Modeling C3 Glomerulopathies on ECM Surface

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Introduction: C3 Glomerulopathies (C3G)

C3G are ultra-rare complement-mediated renal diseases characterized by complement deposits in the glomerulus including the extracellular matrix (ECM) components of the glomerular filtration barrier. Within 10 years of diagnosis, ~70% of children and ~30-50% of adult patients reach end stage renal disease (ESRD). If transplant is offered, C3G will recur in ~60-80% of cases, leading to graft loss in ~50% of cases.

Dysregulation of the C3 convertase of the alternative pathway (AP) of complement (Figure 1) is the main driver of C3G. While existing mouse models of C3G provide fundamental knowledge about C3G pathogenesis, they are ill-suited to model *patient-specific* genetic and acquired drivers of C3G (Figure 2). Here, we present a novel, human-specific *in vitro* model of complement regulation on ECM (MaxGel) surface, upon which we assess patient-specific autoantibodies against the AP C3 convertase (C3Nefs). In this body of work, we elucidate a novel mechanism by which C3Nefs drive C3G through increased AP C3 convertase formation (Figure 7C,D).

Figure 1. The Alternative Pathway of Complement

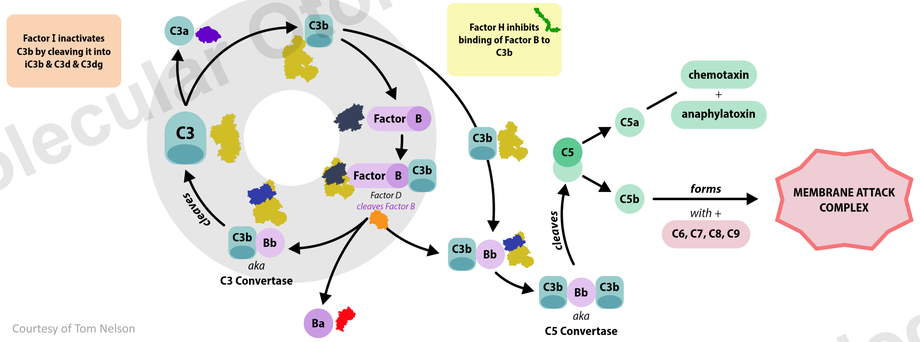
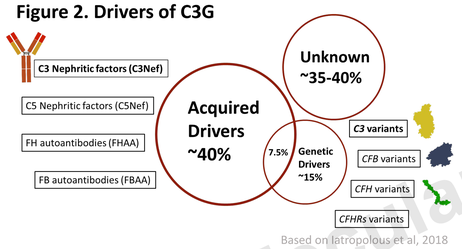
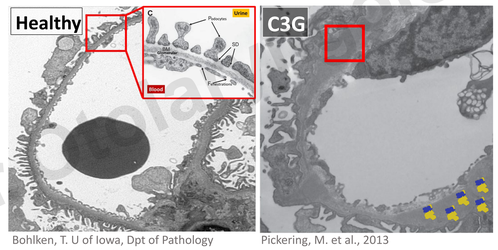


Figure 2. Drivers of C3G





Methods: A novel *in vitro* model of C3G

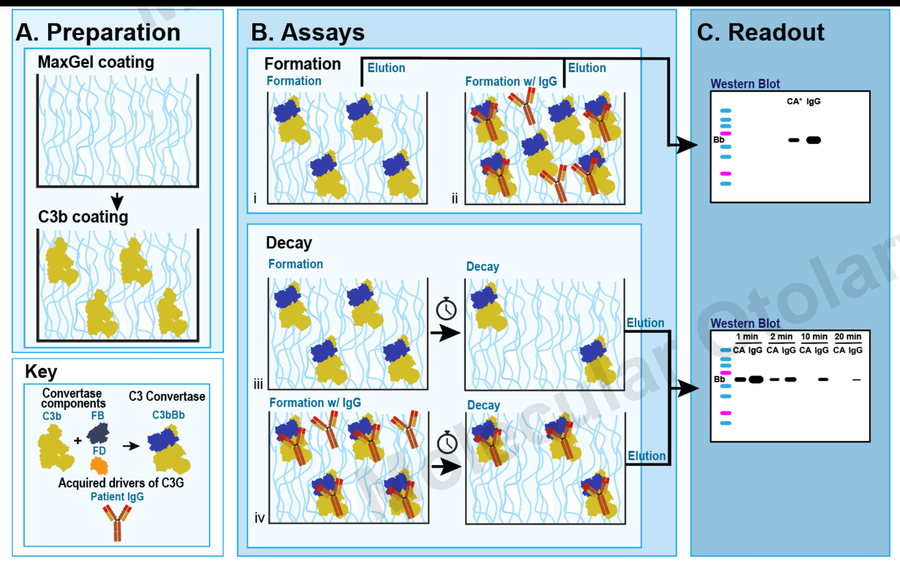


Figure 4. ECM-based model of C3G : C3 convertase assays on MaxGel surface.
(A) MaxGel (ECM) coating, followed by coating with C3b. **(B)** **Formation assay**: Factor B (FB) and Factor D (FD) are added to C3b coated MaxGel, generating C3bBb over the course of 10 min in the absence (**i**) or presence (**ii**) of Patient IgG. **Decay assay**: C3bBb is generated in absence (**iii**) or presence of Patient IgG (**iv**), and then decayed over time. **(C)** C3bBb present on ECM surface at the time of elution is quantitated by western blot against the Bb complement fragment.

Results: C3Nefs can drive C3G by promoting C3 convertase formation

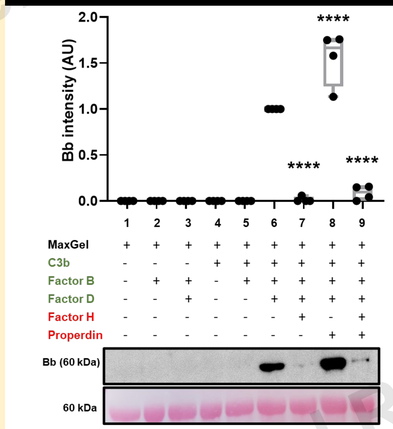


Figure 5. C3 convertase forms and can be regulated on ECM surface. MaxGel does not contain complement proteins necessary to form C3 convertase (lanes 1-5). C3 convertase alone (CA) forms in presence of C3b, FB and FD (lane 6), and is regulated by inhibitory FH (lane 7), stabilizing FP (lane 8) or a mix of FH+FP (lane 9) (n=4)

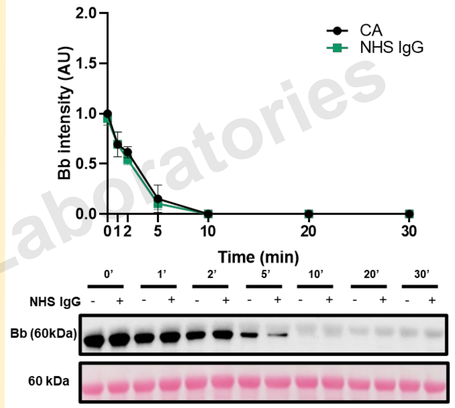


Figure 6. Normal Human Serum (NHS) IgG does not stabilize C3 convertase
Western blot analysis showing C3 convertase formed alone (CA) or in presence of NHS IgG, then decayed for up to 30 min. NHS IgG did not stabilize C3 convertase (NHS IgG $t_{1/2}$=3 min, CA $t_{1/2}$=3.36 min) (n=3)

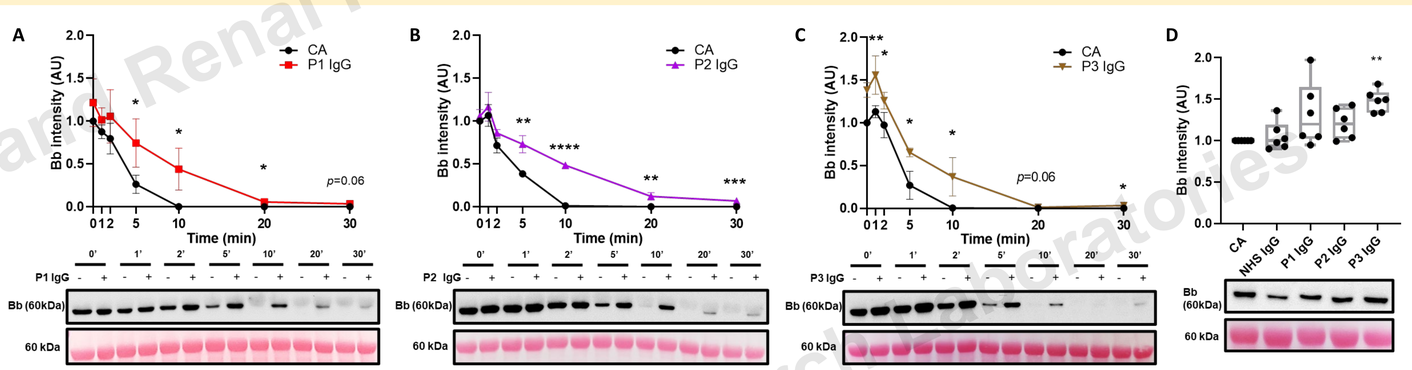


Figure 7. IgG from C3Nef+ C3G patients stabilizes C3 convertase and decreases its decay rate
(A-C) C3 convertase was formed alone (CA) or in presence of IgG derived from C3Nef+ patients, then decayed for up to 30 min. (A) P1 IgG stabilized C3bBb 2-fold; (B) P2 IgG stabilized C3bBb 1.9-fold; (C) P3 IgG stabilized C3bBb 1.7-fold. n=3; (D) CA was formed alone, or in presence of NHS, P1, P2 or P3 IgG. P3 IgG promoted a significant increase in C3bBb formation. (n=6, significance calculated for NHS IgG;* P ≤ 0.05, ** P ≤ 0.01, *** P ≤ 0.001, **** P ≤ 0.0001)

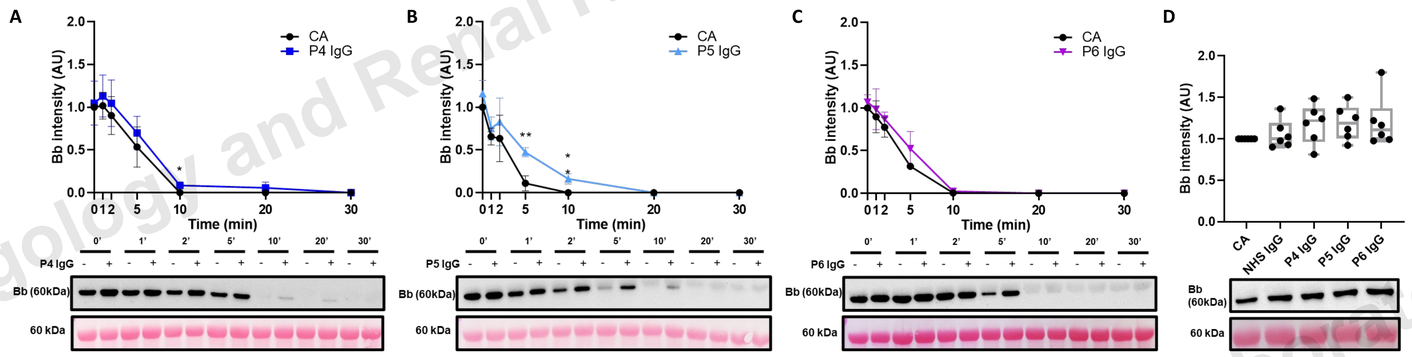


Figure 8. IgG from C3Nef- C3G patients do not promote formation or stabilization of C3 convertase.
(A-C) C3 convertase was formed alone (CA) or in presence of IgG derived from C3Nef- patients, then decayed for up to 30 min. (A) P4 IgG stabilized C3bBb 1.3-fold; (B) P5 IgG stabilized C3bBb 1.7-fold; (C) P6 IgG stabilized C3bBb 1.2-fold (n=3); (D) CA was formed alone, or in presence of NHS, P4, P5 or P6 IgG. IgG from C3Nef- C3G patients did not significantly affect C3bBb formation. (n=6, significance calculated for NHS IgG;* P ≤ 0.05, ** P ≤ 0.01, *** P ≤ 0.001, **** P ≤ 0.0001)

Conclusions

We present an ECM-based *in vitro* model of C3 convertase activity:

- Our assay can be used to interrogate the role of nephritic factors on the C3 convertase.
- It provides a model in which we may study the effect of patient specific point mutations introduced into convertase or complement regulator genes central.
- Our assay offers a potential platform for screening therapeutics in patient-specific manner, providing an estimate of what treatment might benefit the patient most

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