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

Kumar VR, S1, Tato, M1, Liu, Y1, Mulay R, S1, Gruner, S2, Haap, W1, Hartmann, G2, Anders, H1
1Ludwig-Maximilians-Universität München, Nephrologische Zentrum, Munich, Germany; 2Hoffmann La Roche, CV & Metabolism DTA, Basel, Switzerland

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 ROS5459072 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 concomitant 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

ASSESSING THE ROLE OF B7-1 IN DIABETIC NEPHROPATHY

Herrera, M1, Soderberg, M2, Haesman, S1, Baker, D1, Moreno, C1
1MedImmune, Cardiovascular and Metabolic Diseases, Cambridge, United Kingdom; 2AstraZeneca, Drug Safety and Metabolism, Gothenburg, Sweden; 3MedImmune, Pathology, Cambridge, United Kingdom

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 Strepzotocin-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, CTLA4-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.