Autoimmunity and glomerulonephritis

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Review Article

Introduction

Ultrafiltration of blood is perhaps the most important function of kidneys and is accomplished by glomeruli. Hence, the disorders of glomerular structure and function constitute one of the major problems encountered in nephrology practice. The term glomerulonephritis (GN) denotes an inflammatory process involving glomeruli and forms a large and an important group of kidney diseases in man. Glomerulonephritis can occur as a primary disease process involving kidney or can occur in association with systemic disorders. Studies in experimental models as well as widespread use of percutaneous renal biopsy have uncovered to a great extent, the spectrum of glomerulopathic processes and have provided new insights into various possible pathogenic mechanisms initiating and propagating glomerular injury. Eventually, evidence has accumulated over the past few years which suggests that autoimmunity is more widely involved in the pathogenesis of nephritis than previously realized. Consequently, a number of auto-antigens in various renal disorders have been identified. Some of them are listed in Table I. However, the pathogenic role of most of these antigens and of the antibodies against them has not yet been elucidated. Nevertheless, some of them have proven to be important markers of disease activity as well as indices to disease prognosis. Analysis of the autoantibodies can also aid in early, as well as differential, diagnosis of several glomerulonephritides. In the present review, autoantibodies associated with various glomerulonephritis in humans will be discussed.

The classical anti-GBM antibody-induced glomerulonephritis

The classical example of antibody-mediated glomerulonephritis is Goodpasture syndrome, a term coined by Stanton and Tange in 1958 in recognition of a patient described in 1919 by E.W. Goodpasture. This disorder consists of a triad of findings: pulmonary haemorrhage, glomerulonephritis and antibodies to the glomerular basement membrane (GBM). It typically affects young individuals but may appear at any age. Pulmonary haemorrhage may be severe and life threatening or may be extremely mild and easily overlooked. Rapidly progressive glomerulonephritis is the most common clinical feature of the syndrome. Light microscopic studies of renal biopsy most often reveal an extensive extracapillary proliferation (crecents) in glomeruli while immunofluorescence studies show typical linear deposits of IgG along the GBM.

Circulating anti-GBM antibodies are found in over 90% of patients if sera are examined early in the course of the disease by sensitive immunoassays. Anti-GBM antibodies can also be eluted from kidneys of such cases using appropriate techniques. Most often the disease is progressive, occasionally fulminant, leading to irreversible renal failure and sometimes death despite active treatment.

Traditionally the diagnosis of Goodpasture syndrome is confirmed by the finding of linear deposits of immunoglobulins along the GBM on direct immunofluorescence examination. Although fairly simple, it is time consuming and difficulties in interpretation can arise in relatively damaged tis-
Table I Auto-antigens in renal auto-immune disease in man

<table>
<thead>
<tr>
<th>Disease</th>
<th>Auto-antigen</th>
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<tr>
<td>Goodpasture syndrome</td>
<td>NC1 domain of the α3 chain of collagen IV</td>
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<tr>
<td>Primary proliferative GN</td>
<td>Entactin/nidogen</td>
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<td>IgA nephropathy</td>
<td>IgA–fibronectin complex</td>
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<td>Wegener’s granulomatosis and primary small vessel vasculitis</td>
<td>c-ANCA antigen (Proteinase 3)</td>
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<tr>
<td>Idiopathic pauci-immune extracapillary (crenestic) GN</td>
<td>p-ANCA antigen (leucocytic myeloperoxidase (MPO))</td>
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<td>Membranoproliferative GN (MPGN)</td>
<td>MPO</td>
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<tr>
<td>Anti-tubular basement membrane disease</td>
<td>Proteinase 3</td>
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<td>Post-streptococcal GN</td>
<td>C3 convertase of alternate complement pathway (C3bBb)</td>
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<td>SLE and other collagen vascular diseases</td>
<td>TBM antigen/s</td>
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<tr>
<td></td>
<td>Proteoglycans, collagen IV and laminin</td>
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<td>DNA, entactin</td>
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...sue. Moreover, the biopsy tissue may not be adequate on some occasions. This has led to development of methods to estimate circulating anti-GBM antibodies by RIA9,10 and ELISA.16 However, their specificity depends upon the type of antigen used. Recently, there has been considerable progress in identifying and characterizing the antigen involved in Goodpasture syndrome. It is located at the non-collagenous (NC1) globular domain of the carboxyl terminal end of the α3 chain of type IV collagen in the GBM11,12 (Figure 1).

Identification of the antigen involved in Goodpasture syndrome has prompted the development of a highly sensitive, specific and rapid ELISA for the diagnosis of Goodpasture syndrome.5 The assay can be completed in 30 minutes thus permitting early commencement of specific therapy comprising plasma exchange11 or protein A immuno-adsorption,14,15 especially in severely ill patients and contributing to regression in morbidity and mortality from the disease.

Other anti-GBM antibody-associated glomerulonephritides

Although several glomerular antigens have been described in experimental glomerulonephritis in animals; such as 330K and 90K glycoproteins (gp330 and gp90) in Heymann nephritis,16,18 laminin and collagen IV in mercuric chloride-induced nephritis in rats19 and laminin in murine model of graft versus host (GvH) disease,20 the role of possible antigenic determinants of the GBM, other than the Goodpasture antigen, in the pathogenesis of anti-GBM glomerulonephritis remains undefined.9,10,21

Lately, we discovered a middle-aged male patient with a mild form of glomerulonephritis. Direct immunofluorescence examination of the kidney biopsy specimen revealed weak linear immune deposits along the GBM. The patient possessed circulating antibodies directed against the NC1 domain of type IV collagen (Figure 1) but not to the α3 (IV) antigen (Goodpasture antigen). Instead, the antibodies reacted to another subunit in the NC1 region namely α1 (IV). This suggests that anti-NC1 antibodies that are not directed against the Goodpasture antigen α3 (IV) can be associated with glomerulonephritis in humans. However, they are accompanied by trivial renal damage and possibly lead to milder variants of anti-GBM nephritis (Johansson, C. et al., in preparation).

Recently, we observed that almost 40% of more than 200 patients with different types of biopsy-verified glomerulonephritis possessed antibodies directed against antigens present in crude 6M guanidine–HCl extract of GBM.22 Since the crude extract contains a variety of glycoproteins, each one of which could be a potential auto-antigen, it was interesting to isolate these proteins and study the fine specificity of antibodies against them. In this process we isolated entactin (nidogen),23–30 a 150 kD dumbbell-shaped GBM glycoprotein that reacted, in a definite pattern, with sera from several patients with glomerulonephritis in a renal biopsy series.30

Two distinct groups of patients with anti-entactin antibodies were identified. The larger group consisted of young patients (peak incidence 18–30 years) with primary mesangio-proliferative glomerulonephritis associated with significant proteinuria which responded poorly to steroids and immunosuppressive treatment. The other group comprised middle-aged patients (peak incidence 51–60 years) with glomerulonephritis secondary to SLE or SLE-like collagen vascular disease with a milder degree of proteinuria which was well responsive to therapy.30 A highly significant correla-
tion was also observed between the presence of circulating anti-entactin antibodies and the deposition of a corresponding class of immunoglobulins along the GBM on immunofluorescence examination. However, most of these patients had granular immune deposits along the GBM which would traditionally classify them as having immune complex disease. Studies in experimental models of autoimmune nephritis such as mercuric chloride-induced nephritis in Brown Norway rats and the murine model of lupus nephritis have shown the presence of anti-GBM antibodies that are deposited initially in a linear fashion along the GBM but gradually their pattern becomes granular probably because of the reorganization of the antigen–antibody complexes. Such a phenomenon possibly also exists in humans but will require early as well as sequential renal biopsies for its elucidation. Nevertheless, the observations in experimental models implicate that anti-GBM glomerulonephritis can have granular immune deposits along the GBM, which is probably the case with patients possessing circulating anti-entactin antibodies.

To summarize, our observations suggest that entactin may be involved in the pathogenesis of certain forms of glomerulonephritis (non-Goodpasture anti-GBM nephritis) in humans. As one group of patients with anti-entactin antibodies comprises young individuals with relatively severe and progressive disease, identification of such patients may have diagnostic and therapeutic implications.

**Figure 1a** Type IV collagen and the organization of GBM. Type IV collagen forms the backbone of GBM. The classical protomer of collagen IV consists of 2 distinct α helical chains, α1 (IV) (185 kD) and α2 (IV) (175 kD), which associate to form triple helical trimers. However, other α helical chains namely α3, α4 and α5 are also present. Each protomer of type IV collagen consists of 3 domains: a 7S collagenous domain at the amino terminal region; the major collagenous domain in the middle region and a non-collagenous globular domain, NC1, at the carboxyl-terminal region. Four collagen molecules are joined by disulphide bonds in the 7S terminal region, while two collagen molecules are connected to each other at the NC1 region to give rise to a ‘chicken wire’ network. Other components of the basement membrane (laminin, nidogen, proteoglycans) interact among themselves and with the collagen IV to give rise to a highly organized structure of the GBM. Moreover, they assist in the attachment of collagen IV to the adjacent epithelial and endothelial cells.
Circulating IgA–fibronectin complexes in IgA nephropathy

IgA nephropathy is the most common form of glomerulonephritis in the world, usually affecting young males in their second and third decades of life. It is characterized by macroscopic haematuria, often recurrent, with or without proteinuria usually following an episode of pharyngitis or upper respiratory tract infection. Light microscopy typically reveals a varying degree of segmental mesangial proliferation and sclerosis. On immunofluorescence examination, a characteristic deposition of IgA and C3 in the mesangial region is observed. Circulating immune complexes containing IgA have been well documented in this disorder. Recently, a study showed that patients with IgA nephropathy have circulating IgA antibodies that react with structures common to collagen I, II and IV. This binding was later found to be mediated by a collagen-binding site of fibronectin which forms circulating complexes with IgA (Figure 2). Fibronectin (cold insoluble globulin) is present both as a circulating as well as a connective tissue protein. It is composed of two monomeric subunits (210 kD and 230 kD) joined by a disulphide bridge. The N-terminal ends of the two subunits possess collagen binding domains. The fibronectin binding sites on collagen I have been identified as α1-CB7 and α2-CB3,5 fragments after cyanogen bromide digestion. By using the ELISA method of Cederholm et al., a recent study has reported a strong association of circulating IgA–fibronectin aggregates with IgA nephropathy, Henoch Schönlein purpura and recurrent crescentic IgA nephropathy in transplants. Similar results were obtained by Peter et al. using a different modification of this assay system.

Although the pathogenic role of circulating IgA–fibronectin complexes in IgA nephropathy has not been established, the results obtained by the aforementioned studies signify that measurement of circulating IgA–fibronectin complexes by simple ELISA can serve as an important serological marker for the diagnosis and follow-up of patients with IgA nephropathy.
Anti-neutrophil cytoplasm antibodies (ANCA) – associated glomerulonephritis

The association of ANCA with vasculitis and glomerulonephritis has opened new vistas to study pathogenic mechanisms involved in glomerulonephritis. These auto-antibodies which are directed against the constituents of primary (α or azurophilic) granules of human neutrophils and monocytic lysosomes (ANCA) were first described in patients with necrotizing glomerulonephritis and clinical evidence of primary small vessel vasculitis in 1982.43 The auto-antibodies were detected by indirect immunofluorescence staining of ethanol-fixed normal human neutrophils. Since then, two main fluorescence patterns have been characterized. One is a distinct cytoplasmic staining with accentuation at the centre (c-ANCA) while the other is a peripheral perinuclear or nuclear staining (p-ANCA).44 c-ANCA and p-ANCA react with neutrophils and monocytes but not with mature macrophages, lymphocytes or eosinophils.

The c-ANCA antigen is a 29 kD protein present in α granules and lysosomes of neutrophils and monocytes respectively.45 N-terminal sequencing of this 29 kD antigen has revealed that this target antigen is proteinase 3 (PR 3), a recently described human leukocytic serine protease.46 It is identical with myeloblastin, a serine protease that is present in promyelocyte-like leukemia cell line HL-60.47

The p-ANCA antigen is, in 80–90% of cases, found to be the enzyme myeloperoxidase (MPO).48,49 However, p-ANCA is not synonymous with MPO-ANCA. It has been observed that a few patients with p-ANCA have antibodies to other constituents of α granules, mainly human leukocyte elastase (HLE).48,49 It should, however, be noted that perinuclear fluorescence of ethanol-fixed neutrophils observed with p-ANCA is an artefact and disappears when neutrophils are fixed with formalin. Sera from certain patients with collagen vascular diseases, like Felty’s syndrome, have persistent perinuclear fluorescent pattern with formalin-fixed neutrophils. This pattern is referred to as GS-ANA (granulocyte specific anti-nuclear antibodies).

ANCA are mainly associated with idiopathic small vessel vasculitis with or without granuloma (Wegener’s granulomatosis and microscopic polyarthritis respectively) and primary pauci-immune, non-linear extra-capillary GN.49–52 It has been observed that c-ANCA is mainly associated with microvasculitides with widespread systemic involvement whereas most patients with renal restricted idiopathic extracapillary GN have p-ANCA.49–52

Although pathogenicity of ANCA has not been proven so far, several experimental models suggest a pathophysiological role of these antibodies.53 It has been shown that activated neutrophils are involved in the pathophysiology of necrotizing glomerulonephritis by releasing proteolytic enzymes and free oxygen radicals.54 However, under normal circumstances, these proteolytic enzymes are inactivated rapidly by protease inhibitors present in blood. It is assumed that ANCA form complexes with lysosomal enzymes and protect them from proteolytic degradation. The complexes might then be transported to other areas such as kidney and trapped there. If proteinases in these complexes retain their activity, they might then cause local damage.53,54

Analysis of ANCA has proven to be of immense value in early diagnosis of patients with rapidly progressive GN and GN associated with systemic vasculitis. As in the case with Goodpasture syndrome, early diagnosis of ANCA-associated disorders can vastly improve the outcome of these otherwise rapidly progressive medical emergent conditions.

Analysis of ANCA also facilitates differential diagnosis and sub-classification of patients with extracapillary GN. This is exemplified by 16 cases who presented with haemoptysis and rapidly progressive glomerulonephritis (clinically as Goodpasture syndrome). In fact, a majority of them (9 patients) did not have anti-GBM (anti-NC1) antibodies but had ANCA. These findings have clinical implications since anti-NC1 antibodies are associated with more severe disease and require more intensive management than those with ANCA.51

Auto-antibodies against C3b convertase in glomerulonephritis

IgG auto-antibodies to C3 convertase of the alternative complement pathway (C3bBb) have been discovered recently in several patients with hypocomplementemic membranoproliferative GN (MPGN). These autoantibodies are called C3 nephritic factor (C3NeF).55–57 C3NeF stabilizes the labile C3bBb from inactivation, thus resulting in an increased degradation of C3.55–57 This in turn results in depression of haemolytic complement activity of the serum accompanied by low C3 levels in patients with MPGN. However, C3 levels can be normal when C3NeF is present in a high concentration and vice versa.55–57

Not all patients with MPGN possess C3NeF. It is more common in patients with type II MPGN than in patients with type I.55,57,58 It has also been found in some cases of secondary MPGN particularly in patients with SLE, shunt nephritis and idiopathic cryoglobulinemia.59 There is no direct relationship between the levels of C3NeF and renal damage nor is there any correlation between the presence of C3NeF and disease activity.55,57,58
The role of C3NeF in the pathogenesis of MPGN is questionable and it should only be considered as a marker of a subset of MPGN.\textsuperscript{55,57,58}

**Other auto-antibodies in human nephritis**

*Auto-antibodies in post-streptococcal glomerulonephritis*

Recently, Fillit et al.\textsuperscript{59} demonstrated that sera from patients with acute post-streptococcal GN (APSGN) contain antibodies directed against heparan sulphate proteoglycans of the GBM. Kefalides et al.\textsuperscript{60} extended these observations by showing that these sera not only react with heparan sulphate proteoglycans but also with laminin and collagenase-resistant fragment of type IV collagen corresponding to the 7S region (Figure 1). No reaction was observed against fibronectin. They suggested that tissue injury occurring in post-streptococcal GN can release antigen fragments from critical tissue regions into circulation, leading to development of specific auto-antibodies. However, another possibility remains that auto-antibodies in post-streptococcal glomerulonephritis are developed against cross-reactive determinants shared by streptococcus and basement membrane antigens. These cross-reactive determinants need necessarily not be proteins but may also represent cross-reactive carbohydrate epitopes.\textsuperscript{59,60} However, the pathogenic or diagnostic significance of these autoantibodies in APSGN remains unexplored.

Streptococcal neuraminidase activity has also been held responsible for development of autoimmune reactivity in APSGN. Neuraminidase reacts with sialic acid-rich sites found on immunoglobulins and glomerular capillary endothelial and epithelial cells.\textsuperscript{61–64} Sialic acid depletion from these sites may evoke autoimmune response resulting in formation of anti-immunoglobulins (rheumatoid factors) and possibly, antibodies against yet unidentified glomerular antigens.\textsuperscript{61,65}

*Auto-antibodies in tubulointerstitial nephritis*

Tubulointerstitial nephritis (TIN) in humans usually occurs as a secondary process following glomerular or vascular disorders but can also occur as a primary process in a minority of cases. Most of the TIN probably have an immunological basis regardless of the inciting event.\textsuperscript{66} Three different patterns of TIN have been identified on renal biopsy.\textsuperscript{66} One is characterized by cellular infiltration with a negative immunofluorescence pattern. The second is similar to the previous one except that immunofluorescence shows granular immune deposits along the tubular basement membrane (TBM). The third pattern also resembles the first except that a linear immunofluorescence along the TBM is observed. The third form is called anti-TBM disease and is presumed to be associated with antibodies directed against TBM antigens. Although several TBM antigens have been isolated by different workers, like 70 kD antigen by Grandorge and Mahieu,\textsuperscript{67} 58 kD antigen by Fliger et al.,\textsuperscript{68,69} 48 kD antigen by Clayman et al.\textsuperscript{70} and 30 kD antigen by Wakashin et al.,\textsuperscript{71} the immunopathogenesis of anti-TBM nephritis remains incompletely understood.

*Auto-antibodies in lupus nephritis*

Nephritis associated with SLE and other related collagen vascular diseases is a well-known entity and is perceived to arise from autoimmune mechanisms.\textsuperscript{72,73} SLE has been associated with various auto-antibodies, especially anti-nuclear antibodies (ANA) and particularly anti-DNA antibodies.\textsuperscript{72,73} However, the pathogenic role of ANA, anti-DNA antibodies or DNA-anti-DNA immune complexes in lupus nephritis is widely disputed. Several recent experiments have suggested that anti-DNA antibodies probably do not have pathogenic significance in lupus nephritis.\textsuperscript{74–77} Furthermore, in murine models of GvH disease, which resembles SLE, it was observed that antibodies were deposited along the GBM in a linear pattern which later often changed to a granular arrangement.\textsuperscript{20,32,78} This linear phase corresponded to the presence of anti-GBM (anti-laminin) antibodies whereas the granular phase with anti-RTE (renal tubular epithelial antigen) antibodies in the kidney eluates.\textsuperscript{35,78} This, along with our observations of the presence of anti-entactin antibodies in SLE,\textsuperscript{68} suggests the possible pathogenic role of anti-GBM antibodies in lupus nephritis.

*Other probable auto-immune glomerulonephritis in humans*

**Membranous glomerulonephritis**

The autoimmune nature of human membranous glomerulonephritis (MN) has been debated strongly over the past few years. Most discussions are based upon the findings in Heymann nephritis which is regarded as a unique experimental model for human MN. This model was first introduced by Heymann and Lund in 1951 by immunization of rats with homologous kidney cortex homogenate.\textsuperscript{79} The disease is non-inflammatory, associated with nephrotic syndrome and characterized by glomerular aggregates localized exclusively to the epithelial side of the GBM, similar to the MN in man.\textsuperscript{80} The antigen involved is a glycoprotein with a molecular weight of 330 kD (gp 330) and is...
Figure 3 An algorithm showing distribution of various auto-antibodies in primary and secondary glomerulonephritides.

Present on the cell membrane of glomerular visceral epithelial cells. In the early seventies, a passive form of Heymann nephritis was introduced in rats by a single injection of heterologous antibodies directed against gp 330. Bagchs et al. showed that eluates from rat kidneys with passive Heymann nephritis contained antibodies reacting with a glycoprotein with a molecular weight of 90 kD (gp 90), in addition to the antibodies directed against gp 330. Although similar auto-antigens or auto-antibodies have not yet been identified, there is a strong suspicion for the existence of such an antigen–antibody system in human MN.

Summary

Autoimmunity is now unequivocally regarded as the predominant pathogenic process underlying most forms of primary and secondary glomerulonephritis in humans. Most of the investigations so far have been focused upon humoral mechanisms. Consequently, the role of cell-mediated immunity in nephritis is still incompletely understood. Nonetheless, as a result of contemporary studies, a number of previously unidentified auto-antibodies in association with glomerulonephritis have been discovered. However, apart from anti-NC1 antibodies in the classical Goodpasture syndrome, the exact role of these auto-antibodies in the pathogenesis of glomerulonephritis yet remains undefined. This fact, however, does not undermine the relevance of exploring these auto-antibodies. They have been of immense help in sub-classifying glomerulonephritis previously thought homogeneous (Figure 3). Besides, analysis of antibodies has assisted tremendously in the early diagnosis of rapidly progressive glomerulonephritis. This, in turn, has aided in early commencement of therapy thus contributing to regression in morbidity and mortality resulting from these disorders. Moreover, investigation of these auto-antibodies is of enormous value for future studies aimed at understanding the pathogenic mechanisms involved in glomerulonephritis.

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References


