Enzymatic synthesis of a homogeneous antibody glycan

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INTRODUCTION

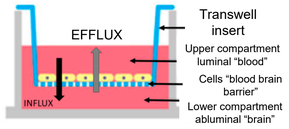
Alzheimer's disease (AD) is a neurodegenerative disorder that affects more than 5.5 million adults in the United States. AD is expected to triple in the next 50 years as the population continues to age[1].

IgG antibodies are a popular immunotherapy drug class used in clinical trials of AD. These antibodies are typically targeted to specific forms of the β-amyloid peptide found in the brain of AD patients.

A major problem with IgG (and other protein drugs) is poor brain delivery.

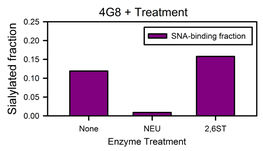
Blood Brain Barrier Studies of IgG Sialic acid

Previous in the Finke lab show that Fab α2,6-sialylated glycans on anti-amyloid IgG antibody 4G8 correlate with lower BBB efflux *but not influx*[2]. IgG sialic acid may enable better IgG drug retention in the brain.



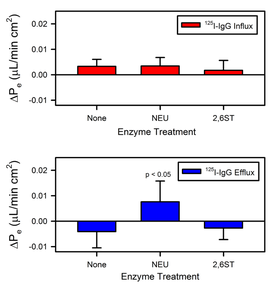
Treatment of 4G8 with neuraminidase

1. Removed all sialic acid (top).
2. Did not alter the influx rate (middle)
3. Reduced the efflux rate (lower).



Treatment of 4G8 with α2,6-sialyltransferase

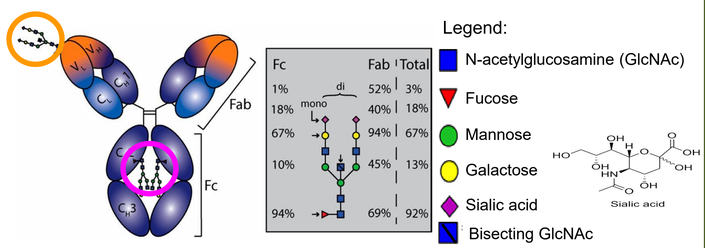
Did not significantly alter these parameters.



Antibodies have a diverse array of Fab glycans

Fab glycans
14% of IgG have them.
Highly processed (long).
Fab sialic acid is easy to detect in IgG (exposed).
PNGase F cannot cleave unless IgG is "denatured".

Fc glycans
All IgG have 2.
Shorter.
Fc sialic acid cannot be detected in intact IgG.
PNGase F will always cleave.

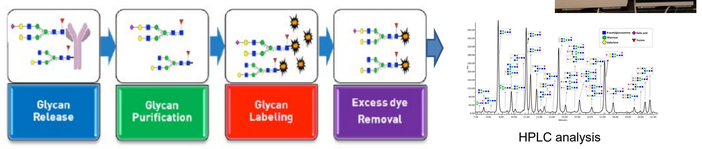


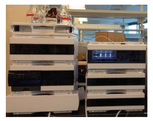
(S with HO or "g" indicates glycolated Sialic acid)

PROJECT GOAL: Make a single sialylated form (G2FS1)

METHODS

Glycan analysis – general process

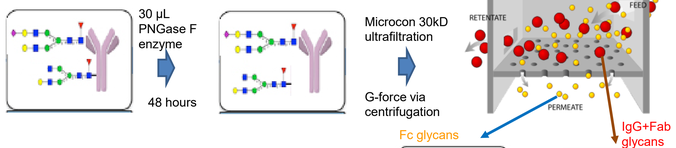




HPLC analysis

Analysis of Fab/Fc glycans

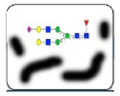
1. Glycan Release of Fc glycans (native conditions).



2. Isolation of cleaved Fc glycans by Microcon 30 kD filtration.

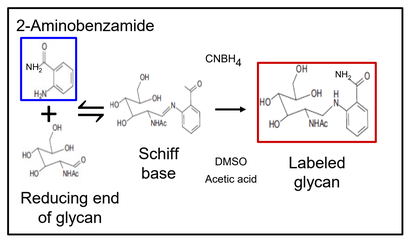
PNGase F
SDS, βme
NP-40

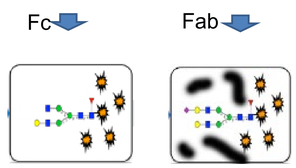
3. PNGase F again on IgG+Fab glycans but with SDS, ß-mercaptoethanol, NP-40 at pH 8.6 (denaturing conditions).



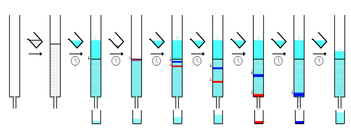
Fab

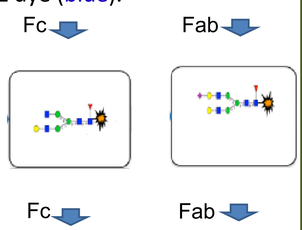
4. Fc and Fab glycans were lyophilized and labeled via reductive amination using 2-aminobenzamide and $CNBH_4$ reductant.



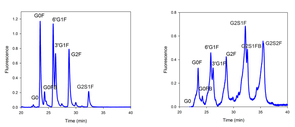


5. Labeled glycans were purified with size exclusion chromatography. Glycans (red) eluted earlier than free ABZ dye (blue).





6. HPLC profile of glycans with polar glycan column. Glycans eluted between 20-40% ammonium formate pH 4.5 in acetonitrile. Measured with ABZ fluorescence.



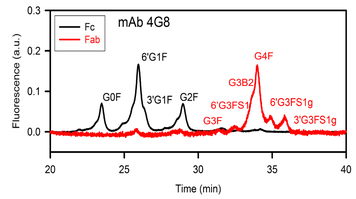
HPLC Profiling of commercial 4G8 and Enzyme-Treated 4G8

Analysis of commercial 4G8

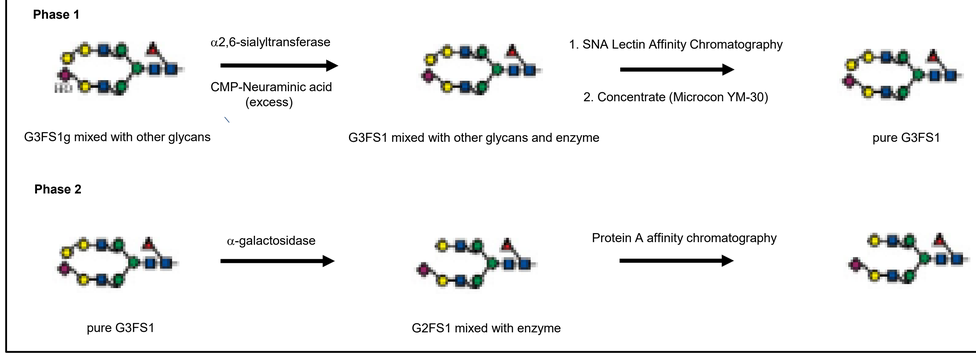
Peak assignments based on LC/MS.

G3 and G4 represent 1 or 2 additional galactose groups added as 3-alpha-galactose to terminal galactose instead of sialic acid. Likely immunogenic to humans.

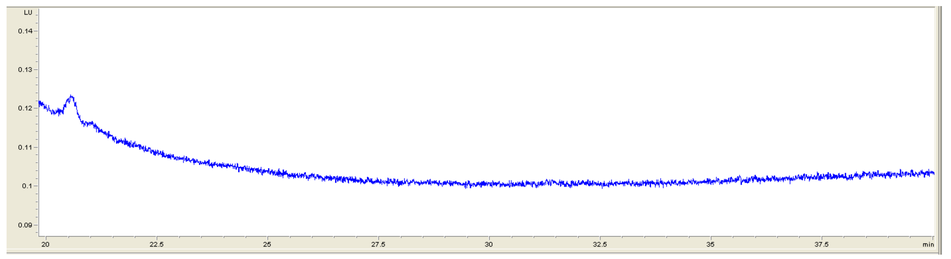
S1g represents glycolated sialic acid. Also likely immunogenic to humans.



Enzyme-Treated 4G8



Results



No peaks detected. Control (above) with untreated 4G8 worked so outcome likely due to low levels of enzyme product after purification steps.

Current work-around:

1. Single enzyme step (α2,6-sialyltransferase + α-galactosidase together).
2. No purification steps (we'll figure this out if we see our product).

REFERENCES

[1]2017 Alzheimer's disease facts and figures. *Alzheimer's Dementia: The Journal of the Alzheimer's Association*. 2017; 3(4):325-373.
[2]Finke, J.M., et al. "Antibody Blood-Brain Barrier Efflux Is Modulated by Glycan Modification." *Biochimica Et Biophysica Acta (BBA)* - General Subjects, 2017, 1861: 2228–2239., doi:10.1016/j.bbagen.2017.06.008.
[3]van de Bovenkamp, Fleur S., et al. "The Emerging Importance of IgG Fab Glycosylation in Immunity." *The Journal of Immunology*, 2016; 196: 1435.
[4]Anumula K.R., "Quantitative glycan profiling of normal human plasma derived immunoglobulin and its fragments Fab and Fc." *Journal of Immunological Methods*, 2012; 382:167-176.
[5]Perdivara et. al., "Glycosylation profiles of epitope-specific anti-β-amyloid antibodies revealed by liquid chromatography–mass spectrometry", *Glycobiology*, 2009; 19:958-970.