(expressed by normal as well as HIV-1 infected T cells) had potential to induce apoptosis/necrosis of infected T cells and thus, providing release of HIV-1 proteins and RNAs for ednocytosis by tubular cells.

Human tubular cells (HK2) were pulsed with HIV-1 (X4 HIV-$1_{92HT599}$) for two hours, followed by rescue of virus by tubular cells. One week later, HIV-1 infected or non- infected CD4+ ive T cells were incubated either with HK2 cells or 293T cells (negative for CD4 and PDL-1) for 48 hours. Subsequently, CD4+ ive T cells were assayed for proliferation and apoptosis by FACS analysis. In addition, HIV-1 proteins and RNAs were assayed in incubation media. Tubular cells probed for HIV-1 mRNA and protein expression.

The population of CD4+ ive T cells dropped significantly after interaction with HK-2 cells, and the effect was more pronounced in HIV-1-infected CD4+ ive T cells. Also, there was increased apoptosis in CD4+ ive T cells on interaction with HK2 indicating that the mechanism of depletion is via apoptosis, and is more significant in HIV-1 infected CD4+T cells. HIV-1 infected CD4+ ive T cells express higher level of PD-1, suggesting involvement of PD-1: PD-L1 pathway. Pretreatment of HK-2 cells with anti-PD-L1 antibody significantly reduced apoptosis on CD4 T cells. HK2 incubated with infected CD4 +ve cells also showed expression of HIV-1 proteins. Altogether, our results indicate that tubular cells may preferentially deplete CD4+ ive T cells especially those infected with HIV-1. Moreover, there may be bidirectional traffic of HIV-1 during interaction of tubular cells and CD4+ ive T cells.

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TH-PO714

Discrete Regulation of VEGF-A and -C in Human Proximal Renal Tubular Cells under Hypoxic and/or Inflammatory Conditions – Inhibitory Effects of Glucocorticoids on Basal and Inducible VEGF Expression Hideki Kimura,[1] Hidehiro Sugimoto,[2] Kazuko Kamiyama,[1] Daisuke Mikami,[1] Kenji Kasuno,[1] Naoki Takahashi,[1] Haruyoshi Yoshida.[1] *[1]Div of Nephrol, Dept of General Med, Fukui Univ, Fukui, Japan; [2]Dept of Clin Lab, Fukui Univ Hosp, Fukui, Japan.*

VEGF-A and C are a main inducer of angiogenesis and lymphangiogenesis which are closely involved in renal fibrosis status after renal injury. Although proximal renal tubular cells produce the two VEGFs and probably affect reconstitution of interstitial vessel networks in the injured kidney, the production alteration and its mechanism have not been fully clarified in the renal cells under hypoxia and/or inflammation and on glucocorticoid (GC) treatments as an essential remedy for severe nephritis.

Confluent human proximal renal tubular epithelial cells (HPTECs) were treated with TGF-β (1-5 ng/ml), TNF-α (10 ng/ml), high glucose (HG; 450mg/dl) and/or GC (0.1-1 μM) for up to 48 h under normoxic or hypoxic conditions. TGF-β and hypoxia induced VEGF-A production (2-3 fold and 1.5 fold) via p38 MAPK but not ERK pathway and Src family tyrosine kinase pathway, respectively, while TNF-α and HG had no influence on the production. In contrast, TNF-α and HG increased VEGF-C production (3 fold and 1.3 fold), while TGF-β and hypoxia did not change the production. Induction of VEGF-C by TNF-α was mediated largely via p38 MAPK pathway but not ERK or Src family pathway. Dexamethasone (DXA) did not affect hypoxia-inducible factor-1α amount and activity under hypoxia, nor did hypoxia influence on DXA-activated GC response element activity. Hydrocortisone (HC) and DXA down-regulated basal VEGF-A and -C production, independently and dependently of the GC receptor, respectively, without VEGF mRNA destabilization. Finally, GC also decreased hypoxia-induced VEGF-A production and TNF-α-induced VEGF-C production.

Theses results suggest that VEGF-A and -C are discretely regulated by hypoxia, inflammatory cytokines, and HG in HPTECs and that GCs suppress hypoxia- or inflammation–stimulated expression of the two factors, potentially leading in part to impairment of orderly neovascularization and ensuing tissue restoration after renal injury.

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TH-PO715

Experimental Sepsis in Tamm-Horsfall Protein-Deficient Mice Hajamohideen S. Raffi,[1] James M. Bates,[1] Zoltan G. Laszik,[2] Satish Kumar.[1] *[1]Medicine, University of Oklahoma HSC and VA Medical Center, Oklahoma City, OK; [2]Pathology, University of California San Francisco, San Francisco, CA.*

Tamm-Horsfall protein (THP) is the most abundant protein in normal urine. In previous studies, we generated THP gene knockout ($THP^{-/-}$) mice by homologous recombination and found them to be more susceptible to experimentally induced urinary tract infection. In this study, we examined the susceptibility of $THP^{-/-}$ mice to systemic sepsis.

Eight $THP^{+/+}$ and $THP^{-/-}$ mice were selected. Baseline urine and blood samples were collected. Sepsis was induced by the cecal ligation and puncture (CLP) method. The mice were euthanized at 24 hrs. The abdomen was opened. Urine was collected by needle aspiration from the bladder and blood by cardiac puncture. Bacteremia and bacteriuria were assessed by blood and urine cultures. Colony forming units (CFU) were counted and expressed as CFU/ml. Systemic response to sepsis was assessed by total and differential white blood cell count. Acute kidney injury was assessed by renal histology and by neutrophil gelatinase-associated lipocalin (NGAL) levels in plasma and urine. Proliferative activity in renal tubules was evaluated by quantitation of proliferating cell nuclear antigen (PCNA) expression. Renal function was assessed by serum cystatin C.

Plasma NGAL levels were lower ($THP^{+/+}$, 336 ng/ml ± 56 vs. $THP^{-/-}$, 171 ng/ml ± 23 p = 0.008) and serum cystatin C levels were higher ($THP^{+/+}$, 569 ng/ml ± 37 vs. $THP^{-/-}$, 667 ng/ml ± 36, p = 0.040) in $THP^{-/-}$ mice at baseline but similar between the two groups 24 hours after CLP. Plasma NGAL levels rose after CLP in both groups but the increase was higher in $THP^{-/-}$ mice ($THP^{+/+}$, 66 ± 93 vs. $THP^{-/-}$, 335 ± 109 p = 0.041). No significant difference were found between $THP^{-/-}$ and $THP^{+/+}$ mice in bacteremia, bacteriuria, blood white cell counts, renal histology, PCNA indices, and urine NGAL levels.

We conclude that absence of THP has minimal effect on the response to sepsis in mice.

Disclosure of Financial Relationships: nothing to disclose

TH-PO716

The Role of IL-6 in Neutrophil Recruitment to Glomerular Endothelial and Epithelial Co-Cultures, a Model of the Glomerular Micro-Environment Sahithi Josna Panchagnula,[1] Simon C. Satchell,[3] Moin Saleem,[3] Lorraine Harper,[1] Helen M. Mcgettrick,[2] Edward Rainger,[2] Samantha Tull,[1] Julie M. Williams,[1] Caroline O. S. Savage.[1] *[1]School of Immunity, Infection and Inflammation, University of Birmingham, Birmingham, United Kingdom; [2]School of Clinical and Experimental Medicine, University of Birmingham, Birmingham, United Kingdom; [3]Academic Renal Unit, Univeristy of Bristol, Bristol, United Kingdom.*

Background: We have examined the hypothesis that glomerular epithelial cells (GEpCs) co-operate with glomerular endothelial cells (GEnCs) to modulate neutrophil recruitment in a simulated inflammatory environment and have investigated the mechanisms involved in the demonstrated cross-talk.

Methods: An *in vitro* static co-culture system was used to study neutrophil recruitment by endothelium in the presence of GEpCs following cytokine stimulation (0-100U/ml TNF-α for 4h). The conditionally immortalized human GEnCs and GEpCs were cultured on opposite sides of 3.0 μm pore transwell filters in double chamber wells allowing cell-cell interactions. Neutrophils were added to the upper chamber in contact with the GEnCs. Soluble mediators released into the cell supernatants were analysed by multiplex assay. Anti–IL-6 monoclonal antibody (5μg/ml, R&D systems) was used to block IL-6 function.

Results: I*n vitro* static co-cultures of TNF-treated GEnCs/GEpCs demonstrated reduced neutrophil recruitment by up to 40 % compared to monocultures of GEnCs. The reduced neutrophil recruitment was dependent on both cell-cell contact and on soluble mediator(s) release. Supernatants from co-cultures showed an 8-fold increase in soluble IL-6 concentrations compared to monocultures following analysis by multiplex assay. Function-neutralising anti–IL-6 antibody added from the initiation of co-culture, reconstituted the neutrophil recruitment to endothelium to control levels.

Conclusions: IL-6 can down regulate neutrophil recruitment during GEnC/GEpC co-culture indicating that it plays a negative role in local inflammatory reactions. Thus, IL-6 may be crucial in neutrophil recruitment during pathogenesis of neutrophil-mediated glomerulonephritic disease.

Disclosure of Financial Relationships: nothing to disclose

TH-PO717

Targeting Keap1-Nrf2 Pathway Ameliorates Renal Inflammation and Fibrosis in Mice with Protein-Overload Proteinuria Carlamaria Zoja,[1] Daniela Corna,[1] Monica Locatelli,[1] Chiara Corna,[1] Sara Cattaneo,[1] Colin Meyer,[3] Giuseppe Remuzzi,[1, 2] Ariela Benigni.[1] *[1]Mario Negri Institute, Bergamo, Italy; [2]Ospedali Riuniti, Bergamo, Italy; [3]Reata Pharmaceuticals, Irving, TX.*

Bardoxolone methyl (RTA 402), a semi-synthetic triterpenoid, is an Antioxidant Inflammation Modulator (AIM) in clinical development for chronic kidney disease. It exerts antioxidant and antiinflammatory activity via activation of the Keap1-Nrf2 pathway, which in turn suppresses NF-kB activity. We previously showed that in experimental proteinuric nephropathies, proteinuria increases NF-kB activity. By regulating the transcription of genes encoding proinflammatory and fibrogenic molecules involved in renal injury, NF-kB is a key determinant of proteinuria-induced tubulointerstitial injury. Here we investigated the effect of a bardoxolone methyl analog RTA 405 in a murine model of protein-overload proteinuria characterized by tubulointerstitial inflammation and fibrosis. Mice (n=20) underwent uninephrectomy. Five days later they received daily i.p. injections of BSA up to 28d and were treated with vehicle or RTA 405 (100mg/kg/d in the chow). In vehicle-mice proteinuria increased within 2d after BSA, peaked at 14d (77±17 mg/d) and remained sustained at 28d (55±16 mg/d). RTA 405 significantly limited proteinuria at 14d (38±10 mg/d, P<0.05) and maintained it at lower level than vehicle-mice at 28d (41±17 mg/d). The presence of a non-responder mouse in the RTA 405 group precluded achievement of statistical significance at 28d. Interstitial accumulation of monocytes/macrophages was lowered by therapy (47±8 vs 70±8 cells/HPF, P<0.01). The antiinflammatory action of RTA 405 was accompanied by an antifibrogenic effect as indicated by significantly reduced peritubular α-SMA staining in RTA 405 compared to vehicle-treated mice (score:1.6±0.1 vs 2.2±0.1). In summary, bardoxolone methyl analog limited proteinuria, interstitial inflammation, and fibrosis in a model of proteinuric nephropathy. Studies are ongoing to assess the mediators of inflammation responsible for the protective effects. These data pave the way for clinical application of AIMs as antiinflammatory agents in progressive nephropathies.

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