C3 Glomerulopathy: A Review with Emphasis on Ultrastructural Features

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Key Points

- While light microscopic patterns in C3 glomerulopathy are quite variable, the diagnosis of all such cases depends on the demonstration of C3-dominant glomerular staining by immunofluorescence microscopy, that is, at least two orders of intensity greater than that of any other immune reactant.
- Electron microscopy is necessary for establishing a diagnosis, in particular for dense deposit disease presenting with varying light microscopic phenotypes.
- Electron microscopy helps distinguish C3 glomerulopathies from their mimickers, especially in cases of postinfectious glomerulonephritis.

Abstract

C3 glomerulopathy (C3G) is a rare disease resulting from dysregulation of the alternative complement pathway, resulting in the deposition of complement component 3 (C3) in the kidney. It encompasses two major subgroups: dense deposit disease and C3 glomerulonephritis (C3GN). Although the alternative complement pathway is typically a very tightly controlled system, dysregulation can be a result of genetic mutations in the fluid phase or membrane-bound inhibitors or accelerators. In addition, de novo/acquired autoantibodies against any of the regulatory proteins can alter complement activation either by negating an inhibitor or activating an accelerator. Triggering events can be complex; however, the final pathway is characterized by the uncontrolled deposition of C3 in glomeruli and the formation of the membrane attack complex. Light microscopic findings can be quite heterogeneous with a membranoproliferative pattern most commonly encountered. Diagnostic confirmation of C3G is based on a characteristic pattern of glomerular immunofluorescence staining, with C3-dominant deposits that are at least 2 orders of intensity greater than staining for any immunoglobulin (Ig) or C1q. Electron microscopy is necessary for diagnosing DDD in particular, but can also help to distinguish C3GN from other glomerular disease mimickers.

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Definition and Nomenclature

C3 glomerulopathy (C3G) is a relatively newly recognized disease entity that, by definition, encompasses a group of glomerular lesions resulting from uncontrolled deposition of complement factor 3 (C3) due to dysregulation of the alternative complement pathway [1, 2]. Impor-
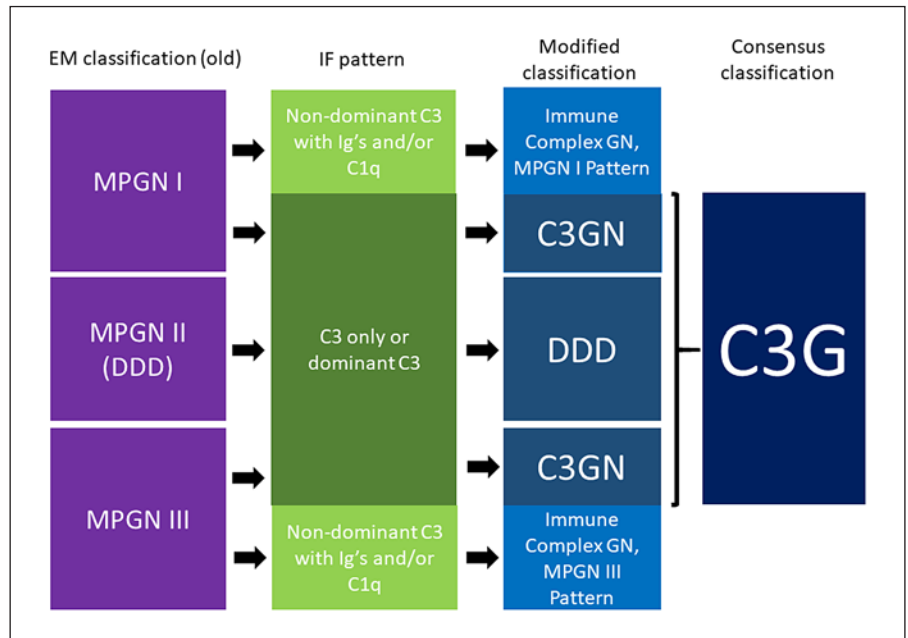
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Fig. 1. Evolution of C3G classification. Historical classifications based on ultrastructural findings were re-evaluated according to IF findings. Cases once classified as MPGN types I and III demonstrating only C3 or C3-dominant staining by IF are now subcategorized as C3GN. MPGN II, which frequently shows only C3 staining with virtually all remaining cases being C3 dominant, retained the designation DDD. The general category of C3G encompasses cases of DDD and C3GN.

tantly, C3G was defined in such a way that it incorporated a subset of glomerular pathologies that had been previously classified according to ultrastructural appearances only, without reference to the underlying etiology or mechanism of glomerular injury. Many of these early cases displayed diffuse mesangial and endocapillary hypercellularity with glomerular basement membrane (GBM) duplication, i.e., a "membranoproliferative" pattern. Based on this histologic pattern of glomerular injury and the distribution of glomerular immune deposits, primarily with respect to the glomerular basement membranes (GBMs) noted by electron microscopy (EM), these cases were historically classified as membranoproliferative glomerulonephritis (MPGN) types I, II, and III [3, 4]. While light microscopic (LM) findings are quite heterogeneous, specific immunofluorescence (IF) patterns emerged over time. The IF pattern in MPGN types I and III was most commonly characterized by variably positive staining for complement proteins and different classes of Igs, while cases of MPGN type II frequently stained exclusively or almost exclusively for C3, with very little or no staining for Igs or other complement proteins. MPGN II was also characterized by elongated or "sausage-like" intramembranous accumulation of homogeneously electron-dense material and thereby becoming known as dense deposit disease (DDD). The pathogenesis of DDD was eventually linked to defects in alternative complement pathway regulation and served as the prototypic disease for the devel-

opment of IF criteria to define C3G [5]. Over time, these IF criteria were amended [6], and diagnostic guidelines for C3G were expanded to not only include DDD but also those glomerular lesions characterized by dominant C3 staining, defined as at least two orders of intensity (on a 0–3+ scale) greater than any Ig or other complement protein. These newly encompassed cases were subsequently termed C3 glomerulonephritis or C3GN (Fig. 1).

Alternative Complement Pathway and Pathogenesis of C3G

It has been noted that the kidney seems to be particularly susceptible to injury resulting from complement dysregulation, manifestations of which include atypical hemolytic uremic syndrome and C3G [7]. Dysregulation and excessive activation of the alternative complement pathway underlie the key pathogenic mechanism of C3G. This pathway normally maintains a low degree of baseline constitutive activity via a spontaneous hydrolysis reaction occurring in the fluid phase that converts C3 to $C3(H_2O)$, known as C3 "tick-over." $C3(H_2O)$ interacts with factor B and factor D to form an intermediate enzyme $C3(H_2O)Bb$, which acts as a weak initial C3 convertase in the alternative complement pathway. $C3(H_2O)Bb$ then cleaves C3 to C3b, which can bind to target surfaces and also interacts with factor B and factor D to form the

C3 convertase C3bBb. C3bBb in turn can propagate and amplify the C3 tick-over reaction to produce more C3b [8–10]. This positive feedback mechanism eventually generates enough C3b to bind with C3bBb and form the C5 convertase (C3bBbC3b), which initiates the activation of the terminal complement pathway leading to the generation of C5b-9, the membrane attack complex that disrupts cell membranes to cause cell injury and lysis [9, 11, 12]. Although C3 and factor B are both abundant circulating plasma proteins, they do not spontaneously interact without the conversion of C3 to C3b from the tightly regulated C3 tick-over reaction [13]. Factor H regulates complement activity by promoting the proteolytic inactivation of C3b and the decay of C3 and C5 convertases. Factor I can combine with Factor H or complement receptor 1 (CR1) to inactivate C3b in the fluid phase and on cell surfaces, respectively [13, 14]. There are several complement factor H-related proteins (CFHR 1–5) with common structural motifs that are thought to be able to compete with factor H-binding and thereby alter factor H-mediated regulatory control [13, 14]. Properdin is a plasma protein that upregulates the alternative complement pathway by stabilizing C3bBb and thus promoting the formation of C3bBbC3b and downstream MAC assembly [9, 15].

In C3G, the C3 convertase activity can be upregulated via either the abnormal stabilization of C3bBb by autoantibodies collectively known as C3 nephritic factors (C3NeF), the loss of inhibitory function by factor H or factor I, or mutations of different CFHR that disrupt factor H-mediated regulation [13, 14]. These pathogenic mechanisms were originally demonstrated in animal models and are supported by genetic analysis and tissue biopsy studies of C3G patients [11–13, 16, 17]. Patients may also have variant alleles for complement proteins or regulatory factors that confer an increased risk of C3G [11, 18], while others may have inhibitory autoantibody production against factor H [19]. Rare cases of monoclonal gammopathy with a paraprotein exhibiting an inhibitory effect against factor H have also been reported [20].

The mechanism(s) of glomerular complement accumulation leading to deposits in C3GN and DDD are not well understood and there is no clear genotype-phenotype correlation. The kidney may be particularly susceptible to complement-mediated injury due to the hemoconcentrated microenvironment in glomeruli that exposes the GBM containing abundant amine or hydroxyl groups for C3b binding to higher concentrations of complement proteins [10, 21]. Mass spectrometry analyses of laser microdissected glomeruli from renal biopsy tissue in C3G patients have revealed similar C3 proteomic profiles between C3GN and DDD and identified the thioester-containing C3dg fragment as the most abundant C3 cleavage product within glomerular deposits in both C3GN and DDD [21]. As the thioester bond is responsible for covalently attaching C3b to target surfaces (such as the GBM), the accumulation of C3dg fragments in glomeruli may reflect factor I-mediated cleavage of C3b that releases C3c into the fluid phase [21]. The current detection method for C3 deposits in renal biopsies may also be limited as most laboratories use anti-C3c for routine IF that does not detect C3dg [2, 21]. Some studies have suggested that the balance of C3 convertase versus C5 convertase dysregulation may influence the phenotype of C3G [22, 23]. Properdin deficiency is associated with a predominance of C3 convertase dysregulation over C5 convertase dysregulation and leads to an aggressive murine C3G phenotype with ultrastructural features similar in appearance to those seen in human DDD [22, 23]. Patients with stabilizing autoantibodies against C5 convertase (C5 nephritic factors) were shown to be more likely to have C3GN than DDD and have higher serum levels of soluble C5b-9 [12].

Clinical Manifestations

C3Gs are rare diseases with an estimated incidence of 1 to 3 patients per million, with C3GN reportedly being between 3 and 9 times more common than DDD [24–26]. Patients with DDD are typically diagnosed at a younger age than those with C3GN; DDD primarily affects children and young adults, although it has been reported in older adults [27]. Older patients diagnosed with C3G should be screened for monoclonal gammopathy as many reported C3G cases in older adults were found to be associated with a monoclonal paraprotein [13, 28].

Patients with C3G (both C3GN and DDD) can have variable clinical presentations ranging from asymptomatic and low-grade proteinuria to nephrotic syndrome, nephritic syndrome, or rapidly progressive glomerulonephritis [24]. Most C3G patients have selectively decreased serum C3 levels, which is more commonly reported in DDD than in C3GN [24–26]. A minority of patients can also have decreased serum C4 levels [24–26]. Complement autoantibodies including C3NeF, factor H antibody, and factor B antibody have been identified in patients with varying frequencies, although they are more commonly reported in DDD than in C3GN [16, 24–26]. Patients tested for complement abnormalities can have a
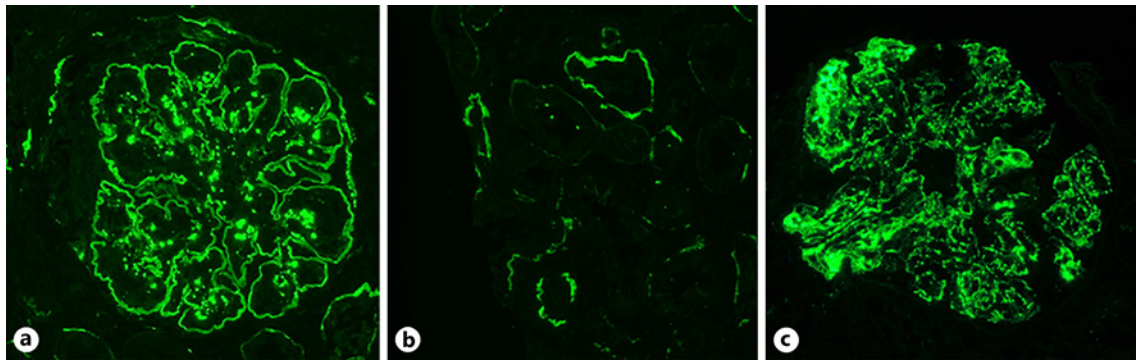
Fig. 2. IF staining patterns in C3G. Staining for C3c in DDD reveals the characteristic confluent granular to linear staining of GBMs (**a**), with occasional linear TBM staining (**b**). Mesangial staining in all cases of C3G (including DDD) can be quite variable ranging from discrete, large rounded deposits (**a**) to coarsely granular (**c**) or confluent granular, all with variable accentuation of capillary walls. All panels: anti-C3c fluorescein isothiocyanate-conjugate, ×400.

variety of complement factor genetic variants, many of which are of unknown significance [24, 26]. DDD, but not C3GN, has been associated with extrarenal manifestations including acquired partial lipodystrophy and drusen-like macular deposits similar to those seen in age-related macular degeneration [29, 30].

Whenever possible, all patients diagnosed with C3G are recommended to undergo thorough complement testing that includes screening for autoantibodies against complement regulators, serum levels of complement proteins and split products, and overall complement activity [2, 13, 31]. These results may offer diagnostic and/or prognostic information and help guide treatment, especially when new targeted therapies against complement proteins or regulators become available in the future [2, 13]. Importantly, genetic testing for mutations in regulators of the alternative pathway, particularly CFH and CFHR, may help uncover familial cases of C3G that could help in the diagnosis of affected family members [14].

Treatment for C3G has not been well established and should be tailored towards each patient based on the clinical presentation and underlying complement disorder. Therapeutic approaches include general modalities such as blood pressure control, blockade of the renin-angiotensin system, and lipid-lowering agents, as well as more specific and aggressive interventions such as immunosuppressive therapy, plasma exchange, and targeted anti-complement therapy (eculizumab) [13, 14, 31].

IF Findings and Diagnostic Criteria

With recognition that C3G resulted from dysregulation of the alternative complement pathway, new IF criteria were proposed in 2014 [6] to improve diagnostic decision-making and to identify potential patients who might benefit from a comprehensive complement workup. In order to cast as broad a diagnostic net as possible, IF criteria stipulated that cases of C3G should include not only those with exclusive IF staining for C3 but also those with dominant C3 staining that was at least two orders of intensity (on a 0–3+ scale) greater than that for any other immune reactant (IgG, IgA, IgM, or C1q). IF detection of C3 in most laboratories is performed using an antibody to C3c (a physiological breakdown product of activated C3) [2]. These criteria were subsequently incorporated into the consensus diagnostic recommendations for C3G [2].

The pattern of C3 staining can vary considerably. While deposits typically display linear, band-like staining of GBMs and some tubular basement membranes (TBMs) in DDD (Fig. 2a, b), the deposits can also form coarse granular to larger, rounded ("ring form") deposits in the mesangium (Fig. 2a) [32]. There can also be variable GBM staining ranging from finely or coarsely granular to semiconfluent with peripheral accentuation (Fig. 2c). Where present, lower grade staining for Igs and/or C1q typically colocalizes with C3. When there is light chain staining, this should generally be polytypic. To exclude the possibility of immune complex-mediated GN with masked monotypic Ig deposits, it is important to consid-

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Fig. 3. The characteristic LM finding in DDD is that of prominent ribbon-like thickening of glomerular capillary walls (arrows), resulting from C3 deposition (**a**). Similar deposits can be seen in TBMs as well (arrows, **b**). Hematoxylin and eosin stain, magnified from original ×600 image.
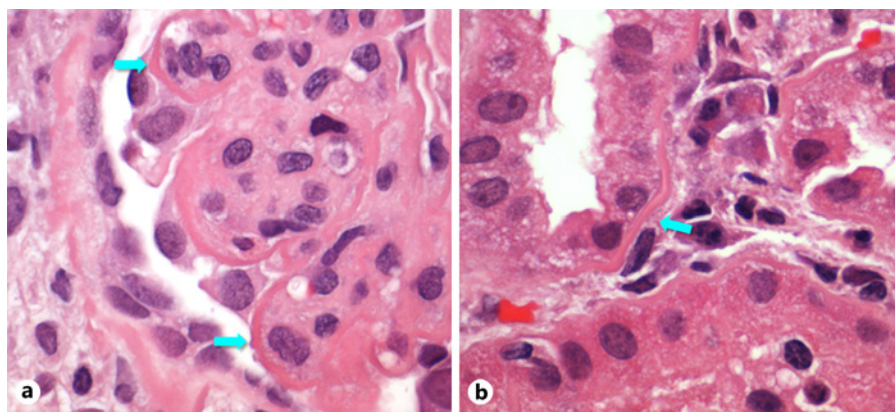
er performing IF on pronase-digested paraffin sections in cases with C3-dominant staining on routine frozen IF, especially in patients with paraproteinemia [31, 33]. It has been suggested that staining for C4d may be useful in discriminating between C3G and immune complex-mediated glomerulonephritis. Sethi et al. [30] demonstrated that glomerular C4d staining was often positive in cases of immune complex-mediated glomerulonephritides likely due to activation of the classical and/or lectin pathways of complement but that C4d staining was very weak or absent in cases of C3G with disturbances in the alternative complement pathway.

LM Findings

The LM findings in C3G are quite heterogeneous; as such it is generally not possible to predict which cases of a proliferative GN will be C3-dominant. Often a MPGN pattern is seen with GBM duplication, and double contours as a response to subendothelial immune complex deposition [3]. In some cases of DDD, the intramembranous accumulation of C3 can be histologically apparent as thickened glomerular capillary walls demonstrating a homogeneous, eosinophilic, glassy appearance on hematoxylin and eosin stains (Fig. 3a), and a variably fuchsinophilic appearance with the Masson's trichrome stain. The deposits are also nonargyrophilic, which can help differentiate these from normal basement membrane material that is silver positive. Occasionally, similar changes may also be seen in TBMs (Fig. 3b). However, the deposits of DDD are most often quite subtle and are frequently missed on initial histologic evaluation, only to be detected by IF. C3 deposits in the non-DDD cases of C3G, or C3GN, display similar tinctorial staining characteristics, although the glomerular location of the deposits can vary.

Cases of C3G (including both DDD and C3GN) can display a wide array of glomerular histologic features ranging from a mesangial proliferative, membranoproliferative, and/or endocapillary proliferative glomerulonephritis, with or without active crescents and fibrinoid necrosis (Fig. 4a–c). While membranoproliferative features are often present, they are not a diagnostic requirement. In the largest cohort of reported C3G cases in the USA [24], the most common histologic pattern was that of MPGN, accounting for more than half of the 111 cases. Mesangial proliferation was the second most common histologic pattern, with slightly fewer cases demonstrating a diffuse endocapillary proliferative pattern. Active (cellular and/or fibrocellular) crescents were present in approximately 10% of cases in the entire cohort. A C3 Glomerulopathy Histologic Index has been proposed, which utilizes different histologic features of activity and chronicity as predictors of renal outcome [24]. While higher total activity and chronicity scores were predictors of a poor clinical outcome by multivariate analysis, crescents were the only individual histologic feature demonstrating such correlation by univariate analysis [24]. However, when this histologic scoring index was recently validated in a large cohort of patients, it was determined that the chronicity score was the principal histologic correlate for renal failure [34].

EM Findings

Although C3-dominant IF staining is constant in C3G, the distribution of the C3 deposits and the associated histologic changes can be quite heterogeneous. Likewise, the location and ultrastructural appearance of the C3 deposits can also display considerable variability. Generally, the deposits are most commonly and easily identified in mesangial regions, with variable subendothelial and subepi-
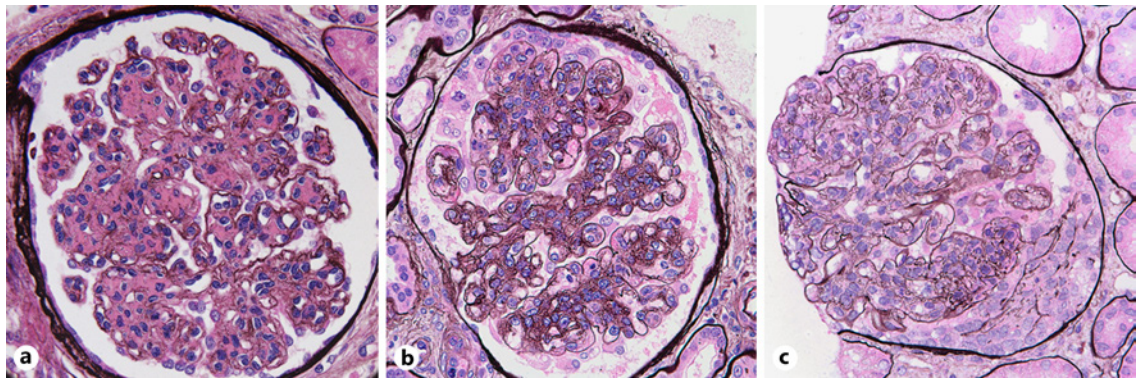
Fig. 4. Heterogeneous histologic appearances of C3G: Mesangioproliferative with silver negative deposits (**a**); membranoproliferative features (**b**), and diffuse endocapillary proliferative with crescentic involvement (**c**). All panels: Jones methenamine silver stain, ×600.
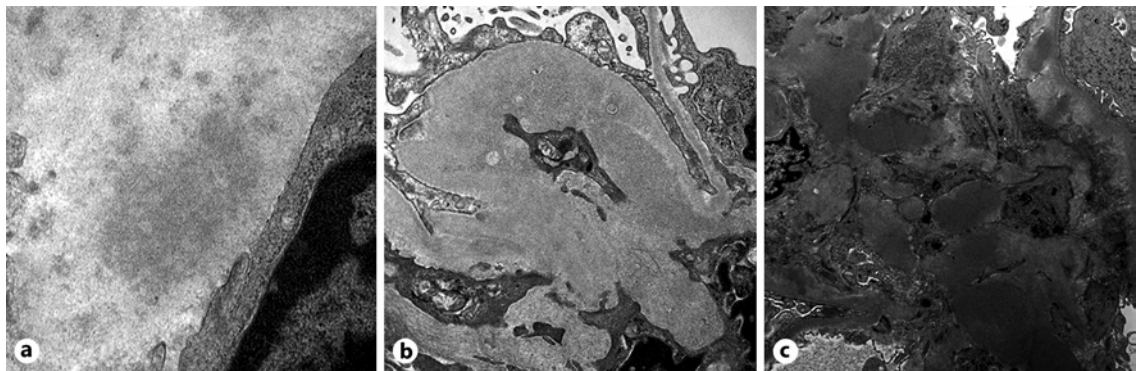

Fig. 5. EM of mesangial C3 deposits of varying size ranging from small (**a**) to large. The C3 deposits are less electron dense than immunoglobulin-containing deposits (see Fig. 6) and can blend into the surrounding mesangial matrix material (**b**). **c** Other deposits can be "smudgy" in appearance. Uranyl acetate and lead citrate stains, original magnifications: **a** ×60,000, **b** ×25,000, and **c** ×10,000.

thelial involvement. These deposits can be in addition to the typical elongated intramembranous GBM transformation of DDD, and the combination of these glomerular deposits generates a pattern historically referred to as MPGN III. For this reason, EM is absolutely necessary to distinguish DDD from C3GN [35, 36].

Several descriptors have been used to describe the fine ultrastructural appearance of the C3 deposits. While typical immune complex deposits composed of immunoglobulins often have well-defined, sharp borders with a dense and granular EM appearance, C3 deposits (Fig. 5) are often described as smudgy and "cloudy" with ill-defined borders [14]. Unlike the highly osmiophilic and finely granular immunoglobulin containing deposits such as those seen in IgA nephropathy (Fig. 6), C3 depos-

its often display an ultrastructural texture slightly denser than that of the mesangial matrix material. As such, the borders can gradually blend into the surrounding matrix and be difficult to delineate (Fig. 5b). In the mesangium, the deposits can form discrete and rounded, spherical, or "globular" aggregates located within the mesangial matrix material that can vary in size from small to quite large, with the associated widening of the mesangial areas (Fig. 7). In cases with abundant mesangial C3, the deposits can appear to be confluent, pushing outwards and up against the overlying paramesangial basement membranes with impingement into capillary lumina and considerable mesangial expansion (Fig. 8a). The deposits can also display heterogeneity within the same biopsy, ranging from evenly dispersed (Fig. 8b) to a geographic ap-
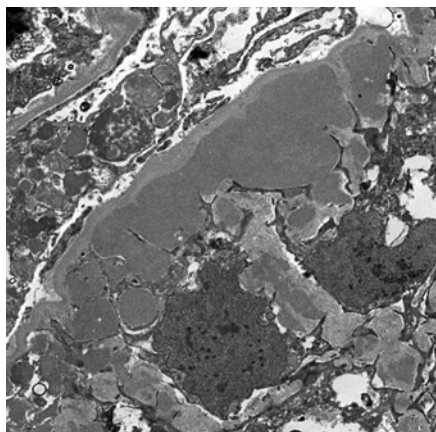
Fig. 6. EM of typical immunoglobulin-containing immune complex deposits in IgA nephropathy. Note the well-defined borders and distinct dense texture of the deposits, as compared with the ill-defined borders and "smudgy" appearance of C3 deposits (see Fig. 5). Uranyl acetate and lead citrate stains, original magnification ×3,000.
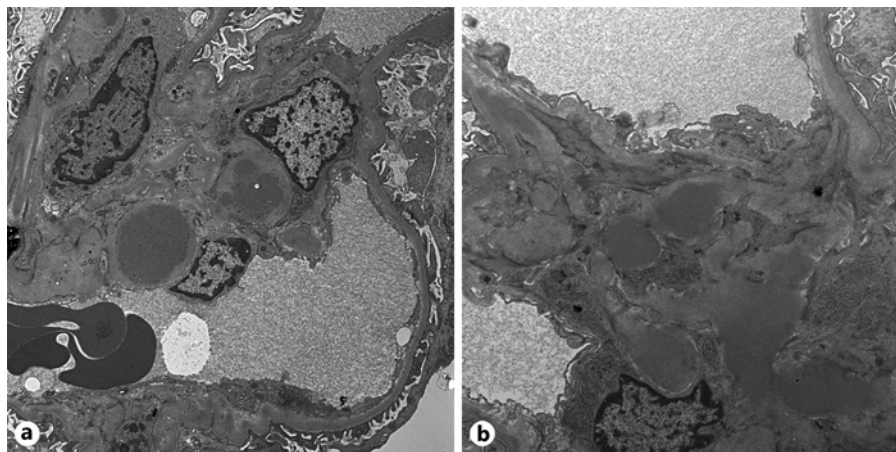

Fig. 7. C3 deposits can form discrete rounded and "globular" mesangial deposits (**a**), as well as deposits with ill-defined, blending borders (**b**). Note the "smudgy" appearance of the C3 deposits (**b**). Uranyl acetate and lead citrate stains, original magnifications: **a** ×6,000, **b** ×10,000.
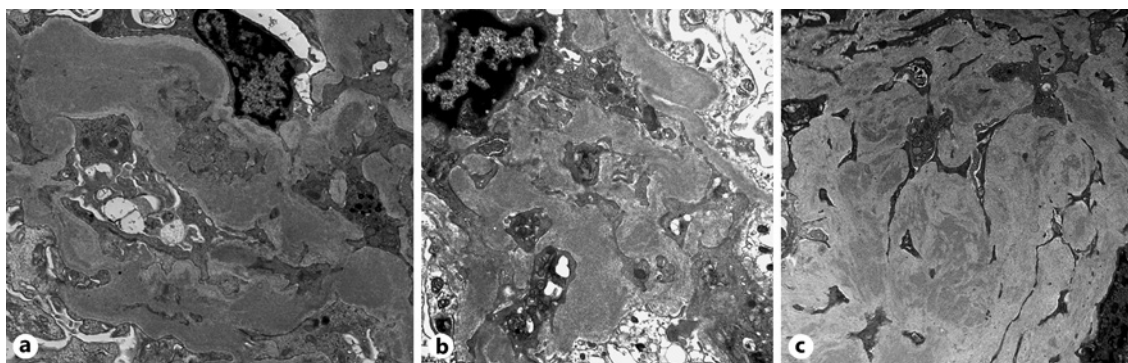

Fig. 8. Extensive, confluent mesangial C3 deposits with mesangial expansion (**a**). Confluent mesangial deposits may also have a more evenly dispersed texture (**b**) or assume a more geographic appearance (**c**), composed of irregularly shaped and variably sized aggregates. Uranyl acetate and lead citrate stains, original magnifications: **a** ×4,000, **b** ×14,000, **c** ×10,000.

pearance with irregularly shaped and angulated aggregates of varying size (Fig. 8c).

Excluding DDD, which typically shows more uniform involvement of GBMs, capillary wall involvement in C3GN can also be quite variable from capillary to capillary. In some biopsies with predominantly mesangial involvement, subendothelial C3 deposits with a typical smudgy appearance can be small and discrete (Fig. 9a) or assume a rounded appearance (Fig. 9b), similar to what

may be seen in the mesangium. Large and discrete glomerular subendothelial deposits have not been commonly described [36, 37] but can also be observed (Fig. 9c). In other cases, the subendothelial deposits can become confluent and assume an elongated appearance, with considerable involvement of capillary loops (Fig. 9d, e). Intramembranous deposits are also common, ranging in appearance from small and discrete to elongated (Fig. 10), sometimes with evidence of glomerular basement remod-
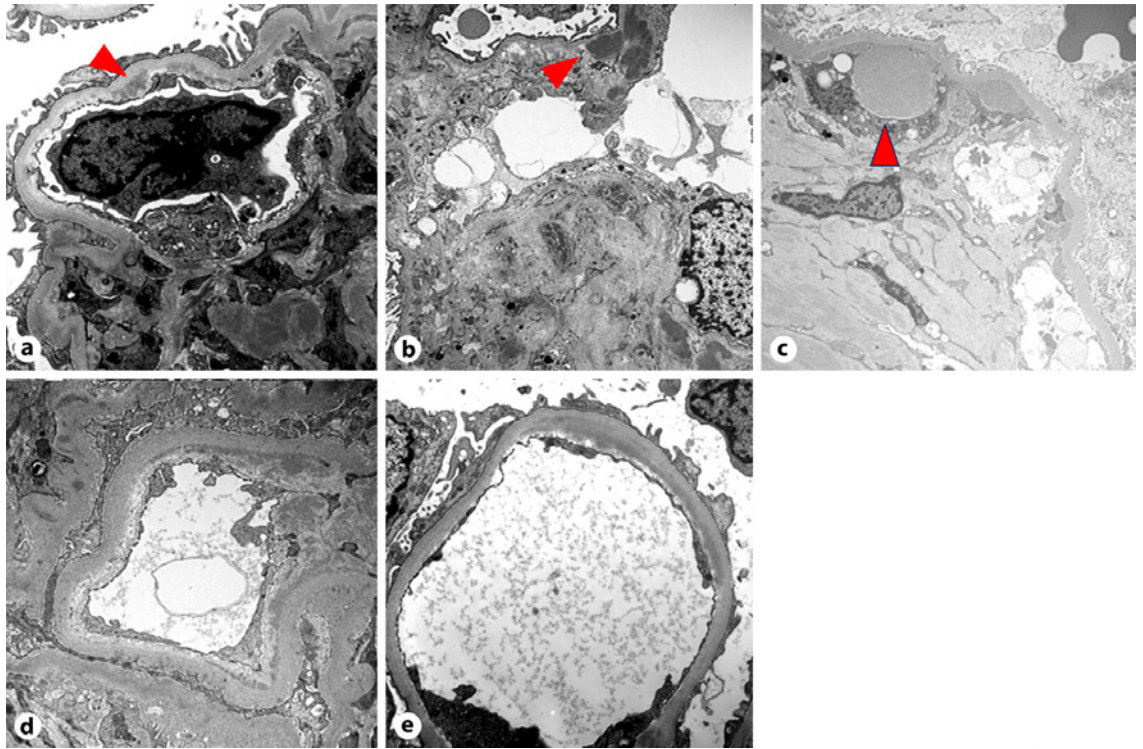
Fig. 9. Varying appearance of subendothelial deposits in C3GN. C3 deposits can form small, discrete subendothelial deposits (arrow, **a**), occasionally with a rounded appearance similar to what is seen in mesangial deposits (arrow, **b**). Although large, rounded subendothelial deposits are rare, they can sometimes be observed (arrow, **c**). **d**, **e** In other cases, the subendothelial deposits can be confluent and elongated, with considerable and variable involvement of capillary loops. Uranyl acetate and lead citrate stains, original magnifications: **a** ×6,000; **b** ×7,500; **c** ×10,000; **d** ×12,000, **e** ×6,000.
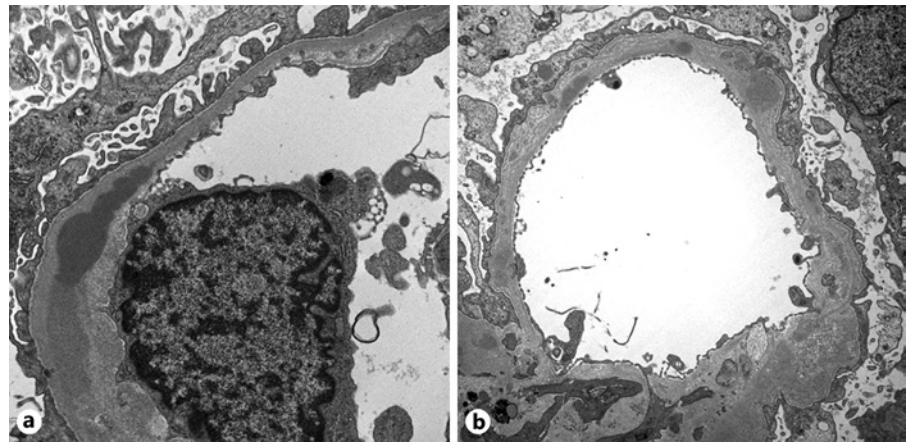

Fig. 10. Intramembranous deposits in C3G can range in appearance from elongated, with evidence of GBM remodeling (**a**, **b**), to small and discrete (**b**). Uranyl acetate and lead citrate stains, original magnifications: **a** ×10,000, **b** ×10,000.

eling and deposits in various stages of resorption (Fig. 10b).

The appearance and distribution of the intramembranous deposits on high magnification are crucial in differentiating DDD from C3GN. In DDD, there is deposition and transformation of the lamina densa by homogeneous, hyperosmiophilic material [37]. In well-established DDD cases, the intramembranous C3 deposits in the GBMs are confluent, elongated, and linear. The deposits can show variability in thickness ranging from segmentally thin to
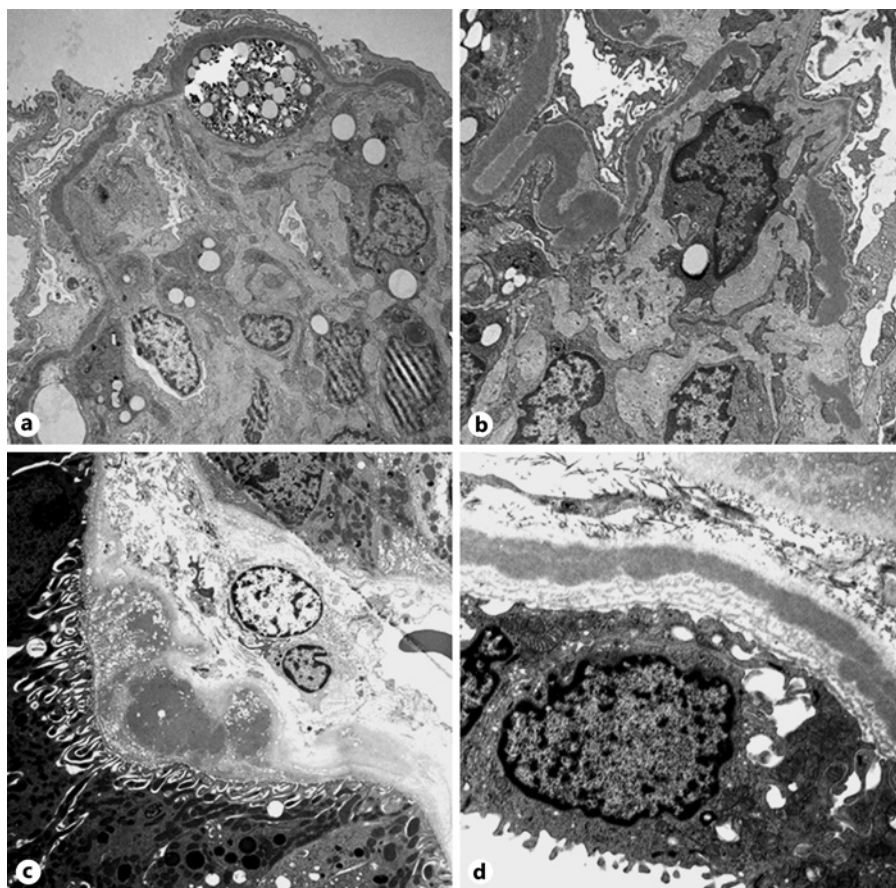
Fig. 11. DDD is defined by the ultrastructural appearance and distribution of the C3 deposits in GBMs and TBMs. **a** Elongated intramembranous deposits display variable thickness ranging from segmentally thin to thick, sometimes imparting a "sausage on a string" appearance. **b** Intramembranous deposits involve both glomerular and paramesangial basement membranes and can show segmental discontinuities. C3 deposits can also be seen in TBMs, where they can exhibit a discrete (**c**) and/or elongated (**d**) appearance. Uranyl acetate and lead citrate stains, original magnifications: **a** ×5,000, **b** ×7,500, **c** ×4,800, **d** ×14,000.
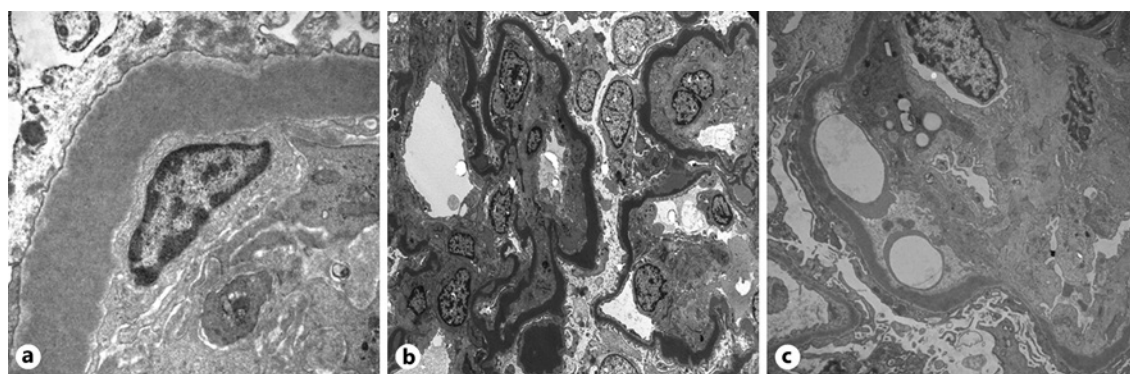

Fig. 12. a C3 deposits in DDD lack substructure, even at extremely high magnifications. **b** In some cases, the deposits can involve and transform the entire thickness of GBM (**b**), and can show hyperdense transformation which is almost exclusively limited to GBMs. **b**, **c** Typical variation in thickness of the C3 deposits resulting in a "sausage on a string" appearance are present. Uranyl acetate and lead citrate stains, original magnifications: **a** ×25,000, **b** ×4,800, **c** ×7,500.

thick, sometimes imparting a "sausage on a string" appearance (Fig. 11a), occasionally with segmental discontinuities which may be observed at the paramesangial reflection (Fig. 11b). Segmental to global mesangial deposits can also be seen, some of which are large and rounded "ring-form" deposits [32]. Similar deposits can also be seen in Bowman's capsule and TBMs (Fig. 11c, d), in discrete and/or elongated configurations [13]. The deposits
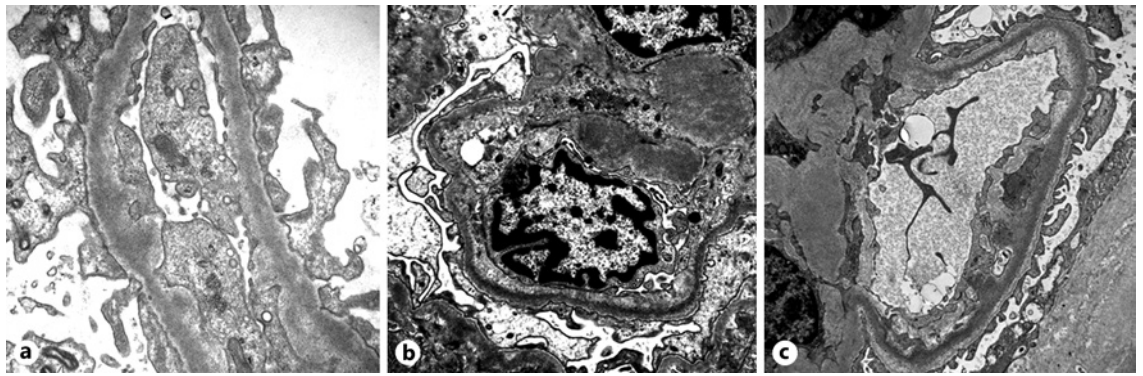
Fig. 13. The early stages of lamina densa transformation by C3 deposits can be evenly dispersed and ill-defined (**a**), or form discrete, well-defined band-like deposits (**b**, **c**). Uranyl acetate and lead citrate stains, original magnifications: **a** ×30,000, **b** ×6,000, **c** ×12,000.

of DDD lack substructure, even at extremely high magnifications (Fig. 12a). In a well-established disease, the deposits can span the entire thickness of the GBM (Fig. 12b) and can show almost exclusive GBM involvement (Fig. 12c). Early stages of lamina densa transformation by the C3 deposits can also be seen, with evenly dispersed and ill-defined intramembranous C3 deposits (Fig. 13a), or narrow but uniform band-like deposits in affected GBMs (Fig. 13b, c). Distinction between C3GN and DDD may not always be possible even with EM, as some cases show an overlap of ultrastructural findings in C3GN and DDD with a mixture of intramembranous elongate hyperdense deposits typical of DDD as well as discrete subendothelial and mesangial deposits typical of C3GN [35, 38].

The underlying genetic and functional abnormalities that determine the location and ultrastructural appearance of the C3 deposits in C3GN versus DDD are not completely understood. Some associations drawn from animal experiments and human studies have demonstrated key roles of several complement regulatory proteins including CFH, properdin, and C3NeF, and also highlighted the heterogeneous properties of C3NeF in activating the alternative complement system [23]. Genetic or pharmacological blockade of properdin converted the mild C3GN phenotype of CFH-deficient mice (Cfh$^{-/-}$) into a lethal C3GN phenotype with glomerular deposits which resembled those of human DDD [39]. CFH-deficient mice (Cfh$^{-/-}$) show depletion of both C3 and C5 in the plasma similar to the complement profile in humans with properdin-dependent C3NeF. Whereas mice deficient in both CFH and properdin (Cfh$^{-/-}$, P$^{-/-}$) show depletion of plasma C3 predominantly, which is similar to the complement profile in humans with properdin-independent C3NeF and may provide a better model for complement dysregulation and renal pathology findings in human DDD [13, 22, 23].

The historical classification of MPGN was further divided into MPGN types I, II (DDD), and III based on the distribution of the immune complex deposits, specifically with respect to the GBMs [40–43]. Cases of C3GN with an MPGN type I pattern display prominent, and sometimes confluent subendothelial C3 deposits and GBM duplication, with or without mesangial cell interposition (Fig. 14a, b). Early rodent studies demonstrated that mice with targeted deletion of CFH alone demonstrated glomerular C3 deposits which were primarily subendothelial in distribution [44] rather than intramembranous as seen in DDD. IF staining for complement factors C3 and C9 preceded the ultrastructural detection of deposits and the relatively late onset of MPGN-like changes by light microscopy. Other studies found that glomeruli with DDD contain various terminal complex components C5, C6, C7, C8, and C9 which appeared to be typical for DDD (and C3GN) and uncommon in IC-mediated MPGN [21, 36, 45].

C3G not only shows intramembranous and/or subendothelial glomerular deposits but can also have subepithelial deposits, sometimes in the absence of MPGN features. The subepithelial deposits can range from segmental and small (Fig. 15a), to global and prominent. In some cases, the subepithelial deposits can display a membranous pattern (Fig. 15b). Subepithelial humps occur but are usually not as prominent as those seen in acute postinfectious glomerulonephritis (Fig. 15c). Historically, an MPGN pattern of glomerular injury with prominent sub-

Fig. 14. C3GN with an MPGN pattern of injury. Large subendothelial deposits with GBM duplication define the MPGN type I pattern of injury (**a**, **b**), which can be accompanied by mesangial cell interposition (**a**). MPGN type III is historically subcategorized into Burkholder variant, with subepithelial deposits which can be separated by intervening spikes of basement membrane material and display a membranous appearance (**c**), and the Strife and Anders variant with complex intramembranous, subepithelial, and transmembranous deposits which are frequently associated with disruption of the lamina densa (**d**). Uranyl acetate and lead citrate stains, original magnifications: **a** ×3,000, **b** ×7,200, **c** ×7,200, **d** ×10,000.
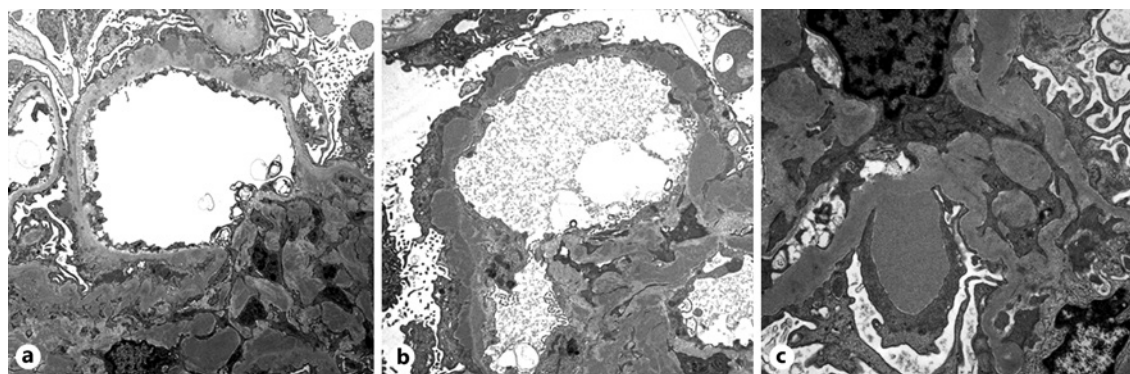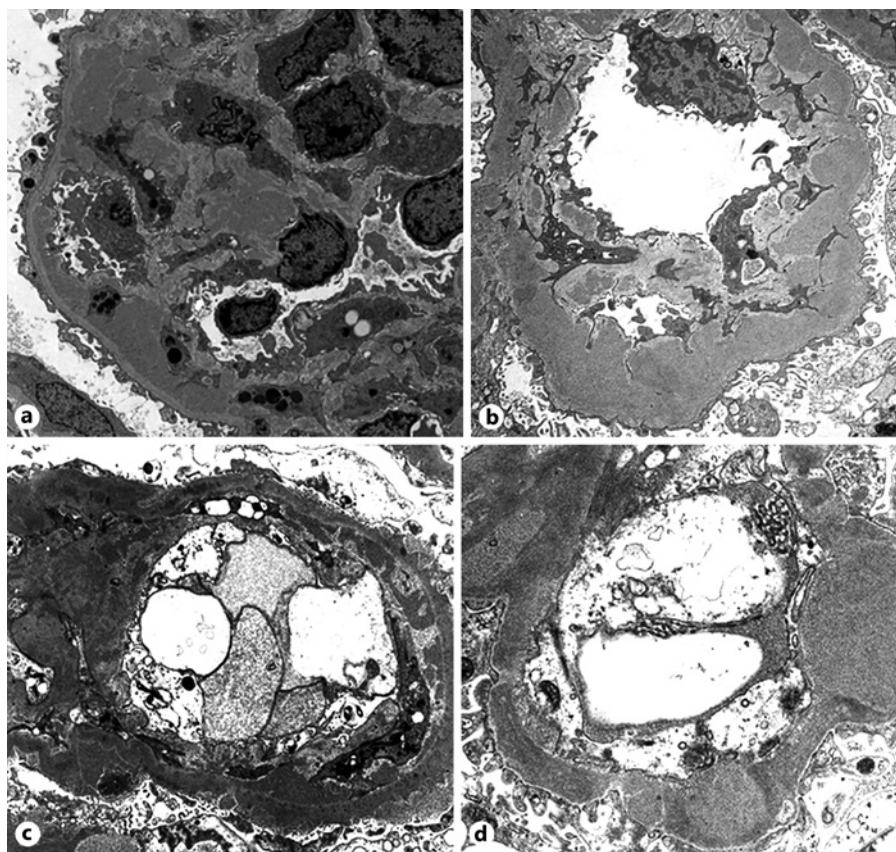

Fig. 15. Subepithelial deposits in the absence of membranoproliferative features can be seen in C3G. The subepithelial deposits may be segmental and discrete (**a**) or can be global and separated by intervening spikes of basement membrane material, with a membranous appearance (**b**). **c** Occasionally, subepithelial "hump"-shaped deposits may also be present, although these tend not to be the dominant ultrastructural appearance of subepithelial deposits Uranyl acetate and lead citrate stains, original magnifications: **a** ×5,000, **b** ×7,200, **c** ×14,000.

epithelial deposits by EM was termed MPGN type III. The Burkholder variant of MPGN III (Fig. 14c) is characterized by the presence of discrete subepithelial deposits which can be separated by spikes of GBM material, superimposed on MPGN type I features. The Strife and Anders variant [35] of MPGN III (Fig. 14d) is characterized by complex subepithelial and intra/transmembranous deposits that cause disruption and fraying of the lamina densa. Of note, cases of C3GN with subepithelial humps and glomerular C3 deposits may be difficult to distin-
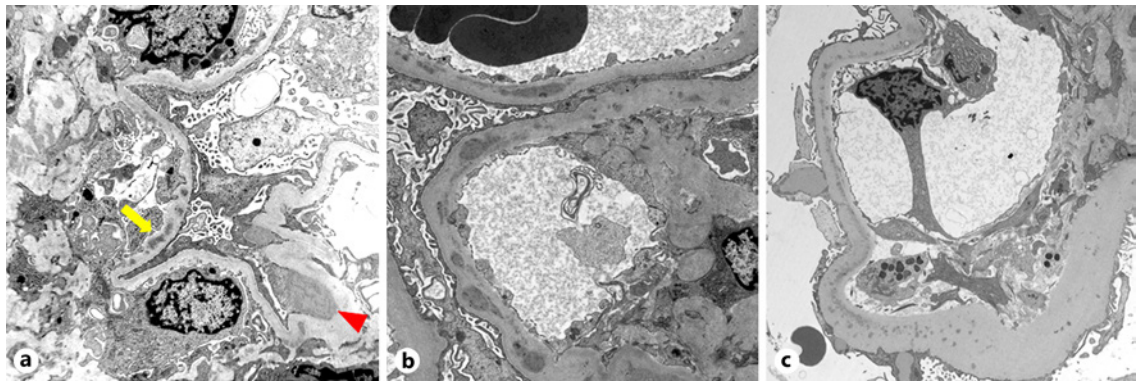
Fig. 16. a Infection-related (postinfectious) glomerulonephritis may display atypical features, with smudgy, elongated intramembranous deposits (yellow arrow) in the presence of typical "hump"-shaped deposits (red arrowhead). Discrete intramembranous deposits (**b**) may grow to confluence (**c**), morphologically similar to what is seen in DDD. Uranyl acetate and lead citrate stains, original magnifications: **a** ×3,000, **b** ×4,800, **c** ×4,800.

guish from infection-related or postinfectious glomerulonephritis, which will be discussed in the following section.

Mimickers

There are situations in which a definitive diagnosis of C3G may not be possible, particularly when the histologic or EM features are atypical. In these cases, the consensus report [2] suggests using the terminology of "glomerulonephritis with dominant C3." In practice, the differential diagnosis in this situation includes both C3G and PIGN. Differentiating the two entities starts with a detailed clinical history. A history of a prior infection, decreased serum C3 and C4, and positive ASO titers are suggestive of PIGN [46]. LM features of acute PIGN may show prominent glomerular neutrophilic infiltration (exudative features), crescents, and numerous fuchsinophilic subepithelial "hump"-shaped deposits on peripheral capillary walls and in mesangial "waist" regions [47, 48]. EM findings of elongated intramembranous hyperdense deposits can help distinguish DDD from PIGN. However, differentiating acute PIGN from hypercellular C3GN may not always be possible, as the prominent subepithelial "hump"-shaped deposits that are considered key ultrastructural findings in acute PIGN may also be present in cases of C3G [8, 49]. The considerable overlap in LM, IF, and EM findings has led some to suggest that C3G and PIGN may represent entities on the same disease spectrum [50].

The distinction may become especially problematic in cases of subacute or resolving PIGN, where the exudative features have disappeared and the subepithelial humps are no longer prominent; however, C3 deposits (mainly mesangial) are still detectable by IF microscopy, and smudgy mesangial deposits are identified by EM. "Atypical" PIGN can have EM findings overlapping with C3G (usually DDD features) such as the presence of elongated, smudgy intramembranous deposits, or detection of dense deposits in the lamina densa reminiscent of early DDD. Figure 16 displays representative images from cases where there was a documented history of infection preceding the renal biopsy, and where EM findings show GBM deposits which demonstrate very focal overlapping features with DDD (Fig. 16). With eventual clinical recovery, there was no further suspicion of a misdiagnosed or "masked" C3G. However, overlapping EM features occurring in a subset of "atypical PIGN" cases may indicate that an infection has triggered or "uncovered" an inherent underlying defect in the alternative complement pathway (genetic or acquired) that leads to C3GN-like clinical presentations and biopsy findings [51].

While the ultrastructural appearance of the GBM deposits can be helpful in distinguishing "typical" PIGN from DDD (subepithelial hump-shaped deposits in PIGN vs. elongated intramembranous deposits in DDD), it may not be practical to definitively distinguish PIGN from C3GN, where the ultrastructural appearance of the deposits can show considerable overlap. This highlights the importance of clinical history in the diagnosis. "Typical PIGN" is a self-limited process of relatively short duration, whereas atypical PIGN/C3G commonly follows a

protracted/chronic clinical course. Rapid normalization of serum complements would favor a diagnosis of PIGN, whereas persistent hypocomplementemia several months following disease onset might favor C3G. Patients with PIGN who are not recovering renal function as expected, or those with persistent isolated depression of serum C3 levels should be tested for an underlying abnormality in the alternative complement pathway. However, it should also be noted that a small subset of PIGN cases and some cases of C3G may not be associated with any alterations in complement levels, and normocomplementemia could indicate either disease [49]. Chauvet et al. [52] demonstrated that a significantly higher proportion of pediatric patients with acute PIGN have autoantibodies targeting factor B at disease onset compared to those with hypocomplementemic C3G, suggesting that screening for anti-factor B autoantibodies during the acute phase may be a potentially useful clinical test to help distinguish acute PIGN from C3G in those difficult cases with overlapping clinicopathologic features.

Pitfalls

The major impediment to the diagnosis of C3G is insufficient (frozen) tissue for IF and unreliable data on C3 staining. Unfortunately, techniques to reprocess formalin-fixed and paraffin-embedded tissue for IF studies, such as enzyme-based antigen retrieval, are not as effective for visualizing complement factor C3 deposits as they are for immunoglobulins or C1q [33]. Although a diagnosis of C3G may be suspected when typical EM findings are present (and serum C3 levels are decreased), a definitive diagnosis cannot be rendered without the diagnostic IF staining pattern. Furthermore, there may be biopsies in which C3-dominant IF staining has been demonstrated, but there are no glomeruli available for ultrastructural evaluation. In these cases, attempts to reprocess paraffin-embedded or frozen tissue for EM can be successful; however, severe tissue reprocessing artifacts and architectural distortion might impact proper diagnostic decision making [53].

Conclusion

While the diagnosis of C3G is dependent on IF studies and dominant staining for C3, EM analysis is necessary for diagnostic confirmation. In addition, clinical correlation with serum complement C3 levels as well as targeted testing for abnormalities in the alternative complement pathway is necessary for proper personalized patient management.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

References

1 Fakhouri F, Frémeaux-Bacchi V, Noël LH, Cook HT, Pickering MC. C3 glomerulopathy: a new classification. Nat Rev Nephrol. 2010; 6(8):494–9.

2 Pickering MC, D'Agati VD, Nester CM, Smith RJ, Haas M, Appel GB, et al. C3 glomerulopathy: consensus report. Kidney Int. 2013;84(6):1079–89.

3 Mandalenakis N, Mendoza N, Pirani CL, Pollak VE. Lobular glomerulonephritis and membranoproliferative glomerulonephritis: a Clinical and Pathologic Study based on renal biopsies. Medicine. 1971;50(4):319–55.

4 Sethi S, Fervenza FC. Membranoproliferative glomerulonephritis--a new look at an old entity. N Engl J Med. 2012;366(12):1119–31.

5 Appel GB, Cook HT, Hageman G, Jennette JC, Kashgarian M, Kirschfink M, et al. Membranoproliferative glomerulonephritis type II (dense deposit disease): an update. J Am Soc Nephrol. 2005;16(5):1392–403.

6 Hou J, Markowitz GS, Bomback AS, Appel GB, Herlitz LC, Barry Stokes M, et al. Toward a working definition of C3 glomerulopathy by immunofluorescence. Kidney Int. 2014;85(2): 450–6.

7 Ricklin D, Reis ES, Lambris JD. Complement in disease: a defense system turning offensive. Nat Rev Nephrol. 2016;12(7):383–401.

8 Cook HT, Pickering MC. C3 glomerulopathies, including dense deposit disease. In: Jeanette JC, Olson JL, Silva FG, D'Agati VD, editors. Heptinstall's pathology of the kidney. 7th ed. Philadelphia: Wolters Kluwer; 2015. 1. p. 341–66.

9 Zhang Y, Nester CM, Martin B, Skjoedt MO, Meyer NC, Shao D, et al. Defining the complement biomarker profile of C3 glomerulopathy. Clin J Am Soc Nephrol. 2014;9(11): 1876–82.

10 Thurman JM. Complement and the kidney: an overview. Adv Chronic Kidney Dis. 2020; 27(2):86–94.

11 Abrera-Abeleda MA, Nishimura C, Smith JL, Sethi S, McRae JL, Murphy BF, et al. Variations in the complement regulatory genes factor H (CFH) and factor H related 5 (CFHR5) are associated with membranoproliferative glomerulonephritis type II (dense deposit disease). J Med Genet. 2006;43(7):582–9.

12 Marinozzi MC, Chauvet S, Le Quintrec M, Mignotet M, Petitprez F, Legendre C, et al. C5 nephritic factors drive the biological phenotype of C3 glomerulopathies. Kidney Int. 2017;92(5):1232–41.

13 Smith RJH, Appel GB, Blom AM, Cook HT, D'Agati VD, Fakhouri F, et al. C3 glomerulopathy: understanding a rare complement-driven renal disease. Nat Rev Nephrol. 2019; 15(3):129–43.

14 Kopel T, Salant DJ. C3 glomerulopathies: dense deposit disease and C3 glomerulonephritis. UpToDate. Weltham, MA: 2018 (accessed July 7, 2019.

15 Kemper C, Atkinson JP, Hourcade DE. Properdin: emerging roles of a pattern-recognition molecule. Annu Rev Immunol. 2010;28: 131–55.

16 Servais A, Noel LH, Roumenina LT, Le Quintrec M, Ngo S, Dragon-Durey MA, et al. Acquired and genetic complement abnormalities play a critical role in dense deposit disease and other C3 glomerulopathies. Kidney Int. 2012;82(4):454–64.

17 Smith RJ, Alexander J, Barlow PN, Botto M, Cassavant TL, Cook HT, et al. New approaches to the treatment of dense deposit disease. J Am Soc Nephrol. 2007;18(9):2447–56.

18 Abrera-Abeleda MA, Nishimura C, Frees K, Jones M, Maga T, Katz LM, et al. Allelic variants of complement genes associated with dense deposit disease. J Am Soc Nephrol. 2011;22(8):1551–9.

19 Zhang Y, Meyer NC, Wang K, Nishimura C, Frees K, Jones M, et al. Causes of alternative pathway dysregulation in dense deposit disease. Clin J Am Soc Nephrol. 2012;7(2):265–74.

20 Jokiranta TS, Solomon A, Pangburn MK, Zipfel PF, Meri S. Nephritogenic lambda light chain dimer: a unique human miniautoantibody against complement factor H. J Immunol. 1999;163(8):4590–6.

21 Sethi S, Vrana JA, Fervenza FC, Theis JD, Sethi A, Kurtin PJ, et al. Characterization of C3 in C3 glomerulopathy. Nephrol Dial Transplant. 2017;32(3):459–65.

22 Lesher AM, Zhou L, Kimura Y, Sato S, Gullipalli D, Herbert AP, et al. Combination of factor H mutation and properdin deficiency causes severe C3 glomerulonephritis. J Am Soc Nephrol. 2013;24(1):53–65.

23 Ruseva MM, Vernon KA, Lesher AM, Schwaeble WJ, Ali YM, Botto M, et al. Loss of properdin exacerbates C3 glomerulopathy resulting from factor H deficiency. J Am Soc Nephrol. 2013;24(1):43–52.

24 Bomback AS, Santoriello D, Avasare RS, Regunathan-Shenk R, Canetta PA, Ahn W, et al. C3 glomerulonephritis and dense deposit disease share a similar disease course in a large United States cohort of patients with C3 glomerulopathy. Kidney Int. 2018;93(4):977–85.

25 Medjeral-Thomas NR, O'Shaughnessy MM, O'Regan JA, Traynor C, Flanagan M, Wong L, et al. C3 glomerulopathy: clinicopathologic features and predictors of outcome. Clin J Am Soc Nephrol. 2014;9(1):46–53.

26 Ravindran A, Fervenza FC, Smith RJH, De Vriese AS, Sethi S. C3 glomerulopathy: ten years' experience at mayo clinic. Mayo Clin Proc. 2018;93(8):991–1008.

27 Cook HTPM. C3 glomerulopathies, including dense deposit disease. In: Jeannette JCOJ, Silva FG, D'Agati VD, editors. Hepinstall's pathology kidney. Philadelphia, PA: Wolters Kluwer; 2015. 1. p. 341–6.

28 Ravindran A, Fervenza FC, Smith RJH, Sethi S. C3 glomerulopathy associated with monoclonal Ig is a distinct subtype. Kidney Int. 2018;94(1):178–86.

29 D'Souza YB, Jones CJ, Short CD, Roberts IS, Bonshek RE. Oligosaccharide composition is similar in drusen and dense deposits in membranoproliferative glomerulonephritis type II. Kidney Int. 2009;75(8):824–7.

30 Sethi S, Nasr SH, De Vriese AS, Fervenza FC. C4d as a diagnostic tool in proliferative GN. J Am Soc Nephrol. 2015;26(11):2852–9.

31 Goodship TH, Cook HT, Fakhouri F, Fervenza FC, Fremeaux-Bacchi V, Kavanagh D, et al. Atypical hemolytic uremic syndrome and C3 glomerulopathy: conclusions from a "kidney disease: improving global outcomes" (KDIGO) controversies conference. Kidney Int. 2017;91(3):539–51.

32 Nasr SH, Valeri AM, Appel GB, Sherwinter J, Stokes MB, Said SM, et al. Dense deposit disease: Clinicopathologic Study of 32 pediatric and adult patients. Clin J Am Soc Nephrol. 2009;4(1):22–32.

33 Nasr SH, Fidler ME, Said SM. Paraffin immunofluorescence: a valuable ancillary technique in renal pathology. Kidney Int Rep. 2018;3(6):1260–6.

34 Caravaca-Fontan F, Trujillo H, Alonso M, Diaz-Encarnacion M, Cabello V, Ariceta G, et al. Validation of a histologic scoring index for C3 glomerulopathy. Am J Kidney Dis. 2021; 77(5):684–95 e1.

35 Barbour TD, Pickering MC, Terence Cook H. Dense deposit disease and C3 glomerulopathy. Semin Nephrol. 2013;33(6):493–507.

36 Sethi S, Fervenza FC, Zhang Y, Zand L, Vrana JA, Nasr SH, et al. C3 glomerulonephritis: clinicopathological findings, complement abnormalities, glomerular proteomic profile, treatment, and follow-up. Kidney Int. 2012; 82(4):465–73.

37 Seif EI, Ibrahim EA, Elhefnawy NG, Salman MI. Dense deposit disease: a 29-years electron microscopy experience. Arab J Nephrol Transplant. 2013;6(3):153–60.

38 Sethi S, Fervenza FC, Smith RJ, Haas M. Overlap of ultrastructural findings in C3 glomerulonephritis and dense deposit disease. Kidney Int. 2015;88(6):1449–50.

39 Lesher AM, Nilsson B, Song WC. Properdin in complement activation and tissue injury. Mol Immunol. 2013;56(3):191–8.

40 Levy M, Gubler MC, Sich M, Beziau A, Habib R. Immunopathology of membranoproliferative glomerulonephritis with subendothelial deposits (Type I MPGN). Clin Immunol Immunopathol. 1978;10(4):477–92.

41 Habib R, Gubler MC, Loirat C, Mäiz HB, Levy M. Dense deposit disease: a variant of membranoproliferative glomerulonephritis. Kidney Int. 1975;7(4):204–15.

42 Strife CF, McEnery PT, McAdams AJ, West CD. Membranoproliferative glomerulonephritis with disruption of the glomerular basement membrane. Clin Nephrol. 1977; 7(2):65–72.

43 Burkholder PM, Hyman LR, Krueger RP. Characterization of mixed membranous and proliferative glomerulonephritis: recognition of three varieties. Perspect Nephrol Hypertens. 1973;1 Pt 1(Pt 1):557–89.

44 Pickering MC, Cook HT, Warren J, Bygrave AE, Moss J, Walport MJ, et al. Uncontrolled C3 activation causes membranoproliferative glomerulonephritis in mice deficient in complement factor H. Nat Genet. 2002;31(4):424–8.

45 Sethi S, Gamez JD, Vrana JA, Theis JD, Bergen HR 3rd, Zipfel PF, et al. Glomeruli of dense deposit disease contain components of the alternative and terminal complement pathway. Kidney Int. 2009;75(9):952–60.

46 Niaudet P.Post streptococcal glomerulonephritis. UpToDate. Waltham, MA; 2018 (accessed July 8, 2019.

47 Nasr SH, Markowitz GS, Stokes MB, Said SM, Valeri AM, D'Agati VD. Acute postinfectious glomerulonephritis in the modern era: experience with 86 adults and review of the literature. Medicine. 2008;87(1):21–32.

48 Montseny JJ, Meyrier A, Kleinknecht D, Callard P. The current spectrum of infectious glomerulonephritis. Experience with 76 patients and review of the literature. Medicine. 1995;74(2):63–73.

49 Khalighi MA, Wang S, Henriksen KJ, Bock M, Keswani M, Meehan SM, et al. Revisiting post-infectious glomerulonephritis in the emerging era of C3 glomerulopathy. Clin Kidney J. 2016;9(3):397–402.

50 Al-Ghaithi B, Chanchlani R, Riedl M, Thorner P, Licht C. C3 glomerulopathy and post-infectious glomerulonephritis define a disease spectrum. Pediatr Nephrol. 2016;31(11):2079–86.

51 Sethi S, Fervenza FC, Zhang Y, Zand L, Meyer NC, Borsa N, et al. Atypical postinfectious glomerulonephritis is associated with abnormalities in the alternative pathway of complement. Kidney Int. 2013;83(2):293–9.

52 Chauvet S, Berthaud R, Devriese M, Mignotet M, Vieira Martins P, Robe-Rybkine T, et al. Anti-factor B antibodies and acute postinfectious GN in children. J Am Soc Nephrol. 2020; 31(4):829–40.

53 Johannessen JV. Rapid processing of kidney biopsies for electron microscopy. Kidney Int. 1973;3(1):46–50.