correlated with the reduction of tubular injury (PAS, Kim-1), renal inflammation (Macrophages, T cells, IL-6, TNF-, RANTES and CCL2) as well as renal fibrosis (SMA, Col1a1, Masson Trichrome).
Conclusions: Therefore, we conclude that BHB ameliorates acute oxalate nephropathy, as well as delays progressive renal failure by inhibiting NLRP3-mediated inflammation. Our findings suggest that ketogenic diets or -hydroxybutyrate formulations can be used therapeutically for the treatment of primary hyperoxaluria related kidney stone disease.

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A NOVEL METHOD FOR HIGH-THROUGHPUT DETECTION AND QUANTIFICATION OF NEUTROPHIL EXTRACELLULAR TRAPS REVEALS ROS-INDEPENDENT NET RELEASE WITH IMMUNE COMPLEXES

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Introduction: A newly-described 1st-line immune defence mechanisms of neutrophils is the extrusion of DNA, called neutrophil extracellular traps (NETs). Known non-infectious triggers of NETosis are phorbol 12-myristate 13-acetate (PMA), IL-8 and immune complexes (ICx). In vitro, PMA and IL-8 are strong inducers of NET release and generally involves all cultured neutrophils. However, ICx induce much lower numbers of neutrophils to release NETs. As such, the in vitro quantification of NETs is challenging with current methodologies (i.e. Picogreen or immunofluorescence). In order to investigate the role of NET release in ICx-mediated autoimmune diseases, we developed a novel method for automated and highly sensitive measurement of NETs, applicable in a high-throughput system.
Methods: Healthy neutrophils are isolated from fresh blood and labelled with the membrane staining PKH26, which serves as a cell marker. Neutrophils are stimulated for 4 hours with various concentrations of heat-aggregated human IgG ICx. After 3¾ hours, Sytox green is added to stain extracellular DNA. Sytox green is incubated for 15 minutes, after which the cells are fixed with 4% PFA. Within 24 hours, the 96-well plate is automatically imaged with the BD Pathway 855. The ratio of the sum of Sytox green area and average PKH area is calculated, representing the amount of NETs in each sample. A higher ratio indicates a higher amount of extracellular DNA present.
Results: Immunofluorescence analysis of ICx-stimulated neutrophils revealed colocalization of extracellular DNA staining with citrullinated histon3 and neutrophil elastase, confirming the release of NETs. The amount of NET release was quantified with ImageJ software, by determining the ratio between positive Sytox green area and the positive PKH26 area, of which we found that it accurately represents the amount of cells imaged. Sensitivity is highly increased since 11% of the total area per well is imaged and a z-stacking system is used to image NETs superimposed on neutrophils. We further characterized NET release upon stimulation with these ICx. When pre-treated with diphenyleneiodonium (DPI), used to block production of reactive oxygen species (ROS), NET release upon ICx stimulation was not affected.
Conclusions: We developed a novel assay that is highly sensitive to detect and quantify in vitro NET release. NET release triggered by human ICx was detected reliably and appeared to be a ROS-independent process. This assay will allow high-throughput and highly sensitive analysis of NET release in ICx-mediated autoimmune diseases (e.g. systemic lupus erythematosus, rheumafactor positive rheumatoid arthritis or cryoglobulinemic vasculitis).

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IL-33 ELICITS INNATE LYMPHOID CELLS AND ALTERNATIVELY ACTIVATED MACROPHAGES THAT REDUCE RENAL ISCHEMIC-REPERFUSION INJURY

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Introduction: IL-33 is an important immune regulator which can promote Th2 immune response-dependent immunity, inflammation, and tissue repair in several important immune-mediated disorders. In the current study, we sought to determine whether IL-33 is an important regulator in renal ischaemic/reperfusion injury (IRI).
Methods: Bilateral renal ischemia was imposed in C57BL/6 mice under isofluorane anesthesia. IL-33 was given by 5 consecutive daily injections starting at day 5 before IRI operation. Adoptive transfer of type 2 innate lymphoid cells into mice with IRI was used to assess their in vivo functions.
Results: Treating IRI mice with IL-33 significantly improved renal function and reduced renal injury. The possible mechanisms underlying the protective effect of IL-33 were examined. Firstly, IL-33 increased the levels of IL-4, IL-5 and IL-13 in serum and kidney and promoted induction of alternatively activated (M2) macrophages in kidney. Moreover, the number of NK cells and neutrophils was significantly reduced in IRI mice treated with IL-33. A novel finding of this study was that IL-33 increased the frequency of type 2 innate lymphoid cells (ILC2) and regulatory T cells (Tregs) in kidney. However, adoptive transfer of ILC2 not only reduced renal functional and histological injury in IRI mice but also induced M2 macrophages in kidney.
Conclusions: In conclusion, our data identify a mechanism whereby IL-33 eliciting ILC2 and Tregs regulate macrophage phenotype in kidney and prevent renal IRI.

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RORÎ³t+ FOXP3+ BITREGS PROMOTE LUPUS NEPHRITIS VIA IL-17 SECRETION AND SUPPRESSION OF TH2 RESPONSES

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Introduction: We have recently characterized Foxp3+ regulatory T cells, co-expressing the Th17 characteristic transcription factor ROR t, as an independent and bi-functional T cell lineage (biTregs). biTregs secrete both, anti-inflammatory (IL-10, IL-35), but also pro-inflammatory (IL-17) cytokines. Studies in a model of acute crescentic glomerulonephritis have suggested that pro- inflammatory biTreg functions are largely mediated by ROR t. This is of high clinical relevance, since multiple ROR t blocking agents are