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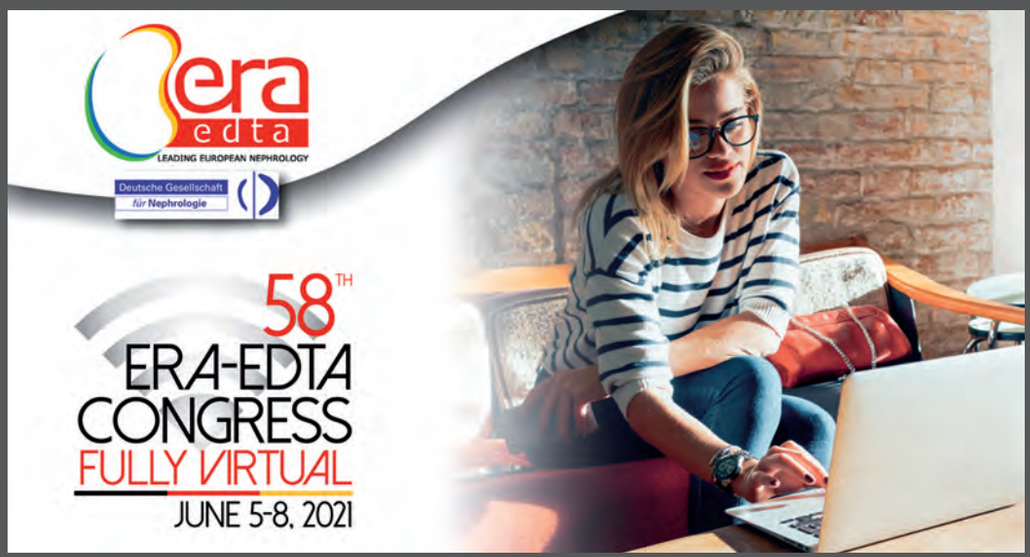

Congress Abstracts

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TREATMENT & OUTCOME OF GLOMERULONEPHRITIS

FC037 NEUTROPHILS PLAY A KEY ROLE IN THE INITIATION OF GLOMERULAR HEMATURIA IN A POSTINFECTIOUS IGAN EXPERIMENTAL MODEL

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BACKGROUND AND AIMS: Hematuria is a common finding in patients with IgA nephropathy (IgAN), occurring mainly after upper respiratory tract infections. Hematuria can lead to acute kidney injury and chronic loss of renal function in IgAN. However, the mechanisms involved in egression of erythrocytes from the glomerular capillaries into the urinary space are unknown. To answer this question, we developed an infection with *Streptococcus pneumoniae* (SP) in a humanized experimental IgAN model (α1KICD89tg mice) that resembles the pathological and clinical findings of disease (IgA1 and soluble CD89 mesangial deposits, complement activation, proteinuria and hematuria).
METHOD: α1KICD89tg mice (12 weeks old) received an intranasal instillation of SP (10^7 bacteria). Blood, urine and renal samples were obtained during 1 month after induction of respiratory infection. The presence of SP in lungs from these mice was confirmed by microbiological analysis. Hematuria was quantified in the urinary sediment and renal function was determined by biochemical analysis. Renal histological characteristics were evaluated by hematoxylin/eosin, masson's trichrome and PAS staining. IgA glomerular deposits, activation of complement system and infiltration of proinflammatory cells was examined by immunohistochemistry or immunofluorescence. Circulating leukocyte populations were studied on a hemocytometer. Renal inflammatory cytokines, metalloproteases, as well as markers of tubular and glomerular damage were determined in kidneys by RT-PCR and western-blot. To further validate the role of neutrophils in this pathological setting, we selective depleted these cells through a single injection of anti-Ly6G mAb (200 µg/kg i.p).
RESULTS: SP-intranasal instillation in α1KICD89tg mice increased hematuria, microalbuminuria and proteinuria, peaking at 48h after induction of the respiratory infection. SP instillation caused disruption of the glomerular basement membrane, with decreased expression of the slit diaphragm proteins nephrin and synaptopodin, as well as higher glomerular accumulation of IgA and proteins of complement system (C3, MBL). Hematuria intensity was positively correlated with the presence of interstitial F4/80+ macrophages, matrix metalloproteinase 9 (MMP-9), inflammatory cytokines and chemokines (IL-1β, IL-6, TNF-α, CCL-2, CCL5 and CX3CL1/CX3CR1) as well as p65 NF-κB activation. Hematuria was negatively correlated with anti-inflammatory IL-10 mRNA expression, Factor H levels and collagen IV content. Notably, SP infection induced expression of the tubular injury markers N-GAL and KIM-1. Increased peripheral neutrophils levels were observed in the SP-infected α1KICD89tg mice. Mechanistically, anti-Ly6G-mediated neutrophil depletion reduced SP-mediated hematuria, proteinuria and albuminuria, prevented loss of synaptopodin and nephrin, decreased renal inflammation and MMP-9 expression in α1KICD89tg mice
CONCLUSION: In a humanized mouse model of IgAN, hematuria bouts following respiratory tract infections are caused by a neutrophil-mediated alteration of the glomerular filtration barrier (podocyte damage, complement deposits and loss of Collagen IV). These findings may help to unveil novel potential therapeutic approaches to combat one of the key elements in the progression of IgAN and related conditions.

FC038 CRESCENTS DERIVE FROM SINGLE PODOCYTE PROGENITORS AND A DRUG ENHANCING THEIR DIFFERENTIATION ATTENUATES RAPIDLY PROGRESSIVE GLOMERULONEPHRITIS

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BACKGROUND AND AIMS: Rapidly progressive glomerulonephritis (RPGN) encompasses a group of diverse disorders characterized by the presence of massive hyperplasia of parietal epithelial cells (PEC) as the main histopathological lesion at kidney biopsy. It is associated with a rapid decline in kidney function referred to altogether as rapidly progressive glomerulonephritis. Typically, crescent formation is the consequence of diverse upstream pathomechanisms involving the specific activation of PEC. PEC normally reside peacefully along Bowman capsule and represent in part renal progenitor cells (RPC). Previous studies observed RPC markers in crescents from patients with different types of glomerulonephritis. Similarities between stem cell niches of bone marrow and kidney, prompted us to hypothesized that crescents result from monoclonal expansion of a single RPC clone conceptually similar to monoclonal diseases originating from hematopoietic stem cells. According to this analogy, we further hypothesized that drugs known to cure monoclonal disease of the hematopoietic stem cells by enforcing their terminal differentiation could also attenuate crescentic glomerulonephritis.
METHOD: To address this hypothesis, we established a RPGN disease model in a conditional transgenic mouse based on the mT/mG and the Confetti reporter that allows lineage tracing and clonal analysis of RPCs. Animals were treated with known pharmacological inhibitors of clonal stem cell proliferation in myeloproliferative disorders. Crescentic lesions were characterized by super-resolution STED microscopy. Finally, we employed single cell RNA sequencing of human renal progenitor cultures to identify the immature progenitor subset-generating crescent in human to identify putative new biomarker(s) of RPNG to validate in biopsy of patients.
RESULTS: We observed that the crescentic lesions originated from the clonal expansion of single RPC, thus suggesting a clonal stem cell disorder. Therefore, we administrated a series of drugs known to ameliorates myeloproliferative neoplasms to our RPGN mouse model as potential therapeutic agents. In particular, treatment with one of the compounds induced a reduction in both proteinuria and crescent formation. 3D confocal microscopy and STED super-resolution imaging of glomeruli showed that this compound turned the uncontrolled hyperplasia of a specific immature PEC subset into a controlled differentiation into new podocytes thereby restoring the injured glomerular filtration barrier.
Single cell RNA sequencing of human renal progenitor cultures identified a new marker of the crescent-generating progenitor cells. Expression of this marker in biopsies of patients with rapidly progressive glomerulonephritis associated with progression toward end stage kidney disease. Treatment of human PEC with the drug that in *in vivo* experiments showed a therapeutic effect on RPGN reduced proliferation of the immature progenitor subset promoting their differentiation into podocytes.
CONCLUSION: These results demonstrate that glomerular hyperplastic lesions derive from clonal amplification of a RPC subset and that shifting proliferation to podocyte differentiation reverses crescent formation and improves clinical outcome.