Inhibition and activation? Molecular characterization of monoclonal nephritic factors reveals a paradox.

Christopher T Culek, Seth J Welsh, Hector Martin Merinero, Renee Goodfellow, Cobey Donelson, Yuzhou Zhang, Richard JH Smith, Carla M Nester

University of Iowa Hospitals and Clinics: Molecular Otolaryngology and Renal Research Laboratories

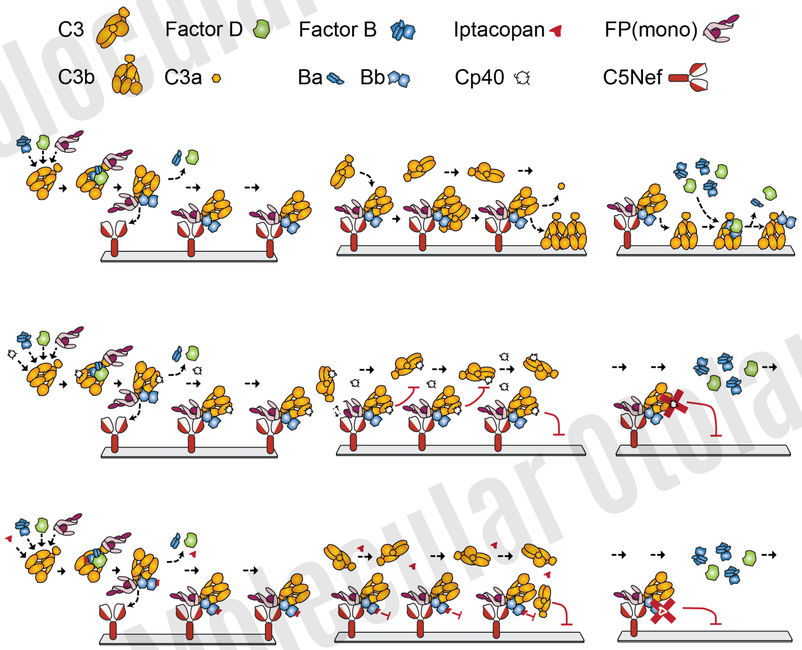
Introduction

Nephritic Factors (Nefs) are autoantibodies that stabilize the C3-convertase and antagonize complement regulator proteins in patients with C3 glomerulopathy (C3G). Nefs are considered pathogenic drivers of complement overactivity and can be properdin-independent (C3Nefs) or -dependent (C5Nefs). Here, we tested the enzymatic activity of the C3-convertase bound to patient-derived monoclonal C3Nefs and C5Nefs to determine their impacts on C3 conversion.

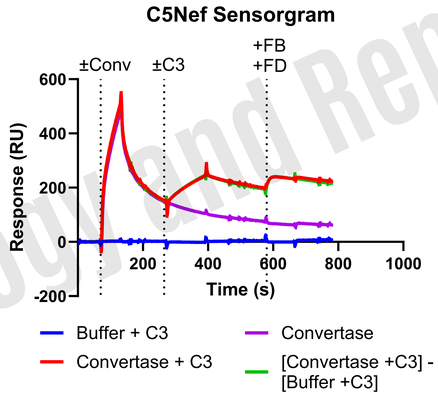
Methods

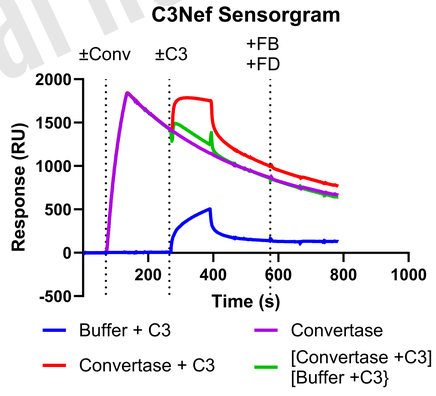
Monoclonal C3Nef or C5Nef was selected for the ability to increase complement activity in hemolytic and immunofixation electrophoresis (IFE) assays, captured on a Protein G-coupled sensor chip, and evaluated by surface plasmon resonance. C3b, factor B (FB), factor D (FD), and +/- monomeric properdin (mFP) were pre-incubated and captured by the Nef, followed by injection of C3. Subsequently, FB and FD were injected to confirm nascent C3b deposition. Assays were performed in the presence or absence of the inhibitors iptacopan or CP40.

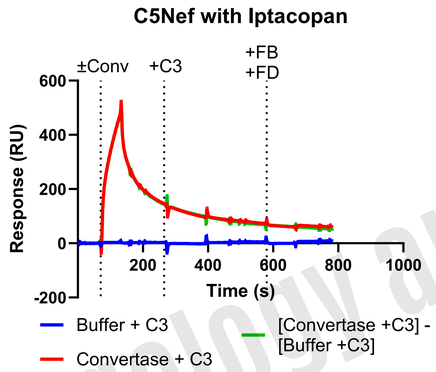
Diagram Legend

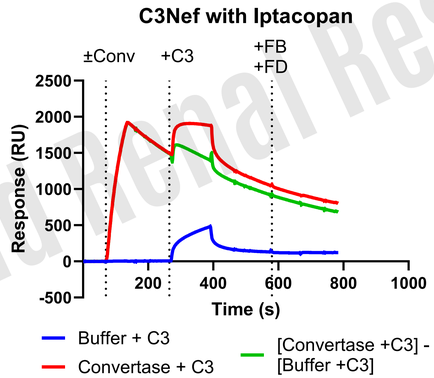


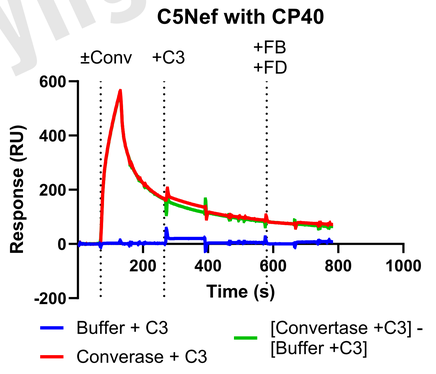
Results

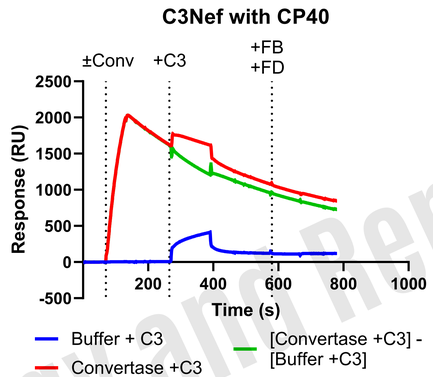


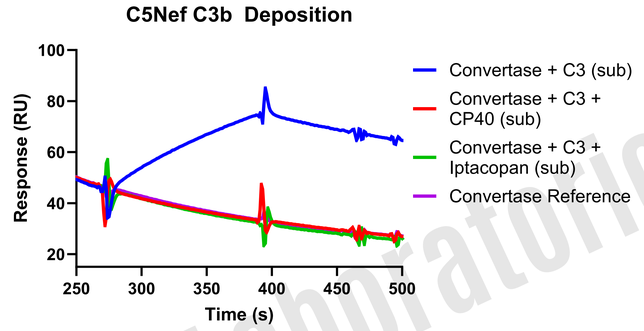


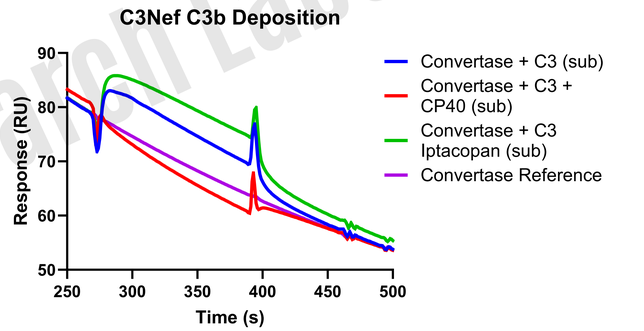












C5Nef captured the mFP-convertase, and C3 could be activated. Neither iptacopan nor CP40 disrupted convertase capture, but both prevented C3 activation by C5Nef. C3Nef captured the convertase, but C3 could not be activated. CP40 blocked C3 binding, whereas iptacopan did not affect C3's interaction with C3Nef-bound convertase.

Discussion

Functional assays showed C3Nef increased complement activity, but paradoxically, C3Nef inhibited C3 activation. These findings highlight the complexity of the complement cascade, which robustly maintains system balance. Nef-induced imbalance likely causes disease mechanisms that are more variable and complicated than currently appreciated. Our findings highlight the need for continued isolation of monoclonal Nefs to best model their pathogenicity and determine treatment.

Acknowledgements

Research is funded in part by the NIH (CMN and RJHS: 1R01DK110023) and generous family-related philanthropy.