed 15 of 16 isolated antibodies with cross-clade neutralization to be FP-site directed

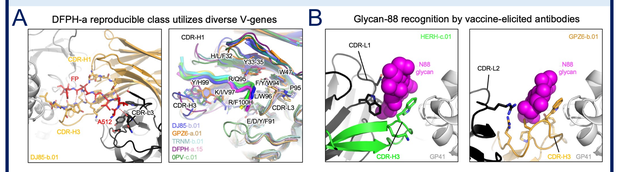


Figure 5. Cryo-EM structures of isolated antibodies provide residue-level detail for HIV-1 neutralization.

A. An expanded lineage of multidonor antibodies directed against the fusion peptide site of vulnerability.
B. Antibodies HERH-c.01 and GPZ6-b.01 focus recognition to N88.

RESULTS

In four macaques with vaccine-induced neutralizing responses (Figs. 1 and 2), SHIV infection boosted plasma neutralization to 45-77% breadth on a cross-clade panel of 208 strains with geometric mean ID50 titers of ~100 (Fig. 3A), a level of potency recently shown to protect against mucosal SHIV challenge from fusion peptide-directed antibodies[5]. Neutralization fingerprinting identified epitope-specific signatures in plasma and observed higher estimates for fusion peptide-directed specificity versus other known specificities (Fig. 3B). By longitudinally plasma viral RNA sequencing, we observed evidence of strong viral selection by post-SHIV week 16 at the N terminus of the fusion peptide and at the structural adjacent regions, including residues nearby glycan 88. Molecular dissection of these responses into component antibody specificities by antibody isolation (Fig. 4) and cryo-EM structure (Fig. 5) determination revealed 15 of 16 isolated antibodies with cross-clade neutralization breadth to be directed towards the fusion peptide-site of vulnerability. In each macaque, isolated monoclonal antibodies recapitulated the plasma-neutralizing response (r= 0.71-0.97) (Fig. 4C), with fusion peptide-binding antibodies reaching breadths of 40-60% (IC50 <50 µg/ml) on a 208 strains panel (Fig. 4B). Longitudinal phylogenetic analysis revealed each of the top macaques to have only 1-2 broadly neutralizing fusion peptide-binding lineages, each induced before SHIV infection.

Immunization scheme

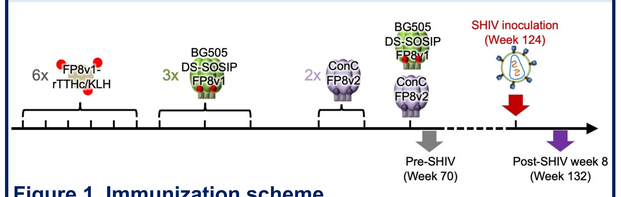
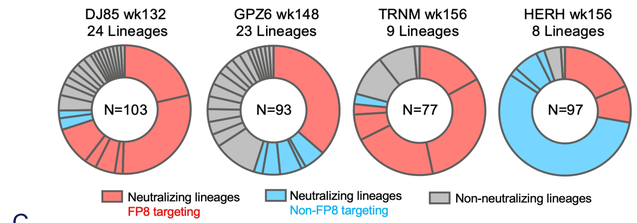


Figure 1. Immunization scheme

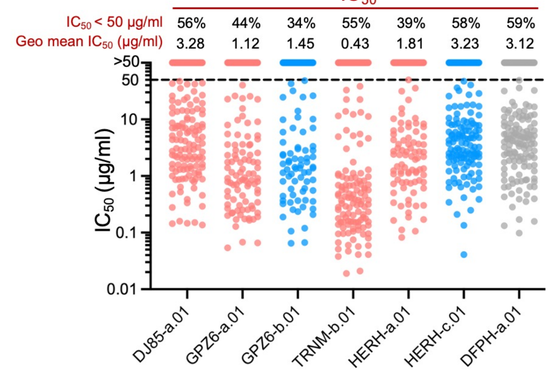
Two groups of 5 rhesus macaques were primed with FP8v1 coupled to a carrier protein and boosted with Env trimers (BG505 DS-SOSIP FP8v1 and ConC FP8v2), followed by SHIV.CH1012 (Group 1) or SHIV.Ce1176 (Group 2) inoculation at week 124.

Neutralization of identified monoclonal antibodies recapitulates corresponding plasma neutralization

A Monoclonal antibodies isolated from top macaques



B



C

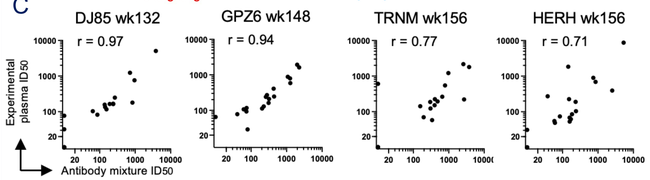


Figure 4. Isolated monoclonal antibodies reveal broadly neutralizing fusion peptide-directed antibodies in the top macaques.

A. Identified antibodies and lineages. Antibodies were isolated by single B cell sorting at plasma neutralization peaks after SHIV-infection boosting.
B. Neutralization IC50 and IC80 of representative antibodies from each macaque with the published antibody, DFPH-a.01, on a 208-strain panel.
C. Correlations (r) between monoclonal simulated plasma and experimental plasma neutralization (ID50) on a 19-strain panel, ID50 values of <20 were at the limit of assay detection (these have been graphed on the axes with calculations assuming ID50=10).

CONCLUSIONS

These results provide explicit in-vivo molecular examples for one or few B-cell lineages affording potent cross-reactive plasma-neutralizing responses. While increased titers in the current study resulted from SHIV-infection boosting, it will be interesting to test fusion peptide-Env trimer immunogens not only with altered boosting regimens, but also with altered priming regimens, such as with the escalating prime, extended-boost regimen.

KEY REFERENCES

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