

Defining the Complement Biomarker Profile of C3 Glomerulopathy

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Abstract

Background and objectives C3 glomerulopathy (C3G) applies to a group of renal diseases defined by a specific renal biopsy finding: a dominant pattern of C3 fragment deposition on immunofluorescence. The primary pathogenic mechanism involves abnormal control of the alternative complement pathway, although a full description of the disease spectrum remains to be determined. This study sought to validate and define the association of complement dysregulation with C3G and to determine whether specific complement pathway abnormalities could inform disease definition.

Design, setting, participants, & measurements This study included 34 patients with C3G (17 with C3 glomerulonephritis [C3GN] and 17 with dense deposit disease [DDD]) diagnosed between 2008 and 2013 selected from the C3G Registry. Control samples ($n=100$) were recruited from regional blood drives. Nineteen complement biomarkers were assayed on all samples. Results were compared between C3G disease categories and with normal controls.

Results Assessment of the alternative complement pathway showed that compared with controls, patients with C3G had lower levels of serum C3 ($P<0.001$ for both DDD and C3GN) and factor B ($P<0.001$ for both DDD and C3GN) as well as higher levels of complement breakdown products including C3d ($P<0.001$ for both DDD and C3GN) and Bb ($P<0.001$ for both DDD and C3GN). A comparison of terminal complement pathway proteins showed that although C5 levels were significantly suppressed ($P<0.001$ for both DDD and C3GN) its breakdown product C5a was significantly higher only in patients with C3GN ($P<0.05$). Of the other terminal pathway components (C6–C9), the only significant difference was in C7 levels between patients with C3GN and controls ($P<0.01$). Soluble C5b-9 was elevated in both diseases but only the difference between patients with C3GN and controls reached statistical significance ($P<0.001$). Levels of C3 nephritic factor activity were qualitatively higher in patients with DDD compared with patients with C3GN.

Conclusions Complement biomarkers are significantly abnormal in patients with C3G compared with controls. These data substantiate the link between complement dysregulation and C3G and identify C3G inter-disease differences.

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Introduction

The term “C3 glomerulopathy” (C3G) has evolved to describe a set of diseases with distinct renal biopsy findings: dominant glomerular C3 fragment deposition on immunofluorescence and characteristic densities on electron microscopy (EM) (1–3). The emergence of this term has been driven by a number of key developments, including (1) the identification of well characterized pedigrees that provided the first glimpses of disease definition and spectrum, (2) a movement to accurately group patients into a pathologic category that may have both mechanistic and treatment significance, (3) advances in the sophistication and availability of assays of complement function and, (4) improved accessibility of comprehensive screens of the complement genes.

Two major subgroups of C3G are recognized: dense deposit disease (DDD) and C3 glomerulonephritis

(C3GN). DDD and C3GN by definition have in common C3 dominance on renal biopsy immunofluorescence. Although the term “dominance” has yet to be fully agreed upon, it generally implies a C3 fragment immunofluorescence intensity of at least two orders of magnitude greater than any other immune reactants (*i.e.*, IgG) on a scale of 0–3 (including 0, trace, 1+, 2+, and 3+) (4). There is no light microscopy requirement in the current definition of C3G.

EM distinguishes DDD from C3GN (Figure 1). The complexity and location of the electron-dense deposits in C3G are varied; however, it is generally accepted that the character of the intramembranous immune deposits on EM differs between DDD and C3GN. In DDD, the glomerular intramembranous deposits are very dense, band-like, or sausage-like shapes interspersed with more normal-appearing glomerular basement

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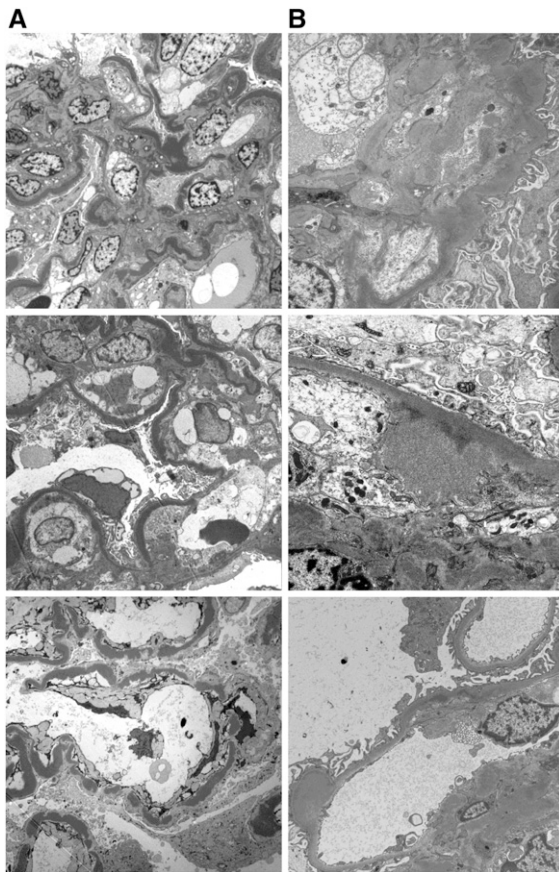


Figure 1. | Electron microscopic distinction between DDD and C3GN. (A) Representative electron microscopy from three patients in the DDD cohort shows the characteristic extremely electron-dense well defined osmiophilic deposits that are typically located in the lamina densa of the glomerular basement membrane. (B) By contrast, representative electron microscopy from three patients in the C3GN cohort shows less well defined deposits that may lead to massive expansion of both the glomerular basement membrane and the mesangium. The reason for these underlying differences is not known. C3GN, C3 glomerulonephritis; DDD, dense deposit disease.

membrane (5). By contrast, the deposits of C3GN are less electron dense (more amorphous appearing) and do not form the characteristic bands seen in classic DDD (6). Therefore, the term C3GN currently applies when there is C3 dominance on immunofluorescence but the deposits do not appear as those described above for DDD. A more precise definition of the distinction between these two entities awaits clarification.

The primary pathogenic process in C3G is presumed to be uncontrolled complement activation, deposition, or degradation. Knowledge of a normally functioning complement cascade combined with a biomarker analysis of the various steps of the cascade in disease can inform our understanding of the critical steps in disease pathology. Complement activity is typically triggered through one of three activating pathways: the classic, lectin, or alternative pathways. Both the classic and lectin pathways are recognition-dependent pathways with activity being triggered by Igs and polysaccharides, respectively. The alternative pathway, by contrast, is constitutively active at low levels as a chemical consequence

of a reactive thiol ester in the C3 molecule located in the thiol ester domain of the protein.

Spontaneous hydrolysis of the thiol ester converts C3 to C3(H₂O) (also known as iC3) in a process known as “tick-over.” C3(H₂O) reacts with factors B (FB) and D to form C3 (H₂O)Bb, an intermediate enzyme and the initial C3 convertase of the alternative pathway. C3(H₂O)Bb cleaves C3 to C3b, forming the nascent C3 convertase C3bBb. Tight control of C3 convertase activity by the regulators of complement activity prevents excessive activity and unintentional complement-mediated damage. However, on pathogenic surfaces or in the absence of appropriate control on self-tissue, large amounts of C3 convertase form and complement activity continues unchecked.

C3 is one of the most abundant plasma proteins (approximately 2% of total plasma protein), and tremendous amounts of C3b are generated with C3 convertase dysregulation. Excess C3b is handled by one of two concurrent pathways: (1) C3b is cleaved in multiple sequential steps by factor I-mediated activity to generate inactive C3b and ultimately the C3 breakdown products C3d and C3g; and (2) C3b combines with extant C3bBb to form the C5 convertase C3bBbC3b, which is the second important enzyme of the complement pathway. C5 convertase cleaves C5 to C5a and C5b. C5b triggers the terminal complement cascade, which culminates in formation of the membrane attack complex (Figure 2).

C3G is driven by dysregulation of the C3 and C5 convertases. Because there have been no comprehensive assessments of the degree of dysregulation of the convertases in C3G compared with controls, we sought to determine whether we could define and/or distinguish the complement activity in DDD and C3GN by biomarker profiling. We hypothesized that specific biomarkers may provide a more detailed and mechanistic understanding of the underlying complement pathology and therefore add to disease definition. By defining a complement pathway signature, we predict that biomarkers may be useful metrics of disease activity and/or severity in the clinical setting.

Materials and Methods

Study Population

Thirty-four patients with biopsy-proven C3G (17 with DDD and 17 with C3GN) diagnosed between 2008 and 2013 were selected from our C3G Registry for inclusion in this study based on the availability of sufficient sera and plasma samples to complete all assays multiple times, as well as histopathologic data (light microscopy, immunofluorescence, and EM) to review the pathologic diagnosis (Figure 3). All patients had biopsy-proven disease consistent with the description provided in the C3G consensus article, and all qualifying biopsies were confirmed by the research team and an independent pathologist (4). Posteculizumab samples were not included in this study. The University of Iowa Institutional Review Board approved all procedures and all patients gave informed consent before donating samples.

Serum and Plasma Samples

Serum and plasma samples were prepared by centrifugation (1000×g/10 minutes) of whole blood allowed to clot, respectively, in either plain (untreated) tubes for 45 minutes or EDTA tubes for <20 minutes. Collected samples were

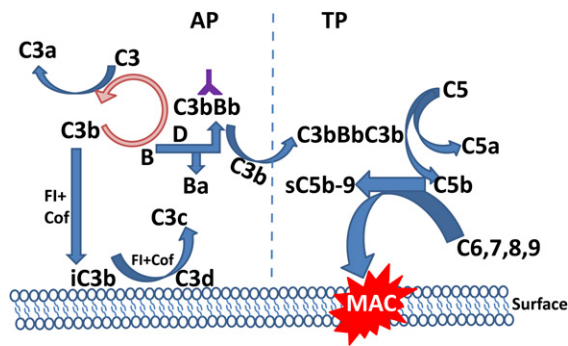


Figure 2. | The alternative and terminal pathways of complement. The alternative pathway is constitutively active due to spontaneous C3 hydrolysis, a process known as tick-over that leads to the generation of C3(H₂O) (also known as iC3). C3(H₂O)Bb then forms, leading to creation of the C3 convertase of the alternative pathway. The C3bBb amplification loop is represented by the pink circular arrow. If positive feedback continues unchecked or if C3bBb is stabilized by an autoantibody like C3Nef (shown in purple), the alternative pathway components are consumed and their breakdown products are generated. In the presence of FI and its cofactors (like FH and CR1), C3b is broken down to iC3b and then by additional cleavages, to C3c, C3d and C3g. Excessive C3 convertase promotes the formation of C5 convertase by the recruitment of additional C3b molecules. Note the similarity between the C3 and C5 convertases. C5 convertase cleaves C5 to C5a and C5b and initiates the assembly of the membrane attack complex by the sequential addition of C6, C7, C8, and C9 or of soluble (C5b-9) in the fluid phase. AT, alternative pathway; C3Nef, C3 nephritic factor; Cof, cofactors; CR1, complement receptor 1; FH, factor H; FI, factor I; MAC, membrane attack complex; TP, terminal pathway.

immediately frozen and stored at -80°C . Upon receipt by the Molecular Otolaryngology and Renal Research Laboratories, samples were thawed, aliquoted on wet ice into screw-top Eppendorf tubes for future use (serum samples, 250 μl ; plasma samples, 50 μl), and stored at -80°C . Samples used for complement testing were never refrozen; the unused portion was discarded. Normative data were generated from serum and plasma samples from 100 healthy blood donors (1:1 ratio of men to women; 97% of participants were European American and 3% were of other ethnicity).

Complement Protein Assays

Plasma samples were analyzed using a variety of commercially available assays (Supplemental Table 1). Direct sandwich ELISA (detection antibody directly coupled with horseradish peroxidase) was used to measure Ba, Bb, C3a, C3d, C5a, and soluble C5b-9 (sC5b-9) (Quidel Corporation, San Diego, CA; ProGen Biologics, Saint Louis, MO). Indirect sandwich ELISA (biotinylated detection antibody followed by streptavidin-conjugated horseradish peroxidase) was used for properdin, FB, C5, C6, C7, C8, and C9 (Abcam, Cambridge, MA; Hycult Biotech, Plymouth Meeting, PA). Total C3 was measured by radial immunodiffusion (The Binding Site, Birmingham, UK) and C3c was measured as described (7). Results were interpreted using a microplate reader at $\lambda 450$ (Bio-Rad Life Science, Hercules, CA) and calculated by Microsoft Excel software or by four-parameter

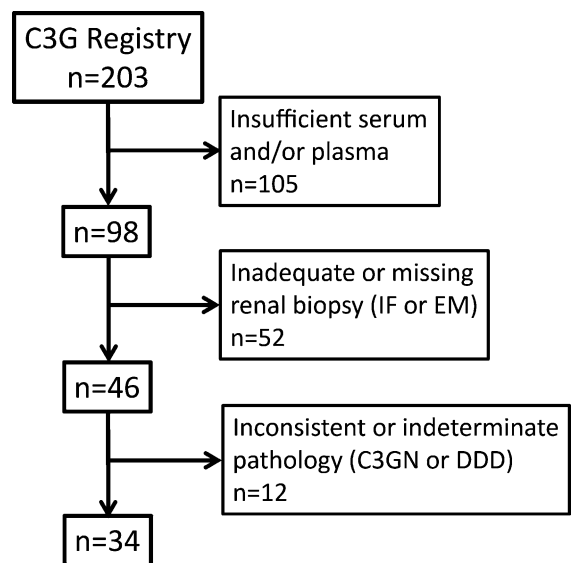


Figure 3. | Patient recruitment. Thirty-four patients with biopsy-proven DDD or C3GN were included in this study. Because 19 different complement assays were performed and each assay was completed at least three times, the initial inclusion criterion was the availability of sufficient sera and plasma. We estimated the sera requirement at 1 ml/patient and the plasma requirement at 3 ml/patient. Next, light microscopy, immunofluorescence, and electron microscopy results were reviewed to confirm the pathologic diagnosis of C3G. In 52 patients, the immunofluorescence and/or electron microscopy results were not available and thus these patients were excluded. In 12 patients, the diagnosis was equivocal (C3GN versus DDD) and thus these patients were also excluded. EM, electron microscopy; IF, immunofluorescence.

logistic regression (www.myassay.com). R^2 exceeded 0.98 for each ELISA assay.

Autoantibody Assays

Serum samples were analyzed for C3 nephritic factors (C3Nefs) and factor H autoantibodies as described (8).

DNA Extraction and Genetic Testing

Genomic DNA was extracted from blood samples using standard protocols (PAXgene Blood DNA Kit; Qiagen, Valencia, CA). Coding regions and intron-exon boundary junctions of multiple complement genes were amplified and screened using bidirectional sequencing as described (9).

Statistical Analyses

Data were collected in triplicate on different testing days using aliquots of a single serum or plasma sample. All data were presented as box plots with values greater than $1.5\times$ the interquartile range (IQR) considered as outliers. The t test or the Mann-Whitney U test was performed using SigmaPlot (Systat Software Inc, San Jose, CA). A P value <0.05 was considered significant.

Results

Study Population

Demographic details and basic clinical information are listed in Table 1. Complement assays were performed on

34 patients and 100 controls. The age range for patients was 7–62 years (mean 23±13). The mean duration of disease at the time of specimen procurement was approximately 5 years. All but two study patients were European American. Patients with C3GN were predominantly women and patients with DDD were predominantly men.

Complement Assays

Alternative Pathway. In both DDD and C3GN, serum C3 levels were significantly lower than in controls (DDD: median 0.52 g/l [IQR, 0.2–0.82]; C3GN: 0.71 g/l [0.44–1.06]; controls: 1.34 g/l [1.21–1.49]; $P<0.001$ for both). Serum FB, a component of the C3 convertase, was also reduced (DDD: 167 mg/l [IQR, 135–207]; C3GN: 143 mg/l [126–179]; controls: 203 mg/l [188–219]; $P<0.001$ for both), whereas its breakdown products Ba (DDD: 1.0 mg/l [IQR, 0.84–1.71]; C3GN: 1.06 mg/l [0.55–1.82]; controls: 0.63 mg/l [0.49–0.72]) and Bb (DDD: 1.28 mg/l [IQR, 1.14–1.52]; C3GN: 1.33 mg/l [1.16–3.10]; controls: 0.85 mg/l [0.75–1.03]) were significantly elevated in C3GN and DDD ($P<0.01$ and $P<0.001$, respectively, for Ba; $P<0.001$ for both for Bb). Properdin binds to and stabilizes C3bBb, increasing its $t_{1/2}$ approximately 10-fold. Whereas properdin serum levels were reduced in both DDD and C3GN, properdin levels were generally lower in C3GN compared with DDD (DDD: 17.4 mg/l [IQR, 12.4–20.2]; C3GN: 10.2 mg/l [7.4–14]; controls: 21.5 mg/l [17.5–25.2]; $P<0.05$ for DDD versus controls; $P<0.001$ for C3GN versus controls). Interdisease comparison of DDD and C3GN for these complement proteins identified only the decrease in properdin as significant between diseases (DDD versus C3GN, $P<0.01$). Measured breakdown products of C3 included C3a, C3c, and C3d (see Figure 2), with only the increase in C3d significantly different in DDD and C3GN versus controls (DDD: 0.88 mg/l [IQR, 0.71–1.52]; C3GN: 1.05 mg/l [0.86–1.14]; controls: 0.47 mg/l [0.44–0.54]; $P<0.001$ for both). There were no significant interdisease differences for any measured cleavage products of C3 (Figure 4A).

Terminal Pathway. Activation of the terminal complement pathway is initiated by activity of the C5 convertase, C3bBbC3b, which cleaves C5 to C5a and C5b. In both DDD and C3GN, C5 levels were reduced compared with controls (DDD: 70 mg/l [IQR, 59–77]; C3GN: 65 mg/l [54–79]; controls: 91 mg/l [76–102]; $P<0.001$ for both) and although C5a levels tended to be elevated, only differences between C3GN and controls were significant (DDD: 9.6 μ g/l [6.0–14.2]; C3GN: 12.9 μ g/l [7.9–16.9]; controls: 8.8 mg/l [4.3–9.9]; C3GN versus controls, $P<0.05$). With the sequential

addition of C6–C9 to C5b, the membrane attack complex and soluble C5b-9 are formed. C6, C8, and C9 levels were not reduced in either DDD or C3GN, but serum levels of C7 were decreased in C3GN compared with both controls and DDD (DDD: 79 mg/l [65–103]; C3GN: 69 mg/l [55–78]; controls: 83 mg/l [73–93]; C3GN versus controls, $P<0.01$; C3GN versus DDD, $P<0.05$). Soluble C5b-9 was increased in both diseases, although the increase was greater in C3GN (DDD: 0.18 mg/l [0.13–0.18]; C3GN: 0.63 mg/l [0.19–0.92]; controls: 0.14 mg/l [0.05–0.19]; C3GN versus controls, $P<0.001$; C3GN versus DDD, $P<0.05$) (Figure 4B).

Autoantibodies. C3Nefs are autoantibodies to the C3 convertase that can be detected using direct and indirect assays. Direct assays test patient-purified Igs after their isolation using a Melon Gel IgG purification kit (Pierce Protein Biology Products, Thermo Fisher Scientific Inc, Rockland, IL) in assays of C3 convertase activity with or without the addition of properdin (C3 convertase stabilizing assay with or without properdin [C3CSAP and C3CSA, respectively]). Indirect assays, by contrast, measure the activity of C3 convertases (presumed to be stabilized by C3Nefs) by detecting C3b breakdown products when patient and control sera are mixed (immune-fixation electrophoresis [IFE]). C3Nefs were significantly present in patients with C3G compared with controls. In the three assays we used (C3CSAP, C3CSA, and IFE), C3Nef activity was always greater in DDD compared with C3GN (DDD versus C3GN: $P<0.001$ for C3CSAP, $P<0.001$ for C3CSA, and $P<0.01$ for IFE). No FH autoantibodies were detected in either group (Figure 4C, Supplemental Tables 2 and 3).

Genetic Testing

Three patients with DDD (17.6%) carried genetic variants. In two patients, three *FH* variants were identified (heterozygous c.2821G>A, p.V941F; compound heterozygous c.2509G>A, p.V837I; and c.3435G>C, p.E1145D), whereas the third patient carried a *FI* variant (heterozygous c.1217G>A, p.R406H). One patient with C3GN (5.9%) carried an *FH* variant (heterozygous c.1699A>G, p.R567G). There was no difference in the frequency of *FH* and C3 risk alleles between groups (DDD versus C3GN: $P>0.05$) (Supplemental Tables 2 and 3).

Discussion

The recently adopted disease designation C3G emerged in part because it has become clear that pedigrees with glomerular disease associated with identifiable complement

Table 1. Demographic parameters for the study cohort

Parameter	C3GN	DDD	Controls
Patients, <i>n</i>	17	17	100
Age, yr	31.4±16.1	21.2±8	20±1
Men/women	6/11	11/6	50/50
Non-Caucasian/Caucasian ethnicity	1/16	1/16	3/97
Duration of disease, yr	5.1±3.4	4.9±3.6	N/A

Data are presented as the mean±SD unless otherwise indicated. DDD, dense deposit disease; N/A, not applicable.

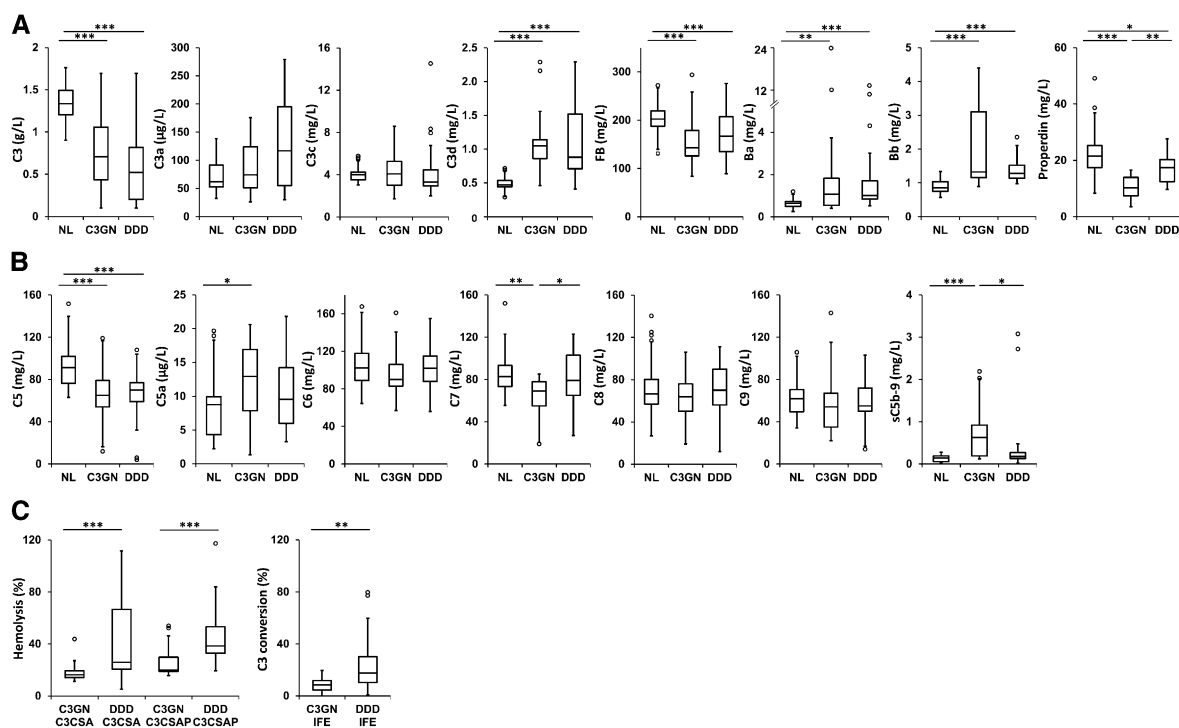


Figure 4. | Complement biomarker assessments in the C3G cohort. (A) Eight alternative pathway biomarkers (C3, FB, properdin, and their associated degradation products C3a, C3c, C3d, Ba and Bb) were assessed in 17 patients with C3GN, 17 patients with DDD, and 100 controls (normal). Serum C3 and FB levels were significantly lower in patients with C3G than in controls, whereas the breakdown products C3d, Ba, and Bb were significantly elevated. Properdin serum levels were reduced in both DDD and C3GN although properdin levels were generally lower in C3GN compared with DDD. (B) Seven terminal pathway biomarkers (C5–C9) and the activation products C5a and sC5b-9) were assessed in the same cohorts. C5 levels were reduced compared with controls in both DDD and C3GN. Although C5a levels tended to be elevated in both diseases, only the elevation in C3GN versus controls was significant. Whereas C6, C8, and C9 levels were not reduced in DDD or C3GN, serum levels of C7 were decreased in C3GN compared with both controls and DDD. Soluble C5b-9 was elevated in both diseases, with the elevation being greater in C3GN. (C) C3Nefs were measured by two direct assays (C3CSA and C3CSAP) and one indirect assay (IFE) (8). No control was positive for any C3Nef assay. In all assays, C3Nef activity was greater in DDD compared with C3GN. For all box-and-whisker plots, the first and third quartiles are represented by the bottom and top ends of the box, the median is represented by the horizontal line in the interior of the box, and the whiskers represent $1.5 \times$ the interquartile range. Open circles are outliers. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. C3CSA, C3 convertase stabilizing assay; C3CSAP, C3 convertase stabilizing assay with properdin; FB, factor B; IFE, immunofixation electrophoresis; NL, normal; sC5b-9, soluble C5b-9.

mutations have a specific immunofluorescence pattern on renal biopsy. In addition, it is now understood that historical renal pathology descriptions were not sufficiently informative about renal disease (*i.e.*, patients with membranoproliferative GN II did not always have membranoproliferative GN). The final advance, the expanded development and availability of both complement functional assays and complement gene analyses, was a necessary advancement in making it reasonable to rethink disease descriptions.

Immunofluorescence findings that are strongly positive for C3 and negative or only weakly positive for Igs suggest an abnormality in the complement system and thus, as recommended by consensus, identify a group of patients in whom genetic and functional testing of the alternative pathway and terminal complement system are warranted (4). Our study confirms the validity of this recommendation in the first comprehensive attempt to characterize in a stepwise fashion the unique complement abnormalities in patients with C3G. It is our expectation that this type of granular approach to identifying the specifics of a given patient's complement abnormality will not only be informative for pathobiology but will also prove integral to the development of effective

on-target treatment options. The ultimate goal will be to aid the clinician in choosing a tailored therapy as additional anticomplement therapeutics become available.

Given the history of C3G and our *a priori* assumption that C3G is a complement-mediated disease, it was not surprising that serum C3 was significantly reduced in C3G compared with normal controls. Although the reduction in C3 tended to be greater in DDD, the difference between DDD and C3GN was not statistically significant. Also in keeping with the belief that a continuously active alternative pathway is central to disease, it was not unanticipated that there was an apparent consumptive decrease in FB with an increase of FB split products Ba and Bb. The reduction in properdin found in both diseases is noteworthy because properdin stabilizes the C3 convertase (C3bBb), increasing its $t_{1/2}$ about 10-fold and favoring formation of the C5 convertase (C3bBbC3b). The intergroup difference in properdin reduction suggests that the generation of C5 convertase is greater in C3GN than in DDD (Figure 4A) (10).

Biomarker studies of C5–C9 also support greater terminal pathway activity in C3GN compared with DDD. In both diseases, C5 is reduced compared with normal controls,

although its split product C5a is elevated only in C3GN. In addition, C7 is consumed, although more so in C3GN than in DDD. Finally, and arguably the most important finding from a clinical perspective given the mechanism of the currently available anticomplement therapeutic option, soluble C5b-9 is more likely to be elevated in C3GN than in DDD (C3GN versus normal, $P < 0.001$; C3GN versus DDD, $P < 0.05$). The combination of an elevated C5a, a potent proinflammatory anaphylotoxin, and an elevated C5b-9 level may define a group of patients more likely to respond to anti-C5 therapeutics as suggested by Bomback *et al.* (11).

C3Nef activity was significantly elevated in patients with C3G compared with normal controls. Because C3Nefs stabilize the C3 convertase, this finding suggests a relative upregulation of this enzyme, which appeared to be more pronounced in DDD compared with C3GN.

In aggregate, our data can be interpreted to mean that C3G is characterized by dysregulation of the C3 and C5 convertases. Because these convertases are extremely similar (C3 convertase=C3bBb; C5 convertase=C3bBbC3b), we do not believe that a “second hit” needs to be invoked for dysregulation of both convertases to occur. Rather, we propose a model in which the inciting factor or factors (genetic and/or acquired) have a differential effect on the two convertases given their similarity, with the observed differences between DDD and C3GN being defined, at least in part, by differences in the degree of convertase dysregulation.

Dysregulation of the C3 convertase tends to be greater in DDD, whereas dysregulation of the C5 convertase tends to be greater in C3GN. The consequence of C3 convertase dysregulation (only or predominantly) appears on EM as the darker/denser, sausage-like, or band-like midlayer deposits and may represent a constant, slow build-up of C3 breakdown products. This hypothesis is consistent with the fact that C3 deposition in DDD renal transplants begins very early, although clinical disease may follow by years if at all. When C5 convertase dysregulation is high, the appearance on EM shifts toward the less electron-dense and more amorphous-appearing deposits of C3GN. There is associated elevation in C5a and a very inflammatory response, the acuity of which may facilitate an intervention response.

A prospective study defining the biomarker profile of patients with C3G during different phases of their disease will be instrumental in establishing the uniformity of our findings. It is likely that the biomarker differences we documented reflect both the nascent activity of a specific patient’s complement system as well as intrinsic characteristics of the marker in question. For example, C5a has a $t_{1/2}$ of 3 minutes and the rate of disappearance of C3d is much slower than that of C3c (12,13). The concept that disparate convertase dysregulation is phenotypically important is well supported by a handful of familial cases of C3G segregating specific point mutations or genetic rearrangements (14–22). These cases provide crucial insight into the phenotypic consequences of variable degrees of convertase dysregulation, whereas biomarker profiling of C3G illustrates the spectrum of complement dysregulation that occurs, highlighting differences in the relative degrees of unchecked activity of the C3 and C5 convertases.

The evaluation of complement biomarkers in C3G is likely to be clinically valuable for three reasons. First, there

are currently no effective disease-specific therapies for C3G. As a consequence, treatment is focused on a variety of supportive measures with occasional trials of plasma therapy and/or cellular immune suppression. It remains difficult to determine the relative effectiveness of this type of therapy. Second, the availability of a licensed complement inhibitor (eculizumab; Alexion Pharmaceuticals) and isolated case reports of its use in C3G support efforts to identify patients who may benefit from the off-label use of this expensive drug. From a drug mechanism standpoint, it is likely that eculizumab could be a useful intervention if soluble C5b-9 is elevated. However, given the aforementioned disparate regulation of the convertases, eculizumab should be considered with the *proviso* that upstream dysregulation of the C3 convertase will not be affected by this anticomplement drug, thus potentially affecting long-term drug response. Third, there are many complement inhibitors in clinical development, some of which may benefit patients with C3G. Because DDD and C3GN are ultra-rare diseases precluding the identification of large numbers of patients for double-blind placebo-controlled studies, clinical trials with these new inhibitors must focus on predicting their effect *a priori* based on their site of function in the complement cascade. Each patient’s biomarker profile should be defined in these studies. Changes in biomarkers can then be utilized as primary efficacy end points of therapy and correlated with secondary clinical outcomes such as changes in proteinuria or CKD stage.

In summary, we have shown that complement biomarkers are significantly abnormal in patients with C3G compared with controls. These abnormalities expand our understanding of C3G by substantiating the link between complement dysregulation and C3G and identifying C3G inter-disease differences.

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Disclosures

None.

References

1. Barbour TD, Pickering MC, Terence Cook H: Dense deposit disease and C3 glomerulopathy. *Semin Nephrol* 33: 493–507, 2013
2. Nester CM, Smith RJ: Diagnosis and treatment of C3 glomerulopathy. *Clin Nephrol* 80: 395–403, 2013
3. Servais A, Noël LH, Frémeaux-Bacchi V, Lesavre P: C3 glomerulopathy. *Contrib Nephrol* 181: 185–193, 2013
4. Pickering MC, D’Agati VD, Nester CM, Smith RJ, Haas M, Appel GB, Alpers CE, Bajema IM, Bedrosian C, Braun M, Doyle M, Fakhouri F, Fervenza FC, Fogo AB, Frémeaux-Bacchi V, Gale DP, Goicoechea de Jorge E, Griffin G, Harris CL, Holers VM, Johnson S, Lavin PJ, Medjeral-Thomas N, Paul Morgan B, Nast CC, Noel LH, Peters DK, Rodríguez de Córdoba S, Servais A, Sethi S, Song WC, Tamburini P, Thurman JM, Zavros M, Cook HT: C3 glomerulopathy: Consensus report. *Kidney Int* 84: 1079–1089, 2013
5. Walker PD, Ferrario F, Joh K, Bonsib SM: Dense deposit disease is not a membranoproliferative glomerulonephritis. *Mod Pathol* 20: 605–616, 2007
6. Servais A, Noël LH, Roumenina LT, Le Quintrec M, Ngo S, Dragon-Durey MA, Macher MA, Zuber J, Karras A, Provot F,

- Moulin B, Grünfeld JP, Niaudet P, Lesavre P, Frémeaux-Bacchi V: Acquired and genetic complement abnormalities play a critical role in dense deposit disease and other C3 glomerulopathies. *Kidney Int* 82: 454–464, 2012
7. Palarasah Y, Skjodt K, Brandt J, Teisner B, Koch C, Vitved L, Skjoedt MO: Generation of a C3c specific monoclonal antibody and assessment of C3c as a putative inflammatory marker derived from complement factor C3. *J Immunol Methods* 362: 142–150, 2010
 8. Zhang Y, Meyer NC, Wang K, Nishimura C, Frees K, Jones M, Katz LM, Sethi S, Smith RJ: Causes of alternative pathway dysregulation in dense deposit disease. *Clin J Am Soc Nephrol* 7: 265–274, 2012
 9. Maga TK, Nishimura CJ, Weaver AE, Frees KL, Smith RJ: Mutations in alternative pathway complement proteins in American patients with atypical hemolytic uremic syndrome. *Hum Mutat* 31: E1445–E1460, 2010
 10. Kemper C, Atkinson JP, Hourcade DE: Properdin: Emerging roles of a pattern-recognition molecule. *Annu Rev Immunol* 28: 131–155, 2010
 11. Bombback AS, Smith RJ, Barile GR, Zhang Y, Heher EC, Herlitz L, Stokes MB, Markowitz GS, D'Agati VD, Canetta PA, Radhakrishnan J, Appel GB: Eculizumab for dense deposit disease and C3 glomerulonephritis. *Clin J Am Soc Nephrol* 7: 748–756, 2012
 12. Weisdorf DJ, Hammerschmidt DE, Jacob HS, Craddock PR: Rapid in vivo clearance of C5ades arg: A possible protective mechanism against complement-mediated tissue injury. *J Lab Clin Med* 98: 823–830, 1981
 13. Teisner B, Brandslund I, Grunnet N, Hansen LK, Thellesen J, Svehag SE: Acute complement activation during an anaphylactoid reaction to blood transfusion and the disappearance rate of C3c and C3d from the circulation. *J Clin Lab Immunol* 12: 63–67, 1983
 14. Licht C, Heinen S, Józsi M, Löschmann I, Saunders RE, Perkins SJ, Waldherr R, Skerka C, Kirschfink M, Hoppe B, Zipfel PF: Deletion of Lys224 in regulatory domain 4 of Factor H reveals a novel pathomechanism for dense deposit disease (MPGN II). *Kidney Int* 70: 42–50, 2006
 15. Wu J, Wu YQ, Ricklin D, Janssen BJ, Lambris JD, Gros P: Structure of complement fragment C3b-factor H and implications for host protection by complement regulators. *Nat Immunol* 10: 728–733, 2009
 16. Martínez-Barricarte R, Heurich M, Valdes-Cañedo F, Vazquez-Martul E, Torreira E, Montes T, Tortajada A, Pinto S, Lopez-Trascasa M, Morgan BP, Llorca O, Harris CL, Rodríguez de Córdoba S: Human C3 mutation reveals a mechanism of dense deposit disease pathogenesis and provides insights into complement activation and regulation. *J Clin Invest* 120: 3702–3712, 2010
 17. Chen Q, Wiesener M, Eberhardt HU, Hartmann A, Uzonyi B, Kirschfink M, Amann K, Buettner M, Goodship T, Hugo C, Skerka C, Zipfel PF: Complement factor H-related hybrid protein deregulates complement in dense deposit disease. *J Clin Invest* 124: 145–155, 2014
 18. Athanasiou Y, Voskarides K, Gale DP, Damianou L, Patsias C, Zavros M, Maxwell PH, Cook HT, Demosthenous P, Hadjisavvas A, Kyriacou K, Zouvani I, Pierides A, Deltas C: Familial C3 glomerulopathy associated with CFHR5 mutations: Clinical characteristics of 91 patients in 16 pedigrees. *Clin J Am Soc Nephrol* 6: 1436–1446, 2011
 19. Medjeral-Thomas N, Malik TH, Patel MP, Toth T, Cook HT, Tomson C, Pickering MC: A novel CFHR5 fusion protein causes C3 glomerulopathy in a family without Cypriot ancestry. *Kidney Int* 85: 933–937, 2014
 20. Malik TH, Lavin PJ, Goicoechea de Jorge E, Vernon KA, Rose KL, Patel MP, de Leeuw M, Neary JJ, Conlon PJ, Winn MP, Pickering MC: A hybrid CFHR3-1 gene causes familial C3 glomerulopathy. *J Am Soc Nephrol* 23: 1155–1160, 2012
 21. Tortajada A, Yébenes H, Abarrategui-Garrido C, Anter J, García-Fernández JM, Martínez-Barricarte R, Alba-Domínguez M, Malik TH, Bedoya R, Cabrera Pérez R, López Trascasa M, Pickering MC, Harris CL, Sánchez-Corral P, Llorca O, Rodríguez de Córdoba S: C3 glomerulopathy-associated CFHR1 mutation alters FHR oligomerization and complement regulation. *J Clin Invest* 123: 2434–2446, 2013
 22. Goicoechea de Jorge E, Caesar JJ, Malik TH, Patel M, Colledge M, Johnson S, Hakobyan S, Morgan BP, Harris CL, Pickering MC, Lea SM: Dimerization of complement factor H-related proteins modulates complement activation in vivo. *Proc Natl Acad Sci U S A* 110: 4685–4690, 2013
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