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Technical note

A strategy for phage display selection of functional domain-exchanged immunoglobulin scaffolds with high affinity for glycan targets

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ABSTRACT

Monoclonal antibodies are essential reagents for deciphering gene or protein function and have been a fruitful source of therapeutic and diagnostic agents. However, the use of anticarbohydrate antibodies to target glycans for these purposes has been less successful. Glycans contain less hydrophobic functionality than do proteins or nucleic acids, thus individual glycan–antibody interactions are relatively weak. Information encoded by glycans often involves subtle variations of branched oligosaccharides that cannot be detected with conventional antibodies. Here we describe a new phage display selection strategy for identification of high-affinity and specific glycan antibodies. We designed and characterized a phage clone that functionally displays the unique architectural scaffold of 2G12, an antibody that targets oligomannoses on the HIV-1 glycoprotein gp120. The two heavy chain variable domains of 2G12 exchange positions to create an extended recognition surface containing four oligomannose binding sites per IgG molecule. We designed and characterized a phage clone in which this domain exchange architecture was recapitulated as an antigen binding fragment dimer [$(Fab)_2$] on the phage surface by protein engineering. The functional display of the 2G12 $(Fab)_2$ fragment was validated by Western blot and phage enzyme-linked immunosorbent assay. Furthermore, we demonstrate that this 2G12 $(Fab)_2$ display system is amenable to selection of functional clones using a mock selection. These results provide proof-of-concept that the privileged 2G12 domain-exchanged scaffold can be used for design of novel antibody libraries that are biased toward glycan recognition.

1. Introduction

Glycans (oligosaccharides) are critical information carriers in biology, yet progress toward understanding their roles has been hampered by lack of reagents that can detect subtle variations in glycan composition (Collins and Paulson, 2004; Prescher and Bertozzi, 2006). Antibodies and glycan-binding proteins (e.g., lectins) that recognize specific terminal sugars exist and are widely used, but these reagents have low affinity and are unable to distinguish among branched glycans. Subtle changes in the glycan composition at cellular surfaces, which can only be detected by discrimination of chemically similar high molecular weight branched oligosaccharides, are thought to signal major biological events and are associated with various disease states (Collins and Paulson, 2004; Prescher and Bertozzi, 2006). Therefore, reagents that can distinguish branched oligosaccharides from one another would be of high value in glycobiology research. Furthermore, such reagents have great potential for diagnostic and therapeutic applications. Antibodies with these capabilities are difficult to obtain using hybridoma methods because glycans themselves tend to be

Abbreviations: Fab, antigen binding fragment;CDR, complementarity determining region;VL, light chain variable domain;CL, light chain constant domain;VH, heavy chain variable domain;CH, heavy chain constant domain;ss-dU-DNA, single-stranded uridine-enriched DNA;PBS-T, phosphate-buffered saline containing 0.05% (v/v) Tween 20;scFv, single-chain variable fragment;iu, infectious units;HRP, horseradish peroxidase;TBS-T, Tris-buffered saline containing 0.05% (v/v) Tween 20;ELISA, enzyme-linked immunosorbent assay;ORF, open reading frame;RBS, ribosome binding site.

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poorly immunogenic, and it is difficult to target antibody response to regions that permit the desired level of discrimination among glycosylation patterns. Glycans have much less hydrophobic functionality than do proteins and nucleic acids; therefore, glycan–protein interactions tend to be lower affinity than protein–protein or protein–nucleic acid interactions (Collins and Paulson, 2004).

Recent progress in protein engineering has enabled identification of antibody fragments against various targets from de novo designed repertoires (Fellouse et al., 2007; Liu et al., 2011; Sidhu and Fellouse, 2006). In this approach, the diversity for such libraries is encoded by synthetic oligonucleotides ('synthetic antibodies'); the position and nature of the diversity elements are tailored to reflect amino acid compositions that have optimal physicochemical properties for antibody-antigen interactions (Fellouse et al., 2007). Therefore, the synthetic antibody approach circumvents the requirement for cloning variable domain segments from a natural immune repertoire. As a result, synthetic antibody libraries are not subject to biases of natural immune repertoires and the resulting antibodies can have enhanced properties. For example, synthetic antibodies with exquisite conformational or structural specificity have been isolated against several protein targets (Brawley et al., 2010; Gao et al., 2009). In addition, synthetic antibodies have been used to target post-translational modifications in high specificity (Newton et al., 2008), as well as nucleic acids, a class of antigens that has resisted traditional antibody isolation methods (Ye et al., 2008). The synthetic antibody approach is dependent upon a stable immunoglobulin framework that serves as a template for library design. The framework is chosen for desirable properties such as structural stability, tolerance to mutation, ease of expression, and predisposition toward particular antibody-antigen interactions. The scaffold of antibody 4D5, which appears highly biased toward protein-protein interactions, has served as the template for many protein-directed synthetic antibody libraries (Fellouse et al., 2007; Lee et al., 2004). Other frameworks that contain alternative CDR loop lengths and conformational propensities appear to have unique recognition properties (Da Silva et al., 2010; Shi et al., 2010). Specific scaffolds have been reported for use against peptide targets, which require a concave antigen binding site (Cobaugh et al., 2008), and single domain antibody scaffolds have also been described against several targets (Gilbreth et al., 2011; Wojcik et al., 2010).

The HIV-1 neutralizing antibody 2G12 is unique in its ability to target a complex glycan (the high molecular weight oligomannose residues on the envelope glycoprotein gp120) with high affinity (reported K_D of 5.6–16.1 nM for binding of gp120) and specificity (Calarese et al., 2003, 2005; Hoorelbeke et al., 2010). The structural basis for this selectivity arises from an unusual IgG1 architecture in which the two heavy chain variable domains on adjacent antigen binding fragments (Fabs) are domain-exchanged to form an extended and polyvalent glycan binding surface (Calarese et al., 2003). We surmised that this privileged scaffold could serve as a starting point for development of phage display synthetic antibodies biased toward glycan recognition. We report a selection strategy based on the 2G12 scaffold for identification of high-affinity glycan binding antibodies.

2. Materials and methods

2.1. Vector construction and mutagenesis

The bivalent Fab display phagemid pAS-Fab$_2$zip from Liu et al. (2011) was modified to generate bivalent 2G12 (Fab)$_2$ phagemid (p2G12-Fab$_2$zip). Splice overlap extension polymerase chain reaction was performed to generate DNA fragments containing the 2G12 light chain variable and constant domains (VL and CL, respectively) and the heavy chain variable and constant domains (VH and CH, respectively), which were subcloned into pAS-Fab$_2$zip. Kunkel mutagenesis was performed to generate mutants of p2G12-Fab$_2$zip (p2G12-Ala, p2G12-Gly, and p2G12-Arg, see Results and discussion) as previously described (Da Silva et al., 2010; Liu et al., 2011). Briefly, single-stranded, uridine-enriched DNA (ss-dU-DNA) of p2G12-Fab$_2$zip was prepared in *E. coli* CJ236 cells (New England Biolabs, NEB, Ipswich, MA) using standard protocols. Kunkel mutagenesis was performed using 5′-phosphorylated primers containing the desired mutations. Typical Kunkel mutagenesis reactions contained 3–5 μg of ss-dU-DNA, a three-fold excess of mutagenesis primer, three units of T7 DNA polymerase (NEB), and two units of T4 DNA ligase (Invitrogen, Carlsbad, CA). Reaction components were mixed and incubated at room temperature overnight and the products purified using a QIAgen (Valencia, CA) PCR purification kit. For the triple mutant p2G12-TriMut, a synthetic DNA fragment encoding the heavy chain fragment with mutations was obtained from a commercial supplier (Genewiz, South Plainfield, NJ) and subcloned as before.

2.2. Phage display

E. coli XL1-Blue (Stratagene Agilent Technologies, Santa Clara, CA) harboring p2G12-Fab$_2$zip were grown for ~4 h in 2xYT broth supplemented with 5 μg/mL tetracycline and 50 μg/mL carbenicillin at 37 °C. Helper phage M13-K07 (NEB) were added to ~10^{10} plaque-forming units (pfu)/mL, followed by 25 μg/mL kanamycin. The culture was grown for 16–18 h at 30 °C, the cells removed by centrifugation, and phage precipitated by addition of 3% (w/v) NaCl and 4% (w/v) PEG 8000. The phage were pelleted by centrifugation and resuspended in phosphate-buffered saline/0.05% (v/v) Tween 20 (PBS-T) containing 0.5% (w/v) BSA. Expression of an irrelevant single-chain variable fragment (scFv, pKZ52-scFv) was similar except the expression culture was grown at 37 °C.

2.3. Western blots

The p2G12-Fab$_2$zip phage (~10^{11} infectious units (iu)/mL) were denatured by incubation at 100 °C for 10 min in SDS-PAGE buffer with or without β-mercaptoethanol. The solutions containing denatured phage particles were run on a 10% Tris–glycine HCl SDS-PAGE polyacrylamide gel and subsequently electrotransferred to polyvinylidene fluoride filter paper. Next, the filter paper was blocked with blocking buffer (Sigma, St. Louis, MO) for 10 min and then incubated with an anti-FLAG/horseradish peroxidase (HRP) conjugate (Sigma) in a 1/2500 dilution from stock in the blocking buffer for