

Immune mechanisms of ischemia-reperfusion injury in transplantation*

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ABSTRACT

All allografts suffer a number of unavoidable ischemic insults. These, starting with brain death and ending with reperfusion, are very troublesome, as ischemia-reperfusion injury (IRI) is demonstrated to be a major cause of allograft damage in various types of transplantations. To counter the threat this poses to allograft function, investigators have worked diligently over the past decades in clinical settings and in the laboratory to understand the pathophysiology and immune mechanism underlying IRI hoping to ultimately devise strategies that lessen its detrimental effects on allografts. Herein, we review the major immune components of the IRI dynamic process. Better understanding of the cellular pathophysiological processes underlying IRI will hopefully result in the design of more targeted therapies to prevent the injury, hasten repair, and minimize chronic progressive allograft damage.

Keywords: Ischemia-Reperfusion; Reactive Oxygen Species; Allograft Damage; Solid Organ Transplantation; Immune Mechanisms

1. INTRODUCTION

During transplantation procedures, allografts are exposed to various periods of complete ischemia; ischemic insults start with brain death and its associated hemodynamic disturbances (elevated intracranial pressure; bradycardia; decreased cardiac output) continue during donor organ procurement, cold preservation, and implantation. Following reperfusion, ischemia-reperfusion injury

(IRI) is triggered; this could potentially lead to allograft damage (delayed graft function, acute and chronic rejection), posing serious threats to transplant recipients. Along the cascade of pathogenic events that accompany ischemic insults and cause IRI, there has been an appreciation for various immune mechanisms within the allograft itself and their role in priming the allograft for further injury. Free radical-mediated injury releases pro-inflammatory cytokines and activates the innate immune system ultimately triggering adaptive immune responses and resulting in tissue damage. The outcome of the organ depends on whether cell death or regeneration prevails. The aim of this review is to revisit these immune mechanisms at the cellular and molecular levels, and provide useful clinical recommendations aiming at overcoming the challenges of IRI.

2. BODY

2.1. General Overview of Immune Injury in Ischemic-Reperfusion

Studies in solid organ transplantation (SOT) have shown that IRI is a potent activator of the immune system, and therefore leads to poor functional outcomes that are directly related to increasing ischemia times [1-4]. Yet ischemia is only one of several factors shown to contribute to acute and chronic rejection, as reperfusion injury further propagates and intensifies the immune response [5]. Specifically, the restoration of blood flow to the previously ischemic allograft compromises the viability of the transplanted tissues via the generation of reactive oxygen species (ROS), activation of the complement system, and the production of pro-inflammatory cytokines that intensify damage in the graft [6]. Furthermore, the expression of MHC antigens is increased after IRI with a concomitant activation of the innate immune system and production of an inflammatory state

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that ultimately leads to acute rejection, increased propensity towards chronic graft deterioration, and decreased potential for tolerance induction and immune regulation [5].

The strong inflammatory response induced by IRI both activates the immune system and mediates tissue injury via activation of leukocytes and endothelial cells, generation of ROS, and upregulation of adhesion molecules and inflammatory cytokines [7]. Cell adhesion molecules on activated leukocytes interact with their ligands on the injured endothelium of the previously ischemic graft, which leads to diapedesis of these cells into the interstitial space. The activated leukocytes also release proteolytic enzymes and generate ROS thereby damaging tissues and propagating the injury response. Notably, the duration of ischemia is directly correlated to the intensity of immune activation, with increased ischemic times corresponding to increased expression of inflammatory cytokines, such as IL-1, IFN- γ , and TNF- α [7,8]. Ischemia causes further damage to the allograft as oxygen-starved tissues increase glycolysis leading to lactic acid accumulation, which reduces the pH and impairs membrane transport functions [9]. If perfusion is not restored, the ischemic tissue will undergo necrosis; but on the other hand, reperfusion causes production of ROS in the mitochondria which leads to injury beyond that caused by the ischemia [10].

2.2. Role of the Innate Immune System

The activation of the immune system is mediated by endogenous stress signals and a class of primitive proteins expressed by the innate immune system. Specifically, ischemically injured tissues generate damage-associated molecular patterns (DAMPs), which are recognized by pattern-recognition receptors (PRRs) of the innate immune system leading to upregulation of inflammatory cytokines and cell adhesion molecules [11]. This contributes to the recruitment of leukocytes into the graft and, ultimately, to transplant vasculopathy [11,12]. The innate immune system is further triggered by the generation of ROS, which activate heat shock proteins (HSP) that signal toll-like receptors (TLRs) on macrophages and B cells, increasing the propensity towards acute and chronic rejection [13]. Notably, the link between non-specific injury, such as IRI, and the innate immune system is still speculative, and although there is biological plausibility, unequivocal evidence does not yet exist [14].

2.3. Role of the Adaptive Immune System

The adaptive immune system also contributes to the rejection process with the participation of TLRs that sensitize and activate antigen-presenting cells (APCs). The sensitization of APCs leads to a significant increase of

effector T cells that further augments the pro-inflammatory cytokine milieu induced by IRI [12,15,16]. An elegant explanation came from Matzinger conducted a series of elegant experiments to investigate the relationship between tissue damage, innate immune responses, and relaying danger signals to the adaptive immune system via TLRs and APCs [17]. These “danger signals” or “alarmins” released during ischemia and reperfusion consist of various graft-derived molecules [DNA, RNA, oxidized proteins and lipids, high-mobility group box-1 (HMGB1), uric acid, and calcium pyrophosphate crystals] [18]. Recent experiments have proven that HMGB1 is actively secreted from at-risk cells via a free-radicals-dependent pathway in the context hepatocyte ischemia-reperfusion. This process requires intact TLR4 signaling and calcium-dependent kinases [19,20]. In another setting, TLR4 is overexpressed in tubular epithelial cells following IRI. Furthermore, TLR4 $^{-/-}$ and MyD88 $^{-/-}$ (MyD88 being TLR4 signaling pathway protein) mice were protected from IRI kidney dysfunction, with no tubular damage, neutrophil and macrophage accumulation, and expression of proinflammatory cytokines and chemokines [21]. Evidently, TLRs and ROS play a dual immunological role, activating the innate and adaptive immune system, and potentially contributing to both acute and chronic rejection.

2.4. Role of the Complement Pathway

Activation of the complement pathway is another critical event leading to tissue injury after IRI. Indeed, complement is considered a key determinant of tissue rejection after SOT as it damages cell membranes through the formation of membrane attack complexes [6,7,9,22, 23]. The byproducts of the complement cascade also contribute to tissue rejection, as chemotactic agents such as C5a attract neutrophils to sites of IRI and anaphylatoxins (C3a, C5a) cause degranulation of mast cell and the release of histamine, which further damages tissues.

2.5. Contribution of the Alloimmune Response

Although the primary factor leading to transplant rejection is undoubtedly the T cell alloimmune response triggered by MHC incompatibility, IRI further intensifies this response, and is believed to be the strongest secondary factor to augment graft allogenicity [24,25]. The danger signals produced by ischemic injury induce an elegant interaction of immune activation and signal transduction that predisposes transplanted tissues to immunologic recognition and rejection through upregulation of MHC II (signal 1) and costimulation (signal 2) by activation of APCs [4]. Each episode of acute rejection induces an inflammatory state that is detrimental to

long-term outcomes of the allograft, as multiple episodes of acute rejection trigger myointimal proliferation, allograft vessel occlusion, and chronic graft deterioration [4,5,26-29]. This is particularly true in vascularized composite allotransplantation, where the highly immunogenic skin component is at high risk for multiple episodes of acute rejection, and this has been shown to lead to composite tissue vasculopathy and degeneration in a rat hind limb transplantation model [30]. Therefore, IRI induces an immune response, which propagates further injury, thereby promoting a new injury response and increased immune recognition [31]. This self-perpetuating feature may lead to irreversible tissue damage and chronic rejection. In this regard, further research in novel immunomodulatory protocols aiming at minimizing immunosuppression post transplantation holds great promise, as not only it alleviates the many metabolic (and neoplastic) side effects of high-dose immunosuppression [32] but also seems to offer substantial immune protection from IRI.

2.6. Direct Implication of Toll-Like Receptors (TLRs)

A clear relationship between the duration of ischemia and allograft survival has been demonstrated in large clinical trials of SOT, and it is hypothesized that this association is mediated by TLRs that are activated by ROS generated by IRI [5,33]. Ample evidence exists implicating TLRs as key contributors to the rate of acute rejection in heart [34], kidney [21], liver [3], lung [5], skin [35], and islet allografts [36]. In clinical studies of cardiac transplantation, TLR gene expression was found to be associated with endothelial dysfunction and vasculopathy [12]. Notably, Genome-wide association studies (GWAS) have shown that transplant recipients with less responsive TLR genes experience improved immunological outcomes illustrated by fewer rejection episodes and improved graft function [1,2,37]. Noris *et al.* studied the role of TLR regulation on allograft survival in a fully MHC-mismatched kidney allograft model, and found that absence of a negative regulator of TLRs led to a more vigorous acute rejection response with enhanced IRI and rapid induction of DC maturation [38]. In the same study, induction of transplant tolerance was impaired and the rate of chronic rejection was amplified due to the absence of this negative regulator of TLRs. Similar to the GWAS studies in cardiac transplantation, human kidney allograft recipients with unresponsiveness to their allografts expressed lower levels of MyD88 (a TLR signal adaptor) than their counterparts that experienced chronic rejection, further implicating TLRs as a key determinant in the rejection process [39]. Along these lines, Walker *et al.* was able to demonstrate this link utilizing a fully MHC-mismatched model of skin allo-

grafts in MyD88 knockout mice [38]. In this study, MyD88 knockout mice experienced indefinite allograft survival after administration of costimulatory blockade (CTLA4-Ig and anti-CD154), while wild-type animals rejected their allografts at an early timepoint. Similarly, systemic administration of a TLR activator (CpG) successfully mitigated tolerance induction in skin allografts [40], which together strongly suggests that TLR signaling impairs transplant tolerance [41]. Therefore, if the consequences of IRI can be mitigated, it may be possible to limit or prevent the activation of the immune system that leads to acute and chronic rejection. Novel biomarkers of IRI taking into advantage the ubiquitous involvement of IRI have a great potential in predicting the development and severity of IRI, potentially serving also as prognostic indicators [42].

2.7. Detrimental and Independent Role of Prolonged Ischemia

Much information has been gleaned from elucidation of the molecular foundations of IRI, and this has served to inform our understanding of the effects of IRI at the macroscopic level.

In cardiac transplantation, for example IRI commonly occurs in the early post-transplant period, especially with prolonged ischemic time, and is characterized by hyperemia in the previously ischemic myocardium, which later becomes prone to coagulative necrosis [43]. Prolonged ischemia in cardiac tissue promotes increased entry of activated T cells, leukocyte migration and accumulation in peripheral tissues, as well as binding of natural IgM antibodies to self-antigens exposed after tissue ischemia—a potent activator of the complement cascade [17,44]. Consequentially, cardiac transplant recipients subjected to prolonged ischemic times are at increased risk for early graft loss, coronary artery vasculopathy, and early death after transplant [45]. Indeed, large clinical trials have confirmed that prolonged ischemia is an independent risk factor for mortality at 1 and 10 years post-transplantation [45].

In kidney transplants, allografts exposed to prolonged ischemia were prone to more acute rejection episodes in animal models and subsequent clinical trials observed the same effect on human renal transplants [24]. In fact, analysis of more than 6000 kidney transplant recipients revealed that patients with prolonged ischemic times suffered increased early acute rejection episodes and decreased 6-year renal allograft survival—findings that were independent of HLA mismatch, panel reactive antibodies, donor/recipient age, and early rejection treatments [46]. Notably, acute rejection episodes were determined to be a significant risk factor for short and long-term graft survival [47].

In lung and liver allografts, IRI leads to higher inci-

dences of acute and chronic rejection [5,27]. Although there are very few studies examining IRI in vascularized composite allotransplantation, it is plausible that vascularized composite allografts may be even more susceptible to IRI given the diversity of tissue components contained within the graft [48].

2.8. Prevention of IRI

In an era where organ shortage is a universal problem with high rates of death among patients on waiting lists, measures to prevent IRI and ensure healthier allografts and safer transplantation procedures are critical. Kidneys recovered from donors should be stored using pulsatile perfusion, allowing better protection during preservation-related ischemia, as well as the measurement of several parameters—flow, resistance, lactate excretion, alfa GST—which may be useful to assess the extent of ischemic injury. Prevention of IRI can even be started before organ recovery by donor pretreatment. Pretreatment with antioxidants holds great promise conferring protective effects against liver IRI in a rat and mice models [49,50]. Moreover, the role of hemoxygenase-1 (HO-1, enzyme converting heme into biliverdin, carbon monoxide, and free Fe) has been extensively studied in protection from ischemia-reperfusion injury. Exposure of liver transplant recipient animals to inhaled CO decreased serum alanine transferase, hepatocyte necrosis, and neutrophil infiltrates in dose-dependent fashion [51]. Noteworthy, HO-1 can be induced by simvastatin preconditioning [52]. Interestingly, through its strong anti-inflammatory effect, nicotine has been shown to reduce tubular damage in experimental models of warm ischemia when administered before reperfusion [53]. As for myocardial ischemia-reperfusion injury, it was shown that the use of the bifunctional platelet GPIIa49-66 ligand confers dose-dependent protective effects in a rat model of acute myocardial ischemia [54].

In the human setting, it has been shown that cyclosporine inhibits permeability transition and, when administered on reperfusion, decreases creatine kinase release and infarct size in humans undergoing percutaneous coronary intervention for acute cardiac ischemia [55]. This needs thorough evaluation in transplant models.

Evidently, a greater understanding of the molecular mechanisms underlying protective pathways will pave the way for clinical trials aiming at testing different strategies for minimizing IRI.

3. CONCLUSION

IRI is a real threat to the success of transplantation and although some period of ischemia is unavoidable, attempts should be made to minimize it to the greatest extent possible. Clear evidence exists, linking prolonged

ischemia to increased episodes of acute and chronic rejection, yet we still do not have a clear understanding of what areas of the immune system can be targeted to mitigate the effects of IRI. Future research trying to bridge this knowledge gap and identify such targets in both the innate and adaptive immune system, as well as characterization of cytokine expression responsible for mediating the effects of IRI may enable longer allograft survival and a better patient quality of life.

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