Glycan derivatization with the Anti-Amyloid-β Monoclonal Antibody 4G8 treatment

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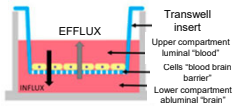
INTRODUCTION

The cause of Alzheimer's disease seems to be deficiently understood. There are many genetic and environmental factors that plays a huge part in how this disease progressed.

Affected people will rely on heavily to their caregivers. With this, it is placing a huge burden and pressure to both people. The antibody drugs may be able to impact Alzheimer's symptoms but cannot be fully actualized due to their inability to be delivered to the brain. This study investigated the glycan modification that could alter IgG BBB permeability to be able to get a better drug retention in the brain for Alzheimer's patient.

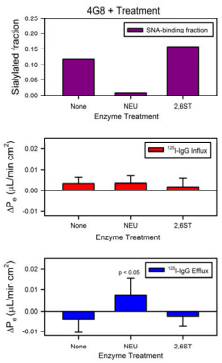
Blood Brain Barrier Studies of IgG Sialic acid

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).



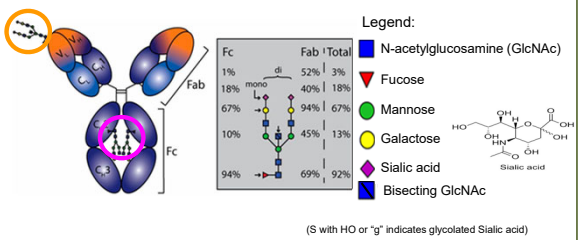
Treatment of 4G8 with α2,6-sialyltransferase

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.

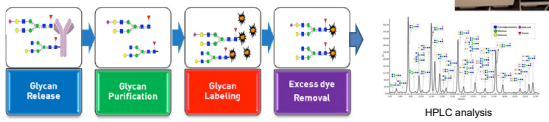


(S with HO or "g" indicates glycolated Sialic acid)

PROECT GOAL: Make a single sialylated form (G2FS1)

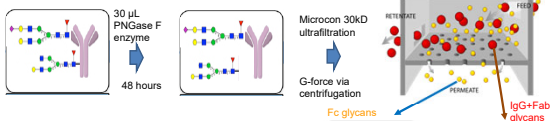
METHODS

Glycan analysis – general process



Analysis of Fab/Fc glycans

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



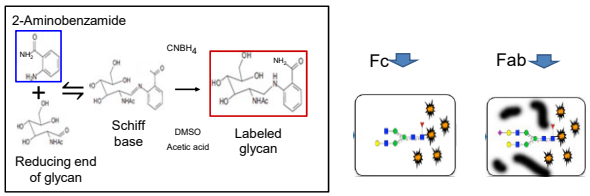
2. Isolation of cleaved Fc glycans by Microcon 30 kD filtration.

PNGase F SDS, βme NP-40

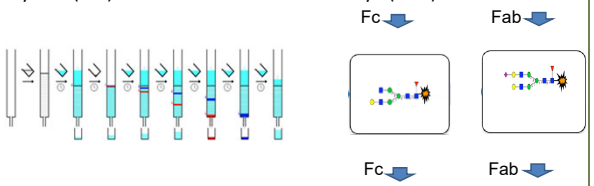
3. PNGase F again on IgG+Fab glycans but with SDS, ß-mercaptoethanol, NP-40 at pH 8.6 (denaturing conditions).

Fab

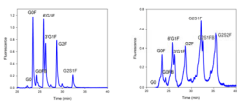
4. Fc and Fab glycans were lyophilized and labeled via reductive amination using 2-aminobenzamide and $CNBH_4$ reductant.



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.



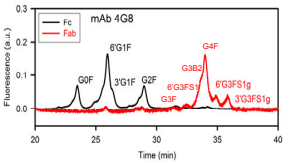
HPLC Profiling of commercial 4G8 and Enzyme-Treated 4G8

Analysis of commercial 4G8

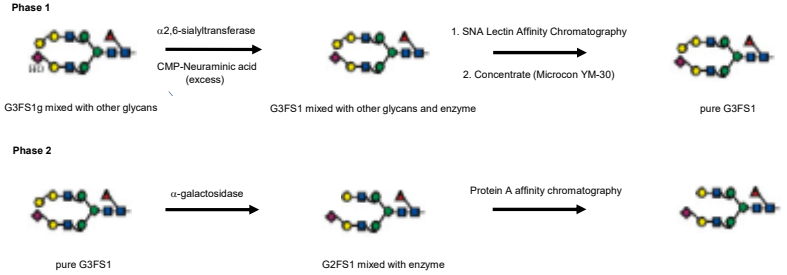
Peak assignments based on LC/MS.

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.

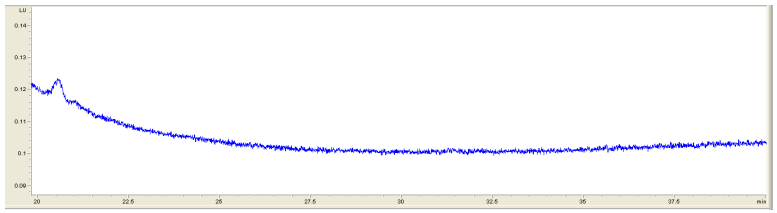
S1g represents glycolated sialic acid. Also likely immunogenic to humans.



Enzyme-Treated 4G8



Results



Using 4G8, a pure sialylated Fab glycan on antibody 4G8 (G2FS2) was produced. With that, Neuraminidase (also called AlphaGal), 26ST added and ECL column to purify.

No peaks were detected. Compare the control above with untreated 4G8. This is likely due to low levels of enzyme product after purification steps.

This analysis showed no functional results has been achieved due to a couple of underlying errors which PNGase must have been expired or had a lesser effect. These results would need further research and modifying some steps where it is suspected that could have affected the outcome to be able to investigate how glycans can be shown in HPLC.

REFERENCES

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FUNDING

National Institutes of Health / NIA R03 AG050184 (JMF, WAB)
Royalty Research Fund (JMF, WAB)
M.J. Murdock Charitable Trust (JMF, ED)