Basic and Translational Concepts of Immune-Mediated Glomerular Diseases

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ABSTRACT
Genetically modified immune responses to infections and self-antigens initiate most forms of GN by generating pathogen- and danger-associated molecular patterns that stimulate Toll-like receptors and complement. These innate immune responses activate circulating monocytes and resident glomerular cells to release inflammatory mediators and initiate adaptive, antigen-specific immune responses that collectively damage glomerular structures. CD4 T cells are needed for B cell–driven antibody production that leads to immune complex formation in glomeruli, complement activation, and injury induced by both circulating inflammatory and resident glomerular effector cells. Th17 cells can also induce glomerular injury directly. In this review, information derived from studies in vitro, well characterized experimental models, and humans summarize and update likely pathogenic mechanisms involved in human diseases presenting as nephritis (postinfectious GN, IgA nephropathy, antiglomerular basement membrane and antineutrophil cytoplasmic antibody–mediated crescentic GN, lupus nephritis, type I membranoproliferative GN), and nephrotic syndrome (minimal change/FSGS, membranous nephropathy, and C3 glomerulopathies). Advances in understanding the immunopathogenesis of each of these entities offer many opportunities for future therapeutic interventions.


Recent reviews of the immune mechanisms that lead to glomerular disease have been published elsewhere.1,2 This review is organized by diseases rather than mechanisms to provide a translational overview of how immune responses mediate the glomerular injury seen by clinicians and pathologists. The processes described derive from studies done in vitro and in an array of animal models of glomerular diseases as well as in humans. Cell cultures are not glomeruli, and mice and rats are not humans, but experience has taught us that mechanisms defined in these settings often translate into better understanding of similar processes seen in human disease. Schematic overviews of the major pathogenic sequences currently believed to be operative in human GN and their interactions are presented in Figures 1 through 4.

OVERVIEW OF BASIC IMMUNE MECHANISMS

The Innate Immune Response

Toll-Like Receptors
Toll-like receptors (TLRs) are ancient and ubiquitous pattern recognition receptors present on all cell membranes and intracellularly between cytoplasm and endosomes (Figure 1).3–6 TLRs recognize conserved immunostimulatory molecular patterns (antigens) like peptidoglycans, LPSs, and bacterial and viral nucleic acids (pathogen-associated molecular patterns [PAMPs]) as well as endogenous cell-derived patterns (danger-associated molecular patterns [DAMPs]). Another related cytoplasmic group of receptors called Nod-like receptors (NLRs) has recently been described.5 TLR ligation is central to activating the non-antigen-specific innate immune system in immediate response to pathogens, but TLR activation is also required for adaptive, antigen-specific immune responses by facilitating conversion of dendritic cells to antigen-presenting cells.4–7 TLRs activate multiple signaling pathways that lead to local release of a variety of cytokines, chemokines, and other inflammatory mediators by all cells, including glomerular cells.4,5 Thus, TLRs and NLRs connect initiating events with mediation of tissue injury in GN associated with infections or autoimmunity or both.

Complement
The complement system and its regulatory proteins are also ancient components of the innate immune system with multiple roles in human GN (Figure 2).7–11 The innate immune response involves immediate complement activation through the mannose binding lectin (MBL) or alternative pathways.7,10 Activation of the MBL pathway proceeds

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381
Figure 1. Schematic overview of both the innate and adaptive immune mechanisms that mediate tissue injury in GN. Etiologic events expose immunostimulatory PAMPs or DAMPs that activate both the innate (red) and the adaptive (antigen specific, blue) immune systems, which also interact with each other. Activation of the innate immune system occurs immediately and involves TLRs or NLRs on both circulating inflammatory cells and resident glomerular cells. TLR activation results in release of inflammatory mediators that activate the alternative pathway beginning directly through the innate immune system. TLRs are also required to activate the adaptive immune system through antigen-presenting cells that promote differentiation of CD4 helper cells, B cell activation, and antibody production. Antibodies lead to circulating complex trapping or in situ formation of immune complexes that can activate both the TLR and complement components of the innate immune system. Complement activation generates the chemotactic factor C5a that attracts circulating inflammatory cells (including neutrophils, macrophages, basophils, and natural killer cells), which release mediators and damage glomeruli and C5b-9 that activates resident glomerular cells to do the same. CD4 Th1 and Th2 cells cause tissue injury primarily through macrophages and basophils, respectively, whereas Th17 cells can mediate glomerular damage directly. CD4 regulatory cells (Tregs) downregulate the adaptive immune response.

when MBL binds to mannose residues on pathogens and activates the serine proteases, MASP-1 and MASP-2, leading to activation of C4 and C2. The alternative pathway is activated spontaneously by hydrolysis of C3 and amplified by defects in complement regulation. Non-Ig zymogens such as damaged cells and bacterial and viral proteins can also activate the alternative pathway beginning directly at C3. The same initiating event may activate more than one pathway.

Complement activation products are the principal mediators of antibody-induced GN (Figures 1 and 2). Usually this involves C1q binding to Ig that leads to classic pathway activation through C4 and C2; however, some Igs, depending on their level of glycosylation, can also bind MBP. IgG subclasses 1 and 3 and IgM are classic complement pathway activators, whereas IgG 2 and 4, IgA, IgD, and IgE activate complement poorly.1,12

All complement activation pathways proceed through cleavage of C3 and C5 leading to release of chemotactic factors such as C5a that attract inflammatory cells (neutrophils, macrophages, and platelets) when abutting the circulation as well as formation of the terminal membrane attack complex (C5b-9) (Figure 2).7–9 Sublytic quantities of C5b-9 can insert into lipid bilayers of adjacent glomerular cell membranes, initiate several signaling pathways, and convert these cells to effector cells, which may proliferate; release a variety of cytokines, growth factors, eicosanoids, oxidants, proteases, and other acute inflammatory mediators; as well as upregulate production of matrix components that contribute to chronic scarring and sclerosis (Figure 1).1,12,13 Complement activation products like C5a can also activate TLRs.14

Complement activation in vivo is tightly regulated by a number of circulating and cell-bound complement regulatory proteins (CRPs), whose functions, mutations and deficiencies are also important in the development of several glomerular diseases.8,9 Abnormalities in serum complement profiles are sometimes helpful in assessing the nature of the underlying disease and its activity, but significant complement-mediated injury may occur locally without alterations in circulating complement components (Table 1).

The Adaptive Immune Response

Ig

CD4 T helper cells stimulate B cells and plasma cells to make antibodies specific for particular antigens (Figure 3). On the basis of older studies of serum sickness in rabbits induced by single (acute) or repeated (chronic) injections of BSA, glomerular immune deposits have long been attributed to the passive trapping of circulating, soluble antigen-IgG antibody complexes (ICs).15,16 Other studies done in antigenic glomerular antibody models (nephrotoxic nephritis; NTN) demonstrate that antibody deposition activates complement through the classic complement pathway generating chemotactic factors that attract circulating inflammatory effector cells, which then cause tissue injury (Figures 1 and 3).17 Typical granular IC deposits can also form locally, or in situ, due to antibody binding to either exogenous planted antigens or endogenous glomerular components (Figure 3).18–23 There are several variables that determine the biopsy findings and clinical consequences in IC GN: (1) where the deposits form—ICs of the same composition in a subendothelial distribution lead to exudative inflammatory cell infiltrates, in the mesangium to mesangial cell proliferation and matrix expansion, and in a subepithelial distribution to a
noninflammatory lesion with podocyte injury, effacement, and heavy proteinuria; (2) the biologic properties of the antibody (or antigen) itself—particularly complement activating capacity, Fc receptor affinity, ability to form lattices, or cryoprecipitability; (3) the mechanism of deposit formation—when ICs form in situ, the process usually induces local tissue injury, whereas passive trapping of ICs formed in the circulation has not been well shown to be nephritogenic (Figure 3); and (4) the quantity—the more deposits form, the more severe the disease.

**T Cells**

In addition to providing help for B cells, some antigen-specific CD4 T cells alone, sensitized to either self or nonself antigens that are localized in glomeruli, can induce antibody-independent tissue injury. Although all subsets of T cells are involved in glomerular immune responses, Th17 cells likely account for much of T cell–induced inflammation (Figure 4). Th17 cells are attracted by mechanisms involving chemokines and their receptors, and release cytokines such as IL9, IL17, IL21, IL22, and TNFα, which induce other cells to produce additional proinflammatory chemokines that attract neutrophils and monocytes and also activate resident glomerular cells. Th17 cells are found in renal biopsies in several forms of human GN. The T cell component of the adaptive immune response is regulated by Tregs.

**Diseases Usually Presenting as GN**

**Postinfectious or Poststreptococcal GN**

The acute, diffuse exudative and proliferative lesion of poststreptococcal GN (PSGN) was long regarded as the human equivalent of the acute, one-shot serum sickness model in rabbits leading to a prolonged search for the nephritogenic streptococcal antigen. Although many candidate proteins have been proposed, most have failed to meet strict criteria for causality. However, streptococcal pyogenic exotoxin B (SpeB) meets most of these criteria, although it has not been implicated in all cases of epidemic PSGN. SpeB is a small (28 kDa), cationic (pK 9.3) cysteine protease with complement-activating and plasmin-binding properties and represents 90% of the secreted extracellular protein made in vivo by nephritogenic strains of group A streptococci. Antibody to SpeB correlates with disease activity in PSGN and co-localizes with IgG and C3 in subepithelial humps. However, the intense exudative glomerular inflammatory response is not well explained by a serum sickness analogy and humps because circulating ICs do not form subepithelial IC deposits directly and subepithelial IC deposits do not produce inflammation. Moreover, IgG is sometimes absent or is only a minor constituent of the deposits, whereas C3 deposition often both precedes and exceeds detectable IgG.

Possible explanations for these apparent contradictions include observations that some subendothelial deposits are also present in PSGN, perhaps because antibody to SpeB also exhibits molecular mimicry with endothelial cells. In addition, SpeB alone can activate complement directly through the MBL pathway independent of IgG. SpeB also exhibits plasmin-binding properties that facilitate complement activation and might cause proteolysis of glomerular basement membrane (GBM), facilitating
IgA Nephropathy

IgA nephropathy (IgAN) is the most common form of GN worldwide and is characterized by focal mesangial proliferation and matrix expansion accompanying diffuse mesangial deposits of IgA, and often IgG, C3, and C5b-9, usually associated with recurrent episodes of GN that often immediately follow mucosal viral infections.33–37 Although assumed to be mediated by mesangial trapping of circulating ICs, no exogenous antigens have been identified consistently.38–40 IgA in mesangial deposits, and in IC form in the circulation, is polymeric (mucosal) IgA1 that exhibits deficient O-linked glycosylation at five sites in the hinge region of the molecule.41–44 The failure to normally glycosylate IgA1 can be inherited in IgA nephropathy and Henoch-Schönlein purpura,45,46 but the defect also seems to occur epigenetically.47 Underglycosylated pIgA1 is produced by mucosal B cells and might also reach the circulation if abnormal trafficking of these cells to the bone marrow occurs.48,49 Underglycosylated IgA1 predicts progression and exhibits altered biologic properties compared with normal IgA1 including increased tendencies to selfaggregate, unmasking of MBL binding sites leading to complement activation, binding to other molecules like fibronectin, IgG, and collagen IV. In circulating macromolecular form, it evades removal from the circulation by asialoglycoprotein CD 89 receptors, and transferrin receptors (TfR, CD71).46,47 TLR activation by IgA aggregates, perhaps containing or accompanied by PAMPs, may account for the recurrent episodes of acute injury with hematuria, particularly those that immediately follow infections.48–50 However, most experimental and clinical studies suggest a role for complement as well.51–53,54,55 C5b-9 generated from complement activation induced by interaction of IgA1 aggregates with MBL, or in situ formation of ICs by IgA antiglycan antibodies, induces mesangial cell transformation to α-smooth muscle actin–expressing myofibroblast-like cells, upregulates genes for collagen type I, and increases production of cytokines and growth factors such as IL1, IL6, TNFα, PDGF, TGFβ, EGF, FGF, CTGF, and HGF, all resulting in mesangial

Table 1. Most common complement profiles and autoimmune features in GN

<table>
<thead>
<tr>
<th>Disease</th>
<th>Serum C Profile</th>
<th>Autoimmune Features</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poststreptococcal GN</td>
<td>AP or MBL normal</td>
<td>Anti-C1q, IgG AECG*, anti-DNA, ANCA, protein</td>
<td>38,44,47–50</td>
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<tr>
<td></td>
<td>C1q, low C3-C9</td>
<td>disulfide Isomerase (PDI), cardiac myosin</td>
<td></td>
</tr>
<tr>
<td>IgAN</td>
<td>Normal</td>
<td>Antiglycan, endothelial cell, mesangial cell, IgG, C1q</td>
<td>54,56,59</td>
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<tr>
<td>Anti-GBM nephritis</td>
<td>Normal</td>
<td>Anti-GBM, ANCA (20%), anti-C1q</td>
<td>87,90,94,96</td>
</tr>
<tr>
<td>ANA-positive GN</td>
<td>Normal</td>
<td>Anti-MPO, PR3, cPR3, NET, DNA, endothelial cell, ? LAMP2</td>
<td>119–122,124,133,134</td>
</tr>
<tr>
<td>Lupus nephritis</td>
<td>CP, low C1q-C9</td>
<td>Anti-dsDNA, annexin, MPO, PR3, nucleosome, IgG, C1q, C1s, C1N9, C4, cardioliopin, MBL, NET, H-ficolin, C3Nef</td>
<td>17,171–173</td>
</tr>
<tr>
<td>MPGN I</td>
<td>CP, low C1q-C9</td>
<td>Anti-C3 Nef, CFH, factor B, C1q</td>
<td>199,282</td>
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<tr>
<td>MCD/FSGS</td>
<td>Normal</td>
<td>None</td>
<td>216,246,217,215</td>
</tr>
<tr>
<td>Membranous nephropathy</td>
<td>Normal</td>
<td>Anti-PLA2R, DNA, NEP, aldose reductase, SOD2, C1q</td>
<td>83,268,255,257,260,281</td>
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<tr>
<td>DDD</td>
<td>AP, normal C1q, low C3-C9</td>
<td>Anti-C3 Nef, C4 Nef, CFH, factor B, C1q</td>
<td>7,198,283,284,290</td>
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<tr>
<td>C3 nephropathy</td>
<td>AP, normal C1q, low C3-C9</td>
<td>C3Nef, CFH, factor B</td>
<td>7,286</td>
</tr>
</tbody>
</table>

CP, classic pathway; AP, alternative pathway; MBL, mannose binding lectin pathway; LAMP2, lysosomal membrane protein 2.

the transit of dissociated subendothelial ICs to form subepithelial humps.37,45,46 Finally, PSGN often exhibits autoimmune features including both IgM and IgG rheumatoid factors with cryoglobulin activity, antiendothelial antibodies, anti-DNA antibodies, and antineutrophil cytoplasmic antibodies (ANCA), although their respective roles in mediating the disease, if any, remain unclear (Table 1).47–50 Other forms of postinfectious GN such as those associated with endocarditis, infected ventricular-atrial shunts, visceral abscesses, and Staphylococcus aureus infection with IgA deposits are clearly mediated immunologically; however, the mechanisms involved have been explored in much less detail.39,41,51,52
cell proliferation and matrix expansion. The pattern of glomerular complement deposition in IgAN includes MBL, C4d, and C5b-9 (but not C1q) that co-localize with IgA1 and suggests both MBL and AP rather than classic pathway activation. Complement deposits correlate with both disease severity and prognosis.

Rapidly Progressive, Crescentic GN
Anti-GBM Nephritis
Anti-GBM nephritis is characterized initially by an acute, focal necrotizing GN with crescents and linear deposition of IgG, usually with C3, along the GBM. When associated with pulmonary alveolar hemorrhage, it is called Goodpasture’s syndrome. The role of anti-GBM antibody deposition inducing complement activation, chemotactic factor release, and neutrophil-mediated injury was defined in NTN models in the 1960s, and the pathogenicity of human anti-GBM antibody was confirmed by the classic primate transfer studies of Lerner et al. in 1967. Studies in C5−/− and C4−/− mice implicate primarily the classical complement pathway activated by IgG1 and IgG3 anti-GBM antibody that correlates with disease activity and recurrence in transplants. Antibodies with apparently similar reactivity (but with lower titers, lower avidity, and primarily of the IgG2 and IgG4 subclasses) can be present in healthy humans.

GBM antigens are also expressed in several extrarenal tissues where they are sequestered by an endothelial cell layer impermeable to IgG. The unique fenestrated endothelium in glomeruli allows free access of IgG to GBM. The GBM antigen itself consists of two normally sequestered or cryptic epitopes, Eα and Eβ, residing on the noncollagenous domain of both the α3 and α5 chains of the NC1 hexamer of type IV collagen. Antibody deposition requires perturbation of the quaternary structure of the α3, α4, α5NC1 hexamer, possibly initiated by oxidant injury, which results in a conformational change in the α3NC1 and α5NC1 domains (an autoimmune conformeropathy).

In rodent models, the nephritogenic GBM antigen has been mapped to as few as three amino acid sequences in a core residue, but both intermolecular and intramolecular epitope spreading occur, suggesting that immune reactivity may extend beyond the initial inducing auto-antigen. Pulmonary toxins such as infections, smoke, and volatile hydrocarbons may damage endothelium and expose antigen in alveolar capillaries accounting for the pulmonary manifestations in Goodpasture’s syndrome. Whether such extrarenal events have any role in autoimmunization is not known.

T cell reactivity to GBM antigens was first demonstrated 40 years ago, and a pathogenic role for GBM antigen-specific sensitized T cells was proposed but given little credence at the time. However, many subsequent studies have confirmed these original observations with newer technologies and documented that nephritogenic GBM antigens can induce a T cell–mediated GN with crescents, proteinuria, and decreased renal function in the absence of anti-GBM antibody. The IL23/Th17 axis is central to the mediation of injury in anti-GBM models. Another unique feature of the T cell response to GBM is the appearance of long-lived Tregs and inversion of the T cell effector/ regulatory cell ratio later in the disease that may account for why recurrences of anti-GBM disease are uncommon compared with other autoimmune glomerulonephritides in which Treg activity is often impaired.

The anti-GBM immune response in humans is strongly linked to HLA DRB1 alleles 1501, 0701, and 0101 with 1501 conferring a relative risk ratio > 8, whereas 0701 and 0101 are protective. Possible triggering events include preceding infections or environmental toxins that might expose antigenic determinants in extrarenal tissue. Most patients have anti-GBM antibodies in the circulation that predate clinical disease. The disease can also be induced experimentally with a small nephritogenic T cell epitope, pCol28–40, from the α3NC1 domain, which exhibits molecular mimicry with PAMPs in some Gram-negative bacteria, especially Clostridia botulinum. Finally, recent studies indicate that glomerular-derived antigenic peptides that enter the urine can be taken up and degraded by tubular cells and then presented to interstitial dendritic cells leading to induction of an immune response in regional lymph nodes. The occurrence of ANCA antibodies and signs of vasculitis in up to 20% of anti-GBM patients, and examples of anti-GBM disease occurring with membranous nephropathy, suggest that some of the proposed etiologic factors in these diseases are operative in anti-GBM disease as well (Table 1).

ANCA-Associated GN
Necrotizing crescentic GN without immune deposits, later called pauci-immune GN, was described in 1979, and a decade later linked to ANCA directed against myeloperoxidase (MPO) and proteinase 3 (PR3). It is characterized by a focal necrotizing and crescentic GN with large gaps in the capillary wall associated with a smoldering, nephritic clinical course, usually in older individuals who may also exhibit extrarenal vasculitic disease. The major entities associated with ANCA and GN are granulomatosis with polyangiitis (formerly known as Wegener’s granulomatosis), Churg-Strauss syndrome, and microscopic polyangiitis, which may be renal-limited. Explorations of how anti-MPO and PR3 antibodies mediate GN without depositing in glomeruli have defined entirely new paradigms of immune glomerular injury.

In vitro studies show that cytokines, released in response to infections, prime neutrophils and upregulate adhesion molecules on neutrophils and endothelial cells (L and E selectins, respectively) to facilitate localization in glomerular capillaries. Cytokine-primed neutrophils redistribute cytoplasmic primary granules containing MPO and PR3 to the cell surface where ANCA IgG binds directly or through Fc, Fab’2, or neutrophil-specific Mac-1 receptors.
activating a respiratory burst with release of cationic MPO and PR3 as well as other proteases and oxidants. Neutrophil extracellular traps (NETs) are also formed containing entrapped MPO, PR3, and MPO DNA in a chromatin web and these can mediate injury directly through TLRs as well as modulate the immune response. In ANCA-GN, NETs are present in the circulation and in glomeruli co-localized with neutrophils and DCs, and anti-NET antibodies are present along with circulating MPO-DNA complexes (nucleosomes). Activation of TLR2 and TLR9 exacerbate experimental crescentic GN. MPO can also cause glomerular injury directly through oxidative mechanisms involving the MPO-H2O2-halide system resulting in halogenation of glomerular structures and severe glomerular injury.

In 2002, Xaio et al. provided the first compelling in vivo evidence for ANCA pathogenicity by transferring spleen cells from an MPO null mouse immunized with murine MPO to an immunologically compromised host to induce a T cell–independent crescentic GN with proteinuria and reduced renal function. Similar studies implicate an immune response to PR3 in pathogenesis. Other models have utilized transfer of MPO+ bone marrow, adjuvants that enhance the immune response and increase cytokine levels, and mice with subclinical GN immunized to human MPO in which the crescentic GN that follows is mediated by the immune response to endogenous MPO. Studies of the Xaio model confirm neutrophil dependence and, despite the absence of antibody deposits, a requirement for alternative complement pathway activation involving C5a and C5a receptors. Both alternative complement pathway proteins and C5b-9 deposits are found in glomeruli in human disease.

Two other ANCA antigens have also been studied. Lysosomal membrane protein 2 exhibits molecular mimicry with the Fim H group of adhesins on some Gram-negative bacteria and is expressed on endothelial cells and neutrophils.

Figure 3. Mechanisms of glomerular immune deposit formation. (A) Glomerular immune deposit formation secondary to passive trapping of circulating immune complexes. Antigen (blue dots) antibody (green) complexes are forming in slight antigen excess. Soluble immune complexes formed in the circulation are then passively trapped in subendothelial and mesangial areas of the glomerulus, where they form lattices and enlarge to become immune deposits detectable by immunofluorescence and electron microscopy. (B) Glomerular immune deposit formation secondary to in situ formation of immune deposits. In the first phase, cationic antigens (blue) localize independently of antibody in subendothelial or mesangial sites (larger antigens) or beneath podocytes in the subepithelial space (smaller antigens). In the second phase, free antibody binds to these planted antigens to form immune complexes in situ. (C) Glomerular in situ immune deposit formation due to autoantibodies to normal glomerular constituents (triangles). Antigens depicted are Goodpasture’s GBM antigen (red), mesangial antigens such as annexin (green), endothelial antigens such as human lysosomal membrane protein 2 (brown), and podocyte antigens such as PLA2R and NEP (blue).
Lysosomal membrane protein 2 antibodies correlate with disease activity and induce a focal necrotizing and crescentic GN without immune deposits in animals. However, these intriguing observations require further confirmation. An antibody directed against a 13 amino acid sequence in complementary PR3 (cPR3), encoded by the anti-sense strand of PR3 DNA, has been detected in a minority (20%) of ANCA patients. Anti-cPR3 IgG elicits an anti-idiotypic antibody response that is reactive with native (sense) PR3, suggesting a role for autoantigen complementarity in initiating the disease. Because amino acid sequences in cPR3 also have homologies with several bacteria and viruses, this could represent another link to potentially etiologic infectious agents and the innate immune system. Anti-cPR3 antibodies are also reactive with plasminogen and delay dissolution of clots in vitro, potentially contributing to the prominent fibrin deposition seen in ANCA GN.

Other groups reason that the absence of antibody deposits in ANCA-positive GN, the limited correlation between ANCA levels and disease activity, and the absence of any detectable ANCA in approximately 10%–20% of patients with typical microscopic polyangiitis suggest a primary role for antibody-independent, T cell–mediated immune mechanisms. Consistent with this hypothesis are persistent activation of T cells and elevation of soluble T cell products that correlate with disease activity, the prominence of traditional Th1 delayed-type hypersensitivity markers like T cells, macrophages, fibroblasts, and occasional granulomas in ANCA-positive GN and T cell reactivity to ANCA antigens in some patients. T cells alone, including Th17 cells, induce focal necrotizing and crescentic GN when sensitized to a planted glomerular antigen as might occur with planted cationic MPO. A recent study used combinations of mice selectively deficient in T cells, B cells, or MPO to demonstrate that active immunization with human MPO (in mice with subclinical glomerular injury) induces crescentic GN without immune deposits that requires the presence of endogenous MPO and T cell reactivity to MPO, but does not require B cells or anti-MPO antibody. Th17 cells and IL17α, as well as TLRs 2 and 9, are also essential to the development of GN in a T cell–dependent model.

Proposed etiologic agents in ANCA disease include environmental toxins such as silica and infectious agents, including Gram-positive (S. aureus) and Gram-negative (Fim H adhesins) bacteria, viral infections, and several drugs. There also have been significant but low-level associations with potential susceptibility genes and their polymorphisms, including ANCA antigens, HLA, immune response proteins, Fc receptors, cytokines and others, but no high-level associations have been described other than DRB1*15 in African Americans. The relatively frequent observation of ANCA antibodies in other autoimmune glomerular diseases including anti-GBM disease, lupus, and membranous nephropathy suggests that common etiologic or susceptibility factors may be present.

**Lupus Nephritis**

In lupus nephritis, IgG, IgM, IgA (full house), and C3 deposits are localized primarily in the mesangial in mild disease (mesangial lupus nephritis, class I and II), along the subendothelial aspect of the capillary wall with increasing proliferative/inflammatory lesions (focal or diffuse proliferative lupus nephritis, class III and IV), or in the subepithelial space with membranous lupus nephritis (class V). The autoimmune responses that underlie lupus have been extensively studied in humans and in mouse strains that spontaneously develop the disease and are beyond the scope of this review. The best-established mechanisms of GN by ANCA antibodies are reported by Dr. H. C. H. van den Hoogen and colleagues in the *J Am Soc Nephrol*.
functional immune abnormalities in lupus are loss of tolerance to numerous self-antigens, B cell hyperactivity with overproduction of autoantibodies, and defective T cell regulation. The most prominent serologic feature of lupus is the presence of IgG anti-double-stranded DNA antibodies (anti-DNA) in serum and in glomerular deposits. The deposits are usually attributed to DNA-anti-DNA ICs trapped from the circulation, although infusing anti-DNA or DNA trapped from the circulation, although in glomeruli. Whether they localize to anti-DNA antibody localization to occur in glomeruli through TLRs as well. In that capacity, they may mimic infectious non-self structures to generate DAMPs that could lead to both loss of tolerance and local inflammation.

Other non-nucleosome autoantibodies have also been implicated in different aspects of the renal lesions in lupus, particularly lupus anticoagulant, antinuclear, antiphospholipid, and anti-β2 glycoprotein I antibodies in glomerular microthrombosis, as well as anti-C1q antibodies, mixed cryoglobulins containing rheumatoid factors, and others (Table 1). Recent studies in both experimental and human lupus also implicate the Th2 immune response with B cell differentiation, activation of haptophils, and production of IgE anti-DNA antibodies that deposit in glomeruli. B cell activating factor (BAFF or BlyS), a cytokine of the TNF ligand superfamily that activates B cells and modulates the immune response by inhibiting B cell apoptosis, is increased in lupus, likely contributes to autoantibody production, and has recently become a potential therapeutic target.

The subepithelial immune deposits in class V (membranous) lupus nephritis could result from dissociation of subendothelial ICs with transit across GBM to reform in a subepithelial location or from deposition of other lupus autoantibodies with specificity for podocyte antigens as occurs in idiopathic membranous nephropathy (see below).

Complement activated by IC deposits is a major mediator of tissue injury in lupus nephritis through both intracellular generation of neutrophil and macrophage chemotactic factors (class II–IV) and formation of C5b–9 (class V). Disease severity is reduced in murine models that lack selected complement proteins and is increased with deficient regulatory proteins. Blocking studies in murine models suggest that the AP of complement is more important in mediating kidney damage than the classic pathway. The observation that deficiencies of classic pathway proteins C1r>C4>C2 are associated with increased risk for lupus suggests protective roles for complement as well. For example, 90% of patients with inherited C1q deficiency develop lupus, and C1q is produced by dendritic cells and involved in tolerance induction and clearance of both apoptotic cells and ICs.

T cells exhibit complex and abnormal phenotypes in lupus. Activated T cells are expanded, provide excess help to B cells, localize in renal cell infiltrates, and produce IL17, which correlates with disease activity, all implying CD4 and Th17 cell involvement. Antigen-specific T cell reactivity to nuclear antigens is well documented in lupus nephritis, and Th17 cells and IL17 are increased in human and murine SLE and correlate with disease activity. IL17-producing T cells, either Th17 or CD4+CD8+ (double negative) T cells, are present in nephritic kidneys, and decreasing IL17 production improves murine lupus nephritis. In addition to increased CD4 activity in SLE, most studies also suggest an accompanying defect in T regulatory cell activity.

The epigenetic events that induce autoimmunity in lupus include environmental exposures such as ultraviolet light and certain drugs and viral infections, especially Epstein–Barr virus. Some of these interact with the immune system through inhibition of DNA methylation, which can lead to overexpression of some genes resulting in hypomethylated CD4 cells, overproduction of some cytokines and Mdm2, and overproduction of IgG by B cells. There also are sufficiently well-established co-occurrences of lupus nephritis with other GNs, including ANCA-positive GN, membranous nephropathy, and even a minimal change-like podocytopathy to suggest common etiologic factors (Table 1).

**Type I Membranoproliferative GN**

Type I membranoproliferative GN (MPGN I) has many clinical and pathologic similarities to a renal-limited lupus
nephritis, including frequent autoantibodies such as rheumatoid factors and antinuclear, anticardiolipin, anti-C1q, anti-C3 convertase (C3NeF), and anti-endothelial antibodies (Table 1).196–199 Hypocomplementemia with a classic pathway profile, and increased disease susceptibility in the presence of C2 and C4 deficiency, is also common to both entities.7,9,10,186,200

The histologic features of capillary wall thickening, cellular proliferation, and infiltrating inflammatory cells associated with primarily mesangial and subendothelial deposits of IgG, IgM, and C3 are similar to lupus nephritis and are also seen in a variety of chronic neoplasias (especially deposits of IgG, IgM, and C3 are similar primarily mesangial and subendothelial targeting in J Am Soc Nephrol.

ular deposits.205 However, in contrast to lupus, MPGN I in adults is seen almost exclusively (>90%) in association with hepatitis C viral (HCV) infection, and the glomerular deposits often have prominent ultrastructural features of cryoglobulins.202–204

The principal nephritogenic HCV antigen seems to be non-enveloped HCV E2 core protein, which is demonstrable in circulating ICs and in glomerular deposits.205–207 IgG3 antibody bound to HCV E2 can interact with the globular domain of C1q, engage B cells through both B cell receptors and TLR7, and elicit production of monoclonal IgMx antibody to polyclonal anti-HCV IgG (rheumatoid factor).196–199,208 These soluble, but cryoprecipitable, aggregates of IgG, IgM, viral proteins/nucleic acids, and C1q constitute the mesangial and subendothelial immune deposits found in glomerulitis and cause local inflammation through direct interaction with TLRs 3, 7, and 9 on both infiltrating inflammatory cells and/or resident glomerular cells as well as by inducing more classic pathway C activation.196–198,209–213 As in lupus, the subepithelial deposits often seen in MPGN I (and sometimes referred to as type III MPGN) may represent subendothelial deposits that dissociate and reform in situ or autoantibodies to as yet unidentified podocyte antigens.45,46

As in lupus, complement likely plays both nephritogenic and protective roles in MPGN I. C1q seems to be important in mediating the initial interaction between IgM, IgG, HCV complexes, B cells, and TLRs,196,197,212 and complement activation by immune deposits through the classic pathway likely aggravates tissue injury,7,10 although overexpression of a complement regulatory protein, Crry, in a well studied murine model did not significantly ameliorate the disease.214 The roles of CD4 effector cells and Tregs in MPGN I are not yet well defined in either animal models or in humans.

Diseases That Usually Present with Nephrotic Syndrome

Minimal Change Disease/Idiopathic FSGS Spectrum

There are many clinical and pathogenetic observations in minimal change disease (MCD) and idiopathic FSGS, which suggest that they may represent different points on the same disease spectrum. Some patients with MCD are steroid resistant and develop FSGS, whereas some patients with biopsy-documented FSGS are steroid responsive and behave like MCD.215–217 Both can be triggered by multiple initiating events, including infections, drugs, malignancies, and others.215–217 Both are diseases of the podocyte that have been associated with circulating permeability factors,215,217–220 can recur immediately in transplants,221,222 and can resolve when affected kidneys are placed in normal environments.223 Thus, differences in disease phenotype and clinical expression could reflect variation in the quantity of a common mediator or group of mediators.

Alternatively, mutations or epigenetic differences in podocyte genes that alter response to, or recovery from, such circulating mediators might also account for differences between MCD and FSGS. Mutations in podocyte genes that regulate the slit diaphragm, cell membrane, and cytoskeleton are increasingly recognized, not only in FSGS but in other forms of GN as well.224 African Americans with nonidiopathic nephropathy express variants in the gene encoding APOLI.225–229 Both clinical and experimental studies document the importance of several other genes, especially ones that regulate the podocyte actin cytoskeleton, in modulating the development of proteinuria, foot process effacement, and sclerosis, including RhoA, urokinase receptor, Pdlim2, and connective tissue growth factor.230–234 Experimentally, podocyte expression of angiopoietin-like-4 is upregulated in experimental MCD and responds to steroids.235 Alternatively, the possibility that MCD and FSGS could involve entirely different pathogenetic mechanisms acting on normal podocytes has not been excluded.

Some evidence suggests that both MCD and idiopathic FSGS reflect the effect on podocytes of circulating, perhaps T cell–derived, non-IgG permeability factors.215,217–220 as suggested first by Shalhoub in 1974.218 Studies by McCarthy et al. demonstrate a factor in the serum of patients with recurrent FSGS that alters the albumin reflection coefficient of normal glomeruli in vitro.220 In MCD, Koyama et al. showed that factors secreted by T cell hybridomas derived from patients with active MCD transfer a MCD-like lesion to normal rats.219 Despite these in vitro and in vivo observations, identification of the responsible factor(s) has proven frustratingly elusive.220 Many cytokines and other mediators—including hemopexin, soluble podocyte urokinase receptor, TNFa, IL13, angiopoietin-like 4, and cardiotrophin-like cytokine 1—are increased in patients with MCD or FSGS and several also increase glomerular albumin permeability in vitro.231,236–238 Soluble urokinase receptor has been implicated in activating podocyte B3 integrins leading to FSGS,231 and mounting evidence suggests that increased plasma levels of soluble podocyte urokinase receptor mediate proteinuria in both active and recurrent FSGS (but not MCD) through a similar integrin-related mechanism.230 Neutralization of cardiotrophin-like cytokine 1 reduces permeability factor activity in FSGS serum as does galactose and normal serum and urine.215–217

Human studies document Th2 polarization and elevated levels of IL13, a Th2 cytokine with podocyte receptors, in active MCD.236,237 IL13 alters podocyte function238,239 and overexpression of IL13 induces albuminuria and foot process effacement.240 Transfer of CD34+
stem cells from patients with active MCD also transfers proteinuria and causes podocyte foot process effacement, although the responsible factor is unclear.241 CD80 (B7.1) is a T cell co-stimulatory molecule involved in antigen processing that is also expressed on podocytes. Podocyte CD80 activation through TLR 3 and 4, independent of T cells, causes proteinuria and foot process effacement.242,243 Recent studies by Garin et al. document increased levels of CD80 in podocytes and in urine in active MCD, but not FSGS,244 although measurement of urinary mRNA encoding CD80 demonstrates higher levels in FSGS than in MCD.245 CD80 also functions as an inhibitory molecule in T cell–DC interaction and is downregulated by CTLA4, which is decreased in both serum and urine in active MCD.244 Thus, an initiating event, or first hit, such as an infectious process, might lead to activation of podocyte CD80 by IL13 or TLR 3 or 4 ligands leading to actin rearrangement and albuminuria with CD80 shedding in the urine.245–247 The second hit would involve defective CD80 regulation by either Tregs or podocyte-derived CTLA4.246 Podocyte overexpression of angiopoietin-like-4, which, like CD80, is increased in serum and podocytes in MCD patients, induces a steroid-sensitive MCD-like glomerular lesion with heavy proteinuria, suggesting a role for this molecule in the podocyte response.248 Finally, recent studies also suggest a role for parietal epithelial cells in formation of sclerotic lesions.248,249

Membranous Nephropathy
Idiopathic membranous nephropathy is a noninflammatory glomerular lesion with exclusively subepithelial deposits of IgG and complement and heavy proteinuria.250,251 Heymann nephritis is a rat model that closely mimics the human disease.252 Studies in both active and passive Heymann nephritis models show that IgG antibodies form subepithelial immune deposits in situ by binding to a podocyte protein complex now called megalin,18,19,253 and that proteinuria is mediated by sublytic C5b-9 attack on podocytes.1,2,83 Sublytic C5b-9 activates several signaling pathways, alters the actin cytoskeleton, and upregulates expression of TGFβ and TGFβ receptors and matrix production leading to GBM thickening and spike formation. Increased podocyte production of oxidants and proteases damages underlying GBM leading to proteinuria.83 C5b-9 also leads to podocyte DNA damage and impaired ability to complete the cell cycle, which may contribute to apoptosis, podocyte apoptosis, shedding of podocytes in the urine, and development of glomerular sclerosis.254

Proof of principle that membranous nephropathy in humans can also result from an analogous autoimmune mechanism was first provided by Debiec et al., who reported alloimmunization of an infant to neutral endopeptidase (NEP) expressed on podocytes, resulting from a maternal NEP deficiency, which led to transfusional transfer of anti-NEP IgG and typical membranous nephropathy in the newborn.255 However, the anti-NEP mechanism is not operative in most cases of adult idiopathic membranous nephropathy.256 Recently, Beck et al., using microdissection and proteomic technology, identified another antipodocyte autoantibody directed against the M-type phospholipase A2 receptor (PLA2R) in 70%–80% of patients with primary membranous nephropathy and showed that IgG anti-PLA2R was present in the glomerular deposits and correlated with disease activity, response to therapy, and recurrence in transplants.257,258 Others have confirmed these findings.259,260 Antibodies reactive with aldose reductase enolase and SOD as well as PLA2R have also been eluted from membranous glomeruli, but these may represent secondary phenomena related to oxidant stress rather than primary pathogenic mediators.259

Whether the role of C5b-9 in mediating proteinuria as established in Heymann nephritis, and in the chronic serum sickness models of membranous nephropathy as well,261,262 mediates podocyte injury and proteinuria in human membranous nephropathy is unclear. Complement-independent mechanisms of proteinuria are also well described with IgG antipodocyte antibodies in several models,263–265 including Heymann nephritis,266,267 although these models do not exhibit the prominent C3 and C5b-9 deposits seen in the complement-dependent Heymann models and in humans.268,269 Despite the prominent complement deposition in membranous nephropathy, deposited anti-PLA2R antibody is predominately of the poorly complement-fixing IgG4 subclass, although complement activation might be induced by the lesser quantities of IgG1 and IgG3 usually present as occurs with anti-NEP IgG.256 However, in both human membranous nephropathy and Heymann nephritis, classic complement pathway components are often absent in glomerular deposits.269 Preliminary studies have reported that IgG4 anti-PLA2R bound to podocytes can activate C via the MBL pathway and induce sublytic podocyte injury analogous to the mechanisms defined in the Heymann models in rats.270

Membranous nephropathy can spontaneously remit,271 but once developed, the glomerular lesion heals very slowly resulting in persistent proteinuria for weeks or months after the immune response has abated and subepithelial deposits no longer are forming.272 This likely explains why only 70%–80% of patients with proteinuria and membranous nephropathy on biopsy have active disease as defined by elevated anti-PLA2R levels.257,258 Glomerular deposition of C3c and urinary excretion of C5b-9 have both been established experimentally as valid biomarkers of ongoing immune deposit formation in membranous nephropathy,272,273 but these should soon be supplanted by direct measurements of anti-PLA2R antibody in serum,274 which correlates with disease activity and response to therapy.275

Although a role for cytotoxic T cells is proposed in complement-independent models of Heymann nephritis,276,277 T cells do not have access to podocytes or the subepithelial space and are rarely seen in most Heymann nephritis models or human membranous nephropathy.269,278 No systematic studies of the role of T cells in human membranous nephropathy have been reported.
C3 Glomerulopathies

Dense Deposit Disease

Dense deposit disease (DDD) has been referred to as type II MPGN because a majority of cases resemble MPGN I by light microscopy and can have a similar glomerular deposits re

normally, low-level spontaneous hydrolysis of C3 to produce C3b leads to formation of the alternative pathway C3 convertase, C3bBb, which then catalyzes more C3 activation (C3 tickover). C3bBb is tightly regulated by circulating complement factor H (CFH), which binds the active Bb site on the convertase to impair degradation of the enzyme and prolong its half-life, leading to hypercatabolism of C3.7–10,198,282–284,288

Over 80% of DDD patients have an IgG autoantibody to the Bb active site of the alternative pathway C3 convertase (C3 nephritic factor, C3Nef) exposed after interaction of factor B with C3b that prevents normal CFH binding.282–284

However, DDD can also be associated with other loss of CFH function conditions independent of C3Nef, including congenital absence or single nucleotide polymorphisms of CFH, neutralization by an anti-CFH antibody288–290 or antibody to factor B,291 and, less commonly, with gain in function mutations in C3 that lead to C3 convertases resistant to CFH regulation.284 In mice, CFH deficiency induces massive complement activation and a DDD phenotype that is ameliorated by administration of CFH or properdin.292,293

Chronic unregulated C3 activation generates a variety of alternative pathway complement activation products that accumulate, perhaps by charge interactions, along the inner GBM to form the classic dense deposits seen by electron microscopy.284,287,290 In turn, this accumulation of proteins modifies filtration barrier structure and integrity, leading to proteinuria and nephrotic syndrome. DDD is associated with other similar disorders of complement regulation such as partial lipodystrophy, but no specific etiologic factors have been identified.

C3 Nephropathy

Glomerular deposits of C3 without Ig also characterize another C3 glomerulopathy variant, sometimes termed C3 nephropathy or C3 deposition glomerulopathy; however, the electron-dense deposits are primarily at mesangial and subendothelial sites rather than within GBM.7,285,286 These lesions also may be associated with a spectrum of histologic abnormalities including MPGN I–like findings.285,286

The disorder seems to affect younger patients who often have hematuria and proteinuria but less commonly exhibit hypocomplementemia, nephrotic syndrome, or progression compared with DDD.288 Evidence of disordered complement regulation in the form of either mutated CRPs (H402 allele of factor H, factor I), anti-CFH or anti-factor B antibodies, or C3Nef is also present in most of these patients.285,286,288–291

A familial form of the disease in people of Cypriot origin due to mutations in CFHR (CFHR5 nephropathy) has recently been described.294 The composition of the deposits and the reason for their different distribution compared with DDD are not known, although studies in murine models of MPGN suggest that abnormalities in complement factor I may play a role.295

OVERVIEW OF IMMUNE MECHANISMS

Recent advances in understanding the pathogenesis of immune glomerular diseases now link infectious processes, especially chronic viral ones, with autoimmunity and GN. Although once viewed primarily as human equivalents of the antibody-mediated serum sickness (IC) or NTN (anti-GBM) models of GN in animals, most human glomerulonephritides are now believed to be primarily autoimmune diseases. They involve both innate and adaptive immune mechanisms, with distinction between the two becoming increasingly blurred, and T cell as well as antibody-driven adaptive immune responses (Figures 1 through 4). Links to etiologic infectious agents more likely proceed through recognition of PAMPs by TLRs and triggering of autoimmune processes than through direct effects of ICs containing exogenous antigens trapped from the circulation. However, progress in translating these scientific advances to better therapies has been slow, and clinicians currently still rely almost entirely on corticosteroids and toxic, nonspecific immunosuppressive agents for treatment.

As the relevant sciences have evolved, three things have remained constant—the
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DISCLOSURES

None.

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