Defining the Impact of Factor H, Factor H-related 1, and Factor H-related 5 on C3b Deposition on Human Glomerular Endothelial Cells

A Heiderscheit[1 2], A Nelson[1], CM Nester[1], Y Zhang[1], RJH Smith[1 2]
[1]*Molecular Otolaryngology and Renal Research Laboratories, Department of Otolaryngology, University of Iowa, USA*
[2]*Graduate Program in Immunology, University of Iowa, USA*

Introduction

The alternative pathway (AP) is a continuously active, low-level process that is the first line of defense against foreign invaders (**Figure 1**). Mediators of the AP, such as Factor H (FH), regulate the amplification loop and prevent excessive activation, while Factor H-related 1 (FHR1) and Factor H-related 5 (FHR5) are thought to enhance the complement response. FH is composed of 20 short consensus repeats (SCRs). SCRs 1-4 are regulatory domains that regulate formation of the C3 convertase. SCRs 6-9 are weak ligand and cell surface recognition domains while SCRs 19-20 strongly recognize ligands and cell surfaces on the glomerular basement membrane (GBM). FHR1 and FHR5 contain unique dimerization SCRs 1-2 and C'-terminal ligand and cell surface recognition SCRs that are similar to SCRs 19-20 of FH (**Figure 2**). Therefore, balance between FH and FHR proteins is critical in controlling the intricate dance between clearance of pathogens and tissue inflammation and damage.

Uncontrolled activation of the AP can lead to excessive C3b deposition on cell surfaces in the glomeruli ultimately impairing filtering function of the kidneys. This can result in a variety of symptoms including hematuria, proteinuria, and decreased kidney function. Therefore, we aimed to determine the impact of FH, FHR1, and FHR5 on C3b deposition on human glomerular endothelial cell (HGEC) surfaces to better understand the renal outcomes. We hypothesized FH would decrease C3b deposition on HGEC surfaces while FHR1 and FHR5 would have the opposite effect.

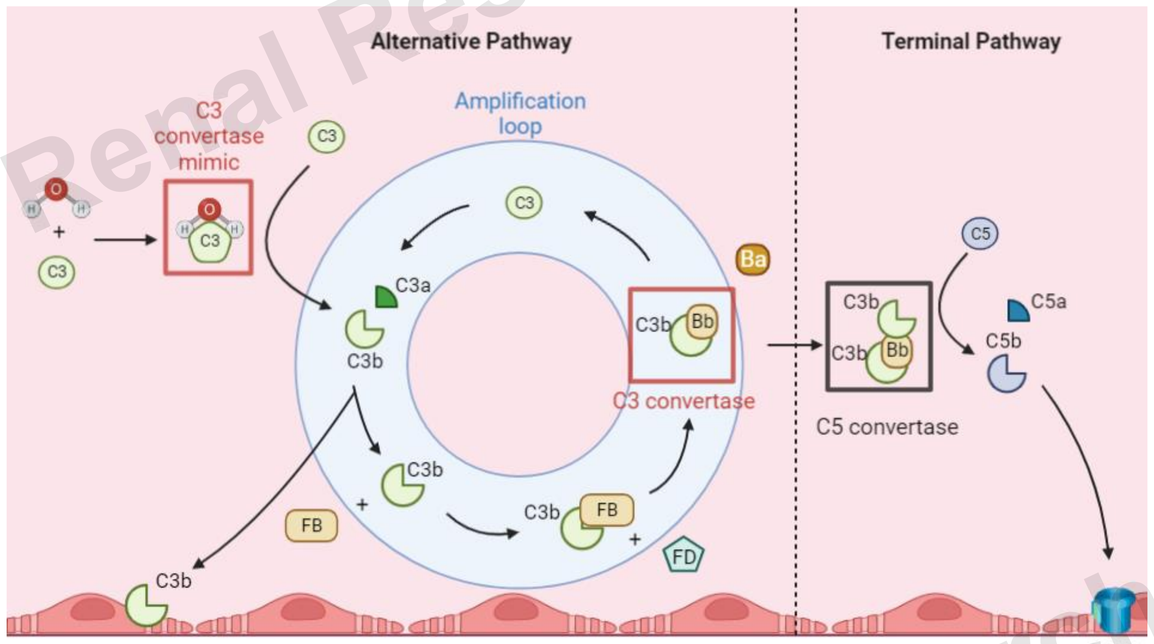

Figure 1. The Alternative Pathway. The AP is activated through spontaneous hydrolysis of thioester bonds on C3, forming a C3 convertase mimic. The C3 convertase mimic can cleave C3 molecules into anaphylatoxin C3a and opsonin C3b, resulting in C3b deposition on cell surfaces. Deposited C3b binds to Factor B (FB), which is cleaved by Factor D (FD) to form the C3 convertase. The C3 convertase can then proceed in one of two directions 1) cleave additional C3 molecules into C3a and C3b, creating an amplification loop resulting in C3b deposition on cell surfaces or 2) enter the terminal pathway by binding C3b to form the C5 convertase. The C5 convertase cleaves C5 into C5a and C5b; C5b interacts with C6, C7, C8, and several C9 molecules to form the membrane attack complex (MAC).

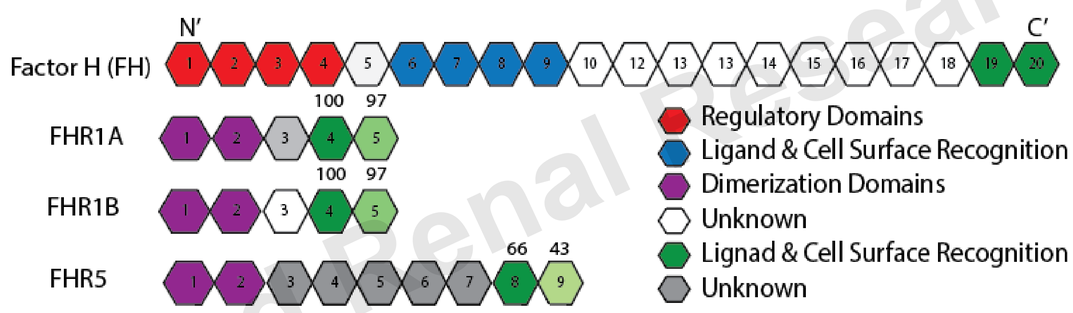

Figure 2. The human factor H family. Hexagons represent SCRs, numbers above hexagons represent amino acid similarity percentages to FH SCRs 19-20; A and B represent acid and basic forms of FHR1.

Methods

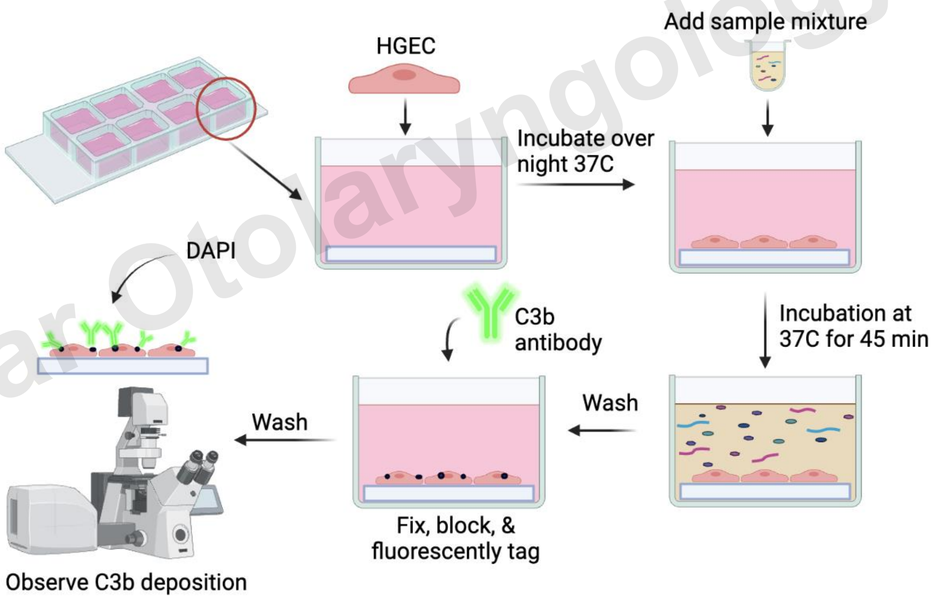

Figure 3. C3b deposition assay. The C3b deposition assay measures complement-mediated C3b deposition on HGEC surfaces through generation of the AP C3 convertase. HGECs are spiked with Factor H, tag-free Factor H-related 1A or tag-free Factor H-related 5 diluted in an AP activation buffer. C3b deposition is visualized on HGEC surfaces using Alexa-488 labeled anti-C3 antibody.

Patient Information

Genetic & Functional Testing	Patient	Interpretation
CFH Gene	p.Tyr352Ter	Pathogenic
FH Level	184ug/ml	Low Normal
FHR1 Level	19.3ug/ml	Normal
FHR5 Level	8.1ug/ml	Normal
C3 Level	1.1g/L	Normal

Table 1. Patient genetic and functional testing. Comprehensive genetic and functional testing was preformed by the Molecular Otolaryngology and Renal Research Laboratory's Clinical Diagnostics Team. C3 glomerulopathy patient DNA, serum and plasma were tested and compared to normal ranges. Genetic testing revealed a pathogenic variant in *CFH* resulting in a low FH level, 184ug/ml (normal range 180ug/ml-420ug/ml).

Results

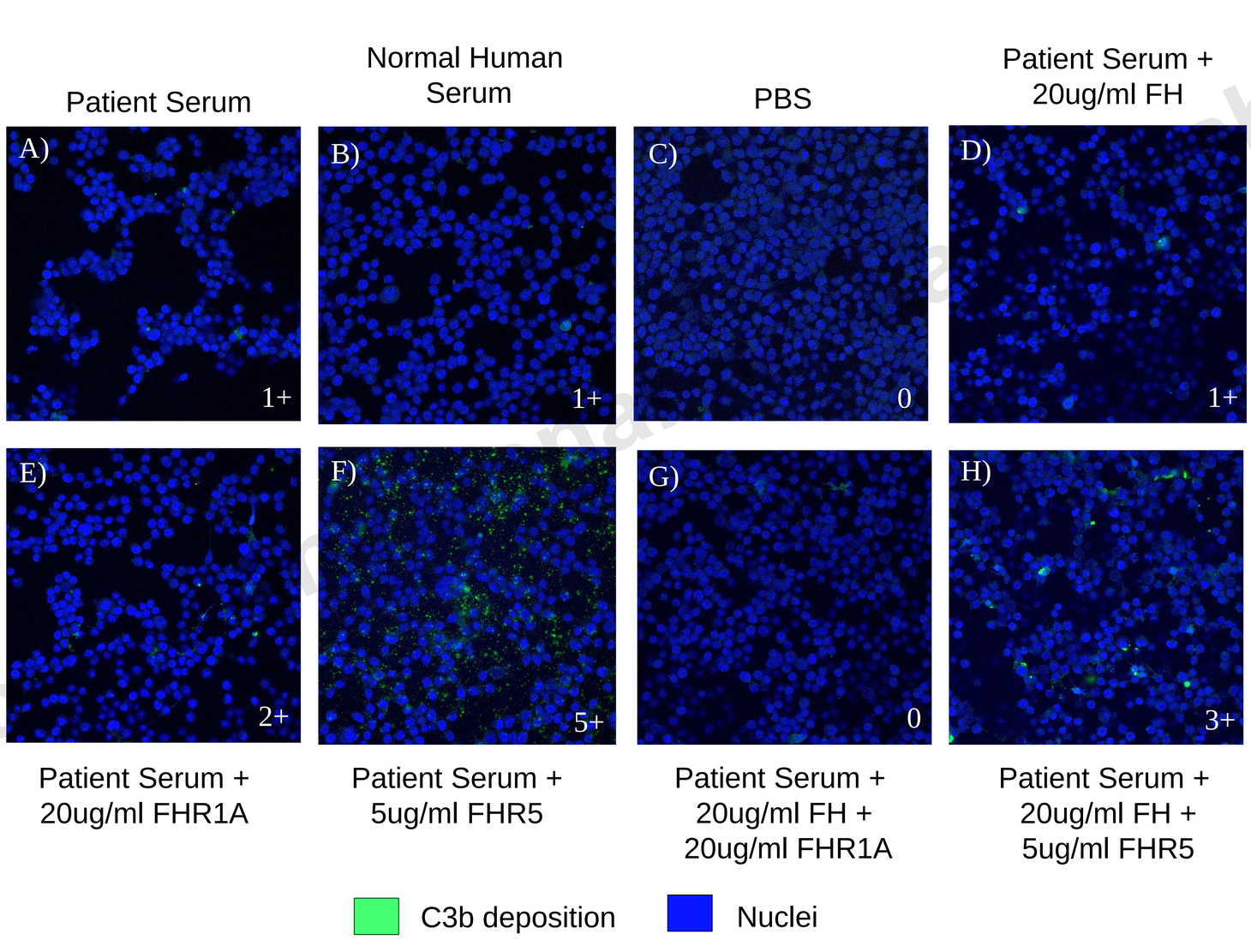

Figure 4. C3b deposition on human glomerular endothelial cells. Human glomerular endothelial cells (HGECs) were incubated with serum, FH, tag-free FHR1, and/or tag-free FHR1 diluted in AP activation buffer. C3b deposition is seen in green and HGEC nuclei in blue. Amount of C3b deposition was quantified numerically on a scale of 0-5+ in the bottom right corner of each image. A) HGECs spiked with serum from a C3G patient caused 1+ C3b deposition. B) HGECs spiked with pooled normal human serum caused 1+ deposition on HGECs. C) HGECs spiked with phosphate buffer solution (PBS) caused no C3b deposition. D) HGECs spiked with patient serum and 20ug/ml human Factor H (FH) did not alter C3b deposition on HGECs. E) HGECs spiked with patient serum and 20ug/ml tag-free Factor H-related 1 acidic isotype (FHR1A) increased C3b deposition on HGECs compared to panel A. F) HGECs spiked with patient serum and tag-free 5ug/ml Factor H-related 5 (FHR5) drastically increased C3b deposition on HGECs compared to panel A. G) HGECs spiked with patient serum, 20ug FH, and 20ug/ml tag-free FHR1A resulted in no C3b deposition compared to panel A and E. H) HGECs spiked with patient serum, 20ug/ml FH, and 5ug/ml tag-free FHR5 increased C3b deposition compared to panel A but decreased C3b deposition compared to panel F.

Conclusions

- FHR1A increases C3b deposition on HGECs
- FHR5 increases C3b deposition on HGECs
- The ratio between FH and FHR1A/FHR5 impacts C3b deposition on HGECs

Acknowledgements/ References

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