induction rate in 1%O2 was same as that in 20%O2. In contrast, media from hypoxic TC kept at 1%O2 activated NET formation. TC-derived histones dose-dependently induced NETosis, which implies a further release of cytotoxic DAMPs that kills renal cells. Histone-induced NET formation was significantly inhibited by anti-histone IgG or heparin. PMA-induced NET formation was inhibited by PADin. Conversely, media of histone-induced NETs induced TC death.

Conclusions: Our data indicate that infiltrating neutrophils undergoes NETosis within the peritubular capillaries, a process that drives ATN via release of cytotoxic DAMPs like histones.

P18

ER-STRESS AND LOSS OF GRP78 EXPRESSION PROVIDES A LINK BETWEEN RENAL ISCHEMIA/ REPERFUSION INJURY AND THE URINARY METABOLOME

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Introduction: Ischemia/reperfusion injury (IRI) profoundly impacts graft survival following kidney transplantation. Epithelial injury is one of the earliest histological alterations of IRI and is especially observed in the corticomedullary junction. In this region oxygen tension is lowest whereas epithelial cells are metabolically very active. Therefore we hypothesized that urinary metabolomics could be a tool for non-invasive assessment of IRI-induced changes.

Methods: Ischemia/reperfusion was induced in Lewis rats by unilateral clamping the left renal artery for 45 minutes and removing the contralateral kidney. Rats were treated with either control antibody or a protective antibody directed against Mannan-binding lectin (MBL). Groups of rats were sacrificed at 2, 5 and 24 h post I/R and samples (serum, urine, tissue) were collected. Markers of renal function (serum creatinine and BUN) and injury (tissue staining and mRNA) were determined. Moreover, the urinary metabolic profiles were analysed using a GC-MS platform.

Results: We demonstrate that this IRI model is characterized by early epithelial injury and an increased expression of KIM-1, NGAL, IL-6 and rise in serum creatinine. Importantly, already at 2 hours a strong reduction of GRP78 protein expression is observed, specifically in the corticomedullary junction. Loss of GRP78, a regulator of the ER-stress response, was accompanied by induction of downstream mediators spliced-XBP1 and CHOP expression. Inhibition of MBL in vivo protected tubular cells from rapid loss of GRP78 expression and consequent tubular injury. Exploratory data analysis of the urinary metabolic profiles showed a dominant effect of time, but not of the protective treatment. The use of PLS regression models in combination with all injury markers as response variables, only revealed a significant association between metabolic changes in urine and tissue expression of GRP78. Exploring the variable importance of projection values we have identified a number of metabolites, including alpha- ketoglutarate, aconitic acid, uric acid, hippuric acid and desaminotyrosine which were significantly contributing to the model and were affected by protective anti-MBL treatment.

Conclusions: In conclusion, we show that loss of GRP78 and induction of the ER-stress response is a very early process in IRI, specifically taking place in the corticomedullary junction. Using a combination of statistical models and specific intervention we could link the metabolic trajectory to the recovery process and show that metabolomics is a valuable tool for the evaluation of IRI at cellular and tissue level.

P19

TISSUE PRIMING OF PLASMACYTOID DENDRITIC CELLS ENHANCES THEIR PHAGOCYTOSIS AND LOWERS THE THRESHOLD FOR SUBSEQUENT TOLL-LIKE RECEPTOR 9 ACTIVATION

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Introduction: Plasmacytoid dendritic cells (pDC) have a pivotal role in clearing viral infections, and can regulate tolerance or immunity depending on their activation status. We have previously demonstrated a strong influx of pDC in the tubulointerstitium of human renal allograft rejection biopsies. In experimental transplant models, pDC were demonstrated to be activators of indirect alloreactivity, suggesting they should be able to take up donor antigens. In this study, we investigated the capacity and requirements of human pDC to ingest and present donor antigen.

Methods: Human pDC were isolated from buffy coats of healthy individuals using negative selection. pDC were cultured overnight in the presence or absence of conditioned medium (CM) of human kidney proximal tubular epithelial cells (HK2), followed by CpG/CMV activation. Phagocytosis of CFSE-labelled apoptotic HK2 cells was determined using flow cytometry. Alternatively, pDC were primed and activated, after which the cytokine/chemokine production, the expression of costimulatory molecules and the T cell stimulatory capacity was assessed.

Results: In absence of any stimulus pDC were unable to ingest apoptotic cells (AC) (mean 2%), which was only slightly increased after activation using TLR9 ligand CpG (mean 15%), in consensus with previous reports. However, priming pDC by CM of HK2 cells, as well as primary tubular epithelial cells, and subsequent TLR9 ligation using CpG or cytomegalovirus (CMV), strongly induced the capacity to ingest AC (mean 44%). Priming by CM led to phosphorylation of the key transcription factor Interferon Regulatory Factor-7, in absence of IFN production. Importantly, upon priming 10-fold lower concentrations of CpG were required to get optimal TLR9 activation. Consequently, activated primed pDC produced vast amounts of IFN (mean 5,354 vs 475 pg/mL) and the chemokines CCL4 and CXCL10. Moreover, priming increased pDC phenotypic maturation (CD40/80/83/86 and CCR7) and TLR7/9 expression, compared to non-primed pDC. As a functional consequence, primed pDC induced a vigorous allogeneic T cell proliferation as compared to their non-primed counterparts (mean 60% vs 4%), as well as inducing a strong TH1 skewing (e.g. IFN mean 2,268 vs 60 pg/mL). Using donor HLA antigen and a CD4 T cell clone with indirect specificity, pDC were shown to be cells with efficient indirect antigen presentation capacity.

Conclusions: In conclusion, we show that factors produced by renal epithelial cells enable the phagocytic capacity of pDC following TLR9 ligation. Moreover, this tissue priming lowers the TLR9 activation threshold by one order of magnitude. Subsequently, TLR9 ligation by CpG or CMV strongly enhances their