# Maturation of decay accelerating activity response across the natural history of C3 Glomerulopathy.

Christopher Culek[1], Monica Hall[1], Yuzhou Zhang[1], Richard J.H. Smith[1,2] and Carla M. Nester[1,2]

[1]Molecular Otolaryngology and Renal Research Laboratories, [2]Stead Family Children's Hospital

University of Iowa Stead Family Children's Hospital

The University of Iowa

## Background

C3 Glomerulopathy (C3G) is an ultra-rare complement-mediated renal disease characterized by dysregulation of the alternative pathway (AP) of complement.[1] Complement dysregulation is often driven by a nephritic factor (C3Nef), an autoantibody to the C3 convertase (C3bBb) of the AP.[2] We have previously shown that the properties of Nefs change over time.[3] *We hypothesized that those changes will be associated with changes in underlying complement dysregulation.*

## Methods

IgG was purified from normal human serum (control) and from sera collected across six disease time points of a well characterized C3G patient. Using SPR (Biacore), the C3 convertase (C3bBb) was formed on a CM5 sensor chip. Purified test or control IgG was injected to form the Nef-C3bBb complex. The ability of native complement regulators to decay the Nef-C3bBb complex (normally resistant to regulators) was assessed by injecting Decay Accelerating Factor (DAF), Complement Receptor 1 (CR1), Factor H (FH), or control reagent. Resistance to decay was determined by the ratio of post- to pre-regulated convertase. Data were compared to time associated complement biomarker results. Additional dilution series tests were completed. DAF was treated to a 5x dilution series between 114nM and 0.18nM, and FH was also treated to a 5x dilution series from 17500nM to 28nM. Data was collected described above. IgG purified from all six patient samples and pooled normal human sera were tested alongside buffer ("No-IgG").

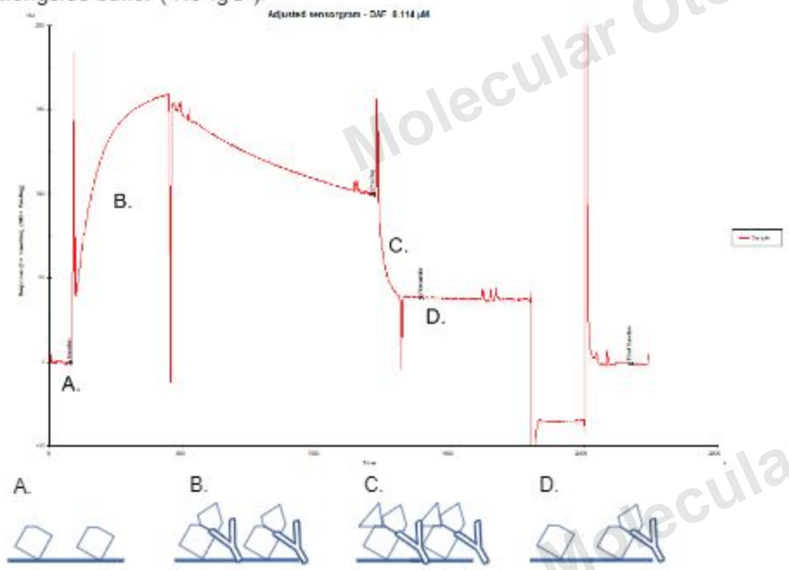


***Fig 1.*** *SPR Injection and Data Collection Scheme.* **A**: C3b-immobilized chip presents sensorgram baseline; RU=0. **B**: injection of FB, FD, and IgG promotes construction of Nef-stabilized C3-convertase complexes; a latent period ends at "Pre-Regulation" RU=100 **C**: injection of DAF promotes DAA of sensitive complexes. **D**: DAF-resistant complexes remain post regulation; RU between 0-100.

## Results

As expected, the presence of Nef conferred resistance to the normal decay accelerating activity (DAA) of DAF, CR1, and FH. Resistance to DAA was highest in the earliest sample (S1, p= <0.0001), with a reduction in subsequent samples (Fig. 2). This change was coincident with reduced complement activity independent of Nef titer (Table 1). Low DAF resistance was maintained in later samples, whereas CR1 and FH resistance gradually increased. Increasing concentrations of DAF and FH resulted in greater complement regulation as indicated by reduced residual convertase (Fig. 3 and 4, respectively). Both regulators show decreased potency against IgG-containing samples. Additionally, both regulators show decreased efficacy when competing against the pathogenic sample S1.

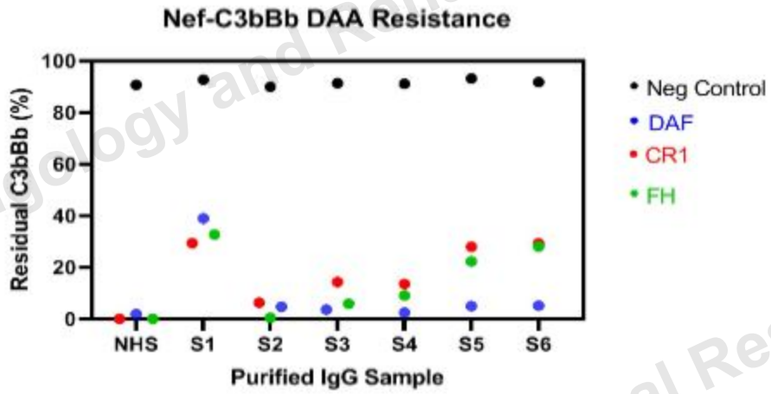


***Fig. 2.*** *Sensitivity to Decay Accelerating Activity for several IgG samples.* The sensitivities of the IgG-C3-convertase complex from six Patient samples and NHS were tested against a buffer, DAF, CR1, and FH. The total convertase present immediately before regulation was normalized to 100%. The y-axis quantifies residual convertase post-regulation. No regulator-induced decay accelerating activity is approximated by 100% residual convertase while total decay is equivalent to 0% residual convertase.

| Biomarker | C3CSA | C3CSAP | CH50 | C3 | Bb | C3c | FH | C5 |
|---|---|---|---|---|---|---|---|---|
| Normal Range | (<20%) | (<20%) | (>70Eq/mL) | (0.9-1.8mg/L) | (<2.2mg/L) | (<1.5 mg/L) | (180-420 mg/L) | (13.5-27.0 mg/dL) |
| S1 (10/15/2016) | 50 | 48 | 39 | 0.8 | 2.3 | 1.4 | 57 | 9.9 |
| S2 (12/1/2017) | 30 | 19 | 129 | 1.04 | 1 | 0.7 | 241 | 14.5 |
| S3 (11/6/2018) | 25 | 17 | 155 | 1.1 | 1.3 | 0.9 | 305 | 13.9 |
| S4 (1/10/2019) | 26 | 19 | 118 | 1.2 | 0.8 | 0.9 | 309 | 14.4 |
| S5 (10/2/2019) | 20 | 17 | 129 | 1.1 | 1.2 | 0.6 | 209 | 11.2 |
| S6 (6/16/2020) | 17 | 14 | 145 | 1.4 | 1.2 | 0.7 | 276 | 20.5 |

**Table 1**. *Sample Cohort Clinical Biomarkers and Sample Chronology.* The results for several well-established complement biomarkers and complement functional assays are shown for each of the six patient samples tested.[4] Note the normalization of data across all eight biomarkers between S1 and S2, which indicates resolution of the underlying complement dysregulation. In this patient, the improved complement activity suggests reduced Nef pathogenicity in samples S2-6 when compared to S1.

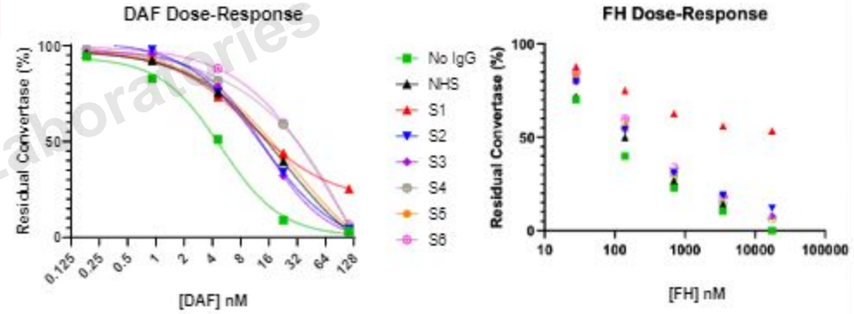


***Fig. 3, 4***. *Dose-Response relationship for several IgG sources against DAF and FH.* The semilog dose-response curves for DAF and FH DAA against the C3-convertase in complex with IgG are shown. The NHS-IgG test condition included IgG from pooled normal human serum and is shown in black. The "No-IgG" test condition exposed no IgG to the convertase and is shown in green. Each of the six Patient 4 IgG samples were tested independently and are color-coded. The y-axis represents residual convertase present after regulation, with 100% residual convertase equivalent to no DAA. DAF DAA is inversely proportional to percent residual convertase, and therefore increased DAF efficacy results in smaller residual convertase values. The baseline RU's present in the test system prior to convertase formation was normalized to 0%.

## Conclusions

- Nef stabilized, C3-convertase resistance to DAA proteins matures over time and is independent of Nef titer.
- Dose response data suggest that the DAA proteins function by noncompetitive inhibition of Nef function in pathogenic sample S1. Whereas less pathogenic S2-6 do not indicate noncompetitive inhibition.
- Changes in response to regulators is accompanied by a relative change in underlying complement dysregulation (as reflected by complement biomarkers).
- The impact of Nef variance (in time and across regulators) on clinical disease course and outcome in patients with C3G warrants further study.
- Whether this phenomenon represents a novel treatment target remains to be seen.

## Future Directions

Future studies will include applying this assay to additional subjects to test the hypothesis that complement regulation changes overtime. Our goal will be to determine if DAA protein interaction may suffice as a predictor of relapse in transplant.

## References

1. *Nat Rev Nephrol.* 15(3), 129-143 (2019)
2. *Kidney Int.* 82, 1084–1092 (2012).
3. *J. Am. Soc. Nephrol. 31, 2020: 565. [Abstract]*
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