Nephritic Factor-Like Autoantibodies are Present in Unaffected Children

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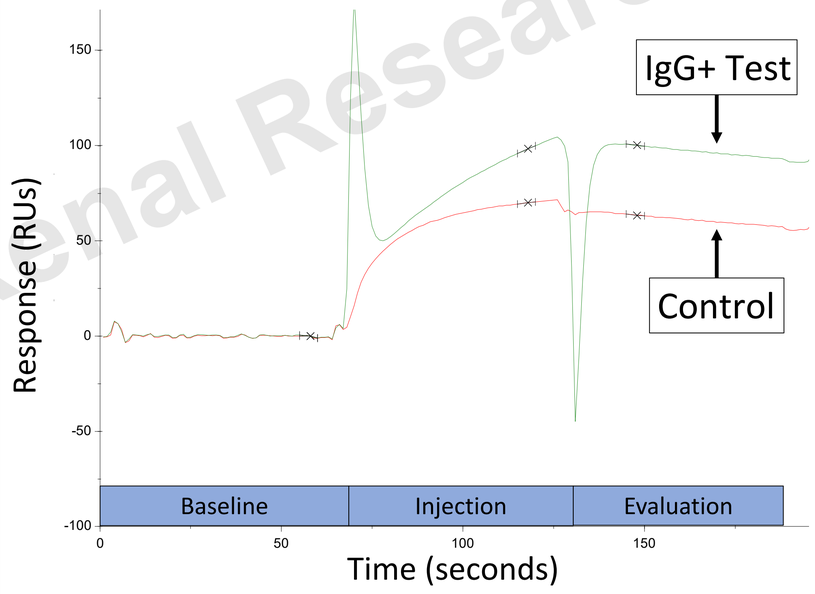
Background

Complement convertase-directed autoantibodies (Nefs) are often associated with C3 glomerulopathy.[1] Their pathogenicity is thought to occur when Nefs amplify complement activity by stabilizing the convertase. The origin of Nefs remains unknown. We previously reported that C3-convertase specific autoantibodies are also highly prevalent in normal young adults (manuscript accepted).[2] Although these autoantibodies did not amplify complement and were not associated with glomerular disease, they may provide an origin for Nefs. In this study, we extend our analysis of benign convertase-directed autoantibodies to healthy pediatric subjects to determine when this autoantibody may emerge.

Methods



This study measured the binding and stabilization of normal human IgG to the C3-convertase and proconvertase. Samples were selected from one, five, and ten-year-olds. Binding was determined using surface plasmon resonance (see left) with C3b as the immobilized ligand. Each IgG sample was tested in four analyte conditions: IgG alone, IgG with FB (proconvertase), IgG with FD, and IgG with FB and FD (convertase). These test conditions were compared to corresponding controls: buffer alone, FB alone, FD alone, or FB with FD. Response (in RUs) was recorded after injection to measure binding and after 200 seconds to evaluate stability. Binding and stability data were analyzed mathematically as described in the results.

Cohort Reactivity Analysis

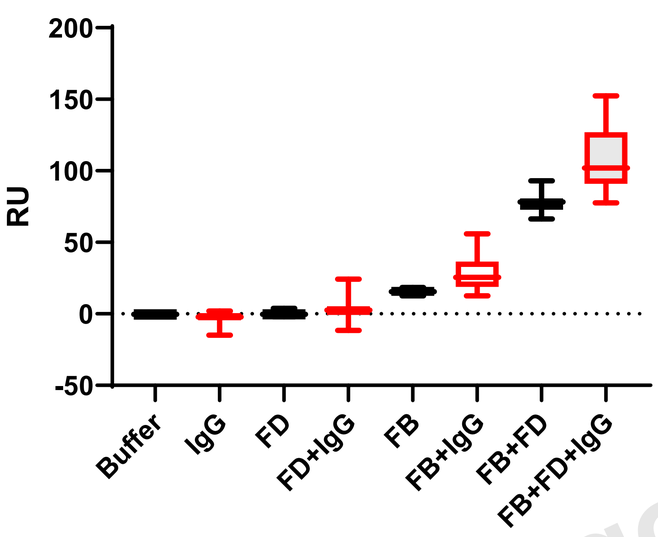


Figure 1. Analyte response to C3b. The response of each analyte to the C3b ligand is shown in RUs. Each box plot represents 29 replicates. No binding was observed in the Buffer and FD control analyte tests, and the introduction of IgG (IgG; FD+IgG) had minimal effect on these results. The FB control analyte showed formation of proconvertase, and the FB+FD control analyte showed formation of convertase. The introduction of IgG increased the response to proconvertase (FB+IgG) and convertase (FB+FD+IgG) in some samples.

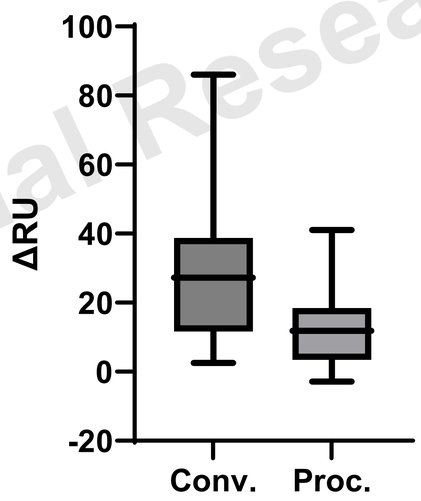


Figure 2. Cohort Reactivity. The difference in response (ΔRU) between corresponding control and test analytes (i.e., FB vs FB+IgG) demonstrates the effect of IgG binding in this test system. The ΔRU of each IgG sample was calculated for the convertase (Conv) and proconvertase (Proc) according to Equation 1 ($\Delta RU_{(Analyte\ Pair)} = RU_{Test} - RU_{Control}$). Each box plot represents all 29 IgG samples. Pending data[2] shows that Negative Control results have a ΔRU of ~0, whereas this pediatric cohort has a median ΔRU of 27.2 and 11.8 for the convertase and proconvertase, respectively.

Sample Specificity Analysis

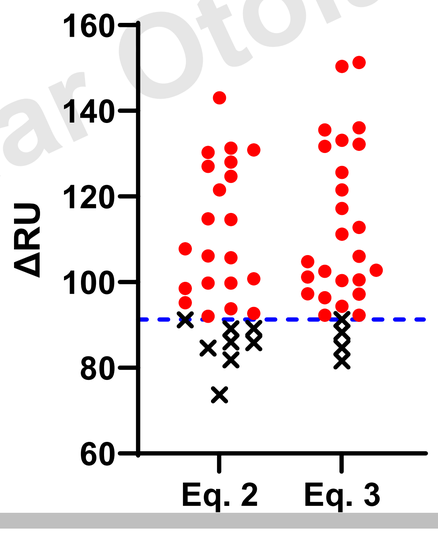


Figure 3. Convertase Specificity. Equation 2 ($\Delta RU = RU_{IgG+FB+FD} - RU_{IgG+FD}$) and Equation 3 ($\Delta RU = RU_{IgG+FB+FD} - RU_{IgG}$) partially control for false negative or false positive interactions in the test system. A ΔRU greater than the threshold value (dotted line) demonstrates a specific positive result. The threshold was calculated from convertase control data: $(\bar{x} + 2s)_{FB+FD}$ = 91.3 RU. Each point represents one sample. Samples indicated with an "x" did not pass the threshold. 25 of 29 samples were positive in at least one of these two control equations and 21 were positive in both (72 to 86%).

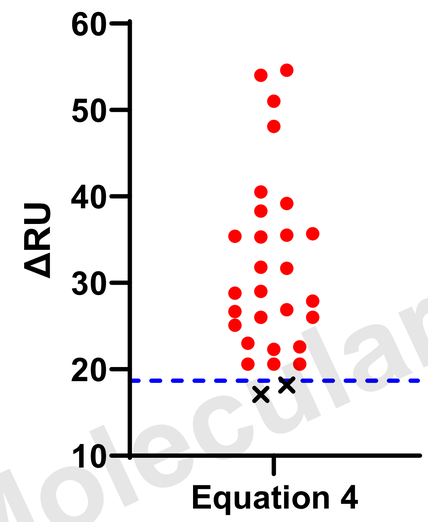


Figure 4. Proconvertase Specificity. Equation 4 ($\Delta RU = RU_{IgG+FB} - RU_{IgG}$) partially controls for false negative or false positive interactions in the test system. A ΔRU greater than the threshold value (dotted line) demonstrates a specific positive result. The threshold was calculated from proconvertase control data: $(\bar{x} + 2s)_{FB}$ = 18.7 RU. Each point represents one sample. Samples indicated with an "x" did not meet the threshold for positivity. Of the 29 samples tested, 27 were positive for the proconvertase (~93%).

Sample Stability Analysis

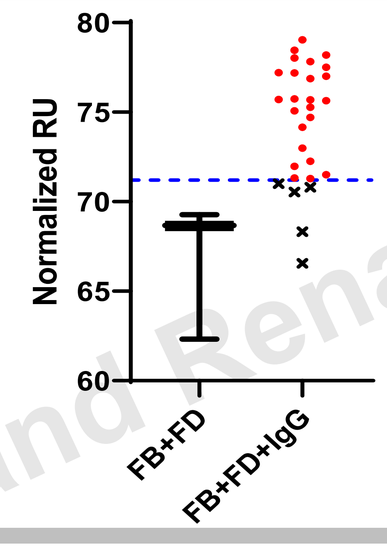


Figure 5. Convertase Stability. The normalized RU at 200 seconds is shown. These values were calculated using Equation 5 [$Normalized\ RU = (RU_{200s}/RU_{20s}) * 100$]. The threshold for stabilization was calculated as $(\bar{x} + 2s)$ of the normalized FB+FD control tests and equaled 71.2 RU. The results show that 24 of the 29 IgG samples had at least some influence on convertase, although several samples are borderline nonstabilizing.

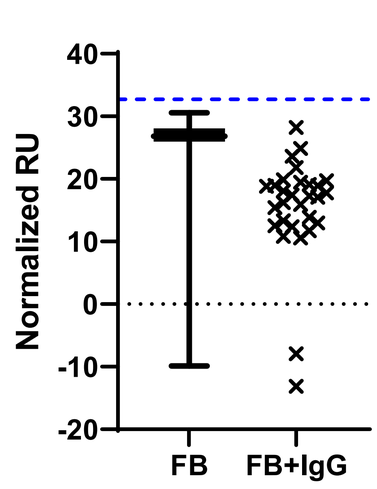


Figure 6. Proconvertase Stability. The normalized RU at 200 seconds is shown. These values were calculated using Equation 5 [$Normalized\ RU = (RU_{200s}/RU_{20s}) * 100$]. The threshold for stabilization was calculated as $(\bar{x} + 2s)$ of the normalized FB control tests and equaled 32.7 RU. The results show that none of the 29 IgG samples influenced proconvertase decay despite their high rate of prevalence (see Figure 4).

Results Summary

- Control analytes resulted in the expected specificity (no response in buffer or FD); Fig 1
- Pediatric IgG (including 1-year-olds) react to the convertase and proconvertase; Fig 2
- 72-86% of pediatric samples are positive for antibodies specific for the convertase; Fig 3
- 93% of pediatric samples are positive for antibodies specific for the proconvertase; Fig 4
- Convertase autoantibodies have a measurable effect on convertase stability, while proconvertase autoantibodies do not; Fig 5 and Fig 6
- The prevalence of these antibodies was less than in the adult cohort (<86% vs >95%)[2]

Discussion

Specific binding to the convertase was observed in a large number of pediatric subjects, including in 67% of one-year-olds, suggesting early emergence of this benign autoantibody. Whether the difference in prevalence between the pediatric and adult populations indicates the *de novo* emergence of this antibody over time or class switching of an IgM to an IgG is currently under investigation. These results provide an interesting parallel to Nefs, which often emerge in pediatric C3G patients.[1]

Funding and References

We are grateful for the support from families with C3G and to the NIDDK: R01 110023

1. Clin J Am Soc Nephrol 7: 265-274, 2012. doi: 10.2215/CJN.07900811
2. Culek C and Nester C. Kidney 360. Publication pending