Novel PGT/2G12 Binders Elicit Antibodies that Cross-react with HIV Env-Associated Glycans

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Abstract

Glycan-dependent epitopes have been identified as one of a few viable targets for developing HIV broadly neutralizing antibodies (bNAbs). We have previously targeted the HIV-1 Env glycan shield by generating a triple mutant (TM) strain of *S cerevisiae* that results in presentation of mainly $Man_8GlcNAc_2$ glycans. TM yeast or TM yeast-derived glycoproteins Gp38 and Pst1 can elicit antibodies that bind to gp120 proteins and neutralize virions when the virions are enforced to retain high mannose N-glycans.

We have advanced our previous success with the TM yeast-based genetic scaffolds by performing genome-wide screens of highly glycosylated proteins using a bioinformatic approach. We have identified a number of proteins that contain a large number and high density of potential N-linked glycosylation sites (PNGS). These genome derived glycoproteins (GGPs), when expressed in the TM yeast, support binding to 2G12 and several PGT bNAbs. Comparison of their binding efficiency with TM yeast proteins indicated that GGP1 exhibits the strongest binding to PGT 126, 128, 130 and 135. Immunization of rabbits with GGP1 elicited antibodies that bind to not only gp120 from diverse HIV-1 strains, but soluble JRFL gp140 trimers. These results reinforce a proof-of-principle that antibodies against HIV-1 Env glycan can be induced with a non-HIV related glycan scaffold immunogen. More importantly, these GGPs represent a new class of molecular scaffolds with greater potential as an immunogen to elicit neutralizing antibodies that target HIV Env-associated glycans.

2G12-reactive TM yeast glycoproteins

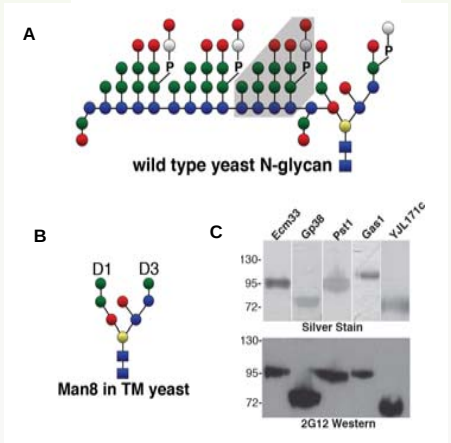

Figure 1 Wild type yeast expressing hyper-mannosylated N-linked glycans (A) was mutated creating triple mutant (TM) yeast that expressed exclusively $Man_8GlcNAc_2$ (B). Five endogenous yeast proteins that support efficient binding to 2G12 were identified from the TM yeast (C).

Genome derived glycoproteins (GGPs)

Table 1. Highly glycosylated proteins from genomes

Name	Acce. No.	Species	Mature protein		PNGS	
			AA No.	MW kDa	No.	%
GGP1	Q9VF46	Drosphila	171	19	11	6.4
GGP2	XP_961675	N. Crassa	188	21	8	4.3
GGP10	Q8SYQ9	Drosphila	178	20	10	5.6

A bioinformatic approach was employed to screen diverse genomes including mammals, insects, worms, fish, fungi and plants for proteins that contain a large number and high density of potential N-linked glycosylation sites (PNGS). The candidate genes were narrowed down using a number of criteria. The genes encoding the proteins were cloned into a yeast expression vector and expressed in the TM yeast. Three genome derived glycoproteins, GGP1, GGP2 and GGP10, were successfully expressed in TM yeast and purified.

2G12/PGT binding of GGPs

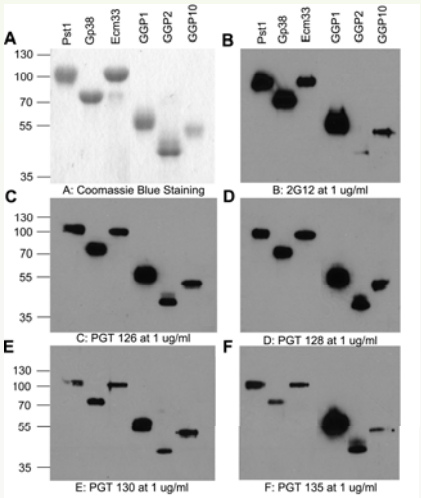

Figure 2 The binding analysis of GGPs to 2G12/PGT bNAbs by WB. The indicated GGPs along with yeast glycoproteins (Pst1, GP38 and Ecm33) were separated on a SDS-PAGE gel, and stained with Coomassie blue (A) and blotted with 2G12 (B) or PGT bNAbs (C-F), respectively.

2G12/PGT binding of GGPs

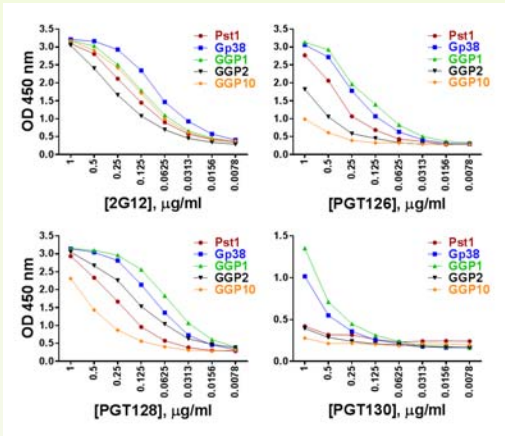

Figure 3 The binding analysis of GGPs to 2G12/PGT bNAbs by ELISA. The indicated GGPs along with TM yeast glycoproteins (Pst1, GP38 and Ecm33) were coated on the ELISA plates and probed with 2G12/ PGT bNAbs.

Induction of gp120-binding sera

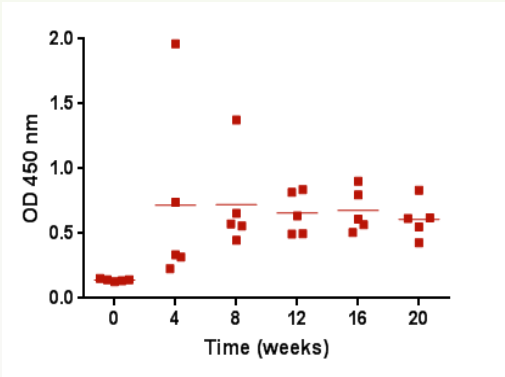

Figure 4 Binding of GGP1-derived immune sera to soluble gp120. Five rabbits were immunized with GGP1 conjugated with a T-cell epitope peptide and coadministrated with TLR2 ligand Pam_3CSK_4 and aluminum salt. Pre- and post-immune sera at 1:500 dilution were tested against the ADA gp120 coated on ELISA plate. The solid lines indicate the mean OD readings from 5 rabbit sera at each time point.

Trimer binding of GGP-derived sera

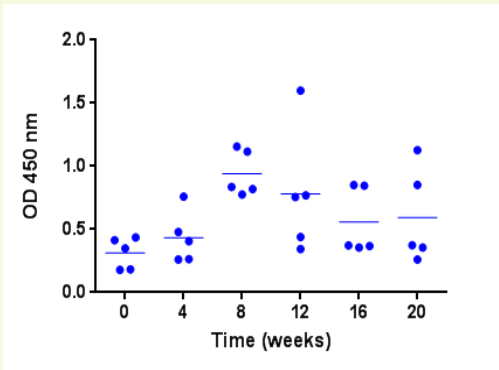

Figure 5 Binding of GGP1-derived immune sera to JRFL gp140 trimers. Rabbit sera at 1:250 dilution were tested for binding to trimeric JRFL gp140-F captured by PG16 to ELISA plate. The solid lines indicate the mean OD readings from 5 rabbit antisera at each time point.

Conclusions

- Three highly glycosylated proteins from Drosophila (GGP1 and GGP10) and fungus (GGP2) were identified through genome-wide screens.
- GGP1, GGP2 and GGP10, when expressed in the TM yeast, support efficient binding to 2G12/PGT bNAbs.
- PGT 126, 128, 130 and 135 recognize GGP1 better than other glycoproteins tested.
- GGP1, when incorporated with T-cell epitope peptide and appropriate adjuvants, can induce glycan-specific HIV-1 Env cross-reactive antibodies.
- GGP1-derived immune sera bind to not only monomeric gp120, but soluble trimeric JRFL gp140-F.
- The GGPs represent a new class of functional genetic scaffolds that target HIV-1 glycan shield.

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