Immune-mediated mechanisms of (mal)adaptive renal tissue repair

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Chapter 8

General discussion

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With the studies presented in this thesis, we intended to provide novel insights into the pathogenesis of acute injury and progression to chronic kidney disease. Specifically, we sought to identify novel targets amongst the plethora of innate immune response activators, which could lay the basis for effective treatment regimes in patients with kidney diseases.

Key findings of this thesis:

1. The damage-associated protein S100A8/A9 is a pivotal player in renal repair following hypoxic damage, through an immune-regulatory role resulting in the alternative activation of macrophages. By controlling excessive M2 polarization, S100A8/A9 fine-tunes the adaptive response of the kidney to IR-induced AKI (**Chapter 2**).

2. In chronically inflamed kidneys, S100A8/A9 contributes to the development of fibrosis possibly through a direct effect on dedifferentiation and apoptosis in tubular epithelial cells. This effect was independent of S100A8/A9-induced inflammation and recruitment of leukocytes into the kidney (**Chapter 3**).

3. The innate immune receptor Triggering receptor expressed on myeloid cells-1 (TREM-1) is not involved in the amplification of acute renal damage and inflammation in preclinical model of acute kidney injury. Additionally, renal transplanted patients carrying the TREM1 gene variant p.Thr25Ser do not show any association with pathological consequences after transplantation (**Chapter 5**).

4. The activating receptor TREM-1 limits the maladaptive repair post IR-induced AKI, through a direct effect on tubular epithelial mitochondrial homeostasis and energy production, empowering cell cycle progression. The metabolic advantage provided by TREM-1 is indispensable for tubular proliferation, which sustains renal repair and accelerates the recovery from IR (**Chapter 6**).

5. TREM-1 and its adapter molecule DAP12 are not involved in the development of renal fibrosis in the preclinical model of chronic kidney disease. DAP12, partly through TREM-1, modulates the renal inflammatory response following unilateral ureteral obstruction (**Chapter 7**).

The complex nature of innate immunity in renal diseases: the fine line between healing and tissue degeneration.

The incidence of renal diseases is dramatically rising in the western world, causing high rates of morbidity and mortality. More specifically, the incidence of Acute Kidney Injury (AKI) is increasing as populations continue to age[1]. AKI accounts for 25% of hospitalized patients and can reach as high as 70% of patients in the intensive care unit. Despite advances in basic research, management of AKI is still supportive and associated with an unacceptably high mortality rate, prolonged hospitalization and dialysis dependence. Renal replacement therapies have extended life expectancy for patients with AKI and terminal CKD, but in general represents only a supportive measure, rather than a curative therapy. Given the kidney's high regenerative capacity, loss of nephrons that fail to regenerate after an episode of AKI, determines the progression to CKD[2]. Research that describes the molecular mechanism underlying this failed regeneration, has high potential for identifying effective therapies for patients at high risk of developing renal failure.

During the last decades, an increased body of evidence suggests that the activation of the inflammatory response is necessary to orchestrate adaptive repair of the kidney, however when the inflammatory response is unbalanced chronic complications can develop[3]. The primary mechanism by which the kidney responds to damage involves the activation of pattern recognition receptors (PRRs), including Toll like receptors (TLRs). During a state of homeostasis TLRs are expressed in resident immune cells and parenchymal cells which when alerted by damage associated molecular patterns (DAMPs) become activated and shape the adaptive immune response. However, a maladaptive inflammatory response is often the cause of a vicious cycle which perpetuates inflammation leading to tissue fibrosis and dysfunction[3,4]. Therefore, the immune system is an integral part of kidney homeostasis, injury and controls the regenerative capacity of the tissue. Danger molecules can be released from any intracellular compartment into the extracellular space, bind to specific PRRs and promote the recruitment and activation of various immune cells, including neutrophils and monocyte/macrophages, that remove cellular debris and secrete many inflammatory factors necessary for tissue repair[5]. Both PRRs as well as DAMPs were described to play a pivotal role in renal inflammation as well as repair in preclinical models of AKI and CKD. However, many PRRs and DAMPs have not yet been studied in the context of acute and chronic kidney diseases. In this thesis we focused on the individual role of the danger protein S100A8/A9 and the activating receptor TREM-1 in the experimental models of ischemia/reperfusion (IR)-induced acute kidney injury (AKI) and unilateral ureteral obstruction (UUO)-induced chronic kidney disease (CKD).

S100A8/A9 is recognized as a DAMP but besides its role in inflammation it is also involved in other biological processes such as development and wound healing[6,7]. In **Chapter 2 and 3** we describe that the danger programs activated by S100A8/A9 drive renal regeneration and fibrosis. We found that long-term inhibition of this inflammatory signal affects renal repair in the preclinical model of AKI, but it is beneficial in the preclinical model of CKD. Therefore, a therapeutic approach that leaves the S1000A8/A9-induced inflammatory response intact during the phase of active repair after AKI is preferable, whereas, in chronically inflamed kidney, S100A8/A9 blockade would preserve the tissue from parenchymal destruction and loss of function.

TLR4 represents one of the best characterized PRRs in the pathophysiology of renal diseases[3]. Functional TLR4 on the renal tubule is required to activate an adaptive immune response during the acute phase of AKI and interfering with its signal dampens the excessive inflammatory response and collateral tissue damage. The double edge sword of blocking TLR4 activation, is the eventual impairment of host defense against pathogens. To overcome this, we investigated the effect of the TLR4 endogenous ligand, S100A8/A9 in the pathogenesis of AKI, expecting a similar role to TLR4 . Our hypothesis was further supported by an early report that showed a detrimental effect of S100A8/A9 in cerebral IR-induced damage, possibly through activation of TLR4-mediated inflammatory response[8].

In sharp contrast **(Chapter 2)** we found that S100A8/A9 is crucially involved in renal adaptive repair after ischemia, by controlling excessive M2 macrophage polarization and preventing renal fibrosis. During the acute phase of injury, an increase in S100A8/A9 protein expression was detected in the kidney, most likely due to the infiltration of granulocytes, which contain S100A8/A9. Unexpectedly, in the absence of S100A8/A9, no differences in the inflammatory cell infiltration nor in renal damage parameters were observed during the acute phase of IR, compared to WT mice. During the early phase of renal IR injury, leukocytes populations have a detrimental role because they promote cell death by releasing reactive oxygen species and activating apoptotic programs. Therefore, the comparable levels of inflammation and renal damage between the two mice strains might be related to the similar influx of leukocytes. Despite this similar phenotype detected in the acute phase, sustained activation of inflammation together with development of fibrosis were observed later during the repair phase in the absence of S100A8/A9. We discovered that the maladaptive repair was elicited by the excessive M2 macrophage polarization in the S100A9 KO animals. Macrophages have an important role in the maintenance of tissue integrity. Depending on the renal inflammatory milieu, macrophage skewing occurs in order to contribute to inflammation

or tissue repair[9]. Specifically, M1 polarization contributes to renal damage[10,11], whereas during renal repair M2 polarization ensures proper tissue healing[12]. Aberrant alternative activation of M2, observed in absence of S100A8/A9 may account for the persistent activation of inflammation and increased renal fibrosis. Although it appears incongruent that the excessive M2 polarization observed in the S100A9 KO animals associates with an M1 signaling activation (namely increased TNFα and IL1β cytokines expression), we believe that this is a secondary response of the kidney which progresses into fibrosis and may activate other cell types with a pro-inflammatory activity, such as dendritic cells[13,14]. Supporting our speculation, Eikmans's group recently described that overexpression of S100A8/A9 in the human monocyte cell line THP-1 does not lead to any difference in TNFα or IL1β expression, when compared to cells transfected with an empty vector[15]. The same group provided the clinical translational potential of our study, by describing that patients with relatively high expression of myeloid-related S100A8 and S100A9 during acute rejection, had an improved long-term outcome. They demonstrated an immuno-regulatory role for S100A8/A9 in orchestrating allogenic T cell activity during rejection. Stimulation of dendritic cells with recombinant S100A8/A9 dampened their maturation and the ability of stimulating the T cell response. This is related to the enhanced ROS production elicited by S100A8/A9, which dampens T cell activity. Apparently, macrophages with high levels of S100A9 have a beneficial immune effect through the activation of the adaptive immune response and anti-inflammatory effects, resulting in reduced tissue damage after transplantation[16]. This body of evidence appears to be in line with our findings, unfortunately, the levels of ROS and dendritic cells activation were not investigated in our study. However, we found that the maladaptive repair observed in absence of S100A8/A9 was associated with enhanced infiltration of CD11C+ cells. Despite being commonly seen as a marker for conventional dendritic cells activation, recent studies also describe CD11C expression in T cells. Additionally, a recent study identified a novel subset of cells in mouse and humans that contains properties of both DC and T cells, including CD11C expression[17,18]. Therefore, it is conceivable that in our preclinical AKI model a similar mechanism can take place.

Finally, we show that S100A8/A9 is crucial for shaping and promoting an adaptive immune response after IR. To conclude, the main message of this study is that some degree of inflammation during the acute phase is crucial for renal tissue repair, as previously described for other PRRs and DAMPs in renal repair.

Given the immune-regulatory effect of S100A8/A9 in macrophages, which are well known to mediate the development of progressive renal fibrosis, we sought to unravel whether this danger protein plays a role in the preclinical model of renal fibrosis: the unilateral ureteric obstruction model (UUO). Interestingly, macrophage infiltration in

this model show predominantly an M2-like phenotype[19] , therefore, we hypothesized a beneficial role for S100A8/A9. In contrast to our finding obtained in the preclinical model of AKI, in **Chapter 3** we demonstrated that S100A8/A9 promotes renal fibrosis by inducing permanent loss of integrity and apoptosis in tubular epithelial cells. We found that S100A8/A9 is expressed in patients with obstructive nephropathy and in the experimental model of UUO. The majority of S100A8/A9 positive cells appear to be granulocytes, as previously described in the preclinical model of AKI[20]. In the absence of S100A9, mice were protected against UUO-induced renal fibrosis, especially during the late stages of damage, which was associated with a decrease in TGFβ activation and plasma creatinine levels. Surprisingly, macrophage infiltration and polarization, as well as the renal inflammatory milieu were unchanged between the two mice strains, contrary to what is described in the study from Fuji et *al*. in the same experimental model[21]. This study suggests that the transcriptional regulator of S100A8/A9, Krüppel-like factor–5 (KLF5), controls S100A8/A9 release from collecting duct cells, resulting in recruitment of inflammatory monocytes to the kidney and promoting their polarization into the M1 inflammatory phenotype. They conclude that KLF5-mediated S100A8/A9 release promotes renal inflammation and tissue remodeling, limiting fibrosis in the UUO model. We believe that the discrepancy with our study is related to the genetic model used in their experiment, which does not entail an endogenous disturbance of S100A8/A9 induction. Additionally, KLF5 has been shown to promote inflammation and cell differentiation, also independently of S100A8/A9. Besides the activation of the inflammatory response, S100A8/A9 can affect endothelial integrity and cell death[22]. In the experimental model of UUO, we excluded a role in renal inflammation, instead, we found that TECs from S100A9 KO mice displayed decreased apoptosis and increased morphological and functional signs of preserved integrity, resulting in decreased fibrosis and TGFβ activation. The functional relationship between S100A8/A9 and TGFβ have been described earlier in relation to proliferation inhibition[23]. Additionally, in renal biopsies of patients with acute rejection not progressing into chronic allograft nephropathy, Eikmans et *al*. described a significant correlation between S100A8/A9 and TGFβ. The transcript levels of both mediators displayed a significant increase, suggesting indeed that during acute rejection they play a beneficial action for long term outcomes[23]. Given the pattern of expression of S100A8/A9 and TGFβ in the experimental model of UUO, it is conceivable that this correlation is also present in our study. Nonetheless, it appears that in chronically damaged kidney the correlation with TGFβ has a different purpose from the aforementioned study. Indeed, we found that extracellular S100A8/A9 has an effect on tubular epithelial cells dedifferentiation and when S100A8/A9 is combined with TGFβ, the synergy results in enhanced apoptosis. S100A8/A9 has already been described to induce apoptosis in several cancer cell lines, but we were the first to demonstrate

it in TECs[24,25]. This effect may be mediated by RAGE, known to be expressed on TECs upon UUO. Supporting our speculation, S100B, another member of the S100 family, mediates myoblast apoptosis in a RAGE- dependent manner[26]. Whether this occurs as well in tubular epithelial cells, remains to be determined. Taken together, this body of evidences suggests that the endogenous S100A8/A9 signaling in macrophages plays a dispensable role in inflammation-driven renal fibrosis, possibly due to redundancy or compensatory pathways activated in immune cells. The two activating receptors that recognize S100A8/A9, namely TLR4 and RAGE, drive the development of renal fibrosis via a direct effect on epithelial cells integrity and function[27,28], but have a mild effect on renal inflammation. The results shown in this study strengthen the theory that the magnitude of inflammation is not correlated with the extend of fibrosis, as described earlier for TLR4 and that TECs contribute to the development of renal fibrosis.

The sharp contrast of roles for S100A8/A9 described in chapter 2 and 3 might be explained by the different primary insult that led to fibrosis in ischemic damage and obstructive nephropathy. For instance, the timing and the activation of the immunological response differ in IR-induced AKI and UUO-induced CKD. Leukocytes that abundantly express S100A8/A9, are recruited and activated in the kidney much earlier upon IR as compared to the UUO model. Therefore, it is not surprising that the S100A8/A9-mediated immune cells play such an important role in the acute model of renal injury. During chronic renal inflammation many other inflammatory pathways can be activated that overrule S100A8/A9's action. Finally, we provide strong evidences that S100A8/A9 mediates renal repair and fibrosis through the regulation of macrophage function and parenchymal damage. What remains to be studied is the relevance of S100A8/A9 as a biomarker for the development of end stage renal disease of the native kidney and whether S100A8/A9 neutralizing antibody can be a potential therapeutic strategy used to hamper tissue deterioration in fibrotic kidney.

In the second part of this thesis we describe the role of an activating PRRs, the Triggering receptor expressed on myeloid cells-1 (TREM-1) in the pathogenesis of sterile inflammatory disorders. Since its discovery in the early 2000's by Colonna and colleagues, TREM-1 has been implicated in the innate immune response activation primarily in infectious disease and considered as a potential target for treatments[29]. TREM-1 works in concert with other PRRs and causes an excessive inflammatory response activation, particularly potentiating TLR-elicited inflammation[30]. Therefore, many TREM-1 inhibiting strategies have been developed to fine tune the immune response as treatment for infectious diseases[31]. However, as we extensively described in **Chapter 4,** the emerging role of TREM1-mediated inflammatory response in non-infectious diseases is

accumulating. Indeed, the same TREM-1 blocking strategies used in infectious diseases, have been shown to dampen excessive inflammation and provide tissue protection in sterile inflammatory disorders such as atherosclerosis, myocardial ischemia, rheumatoid arthritis and many others. However, these studies do not provide any strong evidence for a specific TREM-1 ligand and the underlying mechanism. Elucidation of this mechanism represents an intriguing research line especially in the context of sterile inflammatory disorders.

In the work presented in **chapter 5, 6** of this thesis we found that TREM-1 actually improves tissue repair upon ischemic damage and that its activation is dispensable in the acute phase of renal injury, similarly to what we described earlier for S100A8/A9. Additionally in **Chapter 7** we describe that TREM-1, together with its adapter molecule DAP12, does not seem to play a role in the magnitude of inflammation during the development of renal fibrosis.

This body of evidence suggests that the inflammatory response elicited by PRRs is pivotal in shaping renal regeneration. Despite its well- established role in immune cells, in Chapter 6 we describe a novel role for TREM-1 in TECs proliferation empowering tubular repair.

Early pro-inflammatory signals generated in the kidney upon IR injury mediate the renal response to damage. Fine tuning the inflammatory response by containing excessive activation ensure that the renal microenviroment is primed for adaptive repair and optimal healing. TLR4- elicited inflammation has been shown to be deleterious in renal IR injury and mediates tubular damage[32]. In **chapter 5** we investigated whether the TLR4 synergistic receptor, TREM-1, could be involved in the induction of an exaggerated inflammatory response following IR. Despite the increased number of cells transcribing *Trem1* mRNA in the kidney upon IR, different attempts to modulate TREM-1 function *in vivo* did not result in renoprotection after reperfusion. Targeting TREM1-mediated immune response by short inhibitory peptides or fusion protein seems to be effective in downregulating downstream signaling activation, such as Myd88, but has no effect on the renal inflammatory response, nor renal damage, in the 24 hours following reperfusion. Our findings appear in contrast with studies showing that TREM-1 inhibition dampens inflammation and tissue damage upon ischemia in other organs, but is in line with a similar study conducted in the kidney. We considered that the absence of renoprotection in our model could have been related to the time of intervention, however, Duffield's group demonstrated that daily injection of TREM-1 fusion protein, following renal ischemia or ureteral obstruction, did not alter macrophage activation or renal injury and fibrosis[33]. Whether the inflammatory response elicited by myeloid cells- associated TREM-1 is

redundant during renal IR or plays a tissue specific role, remains to be determined. Our data would reinforce the concept that tubular-specific PRRs are the major determinant in driving the renal inflammatory response, instead of myeloid cells-associated PRRs. Among the different PRRs involved in renal dysfunction in the experimental model of renal IR, TLR4 is the most studied and characterized. Despite the many experimental studies suggesting an immune-regulatory role for TLR4 in allograft tolerance, in various human renal transplanted cohorts, TLR4 single nucleotide polymorphisms (SNPs) were not found to be associated with any pathological consequences after kidney transplantation. This suggests that in the complexity of the human allograft response, TLR4 may represent a minor determinant[34]. Hence, caution is warranted in extrapolating results from experimental studies to human diseases and alternative animal models that mimic different aspects of human AKI are necessary.
The findings that TREM-1 plays a limited role in the renal innate inflammatory response after IR, were corroborated by the results obtained in a human transplantation study. In a relatively large cohort of renal transplanted patients, neither donor or recipient carriers of the non-synonymous *TREM1* variant p.Thr25Ser, showed any association with post-transplant graft outcome. Interestingly, the 5 patients who were homozygous recessive for this variant did not develop delayed graft function, but due to the small number, statistical analysis could not be performed. Whether this variant is associated with a gain of function that could eventually explain the improved graft outcome, supported by the novel role of TREM-1 in tubular regeneration we described in **chapter 6,** warrants further investigation**.**

Herein, we provide an in depth study of the role of TREM-1 in injury and regeneration after IR-induced AKI, by using mice deficient for TREM1/3. In mild renal IR, TREM1/3 KO mice display a maladaptive repair with development of fibrosis and tubular senescence. Remarkably, sham-operated mice in absence of TREM1/3 showed a decreased tubular proliferation, suggesting that the tubular senescence was not a secondary effect, but probably the major cause of the maladaptive repair. The conventional expression of TREM-1 belongs to the innate immune cells, but TREM-1 expression is well described in several epithelia upon inflammation, conferring them a pro-inflammatory character[35,36]. Additionally, TREM-1 is an hypoxia-inducible gene in dendritic cells, empowering their pro-inflammatory activities[37]. Corroborating these findings, we describe an upregulation of TREM-1 protein on TECs after *in vitro*-simulated IR, suggesting indeed a role for TREM-1 in TEC's recovery after hypoxic damage. When we further characterized primary TECs, we found that the absence of TREM1/3 resulted in G2/M arrest, already at steady state. Bonventre's group described an innovative concept about TECs cell cycle and renal fibrosis. They demonstrated that G2/M-arrested TECs upregulate the production of pro-fibrotic

cytokines influencing the renal microenviroment[38]. Interestingly, the cell cycle arrest in TREM1/3 deficient TECs was associated with an upregulation of TGFβ and CTGF transcripts. In order to progress into the cell cycle, cells sense and respond to a bioenergetics demand. Activation of mitochondrial respiration is required for G2/M transition[39]. The cell cycle arrest in TREM1/3 KO TECs was associated with a decreased mitochondria metabolism. Since these cells carry out many tasks in a high energy dependent manner, mitochondrial homeostasis is crucial for their function. During oxidative phosphorylation, mitochondria generate ROS or electron transfer, but paradoxically, increased levels of ROS may attack the mitochondria machinery leading to increased oxidative damage, which is considered a cause of cell and organ dysfunction[40]. TREM1/3 KO TECs displayed increased levels of antioxidants, probably due to the extreme ROS levels we detected in mitochondria, which impaired their ability to carry out many metabolic functions. Supporting the beneficial role of TREM-1 in mitochondria homeostasis, Yang et *al.* described that in bone marrow derived macrophages, TREM-1 regulates mitochondria integrity favoring cell survival[41], through upregulation of Mitofusin-2 (involved in the mitochondria fusion process). However, in TECs this effect appears to be mitofusin-2 independent. When exposed to an extra ROS-generating trigger, such as the *in vitro*-simulated IR, TREM1/3 KO TECs displayed canonical markers of senescence activation, such as the senescence associated-β galactosidase, suggesting that IR was enough to cause a permanent cell cycle arrest. Cellular senescence is referred to as a permanent arrest of cell division, conventionally known as a safety mechanism to defeat cancer development[42]. In the context of tissue repair, senescent cells may be beneficial in the initial phase of injury and help regeneration, however prolonged accumulation of senescent cells delays tissue regeneration and establishment of homeostasis. Additionally, they can spread their senescent state through the senescent associated secretory phenotype (SASP), propagating a vicious inflammatory and pro-fibrotic cycle in the whole tissue. Thus, spreading of senescence can lead to a uniform tissue deterioration, because tubular regeneration cannot be compensated. In light of the aberrant effect of the secretory phenotype on neighbor's cells, senescent TREM 1/3 KO TECs displayed a potentiated pro-inflammatory and fibrotic secretome, which could contribute to the establishment of renal fibrosis. Therefore, we speculate that the prolonged senescence is the root of the maladaptive repair observed *in vivo* and leads to altered wound healing capacities. Additionally, it is conceivable that what was observed in TREM1/3 KO TECs belongs to the category of stress-induced senescence, caused by ROS and DNA damage. Indeed, we described a positive feedback between ROS accumulation, mitochondrial damage and p21 activation, leading to the establishment of senescence as described previously[43]. The increased ROS production observed in the KO animals could be the leading cause of the establishment of senescence. Possibly, as a consequence there is an ongoing DNA

damage response, resulting in cell cycle arrest. Recently, TREM-1 has been described to decrease the DNA damage response (DDR) and stimulate proliferation of leukemic stem cells[44]. Indeed, TREM-1 modulation has attracted attention as potential therapeutic target during cancer in order to prevent tumor progression[31]. Therefore, persistent DDR activation in absence of TREM1/3 may contribute to the cell cycle arrest and decreased proliferation. However we remain unaware of what triggers TREM-1 activation in TECs after IR and how an innate immune receptor localized on the plasma membrane can affect mitochondrial dysfunction and senescence in TECs. We speculate that this effect may be related to the synergistic relationship between TREM-1 and TGFβ[45]. Although TGFβ has been described as a potent inducer of epithelial apoptosis, some studies point to a beneficial role of this growth factor in tubular regeneration after ischemia, which accelerates renal recovery. TECs transiently express TGFβ during proliferation and we speculate that TGFβ may bind to TREM-1, acting as a positive feedback loop for proliferation. In absence of this synergy, TGFβ may become steadily expressed and have fatal consequences, especially for the mitochondrial function and ROS production, as already described[46].

In conclusion, this study highlights a novel role for TREM-1 beyond the innate immune cells. TREM-1 empowers successful proliferation of TECs, possibly by regulating mitochondrial homeostasis and therefore accelerates renal recovery after IR-induced AKI. Additionally, for the first time we show that PRRs are able to modulate tubular senescence, which highlights the power of TECs in dictating the maladaptive repair post-AKI. Again, these basic research findings about TREM-1 in AKI emphasize that a low degree of TREM-1 activation is necessary to orchestrate an adaptive repair. Targeting TREM-1 during active repair may preserve mitochondrial homeostasis and supply the TECs with the energy necessary to proliferate.

In the last chapter (**Chapter 7**) of this thesis, we rule out a role for TREM-1 and its adapter molecule DAP12, in mediating inflammation-driven renal fibrosis, in the UUO model. TREM-1 seems to be implicated in the inflammatory response in many chronic diseases. Although interstitial cells, most likely granulocytes, infiltrating the renal parenchyma following UUO transcribe *Trem1* and *Dap12*, no considerable differences in the inflammatory response or renal fibrosis were found to be mediated by TREM-1. Corroborating our findings, Duffield's group described that daily treatment with TREM-1 fusion protein after UUO, does not result in renoprotection[33]. DAP12 instead (partly through TREM-1), mediates renal inflammation during UUO. Specifically, the reduced infiltration of macrophages observed in theDAP12 KO animals in the advanced stages of renal fibrosis, could be related to the decreased production of macrophage chemoattractant protein-1 or simply to a defect in migration, intrinsic to DAP12 KO

macrophages[47]. It is possible that the effect mediated by DAP12 in renal inflammation entails the activation of other DAP12-associated receptors, which were upregulated in WT mice following obstructive nephropathy. Noticeably, TREM1/3 and DAP12 KO animals displayed increased tubular injury and edema in the early days upon obstruction, a phenotype similar to the TLR4 KO mice, which displayed enhanced renal damage following UUO, as a consequence of decreased tubular integrity but without any effect on interstitial inflammation[27]. Whether this mechanism is also taking place in TREM1/3 or DAP12 KO mice, is still unknown. Unfortunately, tubular integrity or proliferation were not measured in the two animal strains upon UUO. Since impaired mitochondrial homeostasis in TECs can ultimately result in ATP depletion, cytoskeletal changes, loss of the brush border and tubular epithelial cell detachment[48]. Given the protective role of TREM-1 in TEC's mitochondrial energy metabolism described in chapter 6, we speculate that the increased tubular damage and edema observed in the early days upon obstruction, may be a consequence of the mitochondrial dysfunction-driven tubular damage. Our evidence, however, appears to be in contrast with the study from Lo et *al*., which demonstrated that TREM-1 deletion ameliorated renal pathology after UUO by modulating M1 polarization[49]. Their findings were obtained with TREM-1 only KO mice, whereas in our studies we used mice deficient for both TREM-1 and TREM-3, as a model for TREM-1 deficiency[50]. If phenotypes are different in the two murine strains and if TREM-3 plays any role in inflammation and fibrosis, remains to be determined.

In summary, this evidence suggest that TREM-1 activation is dispensable in renal fibrosis, possibly because during the extensive and prolonged renal damage, other PRRs may overrule its function.

Concluding remarks and future perspectives

In this thesis we have described different aspects of S100A8/A9 and TREM-1 functions in the pathology of kidney diseases: from inflammation, fibrosis, wound healing and parenchymal integrity to metabolism and senescence. From the evidence obtained in these 5 years of research it appears clear that both immune cells and TECs are essential in shaping renal immunity and drive regeneration. The recurring theme we found is that constant inhibition of inflammation has the side effect of reduced wound healing and for a high risk of infections. We show that the inflammatory signaling elicited by S100A8/A9 or TREM-1 enhance renal repair; this makes them an attractive novel therapeutic target. Fine tuning the inflammatory response by timing and dosing S100A8/A9 or TREM-1 modulators, appears to be the desirable approach, yet very challenging to accomplish. Defining the timing and the extend of the therapeutic window of opportunity to regulate renal homeostasis, should be the goal of future research.

Interestingly, we unraveled a novel role for the innate immune receptor TREM-1 in preserving mitochondrial homeostasis and proliferative capacities of tubular epithelial cells, which could be targeted to enhance the reparative process. The absence of TREM-1 induces a metabolic reprogramming that predisposes the tubular cells to stress-induced premature senescence.

For the time being where population longevity is increasing, research on senescence has gained more attention. Renal transplant patients that receive a kidney from elderly people, may also inherit an increased risk of age-related disorders and decreased regenerative capacity of the kidney. In the last few years many attempts to target senescent cells in renal diseases have shown contrasting results. Apparently, senescence is important in the acute phase of damage and would support regeneration, whereas chronic senescence appears to be detrimental. Unravelling the pathological mechanisms taking place in senescent tubular cells, such as changes in their metabolism or their secretome, may overcome the issue of "rejuvenate" by elimination. Understanding changes in mitochondria homeostatic processes and the metabolism of senescent TECs may bring to light novel mechanisms that can be targeted to release the brake and supply the energy necessary for cell cycle progression.

As the aging of the immune system matters and immunosenescence represent fertile ground for treatment of acute and chronic disorders, we would not be surprised whether these age-related changes will be displayed by TECs as well, as they fulfil the task of an inflammatory cell, by expressing PRRs and undergoing senescence with ageing. Surely, a more detailed analysis on the role of tubular-associated PRRs and senescence deserves more consideration.

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