Plant-based production of highly potent anti-HIV antibodies with engineered post-translational modifications

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Introduction: Broadly neutralising antibodies (bNAbs) are attractive alternatives and/or as complements to the current regimens for the treatment of HIV infection. Plants, particularly Nicotiana benthamiana are an alternative to the mainstream mammalian cell systems, being well suited for producing efficacious monoclonal HIV antibodies [1–4]. An example, the CAP256-VRC26 bNAb lineage, target the V1V2 region of the HIV-1 gp120 envelope glycoprotein [5,6]. The CAP256-VRC26 bNAbs are characterised by an anionic antigen-binding loop with a protruding *O*-sulfated tyrosine in the CDR H3 loop, the absence of which leads to significantly decreased antigen binding and subsequent loss of function [5,7]. This human type PTM is catalysed by tyrosyl protein sulfotransferases (TPSTs) which *Nicotiana benthamiana* lack [8,9]. This report demonstrates the expression and characterisation of CAP256-VRC26 bNAbs with engineered PTMs to maintain Ab function.

Methodology: A transient expression approach was taken to produce the CAP256-VRC26 bNAbs with and without hTPST1 co-expression in the glycoengineered *N. benthamiana* (ΔXTFT) using deconstructed viral vectors. The sulfation and glycosylation state of the protein A purified CAP256-VRC26 bNAbs, under reducing conditions using liquid chromatography-mass spectrometry. The secondary and quaternary structure of the *N. benthamiana* (ΔXTFT) and HEK293-produced CAP256-VRC26 bNAbs were probed by circular dichroism and intrinsic fluorescence. The TZM-bl neutralisation assay was used to assess the neutralisation efficacy of the sulfated and non-sulfated *N. benthamiana* (ΔXTFT)-produced bNAbs against the HEK293-produced bNAbs using a multi-subtype pseudovirus panel.

Results: Two variants, CAP256-VRC26 08 and 09, were expressed in *N. benthamiana* (ΔXTFT) plants. By *in planta* co-expression of TPST 1, *O*-sulfated tyrosines were installed in CDR H3 of both bNAbs. These exhibited similar structural folding to the HEK293-produced bNAbs, but non-sulfated versions showed loss of neutralisation breadth and potency. In contrast, tyrosine sulfated versions displayed equivalent neutralising activity to HEK293-produced bNAbs retaining exceptional potency against some subtype C viruses.

Discussion and conclusion: This study demonstrates the efficient production of functional anti-HIV bNAbs, CAP256-VRC26 (08 and 09) in *N. benthamiana* (ΔXTFT). Incomplete sulfation and glycosylation of the bNAbs were observed, suggesting that the transiently coexpressed hTPST1 and the native plant oligosaccharyltransferase complexes might not be as efficient as the native machinery of the HEK293 cells. Despite this, the *in vitro* neutralisation efficacy was equivalent to that of the HEK293-produced bNAbs. These results provide a basis for the use of cutting-edge genome editing technology to create a general plant chassis with reduced host cell proteins which is optimised for high level protein production of vaccines with greater levels of the correct post-translational modifications. Together, the data demonstrate the enormous potential of plant-based systems for multiple post-translational engineering and production of fully active bNAbs for application in passive immunisation or as an alternative for current HIV/AIDS antiretroviral therapy regimens.

References

1. Schähs M, Strasser R, Stadlmann J, Kunert R, Rademacher T, Steinkellner H. Production of a monoclonal antibody in plants with a humanized N-glycosylation pattern. Plant Biotechnol J. 2007;5(5):657–63.
2. Castilho A, Beihammer G, Pfeiffer C, Göritzer K, Montero-Morales L, Vavra U, et al. An oligosaccharyltransferase from Leishmania major increases the N-glycan occupancy on recombinant glycoproteins produced in Nicotiana benthamiana. Plant Biotechnol J. 2018;16(10):1700–9.
3. Teh AYH, Maresch D, Klein K, Ma JKC. Characterization of VRC01, a potent and broadly neutralizing anti-HIV mAb, produced in transiently and stably transformed tobacco. Plant Biotechnol J. 2014;12(3):300–11.
4. Loos A, Gach JS, Hackl T, Maresch D, Henkel T, Porodko A, et al. Glycan modulation and sulfoengineering of anti–HIV-1 monoclonal antibody PG9 in plants. Proc Natl Acad Sci. 2015;112(41):12675–80.
5. Doria-Rose NA, Schramm CA, Gorman J, Moore PL, Bhiman JN, DeKosky BJ, et al. Developmental pathway for potent V1V2-directed HIV-neutralizing antibodies. Nature. 2014;509(7498):55–62.
6. McLellan JS, Pancera M, Carrico C, Gorman J, Julien J-P, Khayat R, et al. Structure of HIV-1 gp120 V1/V2 domain with broadly neutralizing antibody PG9. Nature [Internet]. 2011;480(7377):336–43. Available from: http://www.nature.com/doifinder/10.1038/nature10696
7. Rosenberg Y, Sack M, Montefiori D, Labranche C, Lewis M, Urban L, et al. Pharmacokinetics and Immunogenicity of Broadly Neutralizing HIV Monoclonal Antibodies in Macaques. PLoS One. 2015;10(3):1–15.
8. Moore KL. Protein tyrosine sulfation: a critical posttranslation modification in plants and animals. Proc Natl Acad Sci U S A. 2009;106(35):14741–2.
9. Stone MJ, Chuang S, Hou X, Shoham M, Zhu JZ. Tyrosine sulfation: an increasingly recognised post-translational modification of secreted proteins. N Biotechnol. 2009;25(5):299–317.
10. Montero-Morales L, Steinkellner H. Advanced Plant-Based Glycan Engineering. Front Bioeng Biotechnol [Internet]. 2018;6(June):1–8. Available from: https://www.frontiersin.org/article/10.3389/fbioe.2018.00081/full

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