Pathophysiology of the glomerulus: *KI* tells the story

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The glomerular signature in patients with hematuria

Fairley KF, Birch DF. Hematuria: a simple method for identifying glomerular bleeding. Kidney Int. 1982;21:105–108. Our reasoning in glomerular pathology relies on measures of the composition and amount of proteinuria, and the presence of red blood cells (RBCs) in the urine. In the case of isolated hematuria, the question of the origin of RBCs arises. In 1973, Brod was the first to provide some morphologic clue: "red cells of glomerular origin generally bear traces of their long journey [in] the renal tubules."[1] But it was Birch and Fairley who, in 1979, described these changes under phase-contrast microscopy and correlated them with clinical findings.[2] Then they provided a complete description of the RBC morphologic alterations in 88 patients referred for hematuria.[3] A kidney biopsy was performed in all patients who had suggestive manifestations of glomerular disease, whereas the other patients were investigated by cystoscopy and imaging methods. Dysmorphic RBCs were seen in 55 of 58 patients with glomerular disease, and in none of 30 patients with nonglomerular disease. Later, it was shown that cells with a unique deformity, *acanthocytes*, recognized as ring forms with vesicle-shaped protrusions, were closely correlated with glomerular disease (Figure 1).[4] A recent study from the Mayo Clinic[5] challenged this view and concluded that a level of ≥25% of urine-deformed RBCs (mainly acanthocytes) is specific (96.3%) but not sensitive (20.4%) for glomerular diseases. In their cohort, the combined hematuria (>10 RBCs/high-power field) and proteinuria level performed just as well as measures of deformed RBCs plus proteinuria to predict underlying glomerulonephritis, but the cohort only included patients with a clinically indicated kidney biopsy, excluding those with urologic causes of hematuria.

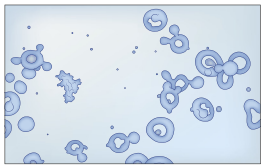

Figure 1 |

The Fairley and Birch studies of the urinary sediment have changed the standard of care. Before those studies, many patients with glomerular bleeding were subjected to unnecessary urologic and radiologic investigations. Searching for acanthocytes in the urine at an early stage of patient investigation has led to a more selective use of these procedures. The use of automated screening might help with standardization and resolve persisting controversies.

Figure 1 is adapted with permission from Fairley KF, Birch DF. Hematuria: a simple method for identifying glomerular bleeding. *Kidney Int.* 1982;21:105–108.[3] Copyright © 1982, International Society of Nephrology.

The glomerular electric field revealed!

Chang RLS, Deen WM, Robertson CR, Brenner BM. Permselectivity of the glomerular capillary wall: III. Restricted transport of polyanions. Kidney Int. 1975;8:212–218. This paper[6] is the logical outcome of a series of elegant studies by Brenner's group on the permselectivity of the glomerular capillary wall. Previous experiments in the isolated perfused

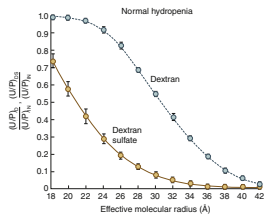

Figure 2 |

Editor's Note

This article is part of the *KI* 60th anniversary series. This month's topic is the pathophysiology of the glomerulus.

The Japanese character 気 and the Chinese character 氣 for *KI* convey the circulating life force, the existence and properties of which are the basis of much of Chinese and Japanese philosophy and medicine.

rabbit ear showed that macromolecular charge may play an important role in restricting the passage of the nonprotein anionic polymer sulfate dextran, compared with neutral dextran.[7] In the kidney, the clearance of albumin relative to that of inulin (0.01) is greatly exceeded by that of uncharged dextrans (0.20), although they have about the same molecular radius (about 36 angstrom units). Given that albumin is a polyanion in physiological solution, the authors suspected that its negative charge could impede passage through the glomerular capillary wall. When they infused sulfate dextran of the same radius, the fractional clearance was decreased to that of albumin. Both dextran and sulfate dextran are neither secreted nor reabsorbed, so the differences observed were not the result of differences in transport across tubule epithelia.

These groundbreaking findings were in keeping with parallel work by Cotran's group showing that perfusion of the rat kidney with polycationic substances such as protamine sulfate was associated with effacement of foot processes.[8] Although it is now well established that the polyanionic coat is partially lost in proteinuric disorders, its nature remains controversial, despite the powerful molecular and genetic tools available to investigate it. For a long time, it was considered that negatively charged heparan sulphate proteoglycans, one of the main components of the glomerular basement membrane, performed a key role in the charge barrier. However, this role has been called into question by studies on the podocyte-specific mutation of agrin,[9] major glomerular basement membrane heparan sulphate proteoglycans, and the knockout of *Ext1*[10] and *Extl3*,[11] which code for enzymes that add glycosylated residues on the core protein of proteoglycans. Other negatively charged proteins, particularly the sialo- and sulfo-protein podocalyxin, have been investigated. Mutation in a key enzyme of sialic acid biosynthesis caused severe glomerular proteinuria that was rescued by N-acetylmannosamine,[12] but podocalyxin might be more important for maintaining podocyte architecture than for contributing to glomerular charge selectivity.[13] The role of glomerular endothelial cell glycocalyx should also be considered.

From a therapeutic standpoint, replacement of the missing charges is attractive, although the beneficial long-term effects of such replacement on chronic kidney disease progression, particularly in diabetes, have not been established.

Figure 2 is adapted with permission from Chang RLS, Deen WM, Robertson CR, Brenner BM. Permselectivity of the glomerular capillary wall: III. Restricted transport of polyanions. *Kidney Int.* 1975;8:212–218.[6] Copyright © 1975, International Society of Nephrology.

The cellular component in rapidly progressive glomerulonephritis: not gone, just forgotten, and now accessible to single-cell RNAseq!

Bolton WK, Innes DJ Jr, Sturgill BC, Kaiser DL. T-cells and macrophages in rapidly progressive glomerulonephritis: clinicopathologic correlations. Kidney Int. 1987;32:869–876. Because of major advances in our understanding of the role of antibodies in the pathogenesis of anti–glomerular basement membrane disease and Heymann nephritis in the 1970s and early 1980s, the implication of T cells has long been ignored. Bolton *et al.*[14] first phenotyped kidney cellular infiltrates with monoclonal antibodies to T cells in various types of acute crescentic rapidly progressive glomerulonephritis. The predominant cellular infiltrates were lymphocytes, mostly T-helper, and macrophages. T cells were found not only in the interstitium, around tubules, in perglomerular localization, but also within glomeruli. T cells, T-helper and suppressor cells, B cells, and macrophages were observed within crescents. Furthermore, the response to methylprednisolone was related to the intensity and composition of these cellular infiltrates.

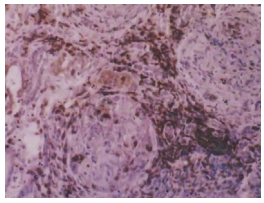

Figure 3|

Considerable work has been devoted since then to the role of lymphoid cells in crescent formation. Chen *et al.*[15] recently developed an elegant experimental model of crescentic glomerulonephritis with which they demonstrated that CD8 T cells penetrate the crescents only at sites of rupture of the Bowman's capsule. The authors developed a "2-hit" hypothesis of crescentic glomerulonephritis progression. The first hit is the damage caused by antibodies causing proteinuria and the activation of parietal epithelial cells with a first generation of crescents and cytokine release. The second hit is triggered by neo-epitopes from damaged podocytes that are released into the urine and taken up by renal medullary dendritic cells, which then migrate to the regional lymph nodes and present the neo-epitopes to

$CD4^+$ and $CD8^+$ T cells. This scenario is supported by recent data showing that the kidney lymph node, particularly its fibroblastic reticular cells, may be the key secondary lymphoid organ responsible for the propagation of the immune response in crescentic glomerulonephritis.[16] The activated T lymphocytes and macrophages migrate to the glomerulus, accumulating around Bowman's capsule until breaches occur. Through these breaches, macrophages and T cells can gain access to the glomerular space, so that $CD8^+$ T cells can then destroy their neo-epitope–expressing target podocytes. Other cell types can participate in the pathogenesis, including the recently identified innate lymphoid cells that were detected in glomeruli, particularly prior to the development of crescents.[17]

We still know very little about mediators and cell interactions. We are confident that single-cell RNA transcriptomics performed on kidney biopsies, glomerular cells shed in the urine, and peripheral blood mononuclear cells will help unravel new pathogenic pathways in clusters of diseased cells, thus opening exciting opportunities for innovative therapeutic intervention.[18]

Figure 3 is adapted with permission from Bolton WK, Innes DJ Jr, Sturgill BC, Kaiser DL. T-cells and macrophages in rapidly progressive glomerulonephritis: clinicopathologic correlations. *Kidney Int.* 1987;32:869–876.[14] Copyright © 1987, International Society of Nephrology.

Glomerular hypertrophy versus glomerular pressure and flow: angiotensin-converting enzyme inhibitor is always the winner

Yoshida Y, Fogo A, Ichikawa I. Glomerular hemodynamic changes vs. hypertrophy in experimental glomerular sclerosis. Kidney Int. 1989;35:654–660. In the 1980s, considerable work was devoted to the pathogenesis of glomerular sclerosis, a key lesion in the progression of chronic kidney disease. Several factors were considered: proteinuria, mesangial deposition of macromolecules, hyperlipidemia, intraglomerular coagulopathy, and above all, increased pressures and flows at the glomerular level.[19,20] Little attention had been focused on glomerular hypertrophy, despite its excellent correlation with sclerosis. Then Yoshida *et al.*[21] used an elegant protocol in Munich-Wistar rats. All left kidneys were subjected to a two-thirds nephrectomy, whereas right kidneys were left untouched, nephrectomized, or ureter-diverted in the peritoneal cavity (that is, renal clearance function was removed while the kidney tissue was kept *in situ*). Micropuncture studies showed that both the glomerular capillary hydraulic pressure and the filtration rate remained at similarly elevated levels in the last 2 groups of rats, compared with the controls. However, glomerular hypertrophy was observed in only the nephrectomized group, thus suggesting an absence of correlation between enhanced hemodynamics and hypertrophy in this rat model. Furthermore, there was a correlation between glomerular hypertophy and sclerosis.

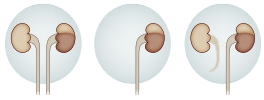

Figure 4|

This work, in conjunction with that of Brenner's group, triggered a lot of controversy and opened new fields of research on the nature of the factors that induce glomerular hypertrophy and the possible mechanisms whereby hypertrophy causes sclerosis. A key aspect of glomerular hypertrophy is that, either as a primary effect of abnormal growth or as a compensatory secondary phenomenon due to loss of other nephrons, it can lead to relative podocytocytopenia, which increases mechanical and other stress on podocytes and eventually promotes podocyte loss.

In subsequent work, Fogo *et al.*[22] showed that glomerular hypertrophy distinguished steroid-resistant nephrotic children who developed focal segmental glomerulosclerosis from those that only had minimal change disease. Glomerular hypertrophy should be taken to be a key factor for glomerular sclerosis in most situations of nephron loss, intrauterine growth retardation, significant prematurity, and a high-protein diet. In those situations, hemodynamic factors also most likely play an important role, but the present paper indicates that hyperfiltration alone is insufficient to induce pathogenic glomerular hypertrophy and subsequent sclerosis. Whatever the respective contribution of hypertrophy and hemodynamics, treatment relies on angiotensin-converting enzyme inhibitors/angiotensin II receptor blockers, the beneficial effects of which are associated with the reduction of glomerular hypertrophy, but better knowledge of the mediators and pathways is urgently needed for more specific and personalized therapy.

Figure 4 is adapted with permission from Yoshida Y, Fogo A, Ichikawa I. Glomerular

hemodynamic changes vs. hypertrophy in experimental glomerular sclerosis. *Kidney Int.* 1989;35:654–660.[21] Copyright © 1989, International Society of Nephrology.

First identification of complement factor H mutation in atypical hemolytic uremic syndrome (HUS) opens Pandora's box

Warwicker P, Goodship THJ, Donne RL, et al. Genetic studies into inherited and sporadic hemolytic uremic syndrome. Kidney Int. 1998;53:836–844. Since the first description of nondiarrheal HUS by Gasser *et al.*,[23] many candidates have been suggested as the cause of the disease. Previous case reports of HUS in patients with factor-H deficiency led the authors to postulate that anomalies of factor H might be involved in the pathogenesis. Warwicker *et al.*[24] took advantage of the rare familial forms of HUS to identify factor-H mutations. In the late 1990s, when genetics technology was still in its infancy, investigators had to overcome many hurdles. They first performed linkage analysis using microsatellite polymorphism markers. They mapped a region that contains the factor H gene. Mutations were sought in the coding region of factor H by single-strand conformational polymorphism and heteroduplex analysis of real-time polymerase chain reaction fragments. They then identified band shifts that were not present in 60 healthy controls. Finally, subcloning and sequencing of the mutant band revealed a mutation in exon 20 (*R*1197G) in 2 families, and a 4–base pair deletion in exon 1 causing a frameshift in a sporadic case. This report illustrates how the amazing development of next-generation sequencing techniques has considerably simplified the whole diagnostic process. One should be aware, however, of the pitfalls of interpreting variants of uncertain significance. The technological problems have been solved, but now the challenges are determining how to interpret the data, establishing the functional significance of the identified variants, and revisiting the concept of variable penetrance using whole-exome sequencing in search of mutations in other complement genes or modifying genes.

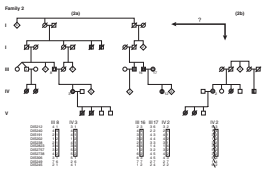

Figure 5 |

This groundbreaking paper established the role of alternative pathway dysregulation and opened the field of genetic complement abnormalities in atypical HUS. It was followed by many studies that underlined the need for genetic screening for all susceptibility factors as part of clinical management of atypical HUS patients[25] and showed that atypical and secondary HUS have no common genetic risk factors.[26] These studies have also induced a paradigm shift in patient care, with the development of complement inhibitors such as anti-C5 antibodies, of which eculizumab is the leader. However, controversies remain regarding the usefulness of complement inhibitors in secondary HUS.

Figure 5 is adapted with permission from Warwicker P, Goodship THJ, Donne RL, et al. Genetic studies into inherited and sporadic hemolytic uremic syndrome. *Kidney Int.* 1998;53:836–844.[24] Copyright © 1998, International Society of Nephrology.

DISCLOSURE

The author declared no competing interests.

REFERENCES

1. Brod J. Hematuria. In: Brod J, ed. *The Kidney*. London: The Butterworth Group; 1973:226–228.
2. Birch F, Fairley KF. Haematuria: glomerular or non-glomerular? *Lancet*. 1979;2:845–846.
3. Fairley KF, Birch DF. Hematuria: a simple method for identifying glomerular bleeding. *Kidney Int*. 1982;21:105–108.
4. Köhler H, Wandel E, Brunck B. Acanthocyturia—a characteristic marker for glomerular bleeding. *Kidney Int*. 1991;40:115–120.
5. Hamadah AM, Gharaibeh K, Mara KC, et al. Urinalysis for the diagnosis of glomerulonephritis: role of dysmorphic red blood cells. *Nephrol Dial Transplant*. 2018;33:1397–1403.
6. Chang RLS, Deen WM, Robertson CR, Brenner BM. Permselectivity of the glomerular capillary wall: III. Restricted transport of polyanions. *Kidney Int*. 1975;8:212–218.
7. Areekul S. Reflection coefficients of neutral and sulphate-substituted dextran molecules in the isolated perfused rabbit ear. *Acta Soc Med Ups*. 1969;74:129–138.
8. Seiler MW, Venkatachalam MA, Cotran RS. Glomerular epithelium: structural alterations induced by polycations. *Science*. 1975;189:390–393.
9. Harvey SJ, Jarad G, Cunningham J, et al. Disruption of glomerular basement membrane charge through podocyte-specific mutation of agrin does not alter glomerular permselectivity. *Am J Pathol*. 2007;171:139–152.
10. Chen S, Wassenhove-McCarthy DJ, Yamaguchi Y, et al. Loss of heparan sulfate glycosaminoglycan assembly in podocytes does not lead to proteinuria. *Kidney Int*. 2008;74:289–299.
11. Aoki S, Saito-Hakoda A, Yoshikawa T, et al. The reduction of heparan sulphate in the glomerular basement membrane does not augment urinary albumin excretion. *Nephrol Dial Transplant*. 2018;33:26–33.
12. Galeano B, Klootwijk R, Manoli I, et al. Mutation in the key enzyme of sialic acid biosynthesis causes severe glomerular proteinuria and is rescued by N-acetylmannosamine. *J Clin Invest*. 2007;117:1585–1594.
13. Miner JH. Glomerular filtration: the charge debate charges ahead. *Kidney Int*. 2008;74:259–261.

14. Bolton WK, Innes DJ Jr, Sturgill BC, Kaiser DL. T-cells and macrophages in rapidly progressive glomerulonephritis: Clinicopathologic correlations. *Kidney Int*. 1987;32:869–876.
15. Chen A, Lee K, D'Agati VD, et al. Bowman's capsule provides a protective niche for podocytes from cytotoxic CD8+ T cells. *J Clin Invest*. 2018;128:3413–3424.
16. Kasinath V, Yilmam OA, Uehara M, et al. Activation of fibroblastic reticular cells in kidney lymph node during crescentic glomerulonephritis. *Kidney Int*. 2019;95:310–320.
17. Okabayashi Y, Nagasaka S, Kanzaki G, et al. Group 1 innate lymphoid cells are involved in the progression of experimental anti-glomerular basement membrane glomerulonephritis and are regulated by peroxisome proliferator-activated receptor α. *Kidney Int*. 2019;96:942–956.
18. Park J, Liu CL, Kim J, Susztak K. Understanding the kidney one cell at a time. *Kidney Int*. 2019;96:862–870.
19. Hostetter TH, Olson JL, Rennke HG, et al. Hyperfiltration in remnant nephrons: a potentially adverse response to renal ablation. *Am J Physiol*. 1981;241:F85–F93.
20. Anderson S, Meyer TW, Rennke HG, Brenner BM. Control of glomerular hypertension limits glomerular injury in rats with reduced renal mass. *J Clin Invest*. 1985;76:612–619.
21. Yoshida Y, Fogo A, Ichikawa I. Glomerular hemodynamic changes vs. hypertrophy in experimental glomerular sclerosis. *Kidney Int*. 1989;35:654–660.
22. Fogo A, Hawkins EP, Berry PL, et al. Glomerular hypertrophy in minimal change disease predicts subsequent progression to focal glomerular sclerosis. *Kidney Int*. 1990;38:115–123.
23. Gasser C, Gautier E, Steck A, et al. Hemolytic-uremic syndrome: bilateral necrosis of the renal cortex in acute acquired hemolytic anemia. *Schweiz Med Wochenschr*. 1955;85:905–909.
24. Warwicker P, Goodship THJ, Donne RL, et al. Genetic studies into inherited and sporadic hemolytic uremic syndrome. *Kidney Int*. 1998;53:836–844.
25. Noris M, Bresin E, Mele C, Remuzzi G. Genetic atypical hemolytic-uremic syndrome. In: Adam MP, Ardinger HH, Pagon RA, et al., eds. *GeneReviews*. Seattle, WA: University of Washington, Seattle; 2007: 1993–2019. Available at: https://www.ncbi.nlm.nih.gov/books/NBK1116. Updated June 9, 2016. Accessed November 15, 2019.
26. Le Clech A, Simon-Tillaux N, Provôt F, et al. Atypical and secondary hemolytic uremic syndromes have a distinct presentation and no common genetic risk factors. *Kidney Int*. 2019;95:1443–1452.