The Binding and Kinetics of Normal versus Pathogenic C3 Convertase Autoantibodies

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Background

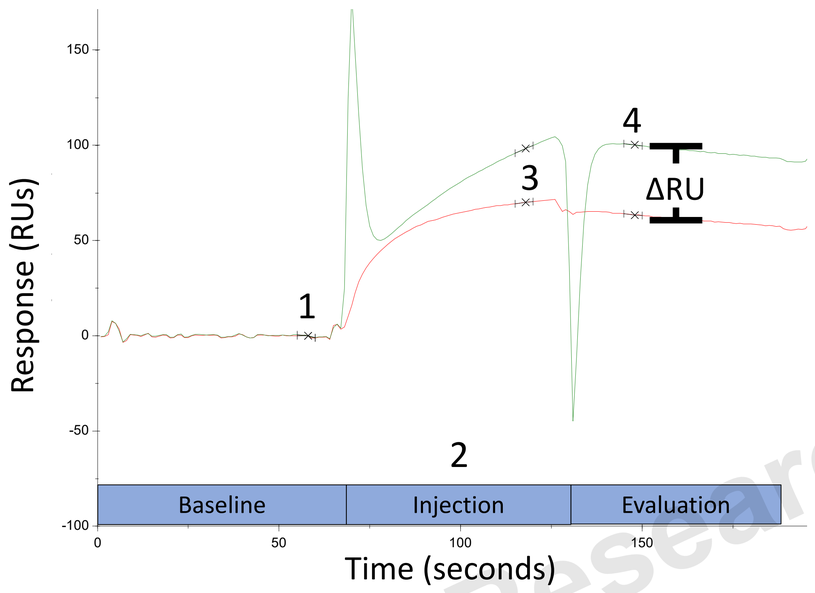
Convertase-directed autoantibodies are hypothesized to exist in the normal population and may be a source of pathogenic Nephritic Factors (Nefs).[1-3] Using a specificity assay, we previously identified an antibody reactive to the C3 convertase (C3CAb) in a high proportion of normal test subjects (prevalence of >95%).[4] These normal test subjects do not display complement dysregulation and their C3CAbs are not detected by hemolytic assay, which suggests C3CAbs are functionally distinct from Nefs. This follow-up study evaluated the kinetics of Nefs and C3CAbs as a function of temperature with a focus at 37C.

Methods

Method 1: Specificity. Sham negative control IgG, Nef+ IgG, and normal human IgG were evaluated by SPR.

1) A C3b-immobilized chip with baseline= 0RU
2) Injection of either FB+FD (red) or FB+FD+IgG (green)
3) Formation of convertase complex. Antibody binding promotes increased response
4) The effect of the antibody is quantified as ΔRU

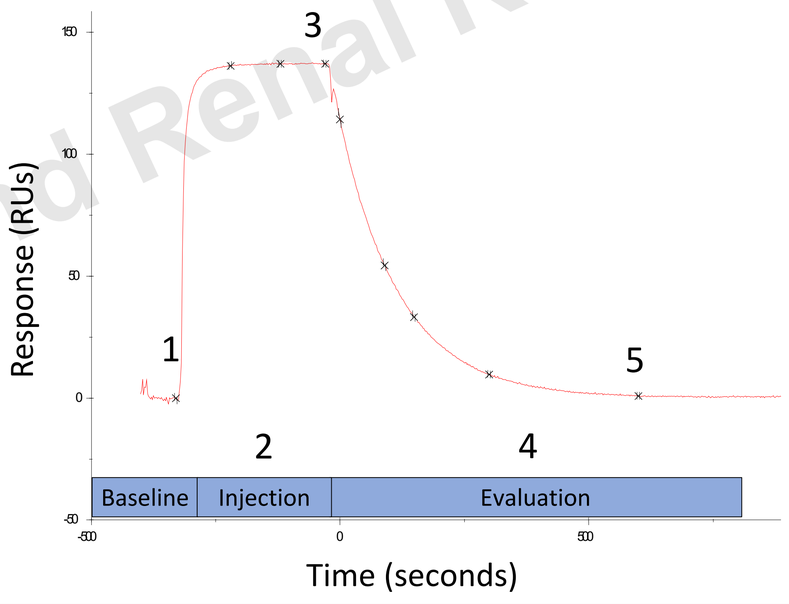
Note: this figure only presents convertase forming test conditions. Additional analytes included buffer, IgG, FD, FD+IgG, FB, and FB+IgG.



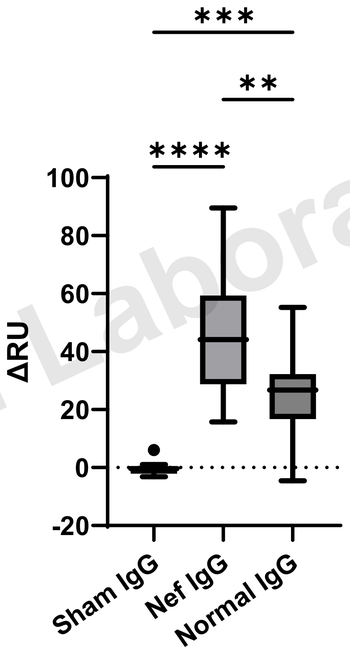
Method 2: Kinetics. Nef+ IgG and C3CAb IgG samples were evaluated on SPR.

1) A C3b-immobilized chip with baseline= 0RU
2) Injection of sample (i.e. FB+FD+Nef)
3) Formation of convertase complex
4) Post-injection dissociation of convertase complex
5) Custom report points are collected to analyze data

Report points include time t= 0, 300, 600, 900, 1200, and 1500 seconds. Data was normalized to report point "600s" = 100RU (for Kinetic Decay at 37C) or report point "0s" = 100RU (for Decay curves by temperature).

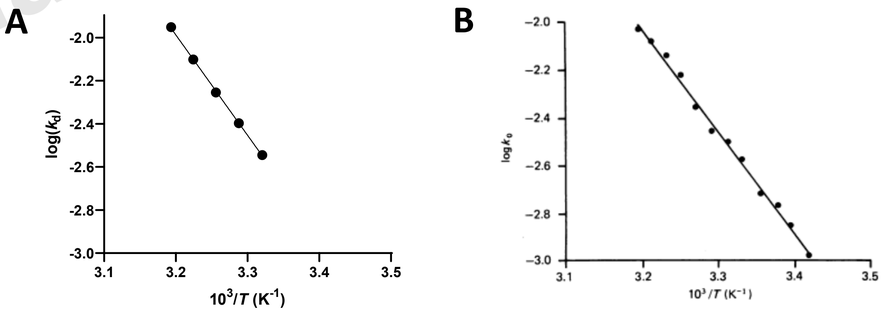


Specificity Results

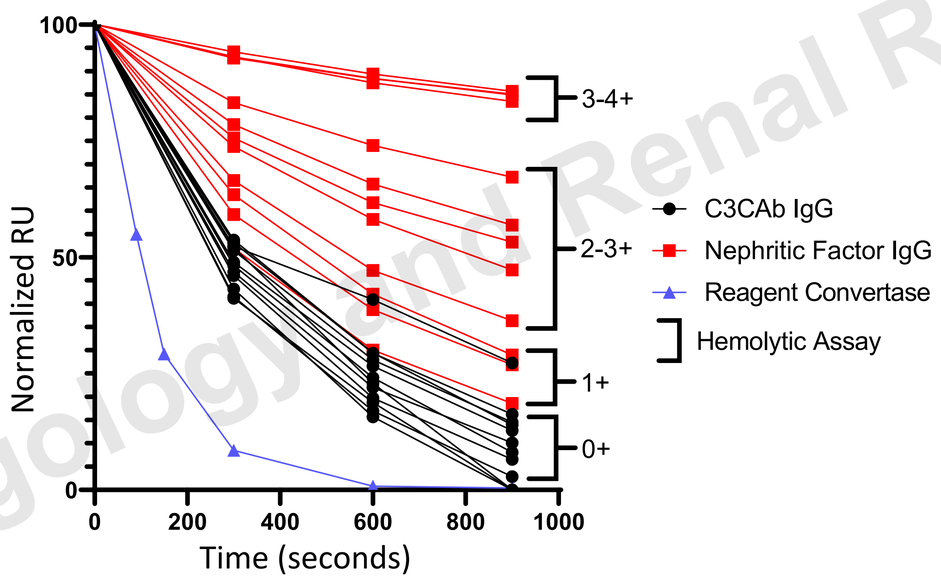


Convertase Reactivity. A statistically significant difference exists between the sham negative control antibody population and both the Nef and normal C3CAb antibody population. The difference suggests anti-convertase antibody reactivity is present in both populations. Additional analysis of a larger cohort of 30 normal human IgG samples (data not shown) revealed a prevalence of >95%.[4]

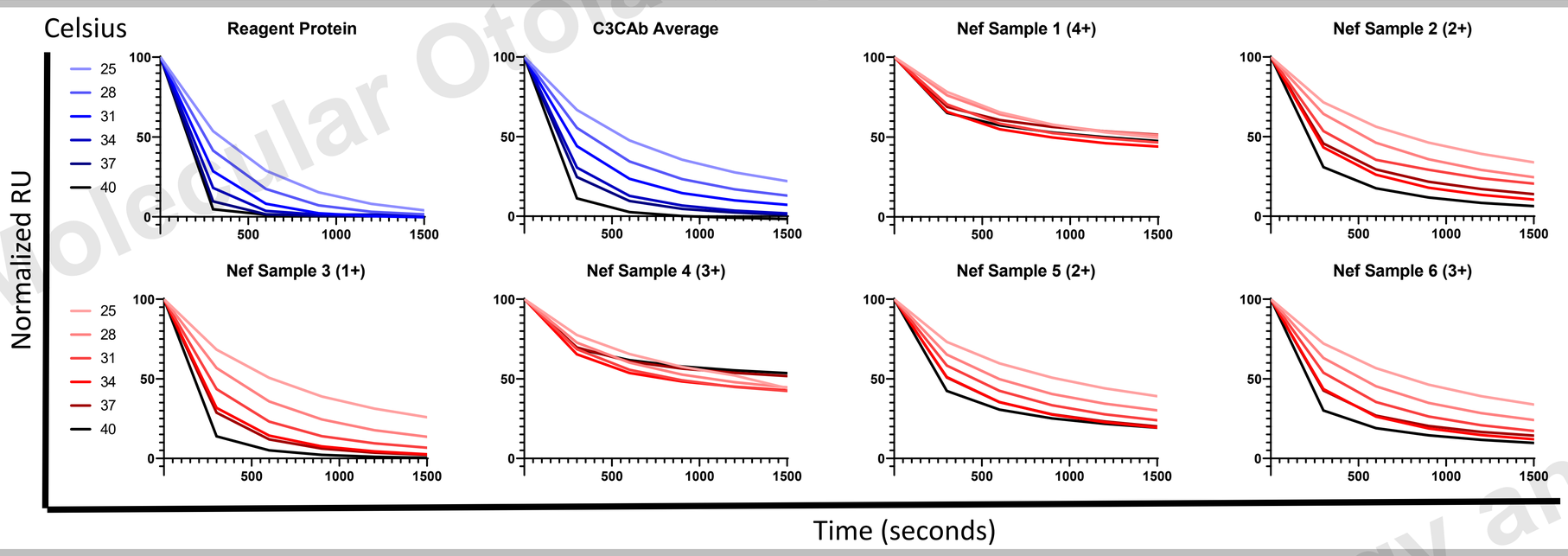
Kinetics Results



Arrhenius plot. The Arrhenius plot ($\log k_d = -(E_a/2.3R)(1/T) + \log A$) for our reagent convertase (**A**) and data published by Pangburn and Müller-Eberhard[5] (**B**). Data for **A** was calculated by estimating the time at 50RU assuming report point 0s = 100 RU. Our calculated E_a = 77.3 kJ/mol is similar to the published data (81.6 kJ/mol).[5]



Kinetic decay at 37C. The normalized data for 24 samples at 37C are shown. Both antibody populations affect decay, however the Nef samples are significantly more stable at physiologic temperature. This is especially true for samples with high hemolytic activity. The estimated reagent convertase $t_{1/2}$ was 84s. The range of estimated C3CAb $t_{1/2}$ was 179 to 415s with a mean at 276s. The Nef $t_{1/2}$ range was 301 to >>1200s. Five Nef samples were too stable to estimate $t_{1/2}$ by this method.



Decay curves by temperature. Increasing temperature accelerates dissociation for most samples. Generally, more active Nefs are less sensitive to temperature, while normal C3CAbs and less active Nefs are more sensitive to temperature. Nef Sample 1 and 4 are particularly resistant to the effects of temperature. Nef Sample 6 is an outlier, which may suggest this sample has greater impact on complement regulator antagonism to promote complement dysregulation. The normal C3CAb samples approach the kinetic profile of reagent convertase at higher temperatures.

Discussion

These results show that C3CAb and C3Nef bind C3 convertase across a range of temperatures. However, unlike C3Nef, C3CAb do not increase complement activity at physiologic temperature. The results from this study suggests this functional difference is due, in part, to the reduced impact C3CAb have on convertase stabilization.

Funding and References

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