Mechanisms of renal injury and repair: role of stem cells, chemokines and the nodosome
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General Discussion
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**Contribution of hematopoietic stem cells to renal repair**

Acute kidney injury (AKI) is still a major clinical problem, with an incidence of 5% in hospitalized patients and a mortality rate of 50-80% in these patients\(^1\). Nowadays, the only supportive treatment option is dialysis, and consequently AKI is a huge burden on our health care expenditures. The kidney has remarkable capacity to regenerate after injury. However, if the damage is long-lasting or too severe repair is no longer possible. Therefore, in the past decade several researchers have focused on stem cells and their capacity to repair tubular epithelium and restore kidney function.

Clinical evidence for the incorporation of bone marrow-derived cells in the kidney was observed in patients that had received a sex-mismatched transplant\(^2-4\), however, this was a rare event and therefore the functional contribution remained uncertain. Strategies to enhance migration of stem cells to the damaged kidney in order to improve its therapeutic potential have been studied extensively. One of the best studied pathways for stem cell migration is the SDF-1/CXCR4-axis, which is thought to be the central signalling axis regulating the trafficking of hematopoietic stem cells (HSC) and progenitor cells. Manipulation of this axis by either blocking SDF-1 or bone marrow-associated CXCR4 or by local administration of recombinant SDF-1 increased homing of stem cells to the liver, spleen, bone marrow, and kidney\(^5-7\).

In contrast, our results show that when using highly purified HSC, manipulation of this axis did not significantly alter the migration of exogenous administered HSC to the ischemic damaged kidney (chapter 2)\(^8\). The main difference between the various migration studies is the origin of the exogenous administered cells. We used pure HSC, while others have used total bone marrow which contains additionally multipotent mesenchymal stromal cells (MSC). Moreover, administration of pure MSC ameliorates tissue damage and enhances anti-inflammatory mediators upon renal I/R injury\(^9-11\). Nowadays, studies investigating the contribution of exogenous cells to renal repair focus on MSC which show greater therapeutic potential compared with HSC.

**Manifold roles of chemokines during AKI**

Chemokines were originally described as chemotactic cytokines responsible for the migration and activation of inflammatory cells. However, besides their role in inflammation, chemokines also play a role in other processes including angiogenesis, homeostasis, development, and wound healing\(^12,13\). Several studies have identified chemokines as participants in renal disease, however, little was known regarding their expression pattern. Therefore we employed microarray analysis to determine the expression of chemokines during the different phases of renal I/R injury.
(chapter 4). CXC chemokines showed a biphasic expression coinciding with the early inflammatory phase and the later reparative phase. Strikingly, all chemokines had the highest expression during this later phase. These results imply that temporal expression of chemokines is a crucial factor in the regulation of I/R injury and repair. It is therefore important to dissect the role of a chemokine during all phases following renal I/R injury before considering it as a therapeutic target. We determined the role of 2 different chemokines, i.e. SDF-1/CXCL12 and MCP-1/CCL2, during renal I/R injury and show alternate mechanisms of action beside chemotaxis. Although we could not observe a role for the chemokine SDF-1 in regulating HSC trafficking to the ischemic damaged kidney, we did observe significant increased expression of SDF-1 upon inducing renal I/R injury (chapter 3). To investigate the role of SDF-1 during renal I/R injury, we depleted renal SDF-1 by antisense oligonucleotides. Mice with depleted renal SDF-1 showed severely increased tubular injury and decreased renal function without affecting migration of HSC, implying that SDF-1 is an important survival factor for tubular epithelial cells (TEC). Our results are in line with other studies that show a direct role for SDF-1 in enhancing survival in wide variety of cells.

MCP-1 is traditionally viewed as the chemoattractant mainly responsible for monocyte influx into damaged tissue. Indeed, accumulation of macrophages into the ischemic damaged kidney coincides with increased renal expression of MCP-1. Surprisingly, mice lacking MCP-1 have increased renal damage and dysfunction without affecting the accumulation of macrophages (chapter 5). Interestingly, just like SDF-1, MCP-1 might serve as a survival factor for various cell types including TEC (chapter 5). Additionally, MCP-1 might play a role in polarization of macrophages towards the anti-inflammatory M2 type (chapter 5). Upon injury, expression of several chemokines is upregulated and moreover they are considered to be involved in the inflammatory response. However, the role of the chemokines SDF-1 and MCP-1 in survival of TEC that we found demonstrates again the diverse actions of these so-called inflammatory mediators. Consequently, it is of vital importance to thoroughly unravel the various biological roles of each chemokine to obtain a complete overview of the pathways in which chemokines are involved. Additionally, time and spatial expression of a certain chemokine likely is an important factor in determining its mode of action.

**Immunomodulatory role for gut microbiota in renal I/R**

The gut microbiota has recently been recognized for its role in systemic immune regulation. Bacterial cell wall components such as peptidoglycan (PGN) and lipopolysaccharide (LPS) translocate from the gut into the circulation, where they can influence the peripheral inflammatory response. Moreover, Clarke et al. have shown that PGN derived from the microbiota can prime granulocytes, and thereby
enhance its function\textsuperscript{36}. During a sterile inflammatory response, however, influx of granulocytes may be detrimental by inducing collateral damage. Depletion of gut microbiota significantly attenuated renal damage and dysfunction following renal I/R injury with concomitant markedly reduced granulocyte influx (chapter 6). These results suggest that the immunomodulatory effect of microbiota on granulocytes is detrimental during renal I/R injury. Although others have shown a role for NOD1 in priming the granulocytes\textsuperscript{36}, we could not confirm this in our model. Beside immunomodulation via translocation of microbial products, other pathways may be involved. One example where gut microbiota regulate immune and inflammatory responses is the interaction of short-chain fatty acids, which are produced by fermentation of dietary fibre by gut microbiota, with the receptor GPR43 which is expressed by granulocytes\textsuperscript{40}. Additionally, a direct effect of the antibiotic-treatment on renal cells cannot be ruled out.

## Nodosome and its role during renal disease

In the past decade the contribution of pattern recognition receptors (PRRs) in renal disease has gained much attention. Although initially described as receptors for pathogens, it became clear that these receptors additionally recognize endogenous stress ligands that are released upon damage. Indeed, a significant contribution to the inflammatory response and consequently renal damage and dysfunction following I/R injury has been described for the PRRs TLR2\textsuperscript{41,42}, TLR4\textsuperscript{43,44}, and NLRP3\textsuperscript{45,46}. Shigeoka \textit{et al.} have reported a protective role for the nodosome in renal I/R injury\textsuperscript{47}. They show preserved renal function, less tubular injury and reduced granulocyte influx in NOD1/2 DKO mice following renal I/R injury as compared with WT mice. However, the mechanism via which the nodosome contributes to renal I/R injury is not investigated. As far as we know, no endogenous stress ligands are described to signal via NOD1 or NOD2. Additionally, we could not confirm a significant contribution of the nodosome during renal I/R injury (chapter 6). The reason for the differences between Shigeoka and our results regarding the involvement of the nodosome in renal I/R injury are currently unclear, but could merely reflect differences in methods and experimental design (e.g. housing, anaesthetics). Another example of sterile renal injury during which endogenous stress ligands are released is progressive renal injury induced by unilateral ureteral obstruction. The PRRs TLR4\textsuperscript{48,49} and NLRP3\textsuperscript{50} contribute to the development of fibrosis, while the PRRs TLR2\textsuperscript{51} and NOD1/2 (chapter 8) do not play a significant role in the development of renal progressive injury and fibrosis. The abovementioned studies highlight the important role of several PRRs during sterile renal injury. However, we feel that scientific evidence for a role of NOD1/2 in sterile injury is not yet convincing. Our major concern is the lack of endogenous stress ligands which can signal via the nodosome. Interestingly, it has been shown recently that NOD1 is
required for PGN-mediated priming of the immune system ($^{36}$) (see above), this would suggest a new role for PRRs in an inflammatory response.

Sepsis-induced renal injury is initiated by detection of microbial products via PRRs. Although NOD1/2 do not play a significant role in the contribution of a systemic inflammatory response following LPS+PGN administration, these receptors are involved in the development of sepsis-induced AKI (chapter 7). This implies that interactions between microbial products and their PRRs are tissue specific. Expression of various PRRs including NOD1 and NOD2 has been described in TECs ($^{47,52,53}$). Therefore, detection of microbial products by TEC-associated PRRs might play a crucial role in regulating the immune response during sepsis-induced renal injury.

Overall, based on their functional and structural homologies with other PRRs it is conceivable that the nodosome is involved in renal disease. Indeed, we identify a role for the nodosome in sepsis-induced renal injury (chapter 7). However, a role for the nodosome during sterile renal injury is not evident.

**Future perspectives**

Several mechanisms of renal injury and repair are investigated in this thesis. What do these new insights add to our knowledge, and in particular, do we have new leads for novel therapies? The future perspectives are discussed below.

We (chapter 2) and others have shown that the contribution of HSC or bone marrow-derived cells to renal repair is very low and hence the physiologic role is questionable. Additionally, we could not alter HSC migration by manipulating the most important HSC migration axis (chapter 2). These results argue against HSC therapy to treat renal injury. Moreover, evidence is accumulating that the most important source of new TEC is most likely the surviving TEC which can dedifferentiate and proliferate in order to restore lost cells. To enhance renal repair it might be more promising to unravel the mechanisms taking place in these dedifferentiating and/or proliferating TEC, so that the intrinsic reparative processes are enhanced.

Various studies have revealed that chemokines are important contributors to the pathogenesis of renal I/R injury and repair. From our results (chapter 3-5), we can learn that the role of chemokines goes beyond its chemotactic properties to attract inflammatory cells to injured tissue. Although we did not find new candidates for a therapeutic target, we gained essential knowledge regarding the role of several chemokines during renal I/R injury. One important finding is that chemokines can directly enhance survival of TEC. However, this function might be difficult to use in a therapeutic strategy as induction of chemokines most likely also affects leukocyte influx adversely. Another important note is that the timing of chemokine induction/blockade is crucial, as suggested by the biphasic expression of CXC chemokines. The main message from the chemokine research is that we need to dissect the
mechanisms via which these small molecules exert their various effects in order to get a complete overview of the roles of chemokines in AKI. The use of chemokine antagonists and inducible knockout mice is essential to unravel these roles in a time-dependent manner.

In chapter 6 we observed a clear protection against I/R injury in absence of intestinal microbiota. The mechanism, however, is not completely clear and further research is necessary to unravel how broad-spectrum antibiotic treatment affects renal I/R injury. Additional mouse experiments are needed to determine whether a single antibiotic or combination of antibiotics is responsible for the observed effect. From a clinical perspective, it would be of great interest to determine whether the composition of intestinal microbiota, which can be manipulated, affects renal outcome. This can be investigated by analyzing the intestinal microbiota of renal transplant recipients with and without acute rejection.

The nodosome (NOD1 and NOD2) was discovered only a decade ago, and although since then a wealth of data has been gathered, we are probably only at the beginning of discovering the full scope of these innate immune receptors. By studying the nodosome deficient mice (chapter 7), we found several new leads, which need to be investigated further to gain more insight into the various roles of the nodosome in biological processes. For instance, it would be very interesting to study the nodosome deficient mice in a model for metabolic syndrome, based on the plasma biochemical analysis of the naive mice. Furthermore to answer whether the nodosome is involved in sterile injury, it is inevitable to determine the endogeous danger ligand(s) which can signal via the nodosome.
References


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