Modifications of IgG Antibody Glycan

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INTRODUCTION

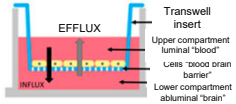
Alzheimer's Disease (AD), is the most common type of dementia and one of the most leading causes of death in the United States. A progressive neurodegenerative disease that involves parts of the brain that controls memory and thought, the prevalence of Alzheimer's Disease is expected to triple by 2050.

One risk factor that is shown to be associated with Alzheimer's Disease is alteration of immunoglobulin G (IgG) found in various regions of the brain. IgG antibodies target forms of beta-amyloid peptides, which are related to the amyloid plaques found in the brains of patients with Alzheimer's Disease.

IgG drugs may be able to prevent the onset of Alzheimer's Disease, therefore this study investigated how alterations of carbohydrates within the IgG antibodies influences the development of the disease in geriatric patients. This study was performed by the enzymatic modification of IgG glycans, followed by analysis involving the isolation and removal of Fab and Fc glycans. The results of this study were unfortunately inconclusive. There were no visible peaks that show the presence of glycan in the HPLC. Additional testing such as the removal of the purification of glycans to prevent the loss of yield may be needed.

Blood Brain Barrier Studies of IgG Sialic acid

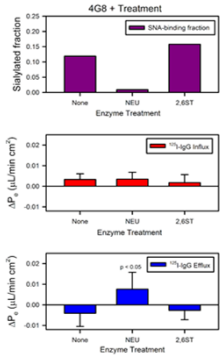
Previous in the Finke lab show that Fab α2,6-sialylated glycans on anti-amyloid IgG antibody 4G8 correlate with lower BBB efflux *but not influx*[2]. IgG sialic acid may enable better IgG drug retention in the brain.



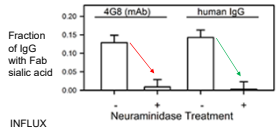
Recent studies show polyclonal human IgG drug IVIG exhibits a different BBB response to sialic acid cleavage than 4G8. Both influx and efflux are increased when sialic acid is removed.

Treatment of 4G8 with neuraminidase

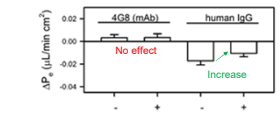
1. The removed all sialic acid (top).
2. Did not alter the influx rate (middle)
3. Reduced the efflux rate (lower).



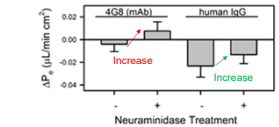
SIALIC ACID



INFLUX



EFFLUX

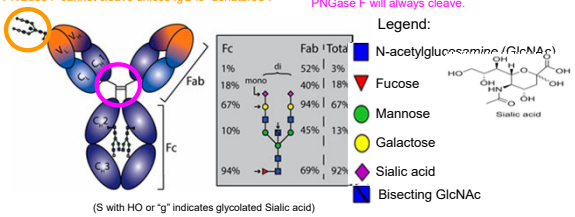


Treatment of 4G8 with α2,6-sialyltransferase did not significantly alter these parameters.

IgG Glycan Profiling – how do 4G8 and IVIG differ?

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".

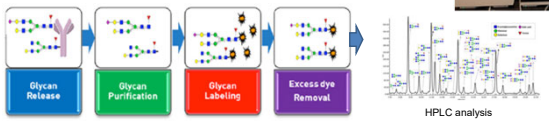
Fc glycans
All IgG have 2.
Shorter.
Fc sialic acid cannot be detected in intact IgG.
PNGase F will always cleave.



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

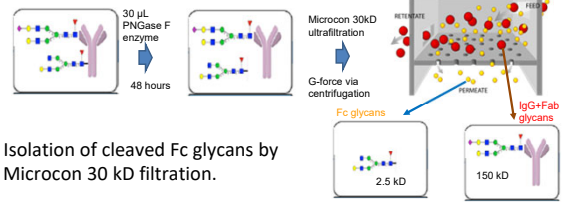
METHODS

Glycan analysis – general process



Analysis of Fab/Fc glycans

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



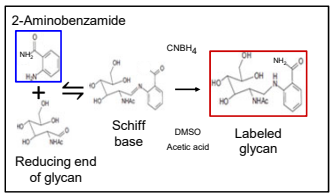
2. Isolation of cleaved Fc glycans by Microcon 30 kD filtration.

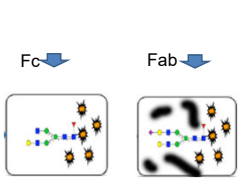
PNGase F SDS, βme NP-40

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

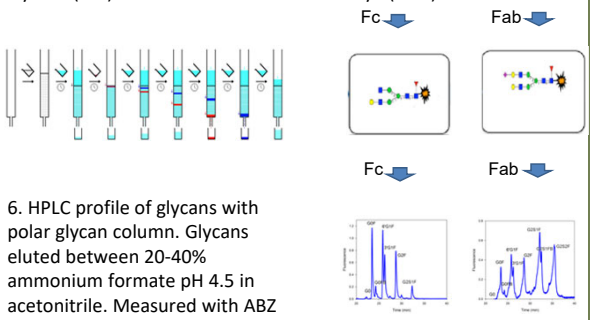
Fab

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).

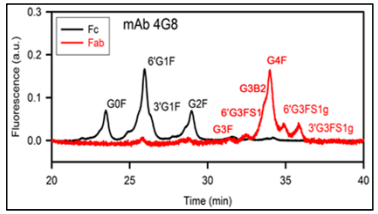


6. HPLC profile of glycans with polar glycan column. Glycans eluted between 20-40% ammonium formate pH 4.5 in acetonitrile. Measured with ABZ fluorescence.

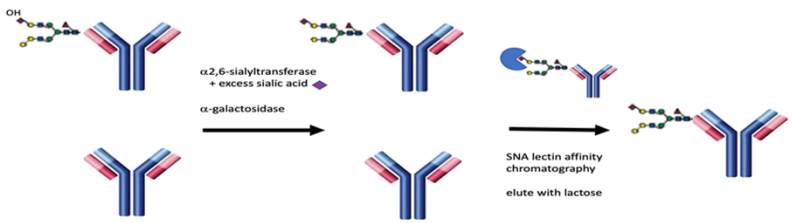
RESULTS: HPLC Profiling of 4G8 and IVIG glycans

Commercial 4G8 Analysis

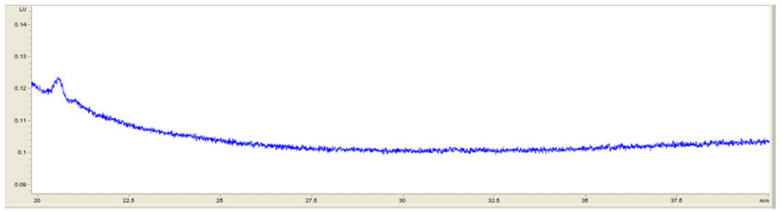
Peak assignments based on LC/MS
G3 and G4 represent 1 or 2 additional galactose groups added as 3-alpha-galactose to terminal galactose instead of sialic acid. Likely immunogenic to humans.
S1g represents glycated sialic acid. Also likely immunogenic to humans.



Enzyme-Treated 4G8 with Purification



Results



Using HPLC, the control (commercial 4G8) gave the expected Fc and Fab peaks. No visible Fc or Fab peaks were found in the enzyme-treated preparation. Since the control gave the expected results, it was concluded that loss from the enzyme preps and purification steps were too great.

To improve the yield, a mixture of 50µL of 4G8, 2µL 26ST, 10µL CAN, and 5µL of **α**Gal was prepared along with water used as a negative control instead of 4G8. No purification steps will be used in order to retain a high antibody concentration sufficient for analysis. Results of this analysis are pending.

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