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Grant Title: Enhancement of IgG function by chemically modified glycosylation

Abstract

The development of technologies enabling the deliberate control of glycosylation is of central importance not only for enhancing antibody functionality but also for establishing quality attributes in regulatory science.

In this study, we investigated whether the structure–function relationships identified in trastuzumab are generalizable to other IgG antibodies. To this end, anti-CD20 antibodies were selected and systematically glycoengineered for comparative analysis. In addition, non-natural glycans—including immunogenic α1,3-Gal structures and chemically modified glycans bearing charged or bulky functional groups—were synthesized and introduced into rituximab to evaluate their impact on antibody function.

A library of 36 structurally defined glyco-engineered rituximab variants, including asymmetric glycoforms, was constructed using an endoglycosidase-based remodeling approach. Native heterogeneous glycans were first removed using EndoS, followed by the transfer of glycan oxazolines using a ENGase mutant (EndoS D233Q). Hemi-glycosylated intermediates were isolated by FcγRIIIa affinity chromatography and further elaborated to generate asymmetric glycoforms. Structural homogeneity was confirmed by ESI-MS.

Binding affinities to FcγRIIIa were quantitatively evaluated using surface plasmon resonance, and dissociation constants were determined for all variants to generate a comprehensive structure–binding map. Representative glycoforms (G2/G2 and G0/G0) were further assessed in cell-based antibody-dependent cellular cytotoxicity assays. The results demonstrated that increasing glycan complexity and galactosylation enhances FcγRIIIa binding and ADCC. Notably, the G2/G2 glycoform exhibited the highest binding affinity and approximately 1.5-fold higher ADCC compared to glycan-heterogeneous rituximab, whereas G0/G0 showed significantly reduced activity.

Non-natural glycan engineering was also explored. α1,3-Gal glycans were chemically synthesized with high stereoselectivity, and novel synthetic routes for scalable production are under development. Comparative analysis between rituximab and trastuzumab revealed both common and distinct features. While increased galactosylation consistently enhanced ADCC, the optimal glycan structures differed between IgGs.

Overall, these findings demonstrate that Fc glycans function cooperatively as paired structural elements rather than independently. The results highlight the importance of integrated glycan pair design—including symmetry and asymmetry—in optimizing antibody functions.

This work provides a foundation for rational glycoengineering strategies toward next-generation antibody therapeutics.