Pathogenesis and treatment of glomerulonephritis—an update
Patoğênese e tratamento da glomerulonefrite - uma atualização

ABSTRACT

This review updates current concepts of the genetic risk factors, etiologic events, nephritogenic responses and treatment of the major immunologically mediated types of glomerulonephritis (GN). These include post-infectious GN, IgA nephropathy, anti-glomerular basement membrane (GBM) antibody disease, ANCA-associated vasculitis (AAV) and lupus nephritis. Although the etiology(s) of most GNs remain undefined, many are now believed to be initiated by environmental insults, particularly infectious processes, that trigger host responses in genetically susceptible individuals which lead to GN. Mechanistic concepts of these diseases have evolved from earlier views that most were consequent to glomerular trapping of preformed immune complexes to the current view that most of these diseases are auto-immune in nature mediated by both antibodies and T cells reactive with self-antigens. Therapy of GN has lagged behind advances in understanding pathogenesis. Newly appreciated roles for older mediators like complement and regulatory proteins offer new therapeutic targets.

Keywords: glomerulonephritis; glomerulonephritis, IgA; lupus nephritis; nephritis.

INTRODUCTION

The past decade has witnessed major advances in understanding the etiology and pathogenesis of glomerulonephritis (GN). Rapidly evolving molecular, genetic and data management technologies have lead to the appreciation that most of the immunologically-mediated forms of GN have an auto-immune basis and are associated with genetic risk factors that determine how an individual will respond to an environmental stimulus and whether that response will include elements that result in immune-mediated injury to the glomerulus (Table 1, Figure 1). Recent reviews by others and myself have focused on the etiologies of each form of GN and on the mechanisms that underlie glomerular disease in these

Resumo

A presente revisão traz os conceitos mais atuais acerca dos fatores de risco genéticos, eventos etiológicos, respostas nefritogênicas e tratamento dos principais tipos de glomerulonefrite (GN) imunomediada. Tais patologias incluem GN pós-infecciosa, nefropatia por IgA, doença por anticorpo antimembrana basal glomerular (anti-MBG), vasculite associada a ANCA (VAA) e nefrite lúpica. Apesar da(s) etiologia(s) da maioria dos casos de GN permanecer indefinida, acredita-se que seu início se deva, em grande parte, a insultos ambientais, particularmente na forma de processos infecciosos que deflagram respostas de hospedeiro em indivíduos geneticamente suscetíveis, levando assim a quadros de GN. A concepção mecanicista em torno dessas patologias evoluiu a partir da visão mais antiga de que a maioria seria consequência do aprisionamento glomerular de complexos imunes pré-formados para a percepção atual de que as mesmas, em sua maioria, são doenças autoimunes por natureza mediadas por anticorpos e linfócitos T reativos a auto-antígenos. O tratamento da GN não tem acompanhado os progressos na compreensão de sua patogênese. Os papéis recentemente atribuídos a mediadores mais antigos como complemento e proteínas reguladoras do complemento lançam luz sobre novos alvos terapêuticos.

Palavras-chave: glomerulonefrite; glomerulonefrite por IGA; nefrite; nefrite lúpica.
entities. Paralleling these advances have been new approaches to therapy that include more specific, and potentially less toxic, agents, particularly biologic agents, that are now showing considerable promise in treating these diseases - indeed some have already been incorporated into current therapeutic guidelines.

The purpose of this review is to summarize and update concepts of the etiopathogenesis and treatment of the major forms of GN (post-infectious GN, IgA nephropathy, anti-GBM nephritis, ANCA-associated GN and lupus nephritis) as they have evolved over the past decade. Diseases presenting primarily as nephrotic syndrome (minimal change/focal sclerosis spectrum, membranous nephropathy, membranoproliferative GNs and C3 nephropathies) are not covered here.

In reviewing these GNs I will also emphasize newer ways of thinking about these diseases that are not always firmly established concepts today but point to new directions in which this field is moving.

**POST-INFECTIOUS GN (PSGN)**

**PATHOGENESIS OF POST-INFECTIOUS GN**

Although still considered the prototype of acute GN, classic post-streptococcal GN (PSGN) has become a rare disease in developed countries. This has been accompanied by an increase in the incidence of non-streptococcal “post-infectious” GNs, or “infection-related” GNs (IRGN). These entities more often present with acute kidney injury (AKI) or nephrotic syndrome than with the typical acute nephritic syndrome associated with PSGN and occur primarily in older male patients with significant associated comorbidities - especially diabetes, HIV infection and malignancy.

The pathogenesis of PSGN has always been assumed to reflect the mechanisms originally defined in acute BSA-serum sickness models in rabbits that involve passive glomerular trapping of circulating immune complexes (CIC) composed of nephritogenic bacterial antigens and IgG antibody to them, activation of complement (C) by IgG through the classical pathway and attraction and activation of neutrophils that release oxidants, proteases and neutrophil extracellular traps (NETs) that inflict the resulting glomerular tissue injury. In fact, PSGN, and other IRGNs, are the only group of GNs in which exogenous antigens, either as a component of passively trapped CICs or as initiators of in situ immune deposit formation, are still regarded as essential mediators of glomerular injury whereas the mechanisms underlying most other forms of GN are now considered to be primarily autoimmune (Table 1).

Autoimmune phenomena are certainly seen in PSGN as well including IgG and IgM rheumatoid factors, cryoglobulins, anti-DNA, anti-C1q, anti-endothelial cell antibody, anti-C3convertase (C3Nef), anti-nephritis-associated plasmin receptor and others. However, none of these findings have been frequent or consistent enough to establish an autoimmune pathogenesis for PSGN.

<table>
<thead>
<tr>
<th>Disease</th>
<th>Serum C profile</th>
<th>Auto-immune features</th>
</tr>
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<tbody>
<tr>
<td>Post-streptococcal glomerulonephritis</td>
<td>AP or MBL normal C1q, Low C3-C9</td>
<td>Anti-C1q, IgG AECAs*, anti-DNA, ANCA, protein disulfide Isomerase (PDI), cardiac myosin</td>
</tr>
<tr>
<td>IgA nephropathy</td>
<td>Normal</td>
<td>Anti-glycan, endothelial cell, mesangial cell, IgG, C1q</td>
</tr>
<tr>
<td>Anti-GBM nephritis</td>
<td>Normal</td>
<td>Anti-GBM, ANCA (20%), anti-C1q</td>
</tr>
<tr>
<td>ANCA-positive glomerulonephritis</td>
<td>Normal</td>
<td>Anti-MPO, PR3, cPR3, NET, DNA, endothelial cell, HLA-MP2</td>
</tr>
<tr>
<td>Lupus nephritis</td>
<td>CP, low C1q-C9</td>
<td>Anti-dsDNA, annexin, MPO, PR3, nucleosome, IgG, C1q, C1s, C1-INH, C4, cardioliopin, MBL, NET, H-ficolin, C3Nef</td>
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Figure 1. Schematic overview of the mechanisms linking initial exposure to an etiologic agent in a genetically susceptible individual to an autoimmune response and glomerular tissue injury. 1. Hereditable risk factors predispose certain individuals to respond to environmental factors in ways that can lead to a nephritogenic autoimmune response (Hit #1). 2. Exposure to infectious etiologic agents in the environment occurs (Hit #2), may be modified by epigenetic factors, and activates the innate immune system through interactions with TLRs and complement. 3. Conversion of a non-antigen-specific innate immune response to an antigen-specific adaptive immune response directed at specific auto-antigens can occur through several pathways that may operate simultaneously and in concert. These include defects in regulation of existing natural autoimmunity, molecular mimicry, epitope spreading, epitope conformational changes, adjuvant/bystander effects and auto-antigen complementarity. 4. The adaptive immune response generates antigen-specific T and B cells, usually directed at antigens that are fixed or “planted” in the glomerulus. These immune reactants, usually through inflammatory effector cells and/or complement, mediate tissue damage. (Reprinted with permission from Reference 1. Couser WG, Johnson RJ. The etiology of glomerulonephritis: roles of infection and autoimmunity. Kidney Int. 2014;86:905-14.)

However, newer serologic and immunopathologic data now suggest the pathogenesis is more complicated and implicate primarily the alternative C pathway (AP) in these diseases. It has long been appreciated that C3 is the dominant component seen by IF with IgG much less prominent and sometimes absent, a phenomenon not seen in traditional immune complex-mediated diseases. Some proposed nephritogenic streptococcal antigens localized in glomeruli, such as pyogenic streptococcal exotoxin B (PSEB), can activate the AP directly through the mannose-binding lectin (MBL) pathway, independently of antibody, a process that might account for the long appreciated co-localization of PSEB and C3 and the dominance of C3 in glomerular deposits. C activation in PSGN is also predominately via the alternative pathway (AP) whereas C activation by IgG-containing ICs usually occurs through the classical pathway. Indeed, both genetic and acquired deficiencies in complement regulatory proteins like complement factor H (CFH) have now been reported in “atypical” PSGN patients who exhibit prolonged, progressive disease rather than complete recovery as is classically seen in childhood PSGN. This supports considering many cases of PSGN as one part of a spectrum of that includes some C3-dominant IRGNs and the recently recognized “C3 nephropathies” rather than as a traditional BSA-like immune complex (IC) disease. Thus we now recognize a spectrum of overlapping entities that include classic PSGN, IRGN, atypical PSGN, and C3 nephropathies in which infections are often precipitating events but deposition of CICs seems unlikely to be the major mechanism, and host abnormalities in C activation and regulation may play more important roles. Whether these newer mechanisms are operative only in unusual cases or play a more generalized role in PSGN awaits further study.

Treatment of Post-Infectious GN

Current guidelines for treatment of PSGN, or other infection-related GNs, involve only supportive care in PSGN and treatment of on-going infection in IRGN. Although some have advocated use of pulse steroids in PSGN patients who present with AKI and crescentic glomerular lesions, there is minimal data to support the benefit of this approach, especially in patients with on-going bacterial infections. However, in response to the changing concepts of disease pathogenesis outlined above, some patients refractory to steroid therapy have been treated with Eculizumab, a humanized monoclonal anti-C5 antibody that inhibits C5 activation, and dramatic benefits have been observed.

Future progress in this area will involve identifying biomarkers that facilitate identification of patients unlikely to experience full spontaneous recovery and early treatment of such patients with biologics such as complement inhibitors to stop on-going inflammation and its long-term consequences. The observation that risk of long term CKD and associated cardiovascular disease is significantly increased in patients who had PSGN and underwent spontaneous “complete” clinical recovery raises the possibility that early therapeutic intervention may ultimately be beneficial even in those patients who are currently treated with only supportive care. These newer observations also raise hope that protective vaccination against nephritogenic bacterial and...
viral molecular patterns may become an option for genetically susceptible individuals in endemic areas in the years ahead.

Because of the favorable prognosis in PSGN, there is no data on recurrent disease in renal allografts.

**IGA Nephropathy (IGAN)**

**Pathogenesis of IGAN**

Major advances in understanding the pathogenesis of IGAN, the most common form of GN in the world, have occurred over the past decade despite the fact that progress in this disease has been hindered by the lack of a good animal model caused by mechanisms similar to those defined in the human disease. New findings include appreciation that the glomerular-deposited IgA is IgA1, normally of mucosal origin, that a fraction of this IgA1 is underglycosylated in the hinge region in both patients and disease-free relatives, and that many patients exhibit an IgG anti-underglycosylated IgA1 antibody (anti-glycan antibody) response that correlates with disease activity, outcome and recurrence. Finally, the IgG anti-glycan antibody is directed at the site of underglycosylation in the hinge region of IgA1, a site that exhibits molecular mimicry with some bacterial antigens such as TN.

Over 100 genes associated with increased risk for IGAN have now been identified and implicate the innate immune system, likely responsible for the immediate hematuria commonly observed following upper respiratory tract or gastrointestinal infections in IGAN patients, autoimmunity (HLA alleles), mucosal barrier function and the complement system. Although still poorly defined, it seems likely there is some connection between the gastrointestinal system and its mucosal-associated lymphoid tissue, intestinal microbiota, the innate immune system and development of IGAN. The presence of predominately IgA deposits in some cases of IGAN and the most common form of GN following infection with methicillin-resistant staph aureus and occasional demonstration of staph antigens in the mesangium in IGAN as well as the observation that IgG anti-glycan antibodies exhibit molecular mimicry with bacterial TN antigens all support a role for an infectious etiology involving the innate and mucosal immune systems. Other non-infectious causes of intestinal inflammation and barrier dysfunction such as inflammatory bowel disease, gliadin or other dietary intolerances may be etiologic as well. Although CICs containing galactose-deficient IgA1 and IgG, IgA or IgM antibody to it are present in the circulation, they do not correlate well with clinical or pathologic features of the disease. It remains unclear if the mesangial IgA deposits reflect primarily these preformed ICs trapped from the circulation as suggested by some authors or in situ formation of ICs due to the inability of asialoglycoprotein receptors in liver and spleen to clear these high molecular weight, glycan-deficient molecules from the serum with consequent uptake in the glomerular mesangium where they serve as “planted” antigens for IC formation. Finally, it is increasingly clear that, although IgA is a poor activator of the classical pathway of complement compared to IgG, complement activation through the MBL or AP is ongoing in IGAN as assessed by increased glomerular deposition of short-lived C3c and increased serum levels of complement activation products that correlate with disease activity and outcomes. Sublytic C5b-9 attack on mesangial cells activates them to proliferate and over-produce oxidants, proteases, cytokines (TGFβ), growth factors (PDGF) and extracellular matrix material that together result in the typical focal proliferative GN with mesangial matrix expansion characteristic of IGAN (3). Thus IGAN joins most other forms of GN in occurring consequent to a genetically-determined autoimmune response to environmental, likely predominately infectious, agents.

**Treatment of IGAN**

Because of its frequency, a number of therapeutic initiatives have been well studied in IGAN. The recent development and validation of a histologic classification system, the Oxford-MEST classification, will facilitate future clinical trials and selection of patients for therapy. The benefit of good blood pressure control and reducing urine protein excretion to < 500 mg/day using 6 mos of conservative therapy with ACE inhibitors and/or angiotensin receptor blockers is well established and is the only therapy needed in over 75% of patients. A course of oral steroids in patients with GFRs of > 30 ml/min and proteinuria > 750 mg/day after 6 months of ACEI/ARB therapy has now been well shown to slow progression and reduce the incidence of ESRD. Recent evidence suggests that giving steroids in a form that targets only the small intestine with minimal systemic absorption (budesamide) is
also beneficial in reducing proteinuria and potentially slowing progression.\textsuperscript{33}

Although additional immunosuppression (aza-thioprine, cyclophosphamide or MMF) has reduced rates of protein excretion in IgAN in some studies, no benefit on preservation of renal function has been shown, and these agents are not generally recommended except in rare patients with rapidly progressive, crescentic disease.\textsuperscript{30,32} Multiple studies have also failed to firmly establish a benefit for fish oil or tonsillectomy in progressive IgAN, although fish oil in a dose of > 3.3 gm/day is recommended in some guidelines.\textsuperscript{4,30} No credible data on the efficacy of biologic agents such as Rituximab and Eculizumab, or PDGF or TGFβ inhibitors, is available yet in IgAN, although several studies are in progress.\textsuperscript{5,30}

IgAN recurs histologically in up to 30% of renal allografts, but usually is manifest only as mesangial deposition of IgA by IF. Recurrence has significant clinical manifestations in about 13% and has a relatively minimal impact on long term graft survival compared to other recurrent GNs.\textsuperscript{34}

**ANTI-GBM NEPHRITIS**

**PATHOGENESIS OF ANTI-GBM NEPHRITIS**

Despite its infrequency, anti-glomerular basement membrane antibody (aGBM) disease remains the prototype of autoimmune disease in man. The disease accounts for about 12% of US patients with rapidly progressive, crescentic GN, and over 80% of patients have crescent formation in > 50% of glomeruli.\textsuperscript{35} Much of what we understand about the immune pathogenesis of acute, inflammatory glomerular diseases in general is derived from studies in animal models of aGBM disease (“nephrotoxic nephritis”, NTN) developed by transferring heterologous anti-GBM IgG into multiple different species (rabbit, sheep, monkey, rat, mouse, guinea pig).\textsuperscript{3,36,37}

The etiology of the disease remains unknown although preceding infections, both bacterial and viral, have been frequently noted clinically, and molecular mimicry between GBM and pathogen-associated molecular patterns (PAMPs) in several bacteria, especially Clostridia botulinum, has been demonstrated.\textsuperscript{1,38} Exposures to pulmonary toxins such as hydrocarbons, formaldehyde and cigarette smoke, as well as some drugs, have also been postulated to be etiologic based primarily on multiple case reports. The disease occurs with increased frequency in association with both membranous nephropathy and anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV), and 20-30% of patients with anti-GBM nephritis in most series have positive ANCAs as well.\textsuperscript{37,39} The molecular mechanisms accounting for these associations between anti-GBM and membranous nephropathy or AAV remain unknown.\textsuperscript{39,40}

There is a strong association with HLA DRB1-1501, which is present in over 80% of patients with anti-GBM disease, and increases risk for the disease by over 8 fold, the strongest association between HLA and any autoimmune disease recognized to date. DBR1-0701 is protective.\textsuperscript{41}

The nephritogenic antigen in man is a 14 amino acid fragment of the NC1 domain of type IV collagen with some reactivity seen with type III collagen as well.\textsuperscript{42} The antigen is sequestered and must undergo conformational change to be accessible to circulating antibody or T cells.\textsuperscript{42} What induces this conformational change, and whether it precedes or follows induction of an immune response to GBM is not known, although a role for oxidant injury has been proposed.\textsuperscript{42} Both IgG1 and IgG3 anti-GBM antibodies that activate complement via the classical pathway, and T helper cells, predominately Th17 cells reactive with GBM T cell epitopes, have been shown to be capable of transferring the glomerular disease independently of each other, and both mechanisms are likely operative in man.\textsuperscript{3,43} An IgG4 variant of anti-GBM disease, often with negative ELISA assays for the antibody, has recently been described in young women who paradoxically have severe disease but good outcomes.\textsuperscript{44} Antibody to a complementary peptide in the alpha III chain of type IV collagen has been shown to be nephritogenic experimentally and present in man, similar to the complementary cPR3 story in AAV (see below), but a pathogenic role for these antibodies in man has not been established.\textsuperscript{45} There are many reports of patients with positive anti-GBM antibody assays in the absence of pulmonary or renal disease, so-called “natural” anti-GBM antibodies.\textsuperscript{46} In most of these patients the specificity of the antibody is similar to that in patients with active clinical disease, and some believe that loss of regulation of these natural antibodies may lead to much higher titers and pathogenicity.\textsuperscript{46} However, some patients also have antibody to apparently non-nephritogenic components of GBM.\textsuperscript{46}
A role for complement activation, through both the classical and alternative pathways, in mediating the antibody-induced portion of the disease has been well established experimentally and suggested in man by glomerular deposition of components of both the classical and alternative pathways and increased serum and urinary levels of complement activation products that correlate with disease severity and outcomes.47,48

**TREATMENT OF ANTI-GBM NEPHRITIS**

Successful therapy of anti-GBM disease requires prompt recognition of the entity before the serum creatinine exceeds about 5.7 mg/dl, anuria develops or dialysis is required.49-51 Although the serum creatinine cut-off for successful therapy of 5.7 is arbitrary, there is no question that anti-GBM nephritis presenting as RPGN is a medical emergency, and the need for therapy is urgent. The recommended treatment regimen consists of steroids, initially given as daily “pulse” steroids, 1000 mg iv three times, oral cyclophosphamide and plasma exchange, usually carried out daily or on alternate days for 2-3 weeks until anti-GBM antibody is no longer detectable in the serum.52-54 Immunoabsorption and double filtration plasma exchange have both been shown to remove antibody somewhat more efficiently than conventional plasma exchange, although no impact of these more expensive and less available therapies on outcomes has yet been established.55,56 Seven case reports of success with B cell depletion using Rituximab have been published with some patients recovering renal function after being on dialysis,57-60 but the time required for B cell depletion and reduction in antibody levels to occur with Rituximab (2-4 weeks) in this rapidly progressive disease that demands immediate therapeutic intervention makes it less attractive as the primary induction therapy.

Transplantation is considered safe and effective if anti-GBM antibody has been undetectable for 6 months and active pulmonary disease is no longer present.14,52,61 In the absence of ANCA, anti-GBM disease rarely recurs, perhaps because of a strong rebound in regulatory T cell populations, and therefore maintenance immunosuppressive therapy is not recommended.32 However, if the patient is one of the 20-30% with dual positivity for both anti-GBM and ANCA antibodies, recurrence of vasculitic symptoms is common and maintenance immunosuppression as described below for AAV should be implemented.37,40,52

**ANCA-ASSOCIATED VASCULITIS (AAV)**

**PATHOGENESIS OF AAV**

Current pathologic classifications of the ANCA-associated vasculidites (AAV) that commonly involve the glomerulus include microscopic polyangitis (MPA), granulomatosis with polyangitis (GPA, formerly Wegener’s granulomatosis) and eosinophilic granulomatosis (EGA, formerly Churg-Strauss disease).62 However, recent genome wide association studies indicate that patients with anti-MPO and anti-PR3 have different genotypes that correlate better with the specificity of the ANCA antibody than with clinical manifestations of MPA or GPA suggesting that clinicians and clinical treatment trials should utilize these genetic or serologic parameters rather than MPA and GPA to define subgroups of AAV for therapeutic purposes.63

The renal manifestations of all of these small vessel vasculidites with positive ANCA antibodies often include focal necrotizing GN without significant glomerular immune deposits (“pauci-immune”), crescents in over 50% of glomeruli and often a rapidly progressive course.40,62 About 10-20% of patients with typical MPA or EGA are ANCA negative in conventional ELISA assays (see below).64-66

There is considerable evidence that infections, both bacterial and viral, are common etiologic agents in AAV, which was originally described in association with Ross River virus infection (reviewed in 1). Non-infectious environmental exposures to drugs (hydralazine, propothiouracil and recently levamisole-adulterated cocaine) have also been implicated.1,64-66 Several mechanisms by which an initial innate immune response to infection can be transformed into an antigen-specific adaptive immune response have been identified in AAV including molecular mimicry, auto-antigen complementarity and epitope conformation.13 The resulting autoimmune response to MPO (or PR3) includes both humoral (ANCA) and T cell components.1,3,64-66

A role for anti-MPO antibody in mediating AAV has been established by both in vitro and in vivo studies.1,64-66 MPO is normally localized in the primary granules of neutrophils but relocates to the cell surface in response to inflammatory cytokines
such as IL1 and TNF. These cytokines also increase expression of leukocyte adhesion molecules on both neutrophils and capillary endothelial cells facilitating neutrophil localization in glomerular capillaries. Anti-MPO IgG then binds to MPO on the neutrophil surface leading to activation of the cell and release of multiple inflammatory mediators including oxidants, proteases, MPO itself and neutrophil extracellular traps (NETs). NETs contain MPO (or PR3) protein and DNA in a histone/chromatin web, are thought to be the primary effectors of neutrophil-mediated injury and can circulate, present antigen to the immune system, promote hypercoagulability and activate the alternative pathway of complement.

In vivo, a mouse model of ANCA-associated vasculitis has been utilized to confirm the role of both neutrophils and complement in AAV with most studies suggesting that C5a and its receptor are the key components.

Other studies have also confirmed the ability of MPO-sensitized T cells to mediate a focal necrotizing GN with crescents in animal models with MPO localization in the capillary wall, and T cells reactive with MPO are present in increased numbers in patients with AAV. Finally, free MPO, which is localized in significant amounts in glomeruli in AAV, can react with a halide to cause halogenation of glomerular structures and severe tissue injury independent of both antibody and T cells. In man it is likely that all three MPO-related mechanisms of injury (antibody, T cells and direct MPO-mediated injury) are operative with antibody perhaps more important in neutrophil localization/activation and complement activation while T cells more likely contribute to focal necrosis and crescent formation.

About 10-20% of patients with clinically typical MPA and EGA are ANCA-negative in conventional commercial assays. Several observations may explain this. A different ANCA antibody directed to HLAMP2, an antigen present on both neutrophils and endothelial cells, has been reported in 67% of AAV patients by one laboratory but not yet confirmed by others. This antibody recognizes the bacterial fimbrial antigen FimH strengthening the case for an infectious/molecular mimicry etiology in these patients, and it transfers disease in rodents thus supporting an anti-endothelial antibody mechanism for AAV. Other ANCA variants that may prove relevant include antibodies reactive with epitopes on MPO that are blocked by circulating ceruloplasmin providing an explanation for some negative results with conventional ANCA assays. Finally, some 30% of patients have an anti-idiotypic antibody directed to a non-pathogenic antibody against the anti-sense strand of PR3 (complementary PR3, or cPR3) (autoantigen complementarity). These anti-idiotypic antibodies are reactive with PR3 and also with pathogen-associated molecular patterns on several bacteria that may be etiologic in AAV. The roles of these several ANCA variants in mediating GN in AAV are currently under investigation.

**TREATMENT OF AAV**

Treatment of AAV, like treatment of lupus nephritis (see below), is divided into induction and maintenance phases. Regardless of its putative role in the pathogenesis of AAV, ANCA antibodies have generally not proven to be reliable biomarkers of disease activity. However, recent studies suggest that in patients with significant renal involvement conversion from negative to positive or a rise in ANCA levels can predict relapse, and it is generally true that major relapses do not occur in ANCA-negative patients. One study has suggested that patients’ subjective feelings about their disease activity more accurately predict relapse than available biomarkers.

Corticosteroids remain a mainstay of both phases of therapy and are usually administered initially as IV “pulse” therapy (1000 mg) for 3 days followed by an oral dose of 1mg/kg for 3-4 mos and tapering to 5-10 mg/day, or on alternate days. Standard induction therapy with steroids and cyclophosphamide results in an initial remission rate of about 80% at one year, a relapse rate of 50% and a mortality rate of 25% in 5 years. Recent therapeutic trials have focused on trying to lower the dose of cyclophosphamide to reduce adverse events, to define “steroid-sparing” approaches to minimize steroid exposure and to identify alternative therapies that are as effective, or more effective, with fewer adverse events. IV and oral cyclophosphamide have been shown to give
comparable short-term results with the iv regimen utilizing lower total doses of cyclophosphamide (CYCLOPS)\textsuperscript{90} but at the expense of a somewhat higher relapse rate.\textsuperscript{91} Recent trials (RAVE, RITUXIVAS) have compared B cell depletion with Rituximab (500-1000 mg iv every two weeks X4) to oral and iv cyclophosphamide induction and demonstrated comparable efficacy (and comparable incidence of significant adverse events).\textsuperscript{92-94} The RAVE results have been maintained out to 18 mos\textsuperscript{95} and in that subset of patients with severe renal involvement.\textsuperscript{96} In most trials, the response of MPO-ANCA patients to immunosuppression (70-80\% sustained remission) has been better than that seen in PR3-ANCA patients (30\% sustained remission) with lower relapse rates as well.\textsuperscript{81,82} Because of the immediate onset of action and the extensive experience with the drug, most clinicians prefer cyclophosphamide as the first choice for induction therapy in patients with severe, acute, crescentic disease.\textsuperscript{97} Currently Rituximab is the first choice for frequently relapsing patients, those with milder disease, especially if fertility or risk of malignancy are issues, and those who fail cyclophosphamide induction, reach maximal cumulative cyclophosphamide exposure (about 36 gms) or relapse following cyclophosphamide induction.\textsuperscript{98}

The role of plasma exchange (PLEX) in induction therapy for AAV is uncertain.\textsuperscript{99} An initial RCT adding PLEX to conventional steroid/immunosuppressive drug therapy in patients with severe disease (serum creatinine > 5.8 mmol/L or on dialysis less than two weeks) (MEPEX trial) showed better short-term outcomes in the PLEX group at 3 and 12 mos,\textsuperscript{100} a finding confirmed by meta-analysis of all existing studies.\textsuperscript{91,93} However, longer-term follow-up confirmed a reduction in end-stage renal disease (ESRD) but no survival benefit in the PLEX group.\textsuperscript{99,101} Hopefully the on-going PEXIVAS trial involving patients with less severe disease will provide more clarity on this issue.\textsuperscript{102}

The maintenance phase of therapy for AAV is designed to prevent relapses, which are clearly associated with poorer outcomes.\textsuperscript{103} Azathioprine was shown in the IMPROVE study to be more effective than mycophenolate mofetil (MMF) in maintaining remission and is usually continued for 12-18 months (with low dose steroids).\textsuperscript{103} Because of the better prognosis in MPO-ANCA patients, low dose steroids alone may be sufficient maintenance therapy if patients are in complete remission and ANCA antibody is absent.\textsuperscript{103} However, recent studies have shown a superiority of Rituximab over AZA as maintenance therapy in AAV and reported dramatic reductions in relapses when Rituximab, usually given in induction doses every 4-6 mos, is continued as maintenance therapy.\textsuperscript{104,105} In one observational study of 172 patients, the long-term survival in AAV patients maintained on Rituximab every 4 months was indistinguishable from the general population.\textsuperscript{106} Although KDIGO guidelines recommend re-treating relapsing patients with the same regimen used for induction, the trend to increased use of B cell depletion and diminishing use of standard immunosuppressive therapy for both induction and maintenance in AAV seems likely to continue as better controlled studies, longer term follow up and newer and more effective B cell depleting or blocking agents become available in the near future. Immunosuppressive therapy for GN should not be continued for more than 4 months after a patient requires dialysis as recovery of renal function is extremely rare and the incidence of adverse events is high.\textsuperscript{52}

Transplantation is considered safe and effective in AAV if signs of active disease have been absent for 12 mos or more to allow recovery from immunosuppressive therapy for the original disease.\textsuperscript{61,107} The recurrence rate is about 9\% and can be renal, systemic or both but has not been documented to alter graft survival.\textsuperscript{61} No serologic parameters preclude transplantation or predict recurrence including elevated ANCA antibody levels.\textsuperscript{52}

**Lupus Nephritis**

**Pathogenesis of Lupus Nephritis**

Lupus nephritis (LN) is another form of autoimmune GN in which concepts of both pathogenesis and treatment have changed over the past decade.\textsuperscript{108-110} Using GWAS, over 50 genetic risk factors (polymorphisms) have been identified in SLE including SNPs involved in IFN1/NFKB signalling, B cell signalling, T cell signalling, the classical complement pathway and apoptosis/debris clearance/IC mechanisms that assure low levels of DNA in extracellular compartments.\textsuperscript{111-113} With a few exceptions such as PDGF and ABIN1, no genetic risk factors specific for LN have been consistently identified.\textsuperscript{114}
Etiologic factors in SLE are diverse and include viral infection, especially EBV, certain drugs and exposure to UV light.\textsuperscript{1,108,109} There is a remarkable similarity between the immune environment in SLE and that stimulated by viral infections that initiate Type I interferon (IFN-\(\alpha\)) signalling pathways resulting in a “signature” of specific gene expression that is observed with both viral infections and SLE.\textsuperscript{115-117} Indeed, IFN-\(\alpha\) has been shown to be essential for development of SLE in both spontaneous and induced animal models.\textsuperscript{117,118}

Regardless of etiology, considerable data implicates defects in apoptotic cell clearing mechanisms leading to presentation of nucleosomes containing DNA in a cationic histone coating to the immune system and provoking an autoimmune response.\textsuperscript{108-110} The anti-DNA and anti-nucleosomal B cell response leads to formation of immune deposits in glomeruli containing these nucleosomal components. Studies of antibody eluted from glomeruli of patients with proliferative LN reveal it to be directed mostly against the components of NETs – nucleosomes, DNA and histones.\textsuperscript{119} Whether these deposits reflect passive trapping of preformed CICs or \textit{in situ} formation of deposits probably initiated by the binding of the highly cationic histone component of nucleosomes to glomerular anionic sites to serve as “planted” antigens is not known in man. However, the degree of inflammation induced by the deposits suggests an \textit{in situ} origin.\textsuperscript{3} Mesangial and subendothelial deposits in Class II-IV proliferative disease likely have similar origins. There is also evidence that some anti-DNA antibodies can react directly with glomerular cells and other components (and that some non-DNA-specific antibodies in LN are directed to glomerular components) and can cause disease.\textsuperscript{111}

The glomerular injury consequent to these deposits is believed to be primarily complement-mediated evidenced by activation of the classical, MBL and APs as judged from levels of complement components and activation products in the serum, urine and biopsy tissue.\textsuperscript{121,122} Although measurements of serum complement component levels and levels of a variety of autoantibodies, including anti-nucleosomes, anti-DNA and anti-C1q, are frequently abnormal, such studies have failed to firmly establish any of these as reliable biomarkers of disease activity in individual patients with LN. Recent reports suggest that anti-C3b levels may identify patients prone to a flare in renal disease activity.\textsuperscript{123}

About 10-20% of patients with LN will have Class V, or membranous, glomerular lesions.\textsuperscript{124} These patients differ significantly from those with more proliferative lesions in pathology, clinical manifestations and probably pathogenesis. Clinically, patients with a Class V lesion are often young women who present with nephrotic syndrome but may initially not manifest serologic parameters suggestive of SLE.\textsuperscript{108,109} In contrast to primary MN in which immune deposits are exclusively subepithelial, in lupus MN IgG deposits are composed of IgG1-3 rather than the IgG4 seen in primary MN, contain other classes of immunoglobulins including IgM, IgA and IgE, can usually be found in subendothelial and mesangial locations rather than exclusively in the subepithelial space and are often accompanied by viral-like tubuloreticular structures.\textsuperscript{125,126} In addition to distinguishing these patients from ones with primary MN, it is also important to distinguish them from “lupus podocytopathy” in which lupus patients present with severe nephrotic syndrome not explainable by the paucity of immune complex deposits present in the biopsy.\textsuperscript{127} These patients are currently believed to have a variant of minimal change nephrotic syndrome (MCNS) superimposed on a relatively mild LN.\textsuperscript{127} Whether the occurrence of MCNS in some lupus patients is coincidental or pathogenetically related is unknown.

**TREATMENT OF LUPUS NEPHRITIS**

Like AAV discussed above, therapy of lupus nephritis is conventionally considered in terms of induction and maintenance phases.\textsuperscript{128,129} Approach to therapy is also based on the findings by renal biopsy classified according to the 2004 ISN/RPA classification, although some modifications and updates of this system have recently been proposed.\textsuperscript{130,131} However, concordance between renal biopsy and clinical findings in LN are imperfect with 33% of patients judged in complete remission clinically having signs of disease activity on the biopsy and 62% of those judged inactive by biopsy still having clinical signs of disease activity.\textsuperscript{132} Current guidelines for therapy are formulated and published by 3 main groups, the US KDIGO guidelines,\textsuperscript{52} the American College of Rheumatology guidelines\textsuperscript{133} and the Joint European League Against Rheumatism and European Renal Association-European Dialysis and Transplant Association (EULAR/ERA-EDTA) recommendations...
for the management of adult and paediatric lupus nephritis with only minor differences between them.

All guidelines recommend that patients with signs of renal involvement receive ACEI/ARB therapy to control blood pressure and reduce urine protein excretion to the lowest possible level, a treatment shown to significantly reduce proteinuria and lower the risk of active renal disease a decade later. Treatment of patients with hydroxychloroquine has also been shown in the LUMINA study to reduce the likelihood of developing ESRD, proteinuria or an eGFR of < 50 ml/min and is generally recommended for all patients. Recommendations for steroid therapy begin with Class II disease and proteinuria exceeding 3.0 g/day and extend to all patients with more severe disease. Steroids are usually initiated with pulse methyl prednisone, 500-1000 mg/day on alternate days for three days followed by oral prednisone or equivalent, 1 mg/kg/day, or 2 mg/kg on alternate days, tapering slowly down to 5-10 mg/day over about 3 months and continuing that dose until a sustained complete response is achieved and maintained and no more immunosuppression is being given. Although most patients with active LN will require additional immunosuppression, a short trial of steroids alone in some patients with early, mild disease is acceptable.

The addition of immunosuppression, primarily cyclophosphamide, has been shown to be more effective in suppressing disease activity and preserving renal function (even if initial renal function is reduced) and treating “flares” than steroids alone, although 4-5 years of follow up was required initially to demonstrate significant benefit. IV cyclophosphamide therapy has been shown to be as effective as oral cyclophosphamide with lower cumulative doses. The most popular cyclophosphamide regimen currently is the “Euro-lupus” protocol which utilizes 500 mg of cyclophosphamide given bi-weekly 6 times (about 3.5 gms) before switching to a less toxic drug such as azathioprine for maintenance. However, for patients with severe, acute disease many clinicians still prefer to begin induction with the higher dose “NIH protocol” (500-1000 mg/m²/month for 6 mos (about 8.5 Gms) which has been better studied in such patients. Plasma exchange has not achieved a place in the treatment of severe LN except when a component of thrombotic microangiopathy or anti-phospholipid syndrome is present.

In the ongoing search for drugs with equivalent or improved efficacy and less toxicity than cyclophosphamide, studies over the past decade have focused primarily on mycophenolate mofetil (MMF) and have now established MMF to be an oral drug with similar efficacy (and similar adverse event rates) to cyclophosphamide, but MMF is much more popular with patients who are spared the hair loss and bone marrow toxicity associated with cyclophosphamide therapy. MMF, usually used for induction in a dose of 2.5-3.0 gm/day, has equivalent efficacy to cyclophosphamide in inducing remission in the short term (50-60%), results confirmed in several populations including adolescents and patients with severe initial disease and eGFR < 30 ml/min. However, MMF does not have a significantly lower adverse event rate than cyclophosphamide, and it appears to be associated with a shorter time to relapse and a higher relapse rate in the longer term. Thus most clinicians still prefer to induce initial remission in severe LN (Class IV disease or > 15% crescents on biopsy, subendothelial deposits by EM, proteinuria > 2 gms/day and decreased GFR in Caucasian patients) with steroids/cyclophosphamide using the Euro-Lupus or NIH protocols. MMF is the preferred induction therapy in patients with milder or relapsing disease, Blacks, Asians, patients with fertility issues or patients who have failed a course of cyclophosphamide or are approaching the long-term cumulative exposure to cyclophosphamide that is associated with increased risk of malignancy (About 36 gms). With appropriate patient selection about 80% of patients with LN can achieve long-term remission. Recent studies have shown that Tacrolimus is equivalent to MMF in Asian patients as induction therapy in LN (although relapses and progression may be higher than with MMF) suggesting another option in patients who cannot tolerate, or do not respond to, cyclophosphamide or MMF.

Unlike AAV where B cell depletion has been shown in two RCTs to be non-inferior to IV cyclophosphamide for induction therapy (see above), in LN two trials failed to show any benefit of Rituximab when added to conventional therapy with MMF or cyclophosphamide. However, meta-analysis of several trials of Rituximab in LN have suggested a benefit with response rates of over 80% in refractory patients with LN and about a 24% relapse rate, and the lupus community...
remains optimistic that a role for B cell depletion in LN will become established with larger and better designed studies.148,149 Meanwhile, some clinicians are employing Rituximab for induction, especially in younger female patients, and the drug is commonly used as rescue therapy in patients who do not respond to induction with cyclophosphamide or MMF, relapse or have contraindications to their use.141

Another possible indication of a future role for Rituximab in LN emerges from the “Rituxilup” study in which patients (40% class III-IV, 43% class V) were treated with 500 mg of steroid pulse therapy and 2 infusions of Rituximab 2 weeks apart followed by MMF maintenance and no oral steroids.151 The results (86% complete or partial remission with a 24% relapse rate at one year) are comparable to those achieved with cyclophosphamide or MMF induction in the ALMS study and better for patients with class V disease.151 More definitive RCTs of this steroid-free regimen are in progress.

About 50% of patients with LN who achieve initial remission following cyclophosphamide/steroids or MMF/steroids induction protocols will relapse.128,129,140,144 There are no serologic parameters that accurately predict relapse better than conventional clinical parameters. Reduction of Uprot to < 1.0 gm/day within 6 mos (or < 0.8 gms at 12 mos) predicts Scr < 1.4 at 10 years.122 Long-term follow-up of the ALMS study subjects comparing steroids/cyclophosphamide with steroids/MMF for induction included comparison of MMF and azathioprine for maintenance therapy to prevent relapses. The results showed a clear benefit of MMF over azathioprine as maintenance therapy in the entire cohort, but equivalence of the two drugs in white patients.153 There is little data on how long maintenance immunosuppressive therapy should be continued in LN, but most guidelines recommend at least one year after a complete remission and 3-4 years after a partial remission.52,129

Available data suggest that the response of patients with Class V LN to induction therapy with cyclophosphamide/steroids and MMF/steroids is about the same at 6 months but less than that achieved in the more proliferative lesions.154 Current recommendations are to treat patients with pure Class V lesions, less than 3.0 gms of proteinuria and stable renal function with supportive, antiproteinuric therapy only reserving cyclophosphamide or MMF induction for patients who do not respond to more conservative therapy, have > 3.0 gms of proteinuria or evidence of progressive loss of GFR.128,129 The response to Rituximab in patients with membranous LN in the Rituxilup study (37% complete remission at one year) suggests a potential role for B cell depletion in these patients, but additional trial data is needed to establish that.151

Transplantation is considered safe and effective for patients with ESRD secondary to LN if signs of active disease and evidence of anti-phospholipid (APL) syndrome are absent.34,61,155 About 50% of patients will display some signs of recurrence in biopsies, but most of these are class I or II, are not associated with clinical manifestations and have a negligible effect on graft survival.61 Several risk factors for recurrent LN have been identified including young age, female sex, African-American or non-Hispanic ethnicity, living related donor and the presence of anti-phospholipid (APL) antibodies.

If these are present, or there are prominent signs of thrombosis in the biopsy, transplantation should be delayed for 6 mos and anticoagulation should be initiated and maintained.34,61,155 There are no other serologic contraindications to transplantation or predictors of recurrence in LN.

**Conclusions**

The past decade has witnessed rapid progress in understanding the pathogenetic mechanisms that cause GN. The role of complement regulatory protein dysfunction in infection-related and other GNs, autoimmune mechanisms like anti-glycan antibodies in IgAN, ANCA antibody and complement in AAV and nucleosomes in LN have been clarified recently and all have therapeutic implications. Clarification of the genetic factors that determine which individuals will exhibit these nephritogenic responses to specific environmental insults and which ones are protected, as well as better understanding of the etiologic events in GN, further strengthen the hope that future therapies can not only be directed selectively at specific nephritogenic immune events in real time but also that the adverse events accompanying application of such therapies can be minimized. Newer, more selective and less toxic, biologic therapies such as B cell depletion and complement inhibition are now finding their way into clinical use in several forms of GN. These agents, and a host of newer ones that
are in the pipeline, promise to finally move therapy of these important renal diseases from the exclusive reliance on corticosteroids and toxic generalized immunosuppression to a new era of steroid-free, personalized renal care with agents that are safer and more effective than the drugs which have been the mainstays of therapy for the past half century.

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