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FULL REVIEW

Recent insights into C3 glomerulopathy

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ABSTRACT

'C3 glomerulopathy' is a recent disease classification comprising several rare types of glomerulonephritis (GN), including dense deposit disease (DDD), C3 glomerulonephritis (C3GN) and CFHR5 nephropathy. These disorders share the key histological feature of isolated complement C3 deposits in the glomerulus. A common aetiology involving dysregulation of the alternative pathway (AP) of complement has been elucidated in the past decade, with genetic defects and/or autoantibodies able to be identified in a proportion of patients. We review the

clinical and histological features of C3 glomerulopathy, relating these to underlying molecular mechanisms. The role of uncontrolled C3 activation in pathogenesis is emphasized, with important lessons from animal models. Methods, advantages and limitations of gene testing in the assessment of individuals or families with C3 glomerulopathy are discussed. While no therapy has yet been shown consistently effective, clinical evaluation of agents targeting specific components of the complement system is ongoing. However, limits to current knowledge regarding the natural history and the appropriate timing and duration of proposed therapies need to be addressed.
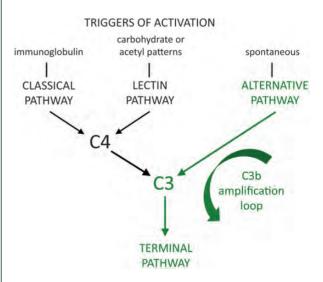
INTRODUCTION

The association between glomerulonephritis (GN) and low serum levels of complement proteins was first reported almost 100 years ago [1]. Sera from two children with a clinical diagnosis of nephritis complicating scarlet fever were found to have markedly reduced haemolytic activity. In the 1960s, an expansion in renal histological techniques and complement biology revolutionized the diagnostic approach to GN. The ability to detect complement C3 in serum [2] and early reports of low serum C3 in patients with lupus nephritis [3] and membranoproliferative GN (MPGN) [4, 5] coincided with the development of an immunofluorescence technique for identifying C3 deposits in renal sections [6]. The existence of a C3 nephritic 'factor' (C3NeF) was inferred from the accelerated C3 breakdown observed *in vitro* following the addition to normal human serum of serum obtained from a patient with 'persistent hypocomplementaemic glomerulonephritis'[7]. A rare glomerular lesion characterized by dense intramembranous deposits was recognized through the use of transmission electron microscopy (EM) [8]. In the 1970s, dense deposit disease (DDD) was taken up in the English-language medical literature [9], where the conjunction of predominant C3 glomerular deposition and low serum C3 levels was attributed to the activation of the alternative pathway (AP) of complement [10]. In the 1980s, several reports in affected families [11–14] indicated a genetic basis for some cases of DDD.

In the past decade, genetic defects in complement factor H (CFH) and C3 have been demonstrated leading to AP complement dysregulation in DDD and several closely related forms of GN, including the novel disease CFH-related protein 5 (CFHR5) nephropathy. These disorders share with DDD the key histological feature of C3 deposits in the glomerulus, with little or no immunoglobulin, the defining criterion for the new disease classification, 'C3 glomerulopathy' [15]. This review summarizes recent insights into the clinical and histological features of C3 glomerulopathy. Genetic and autoimmune mechanisms of disease are discussed, with animal models providing a 'proof of concept' for C3 activation in pathogenesis. Significant limitations exist in current knowledge regarding the natural history of C3 glomerulopathy, with implications for the clinical evaluation of complement-based therapies.

THE COMPLEMENT SYSTEM

The complement system comprises over 30 proteins either circulating in plasma and other body fluids or localized to cell membranes. It plays a physiological role in innate immunity and inflammation leading to the elimination of microbial pathogens (as well as apoptotic host cells and cellular debris) [16]. Complement activation occurs via proteolytic cleavage in three pathways: the classical, lectin and alternative pathways [17, 18] (Figure 1). Whereas the activation of the classical pathway usually requires immunoglobulin, AP activation occurs spontaneously at a low level in the circulation due to hydrolysis of the internal thioester bond of the C3 molecule (so-called 'C3 tickover'). C3 activation generates fragments C3a and C3b, the latter binding complement factor B (Cfb) to form the AP C3 convertase (C3bBb) that amplifies C3 activation in a positive feedback mechanism. The C3b amplification loop (also known as the amplification loop of the complement pathways [19]) is a powerful means through which millions of C3b molecules are generated following the initial activation of C3. The binding of additional C3b molecules to the AP C3 convertase generates a C5 convertase that activates C5, yielding fragments C5a and C5b. C5b initiates terminal pathway activation resulting in the formation of the membrane attack complex (MAC, C5b-9). Fragments C3a and C5a, generated through C3 and C5 proteolysis, respectively, are anaphylatoxins.

The AP is inhibited by several regulatory proteins present both in the circulation and on cell surfaces. CFH is encoded in the regulators of complement activation (RCA) cluster of chromosome 1q32 [20]. CFH competes with CFB for C3b binding and thereby impedes the formation of the AP C3 convertase. CFH also accelerates AP C3 convertase decay and is a cofactor for complement factor I (CFI)-mediated proteolysis of C3b. Membrane cofactor protein (MCP/CD46), encoded in the RCA cluster and expressed exclusively on cellular surfaces, is another complement regulatory protein with CFI cofactor activity. CFI is a serine protease encoded by the *CFI* gene on

FIGURE 1: Complement activation pathways and C3 amplification.

chromosome 4q25. It cleaves C3b in the presence of cofactors, generating iC3b and subsequently C3dg. Unlike C3b, iC3b cannot participate in the C3b amplification loop.

C3 GLOMERULOPATHY

Isolated C3 deposition within the glomerulus is the defining histological criterion for C3 glomerulopathy. This distinguishes C3 glomerulopathy from the more common, immune complex-mediated forms of GN such as post-infectious GN and MPGN Type I, where glomerular C3 together with immunoglobulin is typical. The glomerular morphology as demonstrated by light microscopy (LM) is heterogeneous. EM resolves the C3 deposits and enables definitive separation of DDD from the other subtypes of C3 glomerulopathy, where a spectrum of appearances may be seen (Figure 2). Acquired and genetic defects leading to AP complement dysregulation in patients with C3 glomerulopathy are outlined below. A renal biopsy diagnosis of C3 glomerulopathy should prompt investigation of complement abnormalities including protein levels, gene mutations and autoantibodies (Table 1).

HISTOLOGICAL AND CLINICAL FEATURES

Dense deposit disease

DDD takes its name from the transformation of the glomerular basement membrane (GBM) by extremely dark, ribbon-like electron-dense deposits located within the lamina densa (seen also within the mesangium, tubular basement membrane and Bowman's capsule) [21]. On LM, either a mesangioproliferative [22] or membranoproliferative [23] pattern is most common, while infiltrates of neutrophils and cellular crescents have also been reported in both native disease and post-transplant recurrence [24]. While no mechanistic explanation has been found for this variation, it is clear that the designation of DDD as a subtype of MPGN (Type 2) [10] is inaccurate. Laser microdissection of glomeruli from DDD kidneys has enabled mass spectrometric identification of complement C3, MAC components, CFHR5, vitronectin and apolipoprotein E [25]. The absence of CFB from glomerular tissue is consistent with AP C3 convertase formation leading to excessive C3 activation in the fluid phase, with subsequent deposition of C3 breakdown products.

DDD is usually diagnosed in children although adult cases do occur, and in one series, over one fifth of affected individuals were aged over 60 years [23]. Presenting features comprise any of the following: proteinuria (sometimes with the nephrotic syndrome), haematuria, hypertension and renal failure. Although low serum C3 (but not C4) is a common finding, and reflects uncontrolled C3 activation in the circulation, it is not specific for DDD and does not correlate with disease activity [26]. Individuals with DDD may have acquired partial lipodystrophy, in which subcutaneous fat is lost from the face and upper body, often predating renal clinical manifestations. A common basis in AP activation has long been recognized [27]. DDD is also associated with ocular drusen [28], a lipoproteinaceous deposition of complement-containing debris localized between the retinal pigment endothelium and Bruch's membrane. This pathology is similar to age-related macular degeneration [29]. Monoclonal gammopathy has been noted as a finding in older patients [30, 31], although the incidence may not exceed background rates in an older population. Increased risk of diabetes mellitus type 1 in families with DDD has also been reported [32].

Spontaneous clinical remission of DDD occurs only rarely [33], whereas progression to ESKD despite conventional treatment has been observed in 40–50% of patients with a diagnosis of ≥10 years [34, 35]. The outcomes of renal transplantation are generally favourable, despite histological recurrence being common (possibly universal) and contributing to the increased rates of allograft failure [36].

C3 glomerulonephritis

C3GN is a subtype of C3 glomerulopathy in which C3 deposits are found in the mesangium and capillary wall, where they may be subendothelial or subepithelial. Discontinuous intramembranous deposits are also sometimes seen on EM, but without the osmiophilic, ribbon-like appearance characteristic of DDD. As in DDD, subepithelial 'hump'-like deposits classically associated with post-infectious GN may be present. Mass spectrometry has revealed C3 and MAC components in laser dissected glomeruli, similar to DDD [37]. In the original series from France of 19 patients with C3GN [38], LM revealed MPGN in approximately two thirds of the patients. Clinical and laboratory features resembled those of DDD, with less predilection for childhood. Unlike DDD, no association with acquired partial lipodystrophy or ocular drusen exists for C3GN, although monoclonal gammopathy is sometimes found [39, 40]. Progression to ESKD is less common than in DDD, but does occur, with histological recurrence post-transplantation also reported [38, 41].

CFHR5 nephropathy

CFHR5 nephropathy is a form of C3GN that has been described with autosomal dominant inheritance among Cypriot families [42]. LM may show a mesangioproliferative or membranoproliferative pattern; on EM there are typically subendothelial and mesangial deposits with occasional subepithelial deposits. Microscopic haematuria and episodes of synpharyngitic macroscopic haematuria, clinically similar to IgA nephropathy, occur in up to half of the affected individuals [43]. Serum C3 levels are almost invariably normal, suggesting that excessive C3 activation occurs not in the circulation (as in DDD) but within the glomerulus [44]. Progression to ESKD is common in adulthood and occurs mostly in males (for reasons that are unknown). Ten patients with CFHR5 nephropathy are reported with successful transplantation [43], and one other with disease recurrence following unrelated donor transplantation [45].
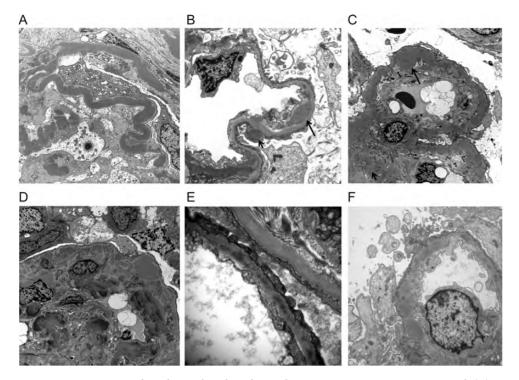
FIGURE 2: Spectrum of C3 glomerulopathy. Glomerular appearances on EM in patients with (**A**) DDD with typical osmiophilic, intramembranous, ribbon-like deposits; (**B**) CFHR5 nephropathy with subendothelial (long arrow) and 'hump'-like subepithelial (short arrow) deposits; (**C**) C3 glomerulopathy with intramembranous (long arrow) and mesangial (short arrow) deposits; (**D**) C3 glomerulopathy with complex, irregular intramembranous and mesangial deposits; (**E**) discontinuous intramembranous deposits in the same patient; and (**F**) C3 glomerulopathy with intramembranous deposits.

PATHOPHYSIOLOGY

Autoantibodies

C3NeF is an autoantibody that binds to a neoepitope on the AP C3 convertase (but not to its individual components). C3NeF stabilizes the convertase against CFH-mediated decay and potentiates its C3 cleaving action, resulting in uncontrolled C3 activation and low serum C3 levels [46]. C3NeF is common in DDD [47, 48], less so in C3GN, and absent in CFHR5 nephropathy. Its role in DDD pathogenesis remains controversial, given that fluctuating levels do not correlate with the course of nephritis. C3NeF is also non-specific for DDD, being found frequently in MPGN Type 1 [48] and rarely in lupus nephritis [49] or individuals without renal disease [50]. Recently, an autoantibody that binds to native Cfb and stabilizes the AP C3 convertase has been reported in a patient with DDD [51]. Two patients with DDD and autoantibodies targeting both CFB and C3b have also been described [52]. Inhibition of CFH by anti-CFH monoclonal light chains [53, 54] or (possibly monoclonal) immunoglobulin [31] has been reported in two patients with DDD, together with a case of C3GN involving CFH autoantibodies [37].

Genetic sequence variation

The genetic basis of a small number of C3 glomerulopathy cases has been demonstrated through family studies showing segregation of complement-related gene defects with the disease phenotype. Two infant Algerian brothers were reported with DDD, both of whom were seronegative for C3NeF but who had low serum CFH, with consequent excessive AP activation and low serum C3 [12, 55]. The genetic abnormality was subsequently identified as a homozygous missense mutation in the *CFH* gene [55]. Familial cases of C3 glomerulopathy (classified morphologically as MPGN Type 3) were also reported in association with resistance of the AP C3 convertase to inhibition by wild-type CFH [11, 14]. In a later DDD pedigree, heterozygous deletion of two codons within the *C3* gene on chromosome 19p13 was found to produce a hyperfunctional C3 molecule [56]. In C3GN, a report in infant sisters from a consanguineous Turkish family demonstrated homozygous deletion of a *CFH* codon resulting in circulating mutant CFH [57, 58] that was predicted to display defective binding to C3b [59]. A recent report of paternal isodisomy leading to homozygous deficiency of CFH in a patient with endocapillary proliferative C3GN is also noteworthy [60].

Genetic association based on studies undertaken in affected individuals and cohorts, but lacking family data, may be less robust. The French C3GN series reported six patients with heterozygous mutations in the *CFH*, *CFI* and *MCP* genes [38]. To these have now been added a report of C3GN (Case 3) and another of well-characterized MPGN Type 1 (Case 2) in patients with homozygous CFH deficiency [61], and further cases of C3GN, MPGN Type 1 and DDD involving heterozygous *CFH* and *CFI* mutations [35]. Two patients with DDD and C3GN involving heterozygous mutations in *CFH* and *MCP*, respectively, were included in a recent small trial of eculizumab [62] (discussed below), while two further cases are

Table 1. Investigations in C3 glomerulopathy

Investigations of the complement cascade
Measurement of complement serum proteins
Complement C3
Complement factor H (CFH)
Complement factor I (CFI)
Complement factor B (CFB)
Testing for the presence of C3NeF
Testing for the presence of autoantibodies
CFH autoantibodies
CFB autoantibodies
Quantifying MCP MCP/CD46 expression on peripheral blood mononuclear cells.
Screening for mutations
Direct exon sequencing of genes encoding complement regulatory proteins and C3 convertase components
CFH
CFI
MCP/CD46
CFHR1-5
CFB
C3
Assessment of copy number variation (CNV) across the *CFH-CFHR* locus
[Reproduced from Fakhouri *et al.* [15] with the permission of the authors.]

reported of C3 glomerulopathy (described as DDD, but without diagnostic EM) and heterozygous *CFH* mutations [63, 64]. Some of these heterozygous mutations have been shown to cause complement dysregulation in patients with atypical haemolytic uraemic syndrome (aHUS) [65] and thus seem likely to confer a common susceptibility to C3 glomerulopathy. However, a mechanistic explanation for C3 glomerulopathy (as opposed to aHUS) due to heterozygous mutations is currently lacking. This is in contrast to the evidence obtained from animal models [66] supporting homozygous CFH deficiency in the pathogenesis of C3 glomerulopathy (discussed below).

In addition to these (rare) mutations, common genetic variants including single nucleotide polymorphisms in the *CFH*, *C3* and *CFHR5* genes have also been recognized as modifying risk of DDD [67]. Complement haplotypes (or 'complotypes') combining polymorphisms in *CFH* [68, 69], *CFHR1* [68] and *MCP* [35] and conferring either increased risk or protection have also been delineated. Such polymorphic variations might be a factor in phenotypic differences between the histology of DDD and C3GN due to *CFH* mutations, for example.

Functional assays have been critical in revealing the mechanisms underlying these genetic associations for C3 glomerulopathy, and proving causality [70]. With the advent of next generation sequencing, a candidate gene approach to C3 glomerulopathy is liable to identify not only genetic variations (both mutations and polymorphisms) that contribute to disease, but also those that are of no functional significance [71]. Hence, functional and structural approaches will assume even greater importance in validating genetic associations in the future.

Genetic structure variation

Genes encoding the five CFHR proteins are positioned in close proximity to the *CFH* gene within the RCA gene cluster on chromosome 1q32, where a high degree of sequence homology predisposes to genomic duplications, deletions and the formation of hybrid genes. These structural changes are detected using copy number variation (CNV) techniques, as in CFHR5 nephropathy, where the heterozygous internal duplication of exons 2 and 3 of the *CFHR5* gene was identified by multiplex-ligation probe amplification (MLPA). The physiological role of CFHR proteins is at present unknown. Homozygous deletion of the *CFHR1* and *CFHR3* genes is a common polymorphism in healthy subjects [72] and is further associated with the presence of CFH autoantibodies in patients with aHUS [73, 74]. In a series of 68 DDD patients, however, none had combined homozygous *CFHR1/3* deletion despite a rate of 3% among control subjects [75]. A genome-wide association study [76] found that this polymorphism was also associated with a reduced susceptibility to IgA nephropathy across three cohorts. The latter is intriguing in light of the clinical similarities between CFHR5 nephropathy and IgA nephropathy.

ANIMAL MODELS—INSIGHTS AND LIMITATIONS

Animal models have provided a 'proof of concept' for excessive C3 activation in the pathogenesis of C3 glomerulopathy. They have also revealed novel disease mechanisms relating to AP complement dysregulation, providing a focus for research and development of targeted therapies. Two experimental models of genetic CFH deficiency, porcine [77–82] and murine [83–88], exhibit low serum C3 levels and renal disease analogous to human C3 glomerulopathy. Whereas the *CFH* mutation in Norwegian Yorkshire piglets occurred in nature, the mouse model was engineered in the laboratory through targeted homozygous *Cfh* gene deletion [83]. Subendothelial electron-dense deposits were preceded in both piglets and mice by accumulation of C3 along the GBM, a sequence that has not been reproduced in some human transplantation series [89]. Administration of murine [87] or purified human [88] CFH to the knockout Cfh−/− mice resulted in normalization of plasma C3 levels and resolution of GBM C3 deposition. Mice with combined homozygous deficiency of Cfh and Cfb (Cfh −/−.Cfb−/−) did not develop these changes, attributable to an

inability in the absence of Cfb to form the C3 convertase that amplifies C3 activation.

In mice with homozygous deficiency of Cfi (Cfi−/−), abnormal mesangial C3 deposits and mesangial expansion were noted but without C3 deposition along the GBM or development of MPGN [86]. This was the case even when Cfi knockout was accompanied by homozygous (or heterozygous) Cfh deficiency, and is accounted for by differences in the AP activation state. In the absence of Cfi, C3b resulting from C3 activation cannot be further cleaved to fragments iC3b and C3dg. As a result, in mice with homozygous Cfi deficiency (irrespective of the Cfh genotype) C3 circulates predominantly in the form of C3b. It appears, therefore, that Cfi-mediated cleavage of C3b is critically important for the development of DDD-like renal disease, implicating C3b metabolites (and specifically iC3b [87]) in pathogenesis. The administration to a Cfh −/−.Cfi−/− double knockout mouse of autologous Cfi led to cleavage of circulating C3b and the concomitant appearance of C3 staining along the GBM. In support of these experimental data, homozygous CFI deficiency has not been reported as a cause of C3 glomerulopathy in humans. Therapeutic strategies that target iC3b, inhibiting its deposition in renal glomeruli, might therefore be an effective means of preventing C3 glomerulopathy, regardless of the specific genetic or autoimmune abnormality. The attempt to recapitulate C3 glomerulopathy due to *CFHR* mutations through animal models has been limited by major differences between the human and rodent *CFHR* gene families.

TREATMENT

Basic measures in the treatment of C3 glomerulopathy include blood pressure control and antiproteinuric therapy especially with ACE inhibitors. While steroids and other immunosuppressants might seem logical based on renal histology showing inflammation, the results have been inconsistent [34]. Moreover, the increased risk of infection associated with these agents is of particular concern in patients with underlying abnormalities of innate immunity, in whom complement activation and inflammation triggered by infection could exacerbate nephritis. Long-term plasma infusion has been reported with success in the sisters with familial C3GN related to circulating mutant CFH [57]. Administration of CFH (if it becomes available) may be efficacious in the rare CFH deficiency states. However, it would not be predicted to influence genetic factors that result in Cfh-resistant C3 convertases [56].

Therapeutic inhibition of complement C3 or C5 holds promise, depending on which of these molecules, once activated, is the principal cause of renal damage (Figure 3). Eculizumab is a monoclonal antibody that prevents C5 activation, and is approved for use in patients with paroxysmal nocturnal haemoglobinuria and aHUS. In DDD, several cases are reported of successful treatment with eculizumab [90, 91], including one patient with post-transplant recurrence associated with progressive renal failure [92]. However, unsuccessful use of eculizumab is also reported [90], suggesting that prevention
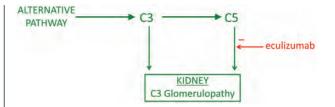

FIGURE 3: Therapeutic complement blockade in C3GN.

of C5 activation may not always be efficacious. This is supported by the results of a recent prospective, uncontrolled trial in six adult patients with DDD or C3GN [62]. At the conclusion of a one-year course of eculizumab, an improvement in clinical and/or histological parameters was observed in four patients, including all three receiving eculizumab (and additional immunosuppressive therapies) for recurrent disease post-transplantation. Two patients with GN in native kidneys (one each with DDD and C3GN) had a marked decline in renal function whilst receiving eculizumab. A putative role for eculizumab in disease flares is suggested by the observation in DDD patients with rapidly progressive GN that glomerular deposits contain C5 [93]. Of note, however, in the mouse model of C3 glomerulopathy, prevention of C5 activation attenuated but did not abrogate disease [84]. The investigators for the eculizumab trial concluded that 'there is a clear need for additional anticomplement therapies that offer the possibility of complement control at the level of the C3 convertase instead of C5' [62].

CONCLUSIONS

A renal biopsy finding of glomerular C3 deposits with little or no immunoglobulin suggests C3 glomerulopathy and should trigger investigation for complement dysregulation. An improved understanding of the natural history of disease would have clear implications for treatment, in terms of identifying those patients who stand to benefit, and the appropriate time points for intervention. While immunosuppressive therapy has not been shown consistently to ameliorate disease, agents targeting specific components of the complement system are undergoing clinical evaluation. Defining the contributions of C3 and C5, respectively, to pathogenesis is thus a key research aim. Recent insights into pathogenetic links between C3 glomerulopathy and much more common forms of GN including IgA nephropathy underline the expanding importance of complement dysregulation in the pathophysiology of GN.

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