Mediation of Immune Glomerular Injury

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ABSTRACT
This paper reviews current concepts of glomerular immune injury of both inflammatory and noninflammatory types. In noninflammatory lesions induced by antibody alone or C5b-9, the glomerular epithelial cell appears to be the principal target of injury. Similar mechanisms are probably operative in human diseases such as minimal change nephrotic syndrome and membranous nephropathy. In inflammatory lesions, circulating effector cells including neutrophils, macrophages, platelets, and probably lymphocytes as well as resident glomerular mesangial cells may mediate tissue injury. Human equivalents of these inflammatory lesions include most diseases associated with mesangial and/or subendothelial immune deposits and/or mesangial cell proliferation. Neutrophil-mediated injury appears to be consequent to both proteinases and oxidants, particularly the myeloperoxidase-H₂O₂-halide system. Platelets may be critically involved in neutrophil mediated injury as well. Platelets also mediate mesangial cell proliferation, probably by a release of platelet growth factors and stimulation of mesangial cell platelet-derived growth factor and platelet-derived growth factor receptor expression. Immunologically induced mesangial cell proliferation is associated with increased production of nephritogenic proteinase in vivo.

Key Words: Glomerulonephritis, glomerulus, epithelium, endothelium, basement membrane

Glomerulonephritis (GN) continues to represent a major cause of end stage renal disease in the United States and throughout the world (1). The frequency of GN as a cause of chronic renal failure has decreased from over 60% in the 1970s to less than 40% today, reflecting in part a better understanding of the mechanisms that underlie this group of diseases and the consequent improvements in their management (2). However, therapy of glomerular diseases remains unacceptably empiric and too often unsuccessful. The 1980s have witnessed a revolution in the basic sciences of cell and molecular biology with many implications for understanding and potential treatment or prevention of kidney disease. It seems likely that advances in knowledge about these disorders during the last decade of the 20th century will equal or surpass most of what has been learned during the past 90 years. In an effort to put this field in perspective as it stands in 1990, the following review summarizes current concepts of the major mechanisms that mediate immune glomerular injury. Whenever possible, I have tried to link descriptions of specific mechanisms of injury with the clinical diseases that are believed to result from them.

IMMUNOPATHOGENESIS OF IMMUNE GLOMERULAR DISEASES
It is not the purpose of this paper to review in detail what little is known of the etiology of glomerulonephritis or the larger topic of mechanisms of glomerular immune deposit formation, subjects that are well reviewed elsewhere (3–5). It is rather my charge to focus on the nature of the mediation mechanisms which are set in motion by these processes and lead to disease. I would offer only two comments on the immune events that activate these mediators. The first is that there is increasing evidence that genetic and immunogenetic factors play a major role in determining not only who gets disease in response to a specific stimulus but also the severity of the disease contracted and therefore its prognosis and response to therapy (6,7). The second is that evidence is also mounting that many more of the antibody-mediated glomerular diseases are autoimmune in nature than previously suspected. The autoimmune nature of antiglomerular basement membrane (GBM) nephritis and the nephritis of systemic lupus have long been recognized. However, recent evidence implicates disorders of immune regulation and autoimmune mechanisms in diseases such as membranous nephropathy (MN) (8,9), IgA nephropathy (10,11), idiopathic crescentic glomerulonephritis (12,13), hemolytic uremic syndrome (14), and several forms of vasculitis.
This leaves only postinfectious glomerulonephritis among the major antibody-mediated glomerular diseases as an example of an exogenous antigen-induced serum sickness-like lesion: some postinfectious sequelae also appear to have autoimmune components (16–18). Thus, our basic understanding of the immunopathogenetic mechanisms of glomerular disease continues to evolve.

Immune glomerular diseases may occur as a consequence of the formation of immune complex deposits in glomeruli or in their absence. When present, glomerular immune deposits may result from: 1) antibody to normal antigens in the glomerular extracellular matrix (anti-GBM nephritis) or glomerular cell membranes (probably idiopathic MN) (4), 2) antibody to antigens not normally present but induced in vessels by inflammatory mediators (as proposed in Kawasaki's vasculitis 15), or 3) antibody to various nonrenal antigens which localize in glomeruli to promote in situ immune complex formation or circulate in soluble immune complex form and are trapped passively in mesangial or subendothelial sites (3,4).

Antibody deposits may cause injury either through various inflammatory (i.e., leukocyte-dependent) mechanisms, as in nephritis induced by mesangial, subendothelial or basement membrane deposits, or by entirely noninflammatory processes as in MN with subepithelial deposits. Similarly, several diseases are believed to be immune in nature but are not associated with antibody deposits, and these too may be inflammatory (idiopathic crescentic glomerulonephritis) or totally noninflammatory (minimal change nephrotic syndrome).

In attempting to focus a review of mediation in a way that will retain relevance to this wide spectrum of clinical glomerular diseases, I have considered a number of consequences of the mediator systems to be discussed including functional abnormalities, such as proteinuria and decrease in glomerular filtration rate, and structural lesions, such as glomerular intracapillary and extracapillary cell proliferation, thrombosis, necrosis, crescents, basement membrane thickening, sclerosis, and mesangial matrix changes. I have chosen for the purpose of this paper to define glomerular injury as an increase in urine protein excretion. Proteinuria is a common denominator of all glomerular diseases. Moreover, alterations in glomerular barrier function have been shown by Myers and colleagues to provide the best single correlate of the severity and prognosis of immune renal injury in several clinical settings (19,20).

In Figure 1, I have depicted the currently established pathways by which immune mechanisms lead to glomerular injury in the form of proteinuria. I would like to distinguish at the outset between mechanisms one and two on the left in Figure 1 (antibody alone and C5b-9) which induce entirely noninflammatory forms of glomerular disease, probably due to injury to the glomerular epithelial cell (GEC), and mechanisms three and four (circulating inflammatory or resident glomerular cells) which induce inflammatory hypercellular lesions involving either glomerular infiltration by circulating cells or resident glomerular cell proliferation, or both, usually with damage to the GBM itself. In the sections that follow, I will review what is known of the mediation mechanisms involved in each of these four pathways in some detail, focusing wherever possible on newer observations and insights from our laboratory and elsewhere.

**NONINFLAMMATORY GLOMERULAR IMMUNE INJURY**

**Glomerular Injury Induced by Antibody Alone**

Although antibody-mediated glomerular disease is traditionally thought to result in proteinuria as a consequence of antibody activation of other humoral and cellular mediators such as complement and leukocytes, it has been clear for some time that damage sufficient to cause proteinuria can be induced by antibody deposition alone. This type of injury is characterized by a marked alteration in glomerular barrier function, no visible structural damage at the light microscopic level, and, ultrastructurally, by GEC pedicel effacement. As such, the structural and functional characteristics of injury induced by antibody alone most closely resemble the glomerular lesions of minimal change disease/focal sclerosis in man in which a marked increase in glomerular permeability to protein is induced acutely without accompanying signs of glomerular proliferation or leukocyte infiltration. Although there is no evidence that minimal change disease is related to glomerular antibody dep-
positional, it now seems probable that better understanding of the mechanisms by which antibody deposition alone can alter the barrier to protein filtration without inflammation may provide important clues to the mechanisms of glomerular injury in minimal change nephrotic syndrome.

Proteinuria induced by antibody alone was first demonstrated using a variety of noncomplement fixing heterologous antibodies, antibody subclasses, and antibody fragments prepared primarily against whole glomeruli or GBM (reviewed in 21). This mechanism seems to be the only one operative in nephrotoxic nephritis in the guinea pig, where all IgG subclasses of both heterologous and autologous anti-GBM antibodies induce a marked and immediate but transient increase in glomerular permeability without involvement of complement, inflammatory cells, or significant glomerular structural lesions (22–24). The absence of complement activation in these lesions has been attributed to a unique structural distribution of GBM antigens which permits antibody binding but precludes the density of immunoglobulin deposition and lattice formation required for complement activation (24,25). Although this lesion has been shown to occur independently of a variety of inflammatory mediators in vivo (24,25), anti-GBM antibody has been most clearly dissociated from circulating humoral and cellular mediators by studies in the isolated perfused rat kidney. In this system, IgG anti-GBM antibody in an acellular albumin perfusate induces a marked increase in the permeability to BSA of normal appearing glomeruli within 1 hour (26,27). Although some of the BSA may reach the urine through nonglomerular pathways (26), there is clear evidence for a glomerular lesion as well including an alteration in dextran sieving curves consistent with a reduction in the charge barrier (27). Although speculation about the mechanism of this direct antibody effect has centered on molecular disorganization of the GBM including charge sites, induced by the binding of anti-GBM antibody, recent studies suggest that the permeability defect may not be due to effects of antibody on the GBM.

In 1978, we reported the first of several descriptions of complement-neutrophil independent proteinuria induced by an antibody directed primarily at the GEC rather than at the GBM (28). Administration of both F(\(\text{Ab}\))\(_2\) and F(\(\text{Ab}\))' fragments of heterologous anti-Fx1A IgG induced a marked, immediate and transient glomerular proteinuria in normal rats with no accompanying renal morphologic changes by light microscopy (28). This finding was in marked contrast to results with equimolar amounts of intact IgG which induces a sustained C5b-9 dependent proteinuria that does not begin until 4 to 5 days following antibody injection (29,30). Since that time, it has become clear that the nephritogenic antibody in anti-Fx1A is directed at antigens expressed on the GEC membrane (31–33).

An apparently analogous phenomenon was reported in 1988 by Mendrick and Rennke who described another model of complement-neutrophil independent proteinuria induced by injecting Freund's adjuvant stimulated rats with a noncomplement fixing monoclonal antibody, K9/9, directed against a GEC membrane antigen (34). In this model, the effect of antibody deposition was not only directly linked to the GEC but was epitope specific and was not seen with monoclonal antibodies directed against other GEC antigens (35). A third study linking the complement-neutrophil independent antibody effect to the GEC was reported by Orikasa et al. who described another example of immediate and transient proteinuria induced by a monoclonal antibody, 5-1-6, directed against an antigenic determinant present on the GEC slit diaphragm (36). Mendrick and Rennke have suggested, and I tend to agree, that proteinuria induced by anti-GBM antibody in the isolated perfused kidney in our earlier studies probably occurred consequent to a similar effect of polyclonal antibodies in this anti-whole glomerular antibody preparation which was directed at the GEC rather than at the GBM (34).

The mechanism by which antibody binding to the GEC, without complement activation, induces this massive but rapidly reversible increase in glomerular permeability remains undefined. Although I have focused here on the ability of antibodies to induce this noninflammatory functional change, nonimmune toxins which also act directly on the GEC, such as aminonucleoside of puromycin, can produce a very similar phenomenon (37,39). The nature of the cellular response induced by antigen–antibody interaction on the GEC membrane is unknown but may be analogous to the response of the GEC to an, as yet, unidentified circulating stimulus, perhaps a cytokine, which leads to minimal change nephrotic syndrome. The process is depicted schematically in Figure 2. The morphologic nature of this response involves a shape change in the epithelial cell podocytes which includes effacement, retraction, and detachment of the GEC from the underlying basement membrane (38,39). The phenomenon of GEC morphologic change with detachment appears to be common to several experimental noninflammatory proteinuric lesions. It has been clearly demonstrated in both the K9/9 and 5-1-6 lesions induced by monoclonal antibodies to GEC (34,36) as well as in lesions induced by antibodies to other GEC-derived GBM constituents including heparan sulfate proteoglycans (40) and laminin (41). Detachment of the GEC and proteinuria are characteristic of glomerular injury induced by systemic protein overload (42), adriamycin (43), and aminonucleoside of puromycin (38,44,45), all lesions
Mediation of Immune Glomerular Injury

![Diagram](image)

**Figure 2.** Suggested mechanism of glomerular injury mediated by antibody alone. Experimentally, antibody to GEC antigen induces GEC shape change, detachment, and proteinuria (dark arrow) which may involve alterations in cell-matrix attachment proteins (integrins) and/or local proteinase release. In clinical settings such as minimal change nephrotic syndrome, analogous cellular alterations may occur in response to an as yet unidentified, nonantibody, circulating mediator; for example, a cytokine.

of the GEC. In aminonucleoside nephrosis, endogenous albumin, IgG, and ferritin all penetrate GBM only in areas of GEC detachment (44,45). Detachment alone is believed to result in proteinuria due to glomerular hemodynamic changes with loss of the hydraulic conductivity barrier and increased water flux leading to enhanced bulk flow of macromolecules, including proteins, through the capillary wall and perhaps through the GEC itself (37,44–47).

The mechanism of GEC detachment is not known. Cell attachment to extracellular matrix is regulated by a variety of cell attachment proteins, many of which are members of the integrin family (48). Integrins are transmembrane receptor proteins consisting of an alpha and beta subunit that are noncovalently linked and usually bind to extracellular matrix ligands that contain an arg-gly-asp (RGD) tripeptide sequence (reviewed in 49). The integrins most likely to be involved in GEC adhesion would include VLA-2 (mediating adhesion to types I–IV collagen), VLA-3 (adhesion to collagen, laminin, and fibronectin), and possibly the VLA-4 and VLA-5 receptors. VLA-3 has been identified in human glomeruli and on kidney epithelial cells (48). An antibody to the common beta I subunit of integrins also stains normal GEC in tissue section (50). Thus, a disorder of expression or function of integrins, or other cell matrix receptors, may contribute to detachment and proteinuria in these noninflammatory, antibody-mediated lesions.

An alternative hypothesis for the effect of antibody alone would be that antibody interaction with GEC membrane antigens in amounts insufficient to cause cell injury may stimulate cells to produce local mediators with a capacity to damage basement membrane or cause detachment. We have noted the capacity of antibody to mesangial cell membrane antigens to stimulate production of prostaglandins by cultured rat mesangial cells (51). In preliminary studies (data not shown), we have identified the presence of a gelatin-digesting, zinc-dependent neutral proteinase with activity against type IV collagen and GBM in supernatants of resting GEC in culture. Recent studies have shown that local release of proteinases with similar properties (elastase and cathepsin G) isolated from neutrophils can produce an immediate and transient proteinuria without morphologic changes which closely resembles both the lesion mediated by antibody to GEC antigen and the glomerular lesions of minimal change nephrotic syndrome (52). Thus, these noninflammatory, antibody-mediated lesions illustrate the importance of the GEC to maintaining glomerular barrier function and may provide very useful tools for the additional investigation of the cellular and molecular mechanisms of noninflammatory proteinuric disorders such as minimal change nephrotic syndrome.

**Glomerular Injury Induced by the C5b-9 Membrane Attack Complex of Complement**

A second major noninflammatory mechanism of immune glomerular injury involves complement activation by antibody deposits resulting in formation of the complement membrane attack complex, or C5b-9. This subject has been reviewed in detail elsewhere (53,54). The human glomerular disease in which this mechanism is most likely operative is MN, but a role is probable in other lesions as well, particularly if subepithelial immune deposits are present (53). The first evidence for C5b-9 induced glomerular injury derived from studies of the mediation of proteinuria in the passive Heymann nephritis (PHN) model of MN in rats. PHN is induced by injection of a heterologous antibody to a proximal tubular brush border fraction termed Fx1A. Studies by several laboratories over the past decade have demonstrated that the subepithelial immune complex deposits that develop in PHN form in situ (55,56) due to IgG antibody reacting with antigens on the membrane of the GEC. The best characterized of these at the present time is GP330 (4,31–33). These antigen-antibody complexes activate complement producing glomerular deposits of the C5b-9 membrane attack complex, but the antigen-antibody complexes themselves are patched, capped, and shed from the cell surface by mechanisms requiring an intact GEC cytoskeleton to form the discontinuous subepithelial immune complex deposits characteristic of MN (57,58). In PHN, proteinuria develops 4 to 5 days after antibody injection when sufficient IgG has deposited to induce
Injury. PHN is morphologically indistinguishable from MN in man in that no circulating effector cells or other inflammatory changes are present (29,30). Despite the lack of inflammatory changes, however, studies in which rats were depleted of complement with cobra venom factor during the 5 days required for proteinuria to develop demonstrated that complement-depletion totally abolished proteinuria in PHN without altering antibody deposition (30). We speculated that this lesion involved a glomerular effect of C5b-9 rather than generation of chemotactic or opsonic factors such as C5a, the only complement-mediated mechanism previously implicated in glomerular injury (30,53).

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 C5b67 complex, an amphiphilic molecule which has binding sites for the lipid bilayer of cell membranes (reviewed in 59). With binding of C8 and multiple C9 molecules, the C5b-9 complex inserts into the lipid bilayer of cell membranes (59). A protective mechanism for C5b-9 injury may be afforded by plasma S-protein (vitronectin), which results in formation of SC5b-7 complexes which can bind C8 and C9 but cannot insert into cell membranes and are therefore considered inactive (60). Membrane insertion of C5b-9 results in rapid lysis of nonnucleated cells, such as erythrocytes, but lysis of nucleated cells is much more difficult to achieve (61).

Confirmation that C5b-9 assembly is critical in the mediation of proteinuria in PHN has been provided now by studies in the intact animal, in the isolated perfused kidney, and in isolated glomeruli. In intact rats, selective depletion of C6 to prevent assembly of C5b-9 can be maintained throughout the 4 to 5 day period required for proteinuria to develop following antibody injection (62). Selective C6 depletion prevents glomerular assembly of C5b-9 complexes as detected by staining for C5b-9 neoantigens and abolishes the development of proteinuria without altering glomerular deposition of antibody or C3 (62). In the isolated perfused rat kidney, proteinuria does not develop when antibody deposition occurs in the presence of serum deficient in C6 or C8 but appears immediately following addition of normal serum containing all complement components (63). In collaboration with Dr. Virginia Savin, we have recently shown in the isolated rat glomerulus that glomerular permeability to albumin, reflected by changes in the reflection coefficient for albumin calculated from the volumetric response of isolated glomeruli to changes in oncotic pressure gradient, is increased within 10 minutes in glomeruli exposed to anti-Fx1A antibody IgG and normal serum as a complement source in vitro (64). No change in permeability is induced by antibody in the presence of C6 or C7 deficient serum (64). Since the principal biologic function of C6, C7, and C8 is the assembly of the C5b-9 membrane attack complex, these studies provide convincing evidence that proteinuria in experimental MN, induced by antibody to GEC antigen, is mediated by C5b-9 in the intact animal, the isolated perfused kidney, and the isolated glomerulus.

A role for C5b-9 has also been established in mediating injury in MN induced by other mechanisms. In the autologous phase of PHN, where injury results from antibody reacting with previously deposited but subnephritogenic quantities of antibody to GEC, injury also appears to be C5b-9 mediated in the intact animal (65,66) and in the isolated perfused kidney (67). Similar results are obtained when subepithelial deposits form in situ due to localization of cationic antigens and antibodies to them. Thus, the onset of proteinuria is delayed in genetically C6 deficient rabbits with cationic BSA induced serum sickness, compared to normal controls, despite comparable amounts of glomerular immune complex deposits (68). Rats selectively depleted of C6 develop significantly less proteinuria in response to a nephritogenic quantity of cationic IgG containing immune complexes compared to controls (69). Evidence for a nephritogenic role for C5b-9 has also been obtained in models of anti-GBM nephritis in rabbits (70,71).

The cellular basis for the C5b-9 effect has not been defined. To better understand this process, we developed a monoclonal antibody directed against a C9 neoantigen present only in the assembled rat C5b-9 complex (72). This antibody stains PHN glomeruli in a pattern similar to staining for IgG (Figure 3) and similar to that reported in human MN and other diseases by Falk and others (73–75). Immunoultrastructural studies in PHN using this reagent demonstrate early C5b-9 localization in subepithelial deposits and along the surfaces of the GEC membrane, particularly in regions of clathrin coated pits where the putative GP330 antigen is expressed (76). The cell bound C5b-9 then undergoes endocytosis and is transported through the GEC in large multivesicular bodies before being exocyted into the urinary space (76). This is in contrast to the cellular handling of antigen–antibody complexes which are primarily patched and capped and then shed from the cell surface where they form granular deposits that become noncovalently bound to the GBM (57,76,77).

Several authors have demonstrated S protein in subepithelial immune deposits in MN (75,78,79). This finding has prompted comment that the glomerular C5b-9 deposits are inactivated, cannot insert into cell membranes, and are therefore likely not pathogenic (75,78,79). We believe this is probably the case with SC5b-9 deposits which remain extracellularly with the immune complexes, and these may represent most of glomerular C5b-9. However, freeze-fracture
promptly when antibody deposition is halted by transplanting a nephritic kidney to a normal host (66,80). Of interest, glomerular C3 deposition follows a very similar course and may also serve as a marker of disease activity (66,81). Similar results have been obtained in the autologous immune complex nephritis (active Heymann nephritis) model (81).

We have attempted to extend these observations in the experimental animal to study MN in man with some success. In studies in 148 patients with proteinuric glomerular diseases, elevated urinary C5b-9 (analyzed as differences from expected values based on urinary C5 excretion) was found only in nine (of 40) patients with idiopathic MN and four (of six) patients with lupus MN and not in a variety of other diseases with comparable levels of proteinuria or in controls (82). These findings have now been confirmed by Coupes et al. (83). Patients with elevated urinary C5b-9 values were generally studied earlier in the course of disease and had higher levels of urine protein excretion than MN patients with normal urinary C5b-9 values, findings consistent with a greater likelihood of disease activity in high C5b-9 excretors (82). These results imply that in at least one subset of patients with idiopathic and lupus MN, the disease mechanism is analogous to the autoimmune process involving antibody to the GEC defined in the Heymann models in rats, and that GEC membrane insertion of C5b-9 also takes place in man. Urinary C5b-9 excretion may serve as a useful marker of immune disease activity in MN in the absence of an assay for the pathogenic antibody.

Despite these advances in understanding the role of C5b-9 in the mediation of MN, the cellular consequences of C5b-9 attack on the GEC remain poorly understood. There appear to be both structural and functional consequences of GEC C5b-9 attack. The structural changes are predominantly an increase in thickness of the GBM, due primarily to an accumulation of laminin along the outer surface, and in the characteristic spikes projecting from the subepithelial surface (84,85). This process progresses to glomerulosclerosis resulting from an accumulation of normal extracellular matrix components, predominantly type IV collagen (86). Functional changes in the barrier to protein filtration consist of the development of large pore defects (19) which appear to represent areas of GEC detachment from underlying basement membrane (87,88). At present, it is unclear what is the relationship, if any, between the structural and functional changes in the basement membrane.

One possibility for the C5b-9 effect might be that C5b-9 induces an abnormality in GEC production of laminin or other constituents of the basement membrane resulting over time in a persistent remodeling of the basement membrane structure with altered
barrier function. Hansch et al. have reported a several fold increase in production of type IV collagen by rat GEC following C5b-9 attack (89), but no studies of laminin production have appeared. However, the alteration which occurs in glomerular permeability in experimental MN induced by C5b-9 can develop in minutes in the isolated kidney and glomerulus (63,64) and therefore seems unlikely to result from an alteration in matrix component metabolism.

As in the noninflammatory lesions induced by antibody alone, which are discussed above, GEC detachment appears to underlie the permeability defect in MN as well (87,88). Thus, a C5b-9 induced alteration in expression or function of integrins, or the matrix components to which they bind, might lead to epithelial cell detachment and proteinuria. One study showed no alteration in expression or distribution of a beta one integrin in human MN (50). However, other integrins and cell membrane receptors have not been explored.

A third mechanism by which C5b-9 might be pathogenic at the cellular level may involve cell activation with release of potentially toxic inflammatory mediators by the GEC itself. Although membrane insertion of C5b-9 leads to lysis of nonnucleated cells such as erythrocytes, nucleated cells are relatively resistant to C5b-9 attack and may actually be stimulated by this process (60). With respect to GEC, Quigg et al. have shown that antibody to GEC membrane, in the presence of sublytic concentrations of complement, induces noncytolytic injury as evidenced by the release of low molecular weight intracellular markers and formation of membrane vesicles similar to those excreted in the urine in PHN (90). Sublytic C5b-9 attack also results in increased intracellular calcium, activation of phospholipase C, increased levels of IP2, IP3, diacyl glycerol and phosphatidic acid with release of arachidonic acid, PGF2α, and thromboxane (91,92). Enhanced prostaglandin production by GEC following sublytic C5b-9 attack has also been reported by Hansch (93). Similar changes occur in mesangial cells in response to C5b-9 (94). This phenomenon of C5b-9 induced cell activation could result in release at the GBM-GEC interface of several cell-derived substances. Three potential inflammatory mediators warrant consideration: prostaglandins, oxidants, and proteinases.

With respect to prostaglandins, Cybulsky et al. reported a reduction in C5b-9 mediated proteinuria in the isolated perfused kidney when a thromboxane synthesis inhibitor was employed (95). Two groups have demonstrated a reduction in proteinuria with indomethacin in models of MN (96,97), although these results may in part reflect a reduction in glomerular filtration rate (97). We have demonstrated a marked increase in glomerular PGE2 and thromboxane production by glomeruli in PHN rats that was complement dependent and may have derived from the GEC (98). However, administration of a thromboxane synthesis inhibitor, which reduced glomerular thromboxane production by over 80%, had no effect on C5b-9 induced proteinuria in PHN (98) or in another model of MN induced with cationized IgG which is believed to be C5b-9 mediated (69,99). Thus, it seems unlikely that alterations in GEC prostaglandin metabolism account directly for the altered permeability to protein induced by C5b-9.

A second possible GEC-derived mediator would be reactive oxygen species. Oxidants including H2O2, superoxide anion, and hydroxyl radical have all been implicated in various forms of inflammatory renal injury (100). Adler et al. have shown that C5b-9 can directly stimulate cultured mesangial cells to produce H2O2 and superoxide anion (60). Although oxidant production by the GEC is relatively low, three studies have described a beneficial effect of administration of the hydroxyl radical scavengers dimethyl sulfoxide and dimethylthiourea (DMTU) on proteinuria in PHN (101–103). Moreover, a beneficial effect of the iron chelating agent deferoxamine suggests participation of the Haber-Weiss reaction to form hydroxyl radical (103). However, a direct effect of C5b-9 on GEC oxidant production remains to be documented.

A final potential candidate for a GEC derived mediator would be a GBM-degrading proteinase. As discussed above, preliminary evidence exists for the production of a proteinase by GEC with type IV collagenase activity. A stimulation of proteinase production by sublytic C5b-9 attack could lead to an immediate and noninflammatory type of glomerular lesion such as that seen in early MN (52).

INFLAMMATORY GLOMERULAR INJURY
Glomerular Injury Induced by Circulating Inflammatory Cells

The prompt glomerular localization of circulating inflammatory cells, particularly neutrophils and macrophages, has long been recognized as the principal mechanism of inflammatory immune injury to glomeruli and was extensively studied decades before the two noninflammatory mechanisms described above were identified (104–106). Experimental studies have shown that inflammatory cell participation in mediating glomerulonephritis depends in large part on the site of glomerular immune deposit formation (107,108). Thus, deposits at subendothelial and mesangial sites are directly accessible to circulating cells and result in their localization and activation, whereas deposits at a subepithelial site are separated from cells in the circulation by the underlying GBM and, in the absence of deposits elsewhere, cause a noninflammatory, C5b-9 mediated lesion (3). Thus, the human equivalents of inflammatory cell
mediated glomerular lesions would include diseases with predominantly mesangial and subendothelial immune complex deposits such as diffuse proliferative lupus nephritis, type I membranoproliferative glomerulonephritis, IgA nephropathy, Henoch Schonlein purpura, and some types of acute postinfectious glomerulonephritis with mesangial and subendothelial deposits.

**Neutrophils.** Mechanisms of neutrophil involvement in glomerular disease have been extensively reviewed elsewhere (100,109–111). Localization of neutrophils in glomeruli occurs in response to a variety of soluble mediators, primarily the C5a chemotactic product of complement activation, but including platelet activating factor, platelet derived growth factor (PDGF), leukotriene B4, and other lipoxygenase products that may be released from damaged cells (reviewed in 100). Once localized to glomeruli, neutrophil adherence can occur through a number of receptors including receptors for Fc, C3b (CR1), the recently described LFA-1, MAC-1 glycoprotein family of leukocyte adhesion molecules, and several others (reviewed in 100). In glomeruli, neutrophil adherence appears to be both Fc and complement-dependent. Neutrophil adherence and attempts to phagocytose deposited immune reactants result in neutrophil activation accompanied by a respiratory burst (100,111). Tissue injury is consequent to local release by the activated neutrophil of toxic inflammatory mediators, primarily proteinases and oxidants (100). Recent studies have clarified how each of these mechanisms work.

Most recent attention has focused on the role of oxidants in neutrophil-mediated glomerular injury. The respiratory burst which follows neutrophil activation generates superoxide anion (O₂⁻) which undergoes dismutation catalyzed by superoxide dismutase (SOD) to H₂O₂. H₂O₂ is then degraded by catalase to H₂O (100,109–111). Three separate studies by Drs. Rehan, Wiggins, and Kent Johnson, in which the renal arteries of rats were either perfused with phorbol myristate acetate, a potent stimulus to neutrophil oxidant release, perfused with cobra venom factor which activates complement and releases C5a, or perfused with nephritogenic anti-GBM antibody, all resulted in neutrophil mediated forms of glomerular injury which were substantially modified by administration of catalase but not SOD (112–114). These studies thus implicate H₂O₂ as the principal oxidant involved in neutrophil mediated injury. H₂O₂ by itself is not a potent oxidant but can combine with Fe²⁺ in the presence of iron to form potentially toxic hydroxyl radicals (OH⁻). H₂O₂ may also react with halides in the presence of neutrophil-derived myeloperoxidase (MPO) to form toxic hypohalous acids which can induce tissue halogenation (100). The nature of glomerular oxidant injury has recently been clarified by Dr. Richard Johnson in a series of studies that implicate the MPO-H₂O₂-halide system as an important potential mechanism. MPO is a highly cationic enzyme derived from the primary granules of neutrophils and monocytes which localizes readily in glomeruli by binding to anionic charge sites (100,115). When MPO is perfused into the renal artery resulting in binding to GBM, followed by exposure to subnephritogenic quantities of H₂O₂ in the presence of a halide, a severe form of glomerular injury occurs with endothelial damage, platelet infiltration, basement membrane halogenation, and later glomerular cell proliferation (Figure 4) (115,116). A similar neutrophil-dependent form of injury can be produced in the intact animal using a model of subendothelial immune complex nephritis induced with concanavalin A and antibody to it and is also characterized by GBM halogenation (117). In contrast, several studies using hydroxyl radical scavengers have not shown significant protective effects in well characterized models of neutrophil-mediated glomerular injury (112–114). Thus, it now appears that oxidant injury mediated by H₂O₂ through the MPO-H₂O₂-halide system is a major mechanism of neutrophil-mediated glomerular tissue damage.

Despite the recent focus on neutrophil oxidant in-

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**Figure 4.** EM autoradiograph of a rat glomerulus perfused with H₂O₂ and Na⁺¹²⁵I (control, A) or MPO followed by H₂O₂ and Na⁺¹²⁵I (experimental, B). Numerous silver grains along the glomerular capillary wall in B document incorporation of Na⁺¹²⁵I into glomerular capillary structures (halogenation). Endothelial cell swelling and focal epithelial cell foot process effacement are also present (original magnification ×2,000). Reprinted with permission from Johnson et al. (115).
jury, it is also clear that proteinase release from inflammatory cells also has the potential to produce severe glomerular disease. Neutrophil-derived proteinases, particularly neutral serine proteinases such as elastase and cathepsin G, have been well shown to degrade GBM in vitro, and GBM fragments are detectable in the urine of animals with neutrophil-mediated lesions (reviewed in 100). However, there has been little evidence until recently that neutrophil-derived proteinases could cause injury in physiological concentrations in vivo. However, Johnson et al. perfused the purified neutrophil-derived proteinases elastase and cathepsin G into the rat renal artery in concentrations calculated to be achievable with the number of neutrophils localized in glomeruli in neutrophil-mediated lesions. They documented a dramatic acute and transient increase in urine protein excretion in the virtual absence of glomerular morphologic changes by both light and electron microscopy (52). This observation may also be relevant to considering the possibility of glomerular injury induced by release of proteinases from resident GEC (see above) or mesangial cells (see below).

Thus, circulating neutrophils can clearly cause glomerular injury by release of both oxidants and proteinases, and both mechanisms are likely operative in neutrophil-mediated diseases. Of particular interest is a recent study that demonstrates an important role for the platelet in one model of neutrophil-mediated glomerular injury (118) (see below). In considering the potential role of oxidants and proteinases in contributing to immune glomerular injury, it is also important to note the studies of Shah and associates demonstrating that oxidants can activate proteinases and thereby potentiate their action (119).

Monocytes and Macrophages. Although not as extensively studied as neutrophils, macrophages can also serve as inflammatory effector cells in certain types of immune glomerular injury (120–123). While they are capable of both proteinase and oxidant production, macrophages lack MPO (100). Macrophages also produce a variety of other potential inflammatory mediators including prostaglandins, leukotrienes, tumor necrosis factor (TNF), polypeptide growth factors including interleukin I and PDGF, and complement components and coagulation factors, any of which may participate in mediating tissue injury (123–125). Like neutrophils, glomerular localization and adherence of monocyte/macrophages occur through a variety of mechanisms (reviewed in 123). Unlike neutrophils, macrophages also respond chemotactically to cytokines produced by lymphocytes in response to specific sensitizing antigens (123,124). Thus, macrophages could serve as the principal effector cell in certain types of glomerulonephritis which may be primarily mediated by sensitized mononuclear cells (reviewed in 123,126). Glomerular macrophages may be resident in the mesangium and exhibit Ia antigen, thereby, presumably, rendering them capable of presenting antigen to sensitized T cells (127,128). Macrophages are particularly prominent in crescentic glomerular lesions where they appear to enter Bowman’s space through rents in the capillary wall and release a tissue factor-like procoagulant material which plays an important role in fibrin deposition and crescent formation (129–131). Finally, macrophages also release a variety of growth factors which may be important in inducing proliferation of resident glomerular cells (124) (see below).

Lymphocytes. Although the T cell arm of the immune response is a well established mediator of several autoimmune diseases (reviewed in 132), a clear role for cell-mediated immunity in glomerular disease has eluded investigators since the original description of cellular immunity to a glomerular antigen in man over 2 decades ago (133). The limited experimental data available on this important mechanism are well reviewed elsewhere (123,126). While T cells specifically sensitized to native glomerular antigens can apparently mediate glomerular hypercellularity independently of antibody deposition in rats (120,134) and chickens (135,136), no clear evidence of cell-mediated injury sufficient to cause proteinuria has emerged. T cells appear to participate in recruitment of macrophages in some models of macrophage-mediated injury (137,138). Of considerable interest is the recent report by Rennke et al. of a granulomatous crescentic glomerulonephritis with proteinuria induced in rats exhibiting cellular immunity to a glomerular-localized exogenous antigen in the absence of glomerular antibody deposition (139). This lesion was adoptively transferred by T cells but not by antibody (139). This success in developing a rat model of cell mediated immune glomerular injury using an exogenous antigen represents a substantial advance in this area and should permit an answer to the question succinctly posed by Dixon 20 years ago of “What are sensitized cells doing in glomerulonephritis?” (140).

Platelets. Although the platelet is a familiar component of many inflammatory glomerular lesions and is well recognized for its role in thrombogenesis, it is an understudied and probably underappreciated participant in mediating acute inflammatory injury (141,142). We have noted the platelet to be a prominent component of glomerular injury induced by neutrophil-dependent mechanisms, such as that resulting from the MPO-H2O2-halide system (115,116), and in acute subendothelial immune complex nephritis induced by deposition of concanavalin A and antibody to it (117,118). We have recently observed that selective platelet depletion in this neutrophil-mediated con A model essentially abolishes neutrophil-
Induced proteinuria without altering glomerular localization of antibody, complement, or neutrophils (118). Subsequent studies using 111In-labeled platelets demonstrated that platelet accumulation occurred immediately in this model within 10 minutes of antibody deposition and was dependent on complement but not on neutrophils that localize independently of platelets (143). These findings imply a functional interaction between the neutrophil and the platelet within the glomerulus itself which is required for neutrophil-mediated injury to occur. The nature of this interaction has not yet been defined, but could involve platelet adenine nucleotide mediated stimulation of neutrophil oxidant release (144), or other enhancements of neutrophil function, or proteinase release by platelet derived products such as platelet factor 4, platelet activating factor, or thromboxane (143-146).

**Glomerular Injury due to Resident Mesangial Cells**

The concept that resident glomerular cells, particularly the mesangial cell, can serve as inflammatory effector cells analogous to the neutrophil and macrophage has received increasing attention with the growing application of cell culture techniques to glomerular disease research (147-150). Unlike the three mechanisms discussed above, there is as yet no clear evidence that mesangial cells, or other glomerular cells, can mediate proteinuria, but it seems probable that this can occur (151). The human diseases in which this mechanism is most likely to participate include any diseases associated with mesangial cell proliferation—for example IgA nephropathy, Henoch Schonlein purpura, lupus nephritis, membranoproliferative glomerulonephritis type I, some cases of steroid-resistant nephrotic syndrome and AIDs nephropathy, and others.

Because of the ease with which mesangial cells can be grown and studied in culture, an extensive literature has developed rather rapidly cataloging the behavior of these cells in response to a variety of inflammatory mediators. In many ways, these cells resemble cells of monocyte/macrophage lineage (150). The list of substances that can activate these cells to proliferate and/or release potential mediators includes immune complexes (152), C5b-9 (60,94), endotoxin (153), growth factors (154,155), and many others (reviewed in 148). There is also an extensive list of potential mediators of disease that can be produced by mesangial cells in response to these stimuli which include prostaglandins (94,156), proteases (157), oxidants (60), tumor necrosis factor (158), plasminogen activator (159), and growth factors (144,155). Extracellular matrix component production by mesangial cells may contribute to mesangial sclerosis (160,161). The list of nephritogenic substances produced by the mesangial cell, and the stimuli that activate these cells, is rapidly expanding. However, interpretation of this *in vitro* data in terms of which observations actually reflect mechanisms operative in renal diseases in the intact kidney remains very problematic.

In an attempt to begin to link observations of mesangial cell biology in tissue culture with diseases involving the mesangium in the intact kidney, we have used a model of mesangial proliferative glomerulonephritis induced by antibody to Thy-1, an antigen expressed on the membrane of mesangial cells (162,163). In the anti-Thy-1 model, there is an initial phase of mesangial cell lysis followed by a striking mesangial cell proliferation (163). Yamamoto and Wilson have shown this proliferative lesion to be mediated by complement but to be independent of neutrophils (163,164). As in other models of acute inflammatory disease, platelets are also conspicuous in the early lesions (165). 111In-labeled platelet studies reveal over 600 platelets/glomerulus 4 hours after disease induction in animals with anti-Thy-1 GN compared to less than 20 platelets/glomerulus in controls (165,166).

To examine the mediation of mesangial cell proliferation, we tested the hypothesis that platelets localized in glomeruli might be responsible for inducing glomerular cell proliferation, possibly through release of platelet-derived growth factors. To quantitate glomerular cell proliferation, an immunoperoxidase stain for the proliferating cell nuclear antigen (PCNA) cyclin was used (167,168). PCNA/cyclin is an auxiliary protein to DNA polymerase delta that is expressed in the cell cycle commencing in the late G1/S phase and extending into mitosis. Proliferating PCNA/cyclin-positive cells are identified by black nuclear staining (Figure 5). To distinguish proliferating mesangial cells from infiltrating leukocytes, which may also proliferate and stain positively for PCNA/cyclin, a double-labeling technique was developed using OX1, a monoclonal antibody to the membrane-associated common leukocyte antigen, CD45 (169). This antigen was detected with an alkaline phosphatase immunocytochemical technique (165). Using this double label staining technique, we demonstrated that over 85% of cyclin-positive cells are OX1 negative and therefore of glomerular origin. When selective platelet depletion was carried out in the anti-Thy-1 model of mesangial proliferative glomerulonephritis, platelet-depleted animals showed a marked reduction in glomerular cell proliferation without changes in complement levels or antibody deposition (165). Thus, in this model of mesangial proliferative disease, platelets appear to have a critical role in mediating the proliferation of glomerular, presumably mesangial, cells. Moreover, complement-
depletion studies demonstrate that complement depletion prevents both platelet localization and cell proliferation (166), suggesting that the effect of complement depletion in modifying proliferation described by Yamamoto and Wilson may have been consequent to the prevention of platelet localization (164).

To additionally investigate the mechanism of this platelet-mediated glomerular cell proliferation, we have used a monoclonal antibody to platelet-derived growth factor B chain (PDGF) (170) (generously provided by Dr. Russell Ross). Immunostaining demonstrates a marked increase in glomerular capillary wall localization of PDGF in anti-Thy-1 animals. The increased amount of PDGF in this model of mesangial proliferative GN may not necessarily reflect the release of PDGF from platelets since PDGF could also derive from circulating monocytes or macrophages or from glomerular endothelial or mesangial cells (155,171). We have recently obtained evidence that this increased glomerular PDGF content in this model may derive largely from cells resident in the glomerulus by demonstrating an increase in PDGF B chain gene expression by northern blot analysis of whole glomerular RNA at 3 and 5 days after disease induction and coincident with the phase of mesangial cell proliferation (172). Similar findings have recently been reported by Gesualdo et al. in a mouse model of mesangial proliferative GN (173). In addition to an increase in glomerular cell PDGF gene expression, we have also recently found a parallel increase in expression of the β subunit of PDGF receptor (172). Enhanced PDGF-β receptor expression has also been reported recently in human glomerulonephritis with mesangial proliferation (174). Taken together, these studies suggest that mesangial cell proliferation in experimental immunologically-mediated mesangial proliferative GN is complement- and platelet-dependent and may involve not only release of platelet-derived growth factors by the platelet but also an increased production of PDGF by resident glomerular cells associated with an increase in PDGF receptor expression as well.

While the above observations clarify the mediation of mesangial cell proliferation, they do not establish a role for proliferating mesangial cells in producing any of the tissue injury observed in these lesions. However, evidence to support such a role has recently emerged from studies done in collaboration with Dr. David Lovett, using an antibody specific for a mesangial cell-derived neutral metalloproteinase which has been found in his laboratory (175–177). This neutral proteinase has been shown to be produced in increased quantities by mesangial cells in culture stimulated by inflammatory mediators (178). Preliminary results of these studies demonstrate little neutral proteinase production by mesangial cells in the normal glomerulus, but a marked increase in staining for neutral proteinase in vivo in mesangial cells of animals with mesangial proliferative glomerulonephritis on day 3 (179). Ultrastructural studies employing an immunogold technique suggest a preferential localization of neutral proteinase in areas of disrupted GBM. No corresponding increase was found in staining for the tissue inhibitor of metalloproteinase, suggesting a marked increase in mesangial production of functional nephritogenic proteinase in these lesions in vivo (179).

While the studies of this pathway of glomerular immune injury are obviously only in their infancy, they do provide preliminary evidence to support the suggestion that inflammatory mediators are released by resident glomerular cells in vivo in response to stimuli similar to those defined in vitro and appear

Figure 5. Light micrograph of glomeruli stained with PCNA/cyclin from rats with anti-Thy 1 induced mesangial proliferative glomerulonephritis. Cyclin-positive cells establish the presence of glomerular proliferation (A), but are markedly reduced by prior platelet depletion (B) (original magnification x350).
to participate in mediating the glomerular damage associated with mesangial immune injury.

CONCLUSION

While this brief overview is necessarily incomplete and emphasizes some areas and concepts at the expense of others, the points made justify several comments regarding progress in this area in the future. One is that dramatic advances are now being made in understanding immunologic mechanisms of glomerular disease. However, additional progress requires, as I have tried to emphasize in this review, the continuous integration of data derived from studies of man, intact animals, isolated kidneys and glomeruli, cultured cells, and molecular studies of gene expression. We are uniquely fortunate in nephrology, in contrast to many other areas of medicine, to have available not only the arsenal of new technologies in cell and molecular biology which has developed in the past decade, but also a library of disease models which closely mimic the spectrum of human glomerular diseases and in which relevant disease mechanisms can be controlled and manipulated with remarkable precision. Moreover, the kidney is an organ in which even minor disturbances in function and structure can be easily detected and precisely quantitated. However, to fully exploit these considerable advantages in extending our knowledge of immune disease mechanisms, it is essential that we maintain continuous excellence in our research capabilities at all levels from human studies to cell and molecular biology. This need to maintain a balanced and integrated approach must be reconciled with the compulsion to clone the next gene if the data derived from molecular studies are ever to be interpreted in the context of human disease.

A final and related point is that, in my opinion, 1990 is not the time to reduce our reliance on the diagnostic renal biopsy in favor of more empiric approaches to the therapy of glomerular disease (180,181). The failure of current therapeutic approaches to benefit substantially from accurate diagnosis in some cases attests only to the need for a much better understanding of what the mechanisms of these diseases are. Ongoing research in this area must have as its goal the application of new technologies and insights to the study of human renal tissue and disease. If we discontinue at this point the careful and systematic study of kidney tissue obtained from man, the exciting advances in renal research that will emerge from continued application of these new scientific technologies in the 1990s and beyond will never be applied to achieve optimal management of the patients with kidney disease who all of us are committed to serve.

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