C5 Nephritic Factor in Proliferative Glomerulonephritis with Monoclonal IgG Deposits

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Introduction

Proliferative glomerulonephritis with monoclonal IgG deposits (PGNMID) is a newly described disease entity under the category of monoclonal gammopathy of renal significance (MGRS). Unlike other MGRSs, only a third of patients with PGNMID have a detectable monoclonal IgG (mIgG) in the circulation. However, renal deposition of mIgGs, mostly IgG3κ, is identifiable in all cases.

Clinical prognosis varies from full recovery to end stage renal failure. Complement biomarker profiling shows dysregulation of the alternative pathways (AP) in ~50% of patients although the underlying mechanisms for this dysregulation is not understood. Here, we describe a case of early-onset PGNMID circulating a clonal IgG3λ that functions as a nephritic factor (C5Nef). The IgG3λ was not detectable by sIFE but was detectable on renal biopsy.

Patient

An 8-year-old boy presented with sub-nephrotic range proteinuria and microscopic hematuria. Serum immunofixation electrophoresis (sIFE) did not detect clonal immunoglobulin expansion. Kidney biopsy revealed glomerular IgG3 deposition with λ restriction.

Figure 1. Search for paraproteins by sIFE. Patient is negative for monoclonal immunoglobulin.

Methods

1. Biomarker and functional testing was performed using a customized panel that includes ELISAs, radial immuno-diffusion and hemolytic assays.
2. Abundancy of Ig subclasses was determined by Luminex multiplex kits (ProcartaPlex Panel, ThermoFisher Scientific).
3. Total IgG was isolated using Melon Gel (ThermoFisher Scientific). IgG3 was purified by Protein A (Seracare, Milford, MA). IgG3 λ and κ were separated by a κ binder (Millipore Sigma, Figure 2).
4. Immunofixation electrophoresis (IFE) detected C3 degradation products by mixing patient serum (or IgG fractions) and normal human serum in a buffer containing EGTA and magnesium. The same mixture in EDTA buffer was used as background control.
5. C5 nephritic factor (C3Nef) activity was assessed using sheep erythrocytes preformed with C3 convertase of the AP. Patient-purified IgG or its fractions was used (Figure 5A).

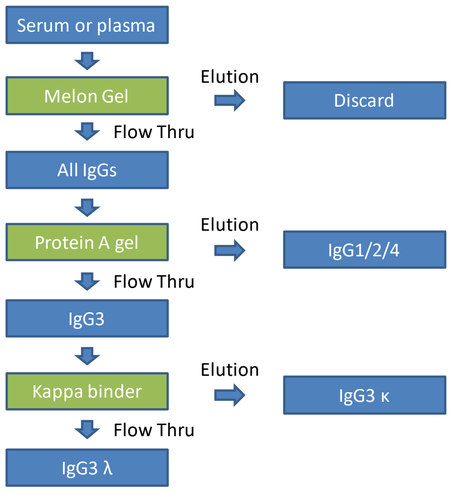


Figure 2. Isolation of IgG3 subclasses.

Results

1) Dysregulation of C3/C5 convertase

	Patient	Reference Range
CH50 (U)	undetectable	30-90
AP functional activity (%)	undetectable	>50
C3 (g/L)	<0.16	0.9-1.8
C4 (g/L)	0.15	0.15-0.57
C3c (mg/L)	>8.0	<1.5
Factor B (mg/L)	36.1	<1.2
Ba (mg/L)	1.0	22-50
Bb (mg/L)	2.6	<2.2
Properdin (mg/L)	2.8	10-33
C5 (mg/L)	2	10-20
Soluble C5b-9 (mg/L)	5.5	<0.3

Table 1. Pathway Activities and Complement Biomarkers. Patient shows undetectable complement activity in both the alternative and classical pathways. Biomarker analysis shows undetectable C3, highly elevated C3c, normal C4, low properdin, low C5 and high soluble C5b-9, consistent with highly dysregulated alternative and terminal pathways.

2) Circulating C5 Nephritic Factor

	Patient	Sample	Reference range
FH autoantibody	undetectable	Serum	<200
FB autoantibody	66	Serum	<200
C3 Nephritic factor	42%	IgG	<20%
C5 Nephritic factor	>100%	IgG	<20%
C4 Nephritic factor	8%	IgG	<20%

Table 2. Acquired Drivers of Diseases. Autoantibody profiling is weakly positive for C3Nefs but highly positive for C5Nefs. The patient was negative for other autoantibodies.

3) Ig Subclasses

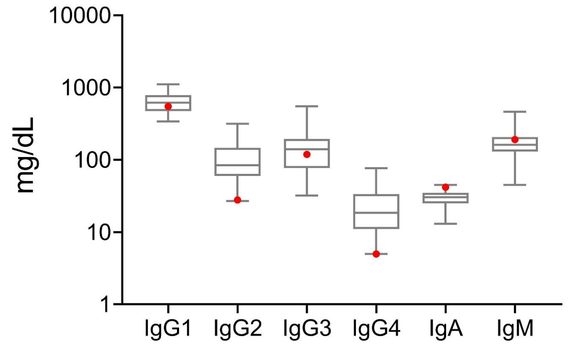


Figure 3. Abundancy of Ig subclasses in patient and controls. Box plot: IgG subclass concentrations from normal controls (n=35). Red dot: IgG subclass concentration for patient. This result shows patient's IgG3 concentration is within normal range.

4) Fluid phase dysregulation by IgG3λ

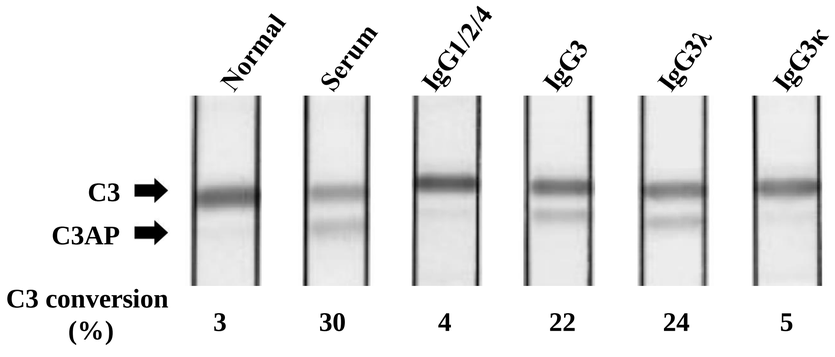
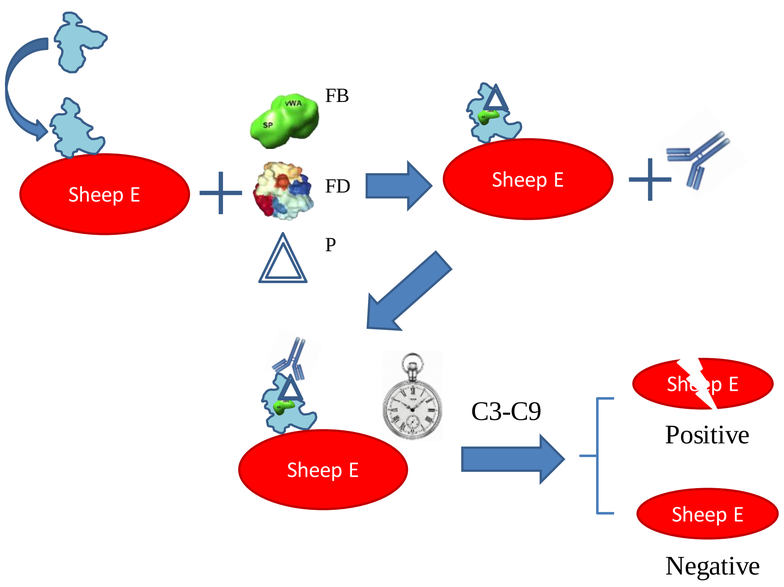


Figure 4. Fluid phase activity determined by IFE. Results indicate that IgG3λ but not IgG3κ results in abnormal convertase activity and lead to C3 cleavage products. (C3AP: C3 activation products)

4) C5 Convertase Stabilizing Activity

A



B

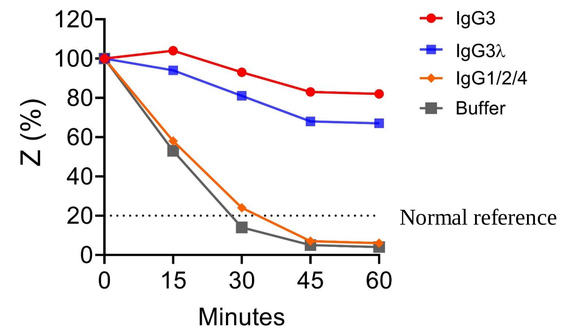


Figure 5. Hemolytic based C5Nef assay. A) Illustration of assay. **B)** Results of C5Nef activity in patient-IgG fractions. IgG1/2/4 fails to stabilize the convertase, as in a normal control. Conversely, IgG3, specifically λ, shows a strong stabilization effect.

Discussion

PGNMID has been recognized recently as a distinct MGRS lesion that affects mostly adult patients (age>20). Pediatric cases are rare. Circulating paraproteins can only be identifiable in ~30% patients. However, monoclonality can be established by renal biopsy as determined by the restrictive deposition of a single Ig light chain and a single IgG subclass (most commonly IgG3κ).

Here we show a pediatric case of PGNMID with restrictive IgG3λ deposition in the kidney. No circulating paraprotein could be identified (Figure 1). However complement dysregulation driven by C5Nefs is prominent. C5Nefs (properdin dependent) stabilize primarily C5 convertase but some can also stabilize C3 convertase (Table 2), leading to activation of both alternative and terminal pathways. The stabilizing activity is found only in the λ fraction of IgG3.

In summary, these results show that C5Nefs can dysregulate the AP in the fluid phase and can be acquired drivers of PGNMID. This case highlights the detailed complement studies that are required to provide insightful information underlying the nature of AP dysregulation in PGNMID.

Acknowledgement

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