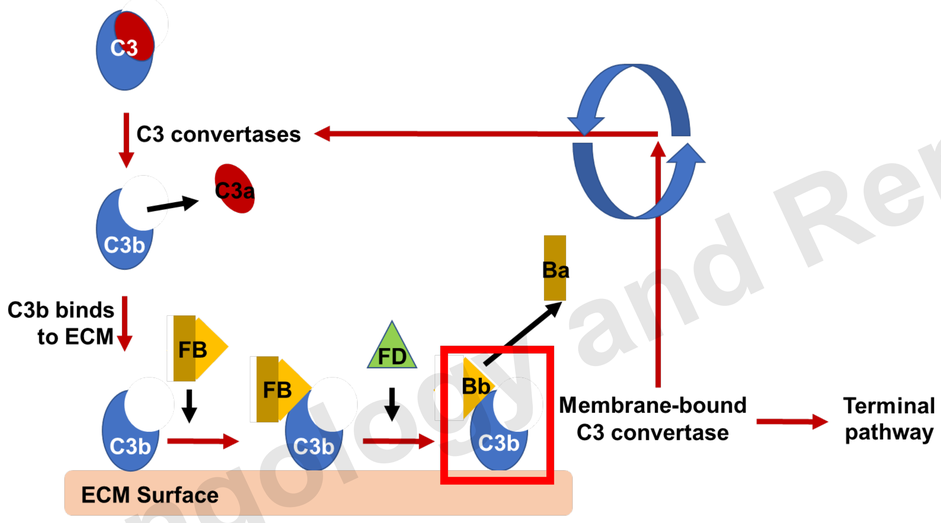
In vitro modeling of the glycomatrix to study kinetics of C3 convertase

Sofiya Pisarenka, Dr. Xue Xiao, Dr. Yuzhou Zhang, Dr. Richard J. Smith

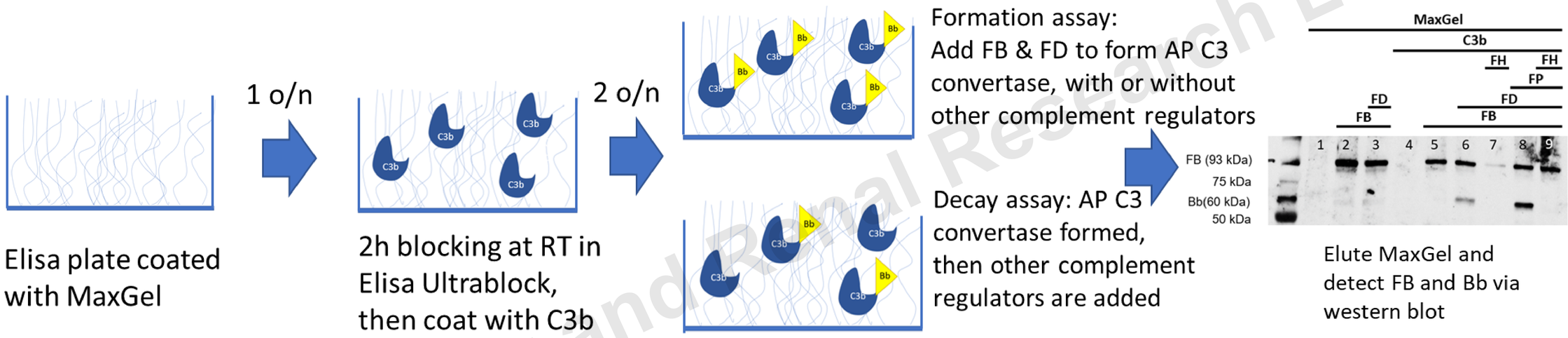
Molecular Otolaryngology and Renal Research Laboratories, Carver College of Medicine, University of Iowa



Introduction

C3 convertase of the Alternative Pathway (AP) is the cornerstone of complement. Dysregulation of the complement cascade can lead to various diseases, including C3 glomerulopathy that is characterized by C3 deposition within the glomerular basement membrane (GBM) in glomeruli. The glomerulus represents a unique microenvironment in the body, due to glomerular endothelial cells being fenestrated. This poses additional challenge to complement control, as not only does its activity need to be controlled in the fluid phase and on the surface of endothelial cells, but also on the glycomatrix (glycocalyx and GBM) that is exposed by the fenestrae. Currently, glycomatrix complement control has not been studied well and is not well understood. In this body of work we are using an *in vitro* model of the glycomatrix (MaxGel) to advance our understanding of the molecular mechanisms of AP regulation in the normal and in the diseased state.

Methods



Results

Figure 1

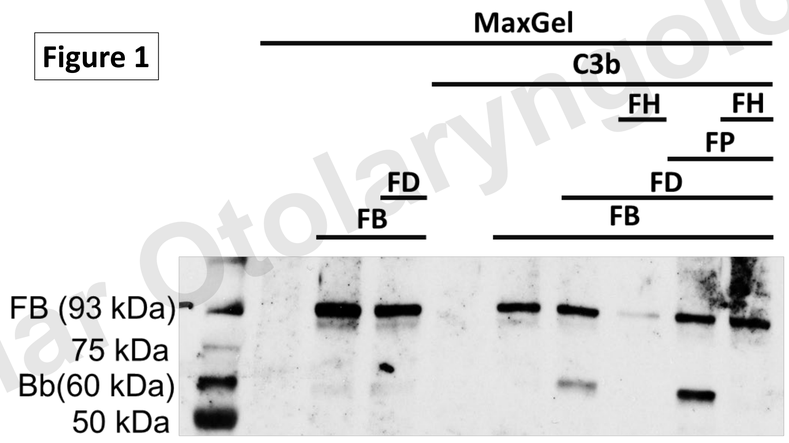


Figure 2

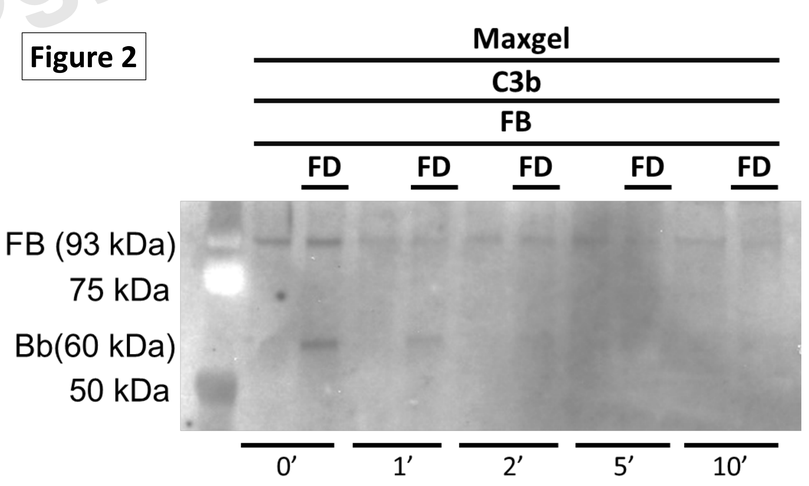


Figure 3

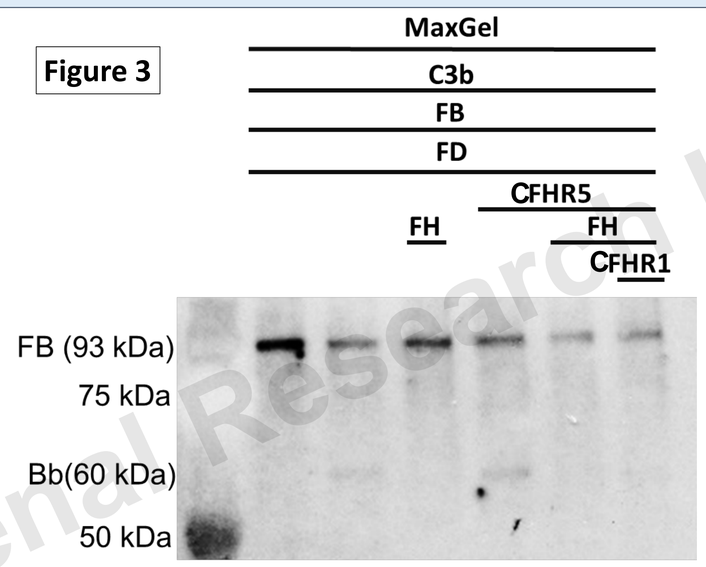


Figure 4

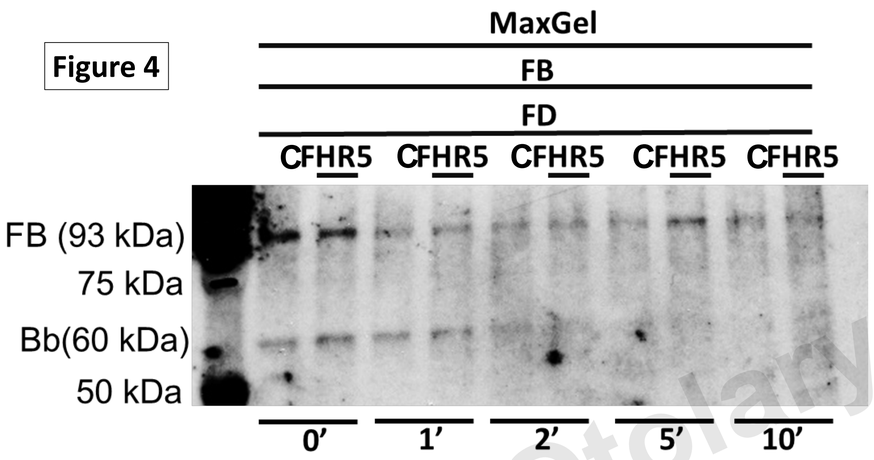


Figure 1: Western blot showing a proof-of-concept AP C3 convertase formation assay using an *in vitro* substitute of glycomatrix (MaxGel). All the complement proteins were used in 1:20 of normal physiological concentration. Convertase successfully formed and was regulated by FH and FP on the surface of MaxGel.

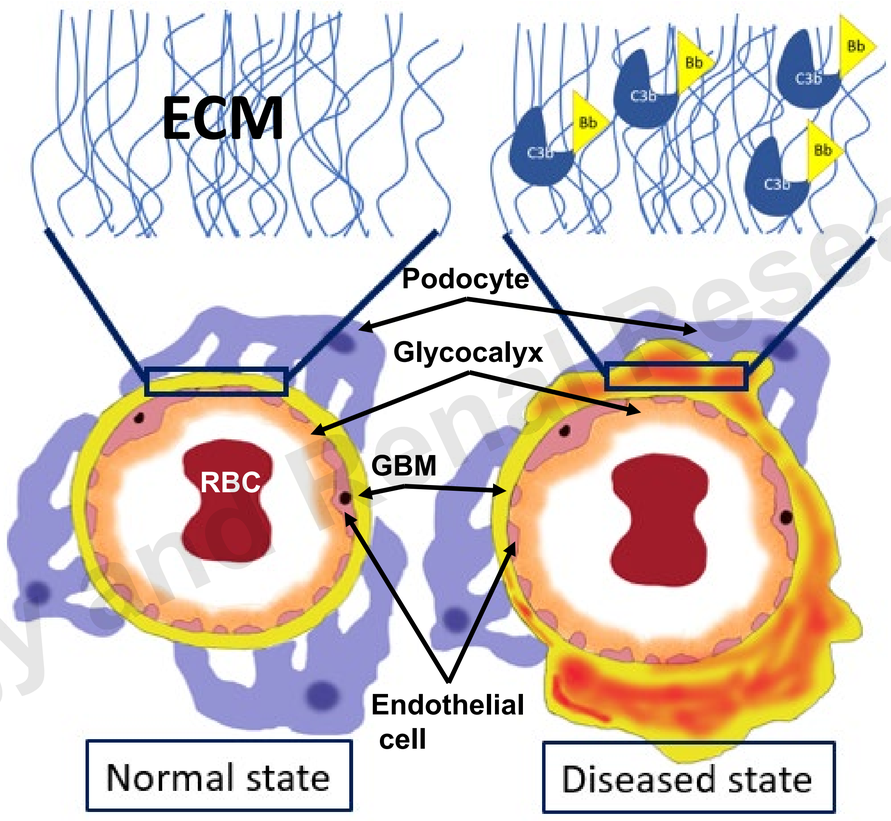
Figure 2: Proof-of-concept AP C3 convertase decay assay, all proteins were used in 1:20 of normal physiological concentration

Figure 3: CFHR5 potentiates formation of AP C3 convertase in the absence of inhibitor of convertase FH.

Figure 4: CFHR5 does not stabilize AP C3 convertase, thus having no affect on decay rates of AP C3 convertase.

Conclusions and Future Directions

In this body of work we show results from a robust assay designed to study formation and decay rates of AP C3 convertase at the surface of the glycomatrix, which in turn allows us to better understand complement dysregulation within the glomerulus that leads to C3 depositions within the GBM of affected patients. Here we show that AP C3 convertase formation is potentiated by the presence of CFHR5 at the surface of the glycomatrix, while decay rates of AP C3 Convertase are not affected by it. Our ongoing experiments, as well as future work will be directed towards modelling the effects of CFHR1 and CFHR5 on AP C3 convertase formation and decay rates in presence and absence of physiological ratios of FH. We will also be looking into the effect of presence of C3 nephritic factors in the sera of patients, as well as mutations in C3, FH and FB proteins on AP C3 convertase .



References and Contact Information

1. Smith RJH, Appel GB, Blom AM, et al. C3 glomerulopathy — understanding a rare complement-driven renal disease. *Nat Rev Nephrol 2019*. 2019:1. doi:10.1038/s41581-018-0107-2
2. Rodríguez De Córdoba S, Esparza-Gordillo J, Goicoechea De Jorge E, Lopez-Trascasa M, Sánchez-Corral P. The human complement factor H: Functional roles, genetic variations and disease associations. *Mol Immunol*. 2004;41(4):355-367. doi:10.1016/j.molimm.2004.02.005
3. Skerka C, Zipfel PF. Complement factor H related proteins in immune diseases. *Vaccine*. 2008. doi:10.1016/j.vaccine.2008.11.021

Please send your questions to Sofiya Pisarenka: sofiya-pisarenka@uiowa.edu