Mechanisms of renal injury and repair: role of stem cells, chemokines and the nodosome
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Summary

The kidney serves important regulatory roles. Most well known is the excretion of excessive fluid and waste products. However, the main function of the kidney is the maintenance of the organism’s homeostatic balance by regulating, among other things, the electrolyte balance, blood pressure, and production of several hormones. Loss of kidney function leads to serious illness, affecting many aspects of well-being. Despite progress made in health care, acute and chronic renal failure is still a major clinical problem. The mechanisms involved in renal injury and repair are not yet fully understood. Therefore, more insight into these mechanisms might result in new leads for novel treatment options. In this thesis, both acute and chronic renal injury is studied by using experimental mouse models. Renal ischemia/reperfusion (I/R) injury is used as a sterile model for acute kidney injury (AKI), mimicking the events taking place during e.g. shock, vascular surgery, and renal transplantation when there is a temporal reduction in blood flow to the kidney. In addition, mouse models of sepsis-induced AKI and obstructive nephropathy are used to study the role of the nodosome in renal injury.

Following renal injury, damaged tubular epithelial cells (TECs) are lost and kidney function is declined. If injury is too severe or is long-lasting, repair is no longer possible. However, if the injury is mild, damaged TECs can be replaced either via dedifferentiation of viable TEC or via the incorporation of stem cells. In chapter 2 we show that hematopoietic stem cells (HSCs) preferentially migrate towards the ischemic damaged kidney. As the number of stem cells that incorporate into the damaged kidney are most likely too low to make a functional contribution, we investigated whether manipulation of the SDF-1/CXCR4-axis might influence HSC migration. SDF-1 belongs to a family of proteins called chemokines, which are chemotactic cytokines involved in the trafficking of leukocytes towards sites of injury. The interaction between SDF-1 and stem cell-associated CXCR4 has shown to be the central signalling axis involved in HSC migration to various tissue including bone marrow and liver. However, we could not alter HSC migration towards the ischemic damaged kidney by manipulating SDF-1/CXCR4-axis using 3 different approaches. The chemokine SDF-1 does not seem crucially involved in HSC migration to the damaged kidney. However, we did observe significant enhanced SDF-1 protein following renal I/R injury. To investigate the role of SDF-1 in renal I/R injury, we depleted renal SDF-1 protein using antisense oligonucleotides and determined the renal response in the post-ischemic kidney (chapter 3). In line with previous results, we did not observe an effect of SDF-1 depletion on HSC migration. However, SDF-1 depletion severely induced renal damage and dysfunction which was accompanied with increased inflammatory response. Several reports have shown that SDF-1 can suppress apoptosis in various cell types. Indeed, we found remarkably increased TEC apoptosis in post-ischemic kidney upon SDF-1 depletion. Together, these results
argue for a role of SDF-1 in survival of TEC rather than a role in the migration of HSC following renal I/R injury.

In addition to SDF-1, several other chemokines might play a role in the pathogenesis of renal I/R injury. In chapter 4 we performed microarray analysis to determine the temporal expression of chemokines and chemokine receptors in the post-ischemic kidney. We expected the highest expression of chemokines during the initial inflammatory response when influx of granulocytes is highest. However, the majority of upregulated chemokines had the highest expression during the reparative phase, suggesting that they play a major role in the resolution of ischemic injury. Moreover, a biphasic expression, coinciding with the acute inflammatory and the latter reparative phase, of CXC chemokines was observed. The expression profiles of chemokines following renal I/R injury provide an important resource for exploring new therapeutic target. We propose a pleiotropic role for chemokines during the different phases after an ischemic insult in the kidney.

One of the upregulated chemokines, MCP-1, was chosen in order to investigate its role during renal I/R injury in more detail. MCP-1 is known as the main chemoattractant for monocytes to the ischemic injured kidney. Additionally few reports describe other properties for MCP-1 including a role in cell survival. In chapter 5 we observed a peak in MCP-1 protein several days following renal I/R injury, coinciding with macrophage accumulation. However, already at day 1 there was a significant elevated MCP-1 protein level in the post-ischemic kidney. We saw a remarkable effect of MCP-1 deficiency on survival following renal I/R injury; 45% of the MCP-1 deficient mice died within 2 days while in wildtype (WT) mice the induced injury did not result in significant lethality. Next we focused on the events taking place at day 1 following renal I/R injury. Increased renal damage markers and TEC apoptosis were observed in post-ischemic kidneys of MCP-1 deficient mice. In an in vitro experiment we could confirm increased apoptosis of MCP-1 deficient TEC. In addition, we observed an effect on the inflammatory response in the post-ischemic kidney. Although macrophage accumulation was comparable between WT and MCP-1 deficient mice, there seemed to be a shift in macrophage polarization towards the more pro-inflammatory (M1) macrophage in MCP-1 deficient mice. Our results indicate that MCP-1 has versatile functions during different phases of renal I/R injury.

Altogether, we have shown that chemokines are important players during renal I/R injury. Moreover, their role goes beyond the classical view that they are involved in the migration of leukocytes towards injured tissue. We have shown that SDF-1 and MCP-1 have direct protective effects on TEC, and MCP-1 may also be involved in macrophage polarization.

In the past years the role of microbiota in shaping the immune system has gained a lot of attention. Next to a local effect, intestinal microbiota also has systemic immunomodulatory effects. Recently, it was shown that peptidoglycan (PGN), which
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is constantly turned over from the microbiota and translocates into the circulation, can prime granulocytes. During sterile injury such as renal I/R, granulocytes which enter the damaged tissue may exacerbate injury by collateral damage due to the release of mediators and enzymes that potentially damage the surrounding tissue. We therefore hypothesized that the intestinal microbiota play a detrimental role during renal I/R injury. Indeed, in chapter 6 we show that depletion of intestinal microbiota by broad-spectrum antibiotic treatment significantly attenuated renal damage and preserved kidney function in WT mice which was accompanied by significantly reduced granulocyte influx. We could not detect bacterial translocation and intestinal integrity did not seem affected during renal ischemia. These results imply that the microbiota do not seem to play a direct role during renal I/R injury, but rather exert immunomodulatory effects which might be regulated via translocation of microbial products (e.g. PGN) from the gut into the circulation. To determine whether the immunomodulatory effects are regulated via NOD1 and NOD2, both receptors for PGN, we subjected mice deficient for both NOD1 and NOD2 (NOD1/2 DKO) with intact or depleted microbiota to renal I/R injury. Comparable to WT mice, antibiotic-treated NOD1/2 DKO mice had reduced renal damage and granulocyte influx and preserved renal function as compared to control NOD1/2 DKO mice suggesting that the detrimental effects of intact microbiota on renal I/R is not regulated via NOD1/2. Altogether, these results argue for a priming role of the microbiota on the immune system.

In chapter 6 we introduced NOD1/2 DKO mice. In order to distinguish between primary and secondary effects of genetic changes, it is indispensable to thoroughly characterize animal models. In chapter 7 we analyzed these NOD1/2 DKO mice under physiological and systemic inflammatory conditions. NOD1 and NOD2 are members of the “Nucleotide-binding domain and Leucine-rich repeat containing Receptor” (NLR) family. Several inflammatory disorders, such as Crohn’s disease and asthma, are linked to genetic changes in either NOD1 or NOD2, suggesting an important role for NOD1/2 in regulating the immune system. NOD1 and NOD2 contribute in a redundant manner to the immune response, as might be expected by their high structural similarity. Thorough screening of 3-months old NOD1/2 DKO and WT mice revealed possible new roles for NOD1/2 under physiological conditions. Liver weight and plasma levels of glucose, triglyceride and total cholesterol were lower in NOD1/2 DKO mice as compared with WT mice, suggesting a role of NOD1/2 in metabolic processes. In addition, NOD1/2 DKO mice showed an increased susceptibility for intestinal permeability while vascular permeability was not affected. Next we subjected WT and NOD1/2 DKO mice to septic shock and analyzed the systemic and renal inflammatory response. Similar pro-inflammatory cytokines and general organ damage markers were found in plasma of WT and NOD1/2 DKO mice. However, renal pro-inflammatory mediators TNFα and KC and anti-inflammatory cytokine IL-10 were significantly increased in NOD1/2 DKO compared with WT mice.
2 hours following induction of septic shock. Twenty-four hours following induction, kidney function was partly preserved and TEC cell damage was lower in NOD1/2 DKO mice compared with WT mice. Overall, our phenotypic screening revealed a possible role for NOD1/2 in metabolic processes and confirmed a role for NOD1/2 in protecting intestinal integrity. Most parameters of inflammation and organ damage were however not affected by NOD1/2 deficiency under physiological or systemic inflammatory conditions. One striking exception is the renal response upon systemic inflammation: our results clearly indicate a role for NOD1 and/or NOD2 in sepsis-induced acute renal disease.

In chapter 8 we determined whether NOD1/2 is also involved in renal injury and fibrosis during obstructive nephropathy. Tissue fibrosis is a leading cause of morbidity and mortality, and renal fibrosis is regarded as the final common pathway for almost all forms of chronic renal disease. No differences were observed between WT and NOD1/2 DKO obstructed kidneys regarding tubular injury, apoptosis, proliferation, and fibrosis. A marginal effect of NOD1/2 deficiency could be detected in the inflammatory response during obstructive nephropathy. Slightly more MCP-1 and concomitant increased macrophage accumulation was observed in NOD1/2 DKO kidneys 7 days following obstruction, however this did not affect the progression of renal fibrosis. Overall, NOD1 and NOD2 do not make a significant contribution to the development of progressive renal injury and fibrosis during obstructive nephropathy.