Cellular Response to Injury in Membranous Nephropathy

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The pathogenesis of membranous nephropathy (MN) involves in situ formation of subepithelial immune deposits that produce glomerular injury by damaging and/or activating podocytes through complement-dependent processes. C5b-9 formation and insertion into podocyte cell membranes causes glomerular injury in MN. C5b-9 in sublytic quantities stimulates podocytes to produce proteases, oxidants, prostanoids, extracellular matrix components, and cytokines including TGF-β. C5b-9 also causes alterations of the cytoskeleton that lead to abnormal distribution of slit diaphragm protein and detachment of viable podocytes that are shed into Bowman’s space. These events result in disruption of the functional integrity of the glomerular basement membrane and the protein filtration barrier of podocytes with subsequent development of massive proteinuria. Complement components in proteinuric urine also induce tubular epithelial cell injury and mediate progressive interstitial disease in MN. Measurements of urinary C5b-9 or podocyte excretion in the urine may be useful in the diagnosis of MN and as measures of disease activity and response to therapy. Recent studies of cell-cycle proteins and DNA damage in podocytes have clarified why podocytes fail to proliferate in response to C5b-9–mediated injury and podocyte loss in MN, resulting in the development of glomerular sclerosis and renal failure. Improved understanding of the role of complement in the pathogenesis of MN and of the cellular response to C5b-9 attack creates several new opportunities for therapeutic intervention that may benefit patients with MN in the future.


Membranous nephropathy (MN) is a common glomerular disease characterized morphologically by glomerular subepithelial immune-complex deposits without inflammation and functionally by a marked increase in urine protein excretion. The disease is the most frequent cause of idiopathic nephrotic syndrome in white adults. The clinical course is usually benign or indolent, but 30 to 40% of patients progress toward end-stage renal failure within 5 to 15 yr. Other articles in this Frontiers in Nephrology deal with the clinical aspects of MN and approach to therapy.

Central to the pathogenesis of MN is the formation of immune deposits in the lamina rara externa of the glomerular basement membrane (GBM) that cause a membrane-like thickening of the capillary wall. The target of injury in MN is the glomerular visceral epithelial cells, or podocytes, beneath which the deposits are formed. Podocytes are highly specialized and terminally differentiated cells that rest on the outside of the GBM with several crucial functions, including maintenance of the glomerular barrier to protein filtration, synthesis of normal GBM, and provision of structural support to the glomerular tufts (1). Subepithelial immune complexes are separated from the circulation by GBM and therefore do not interact with circulating inflammatory cells or cause inflammation but produce glomerular injury by damaging and/or activating podocytes through complement-dependent processes that are reviewed in this article.

Agents of Podocyte Injury in MN

Subepithelial Immune Deposits

Subepithelial immune deposits initiate podocyte injury in MN. The constituents of these immune complexes consist of IgG (often IgG4) and so far largely unidentified antigens (see article by Ronco in this Frontiers in Nephrology). IgG4 is a subclass of IgG produced in the type 2 immune response of helper T cell subsets, and studies of cytokine profiles in patients with MN establish that it is a Th2 predominant disease (2–4). This CD4+ T cell–dependent, humoral immune response results in glomerular Ig deposition and complement activation, which are the hallmarks of the disease.

Although the quest to identify the pathogenic antigen(s) in human MN has been notoriously difficult and unproductive despite many different approaches over many years, the remarkable similarity of the human disease to an experimental model in the rat referred to as Heymann nephritis (5) has enabled us to study molecular pathomechanisms in MN in considerable detail.

On the basis of earlier work by Germuth, Dixon, and others, it was once believed that subepithelial immune deposits in Heymann nephritis and, by analogy, MN resulted from the passive glomerular trapping of preformed, soluble, immune
complexes from the circulation (6). In the 1970s, the laboratories of Hoedemaker and Couser almost simultaneously discovered that immune deposits in the Heymann nephritis model of MN are due instead to autoantibodies directed at podocyte antigens (7–11), thus uncovering a novel mechanism referred to as in situ immune complex formation (12). The autoantigenic target in this rat model was later identified by Kerjaschki and Farquhar as components of the Heymann nephritis antigenic complex (HNAC): a large (516 kD) podocyte-membrane glycoprotein now called megalin (13), bound to a smaller receptor-associated protein (RAP). However, megalin seems to be expressed exclusively in podocytes and proximal tubular epithelial cells of rats and is neither expressed on human podocytes nor detected in subepithelial immune deposits in patients with MN (14). The observations regarding mechanisms of subepithelial immune deposit formation in Heymann nephritis could be interpreted more broadly as suggesting that podocyte-membrane proteins of some kind are likely targets for immune-complex formation in situ in humans. The first reproducible evidence that immune deposit formation in human MN can be analogous to that defined in Heymann nephritis came recently from Ronco and colleagues, who identified neutral endopeptidase (NEP; or metalbuminendopeptidase) on podocytes as a target antigen in neonatal MN, a topic that is updated and reviewed by Ronco in another article in this Frontiers in Nephrology.

NEP probably serves as a target antigen in only a very small number of patients, e.g., the offspring of the rare mothers who lack a functional gene for this enzyme. However, it is likely that there are other podocyte antigens, not megalin or NEP and as yet unidentified, involved in human MN. It remains to be determined whether exogenous antigens that are trapped in the subepithelial space can also induce the disease through in situ immune complex formation in certain subpopulations of MN patients, e.g., secondary MN induced by foreign agents such as viruses. Although antigens of hepatitis B, hepatitis C, and some tumor antigens have been detected in subepithelial deposits in such patients, there is no proof that these antigens are pathogenic, and autoimmune mechanisms could also be involved (15,16).

Chemical Reagents that Induce MN

Mercury induces MN in certain strains of rats, such as the Brown-Norway, and it is speculated that the underlying pathogenetic mechanism is a T cell–dependent polyclonal B cell activation with subsequent production of autoantibodies against proteins on the podocyte (17). Although human cases of MN after exposure to mercury have been reported, it is not known whether similar mechanisms operate in humans. Representative drugs that are known for their association with MN are gold and penicillamine. The pathogenesis of podocyte injury in gold nephropathy may be analogous to Heymann nephritis induced by release of antigens from damaged proximal tubules that are also constituents of podocytes and provoke an autoimmune response (18), but this remains speculative. Mechanisms of penicillamine-induced MN are also unknown. Because penicillamine can dissociate macroaggregates, it may act by breaking down immune complexes in the circulation and by making the podocyte-directed complexes available.

Membrane Attack Complex (C5b-9) in MN

The primary purpose of this article is to review the consequences of antibody-induced injury to the podocyte that lead to functional disease in MN. Strong experimental evidence and some clinical data implicate the terminal portion of the complement system in this process—specifically, an imbalance between complement regulatory proteins and complement attack that results in podocyte membrane insertion of sublytic quantities of C5b-9 that induce podocyte dysfunction and accompanying loss of glomerular barrier function. Cunningham and Quigg in this Frontiers in Nephrology review the central role of complement regulatory proteins in this process. The following discussion is focused on the cellular effects of C5b-9 attack.

Immunofluorescence studies of MN reveal the presence of Ig and complement components in the glomerular deposits (19). Approximately half of patients with MN exhibit staining for C3, predominantly C3c, a short-lived breakdown product of C3 that therefore marks ongoing immune deposit formation and active disease (20). The remainder probably have residual glomerular injury but are no longer forming deposits. Some evidence suggests this immunofluorescent evidence of disease activity also correlates with prognosis and outcome in MN (21).

In 1980, Salant et al. (22) were the first to show, using generalized complement depletion with cobra venom in rats that were given passive Heymann nephritis, that complement is a crucial mediator of podocyte injury in experimental MN. Inhibition of complement deposition was associated with the total absence of proteinuria despite the lack of any effect on antipodocyte antibody deposits. Furthermore, later studies using animals that were selectively depleted of or genetically deficient in C6 established the role of C5b-9 in the development of podocyte injury and proteinuria in the passive Heymann nephritis model of MN, the first evidence of the now well-established role of C5b-9 in mediating tissue injury in the kidney and now many other tissues as well (23–26). Similar results were obtained by Cybulsky and colleagues (25) using an isolated perfused kidney model of Heymann nephritis induced in the absence of C6 and C8.

C5b-9 is a macromolecular complex that results from proteolytic cleavage of C5 to generate C5b, which then combines with C6 and C7 to form the C5b6,7 complex, an amphiphilic molecule that has binding sites for the lipid bilayer of cell membranes. The components of this distal (later than C5) cascade, such as C6, have no known functional roles relevant to tissue injury other than formation of the membrane attack complex and thus have served as targets for selective interventions that test the functional role of C5b-9 by preventing its formation. With binding of C8 and multiple C9 molecules, the C5b-9 complex inserts into the lipid bilayer of cell membranes (27) (Figure 1). When membrane insertion of C5b-9 occurs, non-nucleated cells such as erythrocytes are easily lysed. However, in nucleated cells such as podocytes, the C5b-9 complex is taken up by the cell, and cell membrane repair occurs rapidly. C5b-9 inserted into the membrane of podocytes then is transported...
intracellularly and extruded into the urinary space, where it subsequently appears in the urine (28). Podocytes that undergo C5b-9 attack may become activated rather than damaged (29), and this process has been implicated in glomerular injury. The consequences of C5b-9 attack on podocytes are discussed in the following section.

The observation that urinary C5b-9 excretion is increased in experimental MN induced by both podocyte and exogenous antigens (30) has stimulated attempts to correlate urinary C5b-9 with the presence of MN and to assess disease activity and prognosis in human patients. When properly assayed and corrected for levels of native complement component excretion in proteinuric urine, increased levels of C5b-9 excretion seem to characterize diseases with subepithelial immune deposits including idiopathic and lupus-associated MN (30), parallel disease activity as assessed by measures of renal function, protein excretion, and response to therapy (31,32), and even serve as a prognostic marker (31). Recent studies by Matsuo et al. (33) also demonstrated that urinary C5b-9 excretion is a potent predictor of poor prognosis in a variety of renal diseases, including MN. However, the vagaries of the assays required have prevented this from becoming a readily available clinical tool. The recent observation of increased excretion of viable podocytes in the urine in experimental MN offers the possibility of using a simpler immunocytologic assay to identify C5b-9–containing podocytes in the urine that would likely reflect the same processes that lead to increased urinary excretion of intact C5b-9 with higher specificity for glomerular events (34).

Antibodies to small antigenic determinants on the HNAC, or noncomplement fixing antibodies, can also form subepithelial immune complex deposits but fail to induce complement activation or proteinuria (35,36). One monoclonal antibody against megalin failed to induce Heymann nephritis, whereas another resulted in development of only a mild form of the disease (37). Although one group reported successful development of full-blown active Heymann nephritis by immunization with a 60-kD N-terminal fragment of megalin (38), the prevailing view is that an additional antigen-antibody system that activates complement is required to induce proteinuria in this model. Activation of complement may also depend on the extent of lattice formation by antigen-antibody complexes (39) or on neutralization of complement regulatory proteins on podocytes. Readers are also referred to the review by Quigg in this issue for more details on a role of complement regulatory proteins in the pathogenesis of MN.

**Mechanical Damage**

Glomerular capillary hypertension is a common denominator in various forms of progressive glomerular disease, including MN. When glomerular hypertension overlaps MN, podocytes, which are thought to counteract pressure-mediated capillary expansion, are increasingly challenged by mechanical stretch. Recent studies by Petermann et al. (40) demonstrated that mechanical strain decreases the growth of podocytes through the regulation of specific cell-cycle regulatory proteins. Furthermore, Durvasula et al. (41) from the same group showed an increase in the number of apoptotic podocytes after mechanical stretch, and it is likely that upregulation of local angiotensin II production and expression of angiotensin type 1 receptor in podocytes by mechanical strain is responsible. The biologic importance of this finding was emphasized by a recent report of transgenic rats with overexpression of the human angiotensin II type 1 receptor under the nephrin promoter that developed structural damage in podocytes in association with proteinuria (42). These studies provide a rationale for the inhibition of angiotensin II, a major mediator of increased glomerular pressure, in patients with MN.

Mechanical stretch also induces upregulation of osteopontin (43), cyclooxygenase-2, and E prostanoid 4 receptor (44) as well as cytoskeletal reorganization (45). All of these findings suggest that, in the presence of glomerular hypertension, mechanical
stress may aggravate podocyte injury induced by antibody and C5b-9 in MN.

**Podocyte Response to Sublytic C5b-9–Induced Injury in MN**

**Activation and Overproduction of Pathogenic Molecules**

**Oxidants.** There is accumulating evidence showing that the cellular response to C5b-9 injury is not a simple consequence of just pore formation in the cell membrane but rather is an active process, such as that due to activation of specific signaling pathways and the consequences thereof (46). Adler et al. (47) were the first to show that sublytic C5b-9 could activate glomerular cells, leading to oxidant production, and similar studies have been carried out in podocytes (48). C5b-9 formation damages podocytes via various mechanisms and leads to development of proteinuria (Figure 1). Complement attack on podocytes induces production of reactive oxygen species (ROS) (49,50), which may be mediated by upregulation of NADPH oxidase induced by release of arachidonic acids (51). ROS initiate lipid peroxidation and subsequent degradation of GBM collage IV, leading to proteinuria (49,52). In support of a pathogenic role of oxidant stress in MN, studies that have used antioxidants and oxygen radical scavengers have demonstrated beneficial effects in the Heymann nephritis models (50,53,54) and also in a pilot study of human MN (55).

**Proteases.** Sublytic C5b-9 also stimulates podocytes to produce proteases, which disrupt the GBM. In experimental MN, podocytes exhibit increased expression of metalloproteinase, and the temporal pattern of protease expression correlated with the onset of proteinuria (56). A significant increase in gelatinoletic activity was also observed in cultures of glomeruli from Heymann nephritis rats, and the 98-kD gelatinae was detected in the culture medium of podocytes but not in those of the other resident glomerular cells (57). It now seems likely that C5b-9–activated podocytes are the principal effector cells that mediate the damage to underlying GBM in MN through release of increased quantities of both oxidants and proteases (Figure 1).

**Alterations in Podocyte Slit Diaphragm Proteins.** Nephrin is a recently identified protein that is a key component of the slit diaphragm, a structure with a crucial role in maintaining the glomerular filtration barrier. Nephrin is linked to the actin cytoskeleton via CD2AP. C5b-9 formation leads to cytoskeletal changes of podocytes (58) with subsequent dissociation of nephrin from the actin cytoskeleton and development of proteinuria (59,60). The interaction of the specific neonatal Fc receptor on podocytes with aggregated IgG4 may also induce focal redistribution and extensive loss of nephrin on the podocyte surface in association with the cytoskeleton organization (61). The alteration of nephrin distribution likely contributes to proteinuria in MN (Figure 1).

**Prostanoids.** C5b-9 upregulates cyclooxygenase-2 and induces eicosanoid production (62). Furthermore, C5b-9 activates phospholipase A2 and induces phospholipid hydrolysis in podocytes, resulting in impairment of endoplasmic reticulum membrane integrity and subsequent endoplasmic reticulum stress (63). The physiologic significance of these observations is highlighted by improvement of proteinuria by cyclooxygense-2 inhibition in Heymann nephritis rats (64,65).

**Extracellular Matrix Components.** The morphologic hallmark of established MN is the presence of thickened basement membranes with spike-like extensions of matrix between podocytes that are readily visible by light microscopy with silver methenamine staining (19). Many studies have examined the composition of this material, which seems to consist of normal GBM constituents, particularly collagen IV and laminin (66,67). The increase in matrix proteins causes the characteristic thickening of the GBM, giving rise to the term membranous nephropathy. In vitro studies that have used human podocytes have established the capacity of sublytic C5b-9 attack to markedly upregulate production of laminin and type IV collagen (48), and molecular studies have confirmed an increased gene expression for extracellular matrix, including type I collagen (68,69). That matrix accumulation occurs in the presence of increased production of matrix-degrading proteases suggests that imbalance must exist between production of proteases and protease inhibitors, but protease inhibitors, although produced by podocytes, have been less well studied in MN.

**TGF-β.** In studies to assess mechanisms involved in C5b-9–induced matrix expansion, Shankland et al. (70) documented a marked increase in expression of the TGF-β2 isoform in podocytes in experimental MN as well as upregulation of TGF-β receptors on the podocytes. Thus, matrix expansion, spike formation, and whatever functional abnormalities accompany these processes are likely TGF-β driven. TGF-β has other effects on the podocyte that are probably relevant to MN as well, including effects on cell-cycle proteins favoring hypertrophy over proliferation and the induction of apoptosis (see below).

**PDGF and Secreted Protein Acidic and Rich in Cysteine.** Recent studies by Floege and colleagues (71) demonstrated upregulation of both PDGF-B and PDGF-C in podocytes in MN, which was confirmed by quantitative real-time PCR analysis of microdissected glomeruli. The authors speculated that upregulation of PDGF-C is a sign of dedifferentiation and activation of podocytes in this disease. Studies that have used various animal models also demonstrated a marked increase in secreted protein acidic and rich in cysteine (SPARC) synthesis in association specifically with C5b-9–mediated podocyte injury (72). SPARC is a modular protein with antiproliferative and counteradhesive properties that can interact with growth factors, bind to structural proteins, or interface with cells directly. SPARC is a potent inducer of TGF-β expression (73) (see above). The physiologic significance of PDGF-C and SPARC expression by podocytes remains to be elucidated.

**Podocyte Response to C5b-9 and Glomerulosclerosis**

**Apoptosis.** Normal podocytes are terminally differentiated and quiescent cells. Low levels of proliferation of podocytes may occur in experimental MN (74), but loss of podocytes, in combination with limitations in their compensatory proliferation in response to injury, is thought to underlie the development of glomerulosclerosis in various glomerular diseases, including MN (Figure 2).
Earlier studies failed to document significant podocyte apoptosis. However, recent studies have shown that podocytes undergo apoptosis in glomerular disease induced by genetic overexpression of TGF-β (75) and after toxic injury in the puromycin nephrosis model (76). One explanation for the earlier difficulty in detecting podocyte apoptosis is that apoptotic podocytes are likely flushed out in the urine, making it technically difficult to detect these cells in situ (34). It is likely that apoptosis increases in podocytes under certain circumstances and contributes to the loss of cell number. Recent studies showed that podocyte number is reduced in experimental MN, and this is due to detachment of podocytes (34) and likely apoptosis (77).

C5b-9, like other noxious stimuli, may induce apoptosis of podocytes in MN (1), a process that may involve TGF. Loss of podocytes via apoptosis may occur either directly or indirectly via C5b-9–mediated cellular injury. Podocyte apoptosis may be mediated by ROS or TGF-β, which are also responsible for apoptosis in puromycin-induced podocyte injury (78). In addition, various growth factors and cytokines are known to induce podocyte injury. Deleterious effects of basic fibroblast growth factor on podocytes were demonstrated in passive Heymann nephritis (79). Whereas TGF-β leads to apoptosis of podocytes via the Smad-7–mediated pathway (75), CD2AP is required for early activation of antiapoptotic survival signaling pathways by TGF-β in podocytes (80). This finding is intriguing because recent studies established that the slit diaphragm proteins nephrin, podocin, and CD2AP all participate in cell-signaling pathways as well as being pivotal to the structural organization of the slit diaphragm (81–83). Furthermore, podocytes respond to angiotensin II with an increase of the intracellular calcium concentration via an AT1 receptor (84). Adverse effects of angiotensin II on podocytes are supported by a number of clinical and experimental studies by Remuzzi’s group, demonstrating beneficial effects of inhibition of the renin-angiotensin system in MN (85–90). Singhal and colleagues (91) also showed that angiotensin II induced apoptosis in cultured rat podocytes in a dose- and time-dependent manner.

Podocyte injury in MN may result in a decrease in expression of vascular permeability factor/vascular endothelial growth factor (VEGF-A), which is expressed constitutively in podocytes at high levels. The expression level of VEGF in podocytes is potentially regulated by the GBM matrix-podocyte interaction (92). Clinical studies demonstrated that active MN is associated with diminished expression of VEGF in podocytes, which is reflected by decreased urinary VEGF excretion (93). Recent data from podocyte-specific knockout mice (94) as well as studies using neutralizing antibodies (95) suggest that vascular permeability factor/VEGF-A is critical for the proper maintenance of glomerular filtration barrier and the glomerular endothelial fenestrae. Recent studies also demonstrated that VEGF prevented podocyte apoptosis via phosphorylation of nephrin (96,97). Diminished expression of VEGF in MN therefore may contribute to alterations in glomerular permselectivity as well as podocyte loss, leading to subsequent proteinuria and glomerulosclerosis.

Detachment. Detachment of podocytes from the underlying GBM may also be responsible for an increase in protein permeability as well as a decrease in podocyte number. Older studies by Schneeberger et al. (98) using ultrastructural tracer molecules demonstrated that sites of podocyte detachment corresponded with sites of increased protein permeability in Heymann nephritis. Previous studies mentioned above demonstrating podocytes in urine of patients and experimental animals with glomerular injury support this concept of detachment as an important functional event in MN (34,99) (Figure 2).

Cytoskeletal changes induced by C5b-9, as described above, may cause detachment of podocytes from the GBM, which is aggravated by direct GBM damage from podocyte-derived mediators produced in response to C5b-9 and by mechanical stretch. Furthermore, detachment of podocytes as a result of degradation of GBM by proteases produced by podocytes...
in response to complement-mediated injury in MN. The firm attachment of the podocyte foot processes to the GBM plays a vital role in determining the permselectivity properties of glomeruli to plasma macromolecules (103), and detachment of podocytes contributes not only to development of glomerulosclerosis but also to development of proteinuria.

**Cell Cycle and Lack of Proliferation.** Effects of C5b-9 on the cell cycle have been reviewed elsewhere (46). Sublytic C5b-9 attack on podocytes in vivo is followed by cell activation and DNA synthesis, occasional mitosis, and ploidy (74,78,104). However, podocytes do not readily undergo cytokinesis (cell division), and the podocyte number does not increase in vivo in most podocyte diseases (105). It is likely that this failure of podocytes to proliferate in some forms of glomerular disease, including MN, contributes to glomerular sclerosis and renal failure. After antibody deposition and complement activation, there was a marked upregulation in the cyclin kinase inhibitors p21 and p27 in podocytes of passive Heymann nephritis rats (106). These representative cyclin kinase inhibitors are decreased in diseases with podocyte proliferation such as cellular/collapsing focal segmental glomerulosclerosis (107). A key role for p21 in limiting the proliferative response of podocytes has been demonstrated in studies on p21 knockout mice (108). Furthermore, whereas sublytic C5b-9 attack on podocytes promoted cell-cycle entry in association with upregulation of mitotic proteins such as cyclin B1, B2, and D1 (109,110), C5b-9 arrested podocytes at the G2/M phase, thereby preventing mitosis and cytokinesis in vitro (111). An abnormality in the exit from mitosis results in the presence of bi- or multinucleated podocytes, as observed in Heymann nephritis rats. C5b-9 also induces DNA damage in podocytes that may contribute to the lack of a proliferative response (112). All of these observations contribute to understanding why podocytes fail to proliferate in response to complement-mediated injury in MN.

**C5b-9 and the Pathogenesis of Progression in MN**

The role of C5b-9 in progressive interstitial fibrosis has been reviewed extensively elsewhere (113). Although it has been difficult to isolate the role of C5b-9 in the interstitium from its effects in the glomerulus experimentally, compelling evidence now supports the contention that proteinuria is a major mediator of progressive interstitial fibrosis in any chronic proteinuric disorder (114) and that C5b-9 formation in tubules accounts for most of the nephrotoxic effects of increased excretion of high molecular weight proteins (113,115). Using normal rats and animals that are genetically deficient in C6 and therefore unable to form C5b-9 complexes, we have shown that progressive interstitial fibrosis develops in complement-sufficient rats that were made proteinuric with aminonucleoside of puromycin, whereas C6-deficient rats with equivalent proteinuria are protected from interstitial changes and progression (116). Moreover, in the remnant kidney model of hemodynamically mediated proteinuria and progression, glomerular sclerosis and proteinuria lead to interstitial fibrosis in normal animals, whereas animals that are deficient in C6 develop similar glomerular changes and proteinuria but are protected from interstitial disease (117). The mechanism of this effect likely involves effects of intratubular complement activation, involving both filtered and locally synthesized native complement components, leading to insertion of sublytic quantities of C5b-9 into the brush border membranes of proximal tubular cells with consequences to the tubular cells very similar to those described for the podocyte above and subsequent effects on the interstitial inflammatory response (118). Although the hypothesis that chronic proteinuria leads to C5b-9–mediated interstitial disease in MN specifically is difficult to test experimentally because manipulations of the complement system also alter the glomerular disease, it seems probable that this is correct. Thus, therapeutic maneuvers directed at inhibiting complement activation in MN, such as administration of C5 blocking agents (119), may have beneficial effects by acting at either glomerular or interstitial sites or both.

**Future Research Directions in MN**

MN will not be different from other autoimmune diseases in requiring a much better understanding of how tolerance is broken to selected self-antigens in humans and how that loss of tolerance can be restored to halt the immune process that is the underlying mediator of the disease. What triggers the loss of tolerance to selective podocyte antigens without generalized autoimmunity, what are the genetic and environmental risk factors for this, and how can it be reversed? Obviously the recent advance in understanding the nature of the pathogenic antigen in one form of human MN is a very substantial advance in this area.

The unique role of C5b-9, which seems to account for virtually 100% of the alteration in protein excretion in most forms of experimental MN, clearly offers the opportunity to pursue targeted intervention in the complement system as a potential therapeutic tool. Quigg and Cunningham discuss this option more extensively in their article on complement regulatory proteins in MN in this Frontiers in Nephrology. Technologies that now are in development to target active complement regulatory molecules to specific cell surfaces and to upregulate expression of these molecules in a cell-specific manner hold considerable promise in MN. We believe that such therapies that are targeted to the tubular epithelial cell may be of even more benefit to patients with MN and other chronic proteinuric disorders than targeting the glomerulus, where complement-mediated injury, once developed, is difficult to reverse.

In the past few years, our understanding of podocyte and glomerular biology has expanded rapidly thanks to the establishment of podocyte cell lines, discovery of novel proteins constituting the slit diaphragm, and sophisticated studies using mouse molecular genetics and cell biologic approaches (1). If we cannot yet eliminate the immune response or prevent complement activation, then interference with the nephritogenic responses to C5b-9 reviewed above in ways that prevent the podocyte from becoming an effector cell when targeted by immune events should be an active area of research. Thus, successful development of oxidant and protease inhibitors, agents that act on TGF, etc., will likely benefit this disease.
Of all of the progressive immune glomerular diseases, progress in understanding the pathogenesis of MN has arguably outpaced any others. However, there is a great deal more to be learned and a large leap yet to be made in translating this information into effective and nontoxic therapy for the human disease.

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References


60. Saran AM, Yuan H, Takeuchi E, McLaughlin M, Salant DJ:


84. Zoja C, Benigni A, Camozzi D, Corna D, Longaretti L, Todeschini M, Remuzzi G: Combining lisinopril and L-arginine slows disease progression and reduces endothe-


90. Zoja C, Benigni A, Camozzi D, Corna D, Longaretti L, Todeschini M, Remuzzi G: Combining lisinopril and L-arginine slows disease progression and reduces endothe-


