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GENERAL DISCUSSION

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Immune responses in the kidney are essential to curtail infections. The downside of inflammation is damage induced by kidney infiltrating immune cells and the inflammatory mediators they produce. Tubular injury and subsequent fibrosis will lead to impaired renal function. The detrimental effects of nephritis are illustrated by T cell-mediated rejection (TCMR) of kidney transplants, which is characterized by graft infiltrating cytotoxic lymphocytes and loss of renal function. In this thesis we studied inflammatory processes in the kidney, in particular during cellular transplant rejection and viral infection. In **chapter 3**, we demonstrate that urinary mRNA levels of the cytotoxic genes granzyme (Gzm) A, GzmB and perforin are increased during TCMR. To limit inflammation-mediated damage, immune responses are strictly regulated, for example by suppressive cytokines produced by regulatory T cells (Tregs) ¹. Additionally, renal tubular epithelial cells (TECs) themselves are equipped with various mechanisms to restrict tubular damage. Previously, our group found that the tubular expression of serpinB9 was enhanced during subclinical rejection (SCR) ², suggesting that TECs are protected against GzmB-mediated apoptosis. This finding motivated us to investigate the regulation of serpinB9 and other serine protease inhibitors in primary TECs. To our surprise, serpinB9 expression was enhanced in response to poly(I:C), but not in response to other Toll-like receptor (TLR) ligands or pro-inflammatory cytokines tested (**chapter 4**). Poly(I:C) is a synthetic ligand of TLR3, which is a receptor that senses dsRNA of viral origin. We found that TECs also expressed the cytoplasmic dsRNA receptors melanoma differentiation associated gene 5 (MDA5) and retinoic acid inducible gene-1 (RIG-I) and that their activation induced expression of serpinB9.

Immune and non-immune cells express dsRNA receptors to sense the presence of a virus. Upon ligand binding, dsRNA receptors can activate anti-viral, pro-inflammatory and pro-apoptotic responses ³. We found that renal expression of TLR3, MDA5 and RIG-I was increased during cytomegalovirus (CMV), Epstein-Barr virus (EBV) and BK virus (BKV) infection in kidney transplant recipients (**chapter 6**), suggesting that the dsRNA receptors play a role in the recognition of viral infection in the kidney. Immunohistochemical studies showed that each dsRNA receptor had its unique expression pattern, yet all were expressed in the tubuli. To address the role of dsRNA receptors in TECs, we stimulated primary cells with synthetic ligands for TLR3, MDA5 and RIG-I. We observed that these ligands induced a variety of responses designed to limit viral replication and spread. dsRNA receptor activation induced the production of pro-inflammatory and anti-viral cytokines (**chapter 6**), which can promote activation of the immune system and alert surrounding parenchymal cells. In **chapter 5**, we showed that dsRNA also enhanced the expression of various pro-apoptotic mediators like Noxa and Puma and rendered TECs sensitive to apoptosis mediated by the death receptor CD95. Additionally, dsRNA receptor triggering induced the expression

of several anti-apoptotic proteins, explaining why TECs were resistant to single apoptotic stimuli like dsRNA or CD95-ligand. A better understanding of the regulation of protective proteins, like serpinB9 and inhibitor of apoptosis proteins (IAPs), might help to design therapies that can reduce injury in the kidney caused by allo- or auto-immune reactions, toxic side effects of drugs and/or infections.

The impact of viruses is not restricted to the cells they infect, such as TECs. In **chapter 7**, we demonstrate that CMV also influences cells of the immune system. CMV-induced systemic activation of the immune system characterized by elevated levels of Th1 cytokines such as interleukin (IL) 18 and interferon (IFN) γ and the interferon inducible protein 10 (IP-10), which was maintained during latency. Endothelial cells, which are a known reservoir for CMV, can produce the chemokine IP-10 leading to the recruitment of virus specific CD8⁺ T cells which can in turn enhance IP-10 expression by secreting IFN γ ⁴. The cross-talk between various cell types, including cytotoxic lymphocytes and endothelial cells but also TECs and antigen-presenting cells (APCs), is a crucial factor in the development and propagation of viral infection and interstitial nephritis and thus the severity of tissue injury. Understanding the molecular mechanisms might help to develop treatments to reduce immune responses or to specifically stimulate anti-viral immunity.

Monitoring and diagnosis of inflammation in the renal transplant

To prevent tubular injury it is important to detect tubulointerstitial inflammation in an early phase. Renal allograft function is commonly monitored by analyzing changes in creatinine clearance ⁵. One should keep in mind that renal dysfunction is only apparent when inflammation has led to destruction of a significant percentage of the nephrons. Additional clinical signs of renal inflammation can be fever, nausea, malaise, rash, eosinophilia, abnormal urinary sediment and/or proteinuria ⁶. However, a lot of patients present with only some of these symptoms and they are associated with many other conditions. For that reason, diagnosis of tubulointerstitial nephritis depends on histological examination of renal transplant biopsies, which is characterized by leukocytes infiltrating the renal interstitium and tubuli and edema.

Although considered the golden standard, histological examination of renal biopsies has disadvantages such as inter-observer variability, sampling errors and invasiveness of the procedure ^{7,8}. It would be beneficial for the patient, if inflammation could be detected in an early phase by using a non-invasive method. Therefore, we and many others have explored the potency of biomarkers, which ideally are specific and sensitive and can be measured in a fast, cheap and patient-friendly manner. Methods employed by others include measurement of inflammatory mediators at the transcript or protein level in serum ⁹⁻¹¹, peripheral blood mononuclear cells (PBMCs) ¹²⁻¹⁵, urine ^{9,11,16} and urinary cells ^{14,16-19}. We have analyzed gene-transcription levels of GzmA, GzmB, perforin and serpinB9 in urinary cells. In **chapter 3**, we show that

GzmA mRNA entails a sensitive and specific biomarker to diagnose TCMR even in a subclinical stage.

The identification of the ideal biomarker is hindered by the lack of specificity of many markers, which is not surprising considering that the inflammatory mediators in different forms of renal disease are comparable. We found for example that urinary GzmA was also elevated during CMV infection. CMV infection is easily ruled out by routine PCR. However, we cannot exclude that other viruses, which are not routinely tested, induce an increase in urinary GzmA levels like CMV. Another pitfall is the sensitivity of the test. Inflammation is a gradual process and it is not always clear above which cut-off value treatment is preferred. The detection of GzmA mRNA appears to correlate well with the presence of inflammation in the kidney, in other words the need for therapeutical intervention. Still, sensitivity of urinary GzmA mRNA can be improved, since the biomarker was undetectable in a small number of patients with biopsy confirmed TCMR. Urine seems a perfect compartment to monitor renal function considering the easy and unlimited supply. Nevertheless, it is not possible to analyze patients that lack urine production due to for example delayed graft function.

Currently, none of the proposed biomarkers has made its way to the clinic. The development of new techniques to study multiple genes simultaneously has led to the identification of molecular phenotypes that correspond to for example TCMR and antibody-mediated rejection (ABMR) ²⁰. At the moment, these techniques are expensive and time consuming, but they might help to develop a sensitive and specific test which includes multiple biomarkers in the future.

Subclinical versus acute rejection: differences and similarities

It has long been known that the histological presence of interstitial cellular infiltrates does not always coincide with a reduced renal function ^{21;22}. The incidence of SCR varies and depends on amongst others immunosuppression and composition of the patient cohort studied ^{8;23}. SCR is associated with the development of interstitial fibrosis and tubular atrophy ²⁴⁻²⁸, however the value of protocol biopsies and treatment of SCR is controversial ^{8;29-32}.

The cellular and molecular mechanisms underlying SCR are not well understood, but histologically SCR is indistinguishable from TCMR ^{33;34}. In **chapter 3**, we show that the urinary cell expression pattern of GzmA, GzmB, perforin and serpinB9 is similar in SCR and TCMR. In agreement with these findings, others found no differences in composition and number of graft infiltrating leukocytes between SCR and acute TCMR, including the number of GzmB⁺ and perforin⁺ T cells ³³. It might be that the absent decrease in renal function in SCR simply represents the degree of inflammation, which is determined by the percentage of the kidney involved as well as the activation status of graft infiltrating immune cells. This hypothesis is supported by the notion that expression levels of several inflammatory mediators are elevated during SCR compared to stable grafts, however to a lesser extent than during clinical TCMR. This

gradual expression has been described for chemokines like CXCL9 and CXCL10 (IP-10)³⁵, various urinary proteins³⁶ and transcripts of T cell activation and effector function³³.

On the other hand, one can envision that SCR results from mechanisms that suppress allo-responses or protect the kidney against inflammation induced injury. Specialized cells of the immune system, CD25^{high} FoxP3⁺ Tregs, suppress effector T cell activity and in this way may reduce tubular injury in SCR. Indeed, the proportion of Tregs within the infiltrating CD4⁺ T cells is elevated in biopsies of SCR compared to TCMR³⁷ and a high proportion of Tregs is associated with a better long-term graft function in patients with diagnosed with SCR³⁸. A mild form of immune activation might be required for the induction of immunological tolerance. Transplantation tolerance, defined as the lack of a destructive immune response towards the transplant in absence of immunosuppression, remains the ultimate goal in kidney transplantation³⁹. Various groups have explored the strategies to promote allograft acceptance by for example enhancing the generation of Tregs, unfortunately these strategies are not yet reproducible, safe and effective in human⁴⁰.

Additionally, resident renal cells, which are equipped with various mechanisms to prevent injury and preserve renal function, can determine the degree of renal injury during an allo-immune response. Our group demonstrated that during SCR, tubular serpinB9 expression is increased suggesting that the cells are less sensitive to GzMB-mediated injury⁴¹. In contrast, urinary serpinB9 mRNA expression was not enhanced during SCR (**chapter 3**). The urine sediment consists of multiple cell types including T cells, TECs and granulocytes. During transplant rejection cell number and composition are altered⁴². The presence of serpinB9 expressing lymphocytes might disguise an increased serpinB9 expression in TECs in the urine, explaining the discrepancy found between the histological and urinary studies. Another way by which TECs may prevent injury, is by reducing T cell activity via the expression of co-inhibitory molecules and anti-inflammatory cytokines like transforming growth factor- β (TGF- β)^{43,44}. Of note, these protective mechanisms do have their drawback. For example, TGF- β plays an important role in the development of interstitial fibrosis and tubular atrophy⁴⁵.

In conclusion, the degree of immunopathology and graft dysfunction during cellular allo-immune responses is determined by both immune cells, like Tregs, and non-immune cells, such as TECs, interstitial cells and endothelial cells. Which cell types and mediators preserve renal function during SCR remains to be identified, but insights in these mechanisms may help to develop therapies to reduce renal injury during rejection, ischemia reperfusion (I/R) injury and viral infection.

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TECs are immunoregulatory cells

As mentioned above, TECs are not silent bystanders during an immune response but actively regulate the inflammatory process. One could even consider TECs as an extension of the immune system⁴⁶. First of all, TECs are equipped to sense the presence of pathogens due to expression of a broad set of pattern recognition receptors (PRRs),

including Toll-like receptors (TLRs)⁴⁷, NOD-like receptors (NLRs)⁴⁸ and C type lectin-like receptors (CLRs)⁴⁹. In addition, we found that TECs also expressed RIG-I-like receptors (RLRs) (**chapter 4**). Some of these receptors are preferentially expressed by immune cells; for example TLR3 has first been identified as a dendritic cell (DC)-specific receptor⁵⁰. TLR3 is known to be expressed at low basal levels in various other cell types and tissues. We and others found that expression of TLR3 is induced by its ligand dsRNA (**chapter 4**)^{51;52}. Secondly, TECs contribute to the immune response by secreting effector and regulatory cytokines upon activation. For example during allograft rejection, TECs produce several cytokines and chemokines⁵³⁻⁵⁵. *In vitro*, the same soluble mediators are secreted by TECs after stimulation with pro-inflammatory cytokines like TNF α , IFN γ and IL-1⁵⁶⁻⁵⁸. Thirdly, activated TECs express adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1) and lymphocyte function-associated antigen-3 (LFA-3)⁵⁹ enhancing recruitment of immune cells to the site of inflammation.

Another characteristic of immune cells is presentation of antigens. Tubulitis, a hallmark of tubulointerstitial nephritis, allows close contact between lymphocytes and TECs, which is required for proper antigen presentation. Several studies indicate that under inflammatory conditions TECs have the capacity to act as non-professional APCs. For example, cytokines like TNF α and IFN γ induce the expression of MHC class I and II^{59;60}. Finally, TECs are known to express co-stimulatory molecules including CD40 and CD80^{61;62} and co-inhibitory receptors such as programmed death ligand 1 (PD-L1)⁶³, which enable them to modulate T cell proliferation and effector function. In conclusion, TECs are fully equipped to modulate local immune responses and to regulate the activity of kidney infiltrating lymphocytes.

Viral infection in the kidney

As addressed in the introduction, the most common viral infections in kidney transplant recipients are CMV, EBV and BKV. Despite the fact that all these DNA viruses can infect TECs, their clinical manifestation is diverse. For example BKV infection is commonly associated with nephropathy⁶⁴, while EBV rarely causes interstitial nephritis⁶⁵. These differences can be explained by variations in target cell preference and type of viral life cycle. EBV principally infects B cells⁶⁶, which can lead to post transplant lymphoproliferative disorder⁶⁷, while BKV has a lytic replication in epithelial cells of the kidney and urinary tracts^{64;68}. In addition to these viruses, although less common, adenovirus, SARS-coronavirus, human immunodeficiency virus, hepatitis B and C virus, parvovirus and hantavirus have been associated with the development of various forms of nephropathy in kidney transplant recipients and/or immunocompetent individuals⁶⁹⁻⁷⁵.

Viral recognition

Anti-viral immune responses start with detection of the virus. Immune and non-immune cells are therefore equipped with an array of receptors that recognize virus associated molecular patterns. As stated above, we found that TECs express TLR3, MDA5 and RIG-I (**chapter 2 and 6**). Additionally, TECs are known to express TLR2 and TLR4, which recognize certain viral proteins and thereby contribute to the initiation of an anti-viral immune response ⁷⁶. The expression of the ssRNA sensors TLR7/8 and TLR9 recognizing CpG-rich DNA, is considered to be restricted to specific immune cells like plasmacytoid DCs. Whether TECs express these endosomal receptors is controversial ⁷⁷. We could neither detect TLR7 and TLR9 transcripts in primary TECs, nor did the cells respond to stimulation with their ligands imiquimod and CpG DNA (data not shown), making it quite likely that indeed TECs do not possess TLR7 and TLR9. Recently, two cytoplasmic DNA sensors have been identified: “DNA-dependent activator of IFN-regulatory factors” (DAI, ZBP1) and “absent in melanoma 2” (AIM2) ⁷⁸. The role of these receptors and possible other unidentified cytoplasmic DNA sensors in TECs is unknown. It would be beneficial if the cells possess at least one sensor that recognizes viral DNA, since many viruses known to infect TECs have a DNA genome. Two recent studies have demonstrated that RNA polymerase III can transcribe AT-rich dsRNA into 3pRNA, which in turn activates RIG-I ^{79;80}. This novel DNA-sensing pathway is involved in the transcription of EBV encoded small RNAs and their capacity to activate RIG-I. It might well be that this pathway plays an important role in the recognition of DNA viruses in TECs.

Anti-viral responses

Recognition of the virus is followed by the induction of a range of signaling cascades aimed to suppress viral replication and alert the immune system. Activated APCs migrate to draining lymph nodes to prime virus-specific T cells. Local production of cytokines and chemokines and expression of adhesion molecules ensues recruitment of leukocytes to the site of infection. In **chapter 6**, we show that activation of viral dsRNA receptors induces the production of the pro-inflammatory cytokines IL-6 and TNF α , the chemokine IP-10 and the anti-viral cytokine IFN β in TECs.

In some cell types dsRNA receptor triggering promotes cell death, either directly ⁸¹⁻⁸⁶ or via type I IFNs ⁸⁷. We found that in TECs, viral dsRNA by itself did not induce apoptosis but did sensitize the cells to CD95-mediated cell death (**chapter 5**), which is *in vivo* carried out by CD95-ligand bearing lymphocytes. Next to this death receptor apoptosis pathway, cytotoxic lymphocytes can eliminate virus-infected cells via the granzyme-mediated apoptosis pathway ⁸⁸. In **chapter 4**, we found that activation of dsRNA receptors also induced the expression of serpinB9. In a broader context, this would imply that viral dsRNA sensitizes TECs to death receptor mediated-apoptosis but at the same time protects against Gzmb-mediated cell death, which seems contradictory. It would be interesting to determine whether TECs stimulated with

dsRNA would undergo apoptosis when they encounter CD95-ligand and GzmB/perforin expressing CTLs. Little is known about the relative contribution of the death receptor- and granzyme-mediated apoptosis pathways to the elimination of virus infected human cells *in vivo*. Mouse studies suggest that usage of the apoptotic pathways depends on the type of virus and stage of infection^{89;90}. Both pathways can complement and/or substitute each other, which is useful considering that many viruses have evolved mechanisms to interfere with apoptosis^{91;92}. Timing of the above proposed experiment might be essential, since we observed that the induction of various apoptotic mediators including Noxa and Puma occurred fast, within 2 hours after stimulation (**chapter 5**). In contrast serpinB9 induction seems to lack behind a couple of hours (**chapter 4 and 5**).

Furthermore, we found that in general induction of anti- and pro-apoptotic proteins coincides, for example Noxa and its antagonist Mcl-1. Simultaneous induction of anti-apoptotic proteins might provide a safeguard against uncontrolled cell death, by which tubular integrity is maintained. Moreover, cells that sense dsRNA of non-viral origin, e.g. released from necrotic cells, and do not present viral antigens in MHC molecules are not recognized by CTLs. In this scenario, it might be beneficial to protect these uninfected cells against misdirected GzmB released during the immune response against surrounding infected cells.

In conclusion, activation of viral dsRNA sensors promotes the expression of anti- and pro-apoptotic mediators. Depending on the virus, its capacity to interfere and/or exploit host signaling cascades and on the activation status of the TEC, dsRNA receptor activation will affect the sensitivity of the infected cell to apoptosis triggered directly by the virus or mediated by cytotoxic lymphocytes.

The impact of chronic viral infection

Viruses have a great impact on the cells they infect, but also influence the activity of surrounding parenchymal- and immune- cells. In **chapter 7**, we demonstrate that viral persistence results in a systemic activation of the immune system in both CMV infected kidney transplant recipients and healthy volunteers. We observed increased serum levels of the acute phase proteins C-reactive protein and amyloid A and the Th1 cytokines IFN γ , IL-12 and IL-18, which were maintained during latency. Furthermore, the adhesion molecules ICAM-I and VCAM-I and the chemokine IP-10 were elevated during CMV infection, implying activation of the endothelium. CMV can infect both leukocytes and endothelial cells. Activation of these cells can be the direct result of viral infection or be due to ongoing anti-viral immune responses. CMV is known to trigger various PRRs; envelope glycoproteins of the virus are recognized by TLR2⁹³ and DC-SIGN⁹⁴ and TLR3 and TLR9 are activated in infected cells⁹⁵. Furthermore, CMV itself employs host signaling pathways to its advantage: the early gene US28, an chemokine receptor, can activate NF κ B⁹⁶, while genes expressed later during infection suppress activity of the transcription factor⁹⁷. We observed that the expression of TLR3, MDA5

and RIG-I in kidney transplant biopsies was enhanced during viral infection (**chapter 6**). dsRNA receptor expression can be induced by their own activation as well as by type I IFNs (**chapter 4**). CMV is known to induce type I IFN production in infected cells, also in a TLR3 independent pathway⁹⁸, suggesting that the virus can activate cytoplasmic and/or endosomal nucleic acid receptors. Additional experiments are required to identify via which receptor and signaling pathway CMV promotes dsRNA receptor expression. Studying anti-viral responses to CMV, one ought to keep in mind that CMV evolved several mechanisms to suppress interferon signaling in infected cells⁹⁹. For that reason it will be necessary to include various time points after infection and investigate mediators located at the beginning of the signaling cascade.

The role of inflammasomes in the kidney

In addition to the TLRs and RLRs discussed in the previous paragraph, viral nucleic acids can activate various NLRs¹⁰⁰. NLRs, like NLRP3, form multi-protein complexes, known as inflammasomes in which caspase 1 is activated¹⁰¹. The inflammatory cytokines IL-1 β and IL-18 are produced in a pro-form and require cleavage by caspase-1 to become functional. Several of these NLR members are known to be expressed in the human kidney^{102;103}. Various studies suggest that NLRP3 inflammasome activity contributes to renal injury induced by for example ischemia. NLRP3 expression is indeed enhanced in kidneys of patients with acute or chronic kidney disease and transcript levels of the NLR correlate with serum creatinine values¹⁰⁴. Furthermore, tubular injury and inflammation after renal ischemia are reduced in NLRP3^{-/-} mice¹⁰³⁻¹⁰⁵. The NLRP3 inflammasome is activated by multiple compounds including uric acid crystals, viral DNA, microbial toxins and ATP¹⁰⁶⁻¹⁰⁸. During renal injury, various endogenous danger-associated molecular patterns (DAMPs) are released. It is not precisely known which of those activate NLRP3, but ATP produced by mitochondria released from necrotic cells can activate NLRP3 *in vitro*¹⁰⁵. Although NLRP3 is clearly involved in renal injury, it remains controversial whether NLRP3-mediated production of pro-inflammatory cytokines is essential^{48;109}.

Next to macrophages and DCs, human and murine TECs express NLRP3¹⁰³ and bone marrow chimeras showed that NLRP3 has a biological function in both hematopoietic and renal compartments during renal injury^{103;104}. Furthermore, necrosis and apoptosis was reduced in tubular cells of NLRP3^{-/-} mice suggesting that NLRP3 can promote cell death independent of its capacity to generate active IL-1 β ^{48;104}. Indeed activation of the NLRP3 inflammasome can promote PARP1 cleavage and membrane permeabilization¹¹⁰, which are both hallmarks of apoptosis. Also other NLRs have been shown to contribute to various forms of cell death¹¹¹.

To address the role of the inflammasome in TECs, we stimulated primary TECs with several known NLRP3 ligands like LPS, imiquimod and uric acid crystals. None of these stimuli induced production of pro-IL-1 β or IL-1 β (unpublished findings), which is in agreement with data from others¹⁰⁴. Interestingly, we observed that extracellular

poly(I:C) and TNF α induced the production but not the processing of pro-IL-1 β (Fig. 1). TECs did express pro-caspase 1 and poly(I:C), delivered extracellularly or intracellularly, enhanced its expression, suggesting that the cells are equipped to form a functional NLRP3 inflammasome, yet the proper trigger to activate the complex is currently unknown. Further research is needed to unravel if the NLRP3 inflammasome or alternative inflammasomes play a role in TECs. Recent publications indicate that viruses can trigger IL-1 β secretion, which might be the case in TECs as well. It has been shown that RIG-I can form a complex with ASC leading to caspase 1 activation and that some viruses, but not poly(I:C), can induce activation of the NLRP3 inflammasome via MDA5¹¹². In addition to caspase 1, caspase 8 can process pro-IL-1 β ; activation of TLR3 and TLR4 can trigger this pathway in macrophages¹¹³. Furthermore, viral DNA can activate the AIM2 inflammasome leading to the secretion of IL-1 β and IL-18 and to caspase 1-mediated cell death^{114;115}. In conclusion, pathways that promote IL-1 β secretion in TECs differ from those found in macrophages and DCs. Identification of the pathogen-associated molecular patterns (PAMPs) and/or DAMPs that trigger IL-1 β secretion and of the involved inflammasome compounds in TECs, can help to understand the role of NLRs in the pathology of for example I/R-injury and virus-mediated interstitial nephritis.

The suitability of the cell line HK-2 as model for tubular epithelial cells

HK-2 is a commonly used cell line with a phenotype that resembles epithelial cells from the proximal tubuli. The line originates from primary human TECs immortalized by transduction with the human papilloma virus E6 and E7 genes¹¹⁶. We found significant differences between HK-2 cells and primary TECs regarding the expression and activity of several genes (unpublished observations). First of all, expression levels of serpinB9

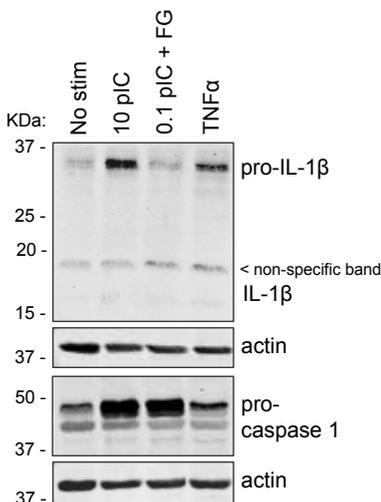


Figure 1: Expression of IL1 β and caspase 1 in primary human tubular epithelial cells. Primary TECs were stimulated with poly(I:C) (pIC, 10 μ g/ml), poly(I:C) and fucose (pIC + FG, 0.1 μ g/ml) or TNF α (100 ng/ml) for 16 h. Expression of (pro-)IL-1 β and pro-caspase 1 was determined by Western blot analysis.

and serpinB2 were absent in HK-2 cells, independent of stimulation with cytokines or TLR-ligands. Next, resting HK-2 cells expressed MDA5 and RIG-I but almost no TLR3. Like in primary TECs, activation of the cytoplasmic sensors upregulated expression of the three dsRNA receptors in HK-2 cells (Fig. 2). However, compared to primary TECs ~100 fold more dsRNA was needed to induce transcription, suggesting that MDA5 and RIG-I signaling is dampened in HK-2 cells. Analysis of cytokine expression in dsRNA stimulated HK-2 cells revealed that NF κ B and IRF signaling pathways were intact but dampened. Finally, we found that overexpression of TLR3 or MDA5 did not restore dsRNA induced serpinB9 expression in HK-2 cells (data not shown). Hence, HK-2 cells might not be a suitable model to address anti-viral responses in TECs.

TECs: one of a kind?

As discussed in the previous paragraphs, TECs are multifunctional cells. Their primary function is reabsorption and secretion of solutes. Considering that TECs form the barrier between urine and blood, it is understandable that they are equipped to recognize pathogens and alert the immune system. In addition, TECs are specialized in controlling damage, which will help to maintain tubular integrity during infection, inflammation and tissue injury. Interestingly, TECs have the capacity to execute immune functions as mentioned before. One may wonder whether this multifunctionality is unique for TECs or that it is a common characteristic shared with other epithelial cells or non-immune cells in general. Many pathways found in TECs are present in other cell types. For example, dsRNA induced the expression of serpinB9 in TECs but also in monocyte derived DCs and fibroblast-like synoviocytes (FLS) (data not shown), indicating that this signaling pathway is present in different cell types including immune and non-immune cells. Another hallmark of TECs, is their resistance to various apoptotic stimuli like CD95-ligand and viral dsRNA. TECs share their relative resistance to CD95-mediated apoptosis with other type II cells like hepatocytes, pancreatic β -cells and FLS¹¹⁷⁻¹¹⁹. We found that dsRNA receptor activation did hardly affect TEC viability. Triggering dsRNA induced the expression of various apoptotic mediators and we did not observe major differences between activation of TLR3, MDA5 and RIG-I (**chapter 5**). Many studies of apoptotic pathways are performed in cell-lines or cancer cells. The expression pattern of apoptotic mediators observed in these cells might significantly differ from the pattern observed in primary cells or *in vivo*. For example, MDA5 and RIG-I activation triggers apoptosis in melanoma cells, but not in human melanocytes due to expression induction of Bcl-XL in the unmutated cells⁸¹. In contrast, melanoma cells remain viable after activation of TLR3, unless protein synthesis or IAPs were blocked⁸⁶.

Expression of PRRs and activation of downstream signaling cascades are subject to the function of the cell, its location and/or the type of pathogens it can encounter. A nice example are two subtypes of DCs in the skin. Dermal DCs express a broad spectrum of TLRs and can sense pathogens of various origins. In contrast, Langerhans

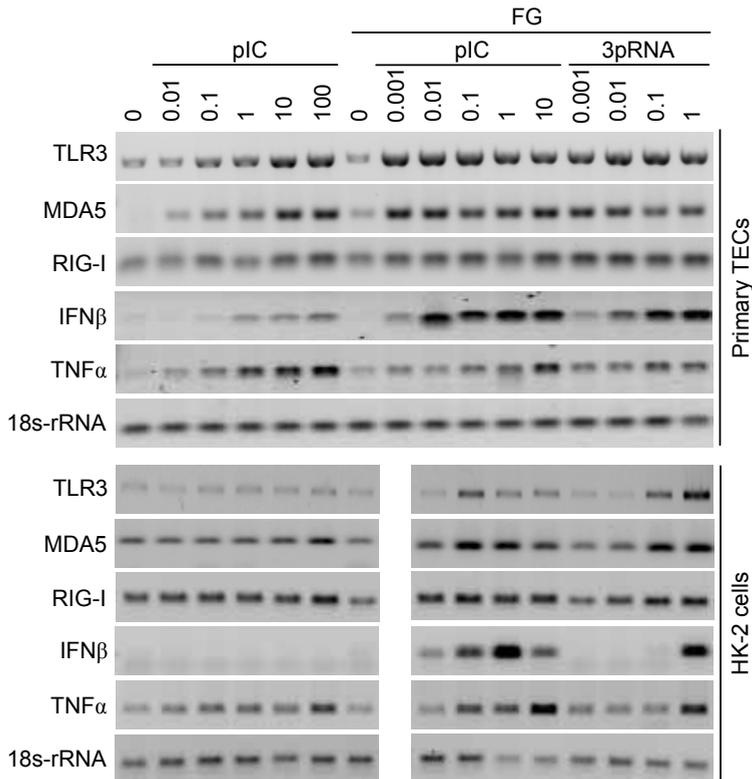


Figure 2: Expression and function of dsRNA sensors in primary TECs and HK-2 cells. Primary TECs and HK-2 cells were stimulated with poly(I:C) (pIC, 0.001-100 $\mu\text{g}/\text{ml}$) or 3pRNA (0.001- 1 $\mu\text{g}/\text{ml}$) for 24 h. Cytoplasmic delivery of the dsRNA mimics was accomplished with the transfection reagent Fugene (FG). Expression of TLR3, MDA5, RIG-I, IFN β and TNF α was analyzed by PCR.

cells, which are located in the epidermis, are specialized in the recognition of viral TLR ligands and do not respond to bacterial PAMPs¹²⁰. In conclusion, cell type specific responses to PAMPs and DAMPs are dependent on the location and function of a cell and whether it is stimulated by cytokines or in a stressed state e.g. due to DNA damage. In this context, the TEC is not one of a kind but it shares responses to pathogens and various kinds of danger with other cell types.

Concluding remarks

This thesis addresses the impact of inflammation on the kidney, which can originate from an allo-immune response, viral infection or I/R-injury. Independent of the etiology, tubulo-interstitial nephritis is characterized by the presence of innate and adaptive immune cells and involves tubular, endothelial and interstitial cells. The amount of tubular injury and decrease in renal function is for an important part

determined by the cross-talk between immune, renal and endothelial cells. Tubular cells are most likely one of the first to encounter a pathogen in case of a viral or bacterial infection. For that reason, TECs serve important roles in alerting the immune system and limiting damage until leukocytes have arrived at the site of inflammation. In addition, the cells are equipped to communicate with immune cells via secretion of cytokines and chemokines thereby modulating immune responses locally. Thus, TECs are no silent bystanders that endure the attack of cytotoxic immune cells without objection.

To fully understand the pathology of tubulo-interstitial nephritis it is essential to identify the pathways that are activated by pathogens, injury and inflammation and to understand how inflammatory, anti-viral and apoptotic responses intertwine. We focused on the responses in TECs and found that apoptosis is a complex process which involved numerous mediators which can reinforce or inhibit each other. Induction of a pro-apoptotic protein always seems to coincide with induction of an anti-apoptotic counterpart. Furthermore, pro-apoptotic responses occurred together with anti-viral immune responses. This delicate balance is important to prevent uncontrolled cell death and maintain tubular integrity, but at the same time make efficient elimination of the pathogen possible.

It would be interesting to investigate how our *in vitro* observations relate to the pathogenesis of interstitial nephritis *in vivo*. These experiments will improve our understanding of the apoptotic and anti-viral signaling cascades that determine the fate of a TEC that has encountered a virus or allo- or auto- reactive T cell *in vivo*. Knowledge about these signaling pathways might provide cues for the design of drugs, which specifically interfere with processes like tubular damage due to infection, inflammation or toxic compounds.

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