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Innate immunity in donor procurement

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Abstract

Purpose of review—Ischaemic kidney injury occurs during organ procurement and can lead to delayed graft function or nonviable grafts. The innate immune system is a key trigger of inflammation in renal ischaemia. This review discusses the components of innate immunity known to be involved in renal ischaemic reperfusion injury (IRI). Understanding how inflammatory damage is initiated in renal IRI is important for the development of targeted therapies aimed at preserving the donor organ.

Recent findings—Much remains to be determined about the role of innate immune signalling in renal ischaemia/reperfusion injury. Recently, discoveries about complement receptors, Toll-like receptors (TLRs), NOD-like receptors (NLRs) and inflammasomes have opened new avenues of exploration. We are also now learning that macrophages, complement and TLR activation may have additional roles in renal repair following IRI.

Summary—A greater understanding of the mechanisms that contribute to innate immune-mediated renal ischaemic damage will allow for the development of therapeutics targeted to the donor organ. New data suggest that treatment limited to specific receptors on specific cells, or localized to specific regions within the kidney, may provide novel approaches to maximize our use of donor organs, particularly those that may have been discarded due to prolonged preimplantation ischaemia.

Keywords

inflammasome; innate immune receptors; kidney ischaemia reperfusion injury; NOD-like receptor; Toll-like receptor

INTRODUCTION

All donors harvested for transplantation undergo obligate injury during procurement. In the case of deceased donors, the injury begins during donor brain death and is worsened during periods of both cold and warm ischaemia. The injuries sustained during renal hypoxia and

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Conflicts of interest

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reperfusion (so-called ischaemia/reperfusion injury or IRI) impair organ viability and increase the risk of rejection after transplantation.

An enormous amount of information generated from basic science laboratories indicates that the innate immune system is rapidly activated after an ischaemic insult. This costimulation-independent, phylogenically conserved defense system is highly expressed in human kidneys and plays a central role in mediating the earliest injury responses in the donor kidney. This review explores the processes by which the innate immune system becomes activated in donor kidneys harvested for transplantation, the inflammatory pathways that are induced, and discusses several novel targets for treating IRI associated with donor organ procurement.

INNATE IMMUNE SYSTEM

The innate immune system serves as the first line of defense against invading pathogens. Infectious microorganisms have highly conserved molecular structures (proteins, lipids and nucleic acids) that trigger the innate immune system by interacting with receptors constitutively expressed on immune and parenchymal cells, called pattern recognition receptors (PRRs). The pathogenic molecules recognized by PRRs are called pathogen-associated molecular patterns (PAMPs). Cells injured by hypoxia also release molecules with similar structural motifs that PRRs recognize. These are called damage-associated molecular patterns (DAMPs). Upon PAMP/DAMP detection by PRRs, inflammatory cytokines are released, leading to the recruitment of innate [monocytes/macrophages, neutrophils, natural killer (NK) cells, NKT cells] and adaptive (T and B cells) effector cells after restoration of blood flow.

CELLULAR EVENTS ASSOCIATED WITH RENAL ISCHAEMIC INJURY

Renal tubular epithelial (RTE) cells residing in the oxygen-sensitive region of the outer stripe of the medulla are highly susceptible to ischaemia [1]. These cells have been shown to undergo depolymerization of the actin cytoskeleton, leading to redistribution of Na^+/K^+ ATPase pumps and subsequent uncoupling of ATP and sodium transport [2]. An inability to maintain adequate ATP levels causes further injury and cell death [1]. In addition, tight junction function is lost, resulting in increased permeability and back leak of glomerular filtrate [1,3,4]. Endothelial cells are also affected by ischaemia. They undergo marked swelling and upregulation of adhesion molecules including P-selectin and E-selectin and intercellular adhesion molecule 1 (ICAM-1), promoting the recruitment/adhesion of inflammatory leukocytes during the reperfusion phase [5].

The complement components C3, C5 and C6 also contribute to ischaemic renal injury [6]. Complement stimulates upregulation of endothelial adhesion molecules, further facilitating inflammatory cell accumulation after reperfusion, leading to microvascular plugging that worsens ischaemia through a 'no-reflow' phenomenon [7]. Complement C3 expression on the donor also plays an important role in donor injury during ischaemia [8]. Complement inhibition in brain dead donors has been shown to improve graft function [9], and several targeted complement inhibition approaches are now being investigated [10*].

Following reperfusion, inflammatory leukocytes worsen the initial ischaemic damage. 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 [11]. Neutrophil recruitment is generally mediated by adhesion molecules including ICAM-1, selectins and CD11/CD18 [12]. Macrophages also accumulate in the kidney following IRI, although there have been conflicting studies over whether they are indeed detrimental [13–15]. A recent study [16^{***}] showed that the use of diphtheria-toxin (DTX)-CD11b treated mice did not protect from renal IRI, whereas clodronate depletion resulted in improved renal function. These results suggest that a protective macrophage population, in addition to the conventional inflammatory population, might exist.

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 [17]. 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 [17]. Blocking or depleting NKT cells has also been shown to prevent neutrophil recruitment and kidney injury, demonstrating a potential role of NKT function in neutrophil recruitment [17]. Although not a part of the innate immune system, Tregs play a vital role in suppressing the inflammation mediated by innate effector cells. Following injury, Tregs traffic to the kidney [18] and mediate suppression through the release of the anti-inflammatory cytokine IL-10 [19]. Mice lacking Tregs have greater numbers of inflammatory leukocytes in the kidney following injury and increased renal damage [18,19].

ACTIVATION OF INNATE IMMUNITY IN RENAL IRI

The innate immune response is triggered by PRR recognition of PAMPs and DAMPs. PRRs are divided into different families on the basis of structural homology: Toll-like receptors (TLRs) and NOD-like receptors (NLRs), which recognize endogenous and microbial particles; C-type lectin receptors (CLRs), which are important for antifungal immunity; and retinoic acid-inducible gene-like receptors, which participate in antiviral immunity [20]. Of the PRRs, the TLRs and NLRs are the best characterized in renal IRI.

During the course of IRI, endogenous molecules (DAMPs) are released from necrotic and damaged cells [21,22]. Intracellular DAMPs are products released by cells and include high-mobility group box 1 (HMGB1), heat shock proteins (HSPs) and purines [23]. Extracellular DAMPs are normally found in the basement membranes, but when degraded can induce inflammation (e.g. fibronectin, biglycan, heparin sulphate and hyaluronan [23–28]). It is not clear whether ischaemic inflammation is mediated by external pathogens (PAMPs) that have found their way into the injured area, rather than DAMPs. PAMPs present during ischaemic injury can include bacterial and viral constituents, such as lipopolysaccharide (LPS), unmethylated DNA, double-stranded RNA and flagellin [29]. PAMPs/DAMPs activate many different cell types, enabling the innate immune system to be activated using a limited number of PRRs.

TOLL-LIKE RECEPTOR EXPRESSION IN KIDNEY

Among the best characterized PRRs in renal IRI are the TLRs, specifically TLR2 and TLR4. Expression of multiple TLRs, including TLR1–4, TLR6, and TLR9, has been documented on RTE cells [30–33]. TLR2 and TLR4 are also expressed on endothelial cells and on podocytes [34,35^{***},36]. TLR3 has been found on glomerular mesangial cells [37,38], and TLR5 on bladder and distal renal epithelium [39]. In humans, TLR9 has also been found on RTE cells, on interstitial cells and within the glomerulus, particularly in models of nephritis [40]. TLR11 has also been found on distal RTE cells and the urinary bladder epithelium [41].

TOLL-LIKE RECEPTOR SIGNALLING

TLR signal transduction relies on a cytoplasmic Toll/interleukin 1 receptor (TIR), which serves as a docking site for other TIR-containing adaptor proteins, such as TIR domain containing adapter-inducing interferon- β (TRIF) and TRIF-related adaptor molecule (TRAM) [42,43^{*}]. Following triggering by PAMPs or DAMPs, all TLRs (with the exception of TLR3) form hetero- or homodimers and engage MyD88 directly or in combination with TRIF/TIRAP [22,42]. Further downstream, activation of MAPK and p38 leads to induction of genes encoding inflammatory chemokines (e.g. CXCL1, CXCL2), cytokines (e.g. IL-10, IL-12b, IL-1 α , IL-1 β), extra-cellular modelling molecules (e.g. MMP-13) and cell adhesion molecules (e.g. VCAM-1) [43^{*}]. The IKK and NF- κ B inflammatory pathways are also activated through TLR stimulation and include chemokines (e.g. CCL2, CCL3, CCL5), Cfb (complement factor b), inflammatory cytokines (e.g. IL-1 β , IL-6) and cell adhesion molecules (e.g. ICAM1, SELE, SELP). A comprehensive list can be found in the review by Newton *et al.* [43^{*}].

TOLL-LIKE RECEPTOR 2

TLR2 is constitutively expressed on RTE cells, glomeruli and renal vasculature [34]. It is upregulated following renal ischaemic injury, leading to increased cytokine and chemokine production [34,44]. In the absence of TLR2, there is decreased leukocyte recruitment and kidney injury [34,44]. The prominence of TLR2 activation in renal ischaemic injury makes it a promising target for treatment of IRI. Mice deficient in TLR2, antisense TLR2 oligonucleotides or pharmacological TLR2 inhibition provide protection from injury [34,44,45]. A deficiency in MyD88, an adaptor molecule downstream of TLR2, also affords protection from ischaemic injury in some studies [34]. Interestingly, TLR2-deficient mice are better protected than MyD88-deficient mice, suggesting that TLR2 signalling might activate inflammatory pathways independent of MyD88 [34]. Indeed, following stimulation by *Staphylococcus aureus*, TLR2 has been shown to initiate a signalling cascade involving Rac1, PI3K and Akt that activates NF- κ B independently of I κ B [46].

Both TLR2 and complement have been implicated in renal IRI, but it is not known whether the two synergize. Mice deficient in both cfb and TLR2 were not protected from IRI, but instead developed severe injury [47^{***}]. These results suggest that a protective mechanism might be mediated through either TLR2 or complement, as inhibiting both appeared to be detrimental to renal injury. TLR2 activation might be important in renal injury and

enhancing repair, as TLR2 activation led to the secretion of inflammatory molecules (e.g. MCP1, IL-6, IL-8, C3) and increased the proliferation rate of adult renal progenitor/stem cells [38]. TLR2 activation might also enhance stem cell differentiation into renal epithelial cells, suggesting that TLR2 has dual roles in renal ischaemia by promoting injury as well as repair [48].

TOLL-LIKE RECEPTOR 4

TLR4 is another major player in IRI. Similar to TLR2, TLR4 expression is increased on RTE cells following injury [49]. TLR4 deficiency alleviated ischaemic injury and decreased expression of proinflammatory cytokines/chemokines and leukocyte recruitment [35²²,49]. TLR4 can also be activated by DAMPs such as HMGB1 to produce IL-6 [35²²,50], which leads to further HMGB1 release, resulting in a feedback loop that perpetuates ischaemic injury [51²]. Histones released by dying RTE cells activate both TLR2 and TLR4 by inducing signalling through MyD88 and NF- κ B [52²²].

Although TLR4 is expressed on leukocytes and renal parenchymal cells, TLR4-induced renal injury might not depend on a single cell type [35²²]. TLR4 expression might also not occur concurrently on all cell types in the kidney following IRI. Endothelial cells from the outer medulla exhibited TLR4 upregulation at 4 h following reperfusion compared with 24 h for the RTE cells [35²²]. Variable TLR4 upregulation within the kidney suggests that TLR4 performs different functional roles at different times of renal IRI.

Many trends and correlations can be drawn between human and mouse studies regarding TLR4 expression. Kidneys harvested from deceased donors are associated with delayed graft function and exhibit upregulation of TLR4 and MyD88 [53²,54]. Some genetic polymorphisms in patient TLR4 (A299G/T399I) expression confer increased protection and improved graft function, due to the decreased ability to respond to LPS [54,55].

NOD-LIKE RECEPTORS

During donor organ procurement, intracellular PRRs also recognize PAMPs/DAMPs. These receptors are the nucleotide-binding, leucine-rich repeat containing receptors (NLRs). The NLR members contain variable caspase recruitment (CARD) or pyrin domains, a nucleotide-binding oligomerization (NACHT) domain, and a leucine-rich repeat domain [43²]. 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)[56]. The noninflammasome family mediates inflammatory function mainly through NF- κ B activation, whereas the inflammasome family induces inflammation through caspase-1 activation. CIITA functions as a transcription factor for MHCII expression [57].

NOD-LIKE RECEPTOR EXPRESSION IN THE KIDNEY

Both NOD and NLRP family members are expressed in murine and human RTE cells [58–60]. Cross-species differences exist, and kidney inflammasome expression levels are higher in mice than in humans [61²].

NOD1/NOD2

The best characterized NOD/noninflammasome family members in renal IRI are NOD1 and NOD2 [58]. The NOD family recognizes bacterial peptidoglycan and induces proinflammatory and antibacterial responses [62–64]. Upon activation, NOD1 and NOD2 undergo self-oligomerization followed by activation of adaptor molecule receptor-interacting protein 2 (RIP2/RICK) [62–64], activating the IKK/NF- κ B and p38/MAPK pathways [57,65]. NOD1 and NOD2 activation leads to production of proinflammatory factors (e.g. IL-6, CXCL3, CXCL1, MIP2, iNOS, Cox-2) and neutrophil recruitment, resulting in ischaemic tissue damage and cell death [62–64, 66–69].

NOD1 and NOD2 play a central role in ischaemia-induced inflammation. Disruption of either the NOD1 or NOD2 signalling pathway diminish inflammation in renal IRI, with NOD2 deficiency affording increased protection over NOD1 [58]. NOD1 and NOD2 are expressed on both leukocytes and parenchymal cells [58], and simultaneous elimination of both NOD1 and NOD2 affords increased protection over the single knockouts [58].

INFLAMMASOMES

The NOD-leucine-rich repeat pyrin domain containing (NLRP) or inflammasome NLR family plays an important role in renal IRI. The NLRP family forms inflammasome complexes by recruiting apoptosis-associated speck-like protein (ASC), and caspase-1 [61[¶]]. Unlike the TLRs or NODs 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 [61[¶]].

The inflammasomes are triggered by many different PAMPs/DAMPs. This makes them optimal sentinels for cellular stress, but the mechanisms behind how they actually sense ligands are unknown. Among the many microbial and endogenous molecules shown to activate NLRPs include ATP, ROS, uric acid, cholesterol crystals, hydroxyapatite crystals, flagellin and DNA [61[¶],70,71]. A review by Anders and Muruve [61[¶]] provides a more comprehensive list.

To date, the best studied inflammasome in renal IRI is NLRP3. Deficiency of NLRP3 protects mice from IRI [60]. Absence of other NLRP3 inflammasome components such as ASC and caspase-1, and factors further downstream such as IL-1 β or IL-18 did not prevent renal IRI to the same extent [60]. This indicates that NLRP3 contributes to ischaemic injury via a pathway independent of the conventional inflammasome mediated pro-inflammatory pathways and that possibly other inflammasome-induced signalling pathways exist.

PREVENTION/PROTECTIVE MOLECULES

There are several novel, promising targets to alleviate renal IRI. One involves the use of anti-inflammatory signalling molecules, such as synthetic adenosine agonists; they have shown protection in many IRI models including the heart, liver and kidney [72,73]. In the kidney, adenosine mediates protection through adenosine receptors such as A2b (Adora2b) on endothelial cells [74^{¶¶}], perhaps by decreasing TNF- α levels [75[¶]]. During hypoxia,

adenosine is available for endothelial A2b activation, but equilibrative nucleoside transporter 1 (Ent1) uptakes adenosine into the proximal tubule cells, opposing protective function of Adora2b. Ent1 can be inhibited by administration of dipyridamole, which leads to enhanced renal protection [74^{***}]. Adora2a is another adenosine receptor involved in protection from kidney IRI mediated through bone marrow derived cells [76,77]. Dendritic cell activation of NKT cells during IRI leads to the production of inflammatory cytokines and initiation of inflammatory cascades [17]. Mice with Adora2a-deficiency solely on dendritic cells sustain renal injury, even with the addition of Adora2a agonists. This indicates that dendritic cells play a central role in mediating NKT cell induced damage during renal IRI [78^{***}]. Treatment of wild-type dendritic cells with Adora2a agonist prevents NKT cell activation and IFN- γ production, even in the presence of NKT cell agonist α -galactosylceramide [78^{***}]. In addition, negative costimulatory molecules were upregulated on the dendritic cells, further suppressing NKT cell activation [78^{***}].

Hypoxia-inducible alpha (HIF-1 α) is another protective molecule that is upregulated during hypoxia. Under normal conditions, HIF-1 α is targeted for proteosomal degradation by the von Hippel-Lindau (VHL) protein, but during injury, HIF-1 α accumulates and activates angiogenesis, energy metabolism, cell proliferation/survival and vascular remodelling to facilitate tissue repair [79]. Inhibition of HIF-1 α in IRI aggravates ischaemic injury, whereas, its accumulation in the kidney protects against damage [80,81]. Indeed, both preconditional and postischemia HIF activation has been found to be beneficial in renal IRI [82]. In addition to the phase of HIF-1 α expression, the location of HIF-1 α expression contributes to its function as well. Systemic treatments of HIF-1 α confer protection during IRI, but transgenic mice with a deficiency of VHL, specifically in the thick ascending limb (TAL), demonstrate attenuated proximal tubular injury as well [83^{***}]. This indicates that specific parts of the kidney might eventually be targeted for treatment.

Another recent study showed that CD47 may also serve as a treatment target [84^{*}]. CD47 is expressed on all cell types and is a thrombospondin-1 (TSP1) receptor. TSP1 is secreted by RTE cells following reperfusion [85]. Both CD47 and TSP1 are upregulated in renal ischaemia, although CD47-deficient mice or inhibition of CD47 protects mice from IRI [84^{*}]. CD47 expression on parenchymal cells appears to be responsible for mediating injury, but the mechanism remains to be determined [84^{*}].

The field of renal IRI research is evolving, as new targets for intervention to mitigate renal injury are discovered. It is difficult to define specific targets for injury that occur to donor organs during the process of IRI because the cellular and molecular mechanisms that contribute to injury are complex. It is clear that innate immune mechanisms are triggered during donor organ procurement, and several potential therapeutic targets to abrogate the initial injurious events will likely emerge. One of these targets may be PRRs either constitutively expressed or regulated during renal IRI. A better understanding of the role of these receptors in renal IRI must be achieved before viable targeted approaches become available.

CONCLUSION

The innate immune system plays a pivotal role in the initial tissue damage and subsequent inflammatory storm following renal IRI. This is particularly important in the setting of kidney donation, as the donor kidney is highly activated, from an immunologic standpoint, by these early innate immune events. There are many questions to be answered before we can approach a reasonable understanding of the innate immune events that activate the donor organ. Achieving an understanding of the mechanisms underlying the initial injury, and also the mechanisms of repair, is critical for the development of therapeutic targets. Recent data indicate that some cell types and receptors may play dual roles by contributing to tissue damage as well as to repair, suggesting that systemic inhibition may not provide the optimal approach to blocking injury associated with ischaemic injury. A greater knowledge of the impact of innate immune activation on organ procurement is essential in the era of limited allografts. The development of therapeutic targets aimed at blocking some of the earliest triggers of renal IRI might provide a mechanism to allow utilization of donors with longer ischaemic times that would otherwise be discarded.

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KEY POINTS

- DAMPs (danger-associated molecular patterns) released by injured cells during donor procurement activate PRRs (pattern recognition receptors) that are expressed on many cell types within the donor kidney.
- Some of the best characterized PRRs in the kidney are Toll-like receptors (TLRs) TLR2 and TLR4, whose activation leads to the production of proinflammatory cytokines, chemokines and recruitment of inflammatory leukocytes.
- The intracellular noninflammasome family of NLRs include NOD1 and NOD2, which induce inflammation through NF- κ B activation. The inflammasome NLRP3 induces inflammation through caspase-1 activation.
- Recent targets identified for abrogation of renal IRI, at least experimentally, include increasing protective molecules such as adenosine and HIF-1 α to alleviate renal IRI.
- Many innate immune receptors and cell types serve as attractive targets for inhibition to alleviate renal IRI induced by procurement, but these receptors may also have important roles in kidney repair, and therefore, their reparative properties must be maintained.