PILR deficient neutrophils showed enhanced adhesion and spreading on IC compared to WT. IC adhesion-dependent H2O2 production of neutrophils was also increased in PILR -/- mice compared to WT animals.

Conclusions: PILR deficiency resulted in deteriorated renal damage in murine antibody-mediated glomerulonephritis compare to WT mice. The present study indicated that PILR negatively regulates antibody-mediated leukocyte recruitment by inhibition of m 2 integrin activation.

P33

IMMUNOSUPPRESSIVE TREATMENT ALTERS SECRETION OF ILEAL ANTIMICROBIAL PEPTIDES AND GUT MICROBIOTA, AND FAVORS SUBSEQUENT COLONIZATION BY UROPATHOGENIC ESCHERICHIA COLI

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Introduction: The immune system controls the gut microbiota. Transplant recipients are treated with immunosuppressive (IS) therapies which could impact host-microbial interactions. We examined the impact of IS drugs on gut microbiota and on the secretion of ileal antimicrobial peptides.

Methods: Mice were treated for 14 days with prednisolone, mycophenolate mofetil, tacrolimus, a combination of these 3 drugs, everolimus or water. Faeces were collected before and after treatment initiation. Ileal samples were collected after sacrifice. Faecal and ileal microbiota was analyzed by pyrosequencing of 16S rRNA genes, and C-type lectins were assessed in ileal tissues by RT-qPCR.

Results: Prednisolone decreased Bacteroidetes and increased Firmicutes in the faeces. While prednisolone disrupted faecal microbial community structure, no single OTU was consistently affected in experimental replicates. In ileal samples, the genus Clostridia sensu stricto was dramatically reduced in the prednisolone and combined IS drug groups. These modifications corresponded to an altered expression of C-type lectins, Reg3 and Reg3. Interestingly, the combined IS treatment enabled a commensal Escherichia coli to flourish, and dramatically increased colonization by uropathogenic E. coli strain 536.

Conclusions: IS treatment alters innate antimicrobial defenses and disrupts the gut microbiota which leads to overgrowth of indigenous E. coli and facilitates colonization by opportunistic pathogens.

P34

DUAL BLOCKADE OF THE HOMEOSTATIC CHEMOKINE CXCL12 AND THE PRO-INFLAMMATORY CHEMOKINE CCL2 IS AS EFFECTIVE AS CYCLOPHOSPHAMIDE IN PROLIFERATIVE LUPUS NEPHRITIS

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Introduction: Induction therapy of proliferative lupus nephritis still requires the use of unselective immunosuppressive drugs with significant toxicities. More specific drugs with equal efficacy but fewer side effects are needed. In search of suitable molecular targets we considered monocyte chemoattractant protein (MCP-1/CCL2) and stromal cell-derived factor (SDF-1/CXCL12), which both contribute to the onset and progression of proliferative lupus nephritis yet through different mechanisms. We hypothesized that dual antagonism of the homeostatic chemokine CXCL12 and the pro-inflammatory chemokine CCL2 could be as potent on lupus nephritis as the unselective and toxic immunosuppressant cyclophosphamide (CYC).

Methods: We used l-enantiomeric RNA Spiegelmer chemokine antagonists, i.e. the CCL2-specific mNOX-E36 and the CXCL12-specific NOX-A12. Female MRLlpr/lpr mice were treated (subcutaneous injection) from week 12 to 24 of age of age either with single regimen of anti-CXCL12 (13.4mg/kg) or anti-CCL2 (14.4 mg/kg) or both along with standard regimen with cyclophosphamide (CYC) (30mg/kg).

Tissues were harvested for histopathological evaluation at the end of the treatment period. Blood and urine samples were obtained at monthly intervals for the estimations of urinary albumin (ELISA: Bethyl Labs, Montgomery, TX, USA) as well as serum and urinary creatinine (Jaffé reaction: DiaSys Diagnostic Systems, Holzheim, Germany). Inflammatory gene profile was determined by RTPCR. All experiments were performed according to German animal protection laws and had been approved by the local government authorities.

Results: Dual blockade was significantly more effective than monotherapy in preventing proteinuria and BUN. Dual blockade was also more effective in controlling the histopathological indices of disease activity and chronicity. Dual blockade reduced IgG immunoglobulins, CD3+ lymphocytes and macrophages more efficiently than monotherapy. Dual blockade also reduced renal IL-6, IL-12p40, CCL5, CCL-2 and CCR2 mRNA expression. Effects of dual blockade on kidney functional parameters are at par with CYC standard regimen.

Conclusions: Dual blockade of CCL2 and CXCL12 can be as potent as CYC to suppress the progression of proliferative lupus nephritis in female MRLlpr/lpr mice probably because the respective chemokine targets mediate different disease pathomechanisms, i.e. systemic autoimmunity and peripheral tissue inflammation.

P35

INCREASED RECRUITMENT OF HUMAN LYMPHOCYTE SUBSETS IN RENAL FIBROSIS AND CHRONIC KIDNEY DISESASE

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Introduction: Lymphocytes occupy pivotal roles in immune-mediated kidney diseases. However the respective contributions of different lymphocyte subsets in diseased human kidneys are not certain, with previous studies limited by the methodology of immunohistochemistry to identify infiltrating cells.

Methods: We developed novel protocols for extracting renal lymphocytes from healthy kidney tissue and diseased biopsies with and without fibrosis. Lymphocyte subsets were identified, enumerated and phenotyped by twelve-colour flow cytometry.

Results: We detected significantly elevated numbers of T cells (CD45+CD3+) in diseased biopsies with interstitial fibrosis compared with healthy kidney tissue. Within this T cell compartment, numbers of T helper cells (CD3+CD4+) and cytotoxic T cells (CD3+CD8+) were elevated in fibrotic kidney tissue. Moreover, numbers of / T cells (CD3+ / +) and natural killer (NK)-T cells (CD3+CD16+) were significantly increased in fibrotic biopsies compared with diseased biopsies without fibrosis and healthy kidney tissue. Of CD3- lymphoid cells, NK cells (CD3-CD56+) were elevated in fibrotic kidney tissue, in particular CD56brightCD16-/+ NK cells, the major cytokine-producing NK subtype in human peripheral tissues and secondary lymphoid organs. B cells (CD3-CD19+) were also increased in fibrotic kidney tissue. Additionally, numbers of / T cells, NK-T, NK and B cells correlated with loss of kidney function (based on eGFR levels). Expression of activation molecule CD69 on renal lymphocytes was increased in fibrotic biopsies compared with healthy kidney tissue, indicative of a pathogenic phenotype.

Conclusions: Collectively, our data show that lymphocyte subsets are differentially recruited into diseased human kidneys. The representation of specific lymphocyte subsets also correlates with the clinical severity of chronic kidney disease. Further identification and functional dissection of these lymphocyte subsets will enable the development of targeted treatment strategies.

P36

CATHEPSIN-S INHIBITION HAS A DUAL THERAPEUTIC EFFECT ON THE SYSTEMIC AND PERIPHERAL PATHOMECHANISMS OF LUPUS NEPHRITIS

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Introduction: The lysosomal cysteine protease cathepsin (Cat)-S processes the invariant chain-MHC II complex inside antigen-presenting cells as a central pathomechanism of autoimmune diseases. In addition, activated myeloid cells release Cat-S and was recently described to activate protease-activated receptor (PAR)-2 in extracellular compartments (Kumar S, et al JASN 2015). We hypothesized that in lupus nephritis Cat-S blockade can target both pathomechanisms and can elicit synergistic therapeutic effects disease outcomes.

Methods: Female MRL-Fas(lpr) mice with spontaneous autoimmune tissue injury were treated with different doses of the oral Cat-S antagonist RO5459072 or mycophenolate mofetil (MMF) or vehicle from week 11 or 15 to 19 of age. To evaluate the Cat-S induced micro-vascular damage, female MRL-Fas(lpr) mice were injected with recombinant Cat-S with or without concomintant Cat-S or PAR2 blockade and urine albumin levels were measured at different time intervals. In-vitro studies with PAR2 expressing endothelial cells were used to confirm the Cat-S induced endothelial activation and dysfunction.

Results: Cat-S blockade dose-dependently protected aberrant systemic autoimmunity, by reducing the plasma cytokine levels, activation myeloid cells and lymphocytes, and hypergammaglobulinemia. Especially IgG auto-antibodies were suppressed. Of note while (MHC-II-independent) IgM antibodies were not affected by Cat-S blockade but strongly suppressed by MMF. Cat-S blockade dose-dependently suppressed immune complex glomerulonephritis together with a profound and early effect on proteinuria, which was not shared by MMF. In fact, intravenous Cat-S injection induced severe glomerular endothelial cell injury and albuminuria, which was entirely prevented by Cat-S or PAR2 blockade. In-vitro studies confirm that Cat-S induces endothelial cell activation and injury via PAR-2. PAR2 silencing in endothelial cells using siRNA significantly protected the endothelial injury in-vitro.

Conclusions: Therapeutic Cat-S blockade suppresses synergistic systemic and peripheral pathomechanisms of autoimmune tissue injury, hence, Cat-S is a promising therapeutic in autoimmune diseases.

P37

ASSESING THE ROLE OF B7-1 IN DIABETIC NEPHROPATHY

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Introduction: Diabetic Nephropathy (DN) remains an unmet medical challenge as its prevalence is projected to continue to increase and specific medicines for treatment remain undeveloped. Activation of the immune system, in particular T-cells, is emerging as a possible mechanism underlying DN disease progression in humans and animal models. We hypothesized that inhibition of T-cell activation will ameliorate DN. Interaction of B7-1 (CD80) on the surface of antigen presenting cells with its binding partners, CTLA4 (CD152) and CD28 on T-cells, is essential for T-cell activation. In this study we used a soluble CTLA4-Fc fusion protein to block cell surface B7-1, preventing the cellular interaction and inhibiting T-cell activation.

Methods: The CTLA4-Fc Abatacept was dosed i.p. in the Streptozotocin-induced Diabetic Nephropathy mouse model in both prevention and intervention modes. Renal damage was assessed by albuminuria and renal B7.1 positive cells and lymphocytes were measured by immunohistochemistry. Gene expression interrogated by real-time PCR and protein expression by Western blotting. Differentiated human immortalized podocytes were used for in vitro studies.

Results: When CTLA4-Fc was dosed in an animal model of DN, it reduced albuminuria in both prevention and intervention modes. The number of T-cells infiltrating the kidneys of DN animals correlated with the degree of albuminuria and treatment with CTLA4-Fc reduced the number of renal T-cells. As B7-1 induction has been recently proposed to underlie podocyte damage in DN, CLTA4-Fc could be efficacious in DN by protecting podocytes. However, this does not appear to be the case as B7-1 was not expressed in: 1) kidneys of DN animals; 2) stimulated human podocytes in culture; or 3) glomeruli of DN patients.

Conclusions: We conclude that CTLA4-Fc ameliorates DN by blocking T-cell activation and not by protecting podocytes.