Carbohydrate-Based HIV Vaccine, from Concept to Mannose-Specific Virion-Binding Antibodies

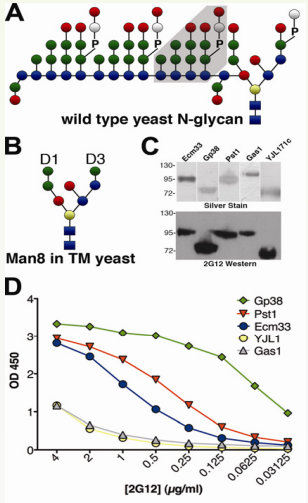
Yu Geng[1], Hu Fu[1], Bingfen Liu[1], Hong Zhang[1], Robert J. Luallen[1], and Robert W. Doms[2]

[1]ProSci Incorporated, Poway, CA 92064; [2]Department of Microbiology, University of Pennsylvania, Philadelphia, PA 19104

Abstract

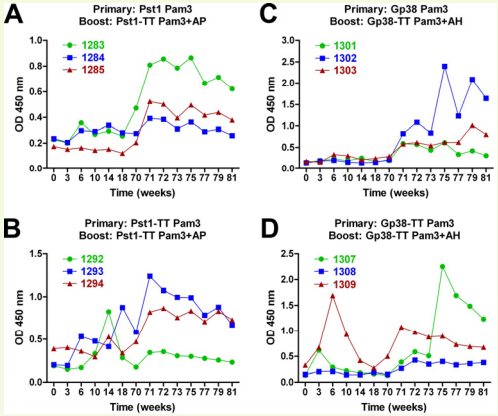
Several lines of evidence indicate that the glycan shield on the HIV-1 envelope may be a rewarding vaccine target. To recapitulate the 2G12 epitope, previously, we generated a triple mutant (TM) strain of *S. cerevisiae* by deleting three genes in the N-linked glycosylation pathway. Glycan profiling shows that the TM yeast cells express almost exclusively $Man_8GlcNAc_2$ N-linked glycans. Five endogenous glycoproteins that efficiently bound to 2G12 were identified from the TM yeast. Like gp120, these yeast proteins contain a large number and high density of N-glycans. Immunization of rabbits with tetanus toxin (TT) peptide conjugated single yeast protein, Pst1 or Gp38 (Pst1-TT or Gp38-TT) in conjunction with Toll-like receptor 2 agonist (Pam_3CSK_4) elicited HIV-1 Env cross-reactive antibodies, which bind to a broad range of gp120 proteins from different HIV subtypes and SIV. Glycan microarray analysis with 610 synthetic and natural glycans indicates that these yeast protein-elicited antibodies bind specifically to the oligosaccharides with terminal Manα1,2-Manα1,2-Mann. Moreover, the purified mannose-specific antibodies bind to not only monomeric gp120 but also functional HIV virions, although the binding affinity is relatively lower compared with that of 2G12. Further optimizing the immungens, adjuvants, and immunization regimen are needed with hopes of inducing 2G12-like neutralizing antibodies.

Yeast glycoproteins bind 2G12



- Wild type yeast expressing hyper-mannosylated N-linked glycans (A) was mutated creating triple mutant (TM) yeast that expressed strictly $Man_8GlcNAc_{c2}$ (B). This resulted in numerous endogenous yeast proteins that could bind to the HIV-1 Mab 2G12 (C).
- Yeast glycoproteins expressing Man8 were individually purified from TM yeast and 2G12 binding was compared by ELISA.

Induction of gp120-binding sera

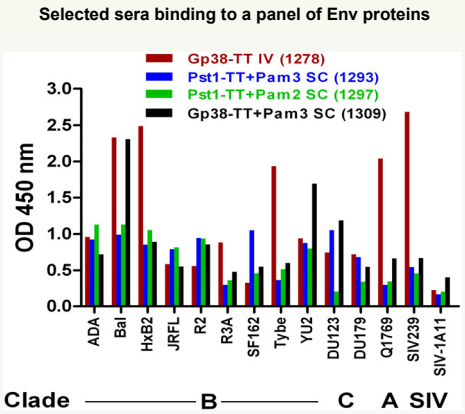


Rabbits were immunized with Pst1(A), Pst1-TT (B), Gp38 (C) or Gp38-TT (D) in conjunction with Pam3. After resting for 52 weeks, the rabbits were boosted with the Pst1-TT (A and B) or Gp38-TT in conjunction with Pam3+AP or Pam3 +AH at week 70. Immune sera (1:500) from all bleeds were tested for binding to 300 ng YU2 gp120 by ELISA.

TT: Tetanus toxin

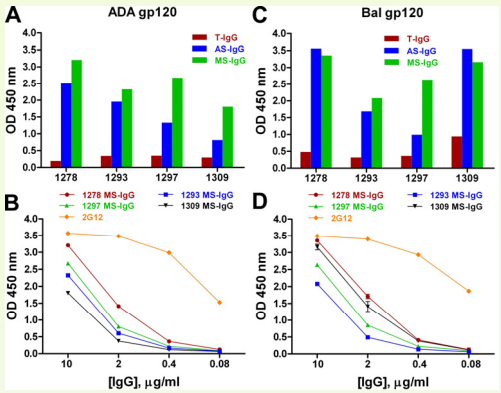
Pam3: Pam3CSK4; AP: Aluminum Phosphate; AH: Aluminum Hydroxide

Breadth of gp120 binding



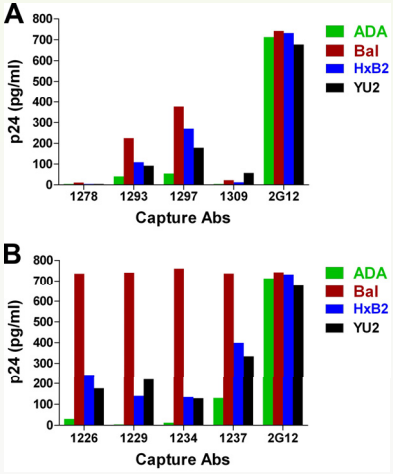
Selected sera (at 1:500) showed binding to a broad panel of mammalian-expressed Env proteins (300 ng) from clade B, C, A of HIV-1 and SIV. Sera from rabbit1278 (week 5),1293 (week18),1297 (week10) and1309 (week 6) post immunizations were tested. An OD450 nm over 0.3 is considered positive.

Binding potency of purified IgG to gp120



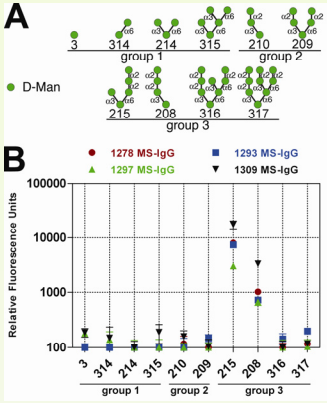
- Total IgG (T-IgG), antigen-specific IgG (AS-IgG) and mannose-specific IgG (MS-IgG) from four rabbits were purified, and their binding to gp120s from strains ADA (A) and Bal (C) were tested in ELISA.
- The binding affinity of MS-IgG toward ADA (B) and Bal (D) gp120 was compared with that of 2G12.

Capture of pseudovirus



Capture of pseudovirus by immobilized single yeast protein-elicited antibodies (A), TM yeast-elicited antibodies (B) and 2G12 (A and B) as measured by p24 ELISA. The capture antibodies (1250 ng per well for purified yeast antibodies; 250 ng per well for 2G12) were coated on ELISA plate, and the pseudotyped viruses bearing the ADA, Bal, HXB2 and YU-2 envelope were added to the wells. The captured virus was quantified by p24 ELISA.

Glycan binding profile of MS-IgG



- Schematic representation of the synthetic mannose-containing carbohydrate structures (A) .
- MS-IgG was tested at 10 μg/ml for binding to the various mannose-containing synthetic carbohydrates (B).

Conclusion

- Mannose-specific HIV-1 Env cross-reactive antibodies can be elicited with 2G12-reactive yeast glycoproteins or whole TM yeast.
- Like TM yeast, single yeast protein-elicited antibodies are capable of binding to a broad range of gp120s from both HIV and SIV.
- Rabbits boosted with single yeast glycoprotein after over a year without immunization elicit better gp120 binding sera.
- The yeast glycoprotein-elicited antibodies are able to capture the pseudovirions, suggesting that the conserved epitope that the broadly reactive antibodies targeted are present on the functional HIV virions.
- The binding affinity of MS-IgG toward gp120s and functional virions was relatively lower than that of 2G12.
- The yeast protein-elicited antibodies exhibited similarity in the carbohydrate specificity to 2G12 by recognizing terminal Manα1,2Manα1,2 trisaccharides.

This work was supported by the International AIDS Vaccine Initiative (IAVI) and NIH grant R43 AI083138