Increasing IgG Permeability in the Blood Brain Barrier by Isolation and Removal of FC and Fab Glycans to Treat Alzheimer's Disease.

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INTRODUCTION

Alzheimer's disease is a type of dementia which progressively impacts memory and other important mental functions through the destruction of brain cell connections. This disease is very common and while treatment can help prevent progression, it is expensive to treat and there is no official cure. Understanding the mechanism behind the development and progression of Alzheimer's disease can aid in finding potential cures, which can improve the lives of millions of families impacted by this disease. Most attempts at finding a cure have not been successful due to drug treatments not penetrating the blood brain barrier (BBB). Some progress has been made on altering the permeability of modified human antibodies through the BBB. Here we sought to use 4G8, a type of mouse monoclonal antibody that consists of IgG antibody with a sialylated Fab glycan, meaning it has a sialic acid on the tip of the molecule. This sialic acid is important to recognizing and binding the amyloid plaque that causes neuronal decay. Previous research shows that glycosylation may improve the possibility of antibody delivery to the brain. We sought to replace the human IgG sialic acid group with that of a 4G8 to get the antibodies to cross the BBB. We found that we were able to successfully replace the sialic acid groups on IgG antibodies, but we were unable to determine whether these antibodies can pass the BBB.

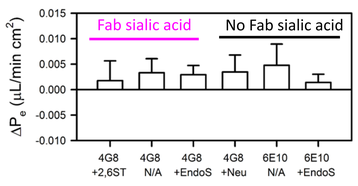
Testing BBB permeability:

Comparing influx and efflux results of the Fab sialic acid presence and absence by testing BBB in vitro.

Influx (into the brain): Altering antibody drugs to increase passage through the BBB.

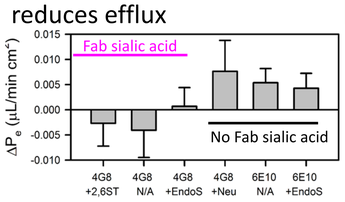
Efflux (out of the brain): Inhibition of efflux prolongs the presence of antibodies and drugs in the brain.

Influx Results: No Difference



(Finke et. al, 2017)

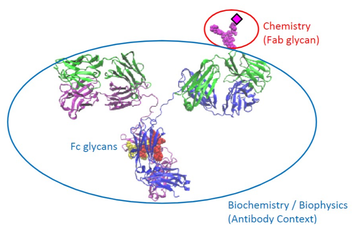
Efflux Results: Fab sialic acid reduces efflux



(Finke et. al, 2017)

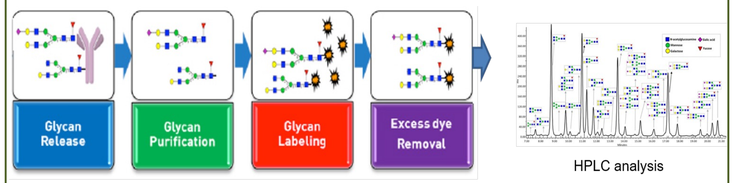
OBJECTIVE

Why does sialylation lower human IgG influx but not 4G8 ?



Make 4G8 homogeneous.
- Take sialic acid off the tips of 4G8 antibody and swap with the sialic acid in IgG antibody

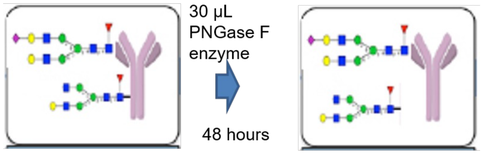
Methods and Materials



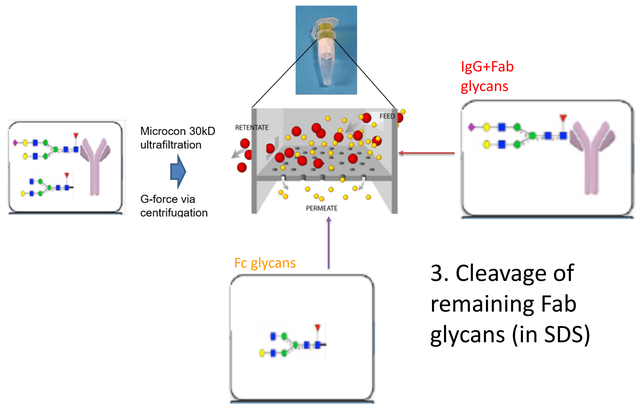
Fab/Fc glycan Analysis

Separating Fab/Fc Glycans

1. Selective Fc glycan cleavage (native conditions):

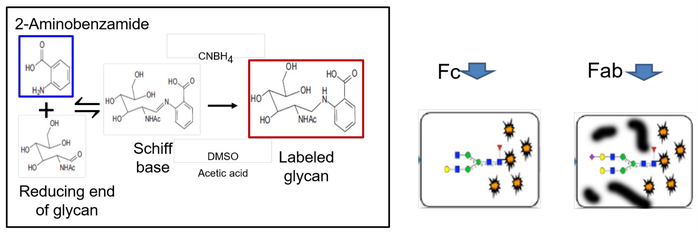


2. Isolation of cleaved Fc glycans:



3. Cleavage of remaining Fab glycans (in SDS)

4. Fc and Fab glycans are glycosylated and labeled via reduction amination using 2-aminobenzamide and CHBH4 reductant:



5. HPLC analysis to verify intended modifications.

RESULTS

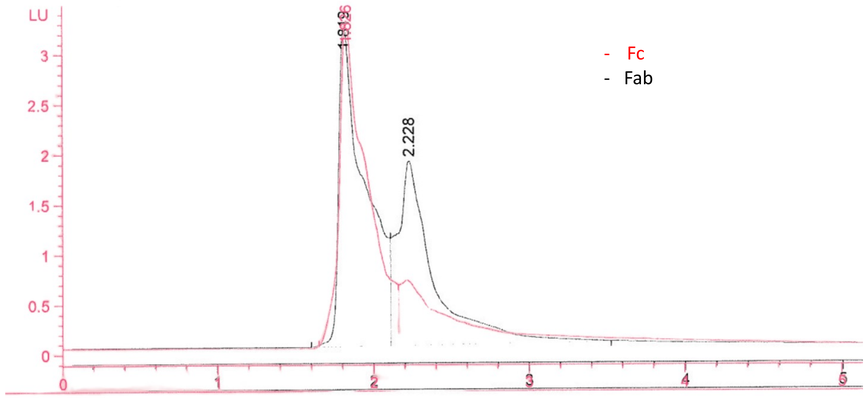


Figure 1. HPLC data shows sialylation of Fc (red) along with Fab glycans (black) with the successful removal from the antibody

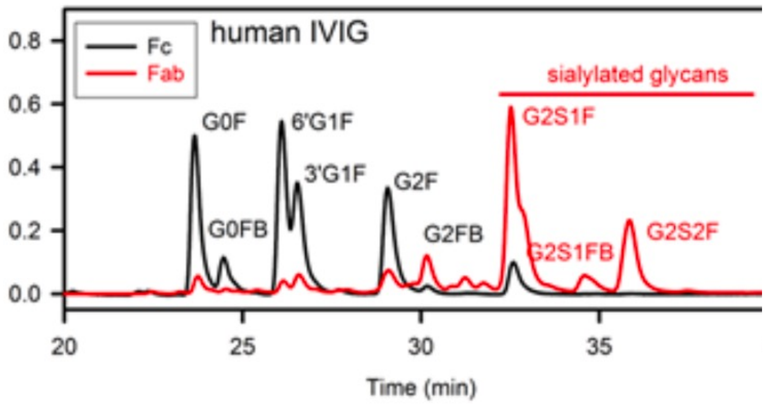


Figure 2: Dr. Finke's research group from 2 years ago (Finke et. al 2022) was not able to successfully sialylate the glycans, as seen in the red peaks above, differing than the black peaks to the right. The peaks in Figure 1 match the red peaks seen in Figure 2, indicating successful sialylation.

REFERENCES

CDC. 2021 Apr 7. What is Alzheimer's Disease? | CDC. wwwcdcgov. https://www.cdc.gov/aging/aginginfo/alzheimers.htm#:~:text=Alzheimer%27s%20disease%20is%20the%20most.

Finke JM, Ayres KR, Brisbin RP, Hill HA, Wing EE, Banks WA. 2017. Antibody blood-brain barrier efflux is modulated by glycan modification. Biochimica et Biophysica Acta (BBA) - General Subjects. 1861(9):2228–2239. doi: https://doi.org/10.1016/j.bbagen.2017.06.008.

ACKNOWLEDGEMENTS

We would like to thank Dr. Finke for the mentorship in doing this experiment.