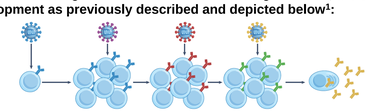
Maturation Pathway of a Rhesus V3-glycan Broadly Neutralizing Antibody Lineage

M Martin[1], T Evangelous[1], R Fenner[1], B Hora[1], M Berry[1], C Jiang[1], K Mansouri[1], RJ Edwards[1], J Lindenberger[1], J Hwang[1], DW Cain[1], H Li[2], KO Saunders[1], K Wiehe[1], P Acharya[1], G Shaw[2], BF Haynes[1], and WB Williams[1].
[1]Duke University School of Medicine, Durham NC; [2]Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA

Poster #370

Introduction

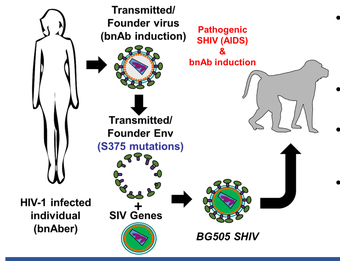
- A key goal of an HIV-1 vaccine is the induction of broadly neutralizing antibodies (bnAbs) that target the envelope (Env) surface protein and prevents infection of multiple different HIV-1 strains.
- HIV-1 vaccine development includes the concept of B cell lineage design whereby envelopes from transmitted founder viruses that initiate infection, and autologous viruses that evolve during infection, are selected as sequential immunogens for bnAb development as previously described and depicted below[1]:



- HIV-1 vaccine immunogens must select for bnAb lineage members with key mutations that confer antibody maturation for neutralization breadth.[1]

Previous Observations

Figure 1: Macaque SHIV-infection Model of BnAb Induction



- Pathogenic SHIV-infected RMs (Figure 1) generated plasma bnAbs that mapped to the V3-glycan bnAb epitope. A V3-glycan bnAb lineage (DH1030) was isolated from one SHIV BG505 monkey (RM08N021) (Williams WB, Haynes BF *et al.*, unpublished).
- Rhesus DH1030 had similar characteristics as human DH270 V3-glycan bnAb previously isolated from a person living with HIV.[2]
- A glycine to arginine mutation (G56R) in the HCDR2 of rhesus DH1030 was associated with neutralization breadth, similar to G57R mutation in human DH270.[2,3]
- R56G revertant mutation in rhesus DH1030.1 and R57G in human DH270[2] V3 glycan bnAbs abrogated neutralization activity.

Purpose of Study

To define a maturation pathway for HIV-1 Env-reactive bnAbs using rhesus DH1030 as a model V3- glycan bnAb lineage

Methods

- B cell isolation via conventional flow cytometry sorting of rhesus antigen-reactive B cells as well as 10X genomics single cell immune profiling assays[4], including LIBRA-seq[5].
- Expression of recombinant monoclonal antibodies (mAbs) via large scale transfections in Expi 293i cells[4].
- MAbs were tested for binding specificities (ELISA), function (TZM-bl neutralization), and structure (Negative Stain Electron Microscopy, NSEM; Modeling based on Cryo-EM data for DH1030 bnAbs).
- Immunogenetics of Ab variable heavy (VH) and light (VL) chain sequences were determined via Cloanalyst.[4]
- Site-directed mutagenesis was performed using a commercially available QuikChange Lightning Multi Site-Directed Mutagenesis Kit (Agilent) by DHVI VGA core facility. Primers were designed for specific sites using kit guidelines.

Figure 2: Phylogram of Rhesus DH1030 V3-glycan BnAb Lineage

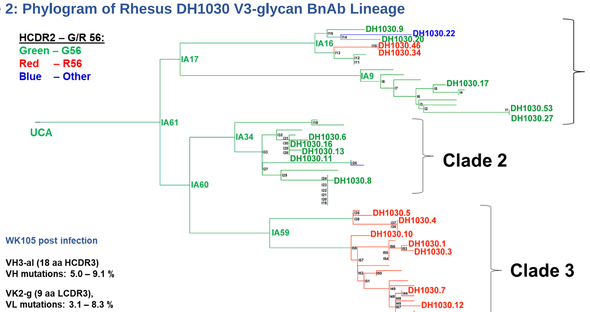


Figure 2: Phylogram displaying 62 clonally related DH1030 Abs isolated at week 105 post infection in SHIV-BG505 infected RM08N021 divided into 3 clades. Labeled on the phylogram branches are representative Abs that have been recombinantly expressed for functional characterization. The branches in red indicate the Abs with arginine at position 56 in the HCDR2, in green are members with glycine at position 56, and in blue are other residues at position 56. **DH1030 Abs clustered in 3 clades based on genetic signatures.**

Results

Figure 3: Neutralization Profile of DH1030 Lineage Abs

IC50, μg/mL: <0.1 | 0.1-1 | 1-10 | 10-50 | >50

Antibody ID	Clade	IC50 (μg/ml) in TZM-bl cells										
		MLV-SVA	Autologous HIV-1 Strain	V3-glycan bnAb precursor-sensitive	Global Panel of heterologous HIV-1 strains							
			BG505.T332N	CH848.10.17 DT	246-F3	TRO.11	BJOX2000	Ce1176	CH119	25710	X1632	Ce0217	CNE55
DH1030_IA16	Clade 1	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
DH1030.9		>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
DH1030.22		>50	>50	0.4	>50	>50	>50	>50	>50	>50	>50	>50	>50
DH1030.20		>50	>50	14	>50	>50	>50	>50	>50	>50	>50	>50	>50
DH1030_34_46		>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
DH1030_IA9		>50	16	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
DH1030.27_53		>50	<0.023	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
DH1030_IA34	Clade 2	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
DH1030.6		>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
DH1030.11_13_16		>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
DH1030.8		>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
DH1030_IA59	Clade 3	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
DH1030.10		>50	>50	0.05	>50	17	5.3	3.2	0.5	>50	>50	>50	> 50
DH1030.1		>50	8.8	< 0.023	>50	5.4	2.0	1.2	0.2	>50	>50	11	>50
DH1030.7		>50	>50	< 0.023	>50	5.8	3.0	1.3	0.2	>50	>50	39	>50
DH1030.12		>50	>50	< 0.023	>50	8.6	4.8	1.6	0.07	>50	>50	>50	>50
DH1030.2		>50	13	0.029	>50	1.8	1.0	0.9	0.2	>50	>50	2.0	>50
DH1030.14		>50	>50	< 0.023	>50	15	6.5	2.4	0.1	>50	>50	>50	>50
DH1030.5		>50	1.7	< 0.023	>50	0.7	0.4	0.2	0.09	6.1	>50	0.1	>50
DH1030.3		>50	>50	< 0.023	>50	8.6	3.5	2.1	0.4	>50	>50	19	>50

Figure 3: Heatmap of IC50 (μg/mL) values for neutralization of DH1030 lineage mAbs tested against heterologous HIV-1 strains from the global panel, autologous HIV-1, and a V3-glycan bnAb precursor sensitive virus CH848 10.17 DT, in TZM-bl cells.[2,4,6] **Clade 3 Abs demonstrated neutralization breadth against heterologous HIV-1 strains.**

Results (continued)

Figure 4: Maturation of DH1030 Neutralization Capacity

Antibody ID	Clade	IC50 (μg/ml) in TZM-bl cells		
		MLV-SVA	-V1 glycans	+V1 glycans
			CH848.10.17 DT	CH848 10.17
DH1030.22	Clade 1	>50	0.4	>50
DH1030.20		>50	14	>50
DH1030.10	Clade 3	>50	0.05	3.8
DH1030.1		>50	< 0.023	1.0
DH1030.7		>50	< 0.023	1.2
DH1030.12		>50	< 0.023	0.8
DH1030.2		>50	0.029	0.4
DH1030.14		>50	< 0.023	1.1
DH1030.5		>50	< 0.023	0.3
DH1030.3		>50	< 0.023	1.3

IC50, μg/mL: <0.1 | 0.1-1 | 1-10 | 10-50 | >50

Non-bnAbs / bnAbs

Figure 4: Heatmap of IC50 (μg/mL) values for neutralization of DH1030 nAbs from Clade 1 and Clade 3 against CH848 10.17 DT (N133DN138T) and CH848 10.17. **Only clade 3 Abs neutralized CH848 10.17 with the Env V1-glycans intact, a characteristic of V3-glycan bnAbs.**

Figure 5: N332-dependence of DH1030 NAbs

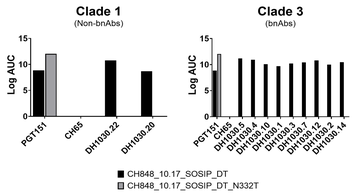


Figure 5: *Left* – Log AUC binding titers from ELISA of clade 1 nAbs (DH1030.22 and DH1030.20) against wild-type (WT) and N332-mutants of CH848 10.17_DT SOSIP. *Right* – Log AUC binding titers of clade 3 bnAbs against WT and N332-mutants of CH848 10.17 DT. **Clade 1 and 3 nAbs demonstrated N332-dependence; a binding profile of V3-glycan Abs.**

Figure 6: Structural Analysis of DH1030 NAbs

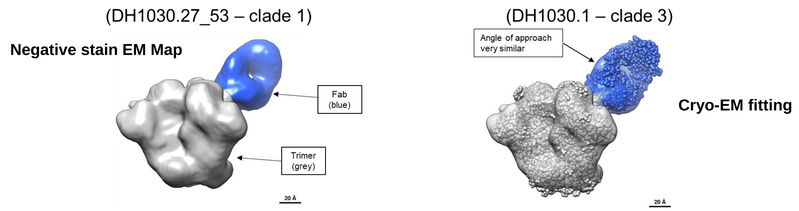
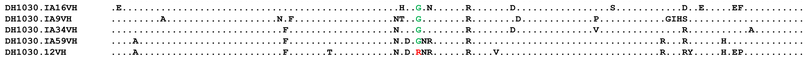


Figure 6: *Left* – 3D reconstructed density map of NSEM data for non-bnAb Fab DH1030.27_53 in complex with BG505.T332N shown in surface view (trimer in gray, Fab in blue). *Right* – cryo-EM model of bnAb Fab DH1030.1 in complex with BG505.T332N fitted into the NSEM map of DH1030.27_53 in complex with the same Env. **Clade 1 autologous nAbs, and clade 3 bnAbs have similar angles of approach for Env recognition.**

Figure 7: Impact of VH-G56R Mutation on DH1030 Intermediate Abs

Question 1: Does G56R mutation in DH1030 intermediate Abs confer binding and neutralizing activities?



B

Ab ID	Clade #	Binding titers (Log AUC)/ HIV-1 Env trimers			
		BG505gp140SOSIP T332N	BG505SOSIP MUT11B	CH848 10.17 SOSIP DT	CH848 10.17 SOSIP
DH1030_IA16	Clade 1	0.07	4.178	1.645	0.550
DH1030_IA16_G56R		0	8.049	1.554	0
DH1030_IA9		10.858	0.032	0	0
DH1030_IA9_G56R		8.138	0	0	0
DH1030_IA34	Clade 2	4.628	2.017	7.099	1.874
DH1030_IA34_G56R		1.125	4.303	9.976	1.667
DH1030_IA59	Clade 3	0	0.751	0.080	0.052
DH1030_IA59_G56R		0.814	9.643	4.677	2.008
DH1030.12		10.482	12.484	11.108	10.076

Figure 7: A. Alignment of VH amino acid sequences for DH1030 lineage intermediate Abs and a representative bnAb (DH1030.12) with the UCA as the reference sequence. Position 56 is colored; green representing glycine and red representing arginine. **B**. Heatmap depicting mAb ELISA binding titers in Log AUC against HIV-1 Env trimers; yellow represents higher binding, and blue represents lower binding. **G56R mutation alone in IA59 conferred similar binding activity to clade 3 bnAbs against select Envs and with lower binding titers. Thus, G56R mutation alone may be necessary, but insufficient to confer complete bnAb activity in DH1030 Abs.**

Figure 8: VH Mutations Associated with Clade 3 BnAb Maturation

Question 2: What are VH mutations in clade 3 DH1030 Abs that impact binding to HIV-1 Envs?



B

Ab ID	Clade #	Binding titers (Log AUC)/ HIV-1 Env trimers			
		BG505gp140SOSIP T332N	BG505SOSIP MUT11B	CH848 10.17 SOSIP DT	CH848 10.17 SOSIP
DH1030_IA59	Clade 3	0	0.751	0.080	0.052
DH1030_IA59_G56R		0.814	9.643	4.677	2.008
DH1030_IA59_S114P		0.192	9.292	7.549	6.616
DH1030.12		10.482	12.484	11.108	10.076
DH1030.12_VGSH		0.539	6.411	11.888	5.264

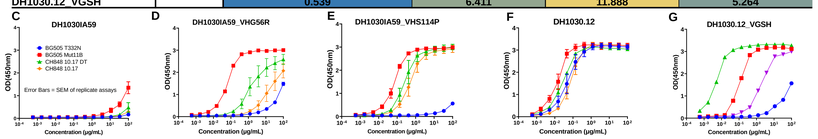


Figure 8: A. Alignment of VH amino acid sequences for wild-type and mutant clade 3 Abs with the UCA as the reference sequence. Positions of interest are circled. **B**. Heatmap depicting mAb ELISA binding titers in Log AUC against recombinant HIV-1 Envs. Yellow represents higher titers and blue represents lower titers. **C-G**. ELISA binding curves for mAbs tested against autologous and heterologous HIV-1 Envs. **S114P mutation conferred similar binding activity to clade 3 bnAbs against select Envs and with lower binding titers. We also identified 4 new sites (VH positions 5, 54, 57 and 100) that may impact DH1030 binding maturation (under further investigation).**

Figure 9: Neutralization profiles of DH1030 mutants and structure

IC50, μg/mL: <0.1 | 0.1-1 | 1-10 | 10-50 | >50

Antibody ID	IC50 (μg/ml) in TZM-bl cells												
	MLV-SVA	246-F3	TRO.11	BJOX2000	Ce1176	BG505.T332N	CH848.10.17 DT	CH848.10.17	CH119	25710	X1632	Ce0217	CNE55
DH1030UCA	>50	>50	>50	>50	>50	>50	>50	NT	>50	>50	>50	>50	>50
DH1030IA59	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
DH1030IA59_G56R	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
DH1030IA59_S114P	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
DH1030IA59_D113ES114P	>50	>50	>50	>50	31	>50	7.5	>50	>50	>50	>50	>50	>50
DH1030IA59_G56RD113ES114P	>50	>50	>50	29	6.8	>50	0.2	13	1.9	>50	>50	>50	>50
DH1030.1	>50	>50	3.5	1.8	1.0	17	< 0.023	1.0	0.2	>50	>50	9.2	>50
DH1030.1_P114S	>50	>50	>50	>50	>50	>50	5.4	48	31	>50	>50	>50	>50

Figure 9: Heatmap of IC50 (μg/mL) values for neutralization of DH1030 lineage mAbs tested against heterologous HIV-1 strains from the global panel, autologous HIV-1 strain, and a V3-glycan bnAb precursor sensitive virus CH848 10.17 DT, in TZM-bl cells. **G56R and S114P are insufficient for neutralization by themselves, but together with D113E they confer broad neutralization.**

Figure 10: VL Mutations that may Impact DH1030 Maturation

Question 4: Does DH1030 VL impact binding to HIV-1 Envs?

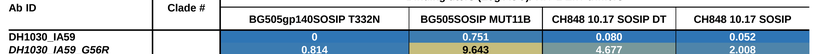
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0 | Log AUC | 15

Ab ID	VH ID	VK ID	Binding titers (Log AUC)/ HIV-1 Env trimer			
			BG505gp140 SOSIP T332N	BG505SOSIP MUT11B	CH848.10.17 SOSIP DT	CH848.10.17 SOSIP
DH1030UCA.2	DH1030UCA VH	DH1030UCA VK	0	0	0	0
DH1030UCA.2_VHG56R	DH1030UCA_G56R VH	DH1030UCA VK	0	0	0	0
DH1030UCA.2-VH-G56R_v2	DH1030UCA_G56R VH	DH1030.1 VK	7.36	7.133	6.451	6.656
DH1030.1	DH1030.1 VH	DH1030.1 VK	9.261	11.375	12.102	11.146
DH1030.1_v2	DH1030.1 VH	DH1030UCA VK	0	0	0	0

Question 5: What are VL mutations that may impact DH1030 binding to HIV-1 Envs?

B



C

Antibody ID	VH ID	VK ID	IC50 (μg/ml) in TZM-bl cells											
			MLV-SVA	246-F3	TRO.11	BJOX2000	Ce1176	BG505.T332N	CH848.10.17 DT	CH848.10.17	CH119	25710	X1632	Ce0217	CNE55
DH1030.1	DH1030.1 VH	DH1030.1 VK	>50	>50	3.5	1.8	1.0	17	< 0.023	1.0	0.2	>50	>50	9.2	>50
DH1030.1_v2	DH1030.1 VH	DH1030UCA VK	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
DH1030.1_v3	DH1030.1 VH	DH1030IA61_60 VK	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
DH1030.1_v4	DH1030.1 VH	DH1030IA59 VK	>50	>50	>50	41	6.8	>50	0.07	4.3	1.5	>50	>50	>50	>50

Figure 10:A.Heatmap depicting mAb ELISA binding titers as Log AUC against HIV-1 Env trimers. **B**. VL amino acid alignment for the listed Ab sequences using the DH1030UCA VK as the reference. Circled amino acids represent two mutations between DH1030.IA60VK and DH1030IA59VK. **C**. Neutralization heatmap for DH1030 mAbs pairing DH1030.1VH with the light chain from intermediates along the bnAb maturation pathway tested against a global panel of HIV-1 strains. **DH1030 VL plays a key role in mediating Ab binding activities, thus candidate VL residues that impact binding are under investigation. Thus far, we have identified mutations N33D and N58K in the light chain that may be contributing to binding and neutralization activity in this lineage.**

Figure 11: Structural Analysis Identifies Contribution of DH1030 Mutations to Neutralization Breadth

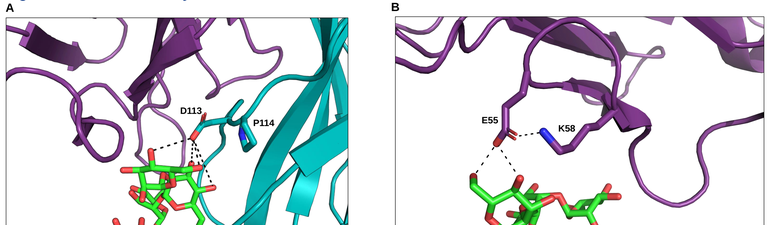


Figure 11:A.Representation of DH1030.1 Cryo-EM structure in complex with BG505 T332N Env. Heavy chain is in cyan, light chain is in purple, N332 glycan is in green stick representation. P114 residue introduces bend in chain that brings D113 residue within H-bonding distance with N332 glycan. **B**. Same representation as Fig. 11A, VK K58 residue forming salt-bridge with E55 residue, which stabilizes steric loop and facilitates H-bonding interaction with N332 glycan. **Thus, VH-P114 and VK-K58 residues in DH1030 lineage may have stabilizing effects that facilitate nearby residue interactions with Env contact sites, which may contribute to binding.**

Summary

- DH1030 Abs clustered into three clades based on genetic signatures with the DH1030 bnAbs clustering together in clade 3 and expressing R56 (HCDR2). DH1030 clade 1 Abs with G56 (HCDR2) demonstrated limited HIV-1 neutralization capacity.
- Autologous HIV-1 neutralizing DH1030 Abs, and DH1030 bnAbs demonstrated N332-dependence for Env recognition.
- NSEM analysis indicated that clade 1 autologous nAbs (VH-G56) and clade 3 bnAbs (VH-R56) demonstrated a similar angle of approach.
- Structural data suggest an indirect stabilizing effect from VH residue P114 (found in all clade 3 bnAbs) involving residue D113, as well as a similar indirect stabilizing effect from VK residue K58 involving residue VK E55.
- DH1030.1VH and DH1030UCAVK chimeric Ab lost binding capacity, suggesting a key role for DH1030VL in bnAb maturation. Residues in DH1030 VL that may impact lineage maturation are under further investigation.

Conclusions and Implications for HIV-1 Vaccine Design

- **V3-glycan bnAb lineage maturation requires a series of mutations in addition to G56R in the HCDR2. VL maturation may impact DH1030 bnAb development as well.**
- **A potential HIV-1 vaccine regimen to elicit bnAbs targeting the V3- glycan epitope will need to select bnAb lineage members with the appropriate mutations that may confer breadth.**

References: [1]Haynes et al, Nat Rev Imm. 23,142–158 (2023); [2]Bonsignori *et al.*, Sci Transl Med 9(381): eaai7514; 2017 ; [3]Wiehe *et al.* Cell Host Microbe. 2018 Jun 13;23(6):759-765; [4]Williams *et al.* Cell. 2021 May 27;184(11):2955-2972.e25; [5]Setliff et al. Cell 2019 Dec;179(7):1636-1646.e15, [6]deCamp et al., J. Virol., 2014

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Contact information: Mitchell Martin: Mitchell.Martin@duke.edu ; Wilton Williams: Wilton.Williams@duke.edu