Adjusting the makeup of Glycan proteins to improve IgG BBB permeability

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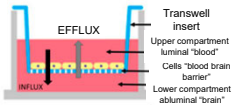
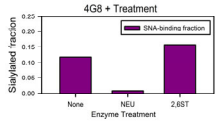
INTRODUCTION

Alzheimer's disease (AD) affects around 10% of the world's population, and it is estimated that within the next 50 years, this diagnosis is expected to to triple. Alzheimer's disease is a neural disorder in which proteins are degrading neural cells and connections. The permeability of antibodies in the brain is very low. This makes it difficult to attack neural diseases in a conventional manner. IgG antibodies are a popular class of immunotherapy and they are used in clinical trials of AD. β-amyloid peptides are found in the brain of AD patients and these kinds of therapies are used to target and eliminate these peptides, as they are the cause of neural degradation. The only issue with this method is that IgG antibodies are not transported effectively in the brain. Dr. Finke in previous studies found that Fab α2,6-sialylated glycans on anti-amyloid IgG antibody 4G8 show a lower BBB efflux without a lower influx.

Blood Brain Barrier Studies of IgG Sialic acid

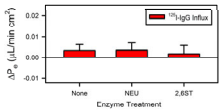


Previous in the Finke lab show that Fab α2,6-sialylated glycans on anti-amyloid IgG antibody 4G8 correlate with lower BBB efflux *but not influx*[2]. IgG sialic acid may enable better IgG drug retention in the brain.



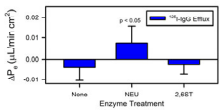
Treatment of 4G8 with neuraminidase

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



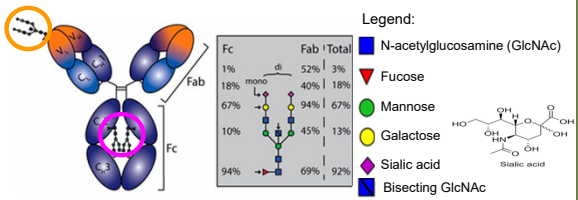
Treatment of 4G8 with α2,6-sialyltransferase

Did not significantly alter these parameters.



Antibodies have a diverse array of Fab glycans

Fab glycans	Fc glycans
14% of IgG have them.	All IgG have 2.
Highly processed (long).	Shorter.
Fab sialic acid is easy to detect in IgG (exposed).	Fc sialic acid cannot be detected in intact IgG.
PNGase F cannot cleave unless IgG is "denatured".	PNGase F will always cleave.

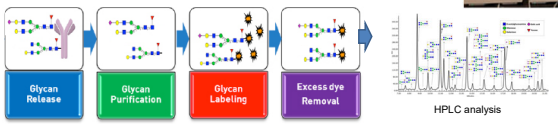


(S with HO or "g" indicates glycolated Sialic acid)

PROECT GOAL: Make a single sialylated form (G2FS1)

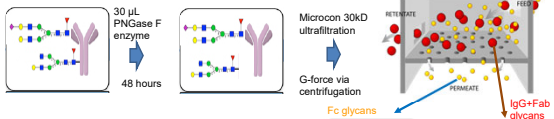
METHODS

Glycan analysis – general process



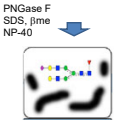
Analysis of Fab/Fc glycans

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

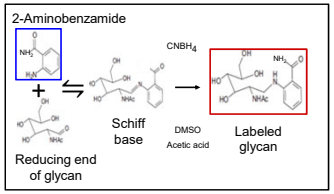


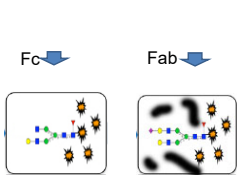
2. Isolation of cleaved Fc glycans by Microcon 30 kD filtration.

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

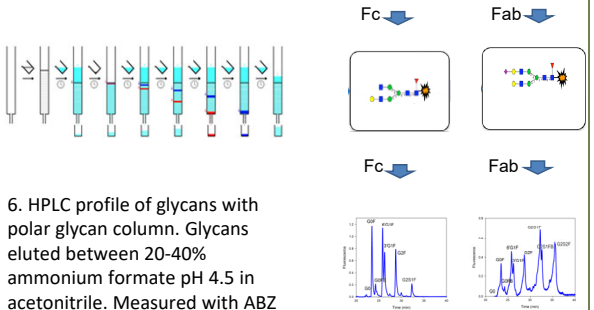


4. Fc and Fab glycans were lyophilized and labeled via reductive amination using 2-aminobenzamide and $CNBH_4$ reductant.





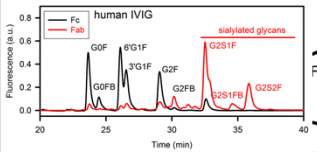
5. Labeled glycans were purified with size exclusion chromatography. Glycans (red) eluted earlier than free ABZ dye (blue).



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.

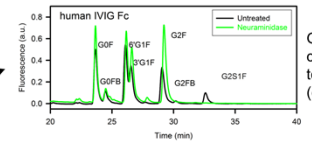
HPLC Profiling of commercial 4G8 and Enzyme-Treated 4G8

Peak assignments based on LC/MS.



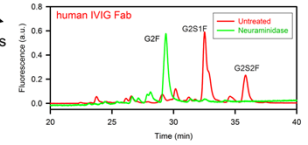
Profiles consistent with standard glycans in published studies[3-5].

Fc Glycans



G2S1F peak converted to G2F (expected).

Fab Glycans

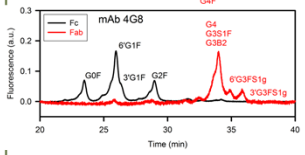


G2S1F and G2S2F peaks converted to G2F (expected).

Effects of neuraminidase (NEU) pre-treatment

Effects of neuraminidase (NEU) OR α2,6 sialyltransferase (26ST) pre-treatment.

Peak assignments based on LC/MS.

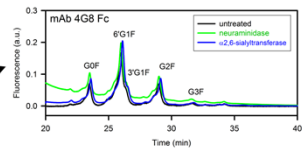


Fc glycans similar to IVIG but lack sialic acid[3-5].

Main asialylated Fab glycans (~70%) are G3F, G4F, G3B2.

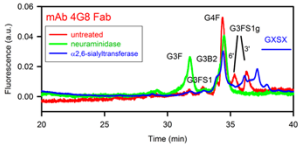
Main sialylated Fab glycans (~30%) are G3S1F with glycolated (S1g or NeuGc) sialic acid.

Fc Glycans



NEU and 26ST show no change in Fc glycans.

Fab Glycans



Results

We didn't register any peaks in our wavelenght graph, meaning there was not any or enough product to be analyzed. Control (above) with untreated 4G8 worked so outcome likely due to low levels of enzyme product after purification steps. These steps reduce our material so much that we get no results from them.

Possible solutions:

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.
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[5]Perdivara et. al., "Glycosylation profiles of epitope-specific anti-β-amyloid antibodies revealed by liquid chromatography–mass spectrometry", *Glycobiology*, 2009; 19:958-970.

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