

## Membranoproliferative Glomerulonephritis Type II (Dense Deposit Disease): An Update

Gerald B. Appel,\* H. Terence Cook,<sup>†</sup> Gregory Hageman,<sup>‡</sup> J. Charles Jennette,<sup>§</sup> Michael Kashgarian,<sup>||</sup> Michael Kirschfink,<sup>¶</sup> John D. Lambris,<sup>#</sup> Lynne Lanning,<sup>\*\*</sup> Hans U. Lutz,<sup>††</sup> Seppo Meri,<sup>‡‡</sup> Noel R. Rose,<sup>§§</sup> David J. Salant,<sup>|||</sup> Sanjeev Sethi,<sup>¶¶</sup> Richard J.H. Smith,<sup>###</sup> William Smoyer,<sup>\*\*\*</sup> Hope F. Tully,<sup>+++</sup> Sean P. Tully,<sup>+++</sup> Patrick Walker,<sup>†††</sup> Michael Welsh,<sup>§§§</sup> Reinhard Würzner,<sup>||||</sup> and Peter F. Zipfel<sup>¶¶¶</sup>

\*Columbia University Department of Nephrology, New York, New York; <sup>†</sup>Division of Investigative Science, Imperial College Faculty of Medicine, London, England; <sup>‡</sup>Department of Ophthalmology and Visual Sciences, University of Iowa, Carver College of Medicine, Iowa City, Iowa; <sup>§</sup>Department of Pathology and Laboratory Medicine, University of North Carolina, Chapel Hill, North Carolina; <sup>||</sup>Department of Pathology, Yale University School of Medicine, New Haven, Connecticut; <sup>¶</sup>Institute of Immunology, University of Heidelberg, Heidelberg, Germany; <sup>#</sup>Department of Pathology and Laboratory Medicine, University of Pennsylvania, Philadelphia, Pennsylvania; <sup>\*\*</sup>Kidneeds, Iowa City, Iowa; <sup>††</sup>Institute of Biochemistry, Swiss Federal Institute of Technology, Zurich, Switzerland; <sup>‡‡</sup>Department of Bacteriology and Immunology, University of Helsinki and Helsinki University Central Hospital, Helsinki, Finland; <sup>§§</sup>Center for Autoimmune Disease Research, Johns Hopkins School of Medicine, Baltimore, Maryland; <sup>|||</sup>Department of Medicine, Boston University Medical Center, Boston, Massachusetts; <sup>¶¶</sup>Department of Pathology, University of Iowa, Carver College of Medicine, Iowa City, Iowa; <sup>###</sup>Department of Otolaryngology, University of Iowa Carver College of Medicine, Iowa City, Iowa; <sup>\*\*\*</sup>Pediatric Nephrology Division, University of Michigan, Ann Arbor, Michigan; <sup>+++</sup>Milagros Research Fund, Chappaqua, New York, New York; <sup>†††</sup>Nephropathology Associates, Little Rock, Arkansas; <sup>§§§</sup>Department of Internal Medicine, University of Iowa Carver College of Medicine, Iowa City, Iowa; <sup>||||</sup>Department of Hygiene, Microbiology and Social Medicine, Innsbruck Medical University, Innsbruck, Austria; and <sup>¶¶¶</sup>Hans Knoell Institute for Natural Products Research, Jena, Germany

Membranoproliferative glomerulonephritis type II (MPGN II) is a rare disease characterized by the deposition of abnormal electron-dense material within the glomerular basement membrane of the kidney and often within Bruch's membrane in the eye. The diagnosis is made in most patients between the ages of 5 and 15 yr, and within 10 yr, approximately half progress to end-stage renal disease, occasionally with the late comorbidity of visual impairment. The pathophysiologic basis of MPGN II is associated with the uncontrolled systemic activation of the alternative pathway (AP) of the complement cascade. In most patients, loss of complement regulation is caused by C3 nephritic factor, an autoantibody directed against the C3 convertase of the AP, but in some patients, mutations in the factor H gene have been identified. For the latter patients, plasma replacement therapy prevents renal failure, but for the majority of patients, there is no proven effective treatment. The disease recurs in virtually all renal allografts, and a high percentage of these ultimately fail. The development of molecular diagnostic tools and new therapies directed at controlling the AP of the complement cascade either locally in the kidney or at the systemic level may lead to effective treatments for MPGN II.

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The membranoproliferative glomerulonephritides are diseases of diverse and often obscure cause and pathogenetic mechanisms that account for approximately 4 and 7% of primary renal causes of nephrotic syndrome in children and adults, respectively (1). On the basis of immunopathology and ultrastructure analysis of the kidney and of the

glomerulus in particular, three subtypes are recognized. Membranoproliferative glomerulonephritis (MPGN) types I and III are variants of immune complex-mediated disease; MPGN II, in contrast, has no known association with immune complexes.

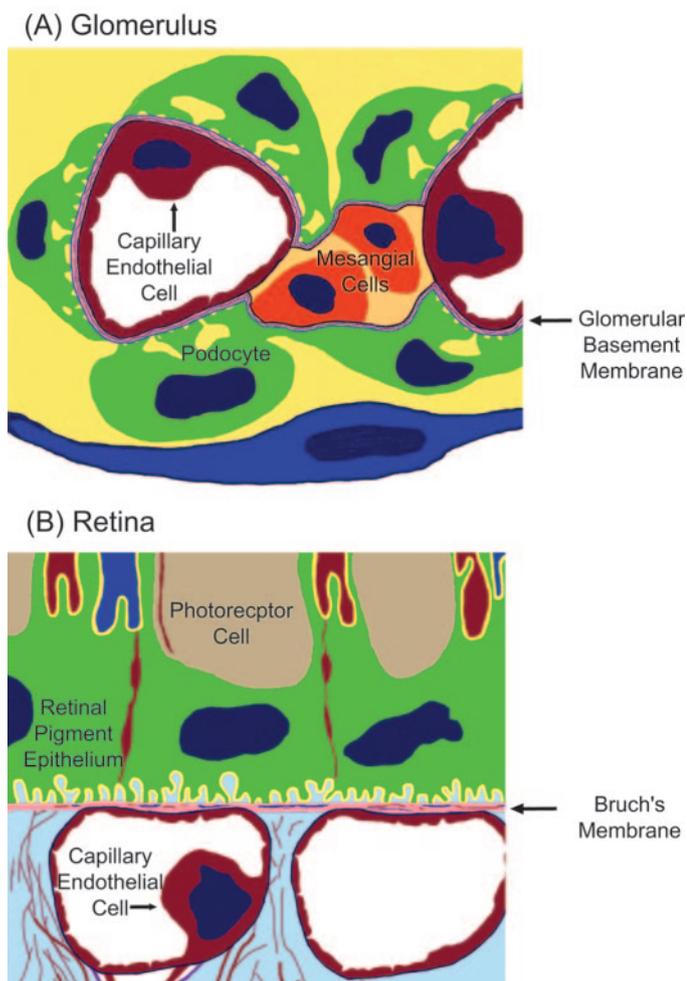
MPGN II is rare. It accounts for <20% of cases of MPGN in children and only a fractional percentage of cases in adults (2). Its morphologic hallmark is the presence of dense deposits within the glomerular basement membrane (GBM) as resolved by electron microscopy. In many individuals with MPGN II, deposits of similar composition and structure occur along the choriocapillaris-Bruch's membrane-retinal pigment epithelial

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Address correspondence to: Dr. Richard J.H. Smith, Department of Otolaryngology, 200 Hawkins Drive, 21151 PFP, The University of Iowa, Iowa City, IA 52242. Phone: 319-356-3612; Fax: 319-356-4108; E-mail: [richard-smith@uiowa.edu](mailto:richard-smith@uiowa.edu)

interface, a region that is morphologically similar to the capillary tuft-GBM-glomerular epithelial interface (Figure 1). Spontaneous remissions are rare, and most affected individuals progress to end-stage renal disease (ESRD), occasionally with the late comorbidity of impaired visual acuity and fields (3–13).

The purpose of this article is to summarize the proceedings of the first meeting of the international MPGN II Focus Group. We provide a comprehensive review of the clinical, histopathologic, and pathophysiologic features of MPGN II, focusing on the role of complement and complement dysregulation in the pathogenesis of this disease so that effective evidence-based treatments may be developed.

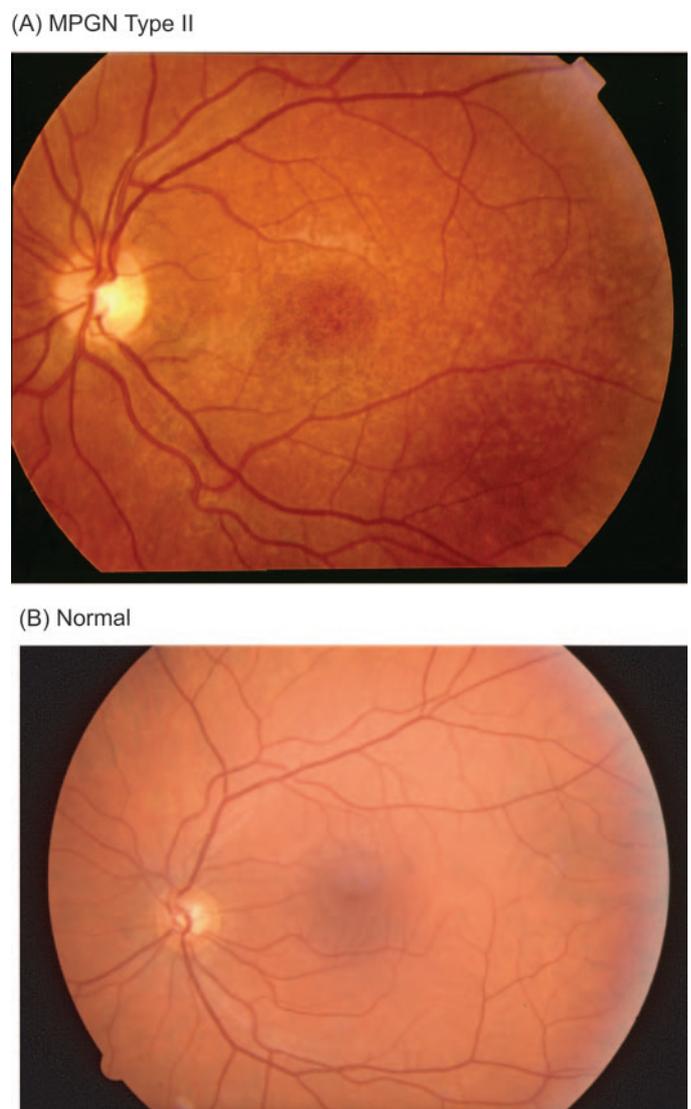


**Figure 1.** Schematic drawings that compare the fenestrated capillary networks in the glomerulus (A) and retina (B). The glomerular podocytes are similar to the retinal pigment epithelial cells, both of which are separated by a basement membrane (either the glomerular basement membrane [GBM] or Bruch's membrane, respectively) from the fenestrated capillary endothelial cells of the glomerular capillary tufts and the choriocapillaris. Both basement membranes are sites of electron-dense deposits in membranoproliferative glomerulonephritis type II (MPGN II).

## Clinical Diagnosis

MPGN II affects both genders equally and is usually diagnosed in children who are between 5 and 15 yr of age and present with one of five findings: Hematuria, proteinuria, hematuria and proteinuria, acute nephritic syndrome, or nephrotic syndrome. Although these findings are nonspecific, >80% of patients with MPGN II are positive for serum C3 nephritic factor (C3NeF), an autoantibody directed against C3bBb, the convertase of the alternative pathway (AP) of the complement cascade (14). Because C3NeF is present in up to one half of people with MPGN types I and III, the definitive diagnosis of MPGN II depends on the ultrastructural demonstration of dense deposits in the GBM.

Patients with MPGN II can develop drusen (Figure 2). These whitish-yellow deposits lie within the ocular Bruch's mem-



**Figure 2.** A funduscopy picture of MPGN II-associated retinal changes (A) as compared with a normal retina (B). The long-term risk for visual problems caused by drusen in MPGN II is approximately 10%. There is no correlation between disease severity in the kidney and the eye.

brane, beneath the retinal pigment epithelium. In contrast to drusen that form in age-related macular degeneration, drusen in individuals with MPGN II occur at an early age and often are detectable in the second decade of life. The distribution of these deposits varies among patients (4,15,16) and initially has little impact on visual acuity and fields. Over time, however, specialized tests of retinal function, such as dark adaptation, electroretinography, and electrooculography, can become abnormal. Vision can deteriorate as subretinal neovascular membranes, macular detachment, and central serous retinopathy develop (4). The long-term risk for visual problems is approximately 10%. There is no correlation between disease severity in the kidney and the eye, and an ophthalmologic examination at the time of diagnosis and periodic fundoscopic assessments should be part of patient treatment (17).

MPGN II can be associated with acquired partial lipodystrophy (APL) (18). The loss of subcutaneous fat in the upper half of the body usually precedes the onset of kidney disease by several years and can result in a strikingly haggard facial appearance. Misra *et al.* (19) reported that approximately 83% of APL patients have low C3 levels and polyclonal C3NeF and that approximately 20% go on to develop MPGN after a median of approximately 8 yr after the onset of lipodystrophy. Compared with APL patients without renal disease, those with MPGN have an earlier age of onset of lipodystrophy ( $12.6 \pm 10.3$  versus  $7.7 \pm 4.4$  yr, respectively;  $P < 0.001$ ) and a higher prevalence of C3 hypocomplementemia (78 versus 95%, respectively;  $P = 0.02$ ). The link between these two entities seems to be related to the effects of dysregulation of the AP of the complement cascade on both kidney and adipose tissue (20). The deposition of activated components of complement in adipose tissue results in the destruction of adipocytes in areas high in factor D (fD; adipsin) content.

Spontaneous remissions of MPGN II are uncommon (2,21). The more probable outcome is chronic deterioration of renal function leading to ESRD in approximately half of patients within 10 yr of diagnosis (22–25). In some patients, rapid fluctuations in proteinuria occur with episodes of acute renal deterioration in the absence of obvious triggering events; in others, the disease remains stable for years despite persistent proteinuria.

In >50% of patients with MPGN II, serum C3NeF persists throughout the disease course (14). C3NeF is nearly always associated with clinical evidence of complement activation such as a reduction in CH50, a decrease in C3, and an increase in C3dg/C3d; however, the relationship among C3NeF, C3 levels, and prognosis is unclear. Some groups report no correlation between C3 levels and clinical course (18,24,26,27), whereas other groups have found persistent hypocomplementemia indicative of a poor prognosis (28,29).

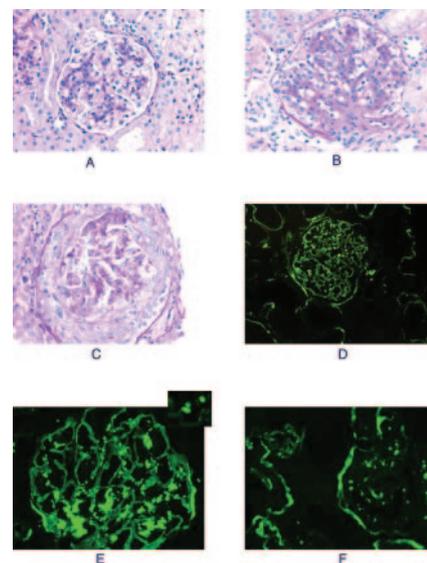
These differences may be reconciled by noting that not all C3NeF are directed against the same epitope and that epitopes can change in an individual over time. Ohi *et al.* (30) provided evidence for the first possibility in their report of six patients with detectable C3NeF in the absence of hypocomplementemia, showing that in these cases, C3NeF did not interfere with factor H (fH)-induced inactivation of C3bBb. Spitzer and Stitzel (31)

documented the second possibility in three people whose C3 levels eventually normalized despite continued C3NeF production. C3NeF isolated from these patients and added to normal sera mediated consumption of C3, as did the addition of normal factor B (fB) to their sera, consistent with a change in the fB autoantigen in these patients.

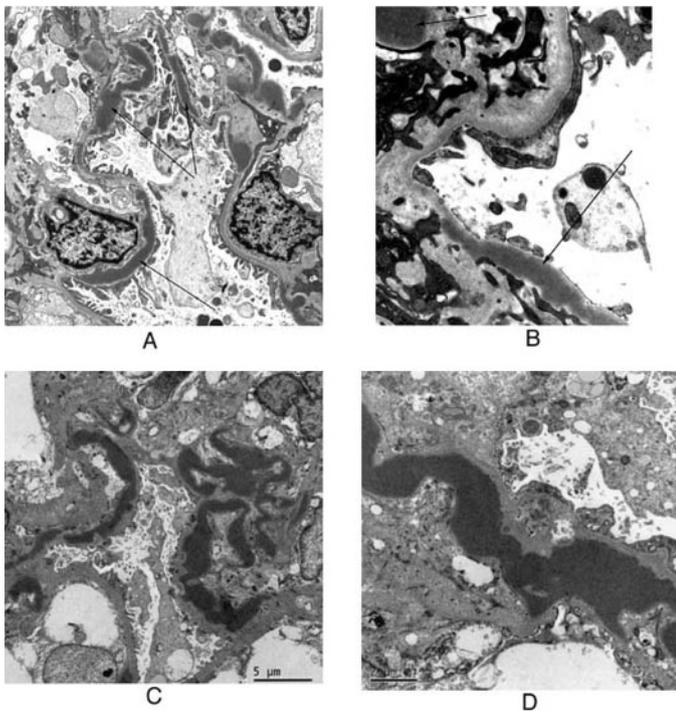
## Histopathology

The term *membranoproliferative glomerulonephritis* is a histologic reference to the thickening of capillary walls, intense glomerular hypercellularity, and increased amounts of mesangial matrix that are usually apparent at the light microscopic level (Figure 3). However, it is the dense intramembranous deposits in the GBM that are the pathognomonic feature of MPGN II (Figure 4). In fact, dense deposit disease is a more accurate descriptive name than MPGN II because dense deposits are diagnostic and are not invariably associated with prominent capillary wall thickening or hypercellularity (Figure 3A).

The normal GBM is built from a three-dimensional scaffold of type IV collagen in the *lamina densa* and provides mechanical stability, a framework for proteoglycans and glycoproteins, and a size-selective filtration barrier to plasma proteins >150 kD (1,32,33). Core proteins and glycosaminoglycans (GAG) concentrate in a regular lattice-like network on either side of the *lamina densa* in the *laminae rarae internae* and *externae* and give the GBM its negative charge. Most abundant is heparan sulfate,



**Figure 3.** The light microscopic appearance of MPGN II varies from mild mesangial hypercellularity (A) through a membranoproliferative pattern (B) to crescent glomerulonephritis (C). C3 is present in an interrupted band pattern along GBM, tubular basement membranes, and the basement membranes of Bowman's capsule (D). C3 in the mesangial areas can result in a prominent spherule or "ring" like pattern (E). Along tubular basement membranes, C3 is present in an interrupted pattern (F). Magnification,  $\times 400$  (periodic acid-Schiff stain) in A through C;  $\times 200$  (fluorescein-conjugated anti-C3) in D;  $\times 400$  (fluorescein-conjugated anti-C3) in E and F;  $\times 1000$  (fluorescein-conjugated anti-C3) in E inset.



**Figure 4.** Electron microscopy reveals an interrupted band pattern of extremely electron-dense material (arrows) along the capillary walls and paramesangial GBM (A). Electron-dense material (arrows) in the mesangial areas sometimes appears as spheres (B). Electron-dense material forming masses with open areas within the mesangial region may correspond to mesangial “rings” seen by immunofluorescence (C; see Figure 3E). Electron-dense material extending from the mesangium into the capillary loop with the basement membrane on either side produces a “tram-track” pattern (D). It is intriguing to speculate that these dense deposits may be the result of continuous complement activation (see Figure 6). Magnification,  $\times 4000$  (uranyl acetate and lead citrate) in A;  $\times 10,000$  (uranyl acetate and lead citrate) in B;  $\times 7500$  (uranyl acetate and lead citrate) in C;  $\times 15,000$  (uranyl acetate and lead citrate) in D.

which contributes approximately 90% of the negative charge of the GBM. It promotes hydration, prevents obstruction, and acts as a charge-selective barrier to small polyanionic plasma proteins of 70 to 150 kD in size (1,33).

The dense deposits associated with MPGN II are distributed in a segmental, discontinuous, or diffuse pattern in the *lamina densa* of the GBM. By light microscopy, they are eosinophilic and refractile, stain brightly with periodic acid-Schiff, and are highly osmophilic, explaining their electron-dense appearance (32) (Figure 4). Even at high magnification, the deposits lack substructure and appear as a very dark homogeneous smudge. Often, they are present in the mesangial matrix, along the basement membranes of Bowman’s capsule, and around small vessels. They also stain brightly with thioflavine-T and wheat germ agglutinin (32,34), suggesting the presence of large amounts of N-acetyl-glucosamine. As compared with normal GBM, there are distinct differences in amino acid and carbohydrate composition in dense deposits with decreased and in-

creased cysteine and N-acetyl-neuraminic acid levels, respectively ( $P < 0.01$  for both) (35). Still, the exact composition of dense deposits remains undetermined.

Mesangial hypercellularity and matrix interposition occur as the disease progresses, with the degree of involvement ranging from minimal to diffuse among different glomeruli even within the same biopsy specimen (36). Podocyte changes also develop, perhaps reflecting either an interference with podocyte-GBM-mesangial cell cross-talk or changes in the negative surface charge on podocytes (37). Although major causes of podocyte injury leading to ESRD include perturbation of the actin cytoskeleton and interference with the slit diaphragm–lipid raft complex, these two events are not thought to be central to the progression of MPGN II. If early damage is not reversed, then severe and progressive changes develop in the GBM, ultimately leading to podocyte detachment, hypertrophy, and death (38).

The characteristic immunopathologic finding in MPGN II is intense deposition of C3 along the glomerular capillary walls in a ribbon-like pattern and in the mesangial regions as coarse granules or spherules. Often, a double contour linear “railroad track” is apparent along capillary walls with a “ring” forming around mesangial deposits as if only the outer surface of the deposits is staining. More specific immunohistology has shown that C3c is the primary constituent of dense deposits in many patients with MPGN II; however, in patients with rapidly progressive MPGN II, dense deposits react with anti-C3d antibodies as well as anti-C3c antibodies. This difference suggests the presence of both C3b and iC3b in patients with rapidly progressive disease, because all C3 breakdown products except C3c react with anti-C3d. Notably absent from dense deposits and other regions of the glomerulus are deposits of IgG, suggesting that C3NeF is not a constituent of dense deposits and that dense deposits do not represent deposition of immune complexes (38) (Figures 3 and 5). Similar deposits are seen in Bruch’s membrane in the eye and in the sinusoidal basement membranes of the spleen (4,15–17,39).

## Complement in MPGN II

The complement system is a complex cascade in which proteolytic cleavage of glycoproteins induces an inflammatory response, phagocyte chemotaxis, opsonization, and cell lysis. It is triggered through three different pathways—the classical, alternative, or mannose-binding lectin—that converge on C3 to ultimately form the membrane attack complex, C5b678 (9). In MPGN II, the alternative pathway (AP) is systematically activated at a high level.

C3 is the most abundant complement protein in serum (1.2 mg/ml). It normally undergoes low levels of continuous auto-activation by hydrolysis of its thioester. Hydrolyzed C3 (C3[H<sub>2</sub>O]) binds fB to form C3(H<sub>2</sub>O)B, which after cleavage to C3(H<sub>2</sub>O)Bb by fD cleaves C3 to C3a and C3b. C3b recruits fB and fD releases Ba to generate C3bBb, the C3 convertase of the AP. The amplifying convertase produces nascent C3b by way of a fleeting intermediate that reacts with water, hydroxyl groups on complex carbohydrates, cell surfaces, immune complexes, and free IgG within a radius of approximately 60 nm from the point of its generation (40).

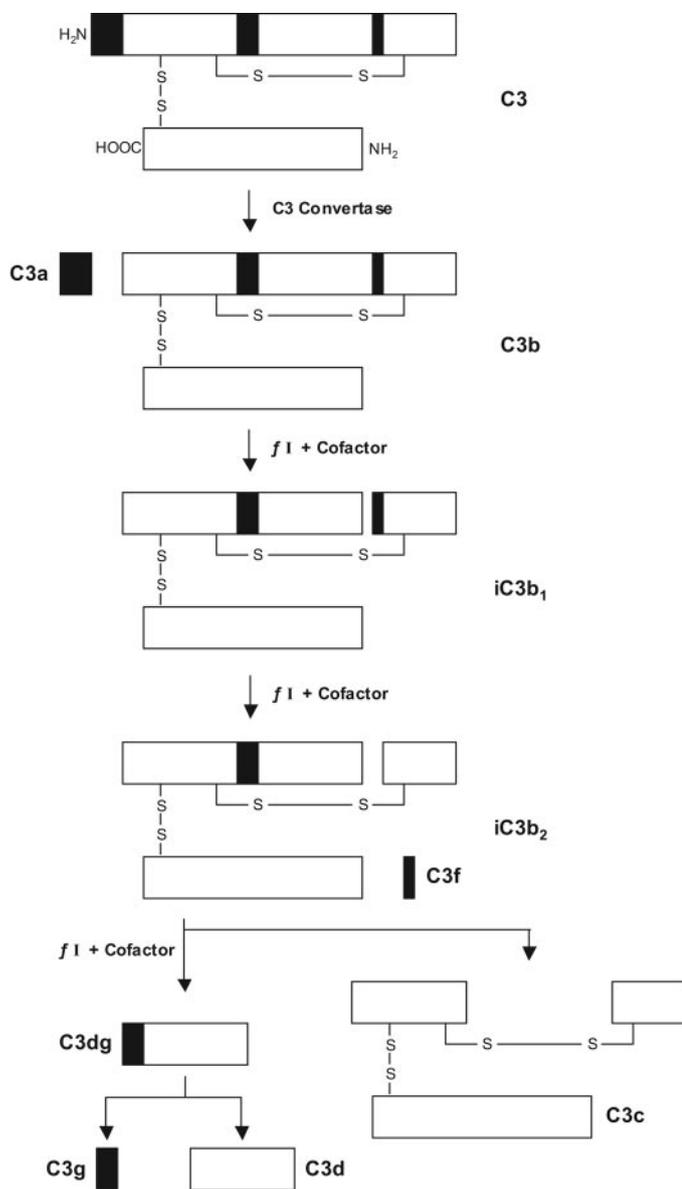


Figure 5. Native C3 consists of two chains joined by a disulfide bond. Activation by C3 convertase cleaves off C3a, an anaphylatoxin, to form C3b. Because C3 is cleaved into many fragments, immunostaining can be done using antibodies to different breakdown products of C3. In many patients with MPGN II, only immunostaining with anti-C3c antibodies is positive; however, in patients with rapidly progressive MPGN II, dense deposits also are recognized by anti-C3d antibodies, suggesting the presence of C3b and iC3b. IgG is absent.

Nascent C3b that reacts with water forms free C3b that has a half-life of <1 s in the presence of fH and fI in the fluid phase. However, nascent C3b that binds covalently to large molecules is partially protected from inactivation. Because IgG is the second most abundant protein in plasma and C3 has a weak affinity for IgG, during systemic activation of the complement cascade in the fluid phase, nascent C3b reacts predominantly with IgG to produce (C3b)<sub>2</sub>-IgG complexes (41). (C3b)<sub>2</sub>-IgG complexes are far better precursors of the C3 convertase of the

AP than free C3b because in addition to being protected from inactivation by fH, they are intrinsically more potent than C3b in assembling a C3 convertase, presumably because they first bind properdin, which facilitates fB binding (42,43) (Figures 5 and 6).

In MPGN II, C3NeF prolongs the half-life of C3 convertase by binding to either C3bBb or IgG-C3b-C3bBb of the assembled convertase. C3NeF slows down dissociation of factor Bb from the C3 convertase precursor, and as a result, this neoenzyme can interact with its substrates for a longer period of time. The exact mechanism by which this stabilization occurs is unknown

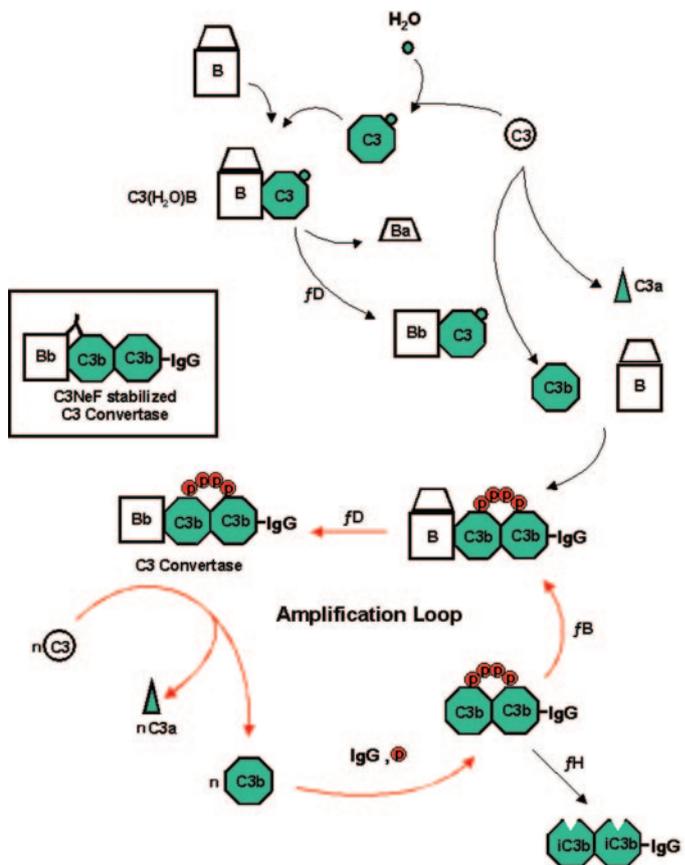


Figure 6. The alternative pathway of the complement cascade is systematically activated at a high level in patients with MPGN II. Normally, continuous low levels of activation of C3 occur by spontaneous hydrolysis. Hydrolysis causes a large conformational change in C3 to make C3(H<sub>2</sub>O) more similar to C3b, although C3a is still attached. The initial convertase, C3(H<sub>2</sub>O)Bb, activates C3 to C3b. C3b has a fleeting half-life, but if it binds to IgG, cells, or basement membranes, then it is somewhat protected from immediate inactivation. C3 has a weak affinity for IgG and so (C3b)<sub>2</sub>-IgG complexes form in the fluid phase. These complexes bind properdin (P), which facilitates factor B (fB) binding and generation of the C3 convertase of the alternative pathway (red arrows, amplification loop). C3NeF (inset) prolongs the half-life of C3 convertase by binding to a neo-epitope on either C3bBb or Bb. In the mouse mutant deficient for both factor H (fH) and fB, C3bBb cannot form, so activation of the alternative pathway of the complement cascade does not occur.

and may vary among patients, consistent with suspected differences in C3NeF itself.

The normal protective and regulatory mechanisms that control C3bBb levels and complement complex deposition on self-cells involve seven proteins. Four of these proteins are present in the serum (fH, factor H-like protein 1 [FHL-1], factor I [fI], and C4 binding protein [C4BP]), and three are cell membrane-associated proteins (membrane co-factor protein [MCP, CD46], decay accelerating factor [DAF, CD55], and complement receptor 1 [CR1, CD35]). With the exception of fI, these proteins belong to the regulators-of-complement-activation (RCA) family of proteins on chromosome 1q32. A striking structural feature shared by the RCA family is homologous 60–amino acid domains known as short consensus repeats (SCR). CR1 has 30, fH has 20, FHL-1 has seven, and CD55 has four of these domains (44).

fH is a soluble glycoprotein present in blood at concentrations ranging from 110 to 615  $\mu\text{g}/\text{ml}$ . It regulates complement both in fluid phase and on cellular surfaces by binding to three sites on C3b destabilizing C3bBb. In fluid phase, this interaction results in dissociation of C3bBb into inactive fBb (ifBb) and C3bfH, which is irreversibly inactivated into iC3b by fI (45). On surfaces, the inactivation of bound C3b is dependent on the chemical composition of the surface to which C3b is bound (46).

Binding of C3bBb by C3NeF makes this complex far more resistant to fH-mediated inactivation than properdin-stabilized convertase (40,47). iC3b that does form can bind to CR1, a polymorphic membrane protein of 190 to 280 kD present on most peripheral cells. CR1 on erythrocytes accounts for almost 90% of the regulator in blood (48). Approximately 15% of healthy people have low CR1 erythrocyte levels, and in a few people, levels are extremely low (49,50). Whether there is an association with this variability and MPGN II is not known. CR1 is also expressed on podocytes, where its biologic function remains speculative. A loss of CR1 on podocytes has been found in various nephropathies, including severe lupus nephritis and crescentic nephritis, and its release as CR1-coated vesicles in the urine is considered a marker of podocyte injury (51). Cleavage fragments of C3b such as C3c and C3dg are found in the plasma of patients with MPGN II (Figure 5).

fH also binds to polyanions, such as heparin on cells and membranes, and protects these surfaces from AP-mediated complement activation (52). This discriminatory activity of fH is dependent on specific SCR, which recognize sialic acid and other negatively charged GAG (Figure 7). The importance of this protective role is highlighted by the fact that MPGN II develops in humans, pigs, and mice that are deficient in fH (36,53–55).

In addition to fH, there are five other members of this protein family, although their functional properties have not been defined fully. fH-related protein 3 (FHR3), two forms of FHR4 termed FHR4A and FHR4B, and FHR5 bind C3b; however, as these proteins do not have SCR homologous to functionally active fH domains, they do not have detectable decay accelerating or fI co-factor activity (46,56). Possibly most interesting with respect to MPGN II is FHR5, which is present in pathologic glomeruli from individuals with kidney disease (57). Its

expression has been documented in podocytes and in *in vitro* studies FHR5 has been shown to associate with surfaces exposed to complement attack with subsequent binding of C3b, suggesting a probable role related to complement activation. The precise relationship between FHR5 and MPGN II has not been defined.

## Genetics and MPGN II

The few patients with inherited mutations of fH and MPGN II have provided valuable insight into disease pathogenesis. One patient, a 13-mo-old Native American, segregated a C518R mutation in fH SCR9 in trans with a C941Y mutation in fH SCR16, the result being retention of fH in the endoplasmic reticulum (55). Two brothers homozygous for R127L in fH SCR2 also developed an MPGN II–like disease (54).

The relationship between fH function and MPGN II has been explored in detail in animals. Norwegian Yorkshire pigs that segregate an I1166R mutation in SCR20 develop MPGN II and die within 7 wk of birth. The I1166R mutation prevents extracellular release of fH, which accumulates intracellularly in disease animals and results in uncontrolled complement activation (36,58). Glomerular disease as evidenced by deposition of complement actually begins *in utero* with C3 and terminal complement complex co-deposition in the GBM. The GBM serves as the nidus of complement activation because it lacks membrane-bound RCA proteins. Morphologic evidence of glomerulonephritis develops later.

The fH-deficient pig model is no longer available (although sperm has been stored), but a mouse with a targeted deletion of fH has been made. Plasma concentrations of C3 in the fH $^{-/-}$  mouse are significantly reduced, with most plasma C3 converted to C3b (53). Heterozygous mouse mutants (fH $+/-$ ) also have depressed levels of C3, suggesting that haploinsufficiency impairs normal C3bBb control mechanisms. Unlike the fH-deficient pig, the fH-deficient mouse has only a 25% 8-mo mortality, but in concordance with the pig model, MPGN develops in all mice and C3 deposition on glomerular capillary walls also precedes the development of glomerulonephritis. It is interesting that the glomeruli are the only site of C3 deposition in these mice, suggesting that the GBM has a unique requirement for the protective role of fH. The mouse mutant null for both fH and fB (fH $^{-/-}$ ; fB $^{-/-}$ ) has a normal renal phenotype (53). The absence of fB in these animals prevents the formation of C3bBb and thereby precludes activation of the AP of complement, making the absence of fH inconsequential.

The central role of tight C3bBb regulation in the prevention of MPGN II is supported by the report of a 57-yr-old woman who developed renal insufficiency and by histopathologic and electronic microscopic analysis of the kidney had both subendothelial and intramembranous dense deposits consistent with MPGN types I and II. Serum C3 and fB levels were reduced, and when patient serum was mixed with control serum, dose-dependent activation of the AP of the complement cascade was observed. A mini-autoantibody in the form of a monoclonal IgA light chain dimer was identified that bound to the SCR3 of fH and the anionic GBM, causing vigorous AP activation and C3 overconsumption (59).

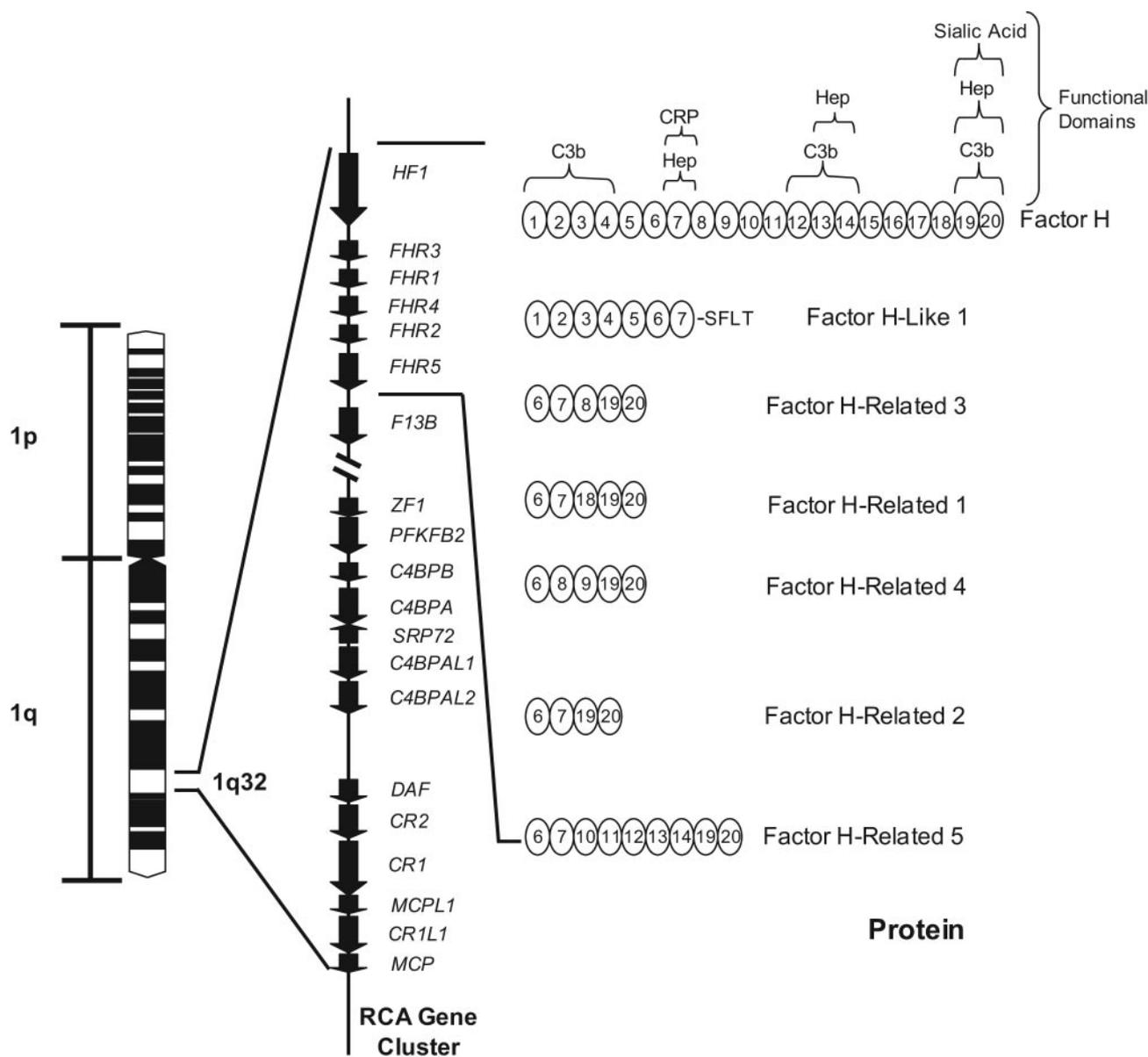


Figure 7. The fH family of proteins contains six members that localize to the regulators-of-complement-activation (RCA) region on 1q32. The functions of some short consensus repeats (SCR) are not known. FHL1 is a splice variant of fH. Each SCR in the fH-related proteins has some (often low) homology to an SCR in fH, as indicated by the number in the ovals. CRP, C-reactive protein; Hep, heparin.

These animal and human data provide compelling evidence that the uncontrolled systemic activation of the AP of the complement cascade results in MPGN II. The initiating triggers can differ, suggesting that the causes of MPGN II are heterogeneous. Some patients develop MPGN II secondary to mutations in fH or to autoantibodies that impede fH function (54,55,58), but in most patients, complement dysregulation is the consequence of the C3NeF autoantibody, which usually binds to C3bBb protecting it from fH-mediated inactivation (46,60).

**C3NeF**

Because most patients with MPGN II develop complement dysregulation associated with the presence of C3NeF, the ap-

pearance of this autoantibody is particularly germane to understanding the pathogenesis of this disease. It is now well recognized that healthy individuals can have autoantibodies associated with many different autoimmune disorders, although titers and prevalence of these autoantibodies are typically very low (61-63). It has been proposed that an idiotype network may regulate this expression and that critical self-epitopes are key to the understanding of self-tolerance and autoimmunity (64-66).

On the basis of Jerne's theory of the idiotypic network, immunization with an antigen leads to a cascade of responses (64). The initial response involves the generation of the antigen-specific antibody (Ab1), which has a unique antigenic site within its variable region to recognize the immunizing antigen.

However, this unique site itself can elicit an antibody response. The second antibody, Ab2, is an anti-idiotypic antibody because the antigenic site that it recognizes is the variable region or idiotype of Ab1. Ab2 in turn induces Ab3 as an anti-anti-idiotypic response, and so on. Because Ab2 recognizes Ab1 and Ab3 recognizes Ab2, Ab3 and Ab1 often have similar binding capacities (67,68).

Consistent with Jerne's idiotypic network theory, both high-affinity C3NeF antibodies (Ab1) and anti-idiotypic antibodies to C3NeF (Ab2) can be identified in newborns and normal adults (69,70). Anti-idiotypic antibodies to C3NeF (Ab2) can also be purified from normal and patient sera (71). The inciting events that can lead to dysregulation of this idiotypic network in patients with MPGN II are unknown.

## Treatment

At this time, there is no universally effective treatment for MPGN II (72–74). Numerous therapeutic regimens have been tried, including the use of corticosteroids and other immunosuppressants, anticoagulants and antithrombotics, and plasmapheresis and plasma exchange. The choice is usually made empirically or in desperation, and until the underlying pathobiology of MPGN II is understood, effective and disease-specific therapies will not exist.

### *Corticosteroids and Other Immunosuppressants*

In children with MPGN types I through III, long-term controlled studies of prednisone therapy have suggested a possible benefit as measured by a decrease in proteinuria and prolonged renal survival (25,72). However, in a randomized, placebo-controlled study, despite evidence of benefit in all patients with MPGN I through III when pooled together, children with MPGN II had no better response to prednisone than to lactose, with treatment failure defined as a creatinine >350 mmol/L (4 mg/dl) in 55.6% (five of nine) and 60% (three of five) of patients, respectively (73). Available data on steroid therapy in adults with MPGN II suggest a similar lack of efficacy (74).

When evaluated in small numbers of patients, the calcineurin inhibitors also do not improve renal survival in MPGN II. *In vitro* studies with cyclosporin and tacrolimus have shown that at therapeutic concentrations, neither drug suppresses C3 transcription (75). Given the evidence that uncontrolled activation of the AP of the complement cascade is the basis of MPGN II, it is not surprising that these drugs are clinically ineffective immunomodulatory treatment modalities.

There are no published data on the use of mycophenolate mofetil in MPGN II. Mycophenolate mofetil selectively blocks inosine 5'-monophosphate dehydrogenase, an enzyme involved in the *de novo* synthesis of guanine nucleotides, and thus inhibits differentiation, maturation, and allostimulatory function of B and T lymphocytes. The use of rituximab, a chimeric IgG1 mAb that specifically targets the CD20 surface antigen expressed on B lymphocytes, has not been studied in MPGN II.

A possibly noteworthy immunosuppressant is triptolide, because it has been shown to decrease renal complement synthesis at therapeutic concentrations (76). Triptolide is an extract of *Tripterygium wilfordii* hook f (Twhf), a woody vine-like shrub of

Southern China and Taiwan commonly called the "thunder god vine." Although its place in traditional Chinese medicine dates back 2000 yr, only after Twhf was reported effective in patients with leprosy and rheumatoid arthritis was its possible value recognized by Western physicians. Studies with triptolide are ongoing, although use probably will be limited by its narrow therapeutic window, which includes severe side effects in approximately one half of treated patients (77).

### *Anticoagulants and Antithrombotics*

One of the most conspicuous features of MPGN II is the increase in extracellular matrix and mesangial cell proliferation, making heparin and heparin-derived GAG potentially interesting therapeutic treatment modalities. Heparin and heparin-derived GAG suppress extracellular matrix turnover, decrease proliferation of mesangial cells, reestablish the negative charge of the GBM and podocytes, and inhibit complement activation (78–81).

Heparin is a large molecule composed of a protein to which GAG side chains of variable composition and number are attached. This heterogeneity makes it difficult to compare different isolates of heparin. There is considerable variation between individual lots in terms of biologic activity and exact chemical content, a heterogeneity that is compounded further in low molecular weight heparins by chemical modifications to alter anticoagulant properties (79).

In a clinical trial using daily subcutaneous injections of heparin for >1 yr, Cade *et al.* (81) reported improved creatinine clearance in nine of 10 patients with chronic proliferative glomerulonephritis. Eight patients had pre- and posttreatment renal biopsies that showed a regression of glomerular hypercellularity. One patient in the treatment group died, as did four of eight patients in a control group that received no therapy. No studies have specifically investigated the efficacy of heparin or heparinoids in patients with MPGN II, although *in vivo* and animal studies suggest that these drugs may have a role in the treatment of this disease (78,79).

### *Plasmapheresis and Plasma Exchange*

Removal of C3NeF from the serum through plasmapheresis has been attempted in a few patients. In one study, one of three adults with MPGN II experienced improvement in serum creatinine during plasmapheresis (82). Another study reported success using plasmapheresis to treat a 5-yr-old boy with recurrent MPGN II after transplantation. Twelve phereses were performed over 24 d, and the patient continued to have improved renal function 1 yr later (83). In another report, a 15-yr-old girl with rapidly progressive recurrent MPGN II in her allograft underwent 73 phereses over 63 wk, stabilizing her creatinine and improving her creatinine clearance. Serial biopsies during this time demonstrated persistent MPGN II without development of tubular atrophy. During the course of therapy, serum C3NeF activity decreased and C3NeF activity was detected in the removed plasma. Because of the morbidity of repeated phereses, treatment was discontinued and graft failure ensued (84).

Plasma exchange is an effective therapy in patients with

MPGN II secondary to protein-inactivating mutations of fH (Peter F. Zipfel and Christoph Licht, Hans Knoell Institute, personal communication, December 2004). This therapy replaces deficient fH with normal fH, correcting the complement defect. Similar results were seen in the fH-deficient pig. Untreated fH<sup>-/-</sup> pigs die by 7 wk of age but develop normally with plasma replacement therapy (36). Most fascinating is the elegant study by Pickering *et al.* (53) in which the MPGN II phenotype in the fH<sup>-/-</sup> mouse mutant was corrected in the fH<sup>-/-</sup>; fB<sup>-/-</sup> double homozygote knockout mouse. Although fH is absent in this mouse, the absence of fB prevents the formation of C3bBb, obviating the need for its inactivation by fH (Figure 6).

Replacement therapy with intravenous gamma globulin (IVIg) to introduce potential blocking antibodies is theoretically possible, although the efficacy of this type of treatment has not been tested in patients with MPGN II. In patients with another autoimmune disease, dermatomyositis, high-dose IVIg has been used to displace nascent C3b away from immune complexes by generating (C3b)2-IgG complexes (85). This displacement attenuates local complement activation by scavenging nascent C3b. Although (C3b)2-IgG complexes are increased and these complexes are extremely potent activators of complement, constant region domains of IgG exert an anti-inflammatory effect through their capacity to bind and neutralize the anaphylatoxins C3a and C5a (86). The net effect is that in patients with dermatomyositis, IVIg attenuates complement amplification to the extent that it even compensates for the extra amounts of C3b that are generated (87).

### Renal Allografts

Dense deposits recur in virtually all renal allografts, and although progression to ESRD is not inevitable, half of allografts ultimately fail (88–91). Studies in the fH-deficient pig have shown that within 24 h of renal allograft placement, recurrence of glomerular complement deposits is demonstrable, presaging the electron microscopic appearance of the dense deposits (36). It is pertinent to note that nephrectomized fH-deficient pigs remain hypocomplementemic, suggesting that the transplanted kidneys do not induce a consumptive hypocomplementemia (92). Unfortunately, long-term studies in fH-deficient pigs that received a transplant were never completed, so although dense deposits recurred in the transplants, long-term outcome was never established (Tom-Eirik Mollnes, Institute of Immunology, University of Oslo, personal communication, December 2004). Whether modifying protocols to include B cell suppression with drugs such as rituximab can increase transplant survival rates in patients with MPGN II is not known. Complement-specific suppression has not yet been tested.

### Nonspecific Therapeutic Measures

Despite the lack of proven specific therapies for MPGN II, nonspecific therapies have been shown to be effective in other chronic glomerular diseases and should be initiated. Angiotensin-converting enzyme inhibitors and angiotensin II type-1 receptor blockers decrease proteinuria in many glomerular dis-

eases and slow progression to renal failure (93,94). Lipid-lowering agents, in particular hydroxymethylglutaryl CoA reductase inhibitors, may also delay progression of renal disease as well as correct endothelial cell dysfunction and alter long-term atherosclerotic risks (95,96). The judicious use of these agents, along with optimal BP control, may be of benefit in patients with MPGN II.

## Conclusions

Available data on MPGN II support the following conclusions:

1. MPGN II is a rare disease that is diagnosed primarily in children between 5 and 15 yr of age. The disease is equally represented among genders. Within 10 yr of diagnosis, ESRD develops in approximately 50% of these children. In contrast to other forms of MPGN, MPGN II is not characterized by immune complex localization in glomeruli.
2. Diagnosis requires renal biopsy, which by electron microscopy shows osmophilic dense deposits in the GBM; C3 but not IgG is demonstrable by immunofluorescence staining. Features of partial lipodystrophy and the development of ocular drusen can accompany MPGN II. Drusen may lead to decreased visual acuity in approximately 10% of patients with MPGN II. In view of the fundus similarities between individuals with MPGN II and age-related macular degeneration, it is conceivable that these disorders may share a common or related cause.
3. The pathophysiologic basis for MPGN II seems to be the uncontrolled systemic activation of the AP of the complement cascade. There are different triggers that result in complement system dysfunction, including mutations in fH, antibodies directed against fH, and an autoantibody directed against C3bBb called C3NeF that is present in most people with MPGN II.
4. All C3NeF are not identical. It is possible that C3NeF is normally present in many healthy people. The triggers that lead to increased and pathologic levels of C3NeF are not known.
5. Most treatments for MPGN II are ineffective. Treatments to remove or suppress C3NeF activity include plasmapheresis, IVIg, and B cell suppression. The first has met with limited success; there is little experience with IVIg and B cell suppression. T cell suppressants are not effective. Consistent with this observation is the recurrence of disease in allografts with the long-term outcome being graft failure in up to half of transplants. In patients with fH mutations, however, plasma exchange can control complement activation and prevent ESRD. Although only a few patients will have fH mutations, genetic screening of fH should be completed on all patients with MPGN II.
6. Whether local control of the complement cascade in the kidney can prevent ESRD in the face of ongoing systemic activation of the AP of complement is not known. If so, then it may be possible to target therapy to the kidney. One example might be the use of heparinoids to protect the GBM from complement activation. Another example would be the

development of therapies specifically directed at controlling the AP of the complement system. Studies that focus on these modalities would seem to be among the best avenues to pursue to develop an effective treatment for MPGN II.

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