GLYCAN-DEFICIENT SHIVs TO ELICIT BROADLY NEUTRALIZING ANTIBODIES IN MACAQUES

Poster Number **#302**

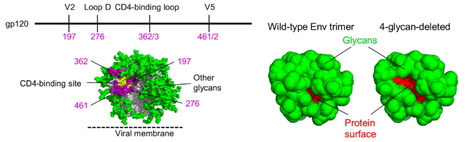
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Background: HIV immunogens engineered to lack glycans surrounding the CD4 binding site (CD4bs) or Fusion Peptide (FP) can immunofocus potent neutralizing antibody responses to these critical epitopes. However, such immunogens do not select for further antibody affinity maturation. The rhesus macaque-SHIV infection model allows us to dissect antibody-antigen co-evolutionary pathways that were important for acquisition of broad neutralization (Roark et al, *Science*, 2021). We hypothesized that SHIVs lacking these glycans would increase the proportion of macaques eliciting neutralizing and broadly neutralizing antibody (bNAb) responses to these epitopes.

1. Immunization with glycan modified Env trimers elicited antibodies with heterologous breadth in glycan deficient backgrounds

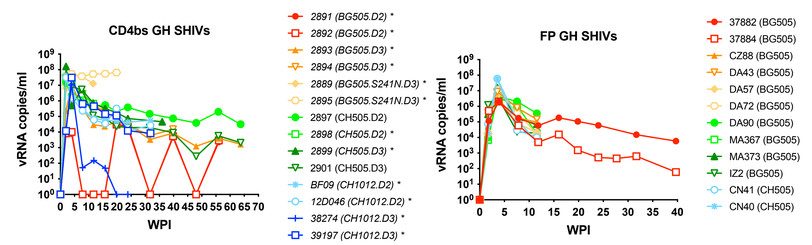


Immunogen	Virus	Clade AE						Clade C											
		CNE5		CNE55		28191-2.48		3168.V4.GC10		0013095-2.11		001428.2.G42		16055		ZM106.9	
	Animal ID	WT	ΔGly4	WT	ΔGly4	WT	ΔGly4	WT	ΔGly4	WT	ΔGly4	WT	ΔGly4	WT	ΔGly4	WT	ΔGly4
CH505 DS.SOSIP. ΔGly4	A13V030	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	118	<10	47	<10	92
	A13V124	<20	<20	<20	<20	<20	3,005	<20	<20	<20	<20	<20	<20	<10	1,222	<10	<10
	A13V099	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<10	15	<10	16
	A13V103	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<10	11	<10	<10
	A11V093	<20	<20	<20	230	<20	21	<20	26	<20	781	<20	2,474	<10	3,232	<10	1,242
	A11V051	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	12,580	<10	78	<10	15

Immunogen	Virus	Clade A		Clade C				Clade B	
		BG505		426c		CH505s.T/F		45_01dG5	
	Animal ID	WT	ΔGly4	WT	ΔGly4	WT	ΔGly4	WT	ΔGly4
BG505 DS-SOSIP	A12V183	<10	<10	<10	<10	<10	54	<10	<10
	A12V193	36	124	<10	<10	<10	181	<10	<10
	A12V049	104	1,531	<10	<10	<10	<10	<10	<10
	A12V045	466	1,434	<10	<10	<10	<10	<10	<10
	A12V165	<10	46	<10	<10	<10	<10	<10	<10
BG505 DS-SOSIP.ΔGly4	A13V171	106	10,000	<10	<10	<10	<10	<10	<10
	A13V173	<10	2,673	<10	<10	<10	<10	<10	<10
	A13V112	<10	3,934	<10	225	<10	<10	<10	<10
	A13V011	<10	26,118	<10	11	<10	<10	<10	<10
	A13V098	<10	20,364	<10	15	<10	<10	<10	<10
	4E-8	<10	2,297	<10	<10	<10	47	<10	<10
426c.DS-SOSIP.ΔGly4	A13V110	<10	<10	<10	1,054,096	<10	<10	<10	<10
	A13V140	<10	<10	<10	245,983	<10	<10	<10	<10
	A13V059	<10	<10	<10	755,489	<10	<10	<10	<10
	A13V160	<10	<10	<10	2,634,355	<10	<10	<10	<10
	A13V008	<10	<10	<10	287,033	<10	15	<10	<10
	1F-8	<10	<10	<10	41,829	<10	<10	<10	<10
CH505 DS-SOSIP.ΔGly4	A13V033	<10	<10	<10	<10	<10	28	1,001	<10
	A13V124	<10	2,697	<10	<10	97	35,899	<10	<10
	A13V099	<10	741	<10	<10	17	17,994	<10	<10
	A13V103	94	3,388	<10	<10	81	12,029	<10	<10
	A11V093	<10	5,859	<10	35	66	34,645	<10	<10
	A11V051	<10	8,826	<10	<10	67	40,700	<10	<10
45_01dG5.DS-SOSIP.ΔGly3	A13V130	<10	<10	<10	<10	<10	<10	<10	3,186
	A13V013	<10	<10	<10	<10	<10	25	<10	1,566
	A13V144	<10	<10	<10	<10	<10	660	5,179	
	A13V045	<10	<10	<10	67	<10	<10	<10	598
	A13V073	<10	<10	<10	11	<10	<10	83	3,052
	6E-8	<10	<10	<10	931	<10	22	457	11,276

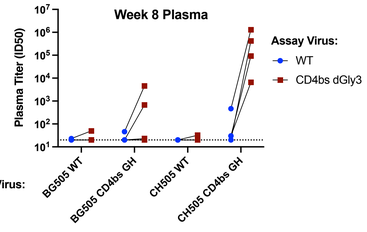
Zhou et al, Cell Reports (2017), found that deleting glycans surrounding the CD4bs increased the antigenicity of Env trimers **(upper left)**. Immunizing animals with these trimers resulted in potent neutralizing responses to the CD4bs **(right)**. Some animals exhibited cross neutralization in glycan deficient backgrounds **(lower left)**. These experiments suggested that creating this glycan hole was a good strategy for a priming CD4bs responses, but further maturation was needed for cross neutralization of WT viruses. Values are reciprocal ID50s.

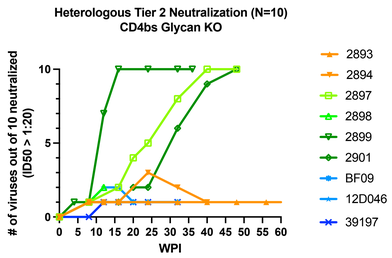
2. Glycan-deleted SHIVs replicate efficiently in vivo with similar viral kinetics as HIV-infected humans



SHIVs that replicated in vitro and had good antigenic characteristics were used to infect pilot cohorts of Indian-origin rhesus macaques. CD4bs glycan-modified SHIVs (**left**) and FP glycan-modified SHIVs (**right**). Most macaques exhibited similar viral loads during peak acute viremia and chronic set-point as observed in HIV-infected humans. Animals denotated with an asterisk were humanely euthanized. Infection with SHIVs CH505.CD4bs.GH or BG505.FP.GH were used for larger experiments.

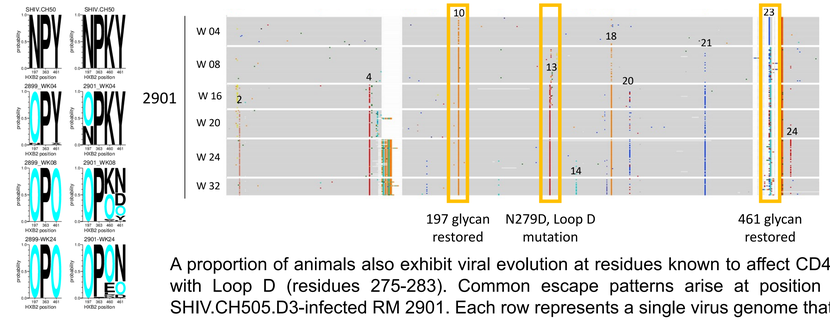
3. Glycan-deleted SHIV infection accelerates and increases the neutralization of these epitopes compared to WT infection





Macaques infected with CD4bs glycan-modified SHIVs exhibited accelerated neutralization to the CD4bs compared to macaques infected with the WT SHIV (**left**). X-axis denotes the inoculum virus whereas the data points compare the neutralization of the WT (**circle**) and CD4bs glycan deficient (**square**) autologous virus. Macaques with ideal viral kinetics (see panel 2) were tested for neutralization of heterologous viruses containing the CD4bs glycan hole (**right**). Animals infected with SHIV.CH505-based constructs (2897, 2898, 2899, 2901) developed superior breadth, 3 of 4 animals neutralized all 10 heterologous viruses.

4. Longitudinal single viral genome sequencing reveals rapid replacement of deleted glycans and on-target antibody pressure



Glycans are reconstituted at these positions in concordance with rising antibodies titers (**left**). Logo plots showing the proportion of viruses identified from single genome sequencing that have filled these glycans back in. Potential N-linked glycosylation sites are labeled as “O” and colored in cyan. Two macaques infected with SHIV.CH505.D3 are representative examples.

A proportion of animals also exhibit viral evolution at residues known to affect CD4bs bNAbs (**right**). Many CD4bs bNAbs interact with Loop D (residues 275-283). Common escape patterns arise at position 279 and 280 which is recapitulated here in SHIV.CH505.D3-infected RM 2901. Each row represents a single virus genome that are aligned to the inoculum as a reference.

5. Broad neutralization targeting the CD4-binding site

Inoculum SHIV	RM ID	WPI	Virus Clade	BG505 A		CH505 C		CH1012 C		CNE5 AE		CNE55 AE		ZM106.9 C		0013095-2.11 C		001428.2.42 C		16055 C		26191-2.48 C		3168.v4.c10 C	
				MLV	WT	dGly3	WT	dGly3	WT	dGly3	WT	dGly4	WT	dGly4	WT	dGly4	WT	dGly4	WT	dGly4	WT	dGly4	WT	dGly4	WT	dGly4
BG505.CD4bs.D3	2893	64	<20	*135*	*3358*	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20
	2894	40	<20	*5350*	*8726*	<20	<20	<20	<20	n.d.	n.d.	<20	<20	65	<20	<20	<20	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	<20	<20
CH505.CD4bs.D2	2897	64	<20	<20	<20	*861*	*3846*	<20	54	<20	217	<20	1217	<20	3614	<20	58	211	7911	<20	232	<20	108	<20	1223
	2898	12	<20	<20	<20	*422*	*62227*	24	24	n.d.	n.d.	<20	<20	<20	<20	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	<20	<20
CH505.CD4bs.D3	2899	36	<25	<25	155	*754*	*17295*	<25	<25	<25	58	<25	164	<25	693	80	188	64	14526	<25	187	<25	915	<25	341
	2901	64	<20	22	243	*878*	*9259*	74	152	40	4168	116	10227	<20	104	<20	68	<20	4250	<20	67	<20	95	<20	792
CH1012.CD4bs.D2	BF09	32	<20	<20	<20	<20	<20	<20	<20	95	*2908*	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20
	12D046	32	<20	<20	<20	<20	<20	*687*	*27902*	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20
CH1012.CD4bs.D3	39197	32	<20	<20	<20	<20	<20	*37*	*4539*	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20
CH505.CD4bs.D3	AA9	32	<20	>20	25	*5612*	*21777*	22	48	<20	<20	<20	<20	<20	<20	<20	<20	817	<20	193	<20	393	<20	<20	
	AR1	32	<20	>20	251	*1247*	*28877*	<20	<20	<20	<20	<20	<20	<20	<20	100	<20	1164	<20	3035	<20	1580	<20	770	
	CL84	32	<20	>20	21	*1406*	*35174*	<20	79	<20	<20	<20	<20	<20	<20	21	<20	1505	<20	438	<20	217	<20	<20	
	CN07	32	<20	<20	<20	*1662*	*35423*	<20	85	<20	<20	<20	<20	<20	<20	55	<20	1958	<20	3194	<20	1428	<20	20	
	CN09	32	<20	<20	221	*610*	*26567*	56	125	<20	2021	<20	452	<20	4473	<20	164	114	21964	<20	22842	<20	8551	<20	490	
	CN17	32	<20	<20	26	*1840*	*24950*	<20	162	<20	<20	<20	<20	<20	<20	192	80	923	<20	1022	<20	710	<20	<20	
	CN28	24	<20	<20	<20	*2333*	*47034*	<20	63	<20	25	<20	<20	<20	<20	55	<20	564	<20	40	<20	358	<20	<20	
	CN37	32	<20	83	11903	*719*	*46970*	<20	<20	52	2222	<20	5488	<20	6435	<20	182	<20	12447	<20	5128	34	10520	<20	2241

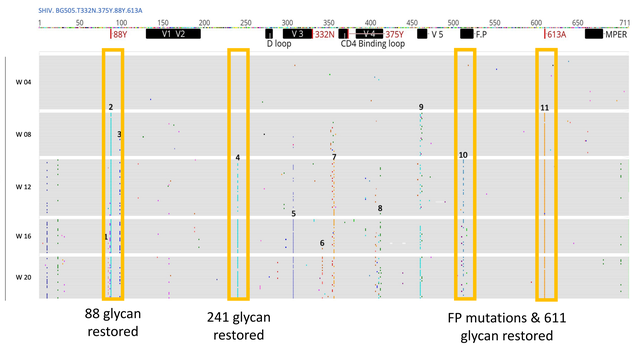
Reciprocal ID50: 20-100 | 100-1000 | 1000-10000 | 10000+

Autologous and heterologous neutralization of glycan-modified SHIV-infected macaque plasma were assessed longitudinally. Values are reciprocal ID50s. The final timepoint prior to euthanasia or most recent bleed for each animal is shown. Autologous neuts are in grey and italics. Animals are grouped by inoculum virus. Pilot animals infected with CH505.CD4bs.D2 or .D3 mounted antibody response capable of neutralizing many heterologous glycan KO viruses and some WT glycan viruses. A larger group of macaques (N=8) were infected with SHIV.CH505.CD4bs.D3 and are developing similar responses as early as week 20 post infection.

6. Broad neutralization targeting the Fusion Peptide

RM 37884 plasma		WK24	WK40	WK48
BG505.332N	FP8_v1	986	716	599
25710	FP8_v1	150	337	184
CH1012	FP8_v1	45	157	n.t.
Q23.17	FP8_v1	43	53	40
900455_A3_4	FP8_v1	40	47	37
ZM233.6	FP8_v1	36	51	80
CE1176	FP8_v1	<20	90	38
CH119	FP8_v1	<20	27	19
BJOX020000	FP8_v1	<20	<20	<20
246.F3	FP8_v1	<20	<20	<20
QB42.D12	FP8_v1	<20	<20	<20
T250-4	FP8_v2	64	25	30
2499M_B10	FP8_v2	<20	43	40
25925-2.22	FP8_v2	<20	22	31
H029.12	FP8_v2	<20	<20	20
X2278	FP8_v2	<20	<20	<20
001428-2.42	FP8_v2	<20	n.t.	<20
3718.v2c6	FP8_v2	<20	<20	<20
3168.v4.c10	FP8_v2	n.t.	n.t.	<20
CNE8	FP8_v5	<20	n.t.	n.t.
CNE55	FP8_v5	<20	<20	<20
SPK-0525.13	FP8_v5	<20	<20	<20
CNE5	FP8_v5	n.t.	n.t.	<20
CH505	AVGNGAVF	<20	68	74
WITO.A160	AVTLGAVF	<20	<20	21
CE0217	AVGNGAVF	<20	<20	<20
TRO11	AVGTLGAMF	<20	<20	<20
X1632	AIGLGTVL	<20	<20	<20
CAP256SU	AVVGLGAVF	<20	<20	<20
CH848	AAGLGALF	<20	<20	<20
706010164 1A7(REV-)	AVGVGAMF	<20	<20	<20
16055-2.3	AVGLGAVI	<20	n.t.	<20
RPW-0510.2	AVIGAMF	<20	<20	<20
HIV-0013095	AVGIGAVI	n.t.	n.t.	<20
HIV-26191	A--ALGAVF	n.t.	n.t.	<20

Reciprocal ID50: 20-100 | 100-1000



Rhesus macaque 37884 was infected with SHIV.BG505.FP.GH. Wildtype heterologous neutralization was observed by 24 wpi that is increasing in breadth and potency through 48 wpi (**left**). Most of the breadth is restricted to viruses containing the same or similar FP8 sequences. Longitudinal single genome sequence of plasma virus in RM 37884 reveals the reconstitution of glycans surrounding the fusion peptide glycan hole (**right**). Additional mutations within the formal fusion peptide known affect FP bNAbs were observed by 12 wpi.

Conclusions: Glycan-modified SHIVs can immunofocus antibody responses to epitopes of interest faster and at a greater magnitude than WT infection. Early bNAb responses have been observed and are being monitored for further maturation. These studies will help inform the design of boosting immunogens hoping to elicit CD4bs or FP broadly neutralizing antibodies

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