Diagnosis and treatment of C3 glomerulopathy

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Abstract. Purpose of review: The purpose of this review is to summarize our current understanding of the principal characteristics of C3 glomerulopathy as a framework for patient evaluation with the goal of setting the stage for a mechanistic approach to treatment. We also review published treatment experience and comment on future initiatives to devise treatment protocols for this rare renal disease patient population. Diagnosis and treatment: Both animal and human data support the role of the alternative pathway of complement in the C3 glomerulopathies. The finding of dominant C3 deposition on renal biopsy, a marker of aberrant complement activity and the primary diagnostic criterion, defines C3 glomerulopathy as a group of diseases that despite variable light and electron microscopy appearance, shares important phenotypic characteristics; namely the presence of genetic mutations in complement genes, the presence of C3 nephritic factors with or without other complement protein abnormalities, and finally a substantial risk for both end-stage renal disease (ESRD) and recurrence after renal transplant. Traditional immune suppressive treatment strategies are often ineffective in this group of patients. Case reports and a single small trial support the efficacy of anti-complement therapy in this setting. Summary: The diagnosis of C3 glomerulopathy is established by renal biopsy and requires a C3 dominant pattern on immunofluorescence in a patient with active glomerulonephritis. Laboratory studies characterizing an individual patient’s complement profile form the basis of an expanded phenotype that has the potential to inform not only the relative activity of disease, but also the risk for adverse outcome or treatment nonresponse. Understanding an individual patient’s complement pathology will facilitate an optimal therapeutic approach to their disease.

Introduction

C3 glomerulopathy (C3G) is the new designation for the group of glomerular diseases that are characterized by a dominant C3 immunofluorescence (IF) pattern on renal biopsy. An underlying assumption, though not part of the formal definition, is that as a group, these diseases are mechanistically related to an aberrantly functioning alternate pathway of complement. The full spectrum of morphological lesions that present as C3G remains to be determined, however a specific light microscopic pattern is not a part of the disease definition (i.e., patients may or may not have a membranoproliferative pattern on renal biopsy). Just as importantly, it is now accepted that there may be small amounts of immunoglobulin present provided the C3 IF intensity is twofold or higher than the immunoglobulin intensity. The original definition of isolated C3 was amended to dominant C3 when a review of the literature revealed that even well characterized dense deposit disease (DDD) patients (i.e., the easiest C3G to recognize) may have small amounts of immunoglobulin on IF (IgG, A and M in 26.7%, 13.3% and 36.7%, respectively) [1].

Two major subgroups of C3G exist and these subgroups are resolved by EM: DDD, defined by the EM findings of intramembranous glomerular basement membrane dense deposits [2] and C3 glomerulonephritis (C3GN), encompassing the remainder of the C3G lesions and defined by some combination of subepithelial, subendothelial and/or less dense, discontinuous intramembranous deposits not characteristic of DDD [3]. Another term often associated with C3G is that of CFHR5 nephropathy, a subcategory of C3GN caused by a genetic rearrangement in CFHR5 that leads to an abnormal protein product [4, 5, 6].
There are many overlapping characteristics between DDD and C3GN, with the primary association being dysregulation of the alternative pathway of complement. C3G patients have in common mutations in alternate complement pathway genes, the presence of C3 nephritic factors and a substantial risk for ESRD and recurrence of disease after renal transplant (Figure 1). It remains to be seen if the similarities amongst the C3Gs support a common therapeutic strategy or whether differences in pathology will necessitate disparate therapeutic approaches.

Complement pathology in C3G

Both animal data and human data support the role of the alternate complement pathway in the pathogenesis of C3G [5, 7, 8, 9, 10, 11, 12, 13, 14]. To understand the pathology behind C3G, it is useful to review the complement system and its major components. Figure 2 depicts the three complement pathways and their relationship to each other. The convertases (common to all of the pathways, though made of slightly different protein constituents) are the key enzymes central to the complement pathways. C3G occurs as a result of dysregulation of the alternate pathway of complement C3 convertase (pink box) and/or the C5 convertase (green box).

The C3 convertase (C3bBb) is the enzyme central to the amplification phase of the complement system. Once the C3 convertase is formed (after cleavage of C3 to C3b, C3b combines with Bb, the active cleavage partner of complement factor B to form the C3bBb), it feeds back to cleave C3, and in a circular fashion leads to more C3b and hence more C3 convertase. This amplification loop and its potential to generate massive amounts of C3b results in a highly active complement system that if left unchecked has at least three consequences: 1) increased availability of anaphylotoxins (C3a and C3b); 2) increased activity of the terminal complement pathway (through the generation of C5 convertase) with subsequent production of the membrane attack complex (MAC); and 3) increased production of inactivated C3 by-products (iC3b). Homeostasis requires that the protein regulators of complement activation (RCA) are present and functioning properly in the fluid phase (i.e., in circulation as opposed to at the surface of tissues) in order to control the aforementioned processes and prevent these consequences and unintended host damage. In the absence of fluid phase control, C3G results, with glomerular deposition of C3b, C3b inactivation products and terminal complement proteins which are resolved as the EM densities in both DDD and C3GN [15].

Driving the disease-causing complement dysregulation are genetic variants in complement regulatory proteins and autoantibodies to the convertases [5, 9, 11, 12, 13, 14, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29]. Mutations have been reported in complement Factor H (CFH), complement Factor I (CFI), MCP (also termed membrane cofactor protein or CD46), complement Factor B (CFB) and CFHR5. The relative distribution of rare and novel variants between DDD vs. C3GN is minimal, as demonstrated in a large French cohort (CFH 17.2% vs. 12.5%, CFI 0% vs. 5.3%, and MCP 0% vs. 1.8%) [10]. However, as mentioned earlier, a specific type of C3GN termed CFHR5 nephropathy has been described in a large Cypriot cohort (91 patients and 61 families) [5, 30]. Gain-of-function mutations have also been reported in CFB [30] and complement Factor C3 (C3) [14].
While it is important to recognize that specific “disease-causing” variants may be found and may direct therapeutic decisions as more complement therapeutics become available, what is far more common in the C3G patient is an enrichment for certain alleles (variations) of the protein components of the complement system. This group of so-called “risk alleles” has been referred to as the C3G “complement haplotype” or “com- plotype”. Even in controls, this complotype is associated with increased basal activity of the complement cascade, and thus this genetic background may predispose to C3G in association with a “triggering” environmental insult [12]. The best studied of these risk alleles is the including H402 variant of CFH [31, 32].

As common as risk alleles in C3G patients are autoantibodies called C3 nephritic factors (C3Nefs) that stabilize the C3 convertase. C3Nefs render C3 convertase resistant to control by Factor H thereby leading to fluid phase convertase dysregulation. Why C3Nefs develop and whether their development reflects molecular mimicry and has any relationship to the C3G complotype is not known. However, C3Nefs are very common and are reported in over 80% of patients with DDD [10] and ~ 50% of patients with C3GN [10]. It is extremely important to emphasize that there are many different C3Nef assays (ELISA is the LEAST sensitive) and the absence of a C3Nef only means that a nephritic factor was not detected by that particular assay and NOT that C3Nefs are absent. We would recommend testing both for the antibody (an IgG-based assay) and its consequence (looking for C3 breakdown products) [33].
The clinical presentation of C3G, with few exceptions, appears to be similar regardless of the subcategory. Much of the phenotypic data comes from two large European cohorts (French [10] and Cypriot [5]) with confirmation from the smaller US cohorts [10, 25, 34]. The male-to-female ratio is essentially equal between the two groups, however the age at presentation is higher for C3GN than for DDD (30.3 ± 19.3 vs. 18.9 ± 17.7) [10]. Hematuria occurs in the large majority of patients (range 64.3 – 75.8%) consistent with C3G being a primary glomerular disease. Proteinuria is also common and is frequently in the nephrotic range. The proteinuria range is slightly lower for C3GN than for DDD (3.6 g ± 3.3 vs. 5.6 ± 4.5). A low serum complement C3 is frequently present (59% in DDD and 40% in C3GN), however, is not a requirement for diagnosis.

In light of the presenting characteristics of C3G, post infectious glomerulonephritis (PIGN) may confound the initial diagnosis. PIGN also presents with hematuria, proteinuria and often a low serum complement C3. Furthermore, on renal biopsy, the IF pattern may be that of isolated or dominant C3 staining consistent with the definition of C3G. Distinction from C3G will depend on the absence of atypical features on light microscopy and EM, and/or on whether the clinical course is typical or atypical for PIGN. However, it is possible that an intercurrent infection, such as one caused by a streptococcal organism, may be a trigger for C3GN when that infection occurs in a person with a C3G-predisposing genotype [35]. We recommend that when presented with a C3 dominant renal biopsy and confusion over whether the pathology should be considered PIGN or C3G (i.e., our current understanding of the pathology fails to distinguish the two), the C3G diagnosis should only be assigned if the serum C3 fails to normalize by 12 weeks.

Another confounding diagnosis is the presentation of a DDD lesion in an elderly patient with a monoclonal gammopathy [36, 37]. The current assumption is that the monoclonal protein plays a role in the pathology of DDD, however there are only limited data to support this cause-and-effect relationship [35]. Nevertheless, the evaluation and treatment of DDD in this setting should be directed at the monoclonal protein (i.e., through hematological therapies) with the assumption that resolution of the monoclonal gammopathy may lead to resolution of the glomerular lesion of DDD.

Recovery from the acute presentation of C3G is unpredictable. Ten year native renal survival for this group of patients appears to be between 50 – 60% with young females having the greatest risk for renal failure [34]. In transplant recipients, Lu et al. [34] reported a 45% renal allograft loss within 5 years of transplant, data confirmed by Servais et al. [6] in their study reporting the graft loss to be 51%. Interestingly, in CFHR5 nephropathy, men are more likely to develop chronic renal failure (80%) than women (21%) and progress to ESRD (78% vs. 22%). From these data, it is clear that C3G is not a benign disease in the majority of patients.

**Treatment**

Measures designed to support the patient’s general health (appropriate nutrition, blood pressure control and chronic kidney disease management) are appropriate in patients with C3G. Extrapolating from other glomerular diseases, the angiotensin converting enzyme inhibitors or angiotensin receptor blockers should be consider for supportive care either for blood pressure control or as an aid to urine protein management. Limited C3G specific data on ACEI or ARB use is offered by the French C3G cohort (a retrospective review) where the use of angiotensin-converting enzyme inhibitors or angiotensin receptor blockers was associated with a better renal survival (p < 0.0001) [10]. Similarly, lipid lowering agents are likely to be useful as needed in C3G [38].

There are no good data to support the use of plasmatherapy as a matter of routine care in C3G patients however some case reports do support its efficacy particularly when there is a known protein abnormality. For example, Licht et al. [20] reported efficacy of plasma therapy in a sibling pair with C3G caused by homozygosity for an in-frame amino acid deletion in factor H that compromises its RCA function. Plasmatherapy has also been reported to be efficacious in the setting of DDD and acute kidney injury [39].
Unfortunately plasma therapy failures are also reported even when C3Nefs have been removed by this treatment [41]. These data suggest that in select cases, plasma therapy may play a role, however, it should be used in the context of a complete patient evaluation with the appropriate biomarker follow-up to determine whether an expected outcome is observed.

Anticellular immune suppression is likely to be entertained early when considering treatment options for C3G (steroid, mycophenolate mofetil, rituximab, cyclophosphamide, etc.). The theoretical benefit to traditional anti-cellular therapy includes limiting the effects of the anaphylotoxins (such as C3a and C5a), inhibiting immune cell reaction or inflammation, and/or reducing antibody production. However, the data to support the successful use of anticellular immune suppression are disappointing. Referring to the larger French cohort [10], the use of such agents offered no renal survival advantage to C3G patients. Steroids have been used in this setting perhaps more than any other agent, however their success rate is also hard to substantiate through a review of the literature: Daina et al. [42] reported a failure to induce a remission of DDD despite a prolonged course of steroids. Combinations of anticellular drugs have also had limited success: McCaughan et al. [41] reported a failure to respond to glucocorticoid, mycophenolate mofetil and rituximab therapy. Similarly, Bomback et al. [25] reported the failure of multiple anticellular immune suppressive agents in their group of patients.

As data implicating complement dysregulation in C3G have become more robust, clinicians have turned to anti-complement therapy as a potential directed therapy. C3G complement blockade disease response has been predicted by animal models [8, 43, 44] and there are now multiple reports describing the efficacy of eculizumab as an anti-C5 therapy in C3G. This agent was seen to mitigate disease in three case reports and in one small trial [25, 41, 42, 45].

Vivarelli et al. [45] reported the case of a 17-year-old female with a 7-year history of DDD (40% glomerular sclerosis on renal biopsy) and normal renal function. She was started on eculizumab when she developed a worsening of nephrotic-range proteinuria. After 18 months of therapy, she had a remarkable improvement in her urine protein. Importantly, when eculizumab was stopped, she had a recrudescence of her urine protein and a re-induction of a relative remission with the restart of eculizumab. Two subsequent renal biopsies showed a progressive reduction of C3 and C5b-9 immunofluorescence and a progressive reduction in mesangial proliferation and glomerular capillary loop thickness – each suggestive of a limited histological recovery of the DDD lesion [45]. This finding is consistent with animal data: the clearance of C3 fragments from glomeruli in CFH-deficient mice through the restoration of complement regulation suggests that the initial process leading to C3 glomerulopathy is dynamic and may be reversible [8, 43].

Daina et al. [42] reported the case of a 22-year-old female with DDD and a long-standing history of nephrotic syndrome unresponsive to the prolonged use of steroids and rituximab. Her laboratory values included a low C3, the presence of a C3Nef, and an elevated terminal complement complex. After 48 weeks of eculizumab, this patient’s serum albumin normalized and her creatinine decreased.

McCaughan et al. [41] reported the efficacy of eculizumab in a case of recurrent DDD post-renal transplant in a 29-year-old female 4 weeks post-operatively (heralded by 6g of urine protein). This recurrence developed while on the standard transplant immune suppression of prednisone, mycophenolate mofetil and tacrolimus. At the time of recurrence, the patient had a low C3 and a positive C3Nef, and despite rituximab therapy and plasmapheresis (with normalization of her C3Nef) she continued to progress. Thirteen weeks after transplant, she was started on eculizumab and her creatinine improved from 4.9 to 1.9 mg/dl.

Finally, a single trial demonstrating efficacy of eculizumab in some patients with C3G has also been published. Bomback et al. [25] performed an open-label, proof-of-concept, efficacy-and-safety study in which they treated 3 DDD (1 with a renal transplant) and 3 C3GN patients (2 with a renal transplant) with eculizumab for 1 year. All had proteinuria > 1 g/d and/or AKI at enrollment. Genetic and complement function testing revealed a
mutation in CFH and MCP in 1 subject each and C3Nefs in 3 subjects. After 12 months of therapy 2 subjects showed significantly reduced serum creatinine, 1 subject achieved marked reduction in proteinuria, and one subject had stable laboratory parameters but was noted to have a histopathologic improvement.

The patients that responded to eculizumab were those patients who had elevated terminal complement activity as represented by an elevated MAC (surveillance of this patient group is ongoing.)

Provided we have a replication of this finding in a broader array of patients, the finding that responders to eculizumab in the study by Bomback et al. [26] were those with an elevated soluble MAC will be important to the rational use of this therapeutic option. Eculizumab binds C5 and prevents C5 convertase cleavage of C5 to C5a and C5b. The direct result of this blockade is a decreased production of soluble MAC and therefore eculizumab is likely to be most effective in patients with severe dysregulation at the level of C5 convertase as measured by an elevation in soluble MAC. As mentioned in the complement pathology section, C3G patients are likely to have varying degrees of...
dysregulation at the level of both the C3 and C5 convertases and so depending on the degree of C3 dysregulation that is present, suppression of the terminal complement cascade may not be enough to facilitate remission. For example, data from the Cfh−/− mouse, a model of C3GN suggests that while anti-C5 therapy reduces inflammation and urine protein, it does not prevent C3 deposition along the glomerular basement membrane [43]. Therefore laboratory tests must evaluate both the potential for dysregulation at the level of the C3 convertase and the C5 convertase. Optimal treatment should then follow based on the findings of this dual evaluation.

**Conclusions**

G3G is a new category of glomerular disease characterized by predominant C3 immune deposits on the renal biopsy of a patient with active glomerular disease. Dysregulation of the alternative and terminal complement pathways either as a result of genetic mutation or acquired autoantibodies (or both) is well described as the disease mechanism in both animal and human studies. The full spectrum of the pathological characteristics of C3G has yet to be defined. Advances in our understanding of the role of complement in C3G has improved our ability to characterize the complement phenotype in individual patients. A more robust understanding of the natural history of C3G will develop as we continue to collect well-described cases, preferably in the form of a registry.

The recent availability of an anti-complement agent has allowed us to reconsider our therapeutic options. Undoubtedly, a combination of clinical presentation, renal morphology, genetic workup and complement abnormality assessment will allow for the most efficacious treatment. In addition, as we advance our understanding of complement abnormalities in this setting, we will be able to take advantage of future therapeutic options.

Questions that remain to be answered: Can we further define complement profiles specific to DDD and C3GN? How frequently will PIGN cases be reassigned to C3GN? What are the other triggers for C3Gs? Will a more precise delineation of complement abnormalities in C3G allow for tailored personal anti-complement therapy as more treatment options become available? Will a better understanding of the morphological characteristics of a patient’s biopsy predict treatment response?

As these questions are answered, new questions will arise, especially focused on longevity of treatment. For instance, once a treatment plan is undertaken, how long should patients be treated? A corollary to this, is C3GN or DDD, a relapsing and remitting disease or is it persistent, slowly progressive in all patients therefore necessitating ongoing therapy? Does treating the anaphylatoxin response offer therapeutic

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**Treatment strategy**

The C3G Consensus Group will soon publish its recommendations on the laboratory studies that should be considered in order to fully evaluate a C3G patient. These recommendations are a direct result of both our current understanding of the pathology of C3G (both DDD and C3GN) and the availability of meaningful complement biomarkers. Based on a complete serologic and genetic assessment of the C3G patient, we believe that it may be possible to devise a successful treatment plan for individual patients. The overall intent is to match the complement abnormality to a treatment approach specific to that abnormality when such treatments are available. We have depicted this strategy in Figure 3.

Plasma therapy should be considered when abnormal proteins are present with plasma infusion (and/or pharmaceutical grade protein factors where available) being preferred for those patients with known factor deficiencies. Anti-cellular therapy may be used to augment the treatment response in this setting. Based on our current understanding of the pathology of C3G and the published successes we have included anti-complement therapy in our algorithm for treating C3G recognizing that additional trial data are required. Based on the mechanism of the currently marketed anti-complement agent (eculizumab) and the limited trial data, this therapy is best suited for those patients with an elevated terminal complement complex assay.
advantage over simply blocking the terminal complement cascade or limiting the production of C3 breakdown products? Would “upstream” (C3 convertase) inhibitors offer a therapeutic advantage over terminal complement blockade?

Taking advantage of the current interest and focus on C3G, both complement scientists and clinicians have the opportunity through rigorous and meticulous study to facilitate improved health for these patients.

Conflict of interest

Dr. Nester is a participant on the C3 Glomerulopathy Advisory Board, sponsored by Alexion, Inc. Dr. Nester and Dr. Smith collaborate with Cellcedex on an investigator initiated study utilizing anti-complement therapy in dense deposit disease patients.

References


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