

Advances in the Pathology of Glomerular Diseases

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Abstract

Glomerular injury can be caused by numerous insults including hemodynamics, toxicity, infections and immunity, hereditary and metabolic diseases. Basic and translational experimental studies in combination with clinical research in patients with renal disease have advanced our understanding of the etiology and pathogenesis of many forms of glomerulonephritis. This new knowledge has facilitated classification and treatment and has contributed to a better outcome of patients with renal disease. Since renal disease almost without exception leads to systemic cardiovascular complications, these advances are also of general health interest. Here, we shall briefly review general principles in the pathology of glomerular injury and discuss recent developments in the study of podocytopathies; membranous glomerulopathy; ANCA-associated vasculitis; C3 glomerulopathies and the role of complement in endothelial injury; and the prognostic value of the renal biopsy in predicting longterm outcome in lupus nephritis, vasculitis and IgA nephropathy.

Introduction

During the 20th century, glomerular pathophysiology and pathology were advanced considerably by the combination of clinical and pathological observations documenting accurately the various glomerular patterns of injury in relation to clinical syndromes such as the acute nephritic syndrome, the nephrotic syndrome and chronic renal disease. Experimental studies provided a rational basis for understanding the etiology and pathogenesis of several forms of renal disease and their evolution over time [1]. The introduction of the renal biopsy made it possible to observe the patterns of renal disease in man by microscopic analysis, including electron microscopy and immunofluorescence techniques. With these new tools, combined with major advances in the field of immunology and cell biology in the second half of the 20th century, diagnostic pathology rapidly advanced. The molecular and cellular basis of the glomerular microcirculation and its permselectivity, and the myriad of tubular functions in health and disease were unravelled.

In diagnostic renal pathology, four patterns of glomerular injury can be distinguished, based on compartmentalization of glomerular injury: epithelial, endothelial, mesangial injury and chronic glomerulosclerosis. The four patterns encompass most glomerular diseases and correlate with four distinct clinical syndromes respectively: the nephrotic syndrome, the nephritic syndrome, hematuria and asymptomatic proteinuria (which in addition to mesangial injury can also be due to structural basement membrane deficiencies), and chronic renal failure.

Epithelial cell injury – podocytopathies and membranous nephropathy

New developments in the study of epithelial cell injury have contributed to a better understanding of minimal change nephropathy (MCNS) and different forms of focal and segmental glomerulosclerosis (FGS). Injury to the podocyte leads to a loss of interdigitating foot processes with detachment from the underlying basement membrane and disruption of the glomerular sieve and charge selective barrier

resulting in massive proteinuria. In MCNS, this injury can only be observed by electron microscopy and is usually sensitive to steroid treatment. MCNS is the most frequent form of nephrotic syndrome in children but can also occur in adults. Clinical and experimental studies have suggested MCNS to be an immune-mediated disease in which podocytes are affected by circulating cytokines with or without an association with hypersensitivity reactions or malignancies such as Hodgkin's lymphoma [2]. However, the molecular basis for MCNS is still uncertain and one of the last enigmas in renal pathology.

The idiopathic form of FGS is considered to be part of the same spectrum as MCNS and is often, in the early phase, sensitive to steroid treatment [3]. In many cases, FGS shows a relapsing pattern of nephrotic syndrome ultimately leading to an unresponsive form of glomerular injury and to end stage renal failure.

Originally, FGS was a pattern of injury described in a wide variety of renal diseases. Over the past three decades and in particular over the last 10 years, this descriptive diagnosis has been unravelled and classified into a number of well characterized podocytopathies, which can be divided into forms based on disruption of podocyte genes encoding cell-adhesion and cell-signalling proteins and into forms based on extrinsic or systemic factors (table 1). In addition, modifying genes such as APOL1 [4] may affect cellular injury of podocytes under stress, facilitating the development of FGS.

The identification of the different causes of podocytopathies has facilitated the management of patients with secondary forms of FGS and has improved the prognostic evaluation, including the prospect of transplantation. Studies on the biology of GVEC have revealed the pathophysiology of cell-cell and cell-matrix interaction, governing the integrity of the cell and the glomerular capillary filter and its malfunction in the nephrotic syndrome in MCNS and FGS [3], including a.o. the significance of mechanosensing, heparan sulphate modulation, proton pump function and cathepsin L activation. A role of the soluble urokinase receptor (suPAR) as a permeability factor in FSGS has been proposed, based on data that in mice suPAR-activated podocyte beta3 integrin causes proteinuria and FSGS-like glomerulopathy and that significantly higher serum levels of suPAR were reported in FSGS patients than in patients with other glomerulopathies [5]. However, in a later study serum suPAR levels were not different amongst idiopathic FSGS, secondary FSGS and MCD nor did they predict responsiveness to steroid therapy, which raised serious doubts on the potential role of suPAR in FSGS pathogenesis [6].

In idiopathic MCNS and FGS currently available treatments include steroids, cyclophosphamide, cyclosporine and anti-CD20 antibodies, but is hampered by the lack of accurate understanding of their etiology.

The pathology of membranous glomerulopathy

Another cause of the nephrotic syndrome due to GVEC injury is membranous nephropathy (MN), a form of immune-mediated glomerular injury, in which immune complexes of immunoglobulins and complement accumulate in a granular pattern along the outer side of the GBM leading to GVEC detachment. The immunoglobulins can be directed against autoantigens, produced by the GVEC or extrinsic antigens accumulating at the specific site due to charge interactions as was first shown by experimental studies and recently proven for a clinical condition involving cationic bovine serum albumin (BSA) [7] (Figure 1). The podocyte in this case is merely an

innocent bystander and likely is injured by the local formation of complement stimulated by the nearby immune complexes. This pattern is more reminiscent of secondary MN that occurs with hepatitis B antigenemia or class V lupus, in which it is hypothesized that positively charged viral proteins or nuclear histones may also target immune deposit formation to the subepithelial side of the GBM. BSA is the first dietary antigen to be unequivocally involved in immune-mediated glomerulopathies, thus pointing to a role for absorbed dietary antigens and more generally for environmental factors in the pathogenesis of these diseases.

In most patients with the idiopathic autoimmune form of MN, the autoantibodies are of the IgG4 and IgG1 type and are directed to the phospholipase A2-receptor (PLA2-R) [8]. These antibodies may affect the GVEC directly through interaction with PLA2-R and they can bind complement components which through different pathways ultimately lead to the formation of the membrane attack complex causing cell injury and death. Distinction between idiopathic and secondary forms of MN has been facilitated by the introduction of assays to determine PLA2-R autoantibodies in serum and PLA2-R antigen in renal biopsies [9, 10]. Further work is needed to establish whether circulating levels of anti-PLA2R1 antibodies can predict disease outcome or recurrence on the grafted kidney after transplantation [11, 12].

Genome wide association studies (GWAS) in large cohorts of MN patients in Europe have revealed the autoimmune response in patients with idiopathic MN to be based on two single nucleotide polymorphisms – one for the gene encoding PLA2-R, and one for the gene encoding the HLA antigen presenting peptide within the HLA-DQA2 complex [13]. This implies a highly restricted autoimmune response based on an inappropriate interaction between the pathogenetic peptide and the complementary antigen-presenting molecule. The cause of the induction of recognition, which often does not occur but at the adult age, is at this moment unknown and could be due to demasking of the epitope by infection, toxicity or degradation processes during cell or tissue renewal.

Further insight in the process underlying PLA2-R self-recognition may facilitate the design of epitope-blocking reagents, which may inhibit autoimmunity, and can be used to monitor and prevent recurrence of the disease after transplantation. After decades of aspecific cytotoxic therapeutic attempts, the anti-CD20 monoclonal antibody Rituximab has been shown to be a promising therapy for idiopathic membranous nephropathy. In patients with persistent nephrotic despite optimized conservative therapy, Rituximab was well tolerated and led to significant reduction of proteinuria and all patients with at least 4 years of follow-up achieved complete or partial remission [14]. This represents a paradigm shift in treatment, allowing fresh insights in pathophysiology.

Furthermore, treatment can be aimed at the level of tissue injury in particular targeting complement component activation and regulation (see below under C3 glomerulopathies).

Other antibodies directed against enolase, SOD2, and aldose reductase have also been identified in the serum [15] but their role in the initial development of MN remains controversial because they recognize cytoplasmic antigens. These antigens can however be routed to the membrane in the aggressed podocyte where they will possibly serve as targets for circulating antibodies.

Endothelial cell injury - ANCA-associated renal pathology

At about the same time as GWAS studies revealed the genetic cause of idiopathic MN, a similar multicentre approach in a large cohort of patients, revealed similar highly restricted polymorphisms in genes encoding HLA-DP and proteinase 3 and SERPINA1 in patients with autoantibodies directed against PR3 suffering from ANCA-associated systemic vasculitis and glomerulonephritis [16]. In this disease, the endothelial compartment of the glomerulus is injured leading to fibrinoid necrosis, capillary wall destruction, and extracapillary proliferation, resulting in permanent damage of the glomerular tuft.

Since the first description of the relevance of ANCA in systemic vasculitis in 1985 [17], the etiology and pathogenesis have been clarified to a major level based on seminal discoveries, such as the identification of proteinase 3 and myeloperoxidase as targets for ANCA, the pathogenesis of endothelial injury and vasculitis due to a direct interaction of ANCA with neutrophils and endothelium, and the role of infection with staphylococcus Aureus and the relevance of prevention by antibiotic treatment [18]. New developments will be aimed at epitope-guided treatment in an attempt to modify the genetic response of this serious form of autoimmune disease.

The role of complement in the ANCA-associated endothelial injury is less well understood and at immunofluorescence only segmental low level deposition of immunoglobulins and complement components can be observed which has led to the designating term *pauci-immune glomerulonephritis*, notwithstanding the undisputed pathogenetic action of the ANCA autoantibodies. Probably regulation of complement activation and breakdown is of importance and treatment aimed at these mechanisms may prove to be successful in limiting tissue injury.

The complement system and glomerular pathology

The serine proteases of the complement system have an important role in opsonisation and removal of microbial antigens and dead cell and tissue fragments. In this process, complement components can be activated by antibodies, by serum proteins of the collectin family and by microbial surface glycoproteins and extracellular matrix components, known as the three routes of activation: the classical, mannan-binding lectin, and alternative pathways (fig. 2) [19].

The complement system can induce removal of debris in a quiescent way through digestion by macrophages and B cells with induction of immune tolerance, but in cases of massive or persistent debris the system may initiate an inflammatory pathway by recruitment of phagocytes through chemotaxis resulting in tissue inflammation and breakdown leading to immunization.

The components of the complement system are held in check by a large number of regulatory and inhibitory proteins in the circulation and at the surface of endothelial cells. Recent experimental and clinical studies have revealed how dysregulation of the complement system may cause induction of SLE by inadequate removal of apoptotic nuclear fragments leading to autoimmunity to DNA and phospholipids [20].

Endothelial cell injury – Complement and HUS

Inappropriate activation of the complement system due to ineffective local inhibition may lead to prolonged tissue damage particularly at the level of the endothelial cells. This mechanism has recently been revealed to underlie the E. Coli associated and atypical forms of haemolytic uremic syndrome (aHUS) [19] and the two idiopathic forms of membranoproliferative glomerulonephritis (MPGN) known previously as type I and type II MPGN (also called dense deposit disease - DDD) [21] .

Sixty percent of aHUS cases are related to mutations in genes encoding complement regulatory proteins, factor H (CFH), membrane-cofactor protein (MCP), factor I (CFI) and thrombomodulin (THBD), or the alternative pathway C3 convertase components C3 and factor B (FB) or to inhibitory anti-CFH autoantibodies. Patients with combined mutations in 2 or 3 different complement genes have also been reported underlying the complexity of aHUS genetics.

A recent immunofluorescence-based classification approach distinguishes those forms of MPGN with isolated C3 deposits -known as C3 glomerulopathies and characterized by defective control of the alternative pathway of complement- from MPGN type I with deposits of immunoglobulin and complement and characterized by activation of the classical pathway by antigen-antibody immune complexes [22]. However such distinction is not universally accepted and evidence is accumulating on involvement of the alternative pathway of complement in MPGN with C3 and immune deposits as well. C3NeF, an autoantibody stabilizing the AP C3-convertase, is found in 50-75% of patients with C3-glomerulopathies but also in about half of patients with MPGN I. Auto-antibodies against CFH and factor B and complement gene mutations have been also reported.

Major therapeutic progress has been made recently in aHUS with the discovery of the efficacy of Eculizumab, an anti-C5 antibody [19, 23].

There is no specific treatment for C3-glomerulopathies. Eculizumab reduced proteinuria in a few patients but others did not respond.

Renal pathology and the prognosis of glomerular disease

The three major glomerular diseases - lupus nephritis, ANCA-associated glomerulonephritis and IgA-nephropathy – have recently been reclassified [24-26] and for all three, clinical observations have revealed a strong correlation of chronic glomerular lesions with poor outcome [27]. More sophisticated quantification of chronic lesions in the glomerular, tubulointerstitial and vascular compartment were reported to have no additional value over the relatively simple analysis of glomerular scars [27]. Recently, molecular and proteomic studies on renal tissue and urine have been initiated to correlate transcription and peptide profiles with outcome in order to allow a more precise intervention in signalling pathways involved in inflammation and repair.

References

1. Weening JJ, Jennette JC. Historical milestones in renal pathology. *Virchows Archiv* 2012; 461:3-11
2. Berg JG, Weening JJ. Role of the immune system in the pathogenesis of idiopathic nephrotic syndrome. *Clin Science* 2004; 107:125-136.
3. D' Agati VD, Kaskel FJ, Falk RJ. Focal segmental glomerulosclerosis. *N Engl J Med* 2011; 365:2398-2411.

4. Genovese G, Friedman DJ, Ross MD, Lecordier L, Uzureau P, Freedman BI, Bowden DW, Langefeld CD, Oleksyk TK, Uscinski Knob AL, Bernhardt AJ, Hicks PJ, Nelson GW, Vanhollebeke B, Winkler CA, Kopp JB, Pays E, Pollak MR. Association of trypanolytic ApoL1 variants with kidney disease in African Americans. *Science* 2010; 329:841-845
5. Wei C, El Hindi S, Li J, Fornoni A, Goes N, Sageshima J, Maiguel D, Karumanchi SA, Yap HK, Saleem M, Zhang Q, Nikolic B, Chaudhuri A, Daftarian P, Salido E, Torres A, Salifu M, Sarwal MM, Schaefer F, Morath C, Schwenger V, Zeier M, Gupta V, Roth D, Rastaldi MP, Burke G, Ruiz P, Reiser J. Circulating urokinase receptor as a cause of focal segmental glomerulosclerosis. *Nat Med* 2011;17:952-960.
6. Maas RJ, Wetzels JF, Deegens JK. Serum-soluble urokinase receptor concentration in primary FGS. *Kidney International* 2012 81:1043-1044.
7. Debiec H, Lefeu F, Kemper MJ, Niaudet P, Deschênes G, Remuzzi G, Ulinski T, Ronco P. Early-childhood membranous nephropathy due to cationic bovine serum albumin. *N Engl J Med*. 2011 Jun 2;364(22):2101-10.
8. Beck LH Jr, Bonegio RG, Lambeau G, Beck DM, Powell DW, Cummins TD, Klein JB, Salant DJ. M-type phospholipase A2 receptor as target antigen in idiopathic membranous nephropathy. *N Engl J Med*. 2009 Jul 2;361(1):11-21
9. Hofstra JM, Debiec H, Short CD, Pellé T, Kleta R, Mathieson PW, Ronco P, Brenchley PE, Wetzels JF. Antiphospholipase A2 receptor antibody titer and subclass in idiopathic membranous nephropathy. *J Am Soc Nephrol*. 2012 Oct;23(10):1735-43.
10. Debiec H, Ronco P. PLA2R autoantibodies and PLA2R glomerular deposits in membranous nephropathy. *N Engl J Med*. 2011 Feb 17;364(7):689-90.
11. Beck LH Jr, Fervenza FC, Beck DM, Bonegio RG, Malik FA, Erickson SB, Cosio FG, Cattran DC, Salant DJ. Rituximab-induced depletion of anti-PLA2R autoantibodies predicts response in membranous nephropathy. *J Am Soc Nephrol*. 2011 Aug;22(8):1543-50.
12. Debiec H, Martin L, Jouanneau C, Dautin G, Mesnard L, Rondeau E, Mousson C, Ronco P. Autoantibodies specific for the phospholipase A2 receptor in recurrent and De Novo membranous nephropathy. *Am J Transplant*. 2011 Oct;11(10):2144-52.
13. Stanescu HC, Arcos-Burgos M, Medlar A, Bockenhauer D, Kottgen A, Dragomirescu L, Voinescu C, Patel N, Pearce K, Hubank M, Stephens HA, Laundry V, Padmanabhan S, Zawadzka A, Hofstra JM, Coenen MJ, den Heijer M, Kiemeneij LA, Bacq-Daian D, Stengel B, Powis SH, Brenchley P, Feehally J, Rees AJ, Debiec H, Wetzels JF, Ronco P, Mathieson PW, Kleta R. Risk HLA-DQA1 and PLA(2)R1 alleles in idiopathic membranous nephropathy. *N Engl J Med*. 2011 Feb 17;364(7):616-26

14. Ruggenenti P, Cravedi P, Chianca A, Perna A, Ruggiero B, Gaspari F, Rambaldi A, Marasà M, Remuzzi G. Rituximab in idiopathic membranous nephropathy. *J Am Soc Nephrol*. 2012 Aug;23(8):1416-25.
15. Murtas Murtas C, Bruschi M, Candiano G, Moroni G, Magistroni R, Magnano A, Bruno F, Radice A, Furci L, Argentiero L, Carnevali ML, Messa P, Scolari F, Sinico RA, Gesualdo L, Fervenza FC, Allegri L, Ravani P, Ghiggeri GM. Coexistence of different circulating anti-podocyte antibodies in membranous nephropathy. *Clin J Am Soc Nephrol*. 2012 Sep;7(9):1394-400.
16. Lyons PA, Rayner TF, Trivedi S, Holle JU, Watts RA, Jayne DR, Baslund B, Brenchley P, Bruchfeld A, Chaudhry AN, Cohen Tervaert JW, Deloukas P, Feighery C, Gross WL, Guillevin L, Gunnarsson I, Harper L, Hrušková Z, Little MA, Martorana D, Neumann T, Ohlsson S, Padmanabhan S, Pusey CD, Salama AD, Sanders JS, Savage CO, Segelmark M, Stegeman CA, Tesař V, Vaglio A, Wieczorek S, Wilde B, Zwerina J, Rees AJ, Clayton DG, Smith KG. Genetically distinct subsets within ANCA-associated vasculitis. *NEJM* 2012; 367:214-223.
17. van der Woude FJ, Rasmussen N, Lobatto S, Wiik A, Permin H, van Es LA, van der Giessen M, van der Hem GK, The TH. Autoantibodies against neutrophils and monocytes: tool for diagnosis and marker of disease activity in Wegener's granulomatosis. *Lancet*. 1985 1:425-9.
18. Kallenberg CG. Pathogenesis of ANCA-associated vasculitis, an update. *Clin Rev Allergy Immunol* 2011;41:224-231.
19. Noris M, Mescia F, Remuzzi G. STEC-HUS, atypical HUS and TTP are all diseases of complement activation. *Nature Rev Nephrol* 2012; 8:622-633.
20. Chen M, Daha MR, Kallenberg CG. The complement system in systemic autoimmune disease. *J Autoimmunity* 2010; 34:J276-286.
21. Sethi S, Fervenza FC. Membranoproliferative glomerulonephritis, a new look at an old entity. *N Engl J Med* 2012; 366:1119-1131.
22. D'Agati VD, Bomback AS. C3 glomerulopathy: what's in a name? *Kidney Int* 2012; 82:379-381.
23. Zuber J, Fakhouri F, Roumenina LT, Loirat C, Frémeaux-Bacchi V; French Study Group for aHUS/C3G. Use of eculizumab for atypical haemolytic uraemic syndrome and C3 glomerulopathies. *Nature Rev Nephrol* 2012; 8:643-657.
24. Weening JJ, D'Agati VD, Schwartz MM, Seshan SV, Alpers CE, Appel GB, Balow JE, Bruijn JA, Cook T, Ferrario F, Fogo AB, Ginzler EM, Hebert L, Hill G, Hill P, Jennette JC, Kong NC, Lesavre P, Lockshin M, Looi LM, Makino H, Moura LA, Nagata M; International Society of Nephrology Working Group on the Classification of Lupus Nephritis; Renal Pathology Society Working Group

on the Classification of Lupus Nephritis. The classification of glomerulonephritis in systemic lupus erythematosus revisited. *Kidney Int* 2004; 65:521-530.

25. Berden AE, Ferrario F, Hagen EC, Jayne DR, Jennette JC, Joh K, Neumann I, Noël LH, Pusey CD, Waldherr R, Bruijn JA, Bajema IM. Histopathologic classification of ANCA-associated glomerulonephritis. *J Am Soc Nephrol* 2010; 21:1628-1636.
26. Working Group of the International IgA Nephropathy Network and the Renal Pathology Society, Cattran DC, Coppo R, Cook HT, Feehally J, Roberts IS, Troyanov S, Alpers CE, Amore A, Barratt J, Berthoux F, Bonsib S, Bruijn JA, D'Agati V, D'Amico G, Emancipator S, Emma F, Ferrario F, Fervenza FC, Florquin S, Fogo A, Geddes CC, Groene HJ, Haas M, Herzenberg AM, Hill PA, Hogg RJ, Hsu SI, Jennette JC, Joh K, Julian BA, Kawamura T, Lai FM, Leung CB, Li LS, Li PK, Liu ZH, Mackinnon B, Mezzano S, Schena FP, Tomino Y, Walker PD, Wang H, Weening JJ, Yoshikawa N, Zhang H. The Oxford classification of IgA nephropathy: pathology definitions, correlations and classification. *Kidney Int* 2009; 76:546-556.
27. Hiramatsu N, Kuroiwa T, Ikeuchi H, Maeshima A, Kaneko Y, Hiromura K, Ueki K, Nojima Y. Revised classification of lupus nephritis is valuable in predicting renal outcome with an indication of the proportion of glomeruli affected by chronic lesions. *Rheumatology* 2008;47:702-707.

Figure legends

Fig. 1 Schematic illustration of the pathogenesis of proteinuria in MN.

Fig. 2. The three pathways of the complement system

Podocytopathies leading to FGS

Gene disruption involving nephrin, podocin, CD2AP, α -actinin, integrin $\alpha3\beta\alpha1$, CD151 tetraspanin, TRPV6, laminin B2, collagen XVII, Ilk, Rhoa, Myo1E, IFN2, MYH9, dystroglycan and heparan sulphate moieties

Extrinsic factors including intracapillary hypertension, viral infection, toxicity, complement-activation, metabolic storage diseases

Table 1. *Two groups of podocytopathies identified as causes for FGS.*