Chemoenzymatically glycan engineered monoclonal IgG antibodies against *Streptococcus pyogenes*

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Summary

We report on the chemoenzymatic glycan engineering of human monoclonal IgG antibodies, including Ab25 and Ab49 targeting *Streptococcus pyogenes* M protein and omalizumab as a control, to generate homogeneous glycoforms that influence antibody functions. Validation via LC-MS confirmed glycoform modifications, and functional assays demonstrated that antibody glycosylation affected bacterial internalization by phagocytes and complement factor C1q deposition, highlighting the significance of glycoform variation in developing IgG-based therapeutics against infections.

Introduction

N-linked carbohydrate structures in the Fc region of human IgG fine-tune effector functions such as antibody-dependent complement activation and Fc-receptor mediated phagocytosis. The Nordenfelt laboratory recently identified and characterized a human monoclonal (mAb) IgG antibody directed towards the M protein, a surface protein and virulence factor of *Streptococcus pyogenes* (GAS). These were isolated from memory B cells from an individual who had recovered from a GAS infection. One of the mAbs, Ab25, not only binds M protein with high affinity, but also promotes efficient phagocytosis of the bacteria in vitro. This is attributed to a natural bispecificity towards two different M protein epitopes.

Chemoenzymatic transglycosylation

Chemoenzymatic engineering was used to remove all Fc glycans on 5 mg of Ab25, Ab49 (monospecific mAb against M protein), and omalizumab (IgG mAb against IgE, used as a control), and then generate approximately 200µg of homogeneous glycoforms (G0, G0-afuc, G2, G2S2) through click chemistry. Validation of glycosylation pre- and post-engineering was performed using LC-MS.

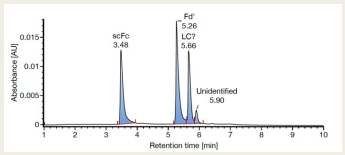
Functional analysis of mAb glycoforms

Binding experiments revealed that only deglycosylation had any major effects on binding to bacteria. Phagocytosis experiments revealed that internalization, but not association, of bacteria to THP-1 cells was influenced by antibody glycoform, and C1q deposition was gradually decreased, correlating with the size and complexity of the glycans.

Conclusions

Human IgG mAbs against GAS, as well as omalizumab, could be converted to homogeneous glycoforms. mAb glycoform clearly influences internalization into phagocytes as well as complement binding. This has important implications for the development of anti-infective mAbs and highlights mAb glycan engineering as a modality when developing IgG-based therapeutic antibodies

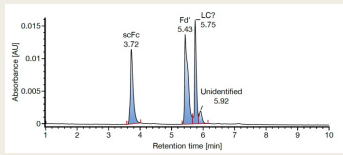
Ab25 unmodified



Glycosylation Profile

Subunit	Glycoform	Percent (%)
scFc	aglycosylated	2.2
scFc	Glycosylation G0F N (1)	45.5
scFc	Glycosylation G1F N (1)	42.8
scFc	Glycosylation G2F N (1)	9.5

Ab25 G2S2

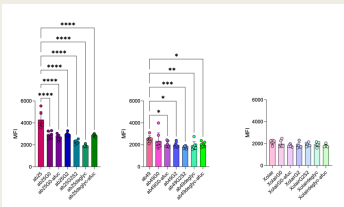


Glycosylation Profile

Subunit	Glycoform	Percent (%)
scFc	Glycosylation G2F+2SA N (1)	100.0

Fig 1. Example of LC-analysis of the Ab25 before and after transglycosylation. Peaks were further analyzed using MS to assign glycoforms. The same procedure was performed for Ab49 and omalizumab where the homogeneous glycoforms G0, G0-afuc, G2 & G2S2 could be generated for further functional analysis.

Binding to SF370



Binding to SF370ΔM1

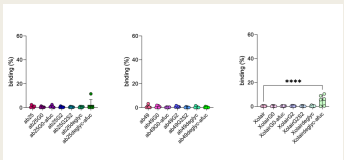


Fig 2. Binding of all glycoforms of Ab25, Ab49 and omalizumab to wild type bacteria with M1 protein on the surface and to SF370ΔM1 lacking M1 protein.

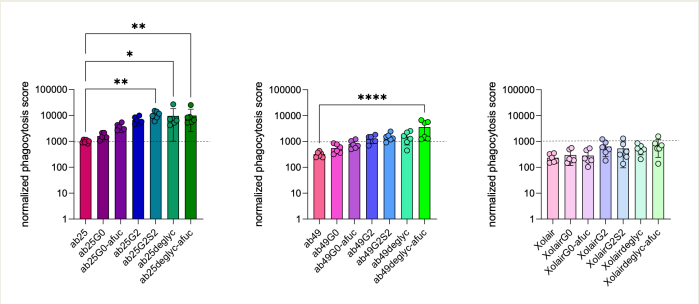


Fig 3. Flow cytometry analysis of phagocytosis of SF370 wild type bacteria by THP-1 cells in the presence of all glycoforms of Ab25, Ab49 and omalizumab. The Multiplicity of Prey (MOP) was 50 (50 bacteria per cell).

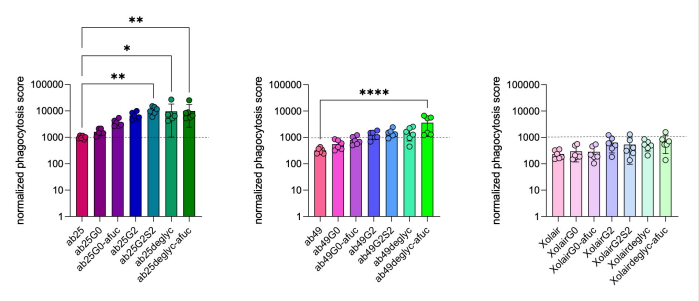


Fig 4. Flow cytometry analysis of deposition of complement factor C1q in in the presence of all glycoforms of Ab25, Ab49 and omalizumab. The Multiplicity of Prey (MOP) was 50 (50 bacteria per cell).