Identification of HIV-1 V3-glycan Supersite-Targeting Nanobodies Using Engineered V3 Ectodomain Probes

Adomah, R. [1,2*]; Chan, P. [2*]; Dominguez, O.[1]; McKee, K.[3]; Olia, A. S.[3]; Zhang, B.[3]; Teng, I.-T.[3]; Pierson, T.[3]; Zhou, T.[3]; Xu, J.[1]

1 The Wistar Institute, 2 Department of Biology, Georgia State University, 3 Vaccine Research Center, NIAID, National Institutes of Health *

HIV-1 imposes a societal and health burden globally, and combating the virus remains challenging due to its high mutation rate and a glycan shield that conceals vulnerable epitopes. The limited breadth and accessibility of conventional broadly neutralizing antibodies (bNAbs) against increasingly diverse HIV-1 envelope (Env) highlight the need for alternative neutralizing agents. Nanobodies derived from camelid heavy chain-only antibodies may provide a solution for preventing HIV-1 infection or even a durable cure. Unlike conventional bNAbs, nanobodies are small, with high affinity, stability, and crevice penetration, allowing access to inaccessible epitopes. To obtain nanobodies that can neutralize HIV-1, we have immunized alpacas with prefusion stabilized Env trimers and constructed a huge phage library of *in vivo* matured nanobodies with repertoire against potentially all epitopes on Env surface. While we have previously identified neutralizing nanobodies targeting CD4-binding site (CD4bs), here, we focused our effort on isolating neutralizing nanobodies targeting V3-glycan supersite. We grafted full length or minimal V3 loop of BG505 strain onto an engineered outer domain (eOD) protein scaffold derived from the gp120 of HXB2 strain. The resultant eOD.BG505fV3 and eOD.BG505mV3 proteins produced well in Expi293 GnTI- cells, presenting V3-glyan epitope that can be recognized by PGT128 and 10-1074. Phage panning of the alpaca nanobody library using eOD.V3 proteins led to the identification of nanobodies that can bind to HIV Env protein JG2, a modified BG505 DS-SOSIP with glycans covering the base. Neutralization activity of ten selected nanobodies was evaluated against three HIV-1 strains: BG505.W6M.C2 (clade A), CH0505s.T/F (clade C), and MW965.26 (clade C). Seven out of ten nanobodies neutralized at least one of the three strains at IC50s below 1 ug/mL. One strong binder (eODV3.Nb5) and one weak binder (eODV3.Nb10) neutralized all three strains at geometric mean IC80s of 0.04 ug/mL and 0.404 ug/mL, respectively. Notably, eODV3.Nb5 neutralized more potently than V3-glycan-targeting bNAbs PGT121, PGT128, and 10-1074, as well as CD4bs-targeting bNAb VRC01. Breadth and potency of these and more nanobodies will be tested on contemporary HIV-1 isolates to avoid overestimating their clinical efficacy. Overall, we demonstrated a novel way of identifying V3-glycan supersite-targeting neutralizing nanobodies using engineered epitope-specific probes.