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EDUCATIONAL REVIEW

A clinical approach to children with C3 glomerulopathy

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Abstract

C3 glomerulopathy is a relatively new clinical entity that represents a challenge both to diagnose and to treat. As new therapeutic agents that act as complement inhibitors become available, many with an oral formulation, a better understanding of this disease and of the underlying complement dysregulation driving it has become increasingly useful to optimize patient care. Moreover, recent advances in research have clarified the role of complement in other glomerular diseases in which its role was less established, namely in immune-complex membranoproliferative glomerulonephritis (IC-MPGN), ANCA-vasculitis, IgA nephropathy, and idiopathic membranous nephropathy. Complement inhibitors are being studied in adult and adolescent clinical trials for these indications. This review summarizes current knowledge and future perspectives on every aspect of the diagnosis and management of C3 glomerulopathy and elucidates current understanding of the role of complement in this condition and in other glomerular diseases in children. An overview of ongoing trials involving therapeutic agents targeting complement in glomerular diseases is also provided.

Keywords Complement · Glomerular disease · C3 glomerulopathy · Children

Introduction

The role of the complement cascade dysregulation in driving kidney diseases has been known for decades. A classic example of this is represented by atypical hemolytic uremic syndrome, a life-threatening thrombotic microangiopathy involving mainly the microvasculature of the kidney. In this disease, dysregulated activation of the alternative and terminal complement pathways leads to endothelial damage and microthrombi with platelet consumption, anemia, and acute kidney injury. The role of genetic mutations in genes coding for alternative pathway (AP) proteins and for regulators of this pathway has been extensively studied, as well as the occurrence of auto-antibodies which inhibit regulatory mechanisms that in physiological conditions keep the pathway in check.

More recently, a number of studies have elucidated how dysregulation of the AP of complement may also drive the pathogenesis of glomerular diseases that were previously considered idiopathic. More specifically, a large group of proliferative glomerulonephritides, mostly but not exclusively with a membranoproliferative histological pattern, have been found to be driven by AP dysregulation which determines C3 deposition within glomeruli in different patterns. This group of glomerular diseases, defined as C3 glomerulopathies, is increasingly recognized and its correct identification is becoming of growing importance as new treatments targeting the alternative, lectin and terminal pathways of complement become available.

Furthermore, a role of complement activation as driver of glomerular damage has been described in a variety of other diseases, such as lupus nephritis [1], anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis [2], idiopathic membranous nephropathy [3], IgA nephropathy [4], and more recently acute post-infectious glomerulonephritis [5]. In many of these conditions, the efficacy of complement inhibition is being assessed in clinical trials [6].

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This review will summarize the current knowledge of primarily C3 glomerulopathy in its different forms, providing an overview of optimal diagnostic strategies and a perspective on future therapeutic approaches.

Basic overview of complement in kidney diseases

The complement cascade is an important component of the innate immune system. Its primary function is generally regarded as protection of the host from infections, but the complement system also helps the body to eliminate injured cells and extracellular debris. The complement cascade is comprised of soluble and cell-surface proteins, and the structure of this system shares several features with the coagulation cascade. Both systems contain zymogens, inactive precursor proteins that acquire serine protease activity when they, themselves, are cleaved. Activation of the complement system causes the formation of the C3 and C5 "convertases," the active enzymes that cleave C3 and C5, respectively. The convertases are critical for generating the downstream effector molecules that mediate biologic activity.

The proteins involved in complement activation circulate in plasma, and when the system is activated several components are cleaved into smaller fragments. Some of the activation fragments remain soluble, whereas other fragments are immobilized on cell and tissue surfaces. Two of the protein fragments, C3b and C4b, can be covalently fixed to cell membranes. C3b and C4b are essential components of the convertases, and C3 fragments are also ligands for complement receptors. Consequently, fixation of these proteins to cell surfaces ("opsonization") focuses complement activation and its inflammatory effects at those sites. Full activation of the complement system also generates the membrane attack complex (MAC) consisting of the complement proteins C5b, C6, C7, C8, and C9. The MAC is a pore through cell membranes that allows the passage of water and ions and can cause cell lysis. Thus, complement activation generates soluble effector molecules that can act throughout the body as well as tissue-bound fragments that direct complement activity to specific targets.

Activation Complement activation is initiated through three different pathways, each of which leads to cleavage of C3. These pathways are triggered by specific "recognition" events and by certain biochemical reactions. The classical pathway is commonly activated by immunoglobulin. When multiple IgG molecules are aggregated on a target, their Fc regions bind to C1q, which then initiates classical pathway activation. The interaction between individual IgG molecules and C1q is weak, so activation requires multiple IgG molecules to be clustered in close proximity. The Fc region of IgM also binds to C1q. Since IgM is pentameric or hexameric, a single IgM molecule can bind to a C1q molecule through multiple Fc regions and activate the classical pathway. Other molecules can also engage C1q and initiate the classical pathway, including C-reactive protein (CRP) and lipopolysaccharide, and double-stranded DNA [7]. The classical pathway is activated in a number of different glomerular diseases, including those in which antibodies bind to glomerular antigens and those where immune-complexes deposit in the glomerular capillaries.

The lectin pathway is similar to the classical pathway insofar as it is activated after recognition proteins bind to specific targets. Lectin pathway activation can be triggered by multiple different proteins, including mannose binding lectin (MBL), ficolins-H, L, and M, and collectins-10 and 11 [8]. Similar to the classical pathway, the lectin pathway also leads to cleavage of C2 and C4 and deposition of C4b on target surfaces. It does not, however, involve C1q. The lectin pathway is suspected to be involved in several kidney diseases. For example, glomerular MBL and L-ficolin deposits are seen in biopsies from a subset of patients with IgA nephropathy [9, 10]. There is also some data that MBL binds to pathogenic IgG4 in membranous nephropathy, activating the lectin pathway [11] and data that show that inhibiting the MBL pathway can prevent complement activation and kidney injury in a mouse model of Shiga toxin-producing *Escherichia coli*-induced HUS [12].

The AP does not require the recognition of target antigens or molecular patterns (Fig. 1). Rather, C3b that is generated by the other pathways can combine with factor B in the circulation to create the AP convertase (C3bBb), which then generates additional C3b. In this way, the AP amplifies the other two pathways. The AP is also activated by the spontaneous hydrolysis of C3. This process, called "tickover," continually generates C3b which can deposit on nearby surfaces. This allows rapid initiation of the AP on pathogens, but requires continuous regulation to prevent inflammatory injury. There is abundant evidence that the AP is involved in multiple different causes of kidney disease. Uncontrolled alternative pathway activation appears to be the primary cause of C3G [13] and atypical HUS [14]. There is also experimental and clinical evidence that the alternative pathway is activated in membranous nephropathy [15], IgA nephropathy [4], ANCA-associated vasculitis [16, 17], and ischemia/reperfusion injury [18].

Biologic effects The primary effector molecules of the complement cascade are C3a, C3b, C5a, and the MAC. C3a and C5a are small, soluble peptide fragments generated by cleavage of C3 and C5, respectively. C3a- and C5a-receptors (C3aR and C5aR) are expressed on leukocytes and kidney parenchyma cells. They contribute to chemokine production by kidney cells, direct chemoattraction of leukocytes, and

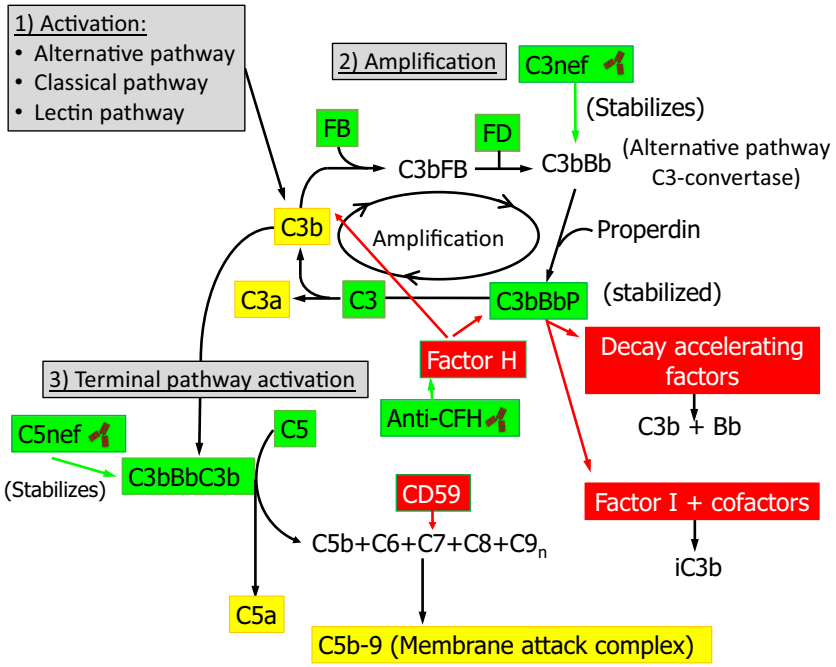

Fig. 1 Alternative pathway amplification. The complement activation pathways and spontaneous hydrolysis of plasma C3 all generate C3b. C3b can combine with factor B (FB), and cleavage of FB by factor D (FD) generates the alternative pathway C3-convertase. The convertase is stabilized by properdin and cleaves additional C3 into C3b. This forms a positive feedback loop. Complement regulatory proteins (indicated with red boxes) control alternative pathway activation by shortening the half-life of the convertase ("decay acceleration") or by cleaving and inactivating the C3b component of the convertase. C3Nef is an autoantibody that binds to the convertase and stabilizes it. Drugs have been developed that block alternative pathway activation by targeting FB, FD, and C3. Complement components or autoantibodies that promote activation are shown in green boxes. The pro-inflammatory fragments generated during activation are indicated in yellow boxes

trigger leukocyte activation. In addition, signaling through these receptors can co-stimulate T cell activation, and blockade of the receptors favors polarization to inducible T regulatory cells [19]. It is too simplistic to label C3a and C5a as simply "pro-inflammatory," as they can have both pro- and anti-inflammatory functions. Nevertheless, they are pathogenic in some kidney diseases and C5a and C5aR antagonists have been developed for clinical use. One of these drugs, Avacopan, has shown promise as an adjunct therapy in ANCA-associated vasculitis [20, 21].

Kidney biopsies are routinely stained for C3 fragment deposits. After C3 cleavage, C3b is fixed to nearby tissues where it is a component of the C3 convertase. C3b is inactivated through proteolytic cleavage, generating smaller fragments (iC3b and C3dg). Although these fragments are no longer catalytically active, they are ligands for complement receptors that are expressed on leukocytes.

Full activation of the complement cascade generates MAC, sometimes referred to as the "terminal complement complex." The MAC is assembled from five separate component proteins (C5b, C6, C7, C8, and C9) that form a pore on the plasma membrane of target cells and can cause cell lysis. Most nucleated cells have mechanisms to remove MAC from the plasma membrane, but even sublytic quantities of MAC deposition can cause cell activation. The role of MAC has been studied in animal models of membranous disease [22], and in vitro studies have shown that MAC deposition on podocytes causes cell activation and injury [23].

Regulation Like the coagulation cascade, the complement cascade is controlled by several different soluble and cell surface regulatory proteins. The regulators are critical for preventing complement-mediated injury to the host. Regulatory proteins expressed on the cell surface inactivate the C3 and C5 convertases. Decay accelerating factor (DAF, or CD55) accelerates the dissociation of the components of the convertases. Membrane cofactor protein (MCP, or CD46) serves as a cofactor for factor I, a protein that can cleave and inactivate C3b, thereby inactivating the convertases. Impaired complement regulation, such as due to inactivating mutations in the complement regulatory proteins, is an important risk factor for several kidney diseases.

Factor H is a soluble complement regulatory protein that has both cofactor activity as well as decay accelerating function. Factor H can perform these functions in plasma, and it can also bind to cell membranes and extracellular matrix, controlling complement activation on those surfaces. Autoantibodies to factor H can also impair complement regulation by this protein, either by directly interfering with its regulatory function or by blocking its ability to bind to tissues. Even though the cell surface regulatory proteins are expressed within the glomerulus, deficiency of factor H is sufficient to

cause complement activation in the kidney [24]. There is also a family of "factor H related proteins" (FHRs) that arose through reduplication of the factor H gene. These five proteins have structural similarities to factor H, but they do not contain the complement regulatory region. Although the function of the FHRs is incompletely understood, they are believed to antagonize binding of factor H to target surfaces. Several mutations in the *CFHR* genes and copy number variants been associated with increased risk of C3G [25–31]. Work has shown that the FHR mutations may increase their ability to antagonize factor H, thereby effectively making affected individuals functionally deficient in factor H.

Soluble properdin (P), mainly produced by leucocytes, is known as the only positive regulator of the AP. Under physiological conditions, the AP convertases are unstable complexes with a short half-life of around 90 s. In association with P, the stability of the AP convertase (C3bBbP) increases up to tenfold.

Although numerous different molecular defects in the complement cascade have been linked with the risk of C3G (discussed below), most of these defects have similar functional effects. Namely, they impair regulation of the alternative pathway.

C3 glomerulopathy: overview and current dilemmas

A rare form of membranoproliferative glomerulonephritis characterized by intramembranous C3 sausage-like deposits and called "dense-deposit disease" (DDD) was linked to AP fluid phase dysregulation [32, 33] by the detection of C3Nef in the serum of two patients with hypocomplementemic membranoproliferative glomerulonephritis in the mid-70s. Subsequent studies identified, in patients with this rare glomerular disease, genetic mutations in regulatory AP proteins and/or the presence of pathogenic auto-antibodies called nephritic factors (mainly C3Nefs) (reviewed in [34]). By light microscopy, in the subsequent 30 years, DDD was found to be more morphologically heterogeneous, with MPGN in 25% of cases in a study published in 2007, other forms of proliferative GN in the remaining 75% [35].The first seminal paper describing a larger group of proliferative glomerulopathies linked to AP dysregulation was published in 2007. Servais et al. identified 19 patients with proliferative glomerulonephritis who had intense C3 glomerular deposition which however did not follow the intramembranous DDD pattern [36]. In these C3 glomerulonephritis (C3GN) patients, the C3 deposits were mainly mesangial and less frequently subendothelial or epimembranous. In all of them, evidence of AP dysregulation, driven by the presence of genetic mutations and/or of C3Nefs, was found. Further studies characterizing by proteomic analysis the glomerular deposits isolated by laser microdissection from kidney biopsies of patients with DDD and with C3GN clearly showed that profiles of the proteomic composition of the deposits were comparable in these two groups of patients [37]. This supported the hypothesis that DDD and C3GN represent two subtypes of a unique proliferative glomerulonephritis which was termed "C3 glomerulopathy" (C3G) [38].

Animal models have confirmed that impaired control of AP activation is sufficient to cause complement activation in the glomerular capillaries [23]. Although it is not fully understood why the kidney is the particular target of activation in this setting, the mechanism of activation seems to be distinct from that in IC-mediated glomerulonephritis. In practical terms, the detection of glomerular C3 in the relative absence of immunoglobulin identifies patients in whom activation primarily involves the alternative pathway, as opposed to IC-mediated disease in which activation is initiated through the classical pathway. The alternative pathway is secondarily activated by the classical pathway, however. Furthermore, transient deposition of ICs within the glomerulus post-infection may trigger C3G in susceptible patients.

Therefore, there is overlap in the etiology and manifestations of C3G and IC-glomerulonephritis (discussed below).

In the years since C3G was first described, we have been able to characterize the clinical, genetic, and serological features of this rare disease more fully. However, many questions remain open:

1) Is the diagnosis of C3G exclusively histological, and if so, what are the precise criteria leading to its identification?
2) Within C3G, is it possible to identify subgroups of patients with distinct clinical features deriving from specific alterations of complement AP regulation?
3) Should all C3G patients be treated in the same way, or can we tailor our therapeutic approach based on the underlying mechanism driving disease in each patient?

In order to address these key issues, patients with this rare glomerulopathy require an extensive work-up of the genetic, serologic, and histologic features and need to be collected in international, independent registries to allow progress in research and therapy.

C3G: clinical presentation

C3 glomerulopathy is a highly heterogeneous disease with varied clinical manifestations and severity. Moreover, some clinical features differ between adults and children. This review will focus on the spectrum of pediatric C3G. At onset, children who are diagnosed with C3G may present with one of the following clinical pictures:

- Intra- or post-infectious macrohematuria with some degree of proteinuria and, occasionally, elevated serum creatinine and hypertension. Circulating C3 is reduced, with mostly normal C4. Urinary sediment shows erythrocytes and casts as in active GN. This picture is identical to acute post-infectious glomerulonephritis. However, circulating C3 remains low (in contrast to post-infectious glomerulonephritis) for more than 8–12 weeks and over time, the disease does not resolve, but rather has a chronic remitting and relapsing course.
- Nephrotic syndrome, often with an active urinary sediment with microhematuria and casts. Circulating C3 is most frequently reduced, whereas C4 and autoantibodies (ANA, anti-dsDNA) are normal. Elevation of serum creatinine and/or hypertension are frequently observed.
- Mild proteinuria and microhematuria in the absence of any clinical sign of disease and with normal kidney function, again with low circulating C3 which can be the only alteration of blood exams.
- Exceptionally, atypical hemolytic uremic syndrome (aHUS) can present in addition to its classic manifestations with nephrotic-range proteinuria with microhematuria and low circulating C3. Histologically, the biopsies can show features of thrombotic microangiopathy coexistent with proliferative glomerulonephritis. This overlap may occur simultaneously or over time in the same patient or in different individuals with the same genetic alteration of the AP of complement.

Following onset, the disease is chronic and tends to have a remitting/relapsing course, with a high degree of variability between individual subjects in terms of severity. In the absence of treatment, proteinuria tends to oscillate. Spontaneous remission is possible [39], and flares are frequently associated with infectious episodes, particularly in children. Circulating C3 tends to remain low, and its normalization is a sign of persistent remission. Prognosis is dependent on factors such as severity of proteinuria at onset, with nephrotic syndrome being a negative prognostic factor, age at onset, with younger age being a positive prognostic factor, and the degree of fibrosis on kidney biopsy. In a recent study on 66 Turkish children with C3G, remission was achieved in 68.3% of cases. At last follow-up (median time 48.3 ± 36.3 months), 10 patients (16.6%) developed stage 5 chronic kidney disease (CKD 5): lower serum albumin and eGFR at diagnosis and low C3 at last follow-up were independent predictors for CKD 5 development [40]. Another recent study conducted in India on 92 children, including 16 with IC-MPGN, 5-year kidney survival was 62.6, 85.5, and 88.5% in patients with DDD, C3GN, and IC-MPGN, respectively. Serum albumin at onset < 2.5 g/dL and persistently low serum C3 were associated with lack of remission [41]. Recently, cluster analysis in a C3G-IC-GN cohort of 173 patients, taking into account clinical, biochemical, and pathology parameters, complement genetic abnormalities as well as the presence of C3 nephritic factor (C3Nef), showed that there is a group of C3G-IC-GN patients who have a worse prognostic outcome. They demonstrated that this group has a unique complement phenotype: low prevalence of genetic complement abnormalities and/or C3Nef and often normal serum C3 and sC5b-9. The presence of genetic abnormalities and/or of circulating C3Nefs was shown to be a relatively more benign prognostic factor [42]. Though historically DDD was considered a particularly severe glomerular disorder, more recent retrospective studies show similar outcomes when comparing DDD and C3GN. In most published retrospective cohorts, around 30% of patients reach CKD within 10 years from disease onset. It is important however to note that these published data refer mostly to historic cohorts which did not benefit from potentially useful more recent therapeutic approaches which will be discussed below.

The disease is driven by circulating factors, and this leads to the possibility of extra-renal symptoms, such as retinal C3 deposits called drusen, which rarely affect vision leading to blind spots, and partial lipodystrophy. Retinal drusen should be assessed at diagnosis and periodically thereafter. The systemic nature of the mechanism driving development of C3 glomerulopathy has another relevant implication: disease can recur following kidney transplantation, with a frequency of > 50% at 5 years post-transplant [43].

C3G diagnostic work-up: kidney biopsy

Diagnosis of C3 glomerulopathy is established by kidney biopsy according to consensus criteria that have been defined by an international task force of experts and published in 2013 [38]. The light microscopy picture is of proliferative glomerulonephritis (Fig. 2, panel a), and can show mesangial hypercellularity (left), endocapillary proliferation, with at times exudative features and less frequently extracapillary proliferation (middle) and membranoproliferative glomerulonephritis (right). Immunofluorescence or immunohistochemistry must show intense C3 positivity in glomeruli. The initial consensus paper [38] stated that C3 intensity with a 2-fold predominance compared to all other stainings (i.e., IgG, IgA, IgM, C1q etc.) is necessary for diagnosis of C3G. However, subsequent work based largely but not exclusively on pediatric patients has shown that the "2-fold predominance" criterion does not always hold true. Patients presenting with idiopathic forms of proliferative glomerulonephritis with a "rich" immunofluorescence (i.e., IgG, IgM, IgA, C1q mild to moderate positivity) in addition to intense C3 positivity are defined as having immune-complex membranoproliferative glomerulonephritis, IC-MPGN.

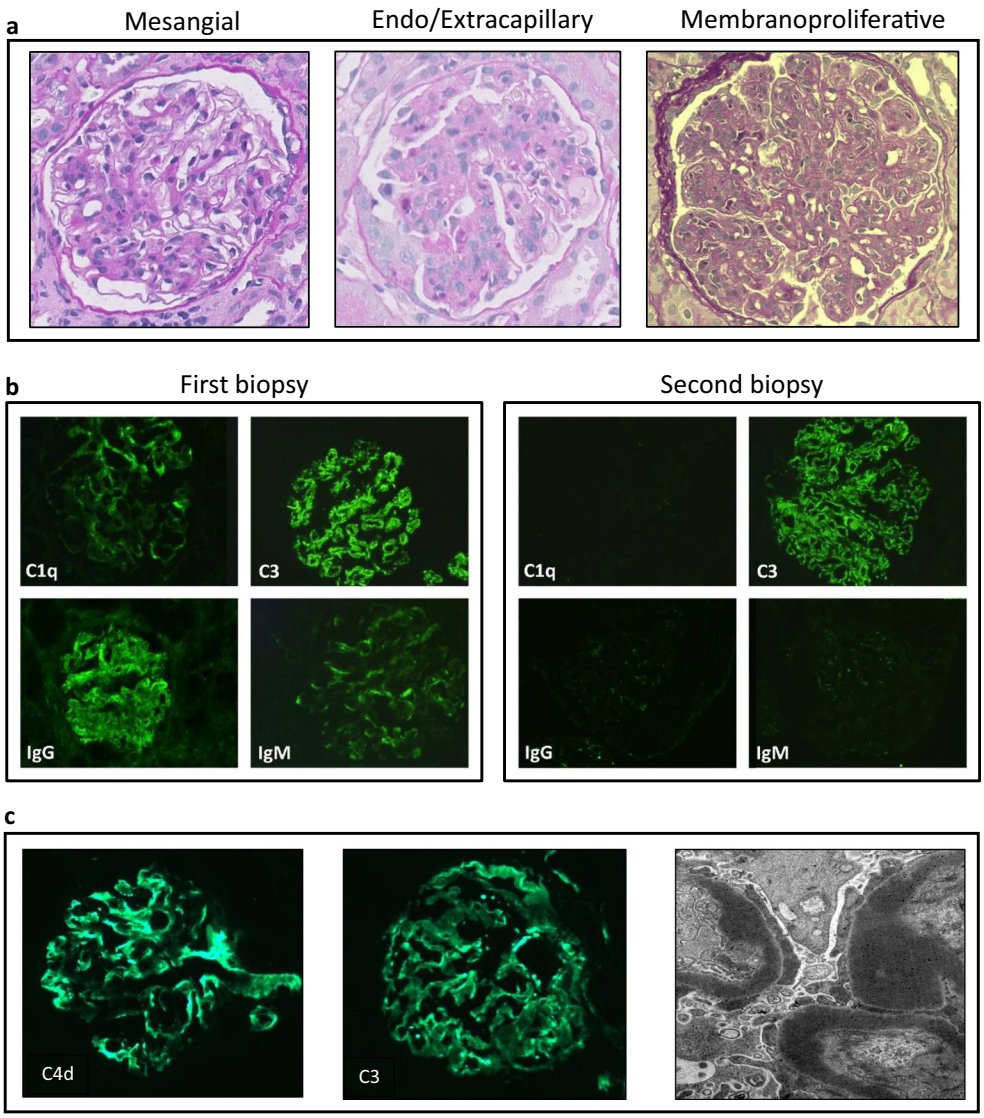

Fig. 2 Kidney biopsy immunofluorescence (IF). Peculiar aspects of glomerulopathies with complement alternative pathway dysregulation. **a** Examples of light microscopy images for IC-MPGN and C3G. Left side: mesangial hypercellularity. Center: endocapillary and extracapillary proliferation. Right side: membranoproliferative glomerulonephritis. **b** Sequential kidney biopsies in a patient with proliferative GN secondary to C3Nef, and to a MCP mutation with low circulating C3 and elevated circulating sC5b9. «First» biopsy is at disease onset, «Second» biopsy was performed 7 months later for persistent proteinuria and low C3, following treatment with oral prednisone and mycophenolate mofetil. **c** C4d IF (left side), C3 IF (center) in a patient with dense deposit disease (electron microscopy shown on the right side)

IC-MPGN patients were recently found to harbor genetic mutations in genes coding for complement proteins or the presence of circulating complement regulatory proteins and/or C3Nefs in comparable percentages to patients with classic C3 glomerulopathy [42, 44]. Consistent with these recent findings, historical papers describing cohorts of patients with features of complement AP dysregulation also show histological pictures that do not fulfill the consensus C3 predominance criteria, with either genetic or acquired AP dysregulation in 53% of patients presenting a "rich" immunofluorescence [45]. Therefore, a subgroup of children with AP dysregulation and proliferative GN who do not fulfill the C3 "2-fold predominance" criterion exists. Some may present with a kidney biopsy characterized by a proliferative GN with a "rich" (positive IgG, IgM, C1q, and C3) immunofluorescence at onset, with a subsequent kidney biopsy following immunosuppression showing C3 predominance (Fig. 2, panel b). This suggests that an infectious trigger at onset may activate the classical and lectin pathway as well as the AP, and that the C3-predominant picture may become more evident over time, as a consequence of classical pathway inactivation due to the infection resolving and/or to immunosuppression deactivating the classical pathway. Recently, glomerular C4d immunofluorescence has been suggested as a potential tool to identify the involvement of the classical and lectin complement pathways in proliferative GNs and to differentiate between immune-mediated forms, such as lupus nephritis, membranous nephropathy, or so-called IC-MPGN and C3G [46]. This finding, which implied that a negative glomerular C4d staining was a marker for C3G, has not been confirmed, particularly in the pediatric population [47–49], suggesting that indeed, particularly initially, an activation of all complement pathways may occur following a trigger, mostly of infectious origin. Also, in our unpublished experience, positive glomerular C4d can be found in the majority (17/30) of pediatric C3G biopsies (Fig. 2, panel c). The detection of glomerular C4d

deposits in some patients with C3G may reflect involvement of the classical or lectin pathways at some point in the evolution of the disease.

Distinction between C3 glomerulopathy's two subcategories, DDD or C3GN, requires electron microscopy. In DDD, the C3 deposits are particularly dense, discrete sausage-like ribbons which are located within the glomerular basement membrane (GBM) walls (intramembranous), leading to its thickening. In C3GN, the deposits are less dense and less discrete and more heterogeneously located within the mesangium on the sub-endothelial and on the sub-epithelial side of the GBM.

Also by electron microscopy, rounded sub-epithelial deposits called "humps" can be found in different forms of C3G, as in acute post-infectious glomerulonephritis, where typically they are most abundant.

Therefore, diagnosing these forms of glomerular disorders requires a kidney biopsy. However, a genetic and serological work-up of the AP of complement function allows pathogenetic elucidation, which may have therapeutic implications.

C3G diagnostic work-up: complement serological tests

Although the diagnosis of C3G is histological, serological complement assays are complementary to histology in confirming diagnosis and are helpful to further define the level of complement involvement at presentation and during the disease course [38]. Moreover, serological markers may be useful as a toolbox to monitor response to therapy as new complement-modulating agents emerge. Complement serological tests employed in C3G as well as their role in the dysregulation of AP are shown in Table 1 [14, 38]. In all suspected C3G patients, serum C3 and C4 must be measured and screening for nephritic factors and autoantibodies against CFH should be performed. A low C3 serum level with normal C4, indicating AP activation, is found in the majority of C3G patients in the acute phase and often persists during the disease. However, normal C3 levels and/or reduced C4 levels do not rule out this diagnosis. In one study including 115 patients, at diagnosis, low C3 levels were noted in 59% of DDD patients and 39.6% of C3GN patients. One patient with DDD and none with C3GN showed low C4 levels [40]. Another series of 80 patients reported low C3 levels in 79% of DDD and 48% of C3GN patients, with suppressed C4 levels in 15% of DDD and 36% of C3GN patients [50]. Elevated serum levels of complement AP breakdown products C3d and Bb, and elevated serum levels of C3bBbP (AP convertase – C3bBb with properdin-P), as well as increased levels of soluble terminal pathway complex, sC5b-9, are indicative of an increase of complement turnover in the AP. These may be more sensitive markers of complement activation than total C3 and C5 levels. For example, a normal serum C3 level in a patient with C3G in combination with an elevated C3d, C3bBbP, and/or sC5b-9 indicates that there still is an activated AP.

A more marked increase in sC5b-9 has been reported in C3GN compared to DDD by Zhang et al. [51]. They also have shown that levels of serum properdin, the positive regulator of the AP convertase, were significantly lower in C3G patients compared to controls. More specifically, C3GN patients had lower properdin levels than DDD, suggesting that the generation of C5 convertase is greater in C3GN than in DDD. Conversely, C3 convertase activation may be more intense in DDD than in C3GN (Table 1).

Auto-antibodies against the AP convertase, C3 nephritic factors and C5 nephritic factors, have been detected in the majority of C3G patients at presentation [32, 52]. These auto-antibodies are able to stabilize the AP convertase and thereby prolong its half-life, leading to an overactivation of the AP. Depending on the epitope that they recognize, two categories of auto-antibodies targeting AP convertases and a rare auto-antibody targeting the classical and lectin pathway (C4 nephritic factor) have been identified in patients with C3G and IC-MPGN:

1) C3 nephritic factor (C3Nef), which recognizes the C3 convertase of the AP (C3bBb),
2) C5 nephritic factors (C5Nef), which recognize the C5 convertase of the AP (C3bBbC3bP), and rarely
3) C4 nephritic factor (C4Nef), which recognizes the C3 convertases of the classical and lectin pathway (C4bC2a) [53].

Although C3Nefs and C5Nefs can be found in DDD as well as in C3GN patients, C3Nefs are more often detected in DDD patients and C5Nefs more frequently seen in C3GN patients [32, 51, 54]. Studies describing the presence and relevance of C4Nefs in C3G and IC-GN are still limited. However, in a recent study performed on 119 patients with IC-MPGN and C3G, 14.3% were found to have positive C4Nef antibody titers, which showed a clear relationship with intense complement activation and consumption (elevated circulating sC5b9, low circulating C3), especially in cases with concomitant C3NeF positivity [53].

In some C3G patients, auto-antibodies against CFH are found, often in the presence of C3Nef. In contrast to antibodies against CFH identified in aHUS patients, there is no association with the homozygous deletions of the *CFHR1* and *CFHR3* genes and the presence of these CFH antibodies in C3G. The binding sites of antibodies against CFH in C3G patients are mostly directed against the N-terminal domain of CFH protein, in contrast to antibodies against CFH seen in aHUS patients, which are directed to the C terminal domains, which interfere with the binding of CFH to the cell surface [55, 56].

Table 1 Serological evaluation of abnormalities of the complement system in C3G

Assays:	
Complement components and regulators	**C3, C4**, C5, CFI, **CFH**, CFB, properdin
Complement breakdown /split products	C3d, C3c, Bb, **sC5b-9**, C3bBbP, C5a
Functional assays	**CH50, AP50**, CFH function, Wieslab assay
Auto-antibodies	**C3Nef**, **C4Nef**, **C5Nef**, **anti-CFH**, anti-CFB, anti-C3b
Patterns of dysregulation in AP complement:	
Activation of AP	Reduced C3, normal C4, reduced factor B, increased C3d, increase Bb, increased C3bBbP
Increased C3 turnover (perhaps more likely in DDD compared to C3GN)	Low C3, increased C3d, increased C3bBbP
Increased C5 turnover (perhaps more likely in C3GN compared to DDD)	Low C5, increased sC5b-9, increased C5a, low properdin

In bold: serological complement investigations recommended in all IC-MPGN and C3G patients

References for this table: [14, 38, 51, 81]

The patterns of dysregulation may vary in patients and over the disease course. Besides C3, C4, AP50, and CH50, most of the above-mentioned serological measurements are mostly only performed in specialized laboratories and need proper handling and processing of the blood taken from the patients [56]. A list of specialized laboratories and complement experts can be found via http://www.ecomplement.org/list-of-diagnostic-labs.html

C3G, C3 glomerulopathy; *CFI*, complement factor I; *CFH*, complement factor H; *CFB*, complement factor B; *sC5b-9*, soluble complement C5b-9 complex; *C3bBbP*, soluble properdin (P) forming a complex with AP convertase (C3bBb); *C3Nef*, C3 nephritic factor; *C4Nef*, C4 nephritic factor; *C5Nef*, C5 nephritic factor

Auto-antibodies against FB and C3b are very rarely detected in C3G [57]. However, pathogenic transient antibodies against FB have recently been detected in a significantly higher proportion in the acute phase of post-infectious glomerulonephritis (31/34; 91%) versus C3G (4/28; 14%) and might help to differentiate between these two glomerulonephritides with low C3 level at onset [5].

Besides C3 and C4, serological measurements of the complement breakdown products (C3d, Bb) complement activations markers (sC5b-9, C3bBbP) and of the acquired auto-antibodies mentioned above are mostly only performed in specialized laboratories and need proper handling and processing of the blood taken from the patients [58]. A list of specialized laboratories and complement experts can be found via http://www.ecomplement.org/list-of-diagnostic-labs.html

Table 1 lists the different assays currently available, and how they can assist in pinpointing at what level the AP dysregulation is occurring in the specific patient analyzed. As we move into an era where more varied complement inhibiting therapeutic agents become available, each one targeting different parts of the cascade, this knowledge can be essential in allowing tailored treatment of increased efficacy and reduced toxicity.

C3G diagnostic work-up: genetics

C3G is characterized by over-activity of the alternative pathway. Many underlying gene mutations have been identified in patients with C3G, although genetic causes are found in only ~ 45% of patients [26]. Causative mutations can decrease factor H function [59]. Moreover, mutations in the genes for C3 and factor B (the components of the alternative pathway C3 convertase) that make this pathway resistant to regulation can occur [60, 61]. Increased copy numbers of the *CFHR* genes and *CFHR* genomic rearrangements have also been identified. Sequencing cannot always detect these genetic variants because of the homology among the *CFHR* genes. Consequently, many clinical laboratories use multiplex ligation-dependent probe amplification (MLPA) to detect rearrangements in this region of the chromosome.

As the diagnostic criteria for C3G are based on the biopsy findings, identification of underlying gene mutations is not essential for diagnosing or managing the disease [14]. However, identification of complement gene mutations may identify patients who are unlikely to respond to treatment with mycophenolate mofetil [62]. Screening for genetic mutations may also be important in patients and related family members when living related transplantation is being considered [14]. Moreover, it supports a diagnosis in forms that do not reach the consensus C3 predominance criteria, and provides a better understanding of genotype–phenotype correlations, paving the way for patient stratification into pathogenetically distinct subgroups. If genomic analysis is undertaken, the genes *CFH*, *CFB*, *C3*, and *CFI* should be included. As several different rearrangements have been identified in the *CFHR* genes, this region should also be analyzed. Interestingly, recent evidence shows that a substantial percentage of patients with C3G/IC-MPGN examined with whole genome sequencing had no genetic mutations in genes regulating the AP of complement, but a common variant locus overlapping the HLA locus, suggesting that many of these patients have an immune-mediated AP dysregulation which may benefit from immunosuppression [63].

C3G therapy: non-targeted

The optimal therapeutic approach to C3 glomerulopathy is based on experience and on relatively small, retrospective cohort studies. An international consensus meeting proposed a sequential treatment scheme [14]. All patients should optimize blood pressure control and receive conservative therapy with low-salt diet and ACE-inhibitors or angiotensin-receptor blockers. In addition to this, moderate disease (proteinuria > 500 mg/24 h, moderate inflammation on kidney biopsy, normal kidney function) may benefit from oral prednisone at high doses for 4 weeks, then tapered and stopped within 6–12 months. Mycophenolate mofetil at standard doses can be added in cases with persistent significant proteinuria, and it has proved beneficial in many cases [62]. Specifically, a recent study evaluating 87 patients with C3G and IC-MPGN showed that MMF added to oral prednisone was preferable both in terms of achieving remission and of preventing CKD compared to patients receiving other immunosuppressants, eculizumab, or conservative treatment alone [64]. In patients with severe disease (proteinuria > 2000 mg/24 h), particularly if severe inflammation with marked endo- and extracapillary proliferation are seen on kidney biopsy and if kidney function is impaired, i.v. methylprednisolone + anti-cellular immunosuppressants (cyclophosphamide) may be considered. When this treatment approach is not effective, complement-targeted therapy must be considered if possible. (Fig. 3).

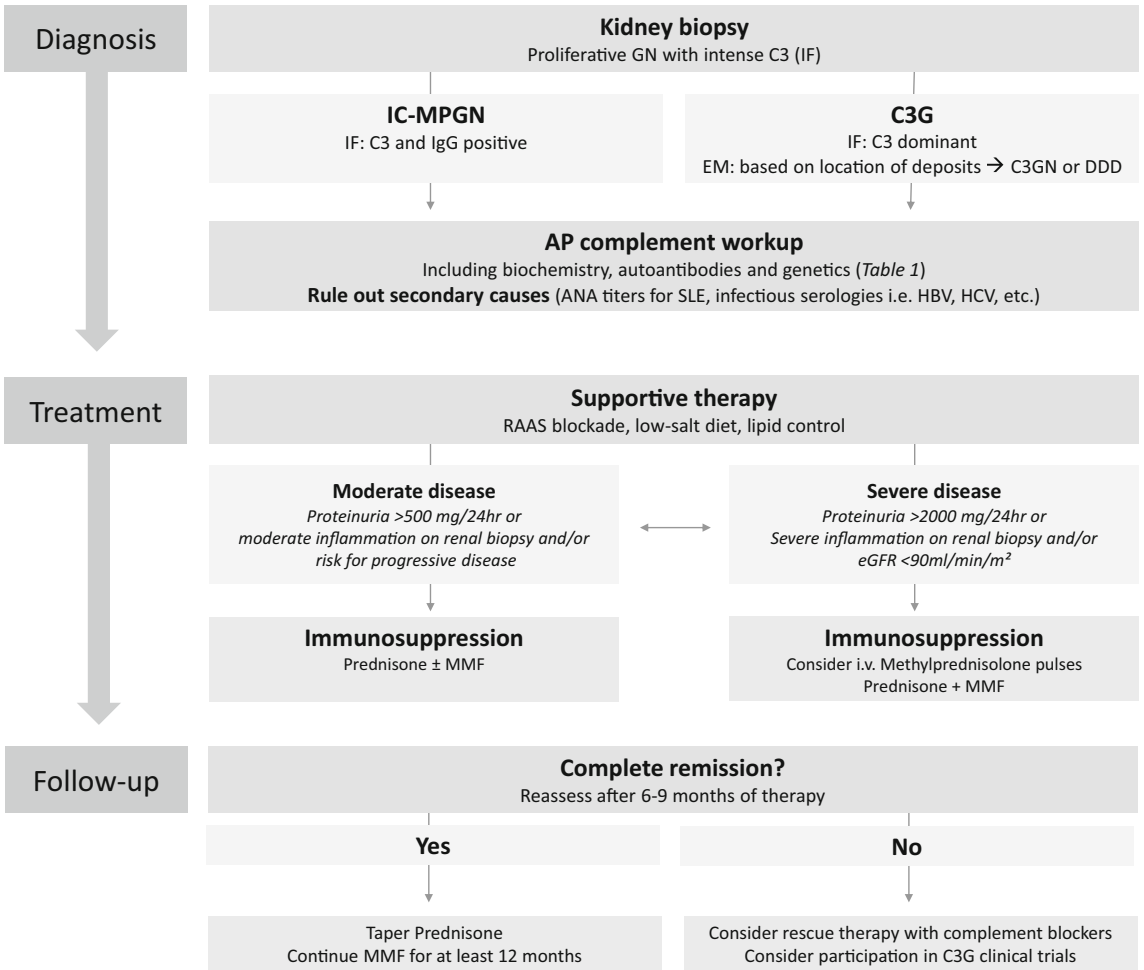

Fig. 3 Management of children with C3G and IC-MPGN. Upon diagnosis by kidney biopsy, exclusion of secondary causes by screening for systemic lupus erythematosus by ANA serology and for infectious causes compatible with clinical presentation (frequently HBV, HCV) followed by genetic and serological work-up of the alternative and terminal pathway of complement are warranted. In terms of therapy, all children should receive supportive measures. The use and intensity of immunosuppression (IS) should be established based on clinical and histological disease activity. Reassessment of response at 6–9 months should lead to IS tapering if response has been achieved, to referral to a specialized center for use of complement inhibiting agents, if possible within a clinical trial, if disease persists

C3G therapy: targeted

Given the central role that the complement system plays in the pathogenesis of C3G, complement inhibition would seem to be the most rational treatment for the disease. Eculizumab has been tested in small clinical trials and case reports [65–71]. Unfortunately, the results have been mixed. Some patients seem to improve. In general, however, the response to treatment is incomplete, and some patients do not seem to benefit at all. Several clinical factors may predict a response to treatment, including white blood cells in the urine [69] and inflammatory lesions on biopsy [68]. It may be that there is greater infiltration of the kidney by leukocytes in those cases where C5a plays an important role. Though initial reports suggested that elevated circulating sC5b9 could predict a positive response to eculizumab treatment in patients with C3G [65], this result has not been confirmed by the only prospective trial evaluating eculizumab in this setting — the EAGLE study [70]. This trial evaluated eculizumab for two sequential 48-week periods separated by one 12-week washout period in 10 patients, (6 with IC-MPGN, 4 with C3G). All of the subjects in this study had normal kidney function, nephrotic range proteinuria (> 3500 mg/24 h) and highly elevated sC5b9 (> 1000 ng/ml). The primary outcome was change in 24-h proteinuria (median of three consecutive measurements) at 24 and 48 weeks. While terminal complement pathway activation was successfully blunted in all patients, only 3/10 patients achieved partial remission of proteinuria. During the first treatment period, median proteinuria, albuminemia, and lipid profile improved, but these mild benefits were lost during the washout period and were not regained in 7/10 patients during the second treatment period.

A large retrospective French study evaluated 26 patients, 13 of whom were pediatric, with a median treatment duration of 14 months [68]. In this study, 6 patients (23%) had a global clinical response, 6 (23%) had a partial clinical response, and 14 (54%) had no response. The patients with a global clinical response had a more rapidly progressive disease and more intense glomerular extracapillary proliferation prior to treatment, suggesting that eculizumab may target glomerular inflammation. Two patients with a mixed aHUS/C3G phenotype who improved after treatment with eculizumab were recently described. This suggests that eculizumab is likely to be beneficial for patients in whom AP dysregulation occurs on the endothelial surface, as in aHUS.

Taken altogether, these findings indicate that currently evidence is insufficient to recommend eculizumab as first-line agent. It may be attempted in cases who have not benefited from untargeted immunosuppression.

Several active clinical trials (reviewed in Table 2) are testing new complement inhibitory drugs in various complement-mediated glomerular diseases. The ongoing trials are mainly focused on C3G/IC-MPGN and include adolescents in some cases. Some of the new drugs target C5a, such as the oral C5aR1 antagonist, CCX168, which blocks anaphylatoxin effect of C5a, thus exerting a strong anti-inflammatory effect. It does not block C5b9 production and it does not affect upstream complement activation. This drug is currently being investigated in phase 2/3 trials in C3G/IC-MPGN enrolling adults and adolescents ≥ 12 years of age. It demonstrated a good safety profile and promising results in terms of efficacy in patients with ANCA-vasculitis [20, 21]. In C3G, in addition to the C5 convertase, fragments of the C3 convertase (C3a and C3b) may also contribute to the disease pathogenesis. If so, then drugs that block complement activation upstream of C5 may be more effective than eculizumab or CCX168, particularly in forms that have a predominant dysregulated activation of the C3 convertase. Several drugs that target C3, the C3 convertase and its regulatory proteins (factor D, factor B), are being tested [6]. In particular, APL2 is a PEGylated cyclic peptide inhibitor of complement C3 which blocks formation of C3 and C5 convertase. It has the potential to block complement activation from all three pathways and to affect all downstream complement activity. It is being investigated in a pilot study involving different glomerular diseases, including C3G patients ≥ 16 years of age (NCT03453619). ACH-4471 and ACH-5528 are two orally administered, thrice daily and twice daily respectively small molecules that act as factor D

Table 2 Complement inhibitory drugs undergoing testing in glomerular disease

Drug	Target	Diseases
LPN023 (Novartis)	FB	C3G, IgAN
ACH4471 (Astra Zeneca)	FD	C3G, MPGN
OMS721 (Omeros)	MASP2	aHUS, C3G, IgAN, Lupus nephritis, MN
AMY-101 (Amyndas)	C3	C3G
APL-2 (Apellis)	C3	C3G, IgAN, Lupus nephritis, MN
Cemdisiran (Alnylam)	C5	IgAN, aHUS
CCX168 (Chemocentryx)	C5aR1	ANCA-associated vasculitis, C3G, IgAN

MASP, mannose associated serine protease; *aHUS*, atypical hemolytic uremic syndrome; *C3G*, C3 glomerulopathy; *IgAN*, IgA nephropathy; *ANCA*, anti-neutrophil cytoplasmic antibody; *C5aR1*, C5a-receptor 1; *MPGN*, membranoproliferative glomerulonephritis; *MN*, membranous nephropathy

inhibitors, blocking the amplification loop at the C3 convertase level. Therefore, they selectively block amplification of the complement alternative pathway, leading to an increase in circulating C3 levels. ACH-4471 has been investigated in small numbers of adults with C3G (vs. placebo NCT03369236) and with C3G/IC-MPGN (open-label NCT03459443). LNP023 is an orally administered twice daily small molecule inhibitor of factor B, which selectively blocks the C3 convertase amplification loop of the AP. It is being investigated in adults with C3G (NCT03832114) and will soon be evaluated also in adolescents.

In terms of safety profile, it is well known that anti-C5 antibodies such as eculizumab carry a risk of meningococcal and other encapsulated bacterial infections, which can be prevented by vaccination against these infections and in high-risk cases prophylactic antibiotic treatment. Compared to eculizumab, CCX168, acting selectively on the C5a receptor, does not affect formation of the MAC and therefore does not impede cell lysis, potentially having less impact on the host defense mechanism. Agents affecting the complement pathway more upstream have a theoretically greater impact on reducing the patient's capacity to recognize, opsonize, and lyse microorganisms. However, in vitro ACH4471, a factor D inhibitor, showed a reduced capacity to impair opsonophagocytosis compared to eculizumab, an anti-C5 antibody [72]. Altogether, the risks of these new therapeutic agents remain to be quantified, especially in younger children, and must not be under-estimated. All children undergoing alternative and terminal complement inhibition should receive anti-meningococcal, anti-pneumococcal, and anti-*Haemophilus influenzae* vaccinations at least 15 days before start and in selected cases antibiotic prophylaxis for the duration of complement inhibiting treatment.

The rarity of these conditions makes it challenging to conduct these trials, but some of the studies are using repeat kidney biopsies or complement biomarkers to help test the efficacy of these drugs at blocking complement activation.

Other glomerular diseases prone to benefit from complement inhibition (IgAN, lupus nephritis, AAV and membranous nephropathy)

Alternative pathway activation is the primary driver of C3G and atypical HUS. However, complement is activated in many other types of kidney disease, including most forms of immune-complex disease. The AP is also activated in the tubulointerstitium of patients with acute tubular injury [73]. Clinical correlations and animal studies provide a rationale for use of complement inhibitors in membranous nephropathy, lupus nephritis, immune-complex-mediated MPGN, IgA nephropathy, and ANCA-associated vasculitis. ANCA-vasculitis is interesting in that complement activation is believed to occur on the surface of neutrophils, rather than within the glomeruli [2]. Thus, complement activation plays an important role in the pathogenesis of this disease even though glomeruli appear "pauci-immune" by histology.

Complement activation fragments can presumably have the same pathologic effects in these other diseases as they do in C3G, although the role of complement in kidney injury may be modified by the specific ultrastructural location where activation occurs. For example, anaphylatoxins generated in the subendothelial space are more likely to enter the bloodstream and attract neutrophils than if they are generated in the subepithelial space [74]. This may explain why neutrophil infiltration is not characteristic of membranous disease, even though there are abundant immune-complexes and complement fragments deposited in the glomeruli. Other disease mechanisms may also add to or modify the complement-mediated effects. Fc receptors can mediate glomerular injury independent of complement activation [75]. Cross-talk between anaphylatoxin receptors and toll-like receptors can also have a strong influence on the inflammatory response [76, 77]. Importantly, C3 fragments lower the activation threshold of B cells [78] and anaphylatoxins serve as co-stimulatory signals for T cells [79]. Consequently, complement can both influence the autoimmune response and mediate antibody-induced tissue injury.

As a downstream effector of immune-complex-mediated injury, complement activation probably plays a role in the pathogenesis of lupus nephritis, immune-complex-mediated MPGN, and cryoglobulinemia. Complement activation also probably causes glomerular injury in membranous nephropathy. The pathogenic antibodies in membranous nephropathy are usually IgG4, however, an isotype that does not activate the classical pathway. The mechanisms of complement activation in this disease are not fully understood, although there is evidence of alternative and lectin pathway activation in some patients [11]. Similarly, IgA does not activate the classical pathway, and the mechanisms of complement activation in IgA nephropathy are not completely clear. There is evidence that IgA can activate the alternative pathway, and frequently, there is co-deposition of complement activating immunoglobin [80]. OMS721, a PEGylated cyclic peptide inhibitor of MASP2, which has the potential to block the lectin pathway, is being investigated mainly in IgA nephropathy, but also in a small pilot study involving adult patients with membranous nephropathy, lupus nephritis, and C3G (NCT02682407).

Conclusions

Complement is a relevant player in driving or amplifying many glomerular diseases and recent evidence has led to an understanding of its pivotal role in diseases such as ANCA vasculitis, IgA nephropathy, and idiopathic membranous nephropathy in which it was not previously well recognized. Moreover, in the last few years, we have identified a growing number of proliferative glomerulophritides secondary to, mainly, a dysregulation of the alternative pathway, characterized by an intense, if not predominant C3 deposition in renal glomeruli, and denominated C3 glomerulopathies. While C3G may be less rare than we previously thought, as our ability to identify it increases, this diagnosis remains at times elusive and requiring expert evaluation. Moreover, as we gain further insight, the heterogeneity of C3G becomes more evident. Collaborative research efforts are the key to achieving a deep geno- and phenotyping of each individual patient, allowing for a dissection of the complement dysregulation at play and, hopefully in the not-too-distant future, a tailored and targeted therapeutic approach.

Multiple choice questions (answers given following the references)

1: A kidney biopsy performed in a child with C3 glomerulopathy can show:

a) Intramembranous dense deposits
b) Mesangial proliferation
c) Subepithelial humps
d) All of the above
e) None of the above

2: Membranoproliferative glomerulonephritis is a histological pattern which may be secondary to alternative pathway of complement dysregulation

a) Very rarely
b) Frequently, especially in children
c) Only in adults
d) Only if there is C3 predominance by IF
e) In all cases

3: The complement system is involved in the pathogenesis of C3G. Which of the following serological tests does not fit in the pattern of increased C3 turnover?

a) low C3 levels
b) normal C3 levels
c) normal factor B
d) auto-antibodies against AP convertase C3bBb
e) all of the above

4: Optimal treatment of C3 glomerulopathy requires

a) Supportive care with RAAS inhibition and low-salt diet
b) Mycophenolate mofetil when proteinuria is ≤ 0.5 g/day
c) Eculizumab
d) Therapy targeting the alternative pathway of complement
e) All of the above

5: Among therapeutic agents targeting complement, which of the following statements is true?

a) Eculizumab is the only agent approved for use in C3G
b) ACH4471 targets Factor B
c) CCX168 is a C5aR1 antagonist
d) APL-2 is orally administered
e) LNP023 targets the C5 convertase

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Declarations

Competing interests MV received funding from the Associazione per la cura del Bambino Nefropatico ONLUS and has served on advisory boards for Novartis, Apellis, Roche, Achillion, Retrophin and participated in the C3G clinical trials by Chemocentrix and Achillion. NK is member of the advisory board of Roche and participant in the clinical trials in C3G by Chemocentryx and Achillion. RL and FD: no funding or conflict of interest to report. JMT receives royalties from Alexion Pharmaceuticals, Inc. and is a consultant for Q32 Bio, Inc., a company developing complement inhibitors. He also holds stock and will receive royalty income from Q32 Bio, Inc. This does not influence the content of this manuscript.

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Answers:1. d; 2. b; 3. c; 4. a; 5. c