

Advances in Membranous and Membranoproliferative glomerulonephritis: Insights into Pathogenesis

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Membranous Glomerulonephritis

Membranous glomerulonephritis is characterised by accumulation of immune complexes on the outside of the glomerular basement membrane. This leads to development of projections of the membrane adjacent to the deposits which eventually become incorporated into the membrane. In about 20% of cases the disease occurs in association with another condition such as SLE, cancer or drug exposure. The remaining cases are referred to as idiopathic. It is the most common cause of idiopathic nephrotic syndrome in Caucasian adults.

In 1959 Heymann and co-workers described a model of membranous glomerulonephritis in the rat (1). Heymann nephritis could be induced by immunising rats with preparations of tubular brush border proteins and was therefore thought to be produced by immune complexes forming between antibodies and tubular antigens and depositing in the glomerulus. However, it was subsequently shown that similar changes could be produced by injection of rabbit anti-rat brush border antibodies or even by perfusing antibodies into the kidney (2;3) indicating that the antibodies reacted with an intrinsic podocyte antigen with *in situ* immune complex formation. The podocyte antigen was identified in the early 1980s as a membrane protein now called megalin (4;5). This protein is a receptor expressed on podocyte foot processes where immune deposits are formed. The model provided considerable insight into the mechanisms of immune complex formation and also the important role for complement activation, and in particular the formation of the membrane attack complex, in causing proteinuria (6).

Following these insights from the Heymann model, researchers have sought a human equivalent of megalin acting as an intrinsic antigen in idiopathic membranous glomerulonephritis. However, it was not until 2002 that the first such antigen was identified by the group of Pierre Ronco (7;8). They studied the development of membranous glomerulonephritis in children born to mothers with genetic lack of neutral endopeptidase (NEP), a membrane associated podocyte antigen. The mothers developed antibodies to NEP that reacted with NEP in fetal glomeruli and led to the development of typical membranous glomerulonephritis. These studies were a proof that an intrinsic podocyte antigen could lead to *in situ* immune complex formation in humans and the search continued for other antigens. A major breakthrough was reported in 2009 from the laboratory of David Salant (9). They showed that 70% of patients with idiopathic membranous glomerulonephritis had circulating antibodies to a 185 kd protein extracted from human glomeruli. The antigen was identified as phospholipase A₂ receptor (PLA₂R). The predominant

subclass of antibody against PLA₂R was IgG4. These studies strongly suggest that in idiopathic membranous glomerulonephritis subepithelial deposits form through binding of autoantibodies to intrinsic podocyte antigens although definitive proof of the mechanism would require transferring disease to non-human primates. It seems likely that other podocyte antigens may also be identified in future. Further questions to be answered include 1. Why do autoantibodies to PLA₂R develop? 2. What are the mechanisms of proteinuria and how important is complement activation?

Membranoproliferative glomerulonephritis

'Membranoproliferative' describes a pattern of glomerular injury characterised by mesangial expansion and hypercellularity, and thickening of glomerular capillary walls. It is a pattern that may be seen with immunoglobulin and/or complement deposits in glomeruli or in other conditions such as chronic thrombotic microangiopathy. The term membranoproliferative glomerulonephritis (MPGN) is best reserved for those cases in which there is glomerular immunoglobulin and/or complement, and electron dense deposits on electron microscopy. These deposits are typically subendothelial and mesangial in location and often associated with duplication of the glomerular basement membrane and mesangial cell interposition. The terms membranoproliferative glomerulonephritis and mesangiocapillary glomerulonephritis (MCGN) are synonymous.

At least 3 variants of MPGN have been described in humans. Type I MPGN is the most common. Light microscopy shows increased glomerular lobulation with mesangial expansion and hypercellularity, and capillary wall thickening with a double contour appearance on silver staining. Electron microscopy demonstrates mesangial and subendothelial electron dense deposits and often scattered subepithelial deposits. Type I MPGN is a pattern of glomerular injury that may be associated with a number of chronic infections including viral hepatitis. It may be seen in mixed cryoglobulinaemia, and lupus glomerulonephritis may also show a membranoproliferative appearance.

Type II MPGN shows a characteristic electron microscopic appearance with dense osmiophilic transformation of the glomerular basement membrane and this appearance is the reason for the alternative name of Dense Deposit Disease (DDD). It is now clear that an MPGN appearance is only one morphological appearance of DDD. DDD typically occurs in the setting of hypocomplementaemia resulting from uncontrolled activation of the alternative pathway of complement.

Type III MPGN has features of type I with, in addition, either conspicuous subepithelial deposits similar to membranous glomerulonephritis (Burkholder type) or disruption and layering of the glomerular basement membrane (Strife type). Many pathologists consider these appearances to be variants of MPGN type I rather than separate entities.

Recent research has shown that many cases of MPGN, particularly those with deposition of C3 in the absence of immunoglobulins may be associated with abnormalities in proteins that control the complement pathway. I shall focus particularly on this area.

Dense Deposit Disease

Dense deposit disease is associated with complement dysregulation. The majority of individuals with DDD have hypocomplementaemia at some stage during their illness and the condition is strongly associated with the presence of C3 nephritic factor (C3NeF) (10). C3NeF is an IgG autoantibody that binds to the C3 cleaving enzyme complex (termed a C3 convertase) of the complement alternative pathway. To understand the action of C3NeF it is necessary to outline the key features of the complement alternative pathway (Figure 1).

Unlike the classical and lectin complement activation pathways, which are triggered by immune complexes and bacterial sugars respectively (Figure 1), the alternative pathway (AP) of complement activation is in a state of constant activation. This is a consequence of spontaneous hydrolysis in plasma of an internal thioester bond in complement C3. When the thioester bond is hydrolysed (denoted by C3i), C3 is able to interact with another complement plasma protein factor B. The complex C3iB is then a substrate for the serine protease factor D which cleaves factor B to form a complex (denoted by C3iBb). This complex has C3 convertase activity i.e. is able to cleave intact C3 to form C3b. C3b, in exactly the same manner as C3i, is able to generate a C3 convertase (C3bBb) through interaction with factor B and D. Therefore, in plasma there is continuous low-level generation of C3b. This pathway has been termed the C3 tick-over pathway. The generation of C3b proceeds at a low-level because of the presence of two plasma complement regulatory proteins: factor H and factor I. Factor H will inhibit the formation of the C3 convertases whilst factor I inactivates C3b by proteolysis. The importance of both factor H and factor I is seen by studying the complement profile of rare individuals with complete deficiencies of either of these proteins. Deficiency of factor H results in unhindered tick-over C3 activation and consequent severe secondary C3 deficiency. Deficiency of factor I results in accumulation of C3b in plasma.

It is now evident that DDD is strongly associated with dysregulation of the complement alternative pathway. C3NeF binds to the C3 convertase and potentiates its C3 cleaving function. The result is secondary C3 depletion in plasma. Furthermore, other distinct causes of excessive C3 convertase activity, all of which result in secondary C3 activation, have been associated with MPGN resembling DDD. These include dysfunctional C3 molecules (11), an anti-factor H autoantibody (12), a dysfunctional factor H molecule (13) and factor H deficiency (14).

Despite these strong associations between C3 dysregulation and DDD, the evidence in humans is predominantly circumstantial. Furthermore, C3NeF and C3 depletion

can be found among individuals without renal disease. Whether or not C3 nephritic factors are directly pathogenic in DDD or epiphenomena has consequently been hotly debated.

Our understanding of the role of factor H in glomerulonephritis has been greatly increased by the use of gene targeted mice lacking factor H developed in our laboratory by Matthew Pickering. These animals were developed by targeted gene deletion of exon 3 of the murine factor H gene in embryonic stem cells. Similar to homozygous factor H-deficient humans, these animals have extremely low levels of plasma C3 as a result of uncontrolled alternative pathway activation (15)

At 8 months all of the homozygous factor H-deficient mice had developed light microscopic features of MPGN with 23% mortality. Although MPGN in the factor H-deficient mice had many similarities with human DDD, particularly the presence of hypocomplementaemia and deposition of C3 and electron-dense material along the GBM, there are some important differences. The glomerular basement membrane electron-dense deposits in the factor H-deficient mice are located predominantly in a sub-endothelial location and linear dense transformation of the basement membrane, as in dense deposit disease, is not seen. Deposits did not develop in the mesangium or in the basement membranes of the tubules or Bowman's capsule. Similar to factor H-deficient pigs and humans, the glomerular basement membrane deposits stain directly for C3 and C9 but not immunoglobulin using immune electron microscopy.

Sequential analysis of the renal histopathology in these mice showed that glomerular C3 deposition preceded the appearance of GBM dense deposits, which in turn, preceded the histological light microscopic appearances of MPGN. Glomerular C3 deposition was evident as early as the first week of life, a time when both the light and electron microscopic appearance of the glomerulus was normal.

Novel mechanistic insights derived from the factor H-deficient mouse model include the very important observation that MPGN in this animal was totally dependent on C3 activation. Hence, preventing C3 activation (achieved by crossing factor H-deficient mice with factor B-deficient animals) completely prevented the development of MPGN (15). Furthermore, preventing C5 activation (achieved by crossing factor H-deficient mice with C5-deficient animals) did not prevent the development of the glomerular basement membrane deposits (16). However the inability to activate C5 was associated with a reduction in glomerular cellularity, serum creatinine levels and mortality. This suggests that whilst it would not be expected to prevent the development of glomerular basement membrane deposits, chronic inhibition of C5 activation in humans with MPGN associated with factor H dysfunction may be beneficial by reducing the glomerular inflammatory response. Whether this would also be applicable to factor H-sufficient individuals with DDD and C3NeF is not clear.

A novel observation has recently come from studies of animals with combined deficiency of both factor I and factor H (17). Factor I-deficient mice develop uncontrolled C3 activation through the alternative pathway together with renal abnormalities characterised by mesangial C3 deposits with nodular mesangial expansion, but importantly no evidence of GBM abnormalities. Human factor I deficiency is similarly associated with uncontrolled C3 activation through the alternative pathway and renal disease is uncommon, and no association with DDD has been described. Remarkably, factor H-deficient mice with deficiency of factor I do not develop MPGN. In these animals the glomerular lesion is identical to that seen in mice deficient in factor I alone. Administration of a source of factor I to mice with combined deficiency of H and I resulted in the appearance of glomerular C3 staining in a pattern identical to that seen in mice with deficiency of factor H alone. These data show that in the setting of factor H deficiency, factor I is required for the deposition of GBM C3 to develop with morphological changes of MPGN. These animal data suggest that plasma C3b targets the mesangium whilst plasma C3b metabolites target the GBM. The implication then is that in situations where GBM C3 is present, strategies that sequester C3b metabolites in the circulation will be therapeutically beneficial.

C3 glomerulonephritis (C3GN)

C3 glomerulonephritis has recently been proposed as a name for glomerulonephritis characterised by the presence of isolated glomerular C3 deposits on immunohistochemistry (18). At the ultrastructural level sub-endothelial and mesangial electron dense deposits are typical. C3GN is not DDD because DDD is characterised by extremely dense intra-membranous GBM transformation. Like DDD, the light microscopic features of C3GN are heterogeneous: in up to 75% of C3GN cases the glomerular changes are those of MPGN (C3GN with MPGN) whilst in others mesangial and subepithelial C3 deposits are present with no membranoproliferative change (C3GN without MPGN). When MPGN is present then C3GN is identical to the lesions described as 'MPGN1 with isolated sub-endothelial deposits of C3'. However, since MPGN is not invariably seen in C3GN it seems illogical to consider C3GN as a sub-type of MPGN. The clinical features of C3GN are also heterogeneous with up to 50% of patients retaining normal renal function whilst as many as 15% progress to end-stage renal disease. Both C3NeF and mutations in the AP regulatory proteins have been detected in C3GN patients and hence like DDD, C3GN is part of the spectrum of glomerular pathology that may develop in the setting of complement dysregulation.

Recently we have characterised two Cypriot families with inherited renal disease characterised by variable glomerular inflammation and sub-endothelial GBM deposits containing C3 but not immunoglobulin. Affected individuals were shown to have a mutation in a complement factor H-related protein (termed complement factor H-related protein 5) (Gale D et al, Lancet, in press). It is noteworthy that complement mutations were specifically sought in these families because of the unusual renal

biopsy appearances: the presence of glomerular inflammation and isolated sub-endothelial deposits of C3.

Mutations in the proteins of the AP have been detected among individuals with MPGN1 where both glomerular complement and immunoglobulin deposition was seen. We speculate that when there is antibody-triggered complement activation through the classical pathway within the kidney, the presence of AP dysregulation could result in uncontrolled amplification of C3 and enhanced complement-mediated renal damage. C3 nephritic factors may be detected in MPGN1 and it is conceivable that in these cases the renal immunoglobulin represents C3NeF which is enhancing local immune complex-triggered complement activation. This is analogous to anti-C1q antibodies which have been shown to potentiate complement activation in immune complex-mediated renal disease.

In summary, there are a group of glomerular pathologies in which there is abnormal accumulation of complement C3 as a consequence of genetic or acquired disorders of complement regulation. The causes of complement dysregulation are heterogeneous and screening individuals is complex (examples of investigations that can be considered are shown in table 1). The glomerular pathology is also heterogeneous with variable glomerular inflammation and GBM deposits that may be sub-endothelial, intra-membranous or sub-epithelial in location although a membranoproliferative pattern is common. We have recently suggested that glomerular lesions with these pathological features could be usefully classified under the term, 'C3 glomerulopathy' (Fakhouri F, submitted)

Table 1. In 2010 examples of specialist investigations that could be considered in patients with C3 glomerulopathy

Complement investigations:	Measurement of serum complement proteins: <ul style="list-style-type: none">• C3• factor H• factor I• factor B <p>C3 nephritic factor</p> <p>Anti-factor H autoantibodies</p> <p>CD46 (membrane cofactor protein) expression on peripheral blood mononuclear cells</p>
Mutation screening:	Complement regulator genes: <ul style="list-style-type: none">• factor H• factor I• CD46 (membrane cofactor protein)• factor H-related protein family (CFHR1, 2, 3, 4, and 5) <p>Complement activation protein genes:<ul style="list-style-type: none">• factor B• C3<p>Assessment of copy number variation across the CFH-CFHR locus*</p></p>

*copy number variations (e.g. whole and internal gene duplications or rearrangements) will not be reliably identified by exon sequencing of genomic DNA.

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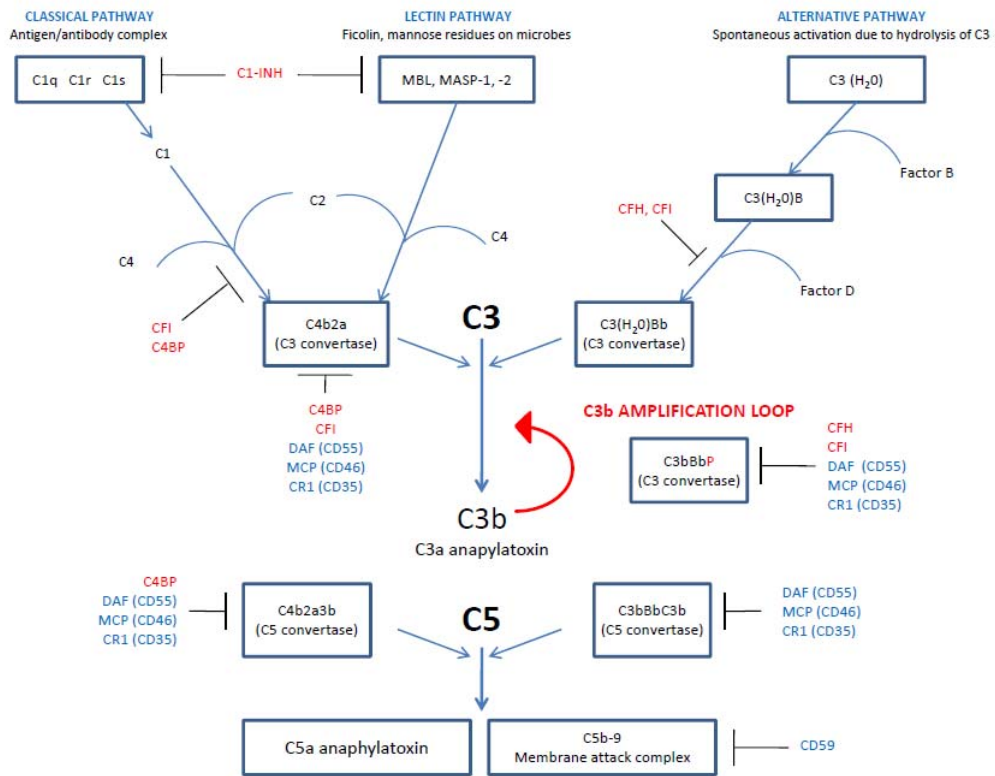


Figure 1