Nephrology Dialysis Transplantation 37 (Supplement 3): i840–i841, 2022

NEW INSIGHTS INTO THE PATHOPHYSIOLOGY OF GN

FC 073 AN EPIGENETICALLY DRIVEN MECHANISM TRIGGERED BY VIRAL AND BACTERIAL RNA REGULATES THE IL-6 LEVELS IN IGA NEPHROPATHY

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BACKGROUND AND AIMS: Several models have been proposed to describe the pathogenesis of Immunoglobulin A nephropathy (IgAN) and, among them, the multihit model where the gut-microbiota may play an important role. These models explain the pathogenesis of IgAN caused by the production of aberrant IgA, but it is believed that further predisposing factors are present, including immunological, genetic, environmental or nutritional factors.

Recently, the role of IL-6 in IgAN pathogenesis is becoming increasingly important. It is essential for the deposition of glomerular immunoglobulin A and the development of renal disease in Cd37-deficient mice, although the pathogenetic mechanisms that determine its increase are not well known.

A possible hypothesis emerges from our recent work on genome-wide DNA methylation screening in patients with IgAN, which identified, among other findings, a hypermethylated region comprising Vault 2–1 RNA (VTRNA2-1), a non-RNA coding also known as a precursor of miR-886 (pre-mi-RNA). Consistently, VTRNA2-1 expression was found downregulated in IgAN patients.

Here we studied the involvement of the VTRNA2-1/PKR/CREB/IL-6 pathway in IgAN.

METHOD: Total RNA were isolated from PBMCs of IgAN patients, transplanted IgAN patients (TP-IgAN), non-IgAN transplanted patients (TP) and healthy subjects (HS). VTRNA2-1, CREB and PKR transcripts were evaluated by RT-PCR. Total and phosphorylated PKR, CREB and Il-6 proteins were evaluated by ELISA. Poly (I: C), a synthetic analogue of dsRNA and Pfizer-BioNTech COVID-19 COMIRNATY vaccine were used to transfect patient PBMCs. PKR inhibitor imoxin (C16) 1 μM was used to stimulate patient PBMCs.

RESULTS: Here we confirm that VTRNA2-1 transcript was down-regulated in native and transplanted IgAN subjects compared to HS and non IgAN transplanted patients, with a decrease of 30- and 100-folds, respectively ($P < 0.05$, and $P < 0.0001$). IgAN patients with downregulated VTRNA2-1 showed a PKR overactivation (fold increase of phosphorilation of 2.6- in IgAN and 2-folds in TP-IgAN patients; $P < 0.05$), coherently with the role played by VTRNA2-1 that binds to PKR and inhibits its phosphorylation. Then, we found that PKR causes the activation of CREB, a classical cAMP-inducible CRE-binding factor (fold increase of phosphorilation of 3- in IgAN and 2.67-folds in TP-IgAN patients; $P < 0.01$). CREB, interacting with a region of the IL-6 promoter, led to IL-6 production. Indeed, in IgAN patients we showed a IL-6 mean increase to 120 pg/mL compared to the respective controls ($P < 0.05$). Moreover, the IL-6 levels correlated with CREB and PKR phosphorylation ($r = 0.97$; $P = 0.0006$ and $r = 0.89$; $P = 0.0064$, respectively, for IgAN and TP-IgAN patients).

Since PKR is normally activated by bacterial and viral RNA, we hypothesized that these microorganisms can further activate the PKR/CREB/IL-6 pathway leading to an excess of IL-6 production. This may explain both the high levels of IL-6, and infection involvement in the disease, and cases of IgAN associated with COVID-19 infection or with COVID-19 RNA-vaccination, and recent data showing microbiota involvement in IgAN. Effectively, we found that IgAN PMBCs stimulated with RNA poly(I: C) or the COVID-19 RNA-vaccine showed a significant increase in IL-6 levels compared to not-stimulated PBMCs ($P < 0.05$), supporting the pathogentic role played by viral RNA in IgAN pathogenesis and explaining the cases of IgAN patients developing episodes of macrohematuria after a COVID-19 infection or vaccination.

Finally, we showed that the IL-6 secretion can be reduced by the PKR inhibitor imoxin (fold decrease of 5-folds in IgAN and TP-IgAN patients; $P < 0.05$).

CONCLUSION: In conclusion, the discovery of the upregulated VTRNA2-1/PKR/CREB/IL-6 pathway in IgAN patients may provide a new pathogenic mechanism in IgAN and may be useful for the development of novel therapeutic approaches, likely by modulating the VTRNA2-1 methylation level in IgAN patients.

FC 074 MINERALOCORTICOID RECEPTOR DRIVES PARIETAL EPITHELIAL CELL ACTIVATION AND GLOMERULAR INJURY DURING CRESCENTIC GLOMERULONEPHRITIS

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BACKGROUND AND AIMS: We have recently demonstrated that targeting specific pathways in parietal epithelial cells (PEC) can markedly alleviate experimental extracapillary glomerular injury (1). Accumulating evidence has indicated the potential contributions of aldosterone and mineralocorticoid receptor (MR) to the pathophysiology of chronic kidney disease. MR is strongly expressed in endothelial cells, glomerular mesangial cells, podocytes and distal tubular cells. Previous studies have shown that administration of mineralocorticoid receptor antagonists, including spironolactone and eplerenone, has beneficial effects in various renal injury animal models, such as unilateral ureteral obstruction, ischemia-reperfusion, cyclosporine-induced nephrotoxicity and hypertensive renal injury. The role of the MR in extra capillary glomerulopathies is still elusive and mechanistically unclear.

METHOD: To investigate the cell-specific role of the MR in PEC in the course of crescentic glomerulonephritis, we generated chimeric mice specifically lacking MR (Pec Cre *Nr3c2* $^{lox/lox}$) in PECs using an inducible Cre recombinase system. Crescentic glomerulonephritis (GN) was induced using the antiglomerular basement membrane nephrotoxic serum (NTS) model. *In* vivo, biological parameters (albuminuria, blood urea nitrogen—BUN), glomerular injury, as well as PEC activation (CD44, CD9 and fibronectin staining) were assessed.

RESULTS: At baseline, Pec Cre *Nr3c2* $^{wt/wt}$ and Pec Cre *Nr3c2* $^{lox/lox}$ mice displayed no kidney morphology and function differences. When challenged using the NTS model, Pec Cre *Nr3c2* $^{lox/lox}$ mice showed less albuminuria and preserved renal function as compared to Pec Cre Nr3c2 $^{wt/wt}$ counterparts. Crescents were also more numerous and organized in Pec Cre *Nr3c2* $^{wt/wt}$ mice.

Next, we examined whether pharmacological MR inhibition could alleviate the severity of crescentic GN. When Pec Cre *Nr3c2* $^{wt/wt}$ mice were orally given eplerenone for 14 days after the onset of the crescentic GN, they were significantly protected from renal injury and failure (decreased proteinuria, normal BUN and reduced number of crescent). Such global action was associated with less activation molecule CD44 on PECs. Thus, genetic disruption of MR in PEC as well as pharmacological inhibition using eplerenone reduced glomerular expression of CD44 and crescent formation.

Furthermore, kidney biopsies of individuals diagnosed with crescentic glomerulonephritis displayed increased expression of MR in PEC and crescents.

CONCLUSION: Altogether, these results indicate the critical role of MR in PEC activation during crescentic glomerulonephritis along with the recently discovered CD9/EGFR/PDGFR pathway. This further supports the idea that the PEC phenotype switch is not a bystander event but plays a targetable critical active pathogenic role in crescentic GN. MR modulation using eplerenone may be a new therapeutic option for the management of such severe disease.

REFERENCE

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