Antibodies in the Healthy Population Influence Convertase Formation

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Background

We previously reported that a large proportion of healthy college students carry autoantibodies that influence alternative pathway convertase formation on C3b-coupled surface plasmon resonance (SPR) assays.[1] These antibodies, which we designated as C3 convertase autoantibodies (C3CAbs), were distinct from Nephritic Factors (Nefs), the well described anti-convertase autoantibodies that are enriched in C3 glomerulopathy patients, in two important ways: 1) they were not detected by routine Nef assays; and 2) they did not amplify complement. In this study, we build on this work by expanding the sample population size and diversity and broadening the method of characterization by SPR.

Methods

Plasma were collected from 141 hospital pathology samples. Inclusion criteria were no evidence of current infection, no evidence of autoimmune or inflammatory disorders, and no evidence of glomerular disease. Additionally, samples spanned the age continuum, ranging from 1 to 75+ years old. All other demographic data were blinded. Polyclonal IgG was purified from plasma samples using Melon Gel (ThermoFisher) and dialyzed into 10mM $MgCl_2$ HBS-EP buffer. IgG was evaluated by SPR in three ways. First, the antibody influence on convertase formation was measured as described in the published manuscript (A).[1] Second, convertase-stabilizing function was tested in a DAF-dependent manner based on previously described techniques (B).[2] Finally, stability was measured at 37 ºC. Samples were compared to Nef+ control IgG and sham negative control monoclonal antibodies (C).

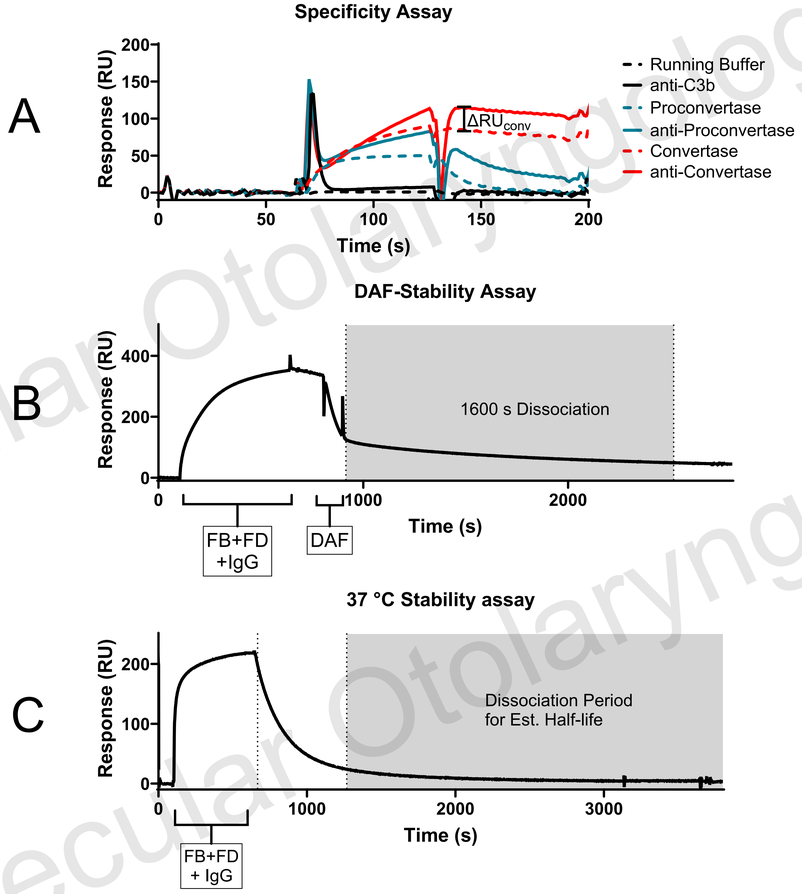

Figure 1 SPR Methods. **A**) Specificity assay. Six conditions were injected over C3b chip. Convertase forming conditions are shown in red. Proconvertase forming conditions are shown in blue. IgG-positive cycles are solid, while IgG-negative reference cycles are dashed. **B)** DAF-Dependent Stability assay. FB+FD with polyclonal IgG are injected over C3b-coupled chip. Subsequently, DAF is injected to dissociate free convertase unbound by IgG. The normalized decay 1600 seconds post-DAF injection is reported in normalized RUs. **C)** 37 ºC Stability Assay. FB+FD with polyclonal Ig are injected over C3b-coupled chip at 37 ºC. After 600s of dissociation, time 0 for the half-life calculation is established. The time to reach 50% residual convertase is calculated from this point.

Results

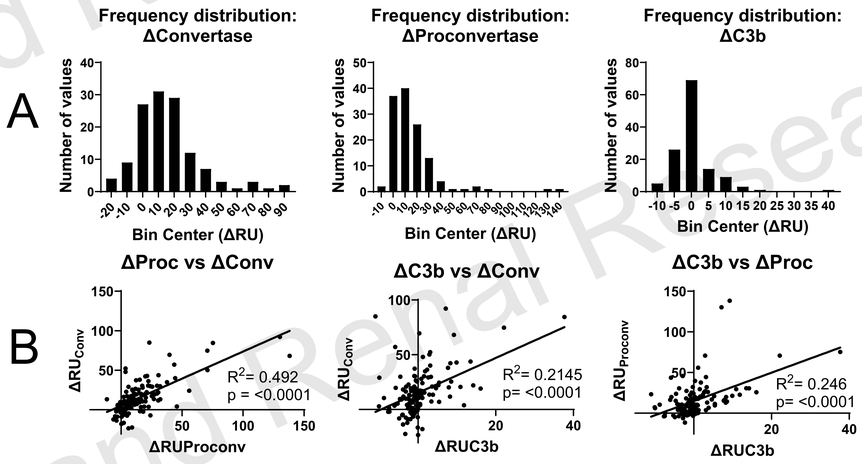

Figure 1 Specificity Assay Results by Antigen. **A)** Binding of polyclonal IgG to Convertase (left), proconvertase (center), and C3b (right) shown as distributions (Bin Center |0| = no response). Some response was observed against all three antigens. **B)** Regressions between antigens show moderate correlations with highest agreement between convertase and proconvertase (left).

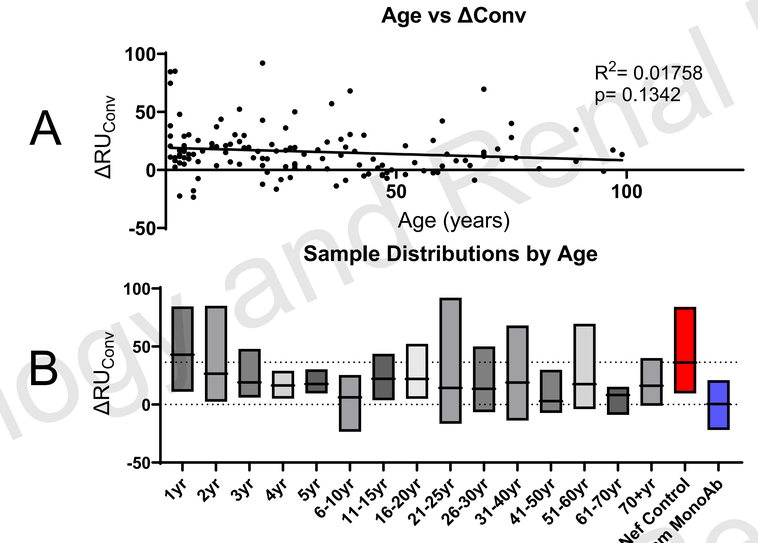

Figure 2 Specificity Assay Results by Age. **A)** A linear regression for convertase response to polyclonal IgG as a function of age shows no significant relationship. **B)** The distribution for replicate results for 15 age cohorts as compared to a Nef positive control. Data suggest that IgG influences convertase formation independently of age of the patient.

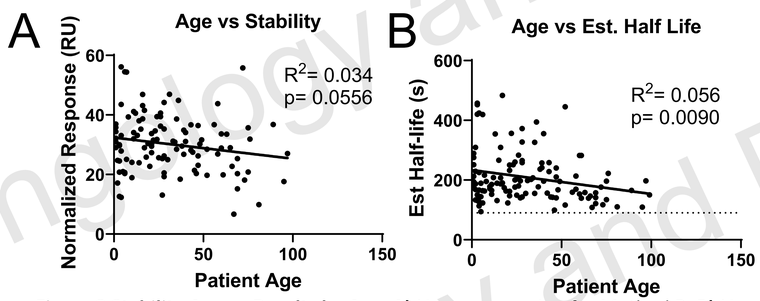

Figure 3 Stability Assays Results by Age. **A)** Linear regression for Method B. **B)** Linear Regression for Method C. Linear regressions show little to no dependence of convertase-antibody stability and age.

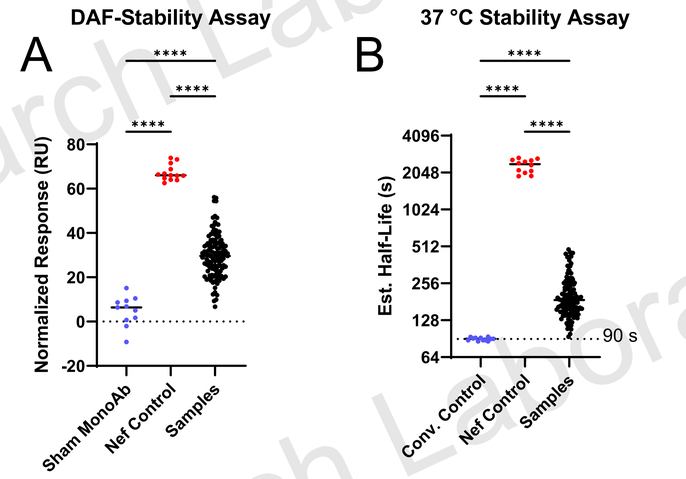

Figure 4 Stability Assay Results by Cohort. **A)** ANOVA results for Method B. **B)** ANOVA results for Method C. Distribution and Kruskall-Wallis with Dunn's post-hoc tests show significant differences in the median value of the respective negative controls and test populations by both stability assays. Differences are also significantly different than the Nef population. The test population varied widely in their stability measurements, which suggests a diverse antibody population.

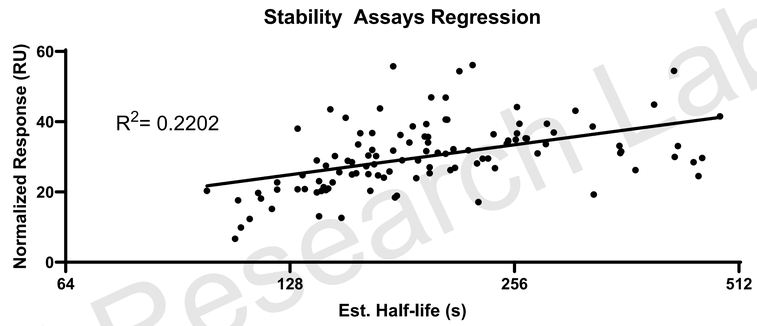

Figure 5 Stability Assays Regression. Nonlinear regression between stability assays shows some agreement. Relationship is semi-log with a moderate correlation coefficient.

Conclusions

This study supports our published findings.[1] C3CAbs were detected in the general hospital population with minimal dependence on age. These antibodies appear to recognize and potentially influence the formation of the convertase. Importantly, they have less impact on convertase stability than Nefs. Low impact may prevent their detection in routine Nef assays and explain their minimal effect on complement activity and hence lack of clinical manifestation. Overall, we believe this antibody population represents a prevalent but benign counterpart to pathogenic Nefs.

Acknowledgements

Research is funded in part by the NIH (CMN and RJHS: 1R01DK110023-01A1) and generous family-related philanthropy.

References

1. Culek and Nester, Kidney360, 2023.
2. Paixao-Cavalcante et al., Kidney Int, 2012.