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Ischemia as a factor affecting innate immune responses in kidney transplantation

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Abstract

Purpose of review—Ischemic injury inevitably occurs during the procurement of organs for transplantation and the injury is worsened by inflammation following reperfusion. The purpose of this review is to describe the role of the innate immune system in ischemia-induced renal injury in kidneys procured for transplantation. The key role of pattern recognition receptors in immune responses to ischemia is described. Innate immune receptors are emerging novel targets for amelioration of ischemic injury of donor kidneys.

Recent findings—Several families of pattern recognition receptors are direct mediators of early injurious events during kidney procurement, and also innate and adaptive immune responses after transplantation. The deleterious events associated with activation of the innate immune system in donor kidneys significantly contributes to short and long-term allograft outcomes.

Summary—Although a number of therapies have been proposed to decrease ischemic donor kidney injury, targeting the innate immune system is an exciting new area that is gaining significant interest in transplantation. As we learn more about how these important receptors are regulated by ischemia, strategies will likely evolve to allow their modulation in ischemic renal injury.

Keywords

renal ischemia; pattern recognition receptor; innate immunity; adaptive immunity

Introduction

Ischemia plays a major role in the initial injury and eventual outcomes of transplanted kidneys. Ischemic injury is of course unavoidable, due to the need to disrupt the renal vasculature during the process of transplantation; and it is worsened by events that occur before and after organ procurement. Donor-specific events (e.g., brain death, renal hypoperfusion, nephrotoxins, cold and warm ischemia, etc.) compound the ischemic insult. After transplantation, a potent inflammatory response mounted by the recipient's immune system can cause even more damage. Many studies have shown that the severity of the

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ischemia-reperfusion injury during the early stages of transplantation ultimately determines long-term graft function (1). This review discusses emerging concepts that have the potential to lead to new targets for amelioration of ischemic kidney injury.

Renal tubular epithelial cells (RTEs), particularly those in the S3 segment of the proximal tubule and medullary thick ascending limb (mTAL) of the loop of Henle, exist in a hypoxic microenvironment. Therefore, it is no surprise that this region of the kidney is significantly impacted by a decrease in oxygen delivery. Upon disruption of the donor vasculature, the ischemic RTEs release their intracellular contents (Figure 1) triggering a potent sterile inflammatory response that causes a cascade of injurious events ultimately impacting survival of the transplanted kidney. Abundant data have shown that RTE cells, interstitial dendritic cells and endothelial cells in the kidney express receptors of the innate immune system, called pattern recognition receptors (PRRs), which are exquisitely sensitive to molecular patterns [danger activated molecular patterns (DAMPs)], such as those found on molecules released from dead and dying cells (including HMGB1, histones, heat shock proteins, uric acid, nucleic acids, etc. (2)).

The PRRs are categorized into families of receptors that recognize structurally conserved molecules, such as those derived from microbes [pattern activated molecular patterns (PAMPs)] and DAMPs. When engaged, PRRs signal the production of proinflammatory cytokines and interferons, and/or induce cell death signaling pathways, thus propagating the initial ischemic insult (Figure 1). These innate immune receptors are now being recognized as potential targets to prevent and treat ischemic renal injury.

Innate immune receptors in the kidney

Among the best-characterized PRRs in kidney are the toll-like receptors (TLRs) (Figure 2). TLRs are transmembrane receptors constitutively expressed on immune and parenchymal cells in rodents and humans. Different TLRs, including TLR1–4, TLR6, and TLR9, have been documented on RTE cells (3–6). TLRs 2, 4 and 3 are also expressed on endothelial cells, mesangial cells and podocytes (4, 7–11) and TLRs 5 and 11 have been found on distal renal tubules and bladder epithelium (12, 13). In humans, TLR9 and TLR7 have also been found within the kidney and are particularly important in models of nephritis and inflammation in the kidney (14, 15). Polymorphisms in TLR3 have been associated with delayed graft function in humans (16) and TLR4 has been shown to be upregulated in kidneys from deceased donors (17) and contribute to ischemia-reperfusion injury (18). Recipients or donors with TLR4 loss-of-function polymorphisms have been reported to have fewer acute rejection episodes (19, 20) although others have found no association (21).

NOD-like receptors

Another family of PRRs has emerged as important in the kidney, the nucleotide binding, leucine-rich repeat containing receptors (NLRs) [Figure 2]. The NLR family can be subdivided into three broad functional categories: the non-inflammasome-activating (NOD1, NOD2, NLRC5, NLRX1), inflammasome-activating (NAIP, NLRP1, NLRP3) and transcriptional regulation (CIITA) (22). The noninflammasome family mediates their function through NF- κ B activation, whereas the inflammasome family relies on caspase-1.

CIITA functions as a transcription factor for MHCII expression (23). Both NOD and NLRP family members are expressed in murine and human RTE cells (24–26).

The best-characterized NOD/noninflammasome family members in renal IRI are NOD1 and NOD2. NOD1 and NOD2 activation leads to the production of proinflammatory factors (e.g. IL-6, CXCL3, CXCL1, MIP2, iNOS, Cox-2) and neutrophil recruitment, worsening ischemic tissue damage and cell death (27–31). Disruption of either NOD1 or NOD2 signaling diminishes inflammation in renal IRI. NOD1 and NOD2 are expressed on both leukocytes and parenchymal cells, and simultaneous elimination of NOD1 and NOD2 affords increased protection over the single knockouts (24).

The NOD-leucine-rich repeat pyrin domain containing (NLRP) or inflammasome NLR family also plays an important role in renal IRI. The NLRP family forms inflammasome complexes by recruiting apoptosis-associated speck-like protein (ASC), and caspase-1 (32). Unlike the TLRs or NODs, which are characterized by induction of NF- κ B and MAPK, the inflammasomes induce cleavage of pro-IL-1 β and pro-IL-18 to their active forms by activating caspase-1. The inflammasomes are triggered by many different molecules that are released from ischemic RTEs making them key sentinels for cellular stress. Among the many microbial and endogenous molecules shown to activate NLRPs include ATP, ROS, uric acid, cholesterol crystals, hydroxyapatite crystals, flagellin and DNA (32–34). To date, the best-studied inflammasome in the kidney is the NLRP3 inflammasome. Deficiency of NLRP3 protects mice from renal ischemia, and absence of other NLRP3 inflammasome components such as ASC and caspase-1, and factors further downstream such as IL-1 β or IL-18, does not prevent renal IRI to the same extent (26). NLRP3 is thought by many investigators to be an important target to prevent ischemic RTE cell injury (2).

Consequences of PRR activation in the transplanted kidney

Within minutes of disrupting the donor blood supply, RTE cells in the S3 and mTAL segments begin to release their intracellular contents, activating PRRs on viable RTEs and setting in place a cascade of events that ultimately extends the initial ischemic injury. There are two primary ways that PRR activation induces injury of RTE cells. The first is through the induction of cell death signaling in previously viable RTE cells and the second is by induction of proinflammatory molecules and interferon proteins, which contribute to inflammatory responses to the injured tissue.

PRR induction of cell death signaling

Many studies have shown that PRR activation can induce cell death signaling pathways, leading to apoptosis and necrosis. Apoptosis (characterized by cell shrinkage, chromatin condensation, membrane blebbing, and nuclear fragmentation) is due to proteolytic activity of caspases and is a regulated, metabolically controlled form of cell death that preserves membrane integrity (35). Endogenous molecules such as ATP and histones released by dying cells can stimulate apoptosis through PRR ligation (36–39). Recent studies have also shown that blocking of apoptosis or components of apoptotic signaling can prevent ischemic renal injury (38, 40–44).

Necrosis

Necrotic cell death is also regulated by PRRs and is characterized by loss of cell membrane integrity and swelling of mitochondria and intracellular organelles. Necrosis is induced by several PRR-mediated intracellular signaling pathways that lead to distinct types of necrotic cell death, which are described below.

Pyroptosis

Pyroptosis is a type of regulated cell necrosis characterized by formation of plasma membrane pores and cell membrane rupture, without nuclear or mitochondrial damage. It is typically triggered by TLRs and NLRs (45). Ligation of these PRRs can result in the formation of two different cytosolic scaffolds, an NLRP3-containing inflammasome and a pyptosome (which is independent of NLRP3). Similar to apoptosis, pyroptosis requires activation of caspase-1, but unlike apoptosis it does not require any other of the classical caspases involved in cell death (46, 47). This form of cell death has been shown to be important in ischemic injury of the liver (48) and heart (49), and although not directly shown to result from ischemia in the kidney, blockade of the Nlrp3 inflammasome prevents renal tubular cell death (26, 50). As yet there are no specific inhibitors of pyroptosis, but many studies have demonstrated that inhibition of inflammasome assembly due to loss of NLRP3 or absence of products from proteolytic activity of inflammasome such as caspase-1 or IL-1 or IL-18 are protective in renal IR injury (2).

Necroptosis

Necroptosis is a regulated form of necrosis executed by large cytosolic caspase-8-containing signaling complexes called ripoptosomes and serine/threonine kinase receptor interacting protein 1 (RIP1) and RIP3 protein complexes called necrosomes. Caspase 8 appears to serve as a switch between whether a cell undergoes apoptosis or necrosis (45). The ripoptosome has been described as a 'docking station', formed upon cellular stress, that can participate in proliferative, inflammatory or cell death signaling pathways (51). To date it is not clear if the necrosome is indeed part of the ripoptosome or whether it signals as an independent protein complex (51). It is clear that ischemic injury can induce necroptosis in the kidney and blocking RIP3 was shown in one study to partially ameliorate IR injury (52).

Necrostatin-1, a blocker of the necroptosome, has also been shown to decrease renal IR injury (53). Necroptosis can be attenuated by small molecule inhibitors such as necrosulfonamide, which binds and inactivates the executioner molecule, mixed lineage kinase like (psedo) kinase (MLKL); this is the effector of necroptosis-induced plasma membrane rupture (54). Clinical targets for the necroptosome are not yet available.

Other forms of necrosis

Several other forms of nonapoptotic (necrotic) orchestrated cell death, each with their own unique biochemical and morphological features, can play a role in ischemic death in the kidney. Parthanatos, a form of caspase-independent cell death is mediated by poly (ADP-ribose) polymerase (PARP), an enzyme involved in the DNA repair process. PARP is one of the most abundant proteins in the nucleus and hyperactivation of PARP results in NAD depletion and subsequent impairment of ATP-generating glycolysis, ultimately leading to

necrotic cell death. PARP-1 inhibitors can provide protection in rat models of ischemia/reperfusion injury (55), although there is likely overlap between necroptotic and PARP-1-mediated processes (56).

Another form of programmed cell death called ferroptosis is due to iron-dependent reactive oxygen species and lipid peroxide accumulation (57). In contrast to necroptosis, ferroptosis causes necrosis of renal tubules *ex vivo* and blockade of ferroptosis provides highly significant protection from ischemic renal tubular injury (58). Finally, incomplete autophagy may cause cell death through a process called autosis (45). Autosis is characterized by accumulation of vacuoles at the nuclear membrane and endoplasmic reticulum rupture. Autosis has been shown to occur in the ischemic brain of rodents *in vivo* (59), but little is known about its role in renal ischemia.

Inflammation as a consequence of ischemia

Once the vascular anastomosis is complete, the inflammatory milieu of the donor organ, induced by ischemia-mediated cell death, directs a potent recipient immune response. In the absence of immunosuppressant medications, this inflammatory response will lead to destruction of the allograft. As mentioned above, intracellular contents released by the necrotic cells as a result of ischemia are thought to be the triggers of the innate and adaptive effectors, through induction of proinflammatory cytokines/chemokines and type 1 interferons (60). Many studies in humans and rodents have shown that immune effector cells, such as neutrophils, macrophages/monocytes and dendritic cells promote further damage by inducing cell death through reactive oxygen species and activating caspases (61–63). Renal tubular epithelial cells are capable of directly contributing to the inflammatory reaction by stimulating resident T cells. MHC class II and co-stimulatory molecule expression is upregulated on RTEs through PRR-induced type 1 interferons (64). Although many reports suggest activated RTEs can act as non-professional-antigen presenting cells to simulate CD4⁺ and cytotoxic CD8⁺ T cells (65, 66), some recent reports indicate that they suppress not only alloreactive human T cell responses but also B cell responses (67, 68). Therefore, further studies need to be performed to address the contrasting nature of RTEs in activating murine and human T and B cell responses.

Immune cells involved in inflammatory response

Inflammatory cells of the recipient that are recruited to the donor kidney worsen the initial ischemic injury. Neutrophils release proteases, reactive oxygen species (ROS), cytokines and chemokines. This potent inflammatory cocktail further increases vascular permeability and reduces tubule epithelial and endothelial cell integrity (69). Neutrophil recruitment is mediated by adhesion molecules, including ICAM-1, selectins and CD11/CD18 (70). Macrophages also accumulate in the kidney following IRI, although there have been conflicting studies over whether they are indeed detrimental (70–72). A recent study showed that diphtheria-toxin (DTX)-CD11b treated mice were not protected from renal IRI, whereas clodronate depletion resulted in improved renal function (73). These results suggest that a protective macrophage population, in addition to the conventional inflammatory population, might exist.

Dendritic cells (DCs) along with macrophages are one of the early initiators of innate immunity, DCs are crucial cells involved in bridging the innate and adaptive arms of immune system. DCs are known to abundantly express PRRs and are implicated in DC activation after IR injury. Activated DCs function as professional antigen-presenting cells and propagate inflammatory responses through direct activation of T cells or indirectly through cytokine secretion (62). The extent of DC function in renal IR injury was described in a recent study where it was shown that preventing DC maturation exerted a therapeutic immunosuppressive effect in renal IR injury (74). Although DCs activate the adaptive immune system they can also induce tolerance and inhibit inflammatory responses (75). In a recent study DCs tolerized with adenosine 2A receptor (A_{2A}R) agonists attenuated renal IRI by suppressing natural killer T cell activation (76).

Other cell types also appear to be important during the reperfusion phase, including NK T cells (NKT) and T regulatory cells (Tregs). NKT cells are a subset of T cells expressing the T-cell receptor and the surface molecule NK1.1. NKT cells are activated by endogenous and exogenous glycolipids presented by Cd1 expressing dendritic cells (77). Upon activation, they produce interferon-gamma (IFN- γ), tumour necrosis factor-alpha (TNF- α), interleukin (IL)-4 and IL-1 β . Following reperfusion, the numbers of NKT cells have been shown to increase in the kidney (77). Blocking or depleting NKT cells prevents neutrophil recruitment and kidney injury, demonstrating a potential role of NKT function in neutrophil recruitment (77). Tregs play a vital role in suppressing the inflammation mediated by innate effector cells. Following injury, Tregs traffic to the kidney (78) and mediate suppression through the release of the anti-inflammatory cytokine IL-10 (79). Mice lacking Tregs have greater numbers of inflammatory leukocytes in the kidney following injury and increased renal damage (78, 79). B lymphocytes are also thought to participate in the early phases of renal IR injury (80). B cells are believed to have an effect during the recovery phase of the renal injury and recent studies of renal transplantation correlates the presence of mature B cells with decreased renal graft survival (81). A recent report suggests that innate immune activation in kidney after IR injury effects systemic B lymphocytes and production of harmful antibodies, and that the absence of B cells alleviates IR injury and promotes renal tubule regeneration (82).

Conclusion

Renal transplantation offers improved survival and quality of life for patients with end stage renal disease. The length of donor organ survival though is limited by ischemic injury that occurs during allograft procurement. Graft ischemia causes a sterile inflammatory response that has its origins in DAMPs, which are ligands for constitutively expressed PRRs on many cells in the kidney, including RTE cells, interstitial DCs and endothelial cells. Activation of PRR signaling pathways trigger secretion of proinflammatory molecules, and they also extend cell death directly by induction of apoptotic and necrotic signaling pathways. There is much interest in in defining the expression and regulation of PRRs in the kidney in order to develop targeted therapies to prevent the downstream consequences of ischemic donor allograft injury. New investigation therapies, targeting the PRR activation of innate immunity during ischemia, may not only prevent early stages of ischemic injury by

decreasing local RTE injury promoting cell death but may also prevent the host inflammatory response after transplantation.

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Glossary

A_{2A}R	A _{2A} adenosine receptor
ASC	Apoptosis-associated speck-like protein containing a caspase recruitment domain
ATP	Adenosine triphosphate
CD11/CD18	Also called LFA-1, a beta 2-type integrin
Cox-2	Cyclooxygenase-2
CXCL	Chemokine (C-X-C motif) ligand
DAMPs	Danger activated molecular patterns
DC	Dendritic cell
DTX	docetaxel
ICAM	Intercellular adhesion molecule
IGNg	Interferon gamma
IL-10	Interleukin 10
IL-18	Interleukin-18
IL-1b	Interleukin-1 beta
IL-1b	Interleukin – 1 beta
IL-4	Interleukin 4
iNOS	Inducible nitric oxide synthase
MAPK	Mitogen-activated protein kinase
MIP2	Macrophage inflammatory protein 2-alpha (also called CXCL2 in humans)
MLKL	Mixed lineage kinase domain-like protein
mTAL	Medullary thick ascending limb
NF-kB	Nuclear factor kappa-light-chain-enhancer of activated B cells
NKT	Natural killer T cell

NLRP3	NOD-like receptor family, pyrin domain containing 3
NLRs	Nucleotide binding, leucine-rich repeat containing receptors
NOD	Nucleotide-binding oligomerization domain receptors
PAMPs	Pathogen activated molecular patterns
PARP	Poly ADP ribose polymerase
PRR	Pattern recognition receptor
RIP	Receptor-interacting protein kinase
ROS	Reactive oxygen species
RTE	Renal tubular epithelial cell
TLRs	Toll-like receptors
TNFα	Tumor necrosis factor alpha
Treg	T regulatory cell

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Key Points

- The pathophysiological events associated with ischemia/reperfusion injury have their origin in signals derived from PRRs expressed in the donor kidney.
- DAMPs released from injured cells serve as ligands for PRRs expressed on many cells in the donor kidney.
- Both membrane-bound PRRs (e.g., TLRs) and intracytoplasmic PRRs (e.g., NLRs) are expressed throughout the normal kidney.
- Activation of PRR signaling in the donor kidney leads to production of proinflammatory cytokines and chemokines as well as cell death signaling, ultimately worsening the initial ischemic injury.
- New therapies targeting PRRs in the kidney are likely to provide novel approaches to prevent or treat ischemic renal disease.

Figure 1. Consequences of donor organ ischemia on innate immune activation

During procurement of the donor kidney, ischemia results in DAMPs being released from dead and dying cells. The DAMPs serves as ligands for pattern recognition receptors that are present on RTE cells, interstitial dendritic cells (iDCs) and endothelial cells. This results in extension of renal tubular epithelial (RTE) cell death, activation of interstitial dendritic cells (iDCs) and injury to endothelial cells resulting in increased endothelial permeability, expression of adhesion molecules on endothelial cells. When the vascular supply is resumed upon anastomosis of donor and recipient vessels, the recipient immune system is activated by proinflammatory molecules and donor dendritic cells release from the donor organ.

Figure 2. Pattern recognition receptors expressed throughout the nephron

Murine and human pattern recognition receptors have been identified in different nephron segments, and appear to be differentially expressed throughout the kidney.