Pattern recognition receptors, sensing re(n)al danger
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Summary

The kidney serves an important role for maintenance of body homeostasis through filtration of blood. Kidney diseases are a major clinical problem associated with a high mortality and therapy still remains generally non-specific or supportive that emphasizes the need to get more insight into the underlying pathophysiological mechanisms which leads to renal diseases. Inflammation is a hallmark of various forms of renal injury, irrespective of the primary nature of the underlying disease, and is an important factor that contributes to the development of many kidney diseases. The current concept comprises that inflammation can be initiated through receptors of the innate immune system, including TLRs and NLRs that are activated by several endogenous danger molecules once released upon tissue or cellular injury. In this thesis we aimed to identify the role of different immune receptors in the pathogenesis of acute kidney injury (AKI) and chronic kidney disease (CKD) by using experimental models of ischemia/reperfusion (I/R) injury and unilateral ureter obstruction (UUO), respectively.

In chapter 2 we investigated the role of TLR4 and the relative contribution of its two intracellular downstream signaling cascades in the pathophysiology of AKI by subjecting TLR4−/−, MyD88−/− and TRIF-deficient mice to renal I/R injury. Our results demonstrated that TLR4 induces an exaggerated and detrimental inflammatory response following I/R injury that negatively affects renal function and morphology. Moreover, bone-marrow chimeric mice and in vitro studies using primary cultured tubular epithelial cells demonstrated an important role for renal-associated TLR4 in the underlying pathology. No preferential usage was observed for either the MyD88- or TRIF-dependent pathways downstream of TLR4 following I/R injury. Together, these data suggest that selective targeting of TLR4 could be beneficial to prevent or reduce an exaggerated inflammatory response following I/R injury that may preserve renal injury and renal function.

In chapter 3 we investigated the role of the intracellular NLRP3-inflammasome in monitoring subtypes of necrotic cells and inducing an effective inflammatory response. We demonstrated that different forms of necrotic cells, including necrotic cells due to hypoxia, trigger NLRP3-inflammasome-mediated cytokine release. The effects observed were in part mediated through the mitochondrial subfraction of necrotic cells, in particular through ATP produced by actively respiring mitochondria. Moreover, in vivo we demonstrated that absence of the NLRP3-inflammasome protected mice against mortality, renal dysfunction and inflammation upon acute tubular necrosis, suggesting that inhibition of inflammasome activity could diminish acute inflammation and damage associated with (renal) tissue injury.
In chapter 4 we investigated the role of the kidney in sensing mitochondria and eliciting inflammatory responses that may lead to renal injury. Mitochondria are regarded as an intracellular pool of DAMPs that may induce inflammation. Indeed, we observed that primary cultured tubular epithelial cells do functionally respond to necrotic cells that is partially dependent on mitochondria. We observed that mice that were subjected to sterile renal injury display enhanced levels of circulating mitochondrial DNA (mtDNA). Systemic administration of purified mtDNA induced renal inflammation but did not lead to renal dysfunction. Moreover, critically ill patients with SIRS demonstrated elevated levels of plasma and urinary mtDNA and proinflammatory cytokines compared to control ICU-patients. The presence of AKI did however not further statistically affect the levels of circulating or urinary mtDNA in SIRS patients. These data demonstrate that the kidney recognizes mitochondrial-derived ligands in order to induce inflammation. In addition, AKI is associated with high circulating mitochondrial-derived DAMPs, corroborating with previous findings that mitochondria may form a link between tissue injury and inflammation. We further suggest that the kidney may actively contribute to SIRS. The presence and increase in levels of plasma or urinary mtDNA may predict the development of systemic inflammation and/or acute kidney injury.

The nuclear protein HMGB1 is passively released by necrotic cells and may act as danger molecule to induce inflammatory responses via activation of innate immune receptors, including RAGE. In chapter 5 we investigated the therapeutic potential of interfering in the HMGB1-RAGE axis and neutralizing HMGB1 following renal I/R injury. Our results clearly demonstrate that inhibiting HMGB1 with an anti-HMGB1 antibody is beneficial in the acute phase upon ischemic injury, as reflected by a preserved renal function, diminished renal injury and a reduced inflammatory response. On the contrary however, RAGE-deficiency did not lead to a preserved renal function or reduction in the levels of renal injury, and as such RAGE-/- mice displayed a comparable phenotype as wild type mice. These results provided evidence that the endogenous danger ligand HMGB1 is released following I/R injury and subsequently contributes to renal inflammation and injury. These effects appear to be mediated through RAGE-independent mechanisms.

In chapter 6 we investigated the role of the endogenous calcium-binding proteins S100A8 and S100A9 during acute kidney injury, as they are described as DAMPs which are elevated in ischemic tissue and hence may induce a, particular TLR4-mediated, exaggerated proinflammatory response. Our results show that S100A8 and S100A9 are expressed on renal tubular epithelial cells and that S100A8/A9 induces a TLR4-dependent proinflammatory response on these cells. Early after renal
I/R injury, total renal S100A8/A9 levels are markedly elevated, but do not contribute to I/R-induced injury. Interestingly, we observed that absence of S100A8/A9 results in sustained renal dysfunction, damage and a fibrotic and chronic inflammatory response later on during repair phase after I/R injury. Moreover, a sustained polarization towards M2-macrophages was observed in the S100A9 deficient mice. Extracellular S100A8/A9 merely induced M1-type macrophage activation, whereas macrophages from S100A9 deficient mice displayed a hyper activation of merely M2-type macrophages. These data implicate that intracellular and extracellular S100A8 and/or S100A9 differentially control intrinsic macrophage activation and polarization. These processes might affect the inflammatory response occurring upon renal damage to ascertain renal repair and/or result into sustained injury.

Progression of renal injury involves a complex cascade of pathophysiological processes, like the accumulation of macrophages and myofibroblasts, renal scarring and tubular atrophy. To investigate whether innate immune receptors are involved in the underlying pathophysiology, in chapter 7 TLR4-/- mice were subjected to the model of UUO that mimics the complex processes following progressive renal injury. Interestingly, our study demonstrated that TLR4 attenuates the level of tubular injury but promotes renal fibrosis upon UUO. TLR4 deficiency was associated with enhanced renal levels of Bambi, a negative regulator of TGFβ signaling, although levels of TGFβ itself were unaffected. Following TGFβ stimulation, we observed that both primary renal TECs and myofibroblasts display a profibrotic response in a TLR4-dependent manner, associated with enhanced Bambi levels in the TLR4 deficient primary cells. Together, the data of this study suggested that TLR4 modulates the susceptibility of renal cells towards TGFβ, and hence might be a therapeutic target for prevention of renal fibrosis.

In chapter 8 we investigated the contribution of the NLRP3-inflammasome upon progressive renal injury by subjecting NLRP3-/- mice to the experimental model of UUO. Our data revealed that NLRP3 protects against the development of tubular injury and attenuates the level of interstitial edema. This might be explained by increased intratubular pressure and an enhanced tubular and vascular permeability due to reduced expression of intercellular junction components observed in NLRP3-/- mice. The decreased epithelial barrier function in NLRP3-/- mice was not associated with increased apoptosis and/or proliferation of renal epithelial cells. In addition, we observed that NLRP3 did not play a role in renal fibrosis and only minor in renal inflammation upon UUO. Together, our data reveal an important role for the NLRP3-inflammasome in preserving renal integrity and protection against tubular injury in the early phase upon progressive renal injury.