The Importance of the Mg^{2+} Ion for Nephritic Factor Function

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Introduction

C3-convertase (C3bBb) is formed from C3bB (proconvertase) by Factor D (FD)-dependent cleavage of Factor B (FB) to release Ba. In proconvertase formation, the divalent-ion Mg^{2+} (or Ni^{2+}) is chelated by FB and creates a coordinate covalent bond with the C-terminus of C3b.[1] This interaction induces conformational changes in FB that promote FD binding and expose the FB scissile bond for cleavage.[2] FB and Mg^{2+} also interact with Cobra Venom Factor (CVF), yet several FB conformation changes do not occur in this interaction (see Table).[3] C3-Nephritic Factors (Nefs) are autoantibodies that bind to and stabilize C3bBb and cause complement dysregulation. We hypothesized that Nef-stabilization is also ion-dependent.

Methods

Using surface plasmon resonance (SPR), we measured the binding of FB, FD, and Nef-positive IgG and the decay of the resulting complexes in various conditions:

1. FB and FD were tested for C3b binding at 0mM Mg^{2+} using Dose-Response curves; (Fig 1)
2. Nef samples were tested for binding specificity to Convertase and Proconvertase in the presence of 10mM Mg^{2+}; (Fig 2)
3. Nef samples were tested for stabilizing function at 0mM Mg^{2+}; (Fig 3)
4. Nef samples were tested for CVF-FB binding and stabilization at 10mM Mg^{2+}; (Fig 4)

Results

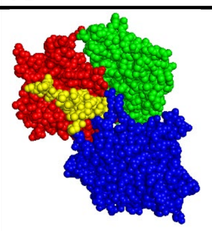
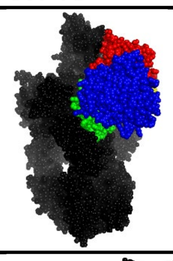
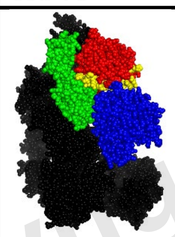
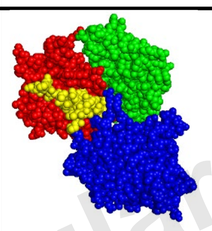
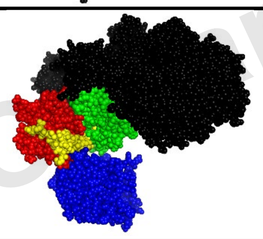
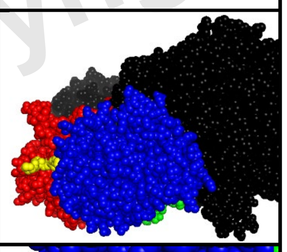
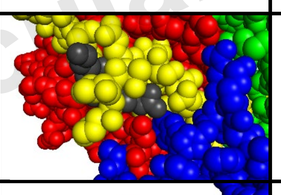
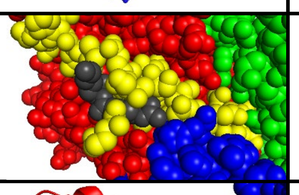
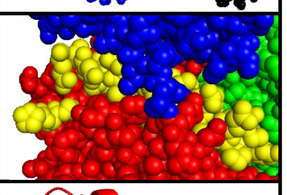
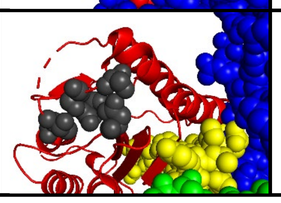
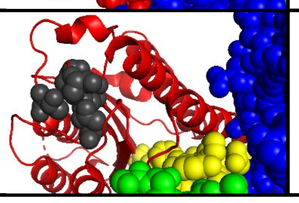
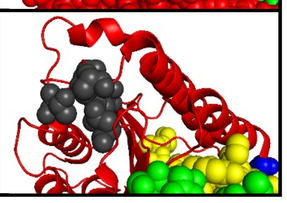
C3b-FB association was detectable on SPR in the absence of Mg^{2+} (Fig 1). This binding represented the rapid first association rate k_{a1}, which was previously shown to be Mg^{2+}-independent.[2] A similar association with FD was not observed. All Nef samples were able to selectively recognize and stabilize C3bB and C3bBb in the presence of Mg^{2+} as seen by the increased binding and slower decay specifically in samples containing both FB and Nefs (Fig 2). No Nef-stabilizing activity was observed in any sample when Mg^{2+} was absent as indicated by the rapid decay (Fig 3), nor was any Nef sample able to stabilize the CVF-FB interaction despite the presence of Mg^{2+} (Fig 4).

Discussion

Our data suggest that Nefs stabilize the proconvertase, C3bB, in addition to the convertase. However, Mg^{2+} was necessary for stabilizing both complexes. Specific conformation changes induced by Mg^{2+} in C3bB (but missing in CVF-FB) may be required for Nef stabilization.

Future Directions

Whether the C3b-Mg^{2+}-FB interaction is functionally enhanced by Nefs or Mg^{2+} promotes necessary conformational changes for Nef binding is under further investigation.

Table	FB[4]	CVF-FB[3]	C3b-FB[2]
Full Structure			
Serine Protease Arrangement			
Scissile Bond			
Mg^{2+}-chelation, MIDAS region			

References

1. *Nat Immunol* **10,** 7 721-727 (2009)
2. *Science* **330**, 6012 1816-1820 (2010)
3. *EMBO J* **28**,16 2469-2478 (2009)
4. *Nat Struct Mol Biol* **14**, 3 224-228 (2007)

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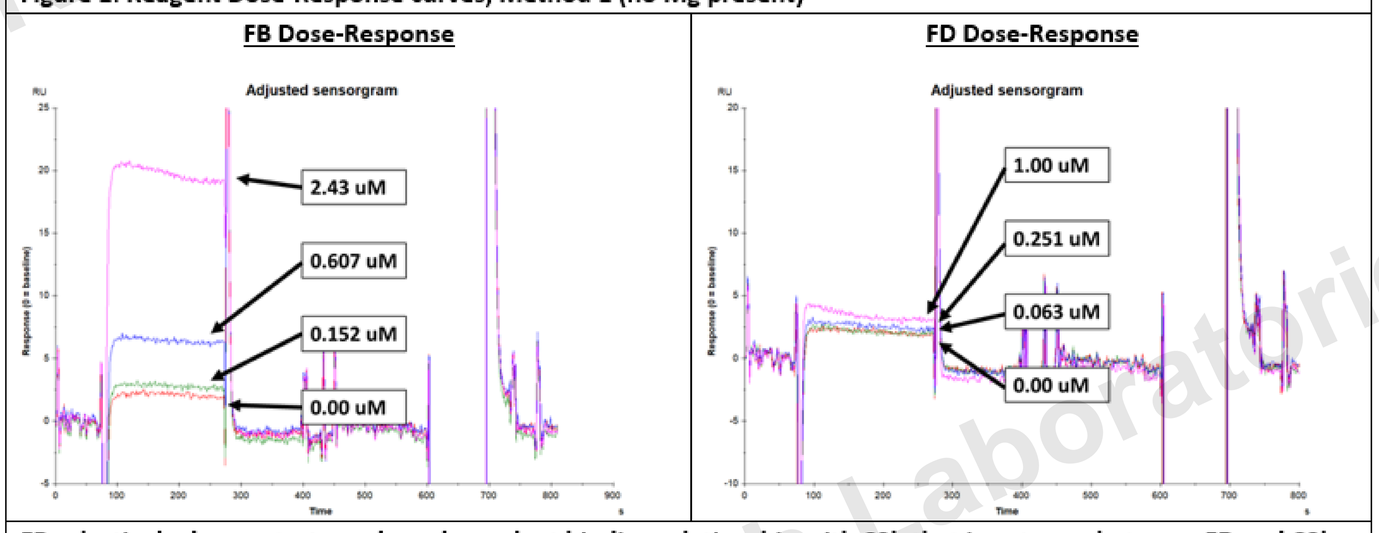
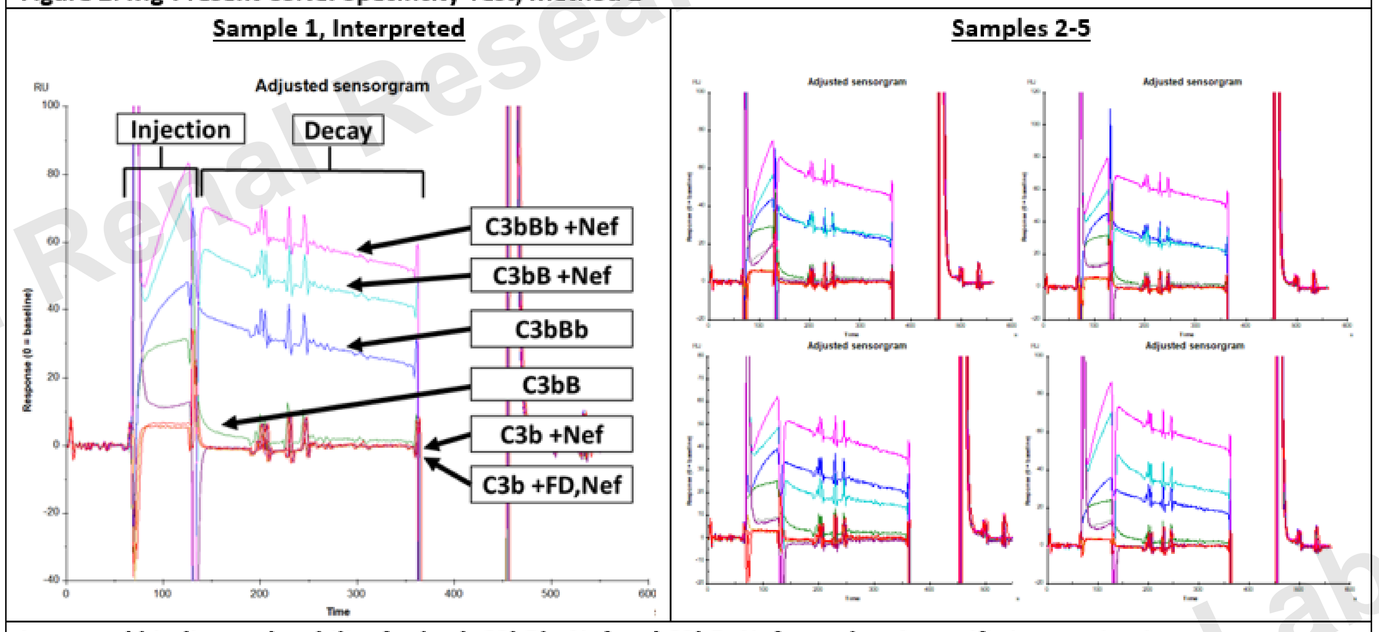
Figure 1: Reagent Dose-Response curves, Method 1 (no Mg present)



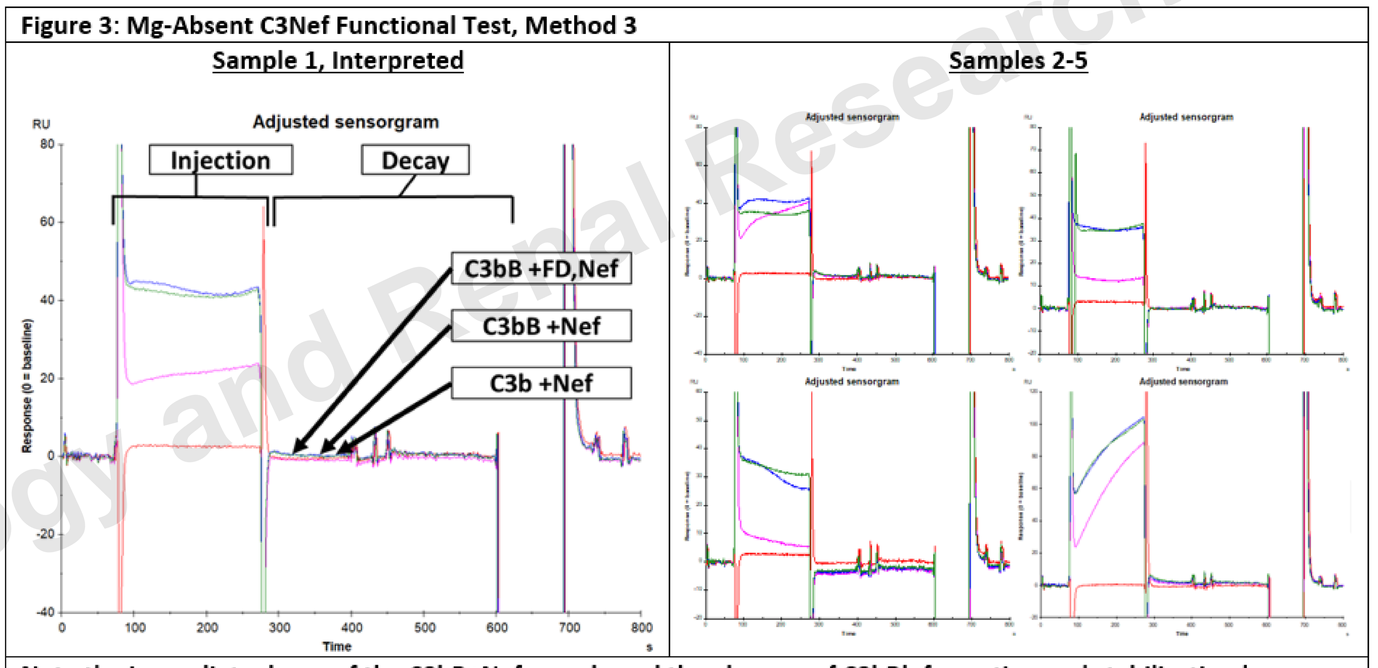
FB selectively demonstrates a dose-dependent binding relationship with C3b that is not seen between FD and C3b. This holds true despite the absence of Mg ions.

Figure 2: Mg-Present C3Nef Specificity Test, Method 2



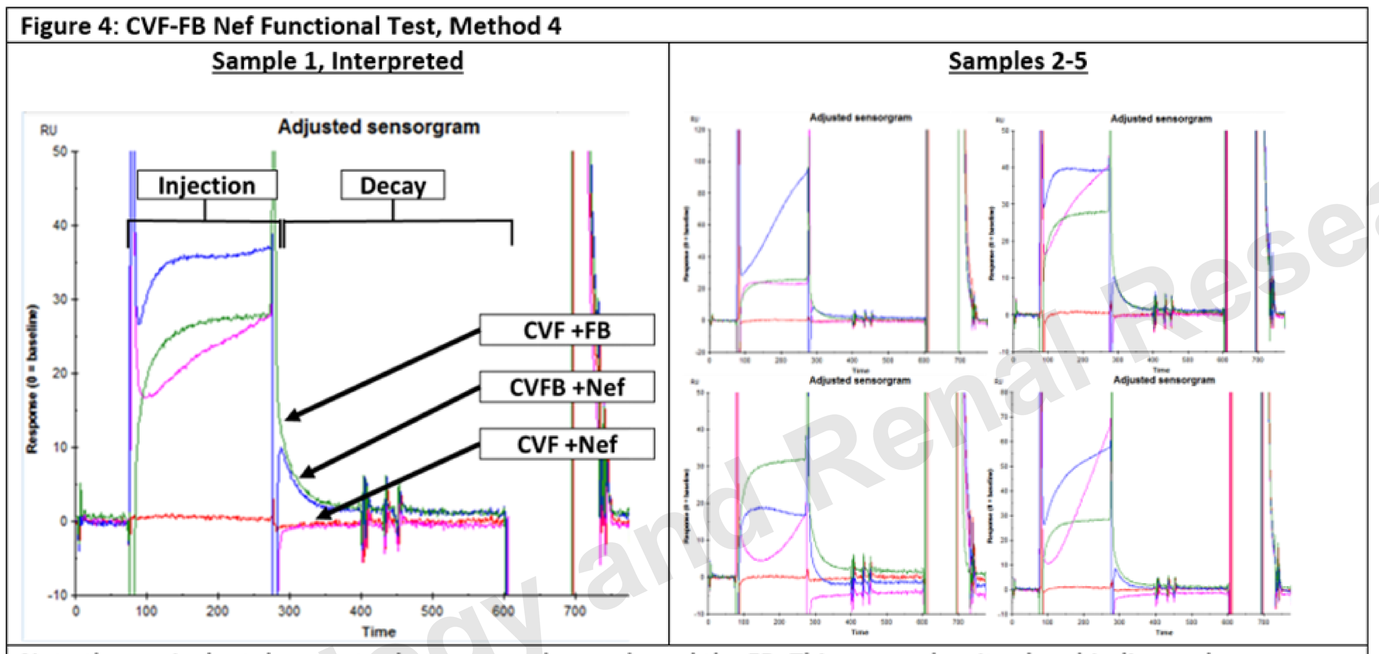
Increased binding and stability for both C3bBb+Nef and C3bB+Nef samples. A specific interaction is not seen between C3b+Nefs or C3b+FD+Nef. This observation was consistent across five Nef-positive samples.

Figure 3: Mg-Absent C3Nef Functional Test, Method 3



Note the immediate decay of the C3bB+Nef sample and the absence of C3bBb formation and stabilization by C3bB+FD+Nefs. The stabilization of both complexes was lost in all five samples tested.

Figure 4: CVF-FB Nef Functional Test, Method 4



Note the equivalent decay rates between each sample and the FB. This occurs despite clear binding and some stabilization of the CVF-FB complex.