L22. Crescent formation: Unraveling local mediators that break glomerular epithelial cell tolerance to immune injury

Introduction: crescentic rapidly progressive glomerulonephritis (RPGN)

With an incidence of 5–10 cases/million population/year worldwide (8000 new cases annually in Europe and North America), rapidly progressive glomerulonephritis (RPGN) is a class of acquired renal disease that has been extensively studied as it remains one of few human autoimmune diseases that represent an acute threat to survival [1–3]. This has stimulated investigation into the immunobiology of the condition in the hope of understanding the pathogenesis not only of anti-glomerular basement membrane disease, but also of other forms of glomerulonephritis in which the aggravating antigen(s) is as yet unknown. Despite aggressive immunosuppressive protocols, the prognosis for retaining kidney function is poor (59% and 70% at 1 year and 5 years, respectively) [4–6]. Patients fortunate to survive RPGN usually experience reduced quality of life due to the requirement for side effect-prone non-specific immunosuppressants and continued dialysis; also significant health care costs. For example, in France, the annual cost of maintenance dialysis is 2.1 billion € (with 70 k€/year per person) of which an estimated 12% (250 M€) is related to care for patients who have suffered RPGN [7]. Focal necrotizing crescentic GN is the renal lesion typically associated with the clinical syndrome of RPGN and is a medical emergency that requires side-effect prone immunosuppressive therapies. Untreated RPGN progresses rapidly to renal insufficiency. The diagnosis of RPGN is made by renal biopsy, where additional layers of cells are visible within Bowman’s space. Severe acute inflammation occurs in the glomerulus, sometimes with formation of ‘crescents’ when the glomerulus is squashed by cells that fill Bowman’s space; these cells are a mixture of infiltrating inflammatory cells and proliferating resident cells. Recent data indicate that podocytes and parietal epithelial cells (PECs) participate to crescent formation. When these proliferating cells obstruct the urinary outflow of the glomerulus, the entire nephron degenerates and irreversible loss of renal function occurs. Whatever the inflammatory cause, similar appearances are seen by light microscopy, and immunohistology is required to identify the likely aetiology. Approximately one-fourth to one-third of patients experience recurrence within a few years. The need for maintained immunosuppressive therapy raises the problem of how to obtain an acceptable balance between adequate immunosuppression and limited toxicity. Most such immunosuppressive regimes are also associated with a significant risk of opportunistic infection and severe metabolic side effects. Loss of renal function due to GN frequently requires kidney transplantation; unfortunately destruction by blood-borne antibodies is often undiminished in transplanted kidneys, leading to rapid recurrence of disease and graft loss [8]. Treatment regimes for GN have significantly improved in the past two decades [9,10]. Although most patients achieve remission, relapses and treatment-related morbidities are common. Hence, there is still room for therapies that: I- would be specific to the pathophysiological mechanism, I- would improve the renal outcome that still remains dramatically poor, III- would be well tolerated, IV- would be pharmacologically compatible with current standard therapies, which may be then alleviated.

Pathogenesis of crescent formation in RPGN: activation of glomerular epithelial cells

Recent work has unraveled an unexpected role of glomerular epithelial cells (podocytes and PECs) in RPGN and also in FSGS...
using cell lineage tracing experiments. The results were validated in multiple animal models as well as in human biopsies. The glomerulus must no longer be seen as a regular capillary sphere whose main function is blood ultrafiltration but as a multicellular structure finely regulating its own vascular permeability [11]. Moreover, most regulators of glomerular capillary homeostasis have additional, non-redundant functions beyond the vascular compartment, in regulating inflammation and mediating tissue repair. These responses can become excessive, such as in formation of the crescent, an irreversible lesion that aggravates endothelial injury and interrupts capillary blood flow, leading to irreversible ischemia and glomerular obsolescence. During crescent formation in mouse models of anti-glomerular basement membrane (GBM) serum-induced RPGN, proliferating cells in cellular crescents originate from PECs and, to a lower extent, from podocytes [12,13]. Podocytes assume a migratory phenotype, attach with their apical membrane onto the parietal basement membrane and proliferate [14,15]. Recent data confirm that podocytes contribute to crescent formation in humans, too [16,17] as well as PECs [12,18]. Since these cells cause the obstruction of the urinary outflow, they may be critical targets for therapeutic interventions.

**Recent evidence: the EGFR signaling pathway is overactivated in RPGN**

We have recently demonstrated de novo expression of heparin-binding epidermal growth factor-like growth factor (HB-EGF) in podocytes and PECs from both mice and humans with RPGN. Such induction of HB-EGF expression correlated with increased phosphorylation of EGFR in podocytes from mice with experimental nephrotoxic serum disease. Glomerular EGFR activation was absent and the course of RPGN markedly improved in HB-EGF-deficient mice. Moreover, conditional deletion of the Egfr gene from podocytes or administration of a clinically available EGFR inhibitor both markedly alleviated proteinuria, crescent formation and renal failure in mice [19]. Strikingly, even delayed inhibition of this pathway after the onset of disease proved effective in stopping the course of the RPGN, suggesting potential therapeutic promise in targeting this pathway. The HB-EGF-EGFR cascade is essential to promote podocyte proliferation and migration in vitro, essential hallmarks of crescent formation.

While screening human kidney biopsies for HB-EGF expression, we found markedly localized de novo expression of the growth factor in areas where PECs and podocytes (GECs) form synechiae (bridges) and crescent. Remarkably, while RPGN is a severe complication of immune-vasculitis caused by unrelated and diverse autoimmune disorders such as systemic lupus erythematosus, granulomatosis with polyangiitis, microscopic polyangiitis, Churg Strauss syndrome, chronic infections, etc., the phenotype switch of GECs is apparently remarkably similar in all human kidney biopsies of patients diagnosed with RPGN that we could screen, as far as HB-EGF expression is concerned. Thus, induction of HB-EGF expression may be a common feature of immune-mediated GN. Antagonizing this pathway may constitute a targeted therapeutic approach to treat immune-mediated GN of diverse etiology [20,21].

In summary, these results suggest that the EGFR signaling cascade is also involved in the activation of glomerular cells in RPGN patients.

**Activation of G-protein coupled receptors (GPCRs) and tyrosine kinase receptors could synergize to switch the phenotype of podocytes and parietal epithelial cells (PECs) from healthy to pathological**

The next important question is about the mechanisms that link activated EGFR pathway to the local pathophysiological context and progression of disease. Is de novo overactivation of the EGFR pathway sufficient to promote GECs phenotype and glomerular demolition? Endothelial activation is obvious in conditions associated with RPGN, and may be a common pathway acting downstream heterogeneous immune disorders. Thus, we suspect that local mediators generated within the glomerulus may not only trigger HB-EGF and a larger pathogenic gene program, but also cooperate in the pathogenic GECs dedifferentiation.
The coagulation cascade is a prominent local candidate for our investigations. In addition to converting fibrinogen to fibrin monomers, serine protease of the coagulation cascade can also directly modulate cellular behavior through protease-activated receptor (PAR) signaling. Four members of this seven-transmembrane domain, G-protein–coupled receptor (GPCR) family have been identified and are designated PAR1-4. In GN, coagulation might be initiated by the abnormal presence of tissue factor (TF), a transmembrane glycoprotein usually excluded from cells that are in direct contact with circulating blood, in glomerular capillaries. TF synthesis can be induced in endothelial cells and macrophages by exposure to proinflammatory cytokines. Alternatively, compromised integrity of the capillary barrier could result in exposure of plasma zymogens to TF expressed or induced on podocytes, mesangial cells, or other cells in Bowman’s capsule [22]. TF activates the extrinsic pathway by complexing with factor VII. In the presence of calcium, the TF/VIIa complex catalyzes the activation of factor X. Factor Xa in turn interacts with factor Va to form a prothrombinase complex, thus leading to conversion of prothrombin to thrombin. All PARs can be activated by coagulation proteases; PAR-1, 3&4 by thrombin and PAR2 by factors VIIa and Xa [23]. In mice, PARs 3&4 are mainly expressed on platelets. Consistent with non-coagulant roles for coagulation proteases in kidney disease, global deficiency of either PAR1 and PAR2 has been shown to confer protection in mouse models of GN. PAR1-deficient [PAR1(-/-)] mice, which have normal coagulation, showed significant protection from crescentic GN compared with wild-type mice. Reduction in crescent formation, inflammatory cell infiltration, and serum creatinine were similar in PAR1(-/-) mice and hirudin-treated mice suggesting that PAR1 may be the major pathogenic thrombin effector in this model [24]. PAR-2(-/-) mice exhibited reduced crescent formation, proteinuria, and serum creatinine compared with wild-type mice 21 days after initiation of RPGN in the same model [25]; whether it is coagulation factors that drive PAR2 activation in this setting is unclear.

In addition to stimulated coagulation cascade, endothelial injury, as expected in vasculitis, is characterized by release of the potent vasoconstrictor endothelin-1 (ET-1), thromboxane, PGE2 and thrombin. Furthermore, chemokines released by recruited immune cells activate a number of GPCRs; some of them being expressed by GECs. Notably, the responsibility of GPCRs on podocytes and PECs for the physiopathology of crescentic diseases has not been established. Further studies may assist clinicians in optimally designing clinical trials for patients at increased risk for CKD.

**Conclusion**

*De novo* expression of Heparin-binding EGF-like growth factor (HB-EGF), a member of the EGF family of proteins, in parietal epithelial cells (PECs) lining the Bowman capsule and in podocytes, provides the first example of a molecular interaction between these two cell types, promoting crescent formation and renal failure.

Activation of the Hbegf gene in PECs and podocytes represents a pathophysiological feature of expression of a more globally abnormal gene program with important consequences for glomerular demolition and tissue response to inflammation. This program should be further analyzed.

Abnormal activation of G-protein coupled receptors (GPCRs) and tyrosine kinase receptors may synergize to switch the phenotype of GECs from healthy to pathological. One likely culprit is activation of the coagulation cascade and dysfunction of the capillary barrier, demonstrated to occur in RPGN, that may provide GPCR ligands to receptors present in target cells such as podocytes and PECs. GPCRs, themselves implicated in the progression of GN, are also known to potently transactivate the EGFR. The EGFR pathway has the potential to amplify actions of GPCRs in GECs, leading to pathogenic calcium and ROS signaling, cytoskeletal rearrangement, and cell death [21,26].

The integration of this knowledge has the potential to improve the identification of patients with vasculitis at risk of detrimental outcome or with RPGN. Furthermore, targeting of these pathways could limit the progression of severe glomerulopathies.

**Disclosure of interest:** the authors declare that they have no conflicts of interest concerning this article.

**References**


L23. Renal transplantation in ANCA-associated vasculitis

Renal transplantation is the treatment of choice for end-stage renal disease. It provides better results with respect to patient survival and morbidity as well as it offers superior possibilities for rehabilitation as compared to various dialysis modalities [1]. Since renal engagement, often healing with scarring, is common in antineutrophil cytoplasm antibody (ANCA)-associated vasculitis (AAV) [2], renal transplantation is a therapeutic option that frequently needs to be considered in AAV patients. There are several publications clearly indicating that AAV patients do fairly well after renal transplantation and that AAV should definitely not be considered as a contra-indication for transplantation [3–12]. However, there are several issues regarding the treatment of AAV before and after transplantation that remain unresolved which will be addressed in this brief review. These issues include the epidemiology of AAV and transplantation, timing of transplantation, risk of relapse, role of ANCA measurements and treatment of relapses after transplantation.

Scope of the problem

There are limited data on the number AAV patients having received a renal transplant and as well as numbers on waiting lists. Understanding of the epidemiology of AAV in end-stage renal disease is hampered by unresolved matters regarding nomenclature and classification of vasculitis [13]. Before the age of prevalent ANCA testing many patients with AAV went into renal failure without a distinct diagnosis. Weidemann et al. reported in 1993 on ANCA among 1277 dialysis patients, about 7% were positive and a distinct AAV diagnosis could retrospectively be made in a substantial portion of them [14]. Many European renal registries use the EDTA codes where patients with renal limited AAV (RLV) could be classified as either "crescentic glomerulonephritis" (EDTA code 17) or "glomerulonephritis not described above" (code 19) depending on the severity of the glomerular lesions, and if no biopsy is performed as "unknown" (code 00). AAV patients with extra-renal symptoms could be listed as either "Wegener’s Granulomatosis" (code 74), "renal vascular disease due to polyarthritis" (code 73) or "other multisystem disease" (code 89). No meaningful epidemiological data on AAV and end-stage renal disease can be extracted from of registries using EDTA codes, but codes as 17 and 74 can be used to identify cohorts to study outcome of AAV after transplantation [7]. Epidemiological studies in AAV have found incidence rates between 15 and 23 cases per million per year in recent publications [15–17]. Data from the US suggest the rates being substantially lower among Afro-Americans [18]. Apart from that there are no convincing data that the incidence of AAV...