

File ID 212297  
Filename Chapter 1: Introduction

---

SOURCE (OR PART OF THE FOLLOWING SOURCE):

Type Dissertation  
Title Recognition of infection and inflammation in the kidney  
Author K.M. Heutinck  
Faculty Faculty of Medicine  
Year 2011  
Pages 208  
ISBN 978-94-90371-72-2

FULL BIBLIOGRAPHIC DETAILS:

<http://dare.uva.nl/record/373821>

---

*Copyright*

*It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use.*

---

# 1

---

INTRODUCTION



## INTRODUCTION

**Acute interstitial nephritis** (Councilman 1898) - An acute inflammation of the kidney characterized by cellular and fluid exudation in the interstitial tissue, accompanied by, but not dependent on, degeneration of the epithelium; the exudation is not purulent in character, and the lesions may be both diffuse and focal <sup>1</sup>.

Interstitial nephritis is a frequent cause of acute kidney failure, accounting for 15-27% of the biopsies performed because of renal dysfunction <sup>2</sup>. The majority of acute interstitial nephritis is drug-induced. Other common etiologies are bacterial or viral infections and systemic diseases like sarcoidosis and systemic lupus erythematosus. A significant proportion of the patients with acute interstitial nephritis, ranging from 30-70%, does not fully recover to baseline renal function after treatment. In the Netherlands, the number of patients with end-stage renal disease is increasing; in 2002 about 1 out of 10.000 people required renal replacement therapy <sup>3</sup>. Up to date, more than 6000 patients depend on dialysis and approximately 8500 patients have a functioning kidney transplant ([www.renine.nl](http://www.renine.nl)). Kidney transplant recipients receive immunosuppressive drugs to prevent rejection, which is successful in the great majority of patients. The downside of these drugs is an increased incidence of infections and toxicity to the kidney, which may manifest itself by the picture of an interstitial nephritis.

This introduction addresses the biological background of kidney inflammation and the general cellular and molecular mechanisms involved. Inflammation and apoptosis are the most important processes contributing to tissue injury and loss of renal function. To understand the pathology of interstitial nephritis, one should be familiar with the signaling pathways that are activated in response to infection and tissue injury. Below I will summarize how cells recognize and respond to signals of pathogens and danger, which cell types play a role in kidney inflammation and how these cell types interact by either activating, suppressing or even killing each other.

### Inflammation of the kidney

The primary task of the immune system is to distinguish non-self from self and to neutralize, eliminate or metabolize that what is foreign <sup>4</sup>. The first immune cells to be activated upon encounter of a pathogen are cells of the innate immune system consisting of granulocytes, natural killer (NK) cells, monocytes and macrophages. In addition mammals have a specific so called adaptive immune system, in which antigen-specific T and B lymphocytes that undergo clonal selection are the key players. The adaptive immune system is characterized by the formation of immunological memory, which ensures a more rapid response upon a second encounter of a pathogen. Pathogens trigger the production of cytokines and chemokines by various

cell types, including macrophages. These soluble mediators initiate a cascade of reactions leading to vasodilatation, increased vascular permeability and influx of leukocytes that release additional inflammatory mediators. This process, known as inflammation, is characterized by in Latin *calor, dolor, rubor, tumor* and *functio laesa* (heat, pain, redness, swelling and loss of function) <sup>4,5</sup>. Inflammatory responses are not restricted to pathogens but can also be induced by for example tissue injury and allo- or auto-antigens.

In the kidney, hypersensitivity reactions to drugs, infection, autoimmune diseases or renal allograft rejection are common causes of tubulointerstitial nephritis, which is characterized by immune cells infiltrating the interstitium and tubuli leading to tubular injury and decline in renal function <sup>6</sup>. The cellular infiltrate consists of T lymphocytes, monocytes, NK cells, macrophages and occasionally plasma cells and eosinophils. The cross-talk between infiltrating lymphocytes and resident renal cells plays an important role in the pathology of interstitial nephritis. Immune and non-immune cells can sense the presence of a pathogens with specialized receptors that recognize a wide spectrum of viruses, bacteria and fungi. These so called pattern recognition receptors (PRRs) are activated by pathogen associated molecular patterns (PAMPs) leading to the local production of chemokines and cytokines that attract and activate immune cells.

Infiltrating lymphocytes subsequently stimulate the expression of MHC molecules and adhesion molecules such as ICAM-I on renal tubular epithelial cells (TECs). The activated TECs successively produce growth factors, extracellular matrix components and additional cytokines and chemokines that enhance the immune response and activate tissue repair mechanisms <sup>7</sup>. The drawback of inflammation is loss of renal function due to tissue damage and scar formation. For that reason mechanisms that confine inflammatory responses are essential. Specialized regulatory T cells prevent excessive immune cell activation via amongst others the secretion of suppressive cytokines IL-10 and TGF $\beta$  <sup>8</sup>. In addition, non-immune cells are equipped with immunosuppressive mechanisms, for example activated TECs express the co-inhibitory molecule PD-L1 to diminish T cell proliferation and cytokine production <sup>9</sup>.

Stressed or dying cells can add an extra element to the immune response via the release of so called danger associated molecular patterns (DAMPs), like heat-shock proteins and nucleic acid structures, which trigger receptors that also recognize PAMPs <sup>10</sup>. It is important to keep in mind that all non-inflammatory forms of kidney injury, caused by for example ischemia, toxic compounds or metabolic stress, will to some extent become inflammatory due to DAMP-mediated production of cytokines and chemokines.

### **Inflammatory complications after kidney transplantation**

The best available treatment for end-stage kidney disease is renal allograft transplantation, which is associated with a better patient survival compared to

long-term dialysis<sup>11</sup>. The long-term goal of transplantation is acceptance of the renal allograft with minimal immunosuppression. To maintain the delicate balance between immunological injury and the adverse effects of immunosuppressive drugs, it is important to have tools to monitor graft status and identify the cause of decreased graft function at an early stage<sup>12</sup>. In transplant recipients, tubulointerstitial nephritis can be allograft-related including T cell-mediated rejection (TCMR) and ischemia reperfusion (I/R) injury or attributable to immunosuppressive drugs such as opportunistic infections and calcineurin inhibitor toxicity<sup>13,14</sup>. Although different forms of tubulointerstitial nephritis can have a comparable clinical manifestation, therapeutic intervention strongly depends on the etiology.

### *Renal allograft rejection*

The recipient-immune system recognizes a renal allograft as non-self. Therefore, rejection of the transplant will occur in the absence of immunosuppression. Rejection is a well known complication after kidney transplantation and can be classified as T cell- or antibody-mediated, based on histopathological analysis of biopsies, and acute or chronic, dependent on time after transplantation<sup>15,16</sup>. It is important to note that cellular and humoral responses commonly occur together. However, the cellular response is more prominent than the humoral response in the majority of the acute rejection episodes<sup>14</sup>.

Antibody-mediated rejection (ABMR) is characterized by donor-specific antibodies directed against HLA-molecules, endothelial cell antigens and/or ABO blood-group antigens<sup>17</sup>. Selection of kidneys based on blood-group and HLA compatibility can reduce the risk of ABMR. Binding of antibodies to antigens, which are present on the endothelium of tubular and glomerular capillaries, induces complement activation and subsequent loss of vascular integrity. Endothelial injury triggers a cascade of events that includes thrombosis formation, ischemia, cytokine production, vasodilatation and the recruitment of leukocytes. Various therapies such as plasmapheresis, immunoadsorption and intravenous immunoglobulins are suitable to treat ABMR. The same treatments are used to prepare patients with pre-existing anti-HLA antibodies for transplantation.

In TCMR, kidney injury is mediated both directly by contact between cytotoxic T lymphocytes (CTLs) and renal cells, like endothelial cells and TECs, and indirectly by pro-inflammatory cytokines such as tumor necrosis factor  $\alpha$  (TNF $\alpha$ ) and interleukin-6 (IL-6) produced by various immune cells<sup>16</sup>. Naïf T cells are activated in lymphoid organs after encounter with donor or recipient derived antigen presenting cells (APCs). Differentiated effector T cells are recruited to the allograft, where they contribute to renal injury and dysfunction. Local inflammation also triggers dedifferentiation of epithelial cells, which leads to further reduction of the renal output<sup>18</sup>.

Next to these acute forms of rejection, a silent form of rejection exists namely subclinical rejection (SCR), which is characterized by the presence of graft infiltrating

lymphocytes without immediate decline in renal function. The mechanism underlying SCR is not well understood but in the long-term, SCR is associated with increased graft loss<sup>19</sup>.

#### *Acute tubular necrosis*

Acute tubular necrosis (ATN) is characterized by morphological changes in proximal tubuli including loss of the brush border and shedding of TECs into the lumen, leading to obstruction and reduced re-absorptive function<sup>20</sup>. Furthermore, ATN is generally associated with interstitial edema and mild to moderate leukocyte infiltration and is a common cause for delayed graft function, which refers to the requirement of dialysis within the first week after transplantation. Factors that affect the incidence of delayed graft function include cold ischemia time, duration of pre-transplant dialysis and donor body-mass-index<sup>21</sup>. Delayed graft function is associated with an increased risk for acute rejection episodes and graft loss, demonstrating that ATN has consequences in the long-term<sup>22</sup>. ATN is the result of damage induced by both ischemia and reperfusion<sup>20</sup>. Ischemia causes shortage of oxygen and nutrients, activates anaerobe glycolysis and leads to the accumulation of waste products and reactive oxygen species (ROS). Reconstitution of the blood flow rapidly restores the anaerobic metabolism resulting in the formation of ATP, which coincides with more ROS generation. The amount of ROS exceeds the capacity of the ROS scavengers present in the cell resulting in activation of the intrinsic apoptosis pathway and cell death. In addition, ROS induce the expression of cytokines, chemokines and adhesion molecules leading recruitment of neutrophils and later on monocytes, NK and T cells. Strategies to minimize I/R-injury include the optimalization of preservation solutions and administration of vasodilatory agents, anti-oxidants and anti-inflammatory drugs.

#### *Viral infections*

Next to rejection, viral infection is a common complication, which is associated with renal allograft failure and is a cause for morbidity and mortality in kidney transplant patients<sup>23</sup>. Viral infection generally occurs within the first year after transplantation, the period with the most stringent immunosuppression, and includes primary infection with allograft-derived viruses and reactivation of viruses latently present in the recipient. Human cytomegalovirus (CMV), Epstein-Barr virus (EBV) and BK virus (BKV) can infect various cell types of the kidney and are frequent in kidney transplant recipients.

CMV is a  $\beta$ -herpes virus that is latently present in 60-80% of the general population<sup>24</sup>. Infection is often asymptomatic but can induce a flu-like disease. The virus uses a glycoprotein complex to enter target cells, which include epithelial and endothelial cells, monocytes and lymphocytes. Leukocytes recruited to the primary site of infection can take up the virus, which is followed by viral spread through the bloodstream. In particular monocytes and DCs contain CMV DNA during latency<sup>25</sup>.

Once acute infection is controlled by the immune system, CMV persists in a latent phase. CMV has evolved multiple mechanisms to evade the immune system and resides inside monocytes and endothelial cells during latency. In kidney transplant recipients, CMV infection can be associated with clinical signs such as fever and has long-term effects on both the allograft and recipient immune system<sup>26</sup>. Prophylactic treatment strongly reduces the incidence of acute infection, however the long-term effects of this treatment are less well understood. CMV can be monitored by measuring CMV DNA in the blood or detecting viral proteins and/or DNA in allograft biopsies, PCR based methods are considered to be more sensitive but do not correlate well with clinical signs of disease<sup>27</sup>. CMV increases the risk of allograft rejection and mortality<sup>28;29</sup> and suppresses the immune system<sup>30;31</sup>, which allows opportunistic infections to flare. Furthermore, CMV has been associated with an increased risk for vascular diseases<sup>32</sup>.

EBV is a  $\gamma$ -herpes virus that infects primarily B cells but also other cells including TECs that express CD21, one of the receptors for EBV<sup>33;34</sup>. The virus is transmitted orally and primary infection is generally asymptomatic during childhood but can result in infectious mononucleosis in adolescents and adults<sup>35</sup>. Although rare, EBV infection has been associated with the development of interstitial nephritis and renal failure<sup>34;36</sup>. Primary infection is followed by life-time latency during which the virus persists in memory B cells avoiding recognition by other immune cells. In 90-95% of the adults antibodies against EBV proteins can be found, demonstrating that they are latently infected. CTLs play the most important role in controlling EBV infection<sup>37</sup>. EBV has the potency to immortalize infected host cells, which can lead to lymphomas predominantly of B cell origin. The EBV gene LMP1 plays an important role in B-cell transformation since it induces the expression of proteins that promote cell-growth and inhibit apoptosis<sup>38</sup>. When T cell function is suppressed, the risk of lymphoma development increases. The overall incidence of post transplant proliferative disorder (PTLD) in EBV infected kidney transplant recipients is 1-3%<sup>39</sup>; especially patients with a primary EBV infection are at risk. The importance of T cells in controlling EBV infection is underlined by the observation that induction therapy with OKT3 and anti-thymocyte globulin (ATG), antibodies that deplete T cells, is associated with an increased incidence of PTLD in kidney transplant recipients<sup>40;41</sup>.

BKV is a small circular dsDNA polyoma virus, which has been identified in 1971 in patient BK<sup>42</sup>. In healthy individuals, BKV infection has no clinical significance and approximately 80% of the population is latently infected. After primary infection, BKV persists in epithelial cells of the urinary tract. BKV enters target cells via binding to N-linked glycoprotein containing  $\alpha(2,3)$ -linked sialic acid, which is followed by caveolae-mediated endocytosis<sup>43</sup>. The frequency of active BKV replication in kidney transplant recipients has increased over the years due to the introduction of third generation immunosuppressive drugs like tacrolimus. BKV-associated nephropathy is histologically characterized by intranuclear viral inclusion bodies inside tubular cells

and severe tubular injury<sup>44</sup>. Tubular damage is the result of the lytic replication of BKV, which coincides with immune activation and influx of lymphocytes. Currently, about 1-10% of the kidney transplant recipients develop nephropathy due to active replication of BKV, which has been reported to result in graft failure in 50-90% of the cases. Better awareness and early diagnosis of BKV associated nephropathy can help to reduce graft loss to less than 10% due to timely reduction and/or adjustment of immunosuppression<sup>45</sup>.

### **Recognition of pathogen associated molecular patterns**

The presence of a virus or other pathogen is detected by immune and non-immune cells via receptors that recognize pathogen associated structures. These receptors are classified into the Toll-like receptors (TLRs), RIG-I-like receptors (RLRs), NOD-like receptors (NLRs) and C-type lectin-like receptors (CLRs), see Table 1.

#### *Toll-like receptors*

TLRs are transmembrane receptors located in the cell membrane or endolysosomal compartment that have N-terminal leucine-rich repeats and a cytoplasmic Toll/IL-1R homology (TIR) domain<sup>46</sup>. Downstream of the TLRs one can distinguish two signaling cascades mediated by myeloid differentiation primary-response protein 88 (MyD88) and TIR-domain containing adaptor inducing IFN $\beta$  (TRIF). These adaptors recruit various members of the TNF receptor associated factor (TRAF) family and induce activation and nuclear translocation of transcription factors such as nuclear factor  $\kappa$ B (NF $\kappa$ B) and interferon regulatory factors (IRF) 1, 3, 5 and 7 (Fig. 1). TLRs are widely expressed in cells of the innate and adaptive immune system; which members are expressed depends on cell type and function<sup>47</sup>. In dendritic cells, TLR activation promotes maturation and migration to lymphoid organs. Non-immune cells also express various TLRs, for example TECs are known to express TLR 1-4 and 6<sup>48</sup>. Non-immune cells respond to TLR-ligands by producing cytokines and chemokines and upregulating activation markers to alert the immune system. In addition, TLRs activate various cellular signaling pathways to help protect the host against the pathogen leading to for example apoptosis and expression of proteins that inhibit viral replication.

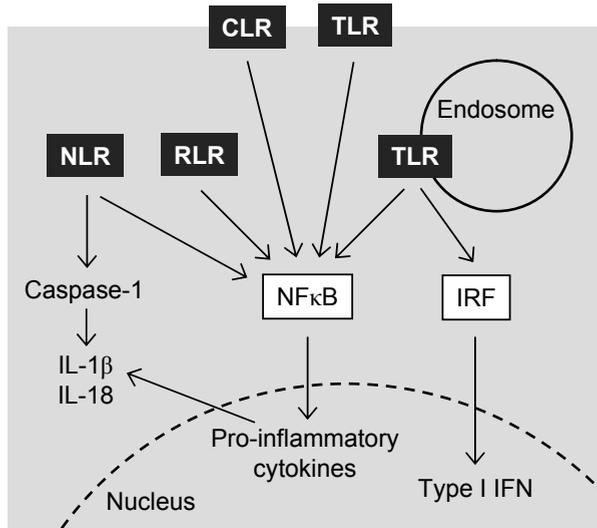
#### *RIG-I-like receptors*

The RLRs are broadly expressed cytoplasmic receptors that recognize viral dsRNA products<sup>49</sup>. The proteins are composed of two N-terminal caspase recruitment domains (CARDs), a DEAD box helicase/ATPase domain and a C-terminal regulatory domain. Retinoic acid inducible gene-I (RIG-I) and melanoma differentiation associated gene 5 (MDA5) bind specific forms of dsRNA and therefore recognize different types of viruses<sup>50-52</sup>, but share downstream signaling pathways leading to the induction of anti-viral and pro-inflammatory immune responses. The function of the third RLR member, LGP2, which lacks CARDs that are essential for interaction with downstream

**Table 1.** Pattern recognition receptors

Group	Members	Ligands	Pathogen	Location
Toll-like receptors (TLRs)	TLR1-TLR2	Triacyl lipoprotein	Bacteria	Plasma membrane
	TLR2	PGN, lipoproteins, porins, HA protein, tGPI-mucin	Bacteria, viruses, parasites	Plasma membrane
	TLR3	dsRNA	Viruses	Endolysosome
	TLR4	LPS, envelope proteins	Bacteria, viruses	Plasma membrane
	TLR5	Flagellin	Bacteria	Plasma membrane
	TLR6-TLR2	Diacyl lipopeptides, LTA, zymosan	Bacteria, fungi	Plasma membrane
	TLR7/8	ssRNA	Bacteria, viruses	Endolysosome
	TLR9	CpG-DNA, DNA, malaria hemozoin	Bacteria, viruses, parasites	Endolysosome
	TLR10	Unknown	Unknown	Endolysosome
	TLR11	Profilin-like molecule	Bacteria, parasites	Plasma membrane
RIG-I-like receptors (RLRs)	RIG-I	5'triphosphate RNA, short dsRNA, ssRNA	Viruses	Cytoplasm
	MDA5	Long dsRNA	Viruses	Cytoplasm
	LGP2	RNA	Viruses	Cytoplasm
NOD-like receptors (NLRs)	NOD1	iE-DAP	Bacteria	Cytoplasm
	NOD2	MDP	Bacteria	Cytoplasm
	NALP1	Anthrax lethal toxin	Bacteria	Cytoplasm
	NALP3	MDP, toxins, ATP, RNA	Bacteria, viruses	Cytoplasm
	IPAF	Flagellin	Bacteria	Cytoplasm
	NAIP	Flagellin	Bacteria	Cytoplasm
C-type lectin-like receptors (CLRs)	Dectin-1	$\beta$ -glucan	Bacteria, fungi	Plasma membrane
	Dectin-2	High mannose	Bacteria, fungi	Plasma membrane
	DC-SIGN	High mannose, fucose	Bacteria, viruses, fungi	Plasma membrane
	Mannose receptor	High mannose, fucose, sulphated sugars	Bacteria, viruses, fungi	Plasma membrane

signaling molecules, is less well understood<sup>53</sup>. RIG-I and MDA5 interact with the adaptor molecule IFN $\beta$  promoter stimulator 1 (IPS-1, also known as MAVS, Cardif and VISA) leading to the activation of the transcription factors NF $\kappa$ B and interferon regulating factor 3 and 7 (IRF3/7)<sup>54</sup>. NF $\kappa$ B induces the expression of pro-inflammatory cytokines while IRF3/7 promote the production of type I IFNs (Fig. 1). Type I IFNs in turn induce the transcription of over 300 IFN-stimulated genes. These genes regulate RNA stability and the synthesis, transport and turnover of proteins thereby inhibiting viral replication<sup>55,56</sup>. Furthermore, IFN $\alpha$  and IFN $\beta$  promote apoptosis in infected cells, enhance antigen presentation and induce the expression of adhesion molecules and



**Figure 1.** Pattern recognition receptor signaling pathways

chemokines, which facilitate the recruitment of leukocytes. Type I IFNs also directly activate dendritic cells and increase the cytotoxic potential of T cells<sup>57-59</sup>. Thus, cytoplasmic dsRNA sensors are potent inducers of anti-viral and pro-inflammatory responses.

Recent studies have revealed another function of viral dsRNA sensors; the induction of apoptosis. In various cancer cells activation of MDA5 or RIG-I by itself can promote cell death by inducing the expression of apoptotic mediators like Noxa<sup>60-63</sup>. Apoptosis of infected cells is an efficient way to restrict viral replication. TLR3, another dsRNA sensor located in endosomes, can enhance apoptosis in certain cell-types<sup>64-66</sup>. The molecular mechanism underlying dsRNA induced apoptosis appears to depend on both the dsRNA receptor and cell-type involved. In summary, activation of cytoplasmic dsRNA sensors in virus infected cells triggers multiple pathways that restrict viral replication and spread. It is essential that viruses are targeted at several levels since they have evolved various mechanisms to suppress the production of type I IFNs and escape recognition by immune cells<sup>67</sup>.

#### *NOD-like and C-type lectin-like receptors*

NLRs are cytoplasmic receptors that recognize bacterial and viral structures<sup>68;69</sup>. NOD1 and NOD2 trigger the transcription of pro-inflammatory cytokines via activation of the transcription factors NFκB and mitogen-activated protein kinase (MAPK). Triggering of NLRP3 and related proteins leads to activation of caspase-1 through formation of a large protein complex, known as the inflammasome. The NLRP3 inflammasome, which is extensively studied in macrophages and DCs, is composed of NLRP3, caspase

1 and the adaptor molecule apoptosis-associated speck-like protein containing a CARD (ASC) <sup>70</sup>.

The production of active interleukin 1 $\beta$  (IL-1 $\beta$ ) requires two signals: the first signal activates NF $\kappa$ B leading to the generation of pro-IL-1 $\beta$  and the second signal triggers the inflammasome formation and caspase 1-mediated cleavage of pro-IL-1 $\beta$  into active IL-1 $\beta$ . Other pro-inflammatory cytokines that are activated by inflammasomes are IL-18 and IL-33.

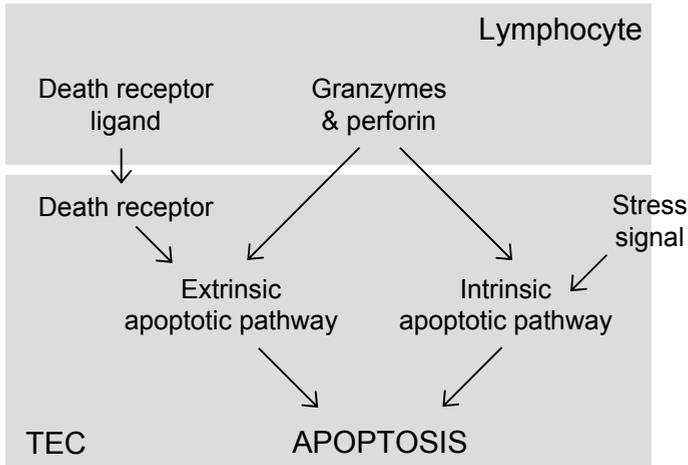
CLRs are trans-membrane receptors that recognize molecular structures of various pathogens, including fungi and mycobacteria, and are expressed by dendritic cells as well as some other types of immune cells. Activation of these receptors determines T cell differentiation either directly by inducing production of cytokines or indirectly by modulating TLR signaling <sup>71</sup>. Since CLRs and NLRs are not further addressed in this thesis, I would like to refer to recent reviews, which discuss these groups of PPRs extensively <sup>68;69;71</sup>.

**Apoptosis** ἀπόπτωση (Kerr et al. 1972) - Used in Greek to describe the “dropping off” or “falling off” of petals from flowers, or leaves from trees. To show the derivation clearly, we propose that the stress should be on the penultimate syllable, the second half of the word being pronounced like “ptosis” (with the “p” silent), which comes from the same root “to fall” and is already used to describe drooping of the upper eyelid <sup>72</sup>.

## Programmed cell death

Apoptosis or programmed cell death is essential for tissue homeostasis and plays an important role in the removal of infected or damaged cells. Apoptosis is characterized by nuclear and cytoplasmic condensation and breaking up of the cell in membrane-bound fragments known as apoptotic bodies <sup>72</sup>. It is a well orchestrated process that can be initiated by various cellular stress triggers for example metabolic stress, nutrient deprivation, ROS and UV-light. In addition, apoptosis can be induced by NK cells and CTLs, which are specialized in eliminating target cells via death receptor and/or granzyme (Gzm) mediated apoptosis (Fig. 2) <sup>73</sup>. NK cells kill cells that lack expression of MHC class I molecules on their surface, while CTLs selectively remove cells with an antigen and MHC class I molecule that is recognized by their T cell receptor (TCR). Granzyme stands for granule associated enzyme <sup>74</sup>; the function of these apoptosis inducing enzymes and their inhibitors is extensively discussed in **chapter 2**.

Death receptors (DR) such as CD95 (Fas), DR4, DR5 and TNFR1 are ubiquitously expressed and activated by their ligands, respectively CD95-ligand, TNF $\alpha$  and TNF-related apoptosis-inducing ligand (TRAIL), which are expressed and or secreted by cytotoxic lymphocytes <sup>73</sup>. Death receptor mediated apoptosis, also known as the extrinsic apoptotic pathway, is characterized by the formation of the death-inducing signaling complex (DISC) in which caspase 8 is activated <sup>75</sup>. Depending on the cell



**Figure 2.** Pathways that can induce apoptosis in TECs

type caspase 8 can either directly activate effector caspases, so called type I cells, or activate the intrinsic/mitochondrial apoptotic pathway via cleavage of Bcl-2 interacting domain (BID), which is the case in type II cells. Truncated BID (tBID) translocates to the mitochondria where it disrupts the balance between anti-apoptotic proteins of the Bcl-2 like family and pro-apoptotic proteins of the BH3-only family. Bcl-2 like proteins like Mcl-1 and Bcl-XL prevent interaction between Bak and Bax, which form a pore leading to mitochondrial outermembrane permeabilization <sup>76</sup>. Subsequent leaking of cytochrome C from the mitochondria induces the formation of another protein complex, the apoptosome, which includes caspase 9, apoptotic protease activating factor 1 (APAF1), cytochrome C and ATP. The apoptosome activates effector caspases like caspase 3 leading to cell death. The mitochondrial apoptotic pathway, also known as the intrinsic pathway, can be activated directly by various stress signals like ROS and nutrient shortage. Apoptosis is a strictly regulated process and proteins such as the inhibitors of apoptosis (IAPs) prevent uncontrolled cell death <sup>77;78</sup>. A schematic representation the extrinsic and intrinsic apoptosis pathway and their inhibitors is depicted at page 114 (chapter 5, figure 5).

### Outline of this thesis

This thesis focuses on inflammatory processes in the kidney, especially those occurring in the transplanted kidney. Our group previously found, that tubular epithelial cells can express serine protease inhibitor B9 (serpinB9). SerpinB9 is a specific inhibitor of GzmB; the role of this cytotoxic enzyme and that of other serine proteases expressed by immune cells is reviewed in **chapter 2**. During SCR, tubular serpinB9 levels were elevated suggesting that serpinB9 expression might help to preserve allograft function

during cellular rejection<sup>79</sup>. Since SCR is not associated with a decline in renal function, it can only be diagnosed by histological analysis of protocol transplant biopsies<sup>80</sup>. In **chapter 3** we aimed to find a non-invasive biomarker to monitor graft status and diagnose rejection even in a subclinical phase. During rejection, cytotoxic lymphocytes infiltrate the renal interstitium and tubuli, which is reflected by shedding of these cells into the urine. We therefore analyzed the transcription levels of GzmA, GzmB, perforin and serpinB9 in urinary cells after renal transplantation with histologically confirmed acute or subclinical rejection, ATN, stable function or CMV infection. In **chapter 4** we aimed to investigate the expression and regulation of serpinB9 in human TECs *in vitro*. SerpinB9 expression in primary TECs was consistently upregulated by the TLR3 ligand poly(I:C), while none of the other tested cytokines or TLR-ligands affected the expression of this GzmB inhibitor. Since TLR3 is specialized in the recognition of viral dsRNA, we also tested the role of the cytoplasmic dsRNA receptors MDA5 and RIG-I.

Little is known about how TECs sense and respond to the presence of a virus. Based on the observation that TECs express TLR3, MDA5 and RIG-I, we studied pro-inflammatory, anti-viral and pro-apoptotic responses in TECs stimulated with dsRNA-receptor ligands or virus. In **chapter 5**, we investigated the effect of dsRNA receptor activation on apoptosis induction in primary TECs and whether dsRNA affected the sensitivity of the cells to other apoptotic stimuli like CD95-ligand. In **chapter 6**, we aimed to study the role of dsRNA receptors in sensing the presence of CMV, EBV and BKV. First, we investigated the expression of TLR3, MDA5 and RIG-I during active viral infection in renal transplant biopsies. In addition, we analyzed the production of pro-inflammatory and anti-viral cytokines in response to dsRNA receptor triggering and influenza infection in primary TECs. The effects of ongoing viral replication are not restricted to the kidney. CMV is a virus that is known to induce a permanent increase of highly differentiated effector T cells<sup>81-83</sup>. In **chapter 7**, we investigated the influence of CMV infection on the activation status of the immune system. For that reason, we analyzed the expression of an array of cytokines, chemokines and acute phase proteins in serum of kidney transplant recipients with a primary CMV infection.

In **chapter 8**, we summarize and discuss our findings in perspective of the general knowledge of the research field.

## REFERENCES

1. Councilman WT: ACUTE INTERSTITIAL NEPHRITIS. *J Exp Med* 3:393-420, 1898
2. Praga M, Gonzalez E: Acute interstitial nephritis. *Kidney Int* 77:956-961, 2010
3. Gansevoort RT, van der Heij B, Stegeman CA, de Charro FT, Nieuwenhuizen MG, de ZD, de Jong PE: Trends in the incidence of treated end-stage renal failure in The Netherlands: hope for the future? *Kidney Int Suppl* 7-10, 2004
4. Janeway CA, Travers P, Walport M, Schlomchik M: *Immuno Biology*, 6 ed, 2004
5. Rather LJ: Disturbance of function (functio laesa): the legendary fifth cardinal sign of inflammation, added by Galen to the four cardinal signs of Celsus. *Bull N Y Acad Med* 47:303-322, 1971
6. Michel DM, Kelly CJ: Acute interstitial nephritis. *J Am Soc Nephrol* 9:506-515, 1998
7. van Kooten C, Daha MR: Cytokine cross-talk between tubular epithelial cells and interstitial immunocompetent cells. *Curr Opin Nephrol Hypertens* 10:55-59, 2001
8. Lopez-Hoyos M, Segundo DS, Fernandez-Fresnedo G, Marin MJ, Gonzalez-Martin V, Arias M: Regulatory T cells in renal transplantation and modulation by immunosuppression. *Transplantation* 88:S31-S39, 2009
9. Starke A, Lindenmeyer MT, Segerer S, Neusser MA, Rusi B, Schmid DM, Cohen CD, Wuthrich RP, Fehr T, Waeckerle-Men Y: Renal tubular PD-L1 (CD274) suppresses alloreactive human T-cell responses. *Kidney Int* 78:38-47, 2010
10. Anders HJ: Toll-like receptors and danger signaling in kidney injury. *J Am Soc Nephrol* 21:1270-1274, 2010
11. Wolfe RA, Ashby VB, Milford EL, Ojo AO, Ettenger RE, Agodoa LY, Held PJ, Port FK: Comparison of mortality in all patients on dialysis, patients on dialysis awaiting transplantation, and recipients of a first cadaveric transplant. *N Engl J Med* 341:1725-1730, 1999
12. Nickerson P: Post-transplant monitoring of renal allografts: are we there yet? *Curr Opin Immunol* 21:563-568, 2009
13. Chapman JR, O'Connell PJ, Nankivell BJ: Chronic renal allograft dysfunction. *J Am Soc Nephrol* 16:3015-3026, 2005
14. Cornell LD, Smith RN, Colvin RB: Kidney transplantation: mechanisms of rejection and acceptance. *Annu Rev Pathol* 3:189-220, 2008
15. Solez K, Colvin RB, Racusen LC, Haas M, Sis B, Mengel M, Halloran PF, Baldwin W, Banfi G, Collins AB, Cosio F, David DS, Drachenberg C, Einecke G, Fogo AB, Gibson IW, Glotz D, Iskandar SS, Kraus E, Lerut E, Mannon RB, Mihatsch M, Nankivell BJ, Nickleit V, Papadimitriou JC, Randhawa P, Regele H, Renaudin K, Roberts I, Seron D, Smith RN, Valente M: Banff 07 classification of renal allograft pathology: updates and future directions. *Am J Transplant* 8:753-760, 2008
16. Nankivell BJ, Alexander SI: Rejection of the kidney allograft. *N Engl J Med* 363:1451-1462, 2010
17. Gloor J, Cosio F, Lager DJ, Stegall MD: The spectrum of antibody-mediated renal allograft injury: implications for treatment. *Am J Transplant* 8:1367-1373, 2008
18. Halloran PF: T cell-mediated rejection of kidney transplants: a personal viewpoint. *Am J Transplant* 10:1126-1134, 2010
19. Moreso F, Ibernón M, Goma M, Carrera M, Fulladosa X, Hueso M, Gil-Vernet S, Cruzado JM, Torras J, Grinyo JM, Seron D: Subclinical rejection associated with chronic allograft nephropathy in protocol biopsies as a risk factor for late graft loss. *Am J Transplant* 6:747-752, 2006
20. Perico N, Cattaneo D, Sayegh MH, Remuzzi G: Delayed graft function in kidney transplantation. *Lancet* 364:1814-1827, 2004
21. Irish WD, Ilsley JN, Schnitzler MA, Feng S, Brennan DC: A risk prediction model for delayed graft function in the current era of deceased donor renal transplantation. *Am J Transplant* 10:2279-2286, 2010
22. Yarlagadda SG, Coca SG, Formica RN, Jr., Poggio ED, Parikh CR: Association

- between delayed graft function and allograft and patient survival: a systematic review and meta-analysis. *Nephrol Dial Transplant* 24:1039-1047, 2009
23. Weikert BC, Blumberg EA: Viral infection after renal transplantation: surveillance and management. *Clin J Am Soc Nephrol* 3 Suppl 2:S76-S86, 2008
  24. Staras SA, Dollard SC, Radford KW, Flanders WD, Pass RF, Cannon MJ: Seroprevalence of cytomegalovirus infection in the United States, 1988-1994. *Clin Infect Dis* 43:1143-1151, 2006
  25. Soderberg-Naucleer C, Fish KN, Nelson JA: Reactivation of latent human cytomegalovirus by allogeneic stimulation of blood cells from healthy donors. *Cell* 91:119-126, 1997
  26. Baron C, Forconi C, Lebranchu Y: Revisiting the effects of CMV on long-term transplant outcome. *Curr Opin Organ Transplant* 15:492-498, 2010
  27. Liapis H, Storch GA, Hill DA, Rueda J, Brennan DC: CMV infection of the renal allograft is much more common than the pathology indicates: a retrospective analysis of qualitative and quantitative buffy coat CMV-PCR, renal biopsy pathology and tissue CMV-PCR. *Nephrol Dial Transplant* 18:397-402, 2003
  28. Hartmann A, Sagedal S, Hjelmesaeth J: The natural course of cytomegalovirus infection and disease in renal transplant recipients. *Transplantation* 82:S15-S17, 2006
  29. Sagedal S, Rollag H, Hartmann A: Cytomegalovirus infection in renal transplant recipients is associated with impaired survival irrespective of expected mortality risk. *Clin Transplant* 21:309-313, 2007
  30. Nachtwey J, Spencer JV: HCMV IL-10 suppresses cytokine expression in monocytes through inhibition of nuclear factor-kappaB. *Viral Immunol* 21:477-482, 2008
  31. Moutafsi M, Mehl AM, Borysiewicz LK, Tabi Z: Human cytomegalovirus inhibits maturation and impairs function of monocyte-derived dendritic cells. *Blood* 99:2913-2921, 2002
  32. Betjes MG, Litjens NH, Zietse R: Seropositivity for cytomegalovirus in patients with end-stage renal disease is strongly associated with atherosclerotic disease. *Nephrol Dial Transplant* 22:3298-3303, 2007
  33. Tanner J, Weis J, Fearon D, Whang Y, Kieff E: Epstein-Barr virus gp350/220 binding to the B lymphocyte CD28 receptor mediates adsorption, capping, and endocytosis. *Cell* 50:203-213, 1987
  34. Becker JL, Miller F, Nuovo GJ, Josepovitz C, Schubach WH, Nord EP: Epstein-Barr virus infection of renal proximal tubule cells: possible role in chronic interstitial nephritis. *J Clin Invest* 104:1673-1681, 1999
  35. Rezk SA, Weiss LM: Epstein-Barr virus-associated lymphoproliferative disorders. *Hum Pathol* 38:1293-1304, 2007
  36. Tsai JD, Lee HC, Lin CC, Liang DC, Chen SH, Huang FY: Epstein-Barr virus-associated acute renal failure: diagnosis, treatment, and follow-up. *Pediatr Nephrol* 18:667-674, 2003
  37. Khanna R, Burrows SR: Role of cytotoxic T lymphocytes in Epstein-Barr virus-associated diseases. *Annu Rev Microbiol* 54:19-48, 2000
  38. Izumi KM, Kaye KM, Kieff ED: The Epstein-Barr virus LMP1 amino acid sequence that engages tumor necrosis factor receptor associated factors is critical for primary B lymphocyte growth transformation. *Proc Natl Acad Sci U S A* 94:1447-1452, 1997
  39. Opelz G, Daniel V, Naujokat C, Dohler B: Epidemiology of pretransplant EBV and CMV serostatus in relation to post-transplant non-Hodgkin lymphoma. *Transplantation* 88:962-967, 2009
  40. Cherikh WS, Kauffman HM, McBride MA, Maghirang J, Swinnen LJ, Hanto DW: Association of the type of induction immunosuppression with post-transplant lymphoproliferative disorder, graft survival, and patient survival after primary kidney transplantation. *Transplantation* 76:1289-1293, 2003
  41. Bustami RT, Ojo AO, Wolfe RA, Merion RM, Bennett WM, McDiarmid SV, Leichtman AB, Held PJ, Port FK: Immunosuppression and the risk of post-transplant malignancy among cadaveric

- first kidney transplant recipients. *Am J Transplant* 4:87-93, 2004
42. Gardner SD, Field AM, Coleman DV, Hulme B: New human papovavirus (B.K.) isolated from urine after renal transplantation. *Lancet* 1:1253-1257, 1971
  43. Egli A, Infanti L, Dumoulin A, Buser A, Samaridis J, Stebler C, Gosert R, Hirsch HH: Prevalence of polyomavirus BK and JC infection and replication in 400 healthy blood donors. *J Infect Dis* 199:837-846, 2009
  44. Nicleleit V, Mihatsch MJ: Polyomavirus nephropathy in native kidneys and renal allografts: an update on an escalating threat. *Transpl Int* 19:960-973, 2006
  45. Ramos E, Drachenberg CB, Wali R, Hirsch HH: The decade of polyomavirus BK-associated nephropathy: state of affairs. *Transplantation* 87:621-630, 2009
  46. O'Neill LA, Bowie AG: The family of five: TIR-domain-containing adaptors in Toll-like receptor signalling. *Nat Rev Immunol* 7:353-364, 2007
  47. Iwasaki A, Medzhitov R: Toll-like receptor control of the adaptive immune responses. *Nat Immunol* 5:987-995, 2004
  48. Anders HJ, Banas B, Schlondorff D: Signaling danger: toll-like receptors and their potential roles in kidney disease. *J Am Soc Nephrol* 15:854-867, 2004
  49. Onoguchi K, Yoneyama M, Fujita T: Retinoic Acid-Inducible Gene-I-Like Receptors. *J Interferon Cytokine Res* 2010
  50. Hornung V, Ellegast J, Kim S, Brzozka K, Jung A, Kato H, Poeck H, Akira S, Conzelmann KK, Schlee M, Endres S, Hartmann G: 5'-Triphosphate RNA is the ligand for RIG-I. *Science* 314:994-997, 2006
  51. Kato H, Takeuchi O, Mikamo-Satoh E, Hirai R, Kawai T, Matsushita K, Hiiragi A, Dermody TS, Fujita T, Akira S: Length-dependent recognition of double-stranded ribonucleic acids by retinoic acid-inducible gene-I and melanoma differentiation-associated gene 5. *J Exp Med* 205:1601-1610, 2008
  52. Rebsamen M, Meylan E, Curran J, Tschopp J: The antiviral adaptor proteins Cardif and Trif are processed and inactivated by caspases. *Cell Death Differ* 15:1804-1811, 2008
  53. Satoh T, Kato H, Kumagai Y, Yoneyama M, Sato S, Matsushita K, Tsujimura T, Fujita T, Akira S, Takeuchi O: LGP2 is a positive regulator of RIG-I- and MDA5-mediated antiviral responses. *Proc Natl Acad Sci U S A* 107:1512-1517, 2010
  54. Kawai T, Takahashi K, Sato S, Coban C, Kumar H, Kato H, Ishii KJ, Takeuchi O, Akira S: IPS-1, an adaptor triggering RIG-I- and Mda5-mediated type I interferon induction. *Nat Immunol* 6:981-988, 2005
  55. Borden EC, Sen GC, Uze G, Silverman RH, Ransohoff RM, Foster GR, Stark GR: Interferons at age 50: past, current and future impact on biomedicine. *Nat Rev Drug Discov* 6:975-990, 2007
  56. Trinchieri G: Type I interferon: friend or foe? *J Exp Med* 2010
  57. Montoya M, Schiavoni G, Mattei F, Gresser I, Belardelli F, Borrow P, Tough DF: Type I interferons produced by dendritic cells promote their phenotypic and functional activation. *Blood* 99:3263-3271, 2002
  58. Agarwal P, Raghavan A, Nandiwada SL, Curtsinger JM, Bohjanen PR, Mueller DL, Mescher MF: Gene regulation and chromatin remodeling by IL-12 and type I IFN in programming for CD8 T cell effector function and memory. *J Immunol* 183:1695-1704, 2009
  59. Kohlmeier JE, Cookenham T, Roberts AD, Miller SC, Woodland DL: Type I interferons regulate cytolytic activity of memory CD8(+) T cells in the lung airways during respiratory virus challenge. *Immunity* 33:96-105, 2010
  60. Besch R, Poeck H, Hohenauer T, Senft D, Hacker G, Berking C, Hornung V, Endres S, Ruzicka T, Rothenfusser S, Hartmann G: Proapoptotic signaling induced by RIG-I and MDA-5 results in type I interferon-independent apoptosis in human melanoma cells. *J Clin Invest* 119:2399-2411, 2009
  61. Lallemand C, Blanchard B, Palmieri M, Lebon P, May E, Tovey MG: Single-stranded RNA viruses inactivate the transcriptional activity of p53 but induce

- NOXA-dependent apoptosis via post-translational modifications of IRF-1, IRF-3 and CREB. *Oncogene* 26:328-338, 2007
62. Goubau D, Romieu-Mourez R, Solis M, Hernandez E, Mesplede T, Lin R, Leaman D, Hiscott J: Transcriptional re-programming of primary macrophages reveals distinct apoptotic and anti-tumoral functions of IRF-3 and IRF-7. *Eur J Immunol* 39:527-540, 2009
  63. Chattopadhyay S, Marques JT, Yamashita M, Peters KL, Smith K, Desai A, Williams BR, Sen GC: Viral apoptosis is induced by IRF-3-mediated activation of Bax. *EMBO J* 29:1762-1773, 2010
  64. Paone A, Starace D, Galli R, Padula F, De CP, Filippini A, Ziparo E, Riccioli A: Toll-like receptor 3 triggers apoptosis of human prostate cancer cells through a PKC-alpha-dependent mechanism. *Carcinogenesis* 29:1334-1342, 2008
  65. Salaun B, Coste I, Rissoan MC, Lebecque SJ, Renno T: TLR3 can directly trigger apoptosis in human cancer cells. *J Immunol* 176:4894-4901, 2006
  66. Weber A, Kirejczyk Z, Besch R, Potthoff S, Leverkus M, Hacker G: Proapoptotic signalling through Toll-like receptor-3 involves TRIF-dependent activation of caspase-8 and is under the control of inhibitor of apoptosis proteins in melanoma cells. *Cell Death Differ* 2009
  67. Bowie AG, Unterholzner L: Viral evasion and subversion of pattern-recognition receptor signalling. *Nat Rev Immunol* 8:911-922, 2008
  68. Kanneganti TD, Lamkanfi M, Nunez G: Intracellular NOD-like receptors in host defense and disease. *Immunity* 27:549-559, 2007
  69. Kanneganti TD: Central roles of NLRs and inflammasomes in viral infection. *Nat Rev Immunol* 10:688-698, 2010
  70. Mariathasan S, Monack DM: Inflammasome adaptors and sensors: intracellular regulators of infection and inflammation. *Nat Rev Immunol* 7:31-40, 2007
  71. Geijtenbeek TB, Gringhuis SI: Signalling through C-type lectin receptors: shaping immune responses. *Nat Rev Immunol* 9:465-479, 2009
  72. Kerr JF, Wyllie AH, Currie AR: Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. *Br J Cancer* 26:239-257, 1972
  73. Chavez-Galan L, renas-Del Angel MC, Zenteno E, Chavez R, Lascurain R: Cell death mechanisms induced by cytotoxic lymphocytes. *Cell Mol Immunol* 6:15-25, 2009
  74. Masson D, Nabholz M, Estrade C, Tschopp J: Granules of cytolytic T-lymphocytes contain two serine esterases. *EMBO J* 5:1595-1600, 1986
  75. Wilson NS, Dixit V, Ashkenazi A: Death receptor signal transducers: nodes of coordination in immune signaling networks. *Nat Immunol* 10:348-355, 2009
  76. Kroemer G, Galluzzi L, Brenner C: Mitochondrial membrane permeabilization in cell death. *Physiol Rev* 87:99-163, 2007
  77. Dubrez-Daloz L, Dupoux A, Cartier J: IAPs: more than just inhibitors of apoptosis proteins. *Cell Cycle* 7:1036-1046, 2008
  78. Srinivasula SM, Ashwell JD: IAPs: what's in a name? *Mol Cell* 30:123-135, 2008
  79. Rowshani AT, Florquin S, Bemelman F, Kummer JA, Hack CE, ten Berge IJM: Hyperexpression of the granzyme B inhibitor PI-9 in human renal allografts: a potential mechanism for stable renal function in patients with subclinical rejection. *Kidney Int* 66:1417-1422, 2004
  80. Wilkinson A: Protocol transplant biopsies: are they really needed? *Clin J Am Soc Nephrol* 1:130-137, 2006
  81. Appay V, Dunbar PR, Callan M, Klenerman P, Gillespie GM, Papagno L, Ogg GS, King A, Lechner F, Spina CA, Little S, Havlir DV, Richman DD, Gruener N, Pape G, Waters A, Easterbrook P, Salio M, Cerundolo V, McMichael AJ, Rowland-Jones SL: Memory CD8+ T cells vary in differentiation phenotype in different persistent virus infections. *Nat Med* 8:379-385, 2002
  82. van Leeuwen EM, Remmerswaal EB, Vossen MT, Rowshani AT, Wertheim-van Dillen PM, van Lier RA, ten Berge IJM: Emergence of a CD4+CD28- granzyme

- B+, cytomegalovirus-specific T cell subset after recovery of primary cytomegalovirus infection. *J Immunol* 173:1834-1841, 2004
83. Gamadia LE, van Leeuwen EM, Remmerswaal EB, Yong SL, Surachno S, Wertheim-van Dillen PM, ten Berge IJM, van Lier RA: The size and phenotype of virus-specific T cell populations is determined by repetitive antigenic stimulation and environmental cytokines. *J Immunol* 172:6107-6114, 2004