with or without 20 μM PTU was determined. Next, we established mouse models with MPO-ANCA production. BALB/c mice were given intraperitoneal (i.p.) injection of PMA (50 ng at day 0 and day 7) and oral administration of PTU (5 mg/day) for 2 weeks. In this model, MPO-ANCA was produced by day 14 though an obvious vasculitic phenotype was not observed. These mice were divided into two groups, namely Group 1 with daily i.p. injection of Cl-amidine (0.3 mg/200 μl/day) (n=7) and Group 2 with daily i.p. injection of PBS (200 μl/day) (n=13). Two weeks later, serum MPO-ANCA titers and amounts of peritoneal NETs were compared between the 2 groups.

Results: In vitro NET formation induced by 20 nM PMA with or without 20 μM PTU was inhibited significantly by 200 μM Cl-amidine. Serum MPO-ANCA titers of Group 1 mice (32.3 ± 31.0 ng/ml) were significantly lower than those of Group 2 mice (132.1 ± 41.6 ng/ml). The amounts of peritoneal NETs in Group 1 mice was significantly smaller than those in Group 2 mice. These findings suggested that the NET formation was inhibited significantly by Cl-amidine both in vitro and in vivo, and that the MPO- ANCA production was also suppressed by Cl-amidine in vivo.

Conclusions: PAD4 inhibitor suppresses MPO-ANCA production through inhibition of NET formation in mouse model so that it could be a novel therapeutic modality for MPO-AAV in humans.

P16

INTERCELLULAR ADHESION MOLECULE-1 K469E(A/G) POLYMORPHISM AND ITS EFFECTS IN THE DEVELOPMENT OF DIABETIC NEPHROPATHY

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Introduction: Recent research has implicated that inflammation may be a key pathophysiological mechanism in diabetic nephropathy (DN), although its pathogenesis is multifactorial. Intercellular adhesion molecule 1 (ICAM-1) is an acute phase marker of inflammation and the ICAM-1 gene is located on chromosome 19p13.2 and resides a linkage region to diabetes and DN. To investigate whether ICAM-1 has effects in the development of DN, we have recently performed genetic and pathological studies of this molecule in Swedish and Malaysian subjects with normal glucose tolerance (NGT), diabetes and DN.

Methods: We genotyped six single nucleotide polymorphisms (SNPs) in the ICAM-1 gene with TaqMan allelic discrimination. We also determined plasma ICAM-1 levels with an enzyme-linked immune-sorbent assay kit.

Results: We found that non-synonymous SNP rs5498 (K469E A/G) was associated diabetes and DN and the G allele had a protective effect. Particularly, we found a high heterozygous index of this polymorphism presenting in both populations. The genotype distribution of this polymorphism was kept in Hardy-Weinberg Equilibrium and no duplicon in the genomic sequence was found. The ICAM-1 K469E(A/G) polymorphism resides in the 5th Ig-like domain of ICAM-1 protein. This domain is essential for dimerization, surface presentation and solubilisation of proteins of the protein and subsequently plays a crucial role in the activity of ICAM-1 protein in the interaction with LFA-1 and the adhesion of B cells. Furthermore, we found the carriers with heterozygous genotype had higher fasting glucose levels among newly diagnosed type 2 diabetes patients compared with the subjects with wild or mutant homozygous genotype. Plasma ICAM-1 levels were increased from the subjects with NGT, diabetes without DN to the patients with DN. Among diabetic patients with DN, the carriers with heterozygous genotype had higher plasma ICAM-1 levels compared with other patients.

Conclusions: Our study provided evidence that ICAM-1 has effects in the development of DN. The patients carrying with heterozygous genotype of SNP rs5498 (K469E A/G) in the ICAM-1 gene have higher risk susceptibility to DN. The combined approach with genotyping this polymorphism and measuring plasma ICAM-1 levels may be useful for prediction of DN in translation medicine.

P17

INTRAVASCULAR NEUTROPHIL EXTRACELLULAR TRAP (NET) RELEASE PROMOTE VASCULAR INJURY AND TUBULAR NECROSIS UPON ISCHEMIA/REPERFUSION INJURY (IRI) OF KIDNEY

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Introduction: Acute tubular necrosis (ATN) is common in severe acute kidney injury (AKI). Infiltrating neutrophils contribute to the crescendo of renal inflammation and kidney injury (necroinflammation), but how neutrophils contribute to ATN is not clear. We hypothesized that infiltrating neutrophils release neutrophil extracellular traps (NETs), which implies the release of cytotoxic DAMPs like histones that accelerate ATN and AKI.

Methods: In vivo; Postischemic AKI was induced in wild type mice by unilateral clamping of the renal pedicle for 35 minutes followed by reperfusion for 6h or 72h. Formation of NETs was identified by immunostaining using citrullinated histone 3 (CitH3) antibody. Intravascular NETs were confirmed by neutrophil elastase (NE)-DNA complex ELISA in plasma. Renalcell death was evaluated in kidney sections by Tunel staining and PicoGreen DNA assay of plasma. To investigate the effect of NETs inhibition in bilateral IRI (ischemic 35min, reperfusion 24h) group of mice were treated with either PAD inhibitor (PADin) or neutrophil depletion and sacrificed 24h after reperfusion. In vitro; To investigate whether hypoxia can directly induce NETs formation or indirectly via hypoxia-induced tubular cell death, 1) human neutrophils were incubated in 1% or 20%O2 for 24h, 2) the media of tubular cell line (TC), which were incubated in 1% or 20%O2 for 24h, and stimulated with neutrophils for 4h. Furthermore, PMA or histone- induced NETs were treated by PADin, anti-histone antibody and heparin. Histone-stimulated neutrophil media were applied to TC. NETs and TC injury in vitro were evaluated by CitH3 staining/MPO-DNA complex and LDH assay, respectively.

Results: IRI kidney showed increased positivity for CitH3 in areas of tubular necrosis of the outer medulla. Plasma levels of the NE-DNA complex were increased in a time-dependent manner as compared to sham-operated mice. NET-induced renal cell death was shown in terms of increased Tunel positive area in kidney and plasma DNA 6h after reperfusion compared to the sham group. Subsequently, NETs in plasma and kidney increased 15～24h after reperfusion. In bilateral IRI, treatment with both PADin and neutrophil depletion significantly reduced the plasma levels of NE-DNA complex and NETs area in kidney compared to the vehicle group. Treatment further improved renal excretory function in terms of reducing plasma creatinine levels. In vitro, the NET-

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