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Innate and adaptive immune responses subsequent to ischemia-reperfusion injury in the kidney

Impact des lésions d'ischémie-reperfusion sur la réponse immunitaire innée et acquise

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Summary

Understanding innate immune responses and their correlation to alloimmunity after solid organ transplantation is key to optimizing long term graft outcome. While Ischemia/Reperfusion injury (IRI) has been well studied, new insight into central mechanisms of innate immune activation, i.e. chemokine mediated cell trafficking and the role of Toll-like receptors have evolved recently. The mechanistic implications of Neutrophils, Macrophages/Monocytes, NK-cells, Dendritic cells in renal IRI has been proven by selective depletion of these cell types, thereby offering novel therapeutic interventions. At the same time, the multi-faceted role of different T-cell subsets in IRI has gained interest, highlighting the dichotomous effects of differentiated T-cells and suggesting more selective therapeutic approaches. Targeting innate immune cells and their activation and migration pathways, respectively, has been promising in experimental models holding translational potential. This review will summarize the effects of innate immune activation and potential strategies to interfere with the immunological cascade following renal IRI. © 2014 Elsevier Masson SAS. All rights reserved.

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MOTS CLÉS

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Résumé

La compréhension des relations entre immunité innée et adaptative est essentielle pour optimiser les résultats de la transplantation d'organe.

En parallèle des mécanismes -déjà explorés - des lésions d'ischémie-reperfusion, le rôle de l'activation l'immunité innée et de ses effecteurs (récepteurs toll-like, cytokines, etc.) est un concept plus récent. Ainsi, de nombreux modèles expérimentaux de déplétion de types cellulaires ont prouvé l'implication des polynucléaires neutrophiles, des monocytes-macrophages, des cellules NK ou encore des cellules dendritiques dans le développement des lésions d'ischémie-reperfusion, suggérant de nouvelles perspectives thérapeutiques sélectives: cibler l'activation et la migration des effecteurs de l'immunité innée semble être une méthode prometteuse.

Notre travail fait le point sur les effets de l'activation de l'immunité innée en transplantation rénale et les différentes stratégies possibles visant à interférer avec la cascade immunitaire déclenchée par l'ischémie-reperfusion.

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Introduction

In the past years, transplantation research has emphasized on understanding the mechanisms of innate immune activation and their initiation of alloimmune responses in organ transplantation. While mechanisms of acute rejections (AR) have been well elucidated and treatment has been effective, innate immune activation early after transplantation has not been targeted successfully, yet. Thus, understanding the consequences of IRI on innate and adaptive immune activation appears critical for an early interference with with the activated immune cascade.

The innate immunity acts as the first line of defense against foreign tissue by detecting so called damage-associated or pathogen - associated molecular patterns (DAMP's, PAMP's) which bind to pattern recognition receptors (PRR) on APC's and other innate immune cells. Mechanical, thermal and pathogen induced cell damage cause a non-specific inflammatory response including TLR activation and proinflammatory cytokine release, thus starting a cascade of neutrophil, DC and T-cell activation [1]. Indeed, surgery itself, brain death and ischemia have all been shown to activate innate immune responses [1-3]. Thus, IRI serves as a non-specific inflammatory injury which elicits a coordinated alloimmune specific response against the graft.

Ischemia Reperfusion injury

IRI is characterized by ATP depletion, lack of glycogen and oxygen supply, all resulting into metabolic changes. As a consequence, renal tubular epithelial cells are injured, tissue resident leukocytes are activated and endothelial cell function is impaired leading to vascular leakage and interstitial edema. Following the upregulation of adhesion molecules (ICAM-1, P-Selectin and others), endo- and epithelial damage is furthermore accelerated by complement activation with subsequent increased cytokine levels resulting into the adhesion of leukocytes to the endothelium capturing red blood cells and platelets in turn [4-6]. Tubular epithelial cells, on the other hand, upregulate TLR-2 and TLR-4 and internalize

the complement inhibitory factor Crry which, in turn, causes deposition of complement, production of chemokines and recruitment of polymorph nuclear leukocytes (PMN's) [7,8].

Besides, reactive oxygen species (ROS) such as superoxide, peroxynitrite or H₂O₂ derived hydroxyl radicals facilitate further DNA damage and activate an ADP polymerase (PARP-1) causing considerable tissue injury. Various inflammatory cells express NADPH oxidases and act as a source of ROS, neutrophils being the most abundant [9]. A randomized clinical trial in kidney transplant patients demonstrated a beneficial effect of the free radical scavenger superoxide dismutase [10]. Amelioration of endothelial cell damage caused by free oxygen radicals lead to significantly improved long-term graft survival and reduced rates of acute rejection (AR) suggesting a causal relation between innate immune injury and chronic immune stimulation.

Furthermore, cell death programs such as apoptosis, autophagy-associated cell death and necrosis are initiated subsequent to IRI [11]. While necrosis causes further immune stimulation, apoptosis is supposed to cause less inflammation. Yet, there are recent reports of an apoptosis associated stimulation of monocytes and macrophages presumably also leading to innate immune activation [12].

Activation of innate immune cells

The innate immune response as a first line response is carried out by neutrophils, macrophages, Dendritic Cells (DC), NK and NKT-cells and T-cells. Following reperfusion, neutrophils adhere to the endothelium and migrate into the tissue. Neutrophils react immediately to unspecific injury, infiltrate the tissue and release proteases, oxygen-free radicals through degranulation and start producing proinflammatory cytokines such as IL-4, IL-6, IFN γ , TNF α [13]. In line with those findings, neutrophil depletion protected mice from IRI [14].

Similarly, macrophages exhibiting an activated phenotype and producing proinflammatory cytokines (IL-1 α , IL-6 IL-12, TNF α) can be found at very early stages of IRI [15,16]. Migration of monocytes/macrophages is mediated by various

chemokines/chemokine receptors, i.e. CX3CR1 or CCR2. Of note, CCR2 - deficient macrophages were unable to mediate injury [15,17]. Likewise, blockade of CX3CR1 and depletion of macrophages abrogated renal IRI showing that monocytes/macrophages play a key role in initiating an early innate response after acute kidney injury [18,19].

Besides, platelets interacting with endothelial cells become activated and cause subsequent activation of plasma factor XII, thereby enhancing the coagulative and inflammatory milieu [20]. The pro-inflammatory cytokine milieu further results into the activation of both, direct and indirect pathways of the complement system. Complement components are released both, systemically (liver, endothelium) as well as locally in the kidney and the deposition of C3, C6 and Mannose-binding Lectin can be detected during reperfusion and after transplantation [21,22]. Complement activation is not only a part innate immune responses but also impacts adaptive immunity. While B-cells are stimulated to produce antibodies, T-cell differentiation is influenced by complement components such as C3a, C5a or decay-accelerating factors modulating T-cell immunity [23-25].

NK cells are an essential part of the innate immune surveillance. NK cells have the capacity to extinct pathogens through granzyme, perforin and FasL dependent cell cytotoxicity. NK cells have been shown to play a central role in renal IRI as they can be detected within 4hrs after reperfusion in the kidney. Perforin dependent killing of tubular cells by NK cells is a major pathway of renal IRI [26]. Moreover, expression of CD137 on NK cells and its ligand on tubular epithelial cells seems crucial for the chemokine mediated recruitment of neutrophils to the site of inflammation [27]. Adoptive transfer of wild-type NK-cells into CD137 deficient mice, in turn, restored IRI, thus demonstrating a crucial role of NK cells in renal IRI. Besides, upon activation and IFN γ secretion in the draining lymph node, NK cells are able to provide fast priming of a Th1 response [28]. Of note, NK-cells interact with the adaptive immune response in many ways, especially through a reciprocal crosstalk with DC's [29]. Moreover, NK cells induce the maturation of DC, which in turn are potent inducers of T-cell activation. On the other hand, NK cells may induce lysis of an excessive amount of immature DC's, thereby preventing an unnecessary T-cell response [30]. Although NK cells are important in the early reperfusion phase and subsequent activation of both, innate and adaptive immune activation, they are unable to reject graft on their own as demonstrated in a murine skin transplant model [31].

DCs can be found within less than an hour after reperfusion of the graft and migrate thereafter into local lymph nodes presenting antigens to adaptive immune cells [32,33]. Following IRI, DCs undergo an antigen-independent maturation process induced by DAMPs and PAMPs. Of note, elevated numbers of DCs can be observed after syngenic renal transplantation [34]. Moreover, renal DCs aggravate IRI through the production of pro-inflammatory mediators such as TNF α , IL-6, MCP-1 and RANTES while DC depletion ameliorated local inflammation [35]. At the same time, DCs are a key link between innate and adaptive immune activation. Upon maturation, they are capable of clonal expansion of an

antigen specific immune response. DC maturation is induced following TLR engagement leading to the expression of a pro-inflammatory receptor profile (CCR4, CXCR4, CCR7), the upregulation of costimulatory molecules (CD80, CD86) and MHC Class II expression. In renal transplantation, oxidative stress induced by brain death caused activation of donor DCs with a subsequent activation of recipient T-cells [36].

Toll-like receptors

Innate immune cells such as monocytes, macrophages, and DCs become activated following PRR engagement, i.e. scavengers and Toll - like receptors (TLR). There is strong evidence that TLRs are essential in initiating the ischemia reperfusion injury.

The TLR family consists of 12 receptors in mice and 10 in humans which are expressed on innate immune cells; TLR and are activated through endogenous ligands (DAMPs) released from necrotic tissue (ATP, heat-shock proteins, high mobility group box - 1 (HMGB-1) or extracellular matrix (fibrinogen, hyaluronic acid, heparin sulfate) [37,38]. Following activation, downstream TLR signaling (except TLR3) is mediated *via* the MyD88 (myeloid differentiation primary response gene 88) and IFN regulatory factor 3 dependent pathway resulting in NF κ B activation and gene induction of various chemokines and cytokines [39]. These changes have been shown to be critical for the activation of naïve T-cells, thereby driving pathogen specific T-cell responses [40]. In mouse models of liver IRI, TLR-4 rather than TLR-2 has been identified as a MyD88 independent key mechanism mediating hepatic inflammation [38,41,42]. In contrast, TLR-2 and TLR-4 have been shown to play a role in murine cardiac IRI, with mechanistic implications of HMGB-1, MyD88 and TRIF in TLR-4 activation and signaling [43,44].

Likewise, TLR 4 signaling plays a major role in IRI after experimental kidney transplantation since both MyD88 and TLR4 deficient mice did not develop IRI [45]. Similarly, renal TLR 2 was shown to initiate inflammatory responses after IRI while TLR-2 antisense treatment protected mice from renal dysfunction and inflammatory effects [46].

In clinical transplantation, TLR 4 expression has been associated with early graft failure in patients undergoing kidney transplantation. Besides the fact that HMGB-1 expression was significantly higher in kidneys from deceased donors compared to those from living donors, kidneys with a functional TLR4 loss had a higher immediate graft function rate than kidneys with a TLR4 wild-type allele [47].

Interestingly, it has been demonstrated that MyD88 deficiency promotes allograft tolerance in a murine kidney transplant model, thereby suggesting a role of an altered Th17/Treg ratio in MyD88 deficient recipients [48]. Similarly, in a murine skin graft model, MyD88 associated abrogation of transplantation tolerance was observed [49]. Moreover, TLR activation has been shown to block the suppressive effects of regulatory T-cells, thus accelerating alloimmune responses [50]. These data highlight the importance of TLR activation and MyD88 signalling in IRI injury and its subsequent impact on the adaptive immune response.

T-cell activation following IRI

While activation of the innate immune system takes places within minutes, the adaptive immune response is generated after a few days. T-cells involved in either antigen-specific or antigen-unspecific responses play a key role in kidney IRI [51]. In contrast, mice lacking T- or B-cells are protected from IRI [52,53]. T-cells can be activated through free oxygen radicals, cytokines or RANTES in an Ag-unspecific fashion while Ag dependent T-cell activation involves presentation of antigens by B-cells, DCs or endothelial cells (reviewed in 54). Consequently, costimulatory blockade inhibiting T-cell activation ameliorated IRI in animal models [55-57]. It is well established that elevated levels of pro-inflammatory cytokines such as $\text{IFN}\gamma$, $\text{TNF}\alpha$, $\text{IL-1}\beta$, IL-2 or IL-6 are involved in T-cell mediated IRI [58]. $\text{IFN}\gamma$ seems to be playing a critical role as splenic T-cells contained more $\text{IFN}\gamma$ after IRI while $\text{IFN}\gamma$ deficient mice were protected from IRI [59-61]. The role of T-cells in IRI is furthermore supported by findings which demonstrate that various immunosuppressant attenuate IRI *via* T-cell inhibition. For instance, FTY720, FK506 and Mycophenolate Mofetil (MMF) decreased IRI in experimental transplant models [62-64].

Moreover, activation of T-cells also translates into long-term immunological and morphological changes subsequent to renal IRI. 6 weeks after murine renal ischemia, morphological deterioration had been associated with the accumulation of neutrophil and CD4^+ T-cell infiltrates. Interestingly, higher numbers of splenic $\text{IFN}\gamma$ positive T-cells suggested a systemic T-cell activation late after IRI [60].

Yet, T-cells may also have protective effects in renal IRI. Regulatory T-cells were shown to exert beneficial effects *via* a IL-10 dependent antagonization of $\text{TNF}\alpha$ and $\text{IFN}\gamma$ secreted by inflammatory cells in an experimental stroke model [65]. Mice deficient of IL-4 known to regulate Th2 differentiation but not IL-12 had a significantly worse graft function and advanced histological alterations suggesting deleterious effects of Th1 cells and beneficial effects of Th2 cells in renal IRI [66]. The adverse role of Th1 cells in renal IRI is furthermore supported by the observation of a predominant Th1 response in kidney transplant patients with Delayed Graft function [67]. However, data on the role of Th2 cells remain controversial since other studies have reported on opposite effects of Th2 associated cytokines in IRI [68]. Interestingly, CD8 T-cells have also been shown to augment the immediate innate response prior to T-cell priming [69]. Hours after graft reperfusion, $\text{IFN}\gamma$ producing CD8 T-cells were shown to regulate early inflammation through the activation of PMNs and endothelial cells.

Recently, IL-17 producing $\gamma\delta$ T cells have been shown to be detrimental in brain ischemia [70]. In this model, depletion of $\gamma\delta$ T cells attenuated IRI, suggesting that T-cell depletion may also be beneficial in transplantation associated IRI.

These studies highlight the multi-faceted role of T-cell subsets during all aspects of immune responses subsequent to IRI.

Targeting IRI

Ischemic preconditioning implies a first period of organ ischemia “tolerizing” the graft to a subsequent second ischemia period. Ischemic preconditioning in patients undergoing

major liver resection was superior over portal clamping and continuous inflow occlusion in protecting from postoperative liver injury [71]. In liver transplantation, ischemic preconditioning of the donor graft has been linked to lower ASAT and ALAT levels but has not improved early organ function [72].

Ischemic preconditioning has been successful in animal models of kidney transplantation, however it has not been translated into clinical transplantation yet. In a recent systematic review and meta-analysis of kidney animal models, ischemic preconditioning has been effective in reducing IRI, especially if conducted >24h prior to ischemia [73]. Yet, the transfer into the clinical setting may be difficult due to the heterogeneity of data, the influence of gender and animal strain differences as well as the unknown impact of co-morbidities and medications on the effects of ischemic preconditioning [74].

Nitric oxide (NO) and its effects on vascular tone and endothelial function have been utilized as therapeutic approaches. Patients inhaling NO during liver transplantation had a better restoration of liver function associated with a decreased apoptosis of hepatocytes [74]. Similarly, administration of nitrite stimulating NO signaling attenuated IRI in a rat kidney transplant model [75].

Adenosine is a well-known anti-inflammatory molecule, a modulator of lymphocyte responses and has been implicated in improved outcomes after acute kidney injury [76]. Activation of the Adenosine receptor A_{2a}R expressed on DCs lead to inhibition of $\text{NF}\kappa\text{B}$ and the transcription of pro-inflammatory cytokines. Recently, it has been shown that administration of the selective A_{2a}R agonist ATL313 attenuated renal IRI *via* tolerizing effects on DCs [77]. Similarly, ATL313 treatment at the time of reperfusion protected mice from liver IRI *via* effects on bone marrow derived cells [78]. Furthermore, ATL313 therapy also reduced alloimmune response after transplantation as skin allograft survival had been enhanced in mice [79].

Toll-like receptors are essential in mediating leukocyte activation and play a critical role in instigating early inflammatory response. Selective TLR 4 blockade by TAK-242 has been effective in attenuating early acute kidney injury in experimental models [80]. TLR 2 and 4 suppression was further implicated in mediating the protective effects of Serp-1, a serine protease inhibitor with anti-inflammatory capacities in a cardiac transplant model [81]. Interestingly, the combination of Serp-1 and subtherapeutic Cyclosporin doses lead to indefinite graft survival in a fully mismatched transplant model, indicating a role of Serp-1 in translating innate into adaptive immune responses. In contrast, a phase II clinical study in septic ICU patients treated with TAK 242 showed only a non-significant reduction in 28 day mortality rate [82].

Erythropoietin (EPO) has also been tested in preventing renal IRI. A study by Imamura et al. demonstrated that EPO increased hypoxia inducible factor-1 α (HIF-1 α) expression and attenuated tubular hypoxia [83]. In an additional study, it has been shown that carbamylated EPO promoted tubular cell proliferation and decreased tubular apoptosis in a rat model of renal IRI [84]. The protective effects of heme oxygenase 1 (HO-1) in renal IRI have also been tested. HO-1 induction by Cobalt-Proto Porphyrin was associated with significantly prolonged graft survival in a rat renal transplant model [85]. In a clinically relevant transplant model, HO-1 induction further attenuated the consequences of donor brain death and increased graft survival [86].

Targeting apoptosis has been another attempt to attenuate IRI. In rodent kidneys, IRI was significantly reduced in mice lacking TSP-1, a matricellular protein causing apoptosis [87].

Conclusion

Dissecting mechanisms of IRI has prompted studies to improve cellular resistance to hypoxia. The growing interest in innate immune responses as the first line of attack following organ transplantation has broadened the understanding of a continuous immunological cascade from IRI to chronic adaptive immune responses. While T-cell research has been in the center of interest for many years, elucidating the functional roles of monocytes/macrophages, NK-cells, DCs and their activation patterns has shed light on novel treatment options. Experimental studies using knock-out animals have revealed interesting opportunities to selectively interfere with the early innate response, thus attenuating antigen-specific immune activation. A better understanding of the role of specific T-cell subsets in IRI, may harbor further potential for cell therapies. While it has become clear that innate responses subsequent to IRI are playing a critical role for the success in organ transplantation, clinical studies in addition to an improved understanding of mechanisms are warranted.

Disclosure of interest

The authors have no conflicts of interest to declare in relation to this review.

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